Accelerated Extraction of the Fat Content in Cheese Using a Focused Microwave-Assisted Soxhlet Device

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A fast method for hydrolysis and extraction of fat in cheese based on the use of a Soxhlet extractor assisted by microwaves is proposed. The use of this type of energy dramatically reduces the time required for both steps (namely, the hydrolysis time was decreased from 1 h to 10 min with avoidance of the neutralization step, and the extraction time was decreased from 6 h to 40 min), providing similar efficiency and reproducibility comparable to or better than that of the conventional Soxhlet methodology. In addition, the proposed method is cleaner than conventional Soxhlet, as 80−85% of the extractant is recycled. The determination of the fatty acid composition by gas−liquid chromatography and quantification of both polymer formation by high-performance size exclusion liquid chromatography and nonpolar triacylglycerol formation by thin-layer chromatography with flame ionization detection in the extracts obtained by the proposed method, conventional Soxhlet and Weibull−Berntrop procedures (Soxhlet with a prior hydrolysis step), showed that the quality of the extracts obtained by the proposed procedure is better than that of the conventional alternatives, possibly due to the shorter time required by the accelerated method.

Keywords: *Solid−liquid extraction; fat; cheese; focused microwaves; Soxhlet*

INTRODUCTION

The definition of total fat content, according to the Nutritional Labeling Act (NLEA) of 1990, includes the sum of all fatty acids obtained from the overall lipid extract expressed as triglycerides (*Federal Register*, 1993). To obtain this content, the NLEA protocol for the determination of this parameter involves a hydrolysis treatment, followed by solvent extraction of lipids, individual separation of fatty acid methyl esters (FAMEs) by chromatography, and quantification of fat after stoichiometric conversion of FAMEs into triglycerides.

Interest in dietary fat is widespread, and the determination of fatty compounds is a basic requirement in testing food materials. Consumers demand reduction of the total fat content and cholesterol in food to improve human health (Chao et al., 1991), thus forcing government agencies to use more precise methods for fat determination that ensure accuracy in labeling products. So far, the determination of fat content has been based on gravimetric measurements after the sample has been leached with the suitable organic solvent. The most widely used procedure for fat removal from solid matrixes is conventional Soxhlet extraction; nevertheless, new extraction methods are displacing conventional Soxhlet because of the drawbacks the latter involves. The most significant of these drawbacks are the strong dependence of the lipid's extraction on the solvent used (one of the most critical steps in fat determination) (Finney et al., 1976; Hubbard et al., 1977), the necessity of a hydrolysis step before extraction (which may lead to high fat values due to the extraction of nonfat material) (Hagan et al., 1967), and the large volumes of organic solvents released into the atmosphere in addition to the slowness of the procedure. Despite these drawbacks, methods based on conventional Soxhlet extraction are at present used in a variety of official methods for the determination of fats (*ISO 659*, 1988; *Métodos Oficiales de Análisis*, 1986), probably due to the fact that it is a well-established, straightforward, and inexpensive technique, whereas the new methods are in a developing step, needing time to reach the status of conventional Soxhlet. Alternative methods, cleaner, faster, and more sophisticated than conventional Soxhlet, such as supercritical fluid extraction (SFE), are in development at present (Luque de Castro et al., 1994); ultrasound methods are used as a way to accelerate the extraction process (Luque de Castro et al., 1997), as are automated commercial devices such as Soxtec HT (Alstin, 1988).

In the past few years microwave energy has also shown its suitability for accelerating the extraction of organic compounds (Ganzler et al., 1986; Onuska et al., 1993; Steinheimer, 1993). Most of the papers appearing in the literature are related to organic pollutant extraction (López-Avila et al., 1994), and only a few of them concern the extraction of fat from alimentary products (Paré et al., 1995; Leroy et al., 1995). This may be due to the dependence of microwave action on the polarity of the irradiated matter: most of the commercial microwave devices are unable to work efficiently with nonpolar organic solvents, and the addition of polar modifiers usually complicates the extraction step by extracting compounds other than fats. The use of a focused microwave-assisted Soxhlet extractor (FMASE) circumvents this problem, as it is able to work with

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10.1021/jf981095f CCC: $18.00
Published on Web 05/18/1999

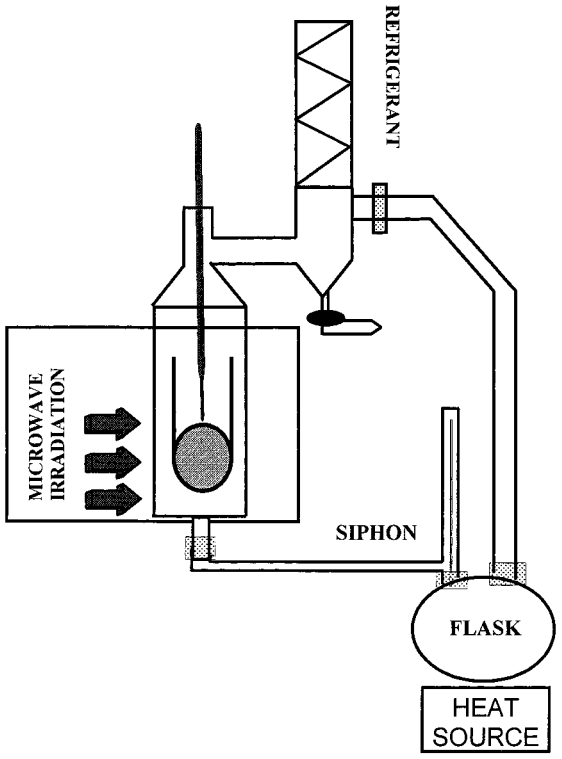

Figure 1. Scheme of the extraction device.

nonpolar solvents. The suitability of FMASE for replacing conventional Soxhlet both in pollutant extraction (García-Ayuso et al., 1998) and in the extraction of fat from food has been demonstrated (García-Ayuso et al., 1999). The key aspect of FMASE is that the system maintains the advantages of the conventional Soxhlet extractor, namely, sample−fresh solvent contact during the whole extraction step, no filtration required after extraction, easy manipulation, well-known procedures, and vast experience in the extraction field for more than a century. In addition, the FMASE circumvents the shortcomings of conventional Soxhlet by accelerating the process and minimizing environmental pollution due to the small amount of solvent used.

MATERIALS AND METHODS

Apparatus. The apparatus and instruments used in the different steps were as follows.

Moisture Determination. A Mettler AB104 analytical balance (Greifensee, Switzerland) and an electrically heated oven with ±2 °C temperature control (Selecta, Barcelona, Spain) were used.

Soxhlet Extraction. A 50 mL Soxhlet extractor (Probus, Barcelona, Spain) and an electrical isomantle (Selecta, Barcelona, Spain) with a rheostat were used.

Hydrolysis. An electrical isomantle (Prolabo, Paris, France) with a rheostat and a 500 mL Erlenmeyer flask were used. After filtration and neutralization, the sample was dried in the electrically heated oven.

FMASE. A conventional Soxhlet extractor was modified to facilitate accommodation of the sample cartridge compartment in the irradiation zone of a Microdigest 301 device of 200-W maximum power (Prolabo). The latter was also modified: an orifice at the bottom of the irradiation zone enabled connection of the cartridge zone to the distillation flask through a glass siphon.

Figure 1 illustrates the operation of the overall device (PCT WO97/44109, 1998). A Megal 500 thermometer (Prolabo) was used to monitor the extraction temperature. Two microprocessor programmers were used to control the microwave unit and thermometer. An electrical isomantle (Prolabo) with a rheostat was used to heat the distillation flask.

Solvent Release. An Eyela rotary vacuum evaporator (Rikakikai Co., Ltd., Japan) was used with this aim.

Chromatographic Step. A Hewlett-Packard model 5880 (Hewlett-Packard, Pittsburgh, PA) capillary gas chromatograph, fitted with split injection and equipped with a fused silica capillary column (0.32 mm i.d. × 30 m, 0.2 μm film thickness) coated with HP−Innowax polyethylene glycol (Hewlett-Packard) was used for the determination of fatty acid composition. A Konik 500 A chromatograph (Konik S.A., Barcelona, Spain) with a 10 μL sample loop, a refractive index detector (Hewlett-Packard) and 100 and 500 A Ultrastyragel columns (Water Associates, Milford, MA) were used for the quantification of polymers. An Iatroscan MK-5 TLC−FID analyzer (Iatron Laboratories, Tokyo, Japan) equipped with a flame ionization detector was used for the quantification of nonpolar triacylglycerols.

Types of Sample. Three types of cheese (namely, fresh, semicured, and cured) were used in this study. The semicured cheese was used in the optimization study, and the results obtained were then checked in the others. The semicured cheese was a commercial powder cheese (∼30% fat in dry extract), used as received. The fresh cheese was a commercial cream cheese (∼30% fat in dry extract) also used as received. The cured cheese (∼50% fat in dry extract) was cut into pieces of ∼1 cm and ground in a mechanical mill. The samples were put into plastic bottles and stored in a refrigerator at 4 °C until use.

Determination of the Moisture and Volatile Matter Contents. Ten grams of cheese was placed on a desiccated, tared capsule that was transferred into an electrically heated oven at 100 °C for 2 h. After this, the capsule was removed from the oven and cooled to room temperature in a desiccator. After weighing, the procedure was repeated until the difference between two consecutive weighings was <10 mg.

Conventional Extraction Method: Weibull−Berntrop Procedure. *Hydrolysis.* The procedure followed in this research was that of the Spanish official method for determination of fat in cheese (*Métodos Oficiales de Análisis*, 1986). Sample (2.5 g) was placed in a 500 mL Erlenmeyer flask containing both 100 mL of ∼3−4 mol/L hydrochloric acid (Panreac, Barcelona, Spain) solution and some pieces of pumice stone (Panreac) as boiling regulators. The mixture was subjected to smooth boiling for 1 h. After that, the mixture was filtered through a wet filter paper (Albet, Barcelona, Spain) and washed with water until neutralization. The filter paper containing the neutralized extracts was transferred into an electrically heated oven at 100 °C for 1 h.

Extraction. The filter paper containing the dried extracts was placed in a cellulose thimble (22 × 88 mm, Albet) that was capped with cotton wool. The thimble was then placed in the Soxhlet chamber, which was fitted to a tared distillation flask containing 100 mL of *n*-hexane (pesticide grade, Prolabo) and some pieces of pumice stone. After extraction for 6 h, the solvent was released in a rotary evaporator, and the last traces were removed by placing the flask with the extract in an oven at 100 °C for 1 h, followed by cooling in a desiccator and weighing. This last step was repeated until the difference between two consecutive weighings was <10 mg.

Microwave-Assisted Extraction Method. *Focused Microwave-Assisted Hydrolysis (FMAH).* Cheese (2.5 g) was placed into a quartz extraction vessel (Prolabo) containing 40 mL of hydrochloric acid solution and some pieces of pumice stone as boiling regulators. The vessel was fitted to a refrigerant and placed in the microwave irradiation zone of a Microdigest 301. The hydrolysis consisted of 10 min irradiation at the maximum power. After that, the mixture was filtered through a wet filter paper, inserted into a cellulose extraction thimble (22 × 88 mm), and dried in the Microdigest 301 during 1 min at 60% of maximum power.

FMASE. One hundred milliliters of *n*-hexane and some pieces of pumice stone were poured into a tared distillation flask. Either 2.5 g of cheese (mixed with 10 g of prewashed sea sand) or the filter paper with the extract obtained in the

Table 1. Optimization of the Number of Cycles Required To Achieve the Best Efficiencies[a]

no. of cycles	% of fat over dry matter	fat amount (g)	% of fat in the sample
3	0.433	17.32	27.19
4	0.455	18.20	28.57
5	0.466	18.64	29.26
6	0.475	19.00	29.83
7	0.483	19.32	30.33
8	0.493	19.72	30.96
9	0.496	19.84	31.15
10	0.496	19.84	31.15

[a] Semicured cheese, irradiation power 99%, and irradiation time 100 s.

Table 2. Experimental Design and Results of the Surface Response Methodology

First Full Factorial Design

coded power	time	decoded power (%)	time (s)	fat amount (g)
−1	−1	30	30	0.448
−1	+1	30	60	0.460
+1	−1	60	30	0.458
+1	+1	60	60	0.476
0	0	45	45	0.460
0	0	45	45	0.461
0	0	45	45	0.465

Path of the Steepest Ascent

increment	coded power	time	decoded power (%)	time (s)	fat amount (g)
origin	0	0	45	45	0.462
Δ	0.67	0.77	10	11	
origin + Δ	0.67	0.77	55	56	0.466
origin + 2Δ	1.34	1.54	65	67	0.480
origin + 3Δ	2.01	2.31	75	78	0.490
origin + 4Δ	2.68	3.08	85	89	0.493
origin + 5Δ	3.35	3.85	95	100	0.495

Second Full Factorial Design

coded power	time	decoded power (%)	time (s)	fat amount (g)
−1	−1	60	60	0.482
−1	+1	60	100	0.488
+1	−1	90	60	0.493
+1	+1	90	100	0.495
0	0	75	80	0.493
0	0	75	80	0.496
0	0	75	80	0.495

Star Points

limits coded power	time	limits decoded power (%)	time (s)	fat amount (g)
+α	0	90	80	0.495
0	+α	75	100	0.495
−α	0	60	80	0.490
0	−α	75	60	0.490
0	0	75	80	0.494
0	0	75	80	0.491

FMAH step 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 an electrical isomantle and connected to the sample vessel by a siphon and a distillation tube. After the extraction step (nine cycles with 85 s of microwave irradiation at 90% of maximum power each cycle), the extractant was collected in a reservoir by actuating a valve, which switched between the usual way of distilling in the Soxhlet extractor and the reservoir. A total of 80−85 mL of the solvent was thus recovered. Removal of solvent traces from the extracted fat and gravimetric determination were performed as in the conventional Soxhlet procedure.

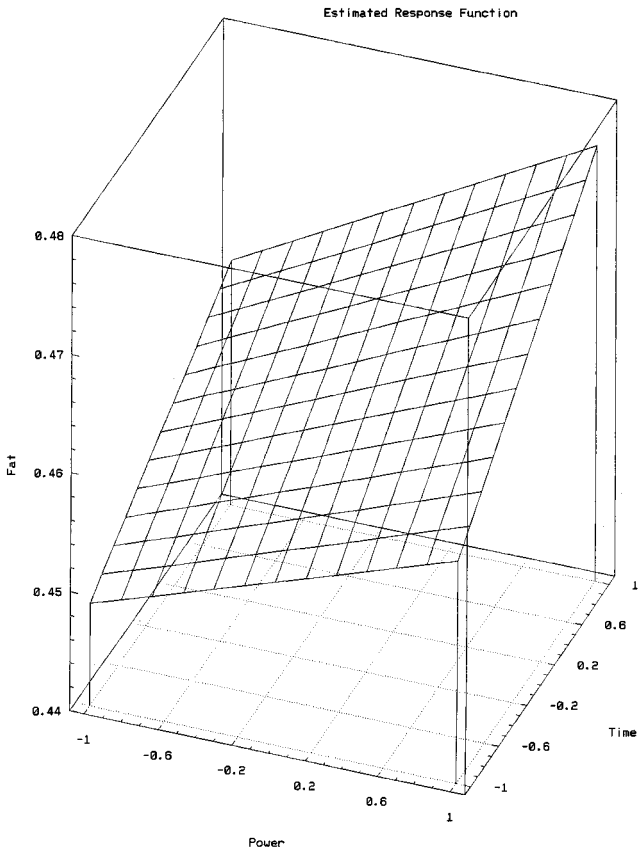

Figure 2. Estimated response surface from the first full factorial design.

Chromatographic Determination. *Determination of Fatty Acid Composition by Gas Chromatography.* FAME derivatives were prepared by base-catalyzed transesterification followed by acid methylation using the NLEA protocol. Samples with high contents of triacylglycerols were also transesterified at room temperature using 2 N methanolic KOH to avoid losses of short-chain FAMEs (IUPAC, 1992a). Methyl esters of fatty acids were analyzed using a capillary gas−liquid chromatograph. After a 3 min hold of the temperature at 60 °C, the gradient was programmed at 10 °C/min to 185 °C and then held at this point for 20 min.

Quantification of Polymers by High-Performance Size Exclusion Chromatography (HPSEC). Polymeric compounds were quantified directly in the samples by HPSEC following the IUPAC standard method (IUPAC, 1992b). Briefly, samples (50 mg/mL) were dissolved in tetrahydrofuran and analyzed in the chromatograph. The column was 25 cm × 0.77 i.d., packed with a porous, highly cross-linked styrenedivinylbenzene copolymer (<10 μm). High-performance liquid chromatography grade tetrahydrofuran at a flow rate of 1 mL/min was used as mobile phase.

Quantification of Nonpolar Triacylglycerols by Thin-Layer Chromatography (TLC) with Flame Ionization Detection. Samples were dissolved in *n*-hexane (15−20 mg/mL), and 1 μL was spotted on Chromarods S-III quartz rods with a coating of silica gel. The Chromarods were developed in light petroleum ether (bp 60−70 °C)/diethyl ether/acetic acid (90:10:2) for 35 min and scanned in an Iatroscan MK-5 TLC-FID analyzer. The Iatroscan was operated under the following conditions: hydrogen flow rate, 150 mL/min; air flow rate, 1500 mL/min; and scanning speed, 0.33 cm/s. Triacylglycerols were quantified as percentage of total area. All samples were analyzed in duplicate.

Experimental Design. A central composite design (CCD) was used as a variate method for optimization of two variables (namely, irradiation power and time). The CCD used consisted of a two-level full factorial design (coded ±1) superimposed on a face-centered star design (coded ±α) with the center

Table 3. Analysis of the Variance (ANOVA)

effect	sum of squares	DF	mean square	*F* ratio	*P* value
A: power	0.0000882	1	0.0000882	23.83	0.0082
B: time	0.0000282	1	0.0000282	7.61	0.0509
AB	0.0000040	1	0.0000040	1.08	0.3572
AA	0.0000124	1	0.0000124	3.36	0.1409
BB	0.0000124	1	0.0000124	3.36	0.1409
lack of fit	0.0000077	3	0.0000026	0.69	0.6040
pure error	0.0000148	4	0.0000037		
total (corr)	0.00018292	12			

$R^2 = 0.877217$ R^2 (adjusted for df) = 0.789516

points (coded 0) of the two designs coinciding. Therefore, the two factors studied had levels set at three separate coded levels due to that, as the star is face centered, α is equal to 1. This design is less advantageous than the rotatable or ortoghonal star, but the features of the system hinder the use of any of these last. The optimization procedure can be divided into the following steps, which belong to the response surface methodology (Montgomery, 1991): (a) first full factorial design (first-order polynomial); (b) checking of this approach (looking for interactions between factors); (c) path of steepest ascent; (d) second full factorial design (first-order polynomial); (e) curvature checks; (f) star design (second-order polynomial); (g) mathematical solution of the second-order equation. The CCD consisted of the second full factorial and the star points. Data interpretation was made using a statistical computer package (Statagraphics, 1993).

RESULTS AND DISCUSSION

The general behavior of the system has been checked in a previous paper (García-Ayuso et al., 1998b) with the following conclusions: (a) a number of factors influence the performance of FMASE; (b) the change of some factors dramatically influences the behavior of the rest; (c) some of these factors can be fixed from the beginning of the optimization study, and the best results can be reached by modifying only the other factors. Factors such as amount of solvent in contact with the sample when the microwave irradiation is applied, sample moisture, speed of cycle, and type of solvent were fixed to a constant value, taking into account the features of the system and the procedure followed. For example, the type of solvent was *n*-hexane because this is the solvent used in the official method; moisture was not modified, and its content was kept in the sample as received; microwave irradiation was applied when the level of solvent in the siphon was a half of the final level, etc. In this way, it was possible to reach better results with FMASE than with the conventional Soxhlet by modifying only three factors, namely, number of cycles, irradiation power, and irradiation time. The study has been developed by taking into account the fact that the total time for extraction should be as short as possible, the speed of each cycle was fixed at the maximum (setting the isomantle at 100% of its nominal value), and the number of cycles was as short as possible. Previous studies showed that the efficiency of the extraction linearly increased when the power and time of irradiation increased. Thus, to know the minimum number of cycles, the irradiation power and time were fixed at the highest values of the extraction device (99% and 100 s, respectively), and the optimization of the number of cycles was carried out. Table 1 illustrates the results obtained and, despite the possibility of reaching the highest extraction efficiency at eight cycles, a value

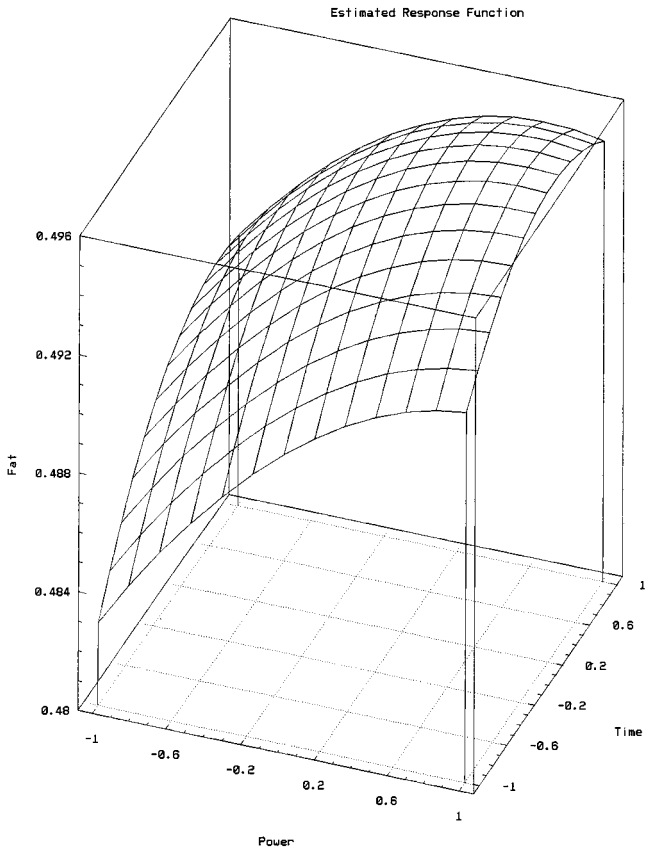

Figure 3. Estimated response surface from the central composite design.

Table 4. Results Obtained Using the Gravimetric Procedure

	Soxhlet					
	1 h	3 h	6 h	Weibull−Berntrop	FMASE	FMAH + FMASE
	Cured Cheese; Dry Extract, 74.5%; Reference Value of the Product, ~50% Fat over Dry Matter					
fat amount (g)	1.002	1.013	1.020	1.029	1.027	1.029
RSD (%) ($n = 5$)	1.7	1.3	1.0	0.7	0.8	0.7
% fat over dry matter	53.8	54.4	54.8	55.2	55.1	55.2
	Semicured Cheese; Dry Extract, 63.5%; Reference Value of the Product, ~30% Fat over Dry Matter					
fat amount (g)	0.419	0.443	0.474	0.534	0.495	0.543
RSD (%) ($n = 5$)	3.1	2.7	2.6	0.7	1.1	0.8
% fat over dry matter	26.4	27.9	29.9	33.6	31.2	34.2
	Fresh Cheese; Dry Extract, 17.3%; Reference Value of the Product, ~30% Fat over Dry Matter					
fat amount (g)	0.047	0.047	0.059	0.111	0.111	0.116
RSD (%) ($n = 5$)	6.4	4.3	3.2	1.5	2.0	1.7
% fat over dry matter	10.9	10.9	13.6	25.7	25.7	26.8

of nine cycles was selected as it provided more reproducible results. The CCD used herein is one of the multivariate methods for optimization more frequently used at present. Multivariate methods provide advantages over traditional univariate methods such as the possibility of finding interactions between variables and the use of a shorter number of experiments. The bivariate optimization involved irradiation power and time for nine cycles using the response surface methodology and semicured cheese as sample.

First Full Factorial Design. The limits chosen for this first-degree approach are shown in Table 2. This study shows both interactions between variables and a linear response of these in the zone included within the limits (Figure 2). The results obtained are also shown in Table 2. This linear response shows that to look for the optimum value in other zone was mandatory.

Path of the Steepest Ascent. The experiments carried out searching for the optimum values are shown in Table 2. These experiments showed that the surface curvature began close to 75% of irradiation power and 80 s of irradiation time. Thus, these conditions were chosen as the center of a new full factorial design.

Second Full Factorial Design. Upper and lower limits for the first-degree approach are shown in Table 2. Lack of fit was observed in this model when a first-degree approach was applied. This was due to the response surface curvature. For this reason, the model was modified by adding the star points and obtaining a second-degree model. The whole of experiments of the second full factorial design, the star points, and the center points constitute the CCD. Table 2 illustrates both the results obtained in the CCD experiments and the overall design, and Figure 3 shows the response surface obtained. Analysis of variance (ANOVA) was performed on the design to assess the suitability of the model used (see Table 3). The F ratio in this table is the mean-squared error to the pure error ratio obtained from the replicates of the central point.

The significance of the F value depends on the number of degrees of freedom (DF), which is shown in the P value (95% confidence level). Thus, the effects <0.05 on this column are significant. R^2 and P values for the lack of fit indicate that the quadratic model exhibits no significant lack of fit at the 95% confidence level. The second-order polynomial for the response surface obtained is as follows:

$$Y = 0.4940 + 0.0038A + 0.0022B - 0.0010AB - 0.0021A^2 - 0.0021B^2$$

where A is the irradiation power and B is the irradiation time.

Table 5. Quantification of Triacylglycerols (Weight Percent) and Polymeric Compounds (Weight Percent)

	triacylglycerols	polymers
Semicured Cheese		
sample		
1. Soxhlet 1 h	96.8	<0.1
2. Soxhlet 3 h	95.4	<0.1
3. Soxhlet 6 h	94.8	0.1
4. Weibull−Berntrop	66.4	7.0
5. FMASE	94.8	<0.1
6. FMAH + FMASE	88.6	0.6
Cured Cheese		
sample		
7. Soxhlet 1 h	96.8	2.3
8. Soxhlet 3 h	90.5	3.6
9. Soxhlet 6 h	89.8	5.5
10. Weibull−Berntrop	55.4	12.1
11. FMASE	96.1	0.5
12. FMAH + FMASE	56.1	7.9
Fresh Cheese		
sample		
13. Soxhlet 1 h	58.4	*a*
14. Soxhlet 3 h	60.5	*a*
15. Soxhlet 6 h	58.6	5.2
16. Weibull−Berntrop	51.3	11.8
17. FMASE	60.1	6.3
18. FMAH + FMASE	53.5	8.7

[a] Not quantified.

Optimal Conditions. It is possible to derive the optimal conditions for extraction from the first derivatives of the second-order polynomial. The procedure involved equalizing the derivatives to zero and solving the resulting equation system. The values thus obtained were $A = 0.827$ and $B = 0.326$. These values must be decoded so $A = 87.4\%$ and $B = 86.5$ s.

The total extraction time was 35−40 min (3 min each cycle plus the initial heating time).

Comparison Study. The optimal conditions (nine cycles, 90%, 85 s) were used in a repeatability study to assess the predictive ability of the model (see Table 4) as compared with (a) the results obtained using the Weibull−Berntrop procedure, (b) the results obtained from conventional Soxhlet (similar to the Weibull−Berntrop procedure without hydrolysis) for 1, 3, and 6 h, and (c) the results obtained from FMAH + FMASE. Table 4 also illustrates the same experiments carried out with the other two types of cheese: fresh and cured.

Quantitative Comparison. It is possible to obtain the following conclusions from the results in Table 4: the use of FMASE improves or equals the results provided by the official method in cured and fresh cheeses, which does not need previous hydrolysis treatment as in the Weibull−Berntrop procedure. Poor reproducibility is achieved in fresh cheese due to the

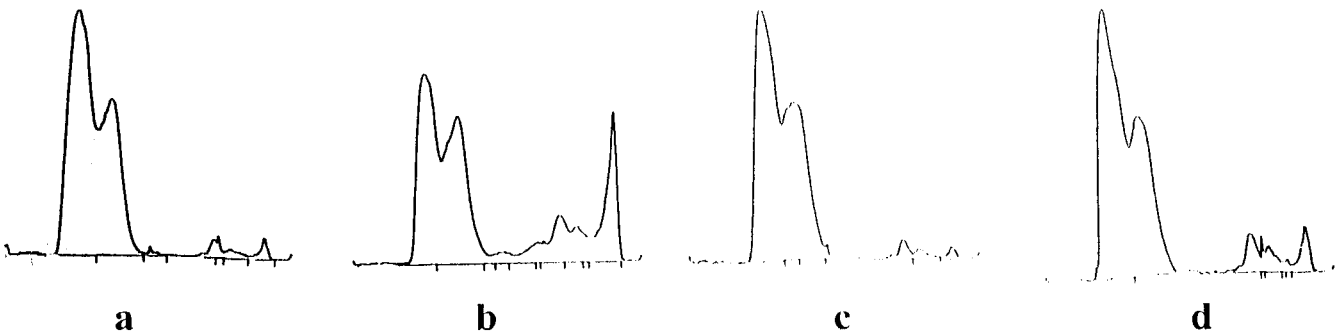

Figure 4. Chromatograms from semicured cheese using TLC-FID in all instances and (a) Soxhlet 6 h, (b) Weibull−Berntrop, (c) FMASE, and (d) FMAH + FMASE.

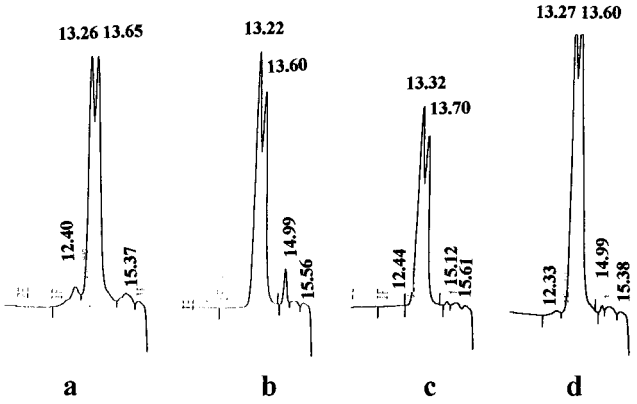

Figure 5. Chromatograms from semicured cheese using HPSEC and (a) Weibull−Berntrop, (b) FMASE, (c) Soxhlet 6 h, and (d) FMAH + FMASE.

high water content of the sample, which prevented accurate knowledge of the dry extract weight. The use of FMASE for semicured cheese improves the results provided by conventional Soxhlet, but the results are lower than those obtained with hydrolysis plus Soxhlet. However, the results are similar to the latter if a microwave-assisted hydrolysis step is applied before FMASE. This behavior could be explained by the highly fusing behavior of the matrix. The latter induces a negative effect by hindering an effective matrix−solvent contact. The presence of different amounts of water in the samples (sample humidity in cured cheese is ∼25.5%, in semicured cheese ∼37%, and in fresh cheese ∼82%) does not constitute any problem for the extraction due to the microwave's ability to remove it during the first cycles.

Table 6. Major Fatty Acid Composition (Percent)

sample	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	others
Semicured Cheese								
Soxhlet 1 h	2.0	3.5	11.7	28.6	13.9	25.9	2.0	12.4
Soxhlet 3 h	1.8	3.2	11.9	30.0	15.0	24.9	1.9	11.3
Soxhlet 6 h	1.8	2.8	10.8	29.2	14.0	27.0	2.8	11.6
Weibull−Berntrop	2.1	3.1	12.0	31.6	15.2	24.3	1.1	10.6
FMASE	1.5	2.6	10.2	28.5	14.0	26.7	2.5	14.8
FMAH + FMASE	1.9	3.3	12.2	32.8	12.1	24.6	1.6	11.5
Cured Cheese								
Soxhlet 1 h	3.1	2.7	9.7	29.9	16.0	28.9	2.8	6.9
Soxhlet 3 h	3.0	2.4	9.3	31.5	19.1	26.5	2.1	6.1
Soxhlet 6 h	3.5	3.0	11.2	33.1	15.1	23.8	2.1	8.2
Weibull−Berntrop	3.2	2.7	11.0	36.8	22.3	15.4	0.7	7.9
FMASE	3.6	2.8	10.7	31.3	13.9	26.7	3.0	8.0
FMAH + FMASE	2.9	2.7	10.6	36.5	22.3	16.6	0.9	7.5

Qualitative Comparison. To know the influence of the extraction procedure on the composition of lipid

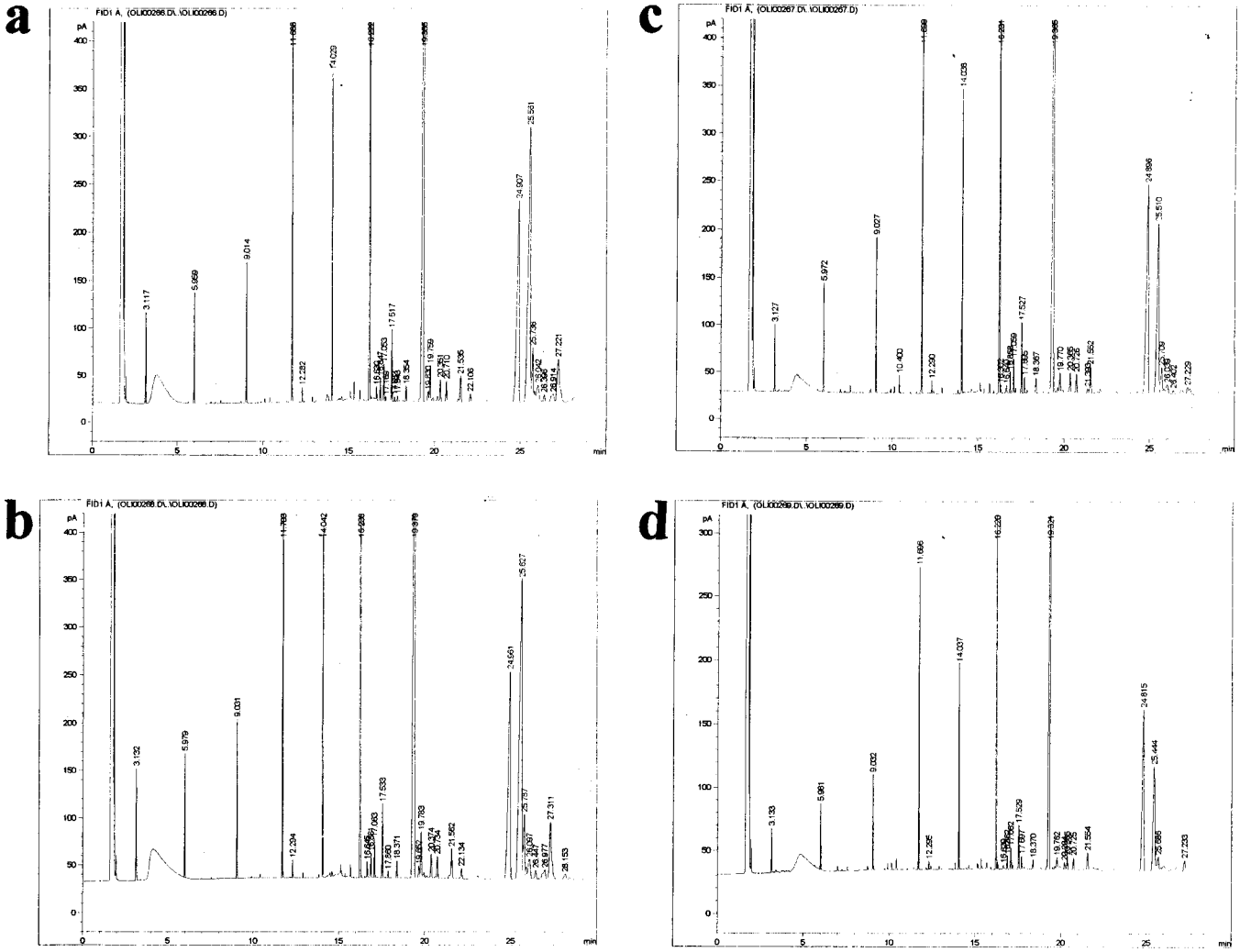

Figure 6. Chromatograms from semicured cheese using GLC and MeONa/H_2SO_4 and (a) Soxhlet 6 h, (b) FMASE, (c) Weibull−Berntrop, and (d) FMAH + FMASE. Retention times were as follows: $C_{4:0}$, 3.145; $C_{6:0}$, 5.990; $C_{8:0}$, 9.036; $C_{10:0}$, 11.696; $C_{10:1}$, 12.295; $C_{12:0}$, 14.040; $C_{14:0}$, 16.237; $C_{14:1}$, 16.641; $C_{iso15:0}$, 16.859; $C_{antiiso15:0}$, 17.059; $C_{15:0}$, 17.530; $C_{iso16:0}$, 18.368; $C_{16:0}$, 19.393; $C_{16:1}$, 19.782; $C_{17:0}$, 20.727; $C_{17:0}$, 21.550; $C_{17:1}$, 22.121; $C_{18:0}$, 24.938; $C_{18:1}$, 25.550; $C_{18:1}$, 25.732; $C_{18:2}$, 27.252.

Table 7. Major Fatty Acid Composition[a] (Percent) of Nonhydrolyzed Samples with High Triacylgycerol Content

sample	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:1}$	others
Semicured Cheese													
Soxhlet 6 h	3.1	1.4	0.8	2.4	3.4	11.4	28.2	1.6	12.9	24.3	2.3	1.7	6.5
FMASE	3.2	1.6	0.8	2.0	2.9	10.7	28.4	1.6	13.2	25.3	2.6	1.4	6.3
Cured Cheese													
Soxhlet 6 h	3.8	1.9	1.6	5.1	3.6	10.9	28.2	1.1	13.2	21.8	1.5	1.0	6.3
FMASE	3.6	3.2	2.4	6.3	3.8	10.6	25.6	1.2	11.4	21.9	2.9	1.3	5.8

[a] Base-catalyzed transesterification (2 N methanolic KOH).

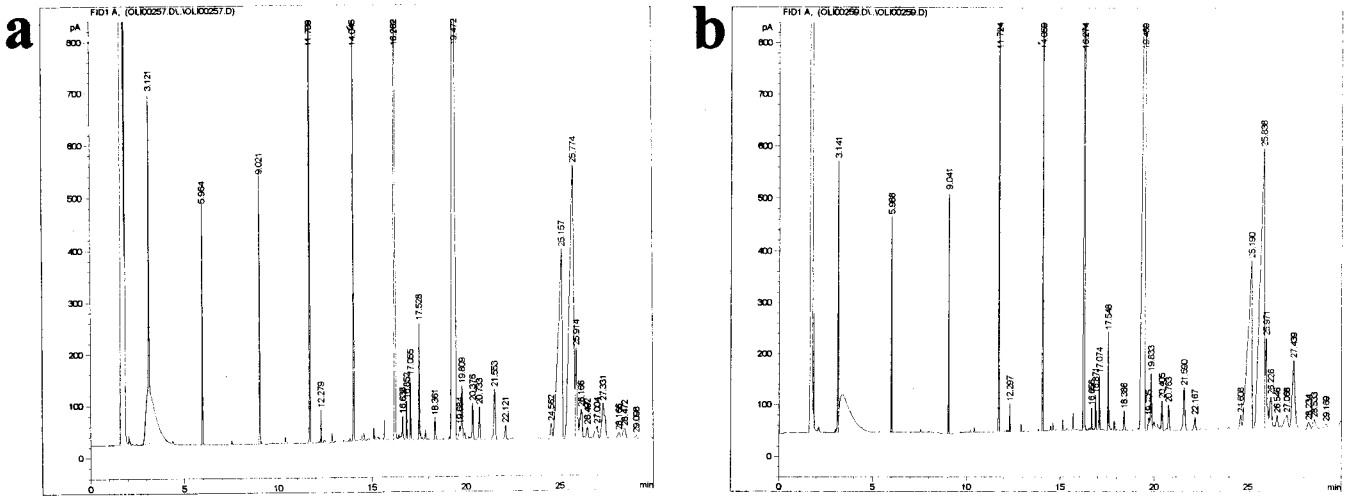

Figure 7. Chromatograms from nonhydrolyzed cured cheese using GLC and 2 N KOH/MeOH and (a) Soxhlet 6 h and (b) FMASE. Retention times were as in Figure 6.

extracts, quantification of major compounds, that is, triacylglycerols (TAG), molecular weight (MW) distribution of lipidic components and FAMEs composition were considered the most useful analyses.

Table 5 shows the quantitative results obtained for both TAG and higher molecular weight compounds by means of TLC-FID and HPSEC, respectively, for cheese samples extracted by conventional Soxhlet (1, 3, and 6 h), Weibull−Berntrop procedure, FMASE, and FMAH + FMASE. As can be observed, nonhydrolyzed samples from cured and semicured cheese had a high content of TAG which, as expected, decreased when samples were subjected to hydrolysis. TLC-FID chromatograms are shown in Figure 4 and illustrate the differences between nonhydrolyzed and hydrolyzed lipid extracts; the two main peaks correspond to TAG. After hydrolysis, peaks of increasing polarity appear mainly due to the formation of partial glycerides and fatty acids. In the case of fresh cheese extracts similar contents of TAG were found before and after hydrolysis, which might be attributed to hydrolysis reactions during extraction; nevertheless, the results are difficult to discuss in depth due to both the high moisture and low fat contents of the fresh cheese samples. Quantitative results for compounds with MW higher than those corresponding to TAG were of special interest. As observed in Table 5, the amounts for nonhydrolyzed samples were much lower than those found for their hydrolyzed counterparts, and also clear differences between the conventional Soxhlet (6 h) and FMASE procedures could be deduced. Figure 5 shows significant parts of the exclusion chromatograms obtained for lipid extracts from semicured cheese. Two aspects are relevant: (a) The retention time for high MW compounds (1800−2000 Da) suggests modification of unsaturated fatty acids and formation of dimeric TAG when the hydrolytic step is developed (Dobarganes et al., 1988). (b) The TAG percentage extracted with conventional Soxhlet (without hydrolysis) and FMASE is similar except for cured cheese, which is higher for FMASE due to the modification of the compounds during the long extraction time of the conventional Soxhlet, which does not occur during the short extraction time required by FMASE. Table 6 and Figure 6 show major FAMEs in the different lipid extracts from cured and semicured cheese obtained by capillary gas−liquid chromatography after transesterification followed by acid methylation (for a general comparison, cold transesterification was avoided as hydrolyzed samples are expected to contain free fatty acids that were not methylated). Results corresponding to extracts from fresh cheese are not given as, due to the low amount of fat available, very poor chromatograms were obtained. As can be seen, the percentage of unsaturated fatty acids, particularly linoleic acid, was much lower for samples subjected to hydrolysis. On the other hand, the results indicate that a lower proportion of unsaturated fatty acids was present when Soxhlet extraction was used for cured cheese. These results would also be in favor of the hypothesis of fatty acid modification due to both the long extraction time used with the conventional system and the conditions used during the hydrolysis step. Table 7 and Figure 7 show the results of nonhydrolyzed samples with high TAG content after cold sample transesterification was performedto avoid losses of the most volatile acids. Conclusions obtained are similar to those of the transesterification followed by acid methylation.

Conclusions. The use of a prototype consisting of a conventional Soxhlet extractor assisted by focused microwaves on the extraction chamber for accelerating the leaching process in the extraction of total fat from different types of cheese provides the following main advantages:

(a) substantial shortening of the extraction time;

(b) extraction efficiencies and reproducibility comparable to or better than those provided by conventional Soxhlet extraction;

(c) extraction of higher percentage of triglycerides for similar efficiencies as compared with the Weibull−Berntrop proceduree, which demonstrates lower transformation due to hydrolysis;

(d) saving of extractant (80−85% of the total volume was recovered by recycling);

(e) use of focused microwave-assisted hydrolysis if needed (the hydrolysis time required is shorter than those of the conventional procedure);

(f) use of the samples as received without the water adjustment usually required in conventional Soxhlet;

(g) avoidance of neutralization after hydrolysis.

It is worth emphasizing that the most remarkable characteristic of FMASE is the use of a new technology for circumventing the drawbacks associated with a well-tested and useful old technique such as conventional Soxhlet. Thus, FMASE has shown its potential to become one of the best alternatives for displacing conventional Soxhlet in routine uses.

ACKNOWLEDGMENT

Prolabo is thanked for providing the prototype.

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Received for review October 8, 1998. Revised manuscript received March 18, 1999. Accepted April 9, 1999. Spain's Comisión Interministerial de Ciencia y Tecnología (CICyT) is gratefully acknowledged for financial support (Project PB96-0505). Prolabo is thanked for providing partial financial support.

JF981095F