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Short communication

Ultrasound-assisted Soxhlet extraction: an expeditive approach for solid sample treatment
Application to the extraction of total fat from oleaginous seeds

J.L. Luque-García*, M.D. Luque de Castro

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba Marie Curie Building (Annex), E-14071 Córdoba, Spain

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Abstract

Conventional Soxhlet extraction assisted in the cartridge by ultrasound has been developed and used to extract the total fat content from oleaginous seeds such as sunflower, rape and soybean seeds. The application of ultrasound to the sample cartridge enormously decreases the number of Soxhlet extraction cycles needed for quantitative extraction of the fat, thus reducing the extraction time at least to half the time needed by the conventional procedures. The results agree well with those obtained by conventional Soxhlet extraction and the ISO reference method, both in terms of efficiency and precision. The repeatability of the proposed approach, expressed as relative standard deviation, was 0.9%; the within-laboratory reproducibility was 1.3%. Qualitative analysis of the extracted fat showed that the application of ultrasound does not change the composition of the oil.

Keywords: Soxhlet extraction; Ultrasound-assisted extraction; Fat extraction; Oleaginous seeds

1. Introduction

Fats are a subclass of lipids but “fat” is often used interchangeably with“lipid”. This is a very common mistake that has been discussed in several papers [1,2] but it is not still clear what lipid compounds should be classified as fat. For nutrition labeling purposes, fat has been defined as triglycerides, substances extracted with ether, or total lipids [3–5]. In order to unify criteria, the US Food and Drug Administration (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 [6]. Hence, a complete extraction of lipids from the sample is a mandatory step.

Seeds are very difficult matrices in which some characteristics such as particle size, moisture, etc. have a decisive influence on extraction. Most of the lipids (75–85%) are easily extractable by the simple use of an appropriate solvent (hexane or ether) but the rest of the lipid matter is strongly bonded to the matrix and exhaustive treatments are needed in order to isolate this fraction.

The determination of the total fat content in oleaginous seeds is of paramount importance in the oil industry as the price of the raw material is a function of its richness in the final, commercial product. Traditionally, this determination has been based on leaching ground seeds with an organic solvent and weighing the residue after solvent evaporation from the extract. The most widely used procedure for fat removal from the solid matrix remains conventional Soxhlet extraction (on which official methods are based) which is straightforward and inexpensive but also slow and tedious. The most severe shortcomings of Soxhlet extraction are the long time involved and the large volumes of organic solvents released into the atmosphere; the Soxhlet procedure is thus far from clean.

A great variety of new approaches based on different principles (namely, supercritical fluid extraction (SFE) [7–10], microwave irradiation [11–13], pressurized liquid extraction (PLE) [14,15], etc.) have been developed to circumvent the shortcomings of conventional Soxhlet extraction. None surpasses it in the extraction of edible oils for reasons such as variable efficiency of SFE as a function of the sample matrix;

* Corresponding author. Tel.: +34-957-218615; fax: +34-957-218615.
E-mail address: q52lugaj@uco.es (J.L. Luque-García).

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the low polar nature of the solvent in microwave-assisted heating of the solvent or sample-solvent partitioning of the analytes in PLE. The ideal approach would be one retaining the advantages of Soxhlet extraction (namely, sample-fresh solvent contact during the whole extraction step, no filtration step, simple manipulation) while circumventing its shortcomings by accelerating the process and minimizing environmental pollution.

With this aim, an ultrasound-assisted Soxhlet extractor has been designed and constructed. The device is based on the same principles as a conventional Soxhlet extractor but modified in order to allow location of the Soxhlet chamber in a thermostat bath through which ultrasounds are applied by means of an ultrasonic probe. The new device has been tested for the extraction of the total fat content from different oleaginous seeds such as sunflower, rape and soybean seeds. A quantitative comparison of the lipid extract obtained by both the official and the proposed method has been carried out. A qualitative comparison has also been performed by chromatographic analysis of the extracts.

2. Experimental section

2.1. Instruments and apparatus

The device used for the ultrasound-assisted Soxhlet extraction (UASE; shown in Fig. 1) consisted of a thermostat water-bath (6000383 P-Selecta, Ultraterm, Barcelona, Spain) modified by making an orifice at the bottom in order to enable connection of a conventional 50 ml Soxhlet chamber (Selecta, Barcelona, Spain) with a 100 ml distillation flask through a Teflon connector. An electrical isomantle with a rheostat (Prolabo, Paris, France) was used to heat the distillation flask. A Branson 450 sonifier (20 kHz, 100 W) equipped with a cylindrical titanium alloy probe (2.54 cm diameter) was immersed in the thermostated water-bath and used to accelerate the extraction process.

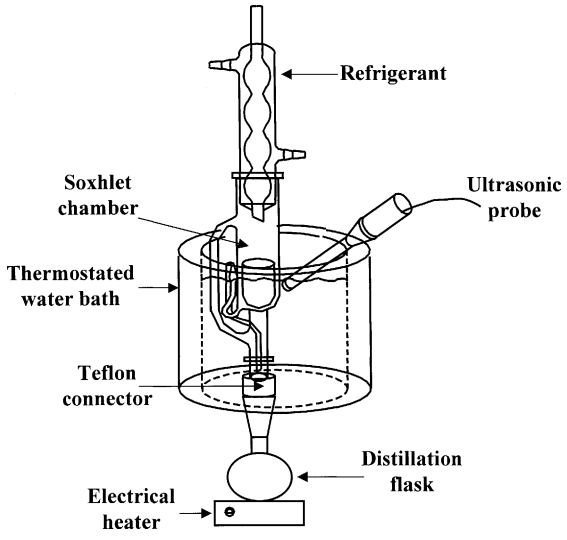

Fig. 1. Scheme of the proposed ultrasound-assisted extractor.

A rotary-evaporator (R-200, Büchi, Zurich, Switzerland) was used to release the solvent after each conventional Soxhlet extraction.

An electrically heated oven (Selecta, Barcelona, Spain), an analytical balance (Explorer Analytical Balance, Ohaus, USA) and a dessicator were used to determine the sample moisture as well as for the gravimetric determination of the extracted oil.

A gas chromatograph HP 5890 Serie II (Hewlett-Packard, Avondale, PA, USA) equipped with a BPX semipolar capillary column 30 m × 0.20 mm i.d. (Sugelabor, Madrid, Spain) and a flame ionization detector (FID) was used for the chromatography analysis of the extracts.

2.2. Samples and reagents

Extractions of the "total oil content" from sunflower, soybean and rape seeds available in the market were carried out. The seeds were milled in an electrical mill, sieved and classified into three different particle size groups: ≤2, ≤1 and ≤0.4 mm. The classified samples were stored at 4 °C until use. Sunflower seeds (≤1 mm) were used in the optimization of the ultrasound-assisted extraction, and the results obtained were applied to other samples.

Analytical grade and chromatographic grade *n*-hexane (Panreac, Barcelona, Spain) were used as extractant and to recompose the extracts for the chromatographic analysis, respectively.

2.3. Determination of the moisture and volatile matter content

Ten grams of the sample were placed on a dessicator tared capsule that was transferred into an electrically heated oven at 100 ± 2 °C for 2 h. After this, the capsule was removed from the oven and cooled to room temperature in a dessicator. After weighing, the procedure was repeated until the difference between two consecutive weights was smaller than 2 mg.

2.4. Conventional ISO extraction method

The conventional extraction procedure followed in this research was that of the International Organization for Standardization (ISO) which involves the gravimetric determination of the oil from the hexane extract (or light petroleum extract) from oilseeds [16]. The hexane extract is called "oil content".

Thus, 10 grams of sample were weighed to the nearest 1 mg (moisture and volatile content should be less than 10% (m/m)) and placed in a cellulose extraction cartridge. The cartridge was plugged with cotton wool and then placed in the Soxhlet chamber which was fitted to a pre-tared distillation flask containing 100 ml of *n*-hexane and 2–3 boiling

glass regulators. After extraction for 4 h, the cartridge was allowed to cool and unloaded in a mortar. An amount of 10 g of sea sand, previously washed with hydrochloric acid and calcined, was then added and the mixture was ground as finely as possible. The mixture was placed back into the cartridge and this into the Soxhlet chamber (that was fitted to a new pre-tared distillation flask) for back-extraction for a further 2 h period (this step was repeated until the amount weighed was smaller than 2 mg).

After each extraction step, the solvent was released in a rotary-evaporator and any traces remaining were removed by placing the flask with the extract in an oven at 85 °C for a preset interval, followed by cooling in a dessicator and weighing: the step was then repeated until the difference between two consecutive weights was smaller than 2 mg.

2.5. Ultrasound-assisted Soxhlet extraction procedure

Ten grams of sample were weighed to the nearest 1 mg and placed in a cellulose extraction cartridge which was then plugged with cotton wool. The cartridge was placed in the Soxhlet chamber and this was placed into a thermostated water-bath at 75 °C and fitted, through a Teflon connector, to a pre-tared distillation flask containing 100 ml of *n*-hexane and 2–3 boiling glass regulators. The sonicator probe was placed at 1 mm from the surface of the Soxhlet chamber with an inclination angle of 45° with respect to the vertical position and at 9 cm height from the bottom of the water-bath. The extraction program consisted of a number of cycles that depended on the extraction kinetics of the target sample. Each cycle involved three steps: (1) filling of the Soxhlet chamber by extractant evaporation from the distillation flask, condensation in the refrigerant, and dropping on the sample; (2) ultrasound irradiation of the cartridge for 10 s (duty cycle 0.5 s, output amplitude 40% of the nominal amplitude of the converter, applied power 100 W); (3) unloading of the Soxhlet chamber content after the extractant reached the siphon height.

After the last cycle, the solvent was transferred to a rotary-evaporator for removal of the solvent traces from the extracted oil. Then, gravimetric determination of the oil was performed as in the conventional procedure.

2.6. Chromatographic determination

Fatty acids were analyzed by gas chromatography after derivatization to fatty acid methyl esters (FAME) with 2 M KOH in methanol, according to the IUPAC standard method [17]. The temperature program used was as follows: 170 °C (5 min), 2 °C min^{-1} to 235 °C (5 min). Samples (0.5 μl) were introduced into the column via a split–splitless injector. The temperatures of the injector and flame ionization detector were 250 and 275 °C, respectively.

Table 1
Experimental values tested for the optimization of the UASE approach (optimal values in italics)

	T (°C)	NC	DC (s)	HP (cm)	IA (°)	IT (s)	*A* (%)
Plackett–Burman design							
Upper value	*75*	10	0.9	*9*	*45*	90	40
Lower value	55	5	0.5	5	0	30	10
Second factorial design							
Upper value	*75*	20	*0.5*	*9*	*45*	30	*40*
Lower value	*75*	10	0.1	*9*	*45*	*10*	80

T, temperature; NC, number of cycles; DC, duty cycle; HP, height of the probe; IA, inclination angle; IT, irradiation time; *A*, amplitude.

3. Results and discussion

3.1. Optimization of the ultrasound-assisted Soxhlet extraction (UASE)

The variables optimized in UASE (Table 1) were the ultrasound radiation amplitude (*A*), the percentage of duty cycle of ultrasonic exposure (DC), the temperature of the water-bath (*T*), the ultrasonic irradiation time (IT), the height of the probe, measured from the bottom of the water-bath (HP), the inclination angle of the probe with respect to the vertical position (IA) and the number of extraction cycles (NC). As seven was the number of variables to be optimized, a Plackett–Burman experimental design was selected for the screening of the main variables affecting the extraction process with a minimum number of experiments. This design involved 12 randomized runs plus three center points in order to evaluate the experimental error of the measurements [18]. The upper and lowest values given to each factor were selected from the available data and experience gathered in the preliminary experiments.

The pareto chart of Fig. 2a shows that after the analysis of this screening design, the number of cycles, the radiation amplitude, the duty cycle and the irradiation time were statistical significant variables for oil extraction, while the temperature of the water-bath, the height of the probe and the inclination angle were not significant within the range studied. The height of the probe and the inclination angle were fixed at the upper values tested (namely, 9 cm height and 45° inclination angle) as their effects on the extraction efficiency were positive (although they were not statistically significant variables). For the temperature of the water-bath, the upper value (75 °C) was also selected as an increase in the temperature of the water-bath produced acceleration of solvent evaporation from the distillation flask to the Soxhlet chamber.

The significant variables were studied more deeply by means of a half-fractioned 2^{4-1} factorial experimental design where higher values for the amplitude and the number of cycles as well as lower values for the irradiation time and the duty cycle were tested. The resulting pareto chart

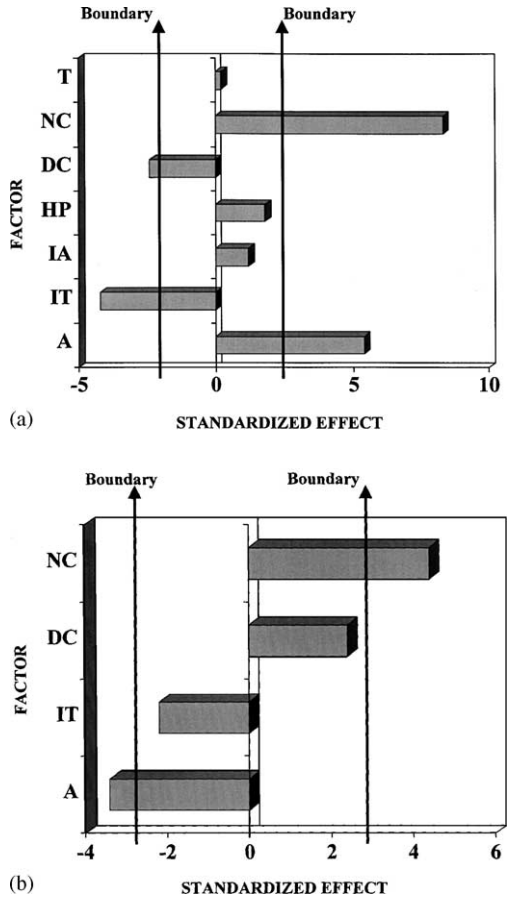

Fig. 2. Pareto chart for the standardized main effects in the experimental designs. The vertical line indicates the statistical significance bound for the effects. (a) Plackett–Burman design and (b) half-fractioned factorial design. *T*, temperature; NC, number of cycles; DC, duty cycle; HP, height of the probe; IA, inclination angle; IT, irradiation time; *A*, amplitude (for details see text).

(Fig. 2b) shows that the irradiation time and duty cycle were not significant so the lowest and highest value tested, respectively, were selected for further experiments as they provided better extraction efficiencies. The ultrasonic radiation amplitude was a significant variable with a negative effect. It means that lower values ought to be tested. However, as lower values were tested in the previous design and the efficiencies were worse, the lowest value tested in this second design (which corresponds to the highest value tested in the first one) was selected as optimum.

3.2. Extraction kinetics: application to different seed samples

In order to establish the number of cycles needed for obtaining quantitative oil extraction from the seeds, a study of the extraction kinetics was performed using sunflower seeds (particle size $\leq$1 mm). Rape and soybean seeds were also tested. The other variables were fixed at the optimum values previously found. After five cycles more than 75% of the total oil content was obtained independently of the type of seed. Twenty-five cycles were necessary for quantitative removal of the oil from sunflower seeds; meanwhile, just 20 cycles were necessary for both rape and soy seeds, which showed similar extraction kinetics.

3.3. Particle size: comparison of UASE, Soxhlet extraction, ISO method and focused microwave-assisted Soxhlet extraction

The efficiency of the extractions by UASE using different number of cycles, by conventional Soxhlet extraction (CSE) at different times, and by the ISO official method for sunflower seeds with different particle size are compared in Table 2.

It is apparent from the table that the seeds contain a readily extractable lipid fraction, around 70% of the total content. This fraction could be extracted by UASE in five cycles ($\approx$15 min), and by CSE in less than 1 h. The main problem in the extraction of seeds is, however, to remove the remaining lipids, i.e. those strongly bound to the matrix. The comparative effectiveness of UASE and CSE is demonstrated in the extraction of this fraction. The data of Table 2 show that the extraction yield of UASE is equal to or better than that of CSE, and with a drastic reduction of the extraction time. CSE requires at least twice as much time as UASE (when using particle size $\leq$0.4 mm). The most remarkable case was for particle size $\leq$2 mm. Thirty UASE cycles ($\approx$90 min) were necessary for obtaining efficiency $\geq$99% as compared with the results obtained by the ISO method; while, 12 h of conventional Soxhlet extraction were necessary to obtain similar efficiency.

Table 2
Fat recoveries using the proposed approach and conventional Soxhlet extraction (the values have been normalized against those obtained with the ISO procedure)

Particle size (mm)	CSE			UASE		
	2 h	6 h	12 h	20c[a]	25c	30c
$\leq$2.0	92 ± 0.3[b]	98 ± 0.1	99 ± 0.2	91 ± 0.4	97 ± 0.9	99 ± 0.2
$\leq$1.0	97 ± 0.3	99 ± 0.4	99 ± 0.2	88 ± 0.3	99 ± 0.5	100 ± 0.4
$\leq$0.4	99 ± 0.2	100 ± 0.3	100 ± 0.1	100 ± 0.6	100 ± 0.7	100 ± 0.3

[a] Number of extraction cycles.
[b] R.S.D. ($n = 5$).

Table 3
Chromatographic results of the fatty acid composition of samples extracted by UASE and ISO reference method

Fatty acid	Soybean 1	Soybean 2	Sunflower 1	Sunflower 2	Rape 1	Rape 2
Myristic	0.12 ± 0.03	0.11 ± 0.01	0.12 ± 0.05	0.12 ± 0.02	0.0 ± 0.0	0.0 ± 0.00
Palmitic	14.2 ± 0.7	11 ± 1	6.7 ± 0.5	6.8 ± 0.4	4.4 ± 0.6	4.5 ± 0.4
Palmitoleic	0.11 ± 0.01	0.11 ± 0.01	0.14 ± 0.03	0.13 ± 0.02	0.31 ± 0.04	0.29 ± 0.03
Margaric	0.14 ± 0.02	0.13 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.01	0.03 ± 0.01
Margaroleic	0.14 ± 0.02	0.14 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.02	0.10 ± 0.01
Stearic	4.7 ± 0.4	4.1 ± 0.3	3.6 ± 0.3	3.6 ± 0.5	2.3 ± 0.2	2.8 ± 0.2
Oleic	25.12 ± 0.09	24.45 ± 0.17	32.58 ± 1.15	32.58 ± 2.02	63.64 ± 3.30	66.01 ± 2.78
Linoleic	50 ± 2	52 ± 2	56 ± 3	56 ± 3	17 ± 1	16 ± 2
Linolenic	5.4 ± 0.4	7.0 ± 0.9	0.12 ± 0.02	0.12 ± 0.04	10.0 ± 0.9	8.2 ± 0.8
Araquic	0.41 ± 0.06	0.44 ± 0.10	0.34 ± 0.01	0.32 ± 0.04	0.74 ± 0.09	0.82 ± 0.01
Gadolenic	0.22 ± 0.05	0.21 ± 0.07	0.10 ± 0.02	0.12 ± 0.01	1.11 ± 0.05	1.14 ± 0.04

Results plus R.S.D. ($n = 5$) are expressed as % over the total weight. "1" indicates samples extracted by UASE and "2" samples extracted by the ISO reference method.

The results provided by the new approach have also been compared with those obtained by using focused microwave-assisted Soxhlet extraction (FMASE) [13]. The results provided by UASE are similar to those obtained by FMASE but with a shorter number of cycles. Thus, the total extraction time was much shorter in the case of UASE. Between 20 and 30 cycles were needed in UASE for quantitative removal (>99% as compared with the ISO reference method) depending on the particle size; while between 50 and 65 were needed in FMASE.

3.4. Evaluation of the precision of the proposed method

In order to evaluate, not only the extraction efficacy of the proposed method but also the precision, within-laboratory reproducibility and repeatability were calculated in a single experimental set-up with duplicates [19]. The experiments were carried out using 10 g of sunflower seeds ($\leq$1 mm particle size) (as in the optimization procedure). In all the experiments the optimal values obtained for the variables were used. Two extractions and measurements of the target compounds per day were carried out on 7 days. The repeatability, expressed as percent relative standard deviation, was 0.9%; meanwhile the within-laboratory reproducibility was 1.3%. These results show that the proposed approach is also comparable in terms of precision with the ISO method.

3.5. Study of the influence of ultrasound on extract composition

In order to evaluate if the oil composition was affected during ultrasound-assisted extraction, GC analyses of the extracts were carried out for both the ISO reference method and UASE using the three types of seeds under study. Table 3 shows that there were no appreciable differences in the extracts obtained by both procedures, which shows that the oil composition is not affected by the use of ultrasound.

4. Conclusions

An approach based on Soxhlet extraction assisted by ultrasounds has been developed. The new device has been tested for the extraction of the total fat content from oleaginous seeds such as sunflower, rape and soybean seeds. Efficiencies similar or even better than those provided by both conventional Soxhlet extraction and the official ISO method have been achieved saving both, time and sample manipulation. The composition of the fat extracts did not change after application of ultrasounds, and the precision of the proposed approach was similar to that obtained by the ISO reference method. Thus, it can be concluded that the present approach constitutes a valuable alternative for the extraction of easily compactable matrices such as seed samples.

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