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# Presentation's effect of granulated or wet barley during the finishing phase on the productive yield of Majorcan Black Pig

J. Jaume\*, S. Joy\* and J. Gonzalez\*\*

\*IBABSA, Esperanto, 8, 07198 Palma de Mallorca (Spain)

\*\*IRTA-Food Technology, 17121 Monells (Spain)

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**Abstract.** Majorcan Black Pig is an autochthonous breed in danger of extinction which is preserved due to its use to elaborate Sobrassada de Mallorca. Sustainability of this production system depends on the economic margin which is generated, thus the use of raw materials avoiding industrial processing is of a great importance. 20 castrated males were distributed in two batches, each one in a 1.5 ha plot placed contiguous, with an initial weight of 105 kg and during 86 days. The experimental design considered as the fixed effect the way the feed was presented: barley that was moistened in a water container during 24 hours vs dry granulated barley. In both cases was used the same barley origin and composition. Animals fed by dry granulated barley showed higher growth rates than those fed by moistened barley (543 vs 471 g/day,  $p < 0.05$ ) and higher feed intakes (2.76 vs 2.55 kg/day,  $p < 0.05$ ). Despite of these results regarding productive efficiency, the economic benefit generated by feeding the animals with moistened barley was 34 € per finishing pig.

**Keywords.** Majorcan Black Pig – Sustainability – Moistened barley.

## **Effet de l'alimentation au cours de la phase de finition avec de l'orge humide ou de l'orge granulée sur le rendement de production du porc noir de Majorque**

**Resumé.** Le porc noir de Majorque est une race autochtone menacée d'extinction qui est utilisée dans la préparation de la Sobrassada de Majorque. La durabilité de leur production dépend de la marge économique obtenue pour la viande et la graisse de ces animaux. À cet égard, et afin de réduire l'impact économique de l'alimentation animale, il est essentiel d'utiliser des matières premières sans transformation. Nous avons utilisé un total de 20 mâles castrés, divisé entre deux parcelles adjacentes similaires et de 1,5 ha dans la zone, avec un poids de départ de 105 kg dans un essai de 86 jours. Le traitement expérimental a été mené dans un seul but, la présentation de l'orge (hydratée pendant 24 h ou granuleuse et sèche). L'origine et la composition de l'orge étaient les mêmes dans les deux traitements. Les animaux nourris avec des granulés secs d'orge ont montré une plus forte croissance (543 g/j vs 471,  $P < 0,05$ ) et consommé plus de nourriture (2,76 kg/j vs 2,55  $P < 0,05$ ). Il n'y avait pas de différences significatives concernant les taux de conversion. En dépit de ces résultats techniques, en termes économiques, la marge brute par porc générée par le régime avec de l'orge trempée était supérieure de 34 € à la marge obtenue par l'orge granulée.

**Mots-clés.** Porc noir de Majorque – Durabilité – Orge humide.

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## **I – Introduction**

The Majorcan black pig is a breed in danger of extinction and is preserved by its use in developing Sobrassada de Mallorca. Porc Negre is profitable for the farmer (Jaume *et al.*, 2006). Its production is extensive and is characterized by the use of endogenous resources in their diet, both as feeder grazing. The grass is supplemented with cereals and legumes. Cereals are usually subjected to a grinding treatment, transformed into flour. This process has an economic cost that is offset by the increased palatability and digestibility of the meal in front of whole grains (Valencia *et al.*, 2008). Milling cost is not insignificant and can make about 10 cents per kg. Sustainability of this production system depends on the economic margin which is

generated, thus, the use of raw materials without processing industry, such as wet barley, is of great importance. The aim of this work was to study presentation's effect granulated or wet barley during the finishing phase on the productive yield of Majorcan Black Pig.

## II – Materials and methods

Diets are composed of barley and peas in the proportions (g/100 g; fresh matter basis) of 80:20. The dietary treatments consisted of two presentations of barley granulated (M) and wet for a day (B). They have established two experimental groups with 10 barrows Majorcan Black Pig bread each. At the start of the test the average weight of animals was 105 kg and they were distributed in two batches, each one in a 1.5 ha plot placed contiguous during 86 days. The experimental design considered as the fixed effect the way the feed was presented: barley that was moistened in a water container during 24 hours vs. dry granulated barley. In both cases was used the same barley origin and composition. The animals were weighed individually at the beginning (Pi) and at the end of the test (Pf) and food supplied daily to each group (Co). We used student t-test statistical package Stratgraphics to analyze whether the differences between batches are significant. It takes  $p < 0.05$  as a minimum level of significance.

## III – Results and discussion

All the pigs remained in good health throughout the experimental period. The presentation's effect are presented in Table 1. During the finishing period (86 days) animals fed by dry granulated barley grew faster than those fed by moistened barley, so the average daily gain (GMD) in group M was 543 g/day and in group 471 g/day. Feed intake was significantly higher in group M than in B (2.76 vs 2.55 kg/day). Consequently, feed conversion ratio (IC) of group M was significantly better (5.21) than those observed in pigs of group M (5.54).

**Table 1. Effect of the presentation of barley in growth performances**

Treatment	N	Pi (kg)	Pf (kg)	GMD (g/day)	IC
M	10	105	151.57 <sup>a</sup>	530.34 <sup>a</sup>	5.21 <sup>a</sup>
B	10	105	145.78 <sup>b</sup>	460.00 <sup>b</sup>	5.54 <sup>b</sup>

a,b Different superscripts across rows indicate significant differences.

It has been estimated gross margin per animal using the technical data of Table 1, and the prices of whole barley (0.130 €/kg) and barley granulated (0.228 €/kg). So it turns out, that gross margin is 34 € more in animals fed wet barley.

## IV – Conclusions

Under the conditions of the present experiment, the use of wet barley has not produced better results in the final growth of the Majorcan Black Pig than barley granulated. Yet economic performance has shown the opposite trend and the gross margin is higher in the case of barley wet. This aspect is very important from the standpoint of sustainability of Majorcan Black Pig.

## Acknowledgements

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# Effect of packaging material on volatile organic compounds (VOCs) of sliced and MAP packaged typical Italian and Spanish dry-cured hams

M.A. Latorre\*, A. Pinna\*\*, A. Montanari\*\* and R. Virgili\*\*

\*Facultad de Veterinaria, Universidad de Zaragoza (Spain)

\*\*Stazione Sperimentale per l'Industria delle Conserve Alimentari, Parma (Italy)

**Abstract.** The volatile organic compounds (VOCs) of Parma and Teruel dry-cured hams were studied, by comparing fresh and MAP (modified atmosphere packaging) sliced products. Two polymeric food-grade packaging materials were used for ready-to-eat packages of pre-sliced dry-cured hams: PET (polyethylene terephthalate) with barrier film PET/EVOH/PE (polyethylene terephthalate / ethylene-vinyl alcohol / polyethylene copolymer) and PLA (polylactic acid) with PLA barrier film. Several differences in VOCs were attributed to dry-cured ham nature, with ethyl esters being a main feature of Parma ham, while compounds generated by amino acids catabolism prevailed in Teruel ham. Packaging in PLA increased VOCs originated from lipid oxidation, more abundant in Parma hams, and from amino acid catabolism, while packaging in PET increased the signals due to branched-chain alkanes (BCAs). VOCs originated from carbohydrate fermentation were negligibly affected by packaging material, while a dependence from the nature of dry-cured ham was observed.

**Keywords.** Dry-cured ham – Packaging material – Volatile organic compounds – Polylactic acid.

**Effet du matériel de conditionnement sur les composés organiques volatils (COV) des jambons italiens et espagnols en tranches et conditionnés en atmosphère modifiée**

**Résumé.** Les propriétés du jambon de Parme et du jambon de Teruel en termes de composés organiques volatils (COV) ont été comparées, par comparaison entre les produits frais en tranches et les produits emballés en atmosphère modifiée. Pour le conditionnement du jambon prétranché on a utilisé deux matériaux d'emballage de qualité alimentaire, consistant en deux polymères différents: PET standard (polyéthylène téréphthalate) avec barrière PET / EVOH / PE (polyéthylène téréphthalate / éthylène-alcool vinylique / copolymère de polyéthylène) et PLA (acide polylactique) avec des films barrière en PLA. Plusieurs des différences de COV observées sont attribuées à la nature du jambon, notamment les esters éthyliques qui caractérisent le jambon de Parme, et au contraire, les composés produits par le catabolisme des acides aminés ont plus d'impact dans le Jambon de Teruel. L'emballage en PLA a montré une augmentation des COV originaires de l'oxydation des lipides et du catabolisme des acides aminés, alors que l'emballage en PET a causé une augmentation de l'intensité d'alcanes à chaîne ramifiée (BCAs). Les COV provenant de la fermentation des glucides ont été négligeablement affectés par le matériel d'emballage, tandis que leur dépendance par rapport à la nature du jambon a été démontrée.

**Mots-clés.** Jambon – Matériaux d'emballage – Composés organiques volatils – Acide polylactique.

## I – Introduction

Protective atmosphere packaged (MAP) pre-sliced dry-cured hams gained in recent years a positive commercial trend (+7.8% in 2009, source: Consortium of Parma ham, 2010). Recent studies (Parolari *et al.*, 2009) reported the occasional onset of changes in MAP packaged dry-cured hams, impairing colour, odour and taste. The standard packaging material used for dry-cured ham is the food-grade polyethylene terephthalate (PET). Meanwhile, new materials have been investigated, taking into account environmental sustainability too. Among them, the biopolymer polylactic acid (PLA) has grabbed attention because it is synthesized from

processed corn and it biodegrades after use. PLA is regarded like a "natural" package with good flavor retention; a limiting factor for PLA is its relatively poor barrier to water vapor and O<sub>2</sub> (Rhimi *et al.*, 2009). In this study, the VOCs were investigated, to focus differences due to ham nature and packaging.

## II – Materials and methods

Four Parma (P) and 4 Teruel (T) dry-cured hams, aged 17-20 months, were sliced and packaged in PLA and PET with N<sub>2</sub>:CO<sub>2</sub> = 70:30 MAP. Each package was filled with 85-90 g of 15 mm-thick slices. VOCs analysis was carried out on fresh and packaged slices (starting 2 weeks after packaging): 3 g, finely cut with a knife, were subjected to HS-SPME-GC-MS with a CAR/PDMS/DVB fiber (Supelco) for 120 min at 40°C, according to the method of Pinna *et al.* (2009). Data were checked for normal distribution and analyzed by the General Linear Model (GLM) procedure of SPSS ver. 11.5. The calculated model included ham nature and packaging as main effects. The Least Square Means (LSM) were estimated and the Bonferroni t-test was performed to statistically separate them. Principal Component Analysis (PCA) was run and scores of ham samples were graphically plotted onto the PC1-PC2 plane.

## III – Results and discussion

Aroma compounds are reported in Table 1 and grouped according to origin mechanism.

**Table 1. Effect of ham nature and packaging type on VOCs (expressed in AU × 10<sup>-4</sup>)**

Volatile compounds <sup>†</sup>	Ham nature (N)		Packaging (P)			Significance			Loadings <sup>††</sup>	
	Parma	Teruel	None	PET	PLA	N	P	O x P	PC1	PC2
<i>Lipid Oxidation</i>										
1-Pentanol	95.8 <sup>a</sup>	46.9 <sup>b</sup>	54.6 <sup>b</sup>	54.8 <sup>b</sup>	105 <sup>a</sup>	***	***	ns	0.63	0.04
Pentanal	72.4 <sup>a</sup>	30.6 <sup>b</sup>	51.2	49.2	54.3	***	ns	ns	0.71	0.04
Hexane	9.70	5.46	7.94	7.76	7.14	*	ns	ns		
1-Hexanol	152	88.6	69.5	119	172	*	*	ns	0.52	0.29
Hexanal	440 <sup>a</sup>	143 <sup>b</sup>	225	248	401	***	*	ns	0.79	0.16
Heptane	27.6 <sup>a</sup>	15.1 <sup>b</sup>	17.1	17.8	29.2	**	ns	ns	0.66	0.05
Heptanal	152 <sup>a</sup>	63.8 <sup>b</sup>	89.7	98.7	136	***	ns	ns	0.79	0.17
2-Heptenal	7.23 <sup>a</sup>	2.82 <sup>b</sup>	4.69	2.99	7.39	**	ns	ns		
3-Heptanone	173	136	97.6 <sup>b</sup>	116 <sup>b</sup>	250 <sup>a</sup>	ns	***	ns		
Heptanoic Acid	268 <sup>a</sup>	103 <sup>b</sup>	139 <sup>b</sup>	159 <sup>ab</sup>	254 <sup>a</sup>	***	**	ns	0.86	0.19
Octane	184.3 <sup>a</sup>	97.1 <sup>b</sup>	100.4	119.6	201.9	**	*	ns	0.66	0.14
Octanal	54.3 <sup>a</sup>	26.5 <sup>b</sup>	41.1	39.2	41.0	***	ns	ns	0.70	0.11
1-Octen-3-ol	54.1 <sup>a</sup>	29.9 <sup>b</sup>	17.8 <sup>b</sup>	30.3 <sup>b</sup>	78.0 <sup>a</sup>	**	***	ns	0.59	0.11
Nonane	5.19 <sup>a</sup>	3.17 <sup>a</sup>	2.88 <sup>b</sup>	3.48 <sup>b</sup>	6.20 <sup>a</sup>	**	***	ns		
2-Nonanone	12.9	9.51	9.53	8.32	15.8	*	ns	ns	0.50	-0.06
2-Decenal	12.2 <sup>a</sup>	4.1 <sup>a</sup>	4.49 <sup>b</sup>	8.53 <sup>ab</sup>	11.4 <sup>a</sup>	***	***	*	-0.38	0.76
Undecane	18.5	24.5	12.1 <sup>b</sup>	33.9 <sup>a</sup>	18.5 <sup>ab</sup>	ns	***	ns	0.63	0.04
<i>Carbohydrate fermentation</i>										
Ethanol	679 <sup>a</sup>	317 <sup>b</sup>	410	485	762	***	ns	ns	0.62	0.28
Acetic Acid	78.2	63.8	63.6	57.8	91.6	ns	*	ns	0.62	-0.08
2-Butanone	40.3 <sup>b</sup>	77.0 <sup>a</sup>	66.0	60.1	49.9	***	ns	ns	-0.64	-0.29
2,3-butanedione	3.27 <sup>b</sup>	4.67 <sup>a</sup>	3.63	3.71	4.56	**	ns	ns		
3-hydroxy-2-butanone	99.1 <sup>b</sup>	165.9 <sup>a</sup>	152.4	138.1	107.0	**	ns	ns	-0.57	-0.03
Butanoic Acid	163 <sup>a</sup>	104 <sup>b</sup>	132	117	151	***	ns	ns	0.71	-0.07

**Table 1. (cont.) Effect of ham nature and packaging type on VOCs (expressed in AU × 10<sup>-4</sup>)**

Volatile compounds <sup>†</sup>	Ham nature (N)		Packaging (P)			Significance			Loadings <sup>††</sup>	
	Parma	Teruel	None	PET	PLA	N	P	O x P	PC1	PC2
<i>Amino acid catabolism</i>										
2-Methyl-propanal	13.1 <sup>b</sup>	24.7 <sup>a</sup>	16.5 <sup>b</sup>	18.1 <sup>ab</sup>	22.2 <sup>a</sup>	***	**	ns	-0.61	-0.19
3-Methyl-thio-propanal	10.5 <sup>b</sup>	17.4 <sup>a</sup>	9.54 <sup>b</sup>	10.5 <sup>b</sup>	21.8 <sup>a</sup>	**	***	**		
2-Methyl-1-butanol	32.8	35.3	21.9 <sup>b</sup>	29.5 <sup>ab</sup>	50.7 <sup>a</sup>	ns	**	ns		
2-Methyl-butanol	127 <sup>b</sup>	254 <sup>a</sup>	161	209	201	***	ns	ns	-0.67	-0.05
3-Methyl-butanol	189 <sup>b</sup>	267 <sup>a</sup>	205	223	257	***	ns	ns		
Dimethyl-sulfide	2.32 <sup>b</sup>	3.75 <sup>a</sup>	4.58 <sup>a</sup>	3.38 <sup>a</sup>	1.15 <sup>b</sup>	**	***	ns	-0.53	-0.24
Dimethyl-disulfide	35.9	56.3	28.0	56.5	53.8	*	*	ns		
Toluene	89.5 <sup>b</sup>	156 <sup>b</sup>	112	112	144	**	ns	ns		
Phenyl-Ethyl-alcohol	11.2 <sup>a</sup>	3.60 <sup>b</sup>	2.40 <sup>b</sup>	6.13 <sup>b</sup>	13.7 <sup>a</sup>	***	***	ns	0.64	0.37
<i>Esterase activity</i>										
Ethyl acetate	4.84 <sup>a</sup>	2.56 <sup>b</sup>	3.26	3.59	4.25	***	ns	ns	0.64	0.25
Ethyl pentanoate	3.99 <sup>a</sup>	0.96 <sup>b</sup>	1.71	2.23	3.50	***	*	*	0.81	0.34
Ethyl hexanoate	53.6 <sup>a</sup>	12.4 <sup>b</sup>	22.3	29.6	47.2	***	ns	ns	0.81	0.33
Ethyl heptanoate	1.39 <sup>a</sup>	0.28 <sup>b</sup>	0.42 <sup>b</sup>	0.72 <sup>ab</sup>	1.36 <sup>a</sup>	***	***	*	0.81	0.35
Ethyl octanoate	9.23 <sup>a</sup>	4.31 <sup>b</sup>	6.29	5.64	8.39	***	ns	ns	0.70	0.18
Ethyl decanoate	6.70 <sup>a</sup>	3.26 <sup>b</sup>	5.27	4.04	5.58	**	ns	ns	0.58	0.12
<i>Packaging contaminants</i>										
2-ethyl-hexene	12.1 <sup>a</sup>	6.47 <sup>b</sup>	11.2 <sup>a</sup>	7.08 <sup>b</sup>	9.53 <sup>a</sup>	***	**	ns	0.51	-0.26
BCA 1	40.4	72.4	39.1 <sup>b</sup>	101 <sup>a</sup>	28.5 <sup>b</sup>	*	***	ns	-0.64	0.54
BCA 2	5.38	8.18	5.11 <sup>b</sup>	11.4 <sup>a</sup>	3.80 <sup>b</sup>	*	***	ns	-0.63	0.67
BCA 3	3.40	4.56	3.46 <sup>ab</sup>	5.93 <sup>a</sup>	2.54 <sup>b</sup>	ns	***	ns	-0.59	0.62
BCA 4	5.7 <sup>b</sup>	12.5 <sup>a</sup>	3.01 <sup>b</sup>	20.8 <sup>a</sup>	3.57 <sup>b</sup>	**	***	ns	-0.60	0.67
BCA 5	47.8 <sup>a</sup>	35.2 <sup>b</sup>	28.4 <sup>b</sup>	67.5 <sup>a</sup>	28.4 <sup>b</sup>	**	***	**	0.07	0.61
BCA 6	40.6 <sup>a</sup>	13.2 <sup>b</sup>	14.3 <sup>b</sup>	30.1 <sup>a</sup>	36.5 <sup>a</sup>	***	***	*	0.79	0.49
BCA 7	15.6	31.1	4.71 <sup>b</sup>	61.7 <sup>a</sup>	3.73 <sup>b</sup>	ns	***	ns	-0.55	0.68
BCA 8	2.91	2.56	1.93 <sup>b</sup>	0.23	0.99 <sup>b</sup>	ns	***	ns	-0.12	0.50
BCA 9	3.20	5.62	1.27 <sup>b</sup>	9.52 <sup>a</sup>	2.43 <sup>b</sup>	ns	***	ns	-0.51	0.73
BCA 10	16.0 <sup>a</sup>	11.7 <sup>b</sup>	14.8	12.3	14.4	**	ns	ns	0.50	0.01
<i>Unknow origin</i>										
Acetal	2.57	2.85	2.72 <sup>ab</sup>	1.44 <sup>b</sup>	3.99 <sup>a</sup>	ns	**	ns		
Ethylbenzene	113	141	69.3 <sup>b</sup>	180 <sup>a</sup>	132 <sup>a</sup>	ns	***	ns		
p-xylene	111 <sup>b</sup>	155 <sup>a</sup>	83.9 <sup>b</sup>	178 <sup>a</sup>	137 <sup>ab</sup>	ns	**	ns	-0.50	0.39
m-xylene	39.0 <sup>b</sup>	67.8 <sup>a</sup>	35.4 <sup>b</sup>	70.9 <sup>a</sup>	53.9 <sup>ab</sup>	***	*	ns	-0.64	0.30
Cyclohexane	3.50	2.97	4.87 <sup>a</sup>	1.70 <sup>b</sup>	3.15 <sup>ab</sup>	ns	***	ns	0.15	-0.66
Butyl-cyclohexane	13.1	12.9	15.4 <sup>a</sup>	8.19 <sup>b</sup>	15.4 <sup>a</sup>	ns	***	ns	0.28	-0.61
3-Methyl-pentyl-cyclohexane	5.96	6.14	8.60 <sup>a</sup>	3.53 <sup>b</sup>	6.04 <sup>ab</sup>	ns	**	ns	0.19	-0.62

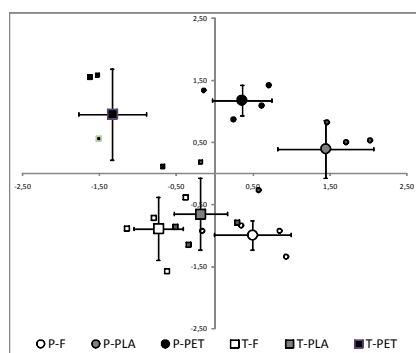
<sup>†</sup>Only VOCs with a significant effect were reported. Significant effect : \* P<0.10; \*\* P<0.05 ; \*\*\* P<0.01. Estimated means within a row with different lower case letters are different (P<0.05).

<sup>††</sup>Only variable loadings ≥ ± 0.5 on PC1 or PC2 are reported.

The compounds originating from the oxidative decomposition of lipids were most abundant in the Parma hams; oxidation compounds proved to be increased by PLA packaging. Dry-cured ham nature accounted for difference in compounds from carbohydrate fermentation. VOCs produced from amino acid catabolism were detected mostly in Teruel hams and were increased



by PLA packaging. According to these results, the "breathable" PLA plays an effective role in enhancing both oxidation and maturation mechanisms. Ethyl esters were a feature of P hams, formed from free fatty acids with the high amount of ethanol found in these hams. Branched chain alkanes (BCAs) could not be identified with the available libraries and were named with subsequent numbers. These compounds increased significantly in PET packaging, as a possible consequence of migration from packaging material in direct contact with the ham slices, favoured by fat content (range 10-18% on wet slice) and high surface/volume ratio. Also ethylbenzene, m- and p-xylene increased in PET packaging. Principal Components Analysis (PCA) was carried out including VOCs listed in Table 1, and the scores of fresh (F) and packaged samples were plotted onto the PC1-PC2 plane (Fig. 1). P-PET and T-PET packaged hams differed from the fresh ones (P-F and T-F) along PC2, mainly discriminated by the BCAs content (Table 1). PLA packaged P (P-PLA) hams differed remarkably from the fresh P hams along PC1 and PC2 (increase of oxidation compounds, see Table 1). F and PLA packaged T samples are grouped closely, showing the stability of T hams in PLA.



**Fig. 1.** Scoreplot of fresh and packaged sliced dry-cured hams. Large symbols represent the mean scores of each ham group, small symbols represent single samples, thin bars represent standard deviations of PC1-PC2 scores within groups.

## IV –Conclusions

VOCs of Parma and Teruel dry-cured hams showed remarkable differences as a consequence of different processing way and formulation. Packaging in PLA material stressed differences between ham types, mainly due to the sharp increase of compounds generated by lipid oxidation in Parma ham. The effect of PET packaging was the rise of BCAs in both ham types, as a possible consequence of migration from packaging material in direct contact with the product. The improvement of safety and quality of ready-to-eat packaged dry-cured meat products will require further research on packaging materials.

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# Meat quality traits of heavy pigs for the production of traditional Tuscan salami

D. Mani\*, G. Balestri\*, A. Pistoia\*, L. Casarosa\*, P. Poli\* and G. Ferruzzi\*

\*Dipartimento di Agronomia e Gestione dell'Agroecosistema, Università di Pisa  
Via S. Michele degli Scalzi 2, 56124 Pisa (Italia)

**Abstract.** The objective of the study was to evaluate the effect of the replacement of corn meal (CoM) by chestnut meal (ChM) in two diets of heavy pigs on the quality of meat and traditional Italian Salami obtained. Sixteen Large White pigs were divided in two groups and reared indoors in Garfagnana Valley (Tuscany, Italy). All animals were slaughtered after 464 days of trial at about 228 kg live weight. The quality of the *Longissimus lumborum* muscle, subcutaneous backfat and salami sausages was evaluated by chemical analysis and fatty acids profile. Data obtained show that ChM group increased the incorporation of unsaturated and monounsaturated fatty acids in muscle and backfat ( $P<0.05$ ), moreover the diet with ChM significantly reduce ( $P<0.05$ ) saturated fatty acids in backfat. Particularly ChM diet produced an increase ( $P<0.05$ ) in the content of C18:1n-9 and significantly ( $P<0.05$ ) reduce the content of C16:0, C18:0 and C20:0 in backfat. Inclusion of chestnut in the diet seems to have no significant effect on the fatty acid profile of salami. It is concluded that feeding a diet with ChM instead of CoM altered the fatty acid composition of *Longissimus lumborum* muscle and backfat without simultaneously affecting characteristics of salami sausages quality.

**Keywords.** Heavy pigs – Chestnut – Meat quality – Salami.

## Qualité de la viande de porc lourd pour la production de charcuterie traditionnelle de la Toscane

**Résumé.** L'objectif de cette étude était d'évaluer l'effet du remplacement de la farine de maïs (CoM) par de la farine de châtaignes (ChM) dans les régimes de porcs lourds, sur la qualité de la viande et du salami italien traditionnel obtenu. Seize porcs Large White ont été divisés en deux groupes et élevés à l'intérieur de la vallée de la Garfagnana (Toscane, Italie). Tous les animaux ont été abattus après 464 jours avec un poids d'environ 228 kg de poids vif. La qualité du muscle *Longissimus lumborum*, du lard et des salami traditionnels a été évaluée par analyse chimique et par profil en acides gras. Les résultats obtenus montrent pour le groupe ChM un niveau plus élevé en acides gras insaturés et monoinsaturés chez le muscle et le lard ( $P<0,05$ ), en plus, le régime à base de ChM a conduit à une diminution significative ( $P<0,05$ ) des acides gras saturés du lard. Particulièrement ChM a conduit à une augmentation ( $P<0,05$ ) du contenu de C18:1n-9 tout en baissant ( $P<0,05$ ) le niveau de C16:0, C18:0 et C20:0 du lard. L'inclusion de la châtaigne dans le régime ne semble pas avoir d'effet sur le profil en acides gras du salami. Nous avons conclu qu'un régime ChM au lieu de CoM modifie la composition en acides gras du muscle et du lard, sans pour autant avoir des interférences sur les caractéristiques de la qualité des salami.

**Mots-clés.** Porc lourd – Châtaignes – Qualité de la viande – Salami.

## I – Introduction

In the territory of Garfagnana (Lucca, Tuscany) pig rearing is carried out for processing meat into high quality traditional sausages (Register of traditional Tuscan products), the technique of production involves the use of animals slaughtered at high live weights (over 200 kg) and fed with by-products of the district derived from the processing of chestnut fruit (*Castanea sativa*) and spelt grains (*Triticum dicoccum*) for human food use. The high availability of these products allows local farmers to reduce feed costs and to typify the processed products.

These feed resources, especially the chestnut meal, confer specific dietary characteristics to meat by changing the lipid fraction increasing the proportion of unsaturated fatty acids (Coutron-Gambotti *et al.*, 1998; Pugliese *et al.*, 2005). Pigs used in this rearing system belong to cosmopolitan breeds indeed carcass traits are highly suitable for the processing into sausages.

The objective of this research was to evaluate the effect of substitution of corn meal with chestnut meal on chemical composition and fatty acids profile of muscle, backfat and a typical Tuscan salami derived from heavy pigs.

## II – Materials and methods

The trial were carried out in a farm located in the Garfagnana district on 16 Large White reared intensively and slaughtered at around 228 kg live weight. In the growing period pigs were fed with a mixed diet (spelt meal, spelt bran, corn meal, soybean meal), while during the finishing period (95 days), heavy pigs were divided in two groups (Table 1) and fed with integration of corn meal (CoM) or chestnut meal (ChM).

**Table 1. Composition of the experimental diets (%)**

	Soybean meal	Corn meal	Chestnut meal	Spelt meal	Spelt bran
CoM <sup>†</sup>	6	44	0	38	12
ChM <sup>††</sup>	6	34	10	38	12

<sup>†</sup>CoM corn meal; <sup>††</sup>ChM chestnut meal.

At the slaughtering, samples of muscle and backfat from each group were taken and stored for the chemical analysis. Traditional *Salame nostrano* were prepared by a local sausage make based from two diets. The lean meat (80%) were obtained from the sizing of ham, shoulder, loin, fillet, cup and fat part (20%) from backfat, cheek, shoulder. Minced into small pieces, the meat was mixed with salt (2.5%), black pepper (0.5%), infusion of garlic, nutmeg and white wine; no additives, sugar or starter were used. The meat was cut into a small pieces and were mixed for obtained a traditional grinding (7 mm). The dry sausages was made by means of a mechanical pressured sausages making machine and packed into natural gut casings (soft pork intestines) and hand-tied. Traditional salami after drying period (7 days) and ripening period (53 days) were prepared; the traditional room temperature ripening ranged from 7°C to 18°C, while the relative humidity ranged from 75% to 80%. At the end of experiment all the salami were weighed an average of about 550 g.

A representative samples of salami were vacuum packaged and stored at -20°C until chemical analysis. On the samples proximate chemical composition were carried out according to the AOAC method (1990), the extraction of total lipid was determined as described by Folch (1957), fatty acids methyl esters were prepared according to the method described by Christie (1982). FAMES were analyzed by gas chromatography using a Thermo Quest (Milan, Italy) GC apparatus equipped with a 100-m high polar fused silica capillary column and a flame ionization detector (i.d. 0.22 mm, 0.25 µm film thickness; Chrompack CP-Sil 88 Varian, Middelburg, The Netherlands). Separated FAMES were identified by comparison with the retention times of pure standards and reported as percentages of total fatty acids. Data were analyzed by one-way analysis of variance with JMP7 software (SAS Institute). Statistical significance was established at the level of  $P < 0.05$  and comparisons between means were conducted using the Tukey's HSD test.

### III –Results and discussion

Results of the proximate chemical composition of diets are presented in Table 2 that were formulated as isoenergetic and isoprotein. CoM diet has a higher content of PUFA, particularly C18:2n-6 and C18:3n-3 while ChM showed a higher content in C18:0 and C18:1n-9 in agreement with other research on Corsican pig (Coutron-Gambotti *et al.*, 1998) where the chestnut has increased the content of oleic acid in the diet.

**Table 2. Chemical analysis (% DM) and major fatty acid composition of diets (% total FA)**

	CoM	ChM
Moisture	9.27	9.06
Crude protein	14.04	13.88
Crude fat	2.72	2.66
Crude fiber	4.89	5.00
Ash	2.39	2.57
DE†	15.10	14.90
C14:0	0.04	0.15
C16:0	13.07	12.63
C16:1n-7	0.17	0.40
C18:0	1.28	1.68
C18:1 n-9	24.70	26.64
C18:2 n-6	54.95	52.93
C18:3 n-3	3.51	2.57
C20:0	0.21	0.35
C20:1 n-9	0.70	0.69
C22:0	0.18	0.20
C24:0	0.06	0.21
SFA	14.95	15.37
UFA	85.05	84.56
PUFA	58.46	55.60

† Digestible Energy expressed as MJ/kg DM.

No statistical differences were observed for the proximate analysis in muscle, backfat and salami among experimental diets (Table 3). The results for *Salame nostrano* were comparable with the data reported in the literature of Italian salami (Moretti *et al.*, 2004).

The fatty acid composition of muscle in pigs fed with chestnut meal showed higher values of MUFA and PUFA (C18:3n-3) according to other research carried out on Cinta Senese and Iberian pig breeds (Pugliese *et al.*, 2005; Andrés *et al.*, 2001; Cava *et al.*, 1999). The fatty acid composition of backfat showed a high values in SFA as a result of higher content in C14:0, C16:0, C18:0, C20:0 in CoM diet, while ChM significantly modified MUFA, particularly oleic acid and the PUFA/SFA ratio. The chestnut meal led to a higher content of C18:3n-3, C20:2n-6 and C20:4n-6 fatty acids, as reported previously by other authors (Diaz *et al.*, 1996). In traditional salami obtained from pig fed with CoM diet the results showed a high level of cis-vaccenic (C18:1n-7) and linoleic (C18:2n-6) acid probably due to the high content of corn meal in diet.

## IV – Conclusions

The integration of chestnut flour in the diet of heavy pig can affect the fatty acid profile of fresh products (backfat and muscle) by increasing the content of unsaturated fatty acids and particularly MUFA. In the processed product (salami) results show that the addition of small amounts of chestnut flour involves minor effects on the lipid fraction.

This research shows that the use of chestnut flour and other by-products of the area can improve the quality aspect and identify local meat products, moreover the use of these feed can help to reduce costs and represent an economic advantage for the farmer.

**Table 3. Proximate analysis (%) and fatty acid composition of muscle, backfat, salami (% total FA)**

	Muscle			Backfat			Salami		
	CoM	ChM	SE	CoM	ChM	SE	CoM	ChM	SE
Moisture	70.25	70.62	0.55	96.25	95.86	0.32	24.96	25.28	1.20
Crude Protein	76.40	76.28	1.88	-	-	-	38.98	35.28	1.43
Total Lipids	2.79	2.78	0.55	40.56	47.07	0.42	21.05	19.70	0.36
Ash	3.54	3.69	0.10	-	-	-	5.52	5.21	0.17
C10:0	0.18 <sup>a</sup>	0.16 <sup>b</sup>	0.01	0.08	0.09	0.01	0.08	0.07	0.01
C14:0	2.09	2.13	0.08	1.89	1.98	0.11	1.37	1.27	0.06
C16:0	21.78	21.88	0.40	22.16 <sup>a</sup>	20.62 <sup>b</sup>	0.36	22.59	23.08	0.27
C16:1 n-7	3.68	4.43	0.39	1.89	1.84	0.36	2.52	2.61	0.08
C18:0	10.54	9.31	0.56	12.25 <sup>a</sup>	10.53 <sup>b</sup>	0.51	11.67	11.81	0.30
C18:1 n-9	43.93	45.36	0.76	40.32 <sup>a</sup>	42.62 <sup>b</sup>	0.94	43.44	43.52	0.40
C18:1 n-7	4.66	5.25	0.30	2.49	2.51	0.30	3.13 <sup>a</sup>	3.22 <sup>b</sup>	0.10
C18:2 n-6	8.21	7.12	0.75	13.65	14.46	0.92	10.56 <sup>a</sup>	10.02 <sup>b</sup>	0.12
C18:3 n-3	0.26 <sup>a</sup>	0.28 <sup>b</sup>	0.22	0.64 <sup>a</sup>	0.74 <sup>b</sup>	0.02	0.51	0.47	0.01
C20:0	0.20	0.19	0.03	0.25 <sup>a</sup>	0.20 <sup>b</sup>	0.01	0.21	0.22	0.01
C20:1 n-9	0.75	0.92	0.16	1.20	1.15	0.15	1.02	1.02	0.01
C20:2 n-6	0.34 <sup>a</sup>	0.33 <sup>b</sup>	0.03	0.79 <sup>a</sup>	0.84 <sup>b</sup>	0.02	0.56	0.52	0.01
C20:4 n-6	1.10	0.60	0.15	0.17 <sup>a</sup>	0.23 <sup>b</sup>	0.20	0.21	0.21	0.01
C22:4 n-3	0.15	0.14	0.02	0.09	0.10	0.02	0.07	0.08	0.06
C22:5 n-3	0.10	0.06	0.03	0.02	0.05	0.03	0.05	0.04	0.01
SFA	35.79	33.67	1.38	36.85 <sup>a</sup>	34.40 <sup>b</sup>	0.59	36.50	37.04	0.49
UFA	63.67 <sup>a</sup>	65.48 <sup>b</sup>	1.34	63.03 <sup>a</sup>	65.46 <sup>b</sup>	0.58	63.38	62.85	0.48
MUFA	53.52 <sup>a</sup>	56.96 <sup>b</sup>	1.65	46.65 <sup>a</sup>	49.38 <sup>b</sup>	0.63	51.24	51.35	0.55
PUFA	10.15	8.52	1.38	15.70	16.81	0.72	12.22	11.60	0.16
PUFA/SFA	0.28 <sup>a</sup>	0.25 <sup>b</sup>	0.02	0.43 <sup>a</sup>	0.49 <sup>b</sup>	0.05	0.33	0.31	0.05
n-6/n-3	19.14	16.94	3.76	18.51	16.45	0.58	16.67	17.19	0.47

a, b within criterion means different ( $P < 0.05$ ).

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# Colour stability during prolonged storage of dry fermented sausages from Iberian pork

M.J. Martín\*, A.I. Andres\*\* and M.C. Sanabria\*

\*Instituto Tecnológico Agroalimentario, Ctra. San Vicente s/n, 06071 Badajoz (Spain)

\*\*Departamento de Producción Animal y Ciencia de los Alimentos, Escuela de Ingenierías Agrarias, 06071 Universidad de Extremadura, Badajoz (Spain)

**Abstract.** 30 units of chorizo and 30 units of salchichón "sarta" were packaged in different gas atmosphere conditions (i) vacuum packaging (Batch 1) (ii) 70% N<sub>2</sub>+ 30% CO<sub>2</sub> (Batch 2) (iii) 100 N<sub>2</sub> (Batch 3) (iv) 70% Argón + 30% CO<sub>2</sub> (Batch 4), and were stored under refrigeration (4±1°C) during nine months. The evolution of several parameters related to colour surface (L\*, a\*, b\*, Croma and Hue angle) were studied all throughout storage period. There were no differences for luminosity (L\*) among packaging treatments for chorizo and salchichón samples (P>0.05). Red colour intensity (a\*) significantly decreased for chorizo "sarta" in every batch, from initial values of 16.89±0.53 to final values ranging 14.17-15.22±0.55-0.49. With respect of differences among packaging systems of chorizo, batch 4 (70% argón+30% CO<sub>2</sub>) showed the highest a\* and C-values after 180 days and batch 2 (70% N<sub>2</sub> + 30% CO<sub>2</sub>) the lowest, indicating more intense oxidation reactions affecting the pigment of samples packed in this gas composition. However, these differences were not evident after 270 days of storage. With respect of salchichón "sarta", a decrease in redness was evidenced after 180 days of storage as well as a reestablishment of red colour at the end of the storage period batch 2 (70% N<sub>2</sub>+30% CO<sub>2</sub>) being the batch with the highest red colour intensity (P<0.001). The results obtained in this study suggest that as far as external appearance, specially colour, is concerned, shelf life of chorizo and salchichón "sarta" could be even longer. With respect of the type of gas atmosphere, this factor was not relevant for chorizo, whereas for salchichón, the gas atmosphere consisting in 70% N<sub>2</sub>+30% CO<sub>2</sub> was the most convenient.

**Keywords.** Chorizo "sarta" – Salchichón "sarta" – Modified atmosphere packaging – Colour.

## *La stabilité de la couleur durant le stockage prolongé des charcuteries crues affinées de porc Ibérique*

**Résumé:** 30 unités de "chorizo" et 30 de saucisson du type "sarta" ont été emballées sous différentes atmosphères : (i) emballage sous vide (Lot 1), (ii) 70% N<sub>2</sub>+ 30% CO<sub>2</sub> (Lot 2), (iii) 100% N<sub>2</sub> (Lot 3), (iv) 70% Argon + 30% CO<sub>2</sub> (lot 4), et elles ont été ainsi stockées sous réfrigération (4±1°C) pendant 9 mois. L'évolution des paramètres en rapport à la couleur de la superficie a été également étudiée (L\*, a\*, b\*, Chrome et Hue) pendant la période de stockage. Il n'y a pas eu de différences de luminosité (L\*) dues aux différentes compositions en gaz dans l'emballage utilisé pour les échantillons de "chorizo" et de saucisson du type "sarta" (P>0.05). Dans le cas du "chorizo sarta" l'intensité de la couleur rouge (a\*) s'est significativement réduite dans tous les lots, depuis les valeurs initiales de 16,89±0,53 jusqu'aux valeurs finales de 14,17-15,22±0,55-0,49. En ce qui concerne les différences dues à la composition en gaz de l'emballage utilisé pour le "chorizo", le Lot 4 (70% argon+30% CO<sub>2</sub>) a présenté les valeurs les plus élevées pour a\* et C, 180 jours après, et le Lot 2 (70% N<sub>2</sub> + 30% CO<sub>2</sub>) les moins élevées en montrant une oxydation plus intense de la pigmentation dans les échantillons emballés dans ce gaz. Néanmoins, ces différences n'étaient pas perçues après 270 jours de stockage. Concernant le saucisson "sarta", on observe une réduction de l'intensité de la couleur rouge après 180 jours d'emballage et sa récupération ultérieure vers la fin de la période d'emballage, le Lot 2 (70% N<sub>2</sub>+30% CO<sub>2</sub>) étant celui qui a présenté une plus grande intensité de couleur rouge dans le produit (P<0,001). Ces résultats nous suggèrent qu'en ce qui concerne l'aspect déterminé par la couleur, la durée de vie utile du "chorizo" et du saucisson du type "sarta" pourrait se prolonger plus longtemps. Et concernant le type d'emballage, ce facteur n'a pas exercé d'effet significatif dans le cas du "chorizo", et dans le cas du saucisson, le mélange 70% N<sub>2</sub>+30% CO<sub>2</sub> a été le plus intéressant.

**Mots clés:** Chorizo "sarta" – Saucisson "sarta" – Emballage en atmosphères modifiées – Couleur.



## I – Introduction

Salchichón and chorizo are the most popular fermented raw cured meat, existing a great variety of them depending on the area of production (Edwards *et al.*, 1999). The kind of variety "sarta" shows a diameter bigger than 22 millimetres and the sausage has got a horseshoe shape.

Currently, it is a more and more frequent practice in the packing of these raw fermented products vacuum or in different gas atmospheres (packed in modified or protector atmospheres, EAM or EAP) with the aim of adapting the sector to the new demands and consumption tendencies. The EAM consists in the replacement of the air that surrounds the food, by a gas or more frequently by an optimum mixture of gases that permits the lengthening of its shelf-life.

The shelf-life of fermented raw cured meat products is determined by their appearance and especially by the colour they show. During the curing process of these products the myoglobin transforms into nitrosylmyoglobin and nitrosylhemochrome, which are more stable pigments than myoglobin. Nevertheless the colour of fermented raw meat products can be altered during the cold storage (Ruiz Pérez-Cacho *et al.*, 2005). The effect of the packed on these characteristics has been studied in other works (Fernández-Fernández *et al.*, 2002). For that reason the aim of this study was to determine the most convenient packing conditions for the optimum preservation of the colour of the chorizo and salchichón samples of the "sarta" kind during a cold storage of 9 months.

## II – Material and methods

For the development of this study 60 (30 and 30) units of chorizo and salchichón "sarta" that weighted between  $0.261 \pm 0.0089$  and  $0.224 \pm 0.0111$  kg respectively were used. These products were manufactured by Montesano SA company according to the standard formulation. Both kinds of sausage present a standardized amount of fat and lean (30% and 70% respectively). The period of ripening was concluded when the decrease of both products reached a 35-37%.

Each unit of product was packed in a bell packaging of the brand Tecnotrip, mod. EV-13-CB, N° 932334 for the vacuum packing lots, an ULMA brand Flow-Pack machine mod. PV 350 LSHIX EMB and N° 1219098 for the rest of the lots and in different conditions: (i) vacuum (Lot 1), (ii) 70% N<sub>2</sub> + 30% CO<sub>2</sub> (Lot 2), (iii) 100 N<sub>2</sub> (Lot 3), (iv) 70% Argon + 30% CO<sub>2</sub> (Lot 4). The vacuum plastic material consisted in PA/PE 30/120 of 150 microns of thickness with an O<sub>2</sub> of 25-30 cm<sup>3</sup>/m<sup>2</sup>/bar/24h at 23°C permeability range and a water vapour transmission of 1,7 g/m<sup>2</sup>/24 h at 23°C and 85% of relative humidity. The samples were stored in refrigeration for 9 months, taking samples in the beginning, after 6 months and in the end of the storing period.

The colour of the surface cut in the chorizo and salchichón "sarta" was immediately measured after the opening of the pack by three times according to the American Meat Science Association (AMSA, 1991) recommendations. The following colour coordinates were obtained: luminosity ( $L^*$ ), red colour intensity ( $a^*$ , red  $\pm$  green) and yellow colour intensity ( $b^*$ , yellow  $\pm$  blue).  $a^*$  and  $b^*$  were used to calculate the hue angle ( $\text{hue} = \arctan [b^*/a^*]$ ) and the colour saturation ( $\text{chroma} = [a^{*2} + b^{*2}]^{0.5}$ ) parameters. The data obtained were analysed by the SPSS (SPSS 13.0) software package.

## III – Results and discussion

The Tables 1 and 2 present the evolution of the colour parameters instrumentally measured ( $L^*$ ,  $a^*$ ,  $b^*$ , colour saturation or C and angle of Hue or h). The values of initial luminosity in the case of chorizo and salchichón "sarta" were of  $32,84 \pm 1,01$  and  $32,37 \pm 0,94$  respectively. During the first 180 days of storage it is possible to observe in a general way that the luminosity values were reduced in comparison to the initial ones, in some cases in a statistically significant way

( $P < 0.05$ ) (Lot 2: 70%  $N_2$  + 30%  $CO_2$  and Lot 3: 100  $N_2$  for the salchichón and lot 1: vacuum, Lot 2: 70%  $N_2$  + 30%  $CO_2$  and Lot 3: 100  $N_2$  for the chorizo). However, after 270 days of storage it is showed that the values of luminosity increase again in the mentioned lots, even if they do not reach the initial values of luminosity registered. In a similar way Rubio *et al.*, (2007) observed that the values of luminosity in samples of salchichón stored for 210 days were even higher in comparison with the initial product ( $P < 0.05$ ).

According to the differences among packed lots, these weren't significant ( $P > 0.05$ ) excepting the chorizo samples after 180 days of storage presenting the lot 3 (100%  $N_2$ ) the maximum values of luminosity and the lot 2 (70%  $N_2$  30%  $CO_2$ ) the minimum. According to the evolution of the red colour in the different studied products, the intensity of red colour ( $a^*$ ) was significantly reduced in the case of chorizo "sarta" in every lot, from initial values of  $16.89 \pm 0.53$  to final ones of  $14.17$ - $15.22 \pm 0.55$ - $0.49$ . The loss of intensity in red colour is mainly owed to the oxidation of nitrosylmyoglobin and the formation of metmyoglobin, due to the presence of residual oxygen quantities (Andersen and Skibsted, 1992).

**Table 1. Instrumental colour evolution ( $L^*$ ,  $a^*$ ,  $b^*$ , C, h) (mean  $\pm$  standard error of the mean ) on chorizo "sarta" stored under modified atmosphere packaging at 4°C during 9 months**

Time (days)	Packaging*	$L^* \pm SE$		$a^* \pm SE$		$b^* \pm SE$		C $\pm SE$		H $\pm SE$	
0		32.84 <sup>1/1/1/-</sup>	1.01	16.89 <sup>1/1/1/1</sup>	0.53	10.22 <sup>1/1/1/-</sup>	0.55	19.77 <sup>1/1/1/-</sup>	0.71	30.83 <sup>-1/-1/1</sup>	0.9
180	1	29.17ab <sup>2</sup>	0.45	14.46ab <sup>2</sup>	0.39	8.86ab <sup>2</sup>	0.29	16.99ab <sup>2</sup>	0.45	31.5	0.6
	2	28.57b <sup>2</sup>	0.38	13.38b <sup>3</sup>	0.34	8.24b <sup>2</sup>	0.3	15.73b <sup>3</sup>	0.43	31.48	0.52
	3	30.66a <sup>12</sup>	0.53	14.05ab <sup>2</sup>	0.35	9.32ab	0.37	16.90ab <sup>2</sup>	0.46	33.24	0.78
	4	30.54a	0.51	15.04a <sup>12</sup>	0.54	9.99a	0.47	18.08a	0.7	33.311	0.55
	P <sub>envasado</sub>	***		*		*		*		NS	
270	1	31.2412	0.63	15.222	0.49	9.1812	0.4	17.792	0.61	30.94b	0.67
	2	31.791	0.61	14.802	0.33	9.611	0.28	17.662	0.41	32.90a	0.5
	3	30.012	0.7	14.172	0.55	9.01	0.53	16.822	0.73	31.96ab	0.67
	4	30.13	0.48	14.332	0.33	9.44	0.3	17.18	0.43	33.17a <sup>1</sup>	0.39
	P <sub>envasado</sub>	NS		NS		NS		NS		*	

Lot 1 = (vacuum); Lot 2= (70%  $N_2$ + 30%  $CO_2$ ); Lot 3=100  $N_2$ ; Lot 4= 70% Argon + 30%  $CO_2$ . Significance levels: ns= $>0.05$ ; \*= $p < 0.05$ ; \*\*=  $p < 0.01$ ; \*\*\*=  $p < 0.001$ . a,b,c: different letters in the same column within the same storage time, mean significant differences between lots ( $P < 0.05$ ).

Test of Tukey 1,2,3: different superscripts in the same column within the same lots, mean significant differences between storage time ( $P < 0.05$ ). Test of Tukey

Regarding the differences related to the gas composition of the pack, the lot 4 (70% argón+30%  $CO_2$ ) showed the maximum values of  $a^*$  and C after 180 days and the lot 2 (70%  $N_2$  + 30%  $CO_2$ ) the minimum, pointing a more intense composition of the pigment in the samples packed in this gas. Nevertheless, these differences weren't perceptible after 270 days of storage.

Regarding the salchichón "sarta", a reduction in the intensity of red colour is observed after 180 days of packing and the following recovering of it in the end of the storage, being the Lot 4 (70% argon+30%  $CO_2$ ) the one that showed a mayor intensity of red colour in the product after 270 days of storage ( $P < 0.001$ ). The loss of intensity in red colour is owed to the formation of metmyoglobin (of brown colour) from the nitrosylmyoglobin (MbFe(II)NO) (Lindahl *et al.*, 2001). The reduction of intensity in red colour and the following recovering of it have been observed in previous studies in dry-cured ham (Andrés *et al.*, 2005) as well as in cured-fermented product (Rubio *et al.*, 2008). Andersen *et al.* (1998) also observed that the mentioned recovering of colour was more important if an adequate exclusion of oxygen in the pack was performed. On

the other hand, it can be supposed that the loss of humidity in the product during its storage can be related.

**Table 2. Instrumental colour evolution (L\*, a\*, b\*, C, h) (mean± standard error of the mean ) on salchichón "sarta" stored under modified atmosphere packaging at 4 °C during 9 months**

Time (days)	Packaging*	L* ± SE		a* ± SE		b* ± SE		C ± SE		H ± SE	
0		32.37 <sup>1/1/1/-</sup>	0.94	11.35 <sup>1/1/1/1</sup>	0.22	4.01 <sup>1/1/1/1</sup>	0.11	12.04 <sup>1/1/1/2</sup>	0.23	19.45 <sup>2/2/2/2</sup>	0.34
180	1	30.86	0.5	8.783	0.17	3.162	0.07	9.333	0.18	19.802	0.25
	2	29.792	0.39	8.333	0.17	2.982	0.08	8.863	0.17	19.792	0.54
	3	29.982	0.46	8.543	0.13	3.033	0.07	9.063	0.15	19.502	0.25
	4	30.43	0.64	8.312	0.16	3.003	0.08	8.843	0.17	19.852	0.38
	P <sub>envasado</sub>	NS		NS		NS		NS		NS	
270	1	30.74	0.54	9.59ab <sup>2</sup>	0.15	3.80a <sup>1</sup>	0.09	10.32ab <sup>2</sup>	0.17	21.57b <sup>1</sup>	0.41
	2	31.0412	0.42	9.82a <sup>2</sup>	0.15	3.82a <sup>1</sup>	0.07	10.54a <sup>2</sup>	0.16	21.26b <sup>1</sup>	0.27
	3	30.9512	0.51	9.21b <sup>2</sup>	0.15	3.64a <sup>2</sup>	0.08	9.91b <sup>2</sup>	0.16	21.59b <sup>1</sup>	0.39
	4	31.77	0.5	8.71c <sup>2</sup>	0.12	3.36b <sup>2</sup>	0.08	9.34c <sup>2</sup>	0.12	21.081	0.45
	P <sub>envasado</sub>	NS		***		***		***		NS	

Lot 1 = (al vacío); Lot 2 = (70% N2+ 30% CO2); Lot 3 = 100 N2; Lot 4 = 70% Argon + 30% CO2. Significance levels: ns =>0.05; \*p<0.05; \*\*= p<0.01; \*\*\*= p<0.001. a,b,c: different letters in the same column within the same storage time, mean significant differences between lots (P<0.05).

Test of Tukey. 1,2,3: different superscripts in the same column within the same lots, mean significant differences between storage time (P< 0.05). Test of Tukey

Chroma or saturation of colour follows similar evolution to the one explained for a\* in salchichón and chorizo, being significantly reduced during the first 180 days of storage and increasing afterwards during the end of the period of storage, although if it doesn't reach the initial values of 12.04±0.23. The mayor value of Chroma on the surface of salchichón from lot 4 (70% argón+30% CO<sub>2</sub>) has to be remarked comparing to the rest of the lots. This parameter represents the brightness of colour on the surface of the product (Sarasibar *et al.*, 1989). b\* is a parameter normally related to the lipid oxidation. In the case of chorizo "sarta" results hardly affected by the time of storage and only after 180 days, differences owed to the mixture of used gases are observed, showing the Lot 4 (70% argón+30% CO<sub>2</sub>) the maximum value. Other authors have observed that the variation of b\* in this product can be related to the presence of carotenoids (β-caroteno and criptoxantina) included in the p  prika pepper, typical spice used in chorizo (Gimeno *et al.*, 2000). The nitrificant salts reduce the intensity and stability of the paprika developing a yellow discoloration of the red paprika of low pH (Sarasibar *et al.*, 1989). In the case of salchich  n "sarta" the maximum values of b\* have to be remarked in lot 4 at the end of the period of storage, because they can point a mayor lipid oxidation.

## IV – Conclusions

The results obtained in this study suggest that regarding the appearance determined by the colour, the-shelf life of the chorizo and salchich  n "sarta" could be extended for a longer time. Regarding the packing time, this factor didn't exert a significant effect in the case of chorizo, and in the case of salchich  n, the mixture 70% argon+30% CO<sub>2</sub> was more convenient.

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# A further look on genetic basis of carcass fat deposition in pigs of 'Casertana' ancient autochthonous genetic type

D. Matassino\*, S. Braglia\*\*, M. Comella\*\*, M. Grasso\*, M. Occidente\*, F. Pane\*,  
C. Annaratone\*\* and R. Davoli\*\*

\*Consortium for Experimentation, Dissemination and Application of Innovative Biotechniques (ConSDABI),  
Italian Sub National Focal Point FAO (Sub NFP.I-FAO) (Mediterranean biodiversity),  
Centre of Omic Science for Nutritional Quality and Excellence (Italy)

\*\*University of Bologna, Faculty of Agriculture, DIPROVAL, Allevamenti zootecnici (Italy)

**Abstract.** 'Casertana' pig ancient autochthonous genetic type (AAGT) has been considered in the years within wide programme of safeguard and rescue promoted by ConSDABI. One of the most relevant aims of this program is to search for gene variation related to quantity and technological, nutritive and organoleptic quality of fat in order to satisfy consumer's request. In the present work, 100 AAGT Casertana pigs were genotyped for 8 SNP at *loci* involved in the regulation of adipose tissue deposition [DECR1, FASN, MC4R, SCD and H-FABP haplotype (H-FABP1, H-FABP2, H-FABP3)] in order to carry out an association study, with some fatness characteristics of carcass. Statistical analysis was performed using the GLM procedure of the SAS package. The results showed association between HFABP haplotype and the majority of considered phenotypic traits. In detail, the effect of this haplotype was significant on the total weight of separable fat in carcass ( $P=0.002$ ), total weight of adipose cuts ( $P=0.006$ ) and back fat thickness measured at level of 1st thoracic vertebra (Th1) ( $P=0.056$ ), 15th thoracic (Th15) ( $P=0.020$ ) and between the 6th lumbar vertebra and sacrum (L6 – S) ( $P=0.007$ ). FASN showed an effect on belly and jowl fat ( $P\leq 0.05$ ). For DECR1 CC genotype influenced the weight of belly cut. Further investigations are ongoing for an operative utilisation of the H-FABP haplotype, FASN and DECR1 genes as molecular markers (candidates) in proper molecular assisted selection (MAS) plans.

**Keywords.** Casertana 'AAGT' – Fat traits – SNPs – Haplotype.

## *Une perspective sur les bases génétiques du dépôt de graisse dans la carcasse des porcs du type génétique autochtone ancien 'Casertana'*

**Résumé.** Le porc 'Casertana', type génétique autochtone ancien (TGAA) du "bioterritorio" de la Campanie, est depuis des années l'objet d'un programme de protection et de valorisation mis en œuvre par le ConSDABI SUB NFP.I - FAO. Parmi les objectifs de ce programme il y a celui d'identifier des variantes des gènes associés à la quantité et à la qualité technologique, nutritionnelle et organoleptique des matières grasses pour répondre aux besoins du consommateur. Dans cet article, nous avons étudié 8 SNP dans les loci candidats au déterminisme quantitatif du tissu adipeux [DECR1, FASN, MC4R, SCD et haplotype H-FABP (H-FABP1, H-FABP2, H-FABP3)]. 100 porcs TGAA 'Casertana' pour lesquels certains relevés étaient disponibles concernant des sections, ont été génotypés afin d'effectuer une étude d'association avec quelques caractéristiques de l'adiposité. L'analyse statistique a été réalisée en utilisant la procédure GML du SAS. Les résultats suggèrent une association entre l'haplotype H-FABP et la plupart des caractères en question. En particulier, l'effet de l'haplotype H-FABP est significatif sur le poids total des morceaux de gras ( $P=0.006$ ), sur les gras séparables ( $P=0.002$ ) et sur l'épaisseur de gras mesurée à la 1ère vertèbre thoracique (Th1) ( $P=0.056$ ), à la 15ème vertèbre thoracique (Th15) ( $P=0.020$ ) et entre la 6ème vertèbre lombaire et le sacrum (L6 – S) ( $P=0.007$ ). En outre, FASN a des associations avec le lard de la poitrine et le lard de la bajoue ( $P\leq 0.05$ ). D'autres recherches sont en cours pour une utilisation opérationnelle des haplotypes H-FABP et des gènes FASN et DECR1 comme marqueurs prometteurs ('candidats') dans les plans de sélection assistée par marqueurs moléculaires (MAS).

**Mots-clés.** TGAA 'Casertana' – Caractéristique de l'adiposité – SNP – Haplotype.

## I – Introduction

'Casertana' (CT) ancient autochthonous genetic type (AAGT) is a black pig of ancient origins. It is object of considerable interest for its many peculiarities, like: good aptitude to grazing with ability to utilize poor feed; appreciable organoleptic and healthy quality of meat which is particularly suitable to obtain valuable local products (Matassino *et al.*, 1968; Colatruglio *et al.*, 1994; Girolami *et al.*, 1996; Matassino *et al.*, 2006; Barone *et al.*, 2008). In the last years, the awareness of the nutritional issue has increased: healthy quality is the element that more concerns the consumer. In this context, the study of quanti-qualitative characteristics of the adipose tissue as well as of the factors influencing them in farm animals, especially of pig, has become increasingly important. Furthermore ongoing research at ConSDABI SUB NFP.I - FAO on pig AAGTs is corroborating the hypothesis to consider pig as a valid model to study human obesity. These pigs, characterized by a thicker back fat than cosmopolite breeds, can constitute an interesting resource to contribute to the knowledge of genetic factors involved in obesity; moreover, AAGTs can constitute a genetic reserve suitable to rescue organoleptic properties penalized in cosmopolite breeds. Different approaches are used to identify molecular markers linked to traits associated to adipose tissue deposition in pig: (i) candidate gene; (ii) QTL identification; (iii) combination of (i) and (ii); (iv) transcriptome analysis (Davoli *et al.*, 2009). Various genes involved in quali-quantitative characteristics of muscle and adipose tissue have been identified. The aim of the present contribute was to evaluate, in CT pig, possible associations between 8 SNPs at *loci* involved in regulation of adipose tissue deposition [DEC1, FASN, MC4R, SCD and H-FABP haplotype (H-FABP1, H-FABP2, H-FABP3)] and some fatness characteristics of carcass; these *loci* are known in literature for their significant association with some fatness traits (Gerbens *et al.*, 1997; Kim *et al.*, 2000; Wimmers *et al.*, 2002; Munoz *et al.*, 2003; Amills *et al.*, 2005; Matassino *et al.*, 2007 and 2009).

## II – Materials and methods

DNA was extracted from blood and muscle samples of 100 subjects of CT AAGT, reared at experimental Farm of ConSDABI SUB NFP.I - FAO. Genotyping for SNPs considered at DEC1, FASN, H-FABP, MC4R and SCD *loci* was carried out by PCR-RFLP method according to literature protocols (Table 1).

**Table 1. SNPs investigated in 'candidate' *loci* object of study**

SNP	LOCUS				BIBLIOGRAPHIC REFERENCE
	ACRONYM	DENOMINATION	FUNCTION	CHROMOSOME	
C(163)G	DEC1	2,4 - Dienoyl reductase 1 mitochondrial	Encodes for an enzyme involved in unsaturated fatty acid beta-oxidation	4	Davoli <i>et al.</i> , 2002
T265C	FASN	Fatty acid synthase	Enzyme key in the conversion of acetyl-CoA and malonyl-coA into long-chain saturated fatty acids	12	Munoz <i>et al.</i> , 2003
C(1811)G H-FABP1 (D/d) T(1324)C H-FABP2 (H/h) T(1970)C H-FABP3 (A/a)	H-FABP	Heart fatty acid - binding protein	Trafficking of fatty acids at level of cardiac and striate muscle as well as lactating mammary gland	6	Gerbens <i>et al.</i> , 1997
G(298)A	MC4R	Melanocortin 4 receptor	Control of uptake capacity and growth rate	1	Kim <i>et al.</i> , 2000
T(233)C SCD1 C(641)T SCD3	SCD	Stearoyl CoA Desaturase	Encodes for an enzyme key in the monounsaturated fatty acid biosynthesis	14	Ren <i>et al.</i> , 2004

For each SNP the allelic and genotypic frequencies were estimated. Concerning H-FABP, 12 out of 27 possible haplotypes were individuated, only 6 of which (HHddAa, HHddAA, HHDdAa, HHDdAA, HHddaa, HhDdAa) were used for statistical elaboration because of the insufficient

numerousness of the others; for each fatness indicator 15 possible pair-wise comparisons were carried out. In the present contribute the following parameters at carcass dissection were considered as adiposity indicators: (i) total weight of adipose cuts (with and without 'jowl'); (ii) total weight of separable fat; (iii) thickness (mm) of back fat measured at level of: 1<sup>st</sup> thoracic vertebra (Th1), 15<sup>th</sup> thoracic vertebra (Th15) and between the 6<sup>th</sup> lumbar vertebra and sacrum (L6 - S).

Statistical elaboration, concerning the association between genotypic and phenotypic data, was performed with the following factorial model of covariance analysis with fixed factors utilizing SAS 9.1v software:

$$Y_{ijk} = \mu + b_1x_1 + b_2x_2 + b_3x_3 + \alpha_i + \text{sex}_j + e_{ijk}$$

where:

$\mu$  = constant common to all observations (overall mean);

$x_1$  = weight of refrigerated carcass after 72 hours of refrigeration (covariate);

$x_2$  = date of slaughtering (covariate);

$x_3$  = age of the pig at slaughtering (covariate);

$\alpha_i$  = fixed effect common to all observation relative to i<sup>th</sup> genotype (i = 1,2,3)/haplotype (i=1, 2, 3, 4, 5, 6);

$\text{sex}_j$  = fixed effect common to all observations related to j<sup>th</sup> sex (j = 1,2);

$e_{ijk}$  = random error.

### III – Results and discussion

From statistical elaboration it emerged a significant effect of some *loci* on carcass fatness. *H-FABP*. The comparison among the considered haplotypes highlighted that the pig with HHDdAA or HHDdAa haplotype, when compared with a subject with HHddAA or HHddAa or HHddaa haplotype, gives a higher adiposity estimated through any detected parameters (Table 2). In particular, the difference of: (i) back fat thickness can vary from about 9 (HHDdAA vs HHddAa; P=0.020) to about 17 mm (HHDdAA vs HHddAA; P=0.002); (ii) total fat cuts (without belly) can range from 6.5 (HHDdAA vs HHddAa; P=0.075) to about 16 kg (HHDdAa vs HHddaa; P=0.002); (iii) of separable cuts can vary from about 6.0 (HHDdAA vs HHddAa; P=0.073) to 13.5 kg (HHDdAa vs HHddaa; P=0.0001).

**Table 2. Some parameters of fatness: significant comparisons between 'H-FABP' haplotypes**

Haplotype		Parameter											
		Backfat thickness, mm						'Fat cuts', kg				'Total separable fat', kg	
Reference (I)	Comparison (J)	'Th1'		'Th15'		'L6-S'		'Total'		'Total without Jowl'			
		$\Delta$ (I-J)	P-value	$\Delta$ (I-J)	P-value	$\Delta$ (I-J)	P-value					$\Delta$ (I-J)	P-value
HHDdAA	HHddAA	12.443	0.031	15.113	0.004	16.949	0.002	8.047	0.028	8.197	0.042	10.542	0.005
	HHddAa	10.958	0.035	11.934	0.011	13.080	0.006	6.565	0.048	6.493	0.075	5.987	0.073
	HHddaa	15.412	0.043	15.277	0.027	16.563	0.017	5.154	0.287	13.485	0.013	6.677	0.173
HHDdAa	HHddAA	11.321	0.031	11.640	0.015	13.332	0.006	9.784	0.004	10.538	0.004	13.490	0.0001
	HHddAa	9.836	0.022	8.461	0.029	9.463	0.016	8.302	0.003	8.835	0.004	8.935	0.002
	HHddaa	14.290	0.043	11.804	0.063	12.946	0.043	6.890	0.126	15.827	0.002	9.625	0.036

The biological and operative importance to consider the effect of a haplotype on a qualitative trait has been widely debated (Matassino *et al.*, 1993; Zullo *et al.*, 1994). Indeed, according to these authors, the global genotype has a semantic value for marker assisted selection (MAS). Within H-FABP, it was believed convenient to individuate a possible effect of



genotype at single *locus* on adiposity using single nucleotide polymorphism. The effect was significant only for D/d polymorphism: the subject with dd genotype gave a significant lower adiposity in comparison with heterozygote for the majority of the parameters considered, with a decrease equal to: (i) about 10 mm for back fat thickness ( $P=0.003$  at Th1 region;  $P=0.001$  at Th15 and L6-S regions); (ii) about 8 kg for total fat cuts and total separable fat ( $P<0.001$ ).

**FASN.** The effect was significant on the weight of belly fat cut ( $P=0.051$ ) and on that of separable fat from jowl ( $P=0.048$ ). It is interesting to observe that the subject with TT genotype, in comparison with that with CC or CT genotype, gives a significant ( $P\leq 0.05$ ): (i) lower weight of belly cut [the difference is equal to 0.895 kg and 1.072 kg for CC vs TT and CT vs TT comparisons, respectively]; (ii) higher weight of separable fat from jowl (difference equal to - 0.439 kg and - 0.454 for CC vs TT and CT vs TT comparisons, respectively).

**DECR1.** The effect of this gene was near to critical limit of significance ( $P=0.072$ ) on jowl; from the comparison between genotypes it emerges that the pig with CC genotype gives a lighter belly than the subject with CG or GG genotype; the comparison was significant ( $P=0.022$ ) for CC vs CG with a difference of -0.902 kg and tendentially significant ( $P=0.135$ ) for CC vs GG with a difference of - 0.608 kg.

**SCD.** This gene tended to influence the weight of separable fat from coppa adipose cut ( $P=0.148$ ), back fat ( $P=0.144$ ) and total separable fat ( $P=0.132$ ).

**MC4R.** A tendency to significance ( $P=0.179$ ) was evidenced for separable fat from jowl.

## IV –Conclusions

In the limits of the observation field, the results suggest that H-FABP haplotype significantly affects the parameters used for the estimation of carcass adiposity, determining differences among subjects with different haplotypes. The pig with HHddAA would be the less physiological obese for the total of adipose cuts without jowl and of separable fat. FASN locus would cause a differential fat deposition probably associated to the effect of TT genotype showing a variable lipogenetic aptitude in different anatomical regions of the body. For DECR1 locus, CC genotype would influence the weight of belly fat. Further investigations are ongoing for an operative utilisation of the H-FABP haplotype and FASN and DECR1 genes as molecular markers (candidates) in proper MAS plans.

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# Effect of feeding and rearing system on growth performance of Sarda breed pig: Preliminary study

S. Porcu\*, M. Decandia\*, S. Pintus\*, P.N. Lei\*, M.A. Sanna\* and S. Ligios\*

\*Dipartimento per la Ricerca nelle Produzioni Animali (DiRPA), Loc Bonassai, 07040 Olmedo, Sassari (Italy)

**Abstract.** Autochthonous Sarda pigs are usually bred in mountainous areas of Sardinia where they use mainly feeding resources in shrublands and forests. The aim of the study was to evaluate the effect of different feeding systems on some performances of Sarda pigs. Twelve, castrated male pigs, homogeneous for body weight ( $98.4 \pm 13.8$  kg) and age (15 months) were randomly assigned to the following three feeding systems: A, reared en *plein-air* system in the woods and fed *ad libitum* with a commercial concentrate; B, reared en *plein-air* in the woods and fed with ground barley (1.8 kg/head/day), with an automatic feeder; C, fed at pasture in the woods and receiving 500 g/head/day of barley grain. Morphological measures and weight were recorded fortnightly until slaughtering (22 months of age). Preliminary results showed differences between feeding systems, with best performance in group A: weight and fat thickness were higher (294 kg and 84.5 mm respectively) than group B (170.7 kg and 29.7 mm) and group C (202.0 kg and 39.7 mm). Therefore extensive feeding systems is a right compromise between good performances and farming profit.

**Keywords.** Sarda breed pig – Growth performance – Feeding system – Rearing system.

## **Etudes préliminaires sur l'influence de l'élevage et de l'alimentation sur l'augmentation pondérale et biométrique des porcs de race Sarde**

**Résumé.** Le porc autochtone de race Sarde, traditionnellement élevé dans les régions montagneuses de Sardaigne, se nourrit principalement des ressources du sous-bois. L'objectif de ce travail était d'étudier l'influence des divers systèmes d'alimentation sur les performances productives de ces porcs. Douze mâles castrés, de poids ( $98,4 \pm 13,8$  kg) et d'âge (15 mois) comparables, ont été soumis à trois régimes alimentaires différents: A, élevés en plein air dans un bois et alimentés *ad libitum* avec un aliment commercial; B, élevés dans le même système et alimentés avec de la farine d'orge (1,8 kg/tête/jour) au moyen d'un distributeur automatique; C, maintenus au pâturage dans un bois et complémentés avec de l'orge en grain (500 g/tête/jour). Les mesures morphologiques et le poids de chacun de ces animaux ont été relevés tous les 15 jours jusqu'à leur abattage à 22 mois. Les résultats préliminaires ont montré des différences significatives ( $P < 0,05$ ) entre ces systèmes d'alimentation avec de meilleures performances pour le groupe A: le poids vif et l'épaisseur de gras des porcs de ce groupe (respectivement 294,0 kg et 84,5 mm) étaient supérieurs à ceux des groupes B (170,7 kg et 29,7 mm) et C (202,0 kg et 39,7 mm). Toutefois, le système d'alimentation extensive représente le meilleur compromis entre performances de production et profit de l'éleveur.

**Mots-clés.** Porc de race Sarde – Gain de poids – Système d'alimentation – Système d'élevage.

## **I – Introduction**

The increased industrialization of agriculture and the greater market demand led the pig farmers to replace the "autochthonous breeds pig", less productive but well adapted to the environment, with the "cosmopolitan breeds pig". This trend started in Italy, since 1872 (Mascheroni, 1927), and caused the gradual replacement of the autochthonous breed pigs.

Among the 21 Italian breed pigs, recognized until the last century, nowadays only 6 survived (Franci *et al.*, 2007). Despite ancient origins of Sarda pig (Porcu, 2006), the study about the

local breed officially recognized (Ministerial Decree No. 21664 of 08/06/2006) has been started recently (Porcu *et al.*, 2007).

The exploitation of the autochthonous species can help the biodiversity safeguard and the fight against the depopulation of the marginal areas (Porcu, 2008). Indeed, in all rural societies, domestic pig breeding has played always an important role because represented, and represent still now, a source of food always available thanks to the spawning characteristics (high fertility and frequency of parts). *Sarda* pig has lived for centuries completely free in the Sardinia mountains showing a great ability to utilize poor food such as spontaneous fruits of woods and only occasionally was supplemented with flour or grains. Such as autochthonous pig (Zumbo *et al.*, 2003), *Sarda* breed is characterized by a strong resistance to illness, a zoo-technical adaptability to different climatic conditions as well as the ability to procure food thanks to its strong inclination to grazing and its high rusticity.

Several authors (Cetti, 1774; Bonadonna, 1960; Porcu, 2004) described the *Sarda* breed pig as a small animal size that hardly could achieve heavy weight.

However Cetti (1774) stated that in some areas of Sardinia some pigs could reach greater size (over 200 kg). So is well-known that the productive and growth performances are influenced both by genetic factors and different environmental conditionings. Among these, the feeding and rearing systems should be considered particularly.

The aim of the study was to evaluate the effect of different breeding and feeding systems on growth performances of *Sarda* pigs.

## II – Materials and methods

The study, lasted seven months (July 2009-February 2010) and was conducted at the experimental farm of AGRIS agency in Foresta Burgos, (Illorai, Sassari; 44°69 lat. North and 4°95 long. East). The experimental site, is characterized by a forest area of 40 ha divided in lots of various sizes and with altitude ranging from 830 to 930 m. Twelve *Sarda* breed castrated male pigs coming from the AGRIS agency (DIRPA), homogeneous for genetic, weight ( $98.4 \pm 13.8$  kg) and age (15 months) were randomly assigned to three experimental groups (A, B and C) that differed in the feeding systems: A, reared en *plein-air* system in a wood area of 4500 m<sup>2</sup> and supplemented with a commercial concentrate (*ad libitum*); B, reared en *plein-air* system in a wood area of 6000 m<sup>2</sup> and supplemented with ground barley (1.8 kg/head/day), through an automatic feeder; C, fed at pasture in a wood area of approximately 20 ha and supplemented with barley grain (500 g/head/day). Chemical composition of principal feedstuff used and main fruits of woody species fed during the experiment was measured (Table 1).

**Table 1. Chemical composition of feed (% dry matter)**

	Moisture	Ash	Crude protein	Ether extract	Crude fiber
<i>Quercus pubescens</i>	54.50	2.47	5.76	2.31	14.83
<i>Quercus ilex</i>	56.91	2.23	4.82	1.70	16.88
<i>Pyrus amygdaliformis</i>	63.62	2.47	3.35	1.1	22.68
Commercial concentrate	14.04	7.69	16.79	2.8	4.91
Ground barley	12.04	2.67	9.11	1.94	7.36

On each animal, fortnightly morphological measurements were determined: live weight, withers and rump height, chest length, chest depth, chest width, rump width and subcutaneous fat thickness at the lumbar level (by RENCO LEAN-Meter instruments). The animals were slaughtered 22 months old.

Data of live weight and morphological parameters were tested by GLM procedure (SAS, 2001) using feeding system as fixed effects. Average daily gains (ADG) were tested by MIXED procedure (SAS, 2001) using feeding system, measurement period and their interaction as fixed effects and animal as random effect.

### III – Results and discussion

As expected, pigs fed *ad libitum* with commercial concentrate (group A) have grown faster than those fed in controlled condition (Fig. 1, Table 2) and between these two groups, higher ADG has been observed in C than B group ( $P<0.001$ ).

**Table 2. Effect of feeding regimen (TR), period (NC) and interaction on average daily gain (AMG) of Sarda pigs submitted to different feeding systems (ls means  $\pm$  S.E.)**

	N	Groups			P<		
		A	B	C	TR	NC	TR*NC
<b>ADG (kg/head/day)</b>	12	0.910 $\pm$ 0.03 a	0.351 $\pm$ 0.03 c	0.469 $\pm$ 0.03 b	0.001	0.001	0.001

a, b values with different letters are different ( $P<0.05$ ).

Preliminary results (Table 2) showed differences between feeding systems, with best performance in group A: weight and fat thickness were higher in group A than group B and group C (Table 2). This is probably due to the higher energetic availability in the A group. Also the fat thickness was higher ( $P<0.01$ ) in group A than group C; this result is in agreement with data of Pugliese *et al.* (2003) on Nero Siciliano pigs reared indoor. The lower backfat thickness found in groups B and C should be considered in relationships with the lower growth rate observed in these groups. Pugliese *et al.* (2003) reported that a slower growth rate usually favours muscle deposition with respect to fat, resulting in leaner carcasses.

**Table 3. Live weight and morphological measurements of Sarda breed submitted to different feeding systems (ls means  $\pm$  S.E.)**

	N	Groups			P<
		A	B	C	
Live weight (kg)	12	294.0 $\pm$ 12.0 a	170.7 $\pm$ 12.0 b	202.0 $\pm$ 12.0 b	0.001
Withers height (cm)	12	89.7 $\pm$ 1.6 a	82.0 $\pm$ 1.6 b	85.7 $\pm$ 1.6 b	0.020
Rump height "	12	91.0 $\pm$ 1.5 a	83.5 $\pm$ 1.5 c	88.0 $\pm$ 1.5 b	0.020
Chest girth "	12	172.2 $\pm$ 3.0 a	129.7 $\pm$ 3.0 c	139.2 $\pm$ 3.0 b	0.001
Chest lenght "	12	135.0 $\pm$ 4.1 a	116.5 $\pm$ 4.1 b	125.0 $\pm$ 4.1 b	0.030
Chest depth "	12	59.0 $\pm$ 1.2 a	45.7 $\pm$ 1.2 b	49.5 $\pm$ 1.2 b	0.001
Chest width "	12	46.0 $\pm$ 0.7 a	35.7 $\pm$ 0.7 b	37.0 $\pm$ 0.7 b	0.001
Rump width "	12	43.7 $\pm$ 0.9 a	31.5 $\pm$ 0.9 c	34.5 $\pm$ 0.9 b	0.001
Fat thickness (mm)	10	84.5 $\pm$ 3.8 a	29.7 $\pm$ 2.7 c	39.7 $\pm$ 2.7 b	0.001

a, b values with different letters are different ( $P<0.05$ ).

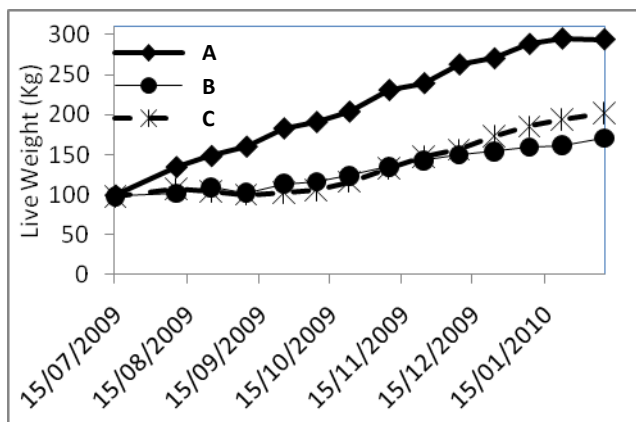


Fig. 1. Live weight trend (kg) of Sarda pigs submitted to different feeding systems (A, reared in plein-air system in the woods and fed *ad libitum* with a commercial concentrate; B, reared in plein-air in the woods and fed with ground barley (1.8 kg/h/d), with an automatic feeder; C, fed at pasture in the woods and receiving 500 g/h/d of barley grain).

## IV – Conclusions

Data show that the performance *in vitam* were significantly influenced by the nutritional level as reported by Liotta *et al.* (2005). The extensive feeding system represents the best compromise between performance and farming profit. The preliminary results presented encourage to pursue research experiments to better understand which feeding and rearing conditions can allow good results by respecting the tradition and productive performance of Sarda breed pig.

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# Biometric and rheologic parameters and qualitative properties of meat from "Sarda" breed pigs: Preliminary results

S. Porcu\*, R. Mazzette\*\*, C. Manca\*\*, M. Decandia\*, G. Busia\*\*, G. Riu\* and S. Ligios\*

\*Dipartimento per la Ricerca nelle Produzioni Animali (DiRPA), Loc Bonassai, 07040 Olmedo, Sassari (Italy)

\*\*Dipartimento di Biologia Animale, Sezione Ispezione degli alimenti di origine animale  
Via Vienna 2, 07100, Sassari (Italy)

**Abstract.** The aim of the present study was to evaluate the impact of different breeding systems on the quality parameters of meat from Sarda pigs. Twelve male pigs, aging 15 months, were subdivided in three groups (A,B,C) homogeneous in number of subjects and mean weight ( $98.4 \pm 13.8$  kg). Groups A and B were reared in *plein-air* and fed with commercial concentrate *ad libitum* and ground barley (1.8 kg/head/day) respectively. Group C was grazed on pasture and received a daily supplement of 500 g/head/day of barley grain. All the subjects were slaughtered after 7 months. A section between L2 to L5 was used to evaluate the lean, fat, rind, connective and bone yield. Samples of muscles *Longissimus dorsi* and *Psoas major* and inner fat were used to determinate colorimetric (CIELab) and rheologic (TPA test: 24h – 7days) parameters. Group A showed highest yields in fat (71.8%) and lowest in connective (1.2%) linked to lowest values in terms of hardness. Group B showed highest yields in lean (41.1%) and connective (2.6%) and lowest in fat (48.9%) linked to highest hardness and lowest springiness values. Group C showed intermediate values in lean (34.6%), fat (55.9%) and connective (1.5%) yields but the samples resulted more firm.

**Keywords.** Sarda breed pig – Colorimetric and rheologic parameters – Meat picking yield – Meat quality.

## **Paramètres biométriques, propriétés rhéologiques et qualitatives de la viande du porc autochtone de race Sarde: Résultats préliminaires**

**Résumé.** L'objectif de ce travail est l'évaluation de l'impact des techniques d'élevage sur les caractéristiques qualitatives de la viande du porc autochtone de race Sarde. Douze mâles (15 mois), divisés en trois groupes (A,B,C), de poids ( $98,4 \pm 13,8$  kg) et d'âge comparables ont été soumis aux techniques d'élevage suivantes: en plein air, avec administration d'un aliment commercial *ad libitum* (A) ou d'une ration d'orge moulue (B); au pâturage dans un bois (C), avec une complémentation quotidienne de 500 g d'orge en grain. À l'abattage (22 mois) ont été mesurés les rendements en maigre, gras, couenne, conjonctif et os sur une section de la région L2-L. Les paramètres colorimétriques (CieLab) et rhéologiques (TPA test: 24h - 7 jours) ont été étudiés sur les échantillons des muscles *Longissimus dorsi* et *Psoas major*. Le groupe A montrait des rendements élevés en gras (71,8%) et faibles en conjonctif (1,2%), corrélés à des valeurs faibles de dureté. Le groupe B présentait des rendements élevés en maigre (41,1%) et conjonctif (2,6%) et faibles en gras (48,9%), qui s'accompagnaient de valeurs de dureté supérieures et de souplesse inférieures. Le groupe C présentait des valeurs intermédiaires en ce qui concerne les rendements en maigre (34,6%), gras (55,9%) et conjonctif (1,5%), mais les échantillons étaient plus compacts.

**Mots-clés.** Porc de race Sarde – Paramètres colorimétriques et rhéologiques – Rendement de carcasse – Qualité de la viande.

## **I – Introduction**

The preservation of the autochthonous breeds is an useful tool for the biodiversity safeguard in the mediterranean areas, where the economic sustainability and productivity of the ecosystem are an essential requirement for their subsistence. Moreover the autochthonous race breeding

affects some important ecological, social and cultural aspects, such as safeguard of the regions, the rural community and their traditions.

Recently, initiatives in safeguard of autochthonous Sarda breed pigs were carried out in Sardinia, (Porcu *et al.*, 2007), aimed at the valorization of meat and meat products. Consumers demand and appreciate these kind of products, where recognize genuineness, sustainability, as well as environment integration. On the other hand, due to the poor productive performance, the survival of this breed is strongly linked to the development of products.

Many researches showed that chemical composition and quality of meat of autochthonous breeds vary in relation to rearing system, age and weight at slaughtering (Lo Fiego *et al.*, 2007), and in general, demonstrated non homogeneous characteristics (Gentry *et al.*, 2004; Gonzales *et al.*, 2007).

Few studies have been carried out about Sarda breed pigs (Porcu *et al.*, 2010), that are usually reared by outdoors systems. Improve the knowledge on the quality of meat is an extremely important issue for its qualification, but you must also identify best breeding conditions to combining yields and quality.

The aim of the present study was to evaluate the impact of different breeding and feeding systems on some meat parameters of Sarda breed pigs.

## II – Materials and methods

Twelve Sarda breed male pigs, with  $98.4 \pm 13.8$  kg of body weight, were subdivided into three groups (A,B,C), each including four animals. Groups A and B were reared in *plein-air* and allowed *ad libitum* access to commercial feed and rationed ground barley respectively. Group C was grazed on woody pasture and received a daily supplement of 500 g/head/day of barley grain. The pigs were weighed weekly during the experiment, which lasted seven months. After slaughtering, a section of the region included between the 2<sup>th</sup> and 5<sup>th</sup> lumbar vertebra (Campodoni *et al.*, 1999; Porcu *et al.*, 2007) was isolated and dissected into the major tissues (lean, fat, connective and bone). Samples of *Longissimus dorsi*, *Psoas major* and inner fat were analyzed for: (i) Colorimetric parameters: one hour after slaughtering, L\*, a\*, b\*, values were determined, in triplicate, by a Chroma Meter Minolta CR400, standard illuminant C; and (ii) Texture analysis: both 24 hours and 7 days after slaughtering, two cylinders (1.5 x 2 cm) were obtained for each sample of the muscle. TPA test was performed using a Universal Testing Machine TAXT plus Texture Analyser (Stable Microsystems Ltd.) with the Texture Exponent software (Vs.2.0.0.7). A double compression cycle test was performed using an aluminum cylinder probe (P/75). A time of 0 s was allowed to elapse between the two compression cycles (bite). Force–time deformation curves were obtained with a 5 kg load cell applied at a cross-head speed of 1 mm/s. The following parameters were evaluated: *hardness* (g, H), maximum force required to compress the sample; *cohesiveness* (Co), extent to which the sample could be deformed prior to rupture; *springiness* (m), ability of the sample to recover its original form after deforming force was removed; *adhesiveness* (g x s), negative parameter which represent the area under the abscissa after the first compression. The results were analyzed using GLM procedure of SAS (2001).

## III – Results and discussion

The evaluation of sample cuts (Table 1) showed the positive effects of different rearing and feeding systems on weight and fat percentage, being the mean values highest in Group A ( $6193.5 \pm 446$  g and  $71.8 \pm 2.5\%$  respectively). Moreover samples from Group A showed lowest percentage in lean tissues (22.9%), connective (1.2%) and bone (4.1%).

**Table 1. Tissue composition of the sample cut of Sarda breed pigs reared with different systems (Is means  $\pm$  S.E.)**

	N	Groups			P<
		A	B	C	
Sample cut weight (g)	12	6193 $\pm$ 446 a	3396 $\pm$ 446 b	4086 $\pm$ 446 b	0.010
Lean (%)	12	22.9 $\pm$ 1.4 c	41.1 $\pm$ 1.4 a	34.6 $\pm$ 1.4 b	0.001
Fat (%)	12	71.8 $\pm$ 2.5 a	48.9 $\pm$ 2.5 b	55.9 $\pm$ 2.5 b	0.001
Connective (%)	12	1.2 $\pm$ 0.3 b	2.6 $\pm$ 0.3 a	1.5 $\pm$ 0.3 b	0.050
Bone (%)	12	4.1 $\pm$ 1.5	7.4 $\pm$ 1.5	8.0 $\pm$ 1.5	0.210

a, b means with different letters within row were significantly different ( $P<0.05$ ).

The results of the colorimetric parameters are shown in Table 2. Low values of  $L^*$  (range 38.7 $\pm$ 1.6  $\rightarrow$  51.9 $\pm$ 2.2) were observed, especially in the *Psoas major*, where the minimum values were showed in samples from Group A. Meat from the Sarda breed pig appears darker and redder than commercial intensive reared breeds (Gentry *et al.*, 2004), and other autochthonous breeds (Gonzales *et al.*, 2007). Indeed the value of  $a^*$ , positively correlated to meat quality, was very high, especially in muscle *Psoas major*, in comparison to other studies (Franci *et al.*, 2004).

**Table 2. Colorimetric parameters (TPA) in meat (muscles *Longissimus dorsi* and *Psoas major* and backfat) of Sarda breed pigs reared with different systems (Is means  $\pm$  S.E.) at 24 h after slaughtering**

	N	Groups			P<
		A	B	C	
<i>Longissimus dorsi</i>	12				
$L^*$		48.3 $\pm$ 2.2	50.1 $\pm$ 2.2	51.9 $\pm$ 2.2	0.53
$a^*$		16.0 $\pm$ 2.0	15.4 $\pm$ 2.0	14.9 $\pm$ 2.0	0.93
$b^*$		9.9 $\pm$ 1.2	10.0 $\pm$ 1.2	10.1 $\pm$ 1.2	0.99
<i>Psoas major</i>	12				
$L^*$		38.7 $\pm$ 1.6 b	41.3 $\pm$ 1.6 b	46.0 $\pm$ 1.6 a	0.02
$a^*$		21.7 $\pm$ 0.8	23.3 $\pm$ 0.8	22.0 $\pm$ 0.8	0.33
$b^*$		9.3 $\pm$ 0.6	10.9 $\pm$ 0.6	11.7 $\pm$ 0.6	0.07
Inner Fat	12				
$L^*$		78.5 $\pm$ 0.9	77.8 $\pm$ 0.9	75.2 $\pm$ 0.9	0.08
$a^*$		1.4 $\pm$ 0.2 c	3.6 $\pm$ 0.2 a	2.8 $\pm$ 0.2 b	0.001
$b^*$		2.5 $\pm$ 0.6	4.7 $\pm$ 0.6	4.3 $\pm$ 0.6	0.07

a, b means with different letters within row were significantly different ( $P<0.05$ ).

The results of instrumental measurement of colorimetric parameters in inner fat showed a very high value of  $L^*$  (mean value  $>75$ ) and a significant difference between the groups regarding the  $a^*$  value, that was higher in fat from samples from group B (3.6 $\pm$ 0.2).

The results of the texture parameters in relation to the study groups, are shown in Table 3. Regarding the TPA test, significant differences between groups were not shown ( $p>0.05$ ) at 24 h nor 7 days after slaughtering. As expected, the hardness value decreased significantly with time in all samples. The springiness was higher in the samples from group A, and underwent a small increase during the maturation of meat at low temperatures. In samples from group C lowest values in terms of hardness were linked to the low percentage of connective. In group B highest hardness and lowest springiness values were correlated with the highest yields in lean

(41.1%), connective (2.6%) and lowest in fat (48.9%). The samples from group C resulted more firm and showed intermediate values in lean (34.6%), fat (55.9%) and connective (1.5%).

**Table 3. Texture parameters (TPA) in meat (muscles *Longissimus dorsi* and *Psoas major*) of Sarda breed pigs reared with different systems (Is means  $\pm$  S.E.) at 24 h after slaughtering**

	N	Groups			P<
		A	B	C	
<i>Longissimus dorsi</i>					
Hardness (g)	12	11229±2764	16888±2764	14628±2764	0.38
Springness	12	0.7±0.05 a	0.5±0.05 b	0.6±0.05 b	0.05
Cohesiveness	12	0.5±0.05	0.4±0.05	0.5±0.05	0.65
Chewiness (g/cm <sup>2</sup> )	12	3284±735	3031±735	3703±735	0.81
Adhesiveness (g x s')	12	-98.51±22	-66.5±22	-73.69±22	0.58
<i>Psoas major</i>					
Hardness (g)	12	13317±1375 a	9300±1375 b	8133±1375	0.05
Springness	12	0.5±0.04	0.5±0.04	0.6±0.04	0.39
Cohesiveness	12	0.4±0.05	0.4±0.05	0.4±0.05	0.98
Chewiness (g/cm <sup>2</sup> )	12	3263±854	1829±854	1756±854	0.41
Adhesiveness (g x s')	12	-60.11±10	-32.28±10	-26.54±10	0.08

a, b means with different letters within row were significantly different (P<0.05).

## IV – Conclusions

Although the preliminary results, Sarda breed pigs are incline to deposit subcutaneous fat when have greater food supply, as demonstrated in other autochthonous breeds. The data resulted comparable with other trials realized under similar conditions. However, further studies, aimed to identify the rearing and feeding systems suitable to obtain meat with high nutritional and technological values, will be carried out.

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# Effects of feeding and rearing system on carcass characteristics of Sarda breed pig: Preliminary study

S. Porcu\*, R. Mazzette\*\*, C. Manca\*\*, M. Decandia\*, D. Meloni\*\* and S. Ligios\*

\*Dipartimento per la Ricerca nelle Produzioni Animali (DiRPA), Loc Bonassai, 07040 Olmedo, Sassari (Italy)

\*\*Dipartimento di Biologia Animale, Sezione Ispezione degli alimenti di origine animale, Sassari (Italy)

**Abstract.** The effects of feeding and rearing systems on carcass characteristics of Sarda breed pigs were studied. Twelve castrated male pigs, homogeneous for body weight ( $98.4 \pm 13.8$  kg) and age (15 months) were randomly assigned to three feeding systems. A e B groups, reared in plein-air system in the woods and fed *ad libitum* with commercial concentrate and fed ground barley (1.8 kg/head/day) respectively; C, fed at pasture in the woods and receiving 500 g/head/day of barley grain. At slaughtering (22 months) carcass weight, biometric measures, pH (at 45' and 24 h after slaughter) and backfat thickness located at the first (1T) and the last (UT) thoracic vertebra and at the top of the *middle gluteus* (GM) were recorded. The live weight (kg) value was 294.0 (A), 170.7 (B) and 202.0 (C). Results showed differences between feeding systems: slaughter yield at 1 h after slaughtering was higher in group A (82.04%) compared to B (78.03%) and C (77.97%); the slaughter yield at 24 h after slaughtering was 80.37% (A) vs 76.27% (B) vs 76.33% (C). Also the fat thickness was higher ( $P < 0.01$ ) in group A.

**Keywords.** Sarda breed pig – Feeding system – Rearing system – Slaughtering yield.

## *Effet du système d'alimentation et d'élevage sur les caractéristiques de la carcasse des porcs de race Sarde : Études préliminaires*

**Résumé.** Ce travail a consisté en l'étude des effets de l'alimentation et du système d'élevage sur le rendement à l'abattage des porcs de race Sarde. Douze mâles castrés, de poids ( $98,4 \pm 13,8$  kg) et d'âge (15 mois) comparables, ont été divisés en trois groupes (A,B,C) et soumis à différentes techniques d'élevage: A et B, élevés en plein air, alimentés respectivement *ad libitum* avec un aliment commercial ou avec une ration de 1,8 kg/tête/jour de farine d'orge (B); C, au pâturage dans un bois avec une complémentation quotidienne de 500 g d'orge en grain. À l'abattage (22 mois) ont été mesurés: le rendement à chaud et à froid de la carcasse, les mesures biométriques, pH à 45' et à 24 h après abattage) et l'épaisseur du gras au niveau de la première et de la dernière vertèbre thoracique et du muscle Gluteus moyen. Le poids vif (kg) était de  $293,4 \pm 7,2$ ;  $171,1 \pm 7,2$  et  $202,0 \pm 7,2$  pour les groupes A, B et C. L'analyse des résultats a montré des différences entre les divers systèmes d'alimentation: le groupe A présentait un rendement à l'abattage supérieur, aussi bien à 1 h [82,04% vs 78,03% (B) et 77,97% (C)], qu'à 24 h [(80,37% vs 76,27% (B) et 76,33% (C)]. L'épaisseur de gras dorsal des carcasses du groupe A était aussi nettement supérieure ( $P < 0,01$ ).

**Mots-clés.** Porc de race Sarde - Système d'alimentation - Système d'élevage - Rendement à l'abattage.

## I – Introduction

The biodiversity safeguard and the fight against the depopulation of the marginal areas is given also by the exploitation of the autochthonous species (Porcu, 2008). Indeed, the use of autochthonous breed pig, some of which are living in absolute freedom, provide high quality production and increased the rate of self-provision of the meat market, limiting importation. Moreover they could allow a wider and more rational exploitation of marginal areas. The autochthonous breeds are the perfect balance between the animal and the environment in which they live increasing the value of the local traditions and diversifying the production



(Goracci, 2008). Sarda pig inserts in this context very well (Porcu *et al.*, 2010).

Available data on productive performances and pork quality about free-range or confinement reared pigs vary widely: several factors could affect the results such as climatic and environmental conditions, genotypes etc. (Filetti *et al.*, 2003). Among these, in particular the nutritional level and qualitative aspects of the food should be considered. Also, the growth of the animal and its productive performance (chemical composition and meat quality) vary in function of rearing system, age and weight at slaughtering. Only recently few researches have been conducted about Sarda breed pig on their growth (Porcu *et al.*, 2010). Establishing the performances of local breeds may involve both consistent differences between rearing environment and interactions between management and breed characteristics (Pugliese *et al.*, 2003).

The aim of the study was to evaluate the effect of different breeding and feeding systems on carcass characteristics of Sarda breed pigs.

## II – Materials and methods

The study lasted seven months (July 2009-February 2010) and was conducted at the experimental farm of AGRIS agency in Foresta Burgos (Illorai, Sassari; 44°59' lat. North and 4°55' long. East). The experimental site is characterized by a forest area of 40 ha divided in lots of various sizes and with altitude ranging from 830 to 930 m. Twelve Sarda breed pigs castrated male coming from the AGRIS agency (DIRPA), homogeneous for genetic, weight ( $98.4 \pm 13.8$  kg), age (15 months) were randomly assigned to three experimental groups (A, B and C) that differed in the feeding systems: A, reared en *plein-air* system in a wood area of 4500 m<sup>2</sup> and supplemented with a commercial concentrate (*ad libitum*); B, reared en *plein-air* in a wood area of 6000 m<sup>2</sup> and supplemented with ground barley (1.8 kg/head/day), through an automatic feeder; C, fed at pasture in the wood area of approximately 20 ha and supplemented with barley grain (500 g/head/day). Just before the slaughtering (22 months of age) live weight of each animal was recorded, then the carcass weight, the white offals weight and red offals weight were detected. The pH values: after 45 minutes (pH<sub>1</sub>), after 24 hrs (pH<sub>u</sub>), *post mortem*, at the top of the *middle gluteus* (GM), at the first (1T) and the last (UT) thoracic vertebra were recorded by a pH-meter (pH600 EUTECH Instrument) equipped with penetration probe.

After 24 hours of refrigeration at 4°C for each subject: carcass weight, and from the right half-carcass, pH<sub>u</sub> and the thickness of dorsal fat located at the top of the middle gluteus (GM), at the first (1T) and the last (UT) thoracic vertebra, carcass length, inner and outer chest depth (Tables 2 and 3) were determined.

## III – Results and discussion

The data (Table 1) showed that slaughter yield was significantly ( $P < 0.001$ ) higher in group A (82.04%) than B (78.03%) and C (77.97%). The slaughter yield at 24 h after slaughtering was 80.37% (A) vs 76.27% (B) vs 76.33% (C). Carcass length and chest depth were not different (Table 1). The fat thickness (Table 2), was higher in group A than the other ones.

Mean values of pH were reported in Table 3. No differences ( $P > 0.05$ ) were observed in pH<sub>1</sub>, where the overall values were considered as normal for the examined muscles (range  $6.15 \pm 0.07 \rightarrow 6.55 \pm 0.11$ ). Significant differences ( $P < 0.05$ ) between mean values of pH<sub>u</sub> were observed (range  $5.64 \pm 0.05 \rightarrow 5.90 \pm 0.04$ ). The pH<sub>u</sub> values were highest in samples from group A ( $> 5.82$ ), while resulted lowest in samples from group B (5.64).

The carcass characteristics showed the positive effects of different rearing and feeding systems on weight and fat thickness as reported by various authors (Liotta *et al.*, 2005; Pugliese *et al.*, 2003) on Nero Siciliano pigs fed *ad libitum* and reared indoor.

**Table 1. Carcass yield at 0 and 24 h after slaughtering (Is means  $\pm$  S.E.)**

	N	Groups			P<
		A	B	C	
Live weight (kg)	12	294.0 $\pm$ 12 a	170.7 $\pm$ 12.0 b	202.0 $\pm$ 12.0 b	0.001
Carcass weight 0 hr	12	241.2 $\pm$ 9.7 a	133.2 $\pm$ 9.7 b	157.5 $\pm$ 9.7 b	0.001
Carcass weight 24 hr	12	236.3 $\pm$ 9.4 a	130.2 $\pm$ 9.4 b	154.2 $\pm$ 9.4 b	0.001
White offals weight (kg)	12	13.6 $\pm$ 1.2	17.1 $\pm$ 1.2	14.6 $\pm$ 1.2	0.18
Red offals weight (kg)	12	5.4 $\pm$ 0.1 a	3.7 $\pm$ 0.1 c	4.5 $\pm$ 0.1 b	0.001
Carcass length (cm)	12	115.0 $\pm$ 2.4	106.1 $\pm$ 2.4	110.4 $\pm$ 2.4	0.08
Chest depth (cm)					
Inner	12	25.6 $\pm$ 1.1	23.4 $\pm$ 1.1	27.1 $\pm$ 1.1	0.12
Outer	12	29.1 $\pm$ 1.5	26.9 $\pm$ 1.5	28.9 $\pm$ 1.5	0.51

a, b means with different letters within row were significantly different ( $P<0.05$ ).

**Table 2. Backfat thickness (mm) of Sarda breed pigs (Is means  $\pm$  S.E.)**

	N	Groups			P<
		A	B	C	
Fat thickness 1T					
Total	12	102 $\pm$ 2.4 a	47 $\pm$ 2.4 c	58 $\pm$ 2.4 b	0.001
Inner	12	71 $\pm$ 2.5 a	31 $\pm$ 2.5 b	35.7 $\pm$ 2.5 b	0.001
Fat thickness UT	12				
Total	12	108 $\pm$ 3.1 a	49 $\pm$ 3.1 b	56 $\pm$ 3.1 b	0.001
Inner	12	79 $\pm$ 4.3 a	32 $\pm$ 4.3 b	41 $\pm$ 4.3 b	0.001
Fat thickness GM					
Total	12	98 $\pm$ 5.5 a	39 $\pm$ 5.5 c	57 $\pm$ 5.5 b	0.001
Inner	12	51 $\pm$ 4.8 a	20 $\pm$ 4.8 b	27 $\pm$ 4.8 b	0.01

a, b means with different letters within row were significantly different ( $P<0.05$ ).

**Table 3. pH values of Sarda pig meat 45' and 24 h after slaughtering (Is means  $\pm$  S.E.)**

	N	Groups			P<
		A	B	C	
pH <sub>1</sub> 1T	12	6.28 $\pm$ 0.07	6.24 $\pm$ 0.07	6.15 $\pm$ 0.07	0.44
pH <sub>1</sub> UT	12	6.29 $\pm$ 0.07	6.21 $\pm$ 0.07	6.19 $\pm$ 0.07	0.30
pH <sub>1</sub> GM	12	6.55 $\pm$ 0.11	6.47 $\pm$ 0.11	6.44 $\pm$ 0.11	0.70
pHu 1T	12	5.90 $\pm$ 0.04	5.73 $\pm$ 0.04	5.83 $\pm$ 0.04	0.08
pHu UT	12	5.82 $\pm$ 0.04 a	5.66 $\pm$ 0.04 b	5.73 $\pm$ 0.04 b	0.03
pHu GM	12	5.86 $\pm$ 0.05 a	5.64 $\pm$ 0.05 b	5.70 $\pm$ 0.05 b	0.04

<a, b means with different letters within row were significantly different ( $P<0.05$ ).

## IV – Conclusions

Data show that feeding and rearing system affected carcass characteristics. The typical backfat content in this local breed can be an important issue. In-fact, in similar conditions, an high content on unsaturated fatty acids was found in adipose tissue of outdoor-pigs, particularly

reared in wood (Pugliese *et al.*, 2005; Cosentino *et al.*, 2003). This aspect could be utilised to promote the quality of Sarda breed pig products. So the extensive feeding system represents the best compromise between performance and farming profit, as well as the valorization of local breed pigs can contribute to the economic and environmental sustainability of the traditional farming. However, being the data presented, the first scientific results obtained on the productive performance of Sarda breed pigs, further studies should be carried out in order to find the optimal rearing and feeding systems.

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# Characterization of dry-cured shoulders: Quality traits

R. Reina\*, J. Ventanas\*, P. López\*\* and C. García\*

\*Tecnología de los alimentos, Facultad de Veterinaria, Cáceres (Spain)

\*\*Tecnología de los Alimentos, Facultad de Veterinaria, Zaragoza (Spain)

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**Abstract.** The present study show some of the chemical and compositional characteristics of dry-cured shoulder from Iberian pigs fed with different diets and dry-cured shoulder produced with the rules of "Protected Designation of Origin Teruel ham". The existence of a quality rule makes the meat industries to follow different guidelines, from the selection of the genetic of the animal, food stuff and processing technology. As well, these rules establish different parameters that must be analyzed to avoid a possible fraud to the consumer. Not significant differences were found in the chemical parameters used by the quality standard, as fatty acids profile, in our samples. For this reason we must search other parameters to justify it. For that the use of products with characteristics very similar to the dry-cured ham which process time is shorter than that, like dry-cured shoulder, can help us to obtain good results which can be extrapolated. The aim of this study is to establish which structure and composition parameters of shoulders are more useful as quality indicators studying different dry-cured shoulders batches (white pigs vs Iberian pigs) depending on the genetic background of the animal and the type of feeding system during the final feeding phase.

**Keywords.** Shoulder – Protein – Lipid – Quality.

## *Une caractérisation des épaules séchées de porc : Des paramètres indicateurs de qualité*

**Résumé.** Dans le présent travail sont montrées certaines des caractéristiques concernant la composition des épaules séchées originaires de porcs Ibériques qui ont reçu divers types d'alimentation (aliments composés vs une alimentation naturelle) et des épaules séchées associées à l'"Appellation d'origine Teruel", qui sont élaborées conformément aux normes établies par leur appellation. La non existence de différences significatives pour quelques paramètres physiques et chimiques utilisés par la norme de qualité pour établir les caractéristiques de qualité que doivent présenter les produits, nous oblige à réaliser une nouvelle recherche de ceux-ci. Pour cela, l'utilisation de produits qui résistent à une élaboration plus rapide, tels que les épaules, peut servir à connaître le comportement d'autres produits comme le jambon d'élaboration la plus prolongée.

**Mots-clés.** Épaule – Protéine – Lipide – Qualité.

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## I – Introduction

Dry-cured shoulders are products of high quality, however the most recognized product are dry-cured hams. Technological characteristics of dry-cured shoulders and sensorial qualities of the final product depend on many factors related to the production process. The effective efficiency related with shoulders production is directly connected with the genetic aptitudes and physiological characteristics (weigh, handing and genetic) where pigs raise.

The high proportion of bone and fat in the foreleg of the pig involves a lower yield and a complex consumption as consumers find it difficult to take advantage of the lean. Nevertheless, the high sensory quality, the nutritional value and the short ripening process make these products a very interesting issue of study for two main reasons. First, from a scientific point of view, the results can be useful as quality indicators of the raw material and the final product can be extrapolated to dry-cured ham which involves a longer ripening process. Secondly, an

integral study of dry-cured shoulders according to its conformation by analysis of weight, measures, muscular topography and composition in order to develop a nutrition labeling (percentage of moisture, proteins, fat ...), can be useful to establish a better utilization of these pieces and for the consumers to gain a greater knowledge of the quality of this product.

## II – Materials and methods

The present study was carried out with three batches of Iberian dry-cured shoulders according to the type of feeding and rearing system during the finish fattening period (90 days prior to slaughter): (i) Country Valdesequera (n=9), (ii) Montanera Salamanca (n=10) and (iii) Normal intensive feeding (n=10), and (iv) another batch of dry-cured shoulder according to the "Protected Designation of Origin Teruel Ham", Teruel shoulders (n=20). Moisture, protein and sodium chloride percentage were determined using official methods (AOAC, 2000). Myoglobin content was evaluated using the method described by Horsney (1956). It was realized a TPA (texture profile) according to the method described by Bourne (1978) for a Universal texturometer TA-XT2i (Stable Micro Systems, Godalming, UK). Intramuscular fat content (GIM) was extracted and quantify using a mixture of chloroform:methanol (2:1) according the method described by Folch *et al.* (1957). Fatty acids methyl esters (FAMES) were prepared by acidic-trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Sandler and Karo, 1992). FAMES were analyzed by gas chromatography using a Hewlett-Packard HP 5890A gas chromatograph, equipped with a flame ionisation detector (FID). Volatile compounds were extracted by using the solid-phase microextraction (SPME) and subsequently analyzed by gas chromatography coupled to mass spectrometry (GC/MS) (gas chromatograph Hewlett-Packard 5890 series II coupled to a mass selective detector Hewlett-Packard HP-5791 A) according to the method described by Jurado *et al.* (2007). Volatile compounds were tentatively identified by comparing their mass spectra with those reported in the Wiley and NIST libraries.

The results from the experiments were used as variables and analyzed using a multivariate analysis (SPSS, 1997) in order to compare physico-chemical parameters between batches. Statistical significance was predetermined at 0.05.

## III – Results and discussion

Table 1 show high significant differences between batches, mainly the less weight and perimeter of the pieces of Montanera batch, it might be by the less initial weight of the pieces, because the yield of the novel pieces is in pure Iberian a 20% smaller than mixed Iberian, like intensive feed batch of this study. Teruel dry-cured shoulder was smaller in longitude and width because the age and size of the animals prior to slaughter was less than the Iberian pigs. Not significant differences were found between Montanera batch and extensive feeding batch (Country Valdesequera) in perimeter and width, probably because this shoulder came from of pure Iberian pigs (that's why these were longer for the same weight than the intensive feed batch).

Chemical composition is shown in Table 2. Not significant differences were found in the fat content between the four batches of the study. It might be because the batches feeding with feed used a prescription very similar than the acorn composition, according to the analysis of the fat composition from different batches. Montanera shoulders moisture was significantly smaller regarding to the other's shoulders, possibly because its morphologic sizes are smaller in this batch so the relation between surface and volume is higher. Myoglobin content showed significant differences being higher in Montanera and Country batches opposite the others two batches. These results are expected in animals raised in extensive. In others papers it had been related with quality attributes (juiciness, intensity of flavor and persistence of flavor), (Ventanas *et al.*, 2007). Higher results of sodium chloride were found, (in Iberian ham are between 3.5%, and more than 4.5% are salty) in three batches of Iberian shoulder, in comparison with Teruel

batch. This is because the three Iberian batches were processed in the same place and in the same conditions, different of Teruel shoulders. Finally, significant differences were found in protein percentage, being higher in Teruel batch.

**Table 1. Morphological characteristics**

	Country Valdesequera	Montanera Salamanca	Normal intensive feeding	Teruel shoulder	p
Weight (kg)	5.4 <sup>c</sup> ± 0.5	4.0 <sup>a</sup> ± 0.5	5.5 <sup>c</sup> ± 0.3	4.6 <sup>b</sup> ± 0.4	***
Lenght (cm)	76.0 <sup>c</sup> ± 3.6	71.4 <sup>b</sup> ± 3.0	72.9 <sup>b</sup> ± 0.5	62.6 <sup>a</sup> ± 0.9	***
Perimeter (cm)	54.3 <sup>ab</sup> ± 3.3	52.6 <sup>a</sup> ± 1.4	56.0 <sup>b</sup> ± 0.9	55.4 <sup>b</sup> ± 1.2	**
Width (cm)	26.2 <sup>a</sup> ± 3.6	26.0 <sup>a</sup> ± 2.2	29.0 <sup>b</sup> ± 3.2	23.9 <sup>a</sup> ± 0.8	***

Statistical significance: (\*\*\*)  $p < 0.001$ ; (\*\*)  $p < 0.01$ ; (\*)  $p < 0.05$ ; (ns) not significant.

**Table 2. Chemical composition**

	Country Valdesequera	Montanera Salamanca	Normal intensive feeding	Teruel shoulders	p
% Moisture	50.5 <sup>b</sup> ± 3.2	46.8 <sup>a</sup> ± 4.0	50.5 <sup>b</sup> ± 3.5	50.5 <sup>b</sup> ± 2.1	*
% Chloride (NaCl) (DM) <sup>†</sup>	11.7 <sup>b</sup> ± 0.6	11.6 <sup>b</sup> ± 1.7	11.6 <sup>b</sup> ± 0.6	8.8 <sup>a</sup> ± 1.1	***
% Fat (DM)	13.6 ± 1.7	14.7 ± 3.5	14.1 ± 4.5	14.4 ± 2.2	ns
% Protein (DM)	78.8 <sup>ab</sup> ± 7.3	73.4 <sup>a</sup> ± 7.1	84.1 <sup>bc</sup> ± 8.9	87.5 <sup>c</sup> ± 2.0	***
Myoglobin (mg Mb/g m)	6.6 <sup>b</sup> ± 0.5	6.7 <sup>b</sup> ± 0.4	3.9 <sup>a</sup> ± 0.8	4.1 <sup>a</sup> ± 0.6	***

Statistical significance: (\*\*\*)  $p < 0.001$ ; (\*\*)  $p < 0.01$ ; (\*)  $p < 0.05$ ; (ns) not significant.

<sup>†</sup>DM, dry matter.

Instrumental texture of Table 3 show the lower hardness of Teruel and Montanera batches in comparison with the others batches, because the higher content in fat make easier to broke the meat during the chewing, and the fewer gumminess and chewinness make higher the sensation of texture similar as "chewing gum".

**Table 3. Instrumental texture**

	Country Valdesequera	Montanera Salamanca	Normal intensive feeding	Teruel shoulder	p
Hardness	3402.7 <sup>b</sup> ± 724.8	2011.0 <sup>a</sup> ± 616.6	3191.0 <sup>b</sup> ± 1067.1	2037.4 <sup>a</sup> ± 490.9	***
Gumminess	1521.6 <sup>b</sup> ± 536.5	952.0 <sup>a</sup> ± 334.1	1520.4 <sup>b</sup> ± 532.8	930.1 <sup>a</sup> ± 201.8	***
Chewiness	959.0 <sup>b</sup> ± 379.1	523.4 <sup>a</sup> ± 236.6	940.3 <sup>b</sup> ± 368.7	571.0 <sup>a</sup> ± 144.0	***

Statistical significance: (\*\*\*)  $p < 0.001$ ; (\*\*)  $p < 0.01$ ; (\*)  $p < 0.05$ ; (ns) not significant.

Table 4 show the total of the fatty acids presents in the intramuscular fat of the 4 batches analyzed. MUFA was significant higher in Montanera and Teruel batch. However the SFA was higher in Intensive feed batch; while in the Country batch the SFA value is similar than the Montanera and Teruel batches, not existing significant differences between them. To explain this, we need to know if the feed used is different for Country and Intensive feed batches. Other possible reasons could be because the fat content is higher in Country batch or because the genetic in this batch (pure Iberian) and the exercise make easier the desaturase activity. It is

important the higher percentage of PUFA in Teruel shoulders, being similar than the Montanera shoulders. We expected these high results in comparison with the other Iberian batches of the study.

**Table 4. Fatty acids profile**

	Country Valdesequera	Montanera Salamanca	Normal intensive feeding	Teruel shoulders	p
SFA	40.35 <sup>ab</sup> ± 2.01	39.15 <sup>a</sup> ± 0.98	42.53 <sup>b</sup> ± 1.83	39.74 <sup>a</sup> ± 2.84	**
MUFA	48.42 <sup>a</sup> ± 1.92	50.01 <sup>b</sup> ± 1.23	49.52 <sup>ab</sup> ± 2.13	50.72 <sup>b</sup> ± 1.22	**
PUFA	9.49 <sup>ab</sup> ± 1.91	10.03 <sup>b</sup> ± 1.94	8.07 <sup>a</sup> ± 2.00	10.07 <sup>b</sup> ± 0.74	*
n-3	0.85 <sup>c</sup> ± 0.21	0.58 <sup>b</sup> ± 0.11	0.39 <sup>a</sup> ± 0.10	0.61 ± 0.11	***
n-6	8.14 <sup>b</sup> ± 1.11	8.30 <sup>b</sup> ± 1.54	6.52 <sup>a</sup> ± 1.70	8.23 <sup>b</sup> ± 0.58	**
n-6/n-3	8.75 <sup>a</sup> ± 1.67	14.44 <sup>b</sup> ± 0.97	17.56 <sup>c</sup> ± 2.65	14.03 <sup>b</sup> ± 0.52	***

Statistical significance: (\*\*\*)  $p < 0.001$ ; (\*\*)  $p < 0.01$ ; (\*)  $p < 0.05$ ; (ns) not significant.

Finally, were found significant differences in the content of n-3 between the four batches of the study, being higher in Country batch follow the others three batches. We expected this for Montanera batch because these pigs were fed with grass, but not for Teruel batch. We can explain this for the used feed; it can change a lot of parameters, like fatty acids or texture profile.

Table 5 shows some of the first results of the total volatile compounds (results in U.A.x10<sup>6</sup>).

**Table 5. Volatile compounds**

	Country Valdesequera	Montanera Salamanca	Normal intensive feeding	Teruel shoulders	p
3-methyl-butanal	9.0 <sup>a</sup> ± 3.8	18.8 <sup>a</sup> ± 10.7	36.3 <sup>ab</sup> ± 30.2	62.6 <sup>b</sup> ± 39.0	***
2-methyl-butanal	4.1 <sup>a</sup> ± 2.3	8.1 <sup>a</sup> ± 4.9	10.8 <sup>a</sup> ± 7.1	24.5 <sup>b</sup> ± 16.3	***
Pentanal	13.5 <sup>a</sup> ± 6.7	13.8 <sup>a</sup> ± 8.7	22.6 <sup>ab</sup> ± 12.7	31.0 <sup>b</sup> ± 17.2	**
Hexanal	106.0 <sup>ab</sup> ± 45.6	113.6 <sup>ab</sup> ± 55.7	143.9 <sup>b</sup> ± 84.5	49.0 <sup>a</sup> ± 40.4	**
Heptanal	10.9 ± 5.2	11.0 ± 4.8	9.4 ± 4.4	8.6 ± 4.7	ns
Octanal	6.7 <sup>a</sup> ± 2.9	6.6 <sup>a</sup> ± 2.5	8.8 <sup>a</sup> ± 3.0	16.4 <sup>b</sup> ± 12.0	**
Nonanal	10.9 <sup>ab</sup> ± 6.0	12.2 <sup>b</sup> ± 4.4	8.8 <sup>ab</sup> ± 3.0	6.2 <sup>a</sup> ± 4.0	**
Decanal	0.6 <sup>a</sup> ± 0.3	0.4 <sup>a</sup> ± 0.1	0.4 <sup>a</sup> ± 0.1	1.4 <sup>b</sup> ± 0.7	***
2,3-butanedione	3.3 <sup>a</sup> ± 0.3	0.7 <sup>a</sup> ± 0.3	1.1 <sup>a</sup> ± 0.3	13.7 <sup>b</sup> ± 6.0	***
2-Heptanone	29.0 <sup>a</sup> ± 14.9	50.0 <sup>b</sup> ± 32.6	26.1 <sup>a</sup> ± 7.3	15.2 <sup>a</sup> ± 9.1	***
Dihydro 2(3H)-5-methyl-furanone	0.8 <sup>a</sup> ± 0.4	0.8 <sup>a</sup> ± 0.1	0.6 <sup>a</sup> ± 0.2	2.4 <sup>b</sup> ± 1.4	***
2-pentyl-furan	2.4 <sup>ab</sup> ± 0.2	5.6 <sup>b</sup> ± 3.0	1.9 <sup>a</sup> ± 0.5	9.4 <sup>c</sup> ± 3.8	***
Dihydro 2(3H)-5-ethyl-furanone	3.4 <sup>ab</sup> ± 1.8	2.4 <sup>ab</sup> ± 0.9	1.7 <sup>a</sup> ± 0.3	3.7 <sup>b</sup> ± 2.0	**
Dihydro 2(3H)-5-butyl-furanone	0.9 <sup>ab</sup> ± 0.4	0.5 <sup>a</sup> ± 0.1	0.8 <sup>ab</sup> ± 0.4	1.2 <sup>b</sup> ± 0.4	**

Statistical significance: (\*\*\*)  $p < 0.001$ ; (\*\*)  $p < 0.01$ ; (\*)  $p < 0.05$ ; (ns) not significant.

The high number of volatile compounds detected, most of them waiting for identification, make us selected some of them because its interest. Is important the higher quantities in volatile

compounds in Teruel shoulders probably because these shoulders had been under storage temperatures during the last stage of ripening, that improve the formation of this compounds like the case of the aldehydes. However, is amazing the hexanal behavior (rancid flavor), being higher in Iberian shoulders in comparison with Teruel shoulder. The high content in Iberian shoulders can be explained for its longer time of process in comparison with the Teruel shoulders, or for its higher concentration of precursors. In the case of ketones, we can emphasize the presence of 2-heptanone, presenting higher concentration in Montanera batch. One of the precursors of this ketone is linoleic acid that shows differences in content slightly higher in Montanera batch. However, this difference is not enough to explain the high difference in this compound between the Montanera batch with the rest of batches. This compound had been identified as odour-active compound showing aromatic notes like almonds or toast (Carrapiso *et al.*, 2002). At the end, 4 furans were identified and described as odour-active. These compounds were higher in Teruel shoulders and it could have an important paper in the aroma of the shoulders due to his presence in ham has related with the typical aroma to "meat" (Flores *et al.*, 1998). The fewer volatile content in the case of Iberian shoulders may be explained for the higher richness of aromatic notes for the presence of others volatile compounds which are analyzing actually.

## IV –Conclusions

For the first time we provide a scientific data about shoulders quality, where the annual production is higher than 5 million of pieces, having a big economic weight in some communities in Spain.

With this study we can conclude that the fatty acids analysis can't use only as indicator parameter about the quality of the product. In our case, we haven't found any significant differences on the fat and the fatty acids proportion, existing the same tendency in the four batches. The same relationship had been found from the point of view of the genetic background and the animals feeding.

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# Comparison between Cinta Senese and Mora Romagnola crossed with Large White pigs

F. Sirtori, A. Croveti, G. Campodoni, R. Bozzi and O. Franci

Department of Biotechnology, Animal Science Section, University of Florence  
Via delle Cascine 5, 50144 Firenze (Italy)

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**Abstract.** The recovery of the "Prosciutto del Casentino", a typical product of the homonymous area located in the Arezzo province, led to the constitution, during 2007, of the "Consorzio del Prosciutto del Casentino". One of the main rules of the Consortium imposes the use of the cross between both Large White or Landrace with the Cinta Senese or Mora Romagnola autochthonous breeds. This first trial foreseen the comparison between two of the possible types of crossbreeding, in reason to the fact that the crossing between Cinta Senese and Large White is well investigated whereas information on the crossing between Mora Romagnola and Large White is lacking. Two different types of crossbreed have been compared for both *in vivo* and *post mortem* performances. Chemical-physical characteristics of raw meat and fat have been determined whereas the hams are at present during the seasoning period. MRxLW subjects produced fatter carcasses with higher thickness of subcutaneous fat than CSxLW (33.8 vs 22.9 mm). Sample joint of MRxLW showed higher fat and lower lean percentage than CSxLW (37.6 vs 25.2; 54.4 vs 64.7). As regards chemical-physical analyses it emerges that the meat of the CSxLW presented higher percentage of moisture (74.30 vs 72.71) and more water losses for pressure (100.05 vs 67.74 mm<sup>2</sup>) than MRxLW.

**Keywords.** Ham – Cinta Senese – Mora Romagnola – Crossbreeding.

## **Comparaison entre Cinta Senese et Mora Romagnola croisés avec Large White**

**Résumé.** La récupération du Prosciutto Casentino, produit typique de la région homonyme dans la province d'Arezzo, a conduit à la création, en 2007, du Consortium de Prosciutto Casentino. Parmi les contraintes imposées par la réglementation se trouve l'utilisation de la première génération de croisements entre les races améliorées Large White et Landrace et les races indigènes Cinta Senese et Mora Romagnola. L'expérimentation a fourni une comparaison entre les deux types génétiques locaux, alors que, tandis que sont disponibles les données de croisement entre les Cinta Senese et Large White, les résultats de Mora Romagnola x Large White manquent. On a fait l'élevage des deux types de croisés, qui ont été comparés pour leurs performances *in vivo* et *post mortem*. Ensuite, on a déterminé les caractéristiques chimiques et physiques de la viande fraîche et de la graisse tandis que la maturation des jambons n'est pas encore terminée. Les animaux MRxLW ont produit des carcasses plus grasses avec une graisse sous-cutanée plus épaisse que CSxLW (33,8 mm vs 22,9 mm) et des coupes plus riches en tissu adipeux et plus pauvres en maigre (37,6 vs 25,2, 54,4 vs 64,7). L'analyse physico-chimique montre que la viande de CSxLW a présenté un pourcentage plus élevé d'eau (74,30% vs 72,71%) et une perte d'eau plus grande avec la méthode de la pression (100,05 vs 67,74 mm<sup>2</sup>).

**Mots-clés.** Jambon – Cinta Senese – Mora Romagnola – Croisement.

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## **I – Introduction**

The use of the crossbreed between Cinta Senese and Large White pig in Tuscany has its origins early last century and provides the so-called "Bigio" or "Tramacchiato". This type of production was resumed last decade following the recovery of the Cinta Senese even if both the Technical Specifications of production for "Suino Cinto Toscano" and niche market, prefer the purebred. It remains that adequate test recently provided some guidance on the productivity of the cross of Large White with the Cinta Senese pig making possible a characterization, particularly in relation to the two parental breeds (Acciaioli *et al.*, 2002; Franci *et al.*, 2003, 2005). The characteristics of Mora Romagnola both as purebred or crossed are less known also

because of its limited diffusion, which has hampered the interest of the research that only recently published results of tests and surveys on the breeding area (Fortina *et al.*, 2005, 2006; Lo Fiego *et al.*, 2007; Zambonelli and Bigi, 2006). The official inclusion of this breed in the Technical Specifications of a traditional production as the Casentino ham, determines the need to clarify the behaviour in relation to farming economy and quality of product. The aim of this study was therefore to assess qualitative differences between the two different crossbreeds Cinta Senese x Large White and Mora Romagnola x Large White.

## II – Materials and methods

Eighteen pigs were used, 10 cross between Cinta Senese boars and Large White sows (CSxLW) and 8 cross between Mora Romagnola boars and Large White sows (MRxLW). The two genetic types, balanced by sex, were kept in two separate pens and fed mixtures *ad libitum*. During the period the subjects were weighed on a regular basis every two months. All the animals were slaughtered within two months starting from the heavier ones. At slaughter the carcasses were dissected in commercial cuts which were weighed to determine their percentage. On sample joint (loin from the 2<sup>nd</sup> to the 5<sup>th</sup> lumbar vertebrae) thickness of subcutaneous fat and area of the *Longissimus lumborum* were measured. Sample joint was dissected into: subcutaneous fat, divided into inner and outer layer, intermuscular fat, *Longissimus lumborum* and *Psoas major* muscles, other lean (muscle portions not identified) and bone. On *Longissimus lumborum* the following physical analyses were performed: (i) Color by Minolta colorimeter (Boccard *et al.* 1981); (ii) water holding capacity (Free water pressure by the method of Grau and Hamm (1952) modified; cooking loss (Boccard *et al.*, 1981), drip loss; and (iii) shear force value by Warner-Bratzler instrument. On *Longissimus lumborum* and *Psoas major* the following chemical determinations were carried out: Moisture, ether extract, crude protein, Ash (AOAC, 1990). Data were processed by GLM procedure of SAS statistical package (2003) using the following models: Weight at slaughter, ADG and age  $Y_{ijk} = \mu + R_i + S_j + \varepsilon_{ijk}$ ; carcass composition, sample joint composition and physical analyses of *L. lumborum* muscle  $Y_{ijk} = \mu + R_i + S_j + b^*(W_{ijk}) + \varepsilon_{ijk}$ ; Chemical analyses of *L. lumborum* and *Psoas major* muscles  $Y_{ijkl} = \mu + R_i + S_j + T_k + b^*(W_{ijkl}) + \varepsilon_{ijkl}$ ; where R = Breed, S = Gender, T = Type of Muscle, W = weight of right side.

## III – Results and discussion

Table 1 shows the *in vivo* performances of the two genetic types. The most evident result is the difference in slaughter weight, with the group CSxLW heavier at slaughter. This data could be affected by the difference in age between the two groups that is due to the choice of the breeder to slaughter the pig at the reaching of the visual maturity point. The most representative value was the ADG showing how the two groups behaved similarly during the trial period.

**Table 1. Weight, ADG and slaughter age**

Item	Genetic type		RSD
	CSxLW	MRxLW	
Live weight (kg)	179.3 a	155.4 b	21.6
ADG (kg)	0.352	0.378	0.06
Age (d)	504a	446b	14.2

As regard the carcass composition (Table 2), there was no difference in the dressing percentage but the results also showed that the CSxLW was basically leaner showing a greater proportion of ham and lesser proportion of kidney fat when compared to MRxLW.

**Table 2. Composition of the right half side**

Item	Genetic type		RSD
	CSxLW	MRxLW	
Dressing percentage	83.57	85.85	3.57
Loin with backfat	23.63 a	25.11 b	0.99
Ham (with feet)	32.17 a	28.94 b	0.93
Shoulder (with feet)	17.79	17.58	0.93
Belly with ribs	15.43	15.97	0.76
Jowl	3.30	3.36	0.46
Kidney fat	2.13 a	4.01 b	0.54
Head	5.55	5.03	0.38

Loin is greater in MRxLW only because this cut included backfat, according to the dissection protocol of the factory. The characteristic of MRxLW to depot more fat is clear also from the analyses of the sample joint (Table 3).

**Table 3. Sample joint composition**

Item	Genetic type		RSD
	CSxLW	MRxLW	
Sample joint (g)	2318.9	2482.1	3.57
Backfat thickness (mm)	22.98 a	33.84 b	4.14
Fat (%)	25.21 a	37.66 b	5.80
Backfat outer layer	12.19 a	15.75 b	2.05
Backfat inner layer	9.64 a	18.43 b	3.75
Intermuscular	3.70	4.27	1.16
Lean (%)	67.78 a	54.48 b	5.24
L. lumborum	40.12 a	33.80 b	3.25
Psoas major	14.98 a	10.59 b	2.03
Other lean	9.30	9.14	1.95
Bone (%)	10.06 a	8.02 b	1.37
Lean/bone	6.52	6.77	1.09

It is noteworthy that the thickness of subcutaneous fat is much higher in the group MRxLW. This parameter is confirmed by the incidence of subcutaneous fat, both as inner and outer layers. Intermuscular fat shows the same trend, even if not significant. Consequently to this behaviour of adipose tissue, the lean and bone components is more developed in CSxLW. Eliminating the masking effect of fat, however, the two genetic types showed similar lean/bone ratio.

The physical-chemical properties are reported in Table 4. The CSxLW showed moister meat, with lower content of protein and higher content of ash. Again, MRxLW showed higher fat content, even if not significant.

The moisture content also appear to affect the parameter of brightness ( $L^*$ ) which is higher in CSxLW subjects whereas the habit of the MRxLW animals to depot more fat didn't occur, showing how the influence of the genetic type is more evident in adipogenesis of the subcutaneous zone.

**Table 4. Chemical analysis of lean**

Item	Genetic type		RSD
	CSxLW	MRxLW	
Moisture (%)	74.30 a	72.71 b	1.01
Protein (%)	21.64 a	23.05 b	0.43
Ether extract (%)	2.35	2.91	1.18
Ash (%)	1.18 a	1.08 b	0.04

As regard the physical analyses of the meat (Table 5), CSxLW showed lower capacity of water retention only when measured as free water demonstrating that the three methods used for this analysis does not always agree among themselves as they are based on different physical principles of water removal.

**Table 5. Physical analysis of *Longissimus lumborum***

Item	Genetic type		RSD
	CSxLW	MRxLW	
Color			
L *	52.01 a	48.37 b	2.16
a *	12.29	13.37	2.40
b *	5.10	4.90	1.04
Water holding capacity			
Drip loss (%)	6.83	7.18	1.89
Cooking loss (%)	24.20	24.88	4.06
Free water (mm2)	100.05 a	67.74 b	18.55
Shear force (kg)			
Wb fresh	10.08	9.29	2.53
Wb cooked	10.17	9.48	1.55

## IV – Conclusions

The results showed that MRxLW crossbreed had greater tendency to depot fat. This trend is very evident in the subcutaneous fat and visceral fat. This could mean that this crossbreed is able to reach the slaughter age earlier, although at lower weights. On the contrary crossbreed including Cinta Senese breed favours lean cuts, especially the ham which is especially important from an economic point of view for the "Prosciutto del Casentino" production.

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# Vertical protein spot chains – proteomic indicators of proteolysis in dry-cured ham?

M. Škrlep\*, M. Čandek-Potokar\*, B. Šegula\*, C. Chambon\*\*1,2 and V. Sante-Lhoutellier\*\*2

\*Agricultural institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana (Slovenia)

\*\*1,2 PFEM- 2UR 370 QuaPA, INRA de TheixSaint Genès Champanelle (France)

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**Abstract.** Proteomic profile of Slovenian "Kraški pršut" dry-cured ham matured for 14 months, was studied. Insoluble protein fraction was extracted from dry-cured *Biceps femoris* muscles. 2-dimensional SDS PAGE gels (24 samples in three technical repetitions) were made and the protein pattern analysed. Several distinctive protein spot patterns (*i.e.* protein spot chains containing spots that differed in molecular weight but not in isoelectric point) were observed. The patterns were highly repeatable between the technical repetitions. The subsequent identifications showed the same protein inside one spot chain. Differences in estimated molecular weight between the spots from the same chain indicate the protein degradation, however, it could not be confirmed by the mass spectrometry (lacking accuracy). For firmer confirmation of our hypothesis, a comparison of proteomic profile of hams in different processing phases is needed.

**Keywords.** Dry-cured ham – Proteolysis – Proteomic profile – Vertical spot chains.

**Des chapelets verticaux de protéines : indicateurs protéomiques de la protéolyse dans le jambon sec ?**

**Résumé.** Le profil protéomique du jambon sec slovène "Kraški pršut" a été établi après 14 mois de séchage. La fraction de protéines insolubles a été extraite du muscle *Biceps femoris*. Les gels d'électrophorèse bidimensionnelle SDS PAGE (24 échantillons en trois répétitions techniques) ont été réalisés ainsi que l'analyse d'image des gels. Plusieurs chaînes verticales de protéines distinctes (c'est-à-dire des spots protéiques différant en poids moléculaire, mais pas en point isoélectrique) ont été observées. L'apparition de ces chaînes était très reproductible entre les répétitions techniques. Les identifications ultérieures ont montré qu'il s'agit d'une même protéine à l'intérieur d'une chaîne. Les différences en poids moléculaire estimé entre protéines de la même chaîne indiquent leur dégradation progressive. Pour confirmer notre hypothèse, un suivi du profil protéomique des jambons au cours du séchage est nécessaire.

**Mots-clés.** Jambon sec – Protéolyse – Profil protéomique – Chapelets verticaux.

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## I – Introduction

The proteolysis of muscle proteins by endogenous enzymes is one of the most important reactions that take place during dry-cured ham processing and is largely responsible for its sensory quality. The process itself begins already early *post mortem* with the breakdown of large cytoskeletal proteins (by calpains) and proceeds through degradation of myofibrillar proteins (mainly by cathepsins) and generation of great amount of small peptides and free aminoacids (by aminopeptidases), which may last for several months during the ham processing (Toldra and Flores, 1998). The degree of proteolysis can be assessed either directly by monitoring a degradation of several large proteins (in particular myofibrillar proteins) or indirectly by protein degradation products (shorter peptides, free amino acids and other amines, overall non-protein nitrogen). To evaluate degree of proteolysis chemical analysis of free amino acids or non protein nitrogen has thoroughly been used (Buscailhon and Monin, 1994a). One-dimensional protein electrophoresis has been used to follow up degradation of main muscle proteins (Toldra *et al.*, 1993; Tabilo *et al.*, 1999; Larrea *et al.*, 2006); however the method does



not allow separation between different proteins of the same molecular weight. This is possible using a two-dimensional electrophoresis (2DE) which separates proteins according to their molecular weight and isoelectric point and which, coupled by mass spectrometry, enables identification of more than 1000 proteins in one gel (Gorg *et al.*, 2000). This so called proteomic analysis represents a valuable tool for identification of molecular markers of food quality. Over the last years, several studies of proteomic research in meat science have been conducted (Hollung *et al.*, 2007). However the studies related to dry-cured ham are rare (Hortos *et al.*, 2004; Di Luccia *et al.*, 2005; Sidhu *et al.*, 2005), moreover these studies are mainly preliminary and difficult to compare due to different methodology and approach. In our recent study (Škrlep *et al.*, 2010a) on dry-cured ham proteomic profile, we noticed several distinctive features on the dry-cured *Biceps femoris* gels, among which the vertical protein spot chains attracted the most of our attention. They could be an indication of progressive protein degradation, however, further characterisation would be needed to confirm that hypothesis, which was the aim of the present research.

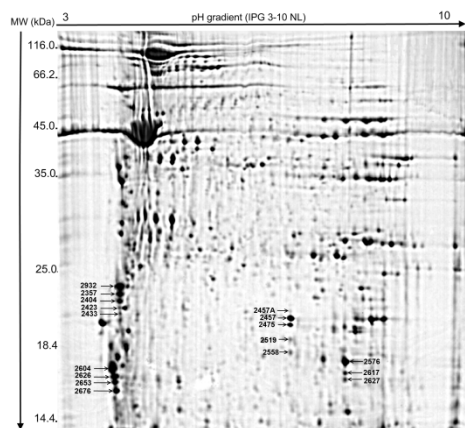
## II – Materials and methods

Material included in the present experiment originated from an extensive study on dry hams performed within EU project Truefood and experimental details are provided in our previous study (Škrlep *et al.*, 2010b). The investigation included also the proteomic analysis of dry-cured *biceps femoris* (BF) muscle, for which a subsample of 24 hams was selected. Sample preparation, protein extraction and two dimensional electrophoresis (2DE) procedure is described in Škrlep *et al.* (2010a) and was performed according to the modified method developed at INRA (Theron *et al.*, 2010). Shortly, insoluble protein fraction was extracted with repeated washing in low ionic strength buffer and loaded (1000 µg) on immobilised pH gradient strips for isoelectric focusing (70.000 Vh). For each sample three technical repetitions were made. SDS-PAGE was performed on 12.5% polyacrylamide gels. For the assessment of molecular weight (MW), protein MW marker #SM0431 (Fermentas Life Sciences, Glen Burnie, MD, USA) was applied prior to running second dimension. The gels were stained with Coomassie Brilliant Blue G250. The gel images were digitalized and spots automatically detected using ImageMaster 2D Platinum 6 software (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The comparison of the images from the present experiment to the images of fresh meat proteome from the available literature revealed some interesting differences, among which several vertical protein spot chains were the most distinctive (see Fig. 1). For the purpose of mass-spectrometry analysis, the spots of interest (spots from protein spot chains) were excised, destained, dehydrated, digested by trypsin and analysed (by peptide mass fingerprinting) using a Voyager DE Pro MALDITOF-MS (Applied Biosystems, Courtaboeuf, France) as previously described (Laville *et al.*, 2009). The obtained spectra were then compared to those from NCBI nr databases *susscrofa* (20090623, 21575 seq) or *mammalia* (20090623, 1263710 seq) using Mascot Software (Matrix Sciences London, V2.2, home license),

## III – Results and discussion

A representative 2DE gel image of insoluble muscle protein fraction of dry-cured BF muscle is shown on Fig. 1. In this article we focused on vertical chains of spots (also designated on Fig. 1). Such patterns could not be seen when compared to the corresponding regions of the gels reported for the fresh pig muscle (Morzel *et al.*, 2004; Hwang *et al.*, 2005; Laville *et al.*, 2005). The chains of spots had almost the same isoelectrical point and different (app 0.5 – 1.0 kDa) estimated molecular weight (see Fig. 1). Some of the most distinctive spots from the chains were subsequently excised (n=16) and analysed by mass spectrometry. The results of the protein identification and peptide matching against the database records are shown in Fig. 2. In the first case (chain 1) all five spots (2932, 2357, 2404, 2423 and 2433) were identified as the

same protein – myosin light chain (MLC1f). Three spots from the second chain (spots 2604, 2626 and 2653) were identified as another myosin light chain (HUMMLC2B) and one spot (2676) as fast skeletal myosin alkali light chain 1. For the remaining two analysed spot chains (spots 2457A, 2457, 2475, 2519 and 2558 in chain 3; spots 2576, 2617 and 2627 in chain 4) were again identified as the same protein, namely  $\alpha$ -B-crystallin (chain 3) and myoglobin (chain 4).



**Fig. 1. Representative gel image of insoluble protein fraction of dry-cured *Biceps femoris* muscle. Identified protein spots from vertical spot chains are denoted with arrows and reference numbers.**

Since the pattern of vertical spot chains was repeatable between the technical repetitions (results not shown) showing clear separation of the spots with progressive decreasing of the estimated molecular weight, one explanation for that could be the gradual proteolytic degradation of the protein molecules. In agreement with our results several studies reported, that dry-cured ham proteins are prone to intensive hydrolysis during the processing period (Cordoba *et al.*, 1994; 1993, Monin *et al.*, 1997; Tabilo *et al.*, 1999). More detailed studies, using one- and two- dimensional electrophoresis for monitoring the course of proteolysis (Di Luccia *et al.*, 2005; Larrea *et al.*, 2006) reported notable degradation or even complete disappearance of several specific myofibrillar proteins (including myosin light chain) during the course of dry-cured ham processing, but direct comparison with the present study is not possible.

However, there are also several facts that speak against our hypothesis. The cleavage of protein spots is expected to cause the shift in isoelectric point, which is not the case for vertical spot chains. This could happen if the cleaved peptide (or aminoacids) were neutral or nonpolar. Furthermore, it could not be undoubtedly confirmed by the mass spectrometry results associated with protein database query that the spots were fragments (see Fig. 2), although the results of matching may indicate such conclusions (e.g. progressive decrease in sequence coverage and number of matched peptides), especially in the case of chains 1 (MLC) and 4 (myoglobin). It is also worth mentioning, that in the case of chain 1, the observed molecular MW of all five spots exceeds the MW of the theoretical match. The database query also did not give any myosin molecules, which would match our search closer in MW, making explanation even more difficult. However, Larrea *et al.* (2006) in a study supported by monodimensional SDS-PAGE, reported comparable myosin light chain of 24.75 kDa, which could match ours. It is worth noting, that we have not made any control gels on fresh ham muscle or muscle during different processing stages, which would be useful to clarify the origin of vertical spot chains.

### CHAIN 1.

Spot 2932 (gi|117660874, MLC1f)  
1 MAPKKDVKKP AAAAAAPAPAP APAPAPAPAP PKEEKIDLSA IKIEFSKEQQ DEFKEAPLLF DRTGECKITL  
71 SGVGDVLRAL GTNPTNAEVK KVLGNPSNEE MNAKKIEFEQ FLPMLQAISN NKDQGSYEDF VEGLRVFDKE  
141 NGTVMGAEL RHVLATLGEK MKEEVEALM AGQEDSNGCI NYEAFVKHIM SI  
Spot 2357 (gi|117660874, MLC1f)  
1 MAPKKDVKKP AAAAAAPAPAP APAPAPAPAP PKEEKIDLSA IKIEFSKEQQ DEFKEAPLLF DRTGECKITL  
71 SGVGDVLRAL GTNPTNAEVK KVLGNPSNEE MNAKKIEFEQ FLPMLQAISN NKDQGSYEDF VEGLRVFDKE  
141 NGTVMGAEL RHVLATLGEK MKEEVEALM AGQEDSNGCI NYEAFVKHIM SI  
Spot 2404 (gi|117660874, MLC1f)  
1 MAPKKDVKKP AAAAAAPAPAP APAPAPAPAP PKEEKIDLSA IKIEFSKEQQ DEFKEAPLLF DRTGECKITL  
71 SGVGDVLRAL GTNPTNAEVK KVLGNPSNEE MNAKKIEFEQ FLPMLQAISN NKDQGSYEDF VEGLRVFDKE  
141 NGTVMGAEL RHVLATLGEK MKEEVEALM AGQEDSNGCI NYEAFVKHIM SI  
Spot 2423 (gi|117660874, MLC1f)  
1 MAPKKDVKKP AAAAAAPAPAP APAPAPAPAP PKEEKIDLSA IKIEFSKEQQ DEFKEAPLLF DRTGECKITL  
71 SGVGDVLRAL GTNPTNAEVK KVLGNPSNEE MNAKKIEFEQ FLPMLQAISN NKDQGSYEDF VEGLRVFDKE  
121 NGTVMGAEL RHVLATLGEK MKEEVEALM AGQEDSNGCI NYEAFVKHIM SI  
Spot 2433 (gi|117660874, MLC1f)  
1 MAPKKDVKKP AAAAAAPAPAP APAPAPAPAP PKEEKIDLSA IKIEFSKEQQ DEFKEAPLLF DRTGECKITL  
71 SGVGDVLRAL GTNPTNAEVK KVLGNPSNEE MNAKKIEFEQ FLPMLQAISN NKDQGSYEDF VEGLRVFDKE  
141 NGTVMGAEL RHVLATLGEK MKEEVEALM AGQEDSNGCI NYEAFVKHIM SI

### CHAIN 2.

Spot 2604 (gi|117660856, HUMMLC2B)  
1 MAPKNARRA AAEAGSSNVFS MFDQTQIQEF KEAFTVIDQN RDGIIDKEDL RDTFAMAGRL NVKNEELDAM  
71 MKEASGPINF TVFLTMFGEK LKGADPEDVI TGAFKVLDP EGKTIKKHFL EELLTTQCDR FSQEEIKNMW  
141 AAFPDVGGN VDYKNICYVI THGDAKDQE  
Spot 2626 (gi|117660856, HUMMLC2B)  
1 MAPKNARRA AAEAGSSNVFS MFDQTQIQEF KEAFTVIDQN RDGIIDKEDL RDTFAMAGRL NVKNEELDAM  
71 MKEASGPINF TVFLTMFGEK LKGADPEDVI TGAFKVLDP EGKTIKKHFL EELLTTQCDR FSQEEIKNMW  
141 AAFPDVGGN VDYKNICYVI THGDAKDQE  
Spot 2653 (gi|117660856, HUMMLC2B)  
1 MAPKNARRA AAEAGSSNVFS MFDQTQIQEF KEAFTVIDQN RDGIIDKEDL RDTFAMAGRL NVKNEELDAM  
71 MKEASGPINF TVFLTMFGEK LKGADPEDVI TGAFKVLDP EGKTIKKHFL EELLTTQCDR FSQEEIKNMW  
141 AAFPDVGGN VDYKNICYVI THGDAKDQE  
Spot 2676 (gi|117660856, fast skeletal myosin alkali light chain 1)  
1 MSFSADQIAE PKEAFLLPDR TGCEKITLSQ VGDVLRALGT NPTNAEVKKV LGNPSNEEM AKKIEFEQFL  
71 PMLQAISNNK DQGSYEDFVE GLRVFDKEGN GTVMGAELRH VLATLGEKMK EVEEVALMAG QEDSNGCINY  
141 EAFVKHIMS

### CHAIN 3.

Spot 2457A (gi|75063982, alpha-B-crystallin)  
1 MDIAIHPWI RRPFFPHSP SRLFDQFFGE HLLESLDFFA STLSPPFYFR PPSFLRAPSW IDTGLSEMRL  
71 EKDRFSVNL VKHFSPEELK VKVLGDVIEV HGKHEERQDE HGFISREFHR KYRIPADVDP LTITSSLSDD  
141 GVLTVNGPRR QASGPERTIP ITREEKPAVT AAPKK  
Spot 2457 (gi|75063982, alpha-B-crystallin)  
1 MDIAIHPWI RRPFFPHSP SRLFDQFFGE HLLESLDFFA STLSPPFYFR PPSFLRAPSW IDTGLSEMRL  
71 EKDRFSVNL VKHFSPEELK VKVLGDVIEV HGKHEERQDE HGFISREFHR KYRIPADVDP LTITSSLSDD  
141 GVLTVNGPRR QASGPERTIP ITREEKPAVT AAPKK  
Spot 2475 (gi|75063982, alpha-B-crystallin)  
1 MDIAIHPWI RRPFFPHSP SRLFDQFFGE HLLESLDFFA STLSPPFYFR PPSFLRAPSW IDTGLSEMRL  
71 EKDRFSVNL VKHFSPEELK VKVLGDVIEV HGKHEERQDE HGFISREFHR KYRIPADVDP LTITSSLSDD  
141 GVLTVNGPRR QASGPERTIP ITREEKPAVT AAPKK  
Spot 2519 (gi|75063982, alpha-B-crystallin)  
1 MDIAIHPWI RRPFFPHSP SRLFDQFFGE HLLESLDFFA STLSPPFYFR PPSFLRAPSW IDTGLSEMRL  
71 EKDRFSVNL VKHFSPEELK VKVLGDVIEV HGKHEERQDE HGFISREFHR KYRIPADVDP LTITSSLSDD  
141 GVLTVNGPRR QASGPERTIP ITREEKPAVT AAPKK  
Spot 2558 (gi|75063982, alpha-B-crystallin)  
1 MDIAIHPWI RRPFFPHSP SRLFDQFFGE HLLESLDFFA STLSPPFYFR PPSFLRAPSW IDTGLSEMRL  
71 EKDRFSVNL VKHFSPEELK VKVLGDVIEV HGKHEERQDE HGFISREFHR KYRIPADVDP LTITSSLSDD  
141 GVLTVNGPRR QASGPERTIP ITREEKPAVT AAPKK

### CHAIN 4.

Spot 2576 (gi|47523546, myoglobin)  
1 MGLSDGEWQL VLNVWGKVEA DVAGHGQEV IRLFKGPET LEKFDKFKHL KSEDEMASE DLKKGNTVL  
71 TALGGILKKK GHHEAELTPL AQSHATKHKI PKYLEFISE ATIQVLQSKH PGDFGADAQG AMSKALELFR  
141 NDMAAKYKEL GFQG  
Spot 2617 (gi|3660246, myoglobin)  
1 GLSDGEWQLV LVNVWGKVEAD VAGHGQEVLI RLFKGPETL EKFDFKFKHL SEDEMASED LKKHGNTVL  
61 ALGGILKKK GHHEAELTPLA QSHATKHKIP VKYLEFISEA IIQVLQSKHP GDGFGADAQGA MSKALELFRN  
141 DMAAKYKELG FQG  
Spot 2627 (gi|47523546, myoglobin)  
1 MGLSDGEWQL VLNVWGKVEA DVAGHGQEV IRLFKGPET LEKFDKFKHL KSEDEMASE DLKKGNTVL  
71 TALGGILKKK GHHEAELTPL AQSHATKHKI PKYLEFISE ATIQVLQSKH PGDFGADAQG AMSKALELFR  
141 NDMAAKYKEL GFQG

Fig. 2. Peptide matching against the database records in case of all four identified protein spot chains (matched sequences designated in bold and underlined).

## IV – Conclusions

Although we could not undoubtedly prove that protein spot chains indicate proteolytic degradation of several dry-cured ham proteins, there is a strong indication towards our hypothesis. However, confirmation of our results is needed, by comparing the proteomic profiles of dry-cured ham in different processing stages.

## Acknowledgments

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# Effect of production system and sex on different carcass traits of Iberian Pigs

D. Tejerina and S. García-Torres

Centro de Investigación "La Orden-Valdesequera", Dpto. Producción Animal, Junta de Extremadura, Guadajira, Badajoz (Spain)

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**Abstract.** Ninety *Retintos* Iberian pigs pure breed (45 males and 45 females) with similar age (~ 12 months old), live weight ( $90 \pm 5$  kg) and the same genetic line (Line Valdesequera), were randomly selected and distributed into three groups (*Montanera*, *Recebo* and *Intensive*,  $n = 15$  males and 15 females in each one) and slaughter at similar weight ( $150 \pm 10$  kg) for to evaluate the effect of production system and sex on some carcass traits. The length and width of rib cage, ham's perimeter, the thickness subcutaneous backfat (I, II and III levels) and the weight of carcass, loins, hams and forelegs were determined. The results showed that the production system had a significant effect on some carcass traits. So, the width of rib cage and the carcass's weight of pigs from *Montanera* were higher than the others systems. The thickness of subcutaneous backfat only shown differences in the measure most caudal, being higher in *Recebo* system and the weight of *Longissimus dorsii* (LD) was higher in *Intensive* system. On the other hand, the sex had effect on weight of forelegs (left and right), being higher in males in both pieces. In conclusion, yield cuts of the main commercial pieces (the hams) of the Iberian pig was not affected by the variables studied and males and females castrated only were different in the yield of forelegs.

**Keywords.** Pig – Ibéricos – Production systems – Carcass – Cutting.

## **Effet du système de production et du sexe sur différentes caractéristiques de la carcasse du porc Ibérique**

**Résumé.** Quatre-vingt-dix porcs de race pure *Retintos Ibériques* (45 mâles et 45 femelles) d'âge similaire (~ 12 mois), de poids vif ( $90 \pm 5$  kg) et de la même lignée génétique (Line Valdesequera), ont été choisis au hasard et répartis en trois groupes (*Montanera*, *Recebo* et *intensif*,  $n = 15$  mâles et 15 femelles pour chacun) abattus à poids similaire ( $150 \pm 10$  kg) pour évaluer l'effet du système de production et du sexe sur certaines caractéristiques de la carcasse. La longueur et la largeur de la cage thoracique, le périmètre du jambon, l'épaisseur sous-cutanée du lard dorsal (niveaux I, II et III) et le poids de la carcasse, des longes, des jambons, plus élevée dans le système *Recebo*, et le poids du *Longissimus dorsii* (LD), qui était plus élevé dans le système *intensif*. D'autre part, le sexe a eu un effet sur le poids des membres antérieurs (gauche et droit), plus élevé chez les mâles pour les deux pièces. En conclusion, les réductions de rendement des principales pièces commerciales (les jambons) du porc Ibérique n'ont pas été affectées par les variables étudiées et les mâles et femelles castrés étaient différents seulement pour le rendement des membres antérieurs.

**Mots-clés.** Porc Ibérique – Systèmes de production – Carcasse – Coupe.

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## **I – Introduction**

The Iberian pig is the most important Mediterranean swine breed, both in population and economic importance (Serra *et al.*, 1998). Most Iberian pig is consumed as high-priced cured products. However, the consumption as fresh meat pork has recently increased in importance (Ramirez and Cava, 2005).



The main production system of Iberian pigs according to Spanish legislation (BOE, 2007) are *Montanera* (the typical free-range rearing system of the Iberian pig with a nutritional strategy based on acorns and grass), *Recebo* (free-range system with nutrition based on a combination of acorn, grass and concentrate), and *Intensive* (indoor system with nutrition based on concentrate).

The carcass is the first step in the process of production of meat. Currently, most commercial transactions in the meat market are made on the carcass. Quality and composition of the carcass depend on several factors among which include the feeding, the production system employed, sex or age. So, the main aim was to study the effect of the production system and sex of Iberian pigs on some traits of the carcass.

## II – Materials and methods

Ninety Retintos Iberian pigs pure breed (45 castrated males and 45 females) with similar age (~12 months old), live weight ( $90 \pm 5$  kg) and the same genetic line (Line Valdesequera), were randomly selected and distributed into three groups ( $n = 15$  castrated males and 15 females in each one): *Montanera* (extensively reared and fed on acorns and grass), *Intensive* (intensively reared and fed on concentrate) and *Recebo* (extensively reared and fed on acorn and concentrate). Animals were slaughtered at  $150 \pm 10$  kg and ~15 months of age by electrical stunning and exsanguinations at a local slaughterhouse. After slaughtering, the carcasses were split longitudinally and weighed (carcass weight, kg). Carcass length was measured as the distance from the first rib to the pubic symphysis (length rib cage, cm) and carcass width was measured as drawing a straight line from the spine to the lower edge of the sternum (width rib cage, cm). On carcass, maximum perimeter ham (cm) was measured and also thickness subcutaneous backfat at three anatomical locations: the first rib (thickness subcutaneous backfat 1), the last rib (thickness subcutaneous backfat 2) and last lumbar vertebrae (thickness subcutaneous backfat 3). The left hand side of the carcass was divided into commercial cuts and commercial pieces weight were measured (kg): *Longissimus dorsii* (LD) muscle, left and right hams and left and right forelegs.

The effect of production system, sex and interaction of both variables was determined by analysis of variance, using the statistical software SPSS for Windows. If the effects studied was significant ( $p < 0.05$ ), then Tukey's test was used at the 5% levels to make comparisons between sample means.

## III – Results and discussion

Results were shown in Table 1. No differences were observed between castrated males and females into the studied variables, except in the values of weight of the forelegs (left and right) which were higher ( $p < 0.01$ ) in castrated males. Mayoral *et al.* (1999) also found no difference between different sex on carcass length, LD's weight or subcutaneous fat thickness. Although, the slaughter weight was similar for all animals, the carcass of pigs from *Montanera* were higher ( $p < 0.001$ ) than the animals from others production systems. In previous studies with other rustic breed (Alentejo pig), Oliveira (2007) not found differences in carcass weight as affected by the production systems (indoor vs outdoor). Carcass measured indicate that length of rib cage and perimeter of ham were not affected by the production system; while the values of width rib cage were higher ( $p < 0.001$ ) in animals from *Intensive* system. Bridi *et al.* (1997) also not found differences in the length of rib cage affected by production system.

Thickness subcutaneous backfat were only affected by the production system in the last lumbar vertebrae (Thickness subcutaneous backfat 3) and the highest thickness was found in animals from *Recebo* while the least thickness in animals from *Intensive* systems. A contrary trend was observed by Chiofalo *et al.* (2007) in previous studies with Nero Siciliano pigs in the anatomical

locations similar to our measure of thickness subcutaneous backfat 2. These differences could be due to both genetic and different production systems used.

Weight of commercial cuts (high value meat cuts) were not significantly affected by the production system except the *LD* muscle weight which was significantly greater ( $p<0.001$ ) in the *Intensive* animals.

**Table1. Effect of production system and sex on different carcass traits of Iberian pigs**

	Production system			Sex		SEM	Significance	
	" <i>Montanera</i> " n=30	" <i>Intensive</i> " n=30	" <i>Recebo</i> " n=30	Male n=45	Female n=45		Production system	Sex
Lenght rib cage (cm)	80.83a	79.32a	78.8a	79.82	79.48	0.49	ns <sup>1</sup>	ns
Width rib cage (cm)	23.8a	25.81b	25.55b	24.82	25.29	0.19	***	ns
Perimeter ham (cm)	75.93a	74.65a	76.25a	77.00	74.21	0.62	ns	ns
Thickness subcutaneous backfat (cm)								
• first rib	8.97a	8.61a	8.8a	8.64	8.95	0.10	ns	ns
• last rib	6.65a	7.6a	7.03a	6.71	7.47	0.52	ns	ns
• last lumbar vert.	5.56ab	5.18a	5.84b	5.59	5.46	0.07	**	ns
Carcass weight (kg)	138.94b	131.04a	134.4a	136.40	133.18	0.79	***	ns
<i>Longissimus dorsi</i> muscle left weight (kg)	1.85a	2.1b	1.83a	1.90	1.95	0.02	***	ns
Ham (kg)								
• left weight	13.01a	12.97a	12.62a	13.14	12.59	0.17	ns	ns
• right weight	12.93a	12.89a	12.57a	13.05	12.54	0.16	ns	ns
Foreleg (kg)								
• left weight	9.07b	8.85a	8.84a	9.26	8.57	0.15	ns	**
• right weight	9.07b	8.84a	8.76a	9.25	8.53	0.14	ns	**

n: Number of samples. SEM: Standard error of the mean. ns: Not significant ( $P>0.05$ ). Significant differences: \*\*\*:  $P \leq 0.001$ ; \*\*:  $P \leq 0.01$ ; \*:  $P \leq 0.05$ ).

## IV – Conclusions

According to our results, castrated males and females showed a similar trend on thickness of subcutaneous backfat, yield of carcass and main commercial pieces (loin and hams), except in forelegs, which were heavier in castrated males than females. On the other hand, the production system affected to carcass and loin yield, being higher in *Montanera* and *Intensive*, respectively. No influences were observed on others commercial pieces yield, such as hams and forelegs. Furthermore, with of rib cage was lower in animals from *Montanera* system.

The descriptive statistics are presented as means (expressed as g water/kg fresh meat); values with the same letters (a, b, c) indicate homogeneous subsets for  $P=0.05$  according to Tukey's HSD test.

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# Comparison of technological quality of meat and nutritional quality of backfat of Krškopolje pigs and commercial fatteners in Slovenia

M. Žemva, Š. Malovrh A. Levart and M. Kovač

Department of Animal Science, Biotechnical Faculty, University of Ljubljana (Slovenia)

**Abstract.** Carcass traits, technological quality of *m. longissimus dorsi* and nutritional quality of back subcutaneous adipose tissue of autochthonous Krškopolje pig (KP) and commercial fatteners were compared. Commercial fatteners were classified according to lean meat content in fatty (51.3%), normal (57.9%) and meaty (64.0%) groups. In KP lean meat percentage was 47.8%. *M. Longissimus dorsi* of KP had the lowest pH<sub>24</sub>, L\* and h\* and the highest a\* values. The backfat in KP had the lowest a\* and c\*. The highest contents of IMF (1.96 and 1.94%) were observed in KP and fatty groups, respectively. Furthermore, the proportion of saturated fatty acid (SFA) in backfat did not vary between the groups. KP had the highest proportion of monounsaturated fatty acid (50.8%). Lower proportion of polyunsaturated fatty acid (PUFA) was also found in KP compared to the fatty group. Additionally, KP contained 5% less n-6 PUFA than the fatty group. The n-6/n-3 PUFA ratio of the meaty group was significantly higher than in KP. There were no differences in ratio of PUFA to SFA and atherogenic indices among the groups. In conclusion, KP had a better technological quality and nutritional quality of backfat compared to the fatty group of commercial pigs. It was very similar to the normal and meaty groups.

**Keywords.** Krškopolje pigs – Commercial fatteners – Technological meat quality – Nutritional quality.

## **Comparaison de la qualité technologique et nutritionnelle de la viande de gras du dos du porc Kraškopolje par rapport à ceux de porcs engraisés industriellement en Slovénie**

**Résumé.** La qualité de la carcasse, la qualité technologique du muscle *Longissimus dorsi* et la qualité nutritionnelle de la longe de tissu adipeux sous-cutané du cochon de race Kraškopolje (KP) ont été comparées à celles de porcs engraisés commercialement. La viande engraisée industriellement a été classée en trois groupes suivant la maigreur de la viande : grasse (51,3%), normale (57,9%) et charnue (viandeuse, 64%). Pour le groupe KP, le pourcentage de viande maigre représente 47,8%. Le muscle *Longissimus dorsi* provenant du groupe KP a les plus faibles valeurs de pH<sub>24</sub>, L\* et h\* mais aussi la plus haute valeur pour a\*. Le gras du dos dans le groupe KP présente les valeurs a\* et c\* les plus basses. Les pourcentages plus élevés d'IMF ont été observés respectivement dans les groupes KP (1,96%) et viande grasse (1,94%). Enfin, la proportion d'acides gras saturés (SFA) contenus dans le gras du dos ne varie pas entre les groupes. Le groupe KP est celui qui contient la plus forte proportion d'acides gras mono-insaturés (50,8%). Enfin, il est à noter qu'une plus faible proportion d'acides gras poly-insaturés (PUFA) est retrouvée dans le groupe KP par rapport au groupe viande grasse. C'est le cas pour n-6 PUFA dont la quantité est plus basse de 5% dans le groupe KP que dans le groupe viande grasse. Le rapport n-6/n-3 PUFA est significativement plus élevé dans le groupe viande charnue que dans le groupe KP. Enfin, aucune différence n'a été mise en évidence pour le ratio PUFA/SFA et les indices athérogènes entre les différents groupes. En conclusion, la qualité technologique et nutritionnelle de la longe de tissu adipeux est meilleure dans le groupe KP que dans le groupe viande grasse des porcs élevés industriellement. Enfin, les résultats et les valeurs obtenues pour les groupes viande normale et charnu sont très similaires.

**Mots-clés.** Porc Kraškopolje – Engraisseurs industriels – Qualité technologique de la viande – Qualité nutritionnelle.

## **I – Introduction**

The Slovenian indigenous Krškopolje pig (KP) is usually under extensive rearing conditions.

Production traits of KP were studied in the past (Ferjan, 1969; Eiselt, 1971). Latter fewer researches were done. In the last decade Kastelic (2001) mentioned meat quality parameters of Krškopolje pigs. Furthermore, Čandek Potokar *et al.* (2003) have compared the carcass traits as well as the technological and sensorial quality of KP with its cross with a modern landrace line – LN 55. However, the composition of KP meat has not yet been investigated.

Technological quality is very important for meat processing. It is determined by technological traits such as pH value, electrical conductivity and colour. Firstly, the effect of pH value and electrical conductivity on technological quality was confirmed by Blendl *et al.* (1991). Further, meat colour, which can be effected by pH value and drip loss, often influences the consumer's choice of product (Ngapo *et al.*, 2004). Technological quality of indigenous pig meat is interesting research topic because of its special meat characteristic.

The nutritional quality of meat has been paid considerable attention in research because of its implications for human health. The World Health Organisation (WHO, 2003) recommended an intake of 15-30% energy from fat, with less than 10% of this amount consisting of saturated fatty acids (SFA), 5-8% consisting of n-6 polyunsaturated fatty acids (PUFA) and 1-2% of n-3 PUFA. The nutritional recommendation for the n-6/n-3 PUFA ratio is less than 4:1 (Enser *et al.*, 2001). The target ratio of polyunsaturated to saturated fatty acids (P/S) is 0.4 or above and the atherogenic index (AI) should be lower than 0.5 (Ulbricht and Southgate, 1991). To avoid consuming too much fat, people want to purchase lean meat. Therefore, reducing carcass fatness was one of the major breeding goals in farm animals for many years. However, it was likely to be accompanied by lower intramuscular fat levels (De Smet *et al.*, 2004), and this had a negative influence on the sensory quality of meat. Dunn (1996) discussed that fatter pigs have more marbling which reflects better meat quality. This is one of the reasons for the better eating quality of meat of indigenous breeds compared to modern breeds.

Very little research has been done on the meat quality of the Slovenian indigenous breed Krškopolje pig. The present study compared the technological of *m. Longissimus dorsi* (LD) and nutritional quality of back subcutaneous fat tissue in Krškopolje pigs and commercial fatteners in Slovenia.

## II – Material and methods

Ten KP originating from a small organic farm in the Pomurje region, were fed with organic feed in outdoor conditions. Commercial fatteners were fed with standard fattening feed mixture in a conventional indoor environment. On the slaughter line 43 commercial fatteners were randomly chosen. In order to compare the meat and fat quality, three groups of commercial fatteners were formed according to lean meat content.

Carcass traits for all animals were measured using the standard Slovenian on-line grading system at slaughter (EC, 2005). After slaughter the warm carcasses were weighted. The pH was obtained 24 hrs ( $\text{pH}_{24}$ ) post mortem by pH meter Metter Toledo (MA130 Ion Meter) in LD and *m. semimembranosus* (SM). The electrical conductivity was measured with conductometer LF/PT-STAR (Matthäus) also in LD and SM 24 hrs post mortem ( $\text{COND}_{24}$ ). Colour ( $L^*$ ,  $a^*$ ,  $b^*$ ) was measured in LD cut at the last rib 24 hrs post mortem by Minolta Chromameter CR300 (Minolta Camera Co., Osaka, Japan).

Samples of LD and back subcutaneous adipose tissue were taken 24 hrs after slaughter at the last rib. They were packed in vacuum bags and stored frozen at  $-21^\circ\text{C} \pm 1^\circ\text{C}$  until chemical analyses. Intramuscular fat content (IMF) in muscle samples was determined by the Weibull-Stoldt method (AOAC, 1997). Fatty acids methyl esters (FAME-s) from samples were prepared using the Park and Goins method (1994). The results were expressed as a percentage of total fatty acids. The statistical model included group effect ( $G_i$ ) and adjustment for carcass weight. Analysis was carried out using the GLM procedure in SAS/STAT (SAS Inst. Inc., 2001).

### III – Results and discussion

The average warm carcass weight varied from 82.5 kg in the meaty group to 93.3 kg in KP (Table 1). The carcass weights of the KP and fatty groups were similar. Lean meat percentage was 47.8% in KP, and 51.3%, 57.9% and 64.0% in the fatty, normal and meaty groups, respectively. Thus, the differences among groups were largely caused by variations in back subcutaneous adipose tissue as well as muscle thickness. Lack of breeding is reflected in thicker backfat and thinner muscle compared to commercial pigs. Although pigs from meaty group were lighter than KP, they have 15 mm thicker muscle, and only one third the back subcutaneous adipose tissue thickness. The carcass weights of KP pigs were similar to those of the fatty group. However, the carcass traits describing body composition were in favour of the fatty group.

**Table 1. Carcass traits in experimental groups of pigs**

	KP (n=10)		Fatty (n=14)		Normal (n=15)		Meaty (n=14)	
	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$	$\bar{x}$	$\pm SD$
Carcass weight (kg)	93.3	$\pm 12.8$	92.9	$\pm 11.1$	88.3	$\pm 13.2$	82.5	$\pm 9.4$
Backfat thickness (mm)	33	$\pm 7$	24	$\pm 5$	16	$\pm 5$	10	$\pm 2$
Muscle thickness (mm)	61	$\pm 5$	67	$\pm 7$	72	$\pm 8$	76	$\pm 9$
Lean meat content (%)	47.8	$\pm 2.5$	51.3	$\pm 2.4$	57.9	$\pm 4.0$	64.0	$\pm 3.2$

KP – Krškopolje pigs.

Technological quality parameters (Table 2) showed no differences in pH value measured 24 hrs after slaughter between groups. Conductivity after 24 hrs in *m. longissimus dorsi* was smaller in KP (3.77 mS/cm) than by fatty (6.36 mS/cm) and meaty (6.40 mS/cm) commercial fatteners.

Result shows better technological quality of KP, because border threshold for normal quality is under 6 mS/cm (Blendl in sod., 1991). Krškopolje pigs had darker and more red meat in comparison with commercial fatteners (Table 2). The result was nearer to desirable value for pig meat 42 – 46 (PIC, 2003) in KP.

Intramuscular fat content in LD of Krškopolje and fatty pigs was around 2% (Table 2). The lowest content of IMF (1.4%) was observed in the meaty group. There were no differences in IMF between KP and the fatty and normal groups. Although KP had greater amount of adipose tissue pigs (Table 1), they do not accumulate IMF (Table 2), possible because barren environmental condition. Kuhn *et al.* (1997) compared a local German breed, the German Saddle Back with commercial Landrace pigs, finding that the former had twice the IMF content of the latter (2.87% and 1.33%). Result confirmed that indigenous breeds have a higher capacity for lipid deposition and are expected to have higher IMF content (Kuhn *et al.*, 1997).

The nutritional quality of subcutaneous adipose tissue is important as backfat is widely used in manufactured meat products (Reichardt *et al.*, 2003). In the current study, the proportion of SFA in the back subcutaneous adipose tissue did not vary between the groups (Table 3). The KP had the highest proportion of MUFA (50.8%) and a lower proportion of PUFA than the fatty group. It has been suggested that an adipose tissue of good nutritional quality should contain less than 15% PUFA (Houben and Krol, 1983). With 13% PUFA the subcutaneous adipose tissue of KP was fulfilled these suggested levels.

**Table 2. Technological quality parameters of *m. longissimus dorsi* and *m. semimembranosus* by KP and commercial pigs**

Variable	KP (n=10)	Fatty (n=14)	Normal (n=15)	Meaty (n=14)	SEM	p - value
pH <sub>24</sub> LD	5.49	5.65	5.57	5.54	0.07	0.3101
pH <sub>24</sub> SM	5.57	5.87	5.77	5.74	0.09	0.0959
COND <sub>24</sub> LD (mS/cm)	3.77 <sup>b</sup>	6.36 <sup>a</sup>	5.80 <sup>ab</sup>	6.40 <sup>a</sup>	0.69	0.0222
COND <sub>24</sub> SM (mS/cm)	8.09	8.53	8.77	7.71	0.88	0.7608
L*	48.10 <sup>b</sup>	51.98 <sup>a</sup>	52.69 <sup>a</sup>	53.83 <sup>a</sup>	1.07	0.0016
a*	9.50 <sup>a</sup>	7.63 <sup>b</sup>	7.36 <sup>b</sup>	7.43 <sup>b</sup>	0.51	0.0085
b*	2.38	3.31	3.35	3.09	0.35	0.1562
c*	9.80	8.37	8.12	8.07	0.56	0.0944
h*	0.25 <sup>b</sup>	0.43 <sup>a</sup>	0.45 <sup>a</sup>	0.42 <sup>a</sup>	0.04	0.0010
IMF content (%)	1.96 <sup>a</sup>	1.94 <sup>a</sup>	1.70 <sup>ab</sup>	1.40 <sup>b</sup>	0.15	0.0462

KP – Krškopolje pigs; SEM – standard error of mean; COND – conductivity; LD – *m. longissimus dorsi*; SM – *m. semimembranosus*; IMF – intramuscular fat; superscripts within the same line are significantly different ( $p < 0.05$ )

**Table 3. Nutritional quality parameters of subcutaneous adipose tissue by KP and commercial pigs**

Variable	KP (n=10)	Fatty (n=14)	Normal (n=15)	Meaty (n=14)	SEM	p -value
Saturated fatty acids	36.13	38.48	39.24	37.63	0.97	0.0781
Monounsaturated fatty acids	50.81 <sup>a</sup>	43.35 <sup>b</sup>	44.70 <sup>b</sup>	43.96 <sup>b</sup>	0.85	<0.0001
Polyunsaturated fatty acids	12.72 <sup>b</sup>	17.85 <sup>a</sup>	16.05 <sup>ab</sup>	17.02 <sup>ab</sup>	1.40	0.0403
n-6 PUFA	11.64 <sup>b</sup>	16.56 <sup>a</sup>	14.85 <sup>ab</sup>	15.90 <sup>ab</sup>	1.29	0.0289
n-3 PUFA	0.97	1.19	1.08	0.97	0.12	0.5974
n-6 PUFA/n-3 PUFA	12.09 <sup>b</sup>	14.86 <sup>ab</sup>	14.77 <sup>ab</sup>	16.56 <sup>a</sup>	0.93	0.0087
PUFA/SFA	0.35	0.47	0.42	0.44	0.05	0.2522
Atherogenic index	0.44	0.47	0.48	0.48	0.02	0.2648

KP – Krškopolje pigs; SEM – standard error of mean; n-6 – omega 6; n-3 – omega 3; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids; superscript within the same line are significantly different ( $p < 0.05$ )

Differences among groups were also found in proportions of n-6 PUFA and in the n-6/n-3 PUFA ratio (Table 3). The KP contained 5% less n-6 PUFA than the fatty group. The n-6/n-3 PUFA ratio of the meaty group was significantly higher than the KP. Furthermore, the n-6/n-3 PUFA ratio of all the groups (Table 3) exceeded the nutritional recommendation of 4:1 (Enser *et al.*, 2001). High n-6 PUFA compared to n-3 PUFA proportions in subcutaneous adipose tissue could be explained by the use of feed components rich in C18:2n-6, such as wheat and barley (Souci *et al.*, 2000). Fatty acids n-3 PUFA are present in many feed ingredients but at lower levels than n-6 PUFA (Wood *et al.*, 2008). There were no differences in P/S and AI indices among the groups (Table 3).

## IV – Conclusions

The organically raised Krškopolje pigs had lower electrical conductivity, more dark and red colour of *m. longissimus dorsi* compared to commercial fatteners. Higher intramuscular fat content were observed in KP and fatty than meaty pigs.

Fatty acid composition of back subcutaneous adipose tissue of KP was nearer to the normal

and meaty groups than fatty group. Krškopolje pigs had higher MUFA content than commercial fatteners, lower PUFA and n-6 PUFA than fatty group and lower n-6/n-3 index than meaty group.

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# Evaluation of the fat content in a small-calibre Salami made with pork from Chato Murciano breed

S. Bañón Arias, L. Méndez Cazorla, E. Almela Pérez and M. Bedia Gea

Research Group on Food Technology, Faculty of Veterinary Medicine, University of Murcia  
Espinardo, 30100 Murcia (Spain)

**Abstract.** Production of Chato Murciano breed, a Mediterranean rustic pig, requires the development of differentiated quality meat products with high commercial value. The aim of this study was to adjust the fat content of a small-calibre Salami manufactured with pork from Chato Murciano breed (castrated, 180 kg live weight and 18 months aged). Three types of Salami with different fat level estimated by near infrared probe (High 20%, Medium 18%; and Low 16% fresh mass) were processed according standard industrial practices, using, *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter culture, and *Penicillium crysogenum* as coverage moulds. The Salami was stuffed into pig casings and then were dried for 12 days (12-14 °C and 90-75% RH). Several quality parameters were determined: proximate composition, drying-ripening rates (dehydration, acidification, fermentation, proteolysis, lipolysis and fat oxidation) and eating quality. The final content of fat was 27.7% for higher, 26.1% for medium and 24.2% for lower fat Salami. Lower fat favoured lactic fermentation and acidification (pH = 4.5), improved the reddening and intensified the proteolysis and the lipolysis. The intensity of aroma and taste of cured-fermented meat was similar in the fat range tested, since Salami showed small variations in firmness, but not in juiciness. Fat level did not affect the acceptance, so we recommend a final fat content of 24% for the Salami made with pork from Chato Murciano

**Keywords:** Salami – Chato Murciano – Fat.

## Évaluation de la teneur en gras de la saucisse sèche du porc Chato Murciano

**Résumé.** La production durable de porcs de races rustiques, comme le Chato Murciano, exige le développement de dérivés de viandes d'une qualité différenciée avec une haute valeur commerciale. L'objectif de l'étude a été d'établir le niveau en graisse idéal pour la saucisse sèche de porc de race Chato Murciano (castré, avec 180 kg de poids vif et 18 mois d'âge). Trois types de saucisses expérimentales furent élaborées avec de la viande maigre et 3 pourcentages de graisse différents estimés par proche infrarouge (élevé 20%, moyen 18% et faible 16% de masse fraîche). La saucisse fut élaborée avec un procédé industriel standard avec une culture initiatrice de *Pediococcus pentosaceus* et *Staphylococcus xylosus*, couverture de *Penicillium crysogenum*. Elle fut emboutie dans des tripes naturelles de porc et fut séchée pendant 12 jours (12-14°C et 90-75% HR). La qualité est définie selon la composition, les indices de séchage-maturation (déshydratation, acidification, fermentation, protéolyse, lipolyse et oxydation des graisses) et l'analyse sensorielle. La teneur finale en graisse fut: 27,7% élevée, 26,1% moyenne et 24,2% faible. La faible teneur en graisse a favorisé la fermentation lactique et l'acidification correcte de la charcuterie (pH=4,5), a amélioré le rougissement et a intensifié la protéolyse et la lipolyse. L'intensité de l'arôme et de la saveur à viande séchée-fermentée fut similaire dans la frange de graisse testée, en observant de petites variations de fermeté et d'onctuosité, mais pas de jutosité. L'acceptation n'a pas été affectée par la teneur en graisse, c'est pourquoi nous recommandons 24% de graisse finale pour la saucisse de Chato Murciano.

**Mots-clés.** Saucisse – Chato Murciano – Graisse.



## I – Introduction

Chato Murciano breed is a Mediterranean rustic pig originated from Iberian trunk. In the early twentieth century this breed was of high socio-economic importance, being traditionally raised at home and in extensive systems with remnants of the orchard. Actually, Chato is being recovered by farmers of Murcia Region. Pigs were raised semi-extensively, fed a balanced diet based on special feeds and optionally local raw materials, and slaughtered around 18 months old and 180 kg live weight. Chato provides heavy carcasses and pork meat with high levels of haematic pigments, proteins, infiltration and oleic acid, being a suitable raw material for dry-cured products (Galián *et al.*, 2008). The sustainable breeding of Chato Murciano would be given for the elaboration of differentiated quality meat products with high commercial value. Local companies from Murcia Region are actually developing pork sausages from Chato Murciano. An example is traditional small-calibre salami (less than 45mm diameter), ripened for 2-3 weeks, which is widely consumed in Spain. High fat infiltration of Chato pork offers the possibility to manufacture salami with no added back fat. This supposes certain technological risk, since fat strongly contributes to eating quality of salami, since fat intensifies the aroma, taste and juiciness. However, excessive fattiness can also limit the commercialization of salami, due mainly to nutritional aspects.

## II – Materials and methods

Salamis were manufactured according industrial practices with pork from Chato Murciano breed. Three fat levels were tested (High 20%, Medium 18%; and Low 16% raw mass) (HF, MF and LF). Fat content was determined by near infrared (NIR) probe. The recipe (g kg<sup>-1</sup>) of salami was: boned pork (880), water (44), sodium chloride (22), black and white pepper (10), dextrose, lactose and sucrose (20), dextrin (20), potassium nitrate (0.25) and sodium nitrite (0.25), sodium isoascorbate (0.5), sodium citrate (0.3) sodium glutamate (2.5) and Ponceau 4R red (0.2). Starter cultures of *Pediococcus pentosaceus*, *Staphylococcus xylosus* and *Penicillium Crysoogenum* were used. The salami was stuffed into pig gut and then was dried for 12 days (12-14°C and 90-75% RH). Several quality parameters were determined: proximate composition drying-ripening rates (dehydration, acidification, fermentation, proteolysis, lipolysis and fat oxidation) and eating quality. The effects of fat level on the quality of salami were determined by simple ANOVA.

## III – Results

Table 1 shows the effects of fat level on the proximate composition and drying-ripening indices of salami. After drying, the fat content of salami reached percentages of 27.7% (high), 26.1% (medium) and 24.2% (low). Salami may contain up to 50% fat (Moretti *et al.*, 2004; Rubio *et al.*, 2008). Appropriated *a<sub>w</sub>* value was reached for all three salamis, although HF had lowers moisture and *a<sub>w</sub>*. Rates of ash and protein were similar in all three salamis, although the MF salami showed a slightly lower protein percentage. LF salami showed more pronounced acidification, reaching pH value of 4.5 at the end of ripening, while pH fell to 4.9 in HF and MF salamis. Reddening, proteolysis and lipolysis were more intense in LF.

Total viable and lactic acid bacteria counts were higher in LF than in MF and HF (Table 2). Low fat level also improved the lactic fermentation of Salami, due to the rate microbiological growth increase at higher *a<sub>w</sub>*. Rapid acidification by LAB in LF was associated with lower counts of *Micrococaceae*.

Table 3 shows the effects of the fat level on the eating quality of Salami. Surprisingly, lean colour scoring was higher in HF and MF than in LF. The intensity of dry-cured colour may increase when Salami contains more lean meat and equal dose of curing agents. Major sensory attributes of Salami such as odour, flavour, juiciness and fattiness were not affected by the fat

level, while minor differences in hardness were found as a function of fat level. Fat increased softness, although the overall acceptance was not affected. Thus, fat reduction can be made without loss of the eating quality of Salami. Acid flavour scoring did not agree with lactic acid content and pH found, getting the higher value in MF Salami.

**Table 1. Effect of fat level on the proximate composition and drying-ripening of Salami prepared with different fat levels**

	Fat level		
	High (M $\pm$ SD)	Medium (M $\pm$ SD)	Low (M $\pm$ SD)
Lipids NIR (g/100 g)	27.7 $\pm$ 0.52 <sup>a</sup>	26.1 $\pm$ 0.68 <sup>b</sup>	24.2 $\pm$ 0.84 <sup>c</sup>
Moisture NIR (g/100 g)	34.3 $\pm$ 0.33 <sup>b</sup>	38.4 $\pm$ 0.87 <sup>a</sup>	37.9 $\pm$ 1.43 <sup>a</sup>
Proteins NIR (g/100 g)	23.7 $\pm$ 1.02 <sup>a</sup>	21.8 $\pm$ 1.02 <sup>b</sup>	23.5 $\pm$ 1.02 <sup>a</sup>
Ash (g/100 g)	5.4 $\pm$ 0.33	5.3 $\pm$ 0.33	5.3 $\pm$ 0.33
<i>a<sub>w</sub></i>	0.89 $\pm$ 0.01 <sup>b</sup>	0.91 $\pm$ 0.01 <sup>a</sup>	0.90 $\pm$ 0.01 <sup>a</sup>
pH	4.88 $\pm$ 0.03 <sup>a</sup>	4.94 $\pm$ 0.07 <sup>a</sup>	4.47 $\pm$ 0.06 <sup>b</sup>
Lactic acid (g lactic 100 g <sup>-1</sup> )	0.67 $\pm$ 0.02 <sup>a</sup>	0.51 $\pm$ 0.03 <sup>b</sup>	0.67 $\pm$ 0.07 <sup>a</sup>
Proteolysis (g NPN/100 g <sup>-1</sup> TN)	11.08 $\pm$ 1.42 <sup>ab</sup>	10.15 $\pm$ 1.31 <sup>b</sup>	12.37 $\pm$ 0.96 <sup>a</sup>
Fat acidity (mg KOH g <sup>-1</sup> )	4.43 $\pm$ 0.36 <sup>c</sup>	6.87 $\pm$ 1.11 <sup>b</sup>	14.36 $\pm$ 1.94 <sup>a</sup>
L* Lightness (CIE units)	44.14 $\pm$ 0.99 <sup>b</sup>	48.42 $\pm$ 1.27 <sup>a</sup>	48.38 $\pm$ 1.58 <sup>a</sup>
a* Redness (CIE units)	15.82 $\pm$ 1.01 <sup>b</sup>	14.55 $\pm$ 0.94 <sup>b</sup>	17.74 $\pm$ 0.94 <sup>a</sup>
b* Yellowness (CIE units)	2.68 $\pm$ 0.65 <sup>b</sup>	3.74 $\pm$ 0.46 <sup>a</sup>	4.48 $\pm$ 0.27 <sup>a</sup>
C* Chroma (CIE units)	16.06 $\pm$ 1.09 <sup>b</sup>	15.02 $\pm$ 0.98 <sup>b</sup>	18.29 $\pm$ 0.95 <sup>a</sup>
° Hue (CIE units)	9.53 $\pm$ 1.80 <sup>b</sup>	14.41 $\pm$ 1.43 <sup>a</sup>	14.18 $\pm$ 0.66 <sup>a</sup>

M: mean; SD: standard deviations; <sup>a, b, c</sup> Fat level effects (P $\leq$ 0.05).

**Table 2. Effects of fat level on the fermentative microflora (log cfu g<sup>-1</sup>) of Salami prepared with different fat levels**

	Fat level		
	High (M $\pm$ SD)	Medium M $\pm$ SD)	Low (M $\pm$ SD)
Total viable counts	8.83 $\pm$ 0.14 <sup>b</sup>	8.72 $\pm$ 0.08 <sup>b</sup>	9.20 $\pm$ 0.09 <sup>a</sup>
Lactic acid bacteria	8.72 $\pm$ 0.08 <sup>b</sup>	8.55 $\pm$ 0.30 <sup>b</sup>	9.15 $\pm$ 0.16 <sup>a</sup>
<i>Micrococacceae</i>	6.86 $\pm$ 0.39 <sup>ab</sup>	7.33 $\pm$ 0.41 <sup>a</sup>	6.45 $\pm$ 0.23 <sup>b</sup>
Moulds and yeasts	5.16 $\pm$ 0.15 <sup>a</sup>	5.32 $\pm$ 0.33 <sup>a</sup>	4.45 $\pm$ 0.22 <sup>b</sup>

M: mean; SD: standard deviations; <sup>a, b, c</sup> Fat effects (P $\leq$ 0.05).

## IV – Conclusions

Fat content of 24% (16% raw mass estimated by NIR) would be adequate to manufacture pork Salami from Chato Murciano breed, stuffed into natural gut and dried-ripened for 12 days. This fat reduction favoured the ripening properties of Salami, including lactic fermentation, without relevant loss of eating quality. Fat reduction could help with the commercialisation of Chato Murciano dry-cured sausages.

**Table 3. Effects of fat level on the eating quality of Salami prepared with different fat levels**

	Fat level		
	High (M ± SD)	Medium (M ± SD)	Low (M ± SD)
Lean colour	4.5±0.10 <sup>a</sup>	4.4±0.10 <sup>a</sup>	3.9±0.17 <sup>b</sup>
Odour	3.2±0.09 <sup>b</sup>	3.5±0.23 <sup>a</sup>	3.3±0.19 <sup>ab</sup>
Pepper odour	2.6±0.18 <sup>ab</sup>	2.4±0.21 <sup>b</sup>	2.8±0.16 <sup>a</sup>
Flavour	3.1±0.14 <sup>b</sup>	3.4±0.09 <sup>a</sup>	3.0±0.19 <sup>b</sup>
Pepper flavour	2.7±0.06	2.8±0.19	2.7±0.26
Acid flavour	3.0±0.19 <sup>b</sup>	3.9±0.13 <sup>a</sup>	3.1±0.15 <sup>b</sup>
Hardness	3.0±0.08 <sup>b</sup>	3.6±0.15 <sup>a</sup>	3.3±0.07 <sup>a</sup>
Juiciness	2.9±0.19	3.0±0.11	2.9±0.21
Fattiness	2.7±0.06	2.8±0.18	2.8±0.16
Acceptance	3.5±0.17	3.4±0.16	3.4±0.20

M: mean; SD: standard deviations; scoring scale: 1-5; <sup>a, b, c</sup> Fat effects (P≤0.05).

## Acknowledgments

José Reverte SL (pig farmer) and Elaborados Cárnicos de Lorca SL (meat industry).

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# Development of Chato Murciano sobrasada prepared using "Appellation of Murcia Origin" paprika

M. Bedia Gea, L. Méndez Cazorla, R. Serrano Espada, A. Aznar González  
and S.J. Bañón Arias

Research Group on Food Technology, Faculty of Veterinary Medicine, University of Murcia, Murcia (Spain)

**Abstract.** Sustainable production of Chato Murciano breed, a Mediterranean rustic pig, requires the development of differentiated quality meat products with high commercial value. The aim was to develop a sobrasada (a fatty dry-cured sausage) manufactured with Chato Murciano pork and AMOP. Sobrasada was prepared with pork from Chato Murciano (castrated and slaughtered at 180 kg live weight). Three types of paprika (Control: 6% standard; Mixed: 3% Standard + 3% AMOP; 6% AMOP) were tested. Sobrasada was stuffed into blind gut and was ripened for 30 days (14-18°C and 75-80% RH). Quality parameters were determined: composition, aw, pH, colour L\*a\*b\*, proteolysis, lipolysis, lipid oxidation, total viable counts, lactic acid bacteria, Micrococcaceae, yeasts, moulds, and eating quality. The use of AMOP hardly affected the technological characteristics of sobrasada, although proteolysis was more intense in samples containing 3% and 6% AMOP. The type of paprika mainly affected the eating quality. The use of AMOP intensified the typical aroma and taste of sobrasada, but provided less intense red-orange colour. It therefore seems advisable to mix AMOP with more pigmented paprika to achieve a good balance between bouquet and colour for sobrasada made with Chato Murciano pigmeat.

**Keywords.** Sobrasada – Chato Murciano – Paprika – Murcia.

## *Développement de la Sobrasada de Chato Murciano élaborée avec du paprika "Appellation d'Origine Contrôlée (AOC) de Murcie"*

**Résumé.** La production durable de porcs de races rustiques, comme le Chato Murciano, exige le développement de dérivés de viande d'une qualité différenciée avec une haute valeur commerciale. L'objectif fut de développer une Sobrasada de porc Chato Murciano élaborée avec du paprika "Appellation d'Origine Contrôlée (AOC) de Murcie". La Sobrasada fut élaborée avec de la viande et du lard de porc de race Chato Murciano (castré et abattu à 180 kg de poids vif). Trois types de paprika furent testés (contrôle: 6% standard, mélangé 3% standard + 3% AOC, et 6% AOC). La Sobrasada a été mise dans des boyaux de porc et a mûri pendant 30 jours (14-18°C et 75-80% HR). Les paramètres de qualité déterminés furent: composition, aw, pH, couleur L\*a\*b\*, protéolyse, lipolyse, oxydation des lipides, aérobies mésophiles totaux, bactéries acido-lactiques, Micrococcaceae, moisissures et levures et attributs sensoriels. L'utilisation de paprika AOC n'a presque pas influencé les caractéristiques technologiques de la Sobrasada, bien que la protéolyse ait été plus intense dans les échantillons avec 3% et 6% de paprika AOC. Le type de paprika affecte surtout la qualité sensorielle. L'utilisation de paprika AOC a intensifié l'odeur et la saveur caractéristique de la Sobrasada, alors qu'elle a donné une couleur rouge-orange moins intense. Donc, nous recommandons le mélange de paprika AOC avec du paprika plus pigmenté ou des oléorésines pour obtenir un bon équilibre entre arôme et couleur pour la Sobrasada fermentée de Chato Murciano.

**Mots-clés.** Sobrasada – Chato Murciano – Paprika – Murcie.

## I – Introduction

Chato Murciano breed is a Mediterranean rustic pig originated from Iberian trunk. Actually, Chato is being recovered by farmers of Murcia, SE Spain. Chato pigs were raised semi-extensively, fed a balanced diet based on special feeds and optionally local raw materials, and slaughtered around 18 months old and 180 kg live weight. The sustainable breeding of Chato

Murciano would be given for the elaboration of differentiated quality meat products with high commercial value. Local consumers and restaurants have begun to demand Chato pork once again and local companies are actually developing Chato sausages. Chato pig provides heavy carcasses with high fattening (Galián *et al.*, 2008), whose excess backfat must be transformed in meat products. Sobrasada, a fatty (40-50% fat) dry-cured sausage prepared with paprika, offers great opportunities in this sense. Paprika is also traditionally produced in the Region of Murcia, being obtained by mixing different varieties of *Capsicum annuum* (Rosselló *et al.*, 1995). Depending of origin, paprika provides particular colouring and flavouring to meat products. Thus, two traditional ingredients from Murcia, Chato pork and paprika, can be used to produce a quality differentiated sausage. The aim was to develop Sobrasada manufactured with Chato Murciano pork and Appellation Murcia Origin Paprika (AMOP).

## II – Materials and methods

Sobrasada was manufactured according industrial practices with pork from Chato Murciano breed. Three types of paprika (Control: 6% standard; Mixed: 3% Standard + 3% AMOP; AMOP: 6% AMOP) were tested. The recipe (g kg<sup>-1</sup>) of sobrasada was: boned pork (365), backfat (325), paprika (60), odorous white wine (6), minor spices (oregano), salt and additives (60). Sobrasada was stuffed into blind gut and was ripened for 30 days (14-18°C and 75-80% RH). Quality parameters were determined: proximate composition, *a<sub>w</sub>*, pH, colour L\*a\*b\*, proteolysis, lipolysis, lipid oxidation, total viable counts, lactic acid bacteria, *Micrococcaceae*, yeasts, moulds and eating quality. The effects of fat level on the quality of sobrasada were determined by simple ANOVA.

## III – Results

Table 1 shows the proximate composition and drying-ripening indices of sobrasada manufactured with different paprika.

**Table 1. Proximate composition and drying-ripening indices for Sobrasada from Chato Murciano prepared with different paprika (standard vs Murcia Origin)**

	Paprika source		
	Control (M ± SD)	Mix (M ± SD)	AMOP (M ± SD)
Moisture (g 100 g <sup>-1</sup> )	29.5±1.54	29.6±0.92	29.3±1.36
Proteins (g 100 g <sup>-1</sup> )	12.9±1.55 <sup>a</sup>	8.65±1.06 <sup>b</sup>	10.5±2.65 <sup>ab</sup>
Lipids (g 100 g <sup>-1</sup> )	51.5±0.34 <sup>b</sup>	54.0±0.32 <sup>a</sup>	53.4±0.30 <sup>a</sup>
Collagen (g 100 g <sup>-1</sup> )	1.50±0.27	1.33±0.28	1.11±0.24
Ash (g 100 g <sup>-1</sup> )	5.44±0.33	5.41±0.31	5.47±0.32
<i>a<sub>w</sub></i>	0.90±0.01 <sup>b</sup>	0.89±0.00 <sup>c</sup>	0.92±0.00 <sup>a</sup>
pH	5.38±0.07	5.51±0.02	5.46±0.13
L* Lightness (CIE units)	48.4±0.72 <sup>b</sup>	48.6±0.35 <sup>b</sup>	50.3±0.52 <sup>a</sup>
a* Redness (CIE units)	35.7±1.33	37.1±0.51	35.9±0.61
b* Yellowness (CIE units)	29.2±2.76	30.5±0.85	31.0±1.57
C* Chroma (CIE units)	49.6±6.01	48.0±0.86	47.4±1.45
° Hue (CIE units)	39.2±1.81	39.4±0.60	40.8±1.04
Proteolysis (g NPN 100 g <sup>-1</sup> TN)	15.9±2.30 <sup>b</sup>	22.9±1.76 <sup>a</sup>	23.0±6.28 <sup>a</sup>
Fat acidity (mg KOH g <sup>-1</sup> )	19.0±0.88	20.3±2.21	18.2±0.72
TBARS (mg MDA kg <sup>-1</sup> )	0.19±0.02	0.18±0.02	0.18±0.04

Paprika sources: 6% standard (Control) : 3% Standard + 3% AMOP (Mix); 6% AMOP (AMOP). M: mean; SD: standard deviations; <sup>a, b, c</sup> Paprika effects (P≤0.05).

After drying, slight differences in proteins and lipids were found for different types of sobrasada. Agreeing this,  $a_w$  value was slight higher in AMOP than in Mix and the Control sobrasadas. Surprisingly (reddening was higher in standard paprika), no chromatic differences were found between the Control, Mix and AMOP sobrasada, although AMOP showed the highest  $L^*$  value. The interaction between paprika and other ingredients, especially fat, may explain these results for colour. On the other hand, sobrasada presented higher proteolysis than the Control and Mix sobrasadas, while no effect of paprika source on fat acidity and lipid oxidation were found. The low counts found for the main fermentative groups (Table 2) indicated that no relevant fermentation takes place during the ripening stage.

**Table 2. Main fermentative groups (log cfu g<sup>-1</sup>) for sobrasada from Chato Murciano prepared with different paprika (Standard vs Murcia Origin)**

	Paprika source		
	Control (M ± SD)	Mix (M ± SD)	AMOP (M ± SD)
Total viable counts	5.78±0.05	5.83±0.04	5.85±0.13
<i>Micrococacceae</i>	3.90±0.31 <sup>ab</sup>	3.95±0.06 <sup>a</sup>	3.44±0.41 <sup>b</sup>
Lactic acid bacteria	5.55±0.26	5.43±0.03	5.46±0.02
Moulds and yeasts	5.73±0.12	5.84±0.21	5.72±0.16

Paprika sources: 6% standard (Control) : 3% Standard + 3% AMOP (Mix); 6% AMOP (AMOP).  
M: mean; SD: standard deviations; <sup>a, b, c</sup> Paprika effects (P≤0.05).

Table 3 shows the sensory scores of sobrasada manufactured with different paprika. Sobrasada was characterized by a typical red-orange colour. In opposite to CIEL\*a\*b\* values, the trained panellists detected colour differences between AMOP-Mix and the Control samples. The intensity of colour was lower scored in AMOP sobrasada than in the Control sobrasada, while Mix sobrasada obtained intermediate scoring. However, the aroma and taste scores were higher in AMOP-Mix than in the Control sobrasada. The use of AMOP at different concentration (3% and 6%) clearly intensified the sobrasada aroma and taste. These results suggest that AMO paprika provided better flavouring and certain discolouration to sobrasada. The physical-chemical, microbiological and sensory data obtained were coherent with those obtained from preliminary studies on Stabilization by chilling of sobrasada from Chato Murciano pigmeat manufactured without preservatives (Martínez *et al.*, 2009).

## IV – Conclusions

The use of AMOP hardly affected the technological characteristics of sobrasada, although proteolysis was more intense in samples containing 3% and 6% AMOP. The type of paprika mainly affected the eating quality. The use of AMO paprika intensified the characteristic aroma and taste of sobrasada, but provided less intense red-orange colour. It therefore seems advisable to mix AMOP with more pigmented paprika to achieve a good balance between bouquet and colour for sobrasada prepared with Chato Murciano pigmeat.

**Table 3. Sensory scoring for sobrasada from Chato Murciano prepared with different paprika (Standard vs Murcia Origin)**

	Paprika source		
	Control M $\pm$ SD	Mix M $\pm$ SD	AMOP M $\pm$ SD
Colour	3.5 $\pm$ 0.6 <sup>a</sup>	3.2 $\pm$ 0.4 <sup>ab</sup>	2.9 $\pm$ 0.5 <sup>b</sup>
Colour homogeneity	2.9 $\pm$ 0.6	2.9 $\pm$ 0.7	2.8 $\pm$ 0.7
Aroma	3.0 $\pm$ 0.6 <sup>b</sup>	3.5 $\pm$ 0.5 <sup>a</sup>	3.4 $\pm$ 0.4 <sup>a</sup>
Paprika aroma	2.8 $\pm$ 0.6	2.9 $\pm$ 0.7	2.8 $\pm$ 0.7
Acid aroma	1.8 $\pm$ 0.6	1.7 $\pm$ 0.6	1.8 $\pm$ 0.6
Taste	2.9 $\pm$ 0.6 <sup>b</sup>	3.4 $\pm$ 0.5 <sup>a</sup>	3.5 $\pm$ 0.5 <sup>a</sup>
Paprika taste	3.0 $\pm$ 0.6	3.3 $\pm$ 0.6	3.2 $\pm$ 0.6
Acid taste	1.7 $\pm$ 0.5	1.8 $\pm$ 0.5	1.9 $\pm$ 0.5
Bitter aftertaste	2.2 $\pm$ 0.8	2.3 $\pm$ 0.8	2.1 $\pm$ 0.5
Fattiness	3.4 $\pm$ 0.8	3.6 $\pm$ 0.4	3.7 $\pm$ 0.4
Creaminess	3.6 $\pm$ 0.7	3.8 $\pm$ 0.4	3.8 $\pm$ 0.5
Fibrous residue	2.3 $\pm$ 0.6	2.3 $\pm$ 0.6	2.4 $\pm$ 0.6

Paprika sources: 6% standard (Control) : 3% Standard + 3% AMOP (Mix); 6% AMOP (AMOP).  
M: mean; SD: standard deviations; <sup>a, b, c</sup> Paprika effects (P $\leq$ 0.05).

## Acknowledgements

José Reverte S.L. (pig farmer) and Elaborados Cárnicos de Lorca SL (meat industry).

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# The weight loss in the production of non-fermented salami "capocollo", "fiocco" and dry cured ham from "casertana" pig ancient autochthonous genetic type (AAGT). Further contribution

D. Matassino\*, G. Gigante\*, M. Grasso\*, C. M. A Barone\*\*

\*Consorzio per la Sperimentazione, Divulgazione e Applicazione di Biotecniche Innovative (ConSDABI) SUB NFP.I., FAO, Centro di Scienza Omica per la Qualità e per l'Eccellenza Alimentare, Piano Cappelle 82100 Benevento (Italy)

\*\*Dipartimento Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni animali Università di Napoli Federico II, Portici, Napoli (Italy)

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**Abstract.** The study was carried out to monitor the weight loss of three unfermented local products (LP), "capocollo Sannita", "fiocco Sannita" and dry cured ham, obtained from males (castrated not less than 40 days before slaughter) and entire females belonging to the "Casertana" pig AAGT, reared at the experimental farm of ConSDABI Sub NFP.I.-FAO. The results, valid within the observation field, showed that for all three products the weight loss during the seasoning, in the same conditions, was statistically greater ( $P < 0.001$ ) in castrated male than that obtained from the entire female and, in particular: (i) the capocollo Sannita [54 (♂♂) and 62 (♀♀)] has decreased by 25% vs 20% at 1 month, 39% vs 33% at 3 months and 45% vs 42% at 6 months; (ii) the fiocco Sannita [51 (♂♂) and 41 (♀♀)] has decreased by 22% vs 17% at 1 month and 43% vs 39% at 12 months; (iii) the dry cured ham [34 (♂♂) and 42 (♀♀)] has decreased by 9% vs 7% at 1 month, 18% vs 14% at 3 months reaching the highest value at 24 months (33% vs 27%).

**Keywords.** Ancient autochthonous genetic type (AAGT) – Capocollo Sannita – Fiocco Sannita – Dry cured ham – Local Products (LP).

**Diminution de poids lors de la production de "capocollo", "fiocco" et "prosciutto" issus du type génétique autochtone ancien (TGAA) "Casertana". Des contributions supplémentaires**

**Resumé.** L'étude a été menée pour surveiller la diminution de poids pendant la maturation de trois produits locaux (PL) non fermentés : "capocollo Sannita", "fiocco Sannita" et "prosciutto Sannita", obtenus à partir de mâles (castrés au moins 40 jours avant l'abattage) et femelles non castrées du TGAA "Casertana" (CT) élevés chez le ConSDABI SUB NFP.I.- FAO. Les résultats, valables dans le champ d'observation, ont montré que pour les trois produits la perte de poids pendant la maturation, en conditions égales, s'est avérée plus élevée pour le mâle castré par rapport à la femelle non castrée et, en particulier: (i) le "capocollo Sannita" [54 (♂♂) et 62 (♀♀)] a diminué de 25% vs 20% à 1 mois, de 39% vs 33% à 3 mois et de 45% vs 42% à 6 mois; (ii) le "fiocco Sannita" [51 (♂♂) et 41 (♀♀)] a diminué en moyenne de 22% vs 17% après 1 mois, et de 43% vs 39 après 12 mois de maturation; (iii) le "prosciutto Sannita" [34 (♂♂) et 42 (♀♀)] a diminué en moyenne de 9% vs 7% après 1 mois, de 18% vs 14% après 3 mois et de 33% vs 27% après 24 mois.

**Mots-clés.** Type génétique autochtone ancien (TGAA) – Capocollo Sannita – Fiocco Sannita – Prosciutto Sannita – Produits locaux.

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## I – Introduction

Casertana (CT) pig, also called "pelatella", for the absence of bristles, or "napoletana" for its



place of origin, is one of the best Italian autochthonous pig population so that Höesch (first half of 10th century) defined it as "pig Italian pride". During the centuries this population underwent alternate events. It contributed, in the past (half of 19th century) to the development of the Yorkshire and Berkshire English breeds, while at the end of 80's years its population size decreased so that it became a genetic type at risk of extinction. Currently, CT pig is reared in Campania, Lazio, Molise and Umbria regions, with a growing population size. Not fermented products, economically more important, obtained from this AAGT are without any doubt "ham" and "fiocco". The aim of this research was to furnish a further contribution to the knowledge of the influence of gender factor on the weight loss during the seasoning of Local product (LP) obtained from meat of CT pig.

## II – Materials and methods

The study involved unfermented local products (LP): "capocollo Sannita", "fiocco Sannita" and dry cured ham obtained from males (castrated less than 40 days before slaughter) and entire females of the Casertana pig AAGT. The animals, reared in multiple boxes at experimental Farm of ConSDABI Sub NFP.I. - FAO, were fed with commercial feed. Net live weights of pigs at the slaughter were 168.8 kg and 163.6 kg respectively for castrated male and entire female. Scheme 1 reports the number of the products analyzed and the period in which the weight variation (weight loss) was registered for each 'LP'.

In particular: (i) the capocollo Sannita was made using neck (starting from atlas-occipital articulation to the 5<sup>th</sup> thoracic vertebra) after trimming; its seasoning takes about six months; (ii) fiocco Sannita, the noble part of the ham, consisting of three muscles (*Semimembranosus*, *Semitendinosus* and *Biceps femoris*) has a peculiar 'pear' shape (typical of "culatello of Zibello"); its seasoning takes about 12 months; (iii) the dry cured ham was obtained from the leg of pig properly prepared until to give it the typical rounded shape; its seasoning takes about 24 months.

The seasoning was realized in proper places controlled for temperature and humidity. Each product was weighted at the start of the process, at the end of drying time, and then periodically (weekly or monthly) until the end of seasoning time (Table 1). Seasoning weight loss was calculated as: [(initial product weight– product weight during process)/ initial product weight ]\*100.

No preservatives (nitrite and nitrate) were used.

**Table 1. Sequence of monitoring for each product**

Product	(♂♂)	♀♀	Seasoning d									
			0	6	15	30	90	180	270	360	540	720
Capocollo	54	62	X	X	X	X	X	X				
Fiocco	51	41	X			X	X	X		X		
Ham	34	42	X			X	X	X	X	X	X	X

The preliminary statistical analyses showed a significant influence of initial weight of product on the trend of weight loss, so the data were processed using the following model of covariance analysis in which initial weight of the product was the covariate and 'gender' was considered as fixed factor (SAS, 1997):

$y_{ijk} = \mu + b_1x_1 + \text{gender}_i + e_{ijk}$ ; where:

$\mu$  = constant common to all the observations (general mean);

$b_1$  = regression coefficients of dependent variable on the weight loss of product ( $x_1$ );

gender<sub>i</sub> = fixed effect of i<sup>th</sup> gender (i = 1, 2);  
e<sub>ijk</sub> = random error and/or unknown effects.

The Student's t test was applied to compare the estimated means.

### III – Results and discussions

The results, valid within the observation field, showed the significant influence of gender to determinate the variation of weight of all three considered products. Tables 2, 3 and 4 evidenced that the products obtained from meat of entire females had a significantly lower weight loss, in comparison with those obtained from meat of castrated males ( $P < 0.001$ ). In particular, for the capocollo Sannita this difference reached the higher value (5.6%) after 90 days of seasoning, as well as fiocco Sannita (6.3%), while for dry cured ham the highest difference was observed at 360 days (6.7%).

**Table 2. Percentage weight loss of "capocollo Sannita" during seasoning period**

Seasoning d	Gender		$\Delta = [(\sigma\sigma) - \text{♀♀}]$
	( $\sigma\sigma$ )	♀♀	
3	5.32	4.55	0.77***
15	16.15	13.41	2.74***
30	24.67	20.17	4.50***
90	38.84	33.26	5.58***
180	45.38	41.59	3.79***

\*\*\* $P < 0.001$ .

**Table 3. Percentage weight loss of "Fiocco Sannita" during seasoning period**

Seasoning d	Gender		$\Delta = [(\sigma\sigma) - \text{♀♀}]$
	( $\sigma\sigma$ )	♀♀	
3	8.75	8.18	0.57**
14	14.47	12.78	1.69***
30	21.58	17.34	4.24***
90	33.80	27.52	6.28***
180	40.30	34.18	6.12***
360	43.04	38.66	4.38***

\*\* $P < 0.01$  \*\*\* $P < 0.001$ .

**Table 4. Percentage weight loss of dry cured ham during seasoning period**

Seasoning d	Gender		$\Delta = [(\sigma\sigma) - \text{♀♀}]$
	( $\sigma\sigma$ )	♀♀	
30	9.04	6.86	2.18***
90	18.50	13.78	4.72***
180	24.49	18.30	6.19***
270	26.62	20.17	6.45***
360	28.59	21.85	6.74***
540	31.30	24.66	6.64***
720	33.19	27.16	6.03***

\*\*\* $P < 0.001$ .

At the end of seasoning time, the average weight loss of dry cured ham was 30.18%, lower than Istrian ham (46.31%) (Karolyi *et al.*, 2005) or Bayonne ham (from 35% to 39%) (Monin *et al.*, 1997) and not more different from Parma ham (about 27%) (Nanni Costa *et al.*, 1999).

## IV – Conclusions

The results, valid within the observation field, highlighted that for the three local products considered, the castrated male had a significantly greater percentage weight loss than entire female. This trend confirms the results obtained by Castellano *et al.* (2006). These authors report that the difference could be attributed to a different texture of fat tissue or to a higher content in intramuscular fat of entire females (not published data). The relation with qualitative data (rheology and colour) taken on both muscular portion and on covering fat of the dry cured ham and fiocco Sannita will may provide useful indications in order to deep factors that influence these differences.

## Acknowledgements

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# The weight loss in the production of dry cured sausages "salsiccia" and "soppressata" from "Casertana" pig ancient autochthonous genetic type (AAGT). Further contribution

D. Matassino\*, G. Gigante\*, M. Grasso\*, C. M. A Barone\*\*

\*Consorzio per la Sperimentazione, Divulgazione e Applicazione di Biotecniche Innovative (ConSDABI) SUB NFP.I., FAO, Centro di Scienza Omica per la Qualità e per l'Eccellenza Alimentare, Piano Cappelle, 82100 Benevento (Italy)

\*\*Dipartimento Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni animali Università di Napoli Federico II, Portici, Napoli (Italy)

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**Abstract.** The study involved 605 "soppressata Sannita" and 121 "salsiccia Sannita" obtained processing meat of Casertana (CT) AAGT pigs [68 castrated males (castrated not less than 40 days before slaughter) and 53 entire females] reared at experimental farm of ConSDABI Sub N.F.P.I.-FAO in order to evaluate the effect of gender on weight loss of the ripened products. The results, valid within the observation field, showed a significant effect of gender on weight loss of both products. In particular: (i) the soppressata Sannita obtained from castrated males had a percentage weight loss greater in comparison with the meat of entire females; at the end of seasoning time (40 days) it lost ( $P<0.01$ ) about the 51% of initial weight (+4% in comparison with that of entire female); (ii) the salsiccia Sannita that at 15 days had a percentage weight loss of 41% if obtained from meat of castrated males and of 34% if obtained from meat of entire female ( $P<0.05$ ), after 30 days had a weight loss of 52% and 47%, respectively ( $P<0.05$ ).

**Keywords.** Casertana pig – Ancient autochthonous genetic type (AAGT) – Salsiccia Sannita – Soppressata Sannita.

**Diminution de poids lors de la production de "salsiccia" et de "soppressata" issus du porc de type génétique autochtone ancien (TGAA) "Casertana". Des contributions supplémentaires**

**Résumé.** L'étude a concerné 605 "soppressate sannite" (saucisson) et 121 "salsicce sannite" (saucisse) préparés avec de la viande de chaque porc [68 mâles (castrés au moins 40 jours avant l'abattage) et 53 femelles non castrées] du TGAA "Casertana", élevés chez le ConSDABI SUB NFP.I.-FAO afin d'évaluer l'effet du sexe sur la diminution de poids des deux produits fermentés. Les résultats, valables dans le champ d'observation, ont montré une significative influence du facteur sexe sur le taux de perte de poids pour les deux produits. En particulier : (i) la soppressata Sannita préparée avec la viande de mâle castré a un pourcentage de diminution constamment plus grand comparé à celui obtenu en utilisant la chair de femelle non castrée ; à la fin du séchage (40 jours) au total on perd ( $P < 0,01$ ) 51% du poids (+4%) par rapport à la femelle non castrée ; (ii) la salsiccia Sannita après 15 jours montre une perte de poids de 41% lorsqu'elle est produite avec de la viande de mâle castré et de 34% lorsqu'elle est produite avec de la viande de femelle non castrée ( $P < 0,05$ ), après 30 jours on perd 52% et 47% respectivement ( $P < 0,05$ ).

**Mots-clés.** Casertana – Type génétique autochtone ancien (TGAA) – Salsiccia Sannita – Soppressata Sannita.

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## I – Introduction

"Casertana" (CT) in the past was reared in particular in Campania and in neighbouring regions and was appreciated, besides for its good productive performances, also for its tendency to accumulate fat. This particular characteristic was one of the reasons for which this AAGT was

replaced with pigs of foreign breeds, selected to make more lean meat. The recent attention to the conservation of the genetic resources and the high demand for local products had allowed to a renewed productive utilization of this AAGT. The valorisation of local products is a desirable and feasible target for cultural, scientific, economic and social reasons. The local products, different from industrial products for ancient origin and for specific organoleptic characteristics, allow to safeguard productive techniques and traditions, to improve the conditions of rural populations, in particular in marginal areas and also to preserve the AAGTs from the eventual extinction. This work is a part of a project aimed at enhancing the use and production of CT pig AAGT; in particular, in this note, we report the results of the monitoring of ripening process evolution of two products, soppressata Sannita and salsiccia Sannita, which enhance the ancient Italian tradition of meat and especially sausage consumer, monitoring the weight loss for 30 and 40 days respectively.

## II – Materials and methods

The study involved 726 individual products obtained from 68 males (castrated at least 40 days before slaughter) and 53 entire females, slaughtered at average live weight of 169 kg and 161 kg respectively. All animals were reared in multiple boxes at experimental farm of ConSDABI Sub NFP.I.- FAO and were fed with commercial feed. The products were made at a salami factory. For the preparation of these LPs the meat was minced and mixed; pH, temperature and humidity at different seasoning times were registered. The seasoning was realized in proper places controlled for temperature and humidity during the 24 hours. Each product was weighed at the end of the drying time and then periodically (daily or weekly) until the end of seasoning time. The dough for the preparation of soppressata Sannita consisted of separable meat obtained from the partial trimming of ham and long back with the addition of lumbar subcutaneous fat derived from trimming of ham, cut manually with knife in an amount equal to about 2- 3% of the total weight of the used meat; for the preparation of salsiccia Sannita, separable meat from jowl, diaphragm, capocollo trimming, partial trimming of ham, shoulder, track, trimming of belly, ribs filet and throat was used.

The length of fresh soppressata Sannita must be about 18 – 20 cm, with a minimum regular circumference of 18 cm when it was fresh.

The length of fresh salsiccia Sannita must be about 50 – 60 cm and it has a typical form of horseshoe bat.

The seasoning included a drying phase (7 days) at controlled temperature (14-18°C) and relative humidity (60-70%) and a second seasoning period (45 and 30 days, respectively for soppressata Sannita and salsiccia Sannita) at lower temperature (10-14°C) and higher relative humidity (70-85%).

At the end of seasoning all two products, after regular brushing and washing with lukewarm water to remove the moulds, were vacuum-packed or preserved under pork fat. The data were processed using the following model of covariance analysis, with initial weight of product and net live weight as covariate for soppressata Sannita and weight of cooled carcass and age at slaughter as covariate for salsiccia Sannita (SAS, 1997):

$Y_{ijk} = \mu + b_1x_1 + b_2x_2 + \text{gender}_i + e_{ijk}$  , where:

$\mu$  = constant common to all the observations (general mean);

$b_1$ , = regression coefficients of the dependent variable from net live weight of pig or weight of 'cooled carcass' ( $x_1$ );

$b_2$ , = regression coefficients of the dependent variable from initial weight of product or 'age at slaughter' ( $x_2$ );

gender<sub>i</sub> = fixed effect of i<sup>th</sup> gender (i = 1,2);

e<sub>ijk</sub> = random error and/or unknown effects.

The significance of differences between the estimated means was tested using Student's t test.

### III – Results and discussion

The weight loss during the seasoning time was significantly higher in all two products obtained from castrated male in comparison with that obtained from entire female; in particular (Tables 1 and 2).

(i) for soppressata at the end of seasoning (44 days) the difference reached 4% ( $P < 0.001$ ), starting from 1.9% at 5 days ( $P < 0.01$ );

(ii) 'salsiccia Sannita' obtained from castrated males registered about 41% of weight loss at 15 days of seasoning ( $P < 0.05$ ) and 52% at the end of seasoning ( $P < 0.05$ ), values higher than that obtained from entire females (about 34% and 47% respectively).

**Table 1. Soppressata Sannita. Percentage weight variation in relation to the seasoning time and gender**

Seasoning d	Sex		$\Delta = [(\text{♂♂}) - \text{♀♀}]$
	(♂♂)	♀♀	
5	20.4	18.4	1.9**
10	28.0	25.4	2.6***
15	34.3	31.2	3.1***
20	38.2	34.5	3.6***
30	47.6	43.7	3.9***
44	51.4	47.4	4.0***

\*\* $P < 0.01$ ; \*\*\* $P < 0.001$

On the average from meat of each castrated male it was obtained 27.5 kg of salsiccia Sannita while from meat of each female it was obtained 23.3 kg. This difference is due to the different live weight of each gender (169,2 vs 160.9 kg).

**Table 2. Salsiccia Sannita. Percentage weight variation in relation to the seasoning time and gender**

Seasoning d	'Sex'		$\Delta = [(\text{♂♂}) - \text{♀♀}]$
	(♂♂)	♀♀	
15	40.8	33.6	7.2*
30	51.8	47.2	4.6*

\* $P < 0.05$ .

Some researchers (Nold *et al.*, 1999; Maiorano *et al.*, 2007) showed that meat obtained from sow has a higher water holding capacity respect to meat of male, and this can be one of the reason that had determined the different weight loss observed by us.

## IV – Conclusions

The results, valid within the observation field, highlighted that, for the two considered local products, the castrated male had a greater percentage weight loss than entire female. This trend may confirm previous results (Barone *et al.*, 2006). This difference could be due to the different adipose tissue's texture (for a probable different amount of saturated fatty acids), as well as to the higher intramuscular fat content and a best water holding capacity of the meat obtained from entire females. This result in association with qualitative data of product (rheology and color traits) may suggest a diversification of the products also in the selling price on the bases of gender of the pig that provide raw material.

## Acknowledgements

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# Selection of staphylococci strains isolated from a Portuguese traditional fermented/dry sausage for potential use as starter cultures

L. Carvalho\*, M.J. Fernandes\*, H. Fernandes\*, T. Semedo-Lemsaddek\*, M. Elias\*\*, A.S. Barreto\* and M.J. Fraqueza\*<sup>1</sup>

\*Faculty of Veterinary Medicine, CIISA, TULisbon, Av. da Universidade Técnica, Polo Univeristário, Alto da Ajuda, 1300-477 Lisbon (Portugal)

\*\*Universidade de Évora, Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Évora (Portugal)

<sup>1</sup>email:mjoaofraqueza@fmv.utl.pt

**Abstract.** In order to evaluate its potential use as starter cultures, 104 isolates of *Staphylococcus* spp. were obtained from sausages and different sampling environment points in workshops A and B, at southern region of Portugal. PCR amplification was carried out to confirm genus and species allocation. From workshop A, 47 isolates were staphylococci, the majority belonging to the specie *S. equorum*, while from workshop B, of the 57 isolates identified as staphylococci, most were *S. xylosus*. The genetic profiles of isolates were further compared using PCR fingerprinting analysis, guiding to the selection of 43 representative strains subsequently characterized for their nitrate reductase, lipolytic and proteolytic activities. Among these strains, 30% revealed proteolytic ability while 42% had lipolytic activity. 65% of the strains reduced nitrate. Subsequently, 10 strains (representatives of different fingerprinting groups) were evaluated for their ability to grow at different temperatures, pH and NaCl concentrations. *S. xylosus* and *S. equorum* showed to grow under all the studied conditions being more effective at 15°C, 20°C and pH 5.5 at 20°C, and NaCl concentrations of 10% and 15%; therefore can be guaranteed their application in technological processes with varying temperatures. In workshop A, strains P05-58 *S. carnosus* and P05-74 *S. equorum* and, from workshop B, P06-01 and P06-26 *S. xylosus* presented the most interesting features.

**Keywords.** *Staphylococcus* coagulase negative – Fermented meat products – Starter culture – Proteolytic activity – Lipolytic activity – Nitrate reductase activity.

**Sélection de souches de staphylocoques isolées à partir de produits carnés traditionnels portugais fermentés pour leur utilisation potentielle comme culture starter**

**Résumé.** Afin d'évaluer leur potentielle utilisation comme culture starter, 104 isolats de *Staphylococcus* ont été obtenus à partir de "chouriços" et de différents points de l'environnement des unités A et B, au sud du Portugal. La confirmation génétique du genre et de l'espèce a été réalisée par PCR en identifiant 47 isolats de staphylocoques dans l'unité A, dont la plupart ont été *S. equorum*, tandis que dans l'unité B les 57 isolats étaient en majorité *S. xylosus*. Par PCR fingerprinting on a sélectionné 43 souches, qui ont été caractérisées concernant les activités nitrate réductase, lipolytique et protéolytique. De ces souches, 30% ont montré une activité protéolytique, 42% avaient une activité lipolytique, et 65% avaient la capacité de réduire les nitrates. Par la suite, 10 souches de différents groupes de fingerprinting ont été évaluées pour leur capacité de croissance à différentes températures, pH et % de NaCl. *S. xylosus* et *S. equorum* ont montré une croissance dans les conditions étudiées, étant plus efficaces à 15°C et 20°C, et dans un pH de 5,5 à 20°C, et dans des conditions de NaCl de 10% et 15%; ainsi on peut garantir leur application dans des procédés technologiques avec des températures variables. Les souches P05-58 *S. carnosus* et P05-74 *S. equorum* de l'unité A, autant que les *S. xylosus* P06-01 et P06-26, de l'unité B, ont démontré être les plus intéressantes.

**Mots-clés.** *Staphylococcus* coagulase négative – Produits carnés fermentés – Culture starter – Activité protéolytique – Activité lipolytique – Activité nitrate réductase.



## I – Introduction

In Portugal there is a wide variety of traditional fermented/dry meat products, produced in small scale units being characterized by having in their manufacture a spontaneous fermentation by means of the so-called "house flora". Such products suffer a slight acidification and sensory characteristics are highly appreciated by consumers. Chorizo is a sausage made with pork, slightly fermented, smoked and dried, with a low pH and a decreased water activity, its production is characterized by a close relationship with the fermentative flora naturally present in the industrial environment. This type of flora is introduced in the meat during slaughter and increased in its concentration during manufacture of the fermented product. Each processing unit has a specific environmental flora, composed of useful microorganisms for the fermentation and the development of sensory characteristics in traditional sausages. The role of *Staphylococcus* as technological culture is well defined and the selection of the most interesting strains is a challenge to improve the hygienic and sensory characteristics of traditional sausages.

This study aimed to characterize the technological flora of *Staphylococcus* spp. isolated from dry fermented sausages and the traditional manufacture environments, in order to assess their potential use as starter cultures.

## II – Material and methods

### 1. Origin of *Staphylococcus* isolates

The experimental work was carried out using 104 staphylococci isolated from the manufacturing environment, samples of traditional fermented and smoked sausage (chorizo) and secondary ingredients (garlic, pimento paste or bell pepper paste and natural casing) at two traditional meat industries (Plants A and B) located in Alentejo. The isolates were previously identified by biochemical miniaturized tests APIStaph (Biomérieux, France) by Fraqueza *et al.* (2006). At each manufacturing plant, samples were taken from the work surfaces (table, knife, grinder, mixer or stuffing machines) and from the fermented/smoked sausages in three different stages of manufacture (product after filling, product with half time smoking period and the final product) and secondary ingredients.

### 2. Extraction of DNA

The isolates were grown in tryptose salt agar to obtain fresh culture by 24 h incubation at 37°C, and the extraction of genomic DNA was performed using the QIAamp DNA Mini Kit 02/2003" from Qiagen (Germany).

### 3. Identification

The isolates were identified as belonging to the species *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylosus* and *Staphylococcus saprophyticus* using a multiplex PCR method described by Morote-Bizot *et al.*, (2004). *Staphylococcus carnosus* and *Staphylococcus simulans* were identified by PCR performed according to the methodology described by Blaiotta *et al.*, (2004) while the identification of *Staphylococcus equorum* was based on the protocol described by Blaiotta, *et al.*, (2004).

### 4. Genetic characterization and strain selection

The selection of isolates to assess for technological interest was based on their genetic profiles using the PCR fingerprinting methodology according to Tenreiro (2007). Representative strains were selected by comparison of the fingerprinting profiles obtained.

## 5. Phenotypic characterization of technological functions

The nitrate reductase activity was determined as described by Miralles *et al* (1996), while proteolytic activity was performed by sowing among PCA with gelatin, peptone and milk powder with subsequent flooding of the plate with a solution of mercuric chloride after incubation. To evaluate the lipolytic activity we used the Spirit Blue Agar medium (France).

Selected representatives ( $n = 10$ ) groups with different genetic profiles were characterized for their ability to grow in different conditions of temperature, pH values and sodium chloride concentrations in order to evaluate their potential use as starter cultures. We investigated 3 incubation temperatures (10°C, 15°C and 20°C), two pH values (5.0 and 5.5), the temperatures of 15 and 20°C and two different concentrations of sodium chloride (10 and 15%).

## 6. Statistical analysis

Descriptive analysis of the results and their graphical presentation was obtained using Microsoft Office Excel 2007. The fingerprinting profiles obtained for each isolate were introduced in the Bionumerics software (version 4.61 [Applied Maths, Kortrijk, Belgium]) and dendrograms were constructed using the Pearson correlation coefficient and clustering method based on the unweighted average distance (UPGMA – unweighted pair group method with arithmetic average).

## III – Results and conclusions

In Plant A, forty-seven *Staphylococcus* isolates were identified as members of the species *S. equorum* while in Plant B, fifty-seven isolates belong to the species *S. xylosus*. The genetic profiles of the isolates were compared by PCR fingerprinting (Figs 1 and 2) and led to the selection of 43 staphylococci which were subsequently characterized for their nitrate reductase, lipolytic and proteolytic activities.

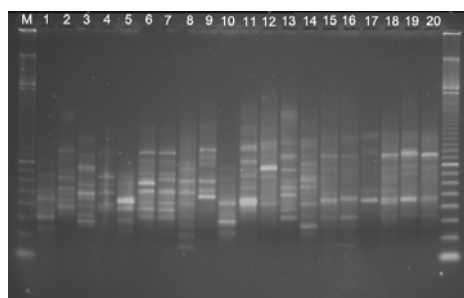


Fig. 1. PCR fingerprinting profiles of *Staphylococcus* strains.

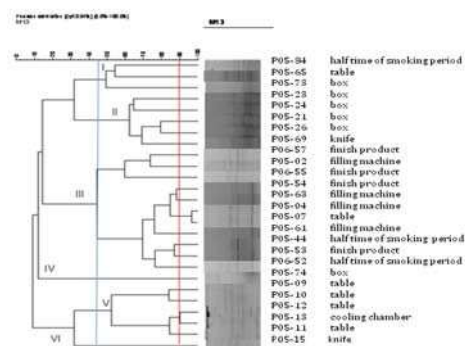


Fig. 2. Dendrogram analysis of the genetic profile of strains of *Staphylococcus equorum*.

By analyzing Fig. 2 we can see that the genetic profiles clustered the isolates in three main groups, with similarities below 45%. Strains P05-04 and P05-07 harbor profiles with a genetic similarity above 90%, being considered identical or highly related.

It was found that 30% of the strains showed proteolytic activity, while 42% showed lipolytic activity. As for the nitrate reductase activity, it was observed that about 65% of the strains reduced nitrate.

Strains of *S. xylosus* and *S. equorum* (n = 10) grew in all the conditions studied, but their development was more effective at 15°C and 20°C, pH 5.5 at 20°C and 10% and 15% concentration of chloride sodium, thus suggesting features that can guaranteed their application in technological processes with varying temperatures (Table 1)

**Table 1 . Evaluation of proteolytic, lipolytic and nitrate reductase activity in strains of *Staphylococcus xylosus* and *Staphylococcus equorum***

	<i>S. xylosus</i> (n=10)		<i>S. equorum</i> (n=8)	
	Positive	Negative	Positive	Negative
Proteolytic Act. (n=18)	3	7	3	5
Lipolytic Act (n=18)	10	-	1	7
Nitrate reductase Act. (n=18)				
30°C/7 h (n=18)	5	5	-	8
20°C/24 h (n=18)	6	4	7	1
15°C/72h (n=18)	2	8	7	1

## IV –Conclusions

Strains of *S. carnosus* P05-58 and *S. equorum* P05-74 from Plant A and strains of *S. xylosus* P06-01 and P06-26 from Plant B, demonstrated by their characteristics to be the most interesting from the technological point of view. These results suggest their potential usage as starter cultures.

## Acknowledgements

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# Quality of the sirloin "presa" of the Iberian pork in two types of package

O. Fernandes\*, S. Ferro Palma\*\*, M.J. Barata de Carvalho\*\*  
N. Alvarenga\*\* and J. Canada\*\*

\*Damicarnes (Portugal)

\*\*ADCTA, Escola Superior Agrária de Beja, Rua Pedro Soares, 7801-902 Beja (Portugal)

**Abstract.** Availability of meat from Iberian pork, which is allowed to graze free range Dehesas (acorn forests) giving pigs fat its unique sweet *flavour*, is limited as its production is seasonable. Freezing may be an option to prolong commercialization periods and further consume of this meat, which represents high economic value. Aim of this study is to compare the quality of frozen meat, "presa" packed under vacuum in PA/PE material and frozen meat packed under vacuum in PA/PE material and stored in carton boxes, taking refrigerated meat as a reference. For this study, 24 samples were freeze using different storage conditions, namely 12 samples were packed individually under vacuum, and 12 samples were packed individually under vacuum and laid up in cardboards. Reference samples were prepared from same batch and stored at refrigerating conditions. After a conservation period of 5 month at -18°C, samples were thawed and its physicochemical properties (pH, color, moisture, water retention capacity), texture (Texture Profile Analysis, penetration) and sensorial analysis (performed by a trained panel), were assessed with the aim of evaluating possible damages caused by freezing. The results show evidences on differences between refrigerated packaging systems. Frozen meat delivered a lower pH and was darker then refrigerated meat which was confirmed by a decrease on L\*. Cut resistance was as well lower for frozen meat. After thermal treatment, moisture content decreases followed by a decrease on meat hardness and adhesiveness. Sensory analysis points out a decrease on *flavour* persistence, overall acceptance, and aftertaste, with significant differences being verified on meat packed only in PA/PE material. This results evidence that packing Iberian pork meat under vacuum and cardboards is the packaging alternative which delivers meat with closest characteristics to the ones observed on refrigerate meat.

**Keywords.** Iberian pig – "Presa" – Meat – Freezing – Packaging.

## **La qualité de la longe ("presa") du porc Ibérique surgelée sous deux formes d'emballage**

**Résumé.** La disponibilité de la viande de porc Ibérique élevé en chênaie, est fonction du rythme saisonnier de sa nourriture (glands). La congélation permet de prolonger sa commercialisation et par conséquent la consommation de cette viande, d'une valeur économique supérieure. Cette étude vise à comparer la qualité de la longe ("presa") du porc Ibérique surgelée en pochette, ainsi que celle de la même viande surgelée en pochette et placée en boîte, par rapport à la viande réfrigérée, et à évaluer le processus de congélation. Nous avons utilisé 24 échantillons surgelés, 12 échantillons emballés individuellement sous vide et 12 échantillons emballés sous vide mais placés en boîtes et 12 échantillons réfrigérés, du même lot, qui ont servi comme référence. Après 5 mois de conservation à une température de -18°C, les échantillons ont été décongelés et leurs propriétés physiques et chimiques (pH, couleur, humidité, capacité de rétention de l'eau), leur texturation (analyse du profil de la texture, test de pénétration simple) et leurs propriétés sensorielles (panel de dégustateurs chevronnés), ont été évaluées, afin d'apprécier les altérations provoquées éventuellement par la congélation. Les résultats démontrent qu'il existe des différences entre les formes d'emballage, notamment la diminution du pH, la diminution de la valeur L\* et la diminution de la résistance à la coupe. Après un traitement thermique, on a constaté une diminution de l'humidité, de la dureté et de l'adhésivité. L'évaluation sensorielle démontre que l'acceptation globale, la persistance de la saveur propre à la viande et le goût résiduel ont diminué, et présentent une différence significative, ceci uniquement dans les échantillons surgelés en pochette. On arrive ainsi à la conclusion que la congélation en pochette avec emballage en boîte est la modalité qui permet le mieux à la longe (« presa ») surgelée, de se rapprocher de la longe réfrigérée.

**Mots-clés.** Porc Ibérique – Longe ("Presa") – Congélation – Surgelé – Emballage.

## I – Introduction

The production of the Iberian pork may be considered seasonal as there are some offering peaks. During the periods of higher distinct production, freezing will be a way of storing the product and so there is a guarantee of adjusting to the market. In industry the portions of a greater economic value were commercialized fresh. When there were no freezing processes, the meat portions were almost used in the manufacture of smoked sausages. The aim of this work is testing the freezing method of the ("presa" / sirloin or portion) ventral, serriform, toracic and cervical muscles of the Iberian pork used in the enterprise "Damicarnes". As we are dealing with meat of high economic value, it is supposed to evaluate the differences between the refrigerated and the frozen product in two distinct forms of package which create differences in the freezing speed of the product. Among all the characteristics of the quality of the meat, texture is extremely important for the consumer (Lawrie, 1998).

The texture of the meat corresponds to sensations of succulence and tenderness which are evaluated by instrumental techniques or by a panel of tasters. The greatest difficulty in evaluating the meat texture is, no doubt, establishing a relationship between the results obtained by the two methods, and above all, because the meat has to suffer a thermal treatment before being consumed, which increases the impact in the meat characteristics (Genot, 2003). Freezing is a preservation system and the technology used tries to limit the damages aiming at causing the slightest number of alterations (Varnan and Sutherland, 1998). Package may confer distinct degrees of protection to the product. The development of flexible pellicles and vacuum package led to the evolution of the meat distribution sector. Meat frozen after vacuum package, keeps its natural quality during a more prolonged period (Price and Schweigert, 1994). The main causes for the deterioration of fat are hydrolysis and oxidation. It is of the utmost importance to freeze as fast as possible after production, under the correct conditions and reduce to the minimum the temperature fluctuations during storage and transportation (Varnan and Sutherland, 1998).

Freezing is the best way to preserve meat at a long term but it must be well done. The maintenance of the characteristics of the product during preservation depends on the temperature of the storage and the type of meat (Prandal *et al.*, 1994). The loss of CRA and the lack of capacity the fibres have to reabsorb the water when thawing and then high ionic force causes denature of the muscle proteins (Lawrie, 1998). The quantity of exsudation varies according to the initial characteristics of the product, the speed of freezing and thawing, the temperature and the period of storage, however, this quantity is lower when the meat is vacuum stored with an impermeable material to oxygen and the flavour of rancidity also decreases. According to Genot (2003), the freezing usually causes a slight increase in the tenderness of the meat, and the force of the muscle cutting which suffered freezing is much lower than in the fresh meat. Pork is slightly more tender and juicy, when it is frozen and it is also more easily chewed. An excessive loss of exsudation is a determining factor in the quality of the meat and unpleasant to the consumer, restricting the acceptability of the product (Varnan and Sutherland, 1998).

## II – Material and methods

The sample used in this study is the sirloin of the Iberian pork. The sirloin is made up of the serriforme, ventral toracic and cervical near the thigh (Mayoral *et al.*, 2003). It is considered a sample, a portion of meat which corresponds to the sirloin and weighs approximately 700 g with the dimensions 20 x 10 x 5 cm, which suffered a process of freezing in a current of forced air and picked at hazard from the right and left side of males and females. Thirty six examples of the sirloin of the Iberian pork were collected which came from the same lot of meat from animals which underwent identical conditions of handling and slaughter and were vacuum stored at +2°C. The plan of sampling followed in this study is the following: 6 samples were used in laboratory analyses and the remaining 6 in a sensorial analysis. The sirloin was packed unit by

unit, in vacuum with a poliamid polyethylene pellicle of 125 µm thickness in a thermoformative machine and frozen according to Table 1. After freezing it was moved to the preservation chamber of frozen products.

**Table 1. Plan of samples**

No. of samples	Refrigerated samples	Samples frozen in a vacuum pouch under a shelf (0°C to -7°C) 4h30; 5 months at -18°C	Samples frozen in a vacuum pouch and packaged in cardboard boxes under a shelf; (0°C to -7°C) 12h; 5 months at -18°C
6+6	CR		
6+6		CBL	
6+6			CCX

pH was determined according to the rule NP 33441 / 1990. The colour was determined by a colorimeter previously calibrated by a plate with a white reference no. 19733057, channel 0 ( $L^* = 97.10$ ;  $a^* = +0.07$ ;  $b^* = +1.83$ ). Result expressed in:  $L^*, a^*, b^*$ ; Relationship  $a/b$ ; Chromaticity  $C = (a^2 + b^2)0.5$ ; Tone  $H^\circ = \arctangent b/a^* \cdot 360^\circ / (2 \cdot 3.14)$  (Minolta, 1991). The percentage of humidity (moisture) was determined according to the procedure of the rule NP 1614/2002. The capacity of water retention was carried out according to the pistometric method (Grau and Hamm, 1953). The rheologic characterization was carried out in a texturometer. The samples were submitted to tests of a simple cutting. Slices of the muscle were cut, 2 cm length in the vertical and each one in parallelepipeds of 1.5 cm. They were grilled in a convector oven at 150°C, during 20 minutes, the grilled meat was kept in a stove at about 55°C until the experiment. The test was carried out with a chopping knife and the speed of the test was 1 mm s<sup>-1</sup> the distance of 10 mm. The test was carried out with a thermal treatment and without a thermal treatment (in the direction of the muscular beam and against it). The characteristics determined were the resistance to the cutting and the work of the strength of the cutting.

The sensorial evaluation of the Iberian pork was tested by a panel of tasters selected and trained by the Agrarian High School in Beja, according to the NP ISO 8586-1 (2001). Tasting occurred according to NP 4258 (1993). To carry out the experiments, slices of meat were cut with 2 cm width and each slice in parallelepipeds of 1.5 cm and they were grilled in a convector oven.

### III – Results and discussion

The average values of pH in the refrigerated meat are superior and with significant differences compared to the values found in the frozen meat inside a pouch and inside a pouch and a box which also show significant differences (Table 2). The average values of CRA don't show significant differences in the different ways of package of the meat frozen in a pouch and a box compared to the refrigerated meat. However, the values showed clearly a fall. Bustabad (1999) refers that vacuum package and the package in cardboards is effective and contributes to reducing the loss of water.

The values of moisture in the meat before being submitted to a thermal treatment don't show any significant differences, however, they don't include the loss of water suffered by the exsudation of the thawed meat (which was about 3%). Farouk *et al.* (2003) showed that the losses of water when thawing were identical to the freezing speeds either high or low, when evaluated after 6 months preservation and increase all along the preservation period. The values obtained in the moisture of the meat after thermal treatment, show significant differences between the refrigerated meat, frozen in a pouch and frozen in a pouch and a box. Under the same conditions under the thermal treatment, the refrigerated meat, managed to keep the water

in a more outstanding way than the meat which was frozen. Lawrie (1998) refers that a pH of 5.9 causes cooking losses superior to the losses when the pH is 6.0, that's to say that the reduction of pH caused an increase of water losses during cooking in a continued way.

**Table 2. Averages, pattern deviations and results of the analysis of the variation of the physic chemistry parameters of the sirloin of the Iberian pork refrigerated (CR) frozen in a pouch (CBL) and frozen in a pouch and a box (CCX)**

	CR	CBL	CCX
pH	6.17 <sup>a</sup> (0.15)	5.93 <sup>b</sup> (0.06)	5.85 <sup>c</sup> (0.11)
CRA %	32.83 <sup>a</sup> (2.12)	29.67 <sup>a</sup> (7.14)	30.00 <sup>a</sup> (3.84)
Meat moisture without thermal treatment	70.85 <sup>a</sup> (1.43)	70.77 <sup>a</sup> (0.86)	69.45 <sup>a</sup> (2.87)
Meat moisture after thermal treatment	57.67 <sup>a</sup> (0.36)	54.68 <sup>b</sup> (0.46)	50.56 <sup>c</sup> (0.18)

The Table 3 shows the values obtained in the colour of the muscle in the lateral face and in the medial face. It is verified that the meat lost its brightness, got dark after freezing and thawing packed in a pouch or in a pouch and box when compared to the values obtained in the refrigerated meat.

**Table 3. Averages, patterns deviations and results of the analysis of the variation of the colour of the muscle in the outside lateral face and medial face, L\*,a\*,b\*, (CIE) of the sirloin of the Iberian pork refrigerated (CR) frozen in a pouch (CBL) and frozen in a pouch and box (CCX)**

	CR	CBL	CCX
L*	38.96 <sup>a</sup> (2.60)	30.98 <sup>b</sup> (3.46)	32.27 <sup>b</sup> (2.32)
a*	17.72 <sup>a</sup> (0.97)	15.67 <sup>b</sup> (2.12)	18.76 <sup>a</sup> (1.12)
b*	4.36 <sup>a</sup> (1.04)	4.21 <sup>a</sup> (1.84)	4.44 <sup>a</sup> (1.19)
a/b	4.28 <sup>a</sup> (1.07)	4.31 <sup>a</sup> (1.52)	4.48 <sup>a</sup> (1.08)
$C=(a^2 + b^2)^{0.5}$	18.27 <sup>a</sup> (1.11)	16.28 <sup>b</sup> (2.24)	19.30 <sup>a</sup> (1.28)
$H^{\circ} = \arctang b/a$	0.24 <sup>a</sup> (0.05)	0.25 <sup>a</sup> (0.09)	0.23 <sup>a</sup> (0.05)
L*	42.47 <sup>a</sup> (4.16)	32.81 <sup>b</sup> (3.33)	33.72 <sup>b</sup> (3.59)
a*	17.98 <sup>a</sup> (2.48)	18.01 <sup>a</sup> (1.75)	18.06 <sup>a</sup> (1.78)
b*	5.15 <sup>a</sup> (1.76)	4.55 <sup>a</sup> (1.17)	4.85 <sup>a</sup> (1.36)
a/b	4.20 <sup>a</sup> (2.75)	4.34 <sup>a</sup> (1.74)	4.02 <sup>a</sup> (1.22)
$C=(a^2 + b^2)^{0.5}$	18.75 <sup>a</sup> (2.689)	18.62 <sup>a</sup> (1.60)	18.74 <sup>a</sup> (1.90)
$H^{\circ} = \arctang b/a$	0.28 <sup>a</sup> (0.08)	0.25 <sup>a</sup> (0.07)	0.26 <sup>a</sup> (0.07)

In the study carried out by Estevéz *et al.* (2003) , the values of L\* fell down just like it happened in this study, which can be explained by the water losses after thawing and hemoglobin concentration which reduces the value of L\* . According to Price and Schweigert (1994) if the temperature of freezing is superior to -57°C it is usually produced a dark colour because metamioglobin is formed. Because of the fall of the pH and CRA , the colour gets dark owing to the passage of mioglobin and metamioglobin (Farraia da Graça,1987) and the effect is greater with values of pH superior to 5.8 (Varnam and Sutherland 1998).

In all the tests of a simple cut in meat without thermal treatment it was observed that the cut resistance and the work of the cut strength showed lower values with significant differences, in the frozen meat inside a pouch or inside a pouch and box, when compared to refrigerated meat (Table 4)

The cellular destruction owing to the formation of intercellular ice crystals, led to the reduction of the cut force in the thawed meat (Lagerstedt *et al.*, 2008). The denatured proteins are particularly sensitive to the attack of proteolytic enzymes, causing the reduction of hardness (Lawrie, 1998).

**Table 4. Averages, pattern deviations and results of the analysis of variance for the parameters of the test of a simple cut in the sirloin of the Iberian pork refrigerated (CR), frozen inside a pouch (CBL), and frozen inside a pouch and a box (CCX), without thermal treatment (direct or against the muscular beam), and with thermal treatment**

	Without thermal treatment for the muscular beam			Without thermal treatment against the muscular beam			With thermal treatment		
	CR	CBL	CCX	CR	CBL	CCX	CR	CBL	CCX
Cut resistance (N)	51.45 <sup>a</sup> (35.79)	29.27 <sup>b</sup> (7.58)	28.09 <sup>b</sup> (8.72)	50.73 <sup>a</sup> (17.63)	36.48 <sup>b</sup> (10.45)	38.04 <sup>b</sup> (11.23)	45.61 <sup>a</sup> (27.06)	38.83 <sup>ab</sup> (5.56)	31.92 <sup>b</sup> (9.79)
Work cut strength (NS)	107.94 <sup>a</sup> (46.11)	72.00 <sup>b</sup> (23.03)	66.13 <sup>b</sup> (24.22)	140.02 <sup>a</sup> (46.14)	97.29 <sup>b</sup> (32.25)	104.82 <sup>b</sup> (40.70)	121.64 <sup>a</sup> (75.44)	133.20 <sup>a</sup> (20.96)	100.95 <sup>a</sup> (39.09)

The average values in the same line differ when affected with different letters to  $\leq 0,05$ .

The values obtained in the cut for the muscular beam show a greater difference between the frozen meat and the refrigerated meat than in the values against the muscular beam, and the joints are more easily destroyed in freezing. According to Farag *et al.* (2009) another factor that influences the texture of the meat is the direction of the muscle fibres. After the thermal treatment it was checked that the cut resistance decreased especially in the meat which suffered freezing compared to refrigerated meat. The panel tasters detected significant differences in the residual taste of the frozen samples in the pouch when compared to the refrigerated ones and with those frozen in a pouch and box (Table 5). For an intermediate freezing speed, the ice is formed outside and inside the cell together with a variable temperature during preservation and leads to damages in the tissues. The CRA of the frozen meat in a pouch is inferior to the one frozen in a pouch and box, which becomes evident in a greater quantity of lost liquids during the thermal treatment, being drawn some precursors of the residual taste that represents the detection of remaining substances (Chamorro and Losada, 2002). In the global appreciation the panel of tasters considered that the samples frozen in a pouch show significant differences from the refrigerated ones but were not significant among the frozen samples.

**Table 5. Average, pattern deviations and results of the analysis of variance for the sensorial parameters in the sirloin of the Iberian pork refrigerated (CR), frozen in a pouch (CBL), and frozen in a pouch and box (CCX)**

	CR	CBL	CCX
Tenderness	5.66 <sup>a</sup> (0.62)	4.79 <sup>a</sup> (0.21)	5.61 <sup>a</sup> (0.15)
Succulence	5.38 <sup>a</sup> (0.24)	3.93 <sup>a</sup> (0.61)	4.29 <sup>a</sup> (1.11)
Flavour of rancidity	0.44 <sup>a</sup> (0.16)	0.79 <sup>a</sup> (0.11)	0.82 <sup>a</sup> (0.16)
Characteristic flavour	6.15 <sup>a</sup> (0.28)	5.68 <sup>a</sup> (0.16)	5.79 <sup>a</sup> (0.60)
Persistence	5.72 <sup>a</sup> (0.12)	4.93 <sup>b</sup> (0.10)	5.04 <sup>b</sup> (0.15)
Residual taste	5.13 <sup>a</sup> (0.12)	4.36 <sup>b</sup> (0.10)	4.93 <sup>a</sup> (0.20)
Global appreciation	6.64 <sup>a</sup> (0.43)	4.50 <sup>b</sup> (0.20)	5.57 <sup>ab</sup> (0.51)

The average values in the same line differ when affected with different letters to  $\leq 0,05$ .



## IV – Conclusions

Freezing in a pouch and box obtained a better result than freezing in a pouch. In the existing conditions it isn't the speed of freezing that interferes in the quality of the frozen meat; this means that the method used at present in the enterprises is the most advisable, however it is important to stabilize the preservation temperatures to minimize the undergone changes. The frozen meat after being thawed and compared to refrigerated meat, shows an inferior aspect, a decrease in pH caused by the development of lactic acid bacteria, a darkness caused by the formation of metamioglobin, a decrease of CRA as well as moisture, hardness, adherence and cut resistance, caused by proteic denature. The tasters classified negatively the residual taste of the frozen sample in a pouch in comparison to the refrigerated one and to the frozen one in a pouch and box. In the global appreciation, tasters preferred refrigerated meat which they consider not showing significant differences with the frozen meat in a pouch and box, only manifesting significant differences in the frozen meat in a pouch. Weight loss during freezing and the storage of frozen meat oxidative and colour changes in meat from three lines of free-range reared Iberian pigs slaughtered at 90 kg live weight and from industrial pig during refrigerated storage.

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# Characterization of surface mycoflora in Nebrodi hams

E. Berni, C. Cacchioli and C. Diaferia

Stazione Sperimentale per l'Industria delle Conserve Alimentari, Viale Tanara 31/A, 43100 Parma (Italy)

**Abstract.** Within the framework of a three-year project, the screening of spontaneous fungal species grown on hams produced with "Nebrodi black pig" meat and seasoned in different environments of the Nebrodi area has been carried out, in order to characterize mycological population of such typical products and to keep under the presence of unexpected changes due to fungal contamination. In most of the aitchbones examined, the prevailing fungal species isolated resulted to be xerotolerant or xerophilic, due to their peculiar ability of adaptation to surface  $a_w$  and to thermohygrometric conditions applied in ripening rooms. With regard to the *Eurotium* strains isolated, *Eurotium herbariorum*, *Eurotium rubrum* and *Eurotium cristatum* have been detected. With regard to the *Penicillium* strains isolated, *Penicillium nalgiovense*, *Penicillium chrysogenum*, *Penicillium griseofulvum*, *Penicillium olsonii*, and *Penicillium aurantiogriseum* have been detected. In any of the aitchbones examined, undesirable *Penicillium* species such as *P. commune* or *Penicillium solitum* (which are considered responsible for the production of the so-called "phenol defect" in hams) and *P. nordicum* (which proved to be one of the greatest Ochratoxin A producer in hams and in protein-based foods) have not been detected.

**Keywords.** Phenol defect – Hams – Moulds – *Penicillium solitum* – *Penicillium nordicum*.

## Caractérisation de la flore fongique de surface dans les jambons de Nebrodi

**Résumé.** On a étudié la mycoflore sur les jambons produits avec la viande de porcs de races autochtones appartenant aux types génétiques Nebrodi et séchés dans les environnements de la région de Nebrodi, afin de caractériser la population fongique sur ces produits locaux et de surveiller la présence de changements anormaux dus à la contamination des moisissures. Dans la plupart des os du bassin («quasi») examinés, les espèces fongiques prédominantes ont été xérotolérantes ou xérophiles, grâce à leur capacité caractéristique de s'adapter à l' $a_w$  superficielle et aux paramètres hygrothermiques utilisés dans les environnements de vieillissement. En ce qui concerne les espèces *Eurotium* isolées, on a constaté la présence de *Eurotium herbariorum*, *Eurotium rubrum* et *Eurotium cristatum*. En ce qui concerne les espèces de *Penicillium* isolées, ont été trouvées *Penicillium nalgiovense*, *Penicillium chrysogenum*, *Penicillium griseofulvum*, *Penicillium olsonii*, et *Penicillium aurantiogriseum*. Dans aucun des os du bassin examinés, n'ont été trouvées d'espèces indésirables de *Penicillium*, comme *P. commune* ou *P. solitum* (qui sont responsables du dénommé "défaut de l'acide phénique" dans les jambons) et *P. nordicum* (qui s'est avéré être l'un des plus grands producteurs d'ochratoxine A dans les jambons et les produits à base de protéines).

**Mots-clés.** Défaut de l'acide phénique – Jambons - Flore fongique – «Quasi» - *Penicillium solitum* - *Penicillium nordicum*.

## I – Introduction

Among factors influencing air-borne contamination of foods, microbial population in environmental air (Heldman, 1974) play a great role. In general, fungal spores proved to represent the prevailing part (56%) of the air-borne microflora (*Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Fusarium*), while *Bacillus* spores, Gram-positive and Gram-negative bacteria represent the remaining one. Nevertheless, in industrial environments where foods such as meat derivatives are produced, fungal population can reach 70% of the total microbial population (Singh *et al.*, 1986).

In aged meats, moulding is directly connected with physico-chemical parameters recorded in productive environments (Baldini *et al.*, 2000). In ripened meats, maturing techniques applied usually allow for the rapid and distinctive colonization by a great number of mycetes, which are considered fundamental to impart both desirable appearance and good organoleptic characteristics to these meats. On the contrary, in dry-cured meats maturing techniques are more and more pointed to obtain products where no or little surface mycoflora has grown: only autochthonous moulds indicating that ripening process is shaping up good and proving to compete and then prevail over undesired species should be tolerated. In general, in hams unexpected changes can occur and even persist in the final product in case thermo-hygrometric parameters reach high values during the resting and dehydration process is not carried out correctly.

A first example of such changes is represented by the so-called "phenol defect", a detrimental effect due to a fungal colonization of the aitchbone area by *Penicillium commune* (Spotti *et al.*, 1988) or by *Penicillium solitum* (Spotti, personal communication) during seasoning in case salt content on ham surface results lower than 17.5% (saturated salt solution) during salting and Relative Humidity (RH) values result higher than 85%. In fact, this area represent the wettest part of the ham and it could be more subjected to spoilage by *Penicillium* species, while the muscle portion, where dehydration occurs in a faster way, is usually more subjected to spoilage by *Eurotium* species, which tend to prevail because of their xerophily (Baldini and Spotti, 1995). To avoid "phenic acid defect" it should be taken into account that: (i) a saturated salt solution on the cut surface allows salt penetration in the aitchbone area during salting; (ii) fast dehydration is essential within the first 15 days of resting; (iii) the control of thermo-hygrometric parameters such as RH is fundamental to avoid fungal growth; (iv) an increasing in dehydration could be applied in case fungal spoilage occurs during resting and pre-ripening.

A further example of the above-mentioned changes is represented by the presence in seasoned hams of ochratoxin A (OTA), a strong, nephrotoxic, secondary metabolite that can be produced in meat products by some fungal species such as *Aspergillus ochraceus* and *Penicillium nordicum* and that can persist in the finished product. The moulds responsible for OTA production can develop on the surface of aged products and start producing OTA in case some variations in thermo-hygrometric parameter occur, so their presence must be always kept under control by means of periodical laboratory tests.

Within the framework of the above-mentioned project, the aim of this work was to screen spontaneous fungal species grown on aitchbones from hams produced with "Nebrodi black pig" meat and seasoned in different environments of the Nebrodi area, in order to characterize mycetical population of such typical products and to keep under the presence of the above-mentioned unexpected changes due to fungal contamination.

## II – Materials and methods

The screening of the mycoflora grown on aitchbones from hams seasoned in traditional environments of the Nebrodi area has been carried out by using sterile swabs, since they allow to scratch out a significant amount of conidia from any of the aitchbones assessed. After collecting the conidial mass, each swab has been plated on Malt Extract Agar (MEA) and on Dichloran 18% Glycerol Agar (DG18). Petri Dishes have been incubated at 25°C for seven days, in order to allow sporification of the fungal species isolated. Fungal identifications have been carried on selective media, according to the methods proposed by Pitt (Pitt and Hocking, 2009) and by Samson (Samson *et al.*, 2004):

- Malt Extract Agar (MEAB), 25% Glicerol Nitrate agar (G25N), Creatine Sucrose Neutral Agar (CSN), CY20S, Yeast Extract Sucrose Agar (YES) incubated at 25°C for seven days;
- Czapek Yeast extract Agar (CYA) incubated at 5°, 25° and 37°C for seven days.

### III –Results and discussion

The screening of the mycoflora grown on aitchbones from products seasoned in traditional environments of the Nebrodi area has been carried out on hams at different stages of their long-term ripening, since thermo-hygrometric parameters recorded in ripening plants proved to be greatly influenced by environmental outdoor conditions. In most of the aitchbones examined, yeasts have been isolated. In fact, they usually can colonise surface layers of cured meats for most of the seasoning time, both contributing to the development of the final typical aroma and avoiding oxidative processes on the ham surface (Martin *et al.*, 2006).

In all the aitchbones examined, (Table 1) the prevailing fungal species isolated resulted to be xerotolerant or xerophilic (capable of growing at water activity ( $a_w$ ) values lower than 0.85). In particular, the presence of *Eurotium* species and the growth of more xerotolerant fungal species belonging to *Penicillium* can be both due to their peculiar ability of adaptation to surface  $a_w$  and to thermo-hygrometric conditions of these plants where RH values range from 85 to 92% and where temperatures range from 10° and 20°C.

**Table 1. Prevalence of fungal species isolated on aitchbones at the end of the seasoning from Nebrodi hams, at different stages of their long-term ripening**

Species isolated	Frequency of contaminated aitchbones (%)		
	Year 2005	Year 2006	Year 2007
<i>Eurotium herbariorum</i>	85.7	63.6	38.5
<i>Penicillium gladioli</i>	71.4	18.0	46.0
<i>Penicillium griseofulvum</i>	71.4	27.0	7.6
<i>Penicillium olsonii</i>	57.0	9.0	23.0
<i>Penicillium chrysogenum</i>	0	9.0	7.6
<i>Penicillium nalgioense</i>	0	9.0	7.6
<i>Penicillium aurantiogriseum</i>	0	27.0	23.0
<i>Eurotium rubrum</i>	0	9.0	30.7
<i>Eurotium cristatum</i>	0	18.0	7.6
<i>Aspergillus candidus</i>	0	9.0	0
<i>Aspergillus versicolor</i>	0	9.0	7.6
<i>Penicillium glabrum</i>	0	0	7.6
<i>Aspergillus sydowii</i>	0	9.0	0
<i>Hyphopichia burtonii</i>	0	9.0	0
Yeasts	85.7	72.7	100.0

With regard to the *Eurotium* strains isolated, *Eurotium herbariorum*, *Eurotium rubrum* and *Eurotium cristatum* have been detected. Their presence can be explained by the fact that: they're markedly xerophiles (so they're able to grow within a wide range of temperature and  $a_w$  values); they tend to form fast-growing colonies that usually prevail over *Penicillium* ones; they reproduce themselves in both a vegetative (via darkish-coloured conidia) and a sexual (via yellowish-coloured cleistotecia containing ascospores) way. Such species, as well as *Aspergillus* ones, can frequently occur on ripened and dry-cured meat, as the surface  $a_w$  is markedly lower on these kind of products.

With regard to the *Penicillium* strains isolated, *Penicillium nalgioense*, *Penicillium chrysogenum*, *Penicillium griseofulvum*, *Penicillium olsonii*, and *Penicillium aurantiogriseum* have been detected. In literature, such species are frequently mentioned as those occurring in seasoning environments, so their presence has been well tolerated.

Among *Penicillium* species, *Penicillium gladioli* has been also detected. As it was isolated yet

within the first part of the project on Nebrodi salami and it was focused on because of its properties as autochthonous "starter" culture, its presence has been well tolerated too.

In addition to this, it must be underlined that some undesirable *Penicillium* species such as *P. commune* or *Penicillium solitum* (which are considered responsible for the production of the so-called "phenic acid defect" in hams) and *P. nordicum* (which proved to be one of the greatest Ochratoxin A producer in hams and in protein-based foods) have never been detected on the examined aitchbones. This means that variations in thermo-hygrometric parameter haven't occurred and that seasoning have been carried out correctly in hams from Nebrodi area.

With regard to other environment-contaminating moulds, contamination by *Cladosporium*, *Aureobasidium* and *Mucor* species has been only rarely detected. The growth of these moulds should be avoided both on hams and salami, as they don't allow homogeneous drying of the product in the first steps of the process and they can form darkish spots on casings of salami or on surface of hams.

Ultimately, *Hyphopichia burtonii* has been detected just once. This species is attributed to Filamentous Fungi and indicated as "yeast-like mould" because of its morphological and reproductive characteristics which enable it to grow widely on the surface of solid media with  $a_w$  values ranging from 0.85 to 0.90. Its presence is usually tolerated as it proved not to produce toxic metabolites, to compete with undesired species and to partially inhibit OTA production (Spotti *et al.*, 2009). For the above-mentioned reasons, its use as possible "competitor" of any undesired *Penicillium* species has been at present taking into account.

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# Comparison of pork quality from pure and crossbred Iberian pig

I. Clemente\*, \*\*, <sup>1</sup>, O. Polvillo\*, \*\*\*\*, M. Juárez\*, \*\*\*\*\*, C. Avilés\*, A. Membrillo\*, A. Horcada\*\*\*\*, E. Diéguez\*\*\*\*\*, J.C. Racero\*\* and A. Molina\*

\*Grupo de Investigación MERAGEM (AGR-158), Dpto. de Genética, Universidad de Córdoba, Córdoba (Spain)

\*\*Centro Tecnológico Andaluz del Sector Cárnico (TEICA), Cortegana (Spain)

\*\*\*Dpto. de Bioquímica y Biología Molecular, Universidad de Córdoba, Córdoba (Spain)

\*\*\*\*Centro de Investigación, Tecnología e Innovación (CITIUS), Universidad de Sevilla, Sevilla (Spain)

\*\*\*\*\*Departamento de Ciencias Agroforestales de la EUITA, Universidad de Sevilla, Sevilla (Spain)

\*\*\*\*\*Asociación Española de Criadores de Ganado Porcino Selecto Ibérico Puro y Tronco Ibérico, Zafra (Spain)

\*\*\*\*\*Lacombe Research Centre, Lacombe, AB (Canada)

<sup>1</sup>iclemente@teica.es

**Abstract.** The Iberian (IB) Pig Breed is the most important Mediterranean swine type, both in population size and economic importance. Most of IB pork is consumed as cured products. However, the consumption of fresh meat has recently increased. Due to the increasing demand of fresh meat, in 2007, a new National Quality Standard (NQS) was published in Spain to regulate the production and marketing of products derived from IB pig carcass, including for first time fresh meat. This Quality Standard included two genetic product types in Iberian pork production: Iberian purebred pork and Iberian x Duroc crossbreeding pork. In fact, Iberian x Duroc (50%) is the most common Iberian crossbreeding pig found in the meat market included in NQS. We have studied the main meat quality parameters of tenderloin (*psaos major* muscle) and *serratus ventralis* muscle, which are the most expensive meat cuts for fresh consumption, from those two genetic pig groups. Meat from IB pig showed different characteristics of that from crossbred pigs. However, more differences were observed in tenderloin than in *serratus ventralis* muscle. Tenderloin from crossbred pigs had lower water holding capacity, intramuscular fat and PUFA contents, and higher SFA content than tenderloin from Iberian purebred pigs. *Serratus ventralis* muscle from crossbred pigs had lower myoglobin content than *serratus ventralis* from IB purebred pigs, but no important differences were observed in other meat quality parameters.

**Keywords.** Meat quality – National Quality Standard – Iberian Pig.

## Comparaison de la qualité de la viande entre la race Ibérique pure et croisée avec Duroc

**Résumé.** Le porc Ibérique (IB) est la race porcine de type méditerranéen la plus importante, autant pour ses effectifs que pour son importance économique. La majorité des produits ibériques sont consommés sous forme de produits secs. Toutefois, la consommation de viande fraîche a augmenté récemment. En raison de la demande accrue de viande pour la consommation, en 2007 a été publiée une nouvelle norme de qualité pour réguler la production et le commerce des produits ibériques, incluant pour la première fois la viande fraîche. Dans cette norme de qualité existent deux types génétiques : produits de porcs purs Ibériques et produits de porcs croisés entre Ibérique et Duroc ; le produit croisé à 50% est le croisement le plus fréquent concernant la commercialisation, inclus dans la norme de qualité. Nous avons étudié les principales caractéristiques de qualité de la viande de l'ailou (Psoas major) et du muscle Serratus ventralis, qui sont les viandes les plus chères, pour ces deux types génétiques. La viande de pur Ibérique a des caractéristiques différentes de celle des porcs croisés. Cependant, il y a plus de différences dans l'ailou que dans le muscle Serratus ventralis. L'ailou des porcs croisés a une moindre capacité de rétention d'eau CRA, une moindre infiltration de graisse intramusculaire, ainsi que des teneurs plus faibles en graisse et acides gras polyinsaturés PUFA, et supérieures en acides gras saturés SFA par rapport à la viande de pur Ibérique. Le muscle Serratus ventralis de porcs croisés contient moins de myoglobine que la viande provenant d'Ibérique pur, mais aucune différence significative n'a été trouvée pour les autres paramètres de qualité de la viande.

**Mots-clés.** Qualité de la viande – Normes de qualité – Porc ibérique.



## I – Introduction

The Iberian pig breed is the most important Mediterranean swine type, both in population size and economic importance. Traditionally, most of Iberian pork is destined to become dry-cured products. However, the consumption of several fresh meat cuts has recently increased, reaching high prices. Due to the increasing demand of fresh meat cuts, in 2007, a new National Quality Standard (NQS) for Iberian products was published in Spain to regulate the production and marketing of products derived from Iberian pig carcass, including for first time fresh meat, instead of dry-cured products only (dry-cured ham, dry-cured shoulder and dry-cured loin) (RD 1469/2007, of November 2). From the point of view of racial origin, that NQS included two genetic product types into Iberian pork production: Iberian Purebred pork and Iberian x Duroc crossbreeding pork. In fact, Iberian x Duroc (50 %) is the most common Iberian pork production found in the meat market included into the NQS. However, into NQS in adapting to Council Directive 88/661/EEC of December 19, the label "Iberian Purebred Pork" is restricted only to the Iberian products from livestock registered in the Studbook, which is a fraction of the total breed. As a result, products from Iberian pigs not registered in the Studbook are sold along with products from Iberian x Duroc crossbreeding, which are labelled as "Iberian" into NQS, without a commercial differentiation between these in the market. This creates a permanent discussion about the appropriateness of the explicit commercial differentiation of products from Iberian x Duroc crossbreeding.

We have studied the main meat quality parameters of tenderloin (*psoas major* muscle) and *serratus ventralis* muscle from those two genetic groups (Iberian Pig and Iberian x Duroc crossbreeding) labelled as "Iberian" into the NQS, due to the importance of these muscles in the Spanish fresh meat market, being actually the most expensive meat cuts of Iberian pork for fresh consumption.

The aim of this study was to compare the meat quality parameters between Iberian and Iberian x Duroc crossbreeding pork, currently undifferentiated in the Spanish market.

## II – Materials and methods

### 1. Preliminary genetic analysis

In order to verify the racial origin of the selected pigs (Iberian and Iberian x Duroc pigs), a preliminary genetic study was conducted. This study was carried out on 25 animals, 15 assigned to Iberian Pig breed and 10 assigned to the Iberian x Duroc crossbreeding. All the animals were genotyped for several SNP of the MC1R and IGF2 genes using RT-PCR. These genes, following the methodology developed by the MERAGEM research group, can be used to differentiate Iberian pig breed from other breeds such as Duroc breed and Iberian x Duroc crossbreeding. In fact, this methodology is officially used by AECERIBER to ensure racial purity of the boars and sows registered in the Studbook, through an agreement with MERAGEM research group.

### 2. Animal management

Twenty-five castrated male pigs were used for this meat quality study, 15 from Iberian breed and 10 from Iberian x Duroc crossbreeding. All pigs were reared under regular semi-extensive management. Iberian and crossbred piglets were weaned at 49-56 days and fattening started at an age of about 12-13 weeks.

### 3. Sampling, carcass and meat quality analysis

The pigs were slaughtered when they reached the commercial live weight (150-170 kg; 10-12 months of age), and they were stunned according to the specifications outlined in the Spanish legislation. All measures (pH, weight percentages of moisture, ash, fat and protein, water

holding capacity, Warner Bratzler shear force, muscle brightness and colour indices, concentration of myoglobin, and total fatty acids) were determined using standard methods.

#### 4. Statistical analysis

Meat quality data were analyzed with the Statistica 7.0 for Windows statistical package (StatSoft, 2007). A general lineal model was used to determinate the significance of the effects of the different racial origins on meat quality traits. Carcass weight was fitted as a lineal covariate.

### III – Results and discussion

#### 1. Genetic analysis

Regarding the genotypes obtained from the study of the DNA molecular markers for the two analyzed genes (MC1R and IGF2) in the sampled animals, we must note that all animals preliminarily assigned to the Iberian pig breed showed the expected characteristic genotypes. On the other hand, all animals preliminarily considered Iberian x Duroc crossbreeding at 50 %, showed heterozygous genotypes (with a characteristic allele from Iberian Pig Breed and the other allele from Duroc Breed, for the two analyzed genes). Therefore, these results confirm a correct sampling of the selected animals.

#### 2. Meat quality analysis

No differences between genetic groups ( $P > 0.05$ ) were observed for pH 24 h in analyzed carcass. The values ranged from 6.09 to 6.14 and from 6.07 to 6.11, in tenderloins and *serratus ventralis* muscles, respectively. These values were similar to those observed for tenderloin by Morcuende *et al.* (2007) and for *semimembranosus* muscle by Serrano *et al.* (2008).

Chemical composition and texture traits of tenderloins and *serratus ventralis* muscles from Iberian and crossbred pigs (Iberian x Duroc crossbreeding) are shown in Table 1. The shown values are similar to those reported by other authors for *longissimus dorsi* muscle of Iberian pigs (Estévez *et al.*, 2003; Cava *et al.*, 2004). Significant differences between Iberian and crossbred pigs were observed for protein, intramuscular fat, moisture and ash contents, as well as for water holding capacity in tenderloins. However, differences between the two analyzed genetic groups were observed only for protein content in *serratus ventralis* muscle. No differences ( $P > 0.05$ ) between Iberian and crossbred pigs were found for shear force in neither of the two studied muscles.

**Table 1. Proximate composition and texture traits of tenderloins and *serratus ventralis* muscles from Iberian and crossbred Iberian pigs**

	Tenderloin			<i>Serratus ventralis</i>		
	Iberian	Crossbreed	Sig.	Iberian	Crossbreed	Sig.
Protein (%)	21.95 ± 0.366	19.84 ± 0.457	**	19.54 ± 0.508	17.98 ± 0.635	*
IMF (%)	4.41 ± 0.328	3.93 ± 0.446	*	4.19 ± 0.341	4.47 ± 0.401	ns
Moisture (%)	72.95 ± 0.354	75.19 ± 0.442	**	71.40 ± 0.390	71.37 ± 0.488	ns
Ash (%)	1.33 ± 0.037	1.06 ± 0.046	***	1.22 ± 0.032	1.18 ± 0.040	ns
WHC (%)	16.23 ± 0.728	12.24 ± 0.909	**	11.95 ± 0.777	12.90 ± 0.971	ns
WBSF (kg/cm <sup>2</sup> )	4.51 ± 0.183	5.02 ± 0.229	ns	5.51 ± 0.358	4.76 ± 0.447	ns

IMF: intramuscular fat; WHC: water holding capacity; WBSF: Warner-Bratzler shear force.

Sig.: significant differences (ns:  $P \geq 0.05$ ; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ).



Physicochemical colour parameters (brightness, colour indices and myoglobin content) between Iberian and crossbred pigs of tenderloin and *serratus ventralis* muscle are shown in Table 2.

**Table 2. Physicochemical colour parameters of tenderloins and *serratus ventralis* muscles from Iberian and crossbred Iberian pigs**

	Tenderloin			<i>Serratus ventralis</i>		
	Iberian	Crossbred	Sig.	Iberian	Crossbred	Sig.
<i>L</i> *	30.91 ± 0.618	38.39 ± 0.772	***	33.64 ± 0.460	38.62 ± 0.575	***
<i>a</i> *	14.48 ± 0.481	10.29 ± 0.601	***	14.18 ± 0.378	10.37 ± 0.473	***
<i>b</i> *	12.82 ± 0.296	6.70 ± 0.370	***	12.67 ± 0.385	8.64 ± 0.481	***
Mb (mg/100g)	4.92 ± 0.152	3.25 ± 0.190	***	5.29 ± 0.126	3.84 ± 0.158	***

*L*\*, *a*\* and *b*\*: muscle brightness and colour indices (CIE, 1976); Mb: myoglobin.

Sig.: significant differences (\*\*\*:  $P < 0.001$ ).

The most significant differences between Iberian and crossbred pigs were found in these meat quality parameters. The redness value (*a*\*), as well as the myoglobin content, were higher ( $P < 0.001$ ) in muscles from Iberian pigs, while the brightness (*L*\*) was lower ( $P < 0.001$ ) in muscles from Iberian than in muscles from crossbred pigs. These data are in accordance with previous studies (Fernández *et al.*, 1999; Estévez, *et al.*, 2003). Iberian pigs have been reported to have higher concentration of oxidative fibres in muscles than less rustic breeds such as Duroc (Serrano *et al.*, 2008). Since muscles from Iberian pigs have more heme pigments (and therefore more iron) than muscles from crossbred pigs, muscles from Iberian pigs have higher redness value and less brightness than muscles from crossbred pigs. These result in an intense dark red colour.

Fat quality parameters, such as intramuscular fat content, marbling and lipid composition, are the main factors affecting consumer acceptability of Iberian fresh meat (Ruiz *et al.*, 2002). Moreover, the study of lipid composition of fat in fresh meat has acquired much importance in recent years mainly due to its correlation with cardiovascular diseases.

Relative percentages of individual fatty acids in intramuscular fat of tenderloins and *serratus ventralis* muscles (results not shown) revealed that the oleic acid (C18:1 n-9) was the most common fatty acid for both analyzed muscles in all sampled animals, followed by the palmitic (C16:0), stearic (C18:0) and linoleic (C18:2 n-6) acids. In general, no significant differences ( $P \geq 0.05$ ) between Iberian and crossbred pigs were found for those majoritary fatty acids in each analyzed muscle, with the exception of palmitic acid ( $P < 0.001$ ) in tenderloin. However, significant differences were found between Iberian and crossbred pigs for smaller fatty acids in intramuscular fat from both analyzed muscles, which may have nutritional and organoleptic influences.

Due to the high variability in the results, no significant differences ( $P \geq 0.05$ ) were found between Iberian and crossbred pigs for the fatty acid main indices in *serratus ventralis* muscle (Table 3). However, compared to crossbred pigs, Iberian pigs had higher PUFA ( $P < 0.05$ ), PUFA/SFA ( $P < 0.05$ ) and UFA/SFA ( $P < 0.01$ ) levels, and lower SFA ( $P < 0.01$ ) values of the intramuscular fat of the tenderloins. In fact, PUFA/SFA ratio of intramuscular fat of tenderloins from Iberian pigs was the only one above 0.4, the international health recommendation (Department of Health, 1994).

**Table 3. Composition of fatty acid indices of intramuscular fat of tenderloins and *serratus ventralis* muscles from Iberian and crossbred Iberian pigs**

	Tenderloin			<i>Serratus ventralis</i>		
	Iberian	Crossbreed	Sig.	Iberian	Crossbreed	Sig.
SFA	38.24 ± 0.550	41.07 ± 0.687	**	38.35 ± 0.476	38.83 ± 0.594	ns
MUFA	43.38 ± 0.679	44.55 ± 0.849	ns	50.14 ± 0.542	49.28 ± 0.677	ns
PUFA	18.38 ± 0.929	14.38 ± 1.161	*	11.50 ± 0.501	11.89 ± 0.625	ns
PUFA/SFA	0.48 ± 0.029	0.35 ± 0.036	*	0.30 ± 0.015	0.31 ± 0.018	ns
UFA/SFA	1.62 ± 0.036	1.44 ± 0.045	**	1.61 ± 0.033	1.58 ± 0.041	ns
n-6/n-3	10.15 ± 0.458	10.26 ± 0.573	ns	8.10 ± 0.447	8.58 ± 0.559	ns

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; n-6/n-3: omega-6 and omega-3 fatty acid ratios (Juárez, 2009). Sig.: significant differences (ns:  $P \geq 0.05$ ; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ).

## IV – Conclusions

Tenderloins from Iberian pigs have different characteristics from that of crossbred pigs, commonly found in the Spanish meat market of Iberian products. These significant differences between Iberian and crossbred pigs in tenderloins would support a better labelling that explicitly differentiate the products from the two genetic groups. However, no meat quality differences between Iberian and crossbred pigs were found in *serratus ventralis* muscles, due to the heterogeneous characteristics and the different metabolism of these muscles compared with the tenderloins. Therefore, according to physicochemical meat quality parameters from Iberian and crossbred pig products, it appears that differences affect certain meat cuts and not the complete carcass. It would be interesting to carry out a study of the complete carcass on a higher number of animals to obtain reliable conclusions.

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# Study of shelf life of liver pâté elaborated from Celta pig breed

J.M. Lorenzo\*,<sup>1</sup>, M.C. García-Fontán\*, G. García\*, P. Valledor\*\* and D. Franco\*

\*Meat Technology Centre of Galicia, Ourense (Spain)

\*\*Porco Celta Fonsagrada, S.L., Lugo (Spain)

<sup>1</sup>Corresponding author e-mail: jmlorenzo@ceteca.net

**Abstract.** In the present work, microbial spoilage, lipid oxidation, increase of non-heme iron content and colour changes occurring during refrigerated storage (75 days/4°C) of liver pâtés from Celta pigs were studied. Psychrotrophs and TVC increased during storage reached final values of 6.64 and 7.69 log cfu/g, respectively. *Brochothrix thermosphacta* was detected after 46 days of storage. During refrigerated storage L\* values were maintained in a range of 63.8-65.4, whereas redness value decrease during the first point of sampling and after maintained a constant value close to 2.45. On the contrary yellowness value increased in the first 14 storage day, reached a value of 15.3, during the rest of storage period this value decrease until 14.4. Fe-heme content decrease during shelf life period from 17.30 to 13.5 mg Fe-heme/kg liver pâté, whereas Fe-total content presented a inverse relationship, because increase from 110 to 223 mg Fe-total/kg liver pâté. However, this high amount of Fe-total did not seem to be related to oxidative process, because even thought TBAR'S values increase during the firsts 60 days of display period, decreasing in the last sampling point, reaching a final values of 0.015 mg malonaldehyde/kg of liver pâté, this final value was indicate that liver pâté did not undergo important lipid oxidation.

**Keywords.** Pâté – Celta pig – Refrigeration – Oxidation stability – Non-heme iron.

## *Étude de la vie utile du pâté élaboré à partir du porc Celta*

**Résumé.** Dans ce travail, l'étude de divers paramètres a été menée sur du pâté élaboré à partir de porc Celta: il s'agit de la contamination microbienne, l'oxydation lipidique, l'augmentation du fer non héminique et les changements de coloration produits au cours de la réfrigération (75 jours à 4°C). Les comptages de psychrotrophes et TVC ont augmenté de manière significative ( $P < 0,001$ ) au cours de l'étape de conservation atteignant respectivement des valeurs de 6,64 et 7,69 log ufc/g. *Brochothrix thermosphacta* fut détecté après 46 jours de conservation. Au cours de la conservation à froid, les valeurs de luminosité sont demeurées constantes à 63,8-65,4, tandis que les indices de rouge ont diminué durant le premier point d'échantillonnage, pour se maintenir constants par la suite à une valeur proche de 2,45. En revanche, l'indice de jaune a augmenté au cours des 14 premiers jours de conservation, atteignant une valeur de 15,3 et régressant à un niveau de 14,4 au cours du restant de la période de conservation. La teneur en fer héminique a diminué au cours de la période de vie utile passant de 17,3 à 13,5 mg de fer haem/kg de pâté, tandis que la teneur en fer total a adopté un comportement inverse puisqu'elle a augmenté de 110 à 223 mg Fe-total/kg pâté. Dans tous les cas, la présence de cette quantité de fer total ne semble pas avoir d'influence sur le processus d'oxydation lipidique, car bien que les valeurs de TBAR's aient augmenté au cours de la période de conservation, atteignant 0,015 mg de malonaldehyde/kg de pâté, elles ne l'ont pas fait de manière assez significative pour pouvoir conclure à une importante oxydation lipidique.

**Mots-clés.** Pâté – Porc Celta – Réfrigération – Stabilité par oxydation – Couleur – Fer non héminique.

## I – Introduction

Apart from microbial spoilage, lipid oxidation is the major factor reducing quality and acceptability of meat and fat products (Morrissey et al., 1998). Lipid oxidation is a complex process whereby polyunsaturated fatty acids are degraded via formation of free radicals, causing flavour, texture, colour and nutritional deterioration of foodstuffs (Gray, 1978). Non-

heme iron (NHI) is considered the most important oxidation promoter in meat systems and, therefore, knowledge of the proportions of the chemical forms of iron is of great importance (Kanner *et al.*, 1991). An increase in the amount of NHI as a result of thermal processes on meat systems has been shown (Lombardi-Boccia, *et al.*, 2002). Miller *et al.* (1994), suggested cooking is not as important as the subsequent refrigerated storage of cooked meats for the release of NHI from myoglobin. The increase of NHI in meats and fish is considered to be a reflection of the decrease of heme iron (HI) as a consequence of the breakdown of the heme molecule during cooking or storage (Gómez-Basauri and Regenstein, 1992a; Gómez-Basauri and Regenstein, 1992b) and this has been linked to the oxidative deterioration of the porphyrin ring of myoglobin (Schricker and Miller, 1983).

The colour of meat products is another important quality attribute that influences consumer acceptance, and a brown-gray colour is preferred for cooked products (Cornforth, 1994). Colour changes in cooked products during refrigerated storage have been linked to oxidation phenomena, and several factors such as the characteristics and amount of fat, the packaging method and the presence of antioxidants have been reported as being influential (Jo and Ahn, 1999).

Liver pâté is a traditional product for which there has been an increasing demand by European consumers in the last 15 years (Rosmini *et al.*, 1996). Liver pâtés contain high amounts of fat and iron, and therefore, oxidative deterioration of liver pâtés during refrigeration is expected. The differences between pâtés from Celta and white pigs in terms of their fatty acid composition and antioxidative status are expected to influence their susceptibility to oxidative deterioration during refrigerated storage.

The aim of the present work was to study the microbial changes of liver pâtés from Celta pigs during refrigerated storage as assessed by lipid oxidation, increase in the amount of NHI and colour deterioration.

## **II – Materials and methods**

### **1. Experimental design**

For the manufacture of the pâtés (1.5 kg), muscles and adipose tissues from Celta pigs were used. In the recipe the ingredients were as follows per 100 g of product: 27 g liver, 26 g adipose tissue, 26 g muscle, 16 g chestnut, 2 g sodium caseinate, 2 g sodium chloride. The procedure for the manufacture of the pâtés has been described by Estévez *et al.* (2004). Liver pâtés were packed in glass containers prior to thermal treatment (80°C/30'). After the containers were allowed to cool at room temperature, they were stored in the dark at 4°C for 75 days from the day of the manufacture (day 0). Liver pâtés were analysed at days 0, 14, 48, 60 and 75 for lipid oxidation, concentration of NHI, instrumental colour and microbial counts. After each of the refrigeration stages, instrumental colour was measured on the surface of the pâtés and then they were stored at -80°C until the analytical measurements were carried out.

### **2. Analytical methods**

#### **A. Microbial analyses**

In each liver pâtés unit, after aseptically removing and discarding the outer plastic, 10 g of the product were aseptically taken and homogenized with 90 ml of sterile 0.1% peptone water also containing 0.85% NaCl and 1% Tween 80 as emulsifier, at 40-45°C for 2 min in a Masticator blender (IUL Instruments, Barcelona, Spain), thus making a 1/10 dilution. Successive decimal dilutions were prepared by mixing 1 ml of the previous dilution with 9 ml sterile 0.1% peptone water.

Phychrotroph microflora was enumerated in Standard Plate Count Agar (PCA) agar (Merck), after incubation at 7°C for 10 d; *Enterobacteriaceae* in violet red bile dextrose (VRBD) agar

(Merck) after incubation at 37°C for 24 h; *Staphylococcus aureus* in Baird Parker agar (Merck) + Egg Yolk Tellurite Emulsion (Biokar Diagnostics) incubated at 37°C for 24 h and Sulfite reducing clostridia in Perfringens Selective Agar (SPS) agar (Merck) after incubation at 44°C for 24 h. Presence or absence of *Salmonella* was investigated by Enzyme Linked Fluorescent Assay (ELFA), VIDAS®-SLM protocol was carried out according to the procedures recommended by the manufacturer. From each sample and on each culture medium, 1 ml of each dilution was inoculated in duplicate on plates and mixed before solidification. Plates of VRBD agar were covered with a layer of the same culture medium before incubation. After incubation, plates with 30-300 colonies were counted.

### B. Iron analysis

HI was measured according to the methodology of Hornsey (1956) with the next expressions (Merck, 1989): Hematin ( $\mu\text{g}$  hematin/ g muscle) = Absorbance  $\times$  342.44 and HI (mg/100 g meat) = (Hematin  $\times$  8.82)/100. Total iron (TI) content was measured following the methodology proposed by Lorenzo *et al.* (2003) and NHI was calculated as difference between TI and HI content.

### C. Colour measurement

A portable colorimeter (Konica Minolta CR-400 Osaka, Japan) was used to measure liver pâtés colour in the CIELAB space (CIE 1978). (lightness, L\*; redness, a\*; yellowness, b\*)

### D. Measurement of TBARs

Lipid stability was evaluated in the liver pâtés using the method proposed by Vyncke (1975) with the modification that samples were incubated at 96 °C in a forced oven (Memmert UFP600, Germany, Schwabach). Results are expressed as (mg malonaldehyde / kg of fresh meat).

## III – Results and discussion

Figure 1 shows microbial spoilage evolution of TVC, psychrotrophs, LAB, enterobacteriaceae and *Brochothrix thermosphacta*. All microbial populations increased during storage at 4°C, reached final values of 7.69, 6.64, 6.74, 5.54 and 3.6 log cfu/g, respectively. *Brochothrix thermosphacta* was detected after 46 days of storage.

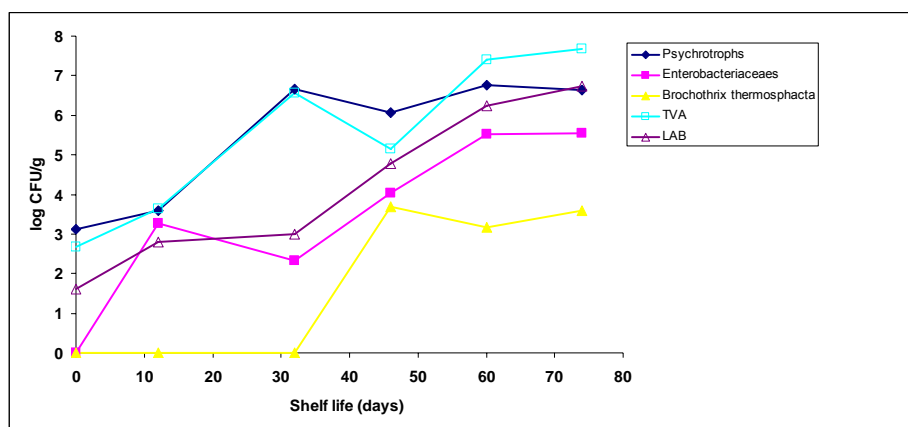


Fig. 1. Evolution of microbial groups (TVC, psychrotrophs, LAB, enterobacteriaceae and *Brochothrix thermosphacta*) on liver pâté from Celta pig under refrigeration storage.

Table 1 shows colour characteristics evolution of liver pâté. During refrigerated storage L\* values was maintained in a range of 63.8-65.4, whereas redness value decrease during the first point of sampling and after maintained a constant value close to 2.45. On the contrary yellowness value increased in the first 14 storage day, reached a value of 15.3, during the rest of storage period this value decrease until 14.4. Compared to pâtés from Iberian pigs of Estévez and Cava (2004) pâtés from our work presented a darker colour (64.52 vs 65.48) with less redness (8.43 vs 2.68) and higher yellowness (12.14 vs 14.39) during 90 days of storage in similar conditions. Obviously differences in the recipe, colour characterises of the meat and adipose tissue, feeding regime and breed explain these differences.

**Table 1. Evolution of colour parameters, ashes, total iron, non-heme and heme iron and lipid oxidation of liver pâté from Celta pig under refrigeration storage**

Colour characteristics	Shelf life (days)				
	0	14	48	60	75
Luminosity (L*)	63.84	63.68	64.15	63.93	65.48
Redness (a*)	3.85	2.60	2.40	2.21	2.68
Yellownes (b*)	12.48	15.33	14.55	14.78	14.39
Ashes-Fe content					
Ashes (%)	1.76	2.04	2.12	2.06	2.22
TI (ppm)	110.8	177.0	201.0	178.1	223.0
NHI (ppm)	93.5	164.7	188.8	163.6	209.5
HI (ppm)	17.3	12.3	12.2	14.4	13.5
Lipid oxidation					
TBARS (mg MDA/kg pâté)	0.149	0.136	0.179	0.318	0.015

NHI content increased during refrigerated storage, from 93 to 209 mg/kg pate from day 0 to day 75, whereas HI content decrease during shelf life period from 17.30 to 13.5 mg /kg liver pâté and TI content presented a inverse relationship, because increase from 110 to 223 mg TI/kg liver pâté (Table 1). Results suggest that some disruption of the porphyrin ring could have occurred during storage that led to the release of iron. For Gómez-Basauri and Regenstein (1992a) and Miller *et al.* (1994) the increase of NHI during refrigeration of meat is a reflection of the degradation of HI. Damage in the porphyrin ring during cooking or storage has been suggested to cause the breakdown of heme molecule and the release of iron from globin (Gómez-Basauri and Regenstein, 1992a). The degradation of HI would reduce the nutritional value of the pâtés in terms of bioavailability of iron, since HI is more available than NHI (Hunt and Roughead, 2000).

However, this high amount of TI did not seem to be related to lipid oxidative process, because TBARS of liver pâtés increasing during 60 days of refrigerated storage, decreasing in the last 15 days of shelf life to a close value to 0. This final value indicates that liver pâté did not undergo important lipid oxidation or also it could be explained by disappearance of primary oxidation products. Secondary peroxidation products such peroxides, could be present in the liver pâté but was not measured.

## IV – Conclusions

According to this study, lipid oxidation, and the increase of NHI during refrigerated storage of liver pâtés could not be closely related. Colour changes seem not to be linked to oxidative processes and microbial counts. This previous results represent a starting point for the promotion elaborated meat products from this endangered pig breed.

## Acknowledgements

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# Manufactured of "chanfaina", from Celta pig breed. Study of shelf life vacuum packaging

J.M. Lorenzo<sup>\*1</sup>, M.C. García-Fontán<sup>\*</sup>, E., Rodríguez<sup>\*</sup>, P. Villedor<sup>\*\*</sup> and D. Franco<sup>\*</sup>

<sup>\*</sup>Meat Technology Centre of Galicia, San Cibrao das Viñas, 32900 Ourense (Spain)

<sup>\*\*</sup>Porco Celta Fonsagrada, S.L., Lugo (Spain)

<sup>1</sup>Corresponding author, e-mail: jmlorenzo@ceteca.net

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**Abstract.** *Chanfaina* is a dry-fermented sausage abundantly produced and consumed in Galicia (NW Spain) elaborated from Celta pig breed. Traditionally, this product is distributed and sold without packaging in the local market. To extend its shelf life and expand the market, some manufacturers have begun to implement vacuum packaging. Total viable count (TVC), psychrotrophs, lactic acid bacteria (LAB), pseudomonas, enterobacteria, moulds and yeasts, *Staphylococcus aureus*, sulfite reducing clostridia, pH and TBAR'S value were analysed during storage at 4 °C. Sulfite-reducing clostridia and enterobacteria were not detected in any sample. Psychrotrophs were the predominant microorganisms reaching a population higher than 8.9 log cfu/g after 60 days of storage. At the same time, LAB becomes the predominant species during storage. The rest of the microbiota did not grow during storage. On the other hand, a lightly increase in pH was noticed during storage. Samples stored in vacuum package remained stable during the whole display period and no significant differences ( $P < 0.05$ ) were observed in TBAR'S values.

**Keywords:** *Chanfaina* – Celta pig breed – Vacuum packaging – Dry-fermented sausages.

## Élaboration de "chanfaina" à partir de porc Celta. Étude de la vie utile après emballage sous vide

**Résumé.** La *Chanfaina* est une saucisse sèche produite à partir de porc Celta en Galice (nord-ouest de l'Espagne) où elle y est hautement consommée. Ce produit est traditionnellement distribué et vendu sans emballage dans les marchés locaux. Dans le but d'accroître sa vie utile et d'élargir son rayon de vente, certains fabricants ont commencé à mettre en œuvre un emballage sous vide. Les comptages de mésophiles aérobies totaux, psychrotrophes, bactéries acido-lactiques, pseudomonas, entérobactéries, moisissures et levures, *Staphylococcus aureus*, clostridia sulfite-réducteurs, pH et TBAR'S, ont été déterminés durant la conservation à 4°C. Aucune trace de clostridia sulfite-réducteurs et d'entérobactéries n'a pu être observée au sein des échantillons. Les micro-organismes prédominants furent les psychrotrophes, avec des valeurs atteignant 8,8 log ufc/g après 60 jours de conservation. Dans le même temps, LAB est apparue comme l'espèce majeure formée durant la conservation. Une légère augmentation du pH fut également observée. Les échantillons conservés sous vide sont demeurés stables durant toute l'étape de conservation et aucune variation significative des valeurs de TBAR'S ( $P < 0,05$ ) n'a pu être mise en évidence.

**Mots-clés.** *Chanfaina* – Porc Celta – Emballage sous vide – Saucisse sèche.

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## I – Introduction

The Celta was the typical breed of pig raised on farms in Galicia (northwest Spain) until the middle of the 20th century, at which time it suffered an important recession in members due to the introduction of improved breeds and their crossbreds. This breed is highly appreciated by consumers because of the succulent meat that results from the profuse infiltration of fat into the lean meat (Franco *et al.*, 2006).

"Chanfaina" is a fermented and dried-ripened sausage abundantly produced from raw Celta pork in Galicia (NW of Spain). For the manufacture of chanfaina, low-quality pork (lean, bacon, lung, heart, jowls) is minced and salt, sweet and spicy paprika, garlic and onion are added. The

resulting mass is left standing for at least 24 h and then, it is stuffed into pork rectum tripe in units of 20-25 cm length. After stuffing, it undergoes a smoking-heating process for 8-10 days and then a drying-ripening process for 15 days.

Oxidation of the lipid fraction is one of the major causes of quality decrease during the shelf-life of sausages. The extent of the overall lipid degradation process may be affected by various factors related to: (i) the storage conditions (García-Esteban *et al.*, 2004; Papadima, and Bloukas, 1999; Zanardi *et al.*, 2002); (ii) the processing technology (Gray *et al.*, 1996; Salgado *et al.*, 2005); (iii) the additives used in the dough formulation (Kanner, 1994; Skibsted, 1992); and (iv) the polyunsaturated fatty acids content of the lipid fraction.

The aim of this study was to evaluate the microbial changes of chanfaina from Celta pigs during refrigerated storage as assessed by lipid oxidation.

## II – Materials and methods

### 1. Samples

Twenty units of chanfaina were manufactured by Porco Celta Fonsagrada, SL following the procedure described in introduction section. Samples were vacuum packed prior to thermal treatment (100°C/15 min). After the samples were allowed to cool at room temperature, they were stored in the dark at 4°C for 180 days.

### 2. Microbial analyses

In each chanfaina unit, after aseptically removing and discarding the outer plastic, 10 g of the product were aseptically taken and homogenized with 90 ml of sterile 0.1% peptone water also containing 0.85% NaCl and 1% Tween 80 as emulsifier, at 40-45 °C for 2 min in a Masticator blender (IUL Instruments, Barcelona, Spain), thus making a 1/10 dilution. Successive decimal dilutions were prepared by mixing 1 ml of the previous dilution with 9 ml sterile 0.1% peptone water.

Phychrotroph microflora was enumerated in Standard Plate Count Agar (PCA) agar (Merck), after incubation at 7°C for 10 d; *Enterobacteriaceae* in violet red bile dextrose (VRBD) agar (Merck) after incubation at 37°C for 24 h; *Staphylococcus aureus* in Baird Parker agar (Merck) + Egg Yolk Tellurite Emulsion (Biokar Diagnostics) incubated at 37°C for 24 h and Sulfite reducing clostridia in Perfringens Selective Agar (SPS) agar (Merck) after incubation at 44°C for 24 h. Presence or absence of *Salmonella* was investigated by Enzyme Linked Fluorescent Assay (ELFA), VIDAS®-SLM protocol was carried out according to the procedures recommended by the manufacturer. From each sample and on each culture medium, 1 ml of each dilution was inoculated in duplicate on plates and mixed before solidification. Plates of VRBD agar were covered with a layer of the same culture medium before incubation. After incubation, plates with 30-300 colonies were counted.

### 3. pH measurement

pH was measured by blending 25 g of product with 225 ml of distilled water for 2 min. A digital pH-meter (Hanna HI 99163, Spain) was used for the measurement.

### 4. Measurement of TBARs

Lipid stability was evaluated in the steaks using a small 2 g portion. Lipid oxidation, measured by aldehydes generated in the process of polyunsaturated fatty acid oxidation, was determined by measuring 2-thiobarbituric acid reactive substances (TBARs) using the method proposed by Vyncke (1975) with the modification that samples were incubated at 96°C in a forced oven. Results are expressed as (mg malonaldehyde / kg of fresh meat).

### III – Results and discussion

#### 1. Microbial characteristics

TVC, psychrotrophs, LAB, moulds and yeasts and pseudomonads are showed in Fig 1. Sulfite-reducing clostridia and enterobacteria were not detected in any of the samples analysed. The aerobic mesophilic bacteria, psychrotrophs and lactic acid bacteria counts were over 6.4, 7.8 and 8.1 log cfu/g, respectively immediately after packaging, while pseudomonads and moulds and yeasts were below over 3 log cfu/g.

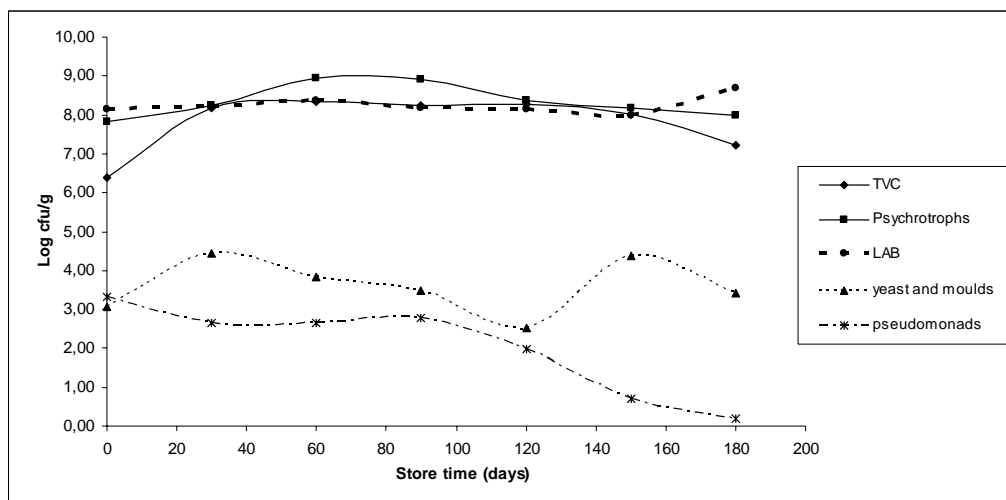


Fig. 1. Evolution of TVC, psychrotrophs, LAB, moulds and yeasts and pseudomonads in chanfaina from Celta pigs under refrigerated storage. Means with different letter in the figure show significant differences ( $P < 0.05$ ; Duncan test) for the effect store time.

#### 2. pH changes

Initial pH values were below 5 for all samples analysed (Fig. 2). A non-significant increase ( $P > 0.05$ ) was observed during all the period of storage. However, it can show a lightly increased in pH values under refrigerated storage reached a mean final pH value of 5.18.

#### 3. Lipid stability

The level of lipid oxidation of chanfaina was estimated on base of the amount of 2-thiobarbituric acid reactive substances (TBAR's values) (Fig. 2). All samples started with low values of about 0.4 mg MDA/kg in fresh meat. Exclusion of the oxygen content in the vacuum package limited oxidation and thus resulted in lower TBAR's values for these *chanfaina* samples. Samples stored in vacuum package remained stable during the whole display period and no significant differences ( $P < 0.05$ ) were observed. This outcome was not surprising, as the meat storage conditions during the display period in a vacuum environment protect the meat from oxygen.

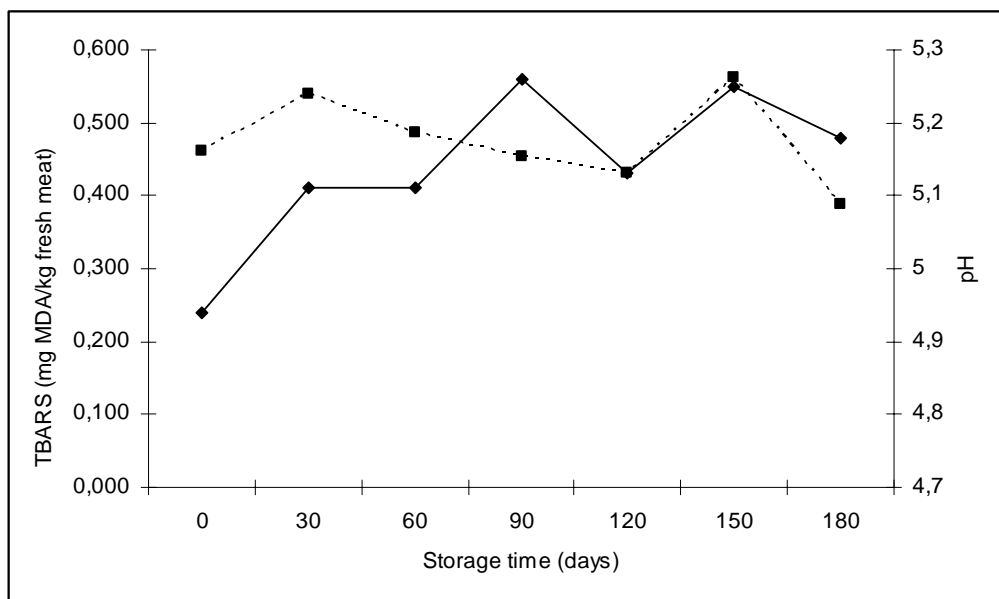


Fig. 2. Evolution of pH and TBAR'S values in chanfaina from Celta pigs under refrigerated storage.

## Acknowledgements

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# Manufactured of "Mestura cocida" from Celta pig breed. Study of shelf life vacuum packaging

J.M. Lorenzo<sup>\*1</sup>, M.C. García-Fontán\*, L. Purriños\*, P. Valledor\*\* and D. Franco\*

\*Meat Technology Centre of Galicia, Ourense (Spain)

\*\*Porco Celta Fonsagrada, S.L., Lugo (Spain)

<sup>1</sup>Corresponding author e-mail: jmlorenzo@ceteca.net

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**Abstract.** "Mestura cocida" is a typical product manufacture and consumed in Galicia (NW Spain) elaborated with dry-cured "lacón", "Galician chorizo" and salted head from Celta pig breed. The elaboration process is strictly controlled to achieve a satisfactory shelf life, so a high quality finally-consumed product is obtained. Physical, chemical, enzymic and microbiological test are performed to ensure this quality. Psychrotrophs, Enterobacteriaceae, *Staphylococcus aureus*, *Salmonella*, sulfite reducing clostridia and TBAR'S value were analysed during storage at 4°C. Psychrotrophs and Enterobacteriaceae showed a increase during storage observed final values 7.88 and 5.12 log cfu/g, respectively after 90 days of storage. *Staphylococcus aureus* and *Salmonella* have not been found in any samples. On the other hand, TBAR'S values increased) during the whole display period reaching a final values of 11.8 mg malonaldehyde / kg of "Mestura cocida".

**Keywords.** Mestura cocida – Celta pig breed – Vacuum packaging.

**Élaboration de "mestura cocida" à partir de porc Celta. Étude de la vie utile après emballage sous vide**

**Résumé.** La "Mestura cocida" est un produit typique produit et consommé en Galice (nord-ouest de l'Espagne) qui est fabriqué à partir de "lacón", de "chorizo galicien" et de tête de porc Celta salée. Afin d'établir la vie utile d'une denrée alimentaire et de s'assurer de sa qualité finale, il existe diverses mesures de type physique, chimique, enzymatique et microbiologique. Les comptages de psychrotrophes, entérobactéries, *Staphylococcus aureus*, *Salmonella*, clostridia sulfite-réducteurs et les valeurs de TBAR'S, ont été déterminés durant la conservation à 4 °C. Après 90 jours d'exposition, une augmentation de psychrotrophes et d'entérobactéries fut observée durant le temps de vie utile, atteignant respectivement des valeurs de 7,88 et 5,12 log ufc/g. Aucune trace de *Staphylococcus aureus* et de *Salmonella* n'a pu être détectée au sein des échantillons. Par ailleurs, une croissance importante des valeurs de TBAR'S fut également observée au cours de la période de conservation, pour atteindre des valeurs finales de 11,8 malonaldéhyde/kg de mestura cocida.

**Mots-clés.** Mestura cocida – Porc Celta – Emballage sous vide.

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## I – Introduction

"Mestura cocida" is a typical product manufacture and consumed in Galicia (Nw Spain) elaborated mainly with dry-cured "lacón", "Galician chorizo" and salted head from Celta pig breed. Dry cured lacón and Galician chorizo are traditional raw-cured meat products made in the northwest of Spain. Dry-cured lacón is elaborated from the foreleg of the pig, using similar manufacturing processes to those used in the production of dry-cured ham, Galician chorizo can be defined as the mixture of minced pork and pork fat, addition of salt, paprika, other spices and additives, mixed and inserted into natural or artificial casings, which undergo a drying-ripening process. The Celta was the typical breed of pig raised on farms in Galicia until the middle of the 20<sup>th</sup> century. This breed is highly appreciated by consumers because of the succulent meat that results from the profuse infiltration of fat into the lean meat.

The elaboration process of this product is controlled to achieve a satisfactory shelf life, so a high quality finally consumed product is obtained. This product is subjected to a pasteurisation process and vacuum-packaging. For this reason these cooked meat products are often post-contaminated because of a packaging and/or slicing step after pasteurisation process. After cooking, the normal flora of the product, is too low to protect the products against the growth of Gram-negative microorganisms (Kotzekidou and Bloukas, 1996). The bacterial flora is gradually selected towards a CO<sub>2</sub>-tolerant but a slowly growing one (Borch *et al.*, 1996). Psychrotrophic lactic acid bacteria are responsible for the spoilage of cooked meat products packed in oxygen-free atmospheres (Debevere, 1989; Borch *et al.*, 1996). Enterobacteriaceae, *Staphylococcus aureus*, *Salmonella* and sulfite reducing clostridia are indicative microorganisms of the hygienic quality of the product and their count can give us an idea from the possible later contamination to the thermal treatment as well as of the effectiveness of the same one.

Oxidation of the lipid fraction is one of the major causes of quality decrease during the shelf life of *mestura cocida*. In particular, the deterioration involves modifications of the organoleptic characteristics, the development of unpleasant odours and tastes and a decrease in the nutritional value of the product due to a lowering of the polyunsaturated fatty acid content, whose beneficial effect on consumers health is well-known (Alexander, 1978; Rose and Connolly, 1999; Berra *et al.*, 2005). Although vacuum packaging can protect meat from contamination and increase the shelf life of the product, the anaerobic conditions may affect the quality. Many studies have revealed a change in the prevailing microflora in vacuum-packed meat products compared to that prevailing before storage (Björkroth *et al.*, 1998; Samelis *et al.*, 2000). This microbiological change could result in some modifications of the sensory properties and it could affect the nutritional value and the chemical compositions of products.

The purpose of the present work was, to assess the quality, during of shelf life, of vacuum-packaging *mestura cocida* elaborated from Celta pig breed. Physical, chemical and microbiological test are performed to ensure this quality.

## II – Material and methods

### 1. Samples

Fifteen units of *mestura cocida* were manufactured by Porco Celta Fonsagrada, SL. For the manufacture of this product muscles and adipose tissues from Celta pigs were used (40% dry-cured "lacón", 40% salted head and 20% "Galician chorizo"). Samples were vacuum packed prior to thermal treatment (80 °C/30 min). After the samples were cooled at room temperature, and stored at 4 °C for 90 days from the day of the manufacture (day 0). "Mestura cocida" samples were analysed at days 0, 30, 45, 60, 75 and 90 for lipid oxidation and microbial counts. In every sample point two units of "mestura cocida" were analyzed.

### 2. Microbiological analysis

In each *mestura cocida* unit, after aseptically removing and discarding the outer plastic, 10 g of the product were aseptically taken and homogenized with 90 ml of sterile 0.1% peptone water also containing 0.85% NaCl and 1% Tween 80 as emulsifier, at 40-45°C for 2 min in a Masticator blender (IUL Instruments, Barcelona, Spain), thus making a 1/10 dilution. Successive decimal dilutions were prepared by mixing 1 ml of the previous dilution with 9 ml sterile 0.1% peptone water.

Psychrotroph microflora was enumerated in Standard Plate Count Agar (PCA) agar (Merck), after incubation at 7°C for 10 d; Enterobacteriaceae in violet red bile dextrose (VRBD) agar (Merck) after incubation at 37°C for 24 h; *Staphylococcus aureus* in Baird Parker agar (Merck) + Egg Yolk Tellurite Emulsion (Biokar Diagnostics) incubated at 37°C for 24 h and Sulfite reducing clostridia in Perfringens Selective Agar (SPS) agar (Merck) after incubation at 44°C for 24 h. Presence or absence of *Salmonella* was investigated by Enzyme Linked Fluorescent Assay

(ELFA), VIDAS®-SLM protocol was carried out according to the procedures recommended by the manufacturer. From each sample and on each culture medium, 1 ml of each dilution was inoculated in duplicate on plates and mixed before solidification. Plates of VRBD agar were covered with a layer of the same culture medium before incubation. After incubation, plates with 30-300 colonies were counted.

### 3. TBAR's determination

Lipid stability was evaluated in the "Mestura cocida" using the method proposed by Vyncke (1975) with the modification that samples were incubated at 96 °C in a forced oven (Memmert UFP600, Germany, Schawabach). Results are expressed as (mg malonaldehyde / kg of mestura cocida).

## III – Results and discussion

Figure 1 shows the evolution of microbial counts in the mestura cocida during the shelf life vacuum packaging. Psychrotrophs bacterias and Enterobacteriaceae showed a increase during storage at 4°C observed final values of 7.88 and 5.12 log cfu/g, respectively after 90 days of storage.

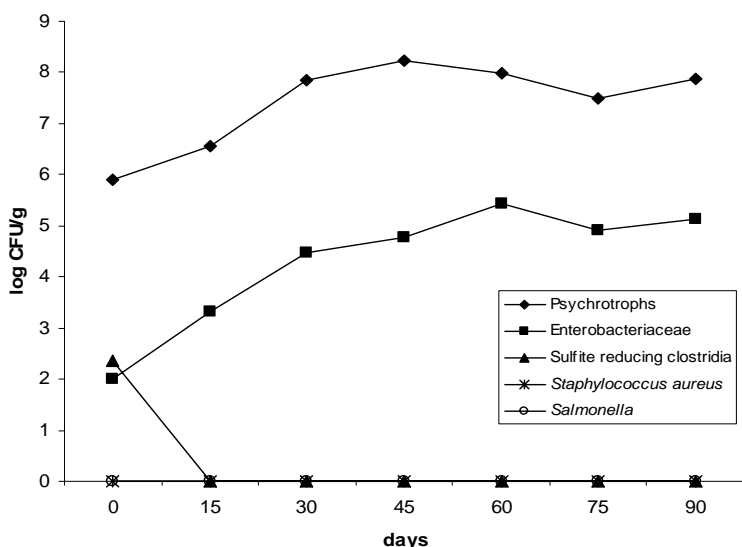


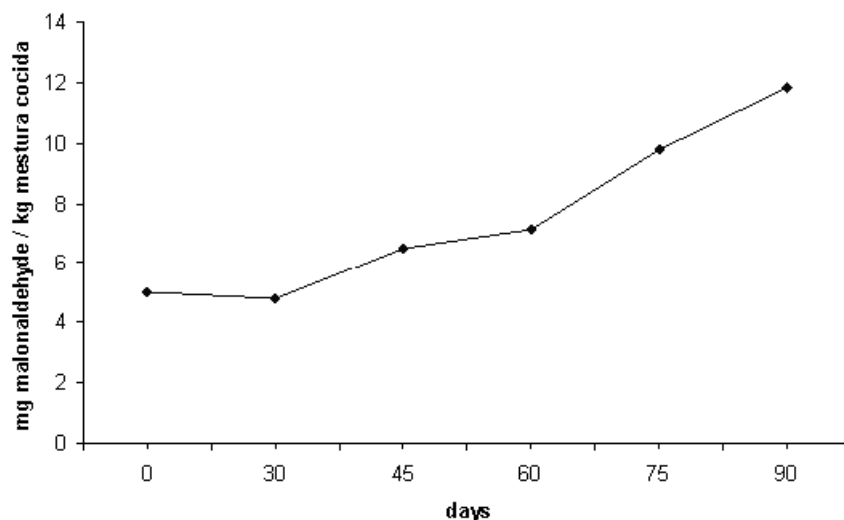
Fig. 1. Changes in log counts of the spoilage microflora in the mestura cocida under vacuum-packaging at 4°C.

In general, mestura cocida samples showed high counts of Enterobacteriaceae and psychrotrophs bacterias. The Enterobacteriaceae populations indicate the hygienic quality of the product, and their presence can be related to contamination of faecal origin and high counts indicate poor hygienic practices or high contamination of the raw materials used in their manufacture.

Sulfite reducing clostridia only was found in the control sample. This fact could be related with the effectiveness of the thermal treatment. *Staphylococcus aureus* and *Salmonella* have not been found in any samples.



Figure 2 shows the development of lipid oxidation in the mestura cocida during the shelf life vacuum packaging. TBAR'S values increased during the whole display period reaching a final values of 11.8 mg malonaldehyde / kg of mestura cocida. Duration of display period affected the overall TBARS formation of the mestura cocida. The amounts of TBARS formed in the course of storage were higher the critical value of 3 mg/kg at which rancidity is detected (Wong *et al.*, 1995).



**Fig. 2.** Changes in TBAR's values in the mestura cocida during shelf life vacuum-packaging at 4 °C.

Vacuum packaging changes the gaseous environment at the sample surface: respiration of microorganisms at the sample surface or the sample itself produces CO<sub>2</sub> and eventually the oxygen concentration within the pack falls below 1% while the CO<sub>2</sub> concentration rises to 20% or more (Eustace, 1981). The compositional changes of gas could have involved the control of oxygen-dependent microorganisms or oxidative degradation of meat in the bag.

## IV – Conclusions

Mestura cocida showed high counts of Enterobacteriaceae and psychrotrophs bacteria. Sulfite reducing clostridia only was found in the control sample. *Staphylococcus aureus* and *Salmonella* have not been found in any samples. The amounts of TBARS formed in the course of storage were higher the critical value of 3 mg/kg at which rancidity is detected.

To extend the shelf life of this product would be advisable: reduction of microbial contamination during production, growth prevention of spoilage bacteria and application of decontamination procedures to product after packaging.

## Acknowledgements

Authors are grateful to FEADER (Project 2008-15) for the financial support. Special thanks to Porco Celta Fonsagrada, S.L. for chanfaina from Celta pig samples supplied for this research.

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# Consumers attitudes to Iberian pork meat

B. Martínez, B. Rubio, M. Vaquero and M.J. Sánchez

Consejería de Agricultura y Ganadería, Junta de Castilla y León, ITACyL  
Estación Tecnológica de la Carne, Guijuelo, Salamanca (Spain)

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**Abstract.** In the last years, Iberian pork consumption has been limited as a result of relations established between fat consumption and cardiovascular diseases. However, recent researches have allowed establishing healthy properties to the intake of these kind of meat, and as a consequence its sale has increased, competing successfully with other meats. The objective of this study was to determine the attitudes of consumers to Iberian pork meat. To assess consumers' behaviour to breed (Iberian and white pig) and anatomical origin of the pieces commercial ("*pluma*", "*presa*", "*secreto*") of Iberian pork meat, discriminant tests were used in order to detect differences. Besides, an affective test was carried out in order to know the consumers preference to feeding of the Iberian pigs ("*bellota*", "*cebo*"). The results obtained in the triangle tests showed that consumers were able to differentiate between Iberian and white pork loin. Also differences were found between different commercial cuts of Iberian pig. However, consumers didn't show preferences regarding feeding of the Iberian pigs.

**Keywords.** Sensory analysis – Meat – Iberian and white pig – Feeding system.

## *Attitudes des consommateurs vis-à-vis de la viande de porc Ibérique*

**Résumé.** Lors des dernières années, la consommation de porc Ibérique a été limitée en raison des relations établies entre la consommation de matières grasses et les maladies cardiovasculaires. Cependant, les récentes recherches ont permis d'attribuer des propriétés santé à la consommation de ces types de viande, ce qui a déterminé un développement de leur vente, concurrençant avantageusement d'autres viandes, grâce à leur qualité nutritive et leurs caractéristiques sensorielles. L'objectif de l'étude était de déterminer les attitudes des consommateurs vis-à-vis de la viande de porc Ibérique. Pour évaluer ce comportement, des tests discriminants ont été réalisés pour détecter des différences dues à la race (porc Ibérique et porc blanc) ou à l'origine anatomique de la pièce ("*pluma*", "*presa*", "*secreto*") et des tests pour déterminer la préférence en ce qui concerne l'alimentation du porc Ibérique ("*bellota*", "*cebo*"). Les résultats obtenus dans le test triangulaire réalisé ont montré que les consommateurs étaient capables de différencier entre échine de porc Ibérique et de porc blanc. Ils ont aussi différencié entre différentes pièces de porc Ibérique. Cependant, ils n'ont montré aucune préférence en fonction de l'alimentation.

**Mots-clés.** Analyse sensorielle – Viande – Porc ibérique et porc blanc – Alimentation.

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## I – Introduction

In the last years, Iberian pork consumption has been limited as a result of relations established between fat consumption and cardiovascular diseases. However, recent researches (García-Rebollo *et al.*, 1998) have allowed establishing healthy properties to the intake of this kind of meat.

Iberian pork comes from genuinely bred Southwest Iberian Peninsula pigs traditionally fattened with acorns and pasture in an extensive production system. Most of Iberian pork is consumed as highly priced cured products. However, the importance of the consumption of fresh meat has recently increased, since consumers have become more concerned about questions such as ethical forms of animal production, animal welfare, traditional production or nutritional and sensory characteristics of the meat.

It is well known that breed, productive system and muscle type influence the fatty acids profiles

and the sensory characteristic of fresh pork meat and meat products. However, the influence of these factors on eating quality of Iberian meat has not been evaluated.

The aim of this study was to determine if the sensory quality of culinary pork meat was affected by breed (Iberian and white pig), retail cuts ("*pluma*", "*presa*", "*secreto*"), and rearing system ("*bellota*", "*cebo*") using different tests with consumers.

## II – Material and methods

### 1. Meat samples

Samples from heavy white pig (loin) were obtained from a regional abattoir (Segovia, Spain) the day after slaughter and samples from Iberian pig (loin, sirloin, "*pluma*", "*presa*", "*secreto*") were obtained from a local abattoir (Guijuelo, Spain) the day of slaughter. All the samples were transferred to Estación Tecnológica de la Carne, where they were vacuum-packed and frozen at -20°C until further analyses.

### 2. Consumers

Consumers tests were performed during the "VI Jornadas del cerdo Ibérico y sus productos" (Salamanca-Spain, 2008). The tasters (n= 85) were mainly farmers, manufacturers and technicians associated with pig meat industry. Before tasting the samples, subjects were asked to specify their age, their gender and their frequency pork meat consumption on the evaluation sheet.

### 3. Analysis

Consumers were only informed that they would evaluate different pork samples.

A triangle test (ISO 4120:2004) was performed on loin (*longissimus lumborum* and *thoracis* muscle), to determine whether there was a difference between samples from Iberian and heavy white pig reared in confinement with a concentrate feed. The triangle test was carried out by the forced-choice option, in which the tasters must choose the sample that, in their opinion, is different. Besides, to complement the triangle test, tasters were asked to indicate their reasons for selecting one particular sample of the three used in the analysis.

A rank order test was carried out between "*pluma*" (rhomboid muscle chest), "*presa*" (serratus, ventral, cervical and thoracic muscles) and "*secreto*" (*latissimus dorsi* muscle) from Iberian pig in order to test the effect of retail cut on the juiciness of the Iberian pork meat.

Finally, a preference analysis between sirloin (*iliopsoas* and *psoas minor* muscle) from Iberian pigs feeding with acorn and grass ("*bellota*") or with concentrates ("*cebo*") was performed by consumers.

### 4. Cooking and serving

Sensory analyses were carried out on samples after about 10 month frozen storage. Vacuum-packed samples were thawed for 24 to 36 h at 4°C. After thawing, the muscles were cut in steaks (1 cm thick) and cooked to a final internal temperature of 72°C using a two-sided electric grill (GV2PSG, Clajosa, Barcelona, Spain).

The samples were immediately served to consumers for their evaluation. For rinsing the mouth between samples, mineral water at room temperature and unsalted toasted bread were served to them.

### III – Results and discussion

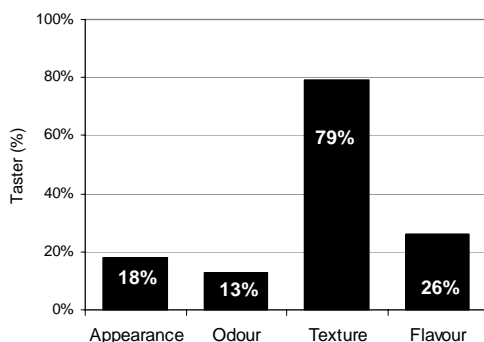
Characteristics of the consumer panel are summarized in Table 1. The tasters were mainly men over 30 years, regular consumers of pork meat.

Results of triangle tests indicated that there was a detectable difference ( $p < 0.01$ ) between the loin from Iberian pigs and heavy white pigs reared under similar conditions.

**Table 1. Demographic characteristics and pork meat consumption frequency (n=85, %).**

Age			Gender		Frequency of consumption			
20-30	31-60	>60	Male	Female	>1/week	1/week	1-3/month	<1/month
19.6	71.4	8.9	82.2	17.8	41.1	21.5	16.0	21.5

According to the tasters, texture characteristics were the main parameter that allowed differentiates the samples (Fig. 1). The tasters pointed out that the Iberian loin was more juicy and tender and showed higher flavour intensity than loin from white pig. These characteristics may be due to variations in intramuscular fat content of meat. Intramuscular fat influence on eating quality of meat resulted in higher saliva excretion during chewing which increase the juiciness of meat (Daszkiewicz *et al.*, 2003). Moreover, intramuscular fat facilitates separation of muscular filament and reduces the cutter force (Wood *et al.*, 1994). As has been pointed out by López-Bote (1998), Iberian breed pig has a high tendency to accumulate fat, whereas the other commercial breeds increases the lean content of the carcass and concomitantly decreases the intramuscular fat content (Ventanas *et al.*, 2007).



**Fig. 1. Sensory differences indicated by the tasters (%) in the triangle test.**

Concerning the results of the rank order test, differences ( $p < 0.05$ ) among the three retail cuts evaluated were detected. Results revealed that subjects considered "*pluma*" as the juiciest. However, there were not significant differences in the juiciness between "*presa*" and "*secreto*". A positive impression of juiciness of meat is related to high fat content (Jaworska *et al.*, 2009). Results from the ranking test may be explained taking into account the inter and intramuscular fat content of the retails cuts evaluated. However, as far as we know, no papers have been devoted to the characterization of this kind of meat.

Regarding the preference tests, differences were not detected ( $p > 0.05$ ). The feeding of Iberian pig with acorn ("*bellota*") or concentrates ("*cebo*") did not suppose any preference of the

consumers. This result would be due to that, today, Iberian pigs reared indoors are fed on monounsaturated fatty acid enriched concentrates (with high oleic acid sunflower oil) for obtaining a similar muscle fatty acid profile to that to pigs fed on acorns.

## IV – Conclusions

On the basis of the results obtained it is possible to confirm that breed and muscle type affect sensory quality of pork meat after heat treatment. However, in this study the feeding on acorns did not contribute to increase consumers' preference of cooked Iberian meat.

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# Influence of genetic type on the characteristics of subcutaneous adipose tissue of pig thighs destined for the PDO production

G. Minelli, A. Cino, M. Comellini, M. C. Ielo, L. A. Volpelli and D. P. Lo Fiego

Dipartimento di Scienze Agrarie e degli Alimenti. Università di Modena-Reggio Emilia (Italy)

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**Abstract.** Italian heavy pig production is founded both on traditional breeds, such as Large White, Landrace and Duroc selected in Italy, and on hybrid pigs coming from specific plans of selection and crossbreeding. The aim of this work was to compare the characteristics of subcutaneous adipose tissue of fresh thighs obtained from pigs belonging to two genetic types and reared in the same farm. Thighs were destined to the production of PDO Italian dry-cured hams. Samples of subcutaneous adipose tissue from 46 thighs of traditional pigs (Italian Landrace x Large White cross) and from 32 thighs of Goland commercial hybrid line, were analysed for water and fat contents, fat iodine value and fatty acid composition. On the whole, hybrids showed, in comparison with traditional pigs, similar lipid content of subcutaneous adipose tissue but different lipid composition: higher levels of polyunsaturated fatty acids (141.1 vs 102.8 mg/g fat;  $P<0.001$ ), lower levels of saturated (334.9 vs 371.0 mg/g fat;  $P<0.001$ ) and mono-unsaturated (420.8 vs 452.5 mg/g fat;  $P<0.001$ ) fatty acids, and greater iodine value (68.9 vs 62.4;  $P<0.001$ ). Females showed lower fat content and higher degree of lipid unsaturation than castrated males.

**Keywords.** Italian heavy pig – Fresh ham – Lipid content – Fatty acid composition.

## *Influence du type génétique sur les caractéristiques du tissu adipeux sous-cutané des cuisses de porc destinées à la production sous AOP*

**Résumé.** La production italienne de porc lourd est fondée à la fois sur des races traditionnelles sélectionnées en Italie (Large White, Duroc et Landrace), et sur des sujets hybrides provenant de plans spécifiques de sélection et de croisement. Le but de ce travail était de comparer les caractéristiques du tissu adipeux sous-cutané des cuisses fraîches obtenues à partir de sujets appartenant à deux différents types génétiques élevés dans la même ferme. Les cuisses étaient destinées à la production de jambon sec italien AOP. Les échantillons de tissu adipeux sous-cutané de 46 cuisses de porc traditionnel (Landrace italien x Large White) et de 32 cuisses de porc hybride commercial Goland, ont été analysés pour la détermination des teneurs en eau et lipides, ainsi que la composition en acides gras et l'indice d'iode. Dans l'ensemble, les hybrides ont montré, en comparaison avec les porcs traditionnels, la même teneur en lipides mais des différences de composition en acides gras: le tissu adipeux sous-cutané des hybrides est plus riche en acides gras polyinsaturés (141,1 vs 102,8 mg/g lipides;  $P<0,001$ ) et corrélativement plus pauvre en acides gras saturés (334,9 vs 371,0 mg/g lipides;  $P<0,001$ ) et monoinsaturés (420,8 vs 452,5 mg/g lipides;  $P<0,001$ ) et l'indice d'iode est plus élevé (68,9 vs 62,4;  $P<0,001$ ). Les femelles ont montré la plus faible teneur en lipides et le plus haut degré d'insaturation des lipides par rapport aux mâles castrés.

**Mots-clés.** Porc lourd Italien – Jambon frais – Teneur en lipides – Composition en acides gras.

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## I – Introduction

The quantity and quality of adipose tissue in the carcass is of utmost importance in the production of Italian heavy pigs, since fat is a basic component of raw cuts destined to processing into valuable products, such as PDO dry cured hams (Lo Fiego *et al.*, 2005; Lo Fiego *et al.*, 2010). It is well known that lipid composition of fat tissues of pigs is directly influenced by the fat composition of diets (Bee *et al.*, 2002, Rossi and Corino, 2002; Kouba *et al.*, 2003), by genetic type (Bout *et al.*, 1988; Lo Fiego *et al.*, 2005) and by carcass fatness (Scott *et al.*, 1981; Lo Fiego, 1996; Lo Fiego *et al.*, 2010). In the past years Italian heavy pig



production has undergone significant changes due to the massive introduction of more recent genetic types, for the largest part commercial hybrids. Moreover, the “traditional” breeds, thanks to constant and continuous selection programs, optimized diets and improved rearing techniques, have themselves been modified, and carcass fat content has been reduced. Both traditional breeds, such as Large White, Landrace and Duroc selected in Italy, and hybrid pigs, coming from specific plans of selection and crossbreeding, have been used since many years for typical productions. Hybrids offer appreciable performance traits, but often show different composition of fat depots in comparison with traditional types: in particular, their growing use in heavy pig production produced an increase of the degree of lipid unsaturation, which is not desired for typical productions. Due to the necessity of constantly monitoring the quality of raw materials obtained from animals which are subject to continuous evolution, the aim of this work was to compare the characteristics of subcutaneous adipose tissue of fresh thighs destined to the production of PDO Italian dry-cured hams, and obtained from pigs belonging to two genetic types and reared in the same condition in the same farm.

## II – Material and methods

For this research, a total of 78 left thighs destined to the PDO Italian ham dry-curing production were used. Samples of subcutaneous adipose tissue from 46 thighs of traditional pigs (Italian Landrace x Large White cross, 34 castrated males and 12 females) and from 32 thighs of Goland commercial hybrid line (22 castrated males and 10 females), reared in the same farm and fed with a standard cereals-soybean meal based commercial feed, were analysed for water content, fat content and fat iodine value as reported by Lo Fiego *et al.* (2005). Moreover, fatty acid composition of lipid was determined using a TRACE™ GC Ultra (Thermo Electron Corporation, Rodano, Milano, Italy) equipped with the Ultra Fast Module (UFM), a Fast Flame Ionization Detector and a UFM-Carbovax column, 5 m long, 0.1 mm i.d, 0.2 µm film thickness as described in a previous paper (Ficarra *et al.*, 2010). The FAME were identified by comparison of each retention time with the known retention times of the corresponding pure standards (Supelco 37 Component FAME mix and PUFA standard n. 2, animal source, Supelco, Bellafonte, PA, USA). For quantification purposes, the response factor was calculated and the method of the internal standard was used. The results were expressed as mg of each fatty acid methyl ester/g of lipids. Data were subjected to analysis of variance using the GLM procedures of the SAS statistical package (SAS, 1996) and a factorial model that considered genetic type and sex as fixed effects.

## III – Results and discussion

Table 1 shows carcass and raw ham weights and fat thickness measurements according to genetic type. The mean values shown in Table 1 for both genetic types represent the optimum for Italian heavy pig production. No statistical difference was found between genetic types as regard both carcass and raw ham refrigerated weight and carcass backfat thickness. The latter was, however, slightly higher in traditional pigs, whose subcutaneous ham backfat thickness was lower in comparison with hybrids (- 4 mm,  $P<0.01$ ), with subsequent lower trimming loss ( $P<0.01$ ). This shows how the dislocation of fat depots in the carcass may vary in different genetic types, and consequently carcass backfat thickness might be not suitable for the estimation of fatness in other cuts. About the effect of sex (data not reported in the tables), females showed lower carcass weight (-10.4 kg;  $P<0.01$ ), lower refrigerated (-0.9 kg;  $P<0.01$ ) and trimmed ham weight (-0.7 kg;  $P<0.05$ ), and thinner subcutaneous fat (- 4.3 mm;  $P<0.05$ ) in comparison with castrated males. Table 2 shows the water and ether extract content and fatty acid composition of the subcutaneous adipose tissue of raw ham according to genetic type. Genetic type did not affect water and ether extract: on the opposite, in a previous work, hybrids, belonging to a different genetic type, showed higher water and lower lipid content in comparison

with traditional pigs reared in the same condition (Lo Fiego *et al.*, 2005). The fatty acid composition of the lipids was significantly influenced by genetic type.

**Table 1. Effect of genetic type on carcass and raw ham traits**

	Genetic type		R -MSE
	Traditional	Commercial Hybrid	
Refrigerated carcass weight (kg)	127.8	128.6	10.96
Carcass backfat thickness <sup>†</sup> (mm)	25.9	24.0	4.83
Refrigerated ham weight (kg)	16.8	16.4	1.34
Trimmed ham weight (kg)	13.9 <sup>a</sup>	13.2 <sup>b</sup>	1.09
Subcutaneous ham fat thickness <sup>††</sup> (mm)	33.9 <sup>A</sup>	37.9 <sup>B</sup>	7.23
Trimming loss (%)	17.9 <sup>A</sup>	18.5 <sup>B</sup>	2.47

<sup>†</sup>Measured between the ¾ last rib at 8 cm from the splitting line of the carcass.

<sup>††</sup>Measured beneath the femur head.

<sup>A, B</sup> =  $P \leq 0.01$ , <sup>a, b</sup> =  $P \leq 0.05$ .

**Table 2. Effect of genetic type on water and ether extract contents (%), lipid fatty acid composition (mg/g of lipids) and iodine value of covering adipose tissue of raw ham**

	Genetic type		R -MSE
	Traditional	Commercial Hybrid	
Water content %	6.57	6.96	1.64
Ether extract content %	90.11	90.91	3.12
Fatty acids composition <sup>†</sup> mg/g			
C14:0	12.55	11.93	1.68
C16:0	220.78 <sup>A</sup>	209.48 <sup>B</sup>	15.14
C18:0	131.40 <sup>A</sup>	108.13 <sup>B</sup>	11.28
C20:0	1.95 <sup>A</sup>	1.59 <sup>B</sup>	0.34
Total saturated	371.04 <sup>A</sup>	334.93 <sup>B</sup>	22.10
C16:1	21.21	22.96	3.58
C18:1	417.96 <sup>A</sup>	386.10 <sup>B</sup>	22.30
C20:1	9.13 <sup>A</sup>	7.69 <sup>B</sup>	1.51
Total monounsaturated	452.53 <sup>A</sup>	420.77 <sup>B</sup>	24.34
C18:2	91.81 <sup>A</sup>	125.33 <sup>B</sup>	14.60
C18:3	4.27 <sup>A</sup>	5.56 <sup>B</sup>	0.78
C20:2	4.50 <sup>A</sup>	5.65 <sup>B</sup>	0.80
C20:4	1.43	1.59	0.40
Total polyunsaturated	102.84 <sup>A</sup>	141.12 <sup>B</sup>	15.63
Iodine value	62.39 <sup>A</sup>	68.91 <sup>B</sup>	2.95
Total fatty acids %			
Saturated	40.18 <sup>A</sup>	37.46 <sup>B</sup>	1.82
Monounsaturated	48.66 <sup>A</sup>	46.80 <sup>B</sup>	1.92
Polyunsaturated	11.16 <sup>A</sup>	15.74 <sup>B</sup>	1.55

<sup>A, B</sup> =  $P \leq 0.01$ ; <sup>a, b</sup> =  $P \leq 0.05$ .

<sup>†</sup>Other fatty acids detected: C<sub>17:0</sub>, C<sub>17:1</sub>, C<sub>18:3\_n6</sub>, C<sub>20:3</sub>.

Traditional pigs produced lipids with a higher content of palmitic (C<sub>16:0</sub>), stearic (C<sub>18:0</sub>) and eicosanoic (C<sub>20:0</sub>) fatty acids ( $P < 0.01$ ) and showed, consequently, a higher level of total

saturated fatty acids (SFA) (452.5 vs 420.8 mg/g of lipids;  $P < 0.01$ ). Total monounsaturated fatty acids (MUFA) were also significantly higher in these subjects ( $P < 0.01$ ) and the differences were mainly due to oleic ( $C_{18:1}$ ) and eicosenoic fatty acids ( $C_{20:1}$ ). The hybrid pigs were found to have a significantly higher polyunsaturated fatty acid content (PUFA) (141.1 vs 102.8 mg/g of lipids;  $P < 0.01$ ), mainly linoleic ( $C_{18:2}$ ) (125.3 vs 91.8 mg/g of lipids), linolenic ( $C_{18:3}$ ) (5.6 vs 4.3 mg/g of lipids) and eicosadienoic ( $C_{20:2}$ ) (5.7 vs 4.5 mg/g of lipids). Consequently, the hybrids also showed a higher iodine value (68.9 vs 62.4;  $P < 0.01$ ). Linoleic and stearic fatty acids are the main components which may influence technological characteristics of lipids, such as firmness and cohesiveness; moreover an excessive content of  $C_{18:2}$  may favor oxidative phenomena during seasoning period. Based on the mean of  $C_{18:0}$  and  $C_{18:2}$  content and iodine value, it can be stated that both genetic types fall within the limits set by Italian PDO ham Consortia (Lo Fiego *et al.*, 2005). As regard the sex (data not reported in the table), females showed lower ether extract content and higher content of total polyunsaturated fatty acids, mainly  $C_{18:2}$ ,  $C_{18:3}$  and  $C_{20:2}$ , than castrated males ( $P < 0.01$ ).

## IV – Conclusions

The results show that the fatty acid composition of subcutaneous adipose tissue of fresh ham from heavy pigs may be considerably influenced by genetic type. The hybrid pigs, confirming the results of previous research on heavy pigs, were found to have a significantly higher polyunsaturated fatty acids content respect to the traditional pigs coming from the national selection programs; but the subjects examined in this research are anyway suitable for PDO production. Thus, taking into account the excellent on-farm performance of hybrids, it seems necessary a strict control of fat depots traits, in order to avoid an excessive increase of unsaturation degree.

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# Effets de la supplémentation alimentaire en acide pantothénique sur la composition en acides gras de la bardière chez le porc lourd en finition

G. Minelli\*, P. Macchioni \*\*, D.P. Lo Fiego\* et P. Santoro\*\*

\*Dipartimento di Scienze Agrarie e degli Alimenti, Università di Modena-Reggio Emilia (Italy)

\*\*Dipartimento di Protezione e Valorizzazione Agroalimentare, Università di Bologna (Italy)

**Resumé.** Cette étude a évalué l'effet de la supplémentation alimentaire en acide pantothénique sur la composition en acides gras (AG) de la bardière chez le porc lourd en finition. Dans ce but, 42 porcs, 21 femelles et 21 mâles castrés de poids vif et d'âge similaires, ont été uniformément allotés en trois groupes de 14 sujets chacun. A partir de 95 kg de poids vif (PV) jusqu'à l'abattage (165 kg PV), les 3 groupes ont reçu le même régime de base mais contenant différents niveaux d'acide pantothénique, sous forme de calcium pantothenate: respectivement 10 (contrôle, C), 60 (T1) et 110 (T2) ppm. Lors de l'abattage, des échantillons de gras de la bardière ont été prélevés individuellement, puis soumis à l'analyse de la composition en AG, par chromatographie capillaire en phase gazeuse, et à la détermination de l'indice d'iode selon la méthode de Wijs. L'utilisation de doses élevées d'acide pantothénique dans la ration conduit à une diminution des AG saturés ( $P=0,061$ ) et monoinsaturés ( $P=0,098$ ) de la bardière, tandis que les AG polyinsaturés et l'indice d'iode augmentent ( $P<0,05$ ). L'acide pantothénique administré à des concentrations supérieures à 60 ppm n'a pas entraîné d'effets significatifs sur la composition en AG de la bardière. Globalement, la qualité technologique du gras de la bardière chez le porc lourd pourrait être négativement affectée par l'utilisation alimentaire de niveaux élevés d'acide pantothénique.

**Mots-clés.** Porc lourd – Ration de finition – Acide pantothénique – Bardière – Acides gras.

## **Effects of dietary pantothenic acid supplementation on fatty acid composition of backfat in the finishing heavy pig**

**Abstract.** This study evaluated the effect of pantothenic acid supplementation to the finishing diet of heavy pigs on backfat fatty acid (FA) composition. To this aim, 42 pigs, 21 females and 21 castrated males of similar weight and age, were evenly divided into three groups of 14 subjects each. From 95 kg live weight (lw) till slaughtering (165 kg lw), the 3 groups received the same basal diet containing either 10 (control, C), or 60 (T1) or 110 ppm (T2) of pantothenic acid, as calcium pantothenate. At slaughter, backfat samples were individually collected and then submitted to FA composition analyses, by capillary GLC. The feeding of vitamin B5 at levels higher than currently recommended led to a decrease of saturated ( $P=0.061$ ) and monounsaturated FA ( $P=0.098$ ) content in backfat lipids, whereas polyunsaturated FA content and the iodine value increased ( $P<0.05$ ). Besides, the feeding of pantothenic acid at levels higher than 60 ppm brought about no further relevant effect on FA content. On the whole, the technological quality of backfat lipids in the heavy pig could be negatively, albeit slightly, affected by high level of pantothenic acid in the finishing diet.

**Keywords.** Heavy pig – Finishing diet – Pantothenic acid – Backfat lipids – Fatty acids.

## **I – Introduction**

Des études récentes ont montré que l'utilise de doses élevées d'acide pantothénique (vitamine B5) dans l'aliment entraîne un effet favorable sur la vitesse de croissance et sur la valeur commerciale de la carcasse avec une augmentation de la proportion des tissus maigres et une réduction de la masse adipeuse (Autrey *et al.*, 2002; Santoro *et al.*, 2006, Lo Fiego *et al.*, 2009). Toutefois cette diminution pourrait comporter une augmentation du degré d'insaturation du tissu

adipeux (Lebret et Mourot, 1998) avec conséquences défavorables sur sa qualité technologique (Lo Fiego *et al.*, 2005). A ce jour, l'effet de la supplémentation alimentaire de la vitamine B5 sur les caractéristiques des tissus adipeux du porc lourd n'a pas été étudié.

L'objectif de cette recherche était donc d'évaluer l'influence des niveaux élevés d'acide pantothénique, dans la ration du porc lourd en finition, sur le profil d'acides gras de la bardière.

## II – Matériels and méthodes

L'essai était conduit sur 42 animaux (Dumeco-Cofok x (LxLW)), dont 21 femelles et 21 mâles castrés, uniformément allotés, par poids et par sexe, en trois groupes de 14 porcs chacun. À partir de 95 kg de poids vif les animaux ont été alimentés avec la même ration composée de céréales, tourteau de soja et mélasse (MAT 15%), mais additionnée avec différents apports de pantothénate de calcium, pour obtenir une dose d'acide pantothénique de 10 (contrôle, C), 60 (T1) et 110 (T2) ppm. Les animaux ont été abattus lorsqu'ils ont atteint un poids vif d'environ 165 kg. Des prélèvements de la bardière ont été effectués dès l'abattage. Les échantillons ont été stockés à -20°C dans l'attente des analyses. Les lipides totaux ont été extraits selon la méthode IUPAC (1979). La composition en acides gras des lipides de la bardière a été déterminée par chromatographie capillaire en phase gazeuse. L'indice d'iode a été déterminé selon la méthode de Wijs. Les résultats ont été analysés à l'aide de l'ANOVA en considérant le niveau alimentaire d'acide pantothénique comme une variable indépendante (SAS, 1996).

## III – Résultats et discussion

De façon générale l'accroissement de l'acide pantothénique dans la ration du porc lourd en finition s'accompagne d'une diminution de l'épaisseur de la bardière (P=0,09) et de l'incidence de morceaux adipeux (P<0,05) de la carcasse (Tableau 1). Ainsi, les données obtenues confirment la capacité de la vitamine B5 à réorienter l'énergie alimentaire au détriment du tissu adipeux. L'utilise des doses élevées d'acide pantothénique dans la ration a influencé la composition en acides gras de la bardière (Tableau 2). Globalement on a constaté une réduction des acides gras saturés (P=0,061), monoinsaturés (P=0,098) et une augmentation des polyinsaturés totaux et de l'indice d'iode (P<0,05), indépendamment du dosage.

Tableau 1. Caractéristiques de la carcasse

	Intégration alimentaire en acide pantothénique (ppm)					
	10	60	110	C vs moyenne T1+T2	T1 vs T2	R-MSE
	C(n=14)	T1(n=14)	T2(n=14)			
Poids de la carcasse (kg)	142,5	138,7	140,4	ns	ns	8,66
V viande maigre (%)	47,9	49,5	48,8	ns	ns	3,05
Epaisseur moyenne de la bardière (mm)	31,5	27,0	28,2	\$	ns	6,26
Morceaux maigres† (%)	64,8	65,7	65,8	ns	ns	2,46
Morceaux gras† (%)	33,3	31,5	30,6	*	ns	3,04

†% de poids de carcasse chaude;

\*= P<0,05; \$=P<0,1; ns = pas significatif.

Plus en détail, l'ajoute de doses élevées d'acide pantothénique a déterminé une diminution de la teneur en acide palmitique (P<0,01), bien connu pour ses propriétés athérogènes. Par contre le niveau d'acide stéarique n'est pas influencé par la teneur en vitamine B5 de la ration. Il montre des valeurs supérieures à 12% des acides gras totaux, c'est-à-dire au dessus de la

teneur minimale recommandée pour satisfaire les caractéristiques technologiques et organoleptiques du tissu adipeux (Lebret et Mouro, 1998).

**Tableau 2. Effet de la teneur en acide pantothénique sur la composition en acides gras de la bardière (mg/g des lipides)**

	Intégration alimentaire en acide pantothénique (ppm)					R-MSE
	10	60	110	C vs	T1 vs T2	
	C(n=14)	T1(n=14)	T2(n=14)	moyenne T1+T2		
<i>Acides gras (mg/g des lipides)</i>						
C14:0	11,26	10,63	10,71	*	ns	0,86
C16:0	222,93	213,56	213,85	**	ns	9,72
C17:0	2,66	2,72	2,52	ns	ns	0,43
C18:0	131,92	131,09	131,23	ns	ns	8,25
C20:0	1,65	1,73	1,86	ns	ns	0,50
Total saturés	370,42	359,73	360,17	\$	ns	15,17
C16:1	17,85	16,48	15,92	**	ns	1,80
C17:1	2,25	2,18	1,85	ns	\$	0,43
C18:1	394,18	384,58	390,82	ns	ns	15,87
C20:1	10,92	8,45	10,54	**	**	1,51
Total monoinsaturés	425,20	411,69	419,13	\$	ns	17,03
C18:2	169,86	183,79	180,33	*	ns	14,99
C18:3	8,93	10,36	9,09	\$	*	1,18
C20:2	7,51	8,41	8,20	*	ns	0,94
C20:3	1,25	1,75	1,61	ns	ns	0,71
C20:4	1,75	2,06	1,82	ns	ns	0,66
Total polyinsaturés	189,30	206,37	201,05	*	ns	16,94
Indice d'iode	70,41	72,26	71,91	*	ns	2,55
<i>Total acides gras %</i>						
Saturés	37,61	36,79	36,74	\$	ns	1,43
Monoinsaturés	43,17	42,10	42,75	ns	ns	1,43
Polyinsaturés	19,22	21,11	20,51	**	ns	1,65

\*\*= P<0,01; \*= P<0,05; \$=P<0,1; ns = pas significatif.

Parmi les acides gras polyinsaturés, l'acide linoléique a montré l'augmentation la plus importante (P<0,05) à des doses élevées d'acide pantothénique avec des valeurs au dessus de 15%, teneur maximale recommandée pour éviter les problèmes lors de la transformation et de la conservation des produits (Wood, 1984). Mais cette valeur était déjà plus élevée même dans le groupe de contrôle.

## IV – Conclusions

Les résultats obtenus avec cette première étude sur l'influence de la supplémentation alimentaire de l'acide pantothénique sur les caractéristiques des tissus adipeux du porc lourd ont mis en évidence que l'addition de doses élevées d'acide pantothénique implique une réduction de l'incidence de morceaux gras et de l'épaisseur de la bardière avec une augmentation du degré d'insaturation des lipides. Dépassant les 60 ppm n'entraîne pas d'autres changements. Cela répond mieux aux besoins spécifiques des consommateurs qui donnent plus d'importance à la composante nutritionnelle des aliments, mais au contraire n'est pas accueilli pour les transformateurs qui sont plus exigeants sur l'aptitude à la transformation et à la conservation des tissus adipeux.

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# Physical measures of the carcass and the chemical composition of *Longissimus dorsi* muscle of Alentejano pigs between 70 and 110 kg LW

J.A. Neves, A. Freitas, J.M. Martins and J.L. Tirapicos Nunes

ICAAM/University of Évora, Évora (Portugal)

**Abstract.** The aim of this work was to determine the relationship between physical measures from the subcutaneous tissue and *Longissimus dorsi* (LD) muscle (area, depth, and width measured between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae, at the last rib, and between the 3<sup>rd</sup> and 4<sup>th</sup> last ribs) and the chemical composition of LD at 70, 80, 90, 100, and 110 kg LW. The content of water, protein, neutral and polar lipids, total and soluble collagen, and total pigments, were determined. Globally, the measures taken and the chemical composition were not affected between 70 and 110 kg, except for the LD depth and width at the 3<sup>rd</sup>-4<sup>th</sup> lumbar vertebrae. At 70 kg, the LD depth was greater than at 110 kg (3.77 vs 2.75 cm,  $P < 0.05$ ) and the width was smaller (8.14 vs 9.82 cm,  $P < 0.05$ ). In conclusion, from 70 to 110 kg: (i) the morphological changes in the lumbar region were due mainly to the width dimension, with no impact on the chemical composition of the muscle; and (ii) the chemical composition did not change drastically, even though the amount of intramuscular fat increased slightly between 70 and 110 kg (5.32 and 6.67%, respectively) suggesting an early intramuscular fat deposition.

**Keywords.** Alentejano pig – Carcass – Meat quality – Live weight.

## **Mesures physiques de la carcasse et composition chimique du muscle *Longissimus dorsi* des porcs Alentejano entre 70 et 110 kg de poids vif**

**Résumé.** Le but de ce travail était de déterminer la relation entre les mesures physiques du tissu sous-cutané et le muscle *Longissimus dorsi* (LD) (superficie, profondeur et largeur mesurées entre les 3<sup>e</sup> et 4<sup>e</sup> vertèbres lombaires, à la dernière côte, et entre les dernières 3<sup>e</sup> et 4<sup>e</sup> côtes) et la composition chimique du LD à 70, 80, 90, 100, et 110 kg de poids vif. Les teneurs en eau, protéines, lipides neutres et polaires, collagène total et soluble, et pigments totaux ont été déterminées. Globalement, les mesures prises et la composition chimique n'ont pas été modifiées entre 70 et 110 kg, sauf pour la profondeur et la largeur du LD au niveau des vertèbres lombaires 3<sup>e</sup>-4<sup>e</sup>. À 70 kg, la profondeur du LD a été supérieure (3,77 vs 2,75 cm,  $P < 0,05$ ) et la largeur inférieure (8,14 vs 9,82 cm,  $P < 0,05$ ) par rapport à 110 kg. En conclusion, entre 70 et 110 kg: (i) les changements morphologiques dans la région lombaire ont été principalement attribuables à la dimension de la largeur, sans impact sur la composition chimique du muscle; et (ii) la composition chimique ne change pas radicalement, même si la quantité de lipides intramusculaires a légèrement augmenté entre 70 et 100/110 kg (5,32 et 6,42/6,30%, respectivement) suggérant un dépôt de gras intramusculaire précoce.

**Mots-clés.** Carcasse – Composition chimique – Alentejano.

## I – Introduction

For centuries, the agriculture in Alentejo region was based in a production system characterised by a strong interdependence between natural fed resources and animal production. In this context, the Alentejano pig breed gained importance by its rusticity and the rational use of fed resources. Comparatively with others, the Alentejano pig shows a slow rate of growth and a high lipogenesis activity at early stages of development. The lipids are deposited mainly in the subcutaneous, renal and pelvic regions. The percentages of fatty cuts can attain more than 50%



of the carcass weight and the backfat thickness at the last rib can reach 60 mm at 120 kg live weight (LW) (Almeida *et al.*, 1993; Neves *et al.*, 2001). Nowadays, the production fulfills a double function: it provides meat for the manufacture of cured products and also for fresh consumption. Considering the volume and the increase of fresh pig meat consumption in Portugal, the farmers have increased their production for this market, traditionally dominated by the meat from precocious pig breeds reared in intensive production systems. Based on high organoleptic quality, pork from Alentejano hogs could be an effective alternative for meat from industrial breeds. However, carcass has too much fat and a bad proportion of lean cuts, which impair the economic rentability of this production system. The animal growth implies chemical, biochemical, and physical changes in the adipose and muscular tissues, mainly due to an increase on the lipid content (subcutaneous and intramuscular) (Mayoral *et al.*, 1999). These changes affect the gross chemical composition. Thus, it was interesting to find if the growth process and fat deposition induce significant changes on muscle chemical composition that could influence its global quality. Since it is possible to measure the depth of LD *in vivo*, it was interesting to study the possible relationship between this measure and the chemical composition of the muscle, particularly with the amount of intramuscular fat at the usual commercial slaughter weights.

## II – Materials and methods

Thirty Alentejano pigs were castrated at the age of 60 days. After weaning, piglets were transferred to individual pens at open air and fed a commercial diet (15% CP; 3100 kcal DE) offered at 85 % of *ad libitum*. At the beginning of the experiment, 5 animals with 40 kg LW were slaughtered. The remaining animals were weighted weekly and slaughtered (5 animals) at 70, 80, 90, 100, and 110 kg LW. After 12 h fasting, the animals were weighted and then slaughtered. Twenty four hours after chill, the left side of each carcass was submitted to commercial cuts, according to the Portuguese Norm N – 2931. The measure of loin muscle depth was done 6 cm lateral to the mid-line of the carcass and resulted from the average of the first measure and two others 0,5 cm apart from it in both directions. The loin muscle area and width were also measured. Those measures were taken at three anatomical sites (between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae, the last rib and between the 3<sup>rd</sup> and 4<sup>th</sup> last ribs). Samples were taken from the loin cut and were vacuum packaged and stored (-30°C) until analysis. Moisture (Portuguese Norm 1614), total protein (Portuguese Norm 1612), total, neutral, and polar lipids (Marmer and Maxwell, 1981), and total pigment (Hornsey, 1956) were determined. Total hydroxyprolin was analyzed (Woessner, 1961) and multiplied by 7.14 (Etherington & Sims, 1981) to obtain the total collagen. The soluble fraction of collagen was also analyzed (Hill, 1966). An ANOVA was carried out and the means comparison was made by SNK test. The correlation between the variables studied was determined by the Pearson coefficient. SPSS statistical program was used.

## III – Results

Globally, the chemical composition and the measures taken weren't affected by live weight between 70 and 110 kg (Tables 1 and 2), except for the depth and width of LD between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar spot (Table 2). At 70 kg the depth of the LD was greater ( $P < 0.05$ ) than at 110 kg LW (3.77 cm vs 2.75 cm) and the width was smaller (8.14 cm vs 9.82 cm). In the other anatomical sites, no significant differences were found. However, the LD area at the last rib tended to be greater ( $P = 0.052$ ) at 110 kg than at 70, 80, and 90 kg LW (21.45 cm<sup>2</sup> vs 17.27, 17.68, and 17.56 cm<sup>2</sup>, respectively). The average area, depth and width presented no significant differences between 70 and 110 kg LW (17.77 cm<sup>2</sup> vs 20.76 cm<sup>2</sup> for area; 3.52 cm vs 3.32 cm for depth, and 7.42 vs 8.37 cm for width). However, the P value obtained for the width was 0.057 which seems to indicate that the increase in the muscle area was due essentially to the increase in width dimension. Daza *et al.*, (2006) observed in Iberian pigs reared extensively

and slaughtered at 152 kg LW, a LD area measured at last rib level of 26.73 cm<sup>2</sup>, and in pigs in confinement slaughtered at 159 kg, 30.31 cm<sup>2</sup>. These values, greater than those observed on this work, could be justified by the greater slaughter weight. Nevertheless, they showed a development pattern similar to the one observed in the present work.

**Table 1. Chemical characteristics of *Longissimus dorsi* muscle of Alentejano pigs at various live weights**

	Slaughter weight groups (kg LW)				
	70	80	90	100	110
Water	69.97 ± 1.7	70.33 ± 0.3	67.39 ± 2.1	67.24 ± 2.5	68.70 ± 0.8
Protein	23.07 ± 0.6	22.41 ± 1.4	22.50 ± 0.9	22.97 ± 0.6	21.86 ± 0.7
Neutral lipids	5.37 ± 2.3	5.23 ± 0.5	6.24 ± 1.3	6.42 ± 1.2	6.67 ± 1.5
Polar lipids	0.85 ± 0.1 <sup>ab</sup>	1.18 ± 0.1 <sup>ab</sup>	0.99 ± 0.2 <sup>ab</sup>	0.73 ± 0.2 <sup>b</sup>	0.86 ± 0.1 <sup>ab</sup>
Total pigment	69.53 ± 2.8	67.32 ± 5.9	84.18 ± 11.7	71.90 ± 17.1	73.78 ± 10.3
Total collagen (ug/mg, DM)	12.47 ± 4.0	9.99 ± 1.0	10.84 ± 1.0	8.98 ± 2.0	10.23 ± 2.0
Soluble collagen (ug/mg, DM)	1.93 ± 1.0	1.20 ± 0.1	0.76 ± 0.3	1.17 ± 0.2	0.97 ± 0.4

Means in the same line with different letter are significantly different (P<0.05).

**Table 2. Physical characteristics of the backfat of Alentejano pigs at a various live weights**

	Slaughter weight groups (kg LW)				
	70	80	90	100	110
3 <sup>th</sup> and 4 <sup>th</sup> LuVA (cm <sup>2</sup> )	18.70 ± 1.9	19.45 ± 2.3	21.32 ± 2.1	20.66 ± 1.7	20.94 ± 4.1
3 <sup>th</sup> and 4 <sup>th</sup> LuVD (cm)	3.77 ± 0.7 <sup>b</sup>	2.99 ± 0.1 <sup>b</sup>	3.12 ± 0.2 <sup>b</sup>	3.00 ± 0.1 <sup>b</sup>	2.75 ± 0.3 <sup>a</sup>
3 <sup>th</sup> and 4 <sup>th</sup> LuVW (cm)	8.14 ± 0.4 <sup>a</sup>	8.73 ± 0.6 <sup>a</sup>	8.41 ± 1.1 <sup>a</sup>	8.54 ± 0.3 <sup>a</sup>	9.82 ± 1.1 <sup>b</sup>
LaRA (cm <sup>2</sup> )	17.27 ± 2.4	17.68 ± 2.8	17.56 ± 1.4	19.42 ± 2.8	21.45 ± 1.7
LaRD (cm)	3.41 ± 0.2	3.43 ± 0.5	3.30 ± 0.5	3.17 ± 0.5	3.63 ± 0.5
LaRW (cm)	7.28 ± 0.5	7.46 ± 0.3	7.08 ± 0.6	7.64 ± 0.5	7.82 ± 0.5
3 <sup>th</sup> and 4 <sup>th</sup> LaRA (cm <sup>2</sup> )	17.35 ± 2.9	18.57 ± 2.9	20.49 ± 2.5	19.35 ± 1.9	19.88 ± 1.4
3 <sup>th</sup> and 4 <sup>th</sup> LaRD (cm)	3.38 ± 0.2	3.57 ± 0.2	3.46 ± 0.4	3.53 ± 0.4	3.59 ± 0.3
3 <sup>th</sup> and 4 <sup>th</sup> LaRW (cm)	6.84 ± 0.2	7.37 ± 0.4	7.37 ± 0.9	6.68 ± 0.2	7.48 ± 0.6

Means in the same line with different letter are significantly different (P<0.05).

LuVA: lumbar vertebrae area; LuVD: lumbar vertebrae depth; LuVW: lumbar vertebrae width; LaRA last ribs area; LaRD: last ribs depth; LaRW: last ribs width.

The amount of neutral lipids showed a ~20% increase between 70 and 100/110 kg LW (5.32% vs 6.42/6.30 %, respectively). The same was observed in a previous work by Neves *et al.*, (1996). However, Estevez *et al.* (2003) reported in three genetic lines intramuscular fat contents between 2.51 and 3.34% in pigs with 90 kg LW. Those amounts, substantially lower than the ones obtained in this work, could be explained by some genetic selection in the lines of the Iberian pigs studied in that work. The correlation analysis showed a negative relation between neutral intramuscular fat and protein (-0.557; P<0.01) of the LD muscle, as expected. The average area showed a greater correlation coefficient with the area of LD measured at the last rib (0.873), than with the areas measured at the two other spots (0.821 and 0.798 for the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae spot and the 3<sup>rd</sup> and 4<sup>th</sup> last ribs spot, respectively). The correlation study between the average area and the individual measure of depth and width in the three spots

shown only significant correlation with the depth of LD at the last rib (0.399; 0.048) and with the width measured between the 3<sup>rd</sup> and the 4<sup>th</sup> lumbar vertebrae (0.464; 0.020). Finally, no correlations were observed between the chemical composition traits and the physical measures taken in the LD muscle.

## IV –Conclusions

Between 70 and 110 kg LW, (i) the lumbar region was the one that showed more morphological changes during growth, and the development of the muscle seems to be due to width more than the depth dimension; (ii) the chemical composition didn't change much, and in the particular case of the amount of intramuscular fat, only a slight increase between 70 and the heavier weights (100 and 110 kg LW) was observed, suggesting a earlier intramuscular fat deposition; and (iii) no statistical significant correlation was found between the chemical composition traits and the physical measures taken from the backfat and from the LD muscle.

## Acknowledgements

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# Consumers perception of iberian cooked meat products

B. Rubio, B. Martínez, C. Vieira and M.D. García

Consejería de Agricultura y Ganadería, Junta de Castilla y León, ITACyL, Estación Tecnológica de la Carne, Guijuelo, Salamanca (Spain)

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**Abstract.** Today, many consumers demand and have available a wide assortment of high quality foods. The quality of meat products is influenced by intrinsic factors such as breed, genotype, reared system, and feeding regime. Thus, cooked products manufactured from raw material from Iberian and white pigs should show different sensory quality. The aim of this study was to evaluate different cooked products made from Iberian and white pigs, in order to establish differences. Samples of different cooked meat products were purchased from local supermarkets (cooked ham, pâté and cooked sausages). In order to know if consumers could distinguish between Iberian and white cooked meat products, different tests (triangle test, duo-trio test and ranking test) were carried out with consumers. The results showed that consumers were able to distinguish between cooked meat products manufactured from Iberian and white raw meat. According to consumers, flavour and juiciness were the attributes that allowed distinguish these products.

**Keywords.** Sensory analysis – Cooked meat product – Iberian and white pig.

## *Perception qu'ont les consommateurs des produits cuits à base de viande de porc Ibérique*

**Résumé.** Les attributs qui sont propres aux produits de la viande et qui les rendent très appréciés par les consommateurs, sont étroitement liés aux caractéristiques des matières premières, notamment la génétique et le système d'exploitation des animaux. Pour cette raison, les produits cuits élaborés avec des matières premières de porc Ibérique devraient présenter des différences par rapport à ceux élaborés avec des matières premières de porc blanc. L'objectif de l'étude a été l'évaluation sensorielle de différents produits cuits élaborés avec du porc Ibérique ou du porc blanc, afin de pouvoir établir des différences. Les résultats obtenus ont montré que les consommateurs ont été capables de distinguer entre les produits élaborés avec une matière première de porc Ibérique et ceux à base de porc blanc. Les attributs qui ont permis d'établir des différences ont été le goût et la teneur en jus.

**Mots-clés.** Analyse sensorielle – Viande de porc Ibérique et de porc blanc – Produits cuits.

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## I – Introduction

Cooked pork meats like pâté, sausages or cooked ham are very consumed in Spain. Even though there is a large variety of cooked products on the Spanish market, most of them are made using meat and back fat from white pigs. From a nutritional point of view, fat from white pigs has a considerable level of saturated fatty acids and cholesterol, with an undesirable n-6/n-3 polyunsaturated fatty acids ratio. Consumer concerns about the relationship between health and nutrition, challenge food technologists to develop new meat and fat-based products with improved characteristics.

In this sense, using meat and adipose tissue from Iberian pigs for the manufacture of cooked products results in a high quality product. Intramuscular fat of these pigs is characterized by large percentages of monounsaturated fatty acids, a small proportion of hypercholesterolemic fatty acids and present lower values of the ratio n-6/n-3 than fat from white pigs (Estevez *et al.*, 2006). The products traditionally obtained from these animals such as dry cured hams and dry-cured loins are highly appreciated by Spanish consumers. However, to date, the iberian cooked meat products are not well known by consumers.

The aim of this study was to evaluate if the consumers are able to establish differences between diverse cooked products made from Iberian and white pigs, in order to increase the knowledge about the consumers' perception to these new products.

## II – Material and methods

To achieve this objective pâté, cooked ham and sausages manufactured from white or Iberian pigs were used. All samples were purchased from local markets and were subject to consumer sensory evaluation.

The tasters (n=84) were mainly farmers, manufacturers and technicians associated with pig meat industries, habitual consumers of meat products. Characteristics of the untrained panel are summarized in Table 1.

**Table 1. Characteristics of the tasters based on age and gender**

Tasters	Age (years)				
	20-30	31-40	41-50	51-60	>60
N <sup>†</sup>	21.4%	27.4%	29.8%	13.1%	8.3%
Male	11.9%	17.9%	22.6%	11.9%	8.3%
Female	9.5%	9.5%	7.2%	1.2%	—

<sup>†</sup>All consumers.

In order to know if consumers could establish differences between cooked meat products manufactured from white or Iberian pork meat, consumers carried out different discrimination tests. First, a triangle test was carried out according to the ISO 4120:2004. Samples, consisting of Iberian pâté and pâté were served on plastic plates to participants. Three coded samples were presented to each panellist and each panellist was asked to pick out which sample they feel different from the other two. Besides, to complement the triangle test, tasters were asked to indicate the reason for selecting one particular sample of the three used in the analysis. Then, consumers carried out a duo- trio test (ISO 10399:2004). Three samples of cooked ham (manufactured from white or Iberian pork meat) were presented to tasters: one sample was labeled "R" (reference) and the other two were coded. One of the coded samples was identical to "R" and panellists were asked to identify the correct sample. Besides, tasters were asked to indicate the reason for selecting one particular sample of the three used in the analysis. Finally, a ranking test (ISO 8587:2006) was carried out with three cooked sausages kind: Frankfurt, Bratwurst and iberian sausages. Panellists received three coded samples and were requested to rank samples for intensity of some specific characteristic. Besides, the subjects were asked to specify the characteristic that determined their preference.

Mineral water at room temperature and unsalted toasted bread were available and tasters were required to consume them before tasting each sample in order to rinse their mouths between samples. Consumer's tests were conducted in a room with white fluorescent lighting and kept at constant temperature of 21±2°C at Salamanca (Spain).

## III – Results and discussion

The results of the triangle test are shown in Table 2 and revealed that tasters perfectly discriminated among the tasted samples.

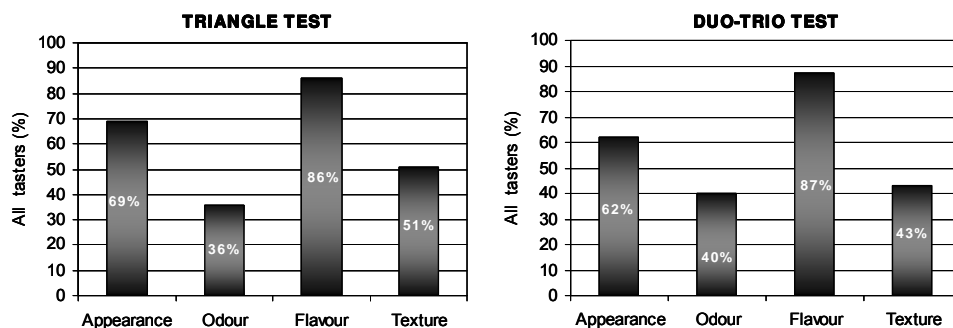
The age and the gender of consumers did not establish differences in the significance level. In general, all tasters chosen the flavour, principally, for selecting the iberian pâté as different sample (Fig. 1).

**Table 2. Results obtained in the triangle test carried out with pâté**

Age	Tasters	No. judgements corrects	Significance level
20-30	N†	18/18	***
	Male	10/10	***
	Female	8/8	***
31-40	N	21/23	***
	Male	13/15	***
	Female	8/8	***
41-50	N	21/25	***
	Male	16/19	***
	Female	5/6	**
51-60	N	11/11	***
	Male	10/10	***
	Female	1/1	—
>60	N	7/7	***
	Male	7/7	***
	Female	—	—

†All consumers.

\*\*\*  $p < 0.001$ .



**Fig. 1. Attributes indicated by the tasters in the triangle and duo-trio tests for selecting the preferred sample.**

Results from the duo-trio test (Table 3) with all consumers pointed out that the consumers ranging 20-60 years significantly differentiated the cooked ham manufactured from white or Iberian pork meat. Taking the gender into account, the number of assessors who correctly identified the different sample was lower for male and higher for female. Similarly to triangle test, all tasters chosen the flavour as attribute for differentiating the iberian cooked ham (Fig. 1).

These results may be due to variations in fatty acids content of meat. Different studies have been focused on the role of fatty acids in meat flavour formation. In this sense, Mottram (1998)

pointed out that the characteristic flavour of cooked meat derives from thermally induced reactions occurring during heating, principally the Maillard reaction and the degradation of lipids. During the oxidation of the fatty acid components of lipids, the reactions occur quickly and provide a profile of volatiles which contribute to desirable flavours. Besides, unsaturated fatty acids undergo autoxidation much more readily than those which are saturated.

**Table 3. Results obtained in the duo-trio test carried out with cooked ham**

Age	Tasters	No. of correct answers	Significance level
20-30	N†	17/18	***
	Male	9/10	*
	Female	8/8	**
31-40	N	21/23	***
	Male	13/15	**
	Female	8/8	**
41-50	N	25/25	***
	Male	19/19	***
	Female	6/6	*
51-60	N	11/11	***
	Male	10/10	***
	Female	1/1	—
>60	N	5/7	N.s
	Male	5/7	N.s
	Female	—	—

†All consumers.

n.s:  $p > 0.05$ ; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

Finally, the results obtained in the ranking test, showed differences ( $p < 0.05$ ) among the three cooked sausages evaluated. The juiciness was the parameter what allowed establish differences. These results revealed that tasters considered Bratwurst sausages as the juiciest and the Iberian sausages as the least juiciness. The fat content of the sausage may explain this result. Matulis *et al.* (1994, 1995) reported that in the manufacture of frankfurters the juiciness differed with the fat content.

## IV – Conclusions

In summary, the Iberian cooked meat products evaluated in this study were differentiated by the consumers. These results show that, probably, these types of products can be accepted by the consumer, although studies with a larger number of subjects should be carried out.

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# Study of the characteristics of conventional cooked hams and organic cooked hams

M.J. Sánchez Iglesias, M. Vaquero Martín, B. Rubio Hernando and B. Martínez Domínguez

Consejería de Agricultura y Ganadería de la Junta de Castilla y León, Instituto Tecnológico Agrario, Estación Tecnológica de la Carne, Guijuelo, Salamanca (Spain)

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**Abstract.** Consumers' concern due to the use of additives in the manufacturing of meat product has focused an increasing demand of the organic products. The organic products are primary or manufactured products obtained without the use of chemical products. So, in the organic cooked meat products the use of phosphates is not permitted. However, the use of nitrites is allowed in a lower amount than for manufacturing conventional products due to the role that nitrites exert on the development of colour. The aim of this work was to study the characteristics of conventional and organic commercial cooked hams. Six different commercial brands of cooked hams were analysed: 3 conventional and 3 organics. In each ham the pH,  $a_w$ , proximal composition, amount of additives (sodium chloride, nitrites and phosphates) and the colour ( $L^*$ ,  $a^*$ ,  $b^*$ ) were evaluated. The main differences found among the cooked ham analysed were in the protein and fat content. The use of pigs with different genetic or with different feed (conventional or organic) could explain these results. Moreover, differences in the amount of phosphates were detected. The use of different concentration of phosphates in the manufacturing of conventional cooked ham and the use of different raw meats might explain these differences.

**Keywords.** Additives – Organic products – Cooked ham – Characterization.

## *Une étude des caractéristiques du jambon cuit conventionnel et du jambon écologique*

**Résumé.** La préoccupation du consommateur à propos de l'inclusion d'additifs chimiques dans les produits à base de viande a entraîné un développement de la demande de produits écologiques. Les produits écologiques sont des produits agricoles primaires ou élaborés, obtenus sans l'utilisation de produits chimiques. Ainsi, dans les produits à base de viande cuits écologiques, l'ajout de phosphates n'est pas permis. Toutefois, l'inclusion de nitrite est autorisée pour favoriser le développement de la couleur, mais en concentrations plus basses que dans l'élaboration des produits conventionnels. L'objectif de ce travail était d'étudier les caractéristiques du jambon cuit commercial écologique et du jambon cuit commercial conventionnel. Pour cela, 6 marques commerciales de jambon cuit ont été analysées : 3 conventionnelles et 3 écologiques. Pour chaque jambon on a déterminé le pH, l' $a_w$ , la composition proximale, la teneur en additifs (chlorures, nitrites et phosphates) et on a réalisé la mesure instrumentale de la couleur. Les principales différences entre les différents jambons analysés concernaient la teneur en graisse et en protéines. L'utilisation de porcs ayant une génétique différente ou recevant une alimentation différente (conventionnelle ou écologique) pourrait expliquer ces résultats. Des différences ont également été détectées dans la teneur en phosphates. L'emploi de différentes concentrations de phosphates pour l'élaboration de jambons conventionnels et l'utilisation de différentes matières premières pourrait être la cause de ces différences.

**Mots-clés.** Additifs – Produits biologiques – Jambon cuit – Caractérisation.

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## I – Introduction

Nowadays, the consumers' demand of additives-free and healthy meat products with quality attributes similar to conventional meat products has focused an increasing demand of the organic products. The organic products are primary or manufactured products obtained without the use of chemical products (DOUE, 2008).



The cooked ham is one of the meat products most demanded in Spain. In the manufacture of this meat product, phosphates (E-450, E-451 and E-452) and nitrites (E-249, E-250) are commonly added. Phosphates improve cohesion of meat pieces and binding of water (Keenan *et al.*, 2010) however, this additive is not permitted for manufacturing organic cooked meat products (DOUE, 2008). Otherwise, the use of nitrites (E-249, E-250) is allowed, due to the role that nitrites exert on the development of colour, but in a lower amount than for manufacturing conventional products. The aim of this work was to study the physico-chemical characteristics of conventional and organic commercial cooked hams.

## II – Material and methods

Six different commercial brands of cooked hams were analysed: 3 conventional (C1, C2, C3) and 3 organics (O1, O2, O3). In all cooked hams evaluated (3 for each commercial brand), different analysis were carried out. The pH value was determined with a Crison 2001 pH meter (Crison Instrument S.A, Barcelona, Spain) equipped with a punction electrode. Water activity ( $a_w$ ) was determined using a hygrometer (Aqua-lab CX2, Decagon, Washington, USA). Moisture, protein, fat and ash content were determined according to ISO standards 1442:1997 (ISO, 1997), 937:1978 (1978), 1443:1973 (ISO, 1973) and 936:1998 (ISO, 1998), respectively. Moreover, NaCl (ISO 1841-1:1996), nitrite (ISO 2918:1975) and phosphate content (ammonium molybdate method, BOE, 1995) were analysed. Surface colour was measured using a reflectance spectrophotometer (CM-2600d/2500d, Konica Minolta, Aquateknia S.A., Spain). Colour coordinates were determined in the CIE-LAB system and the results were expressed as lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ). Data sets were statistically analyzed using one-way variance analysis (ANOVA) in order to determine any significant differences. The means were separated by Tukey-honest significant difference test at 5% level. Principal component analysis (PCA) was performed in order to evaluate the influence of the parameters on total variability. Data analyses were conducted using Statgraphics Plus 4.0 statistical package.

## III – Results and discussion

The results obtained for pH,  $a_w$  and proximal composition are showed in Table 1. Although one of the organic cooked ham brands presented an unusual pH value according to Aymerich *et al.*, (2003), the pH values for the other brands were similar. For  $a_w$ , no differences ( $p > 0.05$ ) were detected among the cooked ham brands evaluated. In general, the values obtained for this parameter were similar than those found by Rubio *et al.*, (2009) in cooked ham.

**Table 1. pH,  $a_w$  and proximal composition in cooked ham (conventional and organic)**

		pH	$a_w$	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Conventional	C1	<sup>b</sup> 6.2	0.972	73.3	<sup>b</sup> 18.9	<sup>ab</sup> 2.9	3.3
	C2	<sup>b</sup> 6.5	0.982	73.4	<sup>b</sup> 18.6	<sup>ab</sup> 3.6	3.5
	C3	<sup>b</sup> 6.2	0.972	75.1	<sup>a</sup> 17.1	<sup>ab</sup> 5.0	4.3
Organic	O1	<sup>b</sup> 6.2	0.973	74.4	<sup>a</sup> 17.5	<sup>b</sup> 4.6	3.2
	O2	<sup>a</sup> 5.1	0.973	73.1	<sup>b</sup> 19.0	<sup>ab</sup> 2.1	2.9
	O3	<sup>ab</sup> 5.6	0.979	74.9	<sup>c</sup> 20.5	<sup>a</sup> 1.4	2.8

<sup>a,b,c</sup> Means within the same column with different letters differ significantly ( $p < 0.05$ ).

With regard to proximal composition, differences for the protein and fat content ( $p < 0.05$ ) were found among the commercial brands analysed. A higher variability was observed for organic

cooked ham in these parameters. The use of raw meat from pigs with different genetic, fed with different diets or slaughtered at different age could affect the composition of raw meat and consequently the composition of meat products (García-Rey *et al.*, 2004). Authors as Cheng, *et al.* (2005) indicated that cooked ham has a moisture, protein and fat content around 65-71%, 21-24% and 1-2%, respectively.

In the Table 2 are showed the results obtained for the content of additives. No differences ( $p > 0.05$ ) were detected for the amount of NaCl. For the nitrite content, it is important to point out that although the maximum amount of nitrites allowed for manufacturing conventional and organic products is different (150 ppm and 80 ppm, respectively), no differences ( $p > 0.05$ ) were found among brands since nitrite is a highly reactive compound at the pH of 5.5-6 (Marco, *et al.*, 2006). As it was expected, some differences were found for the phosphate content ( $p < 0.05$ ) between conventional and organic brands of cooked ham. The conventional cooked hams showed the highest values for this parameter. The intrinsic value of phosphates that meat has is around 4500 and 5000 ppm (Flores, 2001) and, in Spain, the maximum amount of phosphate permitted is 5000 ppm. Therefore, the use of different concentration of phosphates in the manufacturing of cooked hams might explain the differences found in this study. On the other hand, taking into account that in the manufacture of organic cooked ham the addition of phosphate is not allowed, the differences among the three commercial organic brands may be due to the use of different raw meat.

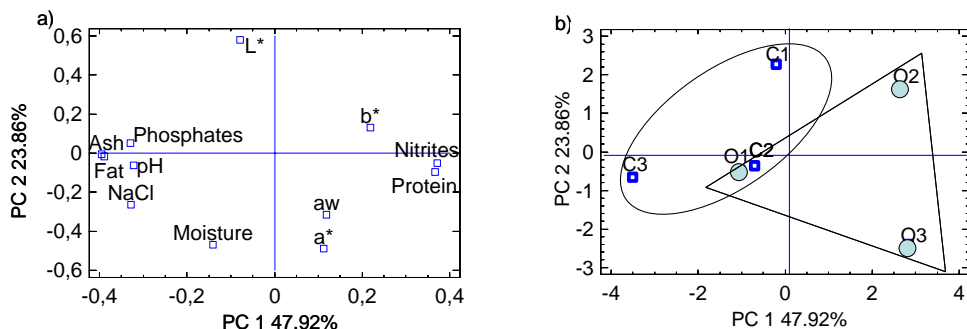
**Table 2. Additives content and colour values obtained in cooked ham (conventional and organic)**

		NaCl (%)	Nitrites (ppm)	Phosphates (ppm)	L*	a*	b*
Conventional	C1	1.7	2.1	<sup>c</sup> 7058	65.9	7.6	<sup>b</sup> 9.0
	C2	2.0	3.3	<sup>a</sup> 5018	59.8	8.1	<sup>a</sup> 5.8
	C3	2.0	1.5	<sup>d</sup> 9742	60.4	10.1	<sup>ab</sup> 6.5
Organic	O1	2.1	3.3	<sup>a</sup> 5087	59.8	9.9	<sup>ab</sup> 8.0
	O2	1.4	5.5	<sup>a</sup> 4968	63.4	9.1	<sup>ab</sup> 7.8
	O3	1.7	4.6	<sup>b</sup> 5772	54.6	12.1	<sup>b</sup> 8.6

a,b,c,d Means within the same column with different letters differ significantly ( $p < 0.05$ ).

Considering colour, only were found differences for b\* ( $p < 0.05$ ) among the conventional commercial cooked hams. The use of different sorts of ingredients and additives in the manufacture of this product or even the use of different raw material, could explain these differences. The L\*, a\* and b\* values were within the range reported by the others authors with regard to this product (Casiraghi *et al.*, 2007)

Finally, the parameters evaluated were subjected to PCA in order to determine the relationships between the different cooked hams. The plot of the two first principal components (Fig 1) explained 71.78% of the total variance. The first component (PC1) explained 47.92% of the variance. The loading plot for PC1 showed that a<sub>w</sub>, a\* values, protein and nitrite had a positive loading, whereas the rest of the parameters evaluated had a negative loading. The second PC (PC2) described 23.6% of the variance, where only L\* and b\* values and phosphate content had a positive loading. The samples distribution on the two first PC plot did not allow clearly separate the two groups. However, differences between them became apparent with respect to PC1. Conventional cooked hams were located on the negative side of the PC1, whereas organic cooked hams were grouped on the positive loading of the PC1. The conventional cooked hams may be distinguished by its phosphate content.



**Fig. 1. Loading plot (a) and score plot (b) after principal component analysis of characteristic of cooked ham analysed, conventional (C1,C2,C3) and organic (O1,O2,O3).**

## IV – Conclusions

On basis of these results, the physico-chemical parameters evaluated did not allow establish clear differences between conventional and organic commercial cooked hams.

## Acknowledgments

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# Gochu Asturcelta semiextensive production: Carcass and meat quality and fatty acid profile

C. Vieira\*, B. Martínez\*, A.M. Fernández\*, C.I., Sánchez, B. Rubio\* and J. Menéndez\*\*

\*Consejería de Agricultura y Ganadería, Junta de Castilla y León, ITACyL, Estación Tecnológica de la Carne, Guijuelo, Salamanca (Spain)

\*\*Asociación de Criadores del Gochu Asturcelta (ACGA) (Spain)

**Abstract.** The breeders association of Gochu Asturcelta (ACGA), has recovered Asturcelta pig breed. The aim of this work is to characterize the carcass and meat produced. Sixteen carcasses were used and carcass weight, dressing percentage and fatness were assessed. Likewise, meat quality parameters as pH, colour ( $L^*$ ,  $a^*$ ,  $b^*$ ), proximal chemical composition, fatty acid profile, water holding capacity, instrumental texture (Warner-Braztler) and sensory analysis were performed. Average carcass weight and dressing percentage were  $180 \pm 30$  kg and  $79 \pm 3\%$  respectively. Thickness subcutaneous fat was higher than common modern breeds ( $44 \pm 9$  mm 6th and  $54 \pm 6$  mm *gluteus medius*). Meat colour measurements revealed lower  $L^*$  and higher  $a^*$  values than common commercial breeds. Chemical composition revealed a high intramuscular fat content ( $9.2 \pm 3.5\%$ FM), but fatty acid profile showed a higher content in MUFA than common modern breeds. Water holding capacity was higher than commercial hybrids. Instrumental texture and sensory analysis show that this meat could be defined as tender, juicy and with good palatability.

**Keywords.** Pork – Carcass – Meat – Fatty acids.

## **Production semi-extensive du Gochu Asturcelta : Qualité de la carcasse et de la viande et profil en acides gras**

**Résumé.** L'association des éleveurs Gochu Asturcelta a repris l'élevage de porc Asturcelta. L'objectif de ce travail est de caractériser la carcasse et la viande de ce produit. Seize carcasses ont été utilisées et le poids de la carcasse, le rendement vrai et l'engraissement ont été établis. De même, les paramètres de qualité de la viande comme le pH, la couleur ( $L^*$ ,  $a^*$ ,  $b^*$ ), la composition chimique proximale, le profil en acides gras, la capacité de rétention d'eau, la texture instrumentale (Warner-Braztler) et l'analyse sensorielle ont été étudiés. Le poids de la carcasse et le rendement vrai étaient de  $180 \pm 30$  kg et de  $79 \pm 3\%$ . L'épaisseur de la graisse sous-cutanée était plus élevée que chez les races commerciales plus communes ( $44 \pm 9$  mm 6th et  $54 \pm 6$  mm sur le *Gluteus medius*). La mesure de la couleur de la viande a révélé un  $L^*$  plus faible et  $a^*$  plus élevé que chez les races commerciales. La composition chimique a montré une graisse intramusculaire élevée ( $9,2 \pm 3,5\%$ ) et le profil en acides gras a montré une quantité plus élevée de MUFA que la moyenne. La capacité de rétention d'eau était plus élevée que chez les races commerciales. La texture instrumentale et sensorielle montre que la viande peut être définie comme tendre, juteuse et savoureuse.

**Mots-clés.** Porc – Carcasse – Viande – Profil en acides gras.

## **I – Introduction**

The changes at world meat markets over the past decade and the improvement in the educational and economical conditions of most consumers have increased the demands in meat they consume. As a consequence, consumers are searching for meat that has characteristics that differ from the most commonly consumed meat. Today, consumers are better informed and more concerned about genetic reserve, production systems and the environment and animal welfare requirements (Fortina *et al.*, 2005; Meinert *et al.*, 2008).

Under this framework, the breeders association of Gochu Asturcelta (ACGA), supported by Principado de Asturias, has recovered Asturcelta pig breed, through a genetic selective program carried out by SERIDA. This Spanish local breed, originated from ancient Celtic breed, characterized by medium-sized and rusticity, is located in the North of Spain. In this area, the natural constraints from the climate and the geography seem to be unfavourable to mass meat production at low cost; consequently, the use of natural sources could be profitable to rear these animals. To produce a high quality meat, however, it is necessary to evaluate variables related to animal production, such as genetic and management properties, as well as variables associated with the processing of meat. In this sense, breed is an important factor that might influence the characteristics of the finished product; therefore, it is presumable that carcass and meat obtained could be different from the most common pork commercialised. Thus, this meat could become an appreciated product that could be sold at high prices in specialty markets and restaurants, and could be considered a profitable alternative for achieving the objective of sustainable meat production, meeting at the same time the European Union requirements about production system, extensification and territory use to contribute to the livelihood of rural population. The aim of this work was to describe the carcass and meat characteristics of Gochu Asturcelta breed.

## II – Materials and methods

Sixteen carcasses of Gochu Asturcelta breed were used. Carcass weight, dressing percentage and backfat thickness (6th rib and M. *gluteus medius* level) were assessed. After the carcasses were cooled for 24 h at 4°C, the M. *longissimus thoracis* muscle between the 6th and 11th ribs was removed from the left carcass side and divided in portions to analyze meat quality.

In this sense, the following meat quality parameters were analyzed: pH, muscle colour (L\*, a\*, b\*) using the m. *longissimus thoracis* at the 6th rib, proximal chemical composition (moisture, protein and fat content), fatty acid profile, water holding capacity (cooking losses) and instrumental texture (Warner-Bratzler). Likewise, in order to perform sensory analysis (descriptive profile), loins were sliced in steaks about 2 cm thick and cooked in double-plate grill (preheated at 220°C for 10 minutes) to an internal temperature of 70°C. After cooking, each steak was wrapped in aluminium foil and kept hot until the time of assessment. Using a increasing 5-points scale, a trained eight-member sensory panel was asked for the following sensory characteristics: fresh colour, cooked colour, odour liking, tenderness, juiciness, flavour liking and overall liking.

Results are shown as means and standard deviations for each parameter.

## III – Results and discussion

Carcass weight, carcass yield, thickness subcutaneous fat and ultimate pH are reported in Table 1. Average carcass weight and dressing percentage agreed to age at slaughter (14-18 months), and are within values commonly observed when modern hybrids are slaughtered at this age.

**Table 1: Carcass characteristics of Gochu Asturcelta breed**

Parameter	Average	Standard deviation
Carcass weight (kg)	180.4	30.2
Carcass yield (%)	79.4	3.4
Backfat thickness, 6 <sup>a</sup> rib level (mm)	44.0	9.0
Backfat thickness, G. <i>medius</i> (mm)	54.6	6.0
pH24 M. <i>longissimus thoracis</i>	5.78	0.17

Backfat thickness measurements were more closely related with Iberian pig values than with common commercial hybrids (Ramírez and Cava, 2007).

Regarding pH values, although average values were in the normal range, some animals showed pH values closed to the threshold of DFD meat, thus, the values were above those observed in commercial hybrids, but this values are in the line of other studies with local European breeds, who proposed an effect of muscular fibre type and an incomplete post mortem glycolysis, caused by a large of number of genetic factors, as hyposthesis to explain such results (Fortina *et al.*, 2005; Ryu *et al.*, 2008).

Table 2 summarizes nutritional characteristics of meat from Gochu Asturcelta. Intramuscular fat content was higher than average values observed in commercial hybrids. These data, even slightly higher than those obtained in other rustic local breeds (Fortina *et al.*, 2005; Gil *et al.*, 2008; Ryu *et al.*, 2008) confirm that these breeds are prone to adipogenesis.

**Table 2: Moisture, fat, protein and fatty acid profile (summary) of Gochu Asturcelta meat**

Parameter	Average	Standard deviation
Moisture (%)	69.1	2.9
Fat content (% FM)	9.2	3.5
Protein content (% FM)	21.4	0.9
Saturated fatty acid (%)	36.8	1.1
Monounsaturated fatty acid (%)	55.9	1.5
Polyunsaturated fatty acid (%)	7.3	1.1

Regarding fatty acid composition, several research studies involving different local Italian breeds (Fortina *et al.*, 2005; Pugliese, 2004; Meinert *et al.*, 2008) reported higher saturated, lower monounsaturated and higher polyunsaturated fatty acid percentages than those observed in our trial. These results could be explained by the different feeding system used. In that regard, in mentioned studies a common diet based on concentrates were offered to animals, whilst, in the present study, the feeding strategy included also concentrates but free access to roughage and some fruits such chestnuts. On the other hand, the lowest content in polyunsaturated fatty acid could be caused by the dilution effect of phospholipids rich in these fatty acids due to a high content of intramuscular fat.

The meat quality instrumental parameters and also the scores of sensory parameters given to Astrucelta pig meat are reported in Table 3. Lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) indicate that Asturcelta pig breed provide darker, redder and yellower meat than commercial breeds such as Landrace, or Large White. In this sense, Gil *et al.*, (2008) reported values ranged 46.0-48.0 for  $L^*$ , 2.7-3.0 for  $a^*$  and 3.6-4.3 for  $b^*$  for these breeds. Likewise, water holding capacity measured as cooking losses was higher than commercial hybrids. This result could be related with the high content of intramuscular fat, as has been reported in other studies comparing different pig breeds (Pugliese *et al.*, 2004; Gil *et al.*, 2008; Meinert *et al.*, 2008)

Regarding Warner-Braztler shear force, values obtained corresponded with tender meat, although the meat studied was not aged, in consonance with the common practice in the pork industry, because only a slight improvement of tenderness is obtained after ageing in pork meat (Meinert *et al.*, 2008). However, our shear force values are lower than those observed in other studies (Pugliese *et al.*, 2004; Meinert *et al.*, 2008). In this sense, several factors are known to affect tenderness, such as connective tissue, intramuscular fat, which have a major impact (Wood *et al.*, 2004; Meinert *et al.*, 2008; Crawford *et al.*, 2010), thus, the higher content of intramuscular fat observed in our study, could have led to more tender meat.

Sensory evaluation provided medium to high scores for the majority of attributes assessed. The raw and cooked meat colour was scored as darker respect to conventionally commercial pork meat, which is consistent with colorimetric measurements. The scores given to pork meat studied (around 3.5 points in a 5 points scale), indicated that Asturcelta pig breed provides meat with sensorial values slightly above respect to the average.

**Table 3. Colorimetric parameters (L\*, a\*, b\*), instrumental texture (Warner-Braztler test) water holding capacity (cooking losses) and sensorial analysis (5 points scale) of Gochu Asturcelta meat**

Parameter	Average	Standard deviation
Lightness (L*)	45.9	4.6
Redness index (a*)	6.7	1.7
Yellowness index (b*)	9.0	1.8
Warner-Braztler (kg)	4.3	1.4
Cooking losses (%)	15.2	3.2
Sensorial analysis		
Colour intensity (raw meat)	3.9	0.7
Colour intensity (cooked meat)	3.0	0.7
Odour liking	3.4	0.4
Tenderness	3.2	0.6
Juiciness	3.2	0.5
Flavour liking	3.6	0.3
Overall liking	3.3	0.4

## IV – Conclusions

Asturcelta pig breed provide heavier and fatter carcasses than commercial breeds. Meat obtained is characterized by high fat content, with higher percentage of monounsaturated fatty acids than commercial breeds. Instrumental measurements and sensory analysis, show that this meat could be defined as tender, juicy and with good palatability.

## Acknowledgments

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# Carcass performance of fresh meat pieces: sirloin, "presa" and "secreto" in Iberian pig finished at montanera

R.C. León Ramírez<sup>1</sup>, V. Rodríguez-Estévez<sup>1</sup>, H. Galán Soldevilla<sup>2</sup>, J.L. López Rivero<sup>3</sup> and M. Rodríguez Sánchez<sup>1</sup>

\*Departamento de Producción Animal

\*\*Departamento de Bromatología y Tecnología de los Alimentos

\*\*\*Departamento de Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Córdoba, Campus Universitario de Rabanales, Córdoba (Spain)

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**Abstract.** Cured products (hams, shoulders and loins) from Iberian pig finished at montanera (free-range with diet based on grass and *Quercus* acorns) rise a high value in the market. Nevertheless other pieces are becoming very valued by consumer of fresh meat. 191 Iberian pigs (Silvela variety) with an average weigh of  $163.87 \pm 11.47$  kg at slaughter (average carcass weigh =  $133.95 \pm 9.83$  kg) have been studied to obtain information about three fresh meat pieces: sirloin (*m. Iliopsoas*, and *m. Psoas minor* muscles), "secreto" (*m. Latissimus dorsi* and *m. Trapezius pars cervicalis* muscles) and "presa" (*m. Serratus ventralis thoracis* and *M. Serratus ventralis cervicis* muscles). The weighs and percentage of carcass of these pieces are: sirloins ( $0.61 \pm 0.01$  kg and  $0.46\% \pm 0.004$ ), "presa" ( $1.14 \pm 0.01$  kg and  $0.85\% \pm 0.009$ ) and "secreto" ( $0.44 \pm 0.01$  kg and  $0.33\% \pm 0.003$ )

**Keywords.** Iberian pig – Montanera – Carcass – Sirloin – Presa – Secreto.

**Rendement de pièces de haute valeur économique chez le porc Ibérique de montanera: le filet, la "presa" et le "secreto"**

**Résumé.** En plus des pièces qu'on prépare traditionnellement par maturation (le jambon sec, l'épaule sèche et le dos sec), il y a d'autres pièces d'un intérêt spécial chez le porc Ibérique de montanera, dû au prix et au prestige qu'elles atteignent sur le marché. 191 porcs Ibériques de la souche Silvela d'un poids moyen de  $163,87 \pm 11,47$  kg à l'abattage (poids moyen de la carcasse =  $133,95 \pm 9,83$  kg) ont été utilisés dans ce travail, qui décrit les performances de trois de ces pièces: le filet (*m. Iliopsoas*, *m. Psoas minor*), le "secreto" (*m. Latissimus dorsi* et *m. Trapezius pars cervicalis*) et la "presa" (*m. Serratus ventralis thoracis* et *m. Serratus ventralis cervicis*). Les résultats nous indiquent que les poids et les performances de la carcasse de porc Ibérique engraisé en montanera sont les suivants: pour les filets ( $0,61 \pm 0,01$  kg et  $0,46\% \pm 0,004$ ), pour la "presa" ( $1,14 \pm 0,01$  kg et  $0,85\% \pm 0,009$ ) et dans le cas du "secreto" ( $0,44 \pm 0,01$  kg et  $0,33\% \pm 0,003$ ).

**Mots-clés.** Porc Ibérique – Montanera – Carcasse – Filet – Presa – Secreto.

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## I – Introduction

Iberian pigs fattened in *montanera* (free-range fattening phase with diet based on *Quercus* acorns and grass) produce the most recognized quality products from the *dehesa* (grasslands on cleared Mediterranean forest) and these rise a high value in the market by virtue of it characteristic flavour and their high content in unsaturated fats, as a healthy food.

The most studied and known pieces are those for cured products: hams, shoulders and loins. Nevertheless other pieces are becoming very valued by consumer of fresh meat (Forero Vizcaino, 2002; Gómez-Nieves and Robina, 2003). However, traditionally during the slaughtering only sirloins were cut to be sold as a pork piece and others were included in the

meat to make traditional Spanish spice-cured sausages, with a lower price in market. Consequently, in order to have a better known and to prevent fraud to consumer, it is important to describe these particular pieces that are being commercialized as fresh meat.

This study is about three of this fresh meat pieces: sirloin (*M. Iliopsoas*, and *M. Psoas minor* muscles), "secreto" (*M. Latissimus dorsi* and *M. Trapezius pars cervicalis* muscles) and "presa" (*M. Serratus ventralis thoracis* and *M. Serratus ventralis cervicis* muscles). These three pieces have been chosen because their increasing interest for consumers and their price in markets (around 15 €/kg). A better known of Iberian pig carcass should contribute to increase its commercial value.

## II – Materials and methods

### 1. Animals

This study was conducted with 120 purebred Iberian pigs (males and females) of the Silvela variety fattened in a *dehesa* of evergreen oaks (*Quercus ilex rotundifolia*) and sacrificed with  $163.87 \pm 11.47$  kg after  $\geq 2$  months of *montanera*. All pigs were castrated following the Spanish regulations, to work with the same kind of pigs of the traditional *montanera* system.

After slaughtering the carcasses were stored to chill them at 4°C during 2 hours to cut these later following the traditional system for Iberian pig.

### 2. Measures and analysis

All the pieces from the carcasses were weight in a scale with a precision of  $\pm 5$  g. The results and values in this study are averages and percentages of the addition of the two pieces of each animal.

SPPS 11.5© was used for statistical analysis (mean  $\pm$  standard error) and Pearson correlations.

## III – Results and discussion

Table 1 shows the carcasses performance with an average weight of  $133.95 \pm 9.83$  kg. The values of weigh and percentages of sirloin, "presa" and "secreto" were respectively:  $0.61 \pm 0.01$  kg and  $0.46\% \pm 0.004$ ,  $1.14 \pm 0.01$  kg and  $0.85\% \pm 0.009$ , and  $0.44 \pm 0.01$  kg and  $0.33\% \pm 0.003$ . The three pieces together represent  $1.64 \pm 0.012$  % of the carcass weight.

**Table 1. Weigh and percentages of sirloins, presas and secretos (two from every carcass)**

	Carcass		Sirloins		"Presas"		"Secretos"		Sirloins, "presas" and "secretos"
	(kg)	(%)	(kg)	(%)	(kg)	(%)	(kg)	(%)	(%)
Mean	133.95	81.53	0.61	0.46	1.14	0.85	0.44	0.33	1.64
Standard error	0.71	0.16	0.01	0.004	0.01	0.009	0.01	0.003	0.012
Minimum value	111.80	76.99	0.40	0.28	0.87	0.66	0.23	0.19	1.24
Maximum value	197.00	89.62	0.89	0.65	2.13	1.67	0.70	0.50	2.45
Percentiles									
25	127.30	80.4238	0.56	0.42	1.05	0.79	0.40	0.30	1.55
50	133.70	81.2607	0.60	0.46	1.13	0.84	0.45	0.33	1.63
75	139.30	82.4608	0.65	0.49	1.20	0.90	0.50	0.36	1.73

Table 2 shows the Pearson correlations. The weights of the three pieces are inversely correlated ( $P < 0.01$ ). However, although these three pieces weights are positively correlated with carcass weight, the correlation between the carcass percentage of these pieces and the carcass weight is negative.

**Table 2. Pearson correlation for carcass weight and performance and different pieces**

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Carcass percentage	0.06							
(2) Sirloins (kg)	0.32 **	0.16						
(3) "Presas" (kg)	0.21 **	0.13	0.24 **					
(4) "Secretos" (kg)	0.41 **	-0.21 *	0.27 **	0.12				
(5) Sirloins (%)	-0.19 **	0.15	0.86 **	0.13	0.06			
(6) "Presas" (%)	-0.28 **	0.10	0.06	0.88 **	-0.09	0.20 **		
(7) "Secretos" (%)	-0.05	-0.25 **	0.13	0.02	0.89 **	0.15 *	0.04	
(8) Sirloins. "presas" and "secretos" (%)	-0.30 **	0.05	0.40 **	0.74 **	0.22 **	0.57 **	0.87 **	0.39 **

(\*\*)  $P < 0.01$ ; (\*)  $p < 0.05$ .

## IV – Conclusions

Sirloins. *presas* and *secretos* from Iberian pigs represent a low percentage of carcass. Hence, having in mind that these are becoming more popular and reach a higher price than other pork products, these should be well described to avoid fraud to consumers.

## Acknowledgements

The authors wish to acknowledge to Turcañada S. L. and Camilo Rios S.L their collaboration.

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# Leaf lard and backfat thickness relation at slaughter in pure breed Iberian pigs finished at montanera

R.C. León Ramírez\*, V. Rodríguez-Estévez\*, C. Díaz Gaona\* and M. Rodríguez Sánchez\*

Departamento de Producción Animal, Facultad de Veterinaria, Universidad de Córdoba,  
Campus Universitario de Rabanales, Córdoba (Spain)

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**Abstract.** Leaf lards (fat deposit surrounding the kidneys) is the most valued fat from Iberian pig. 120 pure breed Iberian pigs, Silvela variety, finished at *montanera* (free-range with diet based on grass and *Quercus* acorns) with mean slaughter weigh of  $162.53 \pm 1.71$  kg have been studied to know if it possible to relate leaf lards weigh ( $llw = 5.15 \pm 0.05$  kg) with backfat thickness (bf), measured at two different levels: last thoratic vertebra level ( $bflt = 6.67 \pm 0.06$  cm) and fourth lumbar vertebra level ( $bf4l = 9.45 \pm 0.08$ ). Linear regression models for each level ( $llw = 2.36 + 0.46 \cdot bflt$ ) and ( $llw = 6.24 + 0.117 \cdot bf4l$ ) have a low  $R^2$  value, hence bf thickness is not related with llw, and consequently these fattening measures are not reliable to estimate leaf lards weigh.

**Keywords.** Iberian pig – Montanera – Back fat – Leaf lard.

**Rapport entre le poids des crêpines (tissu adipeux périrénal) et l'épaisseur de graisse dorsale à l'abattage chez le porc Ibérique à finition montanera**

**Résumé.** La graisse extraite des crêpines (tissu adipeux périrénal) est la graisse la plus estimée de celles qu'on obtient du porc Ibérique. À partir de 120 porcs Ibériques de la souche Silvela à finition montanera, avec un poids moyen à l'abattage de  $162,53 \pm 1,71$  kg, on étudie le rapport possible entre le poids des crêpines ( $llw = 5,15 \pm 0,05$  kg) et l'épaisseur de graisse dorsale (bf) au niveau de la dernière vertèbre thoracique ( $bflt = 6,67 \pm 0,06$  cm) et de la quatrième vertèbre lombaire ( $bf4l = 9,45 \pm 0,08$ ). Les modèles de régression obtenus pour chacun des niveaux: vertèbre thoracique ( $llw = 2,36 + 0,46 \cdot bflt$ ) et quatrième vertèbre lombaire ( $llw = 6,24 + 0,117 \cdot bf4l$ ) ne sont pas utiles pour estimer d'une façon fiable llw à partir du bf, parce qu'elles présentent toutes les deux une valeur de  $R^2$  très faible. Par conséquent, l'épaisseur de graisse dorsale comme mesure d'engraissement n'a pas de rapport avec le poids des crêpines.

**Mots-clés.** Porc Ibérique – Montanera – Crêpines – Graisse dorsale.

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## I – Introduction

Iberian pigs fattened in *montanera* (free-range fattening phase with diet based on *Quercus* acorns and grass) produce the most recognized quality products from the *dehesa* (grasslands on cleared Mediterranean forest). Cured hams and shoulders obtained from these free-range pigs have gained widespread consumer acceptance and a high commercial value by virtue of its characteristic flavour; also, the high content in unsaturated fats of the ham has increased its appreciation as a healthy food. This rearing regime determines the fatty acid composition of pig fat in terms of four main fatty acids: oleic, linoleic, palmitic and stearic (Alonso *et al.*, 2008).

This production is geographically reduced to the South West of the Iberian Peninsula, and mast of acorns is limited to fall and winter seasons. The leaf lard from these pigs is the most estimated lard in the market (Forero Vizcaíno, 2002).

In this study it has been analyzed the relation between the back fact thickness and the weight of the leaf lards in Iberian pigs in order to know if the back fact thickness could predict the weight

of the leaf lards, as method of measuring the greasing degree of the carcasses (Edwards *et al.*, 1992; Medel and Fuentetaja, 2000).

## II – Materials and methods

This study was conducted at a *dehesa* of evergreen oaks (*Quercus ilex rotundifolia*) with 120 purebred Iberian fattening pigs (male and female) of the Silvela variety. Pigs were on average  $111.8 \pm 0.7$  kg of LW at the start of the study and  $162.53 \pm 1.71$  kg at the end, after  $\geq 2$  months ( $69.90 \pm 0.45$  days). All pigs were castrated following the Spanish regulations, to work with the same kind of pigs of the traditional *montanera* system. The stocking rate (0.76 pigs/ha) was established with margins that guaranteed that the acorns would not run out before the fattening was completed (Rodríguez-Estévez *et al.*, 2007, 2008). The carcasses were cut according Iberian pig traditional pork industry.

The leaf lards were weighed individually after slaughtering, and the backfat thickness was measured at two different levels: last thoracic and fourth lumbar vertebra level.

SPSS 11.5© was used for statistical analysis (mean  $\pm$  standard error) and linear regression models.

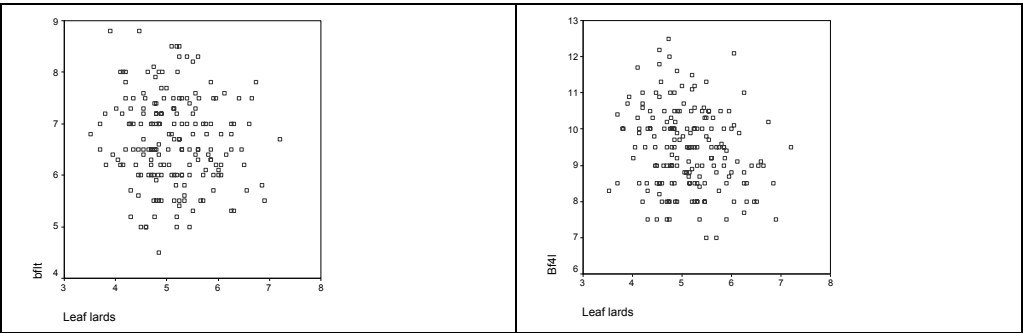
## III – Results and discussion

The leaf lards weighed  $5.15 \pm 0.05$  kg and the back fat thicknesses were:  $6.67 \pm 0.06$  cm at last thoracic vertebra level and  $9.45 \pm 0.08$  cm at fourth lumbar vertebra level.

Table 1 shows the linear regression models for each level; according to the low  $R^2$  values these are not suitable for predictions. Figure 1 represents the weigh of leaf lards and the back fat thicknesses and it is possible to observe the data dispersion.

**Table 1. Linear regression models for leaf lard weighs**

Independent variable	Linear regression	$R^2$	Standar Error	Sig.
Back fat thickness at last thoracic vertebra level (bflt)	$= 2.36 - 0.46 * bflt$	0.342	0.709	0.000
Back fat thickness at fourth lumbar vertebra level (bf4l)	$= 6.23 - 0.117 * bf4l$	0.034	0.69878	0.013



**Fig. 1. Regression graphics of leaf lards weighs (kg) and back thickness (cm) at last thoracic vertebra level (bflt) and fourth lumbar vertebra level (bf4l).**

## IV – Conclusions

The backfat thickness measures at different levels as a fattening measure are not reliable to estimate leaf lard weights in Iberian pig.

## Acknowledgements

The authors wish to acknowledge to Turcañada S. L. and Camilo Rios S.L. their collaboration.

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# Lipids performance of biopsies from the subcutaneous back tissue of Iberian pig along montanera fattening period

R.C. León Ramírez, V. Rodríguez-Estévez, C. Díaz Gaona and M. Rodríguez Sánchez

Departamento de Producción Animal, Facultad de Veterinaria, Universidad de Córdoba,  
Campus Universitario de Rabanales, Córdoba (Spain)

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**Abstract.** Iberian pigs fattened at *montanera* (free-range fattening phase, with diet based on grass and *Quercus* acorns) products are the most recognized from *dehesa*, because their high contents in unsaturated fatty acids. Spanish regulation establishes an official method to separate commercial categories depending of fattening period feed. Fatty acid profile evolution during fattening period helps stockmen to organize grazing and to decide slaughter date. In this study 120 pure breed Iberian pigs have been studied using the "Spring loaded biopsy" method for *in vivo* biopsy; obtaining  $1.45 \pm 0.28$  g of tissue at the beginning of *montanera* (1<sup>st</sup> day) and  $1.26 \pm 0.68$  g one month later. Fat was extracted by microwave to obtain  $0.83 \pm 0.17$  g and  $0.73 \pm 0.23$  g from the first and second biopsy respectively. A regression model has been obtained to predict the amount of extracted fat knowing biopsies weigh ( $EF = -0.218 + 0.767 \cdot BW$  first biopsy and  $EF = 0.046 + 0.533 \cdot BW$  in the second). The amounts of fat obtained are enough for individual fatty profile analysis following the official methods of analysis.

**Keywords.** Iberian pig – Montanera – Back fat.

## **Rendement en gras des biopsies sous-cutanées du porc Ibérique en début et après un mois de montanera**

**Résumé.** Les produits des porcs Ibériques engraisés en montanera sont ceux qui atteignent une valeur commerciale plus élevée, ceci est dû, principalement, à leur teneur en acides gras insaturés. Actuellement, pour déterminer les catégories commerciales, la législation espagnole préconise la méthode de l'analyse du profil en acides gras de la carcasse. La connaissance de l'évolution du profil en acides gras pendant l'engraissement permet d'organiser le pâturage et de programmer les abattages. Dans ce travail, à partir de 120 porcs Ibériques purs, on étudie la quantité de tissu sous-cutané obtenu grâce à la méthode de biopsie *in vivo* "Spring loaded biopsy" ( $1,45 \pm 0,28$  g à l'arrivée en montanera et  $1,26 \pm 0,68$  g après un mois), et la quantité de graisse extraite par micro-ondes ( $0,83 \pm 0,17$  g dans la première biopsie, et  $0,73 \pm 0,23$  g dans la deuxième). On propose des modèles de régression qui nous permettent de connaître la quantité de graisse qu'on obtiendrait à partir du poids de la biopsie ( $EF = -0,218 + 0,767 \cdot BW$  dans la première biopsie et  $EF = 0,046 + 0,533 \cdot BW$  dans la deuxième). Les résultats indiquent que ces quantités de graisse sont suffisantes pour l'analyse individuelle du profil en acides gras.

**Mots-clés.** Porc Ibérique – Montanera – Graisse dorsale.

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## I – Introduction

The Iberian pig constitutes a breed of great economic importance in Spain and Portugal. In the traditional rearing system, pigs are free-reared in an expanse of land of variable area, using natural resources [mainly grass (*Quercus ilex*) and acorns (*Quercus suber*)] only. Cured ham obtained from free-range pigs has gained widespread consumer acceptance and a high commercial value by virtue of its characteristic flavour; also, the high content in unsaturated fats of the ham has increased its appreciation as a healthy food. The free-range rearing system departs considerably from the intensive farming regime, where pigs are confined and fattened with commercial feed. The Spanish official method for discriminating between pig feeding and

rearing regimes determines the fatty acid composition of pig fat in terms of four fatty acids (oleic, linoleic, palmitic and stearic).

The traditional husbandry system of the Iberian pig in the *dehesa* (cleared Mediterranean forest like savannah) is linked to the sustained use of the pasturelands, finishing pigs during the acorn mast-feeding (called *montanera*). Rodríguez-Estévez *et al.* (2009) suggest a daily DM intake of 3.1-3.6 kg acorn kernel and 0.38-0.49 kg grass, which is achieved thanks to the functional characteristics of this breed. Free range Iberian pigs fattened with acorns give the most recognized products from the *dehesa*; however this production is geographically reduced to the South West of the Iberian Peninsula Spain, and acorn production is limited to fall and winter seasons.

*Montanera* products attain the highest prices in the market because of preference by consumers. The main reason for this preference is the high oleic acid content in the fat of these pigs free-range fattened (Daza *et al.*, 2007), mostly due to the high oleic acid content of acorns. This high proportion of oleic acid in the carcass strongly influences the properties of fat, leading a soft and oily lard, which is highly appreciated by consumer, who select these products for special occasions and pays a high price for them (López-Bote, 1998). Moreover, the higher oxidative stability of this pork, because of high  $\alpha$ -tocopherol content of grass and  $\gamma$ -tocopherol of acorns improves technological and sensorial quality of Iberian pigs meat and products (Daza *et al.*, 2007).

To prevent fraud the Iberian pig Spanish regulations establish an official method to evaluate the finishing diet regime (MAPA, 2007), based on the analysis of the subcutaneous backfat tissue fatty acid profile.

The aim of this study is to know the lipid performance of biopsies from subcutaneous back tissue as source of fat to analyze the fatty acid profile evolution during the *montanera* fattening.

## II – Materials and methods

The experimental procedures and animal care conditions were approved by the Animal Experimentation Ethical Committee of the University of Córdoba, Spain.

### 1. Animals and handling

The study was conducted at a *dehesa* of evergreen oaks (*Quercus ilex rotundifolia*) with 120 purebred Iberian fattening pigs (male and female) of the Silvela variety. Pigs were on average  $111.8 \pm 0.7$  kg of LW at the start of the study and  $157.9 \pm 1.7$  kg at the end, after  $\geq 2$  months. All pigs were castrated following the Spanish regulations, to work with the same kind of pigs of the traditional *montanera* system. The stocking rate (0.76 pigs/ha) was established with margins that guaranteed that the acorns would not run out before the fattening was completed (Rodríguez-Estévez, 2007; Rodríguez-Estévez *et al.*, 2008)

### 2. Weighing of the pigs

To calculate body weigh (BW), all the pigs of the herd were weighed individually at the beginning of *montanera*, after a month and the day previous slaughtering. An electronic scale (precision of 100 g) was used for weighing.

### 3. Biopsy collection

The biopsies were taken the first day of *montanera* and one month later. The samples were collected *in vivo* with a *Spring loaded biopsy* instrument, following the method described by (Bosch Puig *et al.*, 2008). Once the sample was extracted, these samples were collected from the cannula and introduced in individual plastic tubes with the animal reference number, cleaning properly the cannula before taking the next sample.

All biopsies were transported at  $\approx 10^{\circ}\text{C}$  to the laboratory, where these were stored at  $-25^{\circ}\text{C}$ , until their processing.

#### 4. Lipid extraction

After completely defrosting each sample was weighed before using the technique of microwave oven described by De Pedro *et al.*, 1997 to extract the fat from these tissue biopsies. Later the fat obtained from each biopsy was weighed and frozen to store it for future analysis of fatty acid composition.

### III – Results and discussion

Descriptive statistics of weights of first and second biopsies (first day and one month later respectively) are showed in Fig. 1 and Table 1.

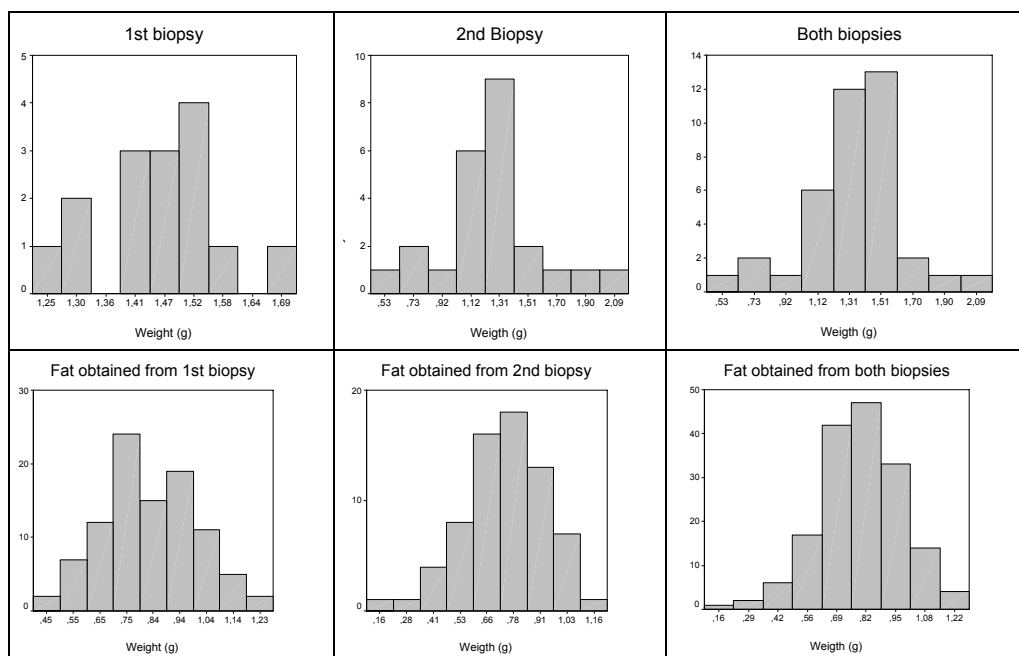


Fig. 1. Weigh of first, second and both biopsies and of fat obtained by microwave extraction.

Table 1. Biopsy weight and fat obtained in the 1<sup>st</sup> and 2<sup>nd</sup> biopsies

	1 <sup>st</sup> Biopsy Weight	Fat obtained	2 <sup>nd</sup> Biopsy Weight	Fat obtained	Both Biopsies Weight	Fat obtained
Mean $\pm$ SE	1.45 $\pm$ 0.28	0.83 $\pm$ 0.17	1.26 $\pm$ 0.68	0.73 $\pm$ 0. 23	1.33 $\pm$ 0.45	0.79 $\pm$ 0. 14
Minimum value	1.27	0.42	0.54	0.15	0.54	0.15
Maximum value	1.67	1.24	2.10	1.17	2.10	1.24
Percentile 25	1.42	0.74	1.08	1.08	1.21	0.68
Percentile 50	1.45	0.83	1.24	1.24	1.34	0.79
Percentile 75	1.52	0.93	1.38	1.38	1.51	0.91

**Table 2. Linear regression models for the weight of fat obtained from biopsies (BW = body weight)**

Effect variable	Linear regression	R <sup>2</sup>	Standard Error	Sig.
Fat obtained (first biopsy)	=-0,218+0,767*BW	0,419	0,102	0,009
Fat obtained (second biopsy)	=0,046+533*BW	0,665	0,128	0,009

As Table 2 shows, it is possible to determine the final fat amount through linear regressions either in the first biopsy (fat obtained = -0,218+0,767\*BW) or the second one (fat obtained = 0.046+533\*BW ).

Results show that it is possible to use this method of fat extraction, because it allows to obtain the necessary amount of fat to determine the fatty acid profile through different methods of analysis, as it is shown in Table 3.

**Table 3. Comparison of different analytical methods to determine the fatty acid profile**

Reference	Method of extraction of fat	Method of analysis	Needed amount of fat extracted
(Carrapiso <i>et al.</i> , 2001)	Dissection	Electronic nose	5 g (adipose tissue)
ISO 15304			250 mg
(Presidencia, 2004) (Oficial method)	Dissection and solvent or microwave extraction.	Gas Chromatography	0.2 g
(Alonso <i>et al.</i> , 2008)	Not specified	Ion Mobility Spectrometry	1 g
(López-Vidal <i>et al.</i> , 2008)	Microwave	Gas Chromatography - Mass Spectrometry and Chemometrics	0.2 g
(Arce <i>et al.</i> , 2009)	Microwave	Infrared spectroscopy	A few microlitres
(Pascual <i>et al.</i> , 2006)	Folch, Lee and Stanley (1957)	Gas Chromatography	0.5 g
(Regueiro <i>et al.</i> , 2006)	ISO-1443 (Soxhlet) Folch, Lee and Stanley (1957) Bligh and Dyer (1959) Accelerated extraction methods Hexano-2, propanol 2:1	Gas Chromatography (FID) -Mass Spectrometry and Chemometrics	

## IV –Conclusions

The combination of the *Spring loaded biopsy* and microwave fat extraction could be used to predict and to categorize animals before finishing and during the *montanera* in order to determine their handling and their slaughter date. It is possible to obtain *in vivo* biopsies and the amounts of fat obtained are enough for individual fatty acid profile analysis following the official methods of analysis.

## Acknowledgements

The authors wish to acknowledge to Turcañada S. L. and Camilo Rios S.L their collaboration.

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# Sex influence in carcass (hams and shoulders) performance of Iberian pigs fattened at montanera

R.C. León Ramírez, V. Rodríguez-Estévez, C. Díaz Gaona and M. Rodríguez Sánchez

Departamento de Producción Animal, Facultad de Veterinaria, Universidad de Córdoba,  
Campus Universitario de Rabanales, Córdoba (Spain)

**Abstract.** Sex factor (castrated males vs castrated females) has been studied in relation with montanera fattening (free-range with diet based on grass and *Quercus* acorns) and carcass performance (pieces which will be transformed by dry-curing process: hams, shoulders and loin) of 81 pure Iberian pigs (Silvela variety) with an average slaughter weigh of  $167.18 \pm 1.08$  kg. The results show that males are more efficient. Results show that fresh hams and shoulders from males before and after cutting are heavier than from females (hams: before 13.80 vs 13.29 kg and after cutting 10.78 vs 10.43 kg; and shoulders: before 9.40 vs 8.88 kg and after cutting 7.19 vs 6.79 kg), and that, after a year of curing process, cured shoulders and hams from males are significantly heavier (cured shoulders: 5.31 vs 4.98 kg; cured hams 8.42 vs 8.19 kg). By other side dry-curing process produces a significant bigger weight loss in female shoulders (26.73 vs 26.14 %) and in male hams (2.41 vs 2.30 kg). In conclusion it is of higher interest to reserve montanera pastures to finish preferment males.

**Keywords.** Iberian pig – Montanera – Ham – Shoulder – Sex.

## **Différences de rendement selon le sexe pour la carcasse du porc Ibérique de montanera : le jambon et l'épaule**

**Résumé.** On étudie l'influence du facteur sexe (mâles castrés vs femelles castrées) sur le rendement de carcasses de pièces de haute gamme (jambons secs, épaules sèches et dos secs) sur 81 porcs Ibériques purs engraisés en montanera, de la souche Silvela, dont le poids moyen à l'abattage était de  $167,18 \pm 1,08$  kg. Les résultats indiquent que les jambons et épaules frais provenant des mâles avant et après le découpage pèsent nettement plus que ceux provenant des femelles (jambons: avant le découpage 13,80 vs 13,29 kg, et après 10,78 vs 10,43 kg ; épaules : avant le découpage 9,40 vs 8,88 kg, et après 7,19 vs 6,79 kg). Nous notons la même différence significative selon le sexe à la fin d'une année de vieillissement (jambons : 8,42 vs 8,19 kg ; épaules : 5,31 vs 4,98 kg). Le pourcentage de perte de poids dans le procédé de vieillissement est supérieur chez les femelles pour les épaules (26,73 vs 26,14 %) et chez les mâles pour les jambons (2,41 vs 2,30 kg). Nous en concluons donc qu'il est plus intéressant de réserver la montanera pour engraisser les mâles.

**Mots-clés.** Porc Ibérique – Montanera – Jambon – Épaule – Sexe.

## **I – Introduction**

Free-range finishing of Iberian pigs based on *Quercus* acorns and grass (called *montanera*) is a strategic production of the *dehesa* (grasslands on cleared Mediterranean forest) in the southwest of the Iberian Peninsula and it is very important for the economy of this agro ecosystem. The study of different factors in relation with efficiency is necessary to maximize profits, preserving traditional breeding and quality products (López-Bote, 1998).

Commercial value of Iberian pigs products in markets comes from his traditional breeding system: pure Iberian breed and free-range finishing, which give to their products (hams and shoulders) a high price because their organoleptic properties which make different from others (León Crespo, 1992).



There are different factors with influence on carcass performance: breed and variety, age, feeding regime, husbandry and sex. Traditionally Iberian pigs of both sexes are castrated while these are piglets to maintain pork quality and to avoid handling problems, as attracting wild boars.

This research has been conducted to study the sex factor (castrated males vs castrated females) in relation with carcass performance (hams and shoulders) of pure Iberian pigs (Silvela variety). To avoid other factors with influence, as variety (Clemente *et al.*, 2008) or pasture differences (Rodríguez-Estévez *et al.*, 2009), all the animals for this experience were of the same variety and were fattened in the same *dehesa* during the same *montanera*.

## II – Materials and methods

### 1. Animals

81 Iberian pigs (pure breed) of the Silvela variety were used (40 castrated males and 41 castrated females). Animals start *montanera* period with an average of  $343 \pm 8$  days of life and a weigh of  $113.5 \pm 3.5$  kg. All these pigs were bred under the same conditions and were finished during  $\geq 60$  days of *montanera*, until these gained the minimum traditional slaughter weigh of 161 kg (14 @), with an average slaughter weigh of  $167.18 \pm 1.08$  kg (Table 1). At the start of *montanera* there were no statistical differences for age and weight between males and females.

During the finishing the animals grazed in a *dehesa* of Fuente Obejuna (Córdoba-Spain) and their diet was based only on *Quercus* acorns grass.

### 2. Measures and analysis

Both hams and shoulders from every carcass were weighed in a scale with a precision of  $\pm 5$  g. Weights correspond to: (i) 24 h after slaughter before cutting; (ii) after cutting to prepare pieces for curing process; (iii) after 1 year of curing process. The results presented here correspond to mean, standard error, minimum and maximum of absolute values and percentages of the two pieces of each animal. SPSS® has been used to statistical analysis. Comparisons between males and females have been made by ANOVA.

## III – Results and discussion

Males and females showed different weight gain; hence these had different slaughter weight (Table 1) ( $P < 0.01$ ). The results show that fresh hams and shoulders from males before and after cutting were heavier than from females (hams: before 13.80 vs 13.29 kg and after cutting 10.78 vs 10.43 kg,  $P < 0.001$ , Table 2; and shoulders: before 9.40 vs 8.88 kg and after cutting 7.19 vs 6.79 kg,  $P < 0.001$ , Table 3). All the same, after a year of curing process, cured hams and shoulders from males were significantly heavier (cured hams 8.42 vs 8.19 kg,  $P < 0.001$ , Table 2; and cured shoulders: 5.31 vs 4.98 kg,  $P < 0.001$ , Table 3). By other side, the dry-curing process gave different weight losses for females and males; so weight losses of hams were higher for males (2.41 vs 2.30 kg,  $P < 0.05$ ) and weight losses of shoulders were higher for females (26.73 vs 26.14 %,  $P < 0.05$ ).

**Table 1. Body weight differences between males and females at slaughter ( $P < 0.01$ )**

Animals	Animals	Mean	Standard error	Minimum	Maximum
Males	40	167.18	1.08	146.10	190.60
Females	41	159.34	1.24	146.40	197.50
Total	81	163.21	0.88	146.10	197.50

**Table 2. Weights of hams according to sex**

		n	Average	Standard error	Minimum	Maximum
Hams before cutting***	Males	80	13.80	0.11	11.62	16.75
	Females	82	13.29	0.12	11.25	16.85
	Total	162	13.54	0.08	11.25	16.85
% of hams about carcass weight	Males	80	20.44	0.10	18.02	22.11
	Females	82	20.53	0.09	18.63	22.42
	Total	162	20.48	0.07	18.02	22.42
Hams after cutting***	Males	80	10.78	0.09	9.00	13.10
	Females	82	10.43	0.09	8.80	13.25
	Total	162	10.60	0.07	8.80	13.25
% of cut hams about carcass weight	Males	80	15.97	0.10	13.81	17.90
	Females	82	16.12	0.10	14.20	18.16
	Total	162	16.05	0.07	13.81	18.16
Weight after 1 year of curing***	Males	80	8.42	0.08	7.05	10.35
	Females	82	8.19	0.07	6.70	10.55
	Total	162	8.30	0.05	6.70	10.55
Weight loss after 1 year of curing (kg)	Males	80	2.41	0.02	2.00	2.95
	Females	82	2.30	0.03	1.51	3.05
	Total	162	2.35	0.02	1.51	3.05
% weight loss after 1 year of curing*	Males	80	22.41	0.17	17.39	25.98
	Females	82	22.01	0.23	14.60	26.87
	Total	162	22.21	0.14	14.60	26.87

Statistical differences: \* for  $P < 0,05$ ; \*\* for  $P < 0,01$ ; \*\*\* for  $P < 0,001$ .

**Table 3. Weights of shoulders according to sex.**

		n	Average	Standard error	Minimum	Maximum
Shoulder before cutting***	Males	80	9.40	0.09	7.43	11.42
	Females	82	8.88	0.07	7.85	11.00
	Total	162	9.13	0.06	7.43	11.42
% of shoulders about carcass weight	Males	80	13.91	0.09	12.29	15.93
	Females	82	13.73	0.08	12.38	15.72
	Total	162	13.82	0.06	12.29	15.93
Shoulder after cutting***	Males	80	7.19	0.07	5.95	9.35
	Females	82	6.79	0.07	5.75	8.75
	Total	162	6.99	0.05	5.75	9.35
% of cut shoulders about carcass weight	Males	80	10.64	0.09	9.11	12.99
	Females	82	10.50	0.08	8.96	12.69
	Total	162	10.57	0.06	8.96	12.99
Weight after 1 year of curing***	Males	80	5.31	0.06	4.35	6.90
	Females	82	4.98	0.05	4.20	6.65
	Total	162	5.14	0.04	4.20	6.90
Weight loss after 1 year of curing (kg)	Males	80	1.79	0.09	-5.05	2.45
	Females	82	1.73	0.08	-4.80	2.15
	Total	162	1.76	0.06	-5.05	2.45
% weight loss after 1 year of curing*	Males	80	26.14	0.20	21.92	29.69
	Females	82	26.73	0.18	21.71	30.27
	Total	162	26.44	0.14	21.71	30.27

Statistical differences: \* for  $P < 0,05$ ; \*\* for  $P < 0,01$ ; \*\*\* for  $P < 0,001$ .

## IV – Conclusions

In conclusion it is of higher interest to reserve the *montanera* (mast of acorns) preferentially to finish castrated males, because these gain more weight and have a highest carcass performance for the most valued pieces from Iberian pig: hams and shoulders.

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# Slaughter weight, carcass performance and backfat thickness relations with losses during the cutting process of hams and shoulders from Iberian pigs fattened at *montanera*

R.C. León Ramírez, V. Rodríguez-Estévez, C. Díaz Gaona and M. Rodríguez Sánchez

Departamento de Producción Animal, Facultad de Veterinaria, Universidad de Córdoba,  
Campus Universitario de Rabanales, Córdoba (Spain)

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**Abstract.** Cutting process of hams and shoulders from Iberian pigs is based in removing some muscle mass, fat and skin to obtain the traditional and standard commercial shape, which makes these different from other commercial cured pieces. There is a significant correlation ( $p < 0.01$ ) between backfat thickness (measured at two different levels: last thoracic vertebra  $6.67 \pm 0.87$  cm and 4th lumbar vertebra  $9.45 \pm 1.12$  cm) and weigh loss in hams and shoulders by cutting process ( $5.95 \pm 1.09$  kg and  $21.40\% \pm 3.32$  in hams vs  $4.30 \pm 0.61$  kg  $23.09 \pm 2.77\%$  in shoulders). Besides there is significant correlation ( $p < 0.05$ ) between ham weigh losses by cutting process and carcass performance, and between shoulder weigh losses by cutting process and slaughter and carcass weights ( $p < 0.01$ ).

**Keywords.** Iberian pig – Montanera – Back fat – Slaughter weigh – Carcass performance – Hams – Shoulder – Cutting process.

**Corrélations entre le poids à l'abattage, le rendement de la carcasse, l'épaisseur de graisse dorsale et les pertes au découpage pour les jambons et les épaules du porc Ibérique pur de "bellota"**

**Résumé.** Le processus de découpage consiste à éliminer une part de la musculature, de la graisse et de la peau, pour obtenir une pièce d'une forme et d'une taille précises, selon les standards commerciaux du jambon et de l'épaule Ibériques, ce qui fait qu'il est différent d'autres types de jambon. Il y a une corrélation significative ( $p < 0,01$ ) entre le poids perdu dans les jambons et les épaules par découpage ( $5,95 \pm 1,09$  kg et  $21,40\% \pm 3,32$  dans les jambons vs  $4,30 \pm 0,61$  kg et  $23,09 \pm 2,77\%$  dans les épaules) et l'épaisseur de la graisse dorsale au niveau de la dernière vertèbre thoracique ( $6,67 \pm 0,87$  cm. d'épaisseur) et de la quatrième vertèbre lombaire ( $9,45 \pm 1,12$  cm d'épaisseur). D'autre part, dans les jambons on constate une corrélation ( $p < 0,05$ ) entre le rendement de la carcasse et les pertes par découpage. Par ailleurs, il existe une corrélation positive ( $p < 0,01$ ) entre les pertes par découpage dans les épaules et le poids vif et le rendement et le poids de la carcasse.

**Mots-clés.** Porc Ibérique – Montanera – Graisse dorsale – Poids à l'abattage – Rendement de la carcasse – Jambons – Épaules – Découpage.

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## I – Introduction

The Iberian pig constitutes a breed of great economic importance in Spain and Portugal. Cured hams obtained from free-range pigs finished using natural resources (basically *Quercus* acorns and grass) has gained widespread consumer acceptance and a high commercial value by virtue of its characteristic flavour and their high content in unsaturated fats. This production is geographically reduced to the South West of the Iberian Peninsula during the fall and winter seasons.

After slaughtering the carcass, hams and shoulders are cut previously to the dry-curing process during a process called cutting (Forero Vizcaíno, 2002). This is based in removing some

muscle, fat and skin in hams and shoulders to obtain the traditional and standard commercial shape, which makes these different from other hams; it mainly is based in a section in V form in muscular and exterior face from the top of the ham until the hoke level (Gómez-Nieves And Robina, 2003)

By other side, the measuring of back fat thickness is the usual method to evaluate the fattening or greasing degree of the carcasses (Edwards *et al.*, 1992; Fortin, 1986; Mayoral *et al.*, 1999; Medel and Fuentetaja, 2000) and usually it is a complementary measurement with the carcass performance (carcass weigh in relation to animal weigh), in order to have more information about the final performance and rentability of different husbandry systems, varieties, strains and genetical lines of Iberian pigs (Ellis *et al.*, 1996; Latorre *et al.*, 2003).

This research has been conducted to know if there is any relation between the carcass performance and the backfat thickness with the weigh of losses during the cutting process of shoulders and hams from the Iberian pig carcass.

## II – Materials and methods

191 Iberian pigs (males and females) of the Silvela variety fattened in *montanera* were used. The animals were slaughtered after  $69.90 \pm 0.45$  days eating only acorns and grass, with a mean weigh of  $162.53 \pm 1.71$  kg according to Spanish regulations and standard commercial procedures. All pigs were castrated following the Spanish regulations, to work with the same kind of pigs of the traditional *montanera* system. The stocking rate (0.76 pigs/ha) was established with margins that guaranteed that the acorns would not run out before the fattening was completed (Rodríguez-Estévez *et al.*, 2008).

The backfat thickness was measured at two levels: last thoracic vertebra and fourth lumbar vertebra level. Hams and shoulders were individually weighed before and after the cutting process; All the pieces from the carcasses were weight in a scale with a precision of  $\pm 5$  g. The results and values in this study are averages and percentages for the addition of the two pieces of each animal.

SPSS 11.5© was used for statistical analysis (mean  $\pm$  standard error) and to model a linear regression.

## III –Results and discussion

The backfat thicknesses measured at the two levels were:  $6.67 \pm 0.87$  cm at the last thoracic vertebra and  $9.45 \pm 1.12$  cm at the fourth lumbar vertebra level. Table 1 shows hams and shoulders weighs and percentages before and after cutting process.

**Table 1. Hams and shoulders weighs and percentages before and after cutting process**

	Minimum value	Maximum value	Mean	Standard error
Fresh shoulders before cutting (kg)	14.90	24.00	18.81	1.46
Fresh hams before cutting (kg)	13.00	34.65	29.35	3.79
Fresh shoulders after cutting (kg)	11.10	18.40	14.33	1.25
Fresh hams after cutting (kg)	17.70	26.15	21.75	1.68
Weigh losses in hams (kg)	0.15	9.55	5.95	1.09
Weigh losses in shoulders (kg)	0.75	5.95	4.3	0.61
% Weigh losses shoulders	4.41	29.46	23.09	2.77
% Weigh losses ham	0.63	31.95	21.40	3.32

Table 2 shows that there is a significant correlation ( $p < 0.01$ ) between backfat thickness (measured at two different levels) and weigh losses in hams and shoulders during the cutting process ( $21.40\% \pm 3.32$  for hams and  $23.09 \pm 2.77\%$  for shoulders). Besides there are significant correlations between ham weigh losses during the cutting process and carcass performance ( $p < 0.05$ ), and between shoulder weigh losses during the cutting process and the slaughter and carcass weight ( $p < 0.01$ ).

**Table 2. Pearson correlations between weigh losses during the cutting process and back fat thickness at last thoracic vertebra (BFTult) and fourth lumbar vertebra levels (BFT4) and carcass weigh and performance**

	BFT4	BF Tult	Slaughter weigh	Carcass weigh	Carcass performance (%)
Weigh losses of hams during the cutting process	0.86(**)	0.75(**)	-0.04	0.02	0.21(*)
Weigh losses of shoulders during the cutting process	0.29(**)	0.21(**)	0.42(**)	0.51(**)	0.15

\*Correlation significance  $P < 0.05$ ; \*\* Correlation significance  $P < 0.01$ .

## IV – Conclusions

Iberian pigs finished at *montanera* present a positive correlation between the fattening degree of the carcass (measured as back fat thickness) and the weigh losses in hams and shoulders during the cutting process.

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The authors wish to acknowledge to Turcañada S. L. and Camilo Rios S.L their collaboration

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# Physico-chemical and sensorial characteristics evolution of vacuum packaged Iberian dry-cured ham stored at refrigerated temperature

M. Elias\*, A.C. Santos\*, M.J. Fraqueza\*\* and A. Barreto\*\*

\*Universidade de Évora, Escola de Ciências e Tecnologia e Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Apartado 94, 7002-554 Évora (Portugal)

\*\*Faculdade de Medicina Veterinária, CIISA, Universidade Técnica de Lisboa, 1300-477 Lisboa (Portugal)

**Abstract.** Two groups of five dry-cured hams from Alentejano pig breed submitted to a post-salting cure of 12 months were studied. One of the groups was processed through craft methods at Barrancos (small city of south-east Alentejo region, Portugal) and the other one in a Portuguese meat plant. Equal groups of slices and unitary portions weighing about 250 g were vacuum packed and stored at 7°C. Physico-chemical analysis (pH,  $a_w$ , chlorides, total volatile basic nitrogen, thiobarbituric acid index and colour ( $L^*$ ,  $a^*$ ,  $b^*$ ) and sensorial evaluation (colour, off colour, aroma, off aroma, tenderness, succulence, taste, off taste, salt intensity and global evaluation) were carried out before packaging (time 0) and after 2, 5 and 8 months of storage. Statistical analysis consisted in analysis of variance considering the factors "process" (artisanal and industrial), "sample presentation" (portions and slices) and "storage time" (0, 2, 5 and 8 months). Physico-chemical analysis exhibit differences, mainly for "process" factor and sensorial evaluation for "storage time" factor. However, results evidence desirable evolution of the samples during storage time. These study allow to conclude that under refrigeration (7°C) is possible to store vacuum packaged high quality dry-cured ham during, at least, 8 months.

**Keywords.** Alentejano dry-cured ham – Quality – Storage time – Vacuum packaged.

## *Évolution des caractéristiques physico-chimiques et sensorielles du jambon de porc Alentejano réfrigéré et conservé sous vide*

**Résumé.** Deux groupes de cinq jambons de cochon Alentejano avec 12 mois de maturation après salage ont été étudiés. Un des groupes a été traité selon un processus artisanal à Barrancos (sud-est du Portugal) et l'autre groupe a été transformé dans une unité industrielle. Des portions unitaires d'un poids approximatif de 250 g et des tranches de 1 mm d'épaisseur du même poids ont été emballées sous vide et entreposées à 7°C. Les analyses suivantes ont été effectuées : analyses physico-chimiques (pH,  $a_w$ , chlorures, azote basique volatil total, index d'acide thiobarbiturique, et couleur ( $L^*$ ,  $a^*$ ,  $b^*$ )) et sensorielles (couleur, couleurs étranges, arôme, arôme étrange, tendreté, teneur en jus, saveur, saveurs étranges, intensité de la salaison et évaluation globale) avant l'emballage (temps 0) et après 2, 5 et 8 mois de conservation. Le traitement statistique consistait en une analyse de la variance en considérant les facteurs "processus" (artisanal et industriel), "mode de présentation des échantillons" (portions et tranches) et "temps de conservation" (0, 2, 5 et 8 mois). L'évaluation physico-chimique a montré qu'il y avait des différences et l'analyse sensorielle a montré des différences, en particulier pour le facteur "temps de conservation". Cependant, les résultats ont montré une tendance positive dans les échantillons au cours de la conservation. Cette étude nous permet de déduire qu'il est possible de conserver des jambons de qualité emballés sous vide et maintenus à une température de 7°C pendant au moins 8 mois.

**Mots-clés.** Jambon de porc Alentejano – Qualité – Conservation – Emballage sous vide.

## I – Introduction

Traditional quality products process should be based on scientific and technological support in order to develop poor regions. Recently the production of dry cured ham of Alentejano pig breed



increased a lot after a long period of low production due to the reduction of pig production. Due to the high nutritional quality of products that are processed with this specific raw material, the dry cured ham has nowadays a high commercial value that obliges to a high sensorial quality standard. Attributes such as color, fat infiltrated proportion, aroma, textures, and taste are, among other, the most important for consumers and that support the decision of buy the product. Dry cured ham is an expensive food product so the market offers deboned pieces packaged in vacuum conditions. So it's an interesting and practical objective to study the evolution of deboned packaged ham portions in order to determine their shelf life.

The specific aim of this work is to evaluate the physico-chemical and sensorial characteristics evolution of dry cured ham portions after 12 months of cure, some obtained by artisanal process and others by industrial process, all of them deboned and vacuum packaged, sliced or cut in small portions, and stored at 7°C along 8 months.

## **II – Materials and methods**

### **1. Sample preparation**

Two groups of five hams of Alentejano pig breed with 12 months of cure were considered to design this research work. One group was processed in a traditional production, region Barrancos, Alentejo, Portugal. Another group was processed in a modern factory. Sample preparation consists on obtained portions of 250g and slices of 1mm thick all of them packaged at vacuum conditions and stored at 7°C. Samples were analyzed before being packaged (day 0) and at 2, 5, and 8 months of storage.

### **2. Physico-chemical analysis**

pH according to NP-3441 (1990), measured with a digital pH meter PTI-9;  $a_w$  (Rotronic Hygroskop DT, was measured with a probe WA-40); chlorides according to NP-1845 (1982); total volatile basic nitrogen-ABVT- according to NP-1848 (1987); thiobarbituric acid index-TBA- according to NP-3356 (1987); colour coordinates  $L^*$ ,  $a^*$ ,  $b^*$  (Minolta CR-210b), were measured on the surfaces of the portions and on the surfaces of the slices.

### **3. Sensorial evaluation**

A panel of 18 panelists evaluated samples made of 1 mm thickness slices. A descriptive quantitative method was used in order to obtain a quantification of different attribute, on structured scale from 0 to 100.

### **4. Statistical analysis**

Statistical analysis consisted in analysis of variance considering the factors "process" (artisanal and industrial), "sample presentation" (portions and slices) and "storage time" (0, 2, 5 and 8 months). For mean comparison LSD test, was used for  $p < 0,05$ .

## **III – Results and discussion**

There were no significant differences on pH values between samples of artisanal ham and industrial ham (Table1). The highest value of pH was observed at the first day of analysis (time 0). This fact can be justified by the action of microaerophilic lactic acid bacteria, that can be responsible by the lower pH values exhibited by the sliced ham (5.76 for sliced samples and 5.92 for portion samples). The higher development of the lactic acid bacteria can be due to the larger specific surface and so a bigger quantity of substrate available for the bacteria. It was observed that the artisanal ham presented higher values of chlorides due to higher water loose during cure process, without environmental control, also was observed lower  $a_w$  values (0.874)

than the industrial ham (0.907). During storage period  $a_w$  values increased from 0.839 at day 0 until 0.892 after 8 months of storage. This change can be justified by the water released from proteolysis.

**Table 1. Means and standard deviation for physico-chemical results**

	Process		Sample presentation		Storage time (month)			
	Artisanal	Industrial	Portions	Slices	0	2	5	8
pH	5.88 ±0.4	5.82 ±0.2	5.92a ±0.3	5.76b ±0.3	6.08a ±0.08	5.74b ±0.03	5.83bc ±0.04	5.87b ±0.02
$a_w$	0.874a ±0.004	0.907b ±0.04	0.899 ±0.004	0.900 ±0.004	0.839a ±0.007	0.914c ±0.004	0.894b ±0.003	0.892b ±0.004
Chlorides (%NaCl)	4.9a ±0.1	4.5b ±0.1	4.8 ±0.1	4.6 ±0.1	4.9a ±0.1	4.3b ±0.1	4.8a ±0.1	5.0a ±0.1
ABVT (mgNH <sub>3</sub> /100g)	230.41a ±14.31	101.34b ±1.97	172.71 ±16.39	156.79 ±12.72	205.67 ±41.15	128.22 ±9.63	165.56 ±15.99	183.95 ±20.27
TBA(mg malonic aldehyde/kg)	0.53a ±0.05	1.40b ±0.27	0.79 ±0.15	1.22 ±0.28	1.33 ±0.49	0.64 ±0.10	0.69 ±0.18	1.47 ±0.43
L*	42.6 ±0.8	41.1 ±1.1	44.2a ±0.9	38.7b ±0.6	39.9 ±1.1	44.9 ±1.1	40.0 ±1.1	41.5 ±1.5
a*	10.8 ±0.5	10.8 ±0.6	9.1a ±0.4	13.1b ±0.4	12.0 ±0.4	12.0 ±0.7	9.7 ±0.7	10.0 ±0.9
b*	5.6a ±0.3	4.2b ±0.4	3.7a ±0.3	6.4b ±0.2	4.2 ±0.6	5.5 ±0.4	4.0 ±0.5	5.4 ±0.6

For each factor and for each physico-chemical variable, different letters represent significantly different means for  $p < 0.05$ .

Probably due to environmental conditions during cure process, more favorable to proteolytic process, were found ABVT values higher in artisanal ham than in industrial ham (230.41 mg NH<sub>3</sub>/100g for artisanal ham and 101.34 mg NH<sub>3</sub>/100g for industrial ham). Although the higher temperatures during the artisanal cure process, oxidation degree was significantly inferior (0.53 mg of malonic aldehyde) when compared with the values obtained for industrial ham (1.40 mg of malonic aldehyde). Considering that pig nutrition was similar for animals used in both processes and that antioxidant quantity used at the ham preparation were also similar, the differences in TBA values could be caused by a strong contamination by moulds, probably lipolytic, during the cure process and a light exposure of industrial ham at the end of that process. Colour coordinates L\* a\* b\* didn't show significant differences during storage time, and sliced ham always present different values when compared with portions, for all the coordinates, L\* with 44.2 for portions and 38.7 for slices, a\* with 13.1 for slices and 9.1 for portions, and b\* with 6.4 for slices and 3.7 for portions. So the sliced ham seems to change more than portions.

About the results of sensorial evaluation (Table 2) it could be observed that values obtained for colour after 5 and 8 months of storage (66 and 67 points respectively) were significantly higher than those at 0 and 2 months (56 and 54 months respectively). Aroma attribute was classified with significantly superior values for artisanal ham (58 points) than industrial ham (53 points) probably due to intensive proteolytic and lipolytic phenomena occurred in artisanal ham. Sliced ham had better classification than portions for the attributes tenderness and taste. Taste was better after 6 or 8 months of storage (62 and 60 points respectively) than at time 0 and 2 months of storage (52 point for both). Global evaluation was almost similar for artisanal ham

and industrial ham (55 and 57 points respectively). However there were noticeable differences between slices and portions for global evaluation with higher values for the first samples, slices. On the other hand time storage improved sensorial global evaluation of ham with (58 points after 8 storage months and 50 points for time 0). Panel noticed strange colours, strange aromas and strange taste but always at low levels. Storage time never caused the increasing of those undesirable attributes. Aroma and strange taste can be related to the presence of acetaldehyde and pentane (results not shown in this work).

**Table 2. Means and standard deviation for sensorial results using a scale from 0 to 100**

	Process		Sample presentation		Storage time (month)			
	Artisanal	Industrial	Portions	Slices	0	2	5	8
Colour	60±2	63±1	62±1	61±2	56a±2	54a±2	66b±1	67b±2
Strange colours	5±1	6±1	6±1	5±1	7ab±1	8b±1	5ab±1	3a±1
Aroma	58a±1	53b±1	53a±1	60b±1	48a±5	50a±3	62b±2	59b±1
Strange aromas	5±1	3±0	4±1	3±1	7a±2	4ab±0	3b±0	3b±1
Tenderness	58±1	58±1	55a±1	62b±1	55ab±0	53b±1	62c±2	61bc±3
Suculence	52±1	49±1	48a±1	52b±1	43a±1	45a±1	57b±2	51c±1
Taste	58±1	56±1	56±1	59±1	52a±1	52a±2	62b±2	60b±2
Strange taste	8±1	4±1	7±1	5±1	10±2	6±1	5±2	6±3
Salt intensity	50±1	43±1	47±1	45±1	43ab±4	42b±2	48ac±2	50c±2
Global evaluation	55±1	57±1	54a±1	59b±1	50a±1	52a±1	61b±1	58b±3

For each factor and for each physic-chemical variable. Different letters represent significantly different means for  $p < 0.05$ .

## IV – Conclusions

Physico-chemical parameters were different for artisanal and industrial hams. The different sample presentation didn't influence a lot the physic-chemical parameters and, considering a practical point of view, the changes observed along the storage time weren't also very expressive.

The sensorial panel didn't notice important differences between the artisanal and industrial hams, neither between sliced ham and portions, however panelists preferred samples stored for a long time. Colour, aroma, tenderness, succulence, flavor became better with the permanence inside the package.

To storage portions and slices of dry cured ham of Alentejano pig bread in vacuum conditions at cold temperature (7°C) is possible for at least 8 months, without noticeable changes in desirable attributes.

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# Effect of fermentation temperature and nitrite nitrate on properties of dry fermented sausage

C. Schivazappa, M.S. Grisenti, M.A. Frustoli and S. Barbuti

Stazione Sperimentale per l'Industria delle Conserve Alimentari (Italy)

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**Abstract.** Sausages, produced with/without nitrite and nitrate, were fermented at 21°C (RH: 65-74%) or 8°C (RH: 75-85%). All sausages were dried until a final weight loss of 44% and analyzed for physicochemical and microbiological data. The removal of  $\text{NO}_2^-/\text{NO}_3^-$  caused an increase in lipid oxidation, although the decrease in the fermentation temperature proved to be effective in controlling the lipid oxidation of ripened nitrite-free sausages. The effect of nitrite on the formation of red and bright colour was evident since the early stages of processing, while its removal resulted in the formation of a less red, less stable and lighter colour, in spite of fermentation temperature. The texture of ripened sausage did not show significant differences among groups. The elimination of  $\text{NO}_2^-/\text{NO}_3^-$  had no effect on the typical microbiota (LAB and SNCP), while differences were observed for *B. thermosphacta*, enterococci and enterobacteria.

**Keywords.** Colour – Dry fermented sausage – Fermentation temperature – Microbiota – Nitrite – pH.

## **Effet de la température de fermentation et de l'élimination des nitrites et nitrates sur les paramètres du saucisson sec**

**Résumé.** Des saucissons produits avec et sans nitrates et nitrites, ont été fermentés à 21°C (RH 69-74%) et à 8°C (RH 75-85%). Les saucissons ont été maturés jusqu'à une perte de poids de 44%. Les analyses physico-chimiques et microbiologiques étaient effectuées au cours de la maturation. L'élimination de  $\text{NO}_2^-/\text{NO}_3^-$  augmente l'oxydation des lipides mais la réduction des températures de fermentation contrôle l'oxydation des saucissons qui ont été produits sans nitrates. L'importance des nitrites dans la formation de la couleur rouge vif est évidente dès le début de leur production; leur élimination, indépendamment des températures de fermentation, détermine la formation d'une couleur rouge moins intense, moins stable et plus claire. Les paramètres de texture du salami mûré n'ont pas mis en évidence de différences significatives entre les groupes. L'élimination de  $\text{NO}_2^-/\text{NO}_3^-$  n'affectait pas le microbiote typique (LAB et SNCP) tandis que des différences ont été observées à propos de *B. thermosphacta*, entérocoques et Enterobacteriaceae.

**Mots-clés.** Couleur – Saucisson sec – Températures de fermentation – Microbiote – Nitrate – pH.

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## **I – Introduction**

The use of nitrite in sausage production is important for its antibacterial, colour-forming, antioxidant and flavouring properties (Shahiti and Pegg, 1992). Despite these properties, there are problems with regard to toxicity of nitrite and nitroso-derivatives for human health (Cassens, 1997). The processing of the sausage is based on a drip and drying phases which reduce water activity; between these two phases there is a fermentation under controlled temperature and relative humidity, during which the growth of lactic acid bacteria, results in a pH decrease, thus the development of texture and colour and the control of spoilage bacteria and pathogens (Hugas and Monfort, 1997; Lucke, 1998). The aim of this research was to determine the effects of fermentation temperature and nitrite nitrate on the physicochemical and microbiological characteristics of dry fermented sausages.

## II – Materials and methods

### 1. Sausage formulation and processing

Three batches of dry fermented sausages, two without  $\text{NO}_2^-/\text{NO}_3^-$  (batches N-) and one with 80 ppm  $\text{NaNO}_2$  and 120 ppm  $\text{KNO}_3$  (batch N+) were produced from a common meat batter (approximately 75% lean pork meat and 25% pork fat). The pork lean and fat were ground through a 7 mm diameter mincing plate and mixed with salt (2.4%), sucrose (0.25%), sodium ascorbate (0.06%), white wine, whole black pepper, ground white pepper and garlic. A commercial starter culture containing *Lactobacillus curvatus*, *Staphylococcus carnosus* and *Kocuria varians* was added. Batters were stuffed into natural casing (80-85 mm diameter), and the final weight for each sausage was 1 kg. A mould starter, SK10 *Penicillium nalgiovensis*, was applied on the surface of sausages. For the batches N- two different processes (P) were applied: "P1" consisting of 3 d at  $21\pm 1^\circ\text{C}$  /65-74% relative humidity (RH) followed by a drying at  $17\pm 2^\circ\text{C}$ /70-80% RH and ripening at  $13\pm 1^\circ\text{C}$ /80-88 % RH; "P2" consisting of 13 d at  $8\pm 1^\circ\text{C}$ /75-85% RH. Afterwards the temperature was increased to  $16\pm 1^\circ\text{C}$  and 80-88% RH for 7 d and finally  $18\pm 1^\circ\text{C}$  and 80-88% RH until the end of the processing. The sausages produced with  $\text{NO}_2^-/\text{NO}_3^-$  (batch N+), were processed with P1 only. All sausages were processed until a final weight loss of 44% was reached. From each batch, samples of the sausage batter (100 g) were collected at days 0 (prior to stuffing) and three sausages were taken at 6, 13 and 29 d. At the end of the process 5 sausages for each batch were taken for analysis.

### 2. Analysis

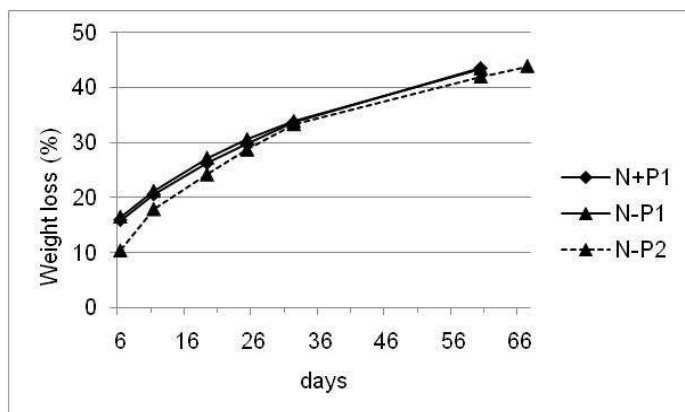
*Water activity* ( $a_w$ ) was measured at  $25^\circ\text{C}$  by means of Aqualab® equipment (Model Series 3TE, Decagon Devices Inc). *pH* was measured using a pH meter WTW (model 330) inserting the electrode (Hamilton) directly into the sausage. *Lipid oxidation* (TBARs) was determined using the 2-thiobarbituric acid method (TBA-test) described by Witte *et al.* (1970) using trichloroacetic acid 5% as solvent. *Textural Profile Analysis* (Bourne, 1978) was performed (Instron Texture Machine mod. 5565) using the central core of two slices of each sample. Each probe (15 mm height and 25 mm diameter) was compressed twice to 50% of original height. The following texture parameters were calculated: hardness (peak force during the 1<sup>st</sup> compression cycle), and cohesiveness (ratio of the positive force area during the 2<sup>nd</sup> compression to that during the 1<sup>st</sup> compression). *Colour* (Minolta CR-508d; illuminant D65), was measured by the CIE  $L^*a^*b^*$  system and the results were expressed as lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ). The sausages were cut into sections 2 cm thick and colour measurements were taken immediately after cutting, and after 24 hr of display in air at  $4^\circ\text{C}$ . Sausages were subjected to the following microbiological analyses: *Enterobacteriaceae* (ISO 7402), Gram-negative bacteria (G-) (violet red bile glucose agar  $30^\circ\text{C}$ /48 hr), Lactic acid bacteria (LAB) (ISO 15214), Micrococci and staphylococci (Mannitol Salt Agar,  $30^\circ\text{C}$ /72 hr) and enterococci (Slanetz Bartley agar  $37^\circ\text{C}$ / 4hr and  $44^\circ\text{C}$ /44 hr).

The chemical-physical and microbiological data were shown as mean values. One-way analysis of variance, (ANOVA, SPSS vr.11.5.0) was run to detect differences among batches at end of ripening (Bonferroni test).

## III – Results and discussion

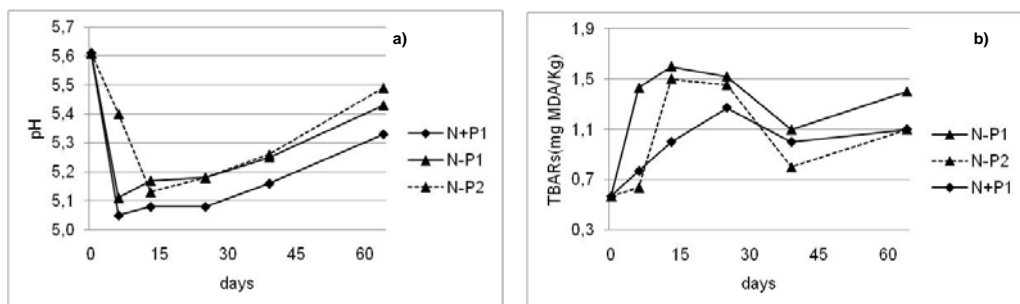
Figure 1 shows the weight losses of sausages throughout the ripening processes: a reduction approximately 44% (w/w) was reached in about 61 d for sausages processed with P1 and 67 d for sausages processed with P2, meaning that the slow ripening conditions of P2 were controlled to minimize differences in drying from a traditional process. During the processing  $a_w$  dropped from the initial value of 0.97 to the final average value of 0.86, without significant differences between the three batches.

All sausages showed at first a pH decrease and a final rise (Fig. 2a). The rate of pH decrease was affected by fermentation temperatures: the higher temperature in P1, favouring formation of organic acids, caused a faster pH decrease than in P2 (6 d vs 13 d). At the end of fermentation and at the end of ripening the pH values of batch N+ were lower ( $p < 0.005$ ) than in batches N-.



**Fig. 1.** Mean values of weight loss in the different batches during the ripening of sausages (N-P1: no  $\text{NO}_2/\text{NO}_3^-$  added sausage, process P1; N-P2: no  $\text{NO}_2/\text{NO}_3^-$  added sausage, process P2; N+P2:  $\text{NO}_2/\text{NO}_3^-$  added sausage, process P2).

For all batches TBARS values showed an increase followed by a reduction (Fig. 2b). The lipid oxidation was more rapidly increased in N-P1 than in N-P2 or in N+P1 batches but, at 13 d the highest TBARS values were observed in both N- batches. At end of ripening process, ANOVA revealed that oxidation of N-P1 sausages was higher ( $p = 0.017$ ) than N+P1 and N-P2.



**Fig. 2.** Mean values of pH (a) and TBARS (b) in the different batches during ripening.

The effect of nitrite on the formation of a red and bright colour was evident since the early stages of processing (data not show), and its removal resulted, at end of ripening, in the formation of a less red, more yellow and lighter colour (Fig. 3). The storage (24 hr at 4°C) of sliced sausages determined an increase of differences in color index among batches N+ and N- despite processing condition.

pH and moisture are major factors affecting texture properties and the increase of hardness (maximum force required to compress the sample) and cohesiveness (strength of the internal bonds making up the body of the sample) observed during ripening. The observed differences of hardness and cohesiveness (Fig. 4) among the batches during the first phases of ripening



could be explained by the differences in the rate of pH decrease and in the weight loss. At end of ripening process, the texture parameters of sausages did not show significant differences among batches.

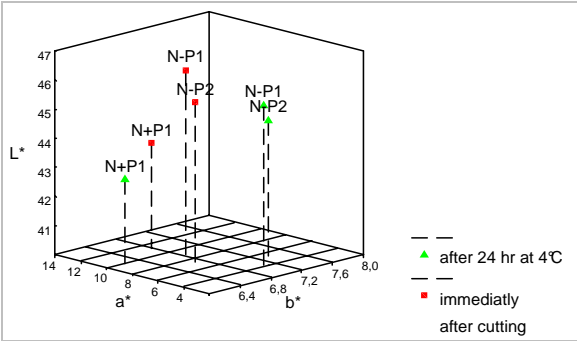


Fig. 3. Colour parameters, after cutting and after 24 hr at 4°C, of the ripened sausage.

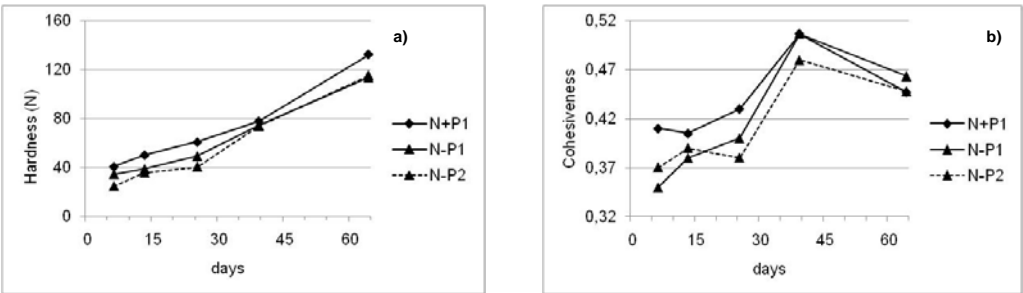


Fig. 4. Mean values of hardness and cohesiveness in the different batches.

Figure 5 shows the fate of typical and spoilage microorganisms during the processing of different batches of sausages. In P1 the elimination of  $\text{NO}_2^-/\text{NO}_3^-$  had no effect on the typical microbiota: LAB increased during the first 10 d, reaching values in the order of  $10^9$  cfu/g, staphylococci not coagulase positive (SNCP), introduced with the starter at level of  $10^6$  to  $10^7$  cfu/g, remained the same for the entire process. Differences were observed for the behaviour of G-, *B. thermosphacta* and enterococci. Trends of G-, in the batch N+P1 revealed, in the first 10 d, a drastic and progressive reduction to reach at the end of process values less than 10 cfu/g; *B. thermosphacta* showed a similar pattern. In batch N-P1 a change was observed in the load of G- that after 30 d, were still around  $10^3$  cfu/g and only at the end of maturing showed values <10 cfu/g. The behaviour of *B. thermosphacta* during the first 30 d is almost similar to that of the batch N-P1 but, at the end of processing, a great variability ( $10$ - $10^4$  cfu/g) among the values, was detected in the different samples. In the N-P2 batch the use of temperature as low as 8°C during the fermentation allowed more control over the evolution of the spoilage bacteria. G-bacteria decreased steadily reaching, after maturing, values <10cfu/g; enterococci remained at the same level until the end of processing. *B. thermosphacta*, after an initial reduction of about 2 log, showed a sharp increase and, at the end, the level was higher than initial one.

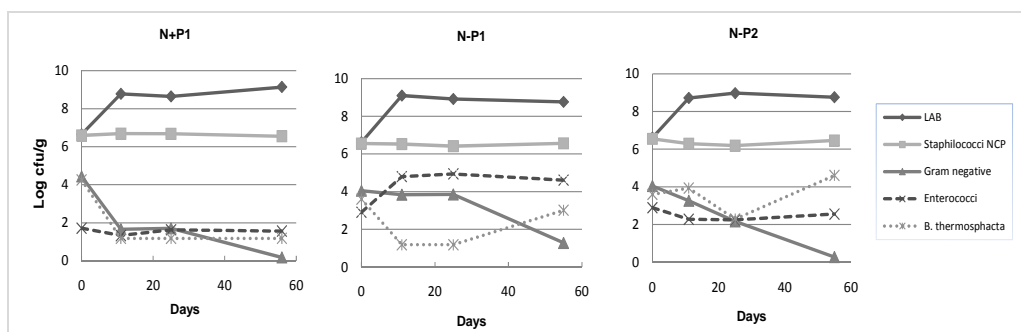


Fig. 5. Performance of the main microbial groups during ripening of sausage.

## IV – Conclusions

Under the conditions of the present study, the production of dry cured sausages without nitrate and nitrite resulted in a not very stable colour and in an uncontrolled lipid oxidation specially at higher temperature. No effect on the typical microbiota was detected whereas spoilage bacteria growth wasn't controlled. The slower rate of acidification at low temperature (8°C) of fermentation did not significantly affect the weight loss texture parameters and it allowed more control of the evolution of Gram negative and enterococci.

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# Volatile hydrocarbon profile of Iberian dry-cured hams. A possible tool for authentication of hams according to the fattening diet

M. Narváez-Rivas\*, E. Gallardo\*, I.M. Vicario\*\*, M.J. Alcalde\*\*\* and M. León-Camacho\*

\*Food Characterization and Quality Department, Instituto de la Grasa (CSIC),  
Avda. Padre García Tejero, 4, 41012 Seville (Spain)

\*\*Department of Food Science and Nutrition, Faculty of Pharmacy, University of Seville (Spain)

\*\*\*Agroforestry Science Department, University of Seville (Spain)

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**Abstract.** The aims of this work were to carry out a comprehensive study of the volatile hydrocarbons of 34 Iberian dry-cured hams and to evaluate the efficiency of these compounds for discriminating hams according to the fattening system: "Montanera" (B) and "Cebo" (C). The samples of hams were obtained by mincing the semimembranosus and semitendinosus muscles from slices of dry-cured ham. The analyses were carried out by gas chromatography-mass spectrometry with a polar capillary column and after a previous extraction by Purge and Trap method. Forty-three volatile hydrocarbons were identified, 26 of them for the first time in Iberian dry-cured ham. Only five compounds showed significant differences between the two types of hams. Among the 33 volatile hydrocarbons, 22 of them allowed a complete discrimination of the two groups of hams according to the fattening system.

**Keywords.** Iberian pig – Slice ham – Volatile hydrocarbons – GC-MS.

**Profil en hydrocarbures volatils chez des jambons ibériques. Un instrument éventuel pour vérifier l'authentification des jambons en fonction de la diète**

**Résumé.** L'objectif de ce travail est de mener une étude concernant les hydrocarbures volatils sur 34 jambons ibériques et d'évaluer l'efficacité de ces composés en vue de différencier les jambons en fonction du système d'alimentation: "Montanera" (B) et "Cebo" (C). Les échantillons de jambons ont été obtenus sur des tranches de jambon, plus précisément des mélanges des muscles Semimembranosus et Semitendinosus. L'analyse a été réalisée par GC-MS avec une colonne polaire après extraction par une méthode de "purge and trap". Quarante-trois hydrocarbures volatils ont été identifiés, parmi lesquels 26 l'ont été pour la première fois chez des jambons ibériques séchés. Seuls cinq composés ont montré des différences significatives entre les deux types de jambons. Sur les 33 hydrocarbures volatils, 22 ont permis que les deux groupes de jambons étudiés se différencient complètement concernant leur système d'alimentation.

**Mots-clés.** Porc Ibérique – Tranche de jambon – Hydrocarbures volatils – GC-MS.

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## I – Introduction

Several authors (López *et al.*, 1992) have identified a large number of volatile compounds such as aldehydes, ketones, aliphatic hydrocarbons, aromatic hydrocarbons, alcohols, carboxylic acids, esters and lactones in the Iberian dry-cured hams. It has been postulated that these compounds arise from numerous chemical or enzymatic reactions such as lipolysis, chemical or enzymatic oxidation, proteolysis, Strecker degradation and Maillard reactions (Toldrá *et al.*, 2000). Most of these studies on volatile compounds have been carried out with the aim of characterizing them or describe their contribution to the flavour of dry-cured hams. Only an attempt to explore the utility of these compounds as classifying factor for the fattening diet has been carried out, but in loins not in hams. The aims of this work were to carry out an exhaustive study of the volatile compound fraction of 34 Iberian ham samples and to explore the utility of

these compounds, mainly short chain hydrocarbons, as discriminating factors for the fattening diet system.

## II – Experimental design

### 1. Ham samples

A total of 34 samples of dry-cured hams from castrated male 14-month-old pure Iberian pigs and processed in an industry for 24 months were used: 23 corresponding to animals of "Montanera" and 12 corresponding to animals of "Cebo". Slices were cut parallel to the femur and to different depths from each ham. Each slice contained semimembranosus and semitendinosus muscles were trimmer by removing the adipose tissue.

### 2. Methods

The volatile hydrocarbons were analysed by the dynamic headspace technique and adsorbed on a Tenax trap, using a Purge and Trap Concentrator apparatus Tekmar velocity XPT (Thousand Oaks, CA, USA), based on the method described by Sabio *et al.* (1998), one of the most useful analytical methods to determine volatile compounds. After, the volatile compounds were desorbed by heating, the Tenax trap at 225°C for 1 min, and sent throw of transfer line (dept at 150 °C) into the chromatograph injector. The GC-ion-trap-MS analyses were performed using a Varian 3800 gas chromatograph coupled to a Saturno 2000 ion trap mass spectrometer (Varian, Palo Alto, CA, USA). The identification and quantification of the volatile hydrocarbons was done comparing the spectra with those from NIST (National Institute of Standards and Technology) and WILEY libraries and verified by standards.

## III – Results and discussion

### 1. Volatile hydrocarbons profile of ham

A total of 43 volatile hydrocarbons have been identified by GC-MS (Fig. 1). The different hydrocarbons identified in the volatile fraction from "Montanera" and "Cebo" samples are shown in Table 2. Together with mean values, standard deviation (S.D.), maximum and minimum values.

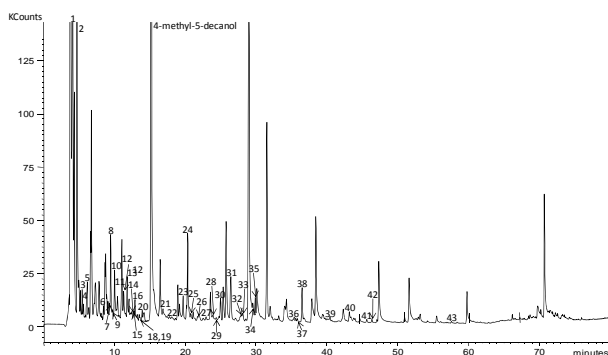


Fig. 1. Chromatograms of the volatile compounds profile of Iberian ham slice samples.

In the group of linear and branched hydrocarbons, 2,4-dimethyl-heptane, 2-octene, 2,2,5,5-tetramethyl-hexane, 2,25-trimethyl-hexane, 2,3,5,8-tetramethyl-decane, 4-methyl-1-decene, 2,4,6-trimethyl-heptane, diisoamilene, 7-methyl-pentadecene, 2,2,3-trimethyl-nonane, 5-(1-

methyl-propyl)-nonane and 3-methyl-5-undecene are observed for the first time in the volatile fraction of Iberian ham.

**Table 1. Volatile hydrocarbons profile of Iberian dry-cured ham samples**

No.	Volatile hydrocarbons	"Montanera"				"Cebo"			
		mean	SD	max	min	mean	SD	max	min
n-alkanes									
5	Nonane	0.36	0.28	0.91	0.00	0.25	0.15	0.45	0.05
8	Dodecane <sup>a</sup>	2.65	3.42	10.10	0.00	0.40	0.28	0.89	0.00
Branched alkanes									
1	3-methyl-hexane	4.64	4.90	23.46	0.56	4.17	1.14	5.93	2.37
2	2,4-dimethyl-heptane <sup>a</sup>	4.65	3.11	11.85	0.89	7.04	3.53	14.28	0.86
7	2,2,5,5-tetramethyl-hexane <sup>b</sup>	0.12	0.28	1.12	0.00	0.48	0.42	1.15	0.00
9	2,2,5-trimethyl-hexane	0.84	1.14	3.20	0.00	0.21	0.25	0.70	0.00
10	2,3,5,8-tetramethyl-decane	1.78	1.74	5.38	0.05	0.98	0.94	2.67	0.00
14	2,4,6-trimethyl-heptane <sup>a</sup>	0.60	0.54	1.77	0.00	0.23	0.19	0.54	0.02
16	7-methyl-pentadecane	0.53	0.59	1.83	0.00	0.38	0.47	1.28	0.00
17	2,2,3-trimethyl-nonane	0.31	0.41	1.51	0.00	0.25	0.35	0.98	0.00
18	5-(1-methyl-propyl)-nonane	0.15	0.17	0.52	0.00	0.10	0.14	0.39	0.00
21	2,6-dimethyl-undecane	0.11	0.12	0.41	0.00	0.05	0.08	0.24	0.00
n-alkenes									
4	2-octene <sup>a</sup>	0.21	0.14	0.49	0.00	0.11	0.08	0.25	0.01
Branched alkenes									
12	4-methyl-1-decene	1.64	1.58	4.83	0.06	0.90	0.66	2.30	0.10
26	3-methyl-5-undecene	0.12	0.18	0.58	0.00	0.14	0.19	0.68	0.00
27	4-methyl-1-undecene	0.18	0.21	0.93	0.00	0.26	0.30	1.08	0.03
Cyclic									
3	1,2-diethyl-cyclobutane	0.17	0.10	0.41	0.00	0.35	0.56	2.06	0.05
6	Butyl cyclopentane	0.16	0.20	0.93	0.00	0.15	0.23	0.87	0.00
20	Heptyl-cyclohexane	0.12	0.26	1.16	0.00	0.29	0.35	0.97	0.00
22	1-ethyl-1-methyl-cyclohexane	0.08	0.16	0.73	0.00	0.10	0.14	0.46	0.00
29	Octyl-cyclohexane	0.06	0.15	0.64	0.00	0.18	0.31	0.99	0.00
37	2-ethenyl-cyclohexane	0.03	0.07	0.29	0.00	0.02	0.02	0.05	0.00
39	Butenyl cyclohexene	0.03	0.04	0.17	0.00	0.02	0.03	0.09	0.00
43	cis-1,2,3,4-tetramethyl-cyclopentane	0.14	0.30	1.25	0.00	0.07	0.12	0.31	0.00
Terpenic									
31	Limonene	0.49	1.14	5.41	0.00	1.12	1.39	4.09	0.03
11	4-carene	0.88	0.95	2.59	0.00	0.36	0.27	1.00	0.06
19	Germacrane B <sup>a</sup>	0.35	0.30	1.05	0.00	0.11	0.13	0.31	0.00
Aromatic									
13	Methyl-benzene	2.10	3.07	13.42	0.34	0.43	0.23	0.96	0.14
15	Diisoamilene	0.34	0.41	1.67	0.00	0.24	0.22	0.67	0.00
23	p-xylene	0.30	0.20	0.79	0.00	0.30	0.25	0.76	0.05
24	m-xylene	0.61	0.72	3.15	0.00	0.37	0.32	1.15	0.00
25	Decahydro-cis-naphtalene	0.31	0.50	2.08	0.00	0.04	0.06	0.18	0.00
28	2-methyl-decahydronaphtalene	0.10	0.19	0.70	0.00	0.08	0.23	0.81	0.00
30	o-xylene	0.14	0.16	0.60	0.00	0.33	0.38	1.16	0.00
32	Propyl-benzene	0.11	0.15	0.73	0.00	0.09	0.14	0.40	0.00

**Table 1. Volatile hydrocarbons profile of Iberian dry-cured ham samples (cont.).**

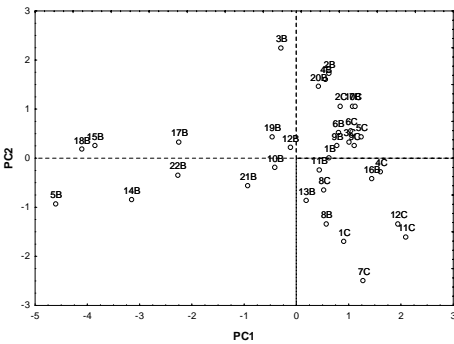
No.	Volatile hydrocarbons	"Montanera"				"Cebo"			
		mean	SD	max	min	mean	SD	max	min
33	Decahydro- <i>trans</i> -naphtalene	0.05	0.08	0.24	0.00	0.11	0.15	0.37	0.00
34	1,3,5-trimethyl-benzene	0.03	0.07	0.30	0.00	0.09	0.14	0.43	0.00
35	1-ethyl-4-methyl-benzene	0.05	0.13	0.61	0.00	0.03	0.09	0.30	0.00
36	1-methyl-3-(1-methyl-ethyl)-benzene	0.04	0.09	0.45	0.00	0.07	0.08	0.23	0.00
38	1,2,4-trimethyl-benzene	0.15	0.20	1.00	0.00	0.12	0.08	0.30	0.05
40	1,2,3-trimethyl-benzene	0.11	0.31	1.50	0.00	0.02	0.02	0.07	0.00
41	4-ethyl-1,2-dimethyl-benzene	0.02	0.04	0.16	0.00	0.00	0.01	0.04	0.00
42	2-ethyl-1,3-dimethyl-benzene	0.01	0.02	0.07	0.00	0.00	0.01	0.04	0.00

<sup>a</sup> For  $p < 0.05$ ; <sup>b</sup> For  $p < 0.01$ .

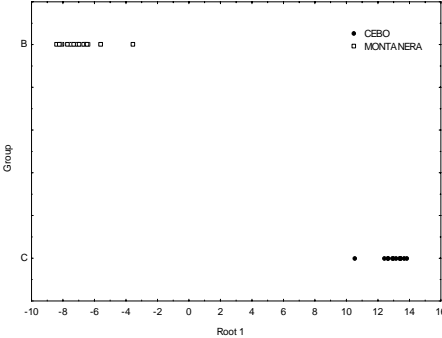
All cyclic hydrocarbons except limonene: 1,2-diethyl-cyclobutane, butyl-cyclopentane, germacrane B, heptyl-cyclohexane, 1-ethyl-1-methyl-cyclohexane, octyl-cyclohexane, 2-ethenyl-cyclohexane, butenyl-cyclohexane and *cis*-1,2,3,4-tetramethyl-cyclopentane compounds have been identified for the first time in the present work. On the other hand, in the present study, we have identified the 4-carene, which has not been described previously.

Most of the aromatic hydrocarbons have been previously described by other authors, however, decahydro-*cis*-naphtalene, decahydro-*trans*-naphtalene, 2-methyl-decahydronaphtalene and 2-ethyl-1,3-dimethyl-benzene have been described for the first time in this work. Besides, (1-methyl-propyl)-benzene, 1-propenyl-benzene and 1-methyl-4-(1-methyl-ethenyl)-benzene compounds were detected and they have not been described at the literature previously.

A PCA was performed (Fig. 2) where it shows a fair separation between "Montanera" (B) and "Cebo" (C) samples. To achieve a better separation of the groups according to fattening diets a linear discriminant analysis (LDA) was carried out. Fig. 3 shows the case discrimination, grouped by fattening diet, according to the first canonical variable or square roots obtained from the classification function. A complete separation between the two groups can be observed.



**Fig. 2. Principal Component Analysis (PCA).**



**Fig. 3. Linear Discriminant Analysis (LNA).**

## Acknowledgments

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## Session 6

### Quality assurance and traceability



# Quality of dry cured ham: Methods for authentication of geographical origin, rearing system and technology

G. Gandemer

Centre INRA de Lille, 2 Chaussée Brunehaut, Estrées-Mons, 80203 Péronne cedex (France)

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**Abstract.** Traceability and authentication of meat and meat products are major concern for consumers, producers and retailers. In Europe, several areas produce high quality dry cured hams under Protected Designation Origin. Efficient and objective methods must be developed to assess origin of dry cured hams. This paper is focused on methods to assess geographical origin, rearing systems and processing conditions. Methods to assess geographical origin are based on multi-analyses of both stable isotopes ( $^{18}\text{O}/^{16}\text{O}$ ,  $^2\text{H}/^1\text{H}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{34}\text{S}/^{32}\text{S}$ ) and trace minerals (Se, Fe, Sr, Cu, Zn...). Genotypes of pigs can be checked by biotechnological methods based on DNA analyses (RFLP, microsatellites). Methods to trace feeding system are based on stable isotope measurement or on quantification of organic components (hydrocarbons, poly phenols, fatty acids, vitamins) in muscles and adipose tissues. Up to now, very few methods have been focused on the authentication of specific processing conditions. Up to now, most of the methods remain at the stage of potential tools and needs to be performed on large numbers of samples to be applied with certainty in routine controls. A proper authentication of dry cured hams requires a combination of methods and multivariate statistical analyses. One of the main challenges for the future is to build wide opened data bases easy to use, aggregating data available on dry cured hams.

**Keywords.** Authentication – Dry cured ham – Geographical origin – Analytical method.

## **Qualité du jambon sec : Méthodes pour l'authentification de l'origine géographique, du système d'élevage et de la technologie**

**Résumé.** La traçabilité et l'authentification des viandes et des produits carnés est un sujet majeur de préoccupation pour les consommateurs, producteurs et détaillants. En Europe, plusieurs régions produisent des jambons secs de grande qualité sous appellation contrôlée. Des méthodes efficaces et objectives doivent être développées pour vérifier l'origine des jambons secs. Cet article est focalisé sur les méthodes destinées à vérifier l'origine géographique, les conditions d'élevage et de transformation. Les méthodes consacrées à l'identification de l'origine géographique reposent sur une analyse multiple d'isotopes stables ( $^{18}\text{O}/^{16}\text{O}$ ,  $^2\text{H}/^1\text{H}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{34}\text{S}/^{32}\text{S}$ ) et de minéraux (Se, Fe, Sr, Cu, Zn...). Le génotype des porcs est vérifié par des méthodes de biotechnologie basées sur l'analyse de l'ADN (RFLP, microsatellites). Les méthodes pour tracer les modalités d'élevage reposent sur la mesure des isotopes stables ou la quantification des composés organiques (hydrocarbures, polyphénols, acides gras, vitamines) dans les muscles et les tissus adipeux. À ce jour, peu de méthodes ont été consacrées à la traçabilité des conditions de transformation. Présentement, la majorité des méthodes restent au stade d'outils potentiels et nécessitent d'être validées sur de grands nombres d'échantillons pour être utilisées en routine. Une identification correcte des jambons secs requiert une combinaison de méthodes et d'analyses statistiques multivariées. Un des enjeux majeurs pour le futur est de construire des bases de données largement ouvertes agrégeant les données disponibles sur les jambons secs de manière intelligible.

**Mots-clés.** Authentification – Jambons secs – Origine géographique – Méthodes analytiques.

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## **I – Introduction**

Traceability and authentication of meat and meat products are a major concern not only for consumers but also for producers and retailers (Karoui *et al.*, 2007). Thus, the globalization of

food market allows a worldwide transportation of both raw material and processed meat that make easy malpractices and substitution of high quality raw material and products by lower quality ones. The international exchanges are also involved in the outbreak of worldwide diseases related to meat consumption (BSE) or animal farming (avian influenza, Foot and Mouth disease).

Consequently, for the last 20 years, the European Union has been reinforcing the policy demanding to all the food companies to develop efficient methods to trace food from farm to fork and also from fork to farm (Schwagele, 2005). In parallel, The EU has promulgate regulation "On the protection of geographical indications and designations of origin for agricultural products and foodstuffs" (Council regulation, 2081/92) for the protection of food names based on geographical origin (Protected Designation of Origin – PDO, Protected Geographical Indication – PGI) or on traditional recipe (Certificate of Specific Character – CSC).

The main goals of these regulations are to:

- (i) Restore the confidence of consumers in meat and meat products giving right information on products to guide their choices;
- (ii) Develop a comprehensive and integrated food safety policy and prevent food crisis;
- (iii) Ensure fair trade and right prices for high quality products (Schwagele, 2005).

If regulations are required to prevent voluntary and involuntary mislabeling of food and food products, they are not sufficient. Effective analytical methods are required to deliver objective proofs that the label products were produced to meet the requirements described in specifications of PDO, PGI or CSC.

Numerous methods have been proposed to authenticate geographical origin or specific process of various foods (Perez *et al.*, 2007; Muller and Steinhart, 2007; Luykx *et al.*, 2008). They can be divided into two groups (Perez *et al.*, 2007). The first one includes methods based on fast and non destructive methods using spectroscopic characteristics of products. These methods provide fingerprints of foods and often require complex data analyses. They remain very difficult to relate to the specifications of products. The second one includes methods based on chemical analyses of raw material or end-product. Often more tedious these methods can be easily interpreted because they are based on an extensive scientific knowledge on the relationship between environment, production system and processing conditions on quality traits of end-products. Up to now, more than thousand papers have been published on the main labeled dry cured hams produced in Europe. They describe the relationships between traits of raw material or dry cured products and genotypes (Gandemer, 2002, Tejeda *et al.*, 2002), rearing systems of pigs (Lebret *et al.*, 1996, Coutron-Gambotti *et al.*, 1999, Andres *et al.*, 2001) and processing conditions (Gandemer, 2002, Andrès *et al.*, 2004). The main requirements written in specifications of labeled dry cured hams are supposed to be involved in the typical traits of dry cured hams of each area of production (Flores, 1997, Gandemer, 2009). Thus, rearing and feeding systems including breed, age at slaughter and consumption of local feeds during fattening largely affect raw material chemical composition (Gandemer, 2002; Ruiz et Lopez-Bote, 2002). The conditions of processing affect the chemical, physical and organoleptic traits of dry cured hams through a set of complex reactions of lipolysis, oxidation and proteolysis, kinetics of those largely determined by temperature and length of the different steps of the process (Gandemer, 2002; Toldra et Navarro, 2002, Toldra, 2006). In contrast, few papers have been published on composition in micronutrients such as minerals or in the ratio of stable isotope in muscle or adipose tissues from pigs and on the environment where they are reared.

Up to now, most of the available methods have been developed to assess the origin of plant foods (Olive oils, wines...) (Kelly *et al.*, 2005; Gonzalez *et al.*, 2009). These for meat and meat products authentication are less numerous (Ballin, 2010). Most of them are not yet effective tools but just potential ones because in many cases they have been established on a too small set of animals or dry cured hams often of well known origins.

## II – Geographical origin

The ratios of stable isotopes of components that constitute all the biological tissues such as muscles and adipose tissues depend on many factors but some of them are strongly related to geographical origin ( $^{18}\text{O}/^{16}\text{O}$ ,  $^2\text{H}/^1\text{H}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{34}\text{S}/^{32}\text{S}$ ) (Karoui and de Baerdemaeker, 2007). Thus,  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  ratios in water depend on parameters such as the altitude, the distance to ocean and the climate.  $^{15}\text{N}/^{14}\text{N}$ ,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{34}\text{S}/^{32}\text{S}$  ratios depend on organic matter in soil and fertilizers. The amount and composition of trace elements in soil (Se, Fe, Sr, Cu, Zn...) are strongly related to the geological underground or specific pollutions from human activities (mining, accident). These elements are incorporated into animal tissues through food chain (Franke *et al.*, 2005).

Measurement of  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  in tissue water is an interesting tool for geographical origin assessment because these ratios are strongly correlated to these in drinking water (Karoui and de Baerdemaeker, 2007; Heaton *et al.*, 2008). Compared to the ratio of these stable isotopes in ocean water, these ratios are lower in altitude or far from the ocean because stable isotopes are discriminated through the successive cycles of evaporation, condensation and precipitation. The ratios of stable isotopes in water are very good indicators of meat origin because they are only slightly affected by feeding systems and main part of the body water come from drinking water. These methods were used with success to discriminate milk products (Heaton *et al.*, 2008; Karoui and De Baerdemacher, 2007) and beef meat from different continents (Boner and Förstel, 2004; Schmidt *et al.*, 2005; Horacek and Min). No data is available on pig meat. But discriminating pig meat from European areas of production of high quality dry cured hams could be very difficult because the main areas of production are close to each other, close to the ocean and in mid-mountains. So the isotope ratio in water could be too close to discriminate geographical origin of meats.

In some studies,  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratio in proteins or lipids of meat were used to discriminate beef and lamb meat according to their geographical origin (Karoui and de Baerdemaeker, 2007; Piasentier *et al.*, 2003). The principle is based on the fact that plants from tropical countries are mainly C4 plants while those from temperate countries are mainly C3 plants. C3 plants discriminate more  $^{13}\text{C}$  and exhibit a lower  $^{13}\text{C}/^{12}\text{C}$  than C4 plants. Consequently, animals eating more C3 plants have a lower  $^{13}\text{C}/^{12}\text{C}$  in their tissues. However, the quantification of  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  suffers of serious drawbacks related to feeding systems (see next part) or to agricultural practice such as fertilizers which increase  $^{15}\text{N}$  in plants (Schmidt *et al.*, 2005; Bahar *et al.*, 2008).

Determination of various minerals in meat has been shown to be efficient tools for geographic origin authentication of meat. Some interesting results were obtained on poultry, lamb and beef meats (Bahar *et al.*, 2008). To be conclusive, it could be assumed that each area of production exhibits a specific profile in some minerals. However, these methods suffer of serious limitations. First, several areas in the world have similar geological undergrounds. Second, some feeds such as cereals and protein sources are commercialized on a worldwide market. Third, some minerals are added in diets of animals through mineral complementation. That is why a multi-elemental analysis coupled with multivariate statistical analysis is required to ensure a good discrimination of geographical origin (Franke *et al.*, 2005).

## III – Rearing conditions

Rearing conditions (outdoor/indoor, age at slaughter, length of fattening) and feeding systems largely affect pig adipose and muscle tissues. These effects are marked in traditional pig production based on local breed (Iberian, Corsican, Basque ...) and fattening diet relied to local food (acorns, chestnuts, grass). Numerous papers describe the chemical traits of pig adipose and muscle tissues as related to many parameters of rearing and feeding in both industrial and traditional pig production (ref). Some of these parameters are of great interest to trace rearing and feeding systems because they are highly variable: lipid content, fatty acid and tri-

acylglycerol composition. Other minor components found in animal tissues, mainly in adipose tissue, are typical of feed source: vitamins, poly-phenols, and hydrocarbons (Prache *et al.*, 2007).

## 1. Breed or genotype

Recent developments in biotechnology open a new field in the traceability and authentication of individuals, lines, genotypes and breeds. The biotechnological methods have been developed very fast for the last 20 years. In theory, these methods are able to give a genetic fingerprint indentifying perfectly each individual and permitting to trace each animal from farm to fork because DNA is specific to each individual. However, the cost of these methods is up to now too high for a routine use (Dalvit *et al.*, 2007; Lockley et Bardsley, 2000).

In contrast, these tools should be very helpful to check the genotypes used for dry cured hams production in PDO where specifications refers to local breed or allows some crossbred genotypes (i.e. Duroc X Iberian) and bans industrial pig genotypes. The development of genetic tools for local breed authentication and their crossbred require a large data base including the typical traits of the main breeds and genotypes used in European pig production. Tracing the local breeds is crucial to the survival of the herds and to defends and valorizes the high quality dry cured hams. Several studies have been devoted to differentiate the Iberian pig breed and line and to control the level of Duroc blood in the crossbreds for detecting mislabeled dry cured hams (Alvez *et al.*, 2002; Fernandez *et al.*, 2004; Ovilo *et al.*, 2000; Garcia *et al.*, 2006). The tools are based on DNA microsatellites and AFLP fragments allow a good differentiation of Iberian from crossbred Duroc X Iberian but are less efficient to distinguish crossbred Duroc x Iberian (50/50) from these with a lower proportion of Duroc blood.

## 2. Feeding systems

As mentioned above, some stable isotope ratios such as  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  are good tracers of feeding systems. Thus  $^{13}\text{C}/^{12}\text{C}$  ratio in meat is related to the proportions of C4 and C3 plants in the diet. In Europe, the main C4 plant used in animal feeding is maize which is included in the diet to increase energy density in feed. So an increased  $^{13}\text{C}/^{12}\text{C}$  in meat is an indicator of a more intensive feed system (Bahar *et al.*, 2005; Boner et Förstel, 2004). Similarly, an increased  $^{15}\text{N}/^{14}\text{N}$  ratio in meat is related to an intensive system of feed production because this increase is related to more intensive use of fertilizers. These isotope ratios give interesting results in discriminating ruminant meat fed grass versus maize or reared onto organic system versus more intensive system (Piasentier *et al.*, 2003). In Iberian pig,  $^{13}\text{C}/^{12}\text{C}$  ratio is higher in adipose tissue in pigs fed on traditional system (acorns and grass) than in pigs fed on more intensive system (concentrate). The higher is the proportion of concentrate, the higher is  $^{13}\text{C}/^{12}\text{C}$  ratio in the tissue (Gonzalez-Martin *et al.*, 1999). This measurement of this ratio could be interesting for discriminating Bayonne hams from these produced in the other areas because maize is largely included in the feed of Bayonne pigs.

## 3. Lipid composition of adipose and muscle tissues

Lipids and lipid fractions have been often used to distinguish animal according to their rearing conditions. Thus it was established that fatty acid composition of both adipose and muscular tissues is strongly related to these of feeds in pigs because it is a monogastric animal. This is of particular interest to distinguish pigs fed on local feeds such as acorns, chestnuts or grass from these fed on concentrate. Regarding fatty acid composition of raw material, genotype is also a major factor of variation. In Europe, the higher quality dry cured hams are produced from local breeds with a slow growth rate which deposit large amount of fat during the fattening period when they are too old to deposit muscle. Consequently lipids contain a high proportion of monounsaturated fatty acids coming from the conversion of starch from diet into saturated and monounsaturated fatty acids. Both fatty acid and triacylglycerol compositions were used to

distinguish pigs according to their breed (Local breeds versus crossbred) or their diet (local feeds versus concentrate). In all the cases, triacylglycerols are more efficient to discriminate pigs because small variations in fatty acid composition are correlated to large variations in triacylglycerol composition (Riaublanc *et al.*, 1999). Several authors have succeeded to distinguish Iberian pigs according to the feeding systems based on fatty acid composition of lipids from adipose tissue, intramuscular fat or liver (Flores *et al.*, 1988; Ruiz *et al.*, 1998; Perez-Palacios, 2009) or on triacylglycerol composition of adipose or muscular tissues (Diaz *et al.*, 1996; Tejeda *et al.*, 2002; Viera-Alcaide *et al.*, 2007). Some minor lipid components can be good indicators of local feed consumption. In various amounts in feeds, they are stored in body fat. Hydrocarbon profiles of adipose tissue were used to distinguish Iberian pigs according to feeding systems. n- alkanes are not efficient (Tejeda *et al.*, 2001a) but some peculiar hydrocarbon such as eut-kaurene (Navaez-Rivas *et al.*, 2008) and neophytadiene (Tejeda *et al.*, 2001b, Perez-Palacios, 2009) coming from grass could be used to discriminate Montanera pigs fed on acorns and grass from other Iberian pigs fed various amounts of concentrate. Tocopherols, namely gamma one, which is in a high amount in acorns (Tejerina *et al.*, 2010), could help to discriminate traditional Montanera feeding system from others containing concentrate (Perez-Palacios, 2009; Tejerina *et al.*, 2010).

#### 4. Age at slaughter

The age of pigs is regarded as one of the main parameters improving meat quality and is included in the specifications of dry cured hams in many areas of production. Up to now, no method allows tracing this physiological parameter.

### IV – Processing

The changes in raw matter during dry cured ham processing are largely involved in the typical sensory traits of end products. These changes involved a complex set of chemical and physico-chemical reactions affecting lipids, proteins, water and salt contents (Gandemer, 2002; Toldra et Navarro, 2002, Toldra, 2006).. The intensity of these changes largely depends on the conditions of processing used in the main area of production in Europe. Many PDO specifications contain specific requirements on the different steps of the process (length, temperature)(Flores, 1997). The changes in chemical and physico-chemical traits of meat and adipose tissues of hams have been largely described and marked differences were observed according to methods of processing. However, very few papers focus on methods to check that the specific requirements on process written in specifications are respected.

The use of thawed meat is prohibited in high quality dry cured ham production. Several papers are devoted to the differentiation of fresh and thawed raw meat. A review of methods indicates that only a combination of several methods allows discriminating fresh from thawed meat including DNA degradation, enzyme profile in juice extracted from meat and microscopy techniques (Ballin and Lametsch, 2008).

Volatile profiles of dry cured hams depend on the length and the temperature of the main steps of the process as well as the chemical traits of the raw material. Many papers described differences in volatile profiles from hams of different countries or feeding systems (Ruiz *et al.*, 1999; Bolzoni *et al.*, 1996; Dirinck *et al.*, 1997). Some volatiles found in aroma of hams come directly from feeds and are tracers of feeding systems. However, quantification of volatiles is very difficult and results vary greatly according to the method of volatile extraction and from one laboratory to another. So, volatile analyses are not proper tools to discriminate hams.



## V – Conclusion

This review shows that the research on the authentication of meat and meat products is in progress. This is a major concern for consumers and producers. However, very little has been done on dry-cured hams. So up to now, we lack of accurate methods to assess that the specifications of dry-cured hams produced under PDO, PGI ou CSC in Europe are strictly applied. Most of methods remain potential tools and are far from their use as standard recognized methods to detect mislabeled products. That is why most of these methods were developed with small sets of samples of well-known origins. These methods must be validated using large numbers of samples of unknown origins and processes including raw meat and dry-cured hams arising from intensive systems of production all around the world. Large opened data bases must be built putting together all the characteristics of dry-cured hams as related to their area and specifications of production. A better characterization of the environment where animals are reared is required to able to mobilize very promising methods based on stable isotope ratios or trace elements quantifications.

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# Chemical composition of dry ham "*Kraški pršut*" predicted by NIR spectroscopy

M. Prevolnik<sup>\*\*\*</sup>, Z. Povše<sup>\*\*\*</sup>, M. Škrlep<sup>\*</sup>, D. Škorjanc<sup>\*\*</sup> and M. Čandek-Potokar<sup>\*\*\*</sup>

<sup>\*</sup>Agricultural Institute of Slovenia, Hacquetova 17, 1000 Ljubljana (Slovenia)

<sup>\*\*</sup>University of Maribor, Faculty of Agriculture and Life Sciences, Hoče (Slovenia)

<sup>\*\*\*</sup>Podlog 29, Šempeter (Slovenia)

**Abstract.** Dry ham "*Kraški pršut*" is a Slovenian traditional meat product with protected geographical designation. This protection implies that certain *consortium* constrains and control of dry-cured ham quality should be respected. For regular checks NIR spectroscopy offers an interesting alternative to conventional chemical methods. The aim of the present study was to test the ability of NIR spectroscopy to predict several chemical constituents of "*Kraški pršut*". Proximate analysis (moisture, salt, protein, non-protein nitrogen, intramuscular fat, free amino acids) was performed in muscles *biceps femoris* (n=135) and *semimembranosus* (n=135) of the final product. The quality of predictive models was assessed on the basis of the coefficients of determination ( $R^2_{CV}$ ) of cross-validation and residual predictive deviation (RPD, i.e. the ratio between standard deviation of the reference data and standard error ( $SE_{CV}$ ) of cross-validation). Highly reliable prediction results were obtained for moisture, protein, salt content and the percentage of salt per dry matter ( $R^2_{CV} > 0.90$ , RPD  $> 3.0$ ). For intramuscular fat, free amino acids content and non-protein nitrogen reasonable calibration models were obtained ( $R^2_{CV}$  between 0.62 and 0.87, RPD between 1.6 and 2.8). Due to good prediction ability and the simplicity of measurement NIR spectroscopy offers good opportunity to replace time-consuming, expensive and/or hazardous laboratory methods.

**Keywords.** NIR spectroscopy – Ham – Chemical composition.

## La composition chimique du jambon sec "*Kraški pršut*" prédite par la spectroscopie NIR

**Résumé.** "*Kraški pršut*" est un jambon sec traditionnel slovène protégé par l'indication géographique. Cette protection implique la certification du produit et le respect de contraintes imposées par le consortium. Pour des contrôles réguliers la spectroscopie NIR offre une alternative intéressante pour remplacer les méthodes chimiques classiques. L'objectif de la présente étude était de tester la capacité de la spectroscopie NIR pour prédire plusieurs constituants chimiques du jambon sec "*Kraški pršut*". L'analyse chimique (humidité, sel, protéines, azote non protéique, gras intramusculaire, acides aminés libres) a été réalisée sur deux muscles du produit final, notamment le Biceps femoris (n = 135) et le semi-membraneux (n = 135). La qualité des modèles de calibrage a été évaluée sur la base de coefficients de détermination de la validation croisée ( $R^2_{CV}$ ) et du coefficient RPD, à savoir le rapport entre l'écart-type des données de référence et l'erreur de la validation croisée ( $SE_{CV}$ ). D'excellents résultats ont été obtenus pour la prédiction de l'humidité, des protéines, de la teneur en sel et du pourcentage de sel dans la matière sèche ( $R^2_{CV} > 0,90$ , RPD  $> 3,0$ ). La précision de la prédiction du gras intramusculaire, de la teneur en acides aminés libres et de l'azote non protéique ( $R^2_{CV}$  entre 0,62 et 0,87, RPD entre 1,6 et 2,8) était un peu inférieure mais acceptable. En raison d'une bonne précision de la prédiction et de la simplicité des mesures, la spectroscopie NIR pourrait remplacer les méthodes chimiques plus laborieuses pour le contrôle régulier du produit.

**Mots-clés.** Spectroscopie NIR – Jambon sec – Composition chimique.

## I – Introduction

"*Kraški pršut*" is a traditional Slovenian dry-cured ham of the carstic region (called Kras in Slovenian language) which belongs to the family of Mediterranean type of dry-cured ham, characterised by dry salting, absence of smoking and long maturation. The product is economically important and highly appreciated among Slovenian consumers (Čandek-Potokar et al., 2004). On the national level "*Kraški pršut*" is protected by geographical designation and

certified, implying that certain *consortium* constraints should be respected in regard to green ham properties, processing losses, chemical and sensory properties of dry-cured hams.

For regular checks near infrared (NIR) spectroscopy offers an interesting alternative over the conventional methods. Since the method enables fast and simple determination of many parameters simultaneously, it could effectively replace lengthy and expensive analyses which are less suitable to be used on a large scale and in particular for assays that are healthy and environmentally damaging (e.g. determination of intramuscular fat content based on hazardous organic solvent extraction). High potential of NIR spectroscopy for the prediction of chemical composition and the quality of raw meat was demonstrated in many previously published studies (for review see Prevolnik *et al.*, 2004; Prieto *et al.*, 2009). The literature reports relating to the application of NIR spectroscopy for the analyses of meat products are not abundant; the majority of them demonstrate good prediction ability (for review see Prieto *et al.*, 2009). The lack of relevant studies is in particular evident in case of dry-cured ham. The few published studies on dry-cured ham were focused mainly on the prediction of sensory characteristics of dry-cured ham (Cruz Ortiz *et al.*, 2006; García-Rey *et al.*, 2005).

Due to the usefulness of NIR spectroscopy for mass analyses in the quality control of dry-cured ham the aim of the present study was to test the ability of the method to predict several chemical constituents of “*Kraški pršut*” dry-cured ham as this would be of high importance for the practice *i.e.* for regular checks in the production of certified product.

## II – Materials and methods

### 1. Sampling and chemical analysis

The research was carried out on 135 dry-cured hams which were processed according to the rules of *consortium* for “*Kraški pršut*”. The analysis is based on pooled data for two muscles, *biceps femoris* and *semimembranosus*. Prior to the chemical analyses, samples were trimmed of superficial fat tissue, cut in small pieces, frozen in liquid nitrogen, grinded to fine dust using a laboratory mill (IKA M120, IKA Werke, Staufen, Germany) and stored in plastic tubes at -20°C until further use. Chemical determinations (moisture, protein, intramuscular fat, non-protein nitrogen, salt and free amino acid content) were carried out in replicates.

For the determination of moisture content (ISO 6496, 1999), 5 g of the sample was mixed with equal amount of quartz sand and dried at 103°C to a constant mass. The loss of mass was recorded and expressed as a percentage of moisture in the sample.

For sodium chloride (salt) content determination (Monin *et al.*, 1997), 1 g of sample was mixed with 80 ml distilled water and boiled at 100°C for one hour. After cooling, 2 ml of 15% potassium ferrocyanide and 2 ml of 30% zinc acetate was added and diluted with distilled water to 100 ml. After filtration, the NaCl content was determined by potentiometric titration using DL53 General Purpose Titrator (Mettler Toledo, Schwarzenbach, Switzerland). Additionally, salt content was expressed as the percentage of moisture or dry matter.

Protein content was calculated from total nitrogen content (ISO 5983-2, 2005) using the Kjeltec 2300 nitrogen analyser (Foss Analytical, Hileroed, Denmark). The organic matter in the samples was degraded by heating with concentrated sulphuric(VI) acid in the presence of catalysts. After the addition of base (NaOH) the resulting ammonia gas was dissolved in boric acid solution and titrated with hydrochloric acid. The total nitrogen content was calculated from the amount of the hydrochloric acid used for the titration. To obtain total protein content total nitrogen content was multiplied with 6.25.

For determination of non-protein nitrogen, 2.5 g of sample was homogenised in 25 ml of distilled water and centrifuged (Monin *et al.*, 1997). Afterwards, 10 ml of 20% trichloroacetic acid was added, stirred well and let to stabilise for 60 min at room temperature. After the centrifugation,

the supernatant was filtered and 15 ml of it used for determination of nitrogen in the same way as described for total nitrogen (ISO 5983-2, 2005). Additionally, non-protein nitrogen was expressed as a percentage of total nitrogen (proteolysis index).

Intramuscular fat content (ISO 1443, 2001) was determined using Büchi Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland). The samples were boiled with dilute hydrochloric acid to free the occluded and bound lipid fractions; the resulting mass was filtered and dried, the fat retained on the filter was extracted with light petroleum. The resulting fat was expressed as a percentage of fat in the sample.

The content of free amino acids was determined according to ISO 13903 (2005) adapted for dry-cured ham (internal laboratory protocol). Free amino acids were extracted with dilute hydrochloric acid. Co-extracted nitrogenous non-amino acid macromolecules were precipitated by adding sulfosalicylic acid and removed by filtration. The pH of the filtered solution was adjusted to 2.20. FAA were separated by ion exchange chromatography and determined by reaction with ninhydrin with photometric detection at 440 nm (for proline) and 570 nm (for other free amino acids) using Agilent 1200 series HPLC apparatus (Agilent technologies, Waldbronn, Germany) equipped with sodium cation exchange column 8  $\mu$ m, 3.0×250 mm (Pickering Laboratories, Mountain View, CA, USA), Pinnacle PCX post column derivatization instrument (Pickering Laboratories, Mountain View, CA, USA) and Agilent 1200 series Diode array and multiple wavelength detector (Agilent technologies, Waldbronn, Germany).

## 2. NIR spectra acquisition and spectral data analysis

Minced samples of analysed muscles were separately put in rectangular quartz cup (47×57 mm<sup>2</sup>) about 3 mm thick, covered by paper disc and placed directly in NIRS apparatus. For each sample one scanning was performed. The samples were scanned with spectrophotometer NIR System model 6500 (Silver Spring, MD, USA) in a wavelength range from 400 to 2500 nm. Absorbance data were collected every 2 nm as  $\log 1/R$ , where  $R$  represents reflectance.

Spectral data processing was performed using WinISI II software. Calibration models were developed using modified partial least squares regression with internal cross-validation. Samples for which the difference between actual and predicted values exceeded 3 standard deviations (SD) were considered as outliers.

The mathematical treatment applied was 1 4 4 1, where the first number indicates the order of the derivative (1 is the first derivative of the  $\log 1/R$ ), the second number is the gap in nm over which the derivative is calculated, the third and fourth number refer to the first and the second smoothing. The "SNV and Detrend" option was used to correct scatter effects in the spectra. Within development of calibration models we tested spectral range (visible spectrum ranging from 400 to 1100 nm, NIR spectrum ranging from 1100 to 2500 nm and the whole spectrum ranging from 400 to 2500 nm). The number of PLS factors was limited to 16, but the actual number of PLS factors was defined separately for every single calibration model respecting the fall of cross-validation errors. The quality of calibration models was assessed through standard error of calibration ( $SE_C$ ), coefficient of determination in calibration ( $R_C$ ), standard error of cross-validation ( $SE_{CV}$ ) and coefficient of determination in cross-validation ( $R_{CV}$ ). Models' performance was additionally evaluated using residual predictive deviation (RPD) which was calculated as the ratio between the SD of reference data and  $SE_{CV}$ .

### III – Results and discussion

#### 1. Material

Basic statistics of analysed chemical constituents in *biceps femoris* and *semimebranosus* muscles is presented in Table 1. Using two muscles we obtained broad range of variability which is of great importance for the development of calibration models.

#### 2. Prediction of chemical composition

In the present study the same sample set was used to develop calibration models and to validate models. Namely, our previous studies (Čandek-Potokar *et al.*, 2006; Prevolnik *et al.*, 2009) showed that external validation (prediction) on the independent set of samples yielded comparable results as the cross-validation. In the present study prediction results are presented as statistical parameters of calibration ( $R_C$  and  $SE_C$ ) and cross-validation ( $R_{CV}$  and  $SE_{CV}$ ). The parameter RPD (the ratio  $SD/SE_{CV}$ ) was also applied as an indicator of models' quality. Namely, RPD evaluates the prediction errors in view of the SD of the reference data and should be over three for accurate predictions (Andrés *et al.*, 2008; Kennedy *et al.*, 1996). Lower RPD values can be attributed either to a narrow variation range of the reference values (giving small SD) or to large NIR prediction error compared to SD of the reference values. Moreover, RPD enables to compare models' quality for the constituents/traits with different variation range where the prediction errors cannot be directly compared.

**Table 1. Basic statistics for chemical constituents of dry ham (two muscles)**

	Mean±SD	Range
Moisture, g/kg	537±51.4	435–652
Salt content, g/kg	71.2±8.19	44.8–94.1
Salt per DM, %	15.7±2.96	8.8–22.2
Salt per moisture, %	13.3±1.49	9.1–17.4
Protein, g/kg	34.2±5.08	26.0–43.9
NPN, g/kg	12.2±1.10	7.0–14.7
Proteolysis index, %	22.8±4.32	13.3–31.1
IMF, g/kg	38±14.0	16–88
FAA, mg/100g DM	7140±895	5237–9507

DM – dry matter; SD – standard deviation; IMF – intramuscular fat;  
NPN – non-protein nitrogen; FAA – free amino acids.

As regards the prediction ability of NIR spectroscopy (Table 2), the results show, that there was a negligible difference in the prediction of chemical constituents based on NIR or the whole spectral range. Visible spectrum was also tested, but the results are not presented as the models showed considerably lower prediction accuracy. On the whole,  $R_{CV}$  for different chemical constituents ranged from 0.65 to 0.96 and RPD from 1.7 to 5.0. Highly reliable results were obtained for salt content and the percentage of salt per moisture or dry matter for which the  $R_{CV}$  were over 0.90 and RPD exceeded three which is indicative of highly reliable predictive models. Satisfactory results were obtained also for moisture, non-protein nitrogen and intramuscular fat content ( $R_{CV}$ =0.80–0.90, RPD=2.2–2.8), while for other constituents (protein, proteolysis index and free amino acids content) moderate results were obtained ( $R_{CV}$ =0.65–0.80, RPD=1.7–2.0).

Good calibration and cross-validation results in NIR (and consequently in the whole) spectral range can be explained by high correlations (up to  $\pm 0.80$ ) between assessed chemical constituents and the absorbance data (data not shown) in wavelength range from 1100 to 2400 nm. In the dry-cured ham water represents 43-65% of the total fresh matter. Absorbance peaks of O-H bonds at 1450 and 1940 nm (Shenk *et al.* 1992) explain satisfactory NIR predictability for water content. Good performance of NIR spectroscopy to predict fat content is due to the strong absorption of C-H bonds in the NIR region at 1000 to 1400, 1700 and 2200-2400 nm (Shenk *et al.* 1992). Regarding protein, specific absorbance of N-H bonds could be found in the NIR region from 1460 to 1570 nm and from 2000 to 2180 nm (Shenk *et al.* 1992). In the case of proteins, it should be mentioned that protein content was calculated on the assumption that all nitrogen in the sample appears in protein, although a part (27%) of nitrogen is in form of non-protein nitrogen. High correlation coefficients between average spectrum and salt content led to good prediction results although NIR spectroscopy is known to be unable to detect inorganic substances unless they are bound to organic substance (Van Kempen, 2001). It is likely that in dry-cured ham salt content is indirectly predicted from other compounds (e.g. correlation coefficient between salt and water content amounts to 0.53). Moreover, NaCl itself shows no absorbance in the NIR region, but the presence of dissolved salts gives rise to the wavelength shifts in the spectrum. This phenomenon has been used to assess the content of NaCl in meat products (Downey and Hildrum, 2004).

**Table 2. Prediction of chemical composition using NIR spectroscopy in two dry ham muscles (*biceps femoris* and *semimembranosus*)**

Constituent	Spectrum nm	n	Mean $\pm$ SD	emin	emax	SE <sub>c</sub>	R <sub>c</sub>	SE <sub>cv</sub>	R <sub>cv</sub>	RPD	PLS
Moisture, g/kg	400-2500	262	538.7 $\pm$ 51.6	383.9	693.6	15.8	0.91	16.3	0.90	3.17	4
	1100-2500	258	539.7 $\pm$ 51.3	385.9	693.6	15.6	0.91	16.1	0.90	3.18	4
Salt, g/kg	400-2500	254	71.69 $\pm$ 7.68	48.66	94.72	2.45	0.90	2.55	0.89	3.01	5
	1100-2500	253	71.70 $\pm$ 7.69	48.63	94.77	2.28	0.91	2.36	0.91	3.25	5
Salt per DM, %	400-2500	257	15.81 $\pm$ 2.88	7.17	24.44	0.85	0.91	0.91	0.90	3.15	5
	1100-2500	254	15.85 $\pm$ 2.89	7.17	24.53	0.86	0.91	0.91	0.90	3.16	5
Salt per moisture, %	400-2500	254	13.33 $\pm$ 1.41	9.10	17.56	0.46	0.89	0.50	0.87	2.80	6
	1100-2500	255	13.33 $\pm$ 1.40	9.12	17.55	0.50	0.87	0.52	0.86	2.69	5
Protein, g/kg	400-2500	264	33.96 $\pm$ 5.05	18.81	49.12	1.42	0.92	1.57	0.90	3.22	7
	1100-2500	262	33.90 $\pm$ 5.01	18.86	48.94	1.46	0.91	1.58	0.90	3.18	6
NPN, g/kg	400-2500	260	12.25 $\pm$ 0.99	9.29	15.21	0.53	0.71	0.57	0.66	1.72	6
	1100-2500	258	12.25 $\pm$ 0.97	9.34	15.17	0.57	0.65	0.59	0.64	1.65	5
Proteolysis index	400-2500	262	22.91 $\pm$ 4.26	10.12	35.69	1.57	0.86	1.69	0.84	2.52	6
	1100-2500	262	22.92 $\pm$ 4.25	10.17	35.66	1.55	0.87	1.70	0.84	2.50	8
IMF, g/kg	400-2500	259	36.6 $\pm$ 12.3	0.0	73.7	7.5	0.63	7.6	0.62	1.63	3
	1100-2500	259	36.6 $\pm$ 12.3	0.0	73.6	7.6	0.62	7.7	0.61	1.60	3
FAA, mg/100g DM	400-2500	258	7120 $\pm$ 879	4484	9757	348	0.84	382	0.81	2.30	6
	1100-2500	258	7124 $\pm$ 873	4507	9742	396	0.79	410	0.78	2.13	4

DM – dry matter; NPN – non protein nitrogen; IMF – intramuscular fat content; FAA – free amino acid content; SD – standard deviation of the reference values (calculated after the elimination of outliers); emin – estimated minimum; emax – estimated maximum; SE<sub>c</sub> – standard error of calibration; R<sub>c</sub> – coefficient of determination of calibration; SE<sub>cv</sub> – coefficient of determination of cross validation; R<sub>cv</sub> – standard error of cross validation; RPD – residual predictive deviation (ratio SD/se<sub>cv</sub>); PLS – number of PLS factors.



### 3. General discussion

Our results demonstrated high potential of NIR spectroscopy to predict chemical constituents and amino acid content of dry-cured ham which is very important for the industry to fulfil the *consortium* requirements. Presently, producers use classical/wet chemistry which is lengthy, expensive, often hazardous and thus less interesting to be used on a large scale. Since NIR spectroscopy enables fast and simple determination of many parameters simultaneously, it could effectively replace regular checking of dry-cured ham chemical constituent prescribed by the *consortium*.

In the literature there are a few literature reports on meat products (Collell *et al.*, 2010; Cruz Ortiz *et al.*, 2006; Gaitán-Jurado *et al.*, 2008; García-Rey *et al.*, 2005; González-Martín *et al.*, 2009; Ortiz-Somovilla *et al.*, 2007), moreover, there is a lack of information regarding the prediction of chemical composition of dry-cured ham. Accuracy of NIR predictive models obtained cannot be directly compared for different meat products because of different matrix, different constituents' variation range, *etc.* Literature reports (Gaitán-Jurado *et al.*, 2008; Ortiz-Somovilla *et al.*, 2007) on meat products (mainly pork sausages) showed successful prediction of fat, moisture and protein with NIR spectroscopy ( $R_{CV}=0.88-0.99$ ,  $RPD=2.9-10.4$ ). Excellent prediction results were published also by Collell *et al.* (2010) for moisture content in fermented pork sausages ( $R_{CV}>0.99$ ,  $RPD>20$ ). Similar as in our study they also managed to predict salt (NaCl) content with high accuracy ( $R_{CV}=0.97$ ,  $RPD=6.2$ ). Ellekjær *et al.* (1992) reported errors in the range of 0.4 to 1.3 g/kg in the prediction of salt content in cooked sausages.

## IV – Conclusions

In the present work NIR spectroscopy proved as highly reliable method for the prediction of studied chemical constituents of dry-cured ham “*Kraški pršut*”. For eventual replacement of (conventional chemical) methods currently used in regular checking of certified products the calibration models should be extended with samples of the whole a slice of dry-cured ham containing several muscles and adjacent fat.

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# Evaluation of a handheld near infrared (NIR) spectrometer for the discrimination of Iberian pigs according to their feeding regime

E. Zamora-Rojas<sup>1</sup>, A. Garrido-Varo, E. De Pedro-Sanz, J.E. Guerrero-Ginel and D.C. Pérez-Marín

Department of Animal Production, Faculty of Agricultural and Forestry Engineering  
University of Córdoba, Córdoba (Spain)

<sup>1</sup>e-mail: g32zaroe@uco.es

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**Abstract.** The aim of this study was to evaluate a handheld near infrared spectroscopy (NIRS) instrument to classify Iberian pig carcasses in different commercial categories for instantaneous quality control and authentication in the industry. Sixty intact subcutaneous adipose tissue samples taken from the tail insertion area in the coxal region of the body were analyzed, twenty per category ("Bellota", "Recebo" and "Cebo"). A discriminant analysis based on the algorithm PLS2 was performed and different spectra pretreatment were evaluated. A Principal Component Analysis (PCA) showed more clear differences between "Cebo" samples and the other classes, while "Bellota" and "Recebo" were spectrally more similar within them. The external validation of the classification models based on a second derivative showed only one "Recebo" sample misclassified as "Bellota". These results show the high potential of the handheld NIRS device evaluated for the individual authentication of Iberian pig carcasses to discriminate the different feeding regime followed by the animal during the growing-finishing period.

**Keywords.** Iberian pigs – NIR Spectroscopy – Classification – MEMS spectrometer – Adipose tissue.

## *Évaluation d'un spectromètre portatif dans le proche infrarouge (NIR) pour la discrimination des porcs Ibériques selon leur régime*

**Résumé.** L'objectif de cette étude était d'évaluer un instrument portatif de spectroscopie dans le proche infrarouge (NIRS) pour classer les carcasses de porcs Ibériques selon les différentes catégories commerciales pour le contrôle de la qualité instantanée et de l'authentification dans l'industrie. Soixante échantillons de tissu adipeux sous-cutané intact prélevés dans la zone d'insertion de la queue dans la région coxale du corps ont été analysés, vingt par catégorie ("Bellota", "Recebo" et "Cebo"). Une analyse discriminante basée sur l'algorithme de PLS2 a été réalisée et les différents spectres de prétraitement ont été évalués. Une analyse en composantes principales (PCA) a montré plus de différences claires entre les aliments des échantillons "Cebo" et les autres classes, tandis que "Bellota" (gland) et "Recebo", spectralement, étaient plus semblables en leur sein. La validation externe des modèles de classification basés sur une dérivée seconde montre un seul échantillon "Recebo" classé à tort comme "Bellota". Les résultats montrent le potentiel élevé de l'appareil de poche NIRS évalué pour l'authentification individuelle des carcasses de porcs Ibériques, afin de discriminer les différents régimes d'alimentation suivis par les animaux pendant la période d'engraissement.

**Mots-clés.** Porcs Ibériques – Spectroscopie NIR – Classement – Spectromètre MEMS – Tissu adipeux.

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## I – Introduction

The feeding regime of the animals during the final period of growing plays an important role in Iberian pig products quality (Garrido and De Pedro, 2007), within other factors that also influence such as the genotype (Ramírez and Cava, 2007), age or rearing conditions (Bonneau and Lebret, 2010). Those factors have a significant impact on the fatty acid composition and

mainly in the high unsaturated/saturated fatty acid ratio which at the end is responsible of the exceptional organoleptic and healthy properties of the Iberian meat products (Cava *et al.*, 2000; Ventana *et al.*, 2007). The Spanish legislation classifies the animals into four commercial categories depending on the feeding regime and production system (BOE, 2007): “Bellota” (i.e. animals in free range fed exclusively with grass and acorns), “Recebo” (i.e. animals fed with acorns and grass supplemented with compound feeds in a outdoor system), “Cebo” (i.e. animals fed with compound feed in an intensive system) and “Cebo de campo” (i.e. animals fed exclusively with compound feed in free range).

Official classification methods (on-farm inspections and gas chromatography of melted fat) are high cost and time-consuming, not providing individual items but batches information of animals groups that can have individual variability. Fast, accurate, objective, low cost and individual analysis methods are demanded by consumers and industry for quality control and authentication of these high market prices products. Since 1992, the potential of Near Infrared Spectroscopy (NIRS) has been highlighted for the analysis of melted fat (De Pedro *et al.*, 1992; García-Olmo *et al.*, 2009), intact adipose tissue (De Pedro *et al.*, 2007) and live Iberian pigs (Pérez-Marín *et al.*, 2009) as a tool for classifying Iberian pig animals into different commercial categories on the basis of the feeding regime. Few applications have been evaluated for on-line analysis (Pérez-Marín *et al.*, 2009) and nowadays there are appearing in the market new handheld devices of low cost and easy analysis presentation, providing instantaneous results readily-available. In this study is evaluated a handheld micro electron mechanical system (MEMS) NIRS spectrometer for classifying Iberian pigs carcasses according to their feeding regime.

## II – Materials and methods

### 1. Sample Set

Sixty Iberian pig adipose tissue samples were measured, taken from the tail insertion area in the coxal region of the body, where traditional gas chromatography biopsy is taken (De Pedro, 2001). The samples were stored at -20°C until 24 hours before the NIRS analysis. The sample set was composed of 20 samples of each commercial category studied (“Bellota”, “Recebo” and “Cebo”). Animal feeding regime was controlled by trained personnel.

### 2. NIRS measurements

A handheld MEMS-NIRS instrument (Phazir 2400, Polychromix Inc., Wilmington, MA, USA) was used to collect reflectance spectra in the range 1600-2400 nm with a resolution of 8 nm (resolution-pixel 8nm, resolution-optical 12nm). A quartz protection was used for preventing dirt accumulation in the instrument. Three spectra per sample were collected and the mean spectrum per sample was used for further analysis.

### 3. Data modelling

As spectral pre-treatments, Standard Normal Variate (SNV) plus Detrending (DT) (Barnes *et al.*, 1989) was used to remove the multiplicative interferences of scatter and two derivative mathematical treatments were performed: window-wise filtering (1,10,5,1) and (2,5,5,1) (ISI, 2000). A Principal Component Analysis (PCA) was performed in order to detect spectral outlier samples and observe possible groups tendency. After outlier detection, the data set was divided in two: a training and validation (5 samples of each category) sets using the SELECT algorithm of the WinISI software. Discriminant analysis based on Partial Least-Squares (PLS2) was applied to classify the subcutaneous adipose tissue in the different commercial categories studied. The optimum number of model factors was selected by cross-validation using 4 groups. Those chemometric analyses were performed using the software WinISI II ver 1.50 (Infrasoft International, Port Matilda, PA, USA). The classification models were statistically evaluated, by calculating the number of animals correctly classified.

### III – Results

Figure 1 shows the mean spectra of each commercial category analyzed. It was observed a similar pattern for all the groups, although it seems that there is a difference in absorbance range. Fat peaks were recorded at around 1720-1760, 2150 and 2310-2340 nm (Williams and Norris, 1987; Osborne, Fearn and Hindle, 1993); characteristic absorption bands at around 1940 nm were water-related (Williams and Norris, 1987; Osborne, Fearn and Hindle, 1993).

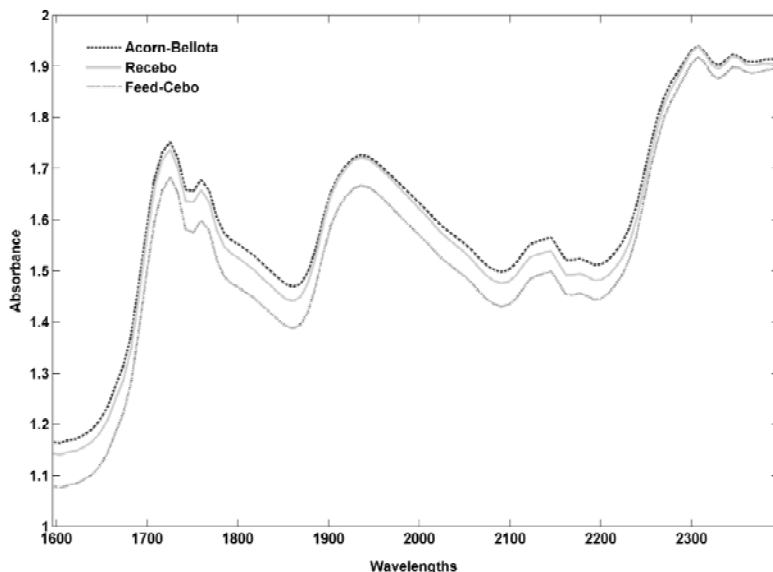


Fig. 1. Raw spectra (mean spectra) for each Iberian pig category studies.

PCA was performed to visualize the main structure of the data set and detect spectral outlier samples. It was observed 7 samples far from the centre of the population or with possible classification error due to the individual variability of the animals. After being removed, a new PCA analysis was performed using 9 Principal Components (PCs) explaining the 98.86%. The score plot (Fig. 2) shows clear differences between “Cebo” cluster and the other categories. “Recebo” and “Bellota” showed an overlap cluster, probably due to they are samples with a fatty acid profile more similar since those animals have eaten both acorns and grass.

Table 1 shows the statistics and number of samples correctly-classified of the training set, after outlier detection, for PLS discriminant. Two spectra pre-treatments were evaluated and a second derivative provided better classification results. A 91.66% of the “Bellota” samples were correctly classified, 63.6% of the “Recebo” samples and 100% of the “Cebo” samples. Table 2 shows the external validation of the model performed with a second derivative. Only one sample of the “Recebo” group was misclassified as an “Bellota” sample.

It should be remarked that any sample of the group “Cebo” (products with lower prices) was misclassified in the other groups (“Bellota” or “Recebo”, products with higher prices) or vice versa. These results confirm the possibility to discriminate Iberian pig adipose tissue from animals reared under different feeding regime using a handheld MEMS-NIRS instrument that can be implemented in on-line applications in the industry.

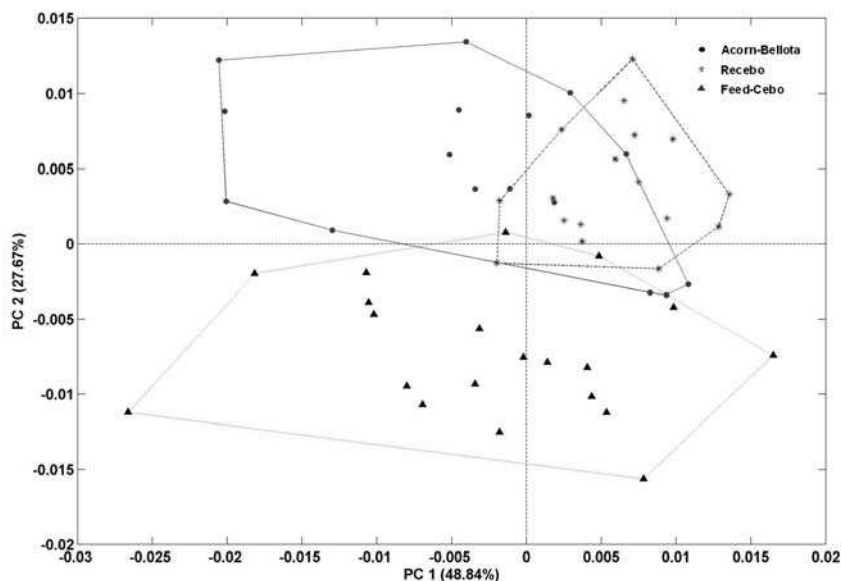


Fig. 2. PCA score plots for the two principal components.

Table 1. Classification results obtained by the PLS discriminant with a second derivative

Origin	No. of samples	Classified as		
		Bellota	Recebo	Cebo
Bellota	12	11	1	0
Recebo	11	4	7	0
Cebo	15	0	0	15

Table 2. External validation of the second derivative PLS discriminant model

Origin	No. of samples	Classified as		
		Bellota	Recebo	Cebo
Bellota	5	5	0	0
Recebo	5	1	4	0
Cebo	5	0	0	5

## IV – Conclusions

The classification of Iberian pig intact adipose tissues using the handheld NIRS device evaluated was successful. PLS discriminant performed better classification predictions with a second derivative with only one sample of the “Recebo” group misclassified for the external

validation. These results indicate the feasibility of performing an individual authentication of Iberian pig carcasses according to the feeding regime of the animals in a fast and with possibility of on-line applications. Further work is required in order to develop robust classification models with larger data sets and transfer the technology from laboratory to an on-line monitoring system.

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# Differential expression of sarcoplasmic protein in 'Casertana', 'Calabrese' and PEN AR LAN Pork

D. Matassino\*, C. Di Prisco\*, F. Inglese\*, F. Romagnuolo\* and A. Di Luccia\*\*

\*Consortium for Experimentation, Dissemination and Application of Innovative Biotechniques (ConSDABI) – Italian Sub National Focal Point FAO (Sub NFP.I-FAO) (Mediterranean biodiversity) – Centre of Omic Science for Nutritional Quality and Excellence (Italy)

\*\*Department of Food Science, Agricultural Faculty, University of Foggia (Italy)

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**Abstract.** The characterization of the water soluble fraction of muscle proteins was carried out on 30 samples of meat taken from a pool of muscles (*Semimembranosus*, *Semitendinosus* and *Biceps femoris*) each representative of the 30 subjects {[20 ancient autochthonous genetic type (AGT) with 'black' coat [10 'Casertana' (CT), 10 'Apulo Calabrese' (Calabrese) (CL)] and 10 PEN AR LAN ('white' coat, from cross breeding Large White and Landrace)}. Protein profile was analyzed by two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry. The comparison of 60 two-dimensional maps was performed by Image Master 2D-Platinum software in order to establish the position and relative intensity, expressed as vol %, of each spot for each gel. In the range of our observation, image analysis showed 32 spots common to all samples analyzed; 17 spots of 32 common differed in relative 'abundance' ( $P < 0.05$ ). These spots, identified by peptide mass fingerprint, were classified as metabolic, cellular defense and other protein types. The results suggest a further possible use of proteomic approach in the tracing back of traditional food.

**Keywords.** MALDI-ToF fingerprint – Pig – Sarcoplasmic proteins – Two-dimensional gel electrophoresis.

## **Expression différentielle des protéines de la fraction soluble dans l'eau pour la viande des TGAA 'Casertana', 'Calabrese' et des hybrides commerciaux PEN AR LAN**

**Résumé.** La caractérisation de la fraction soluble des protéines musculaires a été effectuée sur 30 échantillons de viande provenant d'un pool de muscles (*Semimembranosus*, *Semitendinosus* et *Biceps femoris*) pour chacun des 30 sujets traités {20 de type génétique autochtone ancien (TGAA) à robe 'noire' [10 'Casertana' (CT), 10 'Apulo Calabrese' (Calabrese) (CL)] et 10 PEN AR LAN (à robe 'blanche', d'ascendants Large White et Landrace)}. Le profil protéique a été évalué en utilisant des procédures analytiques telles que l'électrophorèse bidimensionnelle couplée à la spectrométrie de masse MALDI-TOF. La comparaison des 60 cartes a été réalisée avec le software Image Master 2D-Platinum afin de comparer la position et l'intensité relative, exprimée en % vol de chaque spot pour chaque gel. Dans le cadre de l'observation, l'analyse d'image a mis en évidence 32 spots communs à la totalité des échantillons analysés ; 17 spots sur les 32 différent en % vol ( $P < 0,05$ ). Ces spots ont été ensuite identifiés par peptide mass fingerprint et classés comme protéines du métabolisme, de défense cellulaire et autres protéines. Les résultats suggèrent une possible utilisation ultérieure de l'approche protéomique pour des études de caractérisation visant, entre autres, à l'analyse de la traçabilité.

**Mots-clés.** MALDI-ToF fingerprint – Porc – Protéines de la fraction soluble dans l'eau – Électrophorèse bidimensionnelle.

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## **I – Introduction**

Proteomic analysis defines the identity, the structure and the relative abundance of proteins in a given cell type in a specific set of conditions. Proteins are the expression of genetic inheritance which also undergo post-translational modifications (phosphorylation, glycosylation and acetylation). The study of these modifications, by proteomic approach, may assist to differentiate animal species. The proteomic approach can be used for 'molecular

characterization' of raw materials and their products; this 'characterization' can be employed in order to: (i) trace back product; (ii) point out flow chart phases; (iii) characterize possible relationships among protein (and their fragments) 'quantity' and 'quality attributes' of raw materials and their products.

The aim of this contribution was to suggest a proteomic approach to differentiate swine races. Differential analysis of the proteome in relation to the soluble fraction (sarcoplasmic) was carried out on meat samples taken from a pool of muscles (*Semimembranosus*, *Semitendinosus* and *Biceps femoris*) in pigs.

## II – Materials and methods

The study involved 30 meat samples taken from a pool of muscles (*Semimembranosus*, *Semitendinosus* and *Biceps femoris*) each representative of the 30 subjects {[20 ancient autochthonous genetic type (AAGT) with 'black' coat [10 '*Casertana*' (CT), 10 '*Apulo Calabrese*' (Calabrese) (CL)] and 10 PEN AR LAN ('white' coat, from cross breeding Large White and Landrace)}. The slaughter was carried out in a single establishment and the carcass maturation was carried out in refrigerator at a temperature of 2-4 °C for a period of approximately 72 hours.

The analysis covered a total of 60 samples (2 for each sample) processed in parallel by:

### (i) 2D-IPG-SDS-PAGE:

- the first dimension (IEF-IPG) was carried out by Ettan IPGphor II (GE Healthcare) using Immobiline DryStrips gel pH 3-10NL (18 cm) rehydrated with a solution of 8 M Urea, 0.5% CHAPS, 0.2% DTT, 0.5% IPG Buffer.
- the second dimension was carried out in accordance with the procedure of O'Farrell (1975) in polyacrylamide gradient gel electrophoresis (T = 9-18% and C = 2.5%) by using Ettan Twelve System (GE Healthcare).

### (ii) Image analysis of two-dimensional maps (2-DGEm):

- was performed by software Image Master 2D-Platinum (GE Healthcare) quantifying in vol % the expression level of each spot; the spots found in common to three swine races were subjected to statistical analysis Student's t test.

### (iii) Identification by MS:

- each spot was digested *in situ* with trypsin according to the procedure of Shevchenko *et al.* (1996) and tryptic digests were analyzed with Ettan MALDI-Tof/PRO mass spectrometer (GE Healthcare).

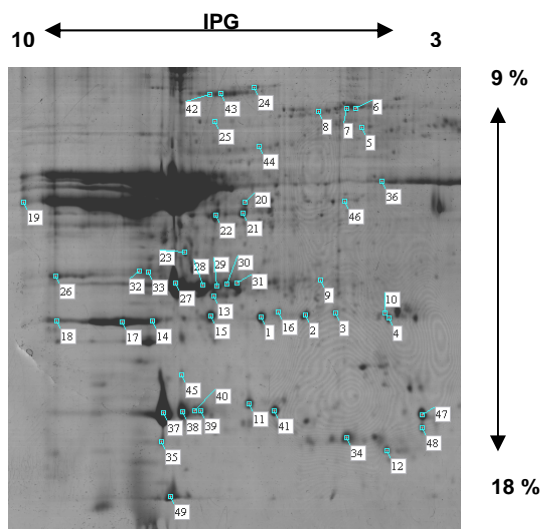
## III – Results and discussions

The measurements carried out by MALDI-Tof mass spectrometer allowed us to identify 49 spots (Figure 1, Table 1). Some proteins appeared heterogeneous with differences in mass [MW (kDa)] and / or isoelectric point (pI) and they were identified as: Adenylate kinase, Myoglobin, Peroxiredoxin, Phosphoglycerate mutase, Similar parvalbumin, Transferrin, Triosephosphate isomerase. This heterogeneity could be due to:

(i) Genetic polymorphism (for example single nucleotide polymorphism).

(ii) Post-transcriptional modifications (for example alternative splicing).

(iii) Post-translational modifications (for example glycosylation and phosphorylation).



**Fig. 1. 'Casertana' AAGT. Two-dimensional map of the sarcoplasmic proteins.**

Considering the three breeds, 32 spots (65% of those identified) resulted in common to totality of subjects analyzed. The proteins identified were grouped by protein function as reported in Table 1:

(i) Metabolic protein:

- adenylate kinase (it catalyzes the reversible transfer of terminal phosphate group between ATP and AMP),
- enolase (it is an enzyme involved in the development and regeneration of striated muscle).

We compared 32 common spots of the three swine races analyzed. The differences observed were analyzed by Student's t test (Figure 2; Table 2) and divided in significant ( $P < 0.05-0.001$ ) and near to significant ( $P < 0.20-0.10$ ):

- CL vs CT: 14 comparisons out of 32 [44%, 6 of which significant ( $P < 0.05-0.001$ ) and 8 near to significance ( $P < 0.20-0.10$ )].
- CL vs PEN AR LAN: 22 comparison out of 32 [69%, 11 of which significant ( $P < 0.05-0.001$ ) and 11 near to significance ( $P < 0.20-0.10$ )].
- CT vs PEN AR LAN: 16 comparison out of 32 [50%, 12 of which significant ( $P < 0.05-0.001$ ) and 4 near to significance ( $P < 0.20-0.10$ )].

**Table 1. Spots identified by MALDI-Tof mass spectrometry. Red spots were common to all two-dimensional maps**

Group	Spot, N	Protein name
Cellular defense proteins	1, 2, 3	DJ-1
	4	Glyoxalase
	5	HSP 60
	6, 7, 8	HSP 70
	9, 10	Peroxiredoxin
	11	Superoxide dismutase
	12	Thioredoxin
Metabolic proteins	13, 14, 15, 16, 17, 18	Adenylate kinase
	19	Aldolase
	20, 21, 22	Enolase
	23, 24	Muscle Creatine kinase
	25, 26	Phosphoglycerate mutase
	27, 28, 29, 30, 31	Triosephosphate isomerase
Transport proteins	32, 33	Carbonic anhydrase
	34	H-FABP
	35	Haemoglobin $\beta$ -chain
	36	Haptoglobin
	37, 38, 39, 40, 41	Myoglobin
	42, 43	Transferrin
Indicators of proteolysis	44	Leucine aminopeptidase
	45	Muscle Creatine kinase fragment
Miscellaneous	46	Zinc finger protein
	47, 48	Similar Parvalbumin
	49	Similar to polyubiquitin

The major number of significant and near to significant critical limit comparisons was found in the comparison CL vs PEN AR LAN (69%) while the lower number (44%) was found in the CL vs CT comparison. It is interesting to note that the major differences in protein expression were detected among race with 'white' coat (PEN AR LAN) and those with 'black' coat (CL and CT). The differences related to the proteins classified by function as:

- (i) cellular defense proteins (DJ-1, HSP 70, thioredoxin),
- (ii) metabolic proteins (adenylate kinase, enolase, triosephosphate isomerase),
- (iii) transport proteins (H-FABP, haemoglobin beta chain, myoglobin),
- (iv) proteolysis indicator [leucine aminopeptidase (LAP)],
- (v) other proteins (parvalbumin).

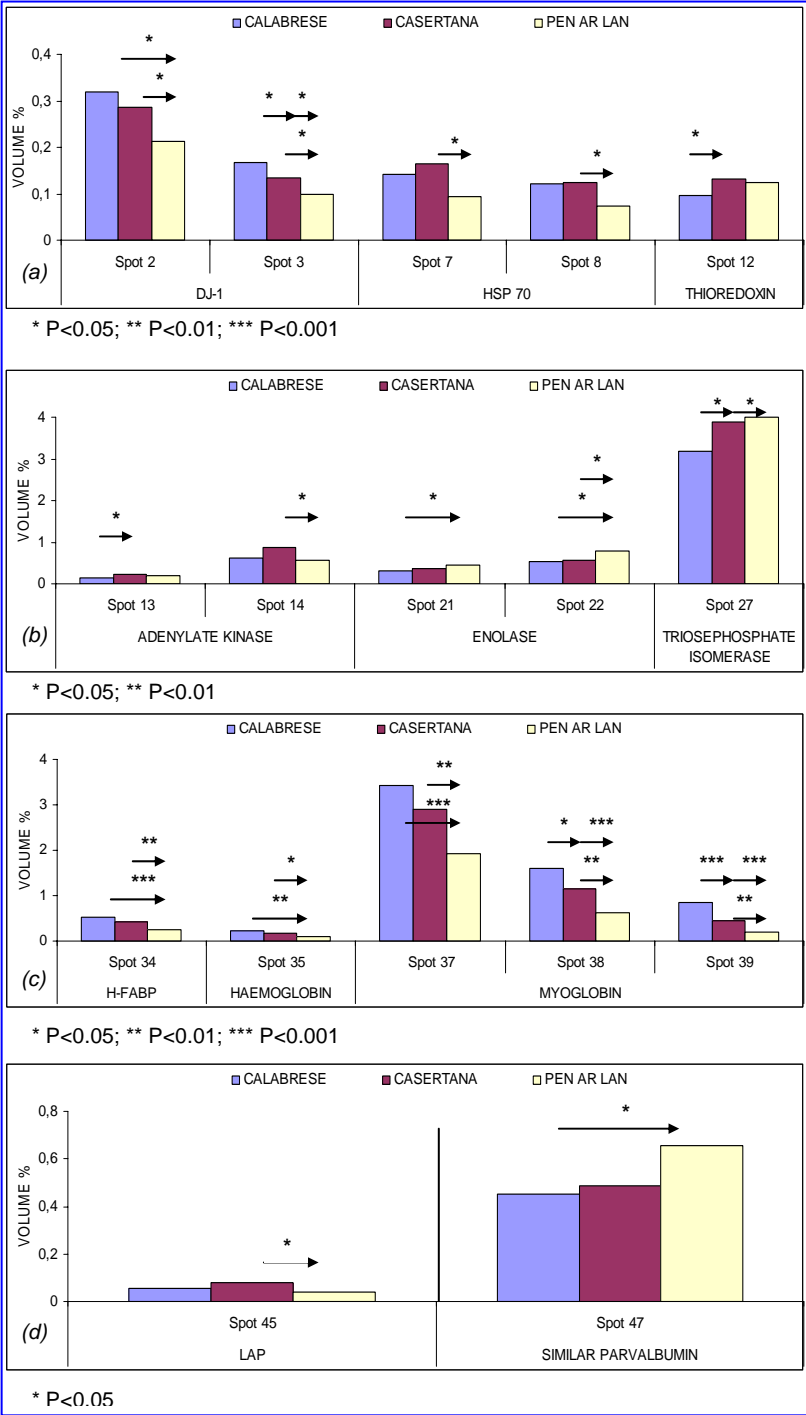
**Table 2. Differentially expressed spots. Comparison between 'Apulo Calabrese' (Calabrese), 'Casertana' and PEN AR LAN. Identification by MALDI-Tof mass spectrometry**

Group	Spot, N	Protein name	CL vs CT	CL vs H	CT vs H
Cellular defense proteins	2	DJ-1	<<<	***	***
	3		**	***	***
	4	Glioxalase	<	<<<	
	5	HSP 60	<<<		<<<
	6	HSP 70		<	
	7			<<	**
	8			<<	*
	12	Thioredoxin	**	<<	
Metabolic proteins	13	Adenylate kinase	**	<<<	
	14		<<<		*
	15		<<<	<<<	
	20	Enolase		<<<	<
	21			*	<<<
	22			*	<<<
	23	Muscle creatine kinase	<<<	<<<	
	27	Triosephosphate isomerase	*	*	
Transport proteins	34	H-FABP	<<	***	**
	35	Haemoglobin $\beta$ -chain		**	*
	37	Myoglobin		***	**
	38		*	***	**
	39		***	***	**
Indicators of proteolysis	44	Leucine aminopeptidase	<<	<<	*
Miscellaneous	47	Similar Parvalbumin		*	<<

CL = 'Apulo Calabrese' (Calabrese); CT = 'casertana'; H = Pen Ar Lan 'Hybrid'

< P<0.20; << P<0.15; <<< P<0.10; \* P<0.05; \*\* P<0.01; \*\*\* P<0.001

Our results highlighted the importance of 'race' for relative quantitative expression (volume, %) of spots considered, as previously showed in sheep and pig by Matassino *et al.* (2010 a,b), and for energy metabolism that may be different depending from coat color ('black' or 'white'). In fact, in the 'white' pig the higher expression of enzymes involved in the glucidic metabolism and the lower expression of myoglobin may point out that muscle cells favour the glycogen catabolism and, so, in order to balance the energy metabolism, they may pre-eminently use glucose rather than fatty acid. The opposite behaviour is observed in 'black' pig in which, an increased expression of myoglobin and H-FABP and a decreased expression of glucose metabolism enzymes would indicate a metabolism mainly based on oxidative chain. This is also confirmed by the predominantly 'oxidative' nature of some muscles of 'black' and 'belted' pigs caused by a higher percentage of Slow Oxidative fibers (SO), as evidenced by previous research (Matassino *et al.*, 1993; Barone *et al.*, 2000, 2005). This would result in a different use of the energy stored by two genetic types of pigs: the 'black' pig would use preferentially energy stored in adipose tissue while the 'white' pig would use energy stored under glycogen form. This difference in the metabolic activity of muscles may be attributed to a different use of the metabolic - energy pathways (glycolysis or oxidative). This differentiation could derive from a diversity of both genetic (for example coat color gene) and hormonal by stress (for example adrenaline) nature; both could influence the structure and the functionality of the muscular fiber.



**Fig. 2. Spots differentially expressed grouped in: (a) cellular defense proteins, (b) metabolic proteins, (c) transport proteins, (d) indicator of proteolysis and miscellaneous.**

This differentiation could derive from a diversity of both genetic (for example coat color gene) and hormonal by stress (for example adrenaline) nature; both could influence the structure and the functionality of the muscular fiber.

These results demonstrated that it was possible to study muscle physiology not only by fiber composition but by molecular level too.

The increased expression of cellular defense proteins in 'black' pig may suggest a better its response to phenomena of environmental stress. This is in agreement with the greater ability to constructivism (Matassino,1989; Lewontin, 1993) hypothesized for 'black' pig in comparison with 'white' pig, being the former also in a initial phase of anthropic conditioning.

## IV – Conclusions

The proteomic approach to the study of sarcoplasmic protein of muscle revealed the differences in energy metabolism between 'black' and 'white' pig. These differences could represent sources of 'molecular characterization' that could allow both to trace back meat of different genetic types and to assess their 'nutritional' and 'extra-nutritional' quality. However, it is necessary to extend sampling and to deepen the study of muscular protein composition.

## Acknowledgements

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# Is it possible the breed origin traceability of Iberian pigs?

E. Alves, A.I. Fernández, L.A. García-Cortés, Á. López, R. Benítez  
C. Rodríguez and L. Silió

\*Departamento de Mejora Genética Animal, INIA, Ctra. La Coruña, Km 7, 28040 Madrid (Spain)

**Abstract.** Two different approaches for the verification of *Iberian* breed origin, in both animals alive and meat products, are jointly described in this study. The former is based on two genes that present polymorphisms with alleles that are exclusive (*MC1R*\*4) or present high frequencies (*IGF2* g.3072A) in *Duroc* breed and are absent in *Iberian*. The use of these markers allows to discriminate *Iberian* purebred from crossbred *Duroc* x *Iberian* but it does not fully detect pigs with a lower proportion of *Duroc* genes. The second approach is centered on the use of single nucleotide polymorphisms (SNP) presenting divergent frequencies in both breeds. After hybridization with PorcineSNP60 BeadChip of samples from different *Iberian* (26) and *Duroc* (15) origins, we selected 96 SNPs with differences of allelic frequencies larger than 0.8 and evenly distributed over the 18 autosomes. Simulations were carried out estimating that 48 out of these SNPs would allow the verification of breed origin, with errors ranging from 1% to 4%, both in purebred and in crossbred animals with different *Duroc* proportion. Two diagnostic analyses based respectively on 750 and 230 samples genotyped for the panel of 96 SNPs have been performed with different purposes and satisfactory results.

**Keywords.** Breed traceability – *Iberian* – *Duroc* – SNP.

## La traçabilité de l'origine des porcs de race Ibérique est-elle possible ?

**Résumé.** Deux approches sont décrites pour vérifier l'origine de la race Ibérique, applicables aux animaux vivants ainsi qu'à leurs produits dérivés, grâce à l'utilisation de marqueurs génétiques. La première est basée sur l'utilisation de deux gènes qui présentent des polymorphismes avec des allèles fixés (*MC1R*\*4) ou qui présentent une fréquence élevée (*IGF2* g.3072A) chez la race *Duroc* et qui sont absents chez la race Ibérique. L'utilisation de ces marqueurs permet de discriminer les génotypes Ibériques purs par rapport aux animaux issus de croisements *Duroc* x Ibérique, mais ne détecte pas complètement les porcs ayant un fond génétique *Duroc* moins important. La deuxième approche est basée sur l'utilisation des polymorphismes de type SNP, présentant des fréquences alléliques différentes chez les deux races. Après hybridation des échantillons de différentes origines Ibériques (26) et *Duroc* (15) sur la puce de génotypage PorcineSNP60 BeadChip, nous avons sélectionné 96 SNPs parmi ceux qui présentaient des différences inter-races de fréquences alléliques supérieures à 0,8 et qui étaient répartis sur les 18 autosomes. Les simulations réalisées estiment que l'utilisation de 48 de ces SNPs permettrait la répartition par race des animaux purs et croisés avec *Duroc* dans des proportions différentes, en obtenant des taux d'erreur compris entre 1% et 4%. Nous avons réalisé deux analyses diagnostiques basées sur le génotypage de 750 et 230 échantillons, respectivement, sur le panel de 96 SNPs, avec des objectifs différents et des résultats satisfaisants.

**Mots-clés.** Traçabilité – Ibérique – *Duroc* – SNP.

## I – Introduction

Iberian pigs are the source of highly priced meat and dry-cured products. The optimum quality of meat and hams is associated with both the combination of purebred *Iberian* genotypes and traditional extensive fattening called *Montanera*. However *Iberian* pigs are commonly crossbred with *Duroc* animals, and even other dark coated breeds like *Large Black*, to improve their efficiency for lean growth. The Spanish regulation of 'Iberian' labelling only admits progenies from *Iberian* sows and boars crosses with males from other breeds than *Duroc*. Moreover cured

products are labelled as 'Ibérico' or 'Ibérico Puro' depending if they proceed from *Duroc* x *Iberian* crossbred or from purebred *Iberian* animals. In the case of products to be exported, additional quality controls could be eventually carried out by customer countries. Full traceability is increasingly being demanded by producers and consumers but traditional tagging systems presents several difficulties, mainly in *Iberian* pigs, because of their extensive management system (López-Bote, 1998).

In this context, genetic markers could be a useful tool to check the breed origin of living animals and products as well as to verify the parentage relations registered on the Herd Book. Microsatellite markers have been proposed for estimate the genetic composition of dry-cured *Iberian* hams (García *et al.*, 2006). However this approach requires the genotyping of a high number of markers (>25) and preliminary analyses on parental populations are needed to determine their allelic frequencies pattern. In addition, these markers can be difficult to score and are not amenable to automation. Single nucleotide polymorphisms (SNPs) present several advantages over microsatellite markers: higher abundance in the genome, more easily to handle and interpret in laboratory and better compatibility with automation. Although SNP are usually bi-allelic and consequently less informative than microsatellites, this disadvantage can be overcome by genotyping a higher number of SNPs. Our group has been working in the search of *Iberian* or *Duroc* exclusive genetic markers and, despite the close genetic relationship between these breeds, some exclusive alleles were reported on nuclear (Fernández *et al.*, 2004) and mitochondrial (Alves *et al.*, 2009) genes. Indeed analysis of *MC1R* and *IGF2* alleles are already in use and mitochondrial DNA markers could result particularly useful for *Iberian* maternal origin validation. The problems arise when samples to analyze carry less than 50% of *Duroc* genes. In these cases neither of the available tests does itself results enough to certify genetic origin (Rodríguez-Ramilo *et al.*, 2008). Advances in high-throughput DNA sequencing and genotyping have led to the recent commercialization of a high density porcine SNP array. The aim of this work was to use the 60K porcine SNP array to develop a low-density panel of evenly spaced SNPs and check its effectiveness for differentiate purebred *Iberian* from crossbred pigs with a wide range of *Duroc* genes.

## II – Materials and methods

### 1. High-density genotyping

In a first step, 41 samples were analyzed including 15 *Duroc* boars from 12 different genetic origins and 26 *Iberian* pigs (both males and females) from 16 breeding nuclei registered in the Herd Book. Genomic DNA was extracted from blood samples according to the standard phenol-chloroform method or from ear tag biopsies using the PureLink™ Genomic DNA kit (Invitrogen, Spain). All these samples were genotyped for 62,163 SNPs, using the Porcine SNP 60 BeadChip (Illumina, San Diego, CA, USA). Genotyping reactions were performed on an "Infinium DNA Analysis Assay" at the Veterinary Service of Molecular Genetics (Universitat Autònoma de Barcelona, Spain).

### 2. SNP selection and evenly spaced low-density genotyping

The porcine SNP60 BeadChip features 62,163 evenly spaced probes with an estimated one marker per 40Kb across the pig genome. Genotyping data were analyzed and those SNPs that failed to produce an amplification product, that have no information about their location or map over sexual chromosomes were eliminated of the study, yielding a total of 45,180 SNPs. A first SNP selection was based on (i) their informativity i.e. the SNP that presented divergent frequencies between the two analyzed breeds with between-breed differences of allelic frequencies larger than 0.80 and (ii) regular distribution over the 18 autosomes. We also incorporated additional probes in order to check the feasibility of genotyping highly informative SNP for breed origin verification (*IGF2*, *MC1R*, *OCA2*) not included in the Porcine SNP 60

BeadChip. The Illumina Assay Design Tool (ADT) was used for evaluating individual loci and creating the most successful custom genotyping assay. The 96 SNP loci finally selected were simultaneously interrogated with the GoldenGate Genotyping Assay. Two analyses were performed with different goals and based respectively on: a) 750 DNA samples of purebred pigs of both breeds and probationalary *Iberian* pigs, and b) 230 DNA samples from *Iberian* sows and *Duroc* boars and controlled F1 *Duroc* x *Iberian* crossbred pigs.

### 3. Simulations and statistical analysis

Previous simulations were performed assuming different number of bi-allelic SNPs (24, 48 and 96) with diverse values (from 0.60 to 0.80) for the allelic differences between *Iberian* and *Duroc* breeds. In each simulation, one hundred reproducers of each breed were simulated with the correspondent number of SNP genotypes. From the alleles of reproducers of each breed we sampled two hundreds F1 crossbred pigs, and a similar procedure was used between purebred and F1 individuals to simulate four hundreds pigs of each one of the backcrosses: F1 x *Iberian* and F1 x *Duroc*. According to its profile of allelic frequencies, each one of the simulated pigs was assigned to one of five clusters using an algorithm similar to STRUCTURE (Pritchard *et al.*, 2000). One hundred replicates were obtained for each simulated case.

For the analysis of actual genotyping data software *Bayesian Analysis of Population Structure* (BAPS) v5 was used (Corander *et al.* 2003).

## III – Results and discussion

### 1. SNP selection from High-Density Genotyping

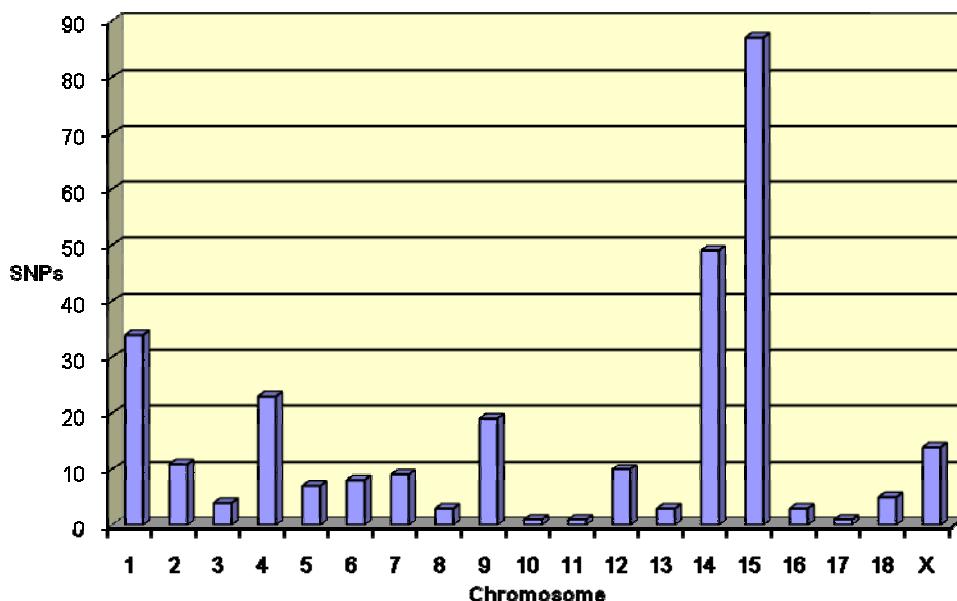


Fig. 1. Distribution over the porcine chromosomes of SNPs showing differences between breeds of allelic frequencies larger than 0.80, obtained from high-density genotyping with SNP60 BeadChip.

The genotyping of 15 *Duroc* and 26 *Iberian* samples with the porcine SNP60 BeadChip revealed a total number of 292 SNPs with allelic frequencies differences larger than 0.80 and mapped on different chromosomes (Fig. 1). The number of SNPs displaying divergent allelic frequencies is proportional to the total number of probes interrogated in each chromosome but it is not related with the size of the chromosome. That is the reason why the highest number of SNPs was observed on SSC14 and SSC15 that are smaller than SSC13. Despite this inconvenient, we tried to carry out the SNP selection taking into account the chromosomes size whenever possible and choice SNPs evenly spaced across the porcine genome. Moreover, as it was said before, we checked the feasibility of genotyping highly informative SNPs for breed origin verification (*IGF2*, *MC1R*, and *OCA2*) not included in the Porcine SNP 60 BeadChip. Assay Design Tool (Illumina) provided satisfactory scores for *OCA2*, *MC1R\*2* and *MC1R\*4* but not for *IGF2* g.3072A polymorphism. Inclusion of mitochondrial polymorphisms also had to be discarded because GoldenGate Genotyping Assay does not allow the genotyping of this kind of variation. The final set of 96 SNPs included 92 probes selected from the Porcine SNP 60 BeadChip, distributed over the 18 autosomes as follows: 11 on SSC1, eight on SSC2, three on SSC3, six on SSC4, six on SSC5, seven on SSC6, five on SSC7, three on SSC8, seven on SSC9, one on each one of SSC10 and SSC11, six on SSC12, four on SSC13, eight on SSC14 and also on SSC15, two on SSC16, one on SSC17 and five on SSC18. Moreover four additional probes were included, two for *MC1R\*2* and one for each one *MC1R\*4* and *OCA2*.

## 2. Simulation results

Results of two of the performed simulations were summarized in Tables 1 and 2. The first case corresponds to the use of a panel of 48 SNPs with remarkable allelic differences between breeds ( $|q_{\text{IBERIAN}} - q_{\text{DUROC}}| > 0.80$ ), and the second to a larger panel of 96 SNPs with lower divergence of frequencies ( $|q_{\text{IBERIAN}} - q_{\text{DUROC}}| > 0.60$ ). In both situations, the method performs very well for assigning adequately each individual to its correspondent genetic group. The observed error rates in these simulations ranged from 1% to 4% using 46 very divergent SNPs (Table 1), and from 0.1 % to 2% using 96 moderately divergent SNPs (Table 2).

**Table 1. Allocation average proportions (over 100 replicates) to the different groups of simulated purebred and crossbred pigs: *Iberian* ( $n = 100$ ), *Duroc* ( $n = 100$ ), F1 *Duroc* x *Iberian* ( $n = 200$ ), F1 x *Duroc* ( $n = 400$ ) and F1 x *Iberian* ( $n = 400$ ) genotyped for 48 SNPs with allelic differences between breeds greater than 0.80**

	Iberian	Duroc	F1 Duroc x Iberian	F1 x Duroc	F1 x Iberian
Iberian	0.992	0.000	0.000	0.000	0.008
Duroc	0.000	0.992	0.000	0.008	0.000
F1 Duroc x Iberian	0.000	0.000	0.960	0.011	0.029
F1 x <i>Duroc</i>	0.000	0.002	0.033	0.965	0.000
F1 x <i>Iberian</i>	0.005	0.000	0.034	0.000	0.961

However, some of the assumptions of the simulations favour these positive results: a) purebred and crossbred pigs are related, the last ones being progenies of the *Iberian* and *Duroc* reproducers considered in the analyses, b) the number of clusters (five) is known and the possible hidden substructure of the purebred populations is ignored, and c) all the genotypes are available without missing marker data. The assumptions a) and c) are clearly unrealistic in practice, and the assumption b) is questionable at least for the *Iberian* breed where a hidden substructure has been previously inferred (Alves *et al.*, 2006). The considered proportions of *Duroc* genes could be directly applied to the situation of *Iberian* products that usually proceed from these crosses. However, a lower proportion of the *Duroc* genome could be present at animals qualified as purebred *Iberian*. These arguments indicate that the ability of these

techniques to solve the practical problems of traceability of *Iberian*-type live pigs and meat products will be lower than the expected according to the simulated results, and the use of panels of at least 96 very divergent SNPs ( $|q_{\text{IBERIAN}} - q_{\text{DUROC}}| > 0.80$ ) seems advisable.

**Table 2. Allocation average proportions (over 100 replicates) to the different groups of simulated purebred and crossbred pigs: Iberian (n = 100), Duroc (n = 100), F1 Duroc x Iberian (n = 200), F1 x Duroc (n = 400) and F1 x Iberian (n = 400) genotyped for 96 SNPs with allelic differences between breeds greater than 0.60**

	Iberian	Duroc	F1 Duroc x Iberian	F1 x Duroc	F1 x Iberian
Iberian	0.999	0.000	0.000	0.000	0.001
Duroc	0.000	1.000	0.000	0.000	0.000
F1 Duroc x Iberian	0.000	0.000	0.972	0.009	0.019
F1 x Duroc	0.000	0.004	0.020	0.976	0.000
F1 x Iberian	0.005	0.000	0.015	0.000	0.980

### 3. Low-density genotyping

The finally selected panel of 96 SNPs was used for genotyping two sets with 750 and 230 DNA samples, respectively, pursuing different objectives. A verification of the purebred *Iberian* origin was performed on a set of analyzed samples proceeding from 82 *Iberian* pigs, 61 *Duroc* and 607 uncertain purebred *Iberian* pigs named here as probationary animals. Fig. 2 represents a graphic summary of the results obtained from one of the performed analysis: admixture based on pre-defined clustering. Purebred pigs from the two breeds were considered as the origin populations and the proportion of alleles proceeding from these populations was estimated for the probationary samples. The presence of *Duroc* genes was observed in different proportions for some of the probationary animals and also for six of the assumed purebred *Iberian*. However, more satisfactory results were obtained from admixture analysis based on the mixture clustering of individuals (results not shown). This analysis assumes the maximum uncertainty about the samples and besides of a cluster grouping the *Duroc* pigs, the clustering of individuals reveals a likely hidden substructure of three different clusters for all the samples (668) of possible *Iberian* origin. The posterior admixture analysis indicates that only a 2.5% of these samples present *Duroc* genes with a probability lower than 0.05.

As it was already mentioned the set of 96 SNPs included *MC1R\*4* and *MC1R\*2* probes that can supply additional information for problematic breeding nucleus. Besides, an additional genotyping of *IGF2* g.3072A/G was carried out (according to the procedure described by Van Laere *et al.*, 2003), for samples proceeding from the herds where *Duroc* genes were detected in putative *Iberian* animals. Both *MC1R\*4* and *IGF2* g.3072A are exclusive alleles of the *Duroc* breed. Their presence in animals from the same breeding nucleus evidence introgression of *Duroc* genes. Moreover, the presence of *MC1R\*2* allele was detected in a few animals which indicates introgression of *Large Black* in those herds.

The goal of the second trial was to validate the *Duroc* x *Iberian* origin of commercial crossbred pigs. The 230 analyzed samples corresponded to seven *Duroc* boars, 50 *Iberian* sows and 173 controlled F1 *Duroc* x *Iberian* animals. In this case, admixture analysis based on pre-defined clustering was performed. The *Duroc* boars and the *Iberian* sows were considered as the source of genes of the crossbred animals, and the proportion of genes proceeding from each one of them was estimated for each individual. The obtained results are summarized on Fig. 3. We could infer the presence of about a 10% of *Duroc* genes on four out of the 50 assumed purebred *Iberian* sows, with a probability lower than 0.001. Results also confirm the crossbred origin of all the *Duroc* x *Iberian* pigs. However, for some of the crossbred animals the inferred

percentage of *Duroc* genes was slightly different than the 50% expected. It can be explained by the low size of the origin breed samples that cannot represent all the within population diversity of these breeds for the selected genetic markers.

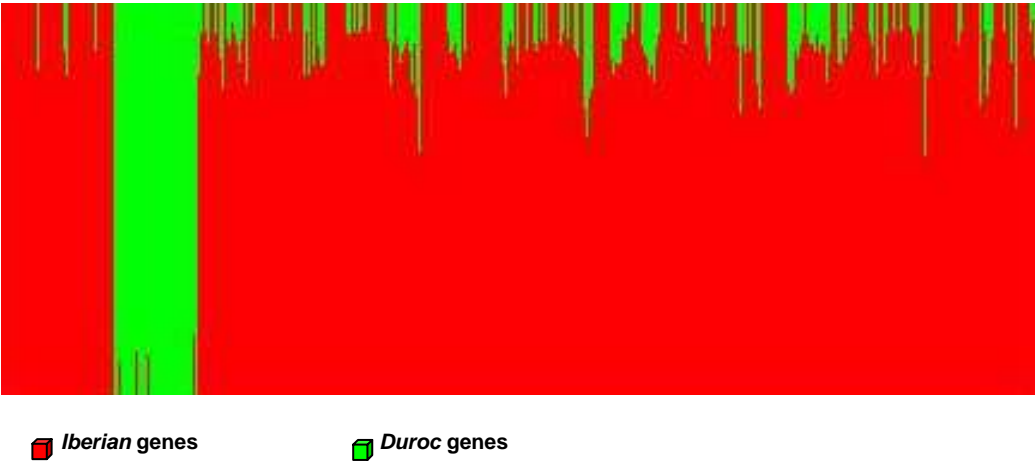


Fig. 2. Proportion of Iberian and Duroc genes on the 750 analyzed samples inferred from admixture based on pre-defined clustering.

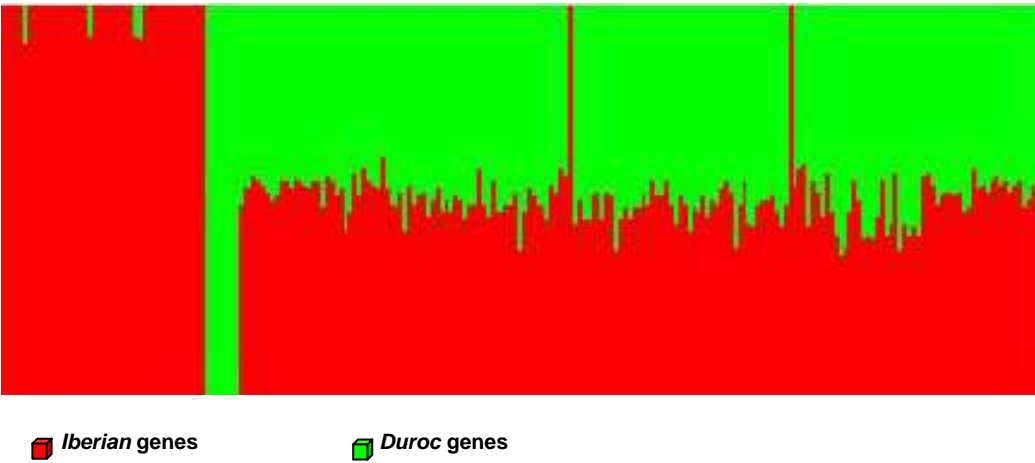


Fig. 3. Proportion of Iberian and Duroc genes on the 230 analyzed samples.

It was mentioned before that the selection of SNPs was based on the ADT SNPScore file output that allows include those assays that are predicted to have a high likelihood of success. However this does not guarantee the complete amplification of all the SNPs because low-density genotyping uses VeraCode technology whereas high-density genotyping uses Infinium technology. Hence, we observed that a number of probes, ranging from 10 to 13 failed to produce amplification. Moreover, taken into account the inclusion of two *MC1R*\*2 probes on the

final set of 96 SNPs the actual number of useful probes varied between 82 and 85 instead the 96 selected SNPs.

## IV – Conclusions

This study exemplifies how the recent advances in SNP discovery and high throughput automated genotyping methods can be applied to solve problems of authentication of genetic origin of *Iberian* pigs and their products. Low density genotyping of a moderate number of SNPs (< 90), with divergent frequencies between the *Iberian* and *Duroc* breeds, may be a powerful tool either to infer the purebred *Iberian* origin, to detect animals with low proportion of *Duroc* genes or to validate the *Duroc* x *Iberian* origin of commercial pigs or meat products. However, further research need to be carried out in order to build adequate databases for improving the usefulness of this procedure. We have planned to study a higher number of purebred and crossbred pigs, and to extend the panel of markers with new SNPs discovered using the next generation sequencing technology (Ramos *et al.*, 2010).

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# Transcriptome analysis of the Iberian pig

G. Dorado\*, A. Membrillo\*\*, I. Clemente\*\*\*, P. Javier Azor\*\*, P. Winter\*\*\*\*, P. Hernández\*\*\*\*\*, A. Roderó\*\* and A. Molina\*\*

\*Dep. Bioquímica y Biología Molecular, Campus Rabanales C6-1-E17. Córdoba (Spain)

\*\*Dep. Genética, Campus Rabanales C5, Córdoba (Spain)

\*\*\*Centro Tecnológico Andaluz del Sector Cárnico (TEICA), Cortegana, Huelva (Spain)

\*\*\*\*GenXPro GmbH, Frankfurt am Main (Germany)

\*\*\*\*\*Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Córdoba (Spain)

eMail: bb1dopeg@uco.es

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**Abstract.** Iberian pig meat is a remarkable constituent of the healthy Mediterranean diet. The organoleptic and nutritional properties of Iberian pig products are influenced by both genetic and environmental factors including the animal's diet. To investigate the potential impact of genotype and diet on gene expression eventually controlling taste and texture of the meat, we performed an in-depth transcriptomics analysis of the Iberian versus Duroc pig muscle from differentially fed animals. Muscle tissue samples were frozen in liquid nitrogen immediately after sacrificing the animals and stored at  $-80^{\circ}\text{C}$ . Total RNA was extracted, retrotranscribed to cDNA and subjected to SuperTag Digital Gene Expression (ST-DGE) functional genomics analysis. The generated sequences were then counted and annotated to entries in public databases to assign potential functions to the expressed genes. To this end we identified significant breed-specific as well as diet-specific expression profiles of known as well as from hitherto unknown genes, involved in metabolic pathways related to the quality of Iberian meat products. These results demonstrate the power of genomics in general and transcriptomics in particular to identify differentially expressed profiles and putative candidate genes for quality control, certification and traceability, helping breeders and farmers to produce both animals and derived products (like ham) with higher nutritional value and improved organoleptic properties, to further enhance the healthy Mediterranean diet.

**Keywords.** Genome – Functional genomics – mRNA – Bioinformatics.

## *L'analyse du transcriptome du porc Ibérique*

**Résumé.** La viande de porc Ibérique est un constituant remarquable de la saine alimentation méditerranéenne. Les propriétés organoleptiques et nutritionnelles des produits du porc Ibérique sont influencées par des facteurs génétiques et environnementaux, y compris l'alimentation de l'animal. Pour étudier l'impact potentiel du génotype et du régime alimentaire sur l'expression génique du goût et éventuellement le contrôle et la texture de la viande, nous avons effectué une analyse en profondeur du transcriptome du muscle du porc Ibérique par rapport aux porcs Duroc à partir d'animaux nourris différemment. Des échantillons de tissus musculaires ont été congelés dans l'azote liquide immédiatement après l'abattage des animaux et conservés à  $-80^{\circ}\text{C}$ . L'ARN total a été extrait, rétrotranscrit en ADNc et soumis à une analyse génomique fonctionnelle pour l'expression des gènes moyens selon la technique SuperTag (ST-DGE). Les séquences ont alors été comptées et annotées pour leur entrée dans des bases de données publiques pour assigner des fonctions potentielles aux gènes exprimés. À cette fin, nous avons identifié des profils d'expression significatifs spécifiques de la race ainsi que de l'alimentation, pour des gènes connus ainsi qu'inconnus jusque-là, impliqués dans les voies métaboliques liées à la qualité des produits de la viande du porc Ibérique. Ces résultats démontrent la puissance de la génomique et de la transcriptomique en général, et en particulier pour l'identification des profils différemment exprimés et des gènes candidats putatifs pour le contrôle, la certification de la qualité et la traçabilité, afin d'aider les éleveurs et les agriculteurs à produire des animaux et des produits dérivés (comme le jambon) ayant une valeur nutritionnelle plus élevée et de meilleures propriétés organoleptiques, pour améliorer encore le sain régime méditerranéen.

**Mots-clés.** Génome - Génomique fonctionnelle – ARNm – Bioinformatique.

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## I – Introduction

The quality is an important parameter of any food product. This is particularly relevant for products with a Protected Designation of Origin (PDO) certification issued by the European Union (EU). Such label corresponds to food products that are specific of a particular region, conveying a particular quality or characteristic which is peculiar of such designated area. The PDO certification requires the food traceability from the origin to the consumer. Likewise, the National Quality Standard (NQS; “Norma de Calidad”) for the Iberian pig products (ham, shoulder blade and loin) in Spain regulates the labeling of such food (“Real Decreto” 1469/2007, of 2nd November). Different approaches have been developed and deployed for food certification and traceability, being particularly efficient and convenient the ones based on DNA molecular markers. Yet, the development of such markers requires the prior knowledge of the DNA sequences differentiating the products to be identified and tracked down.

On the other hand, consumers are demanding with increasing emphasis not only quality-certified food products, but also –and specially– healthy products. The Iberian pig products are remarkable constituents of the healthy Mediterranean diet, which has been included in the United Nations Educational, Scientific and Cultural Organization (UNESCO) list of “Intangible Cultural Heritage of Humanity” since 18th November 2010. The healthy implications of the Iberian pig products have not been completely elucidated, but some studies have shown that their balanced compositions of unsaturated lipids protect against cardiovascular diseases (CVD), effectively reducing the plasma levels of Low Density Lipoprotein (LDL) cholesterol, total cholesterol, triglycerides and fibrinogen (Rebollo *et al.*, 1998; Martín *et al.*, 2009; Jiménez-Colmenero *et al.*, 2010).

Remarkably, the fat composition of the Acorn-Fed Iberian Ham (AFIH) has a surprisingly high content of oleic acid (monounsaturated lipid) for a product of animal origin (50 to 60% of the total fat), depending on the tissue considered. Thus, on the coccyx fat it reaches up to 57% (about 60% monounsaturated lipids), whereas in the intramuscular fat the oleic acid represents 45 to 50% and the saturated fats about 40%. The high oleic acid content of the Iberian ham resembles the one of the olive oil, which ranges from 55.0 to 83.0% of oleic acid content depending on cultivar. In this respect, it should be emphasized that the olive oil has been granted the label of “Qualified Health Claim” by the Food and Drug Administration (FDA) of the United States of America, due to its protective effect against CVD.

The organoleptic, nutritional and healthy properties of the Iberian pig products are influenced by both genetic and environmental factors including the animal's diet (eg., the acorn feeding, as previously indicated; the acorn has a similar lipid composition to the olive oil itself). To investigate the potential impact of genotype and diet on gene expression eventually controlling taste, texture and composition of the meat, we have performed an in-depth transcriptomics analysis of the Iberian *versus* Duroc pig muscle from ham of differentially fed animals. The final goals of this research are both to unravel the genomic, transcriptomic and metabolomic pathways that set apart the Iberian pig from other breeds, as well as to identify differentially expressed genes that can be used as tools for the certification and traceability of Iberian pig products.

Explanatory note: The purpose of this work is not to carry out a statistical analysis of many samples because: i) this kind of genomic experiments use a different methodological approach in the literature; and ii) the prohibitive cost of such statistical approach on genome-wide experiments, not being cost-effective. On the contrary, the purpose of this work is to carry out a genome-wide transcriptomic analysis in order to discover candidate genes that are highly differentially expressed (upregulated or downregulated) between the breeds and feedstuffs used. Such candidate genes are now being subjected to other analysis (validation using many animals from different herds), but such work is out of the scope of the present report. The readers interested on the methodology used in genomic studies are directed to the abundant bibliography on such matter for further details, as previously indicated.

## II – Materials and methods

Female Iberian pigs from the most abundant lineage (Retinto) were selected from the same brood and fatten up for 70 days with either strict “montanera” (acorn-based with grass), standard cereal-based feedstuff without acorn (as well as female Duroc, used as control) or standard cereal-based feedstuff enriched with olein (oleic-acid-rich feedstuff). The standard feedstuff contained (mg/kg): barley ( $14.75 \times 10^4$ ), wheat ( $50.00 \times 10^4$ ), corn ( $21.00 \times 10^4$ ), soybean meal ( $1.20 \times 10^4$ ), lard ( $1.10 \times 10^4$ ), calcium carbonate ( $8.00 \times 10^3$ ), calcium phosphate ( $1.15 \times 10^4$ ), sodium chloride ( $4.00 \times 10^3$ ) and corrector ( $5.25 \times 10^3$ ). The olein-supplemented feedstuff contained (mg/kg): barley ( $47.50 \times 10^4$ ), wheat ( $15.00 \times 10^4$ ), bran ( $15.50 \times 10^4$ ), soybean meal ( $8.50 \times 10^4$ ), pulped beet ( $5.75 \times 10^4$ ), high-oleic sunflower meal ( $5.75 \times 10^4$ ), calcium carbonate ( $9.50 \times 10^3$ ), calcium phosphate ( $7.00 \times 10^3$ ), sodium chloride ( $4.00 \times 10^3$ ), corrector ( $5.00 \times 10^3$ ) and vitamin E mix (250 ppm). The Table 1 summarizes the three feedstuffs used.

**Table 1. Feedstuff composition**

Composition (%)*	“Montanera”		Standard	Olein
	Acorn	Grass		
DM	54.70	10.60	85.62	89.91
DM ash	2.11	13.54	3.89	4.56
DM crude protein	7.97	21.85	16.78	16.94
DM crude fiber	1.82	20.00	3.28	5.67
DM fat	7.06	3.07	3.55	8.54
DM NFE	80.50	46.60	72.57	64.35
SFA	12.12	21.75	25.15	11.15
MUFA	64.65	7.34	32.08	67.50
PUFA	21.60	70.92	42.68	21.52

(\*): DM: Dry Matter; NFE: Nitrogen-Free Extracts; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

The pigs were transported to the slaughterhouse the day before slaughtering, trying to minimize the stress of the animals. Carbon dioxide was used for stunning just before bleeding, according to the specifications outlined in the European Union legislation. The Iberian pigs were slaughtered after 14 months old (150 to 170 kg); the Duroc pig grows faster and thus was slaughtered after 9 months old (157 kg).

The ham muscle tissue samples (*biceps femoris*; femoral biceps) were frozen in liquid nitrogen immediately after sacrificing the animals, and stored at  $-80^\circ\text{C}$  until needed. The total RNA was isolated from samples using Trizol from Life Technologies (Carlsbad, CA, USA). In short, the tissue was ground under liquid nitrogen with a pestle and mortar. A small volume of ground tissue was dissolved in 1 ml Trizol and homogenized. The RNA was isolated after the addition of 1/5 volume of chloroform. Subsequently, the RNA was precipitated with isopropanol, washed and dissolved in diethylpyrocarbonate-treated water. The RNA concentration was determined using a NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). The RNA quality (integrity) was checked by agarose gel electrophoresis. The RNA was retrotranscribed to cDNA with reverse transcriptase and subjected to SuperTag Digital Gene Expression (ST-DGE) functional genomics analysis (Anisimov, 2008; Datson, 2008; Matsumura *et al.*, 2008a,b, 2010; Wang, 2008; Zaretski *et al.*, 2010). The generated sequences were then counted and annotated to entries in public databases to assign potential functions to the expressed genes.

### III – Results and discussion

As previously indicated, the main purpose of this work is not to carry out a statistical study with many animals, but to use the currently validated genomic methodological approaches to carry out a genome-wide transcriptomics analysis. The usefulness of such strategy to identify differentially expressed genes and thus putative candidate genes for further applications is widely supported in the literature. More than a million Expressed Sequence Tags (EST) have been isolated and sequenced, including differentially expressed ones between breeds and feeds. Thus, we have identified breed-specific as well as diet-specific expression profiles of known as well as from hitherto unknown genes, some of them being involved in metabolic pathways related to the characteristics of Iberian meat products.

The transcriptomic profiling of Iberian pigs fed with either acorn, standard feedstuff or olein were compared with the transcriptomic profiling of Duroc pigs fed with standard feedstuff. A total of 15,814 to 18,952 expressed tags were compared for each pair of breeds and feeding, generating gene expression fold changes from +14.58 to +18.09 on one side and -13.71 to -16.58 on the other side. This represents a remarkable wide range of variation.

The analysis of such data has allowed the identification of differentially expressed genes (eg., involved in lipid and peptide metabolism and transport), besides genes that are currently unknown. The expression of genes related with lipid biosynthesis was higher on Iberian vs Duroc pigs. Likewise, for acorn-fed versus standard-fed Iberian pigs. These results indicate that both the genotype as well as the diet determine the higher lipogenic gene expression in the acorn-fed Iberian pig.

These results demonstrate the power of genomics in general, and transcriptomics in particular, to identify differentially expressed genes between pig breeds and feeding conditions. The data generated will contribute to a better understanding of the pig transcriptomics and metabolomics pathways. These results are also being exploited and validated analyzing many individuals from different herds (data not shown, corresponding to future publications) to design DNA molecular markers for quality control, certification and traceability. These developments will help breeders and farmers to produce both animals and derived products (like ham) with higher nutritional value and improved organoleptic properties, to further enhance the healthy Mediterranean diet. Likewise, they can contribute to increase the consumer confidence for products with higher quality and price, which can further assist the promotion and consolidation of demanding markets (Japan, USA, etc).

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# Classification of Iberian pigs as to their nutrition through the ChemSensor technique

J.A. Carrasco \*, L. Giménez \* and J.P.Duque\*\*

\*Departamento de Ingeniería, Instituto del Frío, CSIC, c/ Jose Antonio Novais 10, 28040 Madrid (Spain)

\*\*Delegación en Castilla y León, CSIC, Valladolid (Spain)

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**Abstract.** We have developed a classification method to discriminate the quality of Iberian pigs with different feeding patterns (*bellota*, *recebo*, *extensive* and *intensive* feeding) in view of the large price differences encountered in the market depending on availability of acorns diet or other type of food. We used the *ChemSensor* Technique which combines a gas chromatograph with a mass detector as well as a liquid autosampler and a headspace system. Our starting material consisted of 207 samples of back side bacon extracted from pigs previously fattened with different feeding systems and subsequently fatty acid and volatile compound analysis were performed. Up to 75% of the samples were analyzed in a first step obtaining a prediction model that applies to the remaining 25% of samples. A proper software adds mass abundances in the range of *m/z* scanned (41-550) for further analysis by a chemometric software (Pirouette, by Infometrix Inc.). The classification method used was established with SIMCA (Soft Independent Modelling Class Analogy). Our results indicate a satisfactory classification up to 96% and a prediction of ca. 90% in *bellota*-type pigs and in intensive type and somewhat lower in *recebo* and extensive types.

**Keywords.** ChemSensor – Rating Iberian pigs – Mass Spectrometry – Traceability.

## **Classification des porcs Ibériques selon leur nutrition grâce à la technique du ChemSensor**

**Résumé.** Nous avons développé une méthode de classification pour distinguer la qualité du porc Ibérique élevé selon différents modes d'alimentation (*bellota*, *recebo*, *extensif* et *intensif*) en raison de grandes différences de prix sur le marché en fonction du régime alimentaire ou d'un autre type d'alimentation. Nous avons utilisé la technique *ChemSensor* qui combine un chromatographe en phase gazeuse avec un détecteur de masse ainsi qu'un échantillonneur automatique liquide et un système d'espace-de-tête. Notre matériau de départ se composait de 207 échantillons de lard arrière extraits de porcs engraisés avec différents systèmes d'alimentation et ensuite les analyses des acides gras et des composés volatils ont été effectuées. Dans un premier temps jusqu'à 75% des échantillons ont été analysés, obtenant ainsi un modèle de prévision appliqué aux 25% restants des échantillons. Un software ajoute l'abondance de masse dans la gamme des *m / z* numérisés (41-550) pour une analyse plus approfondie par un software de chimométrie (Pirouette, par Infometrix Inc.). La méthode de classification utilisée était SIMCA (Soft Independent Modelling Class Analogy). Nos résultats indiquent une classification satisfaisante jusqu'à 96% et une prévision de presque 90% chez les porcs de type *Bellota* et de type *intensif* et un peu plus faible pour les types *Recebo* et les modes *extensifs*.

**Mots-clés.** ChemSensor – Evaluation du porc Ibérique – Spectrométrie de masse – Traçabilité.

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## **I – Introduction**

The extensive production systems are appreciated because of the valuable preservation of natural environments as well as the well spread reputation of the meat products obtained. Spain is characterized by having a perfect ecosystem for such production, the *dehesa*, with over a million and a half hectares of cork and oak trees that supply the necessary food for the Iberian pigs. Acorns constitute a unique raw material for the Iberian pork industry, providing high valued products, like ham, paleta and pork loin. However, there is a high differential cost between this



grazing system and the intensive, massive systems used by the bulk of farmers, which affects not only the cost of the animals themselves but the price of manufactured products.

The Spanish government has established quality standards classifying pigs as *bellota*, *recebo*, *extensive* and *intensive* types, depending on the final fattening phase.

There have been developed several analytical methods to assess this type of classification, one of them is presented here as part of a research project supported by the Instituto Nacional de Investigaciones Agrarias y Agroalimentarias (INIA) using the Agilent ChemSensor technique.

## II – Materials and methods

Analysis were performed using a 4440B ChemSensor (*Agilent Technologies*), which combines a gas chromatography-mass spectrometer (GC / MS) with a chemometric software developed by *Infometrix Inc.* The former consists of a 6890 GC with a DB.23 column (*Agilent*), a liquid autosampler for 100 samples (HP 7683) and a 5973N MS mass spectrometer with a 5973 quadrupole detector operating in electron impact mode and scanning of masses in the  $m/z$  41 and 550 range.

The starting material was backfat taken from Iberian pigs proceeding from some farms and livestock farms in Extremadura, Andalusia and Salamanca. The animals were fattened according to the *bellota*, *recebo*, *extensive cebo* and *intensive cebo* diet patterns. The total number of tested samples was 207, mostly pure Iberian breed, with 160 kg average weight. All the samples were originally obtained from the subcutaneous rump fat according to the standards (*ASICI specification*). After fat extraction using microwave and methyl transesterification with KOH in methanol, fatty acid methyl esters were recovered and submitted to the ChemSensor 4440B analyzer obtaining an array of ion mass fragmentation in the range  $m/z$  41 and 550.

The whole number of samples (207 in total) was split as follows. In a first step the procedure involved an initial determination of the classification and predictive mathematical model based on known samples (156 samples, 75% of total) and in a second step it took place the prediction test, using a random set of samples (51 samples, accounts for ca. 25%).

## III –Results and discussion

The chemometric analysis was applied using chemometric software SIMCA (*Soft Independent Modelling Analogy Class*) of *Infometrix Inc.* The first set of samples (156) was classified according to this model into four groups (see Table 1) with distances between groups shown in Table 2. We applied the above mathematical modelisation on the 25% remaining samples, obtaining the prediction results shown in Table 3.

**Table 1. Classification results**

Samples	Classified				% success
	<i>Bellota</i>	<i>Recebo</i>	<i>Cebo ext.</i>	<i>Cebo int.</i>	
<i>Bellota</i>	53	0	0	0	100
<i>Recebo</i>	0	53	1	0	98.15
<i>Cebo ext.</i>	2	1	24	0	88.89
<i>Cebo int.</i>	0	0	0	22	100
Total	55	54	25	22	97.44

**Table 2. SIMCA model relative distances**

	Distance to			
	<i>Bellota</i>	<i>Recebo</i>	<i>Cebo ext.</i>	<i>Cebo int.</i>
<i>Bellota</i>	0	0.9673	0.6596	3.1937
<i>Recebo</i>	0.9673	0	0.8810	3.6142
<i>Cebo ext.</i>	0.6596	0.8810	0	3.3592
<i>Cebo int.</i>	3.1937	3.6142	3.3592	0

**Table 3. SIMCA model predictions**

Actual samples	Prediction				% success
	<i>Bellota</i>	<i>Recebo</i>	<i>Cebo extensivo</i>	<i>Cebo intensivo</i>	
<i>Bellota</i>	16	0	2	0	88.8
<i>Recebo</i>	1	13	1	1	81.2
<i>Cebo ext.</i>	2	5	2	0	22.2
<i>Cebo int.</i>	1	0	0	7	87.5

## IV – Conclusions

The use of ChemSensor for classification purposes proves to be highly reliable in all types of samples, grouped in four classes (*bellota*, *recebo*, *cebo extensivo*, *cebo intensivo*) with an average selectivity greater than 97%.

With the classification model generated with *ChemSensor* some certain predictions have reached 90% reliability. That is the case of *bellota* and *cebo intensivo* types.

Predictions carried out for *recebo*-type animals provided a reliable 81% whereas the *cebo extensivo*-type forecast is only 22%. This one is explained on the basis of the small number of samples used: a single failure over a small set gives necessarily a low percentage.

Compared to other technologies ChemSensor shows a clear advantage in terms of reliability, because it is based on gas chromatography and mass determination, and thus provides up to 500 variables per sample which are easily treated with *Pirouette* chemometrics software. This guarantees a reproducible analysis.

In addition, this technique does not use sensors, which are subjected to a limited lasting or aging. Chemsensor is not affected by environmental humidity and temperature conditions, so results gain accuracy and reproducibility.

The analysis time of each sample is very short, and may use the fatty acids of the sample or volatile compounds directly.

As used for the classification of dead animals is also perfectly suited for the analysis of the derived products such as a ham or loin. This technique is also a valuable tool in traceability tasks in industry.

## Acknowledgments

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# Genetic certification of the Iberian ham

A. Martínez\*, J.L. Vega-Pla \*\*, V. Landi\*, J. Cañón\*\*\* and J.V. Delgado\*

\*Departamento de Genética, Universidad de Córdoba, Campus de Rabanales, Ed. C-5. Córdoba (Spain)

\*\*Laboratorio de Investigación Aplicada, Cría Caballar de las Fuerzas Armadas, Córdoba (Spain)

\*\*\*Laboratorio de Genética, Universidad Complutense de Madrid (Spain)

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**Abstract.** Under current Spanish regulations, the pigs that provide the raw material for the preparation of the country's most appreciated meat-derived product, dry-cured Iberian ham, must be of a specific genetic composition. Only the Duroc breed is accepted for crossing with Iberian pigs, and a maximum of 50% of the Duroc genome is permitted in the animals used to make this ham. This study describes a set of statistical procedures for detecting the breed composition of Iberian ham via the use of multilocus genotypes obtained by the amplification of 25 microsatellite markers. The procedure proposed in this study has been used for several years routinely in our laboratory for various purposes such as to certificate the genetic composition of some Iberian registered trademark hams or to detect commercial fraud in the Iberian ham consumed in Spain.

**Keywords.** STR – Traceability – Individual assignment – Genetic admixture.

## *Certification génétique du jambon Ibérique*

**Résumé.** Les porcs qui produisent la matière première pour la préparation du jambon le plus populaire parmi les produits à base de viande de porc Ibérique doivent être d'une composition génétique spécifique selon la loi espagnole. Seule la race Duroc est acceptée pour le croisement avec le porc Ibérique, pour un maximum de 50% du génome, afin de produire ce jambon. Cette étude décrit un ensemble de procédures statistiques de détection de la composition génétique de la race porcine Ibérique à travers l'utilisation de génotypes multilocus obtenus par amplification de 25 marqueurs microsatellites. La procédure proposée dans la présente étude a été utilisée régulièrement pendant plusieurs années dans notre laboratoire à des fins diverses, comme la certification de la composition génétique de certaines marques de jambon Ibérique ou la détection des fraudes commerciales pour le jambon Ibérique consommé en Espagne.

**Mots-clés.** STR – Traçabilité individuelle – Brassage génétique.

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## I – Introduction

The Iberian pig ham is one of the most important traditional food products of the Spanish culture. The added value of this breed was supported by the necessary conservation of the Mediterranean paddock, known as “dehesa”.

Iberian ham quality is the result of the genetic composition, the animal feeding during the fattening and the artisan management, but to profite this traditional product we have to take into account important additional factors, as the marketing and commercialization.

We have developed a microsatellites based methodology to supervise the assignment of the individuals to the Iberian breed and its varieties, in order to support the conventional mechanism of supervising which is based on the intervention of a qualifier office that use the subjective observation of a morphological phenotype as criterion of assignment. Our methodology is based in the fitness of the genetic individual profile to population genetic profiles, previously defined in animals belonging to the breed herd book or admitted by the breeders association as integrated in the different varieties forming the Iberian breed group.

In the present paper we are showing a real experience using this methodology in the breed

traceability of the products commercialized by Maldonado S.L. enterprise. It could be as a useful model to the sector to protect the genetic patrimony of the Iberian ham at the consumer eyes, the credibility.

## II – Materials and methods

Maldonado S.L. is a traditional enterprise which commercialize only high quality Iberian products. Its “star” product is the called “Albarragena Ham” which is a selection of 100 hams among the thousands produced by the enterprise. This ham has been recognized as one of the most expensive ham of the world by the mass media.

These hundred hams have a lot of special commercial treatments to ensure their differentiated quality, such as a series number stamped in a silver medal, a presentation in an exclusive appearance, etc. This product reaches in the market the prize of 1500 Euro by piece.

One additional proof to support the credibility of the product is the incorporation of a certificate with the probability of assignment to the Iberian breed (Figure 1), and a series number to ensure the genetic traceability of the piece.

DNA extraction was performed from ham samples using the Genomic DNA Purification Kit (Gentra Systems, Minnesota, USA). Samples were screened for 25 pig microsatellites selected from the 27 markers recommended by the FAO for pig biodiversity studies (FAO,1998). Technical procedures of genotyping were described in previous papers (Vega-Pla *et al.*, 2003; Garcia *et al.*, 2006). Probability of assignment of individual genetic were determined using the Bayesian algorithms proposed by Baudouin *et al.* (2004), using Geneclass2 software (Piry *et al.*, 2004) for the calculations. This methodology developed in the context of the EU project Characterization of genetic variation in the European pig to facilitate the maintenance and exploitation of biodiversity (BIO4 CT98-0188, DG XII European Commission, 1998-2000). It gave us the opportunity to access to a wide base of samples from almost all the pig European resources.

## III – Results and discussion

All people involved in the Iberian pig world desire to maintain the credibility on the Iberian ham. There is an interest by the most serious private enterprises, which demanded an objective certification of purity of their products. Our methodology gave successfully response to this demand. Figure 1 shows a scheme of the factorial analysis results, defining the genetic assignment of the Albarragena hams to the Iberian pig genetic profile.

Table 1 is showing an example of the assignment probability of a set of Albarragena hams. This table of results is sent to the enterprise, to support its internal management in regards the selection of animals and pieces; the following up of the donor farms, etc.

Today the Iberian pig is researched intensely, also with the most advanced markers such as SNPs (Padilla *et al.* 2010), but these methodologies, under our point of view are optimal for marker assisted selection, and in the future for genomic selection, but microsatellite typing will continue being the tool of election for all around genetic characterization, breed traceability, and genetic diversity studies in general.

The present paper demonstrated that a tool to ensure the breed traceability of the Iberian pig products, also ensures the credibility of the high quality products.

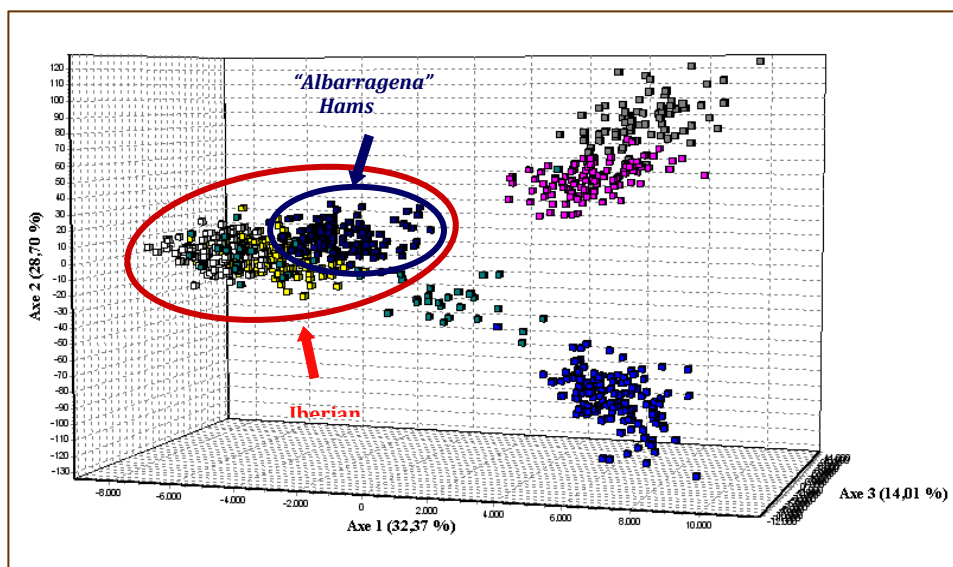


Fig. 1. Factorial analysis results, showing the inclusion of the Albarragena hams in the Iberian genetic profile.

Table 1. An example of the last Albarragena hams certified using the methodology of individual assignment to a population

Ham	P ASIG	Ham	P ASIG	Ham	P ASIG	Ham	P ASIG
0004393	0.983	0004893	0.991	0004839	0.986	0005087	0.992
0004663	0.944	0004878	0.993	0004454	0.988	0004217	0.991
0009386	0.990	0009287	0.923	0005626	0.984	0004010	0.987
0004842	0.991	0004362	0.969	0005118	0.990	0004369	0.967
0009550	0.991	0009435	0.985	0005047	0.993	0009378	0.992
0004344	0.940	0003560	0.969	0004455	0.981	0009392	0.974
0004970	0.989	0005235	0.990	0004329	0.951	0009321	0.990
0004461	0.984	0009454	0.978	0005233	0.987	0003618	0.991
0004871	0.992	0005050	0.984	0009343	0.979	0009445	0.994
0004509	0.972	0003557	0.967	0004951	0.990	0005245	0.993
0005025	0.985	0003930	0.992	0009335	0.961	0003621	0.986
0005259	0.988	0009295	0.949	0003568	0.994	0003492	0.994
0004478	0.984	0005169	0.984	0003652	0.983	0004861	0.993
0005241	0.977	0009437	0.966	0003573	0.973	0004034	0.905
0009545	0.990	0005044	0.996	0005052	0.985	0005084	0.986
0009460	0.956	0003707	0.987	0004915	0.994	0003623	0.966
0004375	0.962	0005177	0.994	0005035	0.990	0004095	0.994
0004920	0.987	0003939	0.992	0004504	0.981	0003650	0.954

## IV – Conclusions

Today, Maldonado S.L. enterprise has enclosed in internal requirements of quality the use of the individual assessment bases on the genetic profiles obtained with a set of microsatellites, as

an objective method to ensure the breed traceability of their Iberian pig products. This common applications is an example to follow to maintain n the credibility of the consumers.

## Acknowledgements

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# Profil en acides gras dans différentes zones de jambons séchés et leur utilisation possible dans le cadre de la prévision de la qualité

J.L. Pérez Almero, C.J. Porras Tejeiro, P. Brun et C. Casas

IFAPA Centro Las Torres-Tomejil, Alcalá del Río, Sevilla (Espagne)

**Résumé.** Malgré le fait que les méthodes de classement selon la qualité de la carcasse des porcs Ibériques soient basées sur le prélèvement d'échantillons sur des animaux vivants ou récemment abattus, il est intéressant, au niveau commercial, de mettre au point de nouvelles méthodes en utilisant des échantillons de produits séchés. Dans cet article, nous présentons les résultats des analyses d'acides gras faites sur des échantillons de graisse de différentes parties de pièces séchées et le classement des pièces conformément à leur qualité. Sur 65 jambons, dont la génétique et l'alimentation sont connues, nous avons déterminé le profil en acides gras pour le lard prélevé sur 6 zones différentes afin de ne pas porter préjudice, ou très peu, à l'intégrité des pièces. Les zones choisies ont été le sommet du "V", la "punta" et la "maza", d'abord dans leur partie externe (VE, PE et ME) et ultérieurement celles qui correspondent aux 3 zones, mais plus à l'intérieur (VI, PI et MI). Il n'existe aucune différence pour les acides gras entre les zones internes et les zones externes. Ainsi, à partir de la 35<sup>ème</sup> pièce, ce sont les zones externes que nous avons analysées, ce qui ne détériore pas les jambons. Le pourcentage oléique est plus élevé de façon significative dans la VE, suivi de la ME et ensuite la PE, en considérant l'ensemble de toutes les pièces, ainsi que dans un groupe alimenté à base de glands. Ce n'est pas le cas chez les animaux élevés au fourrage, chez lesquels il n'y a pas de différences entre les zones. Les résultats de l'analyse discriminante révèlent que le pourcentage des assignations correctes est élevé et pour l'ensemble de toutes les pièces, il varie de 79,4% avec les deux AG de la PE, à 72,6% avec ceux de la ME.

**Mot-clés.** Porc Ibérique – Acides gras – Analyse discriminante.

## *Profile of fatty acids in distinct areas of cured hams and their possible use as quality indicators*

**Abstract.** Despite quality control methods in Iberian pigs being based on the collecting of samples from live or recently slaughtered animals, it is of commercial interest to fine tune other methods using samples from cured products. In this report the results of fatty acid analyses from fat samples from different parts of cured pieces of meat and the classification of the meats according to their quality will be presented. For sixty five hams, of known genetics and diet, the profile of fatty acids of pork fat extracted in six different places has been determined, chosen so that in none or very few cases the integrity of the piece of meat would be affected. The chosen places were in the vertex of the "V", the "punta" (the thigh end of the ham) and the "maza" (the meatiest part of the ham), first in its external part (VE, PE and ME respectively) and then the three interior areas corresponding to the aforesaid areas (VI, PI and MI). There are no differences between the fatty acids in the internal areas and the external areas. Thus, from ham number thirty five upwards samples were only taken from the external areas, which didn't deteriorate the hams. The percentage of oleic acid is significantly higher in the VE samples, followed by ME and then by PE, considering the group of hams as a whole and those bred free-range. This doesn't happen in factory farmed animals, in which there are no differences between the areas. The results of the differentiated analysis show that the percentage of correct assignments is elevated and in the group containing all of the hams it varies from 79.4% with the AG to PE samples to 72.6% with the ME samples.

**Keywords.** Iberian pork – Fatty acids – Discriminate analysis.

## I – Introduction

Dans l'actualité, toutes les méthodes pour vérifier la qualité, en ce qui concerne l'alimentation

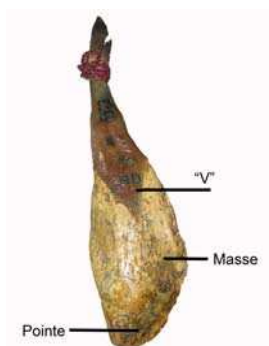


du porc Ibérique, retombent exclusivement sur le premier échelon du secteur, c'est-à-dire, l'éleveur. Rien n'a été néanmoins réalisé au niveau suivant, étant celui du secteur industriel. L'objectif de ce travail est d'analyser l'influence de l'alimentation de l'animal qui donne le jambon séché et qui a subi des régimes alimentaires différents.

## II – Matériel et méthodes

Soixante-cinq jambons ont été utilisés. Ils ont été fournis par diverses industries du secteur et ils correspondent à 7 catégories selon la génétique (100% Ibérique pur, croisé à 50% avec Duroc Jersey, et 0% de sang Ibérique) et le type d'alimentation dans l'étape finale d'engraissement ("Bellota", "Recebo" y "Cebo").

Des échantillons de tissus adipeux de 3 zones différentes de chaque jambon ont été prélevés de manière à ne pas altérer le moins possible le produit commercial. Les zones sélectionnées ont été: de la base du "V", de la Masse et de la Pointe du jambon (Fig. 1). Dans le cas de 30 jambons, les couches externe et interne des échantillons de tissus adipeux prélevés ont été séparées afin de déterminer dans chacune d'elles la composition en tissus adipeux. Dans le reste des échantillons, les deux couches ont été analysées ensemble. Les analyses ont été réalisées au Laboratoire Agroalimentaire de la Junta de Andalucía à Cordoue, selon la normative officielle d'analyses.



**Fig. 1. Zones de prélèvement des échantillons sur le jambon.**

Le pack de programmes statistique SPSS a été utilisé pour l'analyse statistique, comparant les valeurs obtenues dans chaque groupe moyennant un test Duncan, et la caractérisation de chaque groupe, en fonction de leur composition en acides gras, a été réalisée grâce à une analyse discriminante.

## III – Résultats et conclusions

La composition en acides gras des couches de tissu adipeux des jambons, de chaque zone, est recueillie dans le Tableau 1.

Dans le Tableau 2, on peut voir les valeurs (moyenne, maximale et minimale), du contenu en acide oléique de la couche externe, de chacune des zones du jambon, selon le type d'alimentation des animaux. On peut y apprécier que les valeurs les plus élevées en acide oléique sont obtenues dans la couche externe du "V" et les minimales dans la Pointe.

**Tableau 1. Composition des couches de tissu adipeux des jambons de porc Ibérique (n=30)**

Acides gras	Couche	Masse		Pointe		V	
		Moyenne	Signif.	Moyenne	Signif.	Moyenne	Signif.
Palmitique	Externe	23.2	0.69	24.6	0.01	22.2	0.89
	Interne	23.1		23.5		22.3	
Stearique	Externe	12.3	0.93	13.8	0.20	11.0	0.95
	Interne	12.3		13.2		11.0	
Oleique	Externe	50.4	0.51	48.4	0.49	53.1	0.78
	Interne	49.9		48.9		52.9	
Linoleique	Externe	7.8	0.03	7.2	0.00	6.9	0.12
	Interne	8.5		8.2		7.5	

**Tableau 2. Contenu en acide oléique de la couche externe du tissu adipeux de jambons séchés selon le type d'alimentation des animaux**

Type de jambon	Zone	N	Moyenne $\pm$ D.e.	Mínim.	Máxim.
Bellota 100	ME	15	53,2 $\pm$ 1,8 <sup>b</sup>	50	56
	PE	16	51,7 $\pm$ 2,3 <sup>a</sup>	50	58
	VE	16	54,9 $\pm$ 2,3 <sup>c</sup>	51	58
Bellota 50	ME	5	51,6 $\pm$ 2,1 <sup>a</sup>	49	54
	PE	6	50,1 $\pm$ 2,2 <sup>a</sup>	46	52
	VE	6	56,4 $\pm$ 1,0 <sup>b</sup>	55	58
Recebo 100	ME	13	51,7 $\pm$ 2,0 <sup>b</sup>	47	54
	PE	12	49,9 $\pm$ 2,5 <sup>a</sup>	46	53
	VE	12	53,3 $\pm$ 2,5 <sup>c</sup>	48	57
Recebo 50	ME	3	51,1 $\pm$ 2,4 <sup>a</sup>	49	53
	PE	3	47,8 $\pm$ 3,1 <sup>a</sup>	44	50
	VE	3	55,1 $\pm$ 0,1 <sup>b</sup>	55	55
Cebo 100	ME	15	48,4 $\pm$ 2,3 <sup>b</sup>	46	53
	PE	15	46,7 $\pm$ 2,5 <sup>a</sup>	43	53
	VE	15	51,5 $\pm$ 2,6 <sup>c</sup>	47	57
Cebo 50	ME	8	48,6 $\pm$ 1,5 <sup>b</sup>	47	51
	PE	8	46,3 $\pm$ 2,5 <sup>a</sup>	43	51
	VE	7	49,9 $\pm$ 2,4 <sup>b</sup>	47	53
Cebo 0%	ME	3	47,0 $\pm$ 1,9 <sup>b</sup>	46	49
	PE	3	43,7 $\pm$ 1,9 <sup>a</sup>	42	46
	VE	3	46,4 $\pm$ 2,1 <sup>ab</sup>	44	49
Toutes	ME	62	50,6 $\pm$ 2,8 <sup>b</sup>	45,5	56,1
	PE	63	48,8 $\pm$ 3,3 <sup>a</sup>	42,3	58,3
	VE	62	53,0 $\pm$ 3,3 <sup>c</sup>	44,4	58,1

ME: Masse Externe; PE: Pointe Externe; VE: V Externe.

Les résultats de l'application de l'analyse discriminante au total des jambons sont recueillis dans les Tableaux 3, 4 et 5. Dans ces tableaux on peut apprécier que le pourcentage le plus élevé des échantillons correctement classés est obtenu en utilisant la composition des acides gras de la graisse de la couche externe de la pointe (77,8%). Dans la catégorie intermédiaire "Recebo" le pourcentage d'erreurs de classement obtenu a été plus élevé, si l'on considère que ceci est dû à la grande hétérogénéité des animaux.

**Tableau 3. Résultats du classement des jambons selon la composition en acides gras de la graisse externe de la Masse**

Type commercial	N	B100	B50	R100	R50	P100	P50	P0
B100	15	73,3	0,0	13,3	0,0	6,7	6,7	0,0
B50	5	0,0	100,0	0,0	0,0	0,0	0,0	0,0
R100	13	23,1	0,0	61,5	0,0	7,7	7,7	0,0
R50	3	0,0	0,0	0,0	100,0	0,0	0,0	0,0
P100	15	0,0	0,0	6,7	0,0	73,3	6,7	13,3
P50	8	0,0	0,0	12,5	0,0	0,0	87,5	0,0
P0	3	0,0	0,0	0,0	0,0	33,3	0,0	66,7

B: Bellota; R: Recebo; C: Cebo; Classés correctement 77,8 % des échantillons.

**Tableau 4. Résultats du classement des jambons selon la composition en acides gras de la graisse externe de la Pointe**

Type commercial	N	B100	B50	R100	R50	P100	P50	P0
B100	15	87,5	0,0	12,5	0,0	0,0	0,0	0,0
B50	5	0,0	100,0	0,0	0,0	0,0	0,0	0,0
R100	13	25,0	0,0	47,7	0,0	16,7	16,7	0,0
R50	3	0,0	0,0	0,0	100,0	0,0	0,0	0,0
P100	15	6,7	0,0	0,0	0,0	80,0	13,3	0,0
P50	8	0,0	0,0	12,5	0,0	12,5	75,0	0,0
P0	3	0,0	0,0	0,0	0,0	0,0	0,0	100,0

B: Bellota; R: Recebo; C: Cebo; Classés correctement 77,8 % des échantillons.

**Tableau 5. Résultats du classement des jambons selon la composition en acides gras de la graisse externe du V**

Type commercial	N	B100	B50	R100	R50	P100	P50	P0
B100	16	68,8	6,3	12,5	6,3	6,3	0,0	0,0
B50	6	0,0	83,3	0,0	16,7	0,0	0,0	0,0
R100	12	16,7	8,3	50,0	8,3	8,3	0,0	8,3
R50	3	0,0	0,0	0,0	100,0	0,0	0,0	0,0
P100	15	0,0	0,0	6,7	0,0	86,7	0,0	6,7
P50	7	0,0	0,0	14,3	0,0	14,3	28,6	42,9
P0	3	0	0	0	0	0	0	100

B: Bellota; R: Recebo; C: Cebo; Classés correctement 69,4 des échantillons.

Cette méthode d'analyse peut donc être un outil utile pour le classement des jambons séchés

et avec un pourcentage d'erreur comparable à ceux qui se produisent quand on évalue l'alimentation des animaux à travers la composition en acides gras d'échantillons de biopsies du tissu adipeux sous cutané. Ces erreurs sont finalement la conséquence de la fragilité du système basé sur le trait des acides gras pour la détermination de la qualité (Porras et al., 2009), aussi bien dans les biopsies que dans les échantillons de tissu adipeux des propres jambons. La nouveauté de ce système, une fois mis au point, visant à fixer les intervalles des principaux acides gras, permettrait de contrôler la qualité au niveau du séchoir et pas seulement au niveau de l'exploitation, comme on le fait actuellement.

## Remerciements

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# Utilization of tocopherols quantification for the differentiation of Iberian pigs fed in free-range or with feeds in intensive or extensive systems in different geographical regions

A.I. Rey\*, D. Amazan\*, J. García-Cascos\*\* and C.J. López-Bote\*

\*Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Ciudad Universitaria, Madrid (Spain)

\*\*Centro Nacional I+D del Cerdo Ibérico, INIA, Zafra, Badajoz (Spain)

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**Abstract.** The aim of this work was to study the capacity of differentiation of the technique based on the tocopherols quantification in blind samples of fat and muscle coming from animals fed (1) with feed in an intensive system, (2) with an enriched-fat diet in extensive conditions, (3) with a conventional diet in extensive conditions, (4) recebo (low fattening in free-range) and (5) exclusively free-range in different geographical areas. The technique was able to differentiate ( $P < 0.05$ ) samples from animals fed in free-range from the other groups. It was also able to classify ( $P < 0.05$ ) by muscle gamma-tocopherol quantification, animals fed with feed in intensive situations from those fed in recebo or with feed in an extensive system. However, there were not significant differences in the content of gamma-tocopherol in fat and muscle between the recebo pigs (with average weight gained free-range  $<25$  kg) and those fed with feed in extensive conditions.

**Keywords.** Iberian pig – Tocopherols – Carcass classification – Quality.

**Utilisation de la détermination du tocophérol pour la différenciation de porcs Ibériques alimentés au pâturage ou avec du concentré dans les systèmes d'exploitation intensive ou extensive dans différentes régions géographiques**

**Résumé.** L'objet de ce travail est d'étudier la capacité de différenciation de la technique basée sur la détermination des tocophérols que contiennent les échantillons en aveugle de graisses et de muscles en provenance d'animaux engraisés dans des systèmes (1) intensifs, (2) extensifs et alimentés à base de concentrés contenant de la graisse, (3) en engraissement extensif à base de concentrés conventionnels, (4) ré-engraissement, et (5) élevés au pâturage dans différentes régions géographiques. La technique a été capable de différencier ( $P < 0.05$ ) les échantillons provenant d'animaux alimentés au pâturage par rapport au reste du groupe. On a également pu classer ( $P < 0.05$ ) les animaux alimentés à base de concentrés en engraissement intensif par rapport à ceux qui ont été alimentés dans les exploitations de ré-engraissement ou engraissement extensif. Toutefois, il n'y a pas eu de différences significatives dans les teneurs en gamma-tocophérol entre les animaux de ré-engraissement et ceux d'engraissement en exploitation extensive.

**Mots-clés.** Porcs Ibériques – Tocophérol – Classification carcasse – Qualité.

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## I – Introduction

The high price that the Iberian pig products reaches in the market has originated faked practices in order to marketing products coming from Iberian pigs fed with feeds in confinement as if they were from pigs fed free-range with acorns and grass. As quality control measure during the last years has been used the profile of fatty acids to determine the productive origin of the animals, however this technique has stopped to be an approach of classification of full validity. Among the alternative techniques of classification is tocopherols determination. This measurement is

interesting since tocopherols, which are found at high concentrations in acorns (mainly gamma-tocopherol) and grass (mainly alpha-tocopherol), are accumulated in fat tissues (Rey *et al.*, 1997). This accumulation has also been found to depend on the days that pigs stayed in free-range conditions or the kilograms of fattening and so could discriminate the feeding background of the animals and its products classification (Rey *et al.*, 2006). Previous studies (Rey *et al.*, 1997, Daza *et al.*, 2005, Rey *et al.*, 2006) reported differences in the tocopherol concentration between groups fed free-range and those fed in intensive conditions with mixed diets. However, no information is available on the tocopherols accumulation with other feedings such as enriched-fat or conventional diets in combination with extensive conditions in which pigs could utilize some natural resources (mainly grass) (label recently defined in the Iberian pig Quality Norm) (BOE, 2007) and its possible utilization to differentiate these groups from the others. The accumulation of tocopherols at different geographical locations because of grass availability has also to be explored. The aims of this work were to study the capacity of differentiation of the technique based on the tocopherols quantification in blind samples of fat and muscle from animals fed different feedings in intensive or extensive conditions.

## II – Materials and methods

The experimental groups (Table 1) consist of Iberian pigs (pure Iberian or crossed) with ages between 10-18 months fed different dietary treatments.

**Table 1. Data of the Iberian pigs from the different experimental groups**

N	BREED	AGE (months)	FARM	SITUATION	FEEDING	DAYS FREE-RANGE	Fattening free-range (kg)	Grass resources
13	Pure Iberian pigs (males)	18	San Amaro	Olivenza (Badajoz)	Enriched fat diet (extensive)	0	0,00	low
32	Crossed 50 %	10	Turcañada	Fuenteovejuna (Córdoba)	Conventional mixed diet (intensive)	0	0,00	
12	Pure Iberian pigs Valdequesera	12	Valdequesera	Badajoz	Free-range	60	40,15	medium
32	Crossed 75 %	14	Puerto Lobo	Cabeza la Vaca (Sur Badajoz)	Free-range	72	58,39	high
10	Pure Iberian pigs Valdequesera	12	Valdequesera	Badajoz	Conventional mixed diet (extensive)	0	0,00	medium
12	Pure Iberian pigs Valdequesera	12	Valdequesera	Badajoz	Recebo	46	17,13	medium
6	Pure Iberian pigs Valdequesera	12	Valdequesera	Badajoz	Conventional mixed diet (extensive)	0	0,00	medium
14	Pure Iberian pigs Valdequesera	12	Valdequesera	Badajoz	Recebo	46	18,07	medium
29	Pure Iberian pigs (Castrated females)	12	San Miguel	Ciudad Rodrigo (Salamanca)	Free-range	91	55,22	high
7	Pure Iberian pigs Valdequesera	12	Valdequesera	Badajoz	Conventional mixed diet (extensive)	0	0,00	medium
13	Pure Iberian pigs Valdequesera	12	Valdequesera	Badajoz	Recebo	46	23,08	medium

One group was fed with a commercial feed in confinement, a second group was fed outdoors (limited pens with access to grass) with an enriched-fat diet which contained (g/kg) 268 g grease-out wheat meal, 268 g grease-out barley meal, 100 g grease-out corn meal, 150 carob-bean meal, 150 g sunflower seed, 50 g pea, 3 g calcium carbonate, 6 g dicalcium phosphate, 4 g sodium chloride. A third group was fed outdoors (similar conditions that second group) with a conventional diet that contained (g/kg) 120 g corn, 111 g wheat, 110 g barley, 98 g soya-bean meal, 37 g DDG barley, 15 g sunflower seed, 10 g beet root pulp, 7 g dicalcium phosphate, 4 g pork lard, 4 g calcium carbonate, 2 g sodium chloride. Another three experimental groups were fed in “recebo” (free-range for a period of 46 days and then received the same conventional mixed diet that those pigs fed in extensive conditions) and finally another three groups were fed free-range during a variable period. Pigs fed free-range were from different locations in Spain and hence they had variable grass availability (medium and high). Also pigs fed in extensive conditions had different grass resources depending on the pens location.

Pigs were slaughter at the average weight of 164,3 kg ( $\pm 16,4$ ) and samples (fat at the level of the tail and muscle from different areas) were collected and frozen at -20°C until analysis. Tocopherols (gamma and alpha) in muscle were quantified according to the method described by Rey *et al.* (1996) in which samples were homogenised in dibasic sodium phosphate buffer and extraction was made with ethanol and hexane. Tocopherols in fat were analysed by saponification in presence of pyrogallol, potassium chloride and potassium hydroxide as described before (Rey *et al.*, 2006). In both methods tocopherols were dissolved in ethanol prior

to analysis by reverse-phase HPLC (HP 1100, with a diode array detector) (Hewlett Packard, Waldbronn, Germany). Separation of gamma and alpha tocopherols was made by RP-C18 column at a flow rate of 2ml/min. (methanol: water 97:3).

Data were analysed using the general linear model (GLM) procedure contained in SAS version 8 (SAS, 1999). The comparative analysis between means was conducted using the Duncan's test.

Groups of free-range pigs had concentrations of gamma-tocopherol in fat (ug/g: 0.77, 1.51 and 1.80) according to the days in freedom (60, 77 and 91), the weight gained (40, 58 and 55 kg) and the grass resources (medium, high and high, respectively). Alpha-tocopherol was also affected by these factors (ug/g: 8.3, 11.4 and 10.8). In those pigs ("recebo") that stayed in freedom for a short period (46 days) and so gained less weight (17, 18 and 23 kg) the concentration of tocopherols in fat were lower as expected (gamma-tocopherol ug/g: 0.31, 0.26 and 0.58; alpha-tocopherol ug/g: 6.3, 6.6 and 9.5 respectively). In muscle, due to the heterogeneity of the sample and so the different intramuscular fat content differences were not as marked as in fat.

On the other hand, groups fed with a conventional diet outdoors in delimited pens had variable concentrations of tocopherols in fat (gamma ug/g: 0.38, 0.34 and 0.52; alpha ug/g: 6.5, 6.3 and 8.9) and muscle (gamma ug/g: 0.18, 0.13 and 0.15; alpha ug/g: 2.6, 1.9 and 2.5) probably in function of the different natural resources available.

Fat samples (n=180) were classified by 74 % correctly. The main mistake was in the classification of pigs fed outdoors in delimited pens that were considered as those fed extensively in freedom for a short period ("recebo"). Classification of muscle samples (n=188) was correct by 72 % even though the high variability in the sample collection and showed the same trend observed in fat.

Average results of tocopherols concentration in fat and muscle from the experimental groups are presented in Tables 2 and 3. In fat (Table 2), gamma-tocopherol concentration was higher (P=0.0001) in free-range pigs than the other groups. Free-range pigs had also higher concentrations of alpha-tocopherol than the other groups with the exception of those pigs that received an enriched-fat diet, which had similar concentrations. The alpha-tocopherol concentration was also of interest to discriminate between pigs fed with a conventional mixed diet in intensive conditions and the other groups. These pigs fed indoors with feed had the lowest alpha-tocopherol concentration (P=0.0001). Neither gamma-tocopherol nor alpha-tocopherol was different in fat from those pigs fed extensively in freedom ("recebo") or in pens with a conventional mixed diet.

**Table 2. Tocopherols (gamma and alpha) concentration (ug/g) in fat from the experimental groups (average values between similar groups) according to their feeding**

Conditions	Extensive in freedom		Extensive in delimited pens		Intensive	RMSE	P
Feeding	Free-range	Recebo	CMD†	EFD†	CMD		
Gamma-tocopherol (ug/g)	1.501 a	0.381 b	0.415 b	0.351 b	0.367 b	0.37	0.0001
Alpha-tocopherol (ug/g)	10.653 a	7.490 b	7.216 b	10.045 a	4.364 c	1.97	0.0001
Gamma/Alpha (ug/g)	0.142 a	0.050 c	0.058 c	0.036 c	0.088 b	0.04	0.0001

†CMD: Conventional mixed diet; EFD: Enriched-fat diet.



**Table 3. Tocopherols (gamma and alpha) concentration (ug/g) of muscle samples from the experimental groups (average values between similar groups) according to their feeding**

Conditions	Extensive in freedom		Extensive in delimited pens		Intensive		
Feeding	Free-range	Recebo	CMD†	EFD†	CMD	RMSE	P
Gamma-tocopherol (ug/g)	0.476 a	0.153 b	0.150 b	0.137 b	0.0886 c	0.09	0.0001
Alpha-tocopherol (ug/g)	3.925 a	2.948 b	2.341 c	4.022 a	1.7553 d	0.80	0.0001
Gamma/Alpha (ug/g)	0.124 a	0.0546 c	0.066 b	0.037 d	0.0522 c	0.02	0.0001

†CMD: Conventional mixed diet; EFD: Enriched-fat diet.

In muscle, differences between groups were more marked than in fat (Table 3). Gamma-tocopherol concentration was statistically different in those pigs fed in intensive conditions and in free-range in comparison with the other groups fed in extensive conditions either in limited pens or recebo ( $P=0.0001$ ). In muscle as in fat, alpha-tocopherol was the lowest in those pigs fed in intensive conditions while the highest concentrations were detected in both enriched-fat diet in extensive conditions and in free-range groups. Alpha-tocopherol quantification in muscle also allowed the discrimination between pigs fed recebo than those fed a conventional diet in extensive conditions. Hence, those pigs fed recebo showed higher alpha-tocopherol concentrations than those fed a conventional diet extensively (2.9 vs 2.3).

### III –Conclusions

The determination of gamma-tocopherol in fat or muscle from Iberian pig allows the discrimination of free-range pigs from others fed in intensive or in extensive conditions with conventional or enriched-fat diets. Gamma-tocopherol quantification also allows a clear differentiation between free-range pigs and those that stayed a short period and gained low weight (<20-25 kg) in free-range ("recebo"). However, the quantification of both tocopherols (alpha and gamma) in different tissues is needed to differentiate pigs fed mixed diets in extensive or intensive conditions between them and from the other groups.

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# Volatile compounds and sensorial traits of "Toscano" dry-cured ham

F. Sirtori\*, L. Calamai\*\*, W. Giorgi\*\*\*, L. Tardelli\*, C. Pugliese\*

\*Dipartimento di Biotecnologie Agrarie, Sezione di Scienze Animali,  
Via delle Cascine 5, 50144 Firenze (Italy)

\*\*Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, Firenze (Italy)  
and Centro Interdipartimentale di Spettrometria di Massa (CISM), Sesto Fiorentino (Italy)

\*\*\*Consorzio del Prosciutto "Toscano", Firenze (Italy)

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**Abstract.** The aims of this study were to evaluate the effect of seasoning time on aromatic profiles and to describe the sensorial profile of "Toscano" dry-cured ham. At this aim ten hams, cured according to the rule of "Toscano" PDO label, were sampled at the begin of seasoning and at 1, 3, 6 and 12 months. The samples of muscular tissue were submitted to volatile compounds analysis by SPME-GC/MS. At the end of seasoning a sensorial analysis of hams was carried out by a trained panellist group. As regard volatile analysis, about 80 compounds were identified belonging to: aldehydes, organic acids, ketones, esters, alcohols and furans. Aldehydes represent the most important chemical family in "Toscano" ham; they showed a significant increase only in the first 6 months of seasoning. The second quantitatively important chemical family in "Toscano" ham was that of the organic acids that increased along the whole curing time. Ketones dramatically decreased at 12 months of seasoning while esters showed a more constant trend. Significant correlations were found among sensorial traits and aldehydes compounds.

**Keywords.** Volatile analysis – Sensorial profile – Dry-cured ham – Seasoning.

## *Les composés volatils et les caractéristiques sensorielles du jambon sec "Toscano"*

**Résumé.** Les objectifs de cette étude étaient d'évaluer l'effet du temps de mûrissage sur les profils aromatiques et de décrire le profil sensoriel du jambon sec "Toscano". Dix jambons mûris selon la règle du label AOP "Toscano", ont été échantillonnés au début du mûrissage et à 1, 3, 6 et 12 mois. Les échantillons de tissu musculaire ont été soumis à l'analyse des composés volatils par SPME-GC/MS. À la fin du mûrissage l'analyse sensorielle des jambons a été réalisée par un groupe d'experts qualifiés. Pour ce qui concerne l'analyse des composés volatils, environ 80 composés ont été identifiés appartenant à: aldéhydes, acides organiques, cétones, esters, alcools et furanes. Les aldéhydes représentent la famille la plus importante dans le jambon "Toscano", et ils ont montré une augmentation significative seulement dans les 6 premiers mois de mûrissage. La seconde famille de substances chimiques la plus importante quantitativement dans le jambon "Toscano" a été celle des acides organiques qui ont augmenté pendant tout le temps de mûrissage. Les cétones ont diminué considérablement à 12 mois de maturation tandis que les esters ont montré une tendance plus constante. Des corrélations significatives ont été trouvées parmi les caractères sensoriels et les composés aldéhydes.

**Mots-clés.** Analyse de composés volatils – Profil sensoriel – Jambon sec – Mûrissage.

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## I – Introduction

Several studies have been dealing with the flavour of ham of Mediterranean area (Dirinck *et al.*, 1997), either on the relationships between compositional traits and sensory qualities of French and Italian dry-cured ham or on the volatile profile of Iberian hams and on its evolution with ripening, with the identification of the compounds primarily responsible for the typical aromatic characteristics. Also, Italian Parma and San Daniele hams have been characterized for their aromatic composition (Careri *et al.*, 1993, Bolzoni *et al.*, 1996; Gaspardo *et al.*, 2008).

Conversely, the information on the “Toscano” dry-cured ham are, at present, limited to physical-chemical traits as affected by genetic type and rearing system (Franci *et al.*, 1997; Pugliese *et al.*, 2005; Pugliese *et al.*, 2010), while information on its volatile composition are still lacking. There are also few details on its sensory properties. So the aim of this trial is to investigate the evolution of volatile compounds profile of “Toscano” dry-cured ham during 12 months ripening by SPME-GC-MS and, at the same time, to describe its sensorial profile at the end of the 12 months of seasoning.

## II – Materials and methods

Ten hams were seasoned for 12 months according to the “Toscano” PDO Consortium ripening protocol and *Biceps Femoris* muscle was sampled immediately after trimming and after 1, 3, 6, 12 months of ripening. The volatile compound profile was obtained by SPME–GC-MS technique. An Agilent 7890 GC-Chromatograph equipped with a 5975A MSD with EI ionization was used for analysis. Volatile compounds were identified by matching EI mass spectra against NIST 05 or Wiley 07 spectral library and Kovats indices. As regard sensorial analysis samples of cured ham were assessed by a trained panel of 10 members using a descriptive analysis method. A 100 mm unstructured scale was used. Three-four hams were evaluated simultaneously in each evaluation session and the samples order was randomised. Data were subjected to analysis of variance using, as fixed effects, “ham” (10 levels) and “time of ripening” (5 levels) for evolution of aromatic profile or “panellist” (10 levels) for sensorial data. Significant differences were tested after Student’s t test. Principal Component Analysis (PCA) was applied to evaluate the relationships among variables.

## III –Results

Over 60 compounds belonging to esters, aldehydes, organic acids, ketones and alcohols were identified but for lack of space the means of each aromatic compounds were not tabulated while their evolution is shown in Fig. 1.

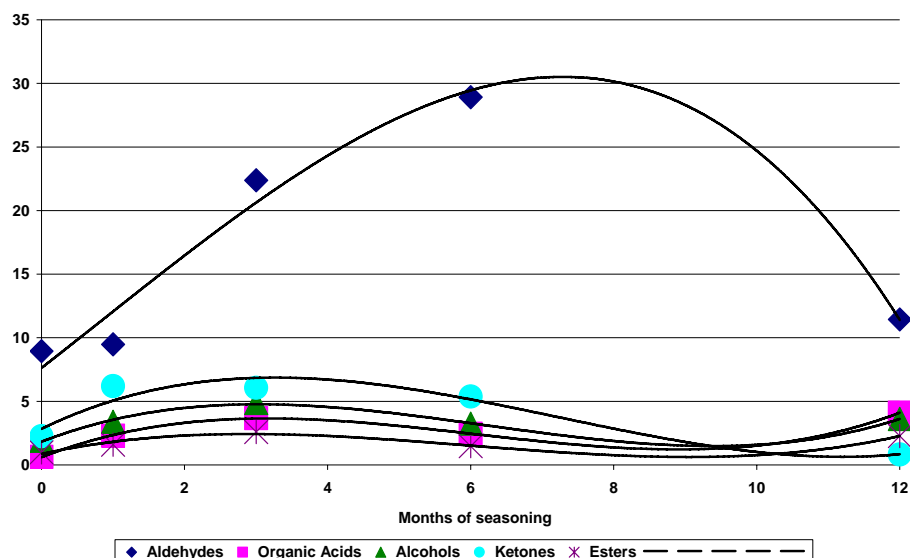


Fig. 1. Evolution of the main chemical families according to seasoning time.

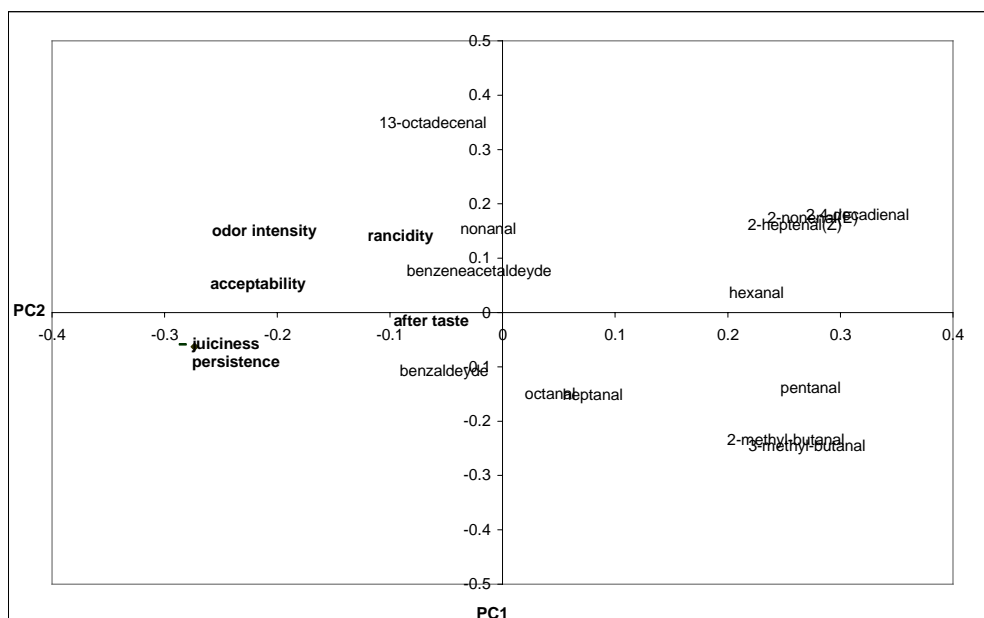
Aldehydes were the most represented compounds in "Toscano" ham, similarly to what reported on lean tissue of Iberian cured ham (Ruiz *et al.*, 1999). The aldehydes and ketones groups reached their greatest relative concentration at 6 and 1 month of ripening, respectively, and then decreased significantly, probably due to further reactions with other compounds. Alcohols, organic acids and esters concentration increased until the third month of ripening, and remained rather constant until 12 months. The described trend of the chemical families was strictly linked to the drying/ripening conditions when most of the biochemical changes become relevant. In the ripening stages a temperature of 16-18°C is adequate for the activity of the lipolytic and proteolytic enzymes, which progressively decrease thereafter, probably due to the contemporary water activity decrease along the process.

Table 1 shows the means and standard deviations of the sensory traits. The variability of the traits was quite high, that means that "Toscano" dry-cured ham is characterized by a medium-low degree of homogeneity. This can be considered a problem from commercial point of view but, on the other hand, it is a guarantee of a really traditional production process.

**Table 1. Sensorial analysis of "Toscano" ham**

Parameter	Means (mm)	Significance of "ham effect"	Standard deviation
Yellowness	33.10	<.0001	21.34
Pinkness	40.49	0.0056	18.08
Oiliness	41.90	0.0106	18.70
Firmness of fat	42.62	0.6815	17.76
Internal fat knob	40.39	0.0050	15.92
Quantity of external fat	41.33	0.1583	16.94
Redness	47.30	0.0005	16.26
Uniformity of color	42.83	0.0024	15.40
Marbling	44.07	<.0001	15.60
Firmness of lean	48.00	0.0015	15.72
Odor intensity	53.83	0.4942	16.67
Saltiness	35.48	0.1439	20.74
After taste	53.06	0.3729	15.84
Rancidity	7.25	0.8018	12.05
Hardness	30.69	0.4049	17.86
Juiciness	50.42	0.0004	22.63
Persistence	54.80	0.3566	19.99
Overall acceptability	56.79	0.0634	17.55

The variability of the data is high only for what concerns the data of color and of fat (quantity and quality). For other parameters, related in particular to aroma and odor, the variability among hams was very small. This fact attests to the validity of the curing process that can provide homogeneous products in these respects. As regards PCA, two principal components were generated that accounted for 60% of the total variance (PC1 = 39% and PC2 = 21%). The score plot of the samples is shown in Fig. 2. For PCA only aldehydes compounds were used because they were the only compounds that showed significant correlations with sensorial traits. As it can be seen in the figure the sensorial traits link to taste are very poor related with aldehydes compounds. The only positive relationships were found with 13-octadecenal, benzeneacetaldehyde, benzaldehyde and nonanal that seems to be related to rancid aroma (García-González *et al.*, 2008).



**Fig. 2. Principal Component Analysis results.**

## Acknowledgements

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# Non-destructive analysis of fresh Iberian pork loins by near-infrared spectroscopy (NIRS)

E. Zamora-Rojas<sup>\*1</sup>, A. Garrido-Varo, D.C. Pérez-Marín, J.E. Guerrero-Ginel and E. De Pedro-Sanz

Department of Animal Production, Faculty of Agricultural and Forestry Engineering,  
University of Córdoba, Córdoba (Spain)  
<sup>1</sup>e-mail: g32zaroe@uco.es

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**Abstract.** A fiber-optic contact probe near infrared spectroscopy (NIRS) instrument is evaluated to predict fat, moisture and protein in intact Iberian pork loins. A total of 173 Iberian pig loin samples were analyzed. Spectra from intact loins were collected with a LabSpec®Pro A108310 spectrometer (Analytical Spectral Device) and from the ground meat with a FossNIRSystem instrument. Spectra attenuation was required for removing very signal noise areas in the intact loin spectra. As reference values were used the NIRS predictions of the sample set estimated with a robust calibration model for ground samples and analyzed in a FossNIRSystem instrument. Modified Partial Least Squared (MPLS) regression was performed and different spectra pretreatments and spectral regions evaluated. The range 450-2300 nm performed the best models for the instrument evaluated, showing a suitable potential of the NIRS instrument for on-line analysis of pork loins.

**Keywords.** Iberian pigs – Loins – NIR Spectroscopy – Fiber optic – Fat – Protein – Moisture.

## **Analyse non destructive d'échines fraîches de porc Ibérique au moyen de spectroscopie à infrarouge proche (NIRS)**

**Résumé.** Un équipement spectroscopique à infrarouge proche (NIRS) doté d'une sonde de fibre optique de contact est évalué pour la prédiction de la graisse, de l'humidité et de la protéine dans des échines intactes de porcs Ibériques. Un total de 173 échantillons d'échines de porcs Ibériques ont été analysés. Les spectres ont eu besoin d'être atténués pour éliminer des aires ayant un signal bruyant. Comme valeurs de référence on a utilisé les prédictions NIRS de l'ensemble des échantillons estimées avec un modèle robuste de calibrage créé pour des échantillons hachés et analysés par un instrument FossNIRSystem. L'algorithme de régression des Moindres Carrés Partiels Modifiés (MPLS) a été utilisé pour évaluer différents pré-traitements et régions spectrales. L'intervalle 450-2300 nm a fourni les meilleurs modèles pour l'instrument évalué, en montrant le potentiel d'analyse en ligne de l'instrument NIRS pour l'échine de porc.

**Mots-clés.** Porcs Ibériques – Rein – Spectroscopie NIR – Fibre optique – Matières grasses – Protéines – Humidité.

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## **I – Introduction**

Iberian pig products have a high rate consumer acceptance, leading to high prices in the market. Consumers assess the quality of those products based on the exceptional organoleptic, healthy and sensory characteristics, but guarantee a target meat product is a complex task that requires control procedures (Prieto *et al.*, 2009a). Fresh meat is a very heterogeneous product and the determination of the major chemical constituents, such as fat, moisture or protein, is interesting for labelling purposes or for preparing good mixtures to produce dry-cured or processed products.

Traditional wet chemistry to determine these parameters are time-consuming, tedious, costly and destructive methods. Nowadays, the meat industry has shown a great interest for new

technologies that enables fast, accurate, non-destructive quality analysis. Near-Infrared Spectroscopy (NIRS) has shown its potential to predict chemical composition in meat products (Prevolnik *et al.*, 2004; Prieto *et al.*, 2009a), even in on-line applications in the industry (Tøgersen *et al.*, 1999; González-Martín *et al.*, 2002; Huang *et al.*, 2008; Prieto *et al.*, 2009b) resolving part of the demands of the industry. Most of the literature has used ground samples which require a sample preparation. In this study is evaluated a fiber-optic contact probe NIRS instrument to predict chemical composition (fat, moisture and protein) of intact Iberian pork loins.

## II – Materials and methods

### 1. Sample set and NIRS measurements

One hundred and seventy three samples of Iberian pork loins were analyzed. The samples were taken from the beginning of the loin in the shoulder area of the animals. Two NIRS analyses were performed with different instruments and samples presentation.

A post-dispersive diode array scanning monochromator spectrometer LabSpec®Pro A108310 (Analytical Spectral Device-ASD Inc., Boulder, Colorado, USA) working from 350-2500 nm (1nm spectral resolution) in reflectance mode was used to analyze intact pork loin samples at the slaughter house, two hours post-mortem. The loins after being analyzed with the LabSpec®Pro were vacuum packaging and frozen at -20°C to be stored. A FossNIRSystem 6500 equipped with a spinning module for standard circular cups working from 400-2500 nm (2 nm spectral resolution) in reflectance mode was used to analyze the same samples set in ground presentation. Before recording the NIRS spectra, the muscles were ground and homogenized by a vertical cutter mixer (Heidolph homogenizer DIAX 900). Two spectra per sample were measured in each case.

### 2. Data modelling

Chemometric data treatment was performed using the software WinISI II ver 1.50 (Infrasoft International, Port Matilda, PA, USA). The Root Mean Squared (RMS) error statistic was used for spectral repeatability evaluation (Shenk and Westerhaus, 1995). The fat, moisture and protein composition of each sample was determined by NIRS using a robust model developed for ground Iberian meat in the range 1100-2500 nm (not published). These predictions were used as reference for further analysis.

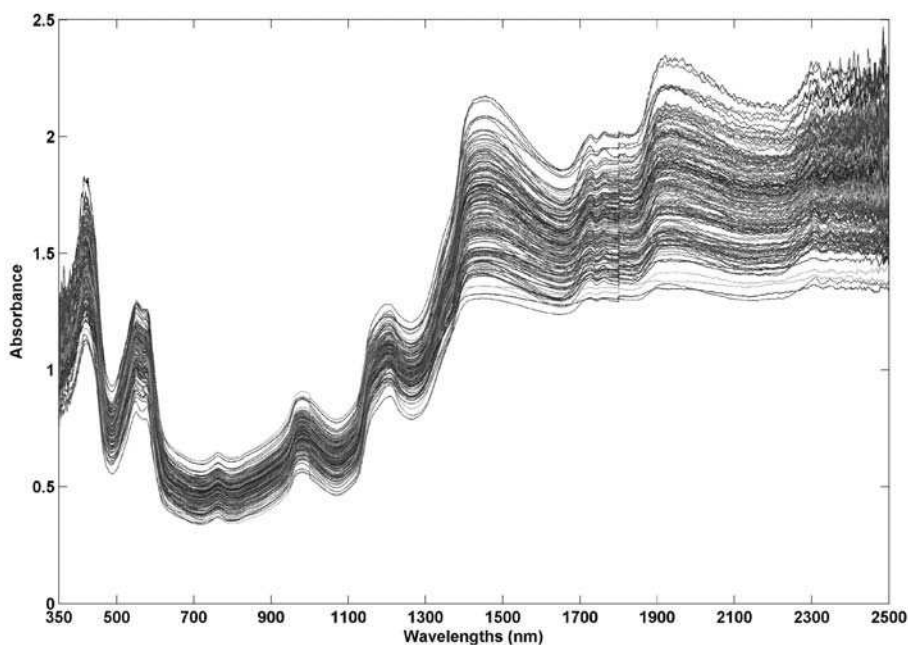
Modified Partial Least Squared (MPLS) regression method (Shenk and Westerhaus, 1991) was used to develop calibration models for predicting fat, moisture and protein with intact pork loin samples analyzed with the fiber optic contact probe instrument. As spectral pre-treatments, Standard Normal Variate (SNV) plus Detrending (DT) (Barnes *et al.*, 1989) was used to remove the multiplicative interferences of scatter and two derivative mathematical treatments were performed: window-wise filtering (1,10,5,1) and (2,5,5,1) (ISI, 2000). The optimum number of model factors was selected by cross-validation using 4 groups.

Signal noise at the beginning and end of the spectral range was eliminated for the LabSpec®Pro spectra measurements. Two spectral regions were selected for performing the calibration models: 450-2300 nm and 1100-2300 nm.

The evaluation of the models was performed with different statistics: the standard error of calibration (SEC), the standard error of cross-validation (SECV) and the coefficient of determination for cross-validation ( $r^2$ ).

### III – Results

The collection of high-quality spectra is a crucial task to develop accurate calibration models. NIRS spectral repeatability using the RMS statistic can help to evaluate the quality of the spectra. In the case of the LabSpec®Pro, the RMS values exceeding the 90,000  $\mu\log 1/R$  for comparisons of spectra from the same sample and location across the whole spectral range (350-2500 nm). By removing spectral areas with a very signal noise, i.e. at the beginning (350-450 nm) and the end (2300-2500 nm) of the spectra, the RMS values were lowered to 86,115  $\mu\log 1/R$ . Figure 1 shows the spectra collected from intact loins with the fiber-optic contact probe and it is observed the high signal noise over 2000nm. In the case of the FossNIRSystem, the RMS values are on the average of 9.941  $\mu\log 1/R$ .



**Fig. 1. Spectra collected from intact pork loins using a fiber-optic NIRS instrument from LabSpec®Pro.**

Figure 2 shows the average spectra of the samples set for the analysis performed for intact pork loins with LabSpec®Pro and for ground pork meat with the FossNIRSystem instrument in the 400-2300 nm range. It is observed strong similarities between both, although the quality of the spectra is different with a smoother pattern in the case of the ground meat, due to the sample presentation and instrument differences. The FossNIRSystem is a laboratory instrument working under controlled-conditions, while the LabSpec®Pro was working on-line in the industry. Ground samples presented a more clearly-defined spectrum with sharper peaks than the intact loins. However, both show different characteristic absorption peaks: around 1200, 1720-1760nm as areas of fat and water-related peaks at 1450 and 1940 nm (Williams and Norris, 1987; Osborne, Fearn and Hindle, 1993).

The spectra of the ground samples were used to predict fat, moisture and protein composition of the Iberian pork loins with a robust model developed for the FossNIRSystem working in the



1100-2500 nm range (not published) over different years and with different ground pork muscles in our research group. Table 1 shows the statistic of that model. The prediction obtained for each sample was used as reference value for developing calibration models for the determination of fat, moisture and protein composition in intact loins using the fiber-optic instrument.

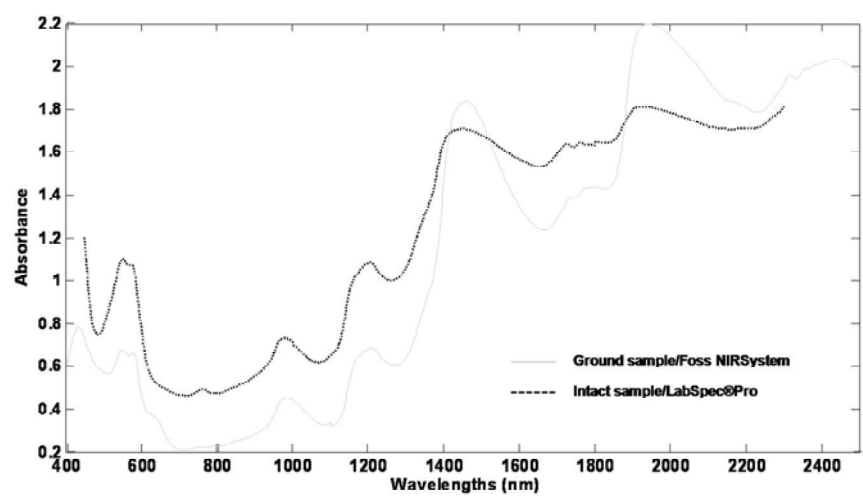


Fig. 2. Average spectra collected with the different instruments and samples presentation.

Table 1. Calibration statistics for the NIRS prediction using ground Iberian meat samples of fat, moisture and protein composition

Parameter	Pre-treatment	No. of samples	Nº of Principal Components	SEC (%)	r <sup>2</sup>	SECV (%)
Fat	SNV + DT (2,5,5,1)	315	4	0.37	0.98	0.40
Moisture	SNV + DT (2,5,5,1)	315	5	0.47	0.96	0.51
Protein	SNV + DT (1,10,5,1)	324	7	0.48	0.91	0.49

Table 2 shows the statistics for the different parameters (fat, moisture and protein) studied of the samples set predicted by NIRS using the Table 1 models. Modified Partial Least Squared (MPLS) algorithm was used to develop calibration models for the prediction of the major chemical composition parameter in intact Iberian pork loins with a remote NIR reflectance fiber-optic contact probe instrument.

Table 2. Calibration statistics for the NIRS prediction using ground Iberian meat samples of fat, moisture and protein composition

Parameter	Training set (173 samples)			
	Minimum	Maximum	Average	Standard Deviation
Fat (%)	3.20	18.95	8.69	2.90
Moisture (%)	63.20	74.97	70.29	2.08
Protein (%)	17.16	21.95	19.68	0.90

The best calibration models obtained for predicting the three main chemical parameter in meat, measured with the fiber-optic contact probe in intact Iberian pork loin, are shown in Table 3 (i.e. after testing second and first derivative for two spectral range: 450-2300 nm and 1100-2300 nm).

**Table 3. Calibration statistics of fat, moisture and protein composition obtained for the NIRS prediction with a remote fiber-optic probe using intact Iberian loin samples**

Parameter	Range (nm)	Pre-treatment	No. of samples	No. <sup>o</sup> of Principal Components	SEC (%)	r <sup>2</sup>	SECV (%)
Fat	450-2300	SNV + DT (1,10,5,1)	173	4	1.42	0.68	1.65
Moisture	450-2300	SNV + DT (2,5,5,1)	168	4	0.83	0.59	1.25
Protein	450-2300	SNV + DT (1,10,5,1)	171	4	0.51	0.56	0.59

As expected, models developed using intact loins are less accurate (Table 3) than those obtained with ground samples (Table 2). Intact loins showed larger sample heterogeneity than ground samples; freezing/drawing of the ground samples can affect mainly the moisture parameter and the quality of the spectra is different between instruments and sample presentation. Nevertheless, the models showed the possible potential of a fiber-optic contact probe NIRS instrument for analyzing chemical composition of intact pork loins. Moreover, an on-line analysis enables instantaneous and without sample presentation measurement providing important industrial advantages for quality and process control. However, there are several topics that require further studies such as the sampling optimization of intact meat products or the choice of the best instrument measurement parameters for an adequate spectra collection.

## IV – Conclusions

The on-line NIRS instrument evaluated in this study for the quantitative chemical composition prediction of intact Iberian pig loins shows a very promising result enabling several practical advantages of the analysis. Further work is required in order to develop more accurate models.

## Acknowledgements

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# <sup>1</sup>H-NMR as a tool to determine the type of feeding of Iberian pigs

J. García-Olmo,\* J.I. Corredor-Coca,\* E. De Pedro-Sanz\*\*

\*Servicio Central de Apoyo a la Investigación (SCAI), Universidad de Córdoba, Campus de Rabanales, 14071 Córdoba, (Spain)

\*\*Dpto. Producción Animal – E.T.S.I.A.M., Universidad de Córdoba, Campus de Rabanales, 14071 Córdoba, (Spain)

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**Abstract.** The fact that animals are often given feed in an attempt to achieve similar carcass characteristics to those of animals exclusively fed with “montanera”, has obliged us to develop methods to differentiate and determine the type of feed consumed by these animals. One of these methods is Nuclear Magnetic Resonance (<sup>1</sup>H-NMR). Iberian pig fat samples were taken in the rump at 10 cm from the tail insertion of Torbiscal pure Iberian pigs fattened with montanera (batch B1; n=44) and with feedcompound (batch P1; n=16). Similar samples were also taken from diverse genetic types of pure Iberian pigs fattened with montanera (batch B2; n=10) or with special feedcompound (batch P2; n=10). The <sup>1</sup>H-NMR spectra of these fat samples were obtained according to routine methods at the NMR Unit of the Central Service for Research Support (SCAI) of the University of Cordoba. The Principal Component Analysis (PCA) of <sup>1</sup>H-NMR spectral data show the possibility to determine the type of feeding of Iberian pigs by using this information.

**Keywords.** Iberian pig – Fat – PCA – <sup>1</sup>H-NMR.

## <sup>1</sup>H-RMN comme outil pour déterminer le type d'alimentation des porcs Ibériques

**Résumé.** Nourrir les animaux avec des aliments composés pour obtenir des carcasses dont les caractéristiques soient semblables à celles des animaux recevant exclusivement une alimentation de type «montanera», nécessite de rechercher des techniques qui permettent de différencier et de reconnaître le type de nourriture qu'ont reçue les animaux. La Résonance Magnétique Nucléaire (<sup>1</sup>H-RMN) est une de ces techniques. Des échantillons de graisse ont été prélevés dans la croupe (à 10 cm de l'insertion de la queue) de porcs Torbiscal purs engraisés selon le mode «montanera» (lot P1, n = 44) ou recevant un aliment composé (lot B1, n = 16). Des échantillons similaires ont été prélevés sur des porcs Ibériques purs de différents types génétiques engraisés selon le mode «montanera» (lot B2, n = 10) ou recevant des aliments spéciaux (lot P2, n = 10). Des spectres <sup>1</sup>H-RMN de ces échantillons de graisse ont été obtenus selon les protocoles d'analyse de l'Unité de RMN du Service Central d'Appui à la Recherche (SCAI), de l'Université de Cordoue. L'analyse en composantes principales (ACP) des données <sup>1</sup>H-RMN montre la possibilité de déterminer le régime alimentaire des porcs de race Ibérique à partir uniquement de ces informations spectroscopiques.

**Mots-clés.** Porc Ibérique – Graisse – PCA – <sup>1</sup>H-NMR.

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## I – Introduction

Pigs used for the production Iberian pork have traditionally eaten the natural resources that abound in the Spanish countryside: fodder, grains and mainly acorns, which have fallen from the *Quercus* trees (oak, cork oak, gall-oak, etc). This system of production (named *montanera*) means that animals fattened in this way produce high quality meat and therefore meat products. The limitation of acorn production on the one hand and the high product price on the other, have led to a search for alternative products to acorns, attempting recreate the characteristics of the animals fattened on acorns and fodder, although the quality of the meat is not as good as that of animals fattened on *montanera*. In order to distinguish which carcasses belong to animals that

have not been fattened on *montanera*, several analytical techniques have been developed to determine certain characteristic parameters for each feeding regime. Some of these parameters are very easy to determine, but have a high error rate, such as tactile sensation, slip temperature and iodine levels. Others, such as the determination of fatty acids, triglycerides or phospholipids, have led to a more accurate identification of the animals' feeding regime (De Pedro, 2001). However, the development of special commercial feeds that create lipids profiles in the animals similar to those provided by acorns, have cast doubt on the reliability of these techniques. Proton Nuclear Magnetic Resonance ( $^1\text{H-NMR}$ ) spectroscopy is one of the more powerful spectroscopic tools for the investigation of the chemistry and physical properties in samples. The use of  $^1\text{H-NMR}$  is becoming universal for a wide range of fields including biochemical, agricultural, medical, materials, chemical, industrial, environmental, and pharmaceutical (Alan and Alan, 2005; Larsen et al., 2006; Hong-Seok et al., 2009; Alonso-Salces et al., 2010). One of the benefits of  $^1\text{H-NMR}$  spectroscopy is the ability to probe complex systems without necessarily requiring a separation of individual components prior to analysis. With the continued development of  $^1\text{H-NMR}$  spectroscopy as an analysis tool, the size and complexity of NMR data sets make them more difficult to analyze simply through operator interaction (Alan and Alan, 2005). Multivariate methods like Principal Components Analysis (PCA) are routinely utilized in other forms of spectroscopy for the analysis of complex mixtures. The use of chemometrics in NMR is more limited, but has quickly become an important tool for the NMR spectroscopist (Alan and Alan, 2005). Hence the aim of this study is to assess how the feeding regime given to Iberian pigs affects their  $^1\text{H-NMR}$  spectra and hence the possibility of using this technique to identify the animals' feeding regime, and therefore, the quality of their carcasses and their products.

## II – Materials and methods

For this study, four batches of Iberian pigs were used. Two batches were fattened at the Dehesón del Encinar Research Centre, which belongs to the Department of Agriculture of Castilla La Mancha; one of them (B1; n=43 pigs) was fed exclusively on pastureland and acorns (the production of acorns was somewhat scarce), whereas the other batch (P1; n=15 pigs) was fed on commercial feed; in both cases, the fattening period was 115 days. The other two batches of animals were part of a genetic study carried out by AECERIBER in two areas of pastureland in Badajoz. One of them (B2; n=9 pigs) was fattened exclusively on *montanera* for 104 days, in which the production of acorns was abundant; the other batch (P2; n=10 pigs) was only fed commercial feed. This feed was special since one of the raw materials used was high-oleic sunflower flour, in order to product high levels of this fatty acid in the animals' subcutaneous fat. Once the animals were slaughtered, a sample of subcutaneous fat was taken from the animals' hindquarters. The sample contained skin, fat between the skin and the lean meat and a little lean meat. A liquid fat sample were extracted from each subcutaneous fat sample by using a microwave oven following the methodology explained by De Pedro et al., (1996). The  $^1\text{H-NMR}$  spectra of liquid fat samples were obtained according to routine methods at the NMR Unit of the Central Service for Reseach Support (SCAI) of the University of Cordoba.

Each liquid fat sample was dissolved in 1 ml of deuterated chloroform and placed in a 5 mm NMR tube. The  $^1\text{H-NMR}$  experiments were performed on a Bruker (Rheinstetten, Germany) Avance 400 WB spectrometer. The spectra were recorded using a 6.5  $\mu\text{s}$  pulse, an acquisition time of 4.0 s (24k data points) and a total recycling time of 3.0 s, a spectral width of 3000 Hz (7.6 ppm), 16 scans. Prior to Fourier transformation, the free induction decays (FIDs) were zero-filled to 32k and a 0.3 Hz line-broadening factor was applied. The chemical shifts are expressed in  $\delta$  scale (ppm), referenced to the residual signal of chloroform (7.24 ppm). XWINNMR were used to perform the processing of the spectra.

The normalized spectral data were analyzed by multivariate technique like PCA with the statistical software package Unscrambler 9.2 (Camo Process AS, Oslo, Norway, 2005).

### III – Results

$^1\text{H}$ -NMR spectra of the 80 liquid Iberian pig fat samples were recorded. Figure 1 shows the full  $^1\text{H}$ -NMR spectral range used in this work (0.5-6.0 ppm). According to this figure, a wide range of highly specific information is obtained by  $^1\text{H}$ -NMR spectroscopy. The spectra include the chemical shifts of the  $^1\text{H}$  signals of the different functional groups of major (triglycerides) and minor components of Iberian pig fat. Figure 2 show the score plot of the sample scores in the space defined by the two first principal components (PC1 and PC2). It can be seen that samples were grouped according to their feeding regime but some clusters were partially overlapped. It occurs with batches fed with similar type of feeding: with acorn (B1 and B2) or with commercial feedcompound (P1 and P2). However, there are more differences between batches fed with different type of feeding (B1 or B2 versus P1 or P2). To confirm these results it is suggested to test a supervised algorithm like SIMCA or Discriminant Analysis to classify these spectra from animals with different feeding. Therefore, it can be concluded that  $^1\text{H}$ -NMR spectroscopy could be a useful technique to verify the animals' feeding regime during the final fattening stage.

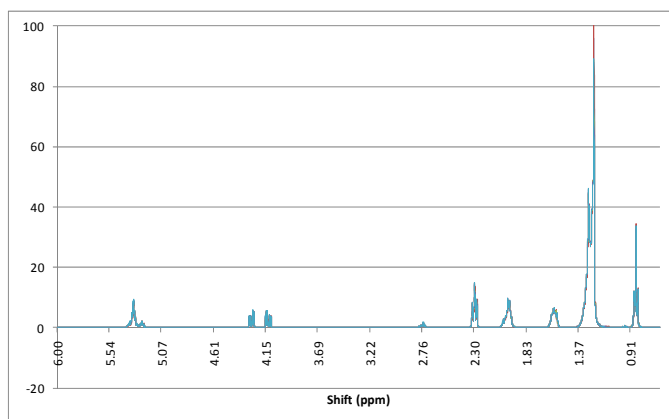


Fig. 1.  $^1\text{H}$  NMR spectra of Iberian pig fat.

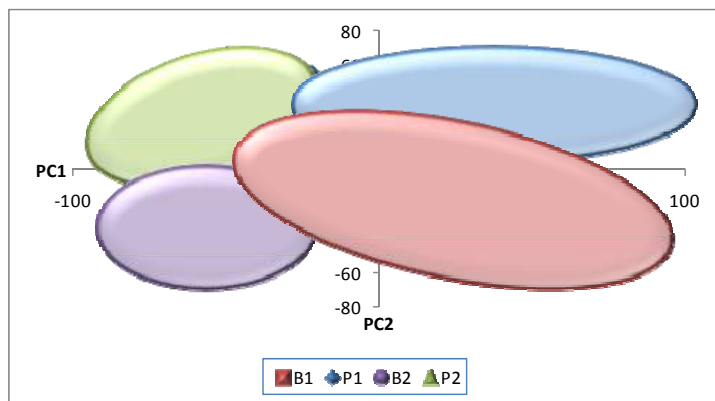


Fig. 2. Score plot.

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# Acidic composition of fresh and dry-cured lard in pigs of Apulo-Calabrese (Calabrese) and Casertana ancient autochthonous genetic types (AAGT)

D. Matassino\*, G. Gigante\*, M. Grasso\*, L. Rillo\*, G. Varricchio\* and A. Di Luccia\*\*

\*Consortium for Experimentation, Dissemination and Application of Innovative Biotechniques (ConSDABI), Italian Sub National Focal Point FAO (Sub NFP.I-FAO) (Mediterranean biodiversity), Centre of Omic Science for Nutritional Quality and Excellence (Italy)

\*\*Department of Food Science, Agricultural Faculty, University of Foggia (Italy)

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**Abstract.** In the last fifteen years the interest in conjugated linoleic acid isomers (CLA) has increased in relation to their effect on the 'nutritional', 'extranutritional' and 'healthy' for humans. This contribute aims to use the subcutaneous adipose tissue ('lard'), 'fresh' and 'dry-cured' as a means of discriminating racial origin of 'lard'. For this aim, 20 subjects of pig ancient autochthonous genetic types (AAGT) [10 of 'Casertana' (CT) and 10 of 'Apulo Calabrese' ('Calabrese') (CL)], reared at the experimental Farm of ConSDABI Sub-NFP.I – FAO and fed a specially formulated feed not supplemented with linoleic acid (LA) and CLA, were used. The quantitative profile showed an interesting content of CLA, a significant variability depending on the genetic type and product group. CL showed a significantly higher content of CLA and linoleic acid on both 'fresh' and 'dry cured'. In conclusion it is considered that the lard ('fresh' and 'dry-cured') has to be re-evaluated to improve human health.

**Keywords.** Casertana – Calabrese – CLA – Ancient autochthonous genetic type – Pig.

**Composition en acides gras du lard, frais et séché, des types génétiques autochtones Apulo Calabrese et Casertana**

**Résumé.** Au cours des quinze dernières années, l'intérêt concernant les isomères d'acide linoléique (CLA) a augmenté en lien avec leur effet sur les propriétés 'nutritionnelles', 'extranutritionnelles' et 'santé' pour l'homme. Cette contribution vise à utiliser le gras sous-cutané ('lard') 'frais' et 'séché' pour discriminer l'origine raciale du 'lard'. À cette fin, on a utilisé 20 sujets, 10 Calabrese et 10 Casertana élevés chez le ConSDABI et engraisés avec un aliment spécialement formulé non supplémenté en acide linoléique (LA) et CLA. Le profil quantitatif a montré une teneur intéressante en CLA, variable de façon significative en fonction du type génétique et du groupe de produits, qui a trait à l'étude de la technologie commerciale dans le type génétique. Le CL met en évidence une teneur significativement plus élevée en CLA et en acide linoléique soit sur le 'frais' soit sur le 'séché'. En conclusion on doit fortement réévaluer le lard ('frais' et 'séché') pour la santé de l'homme.

**Mots-clés:** Acides gras – Casertana – Calabrese – CLA – Type génétique autochtone ancien – Porc.

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## I – Introduction

Linoleic acid in its conjugated form, so called CLA, was detected for the first time in the lipidic component of milk (Pariza *et al.*, 2001) giving rise to a considerable interest for its documented biological effects (Matassino *et al.*, 2006; Secchiari, 2006). The biological effects, as demonstrated in a wide range of animal models, are manifested in the following activities: antiatherogenic, antioxidant, anticancer, antibacterial, antiadipogenic (Lin *et al.*, 1995); protection against diabetes (Belury *et al.*, 2003); promoter of growth factors and



immunomodulator (Hwang, 2000 ; Bassaganya-Riera *et al.*, 2001, Corino *et al.*, 2003). In addition to these biological effects, CLA have the ability to limit the oxidation of polyunsaturated fatty acids (PUFA) in food, reducing the phenomenon of rancidity and thus positively influencing their shelf life (Corino *et al.*, 2003).

As it is known, the lipidic profile and, in particular, the content of CLA, are strongly influenced by feed regimen of the animal; even in pigs the influence of feed regimen on the qualitative-quantitative composition of the lipidic fraction was demonstrated (Dugan *et al.*, 1997).

With this contribute we want to investigate fatty acid composition and the content of CLA in subcutaneous adipose tissue ('lard') in 'Apulo Calabrese' ('Calabrese') (CL) and 'Casertana' (CT) ancient autochthonous genetic types (AAGT) with the intent to detect differential biomarkers.

## II – Materials and methods

The acidic fraction was extracted by the method of Folch from 40 samples of, 'fresh' and 'dry-cured' 'lard' obtained from 20 pigs (10 CT and 10 CL) raised in the same farming conditions at the experimental Farm of ConSDABI SUB NFP.I – FAO. The subsequent transesterification of fatty acids linked to glycerol was obtained by methylation of themselves with methanol and, as catalyst, potassium hydroxide (KOH). The methyl esters of fatty acids were then separated by gas chromatography with FID (flame ionization detector) and on-column injector, equipped with CP-Sil 88 column (100 m, 0.25 mm) to better separation. Fatty acid content was expressed as a percentage of total fatty acids revealed. For the assessment of the 'nutritional' value of 'lard', atherogenic (AI) and thrombogenic (TI) indexes (Ulbricht and Southgaten, 1991) were also calculated.

## III – Results

### 1. Acidic profile

The comparison in saturated (SFA) and unsaturated (UFA) fatty acid of 'fresh' and 'dry-cured' 'lard' from two AAGT's CL and CT is reported in Table 1.

**Table 1. Fresh and dry-cured lard: percentage average content of UFA and SFA and significance of the difference between the two AAGTs**

Fatty acids	Fresh lard				Dry-cured lard			
	TGAA		$\Delta$ (CL-CT)		TGAA		$\Delta$ (CL-CT)	
	CL	CT	Value %	Significance	CL	CT	Value %	Significance
SFA	26.38	34.89	-8.5	0.002	29.30	35.56	-6.26	0.092
UFA	73.62	65.11	8.5	0.003	70.68	64.42	6.26	0.092

As shown in Table 1, the 'lard' of CL evidenced, regardless of the product merceological class ('fresh' or 'dry-cured'), a higher average percentage content of unsaturated fatty acids (UFA) and consequently a lower content in saturated fatty acids (SFA). The superiority of UFA in CL, compared to the CT, is highly significant for the 'fresh' 'lard' ( $P = 0.003$ ) and significant near to the critical limit for the 'dry-cured' 'lard'.

The apparent decrease of UFA in the 'dry-cured' 'lard', may be related to the enzymatic mechanism of lipases, that, in swine, during ripening, act on triglycerides giving priority to positions 3 and 1, that are usually occupied by UFA, causing a decrease of the latter in their esterified form.

In both AAGTs, UFA, which have greater impact on the total UFA content are oleic and linoleic acids. As shown in Table 2, only linoleic acid was significantly different between the two AAGTs both in the 'fresh' ( $P < 0.0001$ ) and 'dry-cured' ( $P = 0.001$ ).

The difference between the two AAGTs, was significant only for the following fatty acids: (i) in the 'fresh' 'lard': myristic acid ( $P < 0.0001$ ), heptadecenoic acid ( $P = 0.002$ ) and stearic acid ( $P = 0.008$ ); (ii) in the 'dry-cured' 'lard': myristic acid ( $P = 0.007$ ) and heptadecenoic acid ( $P = 0.02$ ).

The average CLA content (Table 2) was significantly higher in CL compared to CT in both 'fresh' ( $P = 0.002$ ) and 'dry-cured' 'lard' ( $P = 0.055$ )

**Table 2. Fresh and dry-cured lard: percentage average content of fatty acids and significance of the difference between the two AAGTs**

Fatty acids	Fresh lard				Dry-cured lard			
	AAGT		$\Delta(\text{CL-CT})$		AAGT		$\Delta(\text{CL-CT})$	
	CL	CT	%	Signif.	CL	CT	%	Signif.
Lauric	0.038	0.78	-0.040	0.538	0.072	0.087	-0.015	0.971
Myristic	0.876	1.420	-0.543	<0.0001	0.978	1.464	-0.486	0.007
Palmitic	18.014	22.654	-4.640	0.812	19.408	22.714	-3.306	0.066
Palmitolenic	2.179	2.056	0.123	0.499	1.916	1.990	-0.074	0.926
Heptadecanoic	0.211	0.233	-0.022	0.693	0.225	0.238	-0.013	0.956
Heptadecenoic	0.207	0.220	-0.013	0.002	0.216	0.220	-0.004	0.020
Stearic	7.242	10.509	-3.267	0.008	8.629	10.441	-1.812	0.207
Oleic	41.229	40.346	0.883	0.667	40.115	39.318	0.797	0.987
Cis-12-C-18:1	2.254	1.704	0.550	0.045	1.509	1.525	-0.016	0.809
Linoleic	22.592	16.819	5.773	<0.0001	21.987	17.824	4.162	0.001
$\gamma$ - Linolenic	0.182	0.194	-0.013	0.498	0.208	0.195	0.013	0.544
Linolenic	2.467	2.093	0.373	0.016	2.346	2.218	0.128	0.779
CLA	2.509	1.673	0.836	0.002	2.371	1.773	0.598	0.055

## 2. Atherogenic and thrombogenic indexes

The different fatty acid composition influenced AI and TI in the two genetic types. From Table 3 it is evident that CL pig, than CT, has a significantly lower value of both indices. The values measured for CL are in agreement with those reported by other authors (Matassino *et al.*, 2005) while CT values are slightly higher (Matassino *et al.*, 2008).

**Table 3. Fresh and dry-cured lard: atherogenic and thrombogenic indexes and significance between the two AAGTs**

Indices	Fresh lard				Dry-cured lard			
	AAGT		$\Delta(\text{CL-CT})$		AAGT		$\Delta(\text{CL-CT})$	
	CL	CT	Value %	Signif.	CL	CT	Value %	Signif.
Atherogenic	0.303	0.448	-0.145	0.006	0.342	0.453	-0.110	0.093
Thrombogenic	0.726	1.074	-0.348	0.001	0.837	1.077	-0.240	0.002

## IV – Conclusions

The research showed that some fatty acids can be used to discriminate, with probabilistic approach, both 'fresh' and 'dry-cured 'lard' provided by a given pig AAGT.

In addition, the 'lard' has an atherogenic and thrombogenic index lower in comparison with other animal and plant origin products discretizing many preconceptions about its inclusion in a human food regime.

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# Individual characterization of Iberian pig through NIRS technology: Implementation in Sierra de Sevilla, S.A.

A. Serrano\*, E. De Pedro\*\*, C. Morera\*\*\*, P. Vázquez\*\*\*, A. Moreno\*\*\*, A. Garrido-Varo\*\*, J.E. Guerrero\*\*, D.C. Pérez Marín\*\*, V.M. Fernández-Cabanás\*  
J. García-Olmo\* and N. Núñez-Sánchez\*

\*NIRSoluciones S.L., Avda. Gran Capitán 47, 14006 Córdoba (Spain)

\*\*Department of Animal Production, Faculty of Agricultural and Forestry Engineering  
University of Córdoba, Córdoba (Spain)

\*\*\*Sierra de Sevilla S.A., Sevilla (Spain)

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**Abstract.** The Iberian pig industry has established several quality control programs in order to determine and guarantee the pig feeding system, especially in the last period of growing. These programs consisted of on-farm Iberian pig inspection or fatty acids composition of subcutaneous tissue fat. The potential of NIRS technology in the classification of Iberian pig carcasses in the several commercial categories, according to their feeding system, has been reported by researchers at the University of Cordoba in the last years. However, the implementation of this technology at industrial level has not been yet as widespread as expected in comparison with other industries such as animal feed industry. This communication reports the results obtained in the first year of the project for the implementation of NIRS technology as quality control tool in the "Sierra de Sevilla" Company which elaborates meat products from Iberian pig. The use of NIRS technology as quality control tool allowed a fast, reliable and inexpensive characterization of each animal. Individual characterization is of great importance in "recebo" batches, where a large variability between animals was found. The good results obtained to date have given individual information of its animals to the Company providing a better quality control of their products and also monitoring the farm suppliers.

**Keywords.** Iberian pig – Agro food industry – NIRS technology – Quality control – Feeding system.

**Caractérisation individuelle du porc Ibérique par la technologie NIRS : Mise en œuvre dans l'entreprise "Sierra de Sevilla" S.A.**

**Résumé.** L'industrie du porc ibérique a établi des programmes de contrôle de qualité pour déterminer et veiller sur le système d'alimentation des porcs, en particulier dans la dernière période de croissance. Ces programmes comprennent l'inspection des animaux sur le terrain et l'analyse de la composition en acides gras des tissus adipeux sous-cutanés. Des années de recherche à l'Université de Cordoba ont montré le potentiel de la technologie NIRS pour la classification des carcasses de porc Ibérique en catégories commerciales, en fonction de leur système d'alimentation. Toutefois, en comparaison avec d'autres industries (par exemple, celle des aliments pour animaux), la mise en œuvre de cette technologie au niveau industriel pour le secteur du porc Ibérique est encore très limitée. Cette communication présente les résultats obtenus dans la première année du projet pour la mise en œuvre de la technologie NIRS comme outil de contrôle qualité dans l'entreprise de produits à base de viande provenant de porcs Ibériques «Sierra de Sevilla». L'utilisation de la technologie NIRS comme outil de contrôle de qualité a permis une caractérisation rapide, fiable et peu coûteuse de chaque animal. La caractérisation individuelle est d'une importance cruciale dans les groupes du «recebo», où une grande variabilité entre les animaux appartenant au même groupe a été trouvée. Les bons résultats obtenus à ce jour ont donné des informations individuelles sur les animaux de l'entreprise, en permettant un meilleur contrôle de la qualité de leurs produits et aussi un meilleur suivi des éleveurs fournisseurs.

**Mots-clés.** Porc Ibérique – Industrie alimentaire – Technologie NIRS – Contrôle de la qualité – Système d'alimentation.

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## I – Introduction

Iberian pig ham is one of the most expensive food products produced in Spain. Three commercial categories ("*Bellota* or Acorn", "*Recebo* or Acorn+compound feed" and "*Cebo* or compound feed") are established, according to the pig feeding system, especially during the final phase of the growing period. The classification of these expensive products is of huge interest for industries, authorities and consumers and, for this reason, great efforts have been performed to obtain efficient and reliable methods that allowed a right and unequivocal classification (De Pedro, 2001). In the last years, different methods were applied on the basis of the fatty acid profile in subcutaneous fat or on-farm inspections to classify pig batches in commercial categories (Garrido-Varo and De Pedro, 2007); however these methods provided global information of each batch but individual information of each pig is missing. On the other hand, consumers would have as much nutritional information as possible of these expensive food products and Iberian pig industry has to meet consumer's requirements in order to guarantee the quality of these products and thus satisfy the consumer requests.

The results obtained in the first year of the implementation of NIRS technology as quality control tool in "Sierra de Sevilla" Company allowed a fast, inexpensive and reliable characterization of each pig according to their fatty acid profile. As a result, "Sierra de Sevilla" could improve the maturation process of each piece as well as the quality assurance and traceability of each one of its food products.

## II – Materials and methods

A set of 250 Iberian pig subcutaneous fat samples, from 17 batches with different pig feeding system, were analysed by NIRS in Sierra de Sevilla (Sevilla, Spain) and official gas chromatography data were obtained from a certified laboratory.

NIR absorbance spectra were collected in reflectance mode ( $\log(1/R)$ ) using a FOSS-NIRSystems 5000 spectrometer equipped with a remote reflectance probe (FOSS, Hillerød, Denmark), in the wavelength region between 1100 and 2500 nm (every 2 nm). Each subcutaneous fat sample was taken from carcass of Iberian pigs in the sacrifice line, from the tail insertion area in the coxal region, the same area as that used by the Designation of Origin committees (ref). Subcutaneous fat samples were stored at  $-20^{\circ}\text{C}$  until analyses were performed. NIRS analysis was carried out by placing directly the thawed subcutaneous fat sample on the probe and spectra were collected and recorded using ISIScan™ Routine Analysis Software 3.5 version (Infrasoft International, Port Matilda, PA, USA).

Once the NIRS analysis was performed, adipose tissue sample was heated by microwave energy to melt the fat (De Pedro *et al.*, 1997). After removing the supernatant subcutaneous tissue, the appropriate liquid sample amount was taken to be sent to the laboratory, where the fatty acid composition was determined by gas chromatography, in accordance with the official method, to get the reference data.

NIR and reference data were used to develop modified partial least squares (MPLS) calibration equations by using WINISI software version 1.50 (FOSS NIRSystems Inc., Laurel, MD, USA) to predict fatty acid profile of the fat tissue samples (Williams & Sobering, 1996).

## III – Results and discussion

As animal with different feeding systems had been selected, individual reference data of the 250 samples showed a great variability, (i.e. oleic acid ranged 48 to 58%), and so enough variability has been included in the calibration model to provide reliable predicted NIRS data in all categories.

Table 1 and Table 2 show individual fatty acid profile (4 major fatty acids) of 12 Iberian pigs from a batch of 130 animals fed with grass and acorns (*bellota*) and another 12 Iberian pigs from a batch of 120 animals fed with grass, acorns and supplemented with compound feed (*recebo*), respectively.

**Table 1. Gas chromatography and NIRS predicted data of the 4 major fatty acids in a *bellota* batch**

Sample	Palmitic acid (C16:0)		Stearic acid (C18:0)		Oleic acid (C18:1)		Linoleic acid (C18:2)	
	LAB	NIR	LAB	NIR	LAB	NIR	LAB	NIR
1	21.5	21.4	9.2	9.7	54.9	54.2	8.6	8.5
2	20.5	20.7	9.0	9.3	55.9	55.3	8.9	8.8
3	19.6	19.9	9.7	9.4	56.1	56.2	9.9	8.8
4	19.9	19.7	8.3	8.5	56.6	56.3	9.2	9.2
5	19.3	19.5	8.7	9.2	57.1	56.4	9.5	9.2
6	19.2	19.9	8.3	8.5	57.5	56.8	9.2	9.3
7	18.7	19.4	8.3	9.5	57.7	57.5	9.8	9.1
8	19.1	19.6	8.3	8.1	57.9	57.3	9.2	8.9
9	19.0	19.7	7.6	8.0	57.9	56.6	9.6	9.7
10	19.0	18.9	8.7	8.2	57.9	57.6	8.9	9.2
11	18.8	18.9	7.7	7.9	58.3	57.9	9.6	9.5
12	19.2	19.4	8.4	8.0	58.5	58.3	8.4	8.8
...	...	...	...	...	...	...	...	...
Lab/NIR average (n=12)	19.5	19.8	8.5	8.7	57.2	56.7	9.2	9.1
Lab/NIR Variance (n=12)	0.7	0.5	0.4	0.4	1.1	1.2	0.1	0.1
Batch average (n=130)	19.3		8.1		56.8		9.8	

As can be seen in both Tables, slight differences between official gas chromatography method and NIRS predicted values were found in all samples (12 Iberian pig of each batch) as well as in average values, indicating the good prediction ability of the calibration model. The batch average value, usually employed in the Iberian pig industry to classify Iberian pigs and select the best maturation process of pieces (hams, shoulders and sausages) is also indicated at the bottom of the table.

Individual *versus* batch average analysis of Iberian pig subcutaneous tissue samples allowed detecting differences in the fatty acid profile of animals from a same batch, being these differences more marked in the *recebo* batches than in *bellota* ones. So, oleic and stearic acid values from 50.3 to 57.8 and 8.3 to 11.7, respectively, were found in the *recebo* batch (see Table 2) whereas in the *bellota* batch (see Table 1) these fatty acids only varied from 54.8 to 58.5 and from 7.6 to 9.7, respectively. In general, the magnitude of these differences between animals from a same batch of *bellota* or *recebo* could be observed by using statistical parameters to compare sample sets such as coefficient of variation, standard deviation or variance, among others.

Tables 1 and 2 show variance values found for each fatty acid in *bellota* and *recebo* batches, respectively. In all cases, variance values are higher in *recebo* than in *bellota*, owing to the greatest variability between animals from *recebo* batch.

**Table 2. Gas chromatography and NIRS predicted data of the 4 major fatty acids in a recebo batch.**

Sample	Palmitic acid (C16:0)		Stearic acid (C18:0)		Oleic acid (C18:1)		Linoleic acid (C18:2)	
	LAB	NIR	LAB	NIR	LAB	NIR	LAB	NIR
1	22.9	22.7	12.0	11.7	50.4	50.3	9.0	8.7
2	21.5	21.8	9.9	10.5	52.9	53.8	9.8	9.1
3	21.5	21.3	10.3	10.5	53.4	53.8	8.9	8.6
4	21.5	21.2	9.9	9.7	53.6	54.8	8.7	8.7
5	21.3	21.3	10.7	10.7	53.7	53.1	8.4	8.4
6	20.0	20.1	9.4	9.0	55.0	55.2	9.7	9.3
7	19.2	20.1	10.7	10.1	55.8	55.5	8.6	8.9
8	18.8	19.6	9.6	8.9	56.5	56.1	9.3	9.2
9	19.0	19.4	8.6	8.4	57.0	57.2	9.1	9.0
10	18.9	19.5	8.3	8.2	57.1	57.2	9.4	9.4
11	17.6	17.8	9.8	9.1	57.8	57.4	9.2	9.8
12	18.8	18.3	9.5	9.2	57.2	57.3	9.3	9.6
...	...	...	...	...	...	...	...	...
Lab/NIR average (r=12)	20.1	20.3	9.9	9.7	55.0	55.1	9.1	9.1
Lab/NIR variance (r=12)	1.4	1.2	0.6	0.6	2.2	2.0	0.2	0.2
Batch average (n=120)	19.4		9.4		55.2		9.8	

Taking into account that Iberian pig industries use the 4 major fatty acid values of the batch as quality control parameter for all the animals included in this batch, the implementation of NIRS technology in Sierra de Sevilla, S.A. for the individual characterization of each animal implies a step forward in the quality assurance and traceability of their food products.

## IV – Conclusions

(i) NIRS technology allows a fast, inexpensive and reliable characterization of each pig according to their fatty acid profile of the subcutaneous tissue, providing similar results as the official gas chromatography method.

(ii) Individual *versus* batch average analysis could detect differences between animals included in the same batch, being these differences more marked in the *recebo* batch.

(iii) The implementation of NIRS technology in Sierra de Sevilla S.A. enables individual quality control in their production process monitoring each animal at the reception zone and giving useful information for their optimal maturation process.

## Acknowledgments

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## Session 7

### Socio-economic aspects



# Parma PDO Ham in pork production chain and in Parma economy<sup>1</sup>

G. Bonazzi and M. Iotti

Department of Animal Health, University of Parma, Via del Taglio 10, 43100 Parma (PR) (Italy)

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**Abstract.** Prosciutto di Parma DOP (Parma PDO Ham) is the main product of Italian cold cuts and is produced in the province of Parma. The verification of compliance with its guidelines is implemented by Istituto Parma Quality (IPQ), that together with Istituto Nord Est Qualità (INEQ) operates a monitoring on compliance requirements of the most part of Italian PDO and PGI cold cuts production. In the production area there are 181 production plants where 9 millions of PDO Ham are produced every year and the 71% of factory and the 80% of Parma PDO Ham production is concentrated in four municipal districts in the foothills of the province of Parma. Among the major economic and financial aspects it is to consider production difficulties related to the duration of financial cycle because long maturing period and because of long terms of payment required by large retail chains. Analyzing a sample of firms it emerges the improving technology level and the management strategies with the aim to differentiate production (Parma Grosso ham production), and pre-sliced ham production. These strategies make it possible to generate positive cash flows to serve indebtedness and to reward equityholders.

**Keywords.** Parma PDO Ham – Pork production chain – Cash flow analysis – Parma economy.

## **Le Jambon de Parme AOP dans la chaîne de production porcine et dans l'économie de Parme**

**Résumé.** Le Prosciutto di Parma DOP (Jambon de Parme AOP), produit dans la province de Parme, est le produit le plus important de la charcuterie italienne. Le contrôle du respect du cahier des charges est assuré par l'Istituto Parma Qualità (IPQ), qui, avec l'Istituto Nord-Est Qualità (INEQ), surveille la conformité de l'origine d'une grande partie des AOP et IGP de la charcuterie italienne. Dans le domaine de la production il y a 181 installations qui produisent 9 millions de jambons AOP par an; sur le territoire des quatre municipalités de la province de Parme au pied de la colline sont situés 70% des établissements de fabrication et 80% de la production de Prosciutto di Parma DOP. Parmi les principales difficultés économiques et financières du secteur se trouve la durée du cycle de financement dû à la longue période de maturation et aux longs termes de paiement de la distribution. L'analyse de l'échantillon d'entreprises enquêtées montre la stratégie visant à améliorer la technologie et le processus de gestion, et aussi la variation de la production (production de jambon Parma Grosso), et la production de jambon pré-tranché. Ces stratégies permettent de générer des flux de trésorerie positifs pour payer la dette et de récompenser les détenteurs du capital-risque.

**Mots-clés.** Jambon de Parme AOP – Chaîne de production du porc – Flux de trésorerie – Économie de Parme.

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## **I – Introduction**

In the province of Parma there is an important production chain related to processing of pork meat to produce Prosciutto di Parma DOP (Parma PDO Ham): in the province could be considered others cold cuts productions as Culatello di Zibello DOP, Salame di Felino, Spalla Cotta di San Secondo Parmense, Coppa di Parma, and other productions as not marked hams, with less weight and lower period of maturing. In general, in Italy the pig sector is characterized

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<sup>1</sup>The work, although the result of a joint reflection, was prepared as follows: Giuseppe Bonazzi wrote paragraphs 1, 2, 4, 7, Mattia Iotti wrote paragraphs 3, 5, 6.

by the herd of swine heavy pig (weight of  $160 \pm 10$  kg) as particular animal that is bred to produce pigs to be processed for typical Italian cold cuts products, especially the typical ham (PDO ham); for these hams are used fresh legs of pigs born, raised and slaughtered in a defined area; these pigs must have quality characteristics defined by specific production rules. According to ISTAT data, the Italian production of pigs in 2009 consists of 12,922,000 animals, of which 8,707,362 are to produce PDO (IPQ-INEQ data) cold cuts. The slaughtered animals are 13,593,774 of which 671,774 came from foreign countries. The consumption of pork, in Italy, is 37.68 kg per capita in 2009 so the rate of self-supply in Italy was 68.9%. Genetic selection operates with two separate addresses: for the cold cuts production, it is used Italian Large White (LWI), Italian Landrace (LI) and Italian Duroc (DI) even for production of meat for butcher is used race Petrain (P). It was conducted an important activity for the conservation of native pig breeds, to protect genetic types as Cinta Senese, Mora Romagnola, Nero siciliano, Casertana, Apulo-Calabrese and Sarda. This selection has given the availability of animals with proper genetic definition and with clear breed characteristics for commercial enhancement. The breeding program for the heavy pig is based on the rearing of offspring of boars Italian Duroc and Large White sows for Italian Landrace. The selection objectives are to achieve an efficient conversion of feed, lower losses in livestock, no defects at slaughterhouse to increase production efficiency, plain carcass composition in order to improve meat quality for producing PDO hams and matured meats. The consumption of cured meats in Italy in 2009 was 1.1745 million tons; the ham is the first cold cuts for consumption in Italy, with 280.6 thousand tons, then we have cooked ham, with 275.8 thousand tonnes, Mortadella, with 173.9 thousand tons, and Salame, with 110.4 thousand tonnes. In the Italian cold cuts has an important role in the protected products: in Italy production of cold cuts recognized with PDO and PGI are 33, with a higher concentration in northern regions. In 2009 there were 8,680,611 slaughtered pigs certificates PDO, 17,361,222 available thigh for PDO production, 14,550,654 thighs started to PDO production, of which 9,429,462 for the PDO Prosciutto di Parma and 2,521,213 for the PDO Prosciutto San Daniele.

## II – The PDO chain

The European Community, considering that production, processing and distribution of agricultural products and foodstuffs play an important role in the Community, has implemented a strategy of diversification of farm production in order to achieve a better balance between supply and demand in the markets. Therefore, Reg. (EC) No 510/2006 of 20 March 2006 regulates the protection of geographical indications and designations of origin for agricultural products and foodstuffs. The verification of compliance with specifications (Article 11) shall be performed before selling the product on the market by one or more competent agency and / or one or more control agency within the meaning of art. 2 of Reg (EC) No 882/2004 that operates as a product certification institution. The certification agency of products act in compliance with European standard EN 45011 or ISO / IEC Guide 65. The Istituto Parma Qualità (IPQ), joined with the Istituto Nord Est Qualità (INEQ) has implemented a system that provides control and compliance requirements for origin of raw materials and production process upstream in the chain. In this system of rules and controls, the farms must put the firm code and the month of birth code on both legs, in order to have the slaughter of animals with at least nine months of life; in this way it is possible within thirty days after the birth of the pig to exclude animals born outside the territory of origin. The transfer of animals between farms should be documented so it could be easier the control issued by IPQ and INEQ, even having the pigs supply control. In 2008, the herds for PDO production were 4,819 in 11 regions of central and northern Italy. The highest concentration of farms is 1,936 farms in Lombardia, then we have Piemonte (970) and Emilia Romagna (926), so that 79.5% of herds is located in these three regions. For the distribution of pigs per genotype, on 2008 data, there is the prevalence of pigs from hybrid verro, representing 67.2% of the total, followed by of mixed blood of pigs boars of other breeds with 19.1% value.

The card is also sent to IPQ-INEQ that monitor and insert the data in the respective database. The slaughterhouse must fill out a document for each day of production with a list of all lots of animals received and the number of pigs slaughtered, codes of origin and origin. Moreover, the slaughterhouse puts on each thigh a stamp of approval attesting to the code compliance of origin, provenance and quality.

Slaughterhouses active in this system in Italy (year 2008) are 121 and have slaughtered more than 9 million pigs, the largest number of slaughterhouses has in Lombardia (38), Emilia Romagna (27) and Piemonte (18). All slaughterhouses are inspected at least once a year to control information in the slaughterhouses document that must be prepared every day and of which a sample is detected as for the incoming streams of live pigs and outflows of raw material. This procedure, associated to a selection, allows the exclusion of the fresh pork legs not suitable for production and to keep down the final index of non-compliance. It is to note that slaughtered pigs must be accompanied by a document indicating the code of the firm of origin, the number of pigs, the genetic type and destination.

The fresh raw material arrives at the cold cuts production firm with the stamp of identification and self-certification of slaughter, with a copy of the document issued by the slaughterhouse. The production firm, for each delivery, analyzes the conformity of raw material and mark in an official register all description and identification elements of the product. The raw material is then initiated for processing considering that during the whole process production firm has to ensure that "ID cards and descriptive production package"; IPQ and INEQ include these data in their database and check all the documents produced for each lot of production. It is the responsibility of IPQ and INEQ to check all the quality standards of the cured product by testing and verifying the minimum maturing period, the absence of morphological, technical and taste defects. After these procedures, PDO certification mark is applied to product and it could be marked on the product label.

### **III – Parma PDO Ham**

The Parma Ham is the most important production of Italian cold cuts sector. In 1963, 63 producers founded Parma Ham Consortium (Consorzio del Prosciutto di Parma) that today represents 164 processing firm. The Consorzio del Prosciutto di Parma manages and protects production rules, is responsible for economic policy management in the sector, supervises and ensures the respect of laws and regulations such as the protection of the name "Prosciutto di Parma" and its brand; Consorzio del Prosciutto di Parma is also the guide of trade policies and has the role to enhance the product, conducting advertising actions and fairs to assist firms.

Parma PDO Ham according to EEC Regulation 2081/92 (now Regulation EC 510/06) is produced observing production regulations issued by the Consorzio del Prosciutto di Parma, so Parma PDO Ham is obtained by processing thighs of heavy pig that must be older than 9 months of age, weighing over 150 kg that must be slaughtered "healthy, rested and fasted for at least 15 hours", as per the specification rules.

The pig must be reared in the territory of 10 regions of northern and central Italy but the production process must be done in one part of the province of Parma between the Via Emilia, at a distance of at least 5 km from here, from north, by the river Enza, to the east, and by the river Stirone, from the west. Towards the south we have a limit of production that is an altitude above sea level exceeding 900 meters. In addition to the Consorzio del Prosciutto di Parma, Istituto Parma Qualità (IPQ) conducts necessary checks on the ham as "third and independent part". IPQ performs the functions of control over the ham from 1998 and the activity is performed through unified services control together with Istituto Nord Est Qualità (INEQ) that is this agency involved in Prosciutto di San Daniele PDO control, under a system of division of control activities in the sector of the PDO.

As regards the breakdown by size classes of manufacturing, based on the hams established to production of Parma PDO Ham, there is a concentration of production in a small number of firms (Table 1); with a total production analyzed of 9,429,642 hams processed in 181 plants, with an average production of 52,096 hams per plant, 58.85% of production is concentrated in 25.41% manufacturing plants that are characterized for an annual production of more than 100,000 hams. Manufacturing plants with less than 25,000 hams per year produced 5.62% of the hams, involving 34.25% of plants; moreover it is to note that the plants up to 1,000 pieces per year of production are 8.84% of the total (0.08% of production), while the plants up to 10,000 pieces per year of production are the 22.10% of the total, with only 1,14% of the number of Parma Ham in 2009.

**Table 1. Production plant per rank size (2009)**

Production plant per rank size	Hams (no.)	Hams (%)	Production plants (no.)	Production plants (%)
0 – 1,000	7,652	0.08	16	8.84
1,001 – 10,000	136,870	1.45	24	13.26
10,001 – 25,000	384,970	4.08	22	12.15
25,001 – 50,000	2,067,900	21.93	53	29.28
50,001 – 75,000	1,282,433	13.60	20	11.05
75,001 – 100,000	2,161,614	22.92	25	13.81
100,001 – 200,000	2,316,990	24.57	17	9.39
> 200,000	1,071,033	11.36	4	2.21
Total	9,429,462	100.00%	181	100.00

Source: IPQ.

The consumption of Parma PDO Ham is done for 79% on domestic market and 21% in foreign markets; 2,046,495 hams are exported in 2009 (12,662 tonnes) with an estimated turnover of 181 million euro. In the foreign markets France and the United States prevail, but the European market accounts for 75.05% of exports, while the American continent is 21.30% of exports, of which 18.81% in the USA alone; exports to other states are modest, except Japan, that accounts for 4.28% of exports, approximately 87 thousand hams in 2008. In recent years there has been an increase in consumption of Parma ham sliced and packaged in boxes for sale in the refrigerated counter. During the period 2005/2009 the increase in the number of meats sliced was equal to 83.2%, from 627,344 to 1,149,574 and the relative packages production increased from 30.885 million of 2005 to 54.796 million of 2009. The slicing process performed in the production chain make easier the consumption process, particularly in foreign markets where the process of slicing made at the store or directly from the consumer is not always carried out with the necessary expertise, thus penalizing the sale to final consumer. With regard to market, Parma sliced ham, with a total production of 6,010,930 kg of food (1,865,490 kg for domestic consumption, up to 31.03%, and 4,145,440 kg, 68.97%, for export) confirms the presence of foreign demand for a product with a high level of service. Even with respect to exports of sliced Parma PDO Ham, there is a concentration of demand in some foreign markets, so the top 5 export destination markets are Britain, France, Belgium, Germany and the USA; these markets generate 79.41% exports (49.12% for the first two target markets). Even if the sliced product is concentrated in European market, that consumes 86.55% of exports, is also of relevance the USA market for the sliced product (8.83% of the export market for sliced), so other target markets are, on 2008 data, of marginal importance.

## IV – Prosciutto di Parma DOP in the local socio-economic system

The activities related to agriculture, processing industry and related services are important in the socio-economic system in the province of Parma. The food industry is the first industry in the province, with 2008 sales of 7,500 million euro, 36.6% of total industrial sales in the province, deriving € 973 million from exports. The turnover of the mechanical food plant industry is the third largest industrial sector of the province, behind the general mechanical industry, with a level of 2,200 million euro as per 10.7% of total industry turnover. The sectors of the food and plant for food, taken together, amounted to 47.3% of the industry turnover of the province.

Within the food sector with the highest turnover is the sector of pasta, bread, pastries, frozen foods and related products, as per including the presence of some large companies, that is, in 2008, 3,000 million euro turnover (40.0% of provincial revenues in the food industry and to 14.6% of industry turnover in general). The meat preserved industry generates 900 million euro turnover in 2008 (25.3% of sales in the food industry and 9.3% of industry sales in general). The province of Parma is characterized by a important meat processing activities so the local socio-economic system is therefore characterized by the presence of a large number of firms in processing pork meat for the production of cold cuts. As for the meat processing industry, based on data made available by the Registry of firms at the Chamber of Commerce of Parma, updated at August 2010, are operating 446 businesses and 143 local units of enterprises operating in the meat processing industry in the province of Parma (10.13 main activity code according to the classification ATECO 2007), as for a total of 589 units in the sector in the province of Parma. The firms involved in the sector give job to 4,399 staff employees and 322 independent operators, for a total of 4,721 person. The municipalities in the province with greater presence of these firms are Langhirano (123 companies, 41 local units, 1,140 staff employees and 72 independent operators), Lesignano de' Bagni (38 companies, 10 local units, 352 staff employees, 18 independent operators) Felino (35 companies, 17 local units, 634 staff employees, 44 independent operators), Sala Baganza (26 firms, 17 local units, 609 staff employees, 19 independent operators). The municipality of Parma has 38 companies, 10 local units, 366 staff employees and 21 independent operators.

As for Parma PDO Ham production for the year 2009, out of 181 factories in the municipality of Langhirano were processed 4,032,198 hams, representing 42.76% of the total, in 79 plants, equal to 43.65% of the total. In the first four areas of production (Langhirano, Lesignano de Bagni, Sala Baganza and Felino) it has been done the 80,28% of production (Table 2).

**Table 2. Production plant per municipality (2009)**

Municipality	Production plants (no.)	Production plants (%)	Hams (no.)	Hams (%)
Langhirano	79	43.65	4,032,198	42.76
Lesignano de' Bagni	22	12.15	1,480,612	15.70
Sala Baganza	16	8.84	1,134,104	12.03
Felino	12	6.63	922,619	9.78
Others	52	28.73	1,859,929	19.72
Total	181	100.00	9,429,462	100.00

Source: IPQ / Unione Parmense degli Industriali.

The development of the meat processing sector has encouraged the local employment and the creation of firms active in related production of meats, including craft processing services, production control activities, construction of facilities and machinery activities for meat processing, industrial activities and building, warehousing, as well as study and research



activities involved in meat processing process. In the province of Parma there are the University of Parma with the Faculty of Agriculture, Degree in Food Science and Technology, Veterinary Medicine, Economics and Engineering, particularly focusing on applied research in the meat processing industry. There are also the Experimental Station for the Food processing and preservation (Stazione Sperimentale Industria Conserve Alimentari, as SSICA) as a public applied research center with the purpose of scientific progress in the areas of the Italian canning fruits, vegetables, meats and fish. In Parma there is also the the European Food Safety Authority (EFSA) headquarter, as the European Union agency aiming to control food safety and other agencies involved in supply chain control as Istituto Parma Qualità (IPQ).

## **V – Economic and financial firm data in the Parma PDO Ham industry**

In the area of Parma, ham firms often have difficulties relating to the duration of the financial cycle, because the firms require large investments in start-up activity (Bonazzi *et al.*, 2007), for the acquisition of industrial buildings, plants and equipments. This necessity is inherent with the typical production of ham, which requires large volumes for processing and maturation, and then expanding the need for capital equipment. In addition, the cycle of maturation of the pork leg causes a further expansion of capital requirements in order to sustain the cycle of working capital. Finally, given the sales channel frequently used by firms in the sector for market access, namely large-scale distribution (GDO), there is an increase in average day extension of credits (even this aspect of the financial dynamic improve capital requirement for processing firms). This situation quite often expands the duration of financial cycle in which, even in the face of positive profitability, it is to note unsustainable situations in terms of generating cash flow (unlevered free cash flow) available for debt service; it then becomes important in the sector (Bonazzi *et al.*, 2007) a strategy to contain costs of production, even realizing investments in technologies that could reduce processing cost of raw materials (such as the activities of boning and greasing ham). In order to evaluate these investments, it is possible to use methods of assessment based on values deriving from accounting data (Roi, Roe, Rod), integrating these with assessment methodologies based on cash flow analysis (Npv, Irr, Pbpa), and methodologies to evaluate the business cycle as sustainable (Dscr, Adscr, Llcr). The Parma PDO compartment expresses case of differentiation strategy in production firms; In fact (Bonazzi *et al.*, 2008) there are firms able to diversify its production by having attention to raw material quality also working with a large size choice of pork legs. These firms have production of Parma PDO Ham Parma of high category level, weighing over 9.0 kg (24 maturing months). In this way, firms even with higher production costs for raw materials could be able to obtain on the market a selling price higher than production costs.

## **VI – The sample of firms**

The analysis of annual accounts (year 2008) of a sample of 40 firms in the area of Parma Ham is conducted by analyzing the balance sheet and income statement, on the basis of data made available by the local Chamber of Commerce. The analysis of profitability of business management is performed having the analysis of annual economic accounts (Ferrero, *et al.*, 2005). The annual accounts analysis considers the data presented in the table required by law (Andrei *et al.*, 2006), in patterns defined by the European Union, in accordance with the Fourth EU Directive, in order to make the different firms data comparable even if deriving from different European countries (Andrei *et al.*, 2006). It is therefore useful to analyze the annual accounts data according to reclassification exposure schemes (Iotti, 2009) that aggregate data to increase provided information level, marking additional capital and profit margins (Ceccacci *et al.*, 2008). The reclassification of the income statement using the value-added scheme (Bonazzi *et al.*, 2005) is:

$$(1) \quad S \pm \Delta I - C_e = VA - C_w = \text{EBITDA} - (D + A) = \text{EBIT} \pm I \pm V \pm E \pm T = \Pi$$

In (1) S is sales, I is the stock (inventory),  $C_e$  is external costs, VA is value added,  $C_w$  is the cost of labour, EBITDA is earnings before interest, taxes, amortization, and depreciation, D is depreciation, A is amortization, EBIT is earnings before interest and taxes, I is interest, V is revaluations and depreciation, E is extraordinary income or expenses, T is income tax,  $\Pi$  is profit. The reclassification of the balance sheet (Ceccacci *et al.*, 2008) is conducted according to liquidity level:

$$(2) \quad TA = \alpha WC_t + FA = \alpha WC_c + \alpha WC_i + \alpha WC_{ar} + FA$$

In (2) TA is total asset,  $\alpha WC_t$  is total investment in working capital,  $\alpha WC_i$  is working capital in inventories,  $\alpha WC_c$  is working capital in cash,  $\alpha WC_{ar}$  is working capital in account receivables, FA is fixed assets. Reclassification of balance sheet liabilities is conducted according to the origin of sources of capital:

$$(3) \quad TS = E + D = E + \beta WC_t + DF_s + DF_l$$

In (3) TS total liabilities and shareholders equity, E is equity, D is total debt,  $\beta WC_t$  is total working capital liabilities,  $DF_s$  is short-term liabilities, borrowings (due within 12 months),  $DF_l$  is a medium/long term financial debt (duration 12 months). The difference between  $\alpha WC_t$  and  $\beta WC_t$  is net working capital (NWC). The firm income considers the accrual basis (Andrei *et al.*, 2006) and expresses the moment of creation of value so the income statement is not dependent by the generation of cash flow from operations. It is therefore useful to consider the annual account because this document could be useful to analyze different sources of cash flows (Shireves *et al.*, 2000). To quantify the cash flows could be used an indirect approach, through the cash flows statement (Brealey *et al.*, 2003) in order to calculate unlevered free cash flow and free cash flow to equity:

$$(4) \quad \text{EBIT} + D + A \mp T = CF + \Delta^+ \text{NWC} = \text{OCF} + \Delta^+ \text{FA} = \text{UFCF} - \text{DS} = \text{FCFE}$$

In (4) CF is cash flow, OCF is operating cash flow, UFCF is unlevered free cash flow, FCFE is the cash flow available for shareholders (free cash flow). CF is EBIT corrected with costs that do not cause an outflow of money (D + A) and the impact of income taxes (T), OCF quantifies the absorption of net working capital (NWC) and has, in the case of Parma ham firms, a particular importance for the impact of investments in maturing inventories (hams); UFCF is determined as the sum of OCF and the absorption of capital resulting from investments in fixed assets (FA), as to say that UFCF is the cash flow available for debt service (DS, debt service, defined as  $DS = K + I$ , where K is the principal and I is the cost of debt, as interest). FCFE is the cash flow available for equityholders. Income analysis of 40 firms sample in the 2008 shows that S varies from a minimum of € 1,654,000 to a maximum of € 52,177,000, having that the average sales are € 13,093,000. In the sample, 32 firms generate profits ( $\Pi > 0$ ) and 8 generate losses ( $\Pi < 0$ ); The average ratio  $\Pi/S$  in the sample is 3.62% with a minimum net income/sales ratio of -17.46% and a maximum value of 13.21%, the average RoE of the sample is equal to 2.34%, with a minimum value of -27.09% and a maximum value of 23.83%. The average Roa of the sample is equal to 10.98% with a minimum value of -0.06% and a maximum value of 12.31%. The average Ros in the sample is equal to 12.18% with a minimum value of -0.10% and a maximum value of 17.91%. The average capital turnover in the sample is equal to 0.721, with a minimum value of 0.166 and a maximum value of 1.221, the sample firms are characterized by high capital intensity (as turnover <1). Average ICR is equal to 121.90% with a

minimum of -1.62% and a maximum of 560.51%. The analysis of the balance sheet shows an average TA of EUR 25.781 million to 2.861 minimum and € 68.214 maximum, average  $\alpha WC_t$  / TA is 63,21% and FA / TA è 36,39%;  $\alpha WC_i$  / TA is 41,96%. The other components of working capital ( $\alpha WC_c + \alpha WC_{ar}$ ) / TA, is 21.25%. The analysis around the sources of capital in the sample shows, on average, that L is 5.314 and DER is 4.314 having that in the sample, on average, firms use high debt level to finance their investments so that  $D = 4.314E$ . In order to quantify the sources of liquidity, analyzing the creation of cash flow, data show a situation where average CF is 1.512 million euro, no negative value of CF, OCF average is 0.211 million euro, 11 cases of OCF negative on 40 firms; the average UFCF is -1.430 million €, with 19 cases of negative UFCF on 40 firms; average FCFE is -1.582 million €, with 23 cases of negative FCFE on 40 firms. The analysis shows that the sample firms have difficulty in generating a positive cash flow to serve debt (UFCF) and to distribute dividends to shareholders (FCFE). In particular, working capital has a substantial effect on the absorption of liquidity (0 cases of negative CF and 11 cases of negative OCF).

## VII – Conclusions

Parma PDO Ham is the main product of cold cuts designation of origin in Italy at the level of production. The pork meat processing sector assumes importance in the economy of the province of Parma. The animals preserves sector shows that the turnover generated in the province of Parma, in 2008, is equal to 1,900 million euro, as 25.3% of sales in the food industry and 9.3% of the industry in general. In total of the Parma province, in respect of the meat processing industry, are involved 446 firms and others 143 local units, as for a total of 589 productive units, operate in the local industry, activating directly a total of 4,721 worker. Among the municipalities in the province, Langhirano, Lesignano de' Bagni, Sala Baganza and Felino could be considered territories with an increased level of activity. In those municipalities, with regard to the Parma PDO Ham, are concentrated 71.27% of firm and 80.28% of Parma PDO Ham production. Indeed, firms in the sector are characterized by significant fixed capital investment which should add significant capital equipment needed to support the cycle of maturing along with the long delays of payment required by large retail chains. So the analysis carried out on the sample of firms included in the study shows that there is difficulty in creating financial flows (UFCF) sufficient to support debt service (DS); in fact only firms with high size level of turnover, or production differentiation (as production of Parma Grosso) are able to generate not only profit for the shareholders, but also cash to ensure the sustainability of the business cycle, and the ability to distribute dividends or profits reinvestment to ensure discretionary investments (FCFE).

It emerges therefore that firms adopting strategies aiming to improve technological process to reduce production cost, even increasing profitability and positive cash flow generating capacity to support debt service and to reward equityholders. In addition, higher profit margins can be guaranteed by product differentiation (Parma Grosso) even having a greater focus on the goodwill of the product to the consumer by improving annexed services to the good, as the pre-slicing service that has been shown to have large spaces both on domestic and foreign markets.

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# The Nebrodi Black Pig: socio-economic analysis and perspectives (opportunities) of development

V. Aronica\*, A. Di Rosa\*\*\*\*, G. Spartà\*\*, V. Pruiti\*\*\*, A. Lazzara\*\*\*, M. Russo\*,  
A. Zumbo\*\*\*\* and V. Di Marco\*

\*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Area di Barcellona P.G. (Messina) (Italy)

\*\*Assessorato Agricoltura e Foreste, Servizi allo Sviluppo, Regione Sicilia (Italy)

\*\*\*Regione Sicilia, Distretto Nebrodi U.O. 40, Castell'Umberto (Messina) (Italy)

\*\*\*\*Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Università di Messina (Italy)

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**Abstract.** In recent years, breeding of Nebrodi Black Pigs is taking increasingly an own identity, abandoning that secondary role which saw it as income support and use of marginal areas of the farm. This push to production is due to increased attention and request that the consumer gives to local typical products of excellence. Although registered farms in ANAS are only 36, they are estimated at least a hundred. An exploratory research was conducted, then, on a sample of 36 farms. It provided the first socio-economic data (50% owned firms, average age of 49 years and 83% upper-middle level of education, etc.), farm size and territory (75% farms situated in the mountain, and 95% of those with wooded areas, etc.), end farm management (management of mating and birth, born pigs and period of weaning, feeding and veterinary care, etc.). Besides, results concerning the different farm productions, both farmers (annual production of pigs) and farmers-processors (type of processing and marketing), which represent the 33% of the farm sample under investigation and obtain a better economic performance, are reported. Finally, strategies that farmers hope to apply at the market for better utilization of local typical products and territory development are shown.

**Keywords.** Nebrodi Black Pig – Socio-economic analysis – Territory development.

## **Le Porc Noir des Nebrodi : Analyse socio-économique et perspectives de développement**

**Résumé.** Pendant ces dernières années, l'élevage des porcs noirs des Nebrodi a assumé de plus en plus sa propre identité, en abandonnant le rôle secondaire qui le voyait comme un soutien du revenu et comme une utilisation des zones marginales de l'exploitation. Ce moteur de la production est dû à une plus grande attention et une demande accrue des consommateurs concernant les produits d'excellence du terroir. Bien que les entreprises enregistrées dans l'Aire N.S.A. ne soient que de 36, on en a estimé au moins une centaine. Une enquête a donc été effectuée, sur un échantillon de 36 élevages qui ont fourni les premières données d'ordre socio-économique (50% des entreprises sont de la propriété de l'éleveur, qui a un âge de 49 ans en moyenne et parmi lesquels 83% ont un niveau d'instruction moyen-supérieur, etc.); de dimensionnement des exploitations et du territoire (75% des exploitations sont situées en montagnes et 95% de celles-ci en zones boisées, etc.), et de gestion de l'exploitation (gestion de l'accouplement et de la naissance, porcelets nés, période de sevrage, alimentation, soins vétérinaires, etc.). Le travail rapporte également les résultats concernant les différents produits de la ferme, à la fois pour les éleveurs (production annuelle de porcs) et pour les éleveurs-transformateurs (type de transformation et de commercialisation), qui représentent 33% de l'échantillon des entreprises sous enquête et obtiennent un meilleur résultat économique. Enfin, on montre les stratégies que les agriculteurs souhaiteraient appliquer sur le marché pour une meilleure utilisation des produits du terroir et pour le développement local.

**Mots-clés.** Porc Noir des Nebrodi – Analyse socio-économique – Développement local.

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## **I – Introduction**

The ancient civilization of peasants and shepherds of Nebrodi is reflected in numerous handicrafts. The food products find their highest expression in dairy products as the canestrato

cheese, the pecorino cheese, the provola cheese and the ricotta cheese and the famous cold meats made with meat from Nebrodi Black Pig. In recent decades the influence of globalization on animal production systems has led to a loss of biodiversity. From this awareness society has moved to a rediscovery of ancient production systems of the indigenous breeds considering the influences of the environment in which they have developed and adapted.

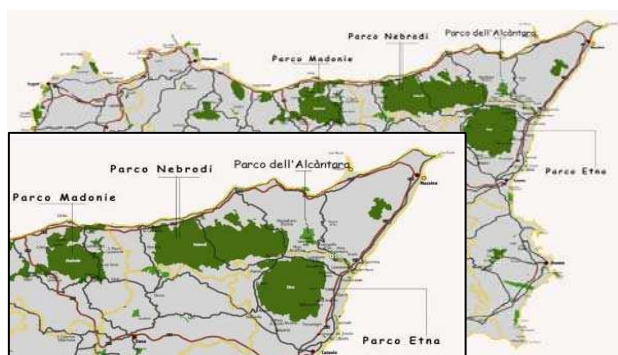
The Nebrodi Black Pig, added to the list of native species in danger of extinction, has very ancient origins and until recently was only a supplementary income business. Marginal areas, not otherwise used as pasture or arable areas, represented by the forests and the Mediterranean scrub, were the primary source of food. The pigs belonging to this breed live in the wild in the densest and most inaccessible woods of mountainous areas of Nebrodi, where they reproduce in their natural state, and because of their characteristics are well adapted to harsh environmental conditions of the territory, where other races would have difficulties to adapt.

The Nebrodi Park, established in 1993, represents, together with the Park of Etna, Alcantara and Madonie, the largest protected area of Sicily with its 85,587 ha, and includes the most important and extensive woodlands in Sicily (Fig. 1). Half of the woodland consists of coppice, and the other by tall trees. The most important tree species are represented by *Fagus sylvatica* (the extreme southern limit of diffusion area), *Quercus cerris* and *Quercus suber*. There are also some formations of *Quercus ilex*, *Taxus baccata*, *Ilex aquifolium* and important lacustrine and rocky environments.

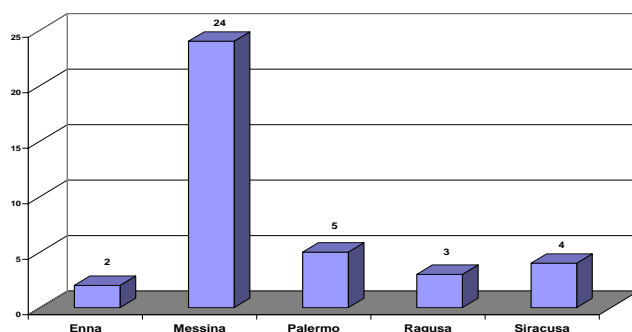
The livestock system of Nebrodi is characterized by mixed farming (cattle, sheep, goats, horses, pigs, etc.) bred in the wild, native species, inaccessible terrain, lack of roads, common grazing and transhumance in some periods of the year. In this context, livestock, agricultural and health management are difficult to implement. In addition, the "livestock Nebrodi system" is a natural and unique symbiosis of factors in which ancient indigenous germplasm, environment and type of nutrition are the basis for the production of excellent local products highly demanded by consumers.

The preciousness and uniqueness of the meat and the typical and natural products derived from the Nebrodi black pig, recognized by consumers, has led by about twenty years, some farmers to change the type of breeding black pigs. Favouring this rise in the market, and in order to increase the number of animals bred and to better organize the chain of production, they tried to optimize the outdoor rearing, using the forest areas at farms' disposal and intervening in feeding by additions during periods of shortage of natural foods. This type of farming evokes the concept of animal welfare and is in line with consumers' demand for those products derived from "stress free" animals. Although the black pig has a slow growth compared to other commercial breeds, the organoleptic characteristics of its meat and fat, used to produce processed meats such as hams and cold meats, are particularly appreciated by consumers.

The products of the forest, in fact, give the meat of these animals qualities of high value, and the increased interest and awareness of farmers to their genetic heritage, has meant that the black pig became a slow food presidium and for its fresh meat was asked the DOP award. Although we said above, the farms registered in the "National Swine Breeders Association" (ANAS), in 2009, appear to be about 40, but they are estimated to be at least a hundred in the territory of Nebrodi (Fig. 2). As shown in Fig. 2, the black pig farming is mainly located in the province of Messina (61.5%). We also remark that in the province of Benevento, there is the presence of a group of Nebrodi black pigs, exclusively for research purposes, belonging to the Consortium for the Testing, Spreading and Application of Innovative Bio-techniques (CONSDABI). The purpose of this survey is to provide information about socio-economic holdings of the Nebrodi Black Pig from a perspective of development of this zootechnic sector.



**Fig. 1. Parks in Sicily.**



**Fig. 2. Diffusion of farms of Nebrodi's Black Pig in Sicily.**

## II – Materials and methods

The survey involves 36 farms of Nebrodi's Black Pig located in municipalities of Sinagra, Mirtò, Militello Rosmarino, S.Fratello, Longi, Frazzanò, Ucria, Castell'Umberto, Tortorici, Alcara Li Fusi, Naso, Caronia, Floresta, Capizzi and S. Lucia del Mela. A large part of them are located within the protected area of the Nebrodi Park (Fig. 3).



**Fig. 3. Georeference of farms and area of Nebrodi Park.**



The recognition of socio-economic aspects of the sample involved was obtained through an interview to the Nebrodi black pig farmers.

The structured interview consists of the following areas of investigation:

- (i) Data about the breeder (the kind of farming management: property, tenancy and mixed; age and school level).
- (ii) Data on companies and territory (size, location and presence of wooded areas and / or pasture).
- (iii) Data about farm management (pig shelters, management of reproduction, age at the time of slaughter, feeding, medical support, production and marketing).
- (iv) Data about the needs / demands of the farmers (enhancement of products and technical assistance).

### III –Results

#### 1. Data on farmers

The details obtained show that the sample farms are 50% owned by the farmers themselves, that their average age is 45.3 years about and that 83% of them have a middle or upper school education level (Fig. 4). From these data it would seem that in this area, the age may be considered an indicator of greater experience and accountability, arousing an interest to undertake the breeding of Nebrodi Black Pigs, in people having a fairly good school level.

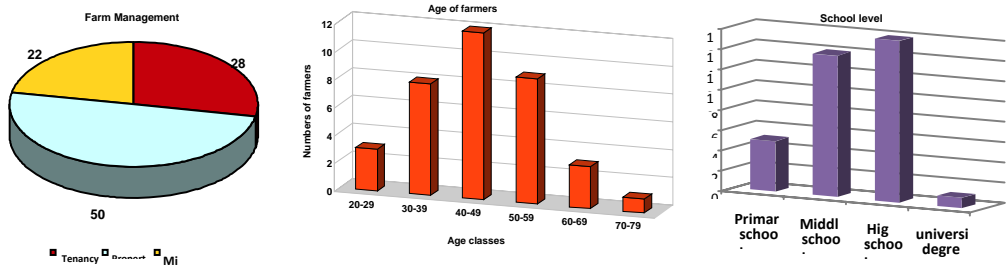


Fig. 4. Distribution of farms by management, age and schooling.

#### 2. Data on the farms and the territory

The analysis shows that 50% of farms are small dimension, localized in the mountains and characterized by the presence of woodland and pastures (Table 1).

The distribution of vegetation in the territory of Nebrodi, includes hills with oaks, chestnut trees and Mediterranean scrub, and mountain areas where, in addition to previous species, there are also turkey oak, beech and holm-oak. In grazing areas, the predominant species are composed largely of wooded and arable areas with prevalence of hay, oats, vetch, barley and field beans, that are used in the farm. The sample of 36 farms is formed as following: 10 farms have only forest areas, 3 farms only grazing areas and 23 farms both forest and grazing areas. Farms with forest areas in their territory are 33 (91.6%: 23 with pastures and 10 with just forest areas). In the 23 farms with woods and pastures has been analyzed the distribution of the prevalence of

these areas, noting that for most farms have forest areas (Table 2). Concerning the extension of the surface, small farms with wooded areas (73%) prevail. Finally, farms having pastures in their territory are 26 (23 with forests and 3 with pastures only). There are many farms having not very extensive pastures.

**Table 1. Distribution of farms by size, location and presence of forests and pastures.**

Farm size	%	Slope	%	Presence of wooded areas and/or pasture	%
1 - 25 ha	50	Mountain	75	Farm with wood and pasture	64
26 - 50 ha	22	Hill	16	Farm with only wood	28
51 - 75 ha	6	Plain	6	Farm with only pasture	8
>75	22				

**Table 2. Prevalence and size of farms with forests and pastures**

<b>Farms with mixed areas</b>	
Prevalence of forest	40%
Equity between forest and pasture	30%
Prevalence of pasture	6%
<b>Farms with wood for area classes</b>	
1-25 ha	73
> 25 ha	27
<b>Farms with pastures for area classes</b>	
1-25 ha	73
> 25 ha	27

### 3. Farm management

The results show that more than half of the farms provides farrowing sows with the presence of shelters or even with specific pig shelters called "Zimme", and in most cases there is not any programmed mating. The litter occurs mainly indoors in individual pens, but there are also farms in which the parturition occurs outside (Fig. 5).

The number of piglets born for each sow is about 9, but the mortality percentage observed is around 16%, especially in farms where the sows litter occurs outside. Usually piglets are weaned between the 35th and the 60th day, and the average reproductive life of sows is 5.4 years (Table 3).

The scatter plot (Fig. 6) shows that the modal value on the slaughter age is about 12 months, and that the weight of the pig is about 70-110 kg., depending on the type of farming and on the type of nutrition.

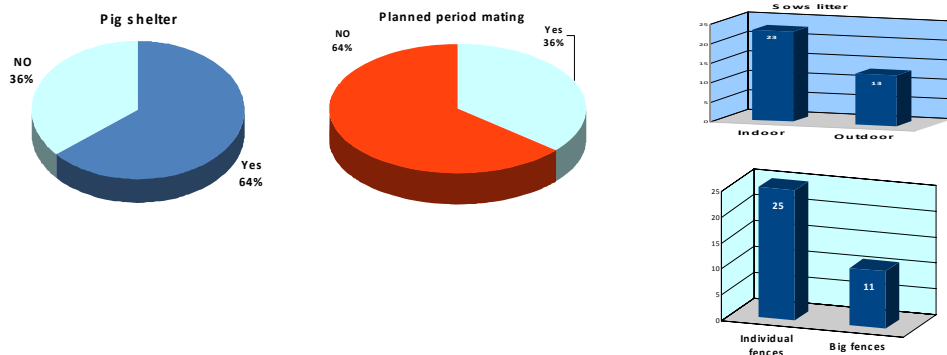


Fig. 7. Presence of pig shelters, mating and parturition

Table 3. Number of piglets born and mortality, age of weaning

Variable	Average	Mode	SD
Piglets born/sow	8.7	8	0.24
Pigletys death rate (%)	15.97	10	1.6
Sow slaughtering (years)	5.4	5	2.46

	No. of farms %	
Age of weaning		
Between 30 and 50 days	14	39
About 60 days	14	39
Between 90 and 120 days	5	14
No weaning	3	8

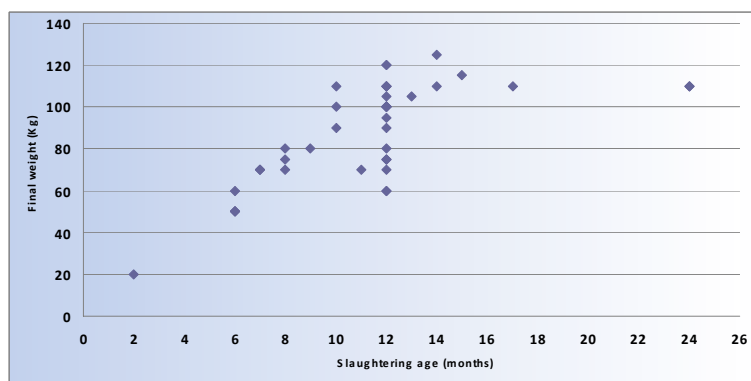


Fig. 6. Age of slaughter and final weight.

## 4. Feeding system

The composition and origin of food is varied and depends on the availability of the farm, on forest areas destined for the use of pasture, on different periods of the year, and on the years with higher or lower production of natural food in the forest. All farms having forest areas at their disposal allow to the animals to graze with the products of the forest and undergrowth. When no food is available for grazing (in winter with snow-covered land, or in summer), pigs are fed on cereals and cereal-based commercial feed. The natural diet of pasture consists mainly of acorns, chestnuts, and products of the Mediterranean scrub. When the integration is realized, it is composed by grains such as barley, germinated barley, field beans, bran, corn and grain. These are given either whole or ground, depending on availability of millstones in the farm. The commercial foods, specially formulated and balanced by category, are composed by mixed cereals and can be both ground and pellets. There is a small percentage of farms, which create their own composition of feed always based on field bean, barley, wheat, corn, carob, bran, supplemented with minerals.

## 5. Veterinary support

Only one farm uses vaccination against colibacillosis for mortality in piglets, and two carry out preventive vaccination against Aujeszky's disease.

The hardiness, the resistance against diseases and adverse climatic conditions, the state of extensive breeding allow almost all farms do not have serious health problems excluding skin, lung and gastrointestinal parasitic infestations, and rare infectious problems to respiratory system.

## 6. Production and marketing

The production activity of the farms is 47.5% only directed to breeding with sale of weaned piglets or pigs at the end of the finishing stage. While 8.3% of the farms carries out both breeding farm and processing.

The marketing is carried out following different models:

### (i) "Long-chain":

Breeder -> traders of animals -> slaughter -> butchers -> seller of fresh product at retail.

In this case the farmer does not address the handling of animals in first person.

Breeder -> slaughter -> butchers -> seller of fresh product at retail.

In this case the farmer addresses the handling of animals and sells them.

### (ii) "Medium chain":

Breeder - transformer -> slaughter <-> farmer - processor -> distribution.

In this case the transformer - farmer relies on other professional figures in the marketing and distribution.

### (iii) "Short chain":

Breeder - transformer -> slaughter <-> breeder - transformer - the seller

In this case the farmer, being transformer, becomes also a seller of his products.

However, some of these transformers are also sellers of their products in accommodation structures (restaurants, specialty shops with tasting, etc.)

With regard to the revenues obtained, the 69.4% represented by the long-medium chains' model, believes that it is not sufficient or just sufficient, while the 27.7%, represented by the short chains' model, is quite satisfied with own economic productivity.

## 7. Demands of farmers

*Strategies for better enhancement of products.* The near unanimity of the interviewed farmers think that is important the creation of a PDO and to be membership of a consortium for development. All respondents agree on the request of a better publicity, diffusion and knowledge of Nebrodi black pig, both at regional and national field. They remark also the need to inform the consumer about the organoleptic characteristics of the products and about high quality of the raw materials used, in order to improve the marketing through a widespread distribution.

*Technical assistance.* About 72% of farmers would like to have more assistance in the management of breeding, specifically concerning the quality and the quantity of food, the decrease of mortality, in addition to a better health care. 10% also requires technical assistance to improve and refine their processing techniques of the products.

## IV – Conclusions

This survey is a description of the of Nebrodi black pig breeding provided by the farmers themselves. The farming system used is mainly a semi-wild type by using the forest resources more in finishing period. The breeding of the Nebrodi Black Pig is a closed-cycle type. The most of respondents is only responsible of the breeding (88%) and relies to other professional figures to the processing and sale of products. It is clear the importance of a suitable health management, that would allow the increase in productivity and would ensure to obtain safe products for the protection of consumers' health, as required by the farmers themselves.

The awareness about the preciousness and uniqueness of the meat and products derived from natural and typical Nebrodi black pig, is associated with the firm conviction that the various phases of the production chain can not be approximately carried out. In this regard, the farmers interviewed emphasize this need due to their awareness about a lack of their organizational structure. In addition, the demand for environmental quality by citizens continues to grow and is becoming even more qualified and functional. In fact, the citizen, as a consumer, adapts its behaviour concerning rural tourism and gourmet or the purchase of agricultural food and products or handicrafts, on the bases of the information received about the environmental quality of territories. European consumers also require high quality food products made using production systems compatible with environmental, landscape and genetic resources conservation. In this regard, traditional foods produced by local farms and traditional practices, that can help to achieve these goals, can have significant effects on innovation and added value for rural societies. In conclusion, the breeding of Nebrodi black pig, is an opportunity for regional development through environmental protection, soil conservation and preservation of biodiversity. In summary, through an innovation, respectful of tradition, young generation can be encouraged limiting the unstoppable phenomenon of rural exodus.

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# Pig breeders in extensive systems based on local breeds: Stakes of their insertion in the development of the territories

F. Casabianca, A. Lauvie and O. Maestrini

INRA LRDE Corte, Corsica (France )

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**Abstract.** Within the Mediterranean QUBIC project centered on innovations developing biodiversity, we approach the points of view of the pig breeders in extensive systems based on local breeds, on their insertion in the dynamics of territorial development. A questionnaire including 4 parts and 12 questions was managed towards 123 farmers carrying on their activities in the 5 areas interested by the project: Italy (Tuscany, Sicily and Emilia-Romagna), Greece (Thessaly) and France (Corsica). Data collected relate to (i) environmental problems, (ii) visions of the territory and local insertion of activities, (iii) local breed seen as a factor of anchorage of the activities, and (iv) professional identity of the stockbreeder of local breed. We carried out an analysis centered on the link between breeds and territory, in order to identify possible points of blocking and levers in the projects of development of these breeds. We identify common features but also marked differences: (i) established systems (Cinta Senese in Tuscany) where a lot of newcomers show some lack of technical culture; (ii) stabilizing systems (Nustrale in Corsica, Nero Siciliano in Sicily) with deep anchorage of activities but weak professional organization; and (iii) emerging systems (Greek in Thessaly, Nero di Parma or Romagnola in Emilia-Romagna) not yet insured in their territorial insertion. Such comparative study allows supplying useful elements for future exchanges at Mediterranean level.

**Keywords.** Pig – Breeders – Local development – Extensive systems.

## **Les éleveurs porcins en systèmes extensifs basés sur des races locales. Enjeux de leur insertion dans le développement des territoires**

**Résumé.** Dans le projet méditerranéen QUBIC centré sur les innovations valorisant la biodiversité, nous approchons les points de vue des éleveurs porcins en systèmes extensifs basés sur des races locales, sur leur insertion dans le développement territorial. Un questionnaire comprenant 4 parties et 12 questions est administré auprès de 123 éleveurs dans les 5 régions du projet : Italie (Toscane, Sicile, Emilie-Romagne), Grèce (Thessalie) et France (Corse). Les données recueillies portent sur (i) les problèmes environnementaux, (ii) les visions du territoire et de l'insertion locale, (iii) la race locale comme ancrage des activités et (iv) l'identité professionnelle de l'éleveur. Nous effectuons une analyse centrée sur le rapport des races au territoire afin d'identifier des points de blocage éventuels et des leviers dans les projets de développement territorial. Une analyse comparative dégage des traits communs mais aussi des différences marquées : (i) des systèmes établis (Cinta Senese en Toscane), où un grand nombre de nouveaux éleveurs montrent un manque de culture technique ; (ii) des systèmes en cours de stabilisation (Nustrale en Corse ou Nero Siciliano en Sicile), avec un fort ancrage territorial mais des faiblesses organisationnelles ; et (iii) des systèmes émergents (Grec en Thessalie et Nero di Parma ou Romagnola en Emilie-Romagne), dont l'insertion territoriale demeure très incertaine. De tels travaux permettent de disposer d'éléments utiles pour de futurs échanges au niveau méditerranéen.

**Mots-clés.** Porc – Éleveurs – Développement local – Systèmes extensifs.

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## **I – Introduction**

Give the floor to the breeders' voice, such intention is not so frequent in our scientific communities (Flamant *et al.*, 1994). What about the breeders' point of view on some main questions we, as scientists, are studying?

The work takes place within the framework of the Mediterranean project centered on the innovations to develop biodiversity (QUBIC): Animal breeding - Quality Biodiversity Innovation Competitiveness. Is that biodiversity good for the future of the production units as Iberian pig is demonstrating (López-Bote, 1998)? Is the local breed an asset at territorial level?

A questionnaire including 4 parts and 38 questions was managed towards 123 farmers carrying on their activities in the 5 areas interested by the project: Italy (Tuscany, Sicily and Emilia-Romagna), Greece (Thessaly) and France (Corsica). In each area, there is one local breed, excepted in Emilia-Romagna where there are two. So, local pig breeds considered in our study are :

- ✓ For Italy, in Tuscany the Cinta senese breed, in Sicily the Nero Siciliano breed, in Emilia-Romagna both the Mora Romagnola breed and the Nero di Parma breed.
- ✓ For Greece, in Thessaly, the Greek breed.
- ✓ For France, in Corsica, the Nustrale breed.

We decided to focus only on some of these data in order to present a more accurate analysis on the specific linkage between local breed and territory according to the breeders' point of view.

## II – Material and methods

### 1. Breeders sampling

As reported in Table 1, the sample of breeders interviewed is quite important with 123 farmers in 5 of the areas of the project.

So these 6 local pig breeds are comparable as census of animals is quite reduced and breeds are still located in the native area.

**Table 1. Sample of breeders according to the country, the region and the breed**

Country	Italy	Italy	Italy	Greece	France
Region	Tuscany	Sicily	Emilia-Romagna	Thessaly	Corsica
Name of the breed	Cinta Senese	Nero Siciliano	Mora Romagnola and Nero di Parma	Greek	Nustrale
Number of interviewed breeders	35	36	19	10	23

### 2. Data recollected

A questionnaire including 4 parts and 38 questions was managed towards the 123 farmers on the following fields:

- Part 1: Environmental problems and the way the farmers face them.  
With a total of 8 questions, only 3 questions are presented.
- Part 2: Visions of the territory and the insertion of breeding activities in the local dynamics.  
A total of 10 questions and 2 questions presented.

- Part 3: The local breed seen as a factor of anchorage of the activities.  
On 10 questions, 5 questions are presented.

- Part 4: The professional identity of the stockbreeder of local breed in the evolutions of the sector.  
Among 10 questions, 3 questions are presented.

Results are expressed as % of YES according to the question.

### III – Results

According to answers, we carried out an analysis field by field. Such analysis is centered on the link between the breeds and their territory within these various located systems, in order to identify possible points of blocking and levers in the projects of territorial development of these breeds.

#### 1. Environmental problems

In the first part of our enquiry, we are dealing with environmental problems and the way the farmers face them. In particular, questions of pollution and sanitary risks of the animals in free range.

*Q 1 – Are you facing some environmental problems in your livestock farming? Which ones? (For example water pollution, soil erosion, plants and trees destruction, animal divagation)*

*Q 2 – What kinds of disease are present?*

*Q 3 – Due to extensive livestock system, are you obliged to consider wild animal diseases in your prevention plan?*

**Table 2. Answers from the breeders about environmental problems**

Answers from the breeders	Cinta Senese	Nero Siciliano	Mora Romagnola Nero di Parma	Greek	Nustrale
Q1	60	0	58	0	56
Q2	Pneumony, Parasites	Parasites	None	None	Aujeszkys
Q3	18	0	5	10	0

The grazing in forest seems to be commonly used and almost a great part of the pigs' life is outdoors. Nevertheless, a majority of the breeders are declaring no environmental problems, but we notice great differences between the various situations.

Parasites are clearly the main kind of disease as mentioned in the answers.

Soil erosion and some trees destruction are also evoked but breeders are complaining to the obligation to put nose ring for avoiding such problems.

And very few breeders have consciousness of the questions of contamination from the wild animals (especially from the wild boars).

As major issue, we can see that the great part of breeders is not aware of environmental problems.



## 2. Insertion within the territory

In the second part of our enquiry, we are looking for the visions of the territory expressed by the breeders and the insertion of their activities in the local dynamics. In particular, we try to approach organizational aspects of the breeds' management and of the product valorization in the territory and the factors of specificity of the extensive breeding in the offer of regional products.

*Q 4 – Do you think breed goodwill and territory linkages represent added value for you?*

*Q 5 – Is the breed well known in the territory and do you use the image of the breed and/or of the territory to sell your products?*

**Table 3. Answers from the breeders about their insertion within the territory**

Answers from the breeders	Cinta Senese	Nero Siciliano	Mora Romagnola Nero di Parma	Greek	Nustrale
Q4	100	100	95	60	100
Q5	95	100	74	70	74

This second part of enquiry is giving less contrast between the various situations.

In general, the local breed is conferring good insertion to the breeder and positive image for its activity.

In addition, some lack of recognition at social level is mentioned by breeders (excepted in Sicily) and the local breed is no sufficient to insure a good position in the local society.

In Corsica, breeders are also mentioning risks of confusion at market level, as products deriving from the local breed have no special identification at the moment.

As main issue, we can assume that the image of the breed is adding value on an effective way. And the breeders are using this image for commercial purposes.

## 3. The local breed as an asset

This third part of the enquiry deals with the local breed seen as a factor of anchorage of the activities. The adequacy of the animals to the systems of breeding and the collective management of the breed are the principal points as well as the question of the possible crossbreeding with other selected races.

*Q 6 - Do you feel that the local breed is insuring you a deep anchorage in the territory?*

*Q 7 - Do you consider the local breed as fully adapted to the local farming system?*

*Q 8 - Do you establish a link between the "good breeder" and the "beautiful animal"?*

*Q 9 - Have you effective practices of crossbreeding?*

In this third part, we can see a real consensus for the questions 6 and 7 as quite all the breeders are considering deep anchorage provided by the local breed and also a good adaptation of their animals to the local farming system.

For the questions 8 and 9, we notice great contrasts among the various situations.

For Cinta senese and Mora Romagnola / Nero di Parma, the crossbreeding is an ancient practice and breeders are considering it without any problem. A special name is given for crossbred animals in Emilia-Romagna. In Greece, as recovering of black pigs is still in progress,

crossbred animals are quite the normal situation. In the other areas, such as Sicily and Corsica, this practice is disappearing moving to the pure local breed as a main stream.

**Table 4. Answers from the breeders about the local breed and the crossbreeding**

Answers from the breeders	Cinta Senese	Nero Siciliano	Mora Romagnola and Nero di Parma	Greek	Nustrale
Q6	100	95	74	60	83
Q7	100	95	90	100	74
Q8	90	10	79	100	17
Q9	36	26	55	50	21
Main issues	Crossbreeding as ancient practice	Rejection of "good breeder"	Crossbreeding = "Borghigiano"	Crossbreeding considered as normal	Rejection of "good breeder"

For Corsican and Sicilian breeders, they reject the notion of "good breeder" and they consider that local breed animals can be diverse according to the breeders' preferences. They assume an internal diversity as breed collective identity.

As main issue, local breed insures deep anchorage to the territory and seems to be well adapted.

#### 4. The local breed as a professional identity basis

In the last part of interviews, we emphasize the professional identity of the stockbreeder of local breed in the evolutions of the sector. Information is in particular collected on the anteriority of the breeding activity, their vision of their trend compared with other types of breeding, as well as the pride to be a producer of local breed.

*Q 10 – Are you claiming to be considered as a distinguished activity compared to exogenous breed farmers?*

*Q 11 – Is the local breed a familial heritage transmitted by the previous generations (not something completely new)?*

*Q 12 – Are you feeling proud to be a local breed promoter?*

**Table 5. Answers from the breeders on the professional identity**

Answers from the breeders	Cinta Senese	Nero Siciliano	Mora Romagnola and Nero di Parma	Greek	Nustrale
Q10	100	100	84	30	30
Q11	14	100	10	40	87
Q12	100	95	68	70	74

Professional identity of the breeders seems to be a crucial point for their territorial insertion. As a majority of breeders is claiming to be differentiated from the exogenous breed farmers, the national situation must be taken into consideration: in Italy, the local pig breeds are giving a strong identity to the farmers.

We notice a lot of newcomers, in Cinta Senese, Mora Romagnola and Nero di Parma, and quite important in Greek. At the contrary, Corsican and Sicilian seems to have mainly familial heritage.

Quite all the breeders are associating pride and local breed activity. This point is very important to be underlined because the future of the local breeds could be facilitated by such a feeling.

As main issue, major part of breeders are proud, but are also claiming to be better recognized.

## IV – Discussion

A comparative analysis makes it possible to identify common features but also marked differences.

As common features, we can identify a low perception of environmental problems by quite all the breeders. Even if the risk of damage to the natural resources is obvious, it seems to be of minor interest in breeders' point of view. This point must become a priority for the extension services in order to avoid further disqualification of outdoor systems for pig production.

The local breed is conferring a deep anchorage to farmers and animals are considered as well adapted to the way of rearing. The image of the local breed is giving some advantage to the breeders and they use such image when marketing the products (Casabianca and Fallola, 1994). All the breeders seem to be proud to be local breed promoters but they are claiming for a better recognition at social level.

As main contrasts, we must distinguish the trajectory of each situation. The evolution of the breed is giving an orientation to the whole sector. We identify:

(i) Established systems such as *Cinta Senese* in Tuscany. With a PDO already recognized at national level, the breed seems to be clearly stabilized. But we notice a lot of newcomers attracted by the reputation of the breed and a lack of knowledge and technical culture in the management of outdoor systems.

(ii) Stabilizing systems as the *Nustrale* in Corsica or the *Nero Siciliano* in Sicily. Both of them are deeply rooted in local culture and applying for a PDO inducing some new questions (Lambert-Derkimba *et al.*, 2011). Breeders show a family heritage and the technical culture is enforced by generations.

(iii) Emerging systems as the *Greek pig* in Thessaly and the *Nero di Parma* or the *Mora Romagnola* in Emilia-Romagna. In such situations, professional organizations are still lacking to ensure the future of the breed.

This type of interviews analysis, mixing qualitative and quantitative data, is useful to identify key topics and stakes for the breeders themselves and for territorial management of local breeds. This type of analysis also allows wide comparative study at a Mediterranean scale.

Those key topics should be further studied thanks to a more qualitative analysis, using for instance semi structured interviews. Such a qualitative analysis should allow gathering breeders' discourse trying to minimize the influence on the orientation of the answers, without a pre-construction of the themes. It could allow understanding better the dynamics of territorial management for local breeds and the stakes on different territories, and complementing usefully this first comparative approach.

## V – Conclusion

According the differences we identified, we carried out a “gap analysis” centered on the link between the breeds and their territory within the various systems, in order to identify possible points of blocking and levers in the projects of territorial development of these breeds.

Some clear conclusions can be provided for each type of situation and breed.

*For Cinta Senese* : The situation is characterized by a lot of newcomers without experience, and some environmental problems not really taken into account by the PDO specification. The processed products should be protected in addition of fresh meat, because of risks of confusion at market level. It should be interesting to compare this situation to the Iberian pig situation where PDO is obtained for a long time.

*For Nero Siciliano and Nustrale* : Some similarities are observed between the two islands as this activity is rooted in the local culture. But farmers seem to face great difficulties to innovate and to organize. Environmental issues should be emphasized and PDO protection should be completed.

*For Mora Romagnola, Nero di Parma and Greek* : The three breeds are not really ensured till now. Breeders show a weak situation to be reinforced mainly at organizational level.

Such comparative studies based upon breeders view points are supplying useful elements for future exchanges at Mediterranean level.

## Acknowledgments

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# Iberian pig price cycle: Extremadura market

A.B. Cabezas, L. Romero, P. Lara and E.J. de Pedro

Department of Animal Production, ETSIAM, University of Cordoba, Cordoba (Spain)

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**Abstract.** This work analyses the pattern of Iberian pig prices at the Extremadura market, in order to find out about the cyclical fluctuations of Iberian pig prices, both for piglets and fattening animals in their different commercial categories: “Bellota”, “Recebo” and “Cebo”. The study looked at prices between 1989 and 2009, using the monthly average prices for those categories at the Extremadura market, and deflating them by the Producer Price Index. These deflated values enabled us to set 12-month Centered Moving Averages to offset the product’s seasonal nature. The prices went through three cycles during the research period. The cycle for the piglet category was seen to fluctuate between 41 and 86 months, with an average of 74.8, and the average duration of the cycle for fattening pigs of the “Cebo” category was found to be similar (between 43 and 87 months, with an average of 74.4 months). A seasonal pattern was observed in the prices for the piglet category, with minimum prices in summer and maximum prices in winter, whereas the fattening animals reached their minimum prices in autumn and maximum prices in winter.

**Keywords.** Iberian pig – Prices – Cycles.

## *Cycle de prix du porc Ibérique : Marché de l'Estrémadure*

**Résumé.** Cet article analyse l'évolution des cours du porc Ibérique dans les halles d'Extremadura, tant pour les porcelets que pour les porcs engraisés, correspondant respectivement aux différentes qualités commerciales de “Bellota”, “Recebo” et “Cebo”, afin de suivre les variations cycliques des cours. La période d'étude porte sur les années 1989 à 2009. Les cours mensuels moyens par catégorie aux halles d'Extremadura, corrigés en fonction de l'indice de prix industriels, ont été utilisés. Ces cours mensuels moyens corrigés ont été utilisés pour déterminer les moyennes mobiles centrées de 12 mois, afin d'éviter les variations saisonnières. Dans la période d'étude il y a trois cycles pour ces cours. Le cycle de la catégorie porcelets oscille entre 41 et 86 mois, avec une moyenne de 74,8. De même la longueur moyenne du cycle des porcs engraisés est de 43 à 87 mois, avec une moyenne de 74,4 mois. Une certaine saisonnalité des cours est observée pour les porcelets, avec des prix minimaux l'été et maximaux l'hiver; et pour les animaux engraisés, avec des valeurs minimales l'automne et maximales l'hiver.

**Mots-clés.** Porc Ibérique – Prix – Cycles.

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## I – Introduction

Pig production in Spain is mainly based on the intensive production of improved white-coat pigs. However, there is also an extensive production based on the Iberian pig, both pure and cross-bred with Duroc Jersey.

In recent decades, this indigenous race has undergone a period of decline, particularly between the 1960s and the 1980s. This has been caused by a number of factors, including zootechnical (lower prolificacy and growth rates than other improved races), social (diminishing agricultural population, leading to a crisis in traditional production methods), dietary (Spanish society has rejected meat that is high in fat), pathological (the Iberian pig was hit hard by African Swine Fever) and economic (other meat products, such as chicken or white pork, offered more competitive prices).

However, it has clearly been making a comeback in recent years. The decline that came about around 1993, as a result of lowering animal prices, was followed by a period of spectacular growth. Furthermore, the demand of Iberian pork products in the Spanish market was on the

rise, with ever increasing appreciation of their quality. Consumers were willing to pay for that quality, as they now had greater spending power.

The marketing of the Iberian pig is characteristically varied and complex. This is due to the range of categories throughout the production cycle, to the races used (pure or cross-bred) and to the different types of feeding used in the final fattening stage: “Bellota” or Montanera (mainly acorns), “Recebo” (mixture of acorns and fodder) and “Cebo” (animals are exclusively fed compound feed, either as part of an intensive or extensive system).

The marketing of the Iberian pig has also been influenced by factors such as the modernisation of farms, which are today managed more rationally and competitively than before; the extended slaughter season, due to improvements in ways of preparing and preserving pork products; and the rise in the standard of living.

Situations in which there is a surplus or deficit in animal production, a shortage or abundance of acorns and pasture, demand for cured products, etc. mean that prices rise and fall on a fairly regular basis, much as they do for other products, and more specifically, in the case of the white-coat pig that is intensively farmed.

The pattern of prices and production of the white pig has been studied on a number of occasions (Zorrilla, 1969; Weinberg and Sobrino, 1958; Caldentey, 1967, 1980; De Pedro *et al.*, 1984; Caldentey and de Haro, 1985; Riopérez and Paz, 1986; Agote, 1991; Muñoz, 1998 and Berrocal, 1999; Dawson P.J., 2009) to discover how it adapts to different production forecasts over relatively wide periods of time.

In the case of the Iberian pig, however, its seasonal production and the irregularity of information about its prices have made it difficult to carry out a market analysis of this type of livestock (Berrocal, 1999, Muñoz, 1998), as well as the fact that only relatively recently has a record been kept of the weekly or monthly prices of the different types of animals sold in farming markets, which makes it difficult to have a clear view of price behaviour.

These circumstances, and the importance of Iberian pig production in south-west Spain, have created an interest in finding out more about the evolution of Iberian pig prices in the main area of pasture production.

The objective of this paper is to analyse the cycle of Iberian pig at the Extremadura market, which will enable us to find out about its pattern and so have an idea about price behaviour.

## II – Material and methods

For this work we have used the average monthly prices for piglets and “Cebo” pigs at the Extremadura market, as these are the only categories of pig that have recorded prices all year round. The average prices were deflated with the Producer Price Index (PPI), with base year 2005, obtained from the [Spanish] National Statistics Institute, in order to counteract the effect of inflation in the period under analysis.

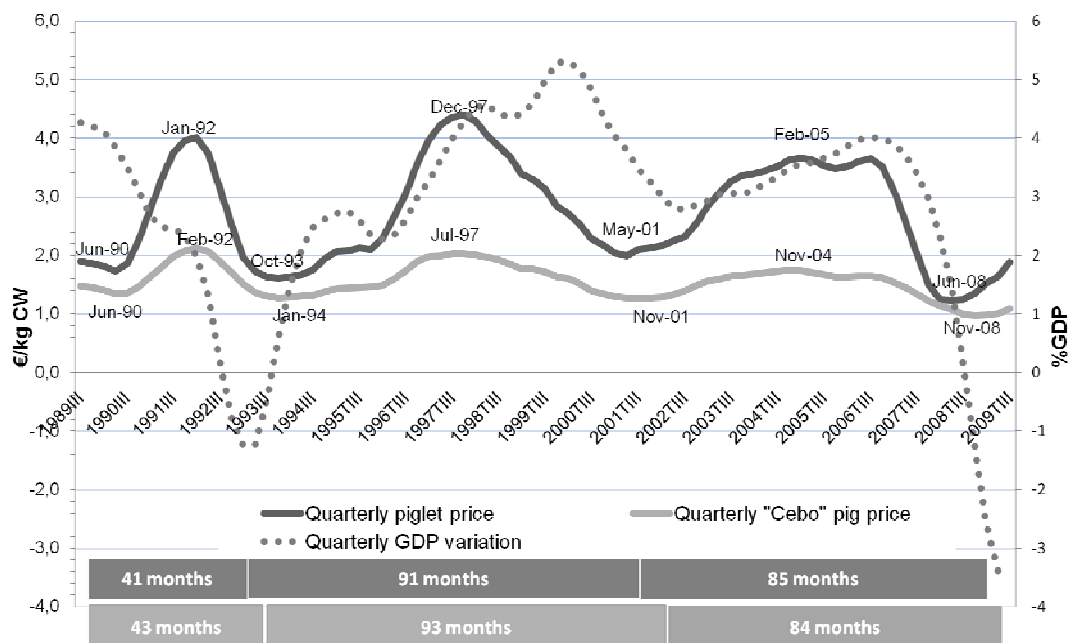
The prices’ seasonality was offset by means of the Centered Moving Averages of the monthly prices that was put forward by Caldentey (1980), applying the following formula:

$$X_t^* = 1/24 * (X_{t-6} + 2X_{t-5} + \dots + 2X_{t-1} + 2X_t + 2X_{t+1} + \dots + 2X_{t+5} + X_{t+6})$$

Where t represents the months considered.

### III – Results

Figure 1 shows the values of the centered moving averages for the piglet and “Cebo” pig prices at the Extremadura market, together with the pattern of the quarterly variations in GDP between 1989 and 2009. In this graph it can also be observed how both series of data behave in a similar way. With regard to the average duration of the two categories’ cycles, there are hardly any differences; in the case of piglets, this duration is 74.8 months, and in the case of “Cebo” pigs it is 74.4 months. In general, however, we observe no coincidence in the maximum and minimum prices for both series, and they also come about with no fixed pattern of differences. Espárrago *et al.* (1999), in a shorter period of time than in our study, also pointed out that the average duration of piglets and “Cebo” pigs was very similar, with an average cycle duration that was just 6 months shorter.



**Fig. 1.** Chart showing the centered moving averages for the quarterly deflated prices of piglets and Cebo pigs and the GDP variation between 1989 and 2009.

In Fig. 1 it is worth pointing out the size of the wave drawn by each cycle. We can clearly see here how, in the case of the piglets, these fluctuations are much more pronounced than in the case of the “Cebo” pigs, where the curve drawn by this series of data is much gentler, which could be due to the rigidity of the short-term supply of piglets, as described by De Pedro *et al.* (1984).

We can see how, for piglets, the price drops are very abrupt, whereas their price recoveries are much slower; however, if we look at the data for “Cebo” pigs, we observe that it is completely the opposite; this could be due to the different production process involved in each category. The need to sell piglets at a given weight would prevent them from being sold at a later point in time, whereas in the case of the “Cebo” pigs, the time at which they are slaughtered may be sooner or later, according to market prospects.



Furthermore, we would like to highlight the rapid fall in prices, both for piglets and “Cebo” pigs, in the period of time between January 1992 and October 1993, compared with the evolution that had come about in subsequent periods of time. We do not have enough information to know what cycles were like prior to June 1990, but in 1991, with the purpose of eradicating African Swine Fever (ASF) in Spain, ASF-“free”, “monitored” and “affected” zones were established ([Official State Gazette] BOE-A-1988-29631, 1988; BOE-A-1991-7267, 1991; BOE-A-1992-26539, 1992). Extremadura was in the affected zone, and was not declared a monitored zone until November 1993. It was not until a year later that it was finally declared an ASF-free zone. These limitations in the animal trade could be one of the causes of that heavy fall in prices in both markets, and the classification as a monitored zone in November 1993 could have been the reason behind the price recovery.

The standstill in 1995, when there were no longer any limitations on the trade of animals and Iberian pork products, coincided with periods of heavy drought and a halt in the GDP growth in Spain. Once this period had passed, the sector once again picked up the growth it had been undergoing in the previous cycle, even reaching higher values than before.

In the second and third cycles (January 1994-November 2001 and November 2001-November 2008, respectively) we can see that the price extension for “Cebo” pigs coincided with a period of high growth in the Spanish economy, which leads us to suppose that the extension of the second cycle was produced as a result of the high demand for animals by industrialists who were motivated by the good economic expectations of the time. This situation meant that the surplus supply that was produced in 1997 was not absorbed in full, as would be expected from looking at the cyclical behaviour of prices in preceding years. Instead, the surplus supply meant that, between December 2002 and October 2006, prices did not reach the maximum values observed in previous cycles, despite the fact that the positive economic situation in the country kept the “Cebo” pig prices high during that period.

## IV – Conclusions

The evolution of Iberian pig prices is conditioned by internal factors (census, production costs, acorn harvest, etc.) and external factors (the country's economic situation). In the period of time analysed here (1989-2009), both in the category of piglets and in the category of intensive “Cebo” pigs, 3 price cycles were produced with similar durations; the duration of the first cycle is approximately half of the other two, largely as a result of the programme to eradicate African Swine Fever, and of the country's economic situation.

It is important for price predictions in the Iberian pig market to know the evolution of the country's GDP, as well as other aspects such as the census and the situations in the farming year.

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# Technology Watch study of the Iberian pig

Y. Benítez Nieto\*, P. Escorsa Castells\*, E. Cruz Jiménez\*, E. Escorsa O'Callaghan\*

\*Iale Tecnología S.L., Barcelona (Spain)

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**Abstract.** The Mediterranean Pig is represented in Spain by a group of varieties, strains or ecological adaptations that make up the Iberian pig. We carried out a study on scientific publications in various strategic areas (genetics, nutrition, health, meat and products quality and traceability) related to the Iberian pig. The application of Technology Watch methodologies allows any industry to anticipate market changes and opportunities, understand and reduce the impact of new technologies, overcome access barriers, develop new business ideas, identify potential partners and minimize risk. Several relevant high quality information sources such as scientific publications have been selected using a high-impact database: FSTA (Food Science and Technology Abstract). The information processing required the use of advanced data mining tools (Analyzer Matheo Software). The main findings are reflected in a Technology Sector Map.

**Keywords.** Technology Watch – Iberian Pig – Technology Map.

## *Veille technologique pour l'étude du porc Ibérique*

**Résumé.** Le porc méditerranéen est représenté en Espagne par un ensemble de races et de variations génétiques différentes, issu d'adaptations écologiques, qui composent le porc Ibérique. Nous avons effectué une étude sur des publications scientifiques dans divers domaines stratégiques sur le porc Ibérique (génétique, nutrition, santé, qualité de la viande et des produits ainsi que traçabilité). L'application des méthodologies de veille technologique permet à l'industrie d'anticiper les changements du marché, d'identifier les opportunités, de comprendre et de réduire l'impact lié à l'arrivée de nouvelles technologies, de surmonter les obstacles bloquant l'accès à un projet, de développer de nouvelles pistes liées à l'investissement, d'identifier des partenaires potentiels et de minimiser les risques. De nombreuses données sont sélectionnées à partir de sources d'informations de haute qualité et de pertinence telles que les bases de publications. Nous avons utilisé une base de données spécialisée dénommée FSTA (Food Science and Technology Abstract). Le traitement de l'information nécessite l'utilisation d'outils de data mining avancés (Matheo Analyzer). Une information efficiente est obtenue grâce à l'analyse de cartes retraçant tous les secteurs technologiques du domaine recherché.

**Mots-clés.** Veille Technologique - Porc Ibérique – Carte de la technologie.

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## I – Introduction

The current challenge for the jamón *ibérico* (Iberian ham) sector is to provide a product with well defined and constant quality. It is therefore necessary to use the scientific and technological knowledge that has been so far generated and can be applied to serve this remarkable sector in Spain [1].

Technology Watch is the integrated and systematic effort of capturing, analyzing and accurately disseminating and pertinently exploiting all relevant technological and legal information that is necessary for the survival and growth of any institution. In today's knowledge economy, the access to information and its treatment is a prerequisite for the successful undertaking of R&D projects leading to new products, processes or services. The aim of the Technology Watch activities is to alert the company's decision makers of any scientific or technical innovation likely to modify their environment.

## II – Methodology

The methodology for this study initiates with the identification of keywords and the narrowing the scope of search. This allows for the retrieval of the relevant information from the selected database. Once a corpus of information is obtained, the analysis and synthesis of the retrieved information is carried out in accordance with the objectives of the study.

The selected database was Food Science and Technology Abstracts, FSTA [2], produced by the International Food Information Service (IFIS). FSTA contains over 500,000 references and covers all areas of food science, food technology, and human nutrition, including basic food science, biotechnology, toxicology, packaging and design. The data mining software Matheo Analyzer [3] was used for the processing of bibliographic records.

## III – Analysis of scientific publications

We performed a retrospective study on the research work that has been produced related to Iberian ham. The study covers scientific research published during the period 2000-2010 (August).

### 1. Scientific indicators

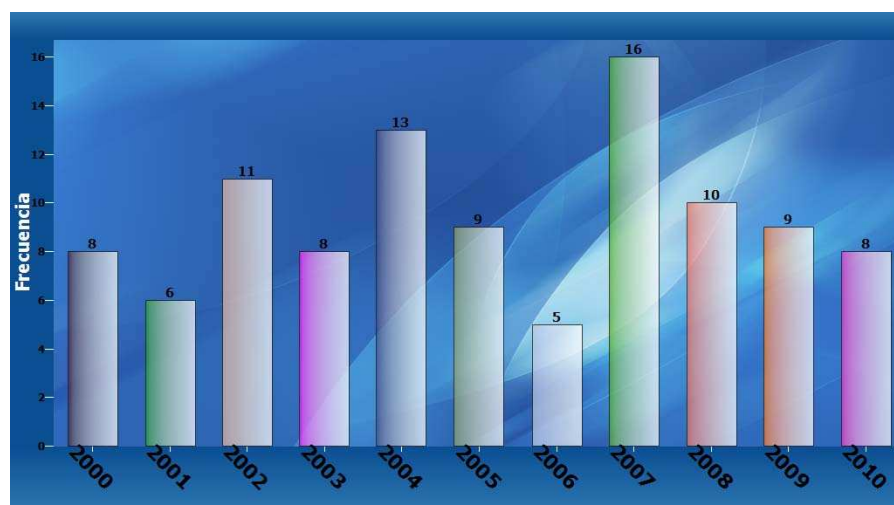


Fig. 1. Evolution of the number of scientific publications (2000-2010).

We obtained 103 scientific publications from FSTA database showing research results related to Iberian ham.

As it can be seen from Fig. 1, the scientific production presents, in general terms, an unstable behavior during the studied period.

Spanish scientific institutions lead the research in the studied area (Table 1); the University of Extremadura holds an absolute leadership, with approximately 59% of all publications retrieved.

Other European institutions that investigate these issues are: Royal Veterinary and Agricultural University and the University of Copenhagen (Denmark), INRA (France) and Fleischerzeugung und fuer Inst Vermarktung (Germany).

Table 2 shows a first group of scientific key terms of the studied area. A total of 251 subject headings describing the different publications research topics were retrieved.

**Table 1. Scientific institutions holders**

<b>Institutions</b>	<b>TOTAL</b>
Univ. Extremadura	61
Instituto de la Grasa (CSIC)	5
Univ. Complutense of Madrid	4
INIA	3
Univ. of Salamanca	3
IFA-CSIC	2
IMIDA	2
Univ. Zaragoza	2

**Table 2. Main scientific content**

<b>Subjects</b>	<b>TOTAL</b>
Fatty acids	22
Feeding	21
Ripening	14
Colour	14
Sensory properties	13
Fats animal	13
Genetics	12
Volatile compounds	11
Oxidation	10
Carcasses	9

More recent work on Feeding, for example, focuses on: (i) the Influence of a diet with probiotic bacteria on the lipid composition of Iberian pigs from different tissues; (ii) Individual phospholipid classes from Iberian pig meat as affected by diet (both from the University of Extremadura, 2010).

Table 3 lists topics of new interest related to the study of Iberian ham. Its only appearance in the studied period (2000-2010) is found within the last three years (2008-2010), according to the data contained in FSTA database. The novelty of these terms should be understood in the context of the Iberian ham as the studied subject.

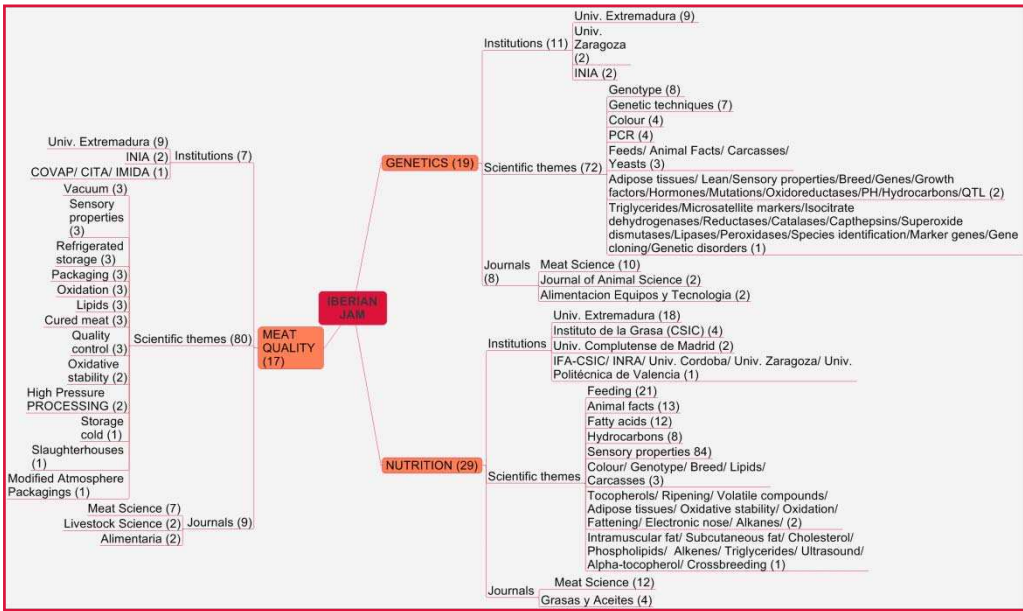
Research on Vacuum, for example, is focused on: (i) modified atmosphere packaging and vacuum packaging for long Period chilled storage of dry-cured Iberian ham; (ii) effect of pressure and holding time on color, protein and lipid oxidation of sliced dry-cured Iberian ham and loin during refrigerated storage; (iii) the effect of HHP treatment (600 MPa) on the oxidative stability of lipids and proteins of vacuum-packaged dry-cured Iberian ham and the Impact on the sensory characteristics of the product was investigated.

**Table 3. Emerging scientific content**

New topic	2008	2009	2010
Vacuum	0	1	2
Phospholipids	0	0	2
Ultrasound	1	0	0
Triacylglycerols	1	0	0
Swine livers	0	0	1
Subcutaneous fat	0	0	1
Polychlorinated biphenyls	0	0	1
Peptides	0	0	1
Nucleic acids	0	0	1

## 2. Technology Map

Figure 2 shows a technological map of the Iberian ham for three areas of special interest such as genetics, nutrition and meat quality.



**Fig. 2. Technology Map.**

## IV – Conclusions

The present Technology Watch study has identified key areas of research in the field of Iberian ham from 2000-2010. It enabled to observe the behavior of several selected indicators (evolution of publications, leading institutions, main scientific subjects as well as emerging areas of interest).

The University of Extremadura is the Spanish scientific institution leading research on this area, according to the studied period and the information obtained from the FSTA database.

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[3] **Matheo Software**, <http://www.matheo-software.com/>





# IBEDROCHES: Socio-economic impact of inter-company cooperation

R. García-Valverde, R. Linares, J. Gómez-Laguna, L. Fernández and M. Hernández

Centro de Investigación y Calidad Agroalimentaria del Valle de los Pedroches  
CICAP, Ctra. de la Canaleja s/n, 14400 Pozoblanco, Córdoba (Spain)

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**Abstract.** Ibedroches is a research project involving industrial partners of Iberian hams. Most of the companies are small and medium-sized enterprises (SMEs), located in an area characterized by a mostly agricultural and livestock economy, with a relatively importance of the services sector. In general, their experience in research activities is weak. The main goals of this project are: to characterize the surface mold populations in hams; and to develop and implement the use of spectral sensors in the industry of Iberian pork. A strong community added value will be raised through the culture of cooperation between companies, enhancing socio-economic impacts in the region of El Valle de Los Pedroches. Ibedroches represents an example of how to enhance the confidence of national and international consumers regarding quality of high-value Iberian hams belonging to the region of Los Pedroches, due to its added value as a product manufactured under healthy and sustainable production systems. The development of a tool to ensure product quality objectively will contribute to drive innovation in the fields of promotion and marketing in Iberian ham area. This project is leading an innovation strategy through a consortium of industrial competitors in order to promote added value, employment and economic development in rural areas.

**Keywords.** Cooperation – Socio-economic impact – Industry – Iberian ham – Rural development.

## **IBEDROCHES : L'impact socio-économique de la coopération entre entreprises**

**Résumé.** Ibedroches est un projet de recherche formé par différentes entreprises de jambon Ibérique. La plupart d'entre elles sont des petites et moyennes entreprises (PYMES). Elles se trouvent dans une zone dont l'économie est principalement basée sur l'agriculture et l'élevage, avec une importance relative du secteur des services. En général, l'expérience patronale en matière d'activités de recherche est faible. Les objectifs principaux du projet sont: caractériser les populations superficielles de moisissures du jambon; développer et implanter l'usage de capteurs spectraux dans l'industrie du porc Ibérique et obtenir finalement des résultats qui fortifient les valeurs socio-économiques dans la vallée de Los Pedroches, grâce à la coopération entre les entreprises du secteur. Ibedroches représente un exemple montrant comment augmenter la confiance des consommateurs nationaux et internationaux concernant la qualité de pièces de jambon exquises, qui ont une haute valeur, produites dans la vallée de Los Pedroches, surtout en raison de leurs valeurs ajoutées de produit sain, provenant de systèmes durables de production. Le développement d'un outil permettant de garantir une qualité plus objective du produit, aidera à l'innovation pour les systèmes de promotion et de commercialisation du jambon Ibérique. Il s'agit d'un projet où l'innovation principale consiste à créer une valeur ajoutée, c'est-à-dire à favoriser l'emploi et le développement socio-économique en zones rurales, au moyen de la R+D+I promue par l'association entre des entreprises qui sont, en principe, concurrentes.

**Mots-cles.** Coopération – Facteurs socio-économiques – Jambon Ibérique – Développement.

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## **I – Introduction**

The livestock population across the EU-27 comprised about 161 million pigs, 95 million sheep, and 88 million head of cattle in 2006, as well as 1.5 billion poultry birds (Eurostat, 2008). Pig production is specialized even across borders, with breeders such as Denmark, fatteners such as Spain and mixed producers such as the Netherlands. Germany, Denmark and the Netherlands form a single pig production area (Marquer, 2010). In 2008 pork production in the EU-27 reached 259.6 million head, of which more than half (54.4%) came from four countries

(Germany, Denmark, Spain and France). Otherwise, Eurostat report (2008) shows that the highest annual apparent consumption among meat products was recorded for pork products, averaging over 40 kg per capita, a level that was higher than the combined total of poultry, cattle, sheep and goats. Spain, Austria, Germany, Denmark and Belgium reported the highest per capita apparent consumption of pig meat, all recording averages in excess of 50 kg.

Pork is produced throughout the EU on several types of farms with considerable variations from one Member State to another. In southern European countries most of the dry-cured meat products come from conventional or improved pig breeds reared indoors and fed on commercial feed mixtures. In some areas of the Mediterranean basin, pig production relies on local breeds (Iberian, Corsican) whose expend a final extensive fattening period in the Mediterranean forest. On the other hand, there is an increasing demand for both fresh and dry-cured products from Iberian pigs reared under intensive nutritional management, due to their high quality at a comparatively lower price (García-Valverde *et al.*, 2008).

The meat industry sector is characterized by a elevated number of small and medium enterprises (SMEs), in particular Iberian hams industries, most of them with a reduced number of employees. The sector is highly atomized (Cruz, 2009). Because of that, together to their strong traditional character, it is difficult to find meat companies that employ part of their resources to R&D activities.

Previous reports strength the interest on a competitive improvement of the meat industries faced to a strongly competitive market and the panorama of economic crisis (UTEDLT, 2008; [www.infocarne.com](http://www.infocarne.com)). Therefore, the promotion of the innovation in the meat industry is a sectorial strategic activity. The development of managerial projects of investment in R&D, individuals or in cooperation, is fundamental in order to assure the survival and long-term viability of the industries.

Ibedroches is a research project involving six industrial partners of Iberian hams in the region of Los Pedroches (Córdoba, Spain). The main goals of this project are to obtain a strong community added value through the culture of cooperation between companies, enhancing socio-economic impacts, as well as through improving the quality and food safety of the Iberian ham.

## **II – Materials and methods**

The consortium involves six meat companies: Camilo Ríos, Celestino Gómez, COVAP, Hnos. Rodríguez Barbancho, IBESA, La Finojosa, under the coordination of CICAP and in collaboration with the Universities of Córdoba and Extremadura.

Five of the six companies are SMEs, located in an area characterized by a mostly agricultural and livestock economy, with a relatively importance of service sector. All of them produce Iberian meat products from pigs reared in the Mediterranean forest. In general, their experience in research activities is weak.

The six companies, with industrial activity in the same geographic area (region of Los Pedroches), are working as a group in two different activities packages: toxigenic molds control and evaluation of spectral technologies EIS and NIRS.

## **III – Results and conclusions**

Ibedroches represents an example of how to enhance the confidence of national and international consumers regarding quality of high-value Iberian hams belonging to the region of Los Pedroches, due to its added value as a product manufactured under healthy and sustainable production systems. The development of a tool to ensure product quality objectively

will contribute to drive innovation in the fields of promotion and marketing in Iberian ham area. This project is leading an innovation strategy through a consortium of industrial competitors in order to promote added value, employment and economic development in rural areas.

The added value obtained from the present study will enhance the quality and food safety of the Iberian ham reducing production costs, becoming in a useful tool for the meat industries. Thus, the technological development derived from the close work of the companies' participants in the project will generate a portable tool that might differ these companies from the competition. This device will be able to be patented by the corresponding economic benefit. All these results expect to open or consolidate new frontiers of the more demanding countries, such as United States or Japan.

## Acknowledgments

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# List of participants

ACCIAOLI, Anna  
Dpto. Agricultural Biotechnology  
Animal Science Section  
University of Firenze  
Via delle Cascine, 5  
50144 Firenze  
ITALY  
anna.acciaioli@unifi.it

AGUDELO, Jorge  
Facultad de Ciencias Agrarias  
Univ. de Antioquia  
Carrera 75 #65-87 (Barrio Robledo),  
of. 46-114  
Medellín  
COLOMBIA  
jorgehat@gmail.com

AGUILERA SÁNCHEZ, José Fernando  
Camino del Jueves s/n  
18100 Armilla, Granada  
SPAIN  
Aguilera@eez.csic.es

ALMEIDA RICO LAVADO, Sergio  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
sergio\_lavado@hotmail.com

ALOISIO, Giuseppe  
APDECCOR  
Calle María Cristina, 13 (of. 201)  
14002 Córdoba  
SPAIN

AMARO LOPEZ, Manuel Angel  
Dpto. Bromatología y Tecnología de los  
Alimentos  
Universidad de Córdoba  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
bt1amlom@uco.es

ANDA UGARTE, Judit  
Dirección General de la Producción Agrícola y  
Ganadera  
Consejería de Agricultura y Pesca  
Junta de Andalucía  
C/ Tabladilla, s/n  
41071 Sevilla  
SPAIN

ASTORGA MÁRQUEZ, Rafael Jesús  
Dpto. Sanidad Animal,  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
sa1asmar@uco.es

ARGAMENTERIA, Alejandro  
SERIDA  
Carretera Villaviciosa-Oviedo, s/n  
33300 Villaviciosa Asturias  
SPAIN  
fh\_mnieves@hotmail.com

ARONICA, Vincenzo  
Istituto Zooprofilattico Sperimentale della Sicilia  
Via S. Andrea 96  
98051 Barcellona Pozzo di Gotto, Messina  
ITALY  
vincenzo.aronica@izssicilia.it

BAENA BLAZQUEZ, Antonio  
Eiras Altas  
7230, Barrancos  
PORTUGAL  
abaena@barrancarnes.com

BAENA MANZANO, Francisca  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa3bamaf@uco.es

BAÑÓN ARIAS, Sancho  
Grupo de Investigación en Tecnología  
Alimentaria. Facultad de Veterinaria  
Universidad de Murcia  
Campus de Espinardo, 30100 Murcia  
SPAIN  
sanchoba@um.es

BARRAGÁN, Carmen  
INIA, Carretera de La Coruña km 7,5  
28040 Madrid  
SPAIN  
barragan@inia.es

BARRETO, António Salvador  
Universidade Técnica de Lisboa  
Av. da Universidade Técnica  
Pólo Universitário, Alto da Ajuda  
1300-477 Lisboa

PORTUGAL  
asbarreto@fmv.utl.pt

BEDIA GEA, Mario  
Grupo de Investigación en Tecnología  
Alimentaria, Facultad de Veterinaria  
Universidad de Murcia  
Campus de Espinardo.  
30100 Murcia  
SPAIN  
mbedia@um.es

BENITEZ NIETO, Yamiladys  
Almirante Lobo, 2, 4ª planta  
41001 Sevilla  
SPAIN  
benitez@iale.es

BERNAL VELA, Francisco José  
C/ Barba y Espinar, 1 bloque 2 1º E  
11540 Sanlúcar de Barrameda, Cadiz  
SPAIN  
francisco.bernal703@gmail.com

BINI, Pier Paolo  
University of Sassari  
Via Vienna 2  
71100 Sassari  
ITALY  
fisvetbi@uniss.it

BONANZINGA, Maurizio  
Sviluppo Rurale, Regione Toscana  
Loc. Ex Enaoli. Rispescia, 58100 Grosseto  
Firenze  
ITALY  
m.bonanzinga@arsia.toscana.it

BONAZZI, Giuseppe  
University of Parma  
Via del Taglio, 8 43100 Parma  
ITALY  
giuseppe.bonazzi@unipr.it

BONDESAN, Valerio  
Veneto Agricoltura  
Viale dell'Università 12  
35020 Legnaro  
ITALY  
valerio.bondesan@venetoagricoltura.org

BONDI, Giulia  
Via San Michele degli Scalzi 2  
56124 Pisa  
ITALY  
gbondi@hotmail.it

BOZZI, Riccardo  
Department of Agricultural Biotechnology

Animal Science Section  
University of Firenze  
Via delle Cascine, 5  
50144, Firenze  
ITALY  
riccardo.bozzi@unifi.it

BRAVO COERTEZ, Adela María  
Dpto. Producción Animal, Universidad de  
Córdoba,  
Campus de Rabanales  
Ctra. Nacional IV, km 396.  
14014 Córdoba  
SPAIN  
edalleperoe90@yahoo.es

BRAVO DE LAGUNA ORTEGA, Fernando  
Nutreco España, S.A.  
Ronda de Poniente, 9  
28760 Tres Cantos, Madrid  
SPAIN  
fernando.bravodelaguna@nutreco.com

BRITO NOBRE FAUSTINO, Nuno  
ACPA  
Rua de Armacao de Pera n. 7 A  
Ourique. Alentejo  
PORTUGAL  
acpaourique@gmail.com

CABELLO, Alejandro  
Diputación de Córdoba  
Centro Agropecuario Provincial  
Plaza de Colón, 15  
14071 Córdoba  
SPAIN  
acr02@dipucordoba.es

CABEZA DE VACA MUNILLA, Francisco  
San Francisco, 51, 1º Derecha  
06300 Zafra, Badajoz  
SPAIN  
fcabezadevaca@terra.es

CABEZAS SERRANO, Ana Belén  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
g72casea@uco.es

CALDENTY ALBERT, Pedro  
Dpto. Economía, Sociología y Política Agrarias  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
es1caalp@uco.es

CALDERONE, Davide  
Consorzio del Prosciutto di Parma  
Via Marco dell'Arpa, 8/b  
43100 Parma  
ITALY  
davide.calderone@prosciuttodiparma.com

CAMPODONI, Gustavo  
Dipartimento di Biotecnologie Agrarie  
Sezione di Produzioni Animali  
University of Firenze  
Via delle Cascine, 5  
50144 Firenze  
ITALY  
gustavo.campodoni@unifi.it

CAMPOS, Javier  
Fundación Bodegas Campos  
c/ Candelaria 2  
14002 Córdoba  
SPAIN

CANDEK-POTOCAR, Marjeta  
Agricultural Institute of Slovenia  
Hacquetova ulica 17  
1000 Ljubljana  
SLOVENIA  
meta.candek-potokar@kis.si

CARBONERO CONTADOR, Francisco J.  
C/ Moreno de Pedrajas, 19  
14440 Villanueva de Córdoba  
Córdoba  
SPAIN  
jj\_carbonero@hotmail.com

CARCANGIU, Vincenzo  
Dipartimento di Biologia Animale  
Università di Sassari  
Via Vienna 2, 71100 Sassari  
ITALY  
endvet@uniss.it

CARRASCO, Librado  
University of Córdoba  
Edificio de Sanidad Animal  
Campus Universitario de Rabanales  
14014 Córdoba  
SPAIN  
librado.carrasco@uco.es

CARRIÓN PARDO, Domingo  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
domingo.carrion@pic.com

CASABIANCA, François  
INRA – LRDE  
Quartier Grossetti  
20250 Corte, Corse  
FRANCE  
casabianca@corte.inra.fr

CASTAÑO ROMERO, Francisco  
BRUKER, Parque Empresarial Rivas Futura  
C/ Marie Curie, 5. Edf Alfa, Planta Baja  
28521 Rivas-Vaciamadrid, Madrid  
SPAIN  
francisco.castano@bruker.es

CASTÓN, Gema  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa3caexg@uco.es

CHARNECA, Rui Miguel  
University of Evora  
Apartado 94  
7002-554 Évora  
PORTUGAL  
rmcc@uevora.pt

CLEMENTE LÓPEZ, Ignacio  
Polígono industrial El Pontón, parcela 136  
21230 Cortegana, Huelva  
SPAIN iclemente@teica.es

CORAZZIN, Mirco  
Dipartimento di Scienze Animali  
Università di Udine  
Via Sondrio 2°  
33100 Udine  
ITALY  
mirco.corazzin@uniud.it

CORRAL JIMENEZ, Juan Manuel  
Finca La Orden-Valdesequera  
Junta de Extremadura  
Autovía A-5  
Km 372  
06187 Guadajira, Badajoz  
SPAIN juanmanuel.corral@juntaextremadura.net

CROVETTI, Alessandro  
Department of Agricultural Biotechnology  
Animal Science Section  
University of Firenze  
Via delle Cascine, 5  
50144 Firenze  
ITALY  
alessandro.crovetti@gmail.com

DE LA GÁNDARA MERINO, Jesús Manuel



D.O.P. Guijuelo  
Filiberto Villalobos, 4  
37770 Guijuelo, Salamanca  
SPAIN

DE LA ROZA, Begoña  
SERIDA  
Carretera Villaviciosa-Oviedo, s/n  
33300 Villaviciosa, Asturias  
SPAIN  
broza@serida.org

DE PEDRO SANZ, Emiliano Jesús  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa1pesae@uco.es

DELGADO PERTIÑEZ, Manuel  
ETSIA  
Departamento de Ciencias Agroforestales  
Universidad de Sevilla  
Ctra. Utrera km, 1  
41013 Sevilla  
SPAIN  
pertinez@us.es

DELGADO VIZCAÍNO, Ricardo  
COVAP  
C/ Mayor 56  
14400 Pozoblanco, Córdoba  
SPAIN  
rdelgadov@covap.es

DIAFERIA, Carlo  
Stazione Sperimentale per l'Industria delle  
Conservazioni Alimentari  
Viale Tanara 31/A  
43100 Parma  
ITALY  
carlo.diaferia@ssica.it

DIÉGUEZ, Elena  
C/San Francisco, 51 – 1º D  
06300 Zafra, Badajoz  
SPAIN

DIETZE, Klaass  
FAO, Animal Production and Animal Health  
Division  
Viale delle Terme di Caracalla  
00153 Roma  
ITALY

DO ROSARIO OLIVEIRA, Antonio  
Instituto Politécnico de Beja  
Rua Pedro Soares, Campus do Politécnico  
7800-295 Beja

PORTUGAL  
aro@ipbeja.pt

DORADO, Gabriel  
Dpto. Bioquímica y Biología Molecular,  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
bb1dopeg@uco.es

DUARTE CORDOVILLA, Juan Luis  
Junta de Extremadura  
Finca la Orden-Valdesequera  
06187 Guadajira, Badajoz  
SPAIN  
juanluisduarte@gmail.com

ELIAS, Miguel  
University of Evora  
Ap. 94  
7002-554 Évora  
PORTUGAL  
elias@uevora.pt

ESPOSITO, Sara  
Dpto. Agricultural Biotechnology  
Animal Science Section  
University of Firenze  
Via delle Cascine, 5  
50144, Firenze  
ITALY  
sara.esposito@unifi.it

FELIX NOBRE CAMACHO DAVID, Filipa  
ACPA  
Rua de Armacao de Pera n. 7 A  
Ourique – Alentejo  
PORTUGAL  
acpaourique@gmail.com

FERNÁNDEZ FIGARES, Ignacio  
Estación Experimental El Zaidín (EEZ-CSIC)  
Camino del Jueves s/n  
Armillá, 18100 Granada  
SPAIN  
ifigares@eez.csic.es

FERNÁNDEZ FERNÁNDEZ, M. Carmen  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa3fefem@uco.es

FERNÁNDEZ MUÑOZ, Almudena  
INIA  
Carretera de La Coruña km 7,5

28040 Madrid  
SPAIN  
afedez@inia.es

FERRAZ DE OLIVEIRA, Maria Isabel  
University of Evora  
Polo da Mitra, Apartado 94  
7002-554 Evora  
PORTUGAL  
mifo@uevora.pt

FERRO PALMA, Silvina  
Instituto Politécnico de Beja  
Rua Pedro Soares  
7801-902 Beja  
PORTUGAL  
sfpalma@ipbeja.pt

FLORES, Katherine  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
katherine1.flores@gmail.com

FLORES LLOVERA, Mónica  
Instituto de Agroquímica y Tecnología de  
Alimentos (IATA-CSIC)  
Apartado 73  
46100 Burjassot  
Valencia  
SPAIN  
mflores@iata.csic.es

FRANCI, Oreste  
Dpto. Agricultural Biotechnology  
Animal Science Section  
University of Firenze  
Via delle Cascine, 5  
50144, Firenze  
ITALY  
oreste.franci@unifi.it

FRANCO MATILLA, Inmaculada  
Universidade de Vigo  
Campus As Lagoas  
32004 Ourense  
SPAIN  
inmatec@uvigo.es

FRANCO RUIZ, Daniel  
Centro Tecnológico da Carne  
Rúa Galicia Nº4  
Parque Tecnológico de Galicia  
San Cibrao das Viñas, 32900 Ourense  
SPAIN  
danielfranco@ceteca.net

FRANCO SOLLEIRO, Francisco Javier

C/Trabajo nº 6, 2ºD  
41010 Sevilla  
SPAIN  
ffrancosolleiro@gmail.com

FRAQUEZA, Maria João  
Faculdade de Veterinária  
Universidade Técnica de Lisboa  
Av. da Universidade Técnica  
Pólo Universitário, Alto da Ajuda  
1300-477 Lisboa  
PORTUGAL  
mjoaofraqueza@fmv.utl.pt

FREITAS ALVES, Estefanía  
INIA  
Carretera de La Coruña km 7,5  
28040 Madrid  
SPAIN  
esalves@inia.es

FREITAS, Amadeu  
Universidade de Évora  
Apartado 94  
7002-554 Évora  
PORTUGAL  
aagbf@uevora.pt

GALLARDO GALLARDO, Emerenciana  
Instituto de la Grasa,CSIC  
Avda. Padre García Tejero, 4  
41012, Sevilla  
SPAIN  
mere@ig.csic.es

GANDEMER, Gilles  
INRA, Centre de Lille  
Chaussée Brunehaut, Estrées-Mons  
80203 Péronne cedex  
FRANCE  
gilles.gandemer@lille.inra.fr

GARCÍA CASCO, Juan  
Centro Nacional de I+D del cerdo Ibérico  
INIA-Junta de Extremadura  
Ctra. Los Santos - Zafra s/n  
6300 Zafra, Badajoz  
SPAIN  
garcia.juan@inia.es

GARCÍA GARCÍA, Generoso  
IBERAICE  
C/ General Rodrigo 6  
28003 Madrid  
SPAIN

GARCÍA VALVERDE, Rosa  
CICAP  
Apto de correos 105  
14400 Pozoblanco, Córdoba  
SPAIN

rgarcia@cicap.es

GARRIDO VARO, Ana  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa1gavaa@uco.es

GIL HENRIQUES CARVALHO, Carla  
ACPA  
Rua de Armacao de Pera n. 7 A  
Ourique, Alentejo  
PORTUGAL  
acpaourique@gmail.com

GÓMEZ CABRERA, Augusto  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa1gocaa@uco.es

GÓMEZ LAGUNA, Jaime  
CICAP  
Apto de correos 105  
14400 Pozoblanco  
Córdoba  
SPAIN  
jgomez@cicap.es

GONZÁLEZ ARMANGUE, Joel  
IRTA  
Granja Camps i Armet, s/n  
17121 Monells  
Girona  
SPAIN  
joel.gonzalez@irta.cat

GONZÁLEZ VALERO Lucrecia  
Estación Experimental El Zaidín (EEZ-CSIC)  
Camino del Jueves s/n  
Armillá, 18100 Granada  
SPAIN  
lucrecia.gonzález@eez.csic.es

GONZÁLEZ DE BULNES LÓPEZ, Antonio  
INIA  
Avda. Puerta de Hierro s/n  
28040 Madrid  
SPAIN  
bulnes@inia.es

GUERRERO GINEL, José Emilio  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales

Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa1gugij@uco.es

GUILLÉN PÉREZ, Fernando  
D.O.P. Jamón de Teruel  
Avda. Sagunto 116 (Edificio CEEI Aragón)  
44002 Teruel  
SPAIN  
fguillen@jamondeteruel.com

GUZMAN GUERRERO, José Luis  
Universidad de Huelva  
Ctra. Palos de la Frontera, s/n  
21819 Palos de la Frontera, Huelva  
SPAIN  
guzman@uhu.es

HERNÁNDEZ GARCÍA, Francisco Ignacio  
Junta de Extremadura  
Finca la Orden-Valdesequera  
06187 Guadajira  
Badajoz  
SPAIN  
francisco.hernandez@juntaextremadura.net

HERNÁNDEZ MATAMOROS, Alejandro  
Dpto de Producción Animal y Ciencia de los  
Alimentos  
Universidad de Extremadura  
Ctra. de Cáceres s/n  
06007 Badajoz  
SPAIN  
fregepower@hotmail.com

HOCHBERG, Elad  
The Institute of Animal Research  
Kibutz Lahav  
85335 Lahav Negev  
ISRAEL  
fabian@shoket.co.il

HUERTA DANA, Miguel  
IBERAICE  
C/General Rodrigo 6  
28003 Madrid  
SPAIN

HUERTAS LORENZO, Belén  
Dpto. Sanidad Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
sa2hulob@uco.es

INGLESE, Filomena  
ConSDABI  
Piano Cappelle

82100 Benevento  
ITALY  
consdabi@consdabi.org

JAUME SUREDA, Jaime  
IBABSA, Instituto de Biología Animal  
Esperanto, 8  
07198 Son Ferriol  
Palma de Mallorca  
SPAIN  
sgte.porqui@ibabsa.net

KAROLYI, Danijel  
Faculty of Agriculture  
University of Zagreb  
Svetosimunska cesta 25  
10000 Zagreb  
CROATIA  
dkarolyi@agr.hr

LA CHICA LOPEZ, Manuel  
Estación Experimental El Zaidín (EEZ-CSIC)  
Camino del Jueves s/n  
18100 Armilla, Granada  
SPAIN  
ifigares@eez.csic.es

LARA VÉLEZ, Pablo  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa1lavap@uco.es

LATORRE GÓRRIZ, M<sup>a</sup> Ángeles  
Dpto. Producción Animal y Ciencia de los  
Alimentos, Facultad de Veterinaria  
Universidad de Zaragoza  
Avda. Miguel Servet, 177  
50013 Zaragoza  
SPAIN  
malatorr@unizar.es

LEBRET, Bénédicte  
INRA UMR SENAH  
35590 Saint-Gilles, Bretagne  
FRANCE  
Benedicte.Lebret@rennes.inra.fr

LEÓN, Rafael  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
veterinariocordobes@gmail.com

LEÓN CAMACHO, Manuel

Instituto de la Grasa, CSIC  
Avda. Padre García Tejero, 4  
41012 Sevilla  
SPAIN  
mleon@cica.es

LINARES BURGOS, Rafael  
CICAP  
Apto de correos 105  
14400 Pozoblanco, Córdoba  
SPAIN  
rlinares@cicap.es

LO FIEGO, Domenico Pietro  
University of Modena and Reggio Emilia  
Via Amendola, 2  
Padiglione BESTA  
42122 Reggio Emilia  
ITALY  
domenicopietro.lofiego@unimore.it

LÓPEZ LÓPEZ, Antonio  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa3loloa@uco.es

LORENZO RODRÍGUEZ, José Manuel  
Centro Tecnológico da Carne  
Rúa Galicia N<sup>o</sup> 4  
Parque Tecnológico de Galicia  
32900 Ourense  
SPAIN  
jmlorenzo@ceteca.net

LUKOVIC, Zoran  
Faculty of Agriculture  
University of Zagreb  
Svetošimunska 25  
10000 Zagreb  
CROATIA  
lukovic@agr.hr

LUQUE MORENO, Inmaculada  
Dpto. Sanidad Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
sa1lumoi@uco.es

MACCHIONI, Paolo  
University of Bologna  
Via F.Lli Rosselli, 107  
42100 Reggio Emilia  
ITALY  
paolo.macchioni@unibo.it

MACEDO NEVES, José Alberto  
ICAM/Universidade de Évora  
Aptd 94, 7002-554 Évora  
PORTUGAL  
jneves@uevora.pt

MAESTRINI, Oscar  
INRA Corte  
Quartier Grosetti, Corte  
20250 Corse  
FRANCE  
oma@corse.inra.fr

MALOVRH Spela  
Dept. of Anim. Sci., Biotechnical Faculty  
University of Ljubljana  
Groblje 3  
SI-1230 Domžale  
SLOVENIA  
spela.malovrh@bf.uni-lj.si

MALPIQUE, João  
Rua D. Antonio Ferreira Gomes 29  
7300-440 Portoalegre, Alto-Alentejo  
PORTUGAL  
joaomalpique@gmail.com

MANI, Danilo  
University of Pisa  
Via S. Michele degli Scalzi, 2  
56124 Pisa  
ITALY  
dmani@agr.unipi.it

MAROTO, Francisco  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
g02mamof@uco.es

MARTÍN MATEOS, María Jesús  
Instituto Tecnológico Agroalimentario  
Junta de Extremadura  
Ctra. San Vicente s/n  
06071 Badajoz  
SPAIN  
mariajesus.martin@juntaextremadura.net

MARTÍNEZ DOMÍNGUEZ, Beatriz  
Estación Tecnológica de la Carne  
Instituto Tecnológico Agrario, Junta de Castilla y  
León (ITACYL)  
Filiberto Villalobos, s/n  
37770 Guijuelo  
SPAIN  
mardomma@itacyl.es

MARTÍNEZ MARTÍNEZ, María Amparo  
Dpto. Genética  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, Km 396  
14014 Córdoba  
SPAIN  
ib2mamaa@uco.es

MATASSINO, Donato  
ConSDABI  
Piano Cappelle, 82100 Benevento  
ITALY  
consdabi@consdabi.org

MATOS FELIX NOBRE, Jose Candido  
ACPA  
Rua de Armacao de Pera n. 7 A  
Ourique, Alentejo  
PORTUGAL  
acpaourique@mail.telepac.pt

MENÉNDEZ FERNÁNDEZ, Juan  
Alvarez Lorenzana nº 15 4ºD  
33006 Oviedo  
SPAIN  
multigestionrural@ya.com

MINELLI, Giovanna  
University of Modena and Reggio Emilia  
Via Amendola, 2  
Padiglione BESTA  
42122 Reggio Emilia  
ITALY  
giovanna.minelli@unimore.it

MORENO TORIL, Bartolomé  
COVAP, C/ Mayor 56  
14400 Pozoblanco  
Córdoba  
SPAIN  
rdelgadov@covap.es

MORERA, Constantino  
Matadero de Sierra Morena  
Huerta Madroñera, s/n  
41360 El Pedroso, Sevilla  
SPAIN  
cmorera@sierradesevilla.com

MORENO UBEDA, Antonio  
Sánchez Romero Carvajal, Jabugo  
Crtra. San Juan del Puerto s/n  
21290 Jabugo, Huelva  
SPAIN

NANNI COSTA COSTA, Leonardo  
Dept. of Protection and Valorisation  
of Agro-Food  
University of Bologna  
Via F.Lli Rosselli, 107

42100 Reggio Emilia  
ITALY  
leonardo.nannicosta@unibo.it

NIETO, Rosa  
Instituto de Nutrición Animal (IFNA), Estación  
Experimental del Zaidín  
Camino del Jueves s/n  
18100 Armilla  
Granada  
SPAIN  
rosa.nieto@eez.csic.es

NOGUERA JIMÉNEZ, José Luis  
IRTA  
Av. Alcalde Rovira i Roure, 191  
25198 Lleida  
SPAIN  
joseluis.noguera@irta.cat  
NÚÑEZ, Nieves  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa2nusan@uco.es

OLMOS LLORENTE, Alejandro  
Montenevado  
Calle San Ignacio, 6  
40270 Carbonero El Mayor  
Segovia  
SPAIN  
alejandros@montenevado.com

ORTIZ MEDINA, Leovigilda  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa2ormel@uco.es

ORTIZ PÉREZ, Juan Luis  
DOP Los Pedroches  
C/ Pozoblanco, 3  
14440 Vva de Córdoba  
Córdoba  
SPAIN  
secretariogeneral@jamondolospedroches.es

ÓVILO MARTÍN, Cristina  
INIA  
Carretera de La Coruña km 7,5  
28040 Madrid  
SPAIN  
ovilo@inia.es

PANIAGUA BREÑA, Mercedes  
Finca la Orden-Valdesequera  
Junta de Extremadura  
Autovía A-5, Km 372  
06071 Guadajira, Badajoz  
SPAIN  
mercedes.paniagua@juntaextremadura.net

PAREJA CASTRO, Verónica  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
veritagro@hotmail.com

PASTORELLI, Grazia  
University of Milan  
Via Trenta Coste 2  
20134 Milano  
ITALY  
grazia.pastorelli@unimi.it

PAVÓN, José Antonio  
DOP Jamón de Huelva  
Plaza Doña Elvira, s/n  
21200 Aracena, Huelva  
SPAIN  
japavon@jamondehuelva.es

PAZ SÁNCHEZ, Yania  
University of Las Palmas de Gran Canaria  
Juan de Quesada, 30  
35001 Las Palmas de Gran Canaria  
SPAIN  
yania.paz102@doctorandos.ulpgc.es

**PEREA REMUJO, Anselmo**  
Dpto. Sanidad Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
sa1perez@uco.es

PEREIRA TONUS, Brumel  
Carrer Humber Torres nº12 piso 1-3  
25008 Lleida  
SPAIN  
BPEREIRA\_90@yahoo.com.br

PÉREZ ALMERO, José Luis  
IFAPA Centro Las Torres-Tomejil  
Junta de Andalucía  
Ctra. Sevilla, Cazalla Km. 12,2  
41200 Alcalá del Río, Sevilla  
SPAIN  
josel.perez.almero@juntadeandalucia.es

PÉREZ ENCISO, Miguel  
Dpto. Ciència Animal i dels Aliments Facultat de  
Veterinària, UAB  
08193 Bellaterra, Barcelona  
SPAIN  
miguel.perez@uab.cat

PÉREZ MARÍN, M<sup>a</sup> Dolores  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa2pemad@uco.es

PÉREZ MARÍN, Carlos  
Dpto. Medicina y Cirugía Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pv2pemac@uco.es

PIREDDA, Giovanni  
Dipartimento per la Ricerca nelle  
Produzioni Animali  
AGRIS Sardegna,  
Loc Bonassai  
07040 Olmedo  
Sassari  
ITALY  
gpiredda@agrisricerca.it

PORCU, Sebastiano  
Dipartimento per la Ricerca nelle  
Produzioni Animali  
AGRIS Sardegna,  
Loc Bonassai  
07040 Olmedo, Sassari  
ITALY  
sporcu@agrisricerca.it

PORRAS, Carlos  
IFAPA Centro Las Torres-Tomejil  
Junta de Andalucía  
Ctra. Sevilla-Cazalla Km. 12,2  
41200 Alcalá del Río, Sevilla  
SPAIN  
carlos.porras@juntadeandalucia.es

PREVOLNIK, Maja  
Agricultural Institute of Slovenia  
Hacquetova 17  
1000 Ljubljana  
SLOVENIA  
maja.prevolnik@kis.si

PUGLIESE, Carolina  
Department of Agricultural Biotechnology

Animal Science Section  
University of Firenze  
Via delle Cascine, 5  
50144 Firenze  
ITALY  
carolina.pugliese@unifi.it

RACERO VALLÉS, Juan Carlos  
TEICA, Centro Tecnológico del Cárnico  
Polígono industrial El Pontón, parcela 136  
21230 Cortegana, Huelva  
SPAIN  
jcracero@teica.es

REGUILLO GRANADOS, Lucía  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
lucireguillogranados@gmail.com

REY MUÑOZ, Ana  
Dpto. Producción Animal  
Facultad Veterinaria, UCM  
Ciudad Universitaria  
28040 Madrid  
SPAIN  
anarey@vet.ucm.es

RICCIOLI, Cecilia  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
z62rirc@uco.es

RILLO, Luigina  
ConSDABI  
Piano Cappelle  
82100 Benevento  
ITALY  
consdabi@consdabi.org

RIOS, Felix  
C/ Carabitos, 4  
14290 Fuente Obejuna, Córdoba  
camilorios@embutidoscamilorios.com

RIVAS, Álvaro  
DOP Dehesa de Extremadura  
Cánovas del Castillo, 16 – 2º  
06800 Mérida, Badajoz  
SPAIN

RODRIGÁÑEZ, Jaime  
INIA  
Carretera de La Coruña km. 7,5

28040 Madrid  
SPAIN

RODRÍGUEZ-ESTÉVEZ, Vicente  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
vrestevez@uco.es

RODRÍGUEZ LÓPEZ, José Miguel  
Estación Experimental El Zaidín (EEZ-CSIC)  
Camino del Jueves s/n  
18100 Armilla, Granada  
SPAIN  
josemiguel.rodriguez@eez.csic.es

RODRIGUEZ SÁNCHEZ-BEATO, Almudena  
Office of Environment and Rural and Marine  
Affairs, Embassy of Spain  
2375 Pennsylvania Ave., NW,  
Washington, D.C. 20037  
USA ARSBeato@mapausa.org

RODRÍGUEZ VALDOVINOS, M. Carmen  
INIA  
Carretera de La Coruña km 7,5  
28040 Madrid  
SPAIN  
valdo@inia.es

ROSSI, Raffaella  
Department of Veterinary Sciences and  
Technologies for Food Safety  
University of Milan  
Via Celoria 10  
20133 Milán  
ITALY  
raffaella.rossi@guest.unimi.it

RUBIO HERNANDO, Begoña  
ITACyL, Estación Tecnológica de la Carne  
Filiberto Villalobos, s/n  
37770 Guijuelo  
SPAIN  
rubherbe@itacyl.es  
RUSSO, Vincenzo  
DIPROVAL  
Sezione di Allevamenti Zootecnici  
University of Bologna  
Via F.lli Rosselli 107  
42123 Reggio Emilia  
ITALY  
vincenzo.russo@stpa.unibo.it

SALAJPAL, Kresimir  
Faculty of Agriculture  
University of Zagreb  
Svetosimunska cesta 25

10 000 Zagreb  
CROATIA  
ksalajpal@agr.hr

SANABRIA TIENZA, Carmen  
Instituto Tecnológico Agroalimentario  
Junta de Extremadura  
Ctra. San Vicente s/n  
06071 Badajoz  
SPAIN  
carmela.sanabria@juntaextremadura.net

SÁNCHEZ CALDERÓN, Manuel  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
up3sacam@uco.es

SÁNCHEZ CAPITÁN MARTÍN, Pilar  
DOP Guijuelo  
Filiberto Villalobos, 4  
37770 Guijuelo  
Salamanca  
SPAIN

SÁNCHEZ ESQUILICHE, Inés  
C/ Monasterio de la Rábida, nº19, Bajo A  
21200 Aracena, Huelva  
SPAIN  
ninesy21@hotmail.com

SÁNCHEZ ESQUILICHE, Fernando  
c/Monasterio de la Rábida, 19 bajo A  
21200 Aracena, Huelva  
SPAIN  
fselcimajo@hotmail.com

SÁNCHEZ LOPEZ, Lourdes  
CICAP  
Apto de correos 105  
14400 Pozoblanco, Córdoba  
SPAIN  
lsanchez@cicap.es



SÁNCHEZ MORENO, Dolores  
Exmo. Ayuntamiento de Villanueva de Córdoba  
C/ Pozoblanco, 3  
14440 Villanueva de Córdoba, Córdoba  
SPAIN

SÁNCHEZ PINILLA, José Luis  
San Francisco, 51, 1º Derecha  
06300 Zafra, Badajoz  
SPAIN

SÁNCHEZ PINEDA DE LAS INFANTAS, M<sup>a</sup>  
Teresa  
Dpto. Bromatología y Tecnología de los  
Alimentos  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
bt1sapim@uco.es

SANS, Pierre  
École Nationale Vétérinaire de Toulouse  
23 Chemin des Capelles  
31076 Toulouse Cedex 3, Midi-Pyrénées  
FRANCE  
p.sans@envt.fr

SANTOS E SILVA, João  
Travessa Senhora Aninhas s/n  
4800-453 Guimarães  
PORTUGAL  
jppss@hotmail.com

SARRA, Pier Giacomo  
Centro Ricerche Biotecnologiche  
Università Cattolica del Sacro Cuore  
V. Milano 24  
26100 Cremona  
ITALY  
pier.sarra@unicatt.it

SCHIVAZAPPA, Cristina  
Stazione Sperimentale per l'Industria delle  
Conservare Alimentari  
V. le Tanara, 31/A  
43100 Parma  
ITALY  
cristina.schivazappa@ssica.it

SCIANÓ, Salvatore  
Sezione di Zoot. e Nutr. Anim.  
Fac. Medicina Veterinaria  
University of Messina  
Polo Universitario dell'Annunziata  
98168 Messina  
ITALY  
salvatore.scian@alice.it

SERRADILLA MANRIQUE, Juan M.  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa1semaj@uco.es

SERRANO, Antonio  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
aserrano@nirsoluciones.com

SEVILLA, F. Javier  
Dpto. Producción Animal  
Universidad de Córdoba  
Campus de Rabanales  
Ctra. Nacional IV, km 396  
14014 Córdoba  
SPAIN  
pa2sepef@uco.es

SILIÓ, Luis  
INIA  
Carretera de La Coruña km 7,5  
28040 Madrid  
SPAIN  
silio@inia.es

SIRTORI, Francesco  
Department of Agricultural Biotechnology  
Animal Science Section  
University of Firenze  
Via delle Cascine, 5  
50144 Firenze  
ITALY  
francesco.sirtori@unifi.it

SKRLEP, Martin  
Agricultural Institute of Slovenia  
Hacquetova ulica 17  
1000 Ljubljana  
SLOVENIA  
martin.skrlep@kis.si

SPARTA, Giuseppe  
Via Passo Casle 25  
91012 Buseto Palizzolo, Trapani  
ITALY  
qubic.med@regione.sicilia.it

TARDELLI, Lapo  
Department of Agricultural Biotechnology  
Animal Science Section  
University of Firenze  
Via delle Cascine, 5

50144 Firenze  
ITALY  
lapo.tardelli@unifi.it

TASSONE, Francesco  
Dept. of Protection and Valorisation of  
Agro-Food  
University of Bologna  
Via F.Lli Rosselli, 107  
42100 Reggio Emilia  
ITALY

TEJERINA, David  
Finca la Orden-Valdesequera  
Junta de Andalucía  
Autovía A-5, Km 372  
06187 Guadajira, Badajoz  
SPAIN  
david.tejerina@juntaextremadura.net

TIRAPICOS NUNES, José Luis  
University of Evora  
Apart. 94  
7002-554 Évora  
PORTUGAL  
jnunes@uevora.pt

TOUZANNE, Armand  
Chanmbre d'Agriculture  
22 place du Foirail  
65000 Tarbes  
FRANCE  
armandtouzanne@aol.com

UTRILLA LUCAS, María Cristina  
Dpto. Química Analítica y Tecnología  
de los Alimentos  
Univ. Castilla La Mancha  
Avda. Camilo José Cela s/n  
13071 Ciudad Real  
SPAIN  
MariaC.Utrilla@uclm.es

VACCA, Giuseppe Massimo  
Dipartimento di Biologia Animale  
Università di Sassari

Via Vienna 2  
71100 Sassari  
ITALY  
gmvacca@uniss.it

VAZQUEZ, Francisco  
Matadero de Sierra Morena  
Huerta Madroñera, s/n  
41360 El Pedroso, Sevilla  
SPAIN  
pvazquez@sierradesevilla.com

VENTANAS BARROSO, Jesús  
Tecnología de los Alimentos  
Facultad de Veterinaria  
Universidad de Extremadura  
Avda. Universidad s/n  
1000 Cáceres  
SPAIN

VIGUERA BOLUMBURU, Javier  
Imasde Agroalimentaria S.L.  
Napoles, 3  
28224 Pozuelo de Alarcón, Madrid  
SPAIN  
jviguera@e-imasde.com

VIRZI, Antonio  
Via Garibaldi 6  
98061 Brolo, Messina  
ITALY  
antonio.virzi@regione.sicilia.it

YESURUN, Fabian  
The Institute of Animal Research  
Kibutz Lahav  
85335 Lahav Negev  
ISRAEL  
fabian@shoket.co.il

ZUMBO, Alessandro  
Servizi allo Sviluppo, Regione Sicilia  
Polo Universitario dell'Annunziata  
98168 Messina  
ITALY  
alessandro.zumbo@unime.it



# OPTIONS méditerranéennes

**SERIES A: Mediterranean Seminars**  
**2012 – Number 101**

## **7th International Symposium on the Mediterranean Pig**

Edited by:  
E.J. De Pedro and A.B. Cabezas

This publication presents the Proceedings of the 7<sup>th</sup> International Symposium on the Mediterranean Pig held in Cordoba (Spain) from 14 to 16 October 2010. More than twenty years have passed since the first symposium was organized in 1989 in Ajaccio (Corsica), with the participation of French, Italian, Portuguese and Spanish researchers. Since then, several symposia have been held and researchers from other European regions and from the rest of the world have joined the group, aiming to share, discuss and spread the scientific progress achieved in pig production linked to sylvopastoral and agroforestry systems.

The objective of the Network on the Mediterranean Pig is to contribute to the progress of this production system, which brings to society all the values it possesses: it is a key element in the economic development of rural areas, it plays a key role in the environmental management of zones associated with sylvopastoral and agroforestry systems, and it offers high-quality products, demanded by consumers and very much desired by other types of livestock production systems.

The pig production system must be studied within a wider context and, thus, issues related to genetics, production management, nutrition, health, product and socioeconomic aspects have been dealt with in the Symposium. This volume contains a total of 114 articles from contributions presented at the Symposium on all those topics. Given that this product quality plays an important role in the survival of these systems, 40% of the work has dealt with this aspect, as well as the traceability of the systems (12%), with the aim of contributing transparency to commercial transactions and giving a guarantee to the consumers in the different stages of the system (stockbreeders, industry, consumers).



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