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Identification and quantification of *trans* fatty acids in bakery products by gas chromatography–mass spectrometry after focused microwave Soxhlet extraction

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Abstract

Trans fatty acids have been determined in fourteen bakery products using derivatisation by ester formation, gas chromatography–mass spectrometry for individual separation and identification/quantification following total fat isolation by Soxhlet extraction accelerated by focused microwave irradiation at the cartridge zone. The detection and quantification limits between 0.98 and 3.93 and 3.23–12.98 μg g^{-1}, respectively, and the linear dynamic ranges between LOQs values and 12,000 μg g^{-1} thus obtained, demonstrated the utility of the approach for this type of analysis thanks to the wide determination range and high information level it provides. The proposed extraction method, which was validated by comparison with the Folch reference method – extraction under very mild conditions, shows that no changes of the original fat are produced under microwave-assisted extraction. The much shorter extraction time – 35 or 60 min vs. 3.5 h of the Folch method – and similar characteristics of the extract make this method an excellent alternative for the treatment of solid samples prior to *trans* fatty acids analysis. The target analytes were determined in fourteen bakery products; thus supporting the validity of the overall process.

Keywords: *Trans* fatty acids; GC–MS; Microwave-assisted Soxhlet extraction; Bakery products

1. Introduction

The definition of 'total fat', as established by the US Food and Drug Determination (FDA) in 1990 through the Nutritional Labeling and Education Act (NLEA), is 'the sum of all fatty acids obtained in the lipid extract, expressed as triglycerides' (Federal Register, 1993). Therefore, edible fats and oils consist almost entirely of fatty acids. Fats and oils of animal origin – such as butter and lard – are composed primarily of saturated fatty acids (Wilkinson, 2003), the high consumption of which, together with that of cholesterol, are mainly responsible for hypercholesterolemia (Kromhout et al., 1995), which in turn is responsible for a number of cardiovascular diseases (Neaton & Wentworth, 1992). In order to reduce the saturated fat content of processed foods, the food industry in developed countries moves progressively from animal fat to vegetable fat sources, which provide oils with a higher content of unsaturated fats, liquid at room temperature (Tavella et al., 2000). Moreover, unsaturated fats are heart-healthy, but they have some undesirable properties, specifically, in air, unsaturated fatty acids can gradually become rancid by absorbing oxygen and forming hydroperoxides that decompose (Wilkinson, 2003). Manufacturers block that deterioration in order to simulate the consistency of saturated fat, through a process of partial saturation called hydrogenation. Hydrogen is bubbled through the fat at elevated temperature in the absence of oxygen and presence of a catalyst such as nickel (Daglioglu, Tasan, & Tuncel, 2000; Dionisi, Golay, & Fay, 2002; Parcerisa, Codony, Boatella, & Rafecas, 1999; Wagner, Auer, & Elmadfa, 2000).

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Prior to hydrogenation, most naturally occurring unsaturated fatty acids take the *cis* configuration at their double bonds. Partial hydrogenation rearranges the double bonds, converting some of them to the *trans* configuration and shifting the double bonds along the carbon chain (Wilkinson, 2003). Several clinical studies have shown that a high *trans* fatty acid (TFA) diet causes adverse changes in the plasma lipoprotein profile, with an increase in low-density lipoproteins (LDL) and decrease in high-density lipoproteins (HDL) (Parcerisa et al., 1999; Pretch & Molkentin, 2000). Some epidemiological studies have also found a relationship association between level of *trans* fatty acid intake and risk of cardiovascular diseases (Ascherio et al., 1994; Ascherio & Willet, 1997). These concerns moved the Food and Agriculture Organization (FAO) and the World Health Organization (WHO, 1994) to recommend that fats for human consumption should contain less than 4% of the total fat as *trans*, and urged the food industry to reduce the presence of *trans* fats in their products to these levels. The Food and Drug Administration (FDA) has also decreed that by January 1, 2006, manufacturers must break the *trans* fats category out of the total fat listing. For this purpose, FDA and Health Canada have proposed food-labeling rules that require the amount of *trans* fat per serving to be added to the amount of saturated fat per serving. Specifically, products that contain >0.5 g per serving would have the asterisked footnote, "*Includes – g *trans* fat" (Ruth, 2002). Also in Europe this is a concern as demonstrated by the Danish legislation – that has established a lower content of these lipids, <2% (w/w) – and the general trend in the EU to include in the label the content of *trans* fatty acids as a quality index.

Lipid extraction is traditionally carried out in different ways and using organic solvents which depend on the sample characteristics (García-Ayuso & Luque de Castro, 1999). Methods such as the Weibull-Berntrop, Röse-Gottlieb, Mojonnier, Folch, Werner-Schmid, and Bligh-Dyer are based on acid, alkaline or enzymatic hydrolysis before solvent extraction but some others involve only the solvent extraction step like Soxhlet as more representative example. However, the long extraction time (16–24 h) and the high temperatures needed for Soxhlet extraction are its main shortcomings and they might involve changes in the extract composition. These alterations can influence the results of some specific types of analysis, as can be the *trans* fatty acids determination owing to *cis*/*trans* configuration. For this reason, the Folch method has been used for total fat extraction prior to the analysis of the *trans* fatty acids content thanks to its very mild conditions – no high temperatures nor pressures are applied.

Despite modifications in solvent mixtures and laboratory practice (Erickson, 1993; Freyburger, Heape, Gin, Boisseau, & Cassagne, 1988), the previous extraction methods have not been greatly improved, and long preparation times with re-extraction steps to ensure complete lipid isolation are required (Nelson, 1991). Looking for avoiding these shortcomings, methods based on new technologies such as supercritical fluid extraction (SFE) (Eller, 1999), closed systems at high temperature and pressure (ASE) (Boselli, Velazco, Caboni, & Lercker, 2001), focused microwave-assisted Soxhlet extraction (FMASE) (Priego-Capote & Luque de Castro, 2005) and dynamic ultrasound assisted extraction (DUAE) (Ruiz-Jiménez & Luque de Castro, 2004) have been reported for total fat extraction.

FMASE operates in a similar way to conventional Soxhlet but the main shortcomings of the latter are circumvented, particularly, the long extraction times, emission to the atmosphere of organic solvents used as extractants and low extraction efficiency for compounds strongly bonded to the sample matrix. Recently, a FMASE method prior to quantification of total *trans* fat content using medium infra-red (MIR) spectroscopy has shown to be an excellent alternative to conventional and reference methods for application in routine analysis. It is worth to emphasis that a shortcoming of the overall method results from the determination step – FTIR, which provides a not low enough limit of quantification – namely, 1.04% w/w – and overall information as quantification corresponds to total *trans* content (Priego-Capote, Ruiz-Jiménez, García-Olmo, & Luque de Castro, 2004).

A way to decrease the limit of quantification and provide a higher level of information is to use a gas chromatograph (GC) equipped with an MS detector, which allows quantification of each individual compound. However, GC does not allow direct individual separation, and the formation of more volatile products from the analytes makes mandatory a derivatisation step, usually to fatty acid methyl esters (FAMEs) (Fritsche & Steinhart, 1998); so the analysis time is considerably increased as compared with IR spectroscopy.

The purpose of this research was the development of an overall analytical method for the fast extraction of fat content from bakery products, based on the use of auxiliary energies, without extract alterations – mainly those that affect to double bonds, and independent identification-quantification of fatty acids using GC–MS with previous derivatisation to FAMEs, including, obviously, the *trans* compounds. FMASE has been used for isolation of fat from the given matrices and the results obtained compared with the Folch reference method in order to demonstrate the advantages of the proposed analysis for *trans* fatty acids. For other side, GC–MS allows to achieve limit of quantification low enough for fulfilling the present and future necessities and provide information in relation to the fat quality used for food elaboration.

2. Experimental

2.1. Instruments and apparatus

The first prototype of a focused microwave-assisted Soxhlet extractor was used in the extraction step (Luque de Castro & García-Ayuso, 1998). This device is composed of a conventional Soxhlet extractor modified in order to

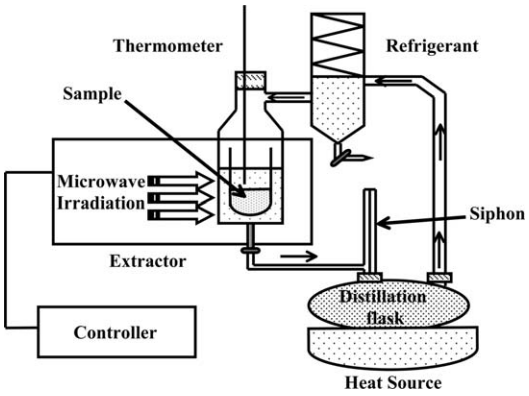

Fig. 1. Scheme of the FMASE prototype operation.

facilitate accommodation of the sample cartridge compartment in the irradiation zone of a Microdigest 301 digestor of 200 W maximum power (Prolabo, Paris, France). The latter, which is also modified, has an orifice at the bottom of the irradiation zone that enables connection of the cartridge zone to the distillation flask through a glass siphon. Fig. 1 illustrates the operation of the overall device.

At present, a commercial automatised version is being developed by Electromedicarin (Barcelona, Spain).

A Megal 500 thermometer (Prolabo) was used to monitor the extraction temperature. Two microprocessor programmers (Prolabo) were used to control the microwave unit and thermometer. An electrical isomantle (Prolabo) with a rheostat was used to heat the distillation flask. The samples were placed in cellulose extraction thimbles (25×88 mm, Albet, Barcelona, Spain).

A rotary-evaporator (Büchi R-200 with Heating Bath B-490, Switzerland) was used to evaporate the solvent after extraction.

A vortex from Ika-Works (Wilmington, USA) and a centrifuge (Selecta, Barcelona, Spain) were used in the derivatisation step.

A Varian CP 3800 gas chromatograph coupled to a Saturn 2200 ion trap mass spectrometer (Sugar Land, TX, USA), equipped with an SP-2380 fused-silica capillary column (60 m × 0.25 mm, 0.2 μm) coated with stabilised poly (90% biscyanopropyl/10% cyanopropylphenyl siloxane), provided by Supelco (Bellefonte, PA, USA), was used for the specific analysis of the *trans* fatty acids from the extracts.

2.2. Reagents and sample preparation

n-Hexane HPLC grade Panreac (Barcelona, Spain) was used as leaching agent for the isolation of fat content in bakery samples by the proposed method. A 2:1 (v/v) ratio of HPLC grade trichloromethane–methanol (Panreac) was used in the Folch extraction reference method. NaCl, $NaClO_4$, and anhydrous Na_2SO_4 (Panreac) were used for partition of the resulting extract and as drying agent of the organic phase, respectively. Sodium methylate (0.5 M) in methanol (Panreac) was used as derivatisation reagent in order to hydrolyse and transform the fat in fatty acid methyl esters (FAMEs). All safety precautions (gloves, mask, hood-fume, etc.) were adopted.

Tetradecanoic acid methyl ester (14:0), hexadecanoic acid methyl ester (16:0), *trans*-hexadecenoic methyl ester (*t*16:1), octadecanoic acid methyl ester (18:0), *trans*-octadecenoic acid methyl ester (*t*18:1), *trans*,*trans*-octadecadienoic acid methyl ester (*tt*18:2), *cis*,*trans*-octadecadienoic acid methyl ester (*ct*18:2), *trans*,*cis*-octadecadienoic acid methyl ester (*tc*18:2), *cis*,*cis*-octadecadienoic acid methyl ester (18:2), eicosanoic acid methyl ester (20:0), *cis*,*cis*,*cis*-octadecatrienoic (18:3) and docosanoic acid methyl ester (22:0) from Sigma–Aldrich (St. Louis, MO, USA) were used as standards. Decanoic acid methyl ester from Fluka (Steinheim, Germany) was used as internal standard in the determination step.

Fourteen bakery products – all them commercial – were used in this study. These products were manufactured in Spain – specifically, Snack Fiber Cheese (1) (Celigüeta, Araia, Alava, Spain), Cheetos (2) (Matutano, Tarragona, Spain), Müesli Multivitamins bifidus effect cookies (3) (Bio Century, Quart, Girona, Spain), Cookies produced using traditional methods (4) (Bjorg, Italy), Snack Corn barbecue-flavored Hacendado (5), Crackers cones Hacendado (6) (Grefusa S.L., Alzira, Valencia, Spain), Built-in doughnut (7) (Santiveri, Barcelona, Spain), Snack Cookies Hacendado (8) (Grupo Siro, Venta de Baños, Palencia, Spain), Home-made cake (9), Bugles 3D's (10) (Matutano), Sancho Panza egg's cakes (11) (Galletas Angulo, Lerma, Burgos, Spain), Hazelnuts and chocolate cookies Hacendado (12) (Arluy, Logroño, La Rioja, España), Corn ham-flavored Hacendado (13) (Grefusa S.L.) and free of salt toasted bread Hacendado (14) (Pimad S.A., Azuqueca de Henares, Spain).

Sample preparation was done according to the protocol established by legislation (Analysis Methods, 1988). The product under study was homogenised; 200 g of sample was crushed in a mincer and then, homogenised again and stored in a hermetic recipient at 4 °C in the dark until use.

2.3. Procedures

All the steps involved in the overall analysis – namely, extraction, derivatisation and separation/determination – are described in this section. In all instances three replicates were made of each sample. It is necessary to point out that the extractant used in the proposed method was *n*-hexane instead of the 2:1 chloroform/methanol mixture of the Folch method. This fact is justified by the impossibility of using solvent mixtures in the FMASE method, where the solvent passes to the extraction chamber as a function of its boiling point. The non-polar character of *n*-hexane provides a more effective extraction of the fat contents than a polar (methanol) and a medium-polar solvent (chloroform) mixture. Moreover, as the derivatisation step

consists of a hydrolysis step, it would not be necessary a solvent with this purpose in the extractant mixture.

2.3.1. Focused microwave-assisted Soxhlet extraction

One hundred and twenty five milliliters of *n*-hexane and some pieces of pumice stone were poured into a distillation flask. Four grams of the sample was mixed with one gram of pre-washed sea sand as a dispersion agent. The mixture was put into a cellulose extraction thimble, which was covered with cotton wool and inserted into the quartz extraction vessel placed in the microwave-irradiation zone. The distillation flask was positioned on the electrical isomantle and connected to the sample vessel by a siphon and a distillation tube. The extraction process consisted of a number of cycles that depended on the extraction kinetics of the target sample. Each cycle involved three steps:

(a) the extractant evaporated from the distillation flask, condensated in the refrigerant and dropped on the sample, filling the sample cartridge vessel. The volume of the extractant put into contact with the sample was controlled by the height into the siphon;
(b) the magnetron started to irradiate the sample cartridge when the solvent reached the preset height in the siphon for 90 s at 100 W;
(c) the extract was unloaded to the distillation flask, after irradiation.

After the extraction step – 12 or 7 cycles, depending on the type of sample matrix (60 or 35 min, respectively), the 75–85% of the extractant was recycled by a new cycle and condensed in the lower part of the refrigerant. So, the extract was evaporated to near-dryness and 90–100 ml of the solvent was thus recovered as a result. The residue was transferred to a 10-ml glass vial, and the last traces of solvent were removed by a nitrogen stream in order to carry out the derivatisation step.

2.3.2. The Folch reference extraction method

This method was selected as reference for fat extraction thanks to its mild working conditions – neither heat nor high pressures are applied, which avoid potential alterations of the fat extracted. Twenty-five grams of sample was mixed with 75 ml of a 2:1 (v/v) chloroform/methanol mixture, which was shaken in a 250 ml Erlenmeyer flask by a magnetic stirrer for 45 min. Then, the mixture was filtered and the solid phase was re-extracted one or two times more, respectively, – depending on the sample matrix – with the same volume of extractant. The liquid phases were combined in a separatory funnel. Thirty-five milliliters of saturated sodium chloride in water and 0.5 g of $NaClO_4$ were added, and the mixture was gently shaken. After phase separation, the chloroform phase was filtered, dried with sodium sulfate and filtered again. Finally, the extractant was evaporated to dryness under an N_2 stream. The total time required was 150 or 270 min, respectively, depending on the sample matrix.

2.3.3. Preparation of fatty acid methyl esters

0.1 g of either the fat extracted was diluted to 5 ml with *n*-hexane and homogenised for 30 s in a vortex. Then, 0.5 ml of sodium methylate in methanol was added and shaken vigorously for 3 min in the vortex and centrifuged for 2 min at 2000 min^{-1}. The supernatant was transferred to a test tube and evaporated to dryness under an N_2 stream. *n*-Hexane (0.5 ml) was used to reconstitute the residue, which was shaken for 1 min. Finally, 1 μl of the solution thus obtained was injected into the chromatograph.

2.3.4. GC–MS separation-detection

Helium at a constant flow-rate of 1 ml min^{-1} was used as carrier gas for the GC–MS analysis of the FAME extracts. The column temperature program was 50 °C, held for 2 min, then increased at 5 °C min^{-1} to 250 °C, and, finally, held for 15 min. The injections (1 μl each) were of the splitless mode with the injector temperature set at 250 °C. As can be seen in Fig. 2, the development of the chromatogram required about 40 min.

The ion trap mass spectrometer was operated in the electron impact ionisation (EI) positive-mode using automatic gain control. For EI experiments the instrumental parameters were set at the following values: a filament emission current of 80 μA, an electron multiplier voltage of 1600 V, modulation amplitude of 4 V using perfluorotributylamine (FC-43) as reference and a multiplier offset of 200 V. The transfer line, the ion trap and the manifold temperatures were kept at 170, 170 and 50 °C, respectively. The storage window was set between 40 *m/z* and 600 *m/z* and selected-ion monitoring (SIM) ion preparation mode was used. The scan time during data acquisition was set at 1.0 s with three microscans per second (Czarniecki, 2003).

3. Results and discussion

Focused microwave-assisted Soxhlet extraction has demonstrated to be an excellent method for isolation of the total fat content in a variety of samples (García-Ayuso, 2000). Furthermore, extracts obtained with FMASE has been used prior to the determination of the TFA overall content, being the Folch reference method used for validation because its mild working conditions enables to ensure the absence of alterations of these acids during extraction (Priego-Capote et al., 2004). However, the applicability of FMASE in a more exhaustive analytical method in terms of information level provided – as is the case of the determination of the fatty acids profile with emphasis on TFA – has not been demonstrated. For this reason, the optimal working conditions obtained for total fat extraction (Priego-Capote & Luque de Castro, 2005) were applied to check the ability of this fast extraction step for the specific analysis of *trans* fatty acids. Previously, the chromatographic conditions were optimised.

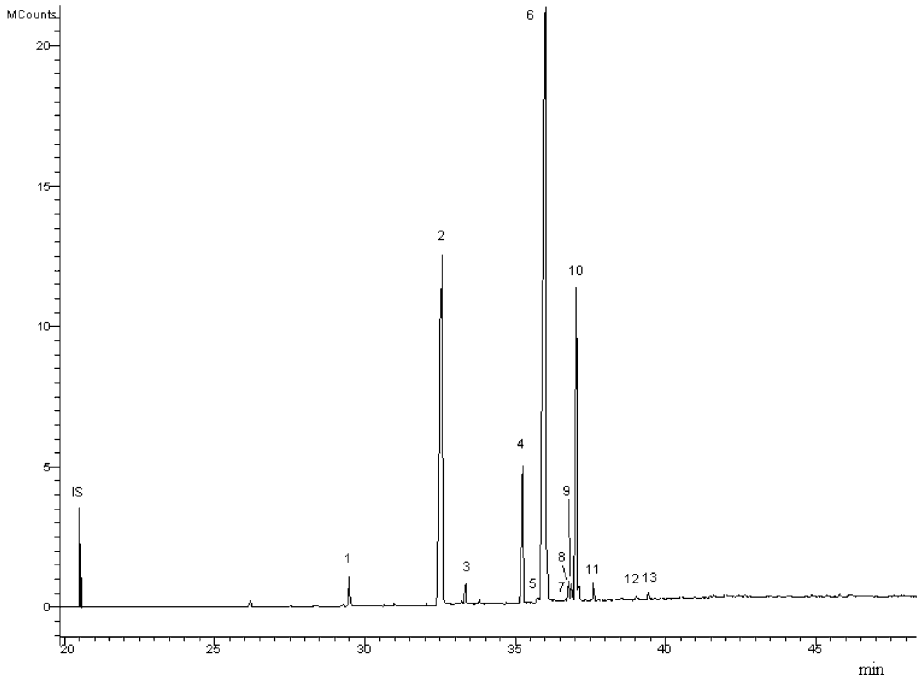

Fig. 2. Chromatogram of a sample after FMASE extraction under the optimal working conditions. (IS) Internal Standard: (1) 14:0; (2) 16:1; (3) *t*16:1; (4) 18:0; (5) *t*18:1; (6) 18:1; (7) *tt*18:2; (8) *tc*18:2; (9) *ct*18:2; (10) 18:2; (11) 20:0; (12) 18:3; (13) 22:0.

3.1. Chromatographic conditions

The optimal values of the experimental chromatographic-detection variables obtained in this study are commented under Section 2. Complete separation of the analytes was achieved within 40 min. Methyl decanoate was used as internal standard (IS) due to its physical and chemical behavior similar to that of the derivatised analytes and its absence in the analysed samples, as demonstrated by the precision of the signal given by the IS in the analyses of different samples, which was 0.82%, expressed as within-laboratory reproducibility. In the case of the presence of this acid in the samples (Daglioglu et al., 2000; Fernández-SanJuan, 2000) another IS, such as methyl undecanoate, methyl heptadecanoate, methyl heneicosanoate, or a mixture of some of them could be used (Czarniecki, 2003; Eder, 1995). The retention time of methyl decanoate (21 min) was not far from that of the first analyte (29 min). The background of both standards and natural samples was not significant.

In this study, 100 μg of methyl decanoate was added before analysis. According to the results obtained, this compound is a suitable IS for this method.

3.2. Features of the quantification method

Calibration plots were run for all analytes using the peak area as a function of the standard concentration of each compound. The calibration curves are shown in Table 1.

The limit of detection (LOD) for each analyte was expressed as the mass of analyte which gives a signal that is 3σ above the mean blank signal (where σ is the standard deviation of the blank signal). The LODs obtained ranged between 0.98 and 3.93 μg g^{-1}. The limits of quantification, expressed as the mass of analyte which gives a signal 10σ above the mean blank signal, ranged from and 3.23 to 12.98 μg g^{-1}. LODs and LOQs were estimated from both extracts and standard solutions and they can be seen in Table 1. The linear dynamic ranges are between the LOQ and 12000 μg g^{-1} for each compound.

3.3. GC–MS analysis

The main difficulties for the analysis of *trans* fatty acids by gas chromatography are encountered in the determination of the position and geometry of the double bonds (DBs) of monounsaturated and polyunsaturated fatty acid methyl esters. Long-chain saturated methyl esters are easily identified (Fritsche & Steinhart, 1998). EI spectra of saturated FAME are dominated by the ion $[CH_2C(OH)OCH_3]^{+\cdot}$ at *m*/*z* 74 caused by McLafferty rearrangement. Losses of neutral aliphatic radicals give rise to a series of ions, $[(CH_2)_nCO_2CH_3]^+$ where *m*/*z* 87 is usually the most abundant. The fragmentation patterns of unsaturated FAMEs are not indicative for the position of double bonds. The most abundant ions in monoenes are a series with molecular formula $[C_nH_{2n-1}]^+$, *m*/*z* 55 $[C_4H_7]$ being usually the base peak. In methylene-interrupted (MI) dienes also exists a series with the molecular formula $[C_nH_{2n-3}]^+$, where *m*/*z* 67 is usually the base peak. In spectra of fatty acids with three or more MI DBs the series with molecular formula $[C_nH_{2n-5}]^+$ is dominating and *m*/*z* 79 $[C_6H_7]^+$ is usually the base peak (Mjos & Pettersen, 2003).

Table 1
Calibration curve, regression coefficient, detection and quantification limits (LOD and LOQ) for each analyte by GC–MS

Compound	Calibration curve	r^2	LOD[a]	LOQ[a]
14:0	$Y = 0.0563X - 0.00443$	0.9986	2.28	7.51
16:0	$Y = 0.0557X + 0.00304$	0.9991	0.98	3.23
*t*16:1	$Y = 0.0160X - 0.00192$	0.9973	2.97	9.79
18:0	$Y = 0.0473X + 0.00813$	0.9991	1.18	3.89
*t*18:1	$Y = 0.0547X + 0.00108$	0.9985	1.01	3.33
18:1	$Y = 0.0491X - 0.00348$	0.9999	1.20	3.97
*tt*18:2	$Y = 0.0136X - 0.00281$	0.9988	2.08	6.86
*tc*18:2	$Y = 0.0165X + 0.00444$	0.9988	1.64	5.41
*ct*18:2	$Y = 0.0635X + 0.00113$	0.9984	0.98	3.23
18:2	$Y = 0.0367X - 0.00943$	0.9988	2.29	7.56
20:0	$Y = 0.0561X - 0.00798$	0.9988	1.10	3.62
18:3	$Y = 0.0933X - 0.00316$	0.9971	1.19	3.93
22:0	$Y = 0.0985X - 0.00174$	0.9973	3.93	12.98

[a] Expressed in $\mu g\ g^{-1}$.

Double bond positions can be determined if unsaturated fatty acids are converted into suitable derivatives. For instance, transformations to pyrrolidine, picolinyl and 4,4-dimethyloxazoline (DMOX) derivatives are proposed for the GC–MS identification of fatty acids with different functional groups. These derivatisations steps are time-consuming – from 30 min in the case of picolinyl derivatives to 3 h for the DMOX derivatives. The moisture level has to be minimised in the case of picolinyl and DMOX derivatives, so an additional step is necessary. Therefore, temperatures equal or higher than 100 °C are necessary for the formation of pyrrolidine derivatives (http://www.lipid.co.uk).

The use of chemical degradation methods such as ozonation, which requires an ozonization equipment, could be a second option for the identification of positional and geometrical isomers (Seppänen-Laakso, Laakso, Backlund, Vanhanen, & Viikari, 1996).

In view of these shortcomings, a third option was tested in this research. The fat extracted was easily derivatised to FAMEs. The latter were directly injected in GC–MS using a specific capillary column for the isolation of the different FAMEs and appropriate standards for their identification/quantification were used. This procedure is shorter, cheaper, and use milder working conditions than procedures based on other derivatisation steps.

3.4. Comparison between the proposed and the Folch extraction method

The optimal working conditions obtained for the proposed method were applied for all samples under study, and the results compared with those provided by the reference Folch method in terms of extraction efficiency as the subsequent steps are identical. Table 2 shows the average extraction efficiencies obtained by the two methods provided by each analyte and the value of % *trans* content for each method – obtained as the ratio between the concentrations of *trans* compounds and the concentration of the total fat.

A two-tailed *t*-test was used to compare the means of related (paired) samples in order to evaluate if both methods yield similar results at the 95% confidence level. The null hypothesis was that both methods yield the same results or, in other words, that the observed differences between the Folch and FMASE methods were not significant. H_0 is formulated as a two-tailed test required:

$$H_0 : \bar{d} = 0 \quad H_1 : \bar{d} \neq 0.$$

The calculated t values are shown in Table 2. These results were compared with the theoretical value at $\alpha = 0.05$ and thirteen degrees of freedom, i.e., 2.16. As the calculated values are smaller than the theoretical value except for 18:0, H_0 is accepted (Massart et al., 1997). This means that at the chosen significance level, the differences between the values obtained for the different fatty acids were within the experimental error. Nevertheless, these values are very similar for 18:1 and 18:2 (2.13 and 2.10, respectively). This fact can be explained taken into account that in the Folch method a saline solution – sodium chloride in this case – is added before the weighing step for removing non-lipids contaminants such as sugars, amino acids and salts, which can be co-extracted with the lipid fraction. Some components, such as glycolipids, can be partially removed in this step (Folch, Lees, & Stanley, 1957). Particularly, the relative standard deviation for all compounds ranged between 0.01% and 7%, see Table 2.

Concerning fatty acids profile for each sample, a clear distinction between samples based on the fatty acid with higher content can be established, namely: (a) samples with high content in exadecanoic (palmitic) acid, a saturated compound which shows the bakery product has been elaborated mainly with fat of animal origin; (b) samples with high of *cis*-octadecenoic(oleic)acid, a monounsaturated compound, demonstrates the use of vegetal fat for product elaboration.

The advantages of FMASE vs. Folch extraction such as a drastic reduction of both the procedure time and sample handling – 35 or 60 min vs. 3.5 h, and less organic solvent needed (75–85% of it was recycled), make FMASE an alternative with reliable possibilities for replacing the Folch

Table 2
Extraction efficiencies (μg g^{-1}) of fatty acids obtained with FMASE and Folch extraction methods from bakery samples

Sample	Method	14:0	16:0	*t*16:1	18:0	*t*18:1	18:1	*tt*18:2	*tc*18:2	*ct*18:2	18:2	20:0	18:3	22:0	%*trans*
1	Folch	523(1.14)	115537(0.45)	1338(5.42)	42926(0.51)	1460(4.1)	31593(2.28)	53(1.08)	75(0.59)	152(4.28)	6541(1.07)	196(2.63)	101(4.21)	460(1.04)	1.53
	FMASE	629(0.85)	130387(2.24)	1620(0.50)	47259(0.25)	1559(1.18)	32917(1.09)	54(0.12)	81(6.9)	173(1.92)	7033(1.74)	257(2.74)	104(2.01)	523(0.62)	1.57
2	Folch	710(1.53)	138482(0.84)	1136(3.44)	30051(0.98)	3640(0.25)	11490(2.37)	126(1.89)	59(2.28)	119(1.35)	8237(0.76)	221(4.52)	201(6.27)	593(0.46)	2.60
	FMASE	705(2.04)	129463(1.89)	1183(1.96)	24873(0.03)	3598(1.64)	10330(2.64)	126(0.47)	62(1.61)	116(2.19)	8404(2.98)	223(0.76)	193(2.60)	593(2.10)	2.83
3	Folch	622(1.76)	141725(1.88)	1738(1.87)	31810(1.42)	931(2.50)	255771(3.13)	81(1.91)	1297(0.33)	16(1.99)	13968(1.50)	320(2.47)	488(2.49)	1285(2.36)	0.92
	FMASE	676(0.94)	124566(0.32)	1681(0.33)	35346(0.84)	981(0.04)	294245(0.71)	94(3.73)	1400(0.05)	18(1.64)	14449(0.66)	344(1.18)	429(0.60)	1292(0.43)	0.85
4	Folch	1780(0.08)	35968(2.31)	3092(1.57)	25058(0.30)	638(0.84)	145886(4.50)	77(2.12)	67(1.39)	115(3.53)	38529(2.83)	1509(0.09)	108(1.51)	25481(0.46)	1.43
	FMASE	1751(1.32)	35592(1.79)	3154(0.01)	25830(0.73)	631(1.75)	151189(1.08)	77(1.11)	68(3.60)	112(1.20)	38574(0.25)	1487(0.18)	100(2.64)	27101(1.64)	1.41
5	Folch	817(1.95)	150250(2.21)	1296(2.98)	35218(1.34)	913(1.77)	11175(2.12)	82(1.83)	1291(2.50)	10(2.33)	9564(1.69)	353(0.29)	115(1.79)	711(1.75)	1.70
	FMASE	812(0.37)	149599(0.13)	1251(0.30)	35472(0.50)	904(0.71)	10785(0.14)	83(0.65)	1298(0.46)	10(1.09)	9417(1.76)	340(1.34)	112(0.84)	709(1.68)	1.68
6	Folch	635(2.32)	137156(0.06)	1581(0.08)	30359(2.19)	4069(1.92)	241137(2.24)	50(0.13)	1069(0.23)	6(3.61)	7487(4.12)	323(0.51)	177(0.12)	526(0.01)	1.60
	FMASE	662(1.78)	138641(0.74)	1614(1.48)	32430(1.43)	3487(0.57)	232744(2.53)	57(0.67)	1064(0.39)	6(3.80)	8931(1.98)	349(2.88)	181(0.14)	555(2.40)	1.45
7	Folch	2019(2.26)	77733(0.11)	1359(1.34)	50025(0.12)	1952(0.37)	10243(1.01)	65(0.92)	367(0.77)	37(0.76)	6580(0.75)	326(0.25)	243(0.34)	977(1.48)	2.49
	FMASE	2330(0.61)	84965(0.22)	1326(0.42)	56863(1.79)	1953(1.74)	9964(0.69)	53(0.14)	377(0.10)	40(0.50)	6624(0.40)	342(0.25)	243(0.10)	1028(1.25)	2.26
8	Folch	63(0.52)	29139(1.61)	567(0.16)	30576(0.49)	180(0.67)	322068(0.37)	231(0.45)	321(0.15)	5(2.52)	17433(2.65)	294(0.68)	278(0.34)	5847(0.78)	0.32
	FMASE	60(0.22)	29306(0.09)	553(2.33)	32009(0.69)	175(0.30)	338060(1.00)	223(0.62)	313(1.62)	28(2.39)	17480(2.18)	302(0.41)	280(0.37)	5925(0.55)	0.30
9	Folch	2854(0.02)	96603(3.00)	2050(0.93)	60642(0.24)	9565(3.39)	43078(1.63)	116(1.39)	33(1.61)	77(0.96)	8826(0.74)	309(0.64)	189(0.38)	688(0.28)	5.26
	FMASE	2765(0.21)	98973(3.51)	2034(0.14)	70135(0.93)	9404(0.24)	51098(2.17)	119(2.30)	39(1.68)	83(0.91)	9043(1.61)	328(1.17)	178(0.39)	676(0.66)	4.77
10	Folch	216(0.77)	52330(1.88)	1003(3.1)	43712(1.99)	3299(1.23)	7281(0.74)	23(3.39)	117(0.30)	10(0.31)	7383(0.43)	431(1.48)	387(1.38)	526(1.48)	3.81
	FMASE	214(1.31)	57816(2.52)	1017(1.67)	45712(1.21)	3261(1.92)	7458(2.18)	21(1.18)	120(0.96)	10(1.11)	7667(0.34)	483(1.55)	340(1.48)	552(1.60)	3.55
11	Folch	392(1.34)	108131(2.53)	1459(1.19)	29872(0.93)	944(1.62)	12013(1.92)	350(1.83)	409(1.63)	16(1.55)	21316(1.82)	295(1.59)	345(1.35)	1448(1.20)	1.80
	FMASE	442(0.99)	111872(0.32)	1450(1.99)	31295(0.52)	999(1.02)	12340(1.00)	328(1.12)	428(0.20)	18(1.56)	21596(1.10)	311(1.35)	358(1.76)	1600(0.46)	1.76
12	Folch	499(0.88)	113614(1.94)	1221(0.39)	23642(0.86)	1214(1.33)	209074(1.86)	223(0.99)	402(2.03)	3(2.38)	8383(0.79)	222(0.85)	209(0.73)	447(0.68)	0.86
	FMASE	496(1.25)	113846(1.35)	1087(0.92)	23345(1.30)	1176(1.26)	208957(1.25)	222(1.67)	789(1.25)	4(2.57)	8185(1.38)	218(1.31)	201(1.32)	414(1.29)	0.93
13	Folch	356(1.70)	62530(1.25)	1216(1.57)	45982(0.58)	3894(1.02)	149955(1.92)	812(0.60)	2719(1.46)	22(0.72)	10026(0.82)	293(1.36)	247(0.52)	1542(0.17)	3.10
	FMASE	346(0.89)	76938(0.09)	1381(1.98)	51220(0.58)	3880(0.98)	187956(0.59)	977(1.33)	2745(1.86)	23(1.22)	10737(0.21)	295(0.71)	280(1.77)	1612(0.56)	2.66
14	Folch	649(0.10)	140458(0.01)	2029(0.70)	31396(0.74)	1880(1.19)	260940(0.15)	100(0.98)	1203(1.17)	7(0.51)	12266(0.40)	332(1.67)	145(0.80)	698(0.63)	1.15
	FMASE	731(0.50)	141587(0.01)	1876(1.14)	33948(1.47)	1109(1.57)	282800(0.25)	123(1.26)	1000(0.59)	10(0.81)	11947(0.01)	365(0.66)	144(1.50)	750(0.05)	1.35
Calculated *t* value		1.38	0.78	0.34	2.64	1.53	2.13	0.09	0.77	1.87	2.10	2.04	1.05	1.31	1.51

Accuracy, in brackets, expressed as relative standard deviation (%, $n = 3$ replicates).

Table 3
Comparison between Folch and FMASE extraction methods in terms of TFA

Sample	Method	*t*16:1	*t*18:1	*tt*18:2	*tc*18:2	*ct*18:2
1	Folch	0.670 ± 0.030	0.730 ± 0.029	0.030 ± 0.001	0.040 ± 0.001	0.080 ± 0.003
	FMASE	0.730 ± 0.004	0.700 ± 0.010	0.020 ± 0.001	0.040 ± 0.001	0.080 ± 0.002
2	Folch	0.580 ± 0.019	1.870 ± 0.005	0.060 ± 0.001	0.030 ± 0.001	0.060 ± 0.001
	FMASE	0.660 ± 0.014	2.000 ± 0.032	0.070 ± 0.002	0.030 ± 0.001	0.060 ± 0.001
3	Folch	0.390 ± 0.007	0.210 ± 0.005	0.020 ± 0.002	0.290 ± 0.001	0.000
	FMASE	0.340 ± 0.001	0.200 ± 0.001	0.020 ± 0.001	0.280 ± 0.001	0.000
4	Folch	1.110 ± 0.017	0.230 ± 0.002	0.030 ± 0.001	0.020 ± 0.001	0.040 ± 0.001
	FMASE	1.100 ± 0.001	0.220 ± 0.004	0.030 ± 0.002	0.020 ± 0.001	0.040 ± 0.001
5	Folch	0.610 ± 0.017	0.430 ± 0.008	0.040 ± 0.002	0.610 ± 0.015	0.000
	FMASE	0.590 ± 0.002	0.430 ± 0.003	0.040 ± 0.001	0.620 ± 0.003	0.000
6	Folch	0.370 ± 0.003	0.960 ± 0.018	0.010 ± 0.001	0.250 ± 0.001	0.000
	FMASE	0.370 ± 0.004	0.810 ± 0.009	0.010 ± 0.002	0.250 ± 0.001	0.000
7	Folch	0.890 ± 0.011	1.280 ± 0.004	0.040 ± 0.002	0.240 ± 0.002	0.020 ± 0.001
	FMASE	0.800 ± 0.003	1.180 ± 0.018	0.030 ± 0.001	0.230 ± 0.002	0.020 ± 0.001
8	Folch	0.140 ± 0.001	0.040 ± 0.001	0.060 ± 0.001	0.080 ± 0.002	0.000
	FMASE	0.130 ± 0.003	0.040 ± 0.001	0.050 ± 0.001	0.070 ± 0.002	0.000
9	Folch	0.910 ± 0.008	4.250 ± 0.165	0.050 ± 0.001	0.010 ± 0.001	0.030 ± 0.001
	FMASE	0.830 ± 0.001	3.840 ± 0.010	0.050 ± 0.001	0.020 ± 0.001	0.030 ± 0.001
10	Folch	0.860 ± 0.026	2.830 ± 0.034	0.020 ± 0.001	0.100 ± 0.001	0.010 ± 0.001
	FMASE	0.820 ± 0.019	2.620 ± 0.050	0.020 ± 0.001	0.100 ± 0.001	0.010 ± 0.001
11	Folch	0.820 ± 0.015	0.530 ± 0.009	0.200 ± 0.004	0.230 ± 0.004	0.010 ± 0.001
	FMASE	0.790 ± 0.016	0.550 ± 0.009	0.190 ± 0.002	0.230 ± 0.001	0.010 ± 0.001
12	Folch	0.340 ± 0.001	0.350 ± 0.005	0.060 ± 0.001	0.110 ± 0.002	0.000
	FMASE	0.300 ± 0.003	0.340 ± 0.004	0.060 ± 0.001	0.220 ± 0.003	0.000
13	Folch	0.430 ± 0.007	1.390 ± 0.014	0.290 ± 0.004	0.970 ± 0.012	0.010 ± 0.001
	FMASE	0.410 ± 0.009	1.150 ± 0.012	0.290 ± 0.003	0.810 ± 0.012	0.010 ± 0.001
14	Folch	0.450 ± 0.003	0.420 ± 0.005	0.020 ± 0.001	0.270 ± 0.005	0.000
	FMASE	0.390 ± 0.009	0.380 ± 0.006	0.030 ± 0.001	0.250 ± 0.002	0.000

Results expressed as % ± SD.
$n = 3$ replicates.

method in official methods in view of the imminent policy of mandatory characterisation of the fat content in foods.

In addition, in the case of specific analysis as TFA determination, the use of auxiliary energy to accelerate the extraction process does not promote *cis*/*trans* interconversion, which would mask the original content of each isomer. This fact has been checked using GC–MS for individual separation-quantification. Table 3 contains the percent of each compound with a *trans* bond for each sample and the two extraction methods. From this table, the absence of *cis*/*trans* interconversions with the FMASE can be assured.

4. Conclusions

Fatty acids analysis, with special emphasis on TFA, has been carried out on fourteen bakery samples using a very fast and effective extraction alternative for total fat isolation based on the use of a focused microwave-assisted Soxhlet extractor. The proposed extraction has been compared with the Folch reference extraction method, and GC–MS has been used for individual separation and detection. The results from the proposed extraction were in agreement with those obtained with the reference method, thus demonstrating the goodness of this approach for fat isolation in routine analysis.

Concerning LODs and LOQs, GC–MS proves to be an excellent option for this type of analysis because it allows the quantification of *trans* compounds at the low $\mu g\ g^{-1}$ level. In this sense, despite GC is more time-consuming than MIR – as the latter does not require a derivatisation step, the former is about 10,000 times more sensitive than MIR – with a quantification limit about 1% w/w for total *trans* content – (Priego-Capote et al., 2004). Another advantage of GC–MS vs. MIR is the possibility of determining fatty acids profiles by individual quantification of each analyte.

Other conclusions of this research are as follows:

(i) no alterations occur in the fat extracted using the method assisted by microwaves as compared with the conventional method in which no high temperature or pressure are applied. This fact has been demonstrated by the similarity between fatty acids profiles;
(ii) it was possible the quantification of the *trans* fatty acid isomers by using both suitable standards and capillary chromatographic column. In this sense, it would be necessary to standardise the type of column used in view of regulating the labeling of food;
(iii) the same extraction method can be allowed to carry out several analyses for food characterisation; for instance: total fat content, fatty acid profile, total *trans* content, *trans* fatty acids;

(iv) the fatty acids profile provides information about both the source of the fat used and the elaboration process.

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