

Changes in basic nutrition and fatty acid composition during production of „Slavonski kulen“

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Scientific paper

Summary

The aim of the study was to examine changes in the chemical and fatty acid composition during the production process of „Slavonski kulen“. During production, samples of Kulen were taken (n=30) on day 1 (raw sausage/stuffing) and during the ripening process (day 30, 60, 90 and 120) and the chemical analysis of the basic nutritional and fatty acids composition were performed. Changes in the basic chemical composition of „Slavonski kulen“ during the phases of production process were in accordance with the literature data. In the finished product, determined ratio of n-6/n-3 fatty acids was 7.61 ± 0.62 , 1.04 ± 0.08 for MUFA/SFA and 0.20 ± 0.02 for PUFA/SFA, represented the ratios of fatty acids characteristic for pork products. It was found that the process of fermentation and three months ripening of „Slavonski kulen“ has no significant effect ($p > 0.05$) on the ratio of n-6/n-3 fatty acids, while for the particular other groups of fatty acids or their ratios, on individual days during the production process, resulted with statistically significant differences ($p < 0.05$).

Keywords: nutritional composition, fatty acid composition, production, „Slavonski kulen“

Introduction

One of the evolutionary characteristics of the modern diet is excessive intake of fat, particularly saturated fatty acids, and at the same time disturbed balance of polyunsaturated fatty acids intake, that is, an increased intake of n-6 in relation to the n-3 fatty acids. These modified ratio n-6/n-3 is associated with disorders of a number of physiological processes that increase the incidence of so-called chronic diseases related to diet, primarily heart disease and disease of cardiovascular system (Cordain et al., 2005).

Since meat and meat products are rich in fat, especially saturated fatty acids, consumers today are advised to reduce consumption of just this type of food (Valsta et al., 2005; Fernandez et al., 2007), while producers are trying to modify these products in order to get them closer to nutritionally acceptable values (Muguerza et al., 2004; Pelsler et al., 2007; Valencia et al., 2006)

At the same time, in European countries the trend is demand of traditional food products, including meat products produced in rural households (Talon et al., 2007), causing more intense researches of their dietary and nutritional value. Research results show that nutritional composition of meat products, in terms of fat mass fraction and fatty acid composition is affected by many factors, from breeds selection, feeding and farming of animals, to technological processes and parameters used during production (Jiménez-Colmenero et al., 2001; Siciliano et al., 2013; Barbir et al., 2014). The pork products generally contain a high proportion of saturated fatty acids (SFA), which is reason to try with changes in the diet to increase the proportion of monounsaturated (MUFA)

and polyunsaturated (PUFA) fatty acids in pork or porks product (Wood et al., 2004; Wood et al., 2008; Woods and Fearon, 2009).

„Slavonski kulen“ is in Croatia the most representative traditional fermented pork sausage, and is produced in rural households with traditional technology and also in industry using a modified recipe (addition of commercial bacterial starter cultures, nitrate and nitrite salts and isoascorbate). In traditional production technology, sausage is made from pork first and second category, previously cleaned of connective tissue, damaged parts, blood vessels and pig claim back fat, with the addition of salt, sweet and hot spicy peppers and garlic and then filled into pork appendix (lat. *intestinum caecum*). After temperature compensation of filling (conditioning), sausage is smoked, fermented, dried and subjected to ripening process for several months. The greatest impact on the sensory properties, aroma and taste of ripe sausage have operations of smoking and adding the garlic, and only after that, compounds resulting from ripening of meat in stuffing, or lipid oxidation and degradation of amino acids (Kovačević, 2001; Kovačević et al., 2010; Kovačević, 2014; Jerković et al., 2010).

The quality of „Slavonski kulen“ is influenced by various factors, from the selection of raw materials and stuffing recipes to production technology, that is, technological processes (conditioning, fermentation, drying, smoking and ripening) and also applied technological parameters (temperature, relative humidity and air/ smoke velocity) (Kovačević, 2014). During the sausage production, due to application of various technological processes, inclu-

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ding the "preservation with obstacles", and because of the technological microflora activities (especially during the fermentation process) and long-term maturation in sausage stuffing, a complex microbiological, physico-chemical and biochemical changes are happening in the fundamental building materials (fats, proteins and carbohydrates), wherein the process is followed by water loss and increase in weight of dry matter. Particularly significant is the process of fat lipolysis, formation of free fatty acids and degradation of short chain fatty acids and their oxidation, since they are key reactions during the ripening process that can affect formation of specific odor and taste of the final product.

The aim of this study was to investigate changes in the nutritional properties of „Slavonski kulen“ during the four-month production process, analyzing the basic chemical and fatty acid composition.

Materials and methods

Sample production and preparation

„Slavonski kulen“ (n=30) was produced under controlled conditions of a laboratory pilot plant using ripening chambers with the option of process programming and automatic control of technological parameters (Kovačević, 2014). Sausage filling was prepared according to traditional recipes and contained pork of first and second category (91.8%), pork back fat (5%), garlic (0.2%), red hot pepper (0.4%) and red sweet pepper powder (0.6%) and salt (2%). Raw mince was stuffed into pig's appendix and the samples were then subjected to the traditional production process of „Slavonski kulen“ production, previously described in the literature (Kovačević i sur., 2010, Babić i sur., 2011). Samples of „Slavonski kulen“ (n=6) were taken during the process of production, at the beginning of that process (day 1), and during the ripening process (30, 60, 90, day 120). After sampling by groups, basic chemical analysis and the analysis of fatty acids composition were performed on each sample.

Sample preparation for analysis

Samples representative for analysis were prepared in accordance with ISO 3100-1:1991. They were homogenized for 15 s at 6000 rpm on homogenizer Grindomix GM 200 (Retch, Germany) and stored in a plastic container filled to the top, due to reduced contact with the air to delay spoilage process. After determination of water content, they are stored at 4 °C until determination of other chemical properties and the fatty acid composition.

Standards and reagents

Standard solution of fatty acids methyl esters (37 fatty acids), concentration of 10 mg/mL, was prepared by resolving standard Supelco™ 37 Component FAME Mix (Bellefonte, Pennsylvania, SAD) in hexan. The solution thus prepared was stored in a freezer at -20 °C and used for identification of fatty acid methyl esters with each analysis.

Hexane and methanol used in the analysis of fatty acids were HPLC grade (JT Baker Derventer, Netherland). All other chemicals used in the analysis were of analytical

grade (Kemika, Zagreb, Croatia). Ultra-pure water with electrolytic conductivity of ≤ 0.05 S/cm was obtained using Milipore Direct-Q 3 UV (Merck, Darmstadt, Germany).

Methods verification

For verification of the analytical methods by determination the parameters of truthness, certified reference material (CRM) with certified values of water content, total protein and fat content, T0149 (FAPAS, England) was used, and for determination of fatty acids composition, CRM with assigned content of seven individual fatty acids, BCR 163 (Institute for Reference Materials and Measurements, Belgium) was also used. CRMs were analyzed six times for each parameter and then mean values were calculated and compared with values certified by the manufacturer.

Basic chemical analysis

Water content was determined gravimetrically (ISO 1442:1997) using thermostat Epsa 2000 (Ba-Ri, Croatia) at 103 °C, and ash content according to ISO 936:1998 by burning the samples at 550 °C in a furnace LV9/11/P320 (Nobetherm, Germany). Total protein content was determined by the Kjeldahl method (ISO 937:1999) with the use of destruction block Unit 8 Basic (Foss, Sweden) and automated device for distillation and titration Kjeltac 8400 (Foss, Sweden). Total fat content was determined by Soxhlet method (EN ISO 1443:1999), which involves digestion of the sample in acid conditions followed by fat extraction with petroleum ether using the Soxtherm 2000 Automatic device (Gerhardt, Germany) and drying in the oven Epsa 2000 (Ba-Ri, Croatia). Carbohydrate content was determined by calculation, based on the determination of water, ash, total protein and fat content.

Results of the analysis are expressed as the mean of two parallel determinations, in percentage (%) of weight, with an accuracy of 0.01%.

Preparation of fatty acids methyl esters

Methyl esters of fatty acids were prepared from the extracted fat according to EN ISO 5509:2000. Ten mL of hexane were added to 100 mg of extracted fat samples and shaken on the HS260 control (IKA, Germany). In addition, 200 ml of 2N methanolic potassium hydroxide solution were added and the samples were shaken for 30 s. The samples were then centrifuged in the centrifuge 320AR (Hettich, Germany) for 15 min at 3000 rpm and temperature of 15 °C, and then 200 ml of each sample was filtered through a PTFE filter into vials to be injected.

Determining the composition of fatty acids by GC-FID

Methyl esters of fatty acids were analyzed by gas chromatography according to EN ISO 5508:1995 using gas chromatographer 7890BA (Agilent Technologies, USA) with the capillary column HP88 of 100 m length, internal capillary diameter 0.25 mm and thickness of stationary phase of 0.20 μ m (Agilent Technologies, USA). The column temperature program was: initial column tempe-

perature of 120 °C, after 1 min temperature increased rate of 10 °C/min until 175 °C, maintaining for 10 min, then temperature increased rate of 5 °C/min until 210 °C and maintaining for 5 min, then again temperature increased rate of 5 °C/min until column final temperature of 230 °C which was maintained for 5 min. A sample (1 ml) was injected in a split-splitless injector with temperature of 250 °C with a partition coefficient 1:50. Carrier gas was helium (99.9999%) with a constant flow rate of 2 ml/min. The components were detected by a flame ionization detector with temperature of 280 °C, with a hydrogen flow of 40 ml/min, air of 450 ml/min and nitrogen of 30 ml/min.

Fatty acids methyl esters were identified by comparison with retention times of 37 fatty acids methyl esters of the standard mixture analyzed under the same condi-

0.05). Since, for determining the difference between the groups in the share of fats and fatty acids, one way ANOVA and Kruskal Wallis test were used, with significance defined at $p < 0.05$.

Results and discussion

Previously validated analytical methods were used for the purpose of this study. Values established from method verification by determining the parameters of trueness were compared with the criteria of the Guidelines for the implementation of analytical methods and the interpretation of results (OG 2/2005) and with criteria of repeatability defined in the used ISO standards. Results of obtained parameters of trueness are shown in Table 1.

Table 1. Results of trueness of analytical methods

Parameter ^a	Assigned value ^b (%)	Obtained value ^c (%)
Water	68,52–70,47	69,00±0,06
Total proteins	17,56–18,88	18,42±0,05
Total fat	2,12–2,87	2,52±0,01
C14:0	2,29±0,04	2,38±0,03
C16:0	25,96±0,30	27,79±0,21
C16:1	2,58±0,16	2,18±0,02
C18:0	18,29±0,17	20,17±0,31
C18:1n-9c	38,30±0,40	36,54±0,31
C18:2n-6c	7,05±0,17	7,16±0,07
C18:3n-3	0,86±0,14	0,52±0,06

^a parameters of the basic chemical composition and fatty acids (seven) with assigned values of CRM

^b assigned values of CRM are given as a range (water, protein, fat) or as mean ± measurement uncertainty

^c obtained values are expressed as mean ± standard deviation

tions. Along with samples and standard in each analysis CRM was also used, prepared and analyzed in the same way as the samples. Results are expressed as a percentage (%) of particular fatty acid on total fatty acids.

Data analysis

Statistical analysis was performed using computer program SPSS 20.0 (SPSS Inc.,USA). Results are expressed as mean ± standard deviation. ShapiroWilks test was conducted to determine whether the results of the analyzed parameters have a normal distribution ($p >$

By comparing obtained results with CRMs certified values and criteria of the Regulations (OG 2/2005) and also criteria of analysis reproducibility defined by norms, methods can be considered eligible for the determination of basic chemical parameters and composition of fatty acids in meat products.

The results of analysis of basic chemical composition of „Slavonski kulen“ by days during production and ripening are presented in Table 2.

Table 2. Basic chemical composition during the ripening of „Slavonski kulen“

Parameter	Mean value ± SD (%)				
	Day 1	Day 30	Day 60	Day 90	Day 120
Water	50,13±2,77 b,c,d,e	40,83±2,41 a,e	40,37±0,72 a,e	36,17±1,59 a,e	29,88±2,51 a,b,c,d
Total fat	16,13±1,76 b,c,d,e	20,00±0,36 a,e	19,77±0,74 a,e	20,60±0,44 a,e	23,04±1,09 b,c,d,e
Total proteins	28,88±0,92 b,c,d,e	34,49±2,30 a,e	34,92±0,46 a,e	37,29±1,23 a,e	40,99±1,33 a,b,c,d
Ash	4,29±0,06 d,e	4,67±0,20 d,e	4,33±0,82	5,39±0,12 a,b	5,65±0,20 a,b
Carbohydrates	< 1	< 1	< 1	< 1	< 1

statistically significant differences ($p < 0.05$) from: ^a day 1, ^b day 30, ^c day 60, ^d day 90, ^e day 120

Statistically significant differences ($p < 0.05$) were determined for water, total protein, fat and ash content between the groups (day 1 to 120) during the production process. Typically, the water content was reduced during ripening process, and already the values determined on day 90 were characteristic for this type of product ($< 40\%$) or in general for products from the group of dry sausages (OG 131/2012). Further ripening leads to additional reduction of water content and the lowest water content of $29.88 \pm 2.51\%$ was determined on day 120 of ripening, when also the highest amount of proteins ($40.99 \pm 1.33\%$) was determined. The results are consistent with the published literature data, which shows that due to prolonged drying and ripening (weight loss up to 50%) and a high share of lean meat used in stuffing preparation, water and protein content in riped „Slavonski kulen“ are on an equal levels (30-40%), indicating a high nutritional value of the finished product (Karolyi, 2011).

Also, continuous increase in share of total fat content to the largest $23.04 \pm 1.09\%$, can be seen from day 1 to 120, proportionally to duration of „Slavonski kulen“ ripening process and dehydration, with continuous reduction of water content in the product. Fat as a substantial component in fermented sausages has multiple functions, presenting a concentrated source of energy (9 kcal/g) and a source of essential fatty acids and fat soluble vitamins (Mela, 1990). Furthermore, it's contributing to fullness of flavor, texture and softness, which are all characteristics

relevant to the quality and acceptability of the product (Olivares et al., 2010). Hydrolysis and oxidation of fatty acids that occur during the process of ripening largely contribute to the taste of fermented sausages (Ordonez et al., 1999).

Related values of basic composition were determined in earlier research of „Slavonski kulen“ (Kovačević et al., 2010; Karolyi, 2011; Senčić et al., 2013). In the study by Kovačević et al. (2010), conducted on Kulen produced by different manufacturers, it was determined fat content of 15.10% to 28.84% and total proteins content from 26.21% to 53.03%. At the same time, significant differences were determined in nutritional composition of this product, according to variations in the quality of its production per households. Such variations can be attributed to differences in the amount of added backfat and choosing more or less fatty meat by individual manufacturers.

Studies have shown that fatty acid composition of meat is on average about 40% of saturated (SFA), 40% of monounsaturated (MUFA) and about 2-25% of polyunsaturated (PUFA) fatty acids, wherein the most common unsaturated fatty acid of all kinds of meat and meat products is oleic acid (C18:1) (Barbir, et al., 2014; Barbir, et al., 2014b). The average fatty acid composition determined during production process of „Slavonski kulen“ is shown in Table 3. Figure 1 shows shares of SFA, MUFA and PUFA by groups of fatty acids and days during production.

Table 3. Fatty acid composition during production of „Slavonski kulen“

Fatty acids	Mass fraction of fatty acids*				
	Mean value \pm SD (%)				
	Day 1	Day 30	Day 60	Day 90	Day 120
C10:0	0,10 \pm 0,00	0,10 \pm 0,00	0,10 \pm 0,00	0,10 \pm 0,00	0,10 \pm 0,01
C12:0	0,10 \pm 0,00	0,10 \pm 0,00	0,10 \pm 0,00	0,10 \pm 0,00	0,10 \pm 0,01
C14:0	1,42 \pm 0,00	1,41 \pm 0,01	1,41 \pm 0,01	1,40 \pm 0,01	1,40 \pm 0,02
C16:0	26,53 \pm 0,02	26,09 \pm 0,10 ^e	26,35 \pm 0,07 ^e	26,45 \pm 0,17	27,22 \pm 1,03 ^{bc}
C17:0	0,28 \pm 0,00	0,27 \pm 0,00	0,28 \pm 0,00	0,28 \pm 0,00	0,29 \pm 0,01
C18:0	14,10 \pm 0,01 ^e	13,78 \pm 0,09 ^e	14,04 \pm 0,09 ^e	14,22 \pm 0,23	14,99 \pm 1,25 ^{bac}
C20:0	0,37 \pm 0,01 ^b	0,43 \pm 0,00 ^{acde}	0,39 \pm 0,01 ^{be}	0,37 \pm 0,02 ^b	0,36 \pm 0,02 ^{bc}
C16:1	2,49 \pm 0,01 ^e	2,51 \pm 0,01 ^e	2,50 \pm 0,01 ^e	2,46 \pm 0,00 ^e	2,10 \pm 0,70 ^{ab,cd}
C18:1n-9t	0,10 \pm 0,00 ^{de}	0,10 \pm 0,00 ^{de}	0,10 \pm 0,00 ^{de}	0,17 \pm 0,01 ^{abc}	0,18 \pm 0,03 ^{abc}
C18:1n-9c	45,19 \pm 0,11	45,16 \pm 0,03	45,14 \pm 0,00	44,70 \pm 0,20	43,92 \pm 1,74
C20:1	0,41 \pm 0,00	0,43 \pm 0,00 ^e	0,41 \pm 0,01	0,41 \pm 0,01	0,39 \pm 0,02 ^b
C22:1n-9	0,10 \pm 0,00	0,16 \pm 0,05	0,10 \pm 0,00	0,12 \pm 0,05	0,15 \pm 0,02
C18:2n-6	8,00 \pm 0,11 ^e	8,28 \pm 0,10 ^e	7,97 \pm 0,09 ^e	7,91 \pm 0,26	7,61 \pm 0,39 ^{abc}
C18:3n-6	0,10 \pm 0,00 ^{bcde}	0,26 \pm 0,00 ^a	0,27 \pm 0,00 ^a	0,27 \pm 0,00 ^a	0,26 \pm 0,01 ^a
Σ n-6	8,10 \pm 0,11	8,54 \pm 0,10 ^e	8,24 \pm 0,09 ^e	8,18 \pm 0,09	7,87 \pm 0,38 ^{bc}
C18:3n-3	1,07 \pm 0,03	1,09 \pm 0,00 ^e	1,08 \pm 0,01	1,08 \pm 0,01	1,04 \pm 0,06 ^b
Σ n-3	1,07 \pm 0,03	1,09 \pm 0,00 ^e	1,08 \pm 0,01	1,08 \pm 0,01	1,04 \pm 0,06 ^b
n-6 / n-3	7,55 \pm 0,31	7,80 \pm 0,09	7,63 \pm 0,11	7,60 \pm 0,11	7,61 \pm 0,62
PUFA/SFA	0,21 \pm 0,00 ^b	0,23 \pm 0,00 ^{ae}	0,22 \pm 0,00 ^e	0,21 \pm 0,00	0,20 \pm 0,02 ^{bc}
MUFA/SFA	1,10 \pm 0,00	1,12 \pm 0,01 ^e	1,11 \pm 0,00	1,10 \pm 0,00	1,04 \pm 0,08 ^b

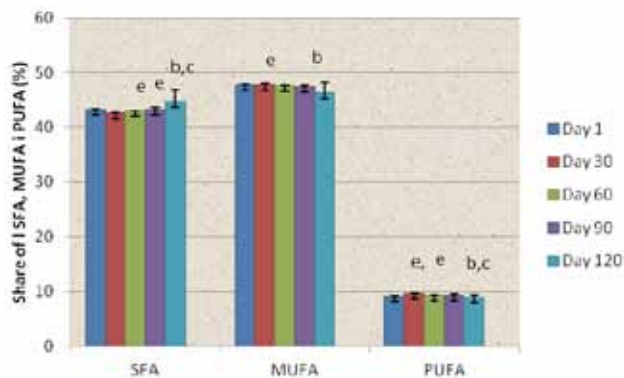
* mass fraction of fatty acid is expressed as the total proportion of fatty acids

statistically significant differences ($p < 0.05$) from: ^a day 1, ^b day 30, ^c day 60, ^d day 90, ^e day 120

SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids

Results of fatty acid analysis of Kulen samples show that the largest share belongs to oleic acid (C18: 1n-9 C), followed by palmitic (C16: 0), stearic (C18: 0) and linoleic (C18: 2n-6) acid. Oleic fatty acid is generally dominant fatty acid in traditional fermented pork sausages from other countries (Casaburi et al., 2007; Visessanguan et al., 2006).

Figure 1. Share of SFA, MUFA and PUFA on days of „Slavonski kulen“ production



Statistically significant differences ($p < 0.05$) from: ^a day 1, ^b day 30, ^c day 60, ^d day 90, ^e day 120

SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids

In this study, a statistically significant difference ($p < 0.05$) in individual fatty acids, as well as group of SFA, MUFA and PUFA by production days of „Slavonski kulen“, was determined. Share of SFA was slightly increased, while PUFA was decreased, what resulted in the lowest PUFA/SFA ratio on day 120 of production process. In terms of the n-6 (C18:2n-6 linoleic acid, C18:3n-6, C20:2n-6 and C20:4n-6 arachidonic acid) and single determined n-3 fatty acid (C18:3n-3 α -linolenic) the slight reduction was shown.

At the end of production process of „Slavonski kulen“ in duration of 120 days, from the total identified fatty acids esters, SFA accounted for $44.75 \pm 2.18\%$, MUFA $46.34 \pm 1.76\%$ and PUFA $8.9 \pm 0.43\%$. Fatty acids from the n-6 group had a share of $7.87 \pm 0.38\%$, and from n-3 group $1.04 \pm 0.06\%$ of the total PUFA, with ratio n-6/n-3 of $7.61 \pm 0.62\%$. At the same time, determined ratio of PUFA/SFA was 0.20 and MUFA/SFA 1.04.

Slightly higher values of PUFA/SFA and MUFA/SFA ratios in samples of traditional (0.41 and 1.27) and industrial (0.38 and 1.25) produced Baranjski kulen (Marušić Radovčić et al., 2014) were determined. Karolyi and Čurić (2012) have determined related ratios of fatty acids groups for the „Slavonski kulen“ and found that there is no significant changes in their composition during the production process, with the exception of C22:5n-3, whose proportion decreased during ripening. Researches of fatty acids composition changes during ripening of indigenous fermented pork sausages from Italy (Moretti et al., 2004) and Spain (Franco et al., 2002) did not show statistically significant changes in composition of fatty acids du-

ring ripening, while in some studies specific reduction in the proportion of PUFA were determined and explained by oxidation of lipids (Gandemer, 2002).

Conclusion

Changes in the basic chemical composition of „Slavonski kulen“ during individual phases of the production process (day 1, 30, 60, 90 and 120) are consistent with literature data. Statistically significant differences ($p < 0.05$) in shares of particular fatty acid or groups such as n-6, n-3, SFA, MUFA and PUFA, were determined in the individual stages of the production process, while the ratio n-6/n-3 at all stages of production was not significantly different ($p > 0.05$). Identified ratios of fatty acids by groups generally are characteristic for pork products.

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4. znanstveno-stručni skup „Okolišno prihvatljiva proizvodnja kvalitetne i sigurne hrane“

Sveučilište Josipa Jurja Strossmayera u Osijeku u suradnji s Poljoprivrednim fakultetom u Osijeku, Prehrambeno-tehnološkim fakultetom Osijek, Poljoprivrednim institutom, Hrvatskom agencijom za hranu, Zavodom za znanstveni i umjetnički rad Hrvatske akademije znanosti i umjetnosti u Osijeku te Gradom Osijekom i Osječko-baranjskom županijom organiziralo je 4. znanstveno-stručni skup "Okolišno prihvatljiva proizvodnja kvalitetne i sigurne hrane" koji se je održao 12. prosinca 2014. na Poljoprivrednom fakultetu u Osijeku, Kralja Petra Svačića 1d, Osijek.



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Želimo Vam čestit Božić i uspješnu Novu 2015. godinu!

