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High-Pressure-Assisted Extraction of Phenolic Compounds from Olive Leaves: optimization and Comparison with Conventional Extraction

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ABSTRACT: The scope of the study was to optimize high-pressure-assisted extraction (HPAE) conditions for the extraction of phenolic compounds from olive leaves and to compare the optimum HPAE conditions with conventional extraction (CE) (at 50 °C for 30 min). In this regard, different treatment pressure levels (300−500 MPa), time (5−15 min), and solid-to-solvent ratios (0.1−0.3 w/v) were used to optimize the total phenolic content (TPC) and oleuropein content by using the Box−Behnken design. According to the results, optimal HPAE conditions were selected as 433.3 MPa for 15 min with 0.1 w/v solid−solvent ratio to obtain the maximum TPC (57.5 mg GAE/g DW) and oleuropein content (18.45 mg/g DW). Compared to CE, HPAE increased the TPC, antioxidant capacity, and oleuropein content significantly ($p \leq 0.05$). Fourier transform infrared spectroscopy results showed no difference between HPAE and CE samples. Based on scanning electron microscopy results, HPAE caused structural changes compared to CE, and this caused to increase the mass transfer rate of phenolic substances.

KEYWORDS: *olive leaves, high hydrostatic pressure, phenolic compounds, optimization, conventional extraction*

INTRODUCTION

The olive tree (*Olea europaea* L.) is accepted as one of the oldest cultivated trees in the Mediterranean countries. Its cultivation has recently increased due to the health benefits of olive oil.[1,2] It was estimated that more than 3.1 million tonnes of olive oil was produced in 2019−2020, and Turkey is one of the most olive oil-producing countries.[3] However, during harvesting or processing, several wastes are produced such as olive leaves, pomace, and olive mill wastewater.[4] Olive leaves can represent nearly 10% of the weight of olives collected for olive oil production.[5] The valorization of olive leaves has become popular recently due to its phenolic composition including hydroxytyrosol, tyrosol, catechin, and caffeic acid.[4] Among these, oleuropein is the most well-known phenolic compound in olive leaves which gives bitterness to both table olives and virgin oil.[6] It has health benefits, such as antiviral, anti-inflammatory, anti-atherogenic, anticarcinogenic, and hepatoprotective effects.[7,8]

High hydrostatic pressure (HHP), a novel processing technique, has been used increasingly in the food industry.[9] It includes using pressures up to 1000 MPa inside a vessel filled with pressure-transmitted medium that is generally water on packaged foods.[10,11] It started to be widely used in the food industry to extend the shelf-life of foods by enhancing the microbiological safety. Conventional heat treatments require a longer treatment time, solvent consumption, and energy. However, previous studies showed that high-pressure-assisted extraction (HPAE) is a good alternative to traditional heat treatment because it decreases the extraction time, solvent consumption, and increases the extraction yield.[12−15] It was also shown that a low-energy input is needed by this technique to compress a sample to 500 MPa compared to heating up to 100 °C.[16] Therefore, HPAE could be accepted as an ecofriendly technology.

As far as we know, this study is the first effort in the literature about optimizing the extraction of phenolic compounds from olive leaves by HPAE. Thus, the focus of this study is to (i) optimize the process conditions with regard to treatment pressure, time, and solid-to-solvent ratio by the Box−Behnken design (BBD) and (ii) characterize and compare the optimum HPAE condition with conventional extraction (CE) in respect of the antioxidant capacity, total phenolic content (TPC), oleuropein content, changes in functional groups, and morphological changes.

MATERIALS AND METHODS

Materials. Olive leaf samples of *O. europaea* L. were kindly provided from a local producer (Zest Ayvalık, Balıkesir, Turkey). The samples were lyophilized (Zhejiang Value Mechanical & Electrical Products Co., Ltd., Wenling City, China). The final moisture content of olive leaves was found to be 5.0 ± 0.3%. Then, the samples were grinded by a Waring commercial blender model 51BL32 and stored at 4 °C till further use.

Conventional Extraction. CE treatment was performed in light of our previous studies with some modifications.[12,17] Briefly, dried

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olive leaves and 70% ethanol solution (0.1 w/v) were prepared and kept in a water bath (WiseCircu, Seoul, Korea) at 50 °C for 30 min. Next, the samples were filtrated by Whatman no. 1 filter paper and kept at 4 °C until analyzed for further analysis.

High-Pressure-Assisted Extraction. HPAE treatment was performed in a 100 mL capacity 760.0118-type pressure equipment (SITEC-Sieber Engineering AG, Zurich, Switzerland), with water as the pressurizing medium. The pressurization time reported in this study did not include the pressure increase and release times because of the short pressure release time (less than 20 s). After extraction, samples were filtrated by Whatman no. 1 filter paper and kept at 4 °C until analyzed for further analysis.

Experimental Design. A BBD with three variables was used to investigate the response pattern and then establish a model. The independent HPAE parameters were pressure (X_1), time (X_2), and solid-to-solvent ratio (X_3). The model parameters are given in Table 1. The model parameters were selected according to the results from

Table 1. Experimental Design of Independent Variables for Olive Leaves

factor levels	independent variables		
	pressure (X_1)	time (X_2)	solid−solvent ratio (X_3)
−1	300	5	0.1
0	400	10	0.2
1	500	15	0.3

our previous studies[12,17,18] and preliminary trials. The extraction temperature and ethanol−water solution were constant in this research at 25 °C and 70%, respectively. Thus, the experimental design included 15 runs with three replicates. The response variables (Y) were TPC and oleuropein amount. Model building, experimental results, and designs were processed using MINITAB version 16.0. A total of 15 experiments including three replicates at the center point were carried out. A second-order polynomial mathematical model was used to measure the relation between the response and the independent variables as shown below

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_i X_i^2 + \sum_{i=1}^{3} x \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

where Y denotes the response variables of TPC and oleuropein content, X_i and X_j are independent factors, and β_0, β_i, and β_{ij} are the regression coefficients of the model.

Model Verification. The predictive extraction model was verified by calculating the relative error of the optimum HPAE condition. The relative error was found according to the following formula

$$\text{error } \% = \left| \frac{X_{\text{Predicted}} - X_{\text{Actual}}}{X_{\text{Actual}}} \right| \times 100 \quad (2)$$

Total Phenolic Content. TPC was measured according to the study of Altemimi et al.[19] A sample of 0.1 mL was mixed with 0.75 mL of 10% Folin−Ciocalteu solution. Next, 0.75 mL of sodium carbonate solution (75 g L^{-1}) was added to the mixture. After waiting for 1 h of incubation time in the absence of light, the absorbance of the mixture was measured at 725 nm in a spectrophotometer. The gallic acid standard solutions were used for calibration, and the results were expressed as milligrams of gallic acid equivalents/gram of the dry weight (DW) of olive leaves (mg GAE/g DW).

Antioxidant Capacity. *DPPH• Radical Scavenging Activity.* The scavenging effect of HPAE and CE extracts was analyzed according to the study of Brand-Williams et al.[20] with some modifications. Briefly, 0.1 mL of extract was mixed with 3.9 mL of DPPH• radical solution (0.1 mM) prepared with 70% ethanol. Then, the mixture was kept at room temperature in dark for 30 min. The absorbance of samples was recorded at 517 nm in a spectrophotometer (Shimadzu UV-1700, Japan). The antioxidant capacity results were given as % inhibition of DPPH• capacity and calculated according to the following equation

$$\%\ \text{inhibition of DPPH}^{\bullet}\ \text{capacity} = [1 - (A_s/A_c)] \times 100 \quad (3)$$

where A_s and A_c are the absorbance values of the sample and control, respectively.

Ferric Reducing Antioxidant Power. Ferric reducing antioxidant power (FRAP) analyses were performed according to the study of Benzie and Strain.[21] FRAP reagent was prepared by mixing 300 mM sodium acetate trihydrate (pH 3.6), triphenyltetrazolium chloride (TPTZ) (40 mM, dissolved in 40 mM HCl), and 20 mM aqueous ferric chloride in a 10:1:1 proportion. After the preparation of the FRAP reagent, 0.1 mL of the sample was mixed with 3 mL of the FRAP reagent. At t = 0 and t = 4 min, the sample's absorbance was measured at 593 nm. To quantify the antioxidant capacity, ferrous sulfate standard solution ($FeSO_4$) was used, and the FRAP results were given as mmol $FeSO_4$/100 g DW.

Scanning Electron Microscopy. SEM analysis was used to analyze the morphological changes of the olive leaves after extraction. Before analysis, the extracts were lyophilized and coated with a thin layer of Au−Pd. Experiments were performed at METU-Central Laboratory. The morphological structures were examined by a QUANTA 400F field emission SEM system at 30. 0 kV with 2000x magnification.

High-Performance Liquid Chromatography. Oleuropein content is quantified by an HPLC instrument (Shimadzu Scientific Instruments, Japan) according to the method of Al-Rimawi.[22] Samples were filtered by 0.45 μm nylon filters before the analysis. The HPLC system consisted of a degasser (DGU-20A5), a pump (LC-20AD), an autosampler (SIL-20AHT), a column oven (CTO-20A), and a PDA detector (SPD-M20A). The analysis was performed with a Hypersil GOLD Phenyl HPLC column (250 × 4.6, 5μm, Thermo Fisher Scientific, USA) by using acetonitrile/water (20/80 v/v) including 0.1% acetic acid as the mobile phase. Isocratic elution was performed with a flow rate of 1 mL/min and 20 μL injection volume. Oleuropein was monitored at 280 nm, and an external calibration curve was prepared in the range of 25−200 ppm. Necessary dilutions were made prior to analysis. The equation of the calibration curve was found as y = 5679.58x + 5633.75 (R^2 = 0.999).

Fourier Transform Infrared Spectroscopy. FTIR analysis of samples was applied with IR spectroscopy (PerkinElmer Instrument, The Spectrum 400, USA). The lyophilized sample was placed over the attenuated total reflection crystal, and the spectra were recorded in the range of 400 to 4000 cm^{-1} with 16 scans at a resolution of 4 cm^{-1}.

Statistical Analyses. Minitab 16.0 software was used to carry out the optimization and statistical analysis. Analysis of variance (ANOVA) test was used to evaluate the significance of statistical terms in the regression equation. Tukey's multiple comparison test was performed to evaluate the significant differences between the experimental mean values. p values of <0.05 and <0.001 were considered statistically significant and highly significant, respectively.

RESULTS AND DISCUSSION

TPC and Oleuropein Content. *Fitting the Model.* The impact of independent variables, namely pressure, time, and solid-to-solvent ratio, on the response variables (TPC and oleuropein content) is shown in Table 2. Polynomial equation coefficients were calculated according to these experimental data used to predict the values of TPC and oleuropein content. The regression equation for the response variables was obtained through BBD

$$\begin{aligned}\text{TPC (mg GAE/g DW)} &= 10.939 + 0.282X_1 + 3.036X_2 - 500.358X_3 - \\ &\quad 0.0004X_1^2 - 0.127X_2^2 + 868.733X_3^2 + 0.003X_1X_2 \\ &\quad + 0.042X_1X_3 - 1.956X_2X_3 \end{aligned} \quad (4)$$

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Table 2. Experimental Results of TPC and Oleuropein Content of Olive Leaves by BBD

	coded variables			responses	
run	X_1	X_2	X_3	TPC (mg GAE/g DW)	oleuropein (mg/g)
1	0	−1	1	11.91 ± 0.20	4.37 ± 0.08
2	1	−1	0	14.14 ± 0.66	4.52 ± 0.11
3	0	1	−1	57.36 ± 0.56	18.27 ± 0.57
4	−1	1	0	21.37 ± 0.80	7.34 ± 0.26
5	0	−1	−1	42.22 ± 0.87	12.90 ± 0.64
6	−1	0	1	13.94 ± 0.25	2.83 ± 0.05
7	1	0	1	18.07 ± 0.65	6.05 ± 0.48
8	0	1	1	21.47 ± 0.10	6.44 ± 0.01
9	−1	0	−1	47.12 ± 0.43	13.46 ± 0.50
10	0	0	0	28.65 ± 0.43	7.48 ± 0.02
11	1	0	−1	48.89 ± 0.83	14.33 ± 0.26
12	1	1	0	30.16 ± 0.28	9.67 ± 0.10
13	−1	−1	0	14.25 ± 0.32	3.80 ± 0.07
14	0	0	0	28.50 ± 0.34	7.49 ± 0.04
15	0	0	0	28.62 ± 0.38	7.50 ± 0.02

$$\text{oleuropein content (mg/g DW)} = 6.749 + 0.086X_1 + 0.421X_2 - 173.512X_3 - 0.0001X_1^2 - 0.001X_2^2 + 287.041X_3^2 + 0.0006X_1X_2 + 0.059X_1X_3 - 1.316X_2X_3 \quad (5)$$

For TPC, coefficient of determination values (R^2) and adjusted determination coefficients (R_{adj}^2) were found as 0.9820 and 0.9774, respectively. R^2 and R_{adj}^2 were found to be 0.9892 and 0.9864, respectively, for oleuropein content (Table 3). At both regression equations, R^2 and R_{adj}^2 values were close to 1; so, the empirical model fit the experimental values well.[23] Also, the lack of fit p-values was insignificant. These showed that a second-order polynomial mathematical model showed a good representation of TPC and oleuropein content. Furthermore, there was a strong correlation between TPC and oleuropein content based on the Pearson correlation (r = 0.93). Irakli et al.[6] reported a strong correlation (r = 0.933) between oleuropein and TPC by using ultrasound-assisted extraction. Şahin et al.[24] also investigated that there was a strong correlation (r > 0.81) between the oleuropein content and TPC by using the automatic solvent extraction method. In experimental values, the highest TPC content was observed at 400 MPa for 10 min with 0.1 w/v solid−solvent ratio (48.89 mg GAE/g DW) although the lowest TPC content was found as 11.91 mg GAE/g DW at 400 MPa for 5 min with 0.3 w/v solid−solvent ratio. The lowest oleuropein content was found as 2.83 mg/g DW at 300 MPa for 10 min with 0.3 w/v, whereas the highest oleuropein content was observed at 400 MPa for 10 min with 0.1 w/v as 14.33 mg/g DW. Response surface plots showed that both TPC and oleuropein content increased with increasing pressure and time (Figures 1 and 2). However, the TPC and oleuropein content decreased with the increasing solid−solvent ratio (Figure 1).

Table 3. Analysis of Variance for Regression Model Equation with HPAE Extraction Conditions on TPC and Oleuropein Content

	TPC		oleuropein	
source	coefficient	F-value	coefficient	F-value
intercept	10.9394		6.74926	
X_1	0.2817	29.24[c]	0.086	72.28[c]
X_2	3.0365	272.5[c]	0.421	347.27[c]
X_3	−500.358	142.94[c]	−173.512	2278.12[c]
X_1^2	−0.0004	35.97[c]	−0.0001	64.13[c]
X_2^2	−0.127	27.13[c]	−0.0011	0.04[a]
X_3^2	868.733	203.17[c]	287.041	372.17[c]
X_1X_2	0.0025	4.39[b]	0.0006	4.49[b]
X_1X_3	0.042	0.51[a]	0.059	17.04[c]
X_2X_3	−1.956	2.79[a]	−1.3157	21.18[c]
lack of fit		0.33[a]		2.71[a]
R^2		0.982		0.989
R_{adj}^2		0.977		0.986

[a]Nonsignificant (p > 0.05). [b]Significant (p ≤ 0.05). [c]Highly significant (p ≤ 0.001).

The dissolving process of bioactive components into the solvent is a physical process. At a higher solvent ratio, more bioactive compounds contact the solvent, and this led to higher leaching rates. In other words, a faster extraction rate was observed with a higher concentration difference between the interior of the solid and solvent.[25−27] Based on the results, the TPC and oleuropein content raised with the increased pressure level from 300 to 500 MPa. Therefore, it was clear that increasing the temperature caused to improve the recovery of the phenolic content. Based on Le Chatelier's principle, the volume of the system tends to decrease during the pressure-promoting period.[25] In this process, the extracting solvent came into cells to integrate with the bioactive components. Furthermore, the pressurized cells showed higher permeability. When the pressure was increased, more solvent could penetrate into the cells, so that more bioactive compounds could be recovered.[12] In the literature, similar findings were found. For green tea leaves, Xi et al.[25] indicated that the extraction yield increased significantly from 15% to 30% with an increase in pressure from 100 to 600 MPa. Shinwari and Rao[28] reported that TPC was raised when the pressure level was increased from 150 to 450 MPa for the recovery of phenolic compounds from saffron.

Extraction time was another important parameter for the extraction of phenolic compounds by HHPE. Based on the results, TPC and oleuropein content were increased with an increase in the extraction time from 5 to 15 min. For discarded blueberries, Briones-Labarca et al.[29] reported that the phenolic content was raised by roughly 21% while the extraction time was increased from 5 to 15 min at 500 MPa. Choi et al.[30] stated that the extraction yield was increased from 8.46 to 10.23% for extracting phenolic compounds from ground turmeric, with the rise in the treatment time from 5 to 15 min.

TPC and oleuropein content were decreased significantly (p ≤ 0.001) while the solid−solvent ratio was increased from 0.1 to 0.3 (w/v). Therefore, it can be said that reducing the solid−solvent ratio could be useful for enhancing the phenolic compounds from olive leaves by using HPAE. With a decrease in the solid−solvent ratio, the chance of bioactive components coming into contact with the extracting solvent increased, and this causes having higher leaching out rates. Jun[31] indicated that the extraction yield was increased from 17 to 30% for the recovery of phenolic compounds from green tea by HPAE when the liquid/solid ratio was raised from 10 to 20 mL/g at 500 MPa.

Verification of the Optimal Predicted HPAE Condition. To verify the reliability of BBD, a verification experiment was

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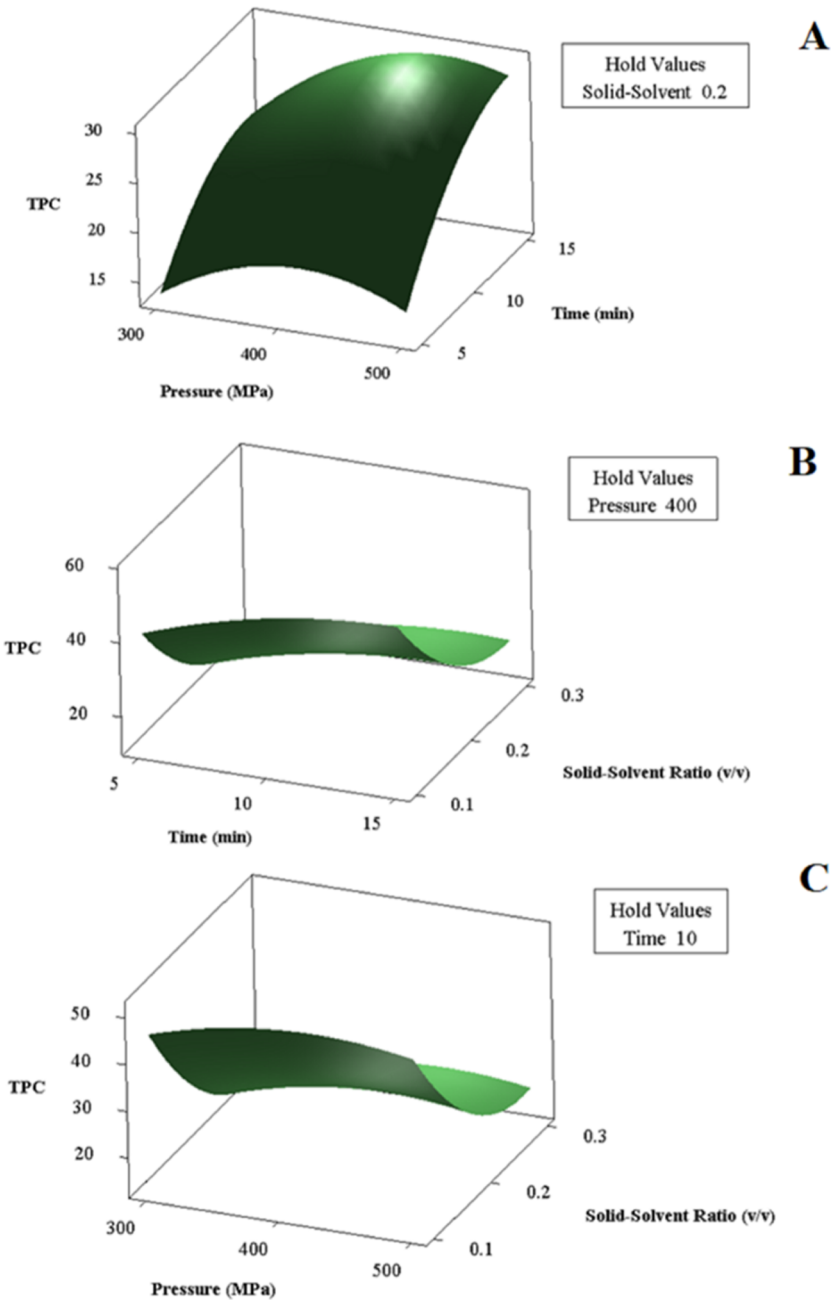

Figure 1. Response surface plots indicating the effect of HPAE on TPC: (A) TPC vs pressure (MPa) and time (min). (B) TPC vs time (min) and solid-to-solvent ratio (%). (C) TPC vs pressure (MPa) and solid-to-solvent ratio (%).

carried out at the optimum HPAE condition estimated from the model. According to the 3D surface plots and regression analysis of the independent variables, the optimum HPAE condition for the extraction of TPC and oleuropein content was found as 433.33 MPa for 15 min, with 0.1 w/v solid−solvent ratio. For TPC, the experimental value was found as 57.5 g GAE/g DW, while the predicted value was 54.91, so the error rate was found as 4.6%. For the oleuropein content, the experimental and predicted values were found as 18.45 and 17.75 g/g DW, respectively. Therefore, the error rate was 3.79%. Overall, the differences between experimental results and predicted values were small. In other words, the model fitted very well with the experimental results.

Comparison with CE. *TPC and Oleuropein Content.* The optimum HPAE condition was compared with CE, and the results are given in Table 4. HPAE increased the TPC and oleuropein content significantly ($p \leq 0.05$). Our previous studies also reported that HPAE improved the TPC compared to CE for sour cherry pomace, olive pomace, and spent coffee grounds.[12,17] Also, Dobrinčić et al.[26] found that HPAE increased the TPC compared to CE in olive leaves. In this study, the highest TPC was found as 73.79 mg/g DW at 500 MPa for 10 min. The reported value was slightly higher than that of our research, but it was found that olive leaves' cultivars were an important parameter for the content of phenolic compounds.[32] Martín-García et al.[4] reported that the highest TPC (158.7 mg/g DW) was observed at 105 °C, with 100% ethanol and 5 min pressurized liquid extraction condition . The result was higher than that of our finding due to the extraction temperature. Irakli et al.[6] found that the optimum extraction

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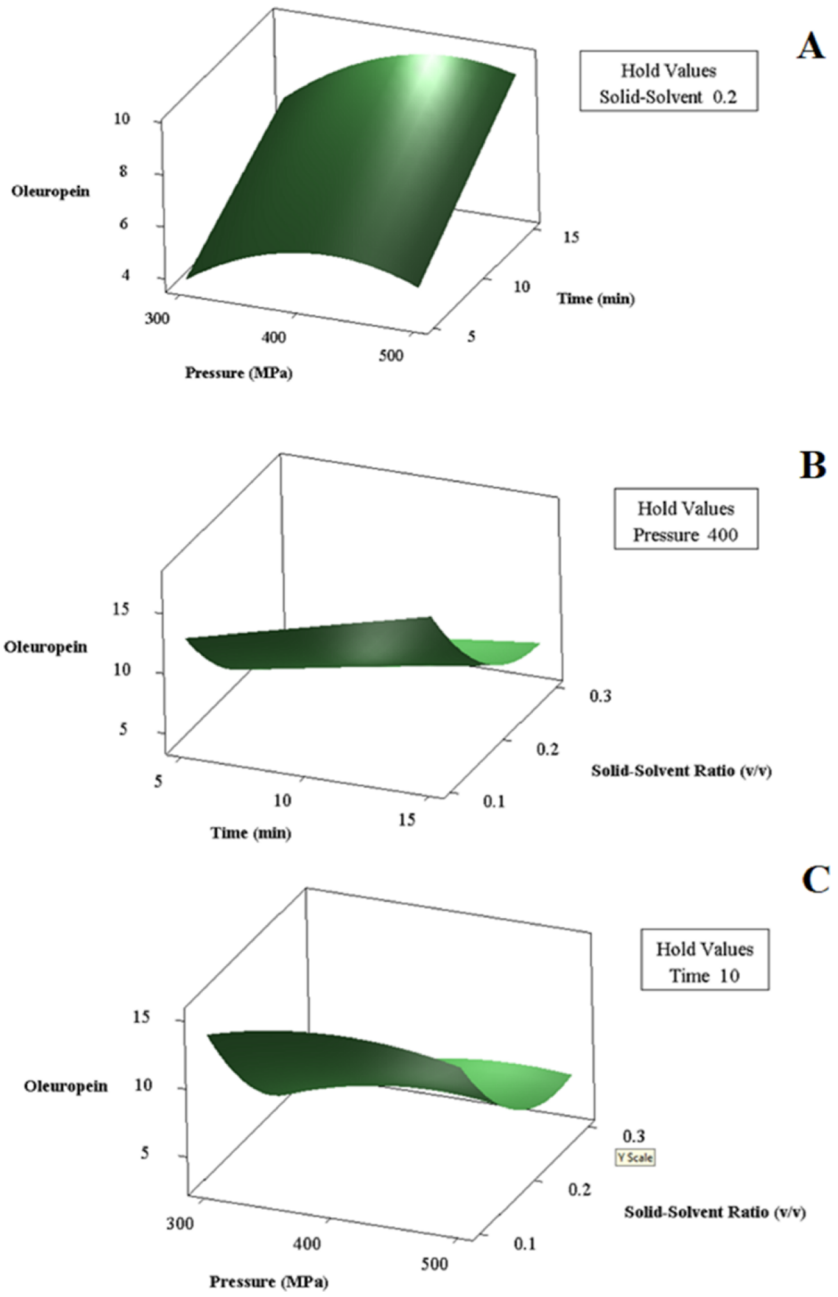

Figure 2. Response surface plots showing the effect of HPAE on oleuropein content: (A) Oleuropein content vs pressure (MPa) and time (min). (B) Oleuropein content vs time (min) and solid-to-solvent ratio (%). (C) Oleuropein content vs pressure (MPa) and solid-to-solvent ratio (%).

Table 4. TPC, Antioxidant Capacity, and Oleuropein Content Results of Phenolic Extracts from Different Extraction Techniques[a]

			antioxidant capacity	
extraction type	TPC (mg GAE/g DW)	oleuropein (mg/g DW)	DPPH•(% inhibition)	FRAP (mmol $FeSO_4$/100 g DW)
CE	11.67 ± 0.13[b]	2.47 ± 0.01[b]	55.70 ± 2.62[b]	0.53 ± 0.02[b]
HPAE	59.5 ± 0.85[a]	18.45 ± 0.13[a]	70.96 ± 0.44[a]	0.88 ± 0.01[a]

[a]Note. Values showed with different superscript letters are statistically different at $p < 0.05$. Total phenolic content (TPC), total phenolic compounds; FRAP, ferric reducing antioxidant power.

condition by ultrasound-assisted extraction (UAE) with 50% acetone for 10 min at 60 °C at a frequency of 37 kHz, for 37.44 mg GAE/g DW. Also, Martin-Garcia et al.[33] indicated that the optimum operation parameter for UAE was 151 W and 55% (v/v) EtOH for 8 min, for 40.9 mg GAE/g DW. These findings were lower than our finding because of olive leaves cultivars' difference, lower treatment time, solvent concentration, or solvent type differences. It was found that the oleuropein content was between 6 and 20 mg/g DW, and our finding was parallel to the reported literature findings.[34−37]

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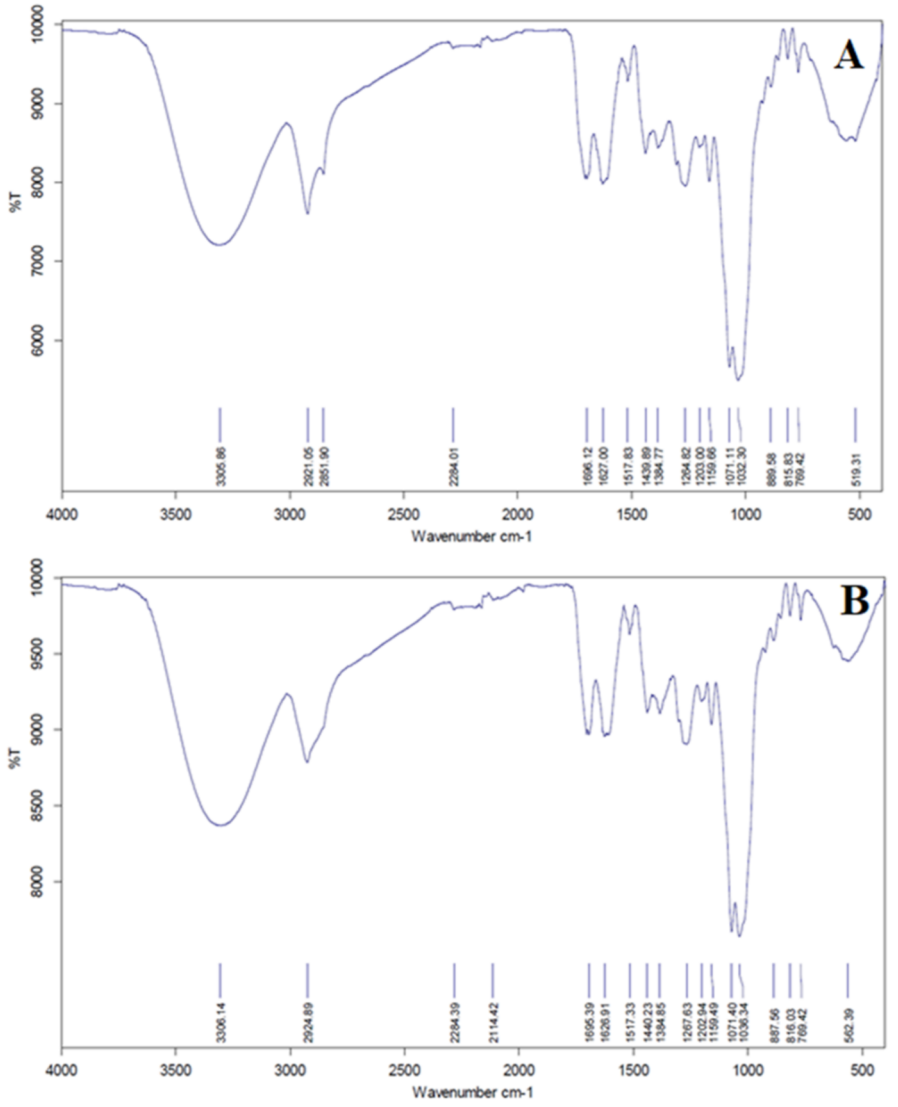

Figure 3. FTIR spectra results of the extracts treated by (A) CE and (B) HPAE (433.3 MPa, 15 min, and 0.1 w/v).

Antioxidant Capacity. The DPPH$^{\bullet}$ radical scavenging capacity and FRAP results are given in Table 4. Based on the results, HPAE raised the antioxidant capacity significantly compared to CE. This result was consistent with our previous findings for sour cherry pomace, olive pomace, and spent coffee grounds.[12,17,18]

In the literature, it was shown that HPAE increased the antioxidant capacity compared to CE. Briones-Labarca et al.[15] reported that the antioxidant capacity of Chilean papaya seeds was increased significantly by using HPAE at 500 MPa for 5, 10, and 15 min. HPAE raised the antioxidant capacity up to 272.8% compared to CE ($p \leq 0.05$). For wild *Lonicera caerulea* berry, it was shown that HPAE within different treatment levels (400 MPa for 20 min, 500 MPa for 15 min, and 600 MPa for 10 min) increased significantly ($p \leq 0.05$) the antioxidant capacity compared to CE (70 °C for 30 min).[38] It was reported that HPAE indicated 3 times higher antioxidant capacity than the heat treatment for blueberry pomace.[39] Moreira, Pintado, and Saraiva[40] stated that HPAE treatment at 500 MPa for 20 min increased the antioxidant capacity up to 48.5% compared to CE for winter savory leaves. For strawberry and blackberry purees, Patras et al.[41] showed that HPAE at 500 and 600 MPa for 15 min showed higher antioxidant capacity compared to CE (70 °C for 15 min). Xi et al.[42] reported that HPAE treatment at 450 MPa for 5 min increased by approximately 1.5 times higher than CE at the boiling temperature of the solvent (50% ethanol) for 4 h.

The antioxidant capacity was proportional to the TPC of the extracts. Therefore, increasing antioxidant capacity could be explained by the HPAE mechanism. HPAE mechanism was described with three different processing stages which were pressure boost, maintaining, and relief stages.[43] At the pressure boost stage, the pressure was raised sharply from the atmospheric pressure to the processing pressure within a short time. This increase caused disruptions of cells, so the mass transfer of the phenolic compounds was enhanced. Moreover, the mass transfer of the phenolic compounds was proportional to the pressure level and resistance of mass transfer. At the maintaining stage, the extraction solvent diffused into cells rapidly due to the first stage situation. At the last stage, relief stage, the pressure was decreased from the operation pressure to the atmospheric pressure. This resulted in dramatic changes in hydrogen bonds, ionic bonds, and hydrophobic interactions that controlled the polymeric structure of the cells. Hence, the cells could expand, and this expansion was related to porous, broken, or loose cellular

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structures, which resulted in improved mass diffusion of bioactive compounds.

Fourier Transform Infrared Spectroscopy. FTIR results are shown in Figure 3. Based on the results, it can be said that CE and HPAE do not have any destructive effects on the functional groups of the extract. This result was also observed in our previous studies for sour cherry pomace, olive pomace, and spent coffee grounds.[12,17,18] The broad band at 3700−3000 cm^{-1} corresponded to OH stretching vibrations.[44] In the literature, this wide band was possibly representing the groups of oleuropein, apigenin-7-glucoside, and/or luteolin-7-glucoside.[45] By looking at the absorbance of this band, it could be concluded that HPAE raised the extraction of oleuropein compared to CE, which was also supported by the HPLC results. The bands between 3000 and 2800 cm^{-1} were related to symmetric and asymmetric C−H stretching vibrations.[46] The bands between the 1800 and 1500 cm^{-1} region were attributed to C=O and C=C stretching vibrations (esters, acid, carboxylate, and aromatic ring). The bands between 1500 and 1200 cm^{-1} were very complex with, especially, CH and OH deformation vibrations as CO stretching vibrations (phenols). The intense bands between 1150 and 950 cm^{-1} relate to the endocyclic and exocyclic C−O stretching vibrations of carbohydrates.[46]

Scanning Electron Microscopy. The extraction rate was related to the physical change of the samples.[47,48] The morphological results of different extraction techniques are given in Figure 4. No cell damage was detected with CE (Figure 4A). Compared to CE, more contact area was observed with HPAE (Figure 4B). This result was also in agreement with our previous studies for sour cherry pomace and spent coffee grounds.[12,17] HPAE treatment enhanced the solvent entrance into the matrix cells by disrupting the cellular walls and the hydrophobic bonds in cellular membranes, leading to a rise in the mass transfer rate of phenolic compounds.[49] In brief, SEM analysis results showed that HPAE treatment caused a rise in the release of phenolic compounds compared to CE because of the morphological changes.

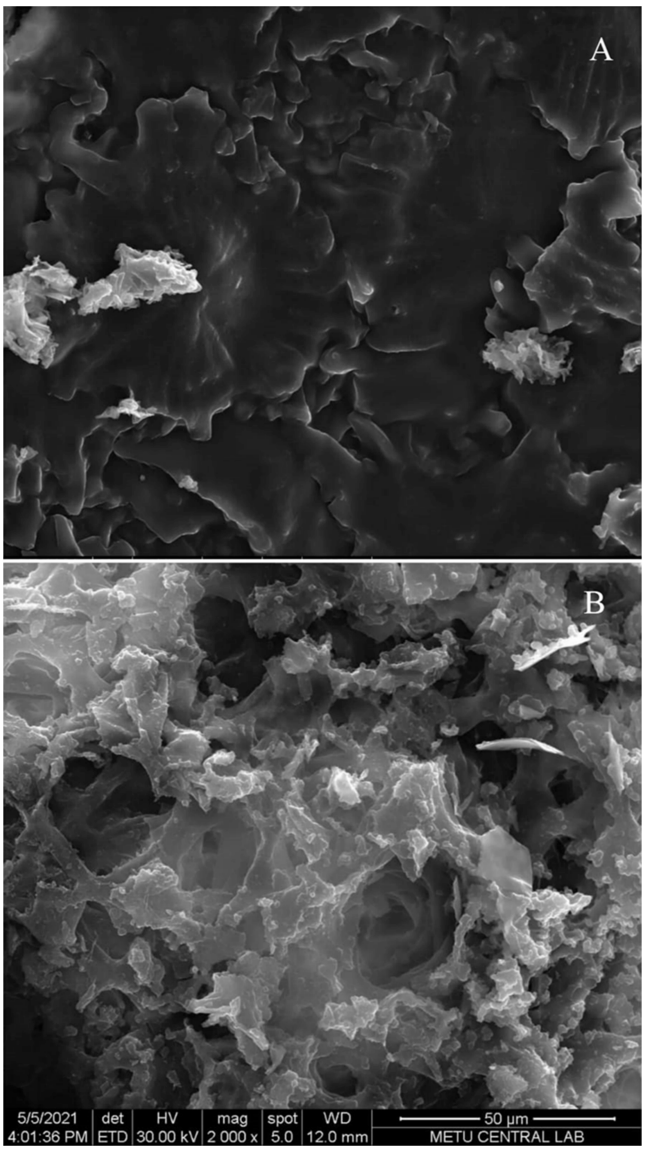

Figure 4. SEM results of extracts treated by (A) CE and (B) HPAE (433.3 MPa, 15 min, and 0.1 w/v).

CONCLUSIONS

In the literature, to the best knowledge of the authors, this study is the first effort to optimize HPAE on the recovery of phenolic compounds from olive leaves extensively. The optimum HPAE condition was selected as 433.33 MPa for 15 min with 0.1 w/v solid−solvent ratio to obtain the maximum TPC (57.5 mg GAE/g DW) and oleuropein content (18.45 mg/g DW). Also, HPAE raised the TPC, antioxidant capacity, and oleuropein content significantly compared to CE ($p \leq 0.05$). According to the FTIR results, there was no significant difference in the chemical structure of phenolic compounds by HPAE. Furthermore, HPAE caused morphological changes compared to CE, increasing the contact area and the release of phenolic compounds. In brief, HPAE has a good potential to be used as a recovery method of phenolic compounds from waste material such as olive leaves based on reducing treatment time, temperature, and solvent consumption.

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Notes

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