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# Phenolic compounds in subcutaneous adipose tissue from Iberian pigs

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**SUMMARY** – The aim of this work was the quantification of polyphenols in subcutaneous adipose tissue from Iberian pigs fed in three different production systems during the final fattening phase ("montanera", "recebo" and "pienso"). There were statistically significant differences between the three feeding systems in total phenol content. Total phenol content was highest in "montanera" (9.11 mg/kg). On the contrary, the pigs that received exclusively a diet based on compound feed (6.74 mg/kg) showed the lowest levels. Pigs fed on acorn in their initial phase of fattening and finished with compound feed (7.95 mg/kg) presented intermediate values. Acorns provide an amount of 9 g of total phenol/kg of acorn ingested. The HPLC profiles are presented at various wavelengths of the phenolic extracts from acorn endosperm and adipose subcutaneous tissue.

**Keywords:** Phenolic compounds, Iberian pig, adipose tissue.

**RESUME** – "Composés phénoliques dans le tissu adipeux sous-cutané de porcs Ibériques". Le but de ce travail était la quantification des polyphénols dans le tissu adipeux sous-cutané de porcs Ibériques alimentés pendant la phase finale d'engraissement selon les trois systèmes de production caractéristiques "montanera", "recebo" et "pienso". Les teneurs totales en polyphénols ont montré des différences statistiquement significatives entre les trois systèmes de production étudiés. Les niveaux les plus hauts de phénols ont correspondu aux porcs alimentés en "montanera" (9,11 mg/kg). Par contre, les porcs alimentés exclusivement avec des aliments composés ont montré les niveaux les plus bas (6,44 mg/kg). Des valeurs intermédiaires (7,95 mg/kg) ont été trouvées chez les porcs alimentés en plein air avec des glands dans la phase initiale d'engraissement et finis avec des aliments composés. Le gland fournit une quantité de 9 g de phénol total/kg de gland ingéré. Dans ce travail les chromatogrammes à diverses longueurs d'onde des extraits phénoliques obtenus par HPLC à partir de l'endosperme de gland et du tissu sous-cutané adipeux sont rapportés.

**Mots-clés :** Composés phénoliques, porcs Ibériques, tissu adipeux.

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## Introduction

The traditional feeding of Iberian pig with "dehesa" natural resources contributes to adipose tissue chemical composition. The pigs can consume daily a great quantity of acorn (7-10 kg) and grass. An increase in the levels of oleic fatty acid (>55%) occurs. The increase of insaturation would cause an increase of the meat oxidations. However, a limited lipid oxidation is produced. An antioxidant action has been attributed to the effect of tocopherols. In particular, the acorn would contribute to the  $\gamma$ -tocopherol content and the grass to the  $\alpha$ -tocopherol content.

At present, pigs are fed with diets rich in oleic acid. This type of feeding leads to a composition of the meat similar to that obtained with traditional "dehesa" feeding, but high oxidations take place. The possible strategy is the use of  $\alpha$ -tocopherol enriched diets in order to increase the level of this antioxidant in muscles, which is an effective mean for reducing lipid oxidation in fresh meat and in meat products (Monahan *et al.*, 1992; Cava *et al.*, 1999; Isabel *et al.*, 2003). Moreover, a lower susceptibility to the oxidation of microsome extracts from Iberian pigs fed on acorn and grass compared with those from pigs fed on mixed diets supplemented with  $\alpha$ -tocopheryl acetate suggests that other dietary constituents from the diet that the pigs receive when fed under free-range conditions may play a role in the stabilization of microsomal lipids (Rey *et al.*, 1998).

For this reason, the interest is being centred in the search of other substances that also have antioxidant capacity like phenolic compounds. The acorn has elevated polyphenols content (Cantos *et*

*al.*, 2003) that, once consumed, could remain deposited in the pig carcass. Thirty-two different phenolic compounds were distinguished in acorns. All of them were gallic acid derivatives, or ellagic acid derivatives (Cantos *et al.*, 2003). Phenolic compounds, which are considered to be compounds with an high antioxidant power, are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation (Shahidi & Wanasundara, 1992).

The aim of this work was the quantification of polyphenols in subcutaneous adipose tissue from Iberian pigs feed on the three different production systems during the final fattening phase.

## Material and methods

### Animals, diets and sampling

Iberian x Duroc (50%) pigs (n=40) were fed on the following systems: "montanera" (n=10), with feeding based on acorns and pasture, "recebo" (n=15), with acorn and pasture but fattening process concluded by feeding with concentrate feed, and "pienso" (n=15), with feeding exclusively with concentrate feeds. The experiment started when the pigs reached 98 kg live weight. The pigs were slaughtered at 173±8 kg live weight at a local slaughterhouse.

Sampling was carried out within 24 h after slaughter. A portion of subcutaneous fat from fresh hams near to the *Gluteo biceps* muscle was taken for analysis.

Acorns from *Q. rotundifolia* Lam., were collected from "montanera" production area. Endosperm from acorns was separated and used for analysis.

### Total phenol content

Phenolic compounds were isolated using the modified method described by Vázquez Roncero, Janer del Valle and Janer del Valle (1973) with triple extraction of a tissue-in-hexane solution with a 80% vol/vol water/ methanol mixture. The concentration of total polyphenols was estimated with Folin-Ciocalteu reagent at 725 nm. Results were expressed as mg of caffeic acid per kg of tissue.

### Phenolic extraction

Phenols were extracted from adipose tissue by following the procedure of Montedoro *et al.* (1992). 2 x 20 ml of methanol/water (80:20 v/v) were added to 10 g of adipose tissue and homogenised for 2 min with a Polytron. The two phases were separated by centrifuging at 3000 rpm for 10 min. Hydroalcoholic extracts were then combined and concentrated under vacuum at temperatures below 35°C until a syrupy consistency was reached. Five ml of acetonitrile were added to the extract and the latter was washed 3 times with 20 ml of hexane. The apolar phases were also purified with 5 ml of acetonitrile. The resulting acetonitrile solution was evaporated under vacuum and dissolved in 5 ml of acetonitrile. Finally, it was evaporated under a stream of nitrogen.

### HPLC analysis of phenolic compounds

The phenolic fraction extracted was dissolved in 200 µl of methanol and analysed by HPLC (loop 40 µl). The HPLC system consisted of a Waters 717, a Waters 600 pump (Waters Inc., Milford, MA). The column was a Inertsil ODS-3 (5 mm, 15 cm<sup>2</sup> 4.6 mm i.d., GL Sciences Inc.) equipped with a Spherisorb S5 ODS-2 (5 mm, 1 cm<sup>2</sup> 4.6 mm i.d., Technokroma, Barcelona, Spain) precolumn. HPLC analysis was performed by following the same procedure as Montedoro *et al.* (1992). The eluents were 0.2% acetic acid (pH 3.1) and methanol and the flow rate was 1.5 ml/min. The total run time was 60 min, the initial composition was 95% acetic acid, 0.2% and 5% methanol, and the gradient changed as follows: the concentration of methanol was maintained for 2 min, then it was increased to 25% in 8 min and finally, the methanol percentage was increased to 40, 50 and 100% in 10 min periods. It was maintained at 100% for 5 min. Initial conditions were reached in 15 min. Chromatograms were obtained at 370, 255, 278 and 339 nm.

## Results

As seen in Table 1 there are statistically significant differences between the three feedings in the total phenol content. These were the highest in *montanera*. On the opposite side, the pigs that received exclusively a feeding based on composed feed showed the lowest content. Pigs fed with acorn in their initial phase of fattening and finished with composed feed showed intermediate values.

Table 1. Effect of feeding on total phenol content (mg/kg) of adipose subcutaneous tissue from Iberian pig

	"Montanera"	"Recebo"	"Pienseo"	SEM <sup>†</sup>	Sig. <sup>††</sup>
	n=10	n=15	n=15		
Total phenol content	9.11 a	7.95 b	6.74 c	0.196	***

<sup>†</sup> SEM: Standard error of the mean.

<sup>††</sup> Significance levels: \*\*\*:  $p < 0.001$ .

The acorn provided an amount of 9 g of total phenol/kg of acorn ingested. The content determined in our study was higher than content previously reported by Cantos *et al.* (2003). The richest source of phenolics was *Q. ilex* that provide an amount of >2 g of phenolics/kg of acorn.

Figures 1 and 2 show the chromatographic profile of the phenolic extracts from acorn endosperm and subcutaneous adipose tissue. With a single maximum at 278 nm, these compounds had a characteristic gallic acid spectrum (Fig. 1). At 339 nm were detected the flavonoids compounds. In addition, several compounds with ellagic acid spectrum were detected at 370 nm and 255 nm (Fig. 2).

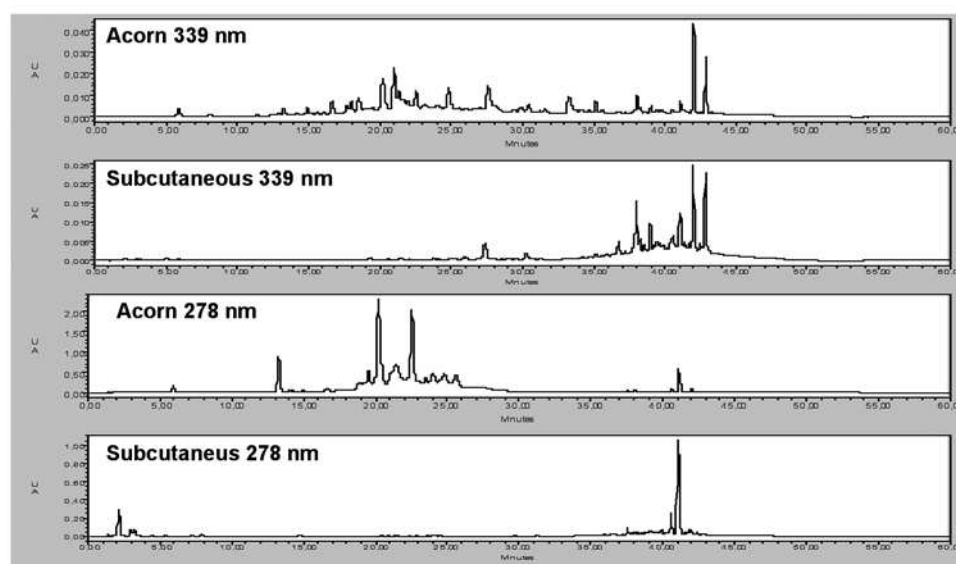


Fig. 1. HPLC chromatograms of acorn endosperm and adipose subcutaneous extracts at different nm 339 and 278 nm.

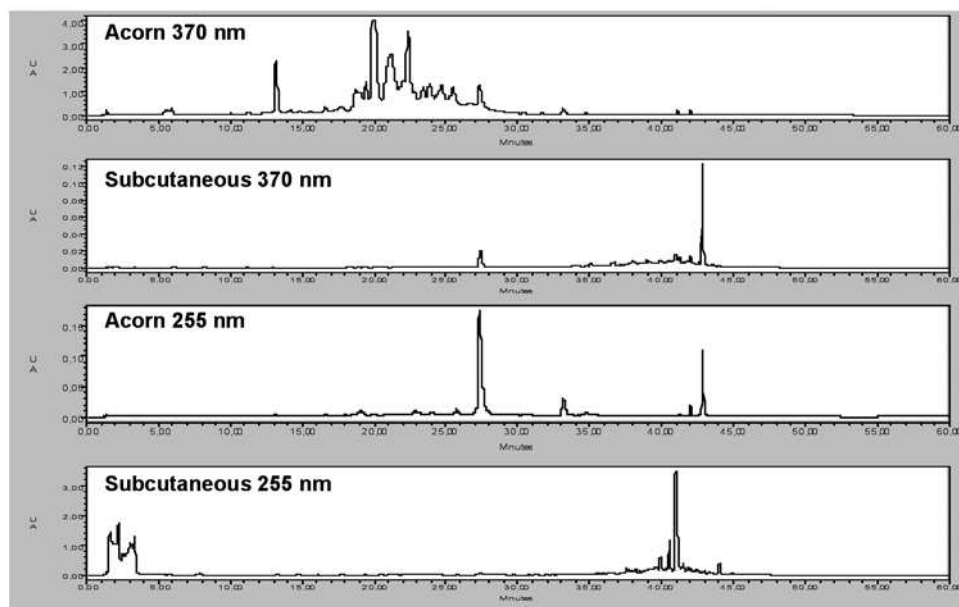


Fig. 2. HPLC chromatograms of acorn endosperm and adipose subcutaneous extracts at 370 and 255 nm.

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