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# Identification and quantification of *trans* fatty acids in bakery products by gas chromatography–mass spectrometry after dynamic ultrasound-assisted extraction

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**Abstract**

*trans* Fatty acids have been determined in 14 bakery products using derivatisation by ester formation, gas chromatography–mass spectrometry (GC–MS) for individual separation, identification and quantification following total fat isolation by dynamic ultrasound-assisted extraction (DUAE). The detection and quantification limits between 0.98 and 3.93 $\mu g\ g^{-1}$ and 3.23 and 12.98 $\mu g\ g^{-1}$, respectively, and the linear dynamic ranges between LOQs values and 12,000 $\mu g\ g^{-1}$ 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—validated by comparison with the Folch reference method—drastically reduces the extraction time as compared with the reference method without degradation of the target analytes by ultrasound irradiation, as demonstrated in the subsequent quantification step. The overall method thus developed could be a valid alternative to the reference method as the present and foreseeable increased demand for the analysis of these analytes makes mandatory faster methods. The number of samples used support the validation process.


*Keywords:* Ultrasound-assisted extraction; Extraction methods; Food analysis; Fatty acids

## 1. Introduction

In 1990, the US Food and Drug Determination (FDA), through the Nutritional Labeling and Education Act (NLEA), defined "total fat" as the sum of all fatty acids obtained in the lipid extract, expressed as triglycerides [1]. 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 by saturated fatty acids. The high consumption of saturated fatty acids and cholesterol is mainly responsible for hypercholesterolemia, which is in turn responsible for the increase in cardiovascular morbidity and mortality of ischemic origin [2]. 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. Vegetable oils have a high content of unsaturated fats, which are liquid at room temperature. Moreover, unsaturated fats are heart-healthy, but they have some undesirable properties, specifically in contact with air, where unsaturated fatty acids can gradually become rancid by absorbing oxygen and forming hydroperoxides that decompose [3].

Manufacturers block deterioration by stimulating the consistency of saturated fat by a process of partial saturation called hydrogenation, in which hydrogen is bubbled through the fat at elevated temperature in the absence of oxygen and presence of a catalyst such as nickel. Prior to this process, most naturally occurring unsaturated fatty acids are endowed with *cis* configuration at their double bonds. Partial hydrogenation rearranges the double bonds, converting some of these acids to the *trans* configuration and shifting the double bonds along the carbon chain. The extent of hydrogenation

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determines how much the process raises the melting point of fats, turning liquid vegetable oil into products ranging from soft margarine to solid shortening [3,4].

Several clinical studies have shown that a high-*trans* fatty acid diet causes adverse changes in the plasma lipoprotein profile, with an increase in low-density lipoprotein (LDL) and a decrease in high-density lipoprotein (HDL) [5]. Epidemiological studies have also found a relationship between the level of *trans* fatty acid intake and risk of cardiovascular diseases [6,7]. Partially due to these concerns, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended in 1994 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 [8]. The FDA decreed that by 1 January 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" [9]. 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 carried out in different ways depending on the sample characteristics. With a view of analysis, organic solvents have traditionally been used for the extraction of fat from food. Some extraction methods (Weibull–Berntrop, Röse–Gottlieb, Mojonnier, Folch, Werner–Schmid, Bligh–Dyer. . .) are based on acid, alkaline or enzymatic hydrolysis before solvent extraction [10–12]. In spite of the fact that several modifications of those methods concerning solvent mixtures and laboratory practice have been proposed, they have not been greatly improved, and long preparation times with a re-extraction step to ensure complete lipid isolation are required [13]. The Folch method has been used for total fat extraction prior to the analysis of the *trans* fatty acid content because its mild working conditions—in terms of no high temperatures nor pressures required—[14].

In view of these problems, some other methods for total fat extraction based on as supercritical fluid extraction (SFE) [15], closed systems at high temperature and pressure (pressurized liquid extraction, PLE) [16], focused microwave-assisted Soxhlet extraction (FMASE) [17] and dynamic ultrasound-assisted extraction (DUAE) [18] might be considered as alternatives in order to substitute the Folch method. Recently, an FMASE method for isolation of total fat and quantification of total *trans* fat content using medium infrared (MIR) spectroscopy has been proposed. The applicability of this approach as an alternative to conventional and reference methods for routine analysis has thus been proved. It is worth to stressing that a shortcoming of the method used for the determination step—Fourier transform infrared (FTIR)—is the limit of quantification it provides: 1.04% [19].

A way to decrease the limit of quantification and provide a higher level of information is to use a gas chromatography (GC) system 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) [20,21]; 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 and independent identification–quantification of fatty acids using GC–MS with previous derivatisation to FAMEs, including, obviously, the *trans* compounds. DUAE 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.

## 2. Experimental

### 2.1. Instruments and apparatus

Ultrasonic irradiation was applied by means of a Branson 450 digital sonifier (20 KHz, 400 W) equipped with a cylindrical titanium alloy probe (12.70 mm diameter), which was immersed into a water bath in which the extraction cell was placed. An extraction chamber consisting of a stainless steel cylinder (13 cm × 8 mm i.d.) closed with screws at either end was used, allowing circulation of the leaching solvent through it. The screw caps were covered with cellulose filters to ensure the sample remained in the extraction chamber. Fig. 1 shows the experimental *set-up* used for the dynamic ultrasound-assisted extraction of fat in bakery products.

A Gilson Minipuls-3 low-pressure peristaltic pump—programmed for changing the rotation direction a preset intervals—and PTFE tubing of 0.8 mm i.d. were used to build

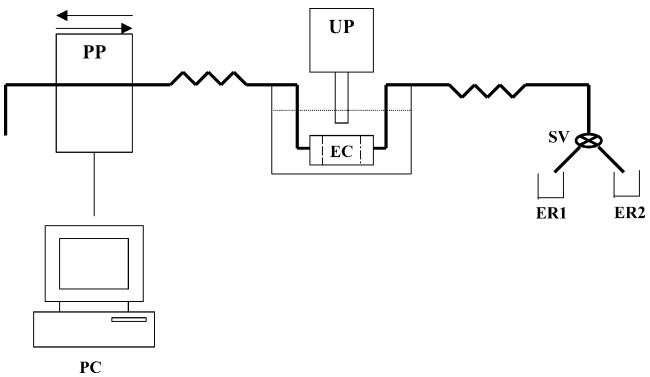


Fig. 1. Experimental set-up used for dynamic ultrasound-assisted extraction. PP, Peristaltic pump; UP, ultrasonic probe; EC, extraction chamber; ER1 and ER2, extract and extractant reservoirs, respectively; PC, personal computer; SV, selection valve.

the flow manifold. The pump was operated through a personal computer and the associated software.

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

HPLC-grade *n*-hexane (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) mixture 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 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 (t16:1), octadecanoic acid methyl ester (18:0), *trans*-octadecenoic acid methyl ester (t18:1), octanodecanoic acid methyl ester (18:1), *trans*,*trans*-octadecadienoic acid methyl ester (tt18:2), *cis*,*trans*-octadecadienoic acid methyl ester (ct18:2), *trans*,*cis*-octadecadienoic acid methyl ester (tc18:2), *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 SA., Azuqueca de Henares, Spain).

Sample preparation was done according to the protocol established by legislation [22]. 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 chloroform–methanol(2:1) mixture of the Folch extraction. 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.

#### 2.3.1. Dynamic ultrasound-assisted extraction (DUAE)

Two grams of the target bakery product was placed in the extraction chamber, which was assembled and filled with the leaching carrier-hexane-aspirated in by the peristaltic pump in order to avoid passage of the organic solvent through the pump tubes. After filling, the extraction chamber was immersed into the water bath at room temperature. The leaching carrier was then circulated through the solid sample for a 6-min preset time under ultrasonic irradiation (duty cycle 0.8 s, output amplitude 100% of the converter nominal amplitude, with the probe placed at 1 mm from the top surface of the extraction cell). During extraction, the direction of the leaching carrier (at 2 ml $min^{-1}$) was changed each 40 s. Only a small volume of extractant (1.25 ml) was used for each extraction cycle. After each 6-min cycle the extract was removed—by draining it to the extract reservoir—and the system was filled with fresh extractant. After 10 or 20 cycles—70 or 140 min, respectively, depending on the sample matrix—the extraction of the fat from the bakery product was complete and the largest part of solvent was released by a rotary-evaporator. Then, the residue was transferred to a 10-ml glass vial, and the last traces of solvent were removed by a nitrogen stream before derivatisation.

#### 2.3.2. The Folch reference extraction method

This method was selected as reference for fat extraction because its mild working conditions, which avoid potential alterations of the fat extracted. Twenty-five grams of sample was mixed with 75 ml of a chloroform–methanol (2:1, v/v) 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 three 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

### 2.4. Preparation of fatty acid methyl esters (FAMEs)

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. 0.5 ml of *n*-hexane 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.

### 2.5. GC–MS separation and 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.

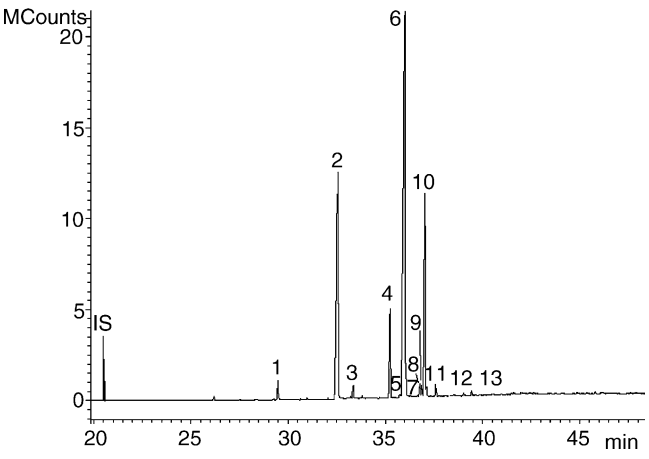


Fig. 2. Chromatogram of a sample after DUAE extraction under the optimal working conditions. (1) 14:0; (2) 16:0; (3) t16:1; (4) 18:0; (5) t18:1; (6) 18:1; (7) tt18:2; (8) ct18:2; (9) tc18:2; (10) 18:2; (11) 20:0; (12) 18:3; (13) 22:0.

## 3. Results and discussion

The dynamic ultrasound-assisted extraction method has already been proposed for the total fat content extraction in bakery products. With that purpose, the DUAE was optimised and validated by comparison with the Soxhlet reference method. However, the applicability of DUAE for the determination of the fatty acids profile with emphasis on *trans* fatty acids, has not been demonstrated. For this reason, the optimal working conditions previously obtained [18] were applied to check the ability of this extraction method for providing extracts appropriate for this specific analysis. In case of obtaining unmodified extracts concerning, the double bonds position and *cis*/*trans* stereochemistry—as compared with the mild Folch method—the proposed method could be suitable for the extraction of *trans* fatty acids prior to their quantification.

Optimisation of the quantification step was necessary for the study of the characteristics of the extract obtained by the DUAE and Folch methods, which are shown in Table 1.

### 3.1. Chromatographic conditions

The experimental GC–MS variables were optimised. The optimal working conditions were those commented under Experimental. 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 0.90%, expressed as within-laboratory reproducibility. In the case of the presence of this acid in the samples [26,27], another IS, such as methyl undecanoate, methyl heptadecanoate, methyl heneicosanoate, or a mixture of some of them could be used [28,29]. 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 2.

Table 1
Extraction efficiencies ($\mu g\,g^{-1}$) of fatty acids obtained with DUAE and Folch extraction methods from bakery samples

| Sample | Method | 14:0 | 16:0 | t16:1 | 18:0 | t18:1 | 18:1 | tt18:2 | tc18:2 | ct18:2 | 18:2 | 20:0 | 18:3 | 22:0 | %*Trans* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Folch | 523 (1.14) | 115 537 (0.45) | 1 338 (5.42) | 42 926 (0.51) | 1 460 (4.1) | 31 593 (2.28) | 53 (1.08) | 75 (0.59) | 152 (4.28) | 6 541 (1.07) | 196 (2.63) | 101 (4.21) | 460 (1.04) | 1.53 |
| | DUAE | 638 (0.86) | 126 659 (2.24) | 1 663 (0.50) | 47 901 (0.25) | 1 788 (1.18) | 32 666 (1.09) | 62 (0.12) | 91 (6.9) | 224 (1.92) | 7 760 (1.74) | 253 (2.74) | 105 (2.01) | 5 618 (0.62) | 1.74 |
| 2 | Folch | 710 (1.53) | 138 482 (0.84) | 1 136 (3.44) | 30 051 (0.98) | 3 640 (0.25) | 11 490 (2.37) | 126 (1.89) | 59 (2.28) | 119 (1.35) | 8 237 (0.76) | 221 (4.52) | 201 (6.27) | 593 (0.46) | 2.60 |
| | DUAE | 639 (2.04) | 118 150 (1.89) | 1 230 (1.96) | 24 971 (0.03) | 3 338 (1.64) | 10 446 (2.64) | 118 (0.47) | 44 (1.61) | 102 (2.19) | 85 348 (2.98) | 223 (0.76) | 172 (2.6) | 461 (2.1) | 2.87 |
| 3 | Folch | 622 (1.76) | 134 532 (1.88) | 1 738 (1.87) | 31 810 (1.42) | 931 (2.50) | 255 771 (3.13) | 81 (1.91) | 1 297 (0.33) | 16 (1.99) | 13 968 (1.50) | 320 (2.47) | 488 (2.49) | 1 285 (2.36) | 0.92 |
| | DUAE | 615 (0.94) | 124 566 (0.32) | 1 663 (0.33) | 30 761 (0.84) | 1 272 (0.04) | 253 771 (0.71) | 75 (3.73) | 1 223 (0.05) | 11 (1.64) | 12 900 (0.66) | 303 (1.18) | 422 (0.60) | 1 225 (0.43) | 0.99 |
| 4 | Folch | 1 780 (0.08) | 35 968 (2.31) | 3 092 (1.57) | 25 058 (0.30) | 638 (0.84) | 145 886 (4.50) | 77 (2.12) | 67 (1.39) | 115 (3.53) | 38 529 (2.83) | 1 509 (0.09) | 108 (1.51) | 25 481 (0.46) | 1.43 |
| | DUAE | 1 782 (1.32) | 36 085 (1.79) | 3 075 (0.01) | 24 753 (0.73) | 624 (1.75) | 1 443 408 (1.08) | 74 (1.11) | 68 (3.60) | 114 (1.20) | 36 750 (0.25) | 1 394 (0.18) | 105 (2.64) | 23 388 (1.64) | 1.45 |
| 5 | Folch | 817 (1.95) | 150 250 (2.21) | 1 296 (2.98) | 35 218 (1.34) | 913 (1.77) | 11 175 (2.12) | 82 (1.83) | 1 291 (2.50) | 10 (2.33) | 9 564 (1.69) | 353 (0.29) | 115 (1.79) | 711 (1.75) | 1.70 |
| | DUAE | 814 (0.37) | 151 376 (0.13) | 1 247 (0.30) | 35 472 (0.50) | 904 (0.71) | 10 785 (0.14) | 83 (0.65) | 1 298 (0.46) | 10 (1.09) | 9 417 (1.76) | 340 (1.34) | 112 (0.84) | 678 (1.68) | 1.67 |
| 6 | Folch | 635 (2.32) | 137 156 (0.06) | 1 581 (0.08) | 303 598 (2.19) | 4 069 (1.92) | 241 137 (2.24) | 50 (0.13) | 1 069 (0.23) | 6 (3.61) | 7 487 (4.12) | 323 (0.51) | 177 (0.12) | 526 (0.01) | 1.60 |
| | DUAE | 653 (1.78) | 136 552 (0.74) | 1 312 (1.48) | 32 787 (1.43) | 2 885 (0.57) | 232 589 (2.53) | 63 (0.67) | 1 227 (0.39) | 5 (3.80) | 8 665 (1.98) | 341 (2.88) | 189 (0.14) | 662 (2.40) | 1.31 |
| 7 | Folch | 2 019 (2.26) | 77 733 (0.11) | 1 359 (1.34) | 50 025 (0.12) | 1 952 (0.37) | 10 243 (1.01) | 65 (0.92) | 367 (0.77) | 37 (0.76) | 6 580 (0.75) | 326 (0.25) | 243 (0.34) | 977 (1.48) | 2.49 |
| | DUAE | 2 647 (0.61) | 107 956 (0.22) | 1 599 (0.42) | 68 663 (1.79) | 2 093 (1.74) | 10 218 (0.69) | 145 (0.14) | 412 (0.10) | 41 (0.50) | 8 905 (0.40) | 372 (0.25) | 238 (0.10) | 1 273 (1.25) | 2.10 |
| 8 | Folch | 63 (0.52) | 29 139 (1.61) | 567 (0.16) | 30 576 (0.49) | 180 (0.67) | 322 068 (0.37) | 231 (0.45) | 321 (0.15) | 5 (2.52) | 17 433 (2.65) | 294 (0.68) | 278 (0.34) | 5 847 (0.78) | 0.32 |
| | DUAE | 52 (0.22) | 27 930 (0.09) | 462 (2.33) | 30 095 (0.69) | 80 (0.30) | 319 714 (1.00) | 183 (0.62) | 260 (1.62) | 5 (2.39) | 16 993 (2.18) | 291 (0.41) | 218 (0.37) | 3 941 (0.55) | 0.25 |
| 9 | Folch | 2 854 (0.02) | 96 603 (3.00) | 2 050 (0.93) | 60 642 (0.24) | 9 565 (3.39) | 43 078 (1.63) | 116 (1.39) | 33 (1.61) | 77 (0.96) | 8 826 (0.74) | 309 (0.64) | 189 (0.38) | 688 (0.28) | 5.26 |
| | DUAE | 1 711 (0.21) | 91 797 (3.51) | 1 953 (0.14) | 58 847 (0.93) | 8 497 (0.24) | 40 998 (2.17) | 61 (2.30) | 35 (1.68) | 69 (0.91) | 7 673 (1.61) | 289 (1.17) | 158 (0.39) | 426 (0.66) | 4.99 |
| 10 | Folch | 216 (0.77) | 52 330 (1.88) | 1 003 (3.1) | 43 712 (1.99) | 3 299 (1.23) | 7 281 (0.74) | 23 (3.39) | 117 (0.30) | 10 (0.31) | 7 383 (0.43) | 431 (1.48) | 387 (1.38) | 526 (1.48) | 3.81 |
| | DUAE | 226 (1.31) | 48 859 (2.52) | 1 384 (1.67) | 45 623 (1.21) | 3 150 (1.92) | 13 239 (2.18) | 21 (1.18) | 124 (0.96) | 11 (1.11) | 7 002 (0.34) | 475 (1.55) | 365 (1.48) | 541 (1.60) | 3.87 |
| 11 | Folch | 392 (1.34) | 108 131 (2.53) | 1 459 (1.19) | 29 872 (0.93) | 944 (1.62) | 12 013 (1.92) | 350 (1.83) | 409 (1.63) | 16 (1.55) | 21 316 (1.82) | 295 (1.59) | 345 (1.35) | 1 448 (1.20) | 1.80 |
| | DUAE | 452 (0.99) | 110 148 (0.32) | 1 481 (1.99) | 30 084 (0.52) | 1 171 (1.02) | 14 405 (1.00) | 351 (1.12) | 448 (0.20) | 21 (1.56) | 20 851 (1.10) | 302 (1.35) | 337 (1.76) | 1 579 (0.46) | 1.91 |
| 12 | Folch | 499 (0.88) | 113 614 (1.94) | 1 221 (0.39) | 23 642 (0.86) | 1 249 (1.33) | 209 074 (1.86) | 223 (0.99) | 402 (2.03) | 3 (2.38) | 8 383 (0.79) | 222 (0.85) | 209 (0.73) | 447 (0.68) | 0.86 |
| | DUAE | 494 (1.25) | 115 232 (1.35) | 1 047 (0.92) | 23 485 (1.30) | 1 176 (1.26) | 209 994 (1.25) | 228 (1.67) | 801 (1.25) | 4 (2.57) | 8 176 (1.38) | 207 (1.31) | 201 (1.32) | 412 (1.29) | 0.90 |
| 13 | Folch | 356 (1.70) | 62 530 (1.25) | 1 216 (1.57) | 45 982 (0.58) | 3 894 (1.02) | 149 955 (1.92) | 812 (0.60) | 2 719 (1.46) | 22 (0.72) | 10 026 (0.82) | 293 (1.36) | 247 (0.52) | 1 542 (0.17) | 3.10 |
| | DUAE | 354 (0.89) | 79 118 (0.09) | 1 367 (1.98) | 49 558 (0.58) | 3 884 (0.98) | 159 263 (0.59) | 966 (1.33) | 2 555 (1.86) | 24 (1.22) | 9 749 (0.21) | 267 (0.71) | 288 (1.77) | 1 485 (0.56) | 2.85 |
| 14 | Folch | 649 (0.10) | 140 458 (0.01) | 2 029 (0.70) | 31 396 (0.74) | 1 880 (1.19) | 260 940 (0.15) | 100 (0.98) | 1 203 (1.17) | 7 (0.51) | 12 266 (0.40) | 332 (1.67) | 145 (0.80) | 698 (0.63) | 1.15 |
| | DUAE | 475 (0.50) | 67 862 (0.01) | 1 544 (1.14) | 16 711 (1.47) | 905 (1.57) | 135 391 (0.25) | 73 (1.26) | 1 000 (0.59) | 5 (0.81) | 5 849 (0.01) | 207 (0.66) | 93 (1.50) | 431 (0.05) | 1.13 |
| Calculated *t*-value | | 0.39 | 0.49 | 0.06 | −0.31 | 0.79 | 0.68 | −0.49 | 0.51 | −0.54 | 0.68 | 0.61 | 0.85 | 0.79 | 0.58 | |

Errors, in parenthesis, are expressed as relative standard deviation ($n = 3$ replicates).

Table 2
Calibration curve, regression coefficient, detection and quantification limits (LOD and LOQ) for each analyte by GC–MS

| Compound | Calibration curve | $r^2$ | LOD | LOQ |
|---|---|---|---|---|
| 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 |
| t16:1 | $y = 0.0160x - 0.00192$ | 0.9973 | 2.97 | 9.79 |
| 18:0 | $y = 0.0473x + 0.00813$ | 0.9991 | 1.18 | 3.89 |
| t18:1 | $y = 0.0547x + 0.00108$ | 0.9985 | 1.01 | 3.33 |
| 18:1 | $y = 0.0491x - 0.00348$ | 0.9999 | 1.20 | 3.97 |
| tt18:2 | $y = 0.0136x - 0.00281$ | 0.9988 | 2.08 | 6.86 |
| tc18:2 | $y = 0.0165x + 0.00444$ | 0.9988 | 1.64 | 5.41 |
| ct18:2 | $y = 0.0635x + 0.00113$ | 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 |

The limit of detection (LOD) for each analyte was expressed as the mass of analyte which gives a signal that is $3\sigma$ above the mean blank signal (where $\sigma$ is the standard deviation of the blank signal). The LODs obtained ranged between 0.98 and 3.93 $\mu g\,g^{-1}$. The limits of quantification, expressed as the mass of analyte which gives a signal $10\sigma$ above the mean blank signal, ranged from 3.23 to 12.98 $\mu g\,g^{-1}$. LODs and LOQs were estimated from both extracts and standard solutions and they can be seen in Table 2. The linear dynamic ranges are between the LOQ and 12,000 $\mu g\,g^{-1}$ 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 [20]. EI spectra of saturated FAMEs are dominated by the ion $[CH_2C(OH)OCH_3]^{+*}$ 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 [23].

Double bond positions can be determined if unsaturated fatty acid 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 derivatisation steps are

Table 3
Comparison between Folch and DUAE extraction methods in terms of *trans* fatty acids

| Sample | Method | t16:1 | t18:1 | tt18:2 | tc18:2 | ct18:2 |
|---|---|---|---|---|---|---|
| 1 | Folch | 0.670 ± 0.030 | 0.730 ± 0.029 | 0.030 ± 0.001 | 0.040 ± 0.001 | 0.080 ± 0.003 |
| | DUAE | 0.750 ± 0.004 | 0.810 ± 0.010 | 0.030 ± 0.001 | 0.040 ± 0.001 | 0.100 ± 0.002 |
| 2 | Folch | 0.580 ± 0.019 | 1.870 ± 0.005 | 0.060 ± 0.001 | 0.030 ± 0.001 | 0.060 ± 0.001 |
| | DUAE | 0.730 ± 0.014 | 1.980 ± 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 |
| | DUAE | 0.390 ± 0.001 | 0.300 ± 0.001 | 0.020 ± 0.001 | 0.290 ± 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 |
| | DUAE | 1.130 ± 0.001 | 0.230 ± 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 |
| | DUAE | 0.590 ± 0.002 | 0.430 ± 0.003 | 0.040 ± 0.001 | 0.610 ± 0.003 | 0.000 |
| 6 | Folch | 0.370 ± 0.003 | 0.960 ± 0.018 | 0.010 ± 0.001 | 0.250 ± 0.001 | 0.000 |
| | DUAE | 0.310 ± 0.004 | 0.960 ± 0.009 | 0.020 ± 0.002 | 0.290 ± 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 |
| | DUAE | 0.780 ± 0.003 | 1.020 ± 0.018 | 0.070 ± 0.001 | 0.200 ± 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 |
| | DUAE | 0.120 ± 0.003 | 0.020 ± 0.001 | 0.050 ± 0.001 | 0.060 ± 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 |
| | DUAE | 0.920 ± 0.001 | 4.000 ± 0.010 | 0.030 ± 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 |
| | DUAE | 1.140 ± 0.019 | 2.600 ± 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 |
| | DUAE | 0.820 ± 0.016 | 0.640 ± 0.009 | 0.190 ± 0.002 | 0.250 ± 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 |
| | DUAE | 0.290 ± 0.003 | 0.330 ± 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 |
| | DUAE | 0.440 ± 0.009 | 1.260 ± 0.012 | 0.310 ± 0.003 | 0.830 ± 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 |
| | DUAE | 0.670 ± 0.009 | 0.390 ± 0.006 | 0.030 ± 0.001 | 0.250 ± 0.002 | 0.000 |

Results expressed in % ± S.D.; $n = 3$ replicates.

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 [24].

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 [25].

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 the GC–MS system 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 1 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 DUAE 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 1. These results were compared with the theoretical value at $\alpha = 0.05$ and fourteen degrees of freedom, i.e. 2.14. As the calculated values are smaller than the theoretical value, $H_0$ is accepted. This means that at the chosen significance level, the differences between the values obtained for the different fatty acids were within the experimental error. Particularly, the relative standard deviation for all compounds ranged between 0.01 and 7%, see Table 1. As can be seen, similar extraction efficiencies, as well as percent of *trans* content were provided by both the proposed method and the Folch reference method. Furthermore, the similar extraction efficiency for each *trans* target compound indicates that alterations of the double bonds do not take place (see Table 3). These good results demonstrated the ability of DUAE for extracting fat for *trans* fatty acids determination, which could substitute the Folch method in routine analysis. As the former is two times faster than the last.

## 4. Conclusions

Fatty acids analysis, with special emphasis on *trans* fatty acids, has been carried out in 14 bakery samples using for fat isolation a dynamic extraction method accelerated by ultrasound irradiation (DUAE), which has been compared with the Folch reference extraction method. Gas chromatography–mass spectrometry has been used for individual separation/determination after derivatisation of the target analytes to their esters in both instances for proper comparison of the extraction step with a view to demonstrate that ultrasound irradiation accelerates the isolation of the target analytes without degradation nor alteration of the double bonds position. The advantages of the proposed extraction method as faster alternative to the Folch method for routine analysis has been thus demonstrated.

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% for total *trans* content—[19]. Another advantage of GC–MS versus MIR is the possibility of determining fatty acids profiles by individual quantification of each analyte.

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