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Original Scientific Paper

Fat and fatty acid content as indicators of declared origin and composition of fermented sausage

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ABSTRACT

An attempt to confirm the declared type of meat from which fermented sausages were manufactured was made. The fat content of the sausages was determined by reference method, and the fatty acid composition of fat was analyzed by gas chromatography. The obtained data were used for statistical processing and confirmation of the declared animal origin of the meat. Also, the data obtained by the above methods were used to verify the compliance of the declared composition. Fermented sausages were obtained from retail, manufacturers and importers in the period 2021-2025, and the research included 189 samples, made with pork meat (n= 151), beef (n=15) and mixed pork and beef (n=23). The fat amount in the sausages varied from 23.70 to 54.10% in pork sausages, 34.80 to 46.00% in beef sausages and 34.80 to 49.30% in mixed pork & beef sausages. Fatty acid content ranges were similar to the reported results for the same products. Principal component analysis provided significant information about origin of raw materials used to make fermented sausages.

1. Introduction

Fermented sausages are highly treasured traditional foods that are widely consumed in many countries of the world, especially in Mediterranean, Middle East and Southeast European countries (*Stajic et al.*, 2013, *Paulos et al.*, 2015). A large number of distinct sausages with widely differing properties are produced from various animals, using different recipes and processing procedures often lacking uniformity (*Pleadin et al.*, 2020). Moreover, the sausages are prepared by mincing animal, one or more species, lean and fatty parts, to which are added spices, additives and permitted condiments. Sausages undergo a drying process which is accompanied by a maturation (temperature ranging from 15 to 25°C, relative humidity in the 65-80 % range during the fermentation process) where some biochemical reactions occur that induce the development of particular organoleptic properties of sausages (*Pavli et al.*, 2020). The world consumption of sausages was estimated to 19.58 million kg by 2019 and is expected to continue growing by 3.3 % per year (*Zhu et al.*, 2020). Average annual production of sausages in Serbia in 2023 was 73803 tons (*Statistical Yearbook*, 2024). Nowadays, the demand for these types of products is rising globally. Traditional recipes used for the

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production of traditional fermented sausages greatly differ, not only across production regions, but across producing households as well (*Lešić et al*., 2020).

The sausages are a complex food matrix, composed mainly of water, proteins, lipids, carbohydrates and spices, so therefore, microbial and chemical alterations can occur during their storage (*Tirado-Gallegos et al*., 2021). The high lipid content and packaging in oxygen semi-permeable materials lead to the main deterioration of the sausage lipids by oxidation (not the microbiota), which impacts the food's nutritional and sensory properties *(Bolívar-Monsalve et al.*, 2019). Fats are important from the physiological point of view because they contain a number of important nutrients, like proteins, liposoluble vitamins and essential fatty acids, and are important source of energy.

Nowadays, consumers are increasingly concerned about the link between diet and health in terms of the quality of foodstuffs offered by the food industry, in particular meat and meat products, which have nutritional high value. However, over recent years, fermented sausage consumption has been associated with potential health hazards due to their high contents of fat, saturated fats, high NaCl content, the presence of nitrite and its degradation products such as nitrosamines, and the use of smoking which can lead to the formation of toxic compounds, e.g., polycyclic aromatic hydrocarbons, in the products. The high levels of fat and salt used to process meat products are known to be associated with the development of obesity, cancer, heart related disease and high blood pressure, respectively (*Brewer*, 2012, *World Health Organization*, 2003, *Alshahrani et al*., 2019, *Virtanen et al.*, 2019). Hazards can also be both of direct microbiological nature, the sausages potentially being contaminated with food pathogens, and of indirect microbiological nature by metabolic activity of microorganisms causing presence of biogenic amines and mycotoxins (*Holck et al*., 2017).

According to national regulation on food labelling (*Official Gazette of the Republic of Serbia*, 2024), as well as EU regulation No 1169/2011 (*EU Regulation*, 2011), it is obligatory for nutrition labels to contain information on the presence of energy and specified nutrients, like fat content, saturated fatty acids (SFA), carbohydrates, sugars, proteins and salt. Part B, Annex 12 of the national regulation, and Annex XIII, Part B of the EU regulation, have defined the reference energy and nutrient intakes as 8400 kJ/2000 kcal, and 70 g of total fat per day.

In this study, an attempt was made to confirm the declared type (species) of meat from which the tested fermented dry sausages were produced. The fat content of the sausages was determined and the fatty acid (FA) composition of that fat was analysed (saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA)). The data obtained by these methods of chemical composition analysis were used as a basis for statistical processing and confirmation of the declared animal origin of the meat from which the sausages were made. Also, the data obtained by the above methods were used to verify the compliance of the declared composition.

2. Materials and methods

2.1. Samples

Fermented sausages were part of regular control of food quality and safety parameters, obtained from retail and from producers and importers in the period 2021-2025. The research included 189 fermented sausage samples, made with pork meat (n=151), beef (n=15) and mixed pork & beef (n=23).

2.2. Analytical procedure

Fat content determination was performed according to *ISO 1444*,1996. Briefly, homogenised sausages were dried, and afterwards, dried homogenates were subjected to Soxhlet extraction using n-hexane. Extracted components in n-hexane were dried and measured, based on which, the free fat contents were calculated.

FA determination was performed by gas chromatography according to *ISO 5509* (2000). Fat extract obtained in the previous step by ISO 1444 (1996) was converted to FA methyl esters (FAMEs) by using 0.25 mol·l-1 trimethylsulfonium hydroxide (TMSH) in methanol (*ISO 5509*, 2000), as described by *Spirić et al*., 2010. FAMEs were determined by capillary gas chromatography on a Shimadzu 2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a capillary HP-88 column 100 m × 0.25 mm × 0.20 μm (J&W Scientific, Folsom, California, USA). The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, Pennsylvania, USA).

2.3. Statistical analysis

Principal component (PCA) analysis of fat and FA content of fermented sausages were performed using the JMP Statistical Discovery 10 software (SAS Institute, Cary, North Carolina, USA). Descriptive statistics and preparation of data for multivariate statistical analysis were performed in MS Office 2016 Excel.

2. Results and discussion

Table 1 presents distribution intervals of fat and FA content (SFA, MUFA and PUFA) in the analysed fermented sausages. Table 2 presents distribution intervals of FA, expressed as % of total fatty acids. The number of noncompliant results according to the fat and SFA content as determined in the fermented sausages, and according to the allowed tolerances between analytically determined and declared values of these nutritional parameters, is shown in Table 3.

The amount of fat present in fermented sausages generally varied widely: from 23.70 to 54.10% in pork sausages, 34.80 to 46.00% in beef sausages and 34.80 to 49.30% in pork & beef sausages. Such variations can be attributed to the differences in the amount of added backfat and the choice of more or less fatty meat made by individual manufacturers (*Pleadin et al*., 2014). These presented results are similar to those obtained for fat content in fermented sausages of similar geographic origin (*Branković et al*., 2019, *Simunović et al*., 2021), but were considerably higher than results for three types of Portuguese fermented sausages, alheira, a certified brand with Protected Geographical Indication (*Teixeira et al*., 2020).

The fat content observed was characteristic for the origin of the raw materials, and differences could be attributed to the production processes, which often are region-specific and increase gradually throughout ripening as a result of water loss (*Lešić et al*., 2020; *Kovačević et al.*, 2014; *Soyer*, 2005; *Polumbo et al*, 1976).

Knowledge of the FA profile of the fat in fermented sausages is an important quality attribute. An unfavourable lipid profile of processed meat products is generally associated with harmful cholesterol levels and heart disease. The content of specific fatty acids plays an important role in prevention and treatment of many chronic disorders, especially cardiovascular diseases. The *World Health Organization* (2003) proposed optimal fat intake, which should be between 15-30% of the total energy intake, while the SFA intake should be up to 10%, PUFA intake between 6 and 10% (n-6: 5-8%, n-3: 1-2%), and an intake of less than 1% trans fatty acids.

Table 1. Fat and fatty acid content in fermented sausages

	PORK (g/100g)	BEEF (g/100g)	PORK & BEEF (g/100g)
FAT	23.70 - 54.10	34.80 – 46.00	34.80 - 49.30
SFA	9.10 - 21.42	16.32 - 23.76	13.73 - 18.10
MUFA	11.56 - 25.21	16.39 - 21.38	13.61 - 25.57
PUFA	1.89 – 12.00	0.60 - 3.82	0.07 - 8.08

Table 2. Fatty acid content, expressed as % of total fatty acids in fermented sausages

	PORK (%)	BEEF (%)	PORK & BEEF (%)
SFA	30.39 - 44.16	42.9 - 51.65	36.32 - 43.52
MUFA	39.28 - 54.27	45.78 - 53.39	36.78 - 58.37
PUFA	3.90 - 24.44	1.65 - 9.46	0.16 - 21.51

Table 3. Number of noncompliant fermented sausages

	PORK	BEEF	PORK & BEEF	TOTAL
FAT	8 (5.3 %)	2 (13.3 %)	0	10 (5.3 %)
SFA	7 (4.6 %)	2 (13.3 %)	0	9 (4.8 %)*

*Number in parenthesis denotes percent of samples per each group

Traditional fermented sausages, prepared from pork meat, were earlier reported to contain SFA from 39.30±1.86% to 43.63±3.04%, MUFA from 47.64±3.27% to 49.93±3.32% and PUFA from 10.72±2.85% to 10.92±2.07% as % of total fatty acids (*Vulić et al*., 2023). These data were similar to the current obtained results: 30.39% to 44.16% (SFA), 39.28% to 54.27% (MUFA), and with much wider range for PUFA of 3.90% to 24.44%. The greater variability ranges in our study were very likely a consequence of the considerable variety of fermented sausage types included in our study.

The quality of fermented sausages' raw material is influenced by animals' genotype, their keeping and feeding, and pre- and post-slaughter conditions. Technological processes also influence the properties of the final product (*Pleadin et al*., 2020). Particular significance is due to the processes of fat lipolysis, free fatty acid formation, and degradation and oxidation of short-chain fatty acids, since these key reactions, taking place during ripening, affect the formation of specific odour and taste of the final product (*Kozačinski et al*., 2006; *Miličević et al*., 2014; *Marušić et al*., 2014). Due to the application of various technological processes, the activity of technological microbiota, especially during the fermentation process and maturation of sausage, complex microbiological, physicochemical and biochemical changes take place in fundamental building materials (fats, proteins and carbohydrates), resulting in water loss and increase in dry matter weight (*Kovačević*, 2014).

Fat content was over the permitted tolerance value (declaration/analytically obtained) in eight samples of pork and two beef fermented sausages (5.3% and 13.3%, respectively per group), and SFA contents were beyond permitted tolerance in seven pork and two beef fermented sausages (4.6% and 13.3% respectively per group). Fermented sausages made from beef & pork meat mix were within permitted tolerances of declared values for contents of fat, SFA, MUFA and PUFA.

Principle component analysis results are shown in Figure 1. The first two principal components (PC1 and PC2) accounted for 94.2 % of the data total variance (68.1% and 26.1% respectively). The confidence ellipses (at a 95% confidence interval) showed complete separation of the data for pork & beef sausages. The pork & beef mixed sausages, in the middle, overlapped with both groups, which is consistent with the composition of these products.

From the PCA results, a high degree of correlation of fat, SFA and MUFA content can be observed (*Pećina et al*., 2018). PUFA content can be considered as the main factor for the separation of our sausage sample groups, while the other three factors were responsible for the variability of data within groups. Sausages declared as prepared from only beef or only pork were distinctly separated from each other, except

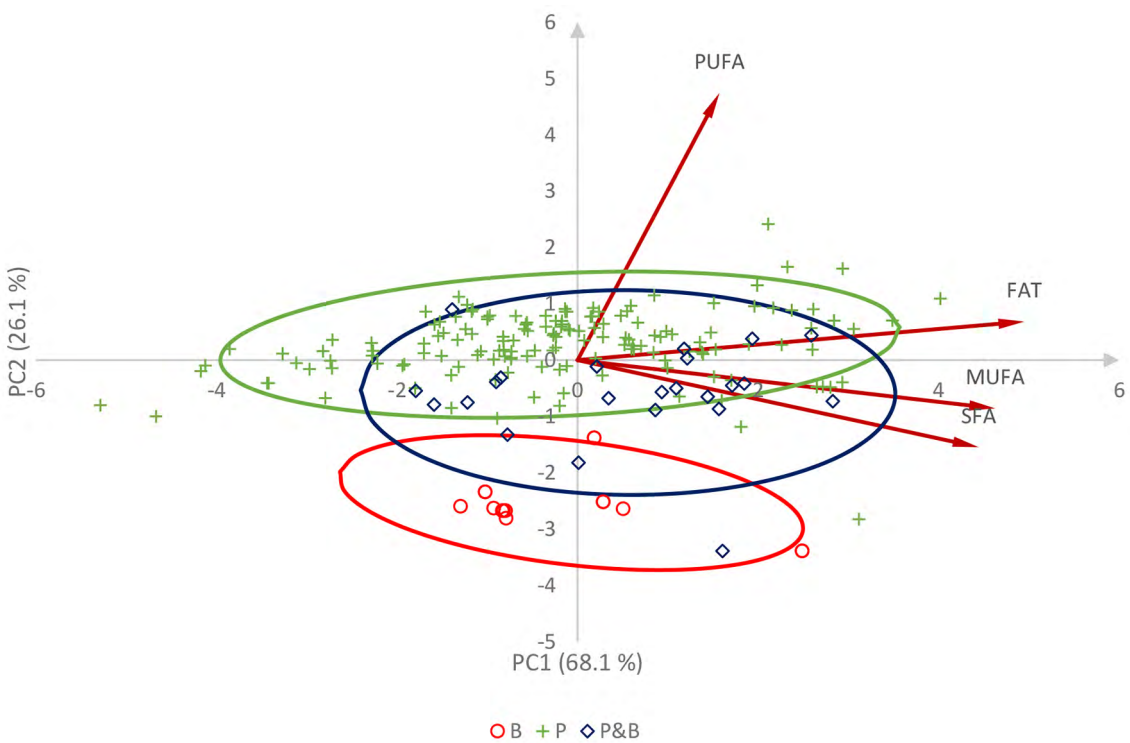

Figure 1. Potential effects of meat-derived BPs on food quality

two sausages, one of which was grouped as beef by our PCA, but was declared as pork only (green cross in lower right quadrant of Figure 1) and one mixed pork & beef (navy blue diamond in the same quadrant). These outlier results could be considered as misdeclaration by the manufacturer for the sausage that was declared as pork only, or, either misdeclaration by manufacturer, or erroneous homogenization of the sample in our laboratory during analysis, in the case of the mixed meat sausage. On the other hand, a sausage declared as made from a mix of pork & beef (navy blue diamond in the upper left part of Figure 1) obviously contained only pork fat, and hence, had a dubious declaration. Declared composition scrutiny based alone on analyses of ingredients cannot achieve such information. Therefore, the introduction of additional, statistical, analysis of the content of measured and declared components is necessary, both to detect economically motivated food fraud and to check the declared composition and nutritional value of products. This is the way to ensure implementation of legal regulative requests, improve the safety and quality of fermented sausages and protect the interests of all subjects in the chain, from manufacturers to retailers to consumers.

3. Conclusion

Along with protein and moisture, fat content is one of the most important quality parameters of dry fermented sausages. The results obtained show that the amount of fat and the FA content in fermented sausages varies widely, but these two parameters were similar to their previously reported contents for similar products. Differences in fat contents could be attributed to the differences in formulations and production processes, which often are region-specific, as well as the nature of the raw materials.

From the point of view of the declared contents of fat and FA, the present results show that fermented sausages sampled from the Serbian market, generally, follow the declared fat and SFA composition. Approximately 5% of all sausages were non-compliant regarding the declared content of both parameters. Fat content analysis showed that the permitted tolerance limit was transgressed in eight pork and two beef fermented sausages (5.3 % and 13.3 %, respectively per group). SFA contents were beyond the tolerance limit in seven pork and two beef fermented sausages (4.6 % and 13.3 % respectively per group). Fermented sausages made from mixed beef & pork meat were in compliance with declared composition of fat and SFA.

PCA results show a high degree of correlation of fat, SFA and MUFA content. PUFA content was main factor for the separation of sausage sample groups. Fat, SFA and MUFA contents were responsible for the variability of data within groups. PCA separated the beef and the pork sausages from each other, with some exceptions. Outlier results could be considered as misdeclaration or, either misdeclaration or errors in sample preparation in the case of a mixed meat sausage. One sausage sample declared as mixed pork & beef, after PCA, was clearly grouped in the pork fat group, and this indicates intentional misdeclaration. This information cannot be achieved without additional, statistical, analysis of the measured content and comparison with the declared components. Thus, it is necessary to implement complementary analytical procedures to detect food fraud, examine the declared composition and nutritional value of products, improve implementation of legal regulative requests, safety and quality of fermented sausages and protect the interests of all subjects in chain, from manufacturers to retailers to consumers.

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