Fat content, fatty acid composition and lipid oxidation in industrial and traditional Baranja kulen

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Summary

Fat content, fatty acid composition and oxidation of lipids (TBA test) was determined in the samples of traditional and industrial type of salami from Baranja - kulen. Fat content in the industrially produced kulen was 8.60%, while in the traditional one it was 9.27%. The content of saturated fatty acids (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in both types of kulen was 37% SFA, 15% PUFA, and 48% MUFA. The ratio of PUFA/SFA in the traditional kulen was 0.41, while in the industrial one it was 0.38 and n-6/n-3 ratio was 12.88 for the traditional and 13.08 for the industrial kulen. TBA value of the traditional kulen was 0.79 mg MDA/kg of sample, while the industrial kulen had slightly higher level of TBA value (1.01 mg MDA / kg sample).

Keywords: *traditional kulen, industrial kulen, fatty acid composition, n6/n3, TBA value*

Introduction

According to the Ordinance on meat products (Official Gazette of the Republic of Croatia No. 131/12), kulen is considered to be a dry fermented sausage. Dry fermented sausages are products made of meat, fatty tissue and additional ingredients which, after processing and filling the casings, are subjected to procedures of fermentation, drying and maturing with or without smoking. They contain 40% water at the most and at least 16% meat protein in a product. They are produced and placed on the market under the following names: kulen, winter salami, čajna sausage, Srijem sausage and others.

Dry fermented sausages must fulfill the following conditions: the casing must adhere well to the sausage mix and the surface of the sausage mustn't be deformed. The sausage mix at the cross section should look like a mosaic consisting of approximately equal small pieces of muscle tissue of red color and fatty tissue of white color. The ingredients of the sausage mix should be equally represented and mutually connected firmly. There mustn't be any cavities and cracks at the cross section of the sausage and it should be easy to slice. Kulen is a product made of a finely chopped pork meat, fatty tissue, table salt, additives, spices and spice extracts, sugar, starter cultures and 10% beef can be added as well. Sausage mix is stuffed into natural or artificial casings.

Baranja kulen is an exceptional delicacy and nutritionally valuable foodstuff which is produced according to the traditional recipe of old Baranja artisans from carefully chosen meat of high quality from their own farms and ground red pepper. Baranja kulen is the holder of the Croatian Creation label and is a nutritionally valuable foodstuff.

The share of fat is the most variable chemical component of meat. It affects taste, texture, shelf life and the price of the product. Except for the share of fat, the fatty acid composition should be emphasized too. The goal of this research was to analyze the share of fat and fatty acid composition, then the degree of lipid oxidation (TBA test) in samples of traditional and industrial Baranja kulen.

Material and methods

Sample and production of industrial and traditional baranja kulen

Six samples of the traditional and six samples of the industrial kulen which were produced according to different recipes were researched for the purposes of this paper. It can be seen in Table 1.

Partially defrosted pork leg meat (≈ 54kg) and solid faty tissue (≈ 6kg) from sows separated from fattening were used for the preparation of stuffing. Total primary and secondary raw material and other materials needed for the production of kulen were obtained from the local meat processing industry Belje JSC in Baranja.

Cutting up of primary raw material to granulation of 8 mm was performed by a meat grinder (wolf). After that, depending on the recipe, minced meat and fatty tissue are homogenized for the period of 10 minutes by an electric mixer along with spices, additions or additives. Stuffing of the pork cecum was performed using electric vacuum filler. Cecums are then ligated manually by a rope, hang on sticks and transported into chambers where the rest of the technological process of kulen production takes place, and it is smoking and drying, i.e. maturing in the period of three months.

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Table 1. Review of recipes for traditional and industrial Baranja kulen

Industrial kulen		Traditional kulen	
Nitrate – nitrite salt (NaNO3 + NaNO2)	max. 1,5 g	Sweet ground red pepper	80 g
NaCl	20 g	Hot ground red pepper	60 g
Dextrose	30 g	White ground pepper	10 g
Sodium erythorbate	max. 15 g	Garlic powder	15 g
Starter – culture	1,25 g	NaCl	20 g
Sweet ground red pepper	80 g	Sucrose	≈10 g
Hot ground red pepper	60 g		
White ground pepper	10 g		
Garlic powder	15 g		

Determining fat content

The share of fat in kulen samples was determined by the Soxlet method (HRN ISO 1443:1999).

Preparing methyl esters of fatty acids

Fat obtained by extraction was used for determining fatty acid composition. Ester-bound fatty acids were converted into methyl esters of fatty acids that are suitable for gas chromatography analysis (ISO 5509, 2000). Around 60 mg± 10 mg of sample is weighed into a glass beaker and 4 mL of isooctane is added. After the sample is completely diluted, 200 µL of methanolic solution of potassium hydroxide is added (13.6 g KOH in 100 mL of methanol) and shaken strongly twice for 30 sec. 1 g of sodium hydrogen sulfate monohydrate is added to the solution for the purpose of neutralization and the solution is shaken twice for 30 sec. When crystals settle, 500 µL of the obtained solution of the sample is transferred to an injection container, 1 mL of isooctane is added and the container is closed and shaken.

Determining fatty acid composition

Fatty acid composition was determined by the gas chromatography method (HRN EN ISO 5508, 1999) using CP-3800 device (Varian, Palo Alto, CA, USA). TriPlus Autosampler (Thermo Scientific, Augustin, TX, USA) was used for injecting. The temperature of the partial loop injector was 250°C and injection volume was 1 µL with partition coefficient of 1:30. The samples were analyzed on a capillary column DB-23 of 60 m length, 0.25 mm of capillary inner diameter and 0.25 µm of layer thickness of selective liquid (Agilent, Walnut Creek, CA, USA). The column temperature program was as follows: column temperature 60°C, temperature increase rate 7°C/min up to the final column temperature of 220°C which was kept for 15 min. Helium was carrier gas with the flow rate of 1.5 mL/min. The temperature of flame ionization detector was 260°C. Star GC Workstation Ver. 6.4 (Varian, Palo Alto, CA, USA) computer program was used for data processing. A more detailed description of the method and its appropriateness for the analysis was presented in the paper by Petrović, Kezić and Bolanča (2010).

Determining the lipid oxidation level (TBA TEST)

Thiobarbituric acid test (TBA test) is used for oxidation detection of unsaturated fatty acids and fats. It depends on the development of red pigment which appears by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). The MDA concentration was determined according to the method by Lemon et al. (1975). 20g of sample is homogenized with 7.5% TCA solution, left to rest for 30 min at room temperature, then filtered. 5 mL of filtrate is taken and added 5 mL 0.02M of TBA solution. It is put for 40 min in water bath at 100°C and then cooled under cool running water. After that, absorbance (538 nm) is read on a spectrophotometer (Helios β, Spectronic Unicam, Cambridge, UK). MDA concentration in samples was calculated by a calibration curve. The results are expressed as mg of MDA/kg of kulen.

Statistical analysis

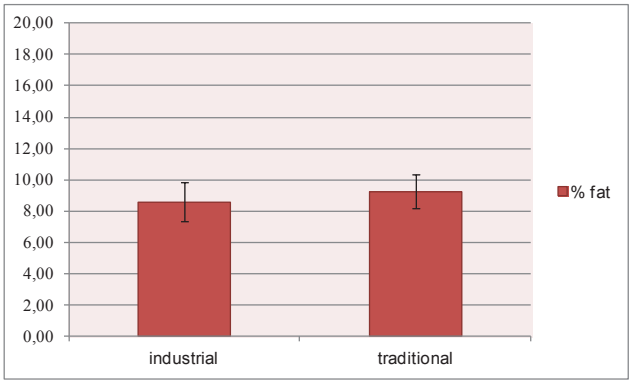
Statistical calculation of the results was determined by one-way ANOVA test with significance level of 5% ($P<0.05$). SPSS computer program was used for statistical data processing

Results and discussion

Fat content

Picture 1 presents fat content in the industrial and the traditional kulen. There wasn't a statistically significant difference between the kulen pieces. Fat content in the industrial kulen was 8.60% and it was 9.27% in the traditional one. Research results indicate to significantly lower values of fat content than the ones in kulen from the neighboring market (43.36%) (Saičić et al., 2010), Salamin fermented sausage (29.71%) (Romero et al., 2013), then Androlla (38.3%) and Botillo (33.6%) (Lorenzo et al., 2000). Lemeški kulen has 14.9% fat at the beginning of maturing and 32.6% at the end of maturing (Vuković et

al., 2012). A large difference is expected in comparison to other kulen pieces and fermented sausages because they had a longer period of maturing than kulen in this research which was maturing for 3 months.



Picture 1. Fat content in industrial and traditional Baranja kulen

Fat content in pork varies the most from chemical content and depends on sex, fat tissue share, slaughter weight, anatomical location, bacon thickness and other (Inmaculada et al., 2002). A high content of fat in fermented sausages at the end of maturation (40-50%) is necessary for sensory characteristics like firmness, juiciness and taste (Olivares et al., 2011). Still, from the aspect of health, an excessive intake of fat is not recommended. For that reason some authors are focused on partial substitution and reduction of fat in fermented sausages (Olivares et al., 2010; Liaros et al., 2009).

Fatty acid composition

Table 2 presents fatty acid composition of the traditional kulen, whereas fatty acid composition of the industrial kulen is presented in Table 3. The traditional kulen contains 37% SFA, 15% PUFA and 48% MUFA, while the industrial one contains 38% SFA, 14% PUFA and 48% MUFA (Picture 2). It follows from the obtained results that both traditional and industrial Baranja kulen pieces have similar fatty acid ratio. The most frequently represented fatty acids in traditional and industrial Baranja kulen pieces are: palmitic (23%), oleic (43%), linoleic (13%) and stearic fatty acid (11%). Fatty acid composition is similar to fatty acid composition in kulen pieces from the neighboring market (Saičić et al., 2010) where the most frequently represented fatty acids are palmitic (25.75%), oleic (43.18%), stearic (11.85%) and linoleic (9.44%).

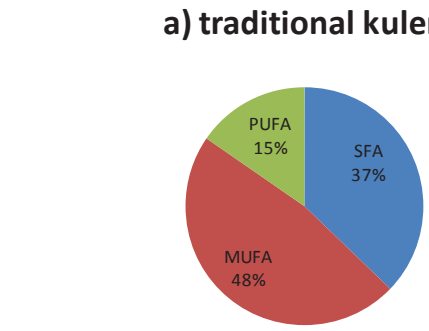
Fermented sausages produced in northern Argentina show similar values. The share of MUFA in Salamin sausages is 44%, whereas it is up to 48% in Morcilla sausage. Oleic acid was the most frequently represented (40%) in Chorizzo sausage, as well as in Morcilla sausage. Palmitic acid is the main saturated fatty acid for Salamin and Chorizzo sausage (22-26%) (Romero et al., 2013).

The share and kind of fatty acids play an important role in prevention and treatment of many chronic disorders, especially cardiovascular diseases.

Table 2. Fatty acid composition in samples of traditional kulen (% of total fat)

Fatty acid	Min	Max	Mean	Stdev
C10:0	0,10	0,10	0,10	0,00
C12:0	0,11	0,11	0,11	0,00
C14:0	1,44	1,51	1,48	0,04
C14:1	0,03	0,15	0,07	0,07
C15:0	0,07	0,16	0,10	0,05
C16:0	21,90	23,50	22,91	0,88
C16:1	2,54	2,84	2,70	0,15
C17:0	0,35	0,40	0,38	0,02
C17:1	0,34	0,39	0,36	0,03
C18:0	10,30	10,81	10,64	0,29
C18:1trans	0,23	0,28	0,25	0,02
C18:1 Cis	40,56	44,57	42,98	2,13
C18:2 Cis	12,48	13,40	13,00	0,47
C18:3n3	0,72	0,82	0,78	0,05
C20:0	0,17	0,17	0,17	0,00
C20:1	0,78	0,85	0,82	0,03
C20:2	0,50	0,57	0,53	0,04
C20:3n6	0,09	0,95	0,38	0,50
C20:4n6	0,31	0,48	0,37	0,10
C20:3n3	0,12	0,14	0,12	0,01
C22:1	0,00	0,69	0,23	0,40
C23:0	0,00	0,20	0,11	0,10
C24:0	0,00	3,65	1,22	2,11
C22:6n3	0,13	0,33	0,20	0,11
SFA	36,66	38,24	37,21	3,49
MUFA	45,37	48,87	47,41	2,84
PUFA	14,39	16,40	15,38	1,27
n-6	13,39	15,11	14,27	1,10
n-3	1,00	1,29	1,11	0,17
n-6/n-3	11,74	13,77	12,88	6,41
PUFA/SFA	0,39	0,43	0,41	0,36
MUFA/SFA	1,19	1,33	1,27	0,81

*SFA- saturated fatty acids; UFA- unsaturated fatty acids; MUFA- monounsaturated faty acids; PUFA-polyunsaturated fatty acids

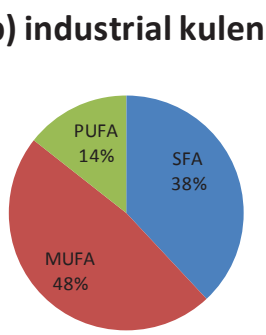


Picture 2. Total share of fatty acids in traditional Baranja kulen

Table 3. Fatty acid composition in samples of industrial kulen (% of total fat)

Fatty acid	Min	Max	Mean	Stdev
C10:0	0,09	0,11	0,09	0,01
C12:0	0,10	0,17	0,13	0,04
C14:0	1,41	1,78	1,55	0,20
C14:1	0,02	0,06	0,04	0,02
C15:0	0,04	0,06	0,05	0,01
C16:0	22,84	25,17	23,63	1,33
C16:1	2,66	2,83	2,73	0,09
C17:0	0,29	0,54	0,42	0,13
C17:1	0,26	0,35	0,32	0,05
C18:0	10,73	11,70	11,13	0,51
C18:1trans	0,21	0,32	0,28	0,06
C18:1 Cis	42,69	43,83	43,34	0,59
C18:2 Cis	11,21	13,88	12,38	1,37
C18:3n3	0,59	0,95	0,78	0,18
C20:0	0,17	0,20	0,19	0,01
C20:1	0,84	0,90	0,86	0,03
C20:2	0,46	0,55	0,49	0,05
C20:3n6	0,09	0,18	0,13	0,05
C20:4n6	0,27	0,46	0,37	0,10
C20:3n3	0,11	0,12	0,12	0,01
C22:1	0,00	0,00	0,00	0,00
C23:0	0,13	1,69	0,67	0,89
C24:0	0,00	0,33	0,17	0,17
C22:6n3	0,11	0,15	0,12	0,02
SFA	36,01	39,23	38,03	1,76
MUFA	46,91	47,98	47,58	0,58
PUFA	12,93	16,01	14,39	1,54
n-6	12,13	14,92	13,37	1,56
n-3	0,81	1,17	1,02	0,22
n-6/n-3	11,16	15,04	13,08	7,21
PUFA/SFA	0,33	0,44	0,38	0,88
MUFA/SFA	1,21	1,33	1,25	0,33

*SFA- saturated fatty acids; UFA- unsaturated fatty acids; MUFA- monounsaturated faty acids; PUFA-polyunsaturated fatty acids



Picture 2. Total share of fatty acids in industrial Baranja kulen

The World Health Organization (WHO, 2003) proposed optimal fat intake which should be between 15-30% of the total energy intake. The saturated fatty acid (SFA) intake should be up to 10%, polyunsaturated fatty acid (PUFA) between 6 and 10% (n-6: 5-8%, n-3: 1-2%), around 10-15% monounsaturated fatty acids (MUFA) and less than 1% trans fatty acids.

Nutritionists nowadays emphasize the importance of PUFA/SFA ratio and n-6/n-3 in comparison to the total share of fatty acids or individual shares of certain fatty acids. Nutrition rich in polyunsaturated fatty acids lowers LDL cholesterol in blood whereas saturated fatty acids have the opposite effect. Numerous researches have proved that a decreased intake of n-6 fatty acids and an increased intake of n-3 fatty acids have a positive effect to human health (Simopoulos, 2002). Hence PUFA/SFA ratio higher than 0.4 is recommended for a healthier diet (UK Department of Health, 1994). The PUFA/SFA ratio in Baranja kulen was 0.41, whereas in the industrial one it was 0.38. The n-6 and n-3 ratio, which should be around 4, is also important (Simopoulos, 2002). The n-6/n-3 ratio in the traditional kulen is 12.88, while it is 13.08 in the industrial one. In researches performed on other kinds of fermented sausages: Serrano, Teruel, Dehesa, Huelva and Guijuelo from Spain, there was determined the n-6/n-3 ratio of fatty acids to be ranging from 9.36 to 13.55 (Fernandez et al., 2007).

It is known that the n-6/n-3 ratio of fatty acids in food lipids that are used nowadays in western countries ranges from 15:1 to 16.7:1, instead of the optimally recommended ratio of 1:1 to 5:1 (Simopoulos, 2004).

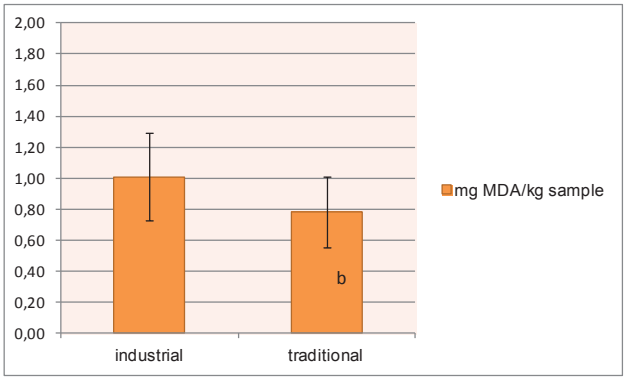
Meat products are a rich source of saturated fatty acids. Except for saturated fatty acids they also contain a low share of polyunsaturated n-3 fatty acids (Fernandez et al., 2007), so a higher ratio of n-6/n-3 ratio of fatty acids is not surprising.

The manner and kind of feeding i.e. the content of a meal, has a crucial effect to the content of fatty acids of intramuscular fat. Fatty acids from the food incorporate into fatty acids of pigs (Toldrá et al, 1996) and the degree of incorporation depends on specific quality of fatty acids and the kind of meal. An example of this is linoleic acid which is taken in by food and passes through pig's stomach unchanged, then it is absorbed from the small intestine into the bloodstream and is incorporated into tissue. In ruminants, linoleic acid which is present in grass and oilseeds is decomposed in rumen to monounsaturated and saturated fatty acids by microbial biohydrogenation and only a small quantity (about 10%) from the food is available for incorporation into lipid tissue (Wood et al., 2008). The changes in pig nutrition have the goal to produce a healthier product so that the intake of SFA is lower and the intake of MUFA or PUFA higher, then a better ratio of n-6/n-3.

TBA test

The level of lipid oxidation is expressed as TBA value. TBA grows at the beginning and continues to grow during maturing (Marco et al., 2006). According to data pro-

vided by many authors, TBA value of fermented sausages varies greatly and amounts from 0.13 to 8.0 mg MDA/kg of sample (Müller, 2006; Marco et al., 2006). TBA value decreases during storage as a consequence of the reaction of malondialdehyde (MDA) with proteins and sugars (Ansorena and Antiasaran, 2004).



* different letters (a-b) indicate to a more significant statistical difference, p< 0.05

Picture 3.
TBA value of traditional and industrial Baranja kulen

Picture 3 presents TBA value of the industrial and the traditional Baranja kulen. TBA value for the traditional kulen amounted 0.79 mg MDA/kg of sample and it was 1.01 mg MDA/kg of sample for the industrial one. A significant difference was determined between our samples of kulen and Lemeški kulen where TBA value at the end of maturing amounts 0.08 mg MDA/kg of sample (Vuković et al., 2012). Dry-cured sausages Androlla have high TBA values (7.02 mg TBA/kg of sample) whereas Botillo sausage has 2.80 mg TBA/kg of sample (Lorenzo et al., 2000).

Conclusion

Fat content is one of the most important quality parameters. The traditional and the industrial Baranja kulen pieces have a low content of fat: 8.60% (industrial) and 9.27% (traditional). The fatty acid composition did not differ much. It was 37% SFA, 15% PUFA and 48% MUFA. The ratio of PUFA/SFA in the traditional kulen was 0.41 while it was 0.38 in the industrial one, and the n-6/n-3 ratio for the traditional kulen was 12.66 and it was 13.08 for the industrial kulen. There is a statistically significant difference ($p<0.05$) in TBA value between the industrial and the traditional Baranja kulen. The TBA value for the traditional kulen was 0.79 mg/kg of sample and it was 1.01 mg MDA/kg of sample.

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