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ARTICLE INFO

ABSTRACT

The influence of ultrasound treatment (US) on cellular damage of olive leaf tissue was studied. Mechanical damage and thermal effect of US were characterized. The level of tissue damage was defined by the diffusivity disintegration index Z_D based on the diffusivity of solutes extracted from olive leaves differently treated. The Arrhenius form using the temperature dependences of the thermal treatment time within the temperature interval 20–90 °C was observed for the thermal process. The corresponding activation energy ΔU_T was estimated as 57 kJ/mol. The temperature dependences of electrical conductivity were measured for extracts of intact and maximally treated olive leaves. Then the diffusivity disintegration index Z_D and total phenolic compounds recovery for three studied US powers were calculated (100, 200, and 400 W). The results evidenced that the mechanically stimulated damage in olive leaf tissue can occur even at a low US power of 100 W if treatment time is long enough (t = 3.5 h). The US treatment noticeably accelerated the diffusion process mechanically in addition to its thermal effect. Trials in aqueous solution revealed the dependence of polyphenols extraction on damage level with respect to the US power applied.

1. Introduction

Olive tree (*Olea europaea* L., Oleaceae) is among the most abundantly cultivated crops in the world, with global production exceeding 16 million metric tons of olives and more than 3 million tons of olive oil annually [1]. Typically concentrated in countries around the Mediterranean basin, olive trees are cultivated for olive and olive oil production. Growth and productivity of olive trees are boosted when they are pruned regularly, when certain branches are selectively reduced or removed. In addition, large quantities of small stems and leaves fall accidentally during olive harvesting, representing around 10% of olives total weight [2], and even more using traditional methods. Consequently, olive leaves are considered one of the major by-products that are generated all along this procedure, yet wasted although they are renowned for being a rich source of high-added value compounds, such as secoiridoids, flavonoids and other phenolics [3]. Directly burned in the fields, or improperly discarded next to the olive mills after separation, disposal of olive leaves remains an important issue in waste management and environmental pollution [4]. Their potential could be better valorized by extracting their valuable constituents. Indeed, numerous studies endorsed the diverse beneficial biological activities of their extracts including antioxidative, anti-diabetic, antiviral, anti-fungal, anti-inflammatory, antimicrobial, anti-carcinogenic, as well as skin protection, anti-aging and anti-obesity [5–10].

Recovery of bioactive compounds using solvents is known as conventional extraction. Plant matrices are soaked in a solvent (or mixture of solvents), usually under a constant agitation, so that the desired molecules are extracted conforming to diffusion and mass transfer phenomena. Conventional extraction presents several drawbacks such as ineffectiveness (recovery and selectivity), high solvent consumption, and lower extraction yield [11]. Various extraction technologies have been reported as an alternative to conventional solvent extraction in

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order to enhance recovery of bioactive compounds from olive leaves. Widely explored, such technologies include microwave-assisted extraction [12], pressurized liquid extraction [13], supercritical fluid extraction [14], infrared-assisted extraction [15], Intensification of Vaporization by Decompression to the Vacuum [16], and ultrasound-assisted extraction [17]. The latter has been validated as a green extraction technique that ensures reduction of energy consumption, solvent usage, processing time, and cuts accumulation of waste and of hazardous substances [18–20].

The fundamental basics of ultrasound relies on physical vibrations passing through a liquid or any liquid-like medium, triggering growth and collapse of generated gas under the effect of acoustic field [21]. The collapse of the microbubbles hereafter formed, known as cavitation bubbles, is accompanied by a local increase in temperature and pressure. Occurring under such extreme physical conditions, this phenomenon engenders substantial mechanical constraints near or onto the outer surface of the treated plant matrix. Their intensity depends on the dynamic interaction between the cavitation bubbles and the exposed plant cells. In order to account for the resulting structural damages, several mechanistic interpretations of the impact of bubble implosion were described suggesting mostly sonoporation or cellular disruption [22–25]. Used for extraction, ultrasonic treatment promotes mass transfer not only by releasing intracellular substances into the extraction medium, but also by strengthening diffusion of the solvent within the cells due to thermal and chemical changes [26–28].

Ultrasound-assisted extraction of bioactive principles from olive leaves has been previously investigated as related to the effects of process parameters (such as emitter surface, wave amplitude, extraction temperature and time, extraction solvent, solid/solvent ratio) on the extraction kinetics and the composition of extracts [29–35]. However, the mechanical damages and the thermal effects caused by ultrasound treatment in leaf tissue, along with their relationship with polyphenols recovery, has not been extensively studied yet. An association between alteration of the microstructure following US and extraction of phenolic compounds was highlighted in the case of maritime pine sawdust [36]. More recently, Rombaut and collaborators correlated between histological modifications in olive leaves (cuticle erosion and hair fragmentation) due to ultrasound and polyphenol extraction enhancement [37].

Within a similar scope, our objective was to contribute to a further understanding of this association. Thereby, the aim of our study was to investigate ultrasonic-induced mechanical damage and thermal effect on olive leaf tissue through the quantification of the diffusivity disintegration magnitude and the subsequent electrical conductivity of the obtained extracts, and to correlate them with polyphenol recovery yield upon extraction. To this end, it was interesting to try to dissociate the mechanical damage from the thermal effect which is the non-mechanical effect induced by just heating the solid matrix.

2. Materials and methods

2.1. Plant material

Olive leaves were obtained from a local olive mill in northern Lebanon El Koura. Leaves were washed with water and then air dried in an oven at 40 °C for 48 h. The dry matter (DM) of raw material was $91 \pm 0.4\%$. Whole dried leaves were stored in sealed packages, away from light, and used in all further analyses.

2.2. Chemicals

All chemicals used in the experiments were analytical grade. Folin-Ciocalteu reagent, sodium carbonate and gallic acid were purchased from Sigma-Aldrich, St-Quentin Fallavier, France.

2.3. Conventional extraction

Conventional extraction was carried out in a glass beaker using a water bath SW22 (Julabo GmbH, Seelbach, Germany) as depicted in Fig. 1.a. Electrical conductivity of olive leaves extracts at different temperatures was studied. The beaker was filled with 20 g of olive leaves and 300 mL of water set at the corresponding treatment temperature, then placed into the water bath kept at the desired temperatures (20, 30, 40, 50, 60, 70, 80, and 90 °C). All experiments were conducted for 2 h in triplicates, and then standard deviations were calculated.

Besides, and according to preliminary trials, it was observed that a treatment at 90 °C for 2 h allowed obtaining a maximum of extracted solutes without disintegration of the solid matrix. In fact, extraction kinetics at 90 °C reached a plateau of solutes recovery at around 2 h, whereas a treatment at more than 90 °C for 2 h induced a collapse of the solid matrix. Based on this consideration, we defined the "maximal electrical conductivity of extract" as corresponding to the maximal amount of solutes extracted from olive leaves, obtained at 90 °C for 2 h in a conventional water bath without disintegration.

2.4. Ultrasound-assisted extraction

In order to study and characterize the mechanical and thermal effects of the ultrasound (US) treatment, a range of parameters variation was designed. Fig. 1.b represents a schematic diagram of the US experimental setup. Ultrasonic treatment of olive leaves, using water as solvent, was carried out directly in a glass beaker using an ultrasonic emitter (UP 400S, 400 W, 24 kHz, Hielscher GmbH, Stuttgart, Germany). A titanium ultrasonic probe H14 (Hielscher GmbH, Stuttgart, Germany) was used, functioning in a continuous mode. The beaker was covered with a heat-reflective metalized foil to avoid corresponding to 100, 200, and 400 W, which resulted in 0.052, 0.103, and 0.206 W/cm² respectively, after taking into consideration the outer surface of the leaves. Various US treatment times were applied accordingly. The temperature within the treated mixture was recorded using a data logger thermometer (YCT, United Kingdom).

The diffusivity of solutes extracted from olive leaves was estimated based on the diffusivity disintegration index Z_D calculated from equation (1) [38]:

$$Z_D = \frac{\sigma_e(T) - \sigma_{ei}(T)}{\sigma_{em}(T) - \sigma_{ei}(T)} \tag{1}$$

where $\sigma_e(T)$ is the actual electrical conductivity of extract obtained from olive leaves during ultrasound treatment, in mS/cm; $\sigma_{ei}(T)$ is the electrical conductivity of extract obtained from control sample, soaked at 10 °C for 10 min without US treatment (intact olive leaves), in mS/cm; and $\sigma_{em}(T)$ is the maximal electrical conductivity of extract obtained by treating whole olive leaves in a conventional water bath at 90 °C for 2 h (maximally treated), in mS/cm. The control sample was adopted upon preliminary trials, which concluded that soaking olive leaves in water at 10 °C up to 10 min does not cause any modification in the water conductivity. Here, the soaking water was cooled down using ice prior to the experimental runs. Electrical conductivities of all studied extracts depend on the temperature and on their content in solutes. Similar procedure used in defining the diffusivity disintegration index Z_D was adopted earlier in order to study solutes' release from sugar beet tissue being treated by pulsed electric field [39].

While ground leaves were used in previous studies [15,40], whole leaves were chosen in the current study in order to isolate and to assess the mechanical effect of US on cell rupture. Application of the equation (1) gives $Z_D = 0$ for solutes release from intact tissue, and $Z_D = 1$ for maximal solutes release obtained. Analytical expression of diffusive mass flux based on Fick's law (for a stirred solution of limited volume for a plane sheet model) was used to describe the mass transfer of polyphenols from the surface of olive leaves to the solvent using equation (2)
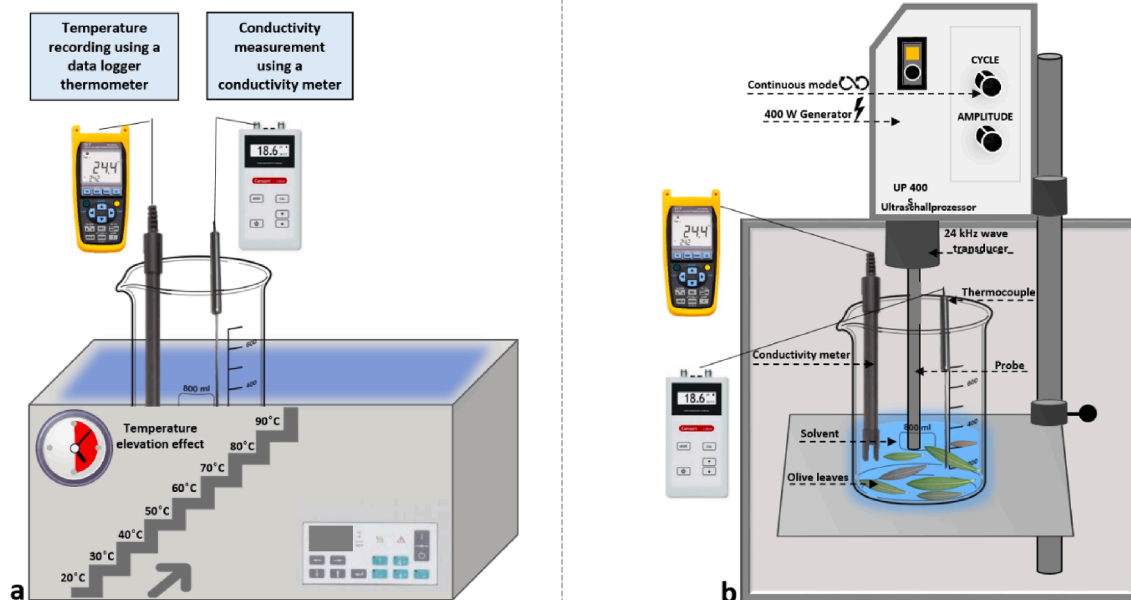
Fig. 1. Schematic representation of the experimental setup of (a) conventional water bath and (b) ultrasound-assisted extraction

as follows [41,42]:

$$j = -D \frac{\Delta C}{\Delta x} = -k_T \Delta C \tag{2}$$

Accordingly, the mass transfer coefficient k_T was calculated using the equation (3) below:

$$k_T = \frac{D}{\Delta x} \tag{3}$$

where j is the flux with respect to the mixture mol m^{-2} s^{-1}, C is the molar concentration mol m^{-3}, x is the distance (position) m, D is the Fick diffusivity m^2 s^{-1}, and k_T is the mass transfer coefficient m s^{-1}.

2.5. Total phenolic compounds

Polyphenol content was assessed to evaluate the effect of the US treatment on the recovery of polyphenols from olive leaves. Total phenolic compounds (TPC) was quantified colorimetrically using the Folin-Ciocalteu method based on oxidation/reduction reactions of phenols [43]. A volume of 0.2 mL of the extracts and 1 mL of ten-fold diluted Folin-Ciocalteu reagent (Sigma-Aldrich, St-Quentin Fallavier, France) were mixed. Then, 0.8 mL of Na$_2$CO$_3$ (75 g/L) (VWR, Fontenay-sous-Bois, France) was added. The mixture was incubated at 60 °C for 10 min then cooled at room temperature. The absorbance was measured at 750 nm by the UV/Vis spectrophotometer (Libra S32, Biochrom, Lagny-sur-Marne, France). Gallic acid (Sigma-Aldrich, St-Quentin Fallavier, France) was used for the calibration curve. Results were expressed as mg GAE/g dry matter (DM). The analyses were performed in triplicates and standard deviations were calculated.

2.6. Statistical analysis

Significant differences between the results were calculated by analysis of variance (ANOVA). Differences at $p < 0.05$ were considered to be significant. The Least Significant Difference (LSD) test was applied to assess differences between the samples.

3. Results and discussion

3.1. Thermally-induced effect in olive leaves

Changes in diffusivity disintegration index Z_D as a function of time were measured in thermally treated olive leaf tissue for different temperatures (Fig. 2.a). The necessary time to reach the maximal extraction is an essential factor determined by the electrical conductivity assessment. Thermal treatment time required to reach one-quarter of the maximal Z_D value $\frac{\tau_T max}{4}$ was also indicated. Arrhenius curve, which corresponds to the thermal treatment time (τ_T) versus inverse temperature ($1/T + 273.15$), was represented in Fig. 2.b. According to the data, the activation energy, or the minimal energy needed to launch the process of membrane alteration (in order to start the extraction phenomenon) in olive leaf tissue, was calculated by the least square fitting of the Arrhenius equation (4) to the line slope [44]:

$$\tau_T = \tau_{T,\infty} \times \exp^{\frac{\Delta UT}{R(T+273.15)}} \tag{4}$$

here, the activation energy ΔUT is 57 kJ/mol, the limiting characteristic thermal treatment time $\tau_{(T, \infty)}$ is 2.79 10^{-6} s, the universal gas constant R is 8.314 J/K/mol and T is the temperature in °C. It was observed experimentally that an increase of 10 °C promoted the interaction between the solvent and the matrix. Nonetheless, the activation energy remained relatively constant with the increasing temperatures (Table 1), which proved that ΔUT has a well-defined and a characteristic value specific to a reaction, intrinsic to the material, yet temperature independent. This finding was corroborated by similar studies conducted on the same material, in which the activation energy value was around 60.97 ± 3.21 kJ/mol [45] or ranged from 24.704 to 53.743 kJ/mol [46].

3.2. Effect of temperature and extraction conditions on the electrical conductivity of olive leaves extracts

Effect of the temperature on electrical conductivity of extracts recovered from the maximally treated (90 °C for 2 h) and intact olive leaves (10 °C for 10 min) is presented as dashed lines in Fig. 3. The beaker containing the olive leaves was placed in the water bath and
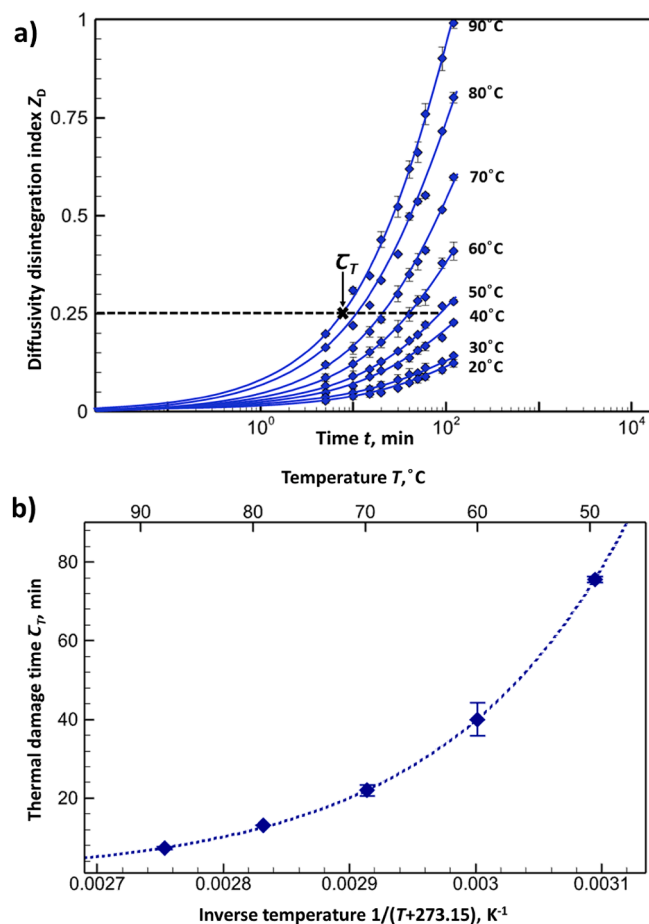
Fig. 2. (a) Diffusivity disintegration index Z_D versus time of thermal treatment (t). (b) Arrhenius plot of the thermal treatment time (τ_T) versus inverse temperature ($1/T+273.15$)

Table 1
Activation energy values in function of temperature variation.

Temperature (°C)	Activation energy (kJ/mol)
90	56.84
80	57.09
70	56.98
60	56.97
50	56.91

maintained at the desired temperature. The electrical conductivity measurements of the extract of intact leaves were carried out within the temperature T range from 0 to 40 °C (latency phase) in order to minimize as much as possible, the impact of thermal degradation, while the temperature considered for the maximally treated leaves ranged from 0 to 90 °C. Using equation (5), the linear dependence of electrical conductivity σ of extracts of intact and maximally treated leaves on temperature T was observed [47]:

$$\sigma = \sigma_0(1 + \alpha \frac{T - T_0}{1 + \alpha T_0}) \tag{5}$$

where σ_0 is the electrical conductivity of extracts at the reference temperature T_0 in °C, and α is the temperature coefficient of the electrical conductivity in °C^{-1}. The values of σ_0 and α were estimated as 55 μS/cm and 0.038 °C^{-1} for the intact olive leaves extract, and 530 μS/cm and 0.033 °C^{-1} for the maximally treated olive leaves extract, respectively (Table 2). With the increasing temperature, the change in electrical conductivity of the extract of maximally treated olive leaf tissue ranged
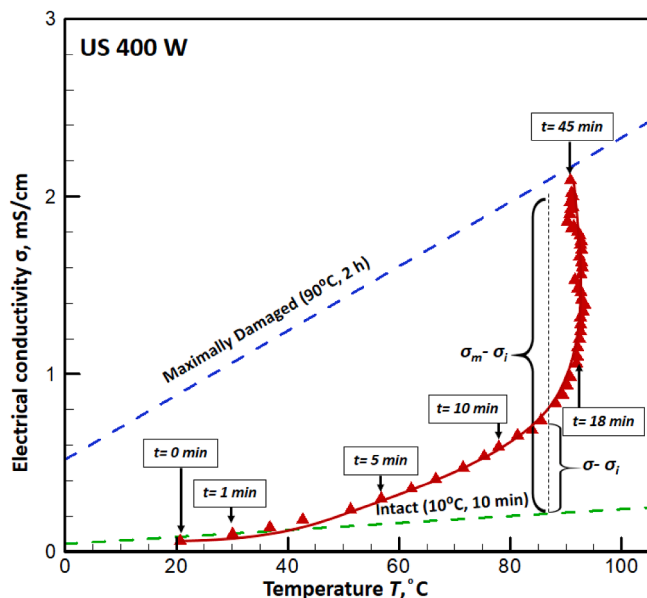

Fig. 3. Typical dependence of the electrical conductivity (σ) of ultrasound treated olive leaves versus temperature (T) at 400 W. Dashed lines show the temperature dependency of conductivities (σ_m and σ_i) of extracts of the maximally treated (90°C; 2 h) and intact (10°C; 10 min) olive leaves, respectively.

Table 2
Values of σ and α for extracts of the intact (10 °C; 10 min) and maximally treated (90 °C; 2 h) olive leaves. Here, σ_0 is the initial electrical conductivity at the reference temperature T_0 in °C, and σ_f is the final electrical conductivity at the reference temperature T_f in °C.

Olive leaves extract	σ_0 (mS/cm)	σ_f (mS/cm)	T_0 (°C)	T_f (°C)	α (°C^{-1})
intact tissue	0.055	0.129	5	40	0.038
maximally treated tissue	0.53	2	5	90	0.033

from 0.53 to 2 mS/cm, almost 20 times more than the extract of intact tissue (between 0.055 and 0.129 mS/cm). These recorded values are in concordance with other studies [48] affirming the positive correlation existing between the temperature and the electrical conductivity.

3.3. Mechanically induced damage in olive leaves during ultrasound treatment

In order to unveil the impact of mechanically induced damage and the cavitation phenomenon coupled with the thermal effect, Fig. 3 emphasizes the effect of ultrasound treatment at 400 W on electrical conductivity σ of the extract. The electrical conductivity was measured every minute in function of US treatment time, while recording the temperature value for each point. The total time extent of the US treatment was 45 min. Dashed lines recall the temperature dependences of electrical conductivity for the maximally treated and intact olive leaves extracts using a linear approach. Remarkably, the conductivity curve of the extract of US-treated tissue surpassed the one drawn for the extract of intact olive leaves. Such observation demonstrates the importance of the mechanical damages coupled with the heating, which may induce a softening of the plant matrix thus amplifying the effect of the cavitation. A synergistic effect is clearly illustrated by the deviation of the US curve from the purely thermal one. A similar behavior was observed with sugar beet tissue using Moderate Electric Field treatment (MEF) that supported the existence of electrically stimulated damage in combination with the thermal effect [47]. Chemat et al. investigated the

importance of the mechanical ultrasonic effect in releasing the soluble compounds from the plant matrix by disrupting cell walls, enhancing mass transfer and facilitating solvent access to cell content [25]. Particularly, Martínez-Patiño et al. confirmed the presence of mechanical and thermal effects that help the solvent to infiltrate within the cell increasing the mass transfer resulting in a better diffusion of the olive leaves cell material [49]. Additionally, when the threshold of 18 min is reached (Fig. 3), the temperature remained at a constant value of 90 °C while the conductivity sustained its elevation, during 27 min, up to 45 min to hit its maximum value of 2 mS/cm. In contrast, two hours of treatment at 90 °C were required to reach an equivalent electrical conductivity using the water bath traditional method. Hence, US reduced by almost three times the treatment duration and consequently energy consumption.

3.4. Effect of ultrasound power on polyphenol extraction from olive leaves

Fig. 4.a compares TPC in olive leaves extracts as a function of temperature for three different ultrasonic power (100, 200, and 400 W) and up to 45 min as treatment time. The same pattern was found for all the studied US powers. During the first minutes of extraction when temperature raised from 10 to 40 °C, a linear correlation was observed between temperature and polyphenol yield; an overlapping between the three curves was noticed. This positive thermal effect on polyphenols recovery could be expected since temperature lowers the viscosity and the surface tension of the solvent, ameliorates the solubility of the solute, thus enhancing the mass transfer phenomenon [50]. Furthermore,

the concentration gradient is very high at the beginning of the extraction process where the solvent is 100% free of ions. This condition favors release of the molecules in the solvent during the first minutes independently from the ultrasonic power, which explains the similarity between the shape of the three curves for the first few minutes of treatment [51]. Afterwards, temperature of the solutions underwent a spontaneous stabilization after a certain extent of time that was longer with higher US power. Indeed, increasing US power induces more heating, thus delaying the balance that occurs between the thermal energy contributed by US and heat exchange with the outside. Another observation can be made is that the temperature level reached upon stabilization increased with higher sonication power. Interestingly, higher amounts of polyphenols were subsequently released in an exponential way even at constant temperatures. For instance, TPC increased from 9.47 to 25.56 mg GAE/g DM between 20 min and 45 min of treatment, at 400 W and at 90 °C. The synergistic thermal and mechanical effects of US is attributed to the US-induced mechanical vibrations. This phenomenon leads to decompression cycles (bubble formation, growth and implosion) in the liquid medium which foster the extraction process [52].

Alternatively, ultrasonic power governs the kinetics of temperature elevation. A temperature elevation of only 8 °C occurred during the first 5 min at 100 W ultrasonic power, whereas a raise of 40° was recorded for the same duration at 400 W. A faster ΔT was observed when the ultrasonic power was heightened which accelerated the process as a function of time. Cell wall/membrane alteration is likely to intensify the extraction process. Moreover, a linear correlation was noticed between the ultrasonic power and the TPC extraction for the same treatment
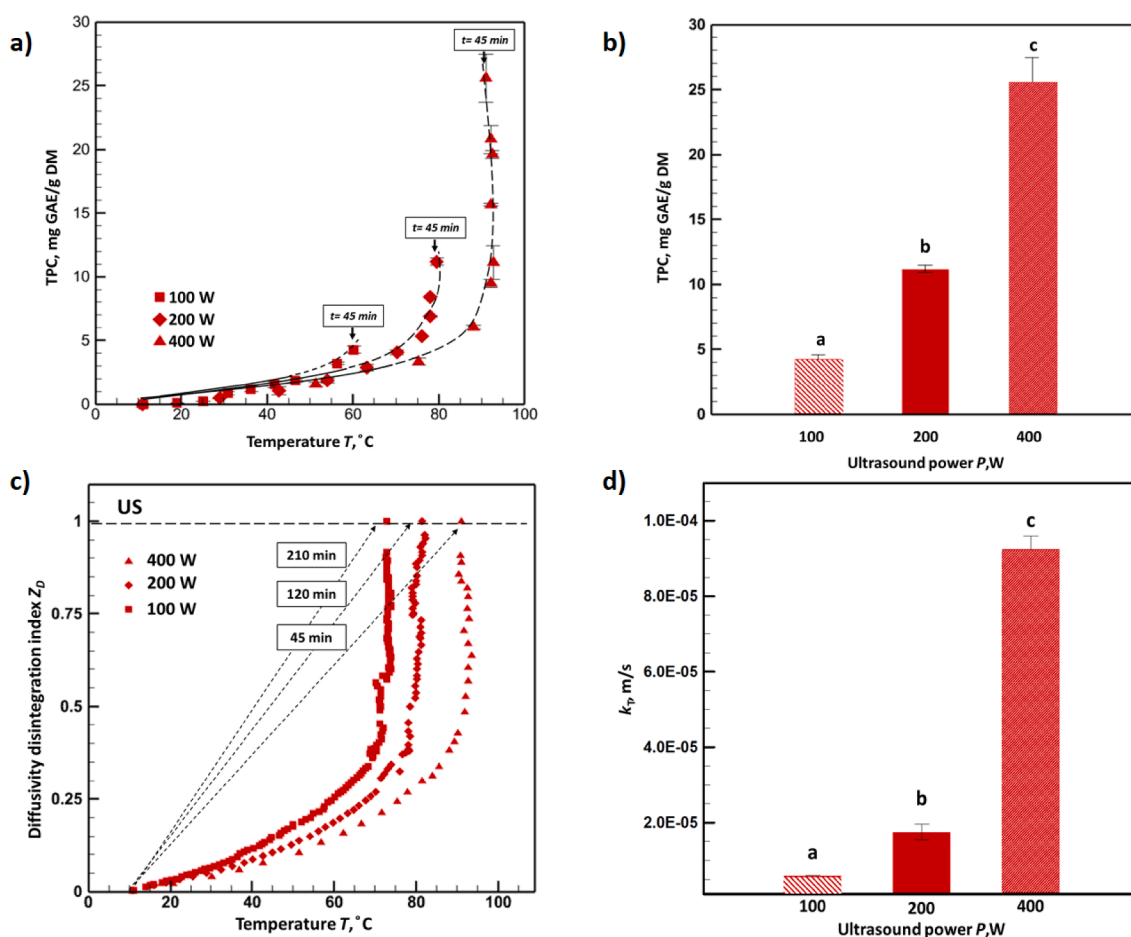

Fig. 4. (a) Effect of temperature (T) on the total phenolic content (TPC) for the different ultrasound treatment powers. (b) Correlation between TPC extracted and the ultrasound power. (c) Effect of temperature (T) on the diffusivity disintegration index Z_D for different ultrasound treatment powers. (d) Transfer coefficient (k_T) for 100, 200, and 400 W ultrasound power.

duration (y = 0.0614x) as depicted in Fig. 4.b.

The second phenomenon taking place is apparently attributed to the mechanical effect of the acoustic cavitation process that is likely to alter the cells. For the three studied ultrasonic powers, 15% of the polyphenols were extracted in a thermally dependent manner, whereas 85% were recovered due to both thermal and mechanical effects. The US mechanism of action is therefore a combination of both thermal and mechanical effects; the latter having a higher impact.

3.5. Effect of ultrasound power on the electrical conductivity of olive leaves extracts

Fis 4.c opposes the curves of diffusivity disintegration index Z_D versus temperature T for the three US powers (100, 200, and 400 W). Damage in cell wall/membrane was quantified by the measurement of Z that reflects the US-induced structural alteration. The diffusivity disintegration index was proportional to the electrical conductivity or ions' release in the solvent. The maximal disintegration index $Z_D = 1$ was achieved after 45, 120, and 210 min for 100, 200, and 400 W US power, respectively. In concordance with TPC results in Fig. 4.a, the linear evolution of Z_D in function of temperature was followed by an exponential increase. Regarding this correlation, the thermal effect affected mostly the first linear stage, whereas the mechanical damage, at a constant high temperature, prevailed during the second exponential stage.

Damaged cells likely release more polyphenols. When increasing US power from 100 to 400 W, the mass transfer coefficient k_T was ameliorated by fifteen times (from $6E^{-6}$ to $9E^{-5}$m/s, respectively) (Fig. 4. d) concurrent with TPC yield improvement by six times (from 4.28 to 25.57 mg GAE/g DM, respectively) (Fig. 4.a). This increase in the mass transfer coefficient can be due to an increase in the mass diffusivity simultaneously to a decrease in viscosity of solvent at high temperatures. The mass transfer coefficient is definitely influenced by the extraction temperature.

4. Conclusion

Regardless the power, ultrasound treatment effectively dislocated the tissues of olive leaves due to the combination between thermal effect and mechanical damage that is typically induced by the cavitation phenomenon. It was demonstrated that damaged tissues can release more polyphenols into the solvent media which highlighted the positive effect of ultrasonic treatment in the intensification of polyphenol recovery from plant material. Nowadays, the choice of an extraction method is imposed by the compromise existing between the efficiency and ease of process, while taking into consideration the cost, time, and safety. All the above-mentioned features focus on the importance and usefulness of this extraction method at laboratory and industrial scale. Further work is required to characterize and fully elucidate the events taking place and leading to mechanical damages in olive leaves tissues.

CRediT authorship contribution statement

Anna-Maria Abi-Khattar: Investigation, Writing – original draft, Writing – review & editing. **Nadia Boussetta:** Conceptualization, Supervision, Formal analysis, Funding acquisition. **Hiba N. Rajha:** Formal analysis, Writing – original draft, Writing – review & editing. **Roula M. Abdel-Massih:** Supervision, Writing – review & editing. **Nicolas Louka:** Conceptualization, Supervision, Formal analysis, Writing – review & editing, Funding acquisition. **Richard G. Maroun:** Supervision, Writing – review & editing, Funding acquisition. **Eugene Vorobiev:** Conceptualization, Supervision, Formal analysis, Writing – review & editing, Funding acquisition. **Espérance Debs:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] International Olive, 2019. International Olive Council production data, collected on Oct. 21, 2019. http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures.

[2] M. Moudache, M. Colon, C. Nerín, F. Zaidi, Phenolic content and antioxidant activity of olive by-products and antioxidant film containing olive leaf extract, Food Chem. 212 (2016) 521–527, https://doi.org/10.1016/j.foodchem.2016.06.001.

[3] D. Majumder, M. Debnath, K.V.L. Kumar, P. Nath, R. Debnath, C. Sarkar, G.B.K. S. Prasad, Y. Kumar, D. Maiti, Metabolic profiling and investigations on crude extract of *Olea europaea* L. leaves as a potential therapeutic agent against skin cancer, J. Funct. Foods 58 (2019) 266–274, https://doi.org/10.1016/j.jff.2019.05.005.

[4] S. Şahin, M. Bilgin, Olive tree (*Olea europaea* L.) leaf as a waste by-product of table olive and olive oil industry: A review, J. Sci. Food Agric. 98 (4) (2018) 1271–1279, https://doi.org/10.1002/jsfa.2018.98.issue-410.1002/jsfa.8619.

[5] Z.J. Suárez Montenegro, G. Álvarez-Rivera, J.A. Mendiola, E. Ibáñez, A. Cifuentes, Extraction and mass spectrometric characterization of terpenes recovered from olive leaves using a new adsorbent-assisted supercritical CO_2 process, Foods 10 (2021) 1301, https://doi.org/10.3390/foods10061301.

[6] Z. Lbay, S. Sahin, K. Büyükkabasakal, A novel approach for olive leaf extraction through ultrasound technology : Response surface methodology versus artificial neural networks, Korean J. Chem. Eng. 31 (2014) 1661–1667, https://doi.org/10.1007/s11814-014-0106-3.

[7] S.N. El, S. Karakaya, Olive tree (*Olea europaea*) leaves: Potential beneficial effects on human health, Nutr. Rev. 67 (2009) 632–638, https://doi.org/10.1111/j.1753-4887.2009.00248.x.

[8] Y.C. Jung, H.W. Kim, B.K. Min, J.Y. Cho, H.J. Son, J.A. Young Lee, J.Y. Kim, S. B. Kwon, Q. Li, H.W. Lee, Inhibitory Effect of Olive Leaf Extract on Obesity in High-fat Diet-induced Mice, In Vivo (Brooklyn) 33 (2019) 707–715, https://doi.org/10.21873/invivo.11529.

[9] G. Difonzo, A. Pasqualone, R. Silletti, L. Cosmai, C. Summo, V.M. Paradiso, F. Caponio, Use of olive leaf extract to reduce lipid oxidation of baked snacks, Food Res. Int. 108 (2018) 48–56, https://doi.org/10.1016/j.foodres.2018.03.034.

[10] P.G. Lins, S. Marina Piccoli Pugine, A.M. Scatolini, M.P. de Melo, In vitro antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes, Heliyon 4 (2018) e00805, https://doi.org/10.1016/j.heliyon.2018.e00805.

[11] F.J. Barba, P. Putnik, D. Bursać Kovačević, M.M. Poojary, S. Roohinejad, J. M. Lorenzo, M. Koubaa, Impact of conventional and non-conventional processing on prickly pear (*Opuntia* spp.) and their derived products: From preservation of beverages to valorization of by-products, Trends Food Sci. Technol. 67 (2017) 260–270, https://doi.org/10.1016/j.tifs.2017.07.012.

[12] A. Taamalli, D. Arráez-Román, E. Ibañez, M. Zarrouk, A. Segura-Carretero, A. Fernández-Gutiérrez, Optimization of Microwave-Assisted Extraction for the Characterization of Olive Leaf Phenolic Compounds by Using HPLC- ESI-TOF-MS/IT-MS 2, J. Agric. Food Chem. 60 (3) (2012) 791–798, https://doi.org/10.1021/jf204233u.

[13] N. Xynos, G. Papaefstathiou, E. Gikas, A. Argyropoulou, N. Aligiannis, A. Skaltsounis, Design optimization study of the extraction of olive leaves performed with pressurized liquid extraction using response surface methodology, Sep. Purif. Technol. 122 (2014) 323–330, https://doi.org/10.1016/j.seppur.2013.10.040.

[14] L. Baldino, G. Della Porta, L.S. Osseo, E. Reverchon, R. Adami, Concentrated oleuropein powder from olive leaves using alcoholic extraction and supercritical CO_2 assisted extraction, J. Supercrit. Fluids 133 (2018) 65–69, https://doi.org/10.1016/j.supflu.2017.09.026.

[15] A.-M. Abi-Khattar, H.N. Rajha, R.M. Abdel-Massih, R.G. Maroun, N. Louka, E. Debs, Intensification of Polyphenol Extraction from Olive Leaves Using Ired-

Irrad®, an Environmentally- Friendly Innovative Technology, Antioxidants. 8 (2019) 1–16, https://doi.org/10.3390/antiox8070227.

[16] A.-M. Abi-Khattar, H.N. Rajha, R.M. Abdel-Massih, R. Habchi, R.G. Maroun, E. Debs, N. Louka, "Intensification of vaporization by decompression to the Vacuum" (IVDV), a novel technology applied as a pretreatment to improve polyphenols extraction from olive leaves, Food Chem. 342 (2020), 128236, https://doi.org/10.1016/j.foodchem.2020.128236.

[17] Q. Yao, Y. Shen, L. Bu, P. Yang, Z. Xu, X. Guo, Ultrasound-assisted aqueous extraction of total flavonoids and hydroxytyrosol from olive leaves optimized by response surface methodology, Prep. Biochem. Biotech. 49 (9) (2019) 837–845, https://doi.org/10.1080/10826068.2019.1630648.

[18] H.N. Rajha, N. Boussetta, N. Louka, R.G. Maroun, E. Vorobiev, A comparative study of physical pretreatments for the extraction of polyphenols and proteins from vine shoots, Food Res. Int. 65 (2014) 462–468, https://doi.org/10.1016/j.foodres.2014.04.024.

[19] B.K. Tiwari, Ultrasound: A clean, green extraction technology, Trends Anal. Chem. 71 (2015) 100–109, https://doi.org/10.1016/j.trac.2015.04.013.

[20] M. Gouda, A. El-Din Bekhit, Y. Tang, Y. Huang, L. Huang, Y. He, X. Li, Recent innovations of ultrasound green technology in herbal phytochemistry: A review, Ultrason. Sonochem. 73 (2021) 105538, https://doi.org/10.1016/j.ultsonch.2021.105538.

[21] M. Ashokkumar, The characterization of acoustic cavitation bubbles - An overview, Ultrason. Sonochem. 18 (4) (2011) 864–872, https://doi.org/10.1016/j.ultsonch.2010.11.016.

[22] C.-D. Ohl, M. Arora, R. Ikink, N. de Jong, M. Versluis, M. Delius, D. Lohse, Sonoporation from jetting cavitation bubbles, Biophys. J . 91 (11) (2006) 4285–4295, https://doi.org/10.1529/biophysj.105.075366.

[23] H.N. Rajha, M. Koubaa, N. Boussetta, R.G. Maroun, N. Louka, N. Lebovka, E. Vorobiev, Selective ultrasound-assisted aqueous extraction of polyphenols from pomegranate peels and seeds, J. Food Process. Preserv. 44 (2020), e14545, https://doi.org/10.1111/jfpp.14545.

[24] H.N. Rajha, A.-M. Abi-Khattar, S. El Kantar, N. Boussetta, N. Lebovka, R. G. Maroun, N. Louka, E. Vorobiev, Comparison of aqueous extraction efficiency and biological activities of polyphenols from pomegranate peels assisted by infrared, ultrasound, pulsed electric fields and high-voltage electrical discharges, Innov. Food Sci. Emerg. Technol. 58 (2019), 102212, https://doi.org/10.1016/j.ifset.2019.102212.

[25] F. Chemat, N. Rombaut, A. Sicaire, A. Meullemiestre, A.-S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications, Ultrason. Sonochem. 34 (2017) 540–560, https://doi.org/10.1016/j.ultsonch.2016.06.035.

[26] E. Roselló-Soto, F.J. Barba, O. Parniakov, C.M. Galanakis, N. Lebovka, N. Grimi, E. Vorobiev, High Voltage Electrical Discharges, Pulsed Electric Field, and Ultrasound Assisted Extraction of Protein and Phenolic Compounds from Olive Kernel, Food Bioprocess Technol. 8 (4) (2015) 885–894, https://doi.org/10.1007/s11947-014-1456-x.

[27] Y. Xu, L. Zhang, Y. Bailina, Z. Ge, T. Ding, X. Ye, D. Liu, Effects of ultrasound and/or heating on the extraction of pectin from grapefruit peel, J. Food Eng. 126 (2014) 72–81, https://doi.org/10.1016/j.jfoodeng.2013.11.004.

[28] A.M. Goula, Ultrasound-assisted extraction of pomegranate seed oil – Kinetic modeling, J. Food Eng. (2012), https://doi.org/10.1016/j.jfoodeng.2012.10.009.

[29] M.H. Ahmad-Qasem, J. Cánovas, E. Barrajón-Catalán, V. Micol, J.A. Cárcel, J. V. García-Pérez, Kinetic and compositional study of phenolic extraction from olive leaves (var. Serrana) by using power ultrasound, Innov. Food Sci. Emerg. Technol. 17 (2013) 120–129, https://doi.org/10.1016/j.ifset.2012.11.008.

[30] I. Khemakhem, M.H. Ahmad-Qasem, E.B. Catalán, V. Micol, J.V. García-Pérez, M. A. Ayadi, M. Bouaziz, Kinetic improvement of olive leaves' bioactive compounds extraction by using power ultrasound in a wide temperature range, Ultrason. Sonochem. 34 (2017) 466–473, https://doi.org/10.1016/j.ultsonch.2016.06.010.

[31] A. Lama-Muñoz, M.del M. Contreras, F. Espínola, M. Moya, I. Romero, E. Castro, Optimization of Oleuropein and Luteolin-7-O-Glucoside Extraction from Olive Leaves by Ultrasound-Assisted Technology, Energies. 12 (13) (2019) 2486, https://doi.org/10.3390/en12132486.

[32] P. Xie, L. Huang, C. Zhang, F. You, Y. Zhang, Reduced pressure extraction of oleuropein from olive leaves (*Olea europaea* L.) with ultrasound assistance, Food Bioprod. Process. 93 (2015) 29–38, https://doi.org/10.1016/j.fbp.2013.10.004.

[33] H. Shirzad, V. Niknam, M. Taheri, H. Ebrahimzadeh, Ultrasound-assisted extraction process of phenolic antioxidants from Olive leaves: a nutraceutical study

using RSM and LC–ESI–DAD–MS, J. Food Sci. Technol. 54 (2017) 2361–2371, https://doi.org/10.1007/s13197-017-2676-7.

[34] C. Alcántara, T. Žugčić, R. Abdelkebir, J.V. García-Pérez, A.R. Jambrak, J. M. Lorenzo, M.C. Collado, D. Granato, F.J. Barba, Effects of ultrasound-assisted extraction and solvent on the phenolic profile, bacterial growth, and anti-inflammatory/antioxidant activities of mediterranean olive and fig leaves extracts, Molecules 25 (2020) 1718, https://doi.org/10.3390/molecules25071718.

[35] S. Şahin, R. Şamlı, Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology, Ultrason. Sonochem. 20 (1) (2013) 595–602, https://doi.org/10.1016/j.ultsonch.2012.07.029.

[36] A. Meullemiestre, E. Petitcolas, Z. Maache-Rezzoug, F. Chemat, S.A. Rezzoug, Impact of ultrasound on solid-liquid extraction of phenolic compounds from maritime pine sawdust waste. Kinetics, optimization and large scale experiments, Ultrason. Sonochem. 28 (2016) 230–239, https://doi.org/10.1016/j.ultsonch.2015.07.022.

[37] N. Rombaut, T. Chave, S.I. Nikitenko, M. El Maâtaoui, A.S. Fabiano-Tixier, F. Chemat, Modification of olive leaves' surface by ultrasound cavitation. Correlation with polyphenol extraction enhancement, Appl. Sci. 11 (2021) 232, https://doi.org/10.3390/app11010232.

[38] N.I. Lebovka, M.I. Bazhal, E. Vorobiev, Estimation of characteristic damage time of food materials in pulsed-electric fields, J. Food Eng. 54 (4) (2002) 337–346, https://doi.org/10.1016/S0260-8774(01)00220-5.

[39] A.B. Jemai, E. Vorobiev, Enhanced leaching from sugar beet cossettes by pulsed electric field, J. Food Eng. 59 (4) (2003) 405–412, https://doi.org/10.1016/S0260-8774(02)00499-5.

[40] A.-M. Abi-Khattar, H.N. Rajha, R.G. Maroun, R.M. Abdel-Massih, N. Louka, E. Debs, Green extraction of polyphenols from olive leaves using Ired-Irrad® as a pretreatment, 2020 5th Int, Conf. Renew. Energies Dev. Countries, REDEC (2020. (2020)), https://doi.org/10.1109/REDEC49234.2020.9163899.

[41] J.A. Wesselingh, R. Krishna, Mass Transfer in Multi-component Mixtures, 12 (2000). http://www.devriesboeken.nl/document/14463850.pdf.

[42] J. Crank, The mathematics of diffusion, Oxford University Press, 1979.

[43] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent, Methods Enzymol. 299 (1999) 152–178, https://doi.org/10.1016/S0076-6879(99)99017-1.

[44] N.I. Lebovka, I. Praporscic, S. Ghnimi, E. Vorobiev, Temperature enhanced electroporation under the pulsed electric field treatment of food tissue, J. Food Eng. 69 (2) (2005) 177–184, https://doi.org/10.1016/j.jfoodeng.2004.08.037.

[45] Z. Erbay, F. Icier, Thin-layer drying behaviors of olive leaves (*Olea europaea* L.), J. Food Process Eng. 33 (2010) 287–308, https://doi.org/10.1111/j.1745-4530.2008.00275.x.

[46] N. Bahloul, N. Boudhrioua, M. Kouhila, N. Kechaou, Convective solar drying of olive leaves, J. Food Process Eng 34 (2011) 1338–1362, https://doi.org/10.1111/j.1745-4530.2009.00432.x.

[47] N.I. Lebovka, M. Shynkaryk, E. Vorobiev, Moderate Electric Field Treatment of Sugarbeet Tissues 96 (2007) 47–56, https://doi.org/10.1016/j.biosystemseng.2006.09.005.

[48] M. V Shynkaryk, T. Ji, V.B. Alvarez, S.K. Sastry, Ohmic Heating of Peaches in the Wide Range of Frequencies (50 Hz to 1 MHz), 75 (2010) E493–E500. 10.1111/j.1750-3841.2010.01778.x.

[49] J.C. Martínez-Patiño, B. Gullón, I. Romero, E. Ruiz, M. Brnčić, J.Š. Žlabur, E. Castro, Optimization of ultrasound-assisted extraction of biomass from olive trees using response surface methodology, Ultrason. Sonochem. 51 (2019) 487–495, https://doi.org/10.1016/j.ultsonch.2018.05.031.

[50] H.N. Rajha, N. El Darra, N. Boussetta, E. Vorobiev, R.G. Maroun, N. Louka, Extraction of total phenolic compounds, flavonoids, anthocyanins and tannins from grape byproducts by response surface methodology. influence of solid-liquid ratio, particle size, time, temperature and solvent mixtures on the optimization process, Food Nutr, Sci. 5 (2014) 397–409, https://doi.org/10.4236/fns.2014.54048.

[51] M.D. Esclapez, J.V. García-Pérez, A. Mulet, J.A. Cárcel, Ca, Ultrasound-Assisted Extraction of Natural Products, Food Eng. Rev. 3 (2) (2011) 108–120, https://doi.org/10.1007/s12393-011-9036-6.

[52] C. Wen, J. Zhang, H. Zhang, C.S. Dzah, M. Zandile, Y. Duan, H. Ma, X. Luo, Advances in ultrasound assisted extraction of bioactive compounds from cash crops - A review, Ultrason. Sonochem. 48 (2018) 538–549, https://doi.org/10.1016/j.ultsonch.2018.07.018.