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Focused microwave-assisted Soxhlet extraction: a convincing alternative for total fat isolation from bakery products

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Abstract

An approach for automated fast extraction of the fat content in bakery products based on focused microwave-assisted Soxhlet extraction (FMASE) and gravimetric determination is proposed. The main factors affecting the extraction efficiency—namely, power of irradiation, number of cycles and irradiation time—were optimized using experimental design methodology. The proposed method was applied to six samples, which were classified in two groups—namely, snacks and cookies. The results obtained agree with those provided by the AOAC 920.39 reference extraction method. No significant differences in the extraction efficiency of the fat content in bakery samples using FMASE versus the official method were found. Moreover, a drastic reduction in both the extraction time (60 and 35 min versus 16 and 8 h, respectively, for the two above commented groups) and sample handling are achieved with similar precision (expressed as repeatability and within-laboratory reproducibility standard deviation) to that provided by the AOAC 920.39 method. In addition, the proposed method is cleaner than the reference method as 75–80% of the extractant is recycled.

Keywords: Fat extraction; Bakery products; Microwave-assisted Soxhlet

1. Introduction

The interest in dietary fat is a growing trend, and the determination of fatty compounds is a basic requirement in testing food material as a result. Consumers demand reduction of the total fat contents in food in order to improve human health [1], thus forcing government agencies to the use of more precise methods for fat determination, which assure accuracy in labeling products.

For nutrition labeling purposes, fat has been defined as triglycerides, substances extracted with ether or total lipids [2–4]. To unify criteria, the US Food and Drug Determination (FDA) through the Nutritional Labeling and Education Act (NLEA) of 1990, defined "total fat" as the sum of all fatty acids obtained in the lipid extract, expressed as triglycerides [5]. Hence, a complete extraction of lipids from the sample is a mandatory step. Lipid extraction is carried out in different ways depending on the sample characteristics [6]. Thus, some extraction methods (namely, Weibull-Berntrop, Röse-Gottlieb, Mojonnier, Folch, Werner-Schmid, Bligh-Dyer methods, etc.,) are based on hydrolysis (either acid, alkaline or enzymatic) before solvent extraction, but some others involve only the solvent extraction step (Soxhlet, Lickens-Nickerson, etc.).

Despite several modifications in solvent mixtures and laboratory practice [7–10], the previous, conventional procedures have not been greatly improved, and long preparation times with a second re-extraction step to ensure complete removal have been required most times [11]. The critical choice of the use of organic solvents and the by-side phenomena—namely, co-extraction of non-lipid material such as sugar or sugar by-products, vitamins, color compounds, etc., [12] and the chemical transformations of triglycerides [13]—associated with the long time and high temperature needed for classical digestion or extraction are the principal shortcomings. These methods provide a lipid extract that is usually quantified by gravimetry, but there also are titration methods as Babcock or Gerber methods.

At present, a tendency towards the use of supercritical fluid extraction (SFE) [14], and accelerated solvent

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extraction (ASE) [15] can be observed. Recently, a dynamic ultrasound-assisted extraction method has been proposed prior to the gravimetric determination of the total fat content in bakery products. Recoveries from 99.7–100.7% and shortening of the extraction time between five and eight times, depending of the type of sample [16], were obtained as compared with conventional Soxhlet. However, in the knowledge of the authors, there are no precedents in the literature about microwave-assisted extraction (MAE) for extracting fat from bakery products.

The use of microwave energy as a heat source in wet ashing procedures was first demonstrated in 1975 [17]. Since then, microwaves have shown their suitability for accelerating extraction processes. A focused microwave-assisted Soxhlet extractor is a patented device [18], which has been checked through several prototypes for the extraction of different compounds from environmental solid samples such as pollutants [19] and also as an alternative to traditional methods for lipid extraction in food analysis [20,21]. The key aspect of focused microwave-assisted Soxhlet extraction (FMASE) is the maintenance and the advantages of conventional Soxhlet extraction—namely, sample fresh solvent contact during the whole extraction step, no filtration required after extraction, easy manipulation, well-known procedures and a large experience in the extraction field for more than a century, and avoidance of the shortcomings of conventional Soxhlet by accelerating the process, minimizing environmental pollution due to the small amount of solvent released into the atmosphere and low degradation of thermolabile analytes. In addition, completeness of analyte extractions not always achieved with conventional methods is assured by this approach [22].

The present contribution focuses on the development of a rapid analytical method for the extraction of fat contents in bakery products using a focused microwave-assisted Soxhlet extractor, and subsequent quantification by gravimetry. The proposed method has been compared with both the ultrasound-assisted method applied to the same samples [16] and the AOAC 920.39 reference extraction method [23], in which a conventional Soxhlet extractor was used.

2. Experimental

2.1. Instrumentation

The first prototype of the focused microwave-assisted Soxhlet extractor was used to perform this research. The device is composed of a conventional Soxhlet extractor modified in order to 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.

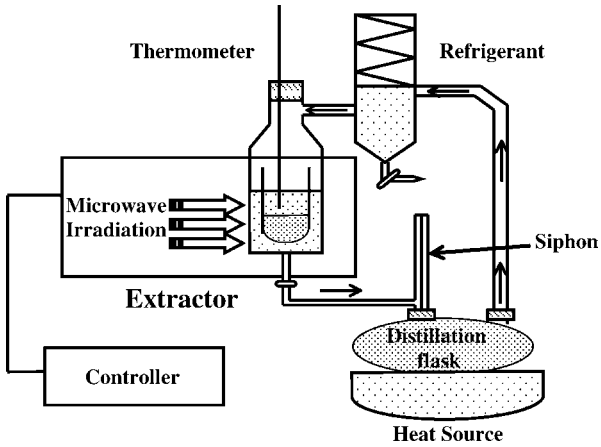

Fig. 1. Scheme of the prototype operation.

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.

A 50-ml Soxhlet extractor (Afora, Barcelona, Spain) was used to carry out the conventional Soxhlet extraction. Cellulose extraction thimbles (25 mm × 88 mm, Albet, Barcelona, Spain) were used both in the proposed and official methods.

A rotary-evaporator (Büchi R-200 with Heating Bath B-490, Switzerland) was used to evaporate the solvent after each extraction and a Selecta 210 Heater was used to eliminate water traces. An Ohaus Explorer analytical balance 110 ± 0.0001 g, (Switzerland) was used to weigh the total fat extracted. Statgraphics Plus for Windows software package (v2.1, 1992; Statgraphics, Rockville, MD, USA) was used in the statistical analysis.

2.2. Reagents and samples

n-Hexane HPLC grade (Panreac, Barcelona, Spain) was used as leaching agent in both the official and the proposed methods. Six bakery products were purchased from local supermarkets and classified in two groups—namely, cookies and snacks—according to the sample matrix. So, Snack Cookies Hacendado (Grupo Siro, Venta de Baños, Palencia, Spain), Cookies produced using traditional methods (Bjorg, Italy), Müesli Multivitamins bifidus effect cookies (Bio Century, Quart, Girona, Spain) and Built-in doughnut (Santiveri, Barcelona, Spain) constituted the cookies group, and Snack Fiber Cheese (Celigüeta, Araia, Alava, Spain) and Cheetos (Matutano, Tarragona, Spain) the snack group.

2.3. Experimental procedure

2.3.1. Sample preparation

The sample was prepared according to the protocol established by legislation [24]. The product under study was homogenized in a mixer, 200 g of sample was crushed in

a mincer and then was homogenized again and stored in a hermetic recipient at 4 °C in the dark until use.

2.3.2. AOAC 920.39 reference extraction method

Five grams of sample was placed in a cellulose thimble, which was capped on with cotton wool. The thimble was then placed in the Soxhlet chamber, which was fitted to a tared distillation flask containing 80 ml of hexane and a boiling glass regulator. After extraction for 16 h, the solvent was released by a rotary-evaporator and the last traces were removed by placing the flask with the extract in a heater at 80 °C overnight. The next day, the flask was cooled in a dessicator and weighed. This heating-weighing step was repeated until the difference between two consecutive weighing was smaller than 10 mg.

2.3.3. Focused microwave-assisted Soxhlet extraction (FMASE) method

One hundred and twenty-five milliliters of *n*-hexane and two pieces of pumice stone were poured into a tared 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 program consisted of a number of cycles (12 or 7 cycles) depending on the type of sample matrix. Each cycle involved three steps:

(1) the extractant evaporated from the distillation flask, condensated in the refrigerant and dropped on the sample, filling the sample cartridge vessel until the level of solvent in the siphon was a 90% of the final level;
(2) the magnetron started to irradiate when the solvent reached the preset height in the siphon for 90 s at 100 W (50% of maximum power); and
(3) after irradiation, the extract in the extraction vessel was unloaded to the distillation flask.

After finishing the last cycle, the 75–85% of the extractant was recycled by a new cycle and condensed on the extraction chamber. So, the extract was evaporated to near-dryness and, 90–100 ml of the solvent was thus recovered. Removal of the solvent traces from the extracted fat and gravimetric determination was performed as in the conventional Soxhlet procedure.

3. Results and discussion

This research focuses on the establishment of a method for the removal of fat from bakery products, which was faster, cleaner and requiring less consumption of reagents than those presently used. Therefore, the optimization of the overall method here proposed was concentrated on the leaching step.

3.1. Optimization of the focused microwave-assisted Soxhlet extraction step

The general behavior of the system has been investigated in previous papers [20–25] with the following conclusions: (i) a number of factors influence the performance of FMASE; (ii) the change of some factors dramatically influences the behavior of the others; (iii) 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 the amount of solvent in contact with the sample when the microwave irradiation is applied, and speed of cycle were fixed to a constant value taking into account the extractor features and procedure. In addition: the solvent was *n*-hexane because this is a less hazardous solvent than petroleum ether used in the official method; moisture content was not modified, so the sample was used as received; microwave irradiation was applied when the level of solvent in the siphon was a 90% of the final level before unloading; 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, power of irradiation and irradiation time. The study has been developed with a view to maximizing the speed of each cycle (setting the isomantle at 100% of its nominal value) and minimizing both the number of cycles and total extraction time.

Therefore, a screening study, using 4-g portions of the Snack cookies Hacendado, of the influence of the main variables affecting the extraction step such as irradiation time, power of irradiation and number of cycles, was performed by means of a two-level factorial 2^3 type V+ resolution experimental design, allowing one d.f. and involving eight randomised runs plus three centre points. The upper and lower values given to each factor were selected from the available data and experience gathered in the preliminary experiments. The tested and optimum values obtained for each variable are shown in Table 1.

The conclusions of this first screening study were that the irradiation power was not a statistically influential factor in the range under study. However, the results showed better recoveries with the minimum value of irradiation power. So,

Table 1
Ranges assessed and optimal values for the variables influencing the extraction step

Variable	Tested range		Optimum value
	First design	Second design	
Power of irradiation (%)	50–100	50	50
Irradiation time (s)	30–60	70–90	90
Number of cycles	4–8	8–10	12[a]

[a] Checked with the univariate study of the number of cycles.

100 W, that is 50% of the power provided by the microwave device, was selected for subsequent experiments.

The other variables, namely irradiation time and number of cycles, were influential factors for isolation of the fat content. Higher values for the number of cycles and the irradiation time were tested using a two-level full factor design 2^2 type V+ resolution allowing zero d.f. involving four randomised runs plus three centre points. In this case, the irradiation time was not an influential factor within the range studied. However, the upper value tested for the irradiation time (90 s) was selected for further experiments as it provided better efficiencies. Analyzing the results of the second experimental design, only the number of cycles was an influential factor with a positive effect on extraction.

The influence of the number of cycles was studied in a univariate way by fixing the other variables at their optimal values. Several number of cycles were tested in order to determine the time necessary for total isolation of the fat. Total removal of fat was obtained after 12 cycles. In view of these results, this number of cycles was selected and used for further experiments.

3.2. Study of the extraction kinetics: a comparison of the proposed method with the Soxhlet method

Four-gram portions of two bakery samples were used to compare the extraction kinetics of the proposed method with both that not assisted by microwave—namely, the same working conditions but without microwave irradiation—and the Soxhlet reference method. The samples used were Snack cookies Hacendado and Cheetos as representative of cookie and snack groups, respectively. FMASE cycles from 2–14 were performed and the extracted fat was quantified as in the proposed method. Fig. 2 shows the focused microwave-assisted extraction kinetics of the fat from the target samples. Twelve cycles were necessary for total removal of the fat from the cookie group sample [Fig. 2(a)]. However, seven cycles were sufficient for the complete extraction of the fat contents in the case of the snack sample [Fig. 2(b)]. This fact can be explained by the different nature of the matrix of the two sample groups based on their ingredients or elaboration process. Thus, the cookie group can be classified as samples with a difficult matrix and the snack group as samples with an easy matrix in terms of extraction facility.

With regard to the non-microwave-assisted method, the fat obtained was 6.93 and 8.21% versus 20.72 and 26.22% provided by the proposed method for Snack cookies Hacendado and Cheetos, respectively. Thus, the influence of microwave irradiation under the optimum conditions was significant in comparison with the non-irradiated process.

Fig. 3 shows the conventional Soxhlet extraction kinetics of fat from the same bakery samples. In this case, the kinetics study was developed between 4 and 20 h in order to know when total extraction was reached. The time required for extraction was much longer than using FMASE, despite

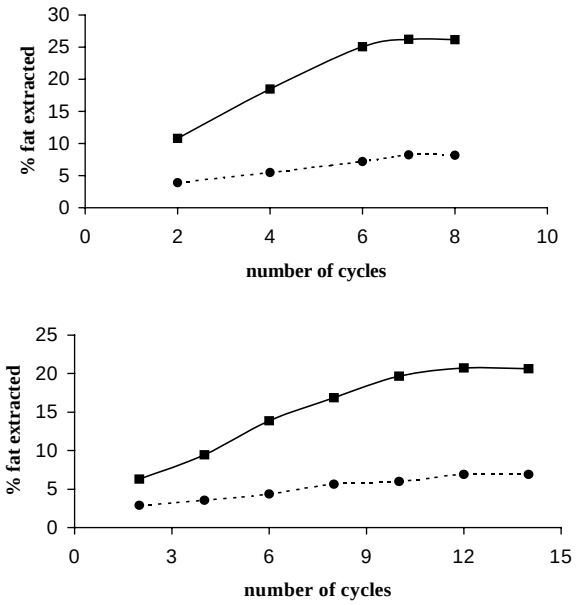

Fig. 2. Comparison of the extraction kinetics between FMASE and without microwave assistance of the process for the extraction of the fat content from two bakery samples: (■), FMASE method; (●), non-irradiated method. (a) Extraction efficiency for Cheetos vs. number of cycles. (b) Extraction efficiency for Snack cookies Hacendado vs. number of cycles.

the duration of each FMASE cycle was equal to that of cycles of conventional Soxhlet. The most interesting aspect of this comparison is that the time necessary for total removal of the fat content by FMASE was 60 and 35 min for Snack cookies Hacendado and Cheetos versus 16 and 8 h, respectively, needed by the reference extractor. This fact shows the high efficiency of microwaves for accelerating extraction. As compared with the ultrasound-assisted method applied to the same bakery products [16], the extraction efficiencies were very similar. However, the times necessary for this step were 3 and 1 h for Snack cookies Hacendado and Cheetos, respectively.

3.3. Evaluation of the precision of the method

With the aim of evaluating the precision of the proposed method, within-laboratory reproducibility and repeatability were estimated in a single experimental set-up with duplicates. The experiments were carried out using 4 g of Snack

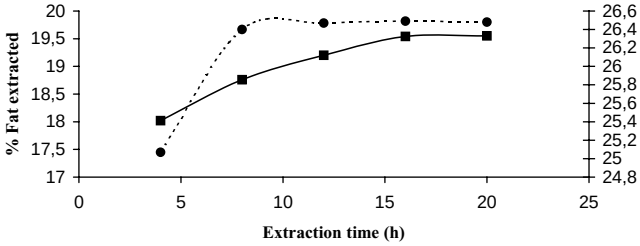

Fig. 3. Study of the extraction kinetics by the conventional Soxhlet extraction method for two samples of bakery products: (■), Snack cookies Hacendado (left axis); and (●), Cheetos (right axis).

Table 2
Comparison of the FMASE and official methods and manufacturers' stated contents of fat for several bakery samples

Sample	Labelled contents	Soxhlet extraction[a]	FMASE[a]	f[b]
Snack cookies Hacendado	20.10	19.55 ± 0.19	20.00 ± 0.21	1.02
Traditional cookies	17.60	17.61 ± 0.09	17.52 ± 0.12	0.99
Müesli Multivitamins cookies	22.00	21.21 ± 0.06	21.23 ± 0.10	1.00
Built-in doughnut	20.00	19.87 ± 0.19	19.91 ± 0.12	1.00
Snack Fiber Cheese	22.00	25.86 ± 0.23	26.09 ± 0.17	1.01
Cheetos	26.00	26.47 ± 0.07	26.32 ± 0.11	0.99

[a] Percentage fat contents ± standard deviation ($n = 3$).
[b] FMASE fat contents (%)/Soxhlet extraction fat contents (%).

cookies Hacendado under the optimum working conditions. Two measurements per day were carried out on 7 days [26]. The repeatability, expressed as relative standard deviation, was 1.29% and the within-laboratory reproducibility (%RSD) was 2.18. These small values can be explained by the simplicity of the proposed method.

3.4. Application of the proposed method to other bakery samples and comparison with the reference method

The proposed FMASE method and the reference extraction method were applied to other bakery samples in order to evaluate if both methods provided similar results. Table 2 shows the fat extracted in both cases as well as the *f* factor defined as the amount of fat extracted by FMASE/amount of fat extracted by the reference method. As can be seen, the *f* factor was 1.00 in the case of Müesli Multivitamins bifidus effect cookies and Built-in doughnut, and close to 1.00 in the other samples.

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 differences between FMASE and conventional Soxhlet were not significant.

The calculated *t*-value was compared with the theoretical value at $\alpha = 0.05$ and five degrees of freedom, i.e. 2.57. As the calculated value (i.e. 0.916) is smaller than the theoretical value; this means that, at the chosen significance level, no differences between the two methods were found.

Thus, similar extraction efficiency was obtained with both methods; so, the excellent agreement found between the two sets of results testifies the applicability of the proposed method. Finally, the results provided were quite consistent with the manufacturers' stated contents of fat. However, in the case of Snack Fiber Cheese, more than 4% higher amounts of the fat labeled was obtained both by the proposed and the Soxhlet reference methods.

4. Conclusions

A prototype consisting of a conventional Soxhlet extractor assisted by focused microwaves on the extraction chamber for acceleration of the leaching process is proposed for isolation of the fat content from bakery samples. The quantitative results obtained from six different samples are in agreement with those provided by both the AOAC 920.39 reference extraction method and the manufacturers' stated contents of fat, except for Snack Fiber Cheese, which had labeled lower amounts of fat (more than 4%).

Focused microwave-assisted Soxhlet extraction provides the following advantages:

(i) Substantial shortening of the extraction time (from 16 and 8 h to 55 and 35 min for Snack cookies Hacendado and Cheetos, respectively).
(ii) Saving of extractant is such a way that only 25–30 ml is consumed per extraction.
(iii) Use of samples as received, without the moisture adjustment, usually required in conventional Soxhlet methods.
(iv) Extraction efficiencies and precision (expressed as repeatability and within-laboratory reproducibility standard deviation) comparable to, or better than, those provided by conventional Soxhlet extraction.

Thus, FMASE has shown its potential to become one of the best alternatives for replacing conventional Soxhlet in routine uses, in this case, food analysis, unlike supercritical fluid extraction (SFE) [14] and accelerated solvent extraction (ASE) [15], FMASE does not require sophisticated equipment nor high pressures and temperatures. The latter feature may lead to the formation of artifacts in the fat extract, such as oxidation products, thus changing the composition of the fat. In comparison with ultrasound-assisted extraction, the extraction time has been considerably shortened with the proposed method for the same bakery samples. An additional shortcoming of ultrasound leaching is that radicals created during sonolysis have an oxidative energy that can also change the composition of the fat extracted.

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