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Physicochemical, hygienic and organoleptic characterisation of Slavonian Kulen, a traditional pork sausage from eastern Croatia

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Abstract. Slavonian Kulen (SK) is traditional dry sausage produced in the region of Slavonia in eastern Croatia. It is made from mince of pork, back fat, spices and salt filled into pork cecum. After stuffing, the sausages are cold-smoked and ripened-dried afterward for several months. Present work aimed to analyse some physicochemical and organoleptic traits of ripe SK as well as the safety of final product. The SK samples (n=12) from different small-scale manufactures were analysed. The following physicochemical attributes were recorded: moisture $38.2\% \pm 3.6$, protein $35.0\% \pm 3.1$, fat $23.7\% \pm 4.6$, moisture/protein ratio 1.1 ± 0.1 , pH value 5.37 ± 0.23 and water activity (aw) 0.82 ± 0.02 . Mean organoleptic scores, on five-point scale, were 3.7 ± 0.6 for surface appearance, 3.4 ± 0.6 for surface smell, 3.8 ± 0.5 for consistency, 3.2 ± 0.4 for inner smell, 3.0 ± 0.7 for cross section quality, 3.3 ± 0.5 for texture, 3.1 ± 0.4 for taste and aroma, 3.0 ± 0.5 for after taste and 3.2 ± 0.4 for overall quality. Regarding the product safety, the following results (per kg) were determined: histamine $330.8 \text{ mg} \pm 126.3$, tyramine $233.9 \text{ mg} \pm 124.7$, nitrite $6.55 \text{ mg} \pm 3.88$ and benzo(a)pyrene $0.05 \text{ } \mu\text{g} \pm 0.03$. *Salmonella* spp. and *L. monocytogenes* have not been found in any sample while counts of *S. aureus*, enterobacteria and sulfite-reducing clostridia were in accordance with regulations.

Keywords. Pork – Dry sausages – Slavonian kulen – Physicochemical traits – Safety.

Caractérisation physico-chimique, hygiénique et organoleptique du Kulen slavonsien, la saucisse de porc haché traditionnelle de Croatie orientale

Résumé. Le Kulen slavonsien (SK) est une saucisse sèche traditionnelle produite dans la région de Slavonie, située dans l'est de la Croatie. Elle est composée de maigre de porc, de graisse, d'épices, et de caecum de porc rempli de sel. Après remplissage, les saucisses sont fumées à froid, et ensuite laissées à sécher pendant quelques mois. Le travail présent visait essentiellement à analyser certaines caractéristiques physico-chimiques et organoleptiques de la production de SK, ainsi que la sécurité des produits finals. On a analysé les échantillons de SK (n=12) produits par différents fabricants en petite série. On a enregistré les attributs physico-chimiques suivants: humidité $38,2\% \pm 3,6$, protéine $35,0\% \pm 3,1$, graisse $23,7\% \pm 4,6$, ratio humidité/protéine $1,1 \pm 0,1$, valeur pH $5,37 \pm 0,23$ et activité de l'eau (ae) $0,82 \pm 0,02$. Les résultats organoleptiques moyens sur une échelle à cinq points sont les suivants: $3,7 \pm 0,6$ pour l'apparition en surface, $3,4 \pm 0,6$ pour l'odeur en surface, $3,8 \pm 0,5$ pour la consistance, $3,2 \pm 0,4$ pour l'odeur intérieure, $3,7 \pm 0,7$ pour la qualité de la coupe transversale, $3,3 \pm 0,5$ pour la texture, $3,1 \pm 0,4$ pour la saveur et l'arôme, $3,0 \pm 0,5$ pour l'arrière-goût et $3,2 \pm 0,4$ pour la qualité générale. En ce qui concerne la sécurité des produits, on a déterminé les résultats suivants (par kg): histamine $330,8 \text{ mg} \pm 126,3$, tyramine $233,9 \text{ mg} \pm 124,7$, nitrite $6,55 \text{ mg} \pm 3,88$ et benzopyrène $0,05 \text{ mg} \pm 0,03$. *Salmonella* spp. et *L. monocytogenes* n'ont été constatés dans aucun échantillon, et par ailleurs les calculs concernant *S. aureus*, entérobactéries et bactérie clostridiale étaient en conformité avec les règlements.

Mots-clés. Porc – Saucisse sèche – Kulen slavonsien – Caractéristiques physico-chimiques – Sécurité.

I – Introduction

The Slavonian Kulen (SK) is a traditional pork sausage from Slavonia region in eastern Croatia that is produced seasonally at many households and small-scale manufactures. It is made from

a mixture of selected and minced pork and back fat, salt and spices such as paprika and garlic filled into pork blind gut (cecum). After stuffing, the SK is cold smoked and ripened-dried afterward for several months until the shelf-stability and typical organoleptic properties are achieved. The quality of SK can be influenced by various factors, like pig breed, rearing and feeding conditions, pre-slaughter handling and post-slaughter conditions which all affect raw pork quality. Several other factors, like selection of lean meat and fat, addition of salt and spices, hygiene and environment (i.e. temperature, humidity, air velocity) during fermentation, smoking, drying and ripening may additionally contribute to diversity of product quality. As a result, the characteristics of final product including its safety may vary, as between producers, so between the years.

The aim of present work was to investigate some physicochemical and organoleptic attributes of traditional SK sausage, in order to better characterize it. In addition, some parameters of hygienic quality and safety of final product were assessed.

II – Materials and methods

Twelve ripe SK sausages aged about 6 months were sampled from different small-scale manufactures in Slavonia. All SK sausages were produced traditionally following the similar manufacturing steps and using the same type of ingredients as presented in scheme in Fig. 1.

After collecting samples were kept in cool until the analyses. The pH values were measured by TESTO 230 pH meter (TESTO®, Germany) by insertion of penetration electrode (type 13) in the core of halved sausage. Water activity (a_w) was measured with the HygroPalm AW1 SET instrument (ROTRONIC®, Germany) using Aw Quick mode in samples, which were taken after coarse homogenisation of 80 g of the core of the sausage. The determination of moisture was done by heating the samples at 105°C until the constant weight. The nitrogen (N) content was determined by Dumas method and proximate protein content (%) was calculated using the conversion factor of $6.25 \times N$. Crude fat (%) was analysed by extraction using the Weibull-Stoldt method. Histamine and tyramine were analysed by thin-layer chromatography according to the procedure described by Macan *et al.* (2006). Nitrite content was determined according to HRN EN 12014-3 method. The microbial safety of product was assessed by the determination of the presence of *Salmonella* spp. and *Listeria monocytogenes* and the number of *Staphylococcus aureus*, enterobacteria and sulfite-reducing clostridia according to HRN EN ISO 6579, HRN EN ISO 11290-1, HRN EN ISO 6888-1, HRN ISO 21528-2 and HRN ISO 15213 method, respectively. Surface moulds were isolated according to the HRN ISO 7954 method. In addition, the contamination by polycyclic aromatic hydrocarbons (PAH) was assessed on sub-sample ($n=5$) by the determination of benzo(a)pyrene (BaP) using the thin-layer chromatography and spectrophotometer.

Organoleptic evaluation of samples was carried out by Faculty Department staff familiar with organoleptic evaluation of SK. Each assessor ($n=4$) was served with one freshly cut slice of SK (thick about 0.5 cm) on a white plastic plate for tasting, whereas the rest of the sausage half was exposed for visual inspection and touch. They were asked to evaluate on a scale from 1 (minimum grade) to 5 (maximum grade) surface appearance, surface smell, consistency, inner smell, cross section quality, texture, taste and aroma and aftertaste. Based on mean score of the particular organoleptic characteristic and the coefficient of importance for that characteristic, the overall quality was calculated by following formula:

$$\text{Overall quality} = 1/17 \times (a + b + c + 3d + e + 3f + 6g + h),$$

where a , b , c , d , e , f , g and h are the mean scores of evaluator's assessments for surface appearance, surface smell, consistency, inner smell, cross section quality, texture, taste and aroma, and after-taste, respectively. During the evaluation, the assessors were offered fresh water and apple slices freely.

For variables analysed the descriptive statistics (minimum, maximum, mean, SD - standard deviation, and CV - coefficient of variation) were calculated.

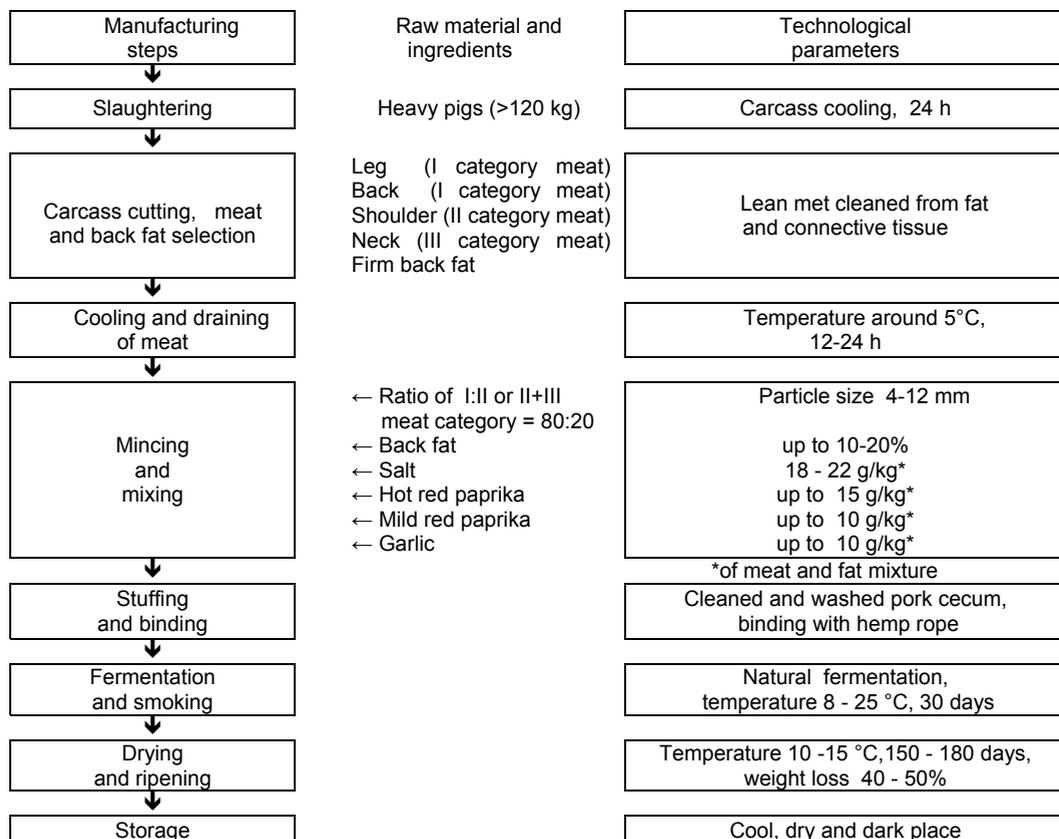


Fig. 1. Schema of traditional manufacturing processes of Slavonian kulen sausage.

III – Results and discussion

The results of organoleptic assessment, physicochemical and hygienic parameters, and microbiological analysis of SK are shown in Tables 1, 2 and 3, respectively.

In SK organoleptic evaluation (Table 1), the highest variability was found for cross section quality (CV=24.7%), while grades for the other traits varied similarly with quite uniform overall quality. The highest graded traits were consistency and surface appearance, and the lowest graded were cross-section quality and after-taste.

The CV of both a_w and pH values (Table 2), which are commonly use in terms of shelf life and safety of dry sausages (Incze, 2007), was very low revealing the homogeneity of SK for these parameters. This is in agreement with result reported by Karolyi (2005). Generally, meat products are considered "shelf-stable" if have pH < 5.2 and a_w < 0.95 or only pH < 5.0 or a_w < 0.91 (Leistner and Rödel, 1975). Hence, the ripe SK with an average a_w value of 0.82 and pH value of 5.37 is shelf-stable product, principally due to extensive dehydration occurred.

European fermented sausages can be roughly classified as Mediterranean, or Southern Europe type, generally characterized by long ripening, slow pH fall with final pH > 5.0 and flavor significantly affected by the use of spices; and Northern Europe type, characterized by fast acidification, final pH < 5.0, smoking and short ripening (Zanardi *et al.*, 2004). Accordingly, SK is similar to long matured low acid Southern Europe type of fermented sausages with the addition of smoking. Similar pH and aw values to those observed for SK were reported for Majorcan sobrasada, which is also stuffed into pork cecum (Rosselló *et al.*, 1995; Martínez *et al.*, 2008).

Table 1. Descriptive statistics for organoleptic traits of Slavonian kulen

Trait	Min	Max	Mean	SD	CV (%)
Surface appearance	2.3	4.5	3.7	0.6	17.4
Surface smell	2.5	4.8	3.4	0.6	17.4
Consistency	3.0	4.8	3.8	0.5	13.0
Inner smell	2.5	3.8	3.2	0.4	12.4
Cross section quality	1.5	4.0	3.0	0.7	24.7
Texture	2.5	4.0	3.3	0.5	14.7
Taste and aroma	2.5	4.0	3.1	0.4	14.0
After taste	2.0	3.8	3.0	0.5	15.5
Overall quality	2.8	3.7	3.2	0.4	10.8

Table 2. Descriptive statistics for physicochemical and hygienic quality traits of Slavonian kulen

Trait	Min	Max	Mean	SD	CV (%)
pH value	5.07	5.75	5.37	0.23	4.3
aw [†]	0.79	0.85	0.82	0.02	2.2
Moisture (% w/w)	31.7	42.8	38.2	3.6	9.4
Total fat (% w/w)	16.4	31.0	23.7	4.6	19.3
Protein (% w/w)	30.3	39.6	35.0	3.1	8.8
M/P ^{††}	1.0	1.3	1.1	0.1	9.4
Nitrite (mg/kg)	2.93	14.30	6.55	3.88	59.2
Histamine (mg/kg)	160.0	560.0	330.8	126.3	38.2
Tyramine (mg/kg)	67.0	400.0	233.9	124.7	53.3
BaP µg/kg ^{†††}	0.05	0.13	0.05	0.03	69.9

[†]Water activity ^{††}Moisture/protein ratio; ^{†††}Benzo(a)pyrene.

The compositional parameters of SK were more variable (Table 2), especially in terms of fat content. This can be attributed to the differences in added fat and selection of more or less lean cuts by certain producers. High fat variability is also reported in other traditional sausages (Ambrosiadis *et al.*, 2004). The moisture in SK (38.2 %) is higher than those cited by Salgado *et al.* (2006) for chorizo and other Spanish traditional sausages what could be explained by large diameter of SK. Compared to sausage of similar size and maturing time, e.g. Majorcan sobrasada (Rosselló *et al.*, 1995; Martínez *et al.*, 2008), the moisture in SK is also higher probably due to higher fat content in Majorcan sobrasada. In contrast, SK has less moisture than botillo sausage from Galicia region in northwest Spain (Lorenzo *et al.*, 2000; García Fontán *et al.*, 2007), which is also stuffed into cecum but it is ripened for much shorter time. Due to long drying and high lean meat share in the mince, the moisture and protein in ripe SK were similar (30-40 %) indicating high nutritional value of product. In fact, the average protein content in SK is higher than those usually found in other traditional sausages (Ambrosiadis *et al.*, 2004; Salgado *et al.*, 2006; Lorenzo *et al.*, 2000; Moretti *et al.*, 2004; Comi *et al.*, 2005). Regarding to

fat content reported for these products, the SK with 23.7 % is generally less fatty. The moisture to protein ratio (M/P), which indicates the extent of drying of lean meat part (Incze, 2007) was 1.1 (Table 2). The M/P, together with pH and a_w , is used to distinguish semidry and dry sausages, i.e. an M/P of 1.2-1.3, which equals $a_w < 0.89-0.90$, is considered as a criterion for dry sausages (Incze, 2007). By that the SK could be clearly distinguished as dry sausage.

The SK is traditionally manufactured without nitrogenous salts. In meat curing, nitrite is widely used for antibacterial, colour and antioxidant purposes. On the other hand, high intake of nitrite presents a risk to human health due to its direct toxicity, or through the endogenous formation of carcinogenic nitrosamines (Sebranek, 2009). The average value of nitrite in ripe SK was 6.55 mg/kg what is lower than residual nitrite levels reported in sausages fermented with the use of nitrite/nitrate (Comi *et al.*, 2005). High level of biogenic amines in foods is another issue of public health concern because of their potentially toxicological effects (Vidal-Carou *et al.*, 2007). Biogenic amines are mainly produced by microbial decarboxylation of amino acids (Silla Santos, 1996) and could be found in various fermented and seasoned foods. The fermentation of sausages in particular offers optimal conditions for biogenic amine accumulation due to availability of free amino acids, the presence of micro-organisms and acidic environment that favours their aminogenic activity (Bover-Cid *et al.*, 1999). The most frequent and most abundant biogenic amine usually found in fermented sausages is tyramine with average concentrations from 100 to 200 mg/kg (Vidal-Carou *et al.*, 2009). Large diameter and long ripening of the sausage can contribute to a greater accumulation of tyramine (Bover-Cid *et al.*, 1999; Parente *et al.*, 2001; Miguélez-Arriazo *et al.*, 2006; Komprda *et al.*, 2009). This could explain the generally high levels of tyramine (Table 2) observed in SK (≥ 200 mg/kg in 75% of samples). The same factors may also be responsible, at least partly for the high accumulation of histamine observed (≥ 200 mg/kg in more than 90 % of samples). However, histamine is rarely found in fermented sausages manufactured under proper hygienic conditions and the occurrence of excessive levels of this biogenic amine is rather an indicator of defective hygienic conditions of raw materials and/or manufacturing processes (Vidal-Carou *et al.*, 2007; Vidal-Carou *et al.*, 2009).

Smoking of SK is done by thermal combustion of hardwoods, e.g. beech, ash, hornbeam. Logs and sawdust are sometimes used wet to lower the temperature of smoke. Smoking is usually in the chamber where the smoke is generated. Direct smoking, however, can lead to greater deposition of potentially unhealthy substances from the smoke, like polycyclic aromatic hydrocarbons (PAH) on the product surface (Andrés *et al.*, 2007). Some PAH, like benzo(a)pyrene (BaP) are classified as possible human carcinogens (US EPA, 2002). The BaP is used as an indicator of total PAH presence in smoked foods and in EU the maximum BaP level of 5 $\mu\text{g}/\text{kg}$ has been established for smoked meats (European Commission, 2005). In present work, the maximum BaP level in SK was far below this margin (Table 2) and lower than those reported for other traditional (Lorenzo *et al.*, 2010) and industrial sausages (Djinovic *et al.*, 2008). Possible explanations for low PAH contamination of SK could be the low temperatures of combustion which generate smoke with less PAH (Šimko, 2009), no use of softwoods which are high in resin that increases the PAH concentration in smoke (Stumpe-Viksna *et al.*, 2008), and the low surface/mass ratio of SK which less favour the PAH adsorption (Lorenzo *et al.*, 2010).

During fermentation and ripening of sausages various microorganisms which are not involved in fermentation are usually progressively eliminated by acidification and drying. As a result, dry fermented sausages are generally safe products which have rarely been involved in food contamination outbreaks. This is particularly true for industry where high sanitary standards and starter cultures are used to control the production. On the other hand, the traditional production is often connected with large variability in raw materials, operation units, fermentation, and/or ripening conditions and hygiene which may result in increased ability of pathogens to survive in end product (Skandamis and Nychas, 2007). For example, investigating the traditional production of sausages in households in Croatia, Kozačinski *et al.* (2008) found the increased total bacterial count at surfaces and equipment used in the sausage preparation including the finding of *S. aureus* and *Enterococcus faecalis* in some cases. The same pathogens, together with enterobacteria and sulfite-reducing clostridia were isolated in high counts from several

samples of raw sausages for cooking, and some of them like *S. aureus* and sulfite-reducing clostridia were found to be exceedingly high in few samples of dry sausages. Close relationship between microbial ecosystems of traditional processing plants (so called "house flora") and produced sausages was also established in studies in other countries (e.g. Lebert *et al.*, 2007).

Table 3. Results of microbiological analysis of Slavonian kulen

Sample	SRC cfu/g	S cfu/25g	E cfu/g	LM cfu/25g	SA cfu/g	Surface moulds
1.	< 10	neg.	< 10	neg.	< 10	neg.
2.	< 10	neg.	< 10	neg.	< 10	<i>Aspergillus flavus</i>
3.	< 10	neg.	< 10	neg.	< 10	neg.
4.	< 10	neg.	< 10	neg.	< 10	<i>Penicillium spp.</i>
5.	< 10	neg.	< 10	neg.	< 10	neg.
6.	< 10	neg.	< 10	neg.	< 10	neg.
7.	< 10	neg.	< 10	neg.	< 10	<i>Aspergillus glaucus</i>
8.	< 10	neg.	< 10	neg.	< 10	neg.
9.	< 10	neg.	< 10	neg.	< 10	<i>Penicillium spp.</i>
10.	< 10	neg.	< 10	neg.	< 10	neg.
11.	< 10	neg.	< 10	neg.	< 10	neg.
12.	< 10	neg.	< 10	neg.	< 10	neg.

SRC - Sulphite-reducing clostridia; S - *Salmonella spp.*; E - *Enterobacteriaceae*; LM - *Listeria monocytogenes*; SA - *Staphylococcus aureus*.

In present study (Table 3), the counts of *S. aureus*, enterobacteria and sulfite-reducing clostridia in all samples were in accordance with Croatian regulations (Narodne novine, 2004), while *Salmonella spp.* and *L. monocytogenes* were not isolated from any sample. Some of the mentioned meat contaminants, like enterobacteria are known for their high ability for biogenic amine production (Vidal-Carou *et al.*, 2007). Hence, the high accumulation of histamine observed in this study may indicate the bacterial contamination of raw materials or hygienic failure during the early steps of sausage production, regardless of the absence or low levels of aminogenic microorganisms in final product. Similar to other fermented products which go through the long ripening, the fungal colonization of surface may also appears in SK.

The natural moulding, which results from contamination by environment-contaminating species, mainly from genera *Penicillium* and *Aspergillus*, may be desirable as it protects against the excessive drying and lipid oxidation and contributes to the flavour development (Spotti and Berni, 2007). On the other hand, many moulds have ability to produce mycotoxins and some of species that were isolated from SK (Table 3), i.e. *Aspergillus flavus*, do have toxigenic potentiality (Bailly and Guerre, 2009).

The presence of certain fungi is not always followed by toxin production as conditions (*aw* in particular) which allow toxin production are more restricted than those which give way to growth (Northolt *et al.*, 1996). However, the presence of mycotoxins in both surface and deeper layers of naturally moulded SK has been recently reported by Frece *et al.* (2010). The contamination of spices and additives used in meat processing may additionally represent a source of mycotoxins (Bailly and Guerre, 2009).

IV – Conclusions

By the manufacturing steps and final characteristics, the SK is similar to long matured Southern Europe type of fermented sausages with the addition of smoking. According to final pH, *aw* and M/P, the SK could be characterised as low-acid dry sausages which shelf-stability is primarily

conditioned by low *a_w* of final product. The organoleptic traits were within the limits of characteristics specific to traditional production. Compared to similar products, the SK has higher protein content and less fat. All samples were microbiologically valid, with low nitrite level and PAH contamination but with high biogenic amines content and, in some cases, with the presence of potentially toxigenic moulds. Hence, from the hygienic and technological point of view, efforts should be made to improve sanitary standards and good manufacturing practice among producers. In addition, better characterization and control of typical microflora during the SK processing is essential in terms of its safety, acceptability and organoleptic quality.

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