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SIMPLE METHOD FOR FATTY ACIDS DETERMINATION IN FOOD, SUPERFOOD AND SPICE SAMPLES BY GC-MS TECHNIQUE

– Research paper –

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Abstract: The aim of the study was to modify and adapt to other matrices the fast and simple method for determining total lipid content expressed as fatty acid methyl esters (FAME) by performing the in situ transesterification. The primary method was published as a technical report for the FAME analysis in algae dry mass. Our modifications included the use of less toxic solvents, the use of an internal triglyceride standard and FAME determination by the gas chromatography technique coupled with the mass spectrometry technique in the Single Ion Monitoring mode (SIM). The modified method was validated for 37 fatty acids (saturated, monounsaturated and polyunsaturated) containing from four to twenty-four carbons in the carbon chain (C4-C24), and was adapted to five food matrices: three solids (yeast, yeast flakes, biscuits), and two liquids (milk thistle (*Silybum marianum* (L.) Gaertner) oil and olive oil). Additionally, 14 samples of spices and superfood samples, rich in unsaturated oils were analyzed. The validation parameters: linearity, precision, recovery, limits of detections and quantifications, were assessed and additionally Certified Reference Material of olive oil was analyzed.

Key words: fatty acids; FAME; gas chromatography; mass spectrometry; SIM mode; food

INTRODUCTION

The composition of fatty acids (FAs) in the food is very important, because lipids are one of its three major constituents. They represent the basic component of phospholipids, triglycerides, diglycerides, monoglycerides and sterol esters. FAs are built of three elements, carbon, hydrogen, and oxygen that form their skeleton structured like a linear carbon chain of a variable length with a carboxyl group attached at one terminal (Bartošová & Štefko, 2017). They are essential to ensure normal activities of the human body. Their main biological functions include energy storage, formation of biological membranes and participation in signal transmission. They also play an important role in cognition, and in social as well as emotional human activities. The central and peripheral nervous system contains very high concentrations of FAs, their total content is estimated at a level of over 60% (Wilczynska & Modrzewski, 2018). Although humans and animals have a variety of metabolic pathways capable of synthesizing and decomposing lipids, some key ones cannot be produced in this way and must be provided with a diet. Also, each essential FA is metabolized along its own pathway. Linoleic acid, following its elongation and desaturation, is transformed into arachidonic acid, a precursor of prostaglandins, and is further metabolized to eventually produce eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Petrović et al., 2010).

Young children and vegetarians are particularly vulnerable consumer groups, for whom the supply of FAs at an appropriate level plays an important role in nutrition. Infant products with a high content of fat support infants' growth and are a suitable medium for those vitamins that are soluble in fat (Nyiri et al., 2017). For vegans, products in which fats are present in the form of unsaturated FAs are desirable. They include mono- (MUFA) and polyunsaturated fatty acids (PUFA) together with the isomers known as omega 3, 6 and 9. The occurrence of MUFA and PUFA in food, and especially FAs ratios, influence human health by reducing the risk of cardiovascular and diet-related diseases (Wilczynska & Modrzewski, 2018).

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The ability to identify and accurately quantify the FAs content in fats, as well as free FAs, is essential for evaluation of the fuel potential and establishing a comprehensive compositional analysis of food products. Different techniques have been proposed for isolation, purification, separation, and detection of FAs in food products, including nuclear magnetic resonance NMR (Barison et al., 2010; Marcone et al., 2013) or high-performance liquid chromatography with ultraviolet detection (HPLC UV) (Carvalho et al. 2012), refractive index detector (HPLC RID) (Syed, 2017), or mass detector (LC–MS) (Blumhorst et al., 2011), Raman spectroscopy (Miranda et al., 2014) or Fourier transform-infrared spectroscopy (FTIR) (Sherazi et al., 2013).

The standard and very popular analytical method used for the FAs analysis is based on gas chromatography with flame ionization detection (GC-FID) (Council of Europe, 2017; Tyburczy et al., 2013). Here, the sample preparation consists of several steps. Fats are extracted from a matrix using a solvent that is nonpolar, and then saponified to obtain free fatty acids salts. In the next step, the free fatty acids are derivatized to the methyl esters, which are then analyzed by gas chromatography (GC). Methylation can be performed using various different methods, such methylation catalyzed by an acid or a base, borontrifluoride methylation, methylation using diazomethane, and silylation, of which the last three require more aggressive reagents (Petrović et al., 2010).

Researchers also forwarded simplified procedures for producing methyl esters, with in situ transmethylation of fats (Schiavon et al., 2016; Van Wychen et al., 2013). The significant advantage characterizing this last approach results from the fact that the extraction and derivatization of FAs can be performed in a single and rapid reaction step. The primary method performing the in situ transesterification was published as a technical report for the fatty acid methyl esters (FAME) analysis in algae dry mass (Van Wychen et al., 2013). We made modifications including the use of less toxic solvents, the use of internal triglyceride standard (ISTD) and determination of FAME by the GC-MS technique in the Single Ion Monitoring mode (SIM), instead of GC-FID. The modified method was used in our laboratory for algae and yeast samples, but full validation data was not published yet (Szpyrka et al., 2020; Potocki et al., 2020; Rogóż et al., 2021; Słowik-Borowiec et al., 2022).

The aim of the study was to amend and adapt to other matrices the fast and simple method for determination of total lipids, expressed as FAME, by performing the in situ transesterification and verified this method by analyses of olive oil certified reference material (CRM).

MATERIALS AND METHOD

Chemical and standards

All reagents were of sufficiently high purity for the GC analysis. Petroleum ether, methanol, and hydrochloric acid were purchased from Chempur (Piekary Slaskie, Poland), dichloromethane was obtained from Honeywell (Seelze, Germany). Standards of fatty acids: 37 Supelco component FAME MIX CRM47885 and ISTD tripentadecanoin, were purchased from Merck KGaA (Darmstadt, Germany).

The certified FAME standard (concentrations of individual FAME within the range of 109-600 μg/mL) was dissolved in 50 mL of petroleum ether to obtain concentrations in the range of 2-12 μg/mL. It was used as stock calibration standard. Next, working standards for calibration were prepared by diluting the standard solution to a volume of 10 mL with petroleum ether, obtaining the following concentrations: 1-6; 0.5-3; 0.25-1.5; and 0.05-0.3 μg/mL. 100 mg of ISTD were dissolved in 100 mL of dichloromethane: methanol (2: 1, v: v) mixture, resulting in a concentration of 1000 μg/mL. All standards were stored at a temperature below -20°C.

Sample analysis and validation

The primary method for determining total lipids, expressed as fatty acid methyl esters (FAME), by performing the in situ transesterification was published as a technical report for the FAME analysis in algae dry mass (Van Wychen et al., 2013). Our modifications included the use of less toxic solvents (dichloromethane instead of chloroform and petroleum ether instead of hexane), the use of triglyceride ISTD tripentadecanoin instead of tridecanoic acid methyl ester, and determination of FAME by the GC-MS instead of GC-FID. We adapted the method to analyze five food matrices including three dry solids (yeast, yeast flakes, biscuits) and two liquids (milk thistle oil and olive oil).

We analyzed the samples in six repetitions: five samples with ISTD (to determine the method recovery) and one without the standard, to

determine the actual trace content of pentadecanoic acid C15:0 in food samples.

The sample preparation and instrumental analysis were done according to procedure described earlier (Van Wychen et al., 2013; Szpyrka et al., 2020; Potocki et al., 2020; Słowik-Borowiec et al., 2022). Shortly, 10 mg of dry (yeast, yeast flakes, biscuits) or 20 μL of liquid (milk thistle oil and olive oil) food samples were weighed, and ISTDs were added. Then transesterification was done by the mixture of dichloromethane: methanol and hydrochloric acid solution, followed by warming to 85°C. After reaction, the FAME were extracted by petroleum ether and analyzed by gas chromatography (7890A model) coupled to mass spectrometry (7000 model, Agilent Technologies, Palo Alto, CA, USA) in the SIM mode. The detailed description for sample preparation and chromatographic acquisition parameters were presented in our earlier works (Szpyrka et al., 2020; Potocki et al., 2020; Słowik-Borowiec et al., 2022). For each FAME, linearity was established using five points calibration curves (Rogóż et al., 2021). Recovery of ISTD, which is added before sample preparation, could be used in calculating the real FAs concentrations in samples.

For an additional check of the method performance, CRM (product ID 47118), matrix olive oil, was analyzed by the modified method.

Real samples

14 samples of spices (black seeds, white mustard, Roman cumin, fenugreek, coriander) and superfood samples (soybeans, linseed, flax seeds, plantain seeds, psyllium seeds, chia seeds, amaranth, milk thistle, hemp seeds) rich in unsaturated oils were analyzed. All samples were bought in local shops.

Statistical analysis

Statistical analysis consisted of calculating average value, standard deviations (SD) and coefficient of variation (RSD).

RESULTS AND DISCUSSION

Method performance

A major advantage of the described method is that in one rapid reaction step, lipids are extracted from a sample and FAs derivatization is performed (in situ transesterification). Some modifications were introduced in relation to the original method (Van Wychen et al., 2013): the use of less toxic solvents (dichloromethane instead of chloroform, and petroleum ether instead of hexane), the use of triglyceride ISTD tripentadecanoin instead of free tridecanoic acid methyl ester, and determination of FAME by the GC-MS technique in the SIM mode instead of GC-FID.

The use of triglyceride ISTD tripentadecanoin instead of free tridecanoic acid methyl ester represents a very important modification. It enables proper determination of the whole method recovery and takes into account the transesterification of triglyceride into methyl ester of pentadecanoic acid, C15:0. This acid is present in food samples, but at a very low level. We analyzed samples in six repetitions: five samples with ISTD added (to determine the method recovery), and one without, to determine the actual trace content of C15:0.

Good linearity (correlation coefficient in the range 0.972 to 1.000) was achieved for all 37 FAME at five calibration levels (Table 1). We analyzed FAs from C4:0 to C24:0, while in the original method FAs from C8:0 to C24:0 were determined. Limits of Quantifications (LOQs) for each FA were assessed as a signal-to-noise ratio (S/N) of 10:1 and Limits of Detections (LODs) were calculated as S/N of 3:1. LOQs reached 0.1 mg/kg for most of FAs, 0.2 mg/kg for 11 FAs, and 0.3 mg/kg for C16:0 (Table 1).

The high sensitivity was achieved by using the SIM mode instead of the full scan or the FID detection. LOQ for individual FAs was in the range of 0.1-0.3 μg/mL (which correspond to 0.01-0.03 %) while in other methods it was within 0.015-0.90 mg/mL (GC-MS) (Bartošová & Štefko, 2017), 0.1% (GC-FID) (Petrović et al., 2010), 2.9-216.5 μg/mL (HPLC-UV) (Carvalho et al., 2012), and 3-100 μg/mL (GC-MS SIM) (Ren et al., 2013).

The recovery of the method was determined by adding ISTD at the beginning of the sample procedure. The analysis of recovery was performed in five repetitions. The best recovery was obtained for liquid samples, with 74.2% for olive oil and 86.5% for oil. For solid samples recoveries amounting to 56% for yeast, 61.9% for yeast flakes, and 66.6% for biscuits were achieved. The precision was calculated as relative standard deviations in the recovery studies for ISTD (Rogóż et al., 2021), and achieved the best values for liquid samples, of 2.6% for milk thistle oil and 4.0% for olive oil. For solid samples, the precision was also good, in the range of 6.4-9.7% (Table 2).

Table 1. A scope of FAs analysis, with their chromatographic and mass spectrometry parameters of analysis.

FA abbreviation name	IUPAC name	Common name	Retention time, min	Quantitative ion	Linearity range, µg/mL	Linearity (R)	LOD, µg/mL	LOQ, µg/mL	LOQ, %
C4:0	butanoic acid	butyric acid	4.007	74.1	0.2-8.0	0.999	0.07	0.2	0.02
C6:0	hexanoic acid	caproic acid	5.768	74.2	0.2-8.0	0.999	0.07	0.2	0.02
C8:0	octanoic acid	caprylic acid	7.049	74.1	0.2-8.0	1.000	0.07	0.2	0.02
C10:0	decanoic acid	capric acid	8.075	74.1	0.2-8.0	0.999	0.07	0.2	0.02
C11:0	undecanoic acid	undecylic acid	8.564	74.0	0.1-4.0	1.000	0.03	0.1	0.01
C12:0	dodecanoic acid	lauric acid	9.053	74.2	0.2-8.0	1.000	0.07	0.2	0.02
C13:0	tridecanoic acid	tridecylic acid	9.591	74.1	0.1-4.0	0.999	0.03	0.1	0.01
C14:1n5	(Z)-tetradec-9-enoic acid	myristoleic acid	10.148	55.1	0.1-4.0	1.000	0.03	0.1	0.01
C14:0	tetradecenoic acid	myristic acid	10.197	74.1	0.2-8.0	1.000	0.07	0.2	0.02
C15:1n5	(*Z*)-pentadec-10-enoic acid	cis-10-pentadecenoic acid	10.862	55.1	0.1-4.0	0.999	0.03	0.1	0.01
C15:0	pentadecanoic acid	pentadecylic acid	10.921	74.1	0.1-4.0	0.999	0.03	0.1	0.01
C16:1n7	(Z)-hexadec-9-enoic acid	palmitoleic acid	11.645	55.1	0.1-4.0	0.999	0.03	0.1	0.01
C16:0	hexadecanoic acid	palmitic acid	11.811	74.1	0.3-12.0	0.999	0.10	0.3	0.03
C17:1n7	(Z)-heptadec-10-enoic acid	cis-10-heptadecenoic acid	12.681	55.1	0.1-4.0	0.998	0.03	0.1	0.01
C17:0	heptadecanoic acid	margaric acid	12.877	74.1	0.1-2.8	0.998	0.03	0.1	0.01
C18:3n6	(6Z,9Z,12Z)-octadeca-6,9,12-trienoic acid	gamma-linolenic acid	13.639	79.1	0.1-4.0	0.998	0.03	0.1	0.01
C18:2n6c	(9Z,12Z)-octadeca-9,12-dienoic acid	linoleic acid	13.825	81.1	0.1-4.0	0.999	0.03	0.1	0.01
C18:2n6t	(9E,12E)-octadeca-9,12-dienoic acid	linolelaidic acid	13.894	55.1	0.1-4.0	0.999	0.03	0.1	0.01
C18:3n3	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	linolenic acid	13.943	79.0	0.1-4.0	0.999	0.03	0.1	0.01
C18:1n9c	(Z)-octadec-9-enoic acid	oleic acid (elaidoic acid)	13.952	55.1	0.2-8.0	0.998	0.07	0.2	0.02
C18:1n9t	(E)-octadec-9-enoic acid	elaidic acid	13.972	55.1	0.1-4.0	0.999	0.03	0.1	0.01
C18:0	octadecanoic acid	stearic acid	14.197	74.0	0.2-8.0	0.999	0.07	0.2	0.02

C20:4n6	(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid	arachidonic acid	16.495	79.1	0.1-4.0	0.990	0.03	0.1	0.01
C20:5n3	(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid	cis-5,8,11,14,17-eicosapentaenoic acid, EPA	16.632	79.1	0.1-2.2	0.990	0.03	0.1	0.01
C20:3n6	(8Z,11Z,14Z)-icosa-8,11,14-trienoic acid	cis,cis,cis-8,11,14-eicosatrienoic acid, DGLA	16.788	79.0	0.1-3.9	0.987	0.03	0.1	0.01
C20:2n6	(11Z,14Z)-icosa-11,14-dienoic acid	eicosadienoic acid	17.111	81.1	0.1-3.6	0.991	0.03	0.1	0.01
C20:1n9	(Z)-icos-11-enoic acid	cis-11-eicosenoic acid	17.189	55.1	0.1-4.0	0.999	0.03	0.1	0.01
C20:3n3	(11Z,14E,17E)-icosa-11,14,17-trienoic acid	cis-11,14,17-eicosatrienoic	17.267	79.1	0.1-3.2	0.986	0.03	0.1	0.01
C20:0	icosanoic acid	arachidic acid	17.639	74.1	0.2-8.0	0.994	0.07	0.2	0.02
C21:0	heneicosanoic acid	heneicosylic acid	19.771	74.0	0.1-4.0	0.989	0.03	0.1	0.01
C22:6	(4Z,7Z,10Z,13Z,16E,19E)-docosa-4,7,10,13,16,19-hexaenoic acid	cis-4,7,10,13,16,19-docosahexaenoic, DHA	20.485	79.1	0.1-2.8	0.981	0.03	0.1	0.01
C22:2n6	(13E,16E)-docosa-13,16-dienoic acid	cis-13,16-docosadienoic acid	21.492	81.1	0.1-4.0	0.972	0.03	0.1	0.01
C22:1n9	(*Z*)-docos-13-enoic acid	erucic acid	21.570	55.1	0.1-4.0	0.994	0.03	0.1	0.01
C22:0	docosanoic acid	behenic acid	22.147	74.1	0.2-8.0	0.986	0.07	0.2	0.02
C23:0	tricosanoic acid	tricosylic acid	24.719	74.0	0.1-3.2	0.983	0.03	0.1	0.01
C24:1n9	(*Z*)-tetracos-15-enoic acid	nervonic acid	26.811	55.0	0.1-4.0	0.995	0.03	0.1	0.01
C24:0	tetracosanoic acid	lignoceric acid	27.447	74.0	0.2-8.0	0.993	0.07	0.2	0.02

The symbol n in abbreviated FAs names shows the location of the last double bond in the chain, thereby type of omega acid.

Table 2. Recovery and precision (expressed as RSD) of the method for different food samples.

Sample	Recovery, %	Precision (RSD, %)
yeast	56.0	6.9
yeast flakes	61.9	9.7
biscuits	66.6	6.4
milk thistle oil	86.5	2.6
olive oil	74.2	4.0

Table 3. Results of CRM analysis.

FA	% in CRM according to certificate	Determined, %	Difference, %
C 16:0	20.6	18.73±0.31	-1.87
C 18:0	6.43	8.16±0.35	1.73
C18:1n9c	68.2	66.49±0.40	-1.71
C18:2n6c	4.77	6.62±0.38	1.85

The AOAC guidelines require a RSD for the precision that does not exceed 15%, except for LOQ, which can have a RSD of 20% (AOAC, 2013). The precision in our method met those criteria. In the FAs analysis, ISTD is added before the analysis, so quantitative results can be recalculated taking into account the recovery for ISTD (Van Wychen et al., 2013; Khoury et al., 2018).

CRM analysis

Table 3 presents the analysis results for CRM (product ID 47118) of olive oil purchased from Merck KGaA (Darmstadt, Germany) by the modified method. The CRM certificate provides area values, as %, for four main components. We analyzed CRM in four replicates. The calculated differences versus certified values were below 2%, which proves a good performance of the method. Figures 1 and 2 present chromatograms for FAME MIX standards at concentration 2-12 μg/mL for individual FAME and for CRM. On Figure 1 good separation of all FAME could be seen. Retentions times for FAME were in the range from 4 minutes to 27.447 minutes. On Figure 2 there are only four peaks as the CRM contain only four FAME. C18:2n6c has close retention time to C18:1n9c, but these peaks were qualified and quantified correctly (Table 3).

FAs content in food samples

Table 4 presents the profile of individual FAs in food matrices, while Figure 3 shows percentage contents of saturated fatty acids (SAFA), MUFA and PUFA. Klug and Daum (2014) stated that in *S. cerevisiae* palmitoleic and oleic acids are main FAs, which are followed by palmitic and stearic acids. The composition of FAs varies, because it depends on growth conditions, and in standard ones, MUFA represent about 80% of total FAs (50% of palmitoleic acid and 30% of palmitoleic acid). Palmitic acid in concentration of 9% is the most dominant SAFA (Martin et al., 2007; Tuller et al., 1999). Our results agree to these values. SAFA contents for yeast and yeast flakes was 15%, while yeast contained more MUFA, especially oleic acid C18:1n9c, than yeast flakes (74% vs. 63%, respectively). Oleic acid is the main FA present in biscuits samples (83%). Yanty et al. (2014) found that composition of FAs in biscuits depends on fats origin (animal or plant) used for their production, and the oleic acid content may exceed 60%. Plant oils are rich sources of MUFA and PUFA. Milk thistle oil contains 50% of PUFA (linoleic acid C18:2n6c) and 29% of MUFA, while olive oil contains 34% of PUFA and 54% of MUFA (mainly oleic acid C18:1n9c). Zhang et al. (2020) found that the predominant FAs in milk thistle seed oils are linoleic (45.83–46.41%) and oleic (30.12–30.59%) acids. Rousseaux et al. (2020) stated that the olive contains 50-60% of oleic acid, depending on cultivar and plant growing region.

FAs content in superfood and spices

Seeds and spices are a rich source of MUFA and PUFA. Flax seeds and chia seeds contain 77% and 86% of PUFA, respectively, and these PUFAs are mainly C18:3n3 (57% and 60%) and C18:2n6c. The omega 3 to omega 6 fatty acids ratio is a very important factor. Ratios from 1: 6 to 1: 4 and even up to 1: 1 are known to prevent cardiovascular diseases, obesity, inflammation, insulin resistance and cancer (Freitas, 2017; Simopoulos, 2008). The most tested superfoods and spices have a beneficial ratio of omega 3 and omega 6 FAs (Table 5). The ratio below 1:1 was found for flax and chia seeds. White mustard seeds contained 41% erucic acid (C22: 1n9), FA of a known negative effect on human health and its content is subject to EU regulations (Schwarzinger et al., 2022).

Table 5 presents the content of individual FAs in superfood matrices and spices, while Figure 4 shows percentage contents of SAFA, MUFA and PUFA.

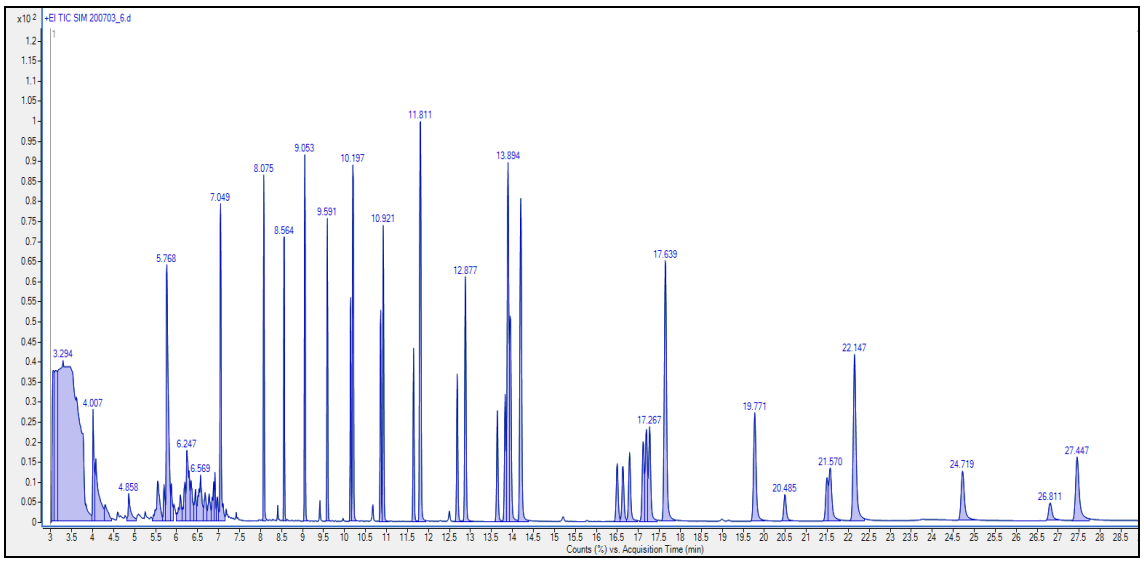

Figure 1. Chromatogram for FAME MIX standards at concentration 2-12 µg/mL for individual FAME.

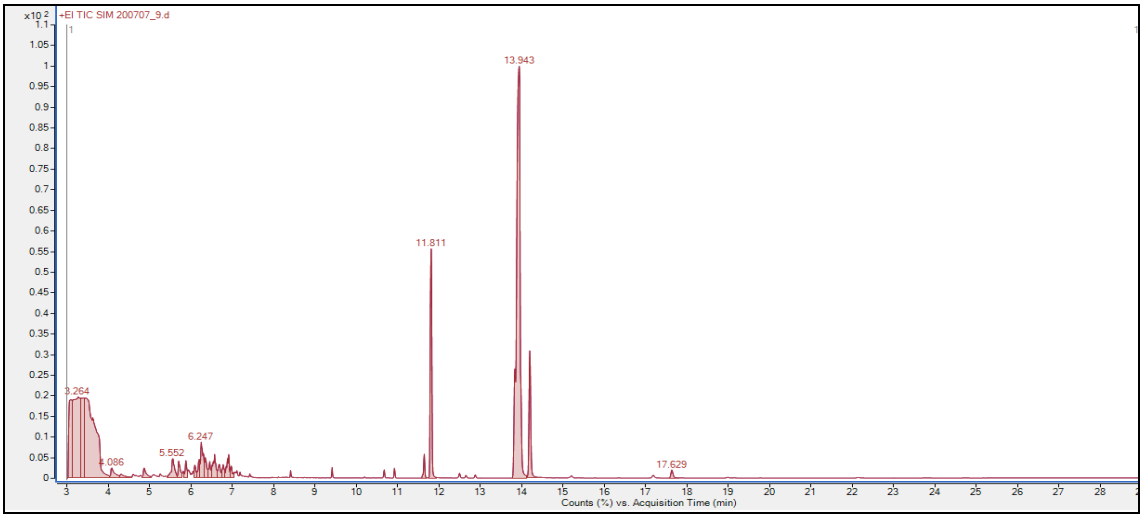

Figure 2. Chromatogram for olive oil CRM (C 16:0 – 11.811 min, C18:2n6c - 13.825 min, C18:1n9c – 13.943 min and C 18:0 – 14.197 min).

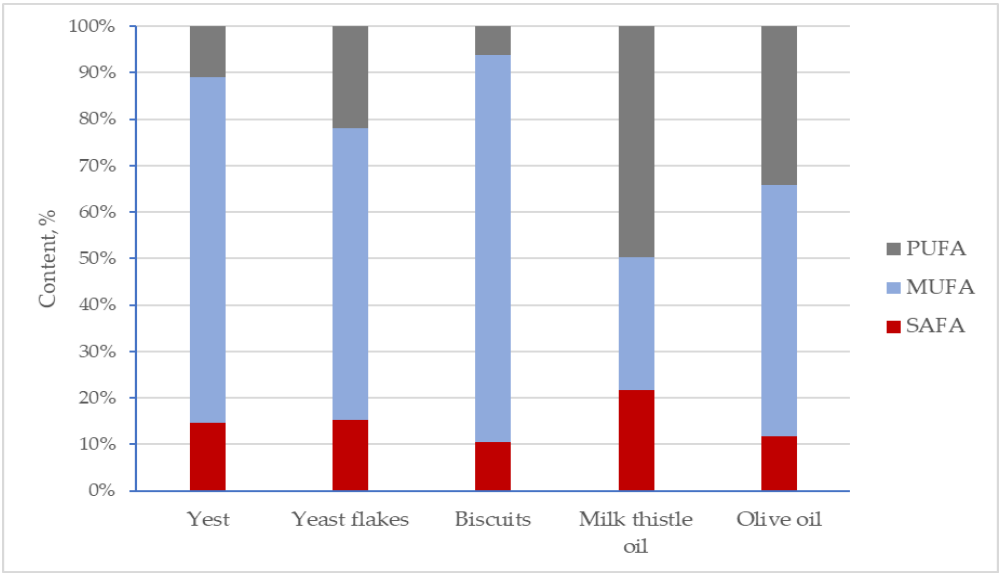

Figure 3. Percentage contents of SAFA, MUFA and PUFA in food products.

Table 4. Profile of FAs (%, average ±SD) in food matrices, n=6.

Sample	Yeast	Yeast flakes	Biscuits	Milk thistle oil	Olive oil
C6:0	–	–	0.13±0.02	–	–
C8:0	–	–	0.06±0.01	–	–
C10:0	–	–	0.32±0.03	–	–
C12:0	0.19±0.03	–	0.47±0.04	–	–
C14:1n5	0.12±0.01	–	0.11±0.01	–	–
C14:0	0.33±0.05	0.20±0.01	1.14±0.10	0.11±0.01	0.02±0.00
C15:1n5	0.10±0.01	–	0.03±0.01	0.01±0.00	–
C15:0	0.14±0.17	0.19±0.42	0.10±0.06	0.02±0.00	0.01±0.01
C16:1n7	18.09±2.66	13.85±1.31	0.21±0.04	0.13±0.01	1.97±0.25
C16:0	9.61±1.39	11.57±1.27	5.47±0.47	3.36±0.20	5.42±0.40
C17:1n7	0.33±0.05	0.28±0.02	0.07±0.01	0.05±0.00	0.31±0.05
C17:0	0.18±0.02	0.19±0.02	0.05±0.01	0.06±0.01	0.14±0.02
C18:2n6c	11.06±1.98	21.86±2.57	6.26±0.64	49.77±3.34	34.10±1.15
C18:1n9c	55.68±6.10	48.69±4.83	82.67±7.04	26.49±1.46	51.17±1.09
C18:0	4.18±0.63	3.16±0.28	2.26±0.24	5.12±0.34	4.76±0.61
C20:1n9	–	–	0.14±0.01	1.93±0.17	0.62±0.09
C20:0	–	–	0.17±0.01	5.77±0.46	0.96±0.14
C21:0	–	–	–	0.06±0.01	0.04±0.01
C22:0	–	–	0.32±0.02	5.57±0.56	0.29±0.06
C23:0	–	–	–	0.05±0.00	0.04±0.00
C24:0	–	–	–	1.50±0.24	0.14±0.02

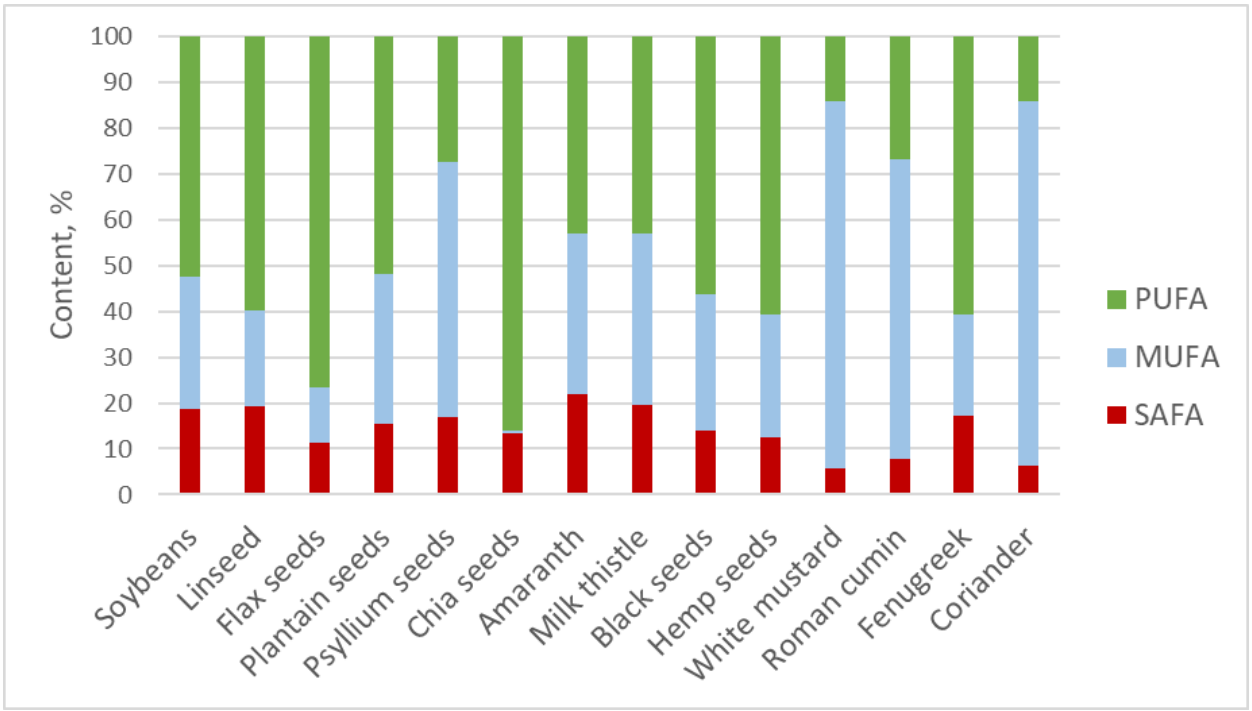

Figure 4. Percentage content of SAFA, MUFA and PUFA in superfood products and spices.

Table 5. Profile of FAs in superfood matrices and spices (%, average±SD, n=3).

Sample	Soy beans	linseed	flax seeds	plantain seeds	psyllium seeds	chia seeds	amaranth	milk thistle	black seeds	hemp seeds	white mustard	Roman cumin	fenugreek	coriander
C6:0	–	–	–	–	–	–	–	–	–	–	–	0.38± 0.02	–	–
C14:1n5	0.03± 0.00	0.04± 0.00	0.04± 0.00	0.07± 0.01	0.11± 0.01	0.03± 0.00	0.07± 0.01	0.04± 0.00	0.03± 0.00	0.02± 0.00	0.02± 0.00	0.04± 0.00	0.07± 0.01	0.03± 0.00
C14:0	–	–	–	–	–	–	–	–	0.27± 0.03	–	–	–	–	–
C15:1n5	0.03± 0.00	0.03± 0.00	0.04± 0.00	0.06± 0.01	0.09± 0.01	0.02± 0.00	0.07± 0.01	0.04± 0.00	0.03± 0.00	0.02± 0.00	0.02± 0.00	0.03± 0.00	0.07± 0.01	0.03± 0.00
C15:0	0.02± 0.00	0.05± 0.00	0.02± 0.00	0.06± 0.00	0.09± 0.01	0.03± 0.00	0.07± 0.01	0.03± 0.00	0.05± 0.00	0.02± 0.00	0.02± 0.00	0.08± 0.01	0.11± 0.02	0.03± 0.00
C16:1n7	0.16± 0.02	0.30± 0.02	0.13± 0.01	0.18± 0.01	0.37± 0.03	0.13± 0.01	0.18± 0.01	0.13± 0.00	0.35± 0.02	0.21± 0.02	0.43± 0.02	0.65± 0.05	0.16± 0.02	0.67± 0.07
C16:0	8.24± 0.23	9.70± 0.08	6.82± 0.42	10.91± 0.50	12.80± 0.09	6.96± 0.10	15.41± 0.90	8.36± 0.54	7.89± 0.42	5.98± 0.30	2.82± 0.03	4.98± 0.09	9.92± 0.10	4.15± 0.41
C17:1n7	0.13± 0.01	0.15± 0.02	0.08± 0.01	0.11± 0.01	0.15± 0.01	0.05± 0.00	0.13± 0.02	0.08± 0.01	0.11± 0.01	0.09± 0.01	0.04± 0.00	0.13± 0.02	0.23± 0.02	0.10± 0.01
C17:0	0.13± 0.00	0.17± 0.00	0.08± 0.01	0.12± 0.02	0.11± 0.01	0.10± 0.00	0.13± 0.01	0.11± 0.00	0.12± 0.01	0.10± 0.02	0.03± 0.00	0.08± 0.00	0.35± 0.02	0.05± 0.00
C18:3n6	–	–	–	–	–	–	–	–	–	0.72± 0.00	–	–	–	–
C18:2n6c	42.31± 1.23	36.32± 1.32	18.80± 0.68	43.63± 2.80	18.87± 1.05	25.24± 1.44	42.70± 2.50	43.11± 3.02	51.55± 3.67	48.48± 2.54	9.23± 0.19	26.92± 1.90	30.90± 2.67	14.11± 1.04
C18:3n3	10.03± 0.87	23.07± 0.90	57.48± 2.40	7.90± 0.45	8.61± 0.70	60.43± 3.66	–	–	–	11.38± 0.58	4.70± 0.30	–	29.38± 0.88	–
C18:1n9c	28.27± 1.20	19.96± 0.90	11.63± 0.39	32.11± 0.54	54.10± 2.33	–	33.95± 2.39	36.03± 1.25	25.38± 1.18	25.62± 1.11	27.00± 1.12	63.63± 3.30	21.07± 1.05	78.34± 3.26
C18:0	4.36± 0.30	7.70± 0.23	2.73± 0.09	2.80± 0.18	1.57± 0.05	5.01± 0.22	3.46± 0.11	5.48± 0.23	4.53± 0.24	4.36± 0.31	0.93± 0.08	0.83± 0.05	3.60± 0.09	0.77± 0.03
C20:2n6	0.11± 0.01	0.16± 0.02	0.16± 0.02	–	–	0.13± 0.011	0.23± 0.02	–	4.45± 0.10	0.14± 0.01	0.25± 0.02	–	0.28± 0.01	–
C20:1n9	0.27± 0.01	0.43± 0.02	0.25± 0.01	0.40± 0.02	0.43± 0.02	0.26± 0.01	0.37± 0.01	0.89± 0.03	3.88± 0.22	0.62± 0.04	7.44± 0.20	0.37± 0.03	0.33± 0.02	0.27± 0.01
C20:3n3	–	0.18± 0.01	0.14± 0.00	0.21± 0.01	–	0.11± 0.01	–	–	–	–	–	–	–	–
C20:0	4.69± 0.23	0.49± 0.02	0.37± 0.01	0.49± 0.02	0.75± 0.03	0.53± 0.03	0.88± 0.04	2.94± 0.11	0.46± 0.02	1.23± 0.06	0.63± 0.02	0.27± 0.01	1.10± 0.02	0.22± 0.01
C21:0	0.11±	0.10±	0.12±	0.21±	0.29±	0.08±	0.23±	0.14±	0.07±	0.07±	0.06±	0.13±	0.28±	0.11±

	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.01	0.00	0.00	0.00	0.02	0.00
C22:2n6	–	–	–	–	–	–	–	–	0.16± 0.02	–	–	–	–	0.14± 0.00
C22:1n9	–	–	–	–	0.36± 0.00	0.09± 0.00	0.25± 0.02	0.16± 0.01	0.07± 0.00	0.09± 0.01	41.22± 2.20	0.58± 0.03	0.32± 0.02	0.14± 0.00
C22:0	0.64± 0.02	0.50± 0.06	0.44± 0.02	0.52± 0.03	0.96± 0.07	0.31± 0.02	0.75± 0.02	2.22± 0.11	0.28± 0.09	0.49± 0.03	0.68± 0.02	0.36± 0.01	0.84± 0.07	0.36± 0.02
C23:0	0.12± 0.01	0.14± 0.01	0.16± 0.01	0.22± 0.02	0.34± 0.02	0.11± 0.00	0.28± 0.02	0.15± 0.01	0.08± 0.00	0.09± 0.01	0.08± 0.00	0.14± 0.00	0.29± 0.02	0.11± 0.01
C24:1n9	–	–	–	–	–	–	–	–	–	–	3.93±0.00	–	–	–
C24:0	0.34± 0.01	0.50± 0.02	0.49± 0.02	–	–	0.38± 0.02	0.82± 0.03	0.08± 0.00	0.24± 0.02	0.27± 0.02	0.48± 0.01	0.41± 0.02	0.72± 0.01	0.38± 0.01
Omega 3:6 ratio	1:4.2	1:1.6	1:0.3	1:5.4	1:2.2	1:0.4	–	–	–	1:4.3	1:2.0	–	1:1.1	–

CONCLUSIONS

The optimized method could be used with satisfying results for fast and simple determination of 37 FAME (saturated, monounsaturated and polyunsaturated) in different food matrices (liquid and solid) in one analytical run. This method allow for quick and precise assessment of food product in terms of nutritional properties. The better recoveries were achieved for liquid samples than for dry solid matrices. Thanks to the application of SIM mode LOQs in the range 0.1-0.3 µg/mL could be achieved.

The superfoods and spices are very good source of MUFA and PUFA which are very important for people health. The best ratio between omega 3 and omega 6 FAs was for flax and chia seeds.

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