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Optimization and Comparison of High-Pressure-Assisted Extraction of Phenolic Compounds from Olive Pomace

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Cite This: *ACS Food Sci. Technol.* 2022, 2, 1862−1869

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ABSTRACT: In this study, it was aimed to find the optimum condition for extraction of phenolic compounds from olive pomace by using high-pressure-assisted extraction (HPAE). In this method, different pressure parameters (300−500 MPa), times (5−15 min), and ethanol concentrations (50−90% v/v) were used. According to Box−Behnken design, 15 experimental runs were performed to find the optimum condition of total phenolic and oleuropein contents. Also, the optimum HPAE condition was compared with that of classical solvent extraction (CSE). Based on the results, the optimum HPAE condition was chosen as 409 MPa for 11.16 min with 63.74% v/v ethanol concentration. According to ANOVA results, pressure, time, and ethanol concentration had significant effects on the total phenolic content and oleuropein content ($p \leq 0.05$). Compared to CSE, it was found that HPAE increased the total phenolic content, antioxidant capacity, and oleuropein content significantly. According to scanning electron microscopy and Fourier transform infrared spectroscopy results, it was found that the mass-transfer rate of phenolic compounds increased because of morphological changes, but there were no chemical changes in phenolic compounds observed when HPAE treatment was applied. Overall, it can be said that HPAE treatment was an effective method to improve the recovery of phenolic compounds from olive pomace compared to the traditional treatment.

KEYWORDS: *olive pomace, high pressure-assisted extraction, optimization, oleuropein, phenolic compounds*

INTRODUCTION

Over the last few years, olive oil consumption has increased worldwide, and nearly 3.2 million tons of olive oil was produced worldwide in 2020.[1] Mediterranean countries such as Spain, Italy, Greece, Portugal, Greece, and Turkey provide 97% of the total olive oil production in the world and represent a major industry in the region.[2,3] Olive pomace, a solid byproduct of olive oil production, is nearly 40% of the total weight of olives being processed in the mill.[4] It includes a high concentration of phenolic compounds, and so olive pomace has a high polluting organic load and a phytotoxic effect, and this results in a significant environmental hazard.[5,6] Therefore, extraction of phenolic compounds from olive pomace is very important to decrease environmental damage, especially for Mediterranean countries with low-cost sources and with beneficial human physiological actions.[7,8]

Conventional solvent extraction (CSE) methods are known to have a low extraction yield and longer extraction time, causes degradation of heat-sensitive phenolic compounds, and consumes high energy.[9,10] Therefore, novel extraction techniques such as high hydrostatic pressure (HHP) have become popular because most of these techniques claimed to improve efficiency and decrease the extraction time and solvent consumption compared to conventional methods.[11−13] High hydrostatic pressure extraction (HHPE), known as cold pasteurization technique, is an emerging technology increasingly used in the food industry, and it is accepted as an alternative method to heat treatment.[14] The fundamental of this technique is to apply high pressure up to 1000 MPa for a certain time and temperature to the food material.[15,16] Furthermore, this technology can be considered as an eco-friendly technology because compressing the food at 500 MPa needs less energy than heating to 100 °C.[17] In the literature, it was found that HHP increased the extraction of phenolic compounds from sour cherry pomace, spent coffee ground, citrus peels, pomegranate, and green tea leaves.[18−22] However, it was found that the phenolic compounds were affected by treatment conditions such as pressure level, treatment time, and solvent concentration. Therefore, optimization was needed to find the best conditions for high-pressure-assisted extraction (HPAE) of phenolic compounds.

To the extent of our knowledge, there has been no published study in the literature related to the optimization of phenolic extraction from olive pomace by using HPAE and comparison with the traditional extraction method. Therefore, the aim of the study is to (i) optimize HPAE in terms of pressure, time, and solvent concentration and (ii) compare the optimum HPAE condition with the conventional method with regard to the total phenolic content (TPC), antioxidant capacity, oleuropein content, morphological changes, and chemical changes in phenolic compounds.

Received: August 22, 2022
Revised: October 27, 2022
Accepted: November 2, 2022
Published: November 11, 2022

MATERIALS AND METHODS

Chemicals. Gallic acid, methanol, ethanol, Folin-Ciocalteu reagent (2 N), DPPH•, sodium acetate, and triphenyltetrazolium chloride (TPTZ) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). $FeCl_3 \cdot 6H_2O$, sodium carbonate, acetic acid, and HCl were purchased from Merck (Darmstadt, Germany). The chemicals mentioned above were of analytical grade. Oleuropein (purity by HPLC, ≥80%) and water used for HPLC analysis and acetonitrile were from Sigma-Aldrich.

Materials. Dried olive pomace was kindly provided by Zest Ayvalık (Balikesir, Turkey). Before extraction, the samples were ground by using a blender (Waring Commercial, Torrington, CT) with a particle size of 1.18 mm obtained via sieve separation. The moisture content of olive pomace samples was 5.1 ± 0.2%, and they were kept 4 °C until usage.

CSE. CSE of olive pomace was performed according to our previous study with some modifications.[21] In brief, the olive pomace sample was mixed with ethanol solution, whose concentration was decided according to the HPAE results, in the ratio of 0.1 w/v. Then, it was kept in a water bath for 30 min at 50 °C. Then, it was filtered and stored at 4 °C till analysis.

HPAE. HPAE was carried out in a laboratory-scale HHP machine (SITEC-Sieber Engineering AG, Zurich, Switzerland). The extraction vessel had 100 mL total internal volume, and water was used as a pressure transmitting medium. For each extraction, 0.1 w/v olive pomace sample and solvent were prepared in 25 mL sterile polyethylene cryotubes (Biosigma Sri, CRYOGENLine, CryoGen Tubes). After HPAE extraction, each sample was filtered by using a Whatman no. 1 filter paper.

Experimental Design. Experimental design was performed by using Box−Behnken design (BBD). The pressure level (X_1), treatment time (X_2), and ethanol concentration (X_3) were model parameters. The model parameters and factor levels are given in Table 1. The treatment temperature and the solid-to-solvent ratio were kept constant (25 °C and 0.1 w/v). The model design was carried out by using MINITAB (Version 16.1.0.0, Minitab Inc., Coventry, UK). The experimental design had 15 runs, and each run had 3 replicates. TPC and oleuropein content were chosen as response variables (Y). A second-order polynomial mathematical model was used to measure the relation between the response and the independent variables as shown below

Table 1. Independent Variables and Selected Levels Used in BBD for TPC and Oleuropein Content for HPAE

	independent variables		
factor levels	pressure (X_1)	time (X_2)	ethanol concentration (X_3)
−1	300	5	50
0	400	10	70
1	500	15	90

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

where Y was the responses that are TPC and oleuropein content, X_i and X_j are independent variables, and β_0, β_i, and β_{ij} are the intercept term, coefficient, and coefficient of interaction effects, respectively.

Model Validation. Relative error was calculated to determine the accuracy of the models. It was calculated for the extraction condition which was found as the maximum TPC and oleuropein content. The error rate was calculated by using the following equation

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

TPC. TPC was measured based on Singleton & Rossi.[23] In brief, 0.75 mL of Folin-Ciocâlteu solution (0.1 v/v) was mixed with 0.1 mL of the sample. After mixing, 0.75 mL of sodium carbonate solution (75 g/L) was added, and the mixture was incubated for 1 h in the dark at room temperature. After this, the absorbance of the sample was recorded at 725 nm by using a spectrometer (Shimadzu UV-1700, Japan). The results were expressed in mg of gallic acid equivalent (GAE) per gram of dry weight (DW) .

Antioxidant Capacity. *DPPH Radical Scavenging Capacity.* The percent radical scavenging activity of samples was quantified by mixing 0.1 mL of the sample to inhibit 3.9 mL of DPPH solution.[24] After the mixture was kept in the dark for 30 min at room temperature, the absorbance of the sample was measured at 517 nm by using a spectrometer (Shimadzu UV-1700, Japan). The results were expressed as Trolox equivalent unit (mg TE/g).

FRAP. For the assay, 0.1 mL of the sample was mixed with 3 mL of ferric reducing antioxidant power (FRAP) reagent prepared by mixing 300 mM sodium acetate trihydrate (pH 3.6), TPTZ (40 mM), and 20 mM aqueous ferric chloride in a 10:1:1 proportion. Then, the absorbance was measured at 593 nm at $t = 0$ and $t = 4$ min by using a spectrometer (Shimadzu UV-1700, Japan).[25] The standard ferrous sulfate solution ($FeSO_4$) curve was used as a standard to quantify the antioxidant activity of the samples.

SEM. To evaluate the morphological changes of olive pomace by HPAE or CSE, scanning electron microscopy (SEM) (Nova NanoSEM 430, FEI, Oregon) analysis was carried out. Samples were lyophilized and coated with a thin layer of Au−Pd before analysis.

Oleuropein Content. Oleuropein content of the samples was measured by high-performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan) with a diode array detector. HPLC included an Eclipse XDB-C18 column (Agilent197 USA) (5 μm, 250 × 4.60 mm) as a stationary phase, with a mobile phase of acetonitrile/water (20:80, v/v) containing 0.1% of acetic acid at a flow rate of 1.0 mL/min was used. Also, oleuropein was quantified at 280 nm.[26] Calibration curves were prepared by external standards with a concentration ranging from 0 to 200 ppm. Calibration equation was found as $y = 5679.58x + 5633.75$ with $R^2 = 0.999$.

FTIR. IR spectroscopy coupled to an attenuated total reflectance (ATR) accessory (Shimadzu Corporation, Kyoto, Japan) was performed to evaluate the chemical changes of phenolic compounds caused by different extraction treatments. Before analysis, the samples were lyophilized to remove spectral bands because of the solvent. A few milligrams were put into ATR cells, and the samples were pressed slightly with a system tip in order to maximize the surface of contact. Spectra were measured in the range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} with 16 scans.

Statistical Analysis. MINITAB software package (Ver 16.1, Minitab Inc., Coventry, UK) was used to analyze the results. To examine the differences between samples, analysis of variance (ANOVA) analysis was performed. Tukey's multiple comparison test at $\alpha \leq 0.05$ was conducted to evaluate the significant differences between samples.

RESULTS AND DISCUSSION

Fitting the Model for TPC and Oleuropein Content. Experimental results of TPC and oleuropein content of olive pomace affected by different HHPE parameters are shown in Table 2. According to the results, TPC was found to be between 6.68 and 15.85 mgGAE/gDW, while oleuropein content was between 19.73 and 42.56 g/100 gDW. These experimental data were used to determine the regression coefficients in the second-order polynomial equation and the ANOVA of the regression models (Table 3). The regression equations for the TPC and oleuropein content were obtained by the following equations

https://doi.org/10.1021/acsfoodscitech.2c00256
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Table 2. TPC and Olueopein Content of Different Conditions of HPAE Based on BBD for Response Surface Analysis

	coded variables			responses	
run	X_1	X_2	X_3	TPC (mgGAE/gDW)	oleuropein (mg/100gDW)
1	0	−1	1	6.68 ± 0.03	19.73 ± 0.05
2	1	−1	0	9.76 ± 0.02	28.47 ± 0.07
3	0	1	−1	13.32 ± 0.03	35.55 ± 0.61
4	−1	1	0	12.23 ± 0.03	29.12 ± 0.06
5	0	−1	−1	10.06 ± 0.02	32.23 ± 0.09
6	−1	0	1	6.81 ± 0.05	16.92 ± 0.07
7	1	0	1	7.77 ± 0.03	23.38 ± 0.08
8	0	1	1	8.59 ± 0.04	20.41 ± 0.08
9	−1	0	−1	11.79 ± 0.06	27.24 ± 0.09
10	0	0	0	15.84 ± 0.04	42.55 ± 0.02
11	1	0	−1	11.18 ± 0.02	31.42 ± 0.09
12	1	1	0	12.79 ± 0.04	36.74 ± 0.03
13	−1	−1	0	10.00 ± 0.03	27.74 ± 0.03
14	0	0	0	15.79 ± 0.04	42.53 ± 0.05
15	0	0	0	15.80 ± 0.03	42.56 ± 0.02

Table 3. Model Summary Statistics: ANOVA, Regression Coefficient, and Coefficient of Determination (R^2) for TPC and Oleuropein Content

	TPC		oleuropein	
source	coefficient	*F*-value	coefficient	*F*-value
intercept	−68.4580		−212.352	
X_1	0.1660	14.13[c]	0.6133	12.23[c]
X_2	1.9932	55.27[c]	5.35	5.48[b]
X_3	1.2048	1310.82[c]	3.1686	393.57[c]
X_1^2	−0.0002	560.11[c]	−0.0007	285.31[c]
X_2^2	−0.0820	435.7[c]	−0.2492	217.56[c]
X_3^2	−0.0097	1550.29[c]	−0.0234	489.49[c]
X_1X_2	0.0004	3.64[a]	0.0008	0.91[a]
X_1X_3	0.0002	17.62[c]	−0.0004	3.52[a]
X_2X_3	−0.0033	12.37[c]	−0.0067	2.71[a]
lack of fit		0.02[a]		0.03[a]
R^2		0.9904		0.9765
R_{adj}^2		0.988		0.9705

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

$$\text{TPC(gGAE/gDW)} = -68.458 + 0.1660X_1 + 1.9932X_2 + 1.2048X_3 - 0.0002X_1^2 - 0.0820X_2^2 - 0.0097X_3^2 + 0.0004X_1X_3 + 0.0002X_1X_3 - 0.0033X_2X_3 \quad (3)$$

$$\text{olueopein content (g/100gDW)} = -212.352 + 0.613X_1 + 5.350X_2 + 3.169X_3 - 0.0007X_1^2 - 0.249X_2^2 - 0.0234X_3^2 + 0.0008X_1X_2 - 0.0004X_1X_3 + 0.0067X_2X_3 \quad (4)$$

It was found that the R^2 and R_{adj}^2 values were 0.9904 and 0.9880 for TPC and 0.9765 and 0.9705 for oleuropein content. According to Pearson correlation results, there is a strong correlation between TPC and oleuropein content ($r = 0.923$) because oleuropein was one of the major phenolic compounds in olives.[27] Also, ANOVA results showed that the overall model for both TPC and oleuropein was highly significant ($p \leq 0.001$). Also, these models had a high F value, and the lack of fit of both models was not significant ($p > 0.05$). Both models had high R^2 and R_{adj}^2 values, so it could be said that there was a higher degree of precision and reliability of the experimental values (Table 2). The generated three-dimensional response surface graphs corresponding to all responses indicated the interactive effects of the variables (Figures 1 and 2).

According to the results, pressure affected the TPC and oleuropein content significantly ($p \leq 0.001$). Pinela et al.[14] reported that pressure was a significant factor in the extraction of phenolic compounds from watercress at the pressure level between 0.1 and 600 MPa. Our previous studies also showed that pressure had a significant effect on phenolic extraction for sour cherry pomace and spent coffee ground for the pressure level between 300 and 500 MPa.[21,22] High pressure increased the solvent strength. It also increased the density and solubility of polar compounds.[28] Furthermore, the high pressure enhanced solvent penetration to the cells by interrupting the cell walls, and this increased the permeability. Thus, high-pressure treatment increased the mass-transfer rate.[29]

The extraction time had a significant effect on the TPC and oleuropein content ($p \leq 0.05$). When the treatment time was increased, the solvent swelled up and more solvent could be permeated to the sample. In other words, more solute and solvent contacted with the sample, and this resulted in increasing TPC and oleuropein content.[10] Our previous study showed that the TPC of sour cherry pomace and spent coffee ground increased significantly when the treatment time was increased.[21,22] Also, Jamaludin et al.[30] showed that the yield of scopoletin and alizarin from noni fruits increased significantly from 67.1 to 74.3% and from 64.1 to 73.0%. Also, Moreira et al.[13] showed that intermediate extraction times (roughly 10 min) were enough to obtain the highest values for TPC for stinging nettle leaves.

Choosing a suitable solvent type is important for the optimization process. Solvents such as ethanol, acetone, and methanol are generally used for the extraction process, especially for less-polar bioactive compounds. Among solvents, ethanol is recommended as the most common solvent for extracting many active compounds from plants due to its nontoxicity and environmental friendly properties.[10,30,31] Based on the fitting results, ethanol concentration had a significant effect on the TPC and oleuropein content ($p \leq 0.001$). It was seen that the TPC and oleuropein content increased when the ethanol content was increased from 50 to 70%. However, the TPC and oleuropein content decreased on increasing the ethanol concentration from 70 to 90%. Corrales et al.[32] showed similar results for grape byproducts under 600 MPa treatment for 1−2 min.[32] Jamaludin et al.[30] found that the optimum ethanol concentration was 65% for obtaining the highest phenolic content from noni fruits treated by HHP.

Verification of the Model. To verify the reliability of the models under the optimal condition, the experiment for the optimum condition was performed. Based on the model, it was found that the optimum condition was found as 409 MPa for 11.16 min with 63.74% v/v ethanol concentration. At this pressure, time, and ethanol concentration, the TPC and oleuropein content were found as 16.05 gGAE/gDW and 43.77 g/100 gDW. For the experimental results, TPC and oleuropein content at the optimum condition were determined

https://doi.org/10.1021/acsfoodscitech.2c00256
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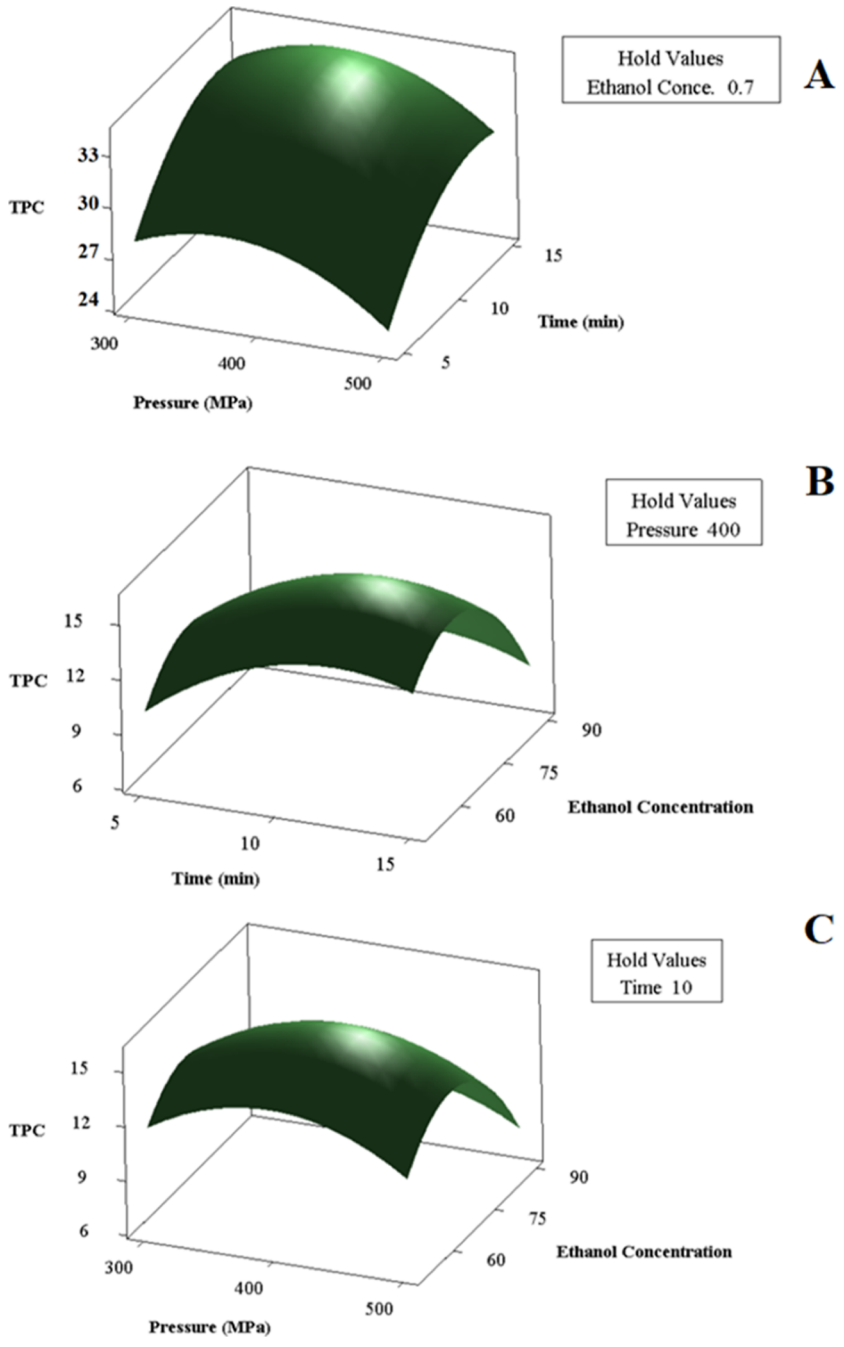

Figure 1. Response surface plots for TPC: (A) TPC versus pressure (MPa) and time (min). (B) TPC vs time (min) to ethanol concentration (%). (C) TPC vs pressure (MPa) and ethanol concentration (%).

as 16.39 gGAE/gDW and 45.90 g/100gDW. Hence, the error rate was calculated as 2.07% for TPC and 4.86% for oleuropein content. As a result, the predicted conditions can be used for further analysis to obtain extraction of phenolic compounds from olive pomace using HPAE. BBD was used to optimize the extraction of phenolic compounds by HPAE from palm dates, kirenol, blueberry pomace, and watercress.[14,33−35]

Comparison of HPAE with CSE. *TPC and Oleuropein Content.* To validate the effectiveness of HPAE in maximizing the contents of total phenolic and oleuropein compounds, a comparative study was performed between HPAE and CSE. The HPAE conditions were as follows: 409 MPa for 11.16 min with 63.74% v/v ethanol concentration. The TPC, and oleuropein content results of these treatments are given in Table 4 and Figure S1. According to the results, HPAE treatment indicated a significant increase in TPC and oleuropein content ($p \leq 0.05$).

In the literature, there were some studies that showed that HPAE increased the TPC compared to CSE. Briones-Labarca et al.[36] found that HHPE treatment for 15 min increased the TPC by 23.3% compared to CSE at room temperature for 16 h for discarded blueberries. Also, Jun[20] reported that HHPE reduced the extraction time compared to heat treatment. The TPC of green tea leaves by HHPE treatment at 500 MPa for 1 min was the same as CSE at 20 °C for 20 h. For extraction of phenolic compounds from chilean papaya seeds, Briones-Labarca et al.[37] showed that HHPE treatment at 500 MPa for 15 min doubled the TPC compared to CSE at 40 °C for 15

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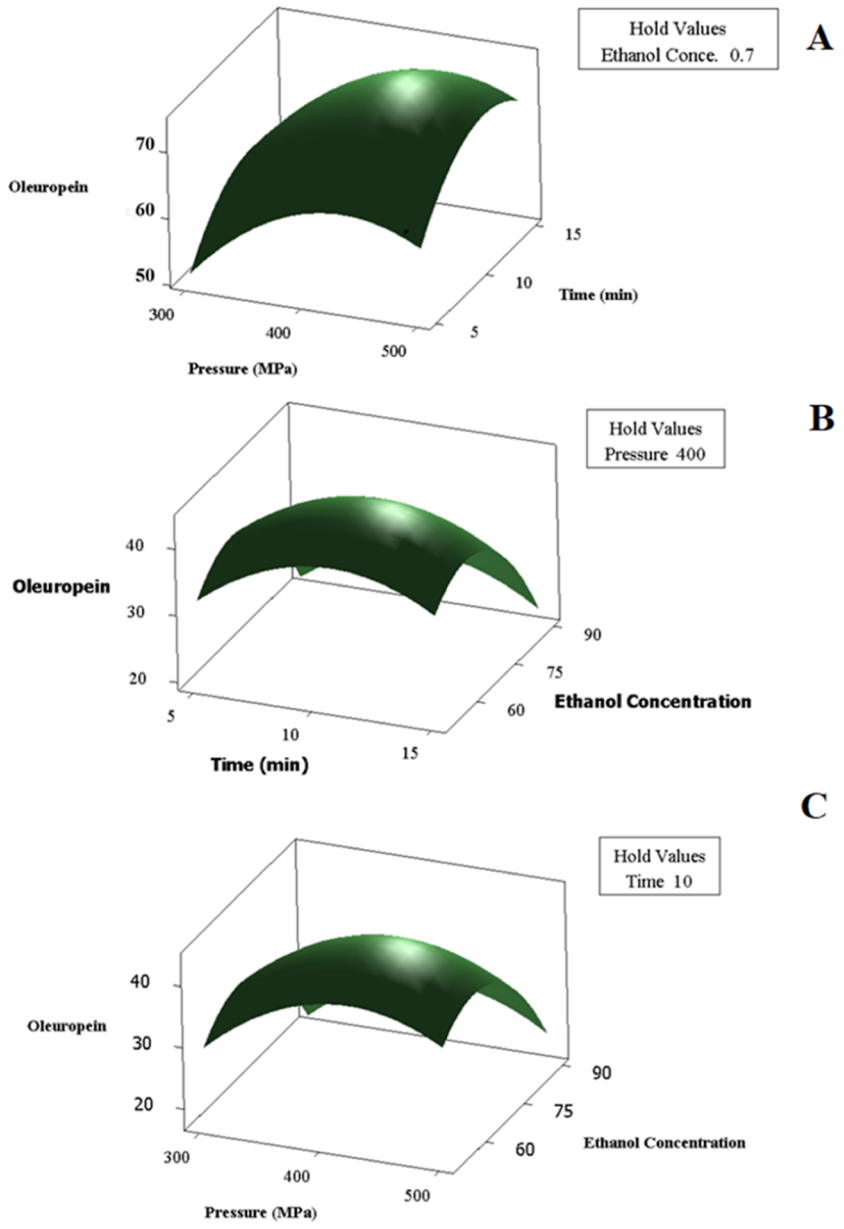

Figure 2. Response surface plots for oleuropein content: (A) Oleuropein content vs pressure (MPa) and time (min). (B) Oleuropein vs time (min) and ethanol concentration (%). (C) Oleuropein vs pressure (MPa) and ethanol concentration (%).

Table 4. TPC, Oleuropein Content, and Antioxidant Capacity of Olive Pomace Extracted by Using Different Extraction Methods

			antioxidant capacity	
extraction type	TPC (mgGAE/gDW)	oleuropein (mg/100gDW)	DRSC (mgTE/g)	FRAP (mmol$FeSO_4$/100 gDW)
CSE	6.08 ± 0.24	14.06 ± 0.14	7.05 ± 0.89	0.35 ± 0.02
HPAE	16.39 ± 0.33	45.90 ± 0.15	18.90 ± 1.15	0.72 ± 0.04

min. For spent coffee ground, Okur et al.[22] stated that HPAE increased the TPC up to 50% compared to CSE at 50 °C for 30 min.

Antioxidant Capacity. DPPH radical scavenging capacity (DRSC) and FRAP results of different extraction techniques are reported in Table 4. Like TPC and oleuropein content, HPAE increased the antioxidant capacity significantly ($p \leq 0.05$). This result was also parallel with that in the literature. For sour cherry pomace, Okur et al.[21] showed that HPAE treatment at pressure levels between 300 and 400 MPa for different treatment times (1−10 min) increased the antioxidant capacity by approximately 20% compared to CSE at 50 °C for 30 min. For chilean papaya seeds, HPAE at 500 MPa for 5, 10, and 15 min increased the antioxidant capacity up to 272.8% compared to CSE.[37] For grape byproducts, Corrales et al.[32] compared different extraction techniques, namely, HPAE (600 MPa for 1 h at 70 °C), ultrasound, pulsed electric field, and CSE (70 °C for 1 h). The authors found that HPAE increased the capacity by around three times higher than CSE.

https://doi.org/10.1021/acsfoodscitech.2c00256
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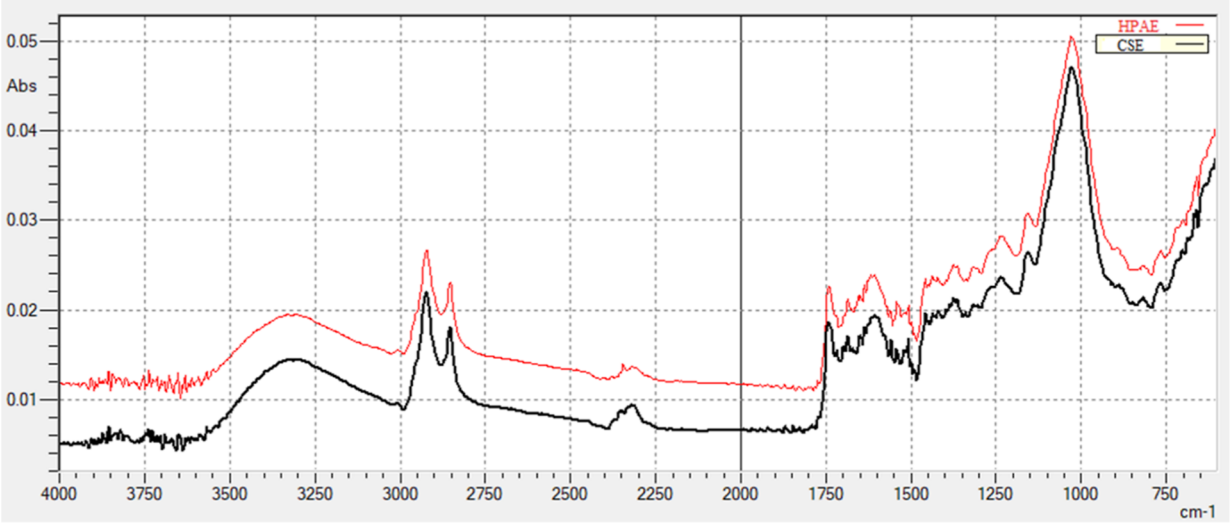

Figure 3. FTIR results of sample extracted by using (A) CSE treatment and (B) HPAE treatment (409 MPa for 11.16 min with 63.74% v/v ethanol concentration).

FTIR. Fourier transform infrared spectroscopy (FTIR) spectroscopy is an important technique for understanding the nature bond structure of substances and the behavior of substances in different applications.[38] FTIR spectra of different extraction techniques are shown in Figure 3. According to the results, CSE and HPAE do not impose any destructive effects on the functional groups of the extract. Similar results were found for spent coffee ground and sour cherry pomace.[21,22] The wide band at 3330 cm^{-1} was related to the hydroxyl group (O−H stretching).[39−41] The peaks at 2920 and 2970 cm^{-1} were attributed to the CH_2 antisymmetric stretch of methyl groups and the C−H asymmetric stretch of $-CH_3$, respectively.[42−44] The clear peak at 1050 cm^{-1} corresponded to the C−OH stretching band.[45] The indicator bands of oleuropein were 1622 and 1698 cm^{-1} and were attributed to the vibration of carbonyl groups in oleuropein.[46,47] Oleuropein is a hydrophilic molecule consisting of numerous OH groups in the structure.[48,49] Because of this, the O−H stretching band could also be used to indicate the bands. By looking at the bands attributed to oleuropein (1622 and 1698 cm^{-1}), it could be said that HPAE increased the extraction of oleuropein compared to CSE. This was also supported by HPLC results.

SEM. The extraction rate contributed to the physical change of the sample.[50,51] The effect of CSE and HPAE on the morphological structure of olive pomace was shown by SEM analysis. In Figure 4A, it can be said that there was no severe fracture in CSE treatment. However, in HPAE treatment, compression and deformation of cells were more noticeable compared to CSE treatment (Figure 4B). This change caused an increase in the solvent and solute transfer through the solid matrix. Thus, HPAE increased the extraction of phenolic compounds from olive pomace, and this result was also parallel with the TPC and oleuropein content results. In the literature, similar results were reported for sour cherry pomace (500 MPa-20 °C-10 min), onions (300 and 600 MPa for 3 min), strawberry (100−500 MPa for 10 min), and spent coffee ground (300−500 MPa for 5−15 min at 25 °C).[21,22,52,53]

In the present study, an optimized HPAE from olive pomace compared to CSE was first designed to improve the combination of phenolic and oleuropein compounds and compared to the extraction techniques based on antioxidant capacity (DRSC and FRAP), morphological changes, and nature bond structure of substances (FTIR). According to the results, the best HPAE condition for recovery of phenolic compounds from olive pomace with the highest TPC (16.39 gGAE/gDW) and oleuropein content (45.90 mg/100 gDW) was found as 409 MPa for 11.16 min with 63.74% v/v ethanol concentration. Pressure, time, and ethanol concentration had a significant effect on the recovery of phenolic compounds from olive pomace ($p \leq 0.05$). Also, compared to CSE results, HPAE increased the phenolic content significantly ($p \leq 0.05$). Based on FTIR results, there were no changes in the chemical bonds of phenolic compounds caused by HPAE treatment. However, HPAE caused morphological changes, and this could be a reason for the increase in the recovery of phenolic compounds from olive pomace. Overall, HPAE is an effective

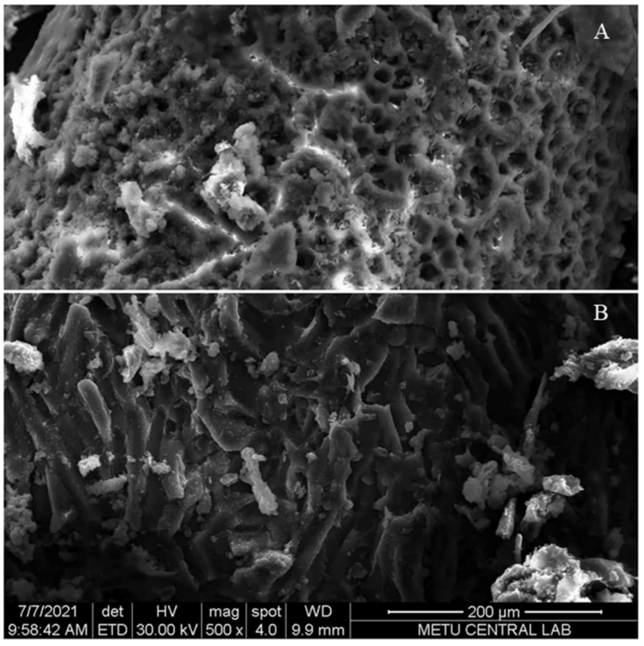

Figure 4. SEM results of sample treated by (A) CSE treatment and (B) HPAE treatment (409 MPa for 11.16 min with 63.74% v/v ethanol concentration).

https://doi.org/10.1021/acsfoodscitech.2c00256
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method to improve the recovery of phenolic compounds from olive pomace compared to the traditional treatment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.2c00256.

HPLC chromatograms (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors want to thank Zest Ayvalık (Balikesir, Turkey) for providing olive pomace.

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https://doi.org/10.1021/acsfoodscitech.2c00256
ACS Food Sci. Technol. 2022, 2, 1862−1869