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Cholesterol content and atherogenicity of fermented sausages made of pork meat from various breeds

Slaviša Stajić[a]*, Dušan Živković[a], Marija Perunović[a], Slađana Šobajić[b], Danijela Vranić[c]

[a]*University of Belgrade, Faculty of Agriculture, Department of Food Technology and Biochemistry,Nemanjina 6, Belgrade 11080, Serbia*

[b] *University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, Belgrade 11221,Serbia*

[c]*Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade 11000, Serbia*

Abstract

Three batches of the Sremska sausage (traditional Serbian fermented sausage) were made: control variant (C) of a Landrace and the other two of primitive breeds: Mangalica (A) and Moravka (B), with the aim of determining the cholesterol content and the content of fatty acids. The cholesterol content in meat, fatty tissue and sausages was determined, in the case of the latter at the beginning of production (day 0), at the end of production (day 14) and after a storage period of the vacuumed product (day 60). The fatty acids content was determined at the beginning and at the end of production. Values were considered significantly different when $p<0.05$. The cholesterol content in meat (mg/100 g of a sample) ranged from 40.58 (B) to 51.54 (C), indicating a significant discrepancy in all samples. As for back fat, there was a notable disparity in cholesterol content between control and primitive breed samples (47.15 (C), 35.54 (B) and 36.41 (A)). The cholesterol content in sausages at the conclusion of the production ranged from 40.41 (A) to 43.33 (B), with no significant difference between the samples. After storage (day 60), the cholesterol content ran from 42.75 (A) to 52.41 (B), with no significant difference only between samples C and A. Fatty acids content was monitored via the index of atherogenicity, and the polyunsaturated/saturated fatty acids ratio. The index of atherogenicity at the end of production was the highest in sample A, 0.61, and significantly differs from other samples, while the lowest value was recorded in sample C, 0.46. PUFA/SFA at the end of production was the lowest in sample A (0.20), with none of the samples exceeding 0.4. No significant advantages of the use of the meat of primitive breeds were noted.

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Keywords: index of atherogenicity; primitive breeds; cholesterol; fatty acids; Sremska sausage

* Coresponding autor, Tel.: +381 11 2615315 ext. 272;
E-mail address: stajic@agrif.bg.ac.rs

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1. Introduction

It has been a recommendation of international medical institutions for several decades that the best way to monitor dietary fat intake is through the quantity of calories and type of fats consumed. In this context, it has been commonly assumed that pork meat and fat have a negative impact on human health, primarily in terms of a higher cholesterol content and the unfavourable composition of fatty acids, which is why consumers were broadly encouraged to enhance their everyday diet by reducing their animal fat intake. It is further advised that cholesterol consumption should not exceed 300 mg per day [1, 2].

Cholesterol levels in blood depend not only on dietary cholesterol, but also on the amount of fat and the fatty acid composition of the diet [3]. More specifically, it appears that saturated fatty acids of 12–16 carbon atoms increase blood total (LDL and HDL) cholesterol concentration and the LDL/HDL ratio, while polyunsaturated ω-6 fatty acids tend to decrease LDL-cholesterol levels, and mono-unsaturated ones are probably essentially neutral with respect to cholesterol [1].

Bearing the above said in mind, the impact of fat on cholesterol concentration can also be observed through the index of atherogenicity (AI), which includes those fatty acids that affect the change of cholesterol (in terms of both increase and decrease).

Obesity and some other chronic diseases of the circulatory system may be prevented by limiting the intake of fat and cholesterol. There also seems to exist a relationship between a high-fat intake, especially of saturated fat, and an increased risk of some cancers (especially colon, breast and prostate cancer) and coronary heart diseases [1, 4].

Man has been making fermented sausages for centuries [5]. From the nutritional viewpoint, sausages are a significant source of proteins of high biological value [4] and of caloric value [1].

Fermented sausages are made of comminuted meat and fatty tissue, with the addition of sugar, salt, nitrates/nitrites, ascorbic acid and various spices (black pepper, paprika, garlic...). The mixture is stuffed into casings of various diameters and then smoked, fermented and dried (ripened) in uncontrolled or controlled conditions (climate chambers) for several weeks. The process is characterised by a series of biochemical transformations related to the development of microflora, the consequences of which are sliceability, structure, colour and flavour of the final product [6].

The most commonly used meats are pork and beef, while countries with Muslim population use sheep, goat and other meats, in addition to beef. Fermented sausages in Europe are mostly made from pork meat and fat and may contain over 40 % of fat, wherefore they are potentially harmful to human health.

The chemical composition of meat and fatty tissue depends on the animal's gender, nutrition, age and breed [7]. Certain data indicates that meat and fat from primitive breeds contain less cholesterol than those of modern breeds. There is also a trend to use the meat of autochthonous breeds (grown in the area where the product is made) for the production of traditional products.

Sremska sausage is an autochthonous traditional sausage home manufactured in the northern, low-lying part of Serbia (Srem region), made of ground (about 8 mm) pig meat and back fat, and mixed with common salt and spices. The mixture is filled in natural pig casings made from small intestines. After being smoked for several days, the product is dried 14–21 days, depending on the conditions in the environment. Traditionally, Sremska sausage was made from the meat of fat pigs of the Mangalica breed, weighing from 160 to 180 kg and up to 12 months of age. This practice was discontinued in the 1960s when this pig breed was completely marginalised by white pigs.

This research aims to determine whether the use of meat and fat tissue of primitive breeds can affect the content of cholesterol and fatty acids in fermented sausages.

2. Materials and Methods

2.1. Sausage manufacture and sampling

Three batches of the Sremska sausage were made. Shoulder and ham meat was used and back fat in the ratio of 75:25. Control variant (C) was of 12-month-old White pigs – Swedish Landrace, and the other two of primitive breeds: 12-month-old Moravka pigs (sample A) and 12-month-old Mangalica pigs (sample B). Meat was processed 24 hours after slaughter and cooling, frozen at the temperature of –20 ^{o}C and stored for 10 days before production. Animals were bred at the test farm of the Institute for Animal Husbandry (Belgrade).

The production process was identical for all variants and carried out on the same day. Meat and fatty tissue were ground in a cutter (Seydelman K60, Germany) to about 8 mm and equal amounts of ingredients were added to all sausage variants: 2.3 % salt, 0.011 % NaNO2, 0.3 % dextrose, 0.20 % garlic and 0.5 % sweet red paprika. Natural casings made of pig intestines (32 mm diameter) were used for filling. The ripening took place in a drying chamber (Maurer, Germany) under controlled conditions and under the following regime: day 1 – relative humidity (RH) 90 % at 21^{o}C, day 2 RH 88 % at 20 ^{o}C with smoking, day 3 RH 85 % at 20 ^{o}C; during the following days RH was reduced daily by 1% at 16 ^{o}C. Fermentation was spontaneous. Once the production was completed after 14 days, the sausages were vacuum-packed and stored at 4–8 °C up to 60 days.

Three sausages were sampled randomly from each batch and examined.

2.2. Determination of cholesterol content

The cholesterol content in meat, fatty tissue and sausages was determined, in the case of the latter at the beginning of production (day 0), at the end of production (day 14) and after a storage period of the vacuumed product (day 60).

Direct saponification method for cholesterol determination was used for cholesterol determination [8] in meat and fatty tissue. To ca. 100 mg of each homogenized meat muscle sample 2 mL of 0.5 M KOH in methanol was added and tubes were vortexed for 30 s. The mixture was directly saponified at 80 °C during 1 h. After cooling, 2 mL of distilled water, saturated with NaCl, were added. The tubes were vortexed for 30 s, followed by the addition of 3 mL diethilether/hexane (1:1, v/v) and were centrifuged for 10 min at 300 g. The upper phase was transferred to a clean tube and the ether/hexane extraction step was repeated twice. All three extracts were combined and evaporated to dryness under a stream of nitrogen. The dry extracts were dissolved in 1000 μL of mobile phase used for HPLC analysis and, after filtration, 10 μl was immediately injected into HPLC.

Cholesterol determination was performed using HPLC/PDA system (Waters 2695 Separation module/Waters photodiode array detector, USA), on a Phenomenex Luna C18(2) reverse/phase column, 150 mm x 3.0 mm, 5μm particle size, with C18 analytical guard column, 4.0 x 2.0 mm, at room temperature. The injected volume was 10 μL. The mobile phase was isopropanol-acetonitrile (20:80 v/v) at a flow rate of 1.2 mL/min, isocraticaly. Detection was performed at 210 nm. Total analysis time lasted 10 min. Quantification of cholesterol was done by external standardization in a linear concentration range from 25 mg/100g to 125 mg/100g. Recoveries of the spiked quantities ranged from 66.30 to 74.80%. Empower Pro software was used to control the HPLC system, as well as for data acquisition and processing.

2.3. Determination of atherogenic index and polyunsaturated/saturated fatty acids ratio

Fatty acids content was monitored via the index of atherogenicity (AI), calculated by the Ulbricht and Southgate formula cited by Vesely et al. [9]:

$$AI = \frac{C12 + 4 \times C14 + C16}{\sum UFA}$$

UFA – unsaturated fatty acids (g/100 g fatty acids)
C12 – lauric acid (g/100 g fatty acids)
C14 – myristic acid (g/100 g fatty acids)
C16 – palmitic acid (g/100 g fatty acids)

Mean values of fatty acids content were used in the calculations.

Also the polyunsaturated/saturated fatty acids ratio (PUFA/SFA) was determined as a ratio of mean values. The fatty acids content was determined at the beginning and at the end of production. The total lipids were extracted from the samples by the modified Bligh-Dyer procedure [10]. Fatty acids were transformed to fatty acid methyl ester by direct transesterification [11]. Fatty acid composition was determined by gas chromatography (GC; VARIAN chromatograph, model 1400; Varian Associates, Walnut Creek, CA), equipped with a flame ionization detector and a 3.0 m × 0.32 cm steel column, packed with LAC-3R-728 (20%; Cambridge Ind. Co., Cambridge, UK) on Chromosorb W/AW(80-100 mesh; Merck, Darmstadt, Germany). Nitrogen was used as a carrier gas (flow rate, 24 mL/min) [12].

2.4. Statistical analysis

Values reported to different parameters resulted from the mean of three sausages, sampled randomly from the same batch. The differences between individual averages were tested using t test. Values were considered significantly different when $p<0.05$. Calculations were done with software Statistica 6.0 PL, for Windows (Statsoft inc.).

3. Results and Discussion

Generally, meat contains a small amount of cholesterol, usually in the range of 100 mg/100 g [13]. The cholesterol content in meat (mg/100 g of a sample) indicates a significant discrepancy in all samples (Table 1).

Table 1. The cholesterol content in meat and back fat of different pig breed (mg/100 g)

variant	meat	back fat
Landrace (C)	51.54±1.23[a]	47.15±3.13[a]
Mangalica (A)	45.20±0.58[b]	36.41±2.18[b]
Moravka (B)	40.58±1.95[c]	35.54±1.31[b]

Values in the same column with different letters are significantly different at P<0.05

Several state-level researches included a large number of samples from retail stores in a number of countries aimed, among other things, at establishing the cholesterol content in various types of meat. Piironen et al. [2] (Finland) received 45–54 mg/100 g for pig meat and stated that similar results were obtained by Honikel et al. (Germany) from 45 to 62 mg/100 g. Buege et al. [14] (USA) reached higher values ranging from 59 (loin chop) to 67 (sirloin chop) mg/100 g, as well as Cormier et al. [15] (Canada) from 59 mg/100 g (tenderloin) to 80 mg/100g (leg inside round).

In their research study, Bragagnolo & Rodriguez-Amaya [3] reported cholesterol content in pork fresh ham of modern pigs to the amount of 49 mg/100 g.

Bearing in mind that our results for the meat of primitive breeds (45.2 (A) and 40.58(B) mg/100 g) are at the bottom level or below the lowest quoted values, and with a presumption that the retail meat originated from modern breeds, it could be presumed that the cholesterol content of observed primitive breeds is lower than that of modern breeds. Bragagnolo & Rodriguez-Amaya [3] report that the cholesterol content in meat and fatty tissue may depend on the animal's age, breed and diet. Contrary to that, Chizzolini et al. [1] state that there are more conspicuous differences between certain parts (muscles) than between breeds or gender. This is illustrated with the data on the average cholesterol content of certain pig muscles: *m. longissimus dorsi* 45.3 mg/100 g, *m. semimembranosus* 49.9 mg/100 g, *m. biceps femoris* 48,6 mg/100 g, neck muscles 62.2 mg/100 g.

There are insufficient data on Mangalica and Moravka. Kovács [16] states that the average cholesterol content in Mangalica is 52 mg/100 g, which is close to our findings (45.2). Petrović et al. [17] find for *m. longisimuss dorsi* of two breeds of Mangalica 61.89 and 62.98 mg/100 g.

Intermuscular and adipose tissue lipids are mainly composed of triacylglycerols and contain a small amount of cholesterol, below 60 mg/100g [13]. It is generally accepted that there is a higher cholesterol content of beef depot fat compared with muscle tissue, but this does not appear to be true for pork [2], which has been affirmed in our case (Table 1). As for back fat, there was a notable disparity in cholesterol content between control and primitive breeds samples (Table 1). Chizzolini et al. [1] reported for back fat 53.6 and 59.3 mg/100g. Our data for primitive breeds is similar to those obtained by Bragagnolo et al. [2] (33 mg/100g), who observed a breed introduced with the aim of offering consumers low-fat and low-cholesterol pork, and which was reared in the pasture, similar to our primitive breeds.

The cholesterol content in fermented sausages may greatly vary depending on the composition of the sausage and its production process (weight loss). Research data on this matter is insufficient. The cholesterol content in sausages at the conclusion of the production process ranged from 40.41 (A) to 43.33 (B), with no significant difference between the samples. After storage (day 60), there were no significant differences only between samples C and A.

Table 2. The cholesterol content in sremska sausages (mg/100 g)

variant	0 day	14 day	60 day
Landrace (C)	40.61 ± 0.79^{a}	41.55 ± 0.87^{a}	44.06 ± 0.11^{a}
Mangalica (A)	36.06 ± 1.48^{b}	40.41 ± 2.25^{a}	42.75 ± 0.74^{a}
Moravka (B)	30.30 ± 1.13^{b}	43.33 ± 0.89^{a}	52.41 ± 0.47^{b}

Values in the same column with different letters are significantly different at $P<0.05$

For salami Milano (produced with shoulder, ham trimmings and belly obtained from experimental animals) Zanardi et al. [18] reported cholesterol content 94.8–110.5 mg/100g. Baggio & Bragagnolo [19] for the Italian type salami reported cholesterol content from 48 to 57 mg/100g and no significant differences between different storage times throughout the entire storage (90 days), which differs from our data. Cholesterol content increased in the final product (14 day) when compared to the beginning of production (0 day), which is normal, considering product weight loss. This change was most pronounced in sample B and may be a result of chemical changes (lower cholesterol oxidation), therefore further research is required.

Petrović et al. [17] reported that kulen (a traditional fermented sausage in Serbia and Croatia) made from the meat of Mangalica breed had a lower cholesterol content then the one made from Moravka (68.84 and 79.62 mg/100 g respectively).

In their study of fermented sausages in Croatia, Pleadin et al. [20], observe that the average cholesterol content of industrial fermented sausages was 58.48–105.24 mg/100g, while that of domestic fermented sausages stood at up to 75.07 mg/100g.

As previously mentioned in the introduction, the level of cholesterol in blood does not only depend on cholesterol intake through food, but also on the ratio between unsaturated and saturated fatty acids. Fatty acid (FA) composition of lipids is important from the nutritional viewpoint, especially the ratio between polyunsaturated fatty acids and saturated fatty acids, the ratio between 'bad' and 'good' FA (AI) and the ratio ω-6/ω-3 FA.

If the AI of certain foods is lower, its atherogenic potential is also lower. The index of atherogenicity at the end of production was the highest in sample A, 0.61, and significantly differs from other samples, while the lowest value was recorded in sample C, 0.46. Index of atherogenicity was not lower in sausages made from the meat and fatty tissue of primitive breeds.

Table 3. **Index of atherogenicity** and PUFA/SFA ratio in Sremska sausages

variant	AI		PUFA		SFA		PUFA/SFA	
	0 day	**14 day**	**0 day**	**14 day**	**0 day**	**14 day**	**0 day**	**14 day**
Landrace (C)	0.42	0.46[a]	21.37±0.12[a]	16.57±0.22[a]	39.42±0.49[a]	41.93±0.51[a]	0.54[a]	0.40[a]
Moravka (B)	0.48	0.51[b]	13.43±0.12[b]	10.64±0.34[b]	40.74±0.67[a]	42.03±0.61[a]	0.33[b]	0.25[b]
Mangalica (A)	0.59	0.61[c]	11.38±0.30[c]	8.69±0.41[c]	43.14±0.75[b]	44.54±0.47[b]	0.26[c]	0.20[c]

Values in the same column with different letters are significantly different at P<0.05

*g/100 g fatty acids

Index of atherogenicity of beef is 0.72, poultry 0.50 and pork 0.60 [21]. Karolyi et al. [22] reported that AI of baby-beef was 0.60 and that it can be changed by diet. They also cited Žlender et al. who reported AI from 0.49 to 0.51 in different muscles of Simmental and Brown bulls.

The PUFA/SFA ratio is nowadays recommended to be above 0.4–0.5 in order to prevent both, an excess of saturated fatty acids with a negative effect on the LDL cholesterol plasmatic level, and an excess of polyunsaturated fatty acids, some of them being precursors of powerful clotting agents and also being involved in the aetiology of some cancers [23].

PUFA/SFA at the end of production (table 3) was the lowest in sample A (0.20), with none of the samples exceeding 0.40. A downfall tendency of the PUFA/SFA ratio during production is conspicuous, and samples from primitive breeds have an unfavourable PUFA/SFA ratio. Such a low PUFA/SFA ratio is a consequence of a significantly lower PUFA in primitive breeds, rather than a higher SFA content.

There is insufficient data on fatty acid content in fermented sausages made from the meat of primitive breeds, but there is some data for meat and back fat. Hollo et al. [24] for *m. semimembranosus* Mangalica reported values for PUFA 14.94 and SFA 32.43 (which makes PUFA/SFA 0.46), for *m. longisimuss dorsi* PUFA 7.37, SFA 36.58 (PUFA/SFA 0.20) and for back fat PUFA 13.90, SFA 40.41 (PUFA/SFA 0.34). Baggio & Bragagnolo [19] ascertained that PUFA/SFA for Italian type salami stood at 0.4 at the end of production and did not change during storage. Research studies, the goal of which was to change the PUFA/SFA ratio by substituting a portion of fatty tissue with linseed oil [23], algae oil [25] or by altering the diet [4, 18], report that the P/S ratio in control variants stood at around 0.40 and can be increased by 0.1–0.3 with the abovementioned treatments.

4. Conclusion

Results of this research indicate a considerably lower cholesterol content in the meat of primitive pig breeds than in the meat of modern breeds. However, these results have not been confirmed for sausages.

Moreover, there are no significant differences in cholesterol content between sausages made from the meat of modern breeds and that of primitive breeds. On the other hand, the AI and PUFA/SFA ratio are more unfavourable in primitive breeds and it has been observed that the higher AI implies a lower PUFA/SFA ratio, that is, sausages made from the meat of primitive breeds have less 'good' and more 'bad' fatty acids. Since the production of fermented sausages requires the meat of older animals, no significant advantages of the use of the meat of primitive breeds were noted; however, further research on this matter is required.

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