

Sono–Soxhlet: In Situ Ultrasound-Assisted Extraction of Food Products

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Abstract An improvement of Soxhlet extraction was designed and developed. The extraction process (Sono–Soxhlet) of oils from crushed dried olives was performed using an ultrasonic horn in situ to provided rapid and complete recovery of analytes from the matrix. The results were compared to conventional Soxhlet extraction using physico-chemical characterization and gravimetric analysis and showed a substantial reduction in the extraction time without interfering on composition and quality of target extracts.

Keywords Soxhlet · Extraction · *N*-hexane · Oil · Ultrasound

Introduction

The usual analytical method of oils and fats recovery from solid samples is the Soxhlet system, which has become the reference technique for a whole range of compounds in solid–liquid extractions (ISO 1988). This method involves repeated percolation of fresh solvent through a solid matrix, which presents a number of advantages over simple solvent extraction such as smaller quantities of solvent used,

reduced isolation time together with ease of handling and better reproducibility. The solid phase is leached repeatedly by a fresh volume of solvent generated from condensed vapors until it reaches a certain level, which triggers the return of the solvent to the reservoir (Fig. 1c, conventional Soxhlet). Despite the acknowledged efficiency of this process, Soxhlet extraction does have certain shortcomings in terms of the length of time required for a complete extraction and an eventual decomposition of some recovered analytes in the boiling solvent reservoir. Over the past few years, some improvements of the orthodox technique have been suggested by either reducing the extraction time, the amount of solvent employed and/or the energy consumption (Luque de Castro and Priego-Capote 2010). In this scenario, Garcia-Ayuso and Luque de Castro (2001), Luque-García and Luque de Castro (2004), and Virost et al. (2007) suggested microwave-assisted extraction of oils whereas Chemat et al. (Virost et al. 2008) have proposed the replacement of hexane by limonene.

The first Soxhlet extraction assisted by ultrasound, named ultrasound-assisted Soxhlet was developed in 2004 by Luque-García & Luque de Castro (2004). The system comprises an ultrasonic probe immersed on a water bath that circulates around the Soxhlet chamber (Fig. 1b). This innovative use of ultrasound in the Soxhlet process leads to a reduced extraction time without degradation of the extracts. This process is analogous to the use of ultrasonics in chemical reactions performed in a vessel containing the reaction system immersed in the ultrasonic bath, i.e., the application of ultrasound is indirect and the energy must be transferred from the water bath through the glassware and then to the solvent surrounding the cellulose cartridge on the Soxhlet chamber.

The present work proposes a modification of a Soxhlet system by inserting an ultrasonic probe directly into the extraction chamber, i.e., ultrasound is applied directly in the extraction reactor. The lipid extracts obtained from the

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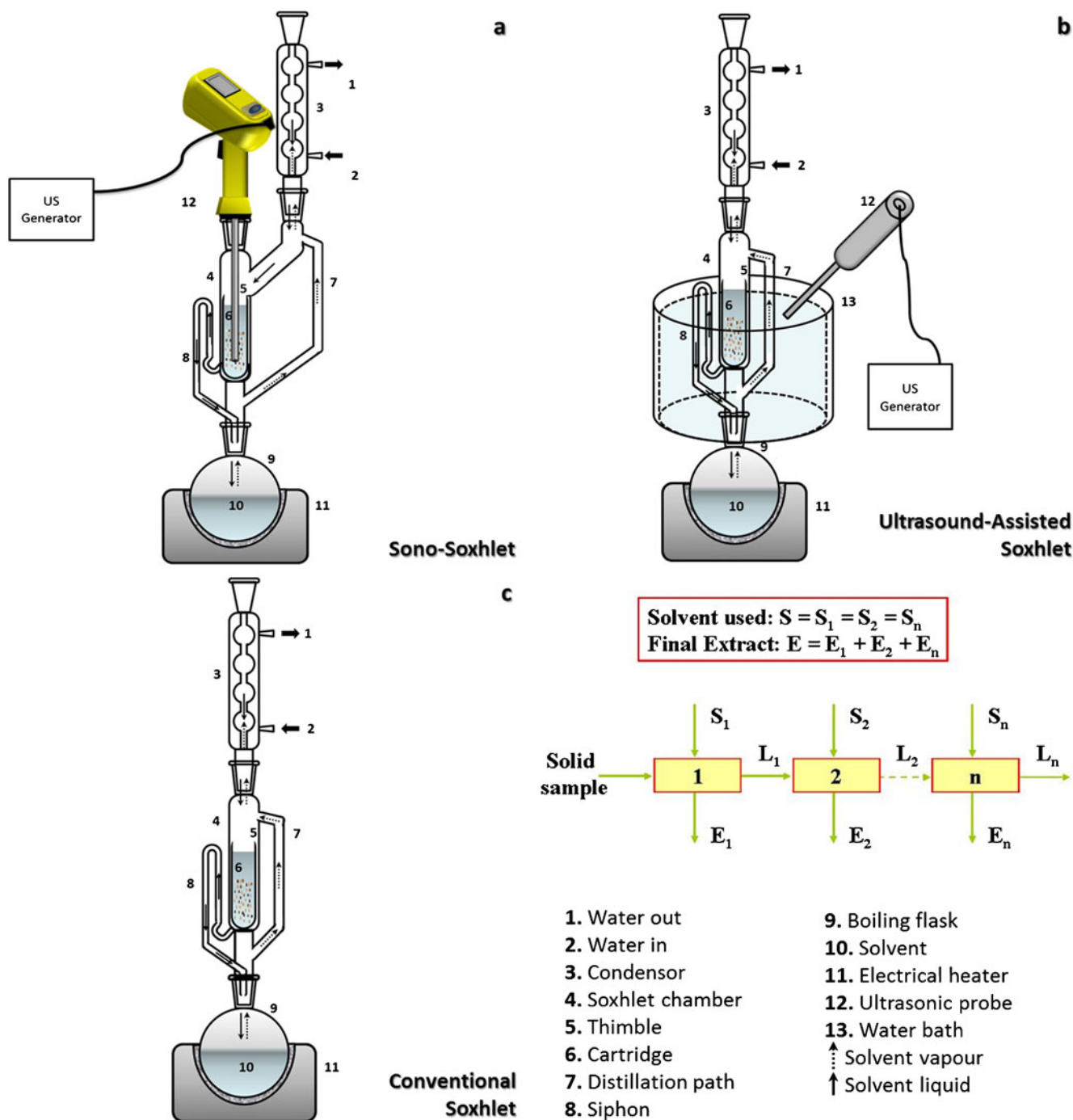


Fig. 1 Schematic Soxhlet apparatuses and extraction (a proposed Sono–Soxhlet, b ultrasound-assisted Soxhlet, c conventional Soxhlet)

Sono–Soxhlet process were analyzed and compared to those obtained by the conventional Soxhlet procedure using gravimetric determination followed by an identification and quantification of fatty acids contained in extracted oils using gas chromatography. Other physicochemical parameters such as free acidity, peroxide value, and conjugated dienes were also measured and compared for both methods.

Materials and Methods

Reagents and Instruments

VWR International (Darmstadt, Germany) provided HPLC grade *n*-hexane, analytical-grade sodium chloride, sodium hydroxide, and BF_3 -methanol reagent (20 % solution in methanol for synthesis). The ultrasonic titanium probe system

(UP200Ht working at 26 kHz and 200 W) was supplied by Hielscher (Germany).

Conventional Extraction

Conventional Soxhlet extractions were performed according to the official ISO procedure (ISO 1988) as follows. Thirty grams of dried and ground sample (weighed to the nearest 10 mg) were placed inside a cellulose thimble, which was loaded into the chamber of the Soxhlet system. A distillation flask containing 300 mL of *n*-hexane and a condenser were then fitted to the Soxhlet apparatus. Extractions under reflux were performed over 8 h, noticing a solvent recycling period of approximately 7 min in the extractor. At the end of the process, the residue in the extraction cartridge was cooled down to room temperature in a desiccator and weighed. In addition, the content of the distillation flask was concentrated to dryness with a vacuum rotary evaporator, cooled to room temperature in a desiccator, then weighed and analyzed.

Sono–Soxhlet Extractions

Sono–Soxhlet extractions were performed using 30 g of dried and ground samples weighed to the nearest 10 mg. The sample was introduced into a cellulose thimble that was loaded into the chamber of a Soxhlet system. The opening reserved for the condenser was used to introduce the ultrasonic probe and another side arm was designed in order to attach the condenser and allow solvent reflux. The ultrasonic device used was the Hielscher UP200Ht ultrasonic processor operating at 26 kHz, 200 W, 70 μm amplitude, and 1.3 W cm^{-2} intensity. A distillation flask containing 300 mL of *n*-hexane was then fitted to the Soxhlet apparatus (Fig. 1a). Some other modifications were made on the apparatus such as the placement of the condenser and the siphon position to preserve a minimum amount of solvent in the Soxhlet chamber for the perfect functioning of the probe. The Sono–Soxhlet extractions were carried out over 30 min with four reflux cycles. In the end, the cartridge residue was collected and cooled down to room temperature in a desiccator then weighed. In parallel, the content of the distillation flask was concentrated to dryness with a vacuum rotary evaporator and then cooled to room temperature in a desiccator before being weighed and analyzed.

Physicochemical Parameters and Gas Chromatography

All the analyzed physicochemical parameters of various extracted oils were carried out according to the AOCS Official method (AOCS 2009). Conjugated dienes were assessed by UV spectrophotometric method (AOCS Official Method Cd 7–58), free fatty acids (AOCS Official Method Ca 5a–40), peroxide value (AOCS Official Method Cd 8–53), polar compounds (AOCS Official Method Cd 20–91),

and preparation and identification of fatty acid methyl esters derivatives (FAMES, AOCS Official Methods Ce 1–62 and Ce 2–66) were performed. For gravimetric determination, the crude oil extract yield of each procedure was expressed as a percentage of the weight of extracted oil relative to the weight of the dry sample before extraction. All experiments were carried out in triplicate and results are expressed as mean value \pm standard deviation.

Gas chromatography (GC) analysis was performed using a Hewlett-Packard 6890 N gas chromatograph equipped with a flame ionization detector. Samples were analyzed on a BPX70 capillary column (50 m \times 0.32 mm \times 0.25 μm film thickness). The oven temperature was programmed to increase from 120 to 200 $^{\circ}\text{C}$ at a rate of 3 $^{\circ}\text{C}/\text{min}$ and was then held at 200 $^{\circ}\text{C}$ for 10 min. Each oil sample was derivatized in order to transform fatty acids into their corresponding FAME, then analyzed in duplicates by GC/mass spectrometry (MS).

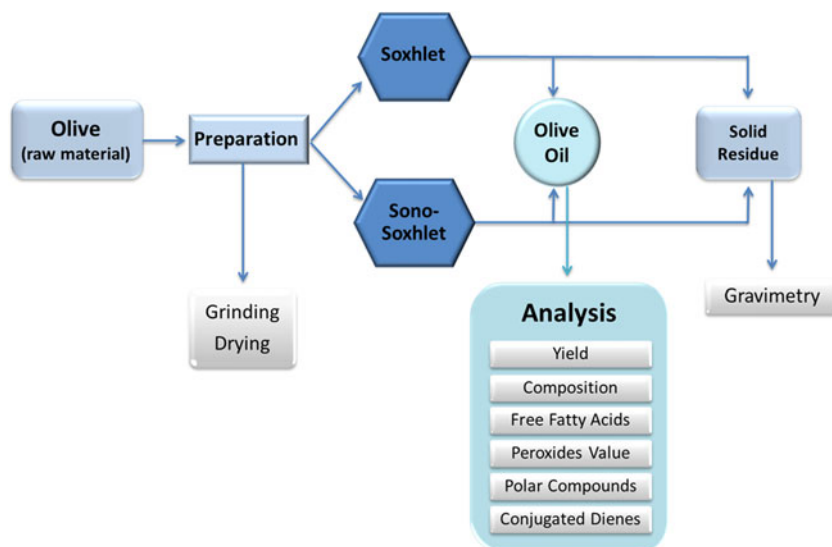
GC/MS analysis was carried out using an Agilent 6890 N coupled to an Agilent 5973 MS. Samples were analyzed on a BPX70 capillary column (50 m \times 0.32 mm \times 0.25 μm film thickness). The oven temperature was programmed to increase from 120 to 200 $^{\circ}\text{C}$ at a rate of 3 $^{\circ}\text{C}/\text{min}$ and was then held at 200 $^{\circ}\text{C}$ for 10 min. Again, each oil sample was derivatized in order to transform fatty acids into their corresponding FAME, then analyzed in duplicates by GC/MS.

Results and Discussion

The extracted oils obtained using both methods were compared (as seen in Fig. 2) using gravimetric and physicochemical analysis; the results are reported in Table 1. For gravimetric results, the data does not show significant differences in final yields between conventional Soxhlet extraction and Sono–Soxhlet extraction (SSE), each representing around 40 % of oil. However, the use of ultrasound reduced the time required to achieve this yield by a factor of 16. These results indicate clearly that the Sono–Soxhlet extraction technique allows a rapid and an accurate determination of the lipid content in a given matrix.

Fatty acids composition, also reported on Table 1, indicates that the main fatty acids present in both samples are palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids and do account for more than 90 % of total fatty acids present in oil. Other compounds such as palmitoleic (C16:1), stearic (C18:0), linolenic (C18:3), and arachidic (C20:0) acids were also identified in smaller proportions. It is interesting to notice here that no significant differences in extract composition were observed between conventional and ultrasonic methods in terms of saturated fatty acids, mono unsaturated fatty acids, and polyunsaturated fatty acids.

In addition, parameters normally used for the evaluation of oil deterioration such as peroxide values, free fatty acids,

Fig. 2 Schematic extraction and analysis steps

conjugated dienes, and polar compounds were investigated (Table 1). The results show similar data for both oil extracts. This is an important finding since some researchers advocated an eventual degradation when sonication is applied given the extreme conditions developed during cavitation collapse (Suslick et al. 2001). However, the literature reports notable background information suggesting that ultrasound is highly

efficient during extraction of biological important plant materials (Vinatoru 2001; Mason et al. 2011). After a sensory appreciation, it was observed that the oil extracted using ultrasound presented a slight rancid/metallic odor. Since the extractions were performed under *n*-hexane boiling point temperature (69 °C) and only a slight difference in cartridge temperature was observed between conventional and Sono–

Table 1 Comparison between Soxhlet extraction and Sono–Soxhlet Extraction for three different olive samples

No.	Fatty Acids	Table olives					
		Green stage		Cherry stage		Black stage	
		SE ^a	SSE ^b	SE ^a	SSE ^b	SE ^a	SSE ^b
1	C16:0	14.2	14.2	13.9	13.9	13.7	13.6
2	C16:1	0.2	0.2	0.2	0.2	0.3	0.3
3	C18:0	3.5	3.4	3.4	3.5	3.2	3.3
4	C18:1	63.2	63.3	62.1	62.2	61.5	61.6
5	C18:2	14.5	14.4	15.6	15.5	15.8	15.7
6	C18:3	2.1	2	2.2	2.2	2.3	2.3
7	C20:0	0.1	0.1	0.1	0.1	0.1	0.1
8	Others	2.2	2.1	2.5	2.4	3.1	3.1
	ΣSFAs	17.8	17.7	17.4	17.5	17.0	17.0
	ΣMUFAs	63.4	63.5	62.3	62.4	61.8	61.9
	ΣPUFAs	16.6	16.4	17.8	17.7	18.1	18.0
	FFA c as oleic acid (%)	0.3	0.3	0.4	0.4	0.5	0.5
	Conjugated dienes (%)	0.65	0.67	0.68	0.69	0.70	0.71
	Peroxides value (meq O ₂ /kg oil)	5.6	5.8	6.1	6.3	6.3	6.6
	Polar compounds (%)	10.2	10.4	10.0	10.5	9.8	9.9
	Lipid yield (% of dry weight)	40.5±0.2	40.6±0.1	40.2±0.2	40.3±0.2	39.9±0.1	39.8±0.2

SFA saturated fatty acid, MUFA mono unsaturated fatty acids, and PUFA polyunsaturated fatty acids

^a Soxhlet extraction

^b Sono–Soxhlet extractions

^c Free fatty acids

Soxhlet extractions (30 and 35 °C, respectively), the slight temperature rise in this case did not induce oil degradation over the short time period of SSE. The most reported effect of ultrasound in oils is the appearance of off-flavor, which have a small odor/flavor threshold (from 0.01 to 33 µg/g depending on the oil composition; Kamal-Eldin 2003) and even though some of these degradation products can be detected on sonicated lipid products, their presence do not imply a direct relation to decrease in oil quality (Pingret et al. 2012; Chemat et al. 2004), which can be observed by the other quality parameters (Table 1). From these observations, we can advocate that ultrasound has contributed to the acceleration of extraction process and has no direct effect on oil quality.

The present work evaluated the impact of direct application of ultrasound during lipid extraction from olives, providing improvements in time over conventional Soxhlet technique. The results show Sono-Soxhlet Extraction can be considered a novel technique, offering a rapid method for the determination of lipid content with recoveries and chemical compositions similar to those obtained by conventional Soxhlet methods and no significant differences in terms of quality indicators are detected.

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