





Fatty acid composition of beef patties with replacement of beef fat by walnut oil and cake

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ABSTRACT

This study investigated the effects of replacing beef fat with walnut oil and walnut cake on the fatty acid composition and nutritional value of beef patties. Nine groups were prepared: four with differing levels of walnut oil, four with walnut cake, and one control without substitution. Fatty acid composition was determined by gas chromatography, and nutritional indices were calculated. Substitution with walnut derivatives reduced saturated fatty acids (SFA) and increased unsaturated fatty acids (UFA), including both monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), with a marked increase in oleic acid (C18:1 n-9) and linoleic acid (C18:2 n-6). Beef patties composed with walnut oil showed a more favourable profile than those with cake, as reflected in lower atherogenic (AI) and thrombogenic indices (TI), and higher values of the hypocholesterolaemic/hypercholesterolaemic ratio (HH), nutritional value index (NVI), and health-promoting index (HPI). The best results were observed in beef patty BP4 (100% fat replacement with walnut oil), which had the highest UFA and PUFA/SFA ratio, and the lowest AI and TI values.

1. Introduction

Burger patties are semi-products made from minced and shaped meat, most commonly from beef and lamb, while pork is used less frequently (Smajić, 2014). According to the *Rulebook on minced meat, semi-products, and meat products* (BiH, 2013), a burger patty is produced by shaping various types of minced fresh meat from domestic ruminants, with the possible addition of other ingredients, such as up to 10% minced onion. Together with ćevapi, pljeskavica is an essential part of local traditional cuisine in Bosnia and Herzegovina. Pljeskavica is considered an extension of the burger, as it contains

more additions and is sometimes referred to as the “poor burger” (Operta, 2016).

Meat products such as patties and hamburgers have gained immense popularity and are considered symbols of the fast-food industry. When grilled, the patty develops a brown crust on the surface while retaining a juicy interior, which can be filled with ingredients such as cheese, ham and onions (Stajić *et al.*, 2024). These products typically contain significant amounts of animal fat, which is high in cholesterol and saturated fatty acids. A high intake of these components, along with a low ratio of polyunsaturated to saturated fatty acids (PUFA/SFA), is recognized

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as a risk factor for coronary heart disease (Pleadin *et al.*, 2016). Accordingly, producers increasingly seek to optimize the fatty acid profile of meat products to enhance their nutritional value and consumer acceptability (Pleadin *et al.*, 2015). One key strategy involves increasing the PUFA/SFA ratio, often by replacing animal fat with vegetable oils. Unlike animal fats, vegetable oils are cholesterol-free and naturally rich in unsaturated fatty acids. Numerous studies have investigated nutritional enhancement of meat products through the inclusion of various vegetable oils such as flaxseed, olive, sunflower, walnut, soybean, sesame, grape seed, avocado, and hemp oil. Improvements in the nutritional profile of beef patties have been reported through the replacement of animal fat with walnut oil (Fadiloglu *et al.*, 2023; Botella-Martinez *et al.*, 2021; Lucas-Gonzalez *et al.*, 2023), sesame oil (Abdul Aali *et al.*, 2021) and flaxseed oil (Anwar *et al.*, 2023). Walnut oil (*Juglans regia* L.) is especially valued for its health benefits and favourable fatty acid profile, being rich in linoleic, linolenic and oleic acids (Saxena *et al.*, 2009; Hussain *et al.*, 2021). Additionally, walnut oil is a source of carotenoids, fat-soluble vitamins, and essential minerals such as zinc, copper, iodine, calcium, magnesium, iron, phosphorus, and cobalt (Popovici *et al.*, 2014). It also contains secondary metabolites like tocopherols, flavones, and polyphenols, which have antioxidant, antidiabetic, anti-inflammatory, and anticancer effects (Yang *et al.*, 2022). Its consumption has been linked to improved lipoprotein profiles and a reduced risk of cardiovascular disease and certain cancers (Rabrenović & Gajić-Krstajić, 2010). The aim of this study was to evaluate the fatty acid composition of beef patties in which beef fat was partially or completely replaced by walnut oil and walnut cake.

2. Materials and methods

2.1 Production and sampling

Walnuts used for oil and cake production originated from the Gajzenhajm-139 variety grown in Bosnia and Herzegovina and were purchased locally. Walnut oil and cake were produced in the laboratory; after removing shells and impurities, the walnuts were pressed using a manual screw press (Piteba, Netherlands). The oil was filtered, and the cake was dried at room temperature. Beef muscle tissue (shoulder and neck) and adipose tissue were collected immediately after slaughter from the Malak farm (Kakanj, Bosnia and Herzegovina). Meat and

fat were ground separately, both using a grinder attachment with a 3.8 mm hole diameter (Bosch, Germany). The ratio of meat to fat in the mixture was 80:20. Common food additives such as salt (2.0%), ground black pepper (0.10%), and baking soda (0.30%) were added, all sourced from a local store. Ground beef tissue was divided into nine parts corresponding to experimental groups with different levels of replacement of beef fat with walnut oil or cake. The sample codes and composition are as follows: BP CON – control with 100% beef fat; BP1, BP2, BP3 – 15%, 30% and 45% replacement with walnut oil; BP4 – 100% replacement with walnut oil; BP5, BP6, BP7 – 15%, 30% and 45% replacement with walnut cake; BP8 – 100% replacement with walnut cake. After adding the food additives, mixing and re-grinding to achieve homogeneity, the mixtures were cooled at 4°C for 24 hours. Subsequently, the appropriate walnut products were added according to the formula. Patties were formed into balls weighing 120–125 g and mechanically shaped (Maxima, Netherlands). Frying was performed on an induction cooktop (Easyline, Italy) with minimal use of sunflower oil until the internal temperature of the patties reached 78°C.

2.2 Methods

2.2.1 Determination of fatty acid composition

Methyl esters of fatty acids were prepared according to the standard method (BAS EN ISO, 2018), which involves dissolving glycerides in iso-octane and transesterification using a methanolic solution of potassium hydroxide. The fatty acid composition was determined by gas chromatography. The fat content in the analyzed samples of beef patties was determined using the reference method (BAS ISO, 2007), which involves fat extraction by the Soxhlet method using organic solvents. The extracted fat was then used for the determination of fatty acid composition. Approximately 60 mg of the extracted fat was weighed into a glass test tube with a glass stopper, followed by the addition of 4 mL of iso-octane. After the fat was dissolved, 200 µL of methanolic potassium hydroxide solution was added. The mixture was vigorously shaken twice for 30 seconds and then left to react at room temperature. Once the mixture clarified, a glycerol layer separated at the bottom of the test tube. To neutralize the mixture, 1 g of sodium hydrogen sulphate monohydrate was added. The solution was again shaken vigorously twice for 30 seconds. After the crystals settled, 500 µL of the

resulting solution was transferred into an injection vial, followed by the addition of 1 mL of iso-octane. The vial was then sealed and shaken. The fatty acid composition was determined using a Shimadzu QP2010 gas chromatograph with a mass spectrometer and a Supelco SP-2560 capillary column (100 m × 0.25 mm × 0.20 µm). Chromatographic conditions were as follows: injector and detector temperatures were 220°C, column temperature was 170°C, and the injection volume was 1 µL. Helium was used as the carrier gas at a flow rate of 0.9 mL/min. Individual fatty acids were identified by comparing their retention times with those of pure commercial standards. The content of fatty acids was expressed as a percentage of the total identified fatty acids.

2.2.2 Determination of nutritional indices

Based on the fatty acid composition, nutritional indices were calculated for the analyzed beef patties, including the atherogenic index (AI), thrombogenic index (TI), and the hypocholesterolaemic/hypercholesterolaemic index (HH). The nutritional indices were calculated according to *Guimarães et al.*, (2013).

2.2.3 Statistical data analysis

Statistical data analysis was performed using the software Past 3.15 (*Hammer et al.*, 2001). Multivariate data analysis was conducted using principal component analysis (PCA). The principal components are intended to explain the variability of the data and visually present the relationships among the samples within the dataset.

3. Results and discussion

Fatty acid analysis (Table 1) showed that UFA predominated in all beef patties. The highest UFA level was found in BP4 (85.98%), where beef fat was fully replaced with walnut oil, consistent with its naturally high UFA content. The lowest UFA level was in BP CON (70.17%) due to the high SFA content in beef fat. Oil-based patties had more UFA than cake-based ones, reflecting the higher UFA concentration in pure oil compared to the cake, which also contains protein and fibre. Among the cake-based patties, BP8 had the highest UFA content (79.07%), and BP5 the lowest (71.70%), likely due to lower fat replacement. *Fadiloglu et al.*, (2023) reported 75.44% UFA in patties with 50% fat replacement using walnut oil, lower

than all our oil-based patties (BP1–BP4), supporting the effectiveness of full replacement. Within UFA, MUFA were more prevalent than PUFA. BP4 had the highest MUFA content (59.95%), and BP CON the lowest (54.25%). Oil-based patties showed slightly higher MUFA than cake-based ones, indicating a stronger enrichment effect from direct oil addition. Oleic acid was the dominant MUFA, ranging from 56.90% (BP4) to 51.97% (BP CON), in line with previous studies. *Fadiloglu et al.*, (2023) reported much lower MUFA content (29.83% and 33.26%), further confirming the success of our fat replacement. The rise in oleic acid mirrored the proportion of walnut oil, which is naturally rich in this fatty acid. The PUFA level ranged from 15.92% (BP CON) to 26.03% (BP4), with linoleic acid as the most abundant. Oil-based patties had higher PUFA and linoleic acid levels than cake-based ones, due to greater availability of polyunsaturated acids in oil. Among cake-based patties, BP8 (100% replacement) had the highest linoleic acid (15.42%), and BP5 the lowest (13.34%), matching the fat replacement level. These trends align with earlier findings showing PUFA increases with higher plant-based ingredient proportions. *Fadiloglu et al.*, (2023) reported linoleic acid levels of 39.27% and 41.46% in their patties, likely higher than in ours due to differences in formulation and raw materials.

The SFA content was highest in BP CON (29.82%) and lowest in BP4 (13.96%), reflecting the fat type, with animal fat being naturally richer in SFA than vegetable oil. Cake-based patties (BP5–BP8) had intermediate SFA levels, lower than BP CON but higher than oil-based ones, due to residual oil and other matrix components in the cake. These results confirm the SFA-reducing effect of both walnut oil and cake, with oil showing a stronger impact. *Fadiloglu et al.*, (2023) reported SFA values of 24.56% and 31.05% in their patties, supporting the trend of reduced SFA with fat substitution. Palmitic acid was the main SFA, highest in BP CON (14.17%) and lowest in BP4 (7.87%), decreasing with greater walnut oil inclusion, consistent with previous findings on vegetable oils. Stearic acid followed the same trend, from 11.47% (BP CON) to 4.83% (BP4). These reductions align with studies showing lower SFA with increased plant-based fat. *Fadiloglu et al.*, (2023) found comparable stearic acid levels (11.68%) at 50% walnut oil replacement in patties, further supporting our results. In our patties, PUFA/SFA ratios ranged from 0.53 to 1.86, all exceeding the recommended minimum of 0.40

(UK Department of Health, 1994; Wood *et al.*, 2004, 2008), indicating good nutritional quality. The highest ratio was in BP4 (100% walnut oil), supporting prior evidence of improved fatty acid profiles with plant-based fat substitution. Patties with walnut cake had lower PUFA/SFA ratios than oil-based ones, but still met nutritional standards. A similar pattern was seen in the ω -6/ ω -3 ratio, which declined as walnut oil or cake increased. The lowest ratio was in BP4

(5.02), a significant drop from the control (22.76). This supports the nutritional benefit of plant-based fats, as recommended ratios range from 1:1 to 4:1 (Simopoulos, 2002, 2004; Wood *et al.*, 2004, 2008). Cardiovascular indices also improved with fat replacement. BP4 had the lowest AI (0.10) and TI (0.24). Fadiloglu *et al.*, (2023) reported similar improvements using walnut oil. Though cake-based patties had slightly higher AI and TI than oil-based

Table 1. Fatty acid composition of beef patties

FATTY ACIDS (%)	BP CON	BP 1	BP 2	BP 3	BP 4	BP 5	BP 6	BP 7	BP 8
C14:0	1.08	1.02	0.82	0.65	0.25	0.94	0.80	0.66	0.39
C15:0	0.67	0.58	0.41	0.39	0.25	0.54	0.53	0.40	0.26
C16:0	14.17	11.60	10.20	9.14	7.87	13.48	13.07	12.60	10.98
C17:0	1.35	1.02	0.82	0.65	0.51	1.35	1.07	0.66	0.39
C18:0	11.47	7.25	6.53	5.87	4.83	11.05	10.67	9.95	8.63
C20:0	1.08	0.73	0.54	0.39	0.25	0.94	0.80	0.66	0.26
C14:1	0.13	0.15	0.14	0.26	0.25	0.27	0.40	0.53	0.65
C16:1	1.21	0.58	0.82	1.04	1.14	1.21	1.33	1.59	1.96
C17:1	0.67	0.58	0.54	0.78	1.02	0.81	1.07	1.13	1.31
C18:1 n-9	51.97	56.84	54.67	55.48	56.90	52.43	52.67	53.32	55.03
C22:1 n-9	0.27	0.15	0.27	0.52	0.64	0.40	0.53	0.66	0.78
C18:2 n-6	13.23	15.95	18.36	18.41	19.30	13.34	13.47	13.93	15.42
C18:3 n-3	0.67	2.18	3.81	4.05	4.32	1.08	1.20	1.33	1.44
C20:4 n-6	2.02	1.45	2.04	2.35	2.41	2.16	2.40	2.52	2.48
SFA	29.82	22.20	19.32	17.09	13.96	28.30	26.94	24.93	20.91
UFA	70.17	77.88	80.65	82.69	85.98	71.70	73.07	75.07	79.07
MUFA	54.25	58.30	56.44	57.88	59.95	55.12	56.00	57.29	59.73
PUFA	15.92	19.58	24.21	24.81	26.03	16.58	17.07	17.78	19.34
ω -6	15.25	17.40	20.40	20.76	21.71	15.50	15.87	16.45	17.90
ω -3	0.67	2.18	3.81	4.05	4.32	1.08	1.20	1.33	1.44
PUFA/SFA	0.53	0.88	1.25	1.45	1.86	0.58	0.63	0.71	0.92
MUFA/SFA	1.82	2.63	2.92	3.39	4.29	1.95	2.08	2.30	2.86
ω -6/ ω -3	22.76	7.98	5.35	5.13	5.02	14.35	13.22	12.37	12.43
AI	0.26	0.20	0.17	0.14	0.10	0.24	0.22	0.20	0.16
TI	0.73	0.45	0.35	0.30	0.24	0.66	0.62	0.57	0.46
HH	4.45	6.06	7.16	8.20	10.21	4.79	5.01	5.36	6.54
NVI	4.48	5.53	6.00	6.71	7.84	4.71	4.85	5.02	5.80
HPI	3.79	4.97	5.98	7.05	9.69	4.16	4.49	4.93	6.30

SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; AI – atherogenic index; TI – thrombogenic index; HH – hypocholesterol/hypercholesterol index; NVI – nutritional value index; HPI – health promoting index; BP CON – beef patty with 100% animal fat; BP1 – beef patty with 15% of animal fat replaced by walnut oil; BP2 – beef patty with 30% of animal fat replaced by walnut oil; BP3 – beef patty with 45% of animal fat replaced by walnut oil; BP4 – beef patty with 100% of animal fat replaced by walnut oil; BP5 – beef patty with 15% of animal fat replaced by walnut press cake; BP6 – beef patty with 30% of animal fat replaced by walnut press cake; BP7 – beef patty with 45% of animal fat replaced by walnut press cake; BP8 – beef patty with 100% of animal fat replaced by walnut press cake.

ones, they still outperformed the control, confirming benefits even with partial replacement. The HH ratio (4.45–10.21) and NVI (4.48–7.84) rose with increasing walnut oil or cake, with BP4 again showing the best values. This is consistent with literature on plant oil enrichment. The HPI, a key fat quality index, was also highest in BP4 (9.69) and lowest in BP CON (3.79), confirming a significantly improved lipid profile. Overall, BP4 showed the most favourable nutritional profile across all indices (AI, TI, HH, NVI, HPI), due to full replacement of animal fat with walnut oil, rich in unsaturated fatty

acids, particularly ω -3 and ω -6. As a result, BP4 had the lowest atherogenic and thrombogenic potential and the highest values for health-promoting indices.

3.1 Principal component analysis (PCA) of the fatty acid composition of beef patties

Principal component analysis (PCA) was conducted based on a correlation matrix including 28 parameters for 9 groups of beef patties in which beef fat was partially or completely replaced with walnut oil and cake.

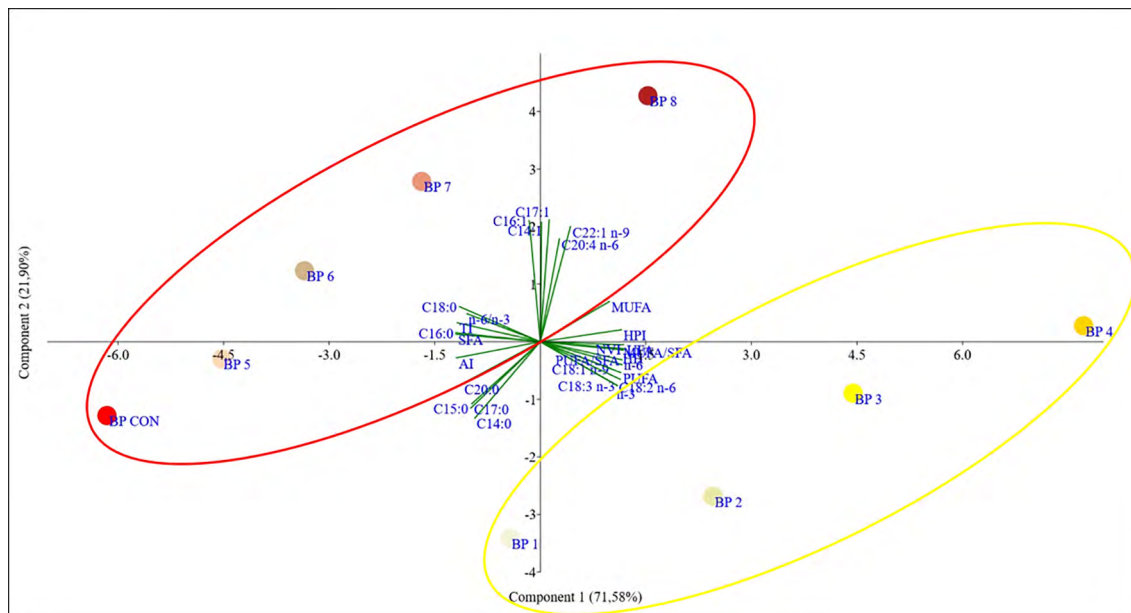


Figure 1. Distribution of acrylamide content category and potato cultivar in 2024

Figure 1 shows that PCA grouped the beef patties according to the type and level of beef fat replacement-walnut oil (yellow ellipsoid) or walnut cake (red ellipsoid). The control group (100% beef fat) was positioned on the left side of the plot and showed the highest levels of SFA, ω -6/ ω -3 ratio, and of the nutritional indices, AI and TI, indicating the least favourable nutritional profile. Beef patties with increasing levels of walnut oil or cake shifted rightwards, associated with higher UFA, MUFA, and PUFA levels, and improved nutritional indices (HPI, NVI, HH). Based on the nutritional indices HPI, NVI, and HH, as well as the PUFA/SFA ratio, the patties with complete replacement of beef fat by walnut oil (BP4) were the most nutritionally acceptable among all examined beef patties. PCA clearly separated the least (BP CON) and most (BP4) favourable beef patties along the first principal component.

4. Conclusion

Fatty acid analysis of beef patties showed that UFA were dominant in all of them, with the highest proportion observed in BP4 (85.98%), where 100% of beef fat was replaced with walnut oil, while the control patties (BP CON) contained 70.17% UFAs. Fat replacement led to a decrease in SFA content, including C16:0 and C18:0, and a simultaneous increase in MUFA (including C18:1 n-9) and PUFA (including C18:2 n-6), with these changes being more pronounced in beef patties constituted with oil compared to those with cake. Consequently, significant improvements in nutritional indices were recorded in the BP4 beef patties, with an increase in the PUFA/SFA ratio, HH, NVI and HPI, and a decrease in AI and TI compared with other patty types. Beef patties containing walnut cake also showed positive changes, although to a lesser extent.

These results are preliminary data on the fatty acid composition and nutritional value of beef patties enriched with walnut oil and cake, contributing to

the development of healthier meat products and the valorization of food industry by-products, thereby supporting sustainability in the food sector.

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