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SPECIES IDENTIFICATION OF THE FATTY ACID COMPOSITION OF THE MARGARINES AND SPREADS

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ABSTRACT

Fat-and-oil products (margarine and spreads) are included in the daily diet and meet the requirements for "wholesome foods". It is a substitute for butter used in cooking. The purpose of a series of studies regarding the fat phase of margarine and spreads was to determine the amounts of fatty acids and their isomers with the highest possible degree of detail in fat-and-oil products, as well as to improve the methodology using gas chromatographic methods in combination with mass spectrometry. Seventeen samples of fat-and-oil products were used for analysis and comparison in this study. All measurements were performed using a triple quadrupole GC-MS/MS Thermo Scientific™ TSQ 8000™ system equipped with GC Thermo Scientific™ TRACE™ 1310 with SSL Instant Connect™ SSL module and Thermo Scientific™ TriPlus™ RSH autosampler. The article shows the possibility of using information about fatty acid composition to establish the species of fat-and-oil products. The study presents the fatty acid composition of margarine and spreads. It is shown that the high-resolution mass spectrometry method for inferring the fatty acid composition is very useful for identifying things that come from plants and animals.

Keywords: dietary fats, fatty acid composition, products of natural origin, species identification.

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INTRODUCTION

Vegetable oils or fats are product of natural origin, which are obtained during the processing of vegetable raw materials. Margarine was developed as an alternative to butter, the fat content in them is 80%. Margarine usually contains suitable ratios of solid vegetable fats from coconut, palm kernels, transesterified vegetable oils and/or hydrogenated vegetable oils (Jala & Kumar, 2018). Basically, in the process of industrial catalytic hydrogenation, some natural fatty acids are destroyed and new artificial *trans* isomers are formed, which behave similarly to saturated fats. These isomers lack the necessary metabolic activity of the starting compounds and they inhibit the enzymatic desaturation of essential fatty acids (Kandhro *et al*., 2008).

Industrial margarines are designed for diverse types of baking. Both butter and spreads are emulsions wherein a matrix of solid fat crystals binds liquid oil and small drops of water containing aromatic compounds. For the manufacture of spreads, a hard, refractory fat is needed (Triantafillou *et al*., 2003). By choosing its distinct types, as well as processing conditions, various spreads are obtained – softer and harder at room temperature and in a cooled form, as well as described by a higher or lower total fatty acid content and saturated fatty acid (SFA) content. Fats with a high SFA content have a higher non-oxidising ability (Garsetti *et al*., 2016; Perna & Hewlings, 2023; Takeuchi *et al*., 2023).

One of the strategies to encourage the replacement of *trans* fatty acids (TFA) with oils with a higher content of polyunsaturated fatty acids

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(PUFA) to establish preferential prices. One of the factors contributing to the frequent use of palm oil as a substitute for TFA is its affordability (Kodali *et al*., 2001).

Industrially produced TFAs remain a global killer and considerably contribute to the development of non-communicable diseases (NCDs). The consumption of TFA in the amount of 2% of the daily calorie intake increases the risk of death from cardiovascular diseases by 20%-32%. High consumption of TFA is one of the main risk factors for the development of coronary heart disease (Roe *et al*., 2013) and causes obesity, type II diabetes (Willett, 2006), oncological diseases, ovulatory infertility, diseases of the nervous, immune systems and gastrointestinal tract (Islam *et al*., 2019; Owais *et al*., 2023; Salemi *et al*., 2023; Xu *et al*., 2022). Based on the accumulated data on the adverse impact of TFA on human health, the Food and Agriculture Organization (FAO)/ World Health Organization (WHO) recommended reducing their consumption to 1% of the daily caloric content of the daily diet, which, with the recommended FAO/WHO daily caloric content of the daily diet (2,000 kcal) is less than 2.2 g/day (The NCD Alliance, 2022; WHO, 2018).

It is estimated that eliminating TFA from food can annually prevent 500,000 deaths from coronary heart disease (CHD) (Restrepo & Rieger, 2016). The highest mortality rates from cardiovascular diseases (CVD) in the region of the Eurasian Economic Union (EAEU) are observed in Russia and Belarus for men and in Kyrgyzstan for women (Wang *et al*., 2016). In Kazakhstan, the average TFA content in publicly available street products was up to 144.3% of the recommended maximum daily intake (Nichols *et al*., 2014).

By using world practices and following the recommendations of the World Health Organization, the European Economic Commission (EEC) introduced restrictions on harmful TFAs in products of vegetable oil and animal fat processing: Margarine, milk fat substitutes, special-purpose fats, vegetable-cream and vegetable-fat spreads, and rendered vegetable-cream and vegetable-fat mixtures. For this, the EEC suggests reducing, or better, eliminating industrially hydrogenated fats from the diet, which will help preserve the health of consumers.

However, the regulations on fat-and-oil products on the content of TFAs do not apply to other types of food products. They are subject to the norms of other technical regulations of the Union. For example, the technical regulation "on the safety of milk and dairy products" (Padrão *et al*., 2019) in creamy vegetable spreads and rendered creamy vegetable mixtures limits the number of *trans* isomers of oleic acid in fat isolated from the product–their mass fraction should not exceed 8%. The technical regulations "on food safety" (Customs Union Commission, 2013) in food products for young children normalise the content of TFA in breast milk substitutes–no more than 4% of the total content of such acids. Therewith, the technical regulations of the Union "on food safety" do not regulate the content of TFAs as a percentage of the total fat content, for example, in cakes, cookies, sweets, and other confectionery products. The high information content and specificity of the fatty acid composition allow using it to identify fat-and-oil products–margarine and spreads.

The purpose of a series of studies on the fat phase of margarine and spreads was to determine the amounts of fatty acids and their isomers with the highest possible degree of detail in fat-and-oil products, as well as to improve the methodology using gas chromatographic methods in combination with mass spectrometry.

MATERIALS AND METHODS

A total of 17 samples of fat-and-oil products were analysed and compared in this study. The samples included margarine, and vegetable-fat spreads since these products mainly contain SFAs and TFAs. Samples were purchased in several Almaty's local supermarkets, including national and imported brands. Standards of *trans*- and *cis*-fatty acid methyl esters of the 37 component FAME Supelco mixture (Supelco, USA) (purity; ≥99% (GC); Sigma-Aldrich, Germany) were purchased from LaborPharm (Almaty, Kazakhstan). All chemicals (methanol, toluene, glacial acetic acid, hydrochloric acid, potassium hydroxide and sodium hydroxide, n-hexane) were pure for analytical reagents and were purchased from LaborPharm (Almaty, Kazakhstan), which had a higher purity (Systerm, Malaysia, for GC ≥99%).

Approximately 1 g of samples of fat-and-oil products were melted in an oven (Binder BD115 thermostat, Germany) at 40°C-50°C to obtain the fat phase. The upper-fat phase was removed after centrifugation at 500 g (centrifugal acceleration) for 4 min (Sigma 2-16P, Germany) (Customs Union Commission, 2011), then dried by adding anhydrous sodium sulphate to remove moisture from the margarine. The fat obtained from the samples of fat-and-oil products was transferred to 5 mL glass vials.

Two mL of hexane were added to 20 µL of fat obtained from samples of fat-and-oil products. Subsequently, 100 mL of sodium methylate solution (2.7 g of sodium metallic Na in 25 mL of methanol CH_3OH) was added, and the mixture was shaken for 30 s (Ika, Vortex Genius 3, Germany). It was incubated at room temperature for 10 min to separate the solution of the transparent layer

containing FAME from the opaque water layer. Then the solution was centrifuged at 3,000 rpm, for 5 min (Çetin *et al.*, 2003).

All measurements were carried out using the Thermo Scientific™ TSQ 8000™ triple quadrupole GC-MS/MS system equipped with the Thermo Scientific™ TRACE™ 1310 GC with SSL Instant Connect™ SSL module and Thermo Scientific™ TriPlus™ RSH autosampler. The method details are presented in *Table 1*.

TABLE 1. TRACE 1310 GC AND TSQ 8000 MS/MS METHOD PARAMETER

TRACE 1310 GC	
Injection mode	Splitless
Splitless time	1.0 min
GC column	Restek™ RTX™-5Sil MS, 60 m × 0.25 mm × 0.25 µm
Carrier gas	He (99.999%)
Flow	1.5 mL/min, constant flow
Temperature programme	50°C, 5 min 5°C/min to 220°C, 20 min
Transfer line temperature	230°C
Total analysis time	14.6 min
TriPlus RSH Autosampler	
Injection volume	1 µL
TSQ 8000 MS/MS	
Ionisation mode	EI, 70 eV
Ion source temperature	285°C
Scan mode	SRM using timed SRM
SRM transition setup	Automatically build-up by AutoSRM software

RESULTS AND DISCUSSION

The results of the fatty acid composition of the analysed samples of fat-and-oil products – margarine (M-A, M-B, M-C, M-D, M-E, M-F, M-G), are presented in *Table 2* and *3*, while spreads (S-A, S-B, S-C, S-D, S-E, S-F, S-G, S-H, S-I, S-J) are presented in *Table 4* and *5*.

Differentiation of vegetable fats of various origins is primarily associated with a certain set of fatty acids that make up their composition and determine the species of vegetable raw materials. Fatty acids are a biological marker that allows determining with a high degree of probability whether a fat belongs to a certain type due to their specificity. They may contain one or more double bonds in their structure, and therefore, are classified into saturated and unsaturated.

The biologically complete fat base of margarine has a balanced fat composition. It is dominated by unsaturated fatty acids, the content of linoleic acid should be about 40.00% and the ratio of polyunsaturated to saturated acids should approach 2:1.

The presence of linoleic acid C18:2 in the studied samples M-B (9.58%) and M-C (4.35%) showed a very low content of fatty acid. In the remaining samples, the content of linoleic acid C18:2 is in the range of 16.24% to 27.54%.

Table 2 demonstrates that the dominant fatty acids among the saturated group in the analysed margarine samples: Capronic C6:0, caprylic C8:0 and capric C10:0 are almost all below the detection limit of 0.10% or completely absent. Lauric fatty acid C12:0 content is in the amount of up to 0.90%. The content of myristic acid C14:0 is in the range of up to 2.00%.

TABLE 2. FATTY ACID COMPOSITION OF MARGARINE SAMPLES (SATURATED FATTY ACIDS, AVERAGE PERCENTAGE)

Component name	Rt	M-A	M-B	M-C	M-D	M-E	M-F	M-G
C4:0	6.22	N/A	N/A	0.00	0.01	N/A	0.02	0.00
C6:0	11.18	N/A	N/A	0.00	0.01	0.00	0.02	0.00
C8:0	16.46	0.01	0.03	0.01	0.04	0.06	0.05	0.03
C10:0	21.07	0.01	0.04	0.02	0.11	0.08	0.08	0.08
C11:0	23.10	N/A	0.00	0.00	0.00	N/A	N/A	N/A
C12:0	25.06	0.18	0.07	0.04	0.41	0.92	0.55	0.45
C13:0	26.86	0.01	0.00	N/A	0.01	0.00	0.00	0.01
C14:0	28.61	1.21	0.42	0.24	1.62	1.36	1.48	1.45
C15:0	30.21	0.06	0.07	0.03	0.13	0.06	0.09	0.09
C16:0	31.82	28.49	15.94	7.90	25.52	30.20	31.05	30.30
C17:0	33.24	0.14	0.14	0.08	0.18	0.12	0.15	0.14
C18:0	34.72	5.41	12.19	6.40	4.92	6.15	5.72	6.70
C20:0	36.63	0.06	0.66	0.56	0.04	0.03	0.03	0.03

TABLE 2. FATTY ACID COMPOSITION OF MARGARINE SAMPLES (SATURATED FATTY ACIDS, AVERAGE PERCENTAGE) (continued)

Component name	Rt	M-A	M-B	M-C	M-D	M-E	M-F	M-G
C21:0	38.54	0.10	0.01	0.68	0.07	0.01	0.01	0.00
C22:0	39.19	0.07	0.03	0.39	0.03	0.00	0.00	0.00
C23:0	40.27	0.88	0.13	4.74	0.77	0.09	0.01	0.03
C22:2	41.03	1.89	0.35	5.94	1.64	0.36	0.08	0.06
C24:0	41.22	0.73	0.08	4.40	0.62	0.05	0.03	0.05
C22:6	44.49	0.18	0.00	2.15	0.18	0.02	0.01	0.01

Palmitic acid (C16:0) is predominant in all samples, with its highest presence in M-F (31.05%) and the lowest in M-C (7.90%). Stearic acid (C18:0) shows variability across samples, ranging from 4.92% in M-D to 12.19% in M-B. Shorter-chain fatty acids, such as C4:0 and C6:0, are either in trace amounts or not detected in the samples. Fatty acids like C23:0, C22:2 and C24:0 show considerably high percentages in the M-C sample, indicating a distinct fat source or processing method for this sample. Although the focus is on saturated fatty acids, it is noteworthy that certain unsaturated fatty acids like C22:2 and C22:6 are listed, indicating the presence of polyunsaturated fats in the samples.

The content of palmitic acid C16:0 does not exceed 35.00%. Fatty acid C17:0 is present in an amount not exceeding 0.20%. The content of stearic fatty acid C18:0 in margarine samples does not exceed the amount of up to 7.00% (Nurgalieva, 2019). The exception is a sample of margarine of the M-B brand, where a sufficiently high content of stearic acid C18:0 12.19% was found, which suggests the presence of animal impurities in this margarine, or rather pork fat (Pedersen & Kirkhus, 2008). The introduction of animal fats complicates the identification of the product and requires additional identification criteria and methods for their determination. The content of arachinic acid C20:0 is in the range of up to 0.60% and begenic acid C22:0 to 0.20%.

Table 3 indicates the content of monounsaturated (MUFA) and PUFA in margarine samples. In the samples of margarine M-B, the content of fatty acid C18:1 turned out to be in the amount of 12.19%, and the content of C18:2 – 10.87% of *trans* isomers, indicating the presence of hydrogenated fats in the composition of the product. In the sample of margarine M-C, the content of fatty acid C18:2 (7.27%) also considerably exceeds the limit, C18:1 within the normal range up to 0.30%. In other samples, *trans* isomers are present in extremely small amounts (up to 0.40%). In the M-A sample, there are no *trans* fats at all.

Sample M-C shows a different composition profile, with higher values of certain fatty acids compared to other samples. High in MUFA: Margarines across the board seem to have a high percentage of MUFA, especially C18:1 n9c (oleic acid). The inclusion of omega-6 and omega-3 fatty acids indicates these margarines could contribute to essential fatty acid intake. Some samples contain TFAs. Consumption of these is generally advised against due to potential health concerns. The varied composition across samples suggests the use of different oils or processing techniques in the production of these margarines. Thus, according to *Table 2*, the spectrum of fatty acids and the ratio of their content indicate the presence of oils with a mass fraction of palmitic acid of more than 17% (Jala & Kumar, 2018). This group includes the liquid fraction of palm oil, referred to in commercial and industrial practice as palm olein, and the solid fraction, known as palm stearin. A fraction that is a substitute for cocoa butter can also be obtained from palm oil by double fractionation. Due to the high content of solid triglycerides, palm oil and its fractions are widely used in the production of margarines and spreads, manifesting the so-called post-crystallisation property–solidification upon storage (Jala & Kumar, 2018).

Analysis of the fatty acid composition of margarine in *Table 2* indicates a complete absence of butter (milk) fat, this is evidenced by the absence of butyric acid C4:0, capronic acid C6:0 and myristoleic acid C14:1 inherent in milk fat. Caprylic C8:0 and capric C10:0 show a fatty acid content of less than 0.1%. In mixtures, vegetable fats are usually in free or hydrogenated form. In hydrogenated forms, the content of TFA that is harmful to health is elevated. When interpreting the data in *Table 2*, the content of *trans* isomers in the studied margarine products showed a result of less than 2.0%. However, two samples M-B (C18:1 n9t – 12.19%, C18:2 n6t – 10.87%) and M-C (C18:2 n6t – 7.27%) contain TFAs in an amount that is higher than the permissible requirements of technical regulations. The fat component of spreads is dominated by unsaturated fatty acids (both MUFA and PUFA), and the amount of linoleic acid varies from 5.00% to 45.00% depending on the type of spread.

TABLE 3. FATTY ACID COMPOSITION OF MARGARINE SAMPLES (UNSATURATED FATTY ACIDS, AVERAGE PERCENTAGE - FAMES)

Component name	Rt	M-A	M-B	M-C	M-D	M-E	M-F	M-G
C14:1	29.46	0.01	0.02	0.01	0.07	0.01	0.03	0.03
C15:1	31.07	0.01	0.00	0.00	0.02	0.03	0.00	0.01
C16:1	32.41	0.27	0.18	0.11	0.33	0.22	0.24	0.23
C17:1	33.88	0.03	0.05	0.06	0.05	0.03	0.03	0.03
C18:1_n9t	34.99	0.00	12.19	0.27	0.19	0.23	0.07	0.38
C18:1_n9c	35.20	25.35	24.62	14.25	23.65	28.02	27.93	27.98
C18:2_n6t	35.57	0.01	10.87	7.27	0.47	0.06	0.56	0.11
C18:2_n6c	36.06	16.24	9.58	4.35	24.04	24.77	27.54	27.03
C18:3_n6c	37.10	0.14	0.96	0.86	0.30	0.21	0.28	0.27
C20:1_n9c	37.34	1.23	1.33	3.26	1.07	0.79	0.78	0.82
C18:3_n3c	37.80	0.44	0.73	1.22	0.42	0.29	0.30	0.30
C20:3_n3	39.57	2.78	2.29	0.16	0.00	1.20	0.76	0.94
C22:1_n9	39.64	2.78	2.29	9.64	2.90	1.22	0.77	0.95
C20:3_n3	39.77	2.78	2.29	9.64	2.90	1.22	0.77	0.95
C20:4_n6	40.68	1.89	0.35	0.29	1.64	0.36	0.08	0.06
C22:2	41.03	1.89	0.35	5.94	1.64	0.36	0.08	0.06
C20:5_n3	42.53	4.42	1.69	8.75	3.78	1.45	0.42	0.42
C24:1_n9c	43.22	2.06	0.37	5.59	1.78	0.39	0.06	0.04
C22:6	44.49	0.18	0.00	2.15	0.18	0.02	0.01	0.01

TABLE 4. FATTY ACID COMPOSITION OF SPREAD SAMPLES (SATURATED FATTY ACIDS, AVERAGE PERCENTAGE)

No.	Component name	Expected RT	Area %									
			S-A	S-B	S-C	S-D	S-E	S-F	S-G	S-H	S-I	S-J
1	C4:0	6.22	N/A	0.48	0.38	0.00	0.54	0.01	0.08	0.04	0.47	0.47
2	C6:0	11.18	0.00	0.49	0.37	0.00	0.53	0.01	0.09	0.04	0.45	0.50
3	C8:0	16.46	0.03	0.45	0.34	0.02	0.47	0.04	0.1	0.09	0.38	0.43
4	C10:0	21.07	0.04	1.29	0.91	0.03	1.26	0.07	0.26	0.23	0.96	1.07
5	C11:0	23.1	N/A	0.03	0.02	N/A	0.02	N/A	0.01	0	0.02	0.02
6	C12:0	25.06	0.53	1.99	1.22	0.32	1.98	0.53	0.61	0.24	1.47	1.43
7	C13:0	26.86	N/A	0.07	0.04	0.00	0.06	0.00	0.01	0.00	0.04	0.04
8	C14:0	28.61	1.10	6.35	4.17	1.39	6.57	2.14	2.12	1.01	4.58	5.16
10	C15:0	30.21	0.05	0.82	0.50	0.07	0.8	0.10	0.20	0.12	0.61	0.67
12	C16:0	31.82	23.15	31.01	22.35	34.47	31.82	0.78	26.31	16.33	24.2	21.33
14	C17:0	33.24	0.15	0.56	0.33	0.15	0.55	0.16	0.22	0.16	0.40	0.44
16	C18:0	34.72	4.03	10.6	13.44	7.11	8.23	0.66	5.37	23.31	7.88	13.09
21	C20:0	36.63	0.06	0.15	0.11	0.07	0.14	0.32	0.09	0.20	0.11	0.09
25	C21:0	38.54	0.64	0.02	0.02	0.01	0.01	N/A	0.01	0.00	0.00	0.01
26	C20:2	38.65	0.64	0.02	0.00	0.01	0.02	N/A	0.01	0.00	0.00	0.01
27	C22:0	39.19	0.39	0.06	0.09	0.02	0.06	1.45	0.11	0.51	0.15	0.44
31	C23:0	40.27	3.71	0.02	0.01	0.00	0.00	0.13	0.01	0.08	0.01	0.06
33	C22:2	41.03	4.59	0.02	0.03	0.01	0.00	0.15	0.00	0.00	0.01	0.01
34	C24:0	41.22	4.59	0.01	0.03	0.02	0.00	0.15	0.01	0.02	0.03	0.03
37	C22:6	44.49	1.82	N/A	N/A	N/A	N/A	0.04	N/A	N/A	N/A	N/A

The right choice of fat compositions and their optimal combination with milk fat in a suitable ratio of fat component and milk plasma allows this product to be considered as an improvement with a more balanced fatty acid composition.

The distinctive features of the composition of fatty acids of natural milk fat are the presence of butyric C4:0, capronic C6:0, caprylic C8:0, capric C10:0, and myristoleic C14:1 acids, as well as minor components (pentadecanoic C15:1, palmitoleic C16:1, and margaric C17:1 acids). In the spread samples, these acids are present in an amount below the permissible limit, which indicates the absence of a fatty phase of milk fat in the composition of this product (butyric acid C4:0 varies from 0.00% to 0.54%, capronic acid C6:0 (0.00% to 0.53%), caprylic acid C8:0 (0.02% to 0.47%), caprine C10:0 (0.03% to 1.29%), myristolein C14:1 (0.00% to 0.74%).

The presence of pentadecanoic C15:1 (from 0.00% to 0.22%), palmitoleic C16:1 (0.20% to 1.15%) and margaric C17:1 acids (from 0.05% to 0.24%) also show a low content. Thus, almost all samples lack butyric acid and other low molecular weight acids, including myristic acid. These spread samples have no fat derived from milk, only fats of non-dairy origin are present.

The following spread samples contain oils with a mass fraction of palmitic acid C16:0 of more than 17%: S-A (23.15%), S-B (31.01%), S-C (22.35%), S-D (34.47%), S-E (31.82%), S-G (26.31%), S-I (24.2%), and S-J (21.33%). The samples of the spreads S-F (0.78%) and S-H (16.33%) have a very low content of palmitic acid.

A sufficiently high content of stearic acid C18:0 in the following spread samples suggests the presence of animal fat in the composition of the product: S-B (10.60%), S-C (13.44%), S-D (7.11%), S-E (8.23%), S-H (23.31%), S-I (7.88%), S-J (13.09%). The introduction of animal fats complicates the identification of the product by its fatty acid composition and requires other identification criteria and methods for their determination.

The samples S-A, S-B, S-C, S-D, S-E, S-G, S-H, S-I, and S-J, with high oleic acid content (C18:1) ranging from 19.38% to 35.67%, indicate the presence of palm kernel oil or its fractions, such as palm stearin, contrasting with palm kernel olein of 22.80% and palm kernel oil with 15.10% oleic acid content.

The analysis of the data in *Table 4* showed a low content of up to 2.00% of fatty acids C6:0-C12:0 in the samples under study. This indicates that the fatty acid composition of these samples does not contain lauric group oils or low molecular weight fatty acids of more than 2.00%.

The content of erucic acid C22:1 is in the range of 0.02 to 2.27%, which indicates the absence in these samples of oils with a high mass fraction of erucic acid, such as rapeseed, cabbage oil (more than 3.00%), mustard (more than 5.00%). The spread samples have an extremely low content of linolenic

TABLE 5. FATTY ACID COMPOSITION OF SPREAD SAMPLES (UNSATURATED FATTY ACIDS, AVERAGE PERCENTAGE – FAMES)

No.	Component name	Expected RT	Area %									
			S-A	S-B	S-C	S-D	S-E	S-F	S-G	S-H	S-I	S-J
9	C14:1	29.46	N/A	0.71	0.42	0	0.74	0.01	0.13	0.07	0.51	0.59
11	C15:1	31.07	0	0.22	0.09	0.01	0.21	0.07	0.02	0.06	0.13	0.15
13	C16:1	32.41	0.23	1.06	0.7	0.25	1.15	0.47	0.43	0.2	0.77	0.76
15	C17:1	33.88	0.06	0.24	0.12	0.03	0.22	0.05	0.07	0.05	0.21	0.17
17	C18:1_n9t	34.99	0.04	0.66	0.05	0.00	0.06	9.32	0.00	0.00	0.00	0.25
18	C18:1_n9c	35.20	19.43	26.23	28.91	33.35	29.06	9.32	25.28	35.67	22.01	19.38
19	C18:2_n6t	35.57	0.34	0.58	11.71	0.64	0.65	32.86	25.28	9.18	22.01	19.38
20	C18:2_n6c	36.06	12.09	11.94	7.39	20.19	13.33	22.27	11.43	5.55	9.36	7.72
22	C18:3_n6c	37.1	0.27	0.32	0.23	0.27	0.37	0.09	0.30	0.28	0.52	0.67
23	C20:1_n9c	37.34	2.81	0.80	0.95	0.82	0.60	0.60	0.30	0.55	0.74	0.92
24	C18:3_n3c	37.8	0.92	0.24	0.34	0.31	0.22	0.31	0.19	0.42	0.21	0.46
28	C20:3_n3	39.57	0.12	0.1	0.03	0.02	0.10	1.20	0.02	0.51	0.05	0.05
29	C22:1_n9	39.64	0.12	1.17	2.27	0.16	0.08	1.59	0.02	0.28	0.06	0.05
30	C20:3_n3	39.77	6.55	1.17	2.27	0.16	0.1	1.58	0.02	0.28	0.06	0.33
32	C20:4_n6	40.68	0.29	0.02	0.03	0.05	0.02	0.14	0.01	0.03	0.02	0.04
33	C22:2	41.03	4.59	0.02	0.03	0.01	0.00	0.15	0.00	0.00	0.01	0.01
35	C20:5_n3	42.53	6.98	0.09	0.07	0.03	0.02	13.18	0.85	4.38	1.53	3.75
36	C24:1_n9c	43.22	4.21	0.00	0.01	N/A	N/A	0.21	0.01	0.09	0.02	0.04
37	C22:6	44.49	1.82	N/A	N/A	N/A	N/A	0.04	N/A	N/A	N/A	N/A

acid C18:3 (0.09%-0.67%). These spreads do not contain oil, with a high content of linolenic acid (from 2.00% to 20.00%). Oils with the maximum mass fraction of linoleic acid C18:2 (26.00%-81.00%) are also not present in these spreads, the content of linoleic acid varies from 5.55% to 22.27%.

When interpreting the data in *Table 4*, the content of *trans* isomers in the samples showed the highest possible content of C18:2 n6t – S-F (32.86%), S-G (25.28%), S-I (22.01%) and S-J (19.38%), S-C (11.71%), S-H (9.18%). In the S-F sample (9.32%), the content of C18:1 n9t *trans* isomers also shows a result above the level allowed by the technical regulations. In the remaining samples of the spreads under study, *trans* fats are found in an amount of less than 2.00%.

The positive health effects of all categories of emulsified fat products (from margarine to spreads) made from mixtures of natural vegetable oils should contain less SFA. C18:1 MUFA and C18:2 PUFA are oxidised, respectively, 10 and 100 times faster than C18:0 PUFA (Kodali *et al.,* 2001). The content of SFA in emulsion fat products should be as low as possible, and PUFA - as high as possible and preferably include both omega-6 and omega-3.

Unsaturated fatty acids of natural origin contained in raw and delicatessen oils and fats have only *cis*-double bonds (*Figure 5*). The main source of TFA is partially hydrogenated oils (PHOs), which are formed as a result of partial hydrogenation. PUFAs are usually converted into MUFA–TFA, and therefore PHOs have increased oxidation resistance. The REPLACE initiative by the World Health Organization (WHO) provides guidelines for regulating food safety issues. It proposes a strategic approach to implementing legislative measures that aim to eliminate industrially produced TFAs from fat products. The ultimate goal is to achieve a worldwide ban on the use of TFAs by 2023 and replace them with oils that have a higher proportion of PUFAs and a lower proportion of SFAs.

Palm oil is rich in saturated fats, specifically palmitic acid, comprising about 44% of its content. Palm oil does not contain *trans* fats, the hydrogenation process used to produce margarine or spreads can lead to the formation of these unhealthy fats. *Trans* fats can increase LDL cholesterol and decrease 'good' cholesterol, known as high-density lipoprotein (HDL), which further contributes to heart disease risk (Urugo *et al.*, 2021).

According to the results of the fatty acid composition of the analysed margarine samples (*Figure 1*), the total content of saturated acids is as follows:

M-A (37.36%), M-B (29.78%), M-C (25.49%), M-D (34.49%), M-E (39.13%), M-F (39.29%), M-G (39.36%), unsaturated fatty acids (both monounsaturated M-A (31.74%), M-B (28.86%), M-C (32.92%), M-D (29.87%), M-E (30.71%), M-F (29.84%), M-G (30.09%) and polyunsaturated M-A (30.86%), M-B (18.25%), M-C (34.04%), M-D (34.97%), M-E (29.88%), M-F (30.25%), M-G (30.04%). Fatty acid *trans* isomers account for M-B (23.06%), M-C (7.54%), M-D (0.66%), M-E (0.29%), M-F (0.63%) and M-G (0.49%).

According to the results of the fatty acid composition of the analysed samples of spreads (*Figure 2*), the total content of saturated acids is S-A (38.47%), S-B (54.4%), S-C (44.33%), S-D (43.68%), S-E (52.5%), S-F (6.55%), S-G (35.61%), S-H (42.34%), S-I (41.76%), S-J (45.28%), unsaturated fatty acids (both monounsaturated S-A (26.86%), S-B (30.43%), S-C (33.47%), S-D (34.62%), S-E (32.06%), S-F (12.32%), S-G (26.26%), S-H (36.97%), S-I (24.45%), S-J (22.06), and polyunsaturated S-A (34.27%), S-B (13.92%), S-C (10.39%), S-D (21.05%), S-E (14.18%), S-F (38.92%), S-G (12.83%), S-H (11.45%), S-I (11.76%), S-J (13.04%).

Fatty acid *trans* isomers account for S-A (0.38%), S-B (1.24%), S-C (11.76%), S-D (0.64%), S-E (0.71%), S-F (42.18%), S-G (25.28%), S-H (9.18%), S-I (22.01%), S-J (19.63%).

As a result of the study performed, the opportunity to use an improved technique for identifying fat traces and objects of biological origin by fatty acid composition using high-resolution mass spectrometry with the highest possible degree of detail in fat-and-oil products is shown (*Figure 2*).

Thus, in the composition of the fat phase of spreads and margarines, the best and most beneficial for human health is the use of mixtures of vegetable oils with a balanced composition of PUFA, which are free of *trans* isomers or presence in minimal amounts (*Figure 1*). To this end, many manufacturers of spreads limit the use of hydrogenated fats, which are the main sources of *trans* isomers. However, the prototypes of most of the spreads showed the highest possible content from 9.18% to 42.18%. In the margarine M-B sample, the content of *trans* isomers is also extremely high at 23.06%.

Gas chromatography-mass spectrometry (GC-MS) is a vital analytical tool for the qualitative and quantitative analysis of complex mixtures. GC-MS is a powerful hyphenated technique that has emerged as an indispensable tool for the analysis of complex mixtures, particularly in identifying and characterising volatile and thermally stable constituents in various samples. GC-MS couples the separating power of gas chromatography with the detection and identification capability of mass spectrometry. In the process, the compounds separated by the GC column enter a mass spectrometer, where they are ionised and fragmented. The resulting ion fragments are then detected and can be used to identify the compound based on its unique mass spectrum. GC-MS is extensively used to detect and quantify pollutants in air, water and soil samples (Nekoei & Mohammadhosseini, 2014).

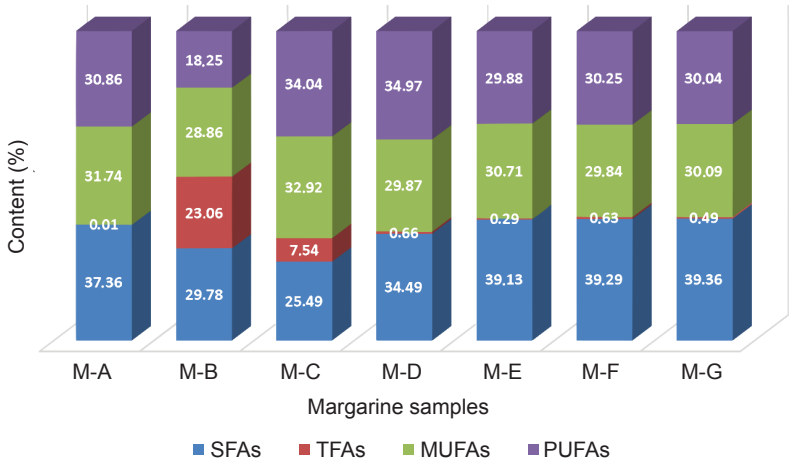

Figure 1. The content of SFAs, TFAs, MUFAs and PUFAs in margarine samples.

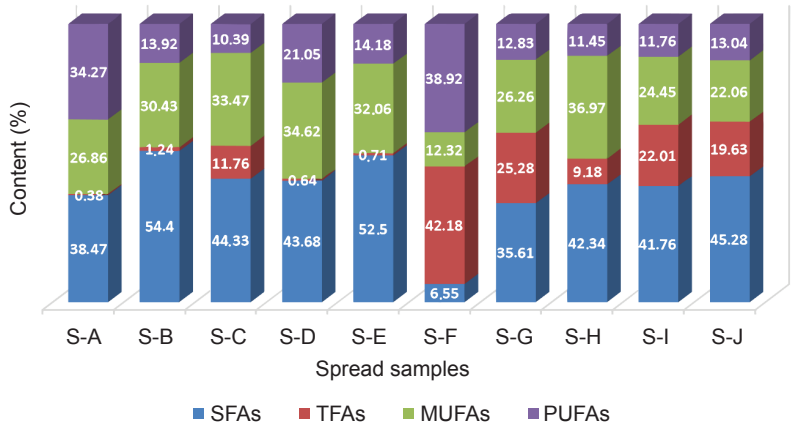

Figure 2. The content of SFAs, TFAs, MUFAs and PUFAs in spread samples.

This technique can identify trace amounts of harmful substances and contaminants, as well as profile the flavour and aroma compounds (Quan *et al.*, 2023). GC-MS can determine substances in blood and urine samples, playing a crucial role in forensic investigations and drug testing (Mohammadhosseini *et al.*, 2016). In drug development and quality control, GC-MS can identify and quantify compounds in formulations. It's a primary tool for identifying constituents of essential oils, plant extracts and other natural products (Mohammadhosseini *et al.*, 2023).

GC-MS stands as a foundational pillar in the realm of analytical methodologies due to its manifold advantages. One of its primary strengths lies in its high sensitivity. This analytical tool is adept at detecting even trace amounts of compounds, making it invaluable in analyses that necessitate the identification of substances present in minute quantities. Coupled with its sensitivity, GC-MS boasts exceptional specificity. This is attributed to the seamless amalgamation of chromatographic separation with mass spectral detection. Such a combination ensures that compounds undergo not just separation but are also pinpointed with precision based on their unique mass spectra.

The versatility of GC-MS is evident in its capacity to analyse a vast spectrum of volatile and semi-volatile compounds. This trait renders it applicable across a myriad of research arenas, encompassing fields like environmental studies, forensics, food analytics and pharmaceuticals. Beyond its qualitative prowess, GC-MS excels in quantitative analysis, furnishing detailed data about the analytes. This capability is bolstered by the rapidity with which modern GC-MS instruments can yield results, facilitating high-throughput analysis. Another salient feature of GC-MS is its advanced software which possesses deconvolution capabilities. This allows for the disentanglement of overlapping peaks, enhancing the potential to discern compounds even in intricate mixtures. Furthermore, the mass spectra gleaned can be juxtaposed against extensive libraries, simplifying compound identification. From a structural perspective, the fragmentation patterns

discerned in the mass spectra can shed light on the structural nuances of unknown compounds. This is further complemented by the fact that many samples, especially those with volatility, demand minimal preparation pre-analysis. Lastly, in the broader context of societal well-being, GC-MS plays an instrumental role. Its proficiency in detecting pollutants and contaminants underscores its importance in safeguarding environmental and health standards. The multifaceted benefits of GC-MS underscore its pivotal position in analytical chemistry, with its influence permeating diverse scientific fields.

CONCLUSION

The conditions for the preparation of samples of emulsion fat products (margarine, spreads) have been optimised: A solvent was selected for the extraction of the fat phase of the product (hexane); the volume of sodium methylate (2 M) required for the esterification reaction (50 μL) and other non-polar and low-polar organic solvents reaction time for complete saponification of triglycerides (15 min). Using the presented methodology, experimental samples of fat-and-oil products (7 samples) and spreads (10 samples) were analysed.

The obtained fatty acid composition is used to deduce whether the sample belongs to a particular type of object of plant or animal origin. The analytical approaches to the identification of objects by fatty acid composition described in this paper can be used to determine and detect the falsification of fat-and-oil products by an instrumental method using high-resolution mass spectrometry. This method is also applicable for the identification of undeclared animal fats in the composition of fat-and-oil products labelled "halal" but requires further scientific research to develop additional approaches and criteria for their identification.

The data of the literature review and the complex investigations conducted on the problem under study suggest that the production of emulsion fat products (from margarine to spreads) made from mixtures of vegetable and animal fats can be the subject of further scientific research and technological developments aimed at ensuring a healthy diet and food safety issues.

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