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A Tasic[1], T Mitrovic[1], K Nesic[1], M Pavlovic[1] and J Kureljusic[1]

E-mail: alekstasic79@gmail.com

Abstract. The aims of this study were to determine the nutritional composition (moisture, protein and total fat) of *peglana* sausages produced in eastern Serbia and to analyze the composition of fatty acids. Determination of fatty acid composition in the sausages was performed after ripening and after 20 days of storage. Also, a sample preparation method for fatty acid analysis after simultaneous microwave-assisted extraction-esterification was implemented and results were compared with conventional extraction. The results obtained show *peglana* sausages have high contents of proteins and saturated fatty acids, but no nitrite; the lack of nitrite makes these sausages a suitable product for consumers trying to avoid this additive. The good agreement between results provided by both fat extraction methods demonstrates the usefulness of both methods as routine methods for the treatment of meat samples prior to fatty acid analysis.

1. Introduction

Meat contains micronutrients and all the essential amino acids and minerals, including selenium, vitamin B6 and B12, and vitamin D [1]. *Peglana* sausage is a popular, traditional specialty in Pirot, Serbia, and which has always been a highly appreciated product among the local people because it is prepared from selected top quality meat. Since thermal treatment and smoke are replaced by air drying and an optimum temperature of -5 °C to 5 °C, free from moisture and frost, then it can rightly be said that *peglana* sausage is truly an ecologically friendly product. It is prepared from beef, goat and sheep meat, but pork is never used, because it contributes to quick deterioration of the sausage. An important principle of the traditional production practice is that producers use a minimum of chemical substances during processing. In terms of household production, they do not use additives and preservatives. The production of this traditional product is not standardized and product is generally monitored subjectively without strict control of the characteristics required for *peglana* sausages. However, if hygienic conditions and the intrinsic properties of foods are maintained, these traditional sausages would be considered safe products.

Fats and fatty acids play an important role in giving specific tastes to the different meat species, which is the result of differences in fatty acid profiles in the different animal species [2]. Fatty acids contained in phospholipids are more unsaturated than those found in triacylglycerols. Thus, phospholipids contain relatively large amounts of linolenic and arachidic acids. In recent years, much attention has been paid to developing meat and meat products with physiological functions to promote health conditions and prevent the risk of diseases [3]. Precise and accurate quantification of the fatty acid profile from meat products is extremely challenging, and relies in a multistep process: adequate storage, meaning fatty acids are well preserved; lipid extraction; derivatization of fatty acids; identification and quantification of the derivatized molecules. Ruminant products are characterized by higher content of saturated fatty acids (SFA) and moderate to lower concentration of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) [4]. In general, the analytical procedure for the determination of fatty acids from meat products comprises three steps: extraction of the fats, esterification and gas chromatographic analysis.

Microwave-assisted extraction is a relatively novel method of extracting soluble products from a wide range of solid materials into a liquid state using microwave energy. Recently, much attention has been given to the application of microwave dielectric heating in analytical chemistry because of the

reduced analysis time, simplified manipulation and higher purity of the final products. Several classes of compounds, such as fats and oils [5], polycyclic aromatic hydrocarbons [6] and metals [7], have been efficiently extracted from a variety of matrices.

2. Materials and methods

Peglana dry sausages were prepared from goat and beef meat. Moisture content in the sausages was determined in triplicate by the AOAC (1991) method [8], and expressed as a percentage of the sample weight. Protein content was determined by the Kjeldahl method (Total N × 6.25) [9]. The nitrite content was determined by the spectrophotometric method [10]. Fat was extracted according to the Soxhlet extraction method [11] and using microwave-assisted extraction, and after extractions, the lipid content of the extracts was gravimetrically determined. Five separate replicates of each fat extraction method were prepared on different days and were used to determine the percentage of fatty acids in the total fat content of the sausages.

Fat and fatty acids were extracted using Soxhlet extraction from dry sausages by the hydrolytic method. Fat was extracted into petroleum, and then methylated to fatty acid methyl esters (FAMEs). An aliquot of lipid extract (50 mg) was dissolved in 2.4 mL of n-hexane. A methanolic solution (600 μL) of 2 M sodium methoxide was then added. The mixture was stirred at room temperature for 2 min, then was acidified with a methanolic solution of 1M HCl and extracted with n-hexane (3 mL). FAMEs were quantitatively measured by gas chromatography (GC). The analyses were performed using an Clarus 680 PerkinElmer equipped with a flame ionization detector. The temperature of the column at the beginning was 80 °C and ramped up at 0.5 °C/min, then 4 °C/min to 220 °C, held for 4 minutes, then ramped up at 4 °C/min to 240 °C and held at 240 °C for 10 minutes. The total run time was 56.5 mins. The temperature of both the injection port and the detector was 240 °C. Fatty acids were identified by comparison of their retention times with those of authentic standards (FAME Reference Standard, FAMQ-005, AccuStandard, USA) and reported as percentage of the total fatty acids determined. C19:0 was used as an internal standard. Results were expressed as saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acid (PUFA). The separation of the 37-component FAME standard mixture was performed on a 30 m×0.25 mm ID, 0.25 μm df PerkinElmer Elite-WAX GC column. The FAMES were quantified as a percentage of total methyl esters. Fatty acids were reported as a percentage of total fatty acids determined.

Microwave assisted extraction was carried out using the Start-E microwave extraction system (Milestone, Sorisole, Italy). Microwave assisted extraction conditions were 500 mg of sample, 10 mL of solvent (hexane:methanol 3:1, v/v), microwave extraction during 10 min at 50 °C and 400 W.

3. Results and discussion

The chemical composition of the dry *peglana* sausages studied is shown in Table 1. The *peglana* sausages had high contents of protein (33.0% wet weight) and fat (30.0%). The nutritional value of meat is mainly due to the protein content, which differs according to the location of the muscle in the animal body.

Table 1. Chemical composition of dry *peglana* sausages (mean±standard deviation)

Parameter	*Conventional preparation*	*Microwave-assisted extraction-esterification*
Moisture, %	27.55±0.5	
Fat, %	30.7±0.8	30.0±0.9
Proteins, %	33.0±0.8	
Nitrite, mg/kg	<0.30	

Table 2 shows the fatty acid composition of *peglana* sausages. FAME analysis was used to characterize the lipid fraction in dry *peglana* sausages. The fatty acid composition of fat is a complex

mixture of SFAs, MUFAs and PUFAs with various carbon chain lengths. The 37-component FAME standard mix is designed to mimic the fatty acid composition of many food samples, and it can be used to identify key FAMEs derived from fatty acids in meat products. This mix contains FAMEs ranging from C4:0 to C24:1, including most of the important saturated, monounsaturated, and polyunsaturated FAMEs. FAMEs were identified by comparing the retention times of the chromatographic peaks with those of the FAME standard.

MUFAs were the major constituents of the fat content in the sausages, ranging from 54.72% to 56.35% of the total fat. Octadecenoic (oleic) acid (C18:1), the predominant MUFA, was found in high amounts, ranging from 31.25% to 31.97%. Palmitic acid (16:0) was the major SFA found, ranging from 23.44% to 24.32%. Good separation was obtained between the fatty acids, except for one pair of C18:1 *cis* and C18:1 *trans* isomers, the peaks of which were obscured.

There is evidence that the type of fat is more important than the total amount of fat in the quantification of cardiovascular disease risk. The analysis of fatty acids has become increasingly important in a modern society with dietary recommendations favoring a low intake of fats. Consequently, there is growing interest in monitoring the composition of fatty acids in sausage and determining the PUFA/SFA ratio [12].

Because water has a major functional and quality impact on processed meats, there are numerous regulations controlling the addition and/or final water content of processed meats. The ionic strength of the salt-water phase, for example, is necessary for the solubilization and extraction of the myofibrillar proteins which are responsible for stabilizing fat in emulsion products, binding of muscle pieces in restructured products and producing product textual properties that result from heat-set gelation of the proteins. Oxidative processes in fermented meat products lead to the degradation of unsaturated fatty acids, cholesterol and proteins (including pigments), although the presence of sodium chloride can have an accelerating effect on lipid oxidation [13]. Table 1 shows a small amount of water in our *peglana* sausages, so the drying process was suitable according to the dry matter and the low content of unsaturated fatty acids (Table 2). Meat contains a high percentage of water. The majority of water in muscle is held within the structure of the muscle itself or within myofibril. Water can be divided into three types in muscle, i.e., bound, entrapped (immobilized) or free water. The content of bound water held closely to protein is a very small portion of the total water in muscle cells. Therefore, water significantly affects the structure and quality of meat, not only after slaughter but also during the storage time. In addition, water affects the sensory and textural properties of meat products, since water is a good solvent for the reactions occurring inside the meat, and is a suitable environment for growth of microorganisms.

Goat meat has a fatty acid profile that is beneficial to consumer health due to the high concentrations of oleic acid, the presence of essential fatty acids, and low concentrations of lauric, myristic, and palmitic acids when compared to the meats of other species. The greater amounts of oleic acid (C18:1) in goat meat could be attributed to the greater animal biosynthesis from stearic acid (C18:0) [14]. Therefore, nutritional strategies that increase the conversion of stearic acid into oleic acid would contribute to an improvement in meat quality.

Table 2. Fatty acid profile (% of total fatty acids; mean±standard deviation) of sausages (n=5) using conventional and microwave-assisted extraction-esterification (ME)

Fatty acid		*Conventional preparation*	*Microwave-assisted extraction-esterification*
Myristic acid	C14:0	1.56±0.05	1.50±0.04
Myristoleic acid	C14:1	0.15±0.03	<0.05
Pentadecanoic acid	C15:0	0.43±0.05	0.38±0.06
cis-10-Pentadecanoic acid	C15:1	0.13±0.03	<0.05

Palmitic acid	C16:0	24.32±0.10	23.44±0.10
Palmitoleic acid	C16:1	1.07±0.04	1.04±0.03
Heptadecanoic acid	C17:0	1.05±0.04	1.02±0.07
cis-10-Heptadecanoic acid	C17:1	0.27±0.03	0.22±0.02
Stearic acid	C18:0	28.99±0.5	28.38±0.5
Oleic acid	C18:1 cis(n9)	31.97±0.6	31.25±0.6
Elaidic acid	C18:1 trans(n9)	4.20±0.06	4.11±0.07
Linoleic acid	C18:2 cis (n6)	3.49±0.06	3.20±0.11
Linolelaidic acid	C18:2 trans (n6)	0.21±0.03	0.10±0.03
γ- Linolenic acid	C18:3n6	0.15±0.02	0.13±0.02
α-Linolenic acid	C18:3n3	0.41±0.03	0.36±0.09
Erucic acid	C22:1(n9)	0.87±0.10	1.80±0.09
cis-13,16-Docosadienoic acid	C22:2(n6)	0.72±0.20	3.07±0.10
	ΣSFA	56.35	54.72
	ΣMUFA	38.66	38.42
	ΣPUFA	4.98	6.86
	Total n-6	4.57	6.50
	Total n-3	0.41	0.36
	Total n-9	37.04	37.16

The results showed that microwave-assisted extraction can be used to study lipids from meat samples without the risk of chemical changes during the extraction process. After microwave-assisted extraction, there was no difference in fatty acid content between the conventional method and sample preparation with microwave-assisted extraction, with the one exception of cis-13,16-Docosadienoic acid content. There were several long-chain n-6 PUFAs in the sausages, but unfortunately, there was only a small amount of long-chain total n-3 PUFAs in the sausages. The n-3 PUFAs are not only essential nutrients for humans, but also are significant in helping protect consumers from inflammatory disease, diabetes, some cancers and behavioral disorders. Therefore, increasing n-3 PUFA in meat contributes to improving consumer health, and would help to combat the negative image of ruminant meat [10].

The PUFA/SFA ratio is one of the major parameters currently used to assess the nutritional quality of the lipid fraction of foods. The recommended PUFA/SFA ratio in human diets is >0.4 [16], but in our study, the PUFA/SFA ratios of *peglana* sausage were unsatisfactorily low, 0.087 and 0.125 using conventional and microwave-assisted extraction methods, respectively.

SFA are considered to raise plasma cholesterol, except for stearic acid which reduces total and low density lipoprotein cholesterol; therefore, the content of stearic acid is subtracted from the SFA fraction when the association between food saturated fatty acids and risk of heart diseases is studied. Moreover, MUFA have a hypocholesterolemic effect, but they do not decrease high density lipoprotein cholesterol, which protects against cardiovascular diseases. It is also possible to find trans fatty acids in beef as they are formed as a result of the biohydrogenation by rumen bacteria.

As the product tested was without additives, the nitrate content was below the limit of quantification (Table 1). Nitrite can cause the formation of carcinogenic N-nitrosamines in cured meat products owing to nitrite's reaction with secondary amines and amino acids in muscle proteins [16].

Microwave-assisted extraction of fat could become the method of choice because of its precision, accuracy and speed. Other advantages of microwave-assisted extraction of fat are the reduction of consumption of organic solvents and the shorter time for analysis than is required using the conventional extraction procedure.

4. Conclusion

The aim of the present research was to implement a sensitive and efficient method for simultaneous extraction and determination of fatty acids from dry sausages. The combination of microwave-assisted extraction with esterification was successfully applied to rapid isolation and pre-concentration of the target analytes prior to analysis by gas chromatography-flame ionization detection. The fatty acid composition measured in *peglana* sausages, and obtained by using the simultaneous microwave-assisted extraction-esterification method and the conventional Soxhlet extraction-esterification method, can be regarded as equivalent.

The presented results show that *peglana* sausage has a desirable chemical composition, high percentages of proteins and fats, but an undesirably high PUFA content. These sausages are typically produced in the eastern Serbia and are a significant part of the culinary traditions of this region.

References

[1] Ferguson L 2010 *Meat Sci.* **84** 308−13
[2] Pereira P and Vicente A 2013 *Meat Sci.* **93** 586−92
[3] Kujawska J, Lachowicz K, Sobczak M, Bienkiewicz G, Tokarczyk G, Kotowicz M and Machcińska E 2016 *Czech J. Food Sci*. **34** 87–92
[4] Ribeiro C, Oliveira D, Juchem S, Silva T and Nalério É 2011 *R. Bras. Zootec.* **40** 121−37
[5] Medina A, Oseas da Silva M, Barbosa H, Arruda M, Marsaioli A and Bragagnolo N 2015 *Food Res. Int.* **78** 124−30
[6] Kamankesh M, Mohammadi A, Hosseini H, and Tehrani Z 2015 *Meat Sci.* **103** 61−7
[7] Tasić A, Ignjatović I, Ignjatović Lj, Ilić M and Antić M 2016 *J. Serb. Chem. Soc.* **81**(9) 1081−96
[8] AOAC 1991 *Official Method 950.46 1991 Moisture in Meat*
[9] ISO 1978 *ISO 937:1978 ISO/TC 34/SC 6 Meat and Meat Products – Determination of Nitrogen Content (Reference Method)*
[10] ISO 1975 *ISO 2918:1975 ISO/TC 34/SC 6 Meat and Meat Products – Determination of Nitrite Content (Reference Method)*
[11] ISO 1973 *ISO 1443:1973 ISO/TC 34/SC 6 Meat and Meat Products – Determination of Total Fat Content*
[12] Liguori A, Belsito E L, Gioia M L, Leggio A, Malagrinò F, Romio E, Siciliano C and Tagarelli A 2015 *Open Food Sci. J.* **9** 5−13
[13] Wójciak K M, Karwowska M and Dolatowski Z J 2014 *Meat Sci.* **96** 750−6
[14] Lopes S, Martins S, Chizzotti M, Busato K, Oliveira I, Machado Neto O, Paulino P, Lanna D and Ladeira M 2014 *Meat Sci.* **97** 602-8
[15] World Health Organization – WHO. 2003 *Diet, Nutrition and the Prevention of Chronic Diseases Technical Report Series* **916** (Geneva: World Health Organization) pp 87−8
[16] Wójciak K M, Karwowska M and Dolatowski Z J 2015 *Scientia Agricola* **72** 124−31