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Traditional meat preparations in the Balkans region

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 Abstract. The aim of this study was to determine the fatty acid (FA) profile and trans fatty acid (TFA) content of the Balkan's traditional meat preparations. Twenty-four meat preparations were examined: fresh sausages, ćevapčići, pljeskavica and hamburger. Gas chromatography of FA and TFA was used. Trans fat was higher than the recommended value of 0.5% in ćevapčići, pljeskavica and hamburger (0.54-0.62%) and lower in fresh sausages (0.26%). There were significant differences ($P < 0.05$) in the atherogenicity index (AI), which was lower in fresh sausages (0.58) and ćevapčići (0.64) and pljeskavica (0.64) and highest in hamburger (0.77). Concerning thrombogenicity indices (TI), there was a significant difference ($P < 0.05$), being the lowest in fresh sausages (1.42), ćevapčići (1.28) and pljeskavica (1.40) and the highest in hamburger (1.82). AI and TI of traditional meat preparations were higher than recommended indices, 0.5 and 1.0, respectively, and so are not desirable for health protection. The n-6/n-3 ratio was significantly ($P < 0.05$) lower in pljeskavica (10.76) and hamburger (7.30) compared with ćevapčići (22.25) and fresh sausages (28.41). Promotion of the Mediterranean diet requires changes in the food systems and public health policies to improve overall diet quality of individuals, communities, and populations.

1. Introduction

Fresh sausages are meat products sold fresh without prior heat treatment. In many countries, they are manufactured on request in butcher shops. The basic raw materials used in fresh sausage manufacture are pork and beef, including their trimmings. Fresh pork sausages are a very popular breakfast item in many European and American homes and restaurants and, throughout the years, have been a leading pork product [1, 2]. Technologically, hamburgers are typical fresh beef meat preparations that are not stuffed into casings. Different techniques and formulae are used in manufacturing fresh sausages in various countries. Fresh sausages are mostly stored and sold in chilled form, although some are sold frozen. The frozen product is bought in the shop or supermarket. Fresh sausages are produced according to different quality standards and, in large parts of the world, they are sold very cheaply. It is absolutely critical that the meat and fat materials processed have low microbiological counts. A bacteria count of $10^2–10^4$/g of meat should be achieved; a maximum count of 10^3/g of meat is often seen as the standard in most processing companies [3].

Ćevapčići or ćevap is a grilled skinless Serbian product, found traditionally in the countries of southeastern Europe (the Balkans). They are considered a national dish in Bosnia and Herzegovina and Serbia, and are also common in Croatia, Montenegro and Slovenia, as well as in Albania, North Macedonia, Bulgaria and Romania. The typical portion consists of 5 pieces of small, rolled patties of mixed ground meats that are heavily seasoned, grilled and served on a plate or in a flatbread, usually with chopped onions, yogurt/sour cream, or potato salad [4, 5]. Pljeskavica is a popular meat patty dish, second only to ćevapčići. It is often served with milk cream, ajvar sauce of peppers and urnebes, a mixed spicy cheese spread. Other popular dishes in the Balkans include roasted sausage and hamburger.

Analysis of the Global Burden of Disease Study 2010 shows that dietary factors are the most important factors that undermine health and well-being in every Member State in the WHO European Region [6]. Excess body weight, excessive consumption of energy, saturated fats, *trans* fats, sugar and

salt, as well as low consumption of vegetables, fruits and whole grains are leading risk factors and priority concerns. Overweight and obesity are also highly prevalent among children and adolescents, particularly in southern European countries. The World Health Organization (WHO) defines four factors in the epidemiology of cardiovascular diseases, cancers, obesity and type 2 diabetes mellitus – poor diet, physical inactivity, tobacco and alcohol use – are of overwhelming importance to public health [6]. Childhood obesity, too, is a growing problem across the world, with physical inactivity a major factor. There are clear associations between physical activity and health and between diet and health, and the two relationships are often linked through obesity [6]. The Mediterranean diet abundant in minimally processed plant-based foods, rich in monounsaturated fat from olive oil, but lower in saturated fat, meats and dairy products, seems an ideal nutritional model for cardiovascular health [7].

Desirable total fat intake in the diet, according to most experts, should amount to only 25-30% of the total daily energy intake (E%) [8, 9], provided that 10% of E% in the form of saturated fatty acids (SFAs) would enable the body to completely fulfil its essential metabolic functions. On the one hand, an intake of 0.5 to 1-2% E% of trans fatty acids (TFA) was proposed [8]. However, there was agreement among the experts that in populations with inadequate total E%, such as is seen in many developing regions, dietary fats are an important macronutrient to increase E% to more appropriate levels. The study also showed a higher prevalence of overweight associated with lower socioeconomic status in some countries. There are not enough data on the fat quality of fresh sausages, ćevapčići, pljeskavica and hamburger. The final goal of these studies is to establish and expand food composition and trans fatty acid (TFA) databases as a priority. Therefore, the aim of this study was to determine the fatty acid (FA) and TFA profiles of the Balkan's traditional meat preparations, and to calculate the atherogenic index (AI) and thrombogenic index (TI) of the products.

2. Materials and Methods

2.1. Samples
Twenty-four traditional meat preparations were examined. Traditional meat preparations were purchased from meat companies from Serbia.

2.2 Fatty acid and trans fatty acids analysis by capillary gas chromatography
The total fat content was determined according to ISO standard method 1443:1973. FA and TFA were extracted from traditional meat preparations by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA). Further, fatty acid methyl esters (FAMEs) and TFA were prepared by transesterification using 0.25 M trimethylsulfonium hydroxide (TMSH) in methanol (EN ISO 5509:2000). Separation and quantification of the FAMEs and TFA was carried out using a gas chromatograph (Shimadzu 2010, Japan) equipped with a flame ionization detector and an automatic sample injector and using fused silica capillary column HP-88 (100m, 0.25 mm i.d., 0.2 μm film thickness). The chromatographic conditions were as follows: initial column temperature 125 °C and ending 280 ºC. The injector and detector were maintained at 250 and 280 °C, respectively. Nitrogen was used as the carrier gas at a constant flow-rate of $1.33 \ mL \cdot min^{-1}$. The split ratio was 1:50 and 1 μL of solution was injected. Individual FAMEs and TFA were identified by comparing their retention times with those of authenticated standards (Supelco 37 component FAME Mix, Supelco, Bellefonte, USA). Data regarding FAME composition were expressed in percentage according to the weight of the total identified FAMEs.

2.3. Calculation of daily intake of total fat, saturated fats and trans fat

The rules on labelling and advertising in Serbia [10] and in the US [11] recommend intakes of fats 70 g d^{-1}, saturated fats of 20 g d^{-1}, and trans fat of 40 g d^{-1}. These values are informative for consumers in interpreting nutritional values of food products.

2.4. Statistical analysis

Data obtained for the FA compositions were subjected to analysis of variance (ANOVA) with the Tukey-Kramer HSD test for the comparisons of means at the 5 % level of significance. Statistical analysis was performed using SAS Institute Inc. JMP 10 software.

3. Result and discussion

The FA compositions and TFA of different traditional meat preparations are presented in Table 1.

Table 1. FA composition (g in 100 g) of traditional meat preparations, AI, TI, fat content and TFA

FAs	Fresh sausage (n=6)	Ćevapčići (n=6)	Pljeskavica (n=6)	Hamburger (n=6)
C14:0	1.49 ± 0.16^{NS}	2.11 ± 0.11^{NS}	2.38 ± 0.02^{NS}	2.49 ± 0.09^{NS}
C15:0	0.11 ± 0.02^{B}	0.27 ± 0.02^{AB}	0.27 ± 0.01^{AB}	0.33 ± 0.01^{A}
C16:0	27.50 ± 0.28^{NS}	27.55 ± 0.20^{NS}	27.05 ± 0.02^{NS}	28.76 ± 0.22^{NS}
C16:1	2.49 ± 0.18^{B}	3.40 ± 0.24^{AB}	4.94 ± 0.03^{A}	3.08 ± 0.13^{B}
C17:0	0.41 ± 0.03^{C}	0.64 ± 0.02^{B}	0.66 ± 0.01^{AB}	0.83 ± 0.01^{A}
C18:0	13.39 ± 0.02^{C}	14.65 ± 0.05^{B}	12.38 ± 0.07^{D}	16.94 ± 0.07^{A}
C18:1cis-9	44.28 ± 0.19^{BC}	44.92 ± 0.10^{AB}	45.76 ± 0.08^{A}	43.61 ± 0.15^{C}
C18:1 trans-9	0.45 ± 0.07^{C}	0.96 ± 0.05^{BC}	1.36 ± 0.09^{B}	2.20 ± 0.07^{A}
C18:2n-6	10.13 ± 0.48^{A}	8.91 ± 0.79^{AB}	5.22 ± 0.07^{BC}	2.63 ± 0.06^{C}
C20:0	0.23 ± 0.01^{A}	0.23 ± 0.01^{A}	0.12 ± 0.01^{B}	0.15 ± 0.07^{B}
C18:3n-6	0.03 ± 0.01	nd	nd	nd
C18:3n-3	0.43 ± 0.02^{NS}	0.36 ± 0.01^{NS}	0.44 ± 0.01^{NS}	0.34 ± 0.01^{NS}
C20:1	0.45 ± 0.03^{A}	0.49 ± 0.04^{A}	0.17 ± 0.01^{B}	nd
C20:2n-6	0.30 ± 0.03^{A}	0.19 ± 0.02^{AB}	0.04 ± 0.01^{B}	0.05 ± 0.01^{B}
C22:0	0.03 ± 0.01	nd	nd	nd
C20:3n-6	0.04 ± 0.01^{NS}	0.03 ± 0.01^{NS}	0.05 ± 0.01^{NS}	0.04 ± 0.01^{NS}
C22:1n-9+C20:4n-6	0.10 ± 0.01^{A}	0.09 ± 0.01^{A}	0.04 ± 0.01^{B}	0.06 ± 0.01^{AB}
C20:5n-3	nd	nd	0.05 ± 0.01^{NS}	0.03 ± 0.01^{NS}
C22:5n-3	nd	0.09 ± 0.01	nd	nd
SFA	43.21 ± 0.44^{B}	46.34 ± 0.50^{AB}	42.33 ± 0.35^{B}	49.08 ± 0.38^{A}
MUFA	46.53 ± 0.20^{C}	47.87 ± 0.04^{BC}	51.03 ± 0.14^{A}	48.27 ± 0.22^{B}
PUFA	11.66 ± 0.56^{A}	6.60 ± 0.36^{B}	5.55 ± 0.21^{B}	3.50 ± 0.20^{B}
n-3	0.43 ± 0.14^{NS}	0.36 ± 0.19^{NS}	0.49 ± 0.03^{NS}	0.49 ± 0.03^{NS}
n-6	10.49 ± 3.05^{A}	5.55 ± 2.58^{B}	5.31 ± 0.42^{B}	5.31 ± 0.41^{B}
n-6/n-3	28.41 ± 7.42^{A}	22.25 ± 8.83^{A}	10.76 ± 0.82^{B}	7.30 ± 1.40^{B}
AI	0.58 ± 0.10^{B}	0.65 ± 0.09^{B}	0.64 ± 0.05^{B}	0.77 ± 0.02^{A}
TI	1.42 ± 0.13^{B}	1.28 ± 0.51^{B}	1.40 ± 0.05^{B}	1.82 ± 0.03^{A}
Fat	23.28 ± 1.23^{A}	22.43 ± 0.02^{AB}	15.48 ± 0.45^{BC}	11.20 ± 0.34^{C}
TFA	0.45 ± 0.14^{C}	0.96 ± 0.13^{BC}	1.35 ± 0.46^{B}	2.30 ± 0.90^{A}

*n, number of samples; results are represented as mean ± SEM; nd = not detected <0.01%. Values in the same row with the same letter are not significantly different (P≥0.05); NS – not significant; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI – atherogenic index; TI – thrombogenic index; TFA – trans fatty acids

Generally, traditional meat preparations were characterized by higher contents of SFAs. The monounsaturated fatty acid (MUFA) content of the traditional meat preparations was the second most predominant fatty acid group, with oleic acid (C18:1n-9) being the most common MUFA. The most common n-6 polyunsaturated fatty acid (PUFA) was linoleic acid (C18:2n-6). The most common n-3 PUFA was α-linolenic acid (ALA, C18:3n-3), which occurred in lower amounts (Table 1). Fat E% was higher in fresh sausages (33%) and ćevapčići (32%) (Table 2) than the recommended value of 25-30% [8, 9]. Saturated fats E% (27-50%) was higher than the recommended value of 10% [8, 9]. Trans fat was also higher than the recommended value of 0.5% for ćevapčići, pljeskavica and hamburger (Table 2), and lower in fresh sausages [8, 9]. Pljeskavica (33%) had similar E% (33.90%) to fine minced pork cooked sausages [12]. Hamburger was a low fat meat with high levels of SFA E% (27%), which is similar to beef luncheon meat (23%), and with high levels of trans fat E% (0.62%) [13]. Fresh sausage (50%) was similar in SFA E% (53%) to pork liver pâté [13].

The lipid indices of AI and TI, calculated from the data on the FA composition, are shown in Table 1 according to equations of Ulbricht and Southgate [14]. These lipid indices indicate the suitability of food for prevention of cardiovascular disease in humans, and for any health benefit, they have to be low. In terms of human health, AI and TI less than 0.5 and 1.0, respectively, are recommended in the diet [15]. There were significant differences (P < 0.05) in the AI between traditional meat preparations we examined, being the lower in fresh sausages (0.58), ćevapčići (0.64) and pljeskavica (0.64) and the highest in hamburger (0.77). The AIs of these Balkan products were higher than in study of pork meat (0.51-0.54) [16] and pork meat reported by Kasprzyk et al. [17] (0.47) but lower than in rabbit (0.90) [18]. Concerning TI values, there was a significant difference between traditional meat preparations (*P* < 0.05) being the lowest in fresh sausages (1.42), ćevapčići (1.28) and pljeskavica (1.40) and highest in hamburger (1.82). The TI values we obtained are lower than were reported in lamb (0.87) [19], chicken (1.14) and turkey meat (0.91) [20, 21]. However, AI and TI of traditional meat preparations were higher than recommended indices [13] and are not desirable for health protection.

The n-6/n-3 fatty acid ratio in fresh sausages was above the recommended levels of 4:1 [22] (Table 1). The n-6/n-3 ratio was significantly (*P* < 0.05) lower in pljeskavica (10.76) and hamburger (7.30) compared with ćevapčići (22.25) and fresh sausages (28.41). However, the n-6/n-3 ratio in fresh sausages and ćevapčići were higher than in pâtés (4.01-21.86), cooked chicken sausages, chopped canned meats (8.55-14.98) [13], pork canned meat pieces (13.80) [12] and pork meat (19.62-20.88) [16]. The estimated percentage of fat daily intake, saturated fat daily intake and trans fat for products is presented in Table 2.

Table 2. Percentage of total fat, saturated fat derived and trans fat from traditional meat preparations in relation to the reference intake of 2,000 kcal per day

Daily intake	Fresh sausages	Ćevapčići	Pljeskavica	Hamburger
Fat E%	33	32	22	16
Saturated fat E%	50	52	33	27
Trans fat E%	0.26	0.54	0.53	0.62

Legend: E% - energy value/intake

By the linear discriminant analysis (LDA), the separation between traditional meat preparations might be improved. LDA clearly differentiates the sampled traditional meat preparations into four groups (Figure 1).
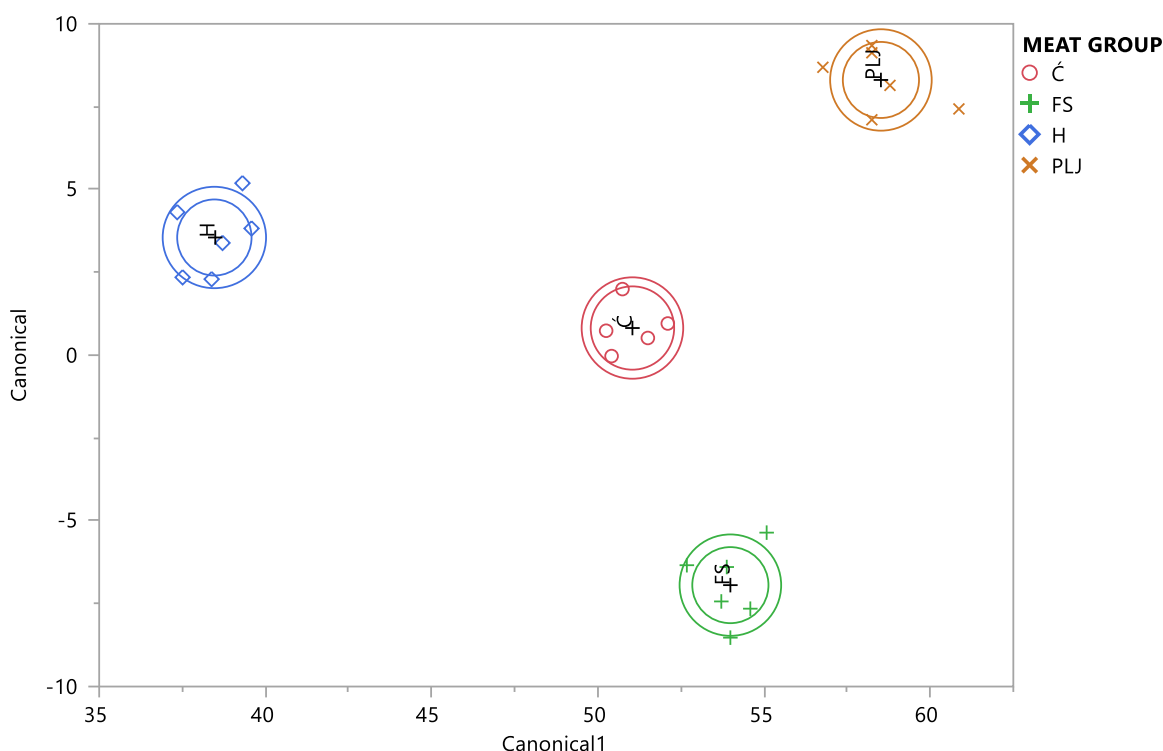
Figure 1. Linear discriminant analysis (canonical plot) of fatty acid profiles of traditional meat preparations

Legend: Ć – ćevapčići/ćevap, FS – fresh sausages, H – hamburger, PLJ –pljeskavica

The results of the classification are very satisfactory and allow 100% of traditional meat preparations to be correctly grouped. Out of 24 samples of traditional meat preparations, all 24 samples were classified according to the fresh meat origin. Data were expressed as discriminant scores along 2 eigenvectors, as a function of group provenance regarding FA content. All FAs herein considered had a direct influence on the differentiation of group origin. The first discriminant eigenvalue explained 61.3% of the total variance and the second eigenvalue explained 33.8% of the total variance. The established Wilks value was equal to 0.0005 ($P < 0.0001$). By canonical correlation, the first and the second discriminant functions were 0.993 and 0.987, respectively. The shortest distance between the points on the canonical plot in Figure 1 represents the smallest differences in the FA profiles of the samples. Pljeskavica was very distant from hamburger, ćevapčići and raw sausages and hamburger far from pljeskavica, ćevapčići and fresh sausages; these results correlate to the type of meat. Based on stepwise variable selection, the fat content of fresh meat did not influence the separation of samples ($P > 0.05$). Our results confirm the statement of other authors [23, 24]. They conclude that the region of South-Eastern Serbia has a population of lower socio-economic status, potentially coupled with malnutrition and poor dietary habits. The results obtained indicate a high intake of saturated fats from consumption of traditional meat preparations in the region. Although this dietary pattern does not appear hazardous, improving nutritional habits is desirable. Education and national/regional policies for advancement of dietary quality in Balkan regions should promote complex carbohydrates and relevant food choices such as whole grains, legumes, fruits and vegetables; as well as optimize n-3 balance through increased intake of fish, seafood, nuts and green vegetables.

4. Conclusion

People in the Balkan region want to eat fresh sausages, pljeskavica, ćevapčići and hamburger. Our research shows these traditional products are full of saturated fat. Although bad food does not really

exist, bad eating habits can impair health in the long-term. The Balkan region has a population of lower socio-economic status with poor dietary habits. There is a lot of strong evidence to support the benefits of the Mediterranean diet on cardiovascular health. Promotion of the Mediterranean diet requires changes in the food environment, the food systems, and public health policies to improve overall diet quality of individuals, communities, and populations.

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