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Original scientific paper

Nutritional quality of selected Croatian traditional dry-fermented sausages

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A R T I C L E I N F O

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A B S T R A C T

The production of dry-fermented meat products, including dry-fermented sausages, has a long tradition in Croatian households. These sausages differ greatly depending on the production region. The aim of the study was to characterize the best-known types of dry-fermented Croatian sausages from Eastern Croatia, including Kulen, Kulenova Seka and Slavonian sausage. The results uncovered significant differences ($p<0.05$) in chemical properties of the above sausages in terms of not only fat, water and ash, but also mineral (sodium and calcium) content. Fatty acid profiles of the sausages under study did not significantly differ, except for the share of total saturated fatty acids and stearic acid. These findings indicate that different recipes and production processes applied to these fermented pork meat sausages affect their physicochemical properties and mineral content, but not their fatty acid profile.

1. Introduction

The production of dry-fermented meat products has a long tradition in Croatian households. These products are characterized by excellent sensory and good nutritional properties, which make them appealing to consumers. Nowadays, the demand for these types of products is rising globally. Traditional recipes used for the production of traditional dry-fermented sausages greatly vary not only across production regions, but across producing households as well (*Lešić et al.*, 2020). In Croatia, the best-known types of dry-fermented sausages are Kulen (Slavonski and Baranjski), Kulenova Seka, Istrian sausage and Slavonian sausage. Their basic ingredients are pork meat, with or without the addition of beef, as well as fat, which are then mixed with different kinds of spices (salt, black pepper, dried red pepper, etc.). The stuffing is then filled into the intestine, pork appendix for Kulen and small intestine for Kulenova Seka/Slavonian sausage in, and left to dry, ferment and cure (*Kovačević et al.*, 2010; *Kovačević*, 2014). Both the ingredients and the chosen production processes are responsible for the differences in physiochemical properties of the final products. Slavonski/Baranjski Kulen and Slavonian sausage are traditional dry-fermented sausages labelled as Protected Geographical Indications (PGI) (*Pleadin et al.*, 2021), while Kulenova Seka is prepared in the same manner, but using narrower casings. All of the above are prepared from pork meat and fat tissue, and supplemented with spices, but differ in their ripening duration (*Ministry of Agriculture*, 2018; *Ministry of Agriculture*, 2019). The production of these sausages can also differ between households, each of them tending to introduce their own special process modifications.

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The study aimed to characterize the nutritional quality of the selected Croatian traditional dry-fermented sausages Kulen, Kulenova Seka and Slavonian sausage, collected from different households situated in Eastern Croatia.

2. Materials and methods

2.1. Dry-fermented sausage samples

Dry-fermented sausages Kulen (n=17), Kulenova Seka (n=17) and Slavonian sausage (n=17) were collected from households situated in Eastern Croatia, which embraces Vukovar-Srijem, Osijek-Baranja, Virovitica-Podravina, and Požega-Slavonia districts. The sampling was carried out during spring, summer, and autumn of 2021 and 2022, the sampling season thereby depending on the sausage type. During both sampling years, the samples were taken from the same localities and in more or less equal quantities (around 1.5 kilos depending on the product size). Samples were ground using a Grindomix GM 200 laboratory mill (Retsch, Germany); the water content was analysed immediately, while the rest of the sample was stored in a refrigerator at 4 °C pending analysis.

2.2. Chemicals and standards

Ultrapure water was obtained from a Direct-Q 3 UV device (Merck, Darmstadt, Germany). High purity chemicals were obtained from Honeywell (Charlotte, NC, USA) and p.a. chemicals from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure nitric acid was obtained from Merck (Darmstadt, Germany). Standard solution of fatty acid methyl esters (FAME) was prepared by dissolving 100 mg of standard SupelcoTM 37 Component FAME Mix (Pennsylvania, USA) in 10 mL of hexane. For the determination of each mineral, the 1,000 μg/mL in 5% nitric acid standard solution (Agilent Technologies, USA) was used for calibration.

2.3. Determination of chemical properties

Compositional analysis was performed using standard and validated internal analytical methods. Determination of water (*ISO 1442*, 1997) and ash (*ISO 936*, 1998) content was performed using gravimetric methods, which made use of a thermostat (UF75 plus, Memmert, Germany) and muffler burning furnace (Program Controller LV 9/11/P320, Nabertherm, Germany). Crude protein content was determined by the Kjeldahl method (*ISO 937*,1978), which involves block digestion of organic matter (Unit 8 Basic, Foss, Denmark) and titration/distillation (Vapodest 50s, Gerhardt, Germany). The Soxhlet method (*ISO 1443*,1973) was employed for crude fat determination (Soxtherm 2000, Gerhardt, Germany). Sugar content was determined by means of enzymatic method using a commercial enzyme kite (Sucrose/D-glucose/D-fructose, R-Biopharm, Germany), while the content of carbohydrates was calculated.

2.4. Determination of minerals

Approximately 0.2 g of each sample was submitted to microwave (Ethos easy, Milestone, Italy) acidic digestion supported by hydrogen peroxide (7 mL of 60 % nitric acid and 3 mL of hydrogen peroxide). The digested samples were transferred into volumetric flasks and diluted with ultrapure water. Sodium (Na), calcium (Ca), potassium (K), magnesium (Mg), copper (Cu), zinc (Zn), and iron (Fe) were analysed by means of flame atomic absorption spectroscopy (200 Series A4 equipped with SPS 4 Autosampler, Agilent Technologies, USA) with wavelengths set at λ=589.0 nm for Na, λ=422.7 nm for Ca, λ=766.5 nm for K, λ=285.2 nm for Mg, λ=324.8 nm for Cu, λ=213.9 nm for Zn and λ=248.3 nm for Fe. A HC coded lamp specific for each mineral (Agilent Technologies, SAD) was used.

2.5. Fatty acid methyl esters (FAME) analysis

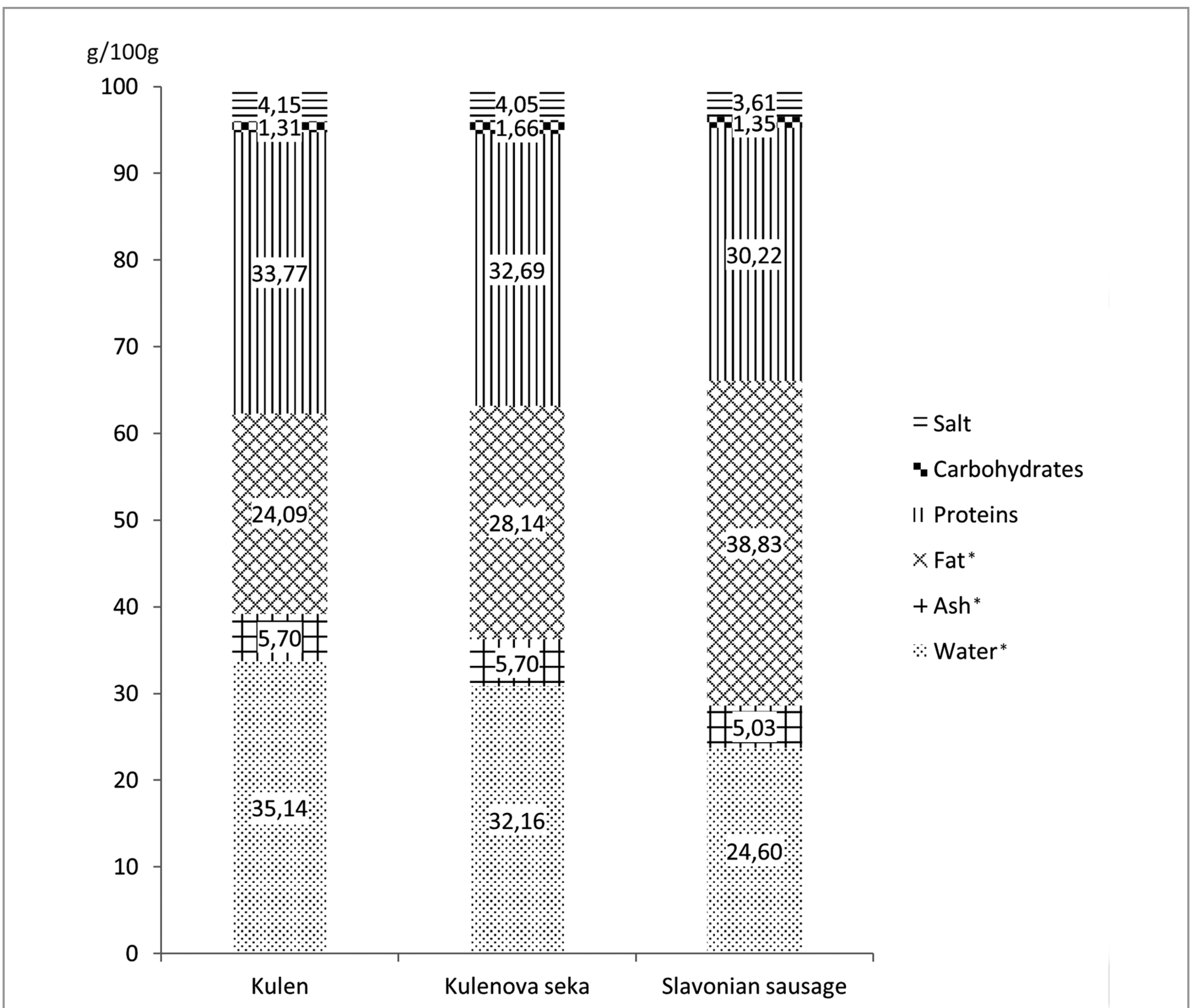
Extracted fat was used for fatty acid methyl esters preparation according to *ISO 12966-2* (2015) with some modifications, as described by *Vulić et al.* (2021). Briefly, the extracted fat was dissolved in isooctane, following which a methanolic transesterification was performed. Afterwards, saturated sodium chloride solution was added. After the separation of layers, the upper isooctane layer was transferred into another tube and anhydrous sodium hydrogen sulphate was added. The aliquot of each sample was filtered through a PTFE filter (0.2 μm pore size) into vials. Methyl esters of fatty acids were analysed by GC-FID (gas chromatography with flame ionisation detector) method using a 7890 A gas chromatograph (Agilent Technologies, USA) equipped with a DB-23 capillary column (60 m length, internal capillary diameter 0.25 mm and stationary phase thickness 0.20 μm (Agilent Technologies, USA). The carrier gas was helium. A split/splitless injector was used to inject one microliter of each sample in split ratio of 1:50. FAME identification was performed by comparing the sample FAME retention time to standard solution mixture FAME. Verification of the method was described earlier by *Pleadin et al.* (2014). The results were expressed as the percentage (%) of a given fatty acid in the total fatty acid share.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY IBM, 2013, Sankt Ingbert, Germany). The differences between the sample groups were established using the analysis of variance (ANOVA) at 95% significance level ($p<0.05$).

3. Results and discussion

The results of the analysis of chemical properties of traditional dry-fermented sausages carried out in this study are presented in Figure 1. Earlier studies, conducted on dry-fermented sausages from different Croatian regions, have shown that chemical composition of this type of sausages varies greatly depending on the recipes and production processes, which are region-specific (*Lešić et al.*, 2020). This study was focused on dry-fermented sausages from the Eastern Croatia, where recipes and production process used are fairly similar. The results of compositional analysis of Kulen, Kulenova Seka and Slavonian sausage showed significant differences in water, ash and fat content, and no difference in protein, carbohydrate and salt content ($p<0.05$).



*statistical significant difference ($p<0.05$)

Figure 1. The average water, ash, fat, protein, carbohydrate and salt content (g/100 g ± SD) found in Kulen, Kulenova Seka and Slavonian sausage

Water content is a parameter that decreases over the production period, especially during drying, and is important from the food spoilage aspect. The lowest water content determined in the tested sausages was that of Slavonian sausage (24.60 g/100g). Product drying and decrease in water content consequently increase ash, protein and fat content. As compared to Kulen and Kulenova Seka, Slavonian sausage had the highest fat content (38.83 g/100 g as compared to 24.09 and 28.14 g/100 g, respectively). Kulen, as mentioned earlier, was the first Croatian dry-fermented sausage designated as protected by geographic indication (PGI), so that its composition in regard to fat, protein and water content is strictly specified. Among the tested sausages, Kulen had the highest protein content (33.77 g/100 g), which is in accordance with the specification and studies published earlier (*Kovačević et al*., 2014; *Bogdanović et al*., 2016). The low amount of carbohydrates determined in the tested sausages could be attributed to the added sugars, which are sometimes used as fermentation enhancers. Salt is an important ingredient of dry-fermented sausages as it enhances organoleptic properties, but also preserves the product from microbiological spoilage. Salt content did not significantly differ among the tested sausages and was around 4.0 g/100 g. As compared to another group of Croatian traditional meat products — dry-cured meat products — these products can be classified as less salty (*Bogdanović et al*., 2016).

The content of sodium, calcium, potassium, magnesium, copper, zinc and iron determined in Croatian dry-fermented sausages is presented in Table 1. A significant difference between sausages under study in regard to the tested minerals was found only for sodium and calcium content. The highest amount of sodium was found in Kulen due to it having the highest salt content. Similarly, the lowest sodium content was found in Slavonian sausage, the salt content of which was the lowest. Differences in sodium content can be linked to the amount of salt added to the stuffing according to different recipes applied to each type of the tested sausages. Calcium content also varied significantly between the tested sausages, with the highest amount determined in Kulen (377.22 mg/kg) and the lowest in Kulenova Seka (281.11 mg/kg). The main source of calcium in these sausages is meat, while spices are added to the stuffing in small amounts. The recipe and the specification of Kulen and Kulenova Seka are the same when it comes to the pork meat category utilised, so that the difference in calcium content is to be attributed solely to the pork origin.

Fatty acid profiles of Kulen, Kulenova Seka and Slavonian sausage are presented in Table 2. The predominating fatty acid in all analysed sausages was oleic acid, with the average values of 42.40 %, 41.71 % and 40.54 % in Kulen, Kulenova Seka and Slavonian sausage, respectively. The highest share of oleic acid is typical of meat (*Dinh et al*., 2021), but can vary across animal species. The only significant differences between the tested sausages were observed for stearic fatty acid (C18:0) and the share of saturated fatty acids on the whole. Fatty acid profile is mainly related to the meat and fat tissue used in sausage production, so that these findings confirm that the same raw materials (i.e., the same pork meat category), were used for the production of the tested sausages.

Table 1. Mineral content (mg/kg) found in dry-fermented sausages from Croatian households

Sausage	Sodium (mg/kg)	Calcium (mg/kg)	Potassium (mg/kg)	Magnesium (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)	Iron (mg/kg)
Kulen	16560.97 ± 2479.57*	372.77 ± 142.08*	5673.59 ± 672.28	319.65 ± 55.76	1.63 ± 0.51	42.57 ± 53.68	17.98 ± 5.53
Kulenova Seka	16179.27 ± 2801.34*	281.11 ± 62.47*	5712.30 ± 1490.31	366.97 ± 71.44	1.83 ± 0.41	39.11 ± 8.86	20.80 ± 6.16
Slavonian sausage	14080.21 ± 2775.30*	318.32 ± 65.18*	5582.62 ± 1168.98	364.80 ± 79.61	1.77 ± 0.47	36.06 ± 8.70	19.90 ± 5.17

The results are expressed as the mean value ± standard deviation; *statistical significant difference ($p<0.05$)

Table 2. Fatty acid profile of the selected Croatian dry-fermented sausages

	Kulen	Kulenova Seka	Slavonian sausage
C10:0	0.07±0.03	0.07±0.04	0.07±0.03
C12:0	0.08±0.01	0.08±0.01	0.08±0.01
C14:0	1.31±0.05	1.33±0.16	1.45±0.45
C15:0	0.05±0.02	0.06±0.04	0.07±0.07
C16:0	24.35±0.93	24.38±1.05	25.07±1.30
C16:1n7t	0.39±0.06	0.42±0.08	0.37±0.08
C16:1n7c	2.59±0.32	2.48±0.37	2.57±0.82
C17:0	0.48±0.10	0.54±0.18	0.54±0.17
C17:1	0.28±0.09	0.28±0.10	0.29±.014
C18:0	12.70±1.07*	13.37±1.34*	14.11±1.82*
C18:1n9t	0.15±0.09	0.19±0.14	0.14±0.13
C18:1n9c	42.40±2.56	41.71±1.67	40.54±2.49
C18:1n7	3.17±0.46	3.00±0.29	2.82±0.41
C18:2n6t	0.12±0.05	0.10±0.07	0.14±0.09
C18:2n6c	9.41±2.63	9.53±1.80	9.32±2.58
C18:3n3 (ALA)	0.40±0.17	0.45±0.16	0.44±0.14
C18:4n3	0.05±0.06	0.06±0.05	0.06±0.08
C20:0	0.22±0.02	0.21±0.02	0.22±0.04
C20:1n9	0.96±0.15	0.90±0.14	0.85±0.19
C20:2n6	0.46±0.10	0.45±0.07	0.44±0.13
C20:3n6	0.05±0.05	0.06±0.05	0.06±0.05
C20:4n6	0.21±0.06	0.24±0.06	0.21±0.08
C20:3n3	0.05±0.07	0.07±0.06	0.05±0.06
C23:0	0.07±0.07	ND	ND
SFA	39.30±1.86*	40.06±2.09*	41.63±3.04*
MUFA	49.93±3.32	48.97±1.96	47.64±3.27
PUFA	10.74±2.95	10.92±2.07	10.72±2.85

The results are expressed as the mean value of the % of total fatty acids ± standard deviation; ND – not detected; LOD – limit of detection = 0.05%; SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids; *statistical significant difference ($p<0.05$)

4. Conclusion

Traditional dry-fermented sausages produced in Eastern Croatia differ in water, ash and fat contents, which can be attributed to the different production processes, especially ripening duration differences. Fatty acid profiles of Kulen, Kulenova Seka and Slavonian sausage are comparable since fatty acids are fat constituents, and the same pork meat categories and fat tissue were used in the production. The differences in sodium content can be attributed to the amount of salt added to the stuffing, while calcium content depends on the pork meat origin. The nutritional quality of Kulen, Kulenova Seka and Slavonian sausage is constant and in agreement with the product specifications.

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References

Bogdanović, T., Pleadin, J., Vahčić, N. & Petričević, S. (2016). Chemical and sensorial properties of fermented meat products. In Zdolec, N. (Ed), *Fermented Meat Products: Health Aspects,* (pp. 359–388), CRC Press.

Dinh, T. T., To, K. V. & Schilling, M. W. (2021). Fatty Acid Composition of Meat Animals as Flavor Precursors. *Meat and Muscle Biology* 5(1), 34, 1–16, https://doi.org/10.22175/mmb.12251

ISO Standard 1442:1997, (1997). Meat and meat products — Determination of moisture content. International Organization for Standardization: Genève, Switzerland, 1997.

ISO Standard 936:1998, (1998). Meat and meat products — Determination of total ash. International Organization for Standardization: Genève, Switzerland, 1998.

ISO Standard 1443:1973, (1973). Meat and meat products — Determination of total fat content. International Organization for Standardization: Genève, Switzerland, 1973.

ISO Standard 937:1978, (1978). Meat and meat products — Determination of nitrogen content. International Organization for Standardization: Genève, Switzerland, 1978.

ISO Standard 12966-2:2015, (2015). Animal and vegetables fats and oils — Gas chromatography of fatty acid methyl esters — Part 2: Preparation of methyl esters of fatty acids. International Organization for Standardization: Genève, Switzerland, 2015.

Kovačević, D., Mastanjević, K., Šubarić, D., Jerković, I. & Marijanović, Z. (2010). Physico-chemical, colour and textural properties of Croatian traditional dry sausage (Slavonian Kulen). *Meso*, 12, 270–275.

Kovačević, D. (2014). Technology of Kulen and Other Fermented Sausages; Faculty of Food Technology, University of J. J. Strossmayer: Osijek, Croatia.

Lešić, T., Vahčić, N., Kos, I., Zadravec, M., Sinčić Pulić, B., Bogdanović, T., Petričević, S., Listeš, E., Škrivanko, M. & Pleadin, J. (2020). Characterization of Traditional Croatian Household-Produced Dry-Fermented Sausages. *Foods*, 9, 990, https://doi.org/10.3390/foods9080990

Ministry of Agriculture, (2018). Regulation on Meat Products. Official Gazette 62/18. Available online: https://narodnenovine.nn.hr/clanci/sluzbeni/2018_07_62_1292.htm

Ministry of Agriculture, (2019). *Slavonian Sausage–Product Specification*; Ministry of Agriculture: Osijek, Croatia, 2019.

Pleadin, J., Krešić, G., Barbir, T., Petrović, M., Milinović, I. & Kovačević, D. (2014). Changes in basic nutrition and fatty acid composition during production of „Slavonski kulen". *Meso,* 16, 487–492.

Pleadin, J., Lešić, T., Kos, I., Krešić, G., Kudumija, N., Dergestin Bačun, L., Vulić, A., Dujić, T. & Vahčić, N. (2021). Sukladnost fizikalno-kemijskih svojstava sa specifikacijama zaštićenih hrvatskih autohtonih mesnih proizvoda. *Meso*, 23(4), 336–344.

Vulić, A., Kudumija, N., Lešić, T., Tanković, S., Jelušić, V., Frizbegović, J. & Pleadin, J. (2021). Chemical composition and fatty acid profile of Alpine and Saanen goat milk from Bosnia and Herzegovina. *Veterinarska stanica*, 52(1), 0-013-21, https://doi.org/10.46419/vs.52.1.12