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Kinetic improvement of olive leaves' bioactive compounds extraction by using power ultrasound in a wide temperature range

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ABSTRACT

In this study, the effect of temperature and ultrasonic application on extraction kinetics of polyphenols from dried olive leaf was investigated. Conventional (CVE) and ultrasonic-assisted extraction (UAE) were performed at 10, 20, 30, 50 and 70 °C using water as solvent. Extracts were characterized by measuring the total phenolic content, the antioxidant capacity and the oleuropein content (HPLC–DAD/MS–MS). Moreover, Naik's model was used to mathematically describe the extraction kinetics. The experimental results showed that phenolic extraction was faster in UAE (ultrasonic-assisted extraction) than in CVE (conventional extraction), being extraction kinetics satisfactorily described using Naik model (include VAR > 98%). Besides, the total phenolic content, the antioxidant capacity and the oleuropein content were significantly ($p < 0.05$) improved by increasing the temperature in both CVE and UAE. Oleuropein content reached 6.57 ± 0.18 being extracted approximately 88% in the first minute for UAE experiments.

1. Introduction

The olive tree (*Olea europaea*) is widely distributed in the Mediterranean basin in which it has provided relevant economic and health-related benefits. Olive leaves, which represent during the harvest around 10% of the total weight of olives, have shown higher antioxidant and bioactive potential than other parts of the tree [1,2]. Oleuropein, in particular, the major phenol in olive leaves, presents antiviral properties, protects enzymes and hypertensive cell death in cancer patients, prevents cardiac diseases and improves the lipid metabolism to limit obesity problems [1]. Thereby, it could be interesting to use olive leaves to obtain high-added-value compounds with high antioxidant power [3].

Conventional extraction by maceration has been traditionally used to extract phenolic compounds from olive leaves. Nonetheless, the main disadvantages of this process include the low extraction efficiency, and the longtime of solid–solvent contact to reach equilibrium [4]. As a consequence, in order to solve these limitations, new techniques have been developed in recent years for the extraction of bioactive compounds from plant materials, including ultrasound-assisted, microwave-assisted and supercritical fluid extraction [5].

Ultrasonic-assisted extraction (UAE) has a reasonably low cost and its operation is easy [5]. High intensity ultrasonic application induces the growth of bubbles inside liquids causing the occurrence of the cavitation phenomenon [6]. The cavitation provokes stirring and thermal effects in the extraction solvent, as well as structural changes in the solid sample [7]. Thus, it affects the cellular structures provoking their disruption which enhances mass transport processes [8]. Besides, high temperatures might increase the diffusion and the solubility of polyphenols resulting, therefore, in the increase of their extraction rate [9]. However, temperature could affect the olive leaf extract composition linked to degradation of thermolabile compounds. As seen from literature, most of the work in relation with the ultrasound assisted extraction has been performed by using ultrasonic probe [10,11] and ultrasonic bath [3,9,12]. Ultrasonic baths are more widely used, however, ultrasonic probes have an advantage of focusing their energy on a localized zone providing therefore more efficient cavitation in the solution [13].

Most of the previous works addressing the use of ultrasound to improve extraction focus on its use as analytical tool to exhaust the raw material [14,15,12]. Moreover, ultrasound has also been tested to speed up the extraction process under different process

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parameters [1,3,9,11], which is highly relevant for industrial purposes. Japón-Luján et al. [12] investigated the ultrasonic assisted extraction of oleuropein and related biophenols from olive leaves. They found that the ultrasound-assisted extraction was faster and more efficient than maceration/stirring. Şahin and Şamli [9] studied the optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Bilgin and Şahin [3] also investigated the effects of geographical origin and extraction methods on total phenolic yield of olive tree (*Olea europaea*) leaves. They observed that the amounts of extract and total polyphenols were higher in olive leaves through homogeniser-assisted extraction compared with ultrasonic assisted extraction. Ahmad-Qasem et al. [10] studied the extraction kinetics of phenolic compounds by measuring the total phenolics content and antioxidant capacity optimizing the ultrasonic performance by modifying the process parameters. However, the extraction kinetics of individual polyphenols with relevant bioactive properties, such as oleuropein, from olive leaves has not been reported yet. In addition, as far as we are concerned, there is no previous work addressing the use of temperatures below standard room conditions for ultrasonic-assisted extraction from olive leaves. Therefore, the aim of this work is to assess the influence of temperature on extraction kinetics of phenolic compounds from olive leaves using conventional and ultrasound assisted extraction.

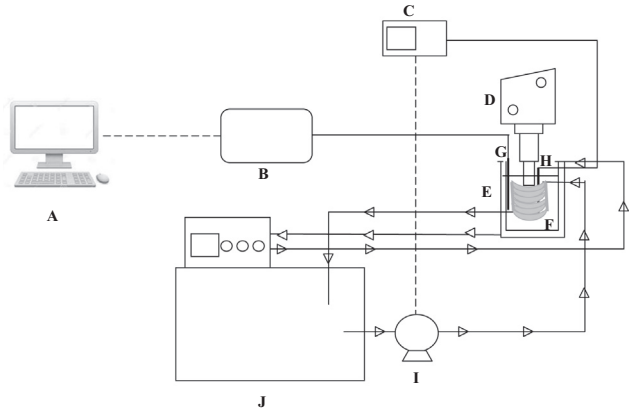

Fig. 1. Experimental set-up for ultrasonic assisted extraction. A: computer; B: Switch unit; C: process controller; D: ultrasonic probe system; E: jacketed extraction vessel; F: copper column; G: thermocouple; H: Pt100 sensor; I: peristaltic pump; J: refrigerated Circ.

2. Materials and methods

2.1. Raw material

Olive leaves (*O. europaea*, cultivar Chemlali) were collected from Sfax (Tunisia) in July, 2014, dried in a tunnel microwave dryer (Adasen, JN-100, China) for 10 min (1200 W, 70 °C), then milled and stored at 4 °C until being analyzed.

2.2. Extraction experiments

2.2.1. Ultrasonic assisted (UAE) and conventional (CVE) extraction

Fig. 1 illustrates the experimental set-up used to perform the ultrasonic assisted extraction. UAE experiments were conducted using an ultrasonic probe system (UP400S, Dr. Hielscher, Teltow, Germany). The ultrasonic emitter, with a diameter of 2.2 cm, was placed in a jacketed vessel and immersed1 cm into the solvent. Extraction temperature was controlled by re-circulating an ethylene glycol solution (40%) through a cooling coil made with a copper tube (4 mm diameter), which was immersed into the solvent, and also through the jacket of the vessel. In order to re-circulate the ethylene glycol solution, a peristaltic pump (302 S, Watson-Marlow, Postfach, Germany) was connected to a cooling reservoir (refrigerated Circ, Model 1190S, USA) and to a process controller (E5CK, Omron, Hoofddorp, Netherlands) to perform an ON-OFF type control. The temperature of the solvent was monitored using a Pt100 sensor, which was also wired to the controller.

Extraction experiments were carried out using distilled water as solvent. A ratio of 2.5% (w/v, weight of dry leaves/volume of water) and a total volume of 400 mL were used for each experiment. Different temperatures were tested (10, 20, 30, 50 and 70 °C) supplying, in every case, 100% of the total power of the system (400 W) as suggested by Ahmad-Qasem et al. [10] in order to speed-up the extraction rate. Extraction time was fixed at 10 min, taking

Table 1
Identified parameters of Naik's model for total phenolic content of olive leaves extracts.

Temperature (°C)	Total phenolic content							
	CVE				UAE			
	Y_∞ (g OE/100 g dm)	R_0 (g OE/100 g min)	*VAR* (%)	*MRE* (%)	Y_∞ (g OE/100 g dm)	R_0 (g OE/100 g min)	*VAR* (%)	*MRE* (%)
10 °C	7.89	6.68	98.26	4.41	9.38	5.66	98.10	5.15
20 °C	8.64	10.18	98.48	3.86	9.69	10.45	99.03	3.13
30 °C	9.56	14.14	99.24	2.73	9.89	21.72	99.35	2.33
50 °C	11.21	21.32	99.08	2.67	10.90	44.20	99.74	1.45
70 °C	12.37	33.85	99.41	2.30	11.81	76.03	99.69	1.45

Table 2
Identified parameters of Naik's model for antioxidant capacity kinetics from olive leaves.

Temperature (°C)	Antioxidant capacity (FRAP)							
	CVE				UAE			
	Y_∞ (mmolTrolox/100 g dm)	R_0 (mmolTrolox/100 g min)	*VAR* (%)	*MRE* (%)	Y_∞ (mmol Trolox/100 g dm)	R_0 (mmol Trolox/100 g min)	*VAR* (%)	*MRE* (%)
10 °C	36.92	13.58	94.56	9.85	34.98	23.33	96.00	8.48
20 °C	35.77	35.49	98.28	4.13	39.34	38.85	99.19	2.89
30 °C	39.66	57.81	97.42	4.43	41.89	90.55	98.44	3.71
50 °C	50.44	47.35	98.22	4.18	44.27	180.39	98.49	3.63
70 °C	53.36	91.12	98.04	3.99	47.40	332.03	98.77	3.04

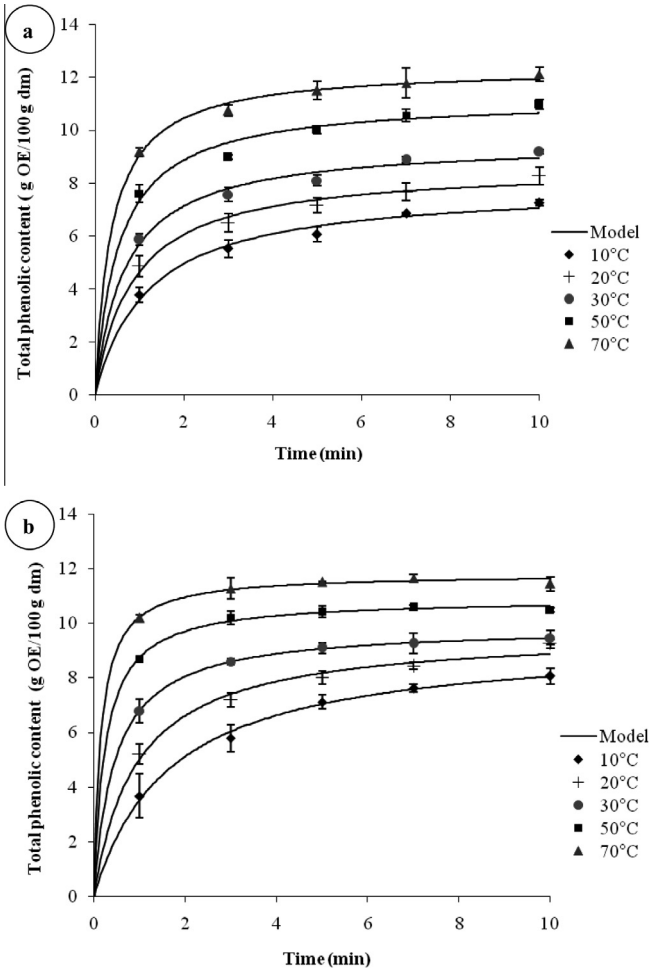

Fig. 2. Influence of extraction temperature on total phenolic content of olive leaves extracts obtained by CVE (a) and UAE (b).

samples (2.5 mL) at preset times (1, 3, 5, 7 and 10 min) to determine the extraction kinetics. Once the sample was taken, the same volume of water was introduced to keep constant the ratio dry leaves/volume of solvent. 2.5 mL of each extract were filtered (0.45 μm) prior to analytical determinations and extraction experiments were performed in triplicate.

CVE experiments were carried out in the same experimental conditions but replacing the ultrasonic tip by a heating magnetic stirrer (F 20520162, VELP Scientifica, Europe) at 1200 rpm.

2.3. Characterization of the ultrasonic field

The calorimetric method was used to estimate the actual ultrasonic power released into the medium. To achieve this purpose, the temperature of the solvent was measured every second for the first 3 min of the ultrasonic application without sample and without controlling the temperature [16]. Then, the ultrasonic power applied (P [W]) was calculated using the temperature rise, as expressed in Eq. (1):

$$P = m \cdot Cp \cdot dT/dt \quad (1)$$

where m (kg) is the solvent mass, Cp(J/kg °C) the specific heat capacity of the solvent (water, 4200 J/kg °C) and dT/dt, the slope of the logged temperature–time curve.

The ultrasonic power was measured, at least in triplicate using two type-K thermocouples wired to a data logger.

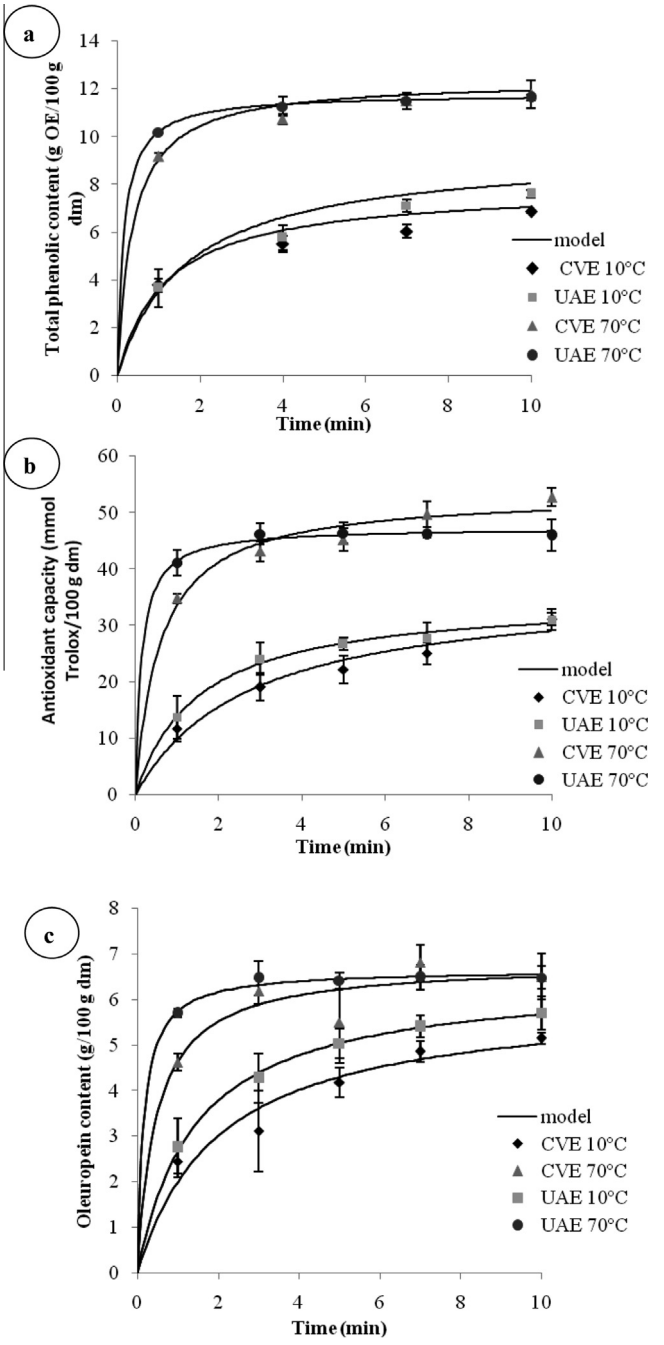

Fig. 3. Total phenolic content (a), antioxidant capacity (b) and oleuropein content (c) comparison between CVE and UAE performed at 10 °C and 70 °C.

2.4. Total phenolic content (TPC)

The TPC[1] was determined by the Folin–Ciocalteu method according to the procedure described by Singleton, Orthofer, & Lamuela-Raventós [17]. Briefly, 100 μL of sample was mixed with 500 μL of Folin–Ciocalteu's phenol reagent. After 1 min, 1 mL of Na_2CO_3 solution (20%, w/v) was added and the mixture was adjusted to 10 mL with distilled water. The reaction was kept in dark for 30 min and the total phenolic content was determined by measuring the absorbance at 765 nm using a spectrophotometer (Helios Gamma, ThermoSpectronic, Cambridge, UK). A standard curve of oleuropein

[1] Total phenolic content.

(Extrasynthese, GenayCedex, France) was previously prepared using solutions of a known concentration in water. Results were expressed in terms of oleuropein equivalent (g OE/100 g of dry matter (dm) of olive leaves).

2.5. Ferric-reducing ability power (FRAP)

The FRAP method was performed according to the procedure described by Benzie & Strain [18] modified by Ahmad-Qasem et al. [10]. Antioxidant capacity (AC) was evaluated through a calibration curve that had been previously determined using the extracted solvent (water) of a known Trolox (Sigma-Aldrich, Madrid, Spain) concentration. Results were expressed in terms of Trolox equivalent (mmol Trolox/100 g dm).

2.6. Identification and quantification of polyphenols by HPLC-DAD/MS–MS

An HPLC instrument (Agilent LC 1100 series; Agilent Technologies, Inc., Palo Alto, CA, USA) controlled by the Chemstation software was used to identify and quantify the main polyphenols present in the UAE and CVE extracts. The HPLC instrument was coupled to an Esquire 3000+ (Bruker Daltonics, GmbH, Bremen, Germany) mass spectrometer equipped with an ESI source and ion-trap mass analyzer, and controlled by Esquire control and data analysis software. A Merck Lichrospher 100RP-18 (5 μm, 250 × 4 mm) column was used for analytical purposes.

Separation was performed through a linear gradient method using 2.5% acetic acid (A) and acetonitrile (B), starting the sequence with 10% B and programming gradient to obtain 20% B at 10 min, 40% B at 35 min, 100% B at 4 min, 100% B at 45 min, 10% B at 46 min and 10% B at 50 min. In order to ensure that the LC–MS pump performed accurately, 10% of organic solvent was premixed in the water phase. The flow-rate was 1 mL/min and the chromatograms were monitored at 240, 280 and 330 nm. The mass spectrometry operating conditions were optimized in order to achieve maximum sensitivity values. The ESI source was operated in negative mode to generate [M−H]− ions under the following conditions: a desolvation temperature of 365 °C and a vaporizer temperature of 400 °C; dry gas (nitrogen) and nebulizer were set at 12 L/min and 70 psi, respectively. The MS data were acquired as full scan mass spectra at 50–1100 m/z by using 200 ms for the collection of the ions in the trap. The main compounds were identified by means of an HPLC–DAD analysis, comparing the retention time, UV spectra and MS/MS data of the peaks in the samples with those of authentic standards or data reported in the literature. Only the main olive leaf polyphenol, oleuropein, was quantified using an external standard (Extrasynthese, GenayCedex, France).

2.7. Modeling of extraction kinetics and statistical analysis

In order to assess the influence of temperature and ultrasonic application, the kinetics of TPC, AC[2] and oleuropein content of extracts were determined during the extraction and modeled using the Naik model [19].

$$Y = Y\infty \cdot t/B + t \quad (2)$$

where Y represents the measured variable (TPC, AC or oleuropein content), t (min) the extraction time, $Y\infty$ the value of the measured variable in the equilibrium, and B (min) the extraction time needed to reach half of $Y\infty$. The model parameters ($Y\infty$ and B) were identified using the Excel™ Solver tool (Microsoft Corporation, Seattle, WA, USA) by minimizing the sum of the squared differences between the experimental and calculated Y. The explained variance (VAR) and the mean relative error (MRE) were calculated to determine the goodness of the model fit to the experimental data:

$$VAR(\%) = (1 - S^2xy/S^2y) \cdot 100 \quad (3)$$

$$MRE(\%) = \left(\frac{100}{n}\right)\sum_{i=1}^{n}(|Yi, exp - Yi, cal|/Yi, exp) \quad (4)$$

where S^2xy is the variance of the estimation and S^2y the variance of the sample, n is the number of experimental data, Yi, exp and Yi, cal refer to experimental and calculated values of experiment i.

Analyses of variance (ANOVA) were carried out using the software SPSS statistics 17. Significant differences ($p < 0.05$) were identified using Duncan's multiple range tests.

3. Results and discussion

3.1. Characterization of the ultrasonic fields

The actual ultrasonic power released into the medium during the ultrasonic application was determined by using a calorimetric method, as explained in Section 2.3. Thus, when supplying 100% of electric power to the transducer (400 W), an effective ultrasonic

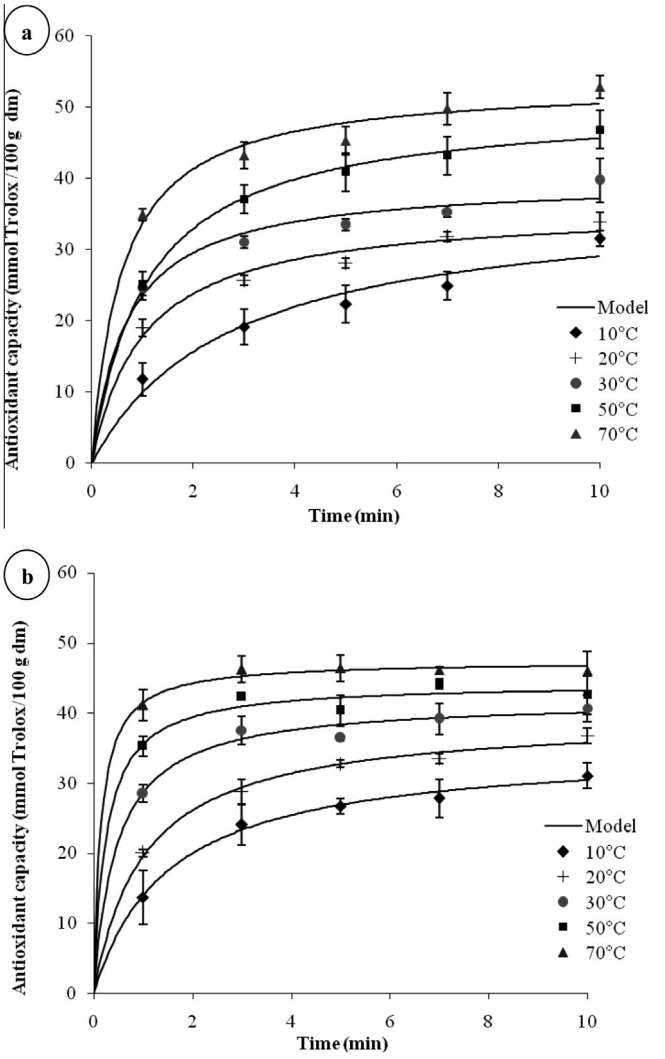

Fig. 4. Influence of extraction temperature on antioxidant capacity (FRAP) of olive leaves extracts obtained by CVE (a) and UAE (b).

[2] Antioxidant capacity.

power of 109.5 ± 1.7 W was introduced into the medium. Consequently, the electric/ultrasonic power yield was of about 27%. It should be emphasized that the calorimetric method computes only the acoustic energy converted into heat, while other mechanical phenomena, such as stirring, oscillating velocities and pressure variations, are not computed. Therefore, the actual electric/ultrasonic power yield should be higher than 27%.

3.2. Evolution of total phenolic content and antioxidant capacity during extraction

TPC and AC kinetics obtained at different temperatures by CVE and UAE are presented in Figs. 2 and 4, respectively. Additionally, the identified kinetic parameters from Naik model are shown in Tables 1 and 2, respectively for TPC and AC. As shown in Table 1 and 2, the explained variance (*VAR*) exceeded 94% and the mean relative error (*MRE*) was lower than 10%. These results indicated that the model fitted satisfactorily to the experimental data. The satisfactory fit of the model to the experimental data is also illustrated in Figs. 2–4, respectively, where experimental values are compared to calculated ones.

The kinetic extraction curves were typically comprised of a fast extraction step (washing stage) and a slow extraction step (diffusion stage) as shown in Figs. 2 and 4. Regardless of the extraction method and temperature, almost 80% of polyphenols and antioxidant compounds (Figs. 2 and 4) were extracted in only 3 min. Afterwards, the extraction rate decreased and it approached a constant value close to its equilibrium (Y_∞). Therefore, the extraction process was mainly controlled by the washing step. The characteristics of washing and diffusion steps in the extraction can be determined by the proportion of broken and intact cells after sample preparation [7]. In fact, the grinding of leaves sample to small particle size improved the washing step of the extraction and made the diffusion phase almost negligible.

Experimental results highlighted that the temperature affected extraction kinetics. As shown in Table 1 and Fig. 2, the kinetic of TPC was faster when the temperature increased from 10 to 70 °C. After 1 min of extraction, TPC obtained at 70 °C was almost 2.5 times higher than that obtained at 10 °C. It was noticeable that the influence of temperature on R_0 was more intense in the case of AC (Table 2) than in TPC (Table 1). Thus, varying the extraction temperature from 10 to 70 °C, R_0 increased from 13.60–91.12 mmol

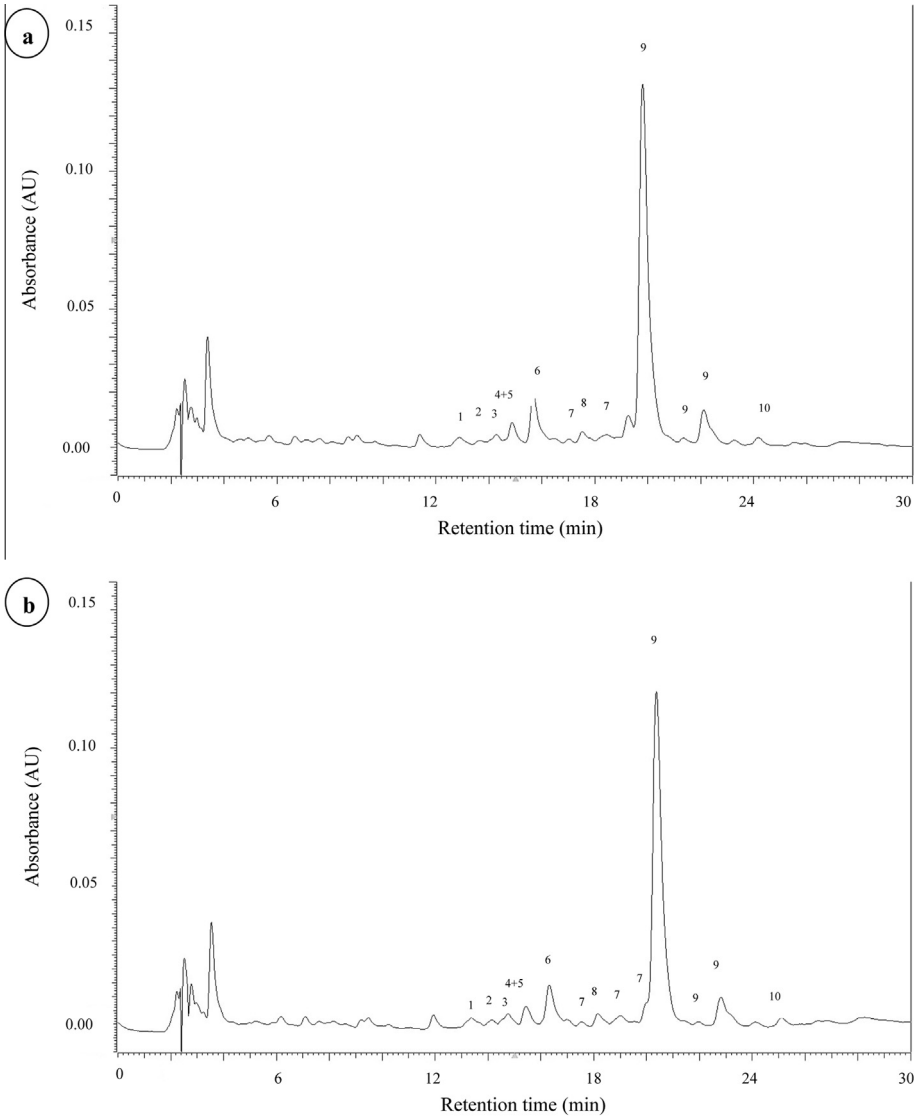

Fig. 5. HPLC chromatograms of olive leaves extracts obtained at 70 °C by CVE (a) and UAE (b) for an extraction time of 10 min.

Trolox/100 g min in CVE. Thus, the higher the extraction temperature, the higher the R_0 for both AC and TPC kinetics is.

Equilibrium contents (Y_∞) for both TPC and AC were significantly ($p < 0.05$) improved by increasing the temperature (Tables 1 and 2). Thus, the maximum TPC was achieved by using an extraction temperature of 70 °C (12.37 g OE/100 g dm). It seems that high temperatures improved the permeation and the solubilization processes to wash the intracellular ingredients out of the matrix. Regarding the AC (Table 2 and Fig. 4), the highest content was reached at 70 °C for CVE experiments (53.36 mmol Trolox/100 g dm). This result proved that high extraction temperature does not provoke the degradation of antioxidant compounds from olive leaves. This finding is entirely consistent with that of Zhang et al. [20], who demonstrated that the extraction yields increased at high temperature (80 °C).

As observed in Fig. 2 and Table 1, in general terms, the application of power ultrasound gave rise to higher initial extraction rates (R_0). As an example, for an extraction temperature of 30 °C, R_0 for UAE and CVE was 21.72 and 14.14 g OE/100 g min, respectively. This fact could be linked to the ultrasound effects in the release and solubilization of phenolic compounds in the medium (washing effect) which, consequently, accelerated the extraction process [10]. As found in CVE experiments, Y_∞ significantly ($p < 0.05$) improved by increasing the temperature in UAE experiments. In this case, the employment of high temperatures led to the rise of the number of cavitation nucleus formed responsible for acoustic cavitation, which enhanced the mass transfer and then the access of solvent to cell components as reported by Jerman, Trebše, & Mozetič Vodopivec [13].

In order to expound the effect of both temperature and extraction method, the TPC and AC kinetics of UAE at the lowest (10 °C) and the highest (70 °C) temperature were compared with the CVE ones (Fig. 3a, b). For TPC, at low temperature (10 °C), there was no significant ($p > 0.05$) difference between CVE and UAE for the first 5 min. However, thereafter the TPC was higher in UAE. Thus, at the end of the extraction, the TPC was 7.26 ± 0.12 g OE/100 g dm and 8.07 ± 0.29 g OE/100 g dm for CVE and UAE, respectively. At high temperature (70 °C), the phenomenon was completely different. Ultrasound assistance improved significantly ($p < 0.05$) the phenolic extraction from the beginning. After 1 min of extraction, CVE reached a TPC of 9.18 g OE/100 g dm where as UAE enhanced it until 10.19 g OE/100 g dm. However, at the end of extraction no significant ($p > 0.05$) differences were found among methods. The same effect was found in AC kinetics. This result might be linked to the capacity of high temperature to contribute to mask the effect of ultrasound energy since the effect of the mechanical energy introduced into the medium could be almost negligible when the level of thermal energy is very high.

3.3. Identification of phenolic compounds and quantification of oleuropein by HPLC-DAD/MS–MS

3.3.1. Identification of phenolic compounds

Extracts obtained by CVE and UAE at 10, 20, 30, 50 and 70 °C were analyzed by chromatography coupled to mass spectrometry in order to identify the main olive leaf phenolic compounds. Fig. 5 presents the HPLC profile of olive leaf extracts obtained at

The main compounds identified in every olive leaf extract were oleuropein, verbascoside, luteolin-3′-7-di-O-glucoside, 10-hydroxy oleuropein, quercetin rutinoside, ligstroside, oleuropein diglucoside, luteolin-7-O-rutinoside, luteolin-7-O-glucoside and apigenin 7-O-rutinoside (Table 3). The phenolic profile detected in this work coincided with those found in previous works [10,21,22]. Chromatograms corresponding to CVE and UAE showed a noticeable similarity (Fig. 5). Therefore, the ultrasound application neither involved the degradation of phenolic compounds nor favored the formation of new phenolic compounds. These results agreed with Ahmad-Qasem et al. [10], who asserted that olive leaf phenolic extraction by applying an ultrasound treatment did not affect the phenolic profile of extracts.

Table 3
Relevant analytical data of compounds identified in olive leaf extracts by HPLC-DAD-MS/MS.

Peak no.	Phenolic compound	Retention time (min)	UV max	[M−H]− *m/z*
1	Luteolin-3′-7-di-O glucoside	12.89	248,267,335	609
2	10-Hydroxy-oleuropein	13.66	240,280	555
3	Verbascoside	14.29	234,329,447	623
4	Luteolin-7-O-rutinoside	14.89	253,348	593
5	Quercetin rutinoside	14.89	350,253	447
6	Luteolin-7-O-glucoside	15.68	253,347	701
7	Oleuropein diglucoside	17.01	237,282,332	577
8	Apigenin-7-rutinoside	17.53	237,266,336	539
9	Oleuropein	19.80	234,280	553
10	Ligstroside	24.15	230,279	523

3.3.2. Extraction kinetics of oleuropein

In order to determine the influence of the extraction method (CVE and UAE) and temperature (10, 20, 30, 50 and 70 °C) on oleuropein extraction kinetics (Fig. 6a and b), this individual compound was monitored. It is important to highlight that, as far as we are concerned, this fact has not been previously reported in the literature.

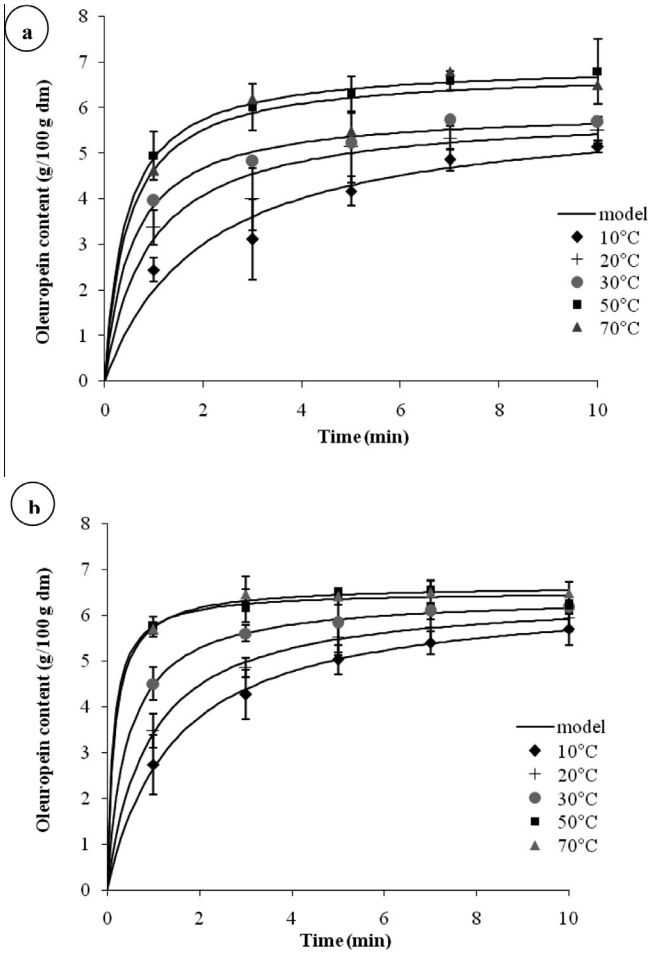

Fig. 6. Influence of extraction temperature on oleuropein content of olive leaves extracts obtained by CVE (a) and UAE (b).

Table 4
Identified parameters of Naik's model for extraction kinetics of oleuropein from olive leaves.

Temperature (°C)	Oleuropein content							
	CVE				UAE			
	Y_∞ (g/100 g dm)	R_0 (g/100 g min)	*VAR* (%)	MRE (%)	Y_∞ (g/100 g dm)	R_0 (g/100 g min)	*VAR* (%)	*MRE* (%)
10 °C	6.03	3.00	92.89	9.68	6.48	4.55	96.77	6.42
20 °C	5.90	6.64	93.35	7.00	6.44	7.37	98.43	4.20
30 °C	5.95	10.94	97.08	4.83	6.42	14.93	98.32	3.41
50 °C	6.96	16.56	97.09	4.73	6.52	49.92	99.13	2.58
70 °C	6.80	14.67	94.32	6.03	6.65	42.00	99.18	2.55

The Naik model fitted adequately to experimental kinetics of oleuropein extraction for both CVE and UAE, since the *VAR* exceeded 92% and the *MRE* was lower than 10% (Table 4). Fig. 6a and b illustrate the goodness of the fit reached for the oleuropein kinetic.

As expected, the oleuropein content in olive leaf extracts was slightly lower than the TPC expressed as oleuropein equivalent (Table 1 and Table 4). This result proved that the oleuropein is the most important compound in olive leaf extract. Indeed, the antioxidant capacity obtained in olive leaf extracts was almost related to oleuropein content.

According to the TPC and AC kinetics, oleuropein extraction kinetics also consisted of two extraction phases: the washing phase, which corresponds to the high extraction rate, and the diffusion phase characterized by the decrease of this rate. Although, the most relevant stage in terms of mass transfer resistance was the solubilization.

For CVE, the initial extraction rate (R_0) increased as the temperature went up. Therefore, high temperature quickened the extraction process. Probably, the increase of the extraction temperature intensified the solubilization of oleuropein. This finding was in accordance with TPC and AC kinetics. However, once exceeding the 50 °C the R_0 slightly decreased.

As regards the oleuropein content achieved at equilibrium (Y_∞), it increased when the temperature rose. Indeed, the increase observed in the oleuropein content may be due to the rise of solvent diffusion into cells and also to the enhancement of desorption and solubility at high temperatures [10]. The influence of temperature was more noticeable in the CVE where asignificant ($p < 0.05$) difference was found between 10 °C and 50 °C. However, increasing the extraction temperature up to 70 °C did not mean a significant ($p < 0.05$) effect on the oleuropein content (Table 4). Chan et al. [7] reported that once the extraction parameters reached their optimum value, further increase in the process parameters will be negligible and in some cases it could be negative in terms of yield. However, Xie et al. [11] demonstrated that the oleuropein extraction yield decreased when the extraction temperature increased from 50 °C to 60 °C.

As observed, the ultrasound application not only improved the R_0 of TPC and AC kinetics but also the oleuropein one. Moreover, the increase in the extraction temperature enhanced the washing stage by increasing the initial extraction rate (R_0) up to 50 °C. Thus, compared with CVE, UAE reached at 50 °C a R_0 of 49.92 g/100 g min while for CVE it was only 16.56 g/100 g min.

Despite the fact that ultrasound-assisted treatment speeded up the initial extraction rate (R_0), it did not have a significant effect ($p < 0.05$) on final oleuropein content (Y_∞). Equilibrium contents determined in the extracts were in the range of 6 and 7 g/100 g dm, being the highest one achieved by CVE at 50 °C. In this context, Shirsath et al. [23] said that ultrasound-assisted procedure shortened extraction time but did not show higher level of polyphenols from flowers of *Delonix regia* trees than stirring-assisted procedure. However, in a previous study [10], it was shown that UAE extracts exhibited a 12% significantly ($p < 0.05$) lower oleuropein content than CVE ones.

As for ultrasound application on oleuropein content kinetics, the performance was different depending on the extraction temperature. As an example, oleuropein content kinetics at 10 and 70 °C were depicted for both CVE and UAE in Fig. 3c. At both temperatures, as previously mentioned, the ultrasound application brought about an increase in the R_0. However, as extraction progressed, some particularities were appreciated. At low temperature (10 °C), extracts obtained from UAE showed a significantly ($p < 0.05$) higher oleuropein content (5.71 ± 0.36 g/100 g dm after 10 min than CVE (5.15 ± 0.13 g/100 g dm). At high temperature (70 °C), no oleuropein content differences were observed among extraction methods. Indeed, the efficiency of ultrasonic application was more detected at low temperatures.

4. Conclusion

In general terms, the extraction kinetics of olive leaves was improved by increasing the temperature. Indeed, Y_∞ and R_0 were enhanced for TPC, AC and oleuropein content. Considering the energy consumption and the slight improvement of the bioactive potential by increasing the extraction temperature from 50 °C to 70 °C, it could be stated that the most suitable temperature was 50 °C for both CVE and UAE. Regarding the extraction method, no clear effect was found on the extract composition. Ultrasound assistance intensified the initial extraction rate but its influence on the evolution and the final content of phenolic compounds, antioxidant activity and oleuropein depended on the extraction temperature.

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