MESMAP – 9
ABSTRACTS & PROCEEDINGS BOOK
03-05 May 2023, Ankara-Türkiye
www.mesmap.com

ORAL PRESENTATION - FULL PAPER

AN EFFICIENT GREEN METHOD FOR RECOVERY OF POLYPHENOLS FROM OLIVE TREE (OLEA EUROPAEA) LEAVES BY MEANS OF LACTIC ACID-BASED DEEP EUTECTIC SOLVENT

İrem Toprakçı and Selin Şahin

[1] *Department of Chemical Engineering, Faculty of Engineering, Istanbul University-Cerrahpaşa, 34320, Istanbul, Türkiye, E-mail: irem.toprakciyuksel@iuc.edu.tr*

Abstract

Biowastes generated during the processing of industrial crops and products are often rich in bioactive compounds like polyphenols. Using biowastes to obtain natural antioxidants is a sustainable and environmentally friendly approach that can promote human health while reducing waste. Olive leaf, which is one of the by-products of the olive oil industry, was used as a raw material in this study due to its rich bioactive content.

The current study is aimed at establishing an efficient green media, using an eco-friendly lactic acid-based deep eutectic solvent (DES) system, consisting of a hydrogen bond donor-HBD (glycerol) and hydrogen bond acceptor-HBA (lactic acic). The molar ratio of lactic acid-glycerol mixture was selected 1:1 depending on our previous study [1]. Ultrasound-assisted extraction (UAE) method, a green extraction method, has been used to recover natural antioxidants from olive leaves. The extraction time and amplitude were determined for ultrasound-assisted extraction of bioactive-rich extract from olive leaves prior to routine studies. The Box–Behnken design type of the response surface method (RSM) was employed to investigate the effects of independent parameters (particle size, solid mass and water content in DES) on the total phenolic content (TPC). Bioactive properties of the olive leaf extracts were also assessed depending on total flavanoid content (TFC) and antioxidant activity measured by 2 different assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) methods.

Key Words: Natural antioxidants, biowaste, deep eutectic solvent, green extraction, Box-Behnken-RSM.

1. Introduction

By-products from the olive oil industry, such as crude olive cake, vegetation water, twigs, leaves and olive leaf (traditionally used as animal feed), collectively account for approximately 10% of the total olive weight [2]. Olive leaf extract has potential as a source of valuable bioactive compounds for the cosmetic, therapeutic, and food industries, as well as a treatment for conditions such as hypertension, atherosclerosis, inflammation, high blood sugar, and high cholesterol [3].

Extraction is a powerful tool for the recovery of valuable compounds from biowastes [4]. Green extraction aims to minimize solvents, energy, wastes, and pollution while maximizing yields through safe and cost-effective methods, including novel technologies that reduce energy costs and greenhouse gas emissions [5]. Ultrasound is a promising technology that can contribute to achieving the goal of sustainable "green" chemistry and extraction. Ultrasonic technology is a green extraction method that offers advantages such as lower costs, reduced operation time, lower energy consumption,

and higher yield, and is commonly used in the food industry to accelerate chemical reactions. The ultrasonic technology leads to structural changes in food matrices, leading to the release of bioactive compounds from plant matrices after cell disruption [6]. This is achieved through the production of cavitation bubbles in the biological matrix, resulting in high yields and extraction rates [5].

Green Chemistry aims to develop safer and more efficient chemical processes with minimal impact on health and the environment, using sustainable solvents such as deep eutectic solvents (DESs) as alternatives to conventional solvents. DESs are formed by combining a eutectic mixture of Lewis or Brønsted acids and bases, which can include various cationic and/or anionic species [7]. DESs have several advantages over conventional solvents, including low toxicity, low volatility, high thermal stability, and biodegradability. These properties make them ideal for use in a wide range of applications, including as a solvent in the extraction of high-added value bioactive compounds for food and pharmaceutical applications. [8]. In this study, a recently developed and quickly growing type of ionic liquid known as deep eutectic solvent (DES) was utilized. A mixture of lactic acid and glycerol in a 1:1 molar ratio was prepared as a DES for the purpose of extracting high-value compounds from olive leaves using ultrasound-assisted extraction (UAE), based on our previous study [1]. The use of lactic acid and glycerol as components of the DES was chosen based on their availability, low toxicity, and potential for biodegradability.

To achieve successful UAE, it is crucial to take into account several process variables, such as ultrasonic power, frequency, output amplitude, extraction time, solvent-sample interaction and particle size [9]. Thus, the main objective of this study was to examine how particle size, solid mass, and water content in the DES system affect the UAE process, using the optimal amplitude and extraction time determined through preliminary trials. Furthermore, the bioactive properties of the olive leaf extracts were evaluated by quantifying the total flavonoid content (TFC) and assessing their antioxidant activity through two in vitro assays: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method. In addition, various kinetic models were employed to evaluate the kinetic behavior of lactic acid-based deep eutectic solvent integrated into UAE system, providing a comprehensive understanding of the kinetics of the extraction process.

2. Material and Methods

2.1. Plant material and chemicals

During the olive harvest season olive leaf samples, which are by-products of olive oil, were collected from Bursa in Turkey. After being dried in the dark, the olive leaves were ground and passed through a sieve system (Endecotts Test Sieves Ltd, London, UK) to classify the particles into a size range of 0.5-2 mm. All chemicals were provided from Merck (Darmstadt, Germany).

2.2. Preparation of deep eutectic solvent

In this study, a lactic acid-based DES was prepared using lactic acid as the hydrogen bond acceptor (HBA) and glycerol as the hydrogen bond donor (HBD) in a 1:1 molar ratio. The preparation process involved stirring the mixture at 80°C using a magnetic stirrer until a homogenous liquid was obtained. By mixing deionized water with lactic acid-based deep eutectic solvents (DESs), solutions with different concentrations were prepared, containing 20%, 50%, and 80% water (v/v) respectively.

2.3. Ultrasound-assisted extraction

The ultrasonic treatment was carried out using an ultrasonicator (Bandelin Sonopuls Mini 20, Bandelin Electronic, Berlin, Germany) with a pulse mode (3/7, on/off) to prevent overheating (Figure 1). The frequency of the ultrasonic treatment was 20 kHz, and the treatment lasted for 3 seconds with a 7-second pause during extraction. The amplitude and extraction time were determined through preliminary trials, and an output amplitude of 30% and an extraction time of 45 minutes produced the best results. Consequently, the extraction proceeded under these specific conditions. After extraction, the extracts were filtered using a 0.45 µm injection filter.

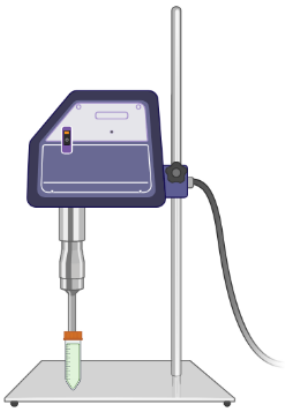


Figure 1. Ultrasound-assisted extraction system

2.4. Measurement of bioactive compounds

The bioactive compounds in the extracts were determined using a UV-visible spectrometer (PG Instruments, T60/Leicestershire, England). The total phenolic content (TPC) was measured at 765 nm using the Folin-Ciocalteau method, while the TFC was measured using the aluminum chloride method at 510 nm [10]. Gallic acid and catechin solutions were used to create calibration curves for determining the TPC and TFC concentrations of the extracts, respectively. The TPC values were expressed as mg-GAE/g-DM in gallic acid equivalent (GAE) per gram dried material (DM), while the TFC values were reported as mg of catechin equivalent (CE) per gram of DM.

2.5. Measurement of antioxidant activity

The antioxidant activity of the extracts was determined by performing DPPH and ABTS assays at 517 nm [11] and 734 nm [10], respectively. Trolox solution was used to create calibration curves. The results were expressed as milligram trolox equivalent antioxidant capacity per gram of dried matter (mg-TEAC/g-DM).

2.6. Kinetic study

Four different mathematical models were used to analyse the kinetic behaviour of system, including the pseudo-first-order model, pseudo-second-order model, film theory and Peleg's model. The equations for each model are provided in Table 1. The kinetic parameters of the models were determined using the TPC values obtained from preliminary trials conducted at three different amplitudes (10%, 30%, 50%). Film theory operates on a two-stage extraction mechanism, with the initial stage being the washing stage and the subsequent stage being the slow extraction stage. These stages are defined by two parameters within the model (Table 1).

Table 1. Application of kinetic model equations to for ultrasound-assisted extraction of olive leaves

Kinetic model	Equation	Model term
Pseudo first-order	$\ln\left(\frac{C_e}{C_e - C_t}\right) = k_1 t + \ln\left(\frac{C_e}{C_e - C_o}\right)$	C_0: initial TPC concentration (mg-GAE L^{-1}) C_e: TPC concentration at equilibrium (mg-GAE L^{-1}) C_t : TPC concentration at time t (mg-GAE L^{-1}) t: time (min)
Pseudo second-order	$\frac{t}{C_t} = \frac{1}{k_2 C_e^2} + \frac{t}{C_e}$	k_1:first order rate constant (min^{-1})
Film Theory	$\ln\left(1 - \frac{C_t}{C_e}\right) = \ln(1-b) - k_3 t$	k_2: second order rate constant (L mg^{-1} min^{-1}) k_3:slow extraction coefficient (min^{-1}) b: washing coefficient
Peleg's model	$C_t = \frac{t}{k_4 + k_5 t}$	k_4: Peleg's rate constant at the beginning (mingmg^{-1} GAE) k_5: Peleg's capacity constant (mg GAE g^{-1} min^{-1})

2.7. Box-Behnken design

The Box-Behnken design (BBD) is a type of response surface methodology (RSM) used in experimental design to identify the relationship between several independent variables and their effect on a response variable. It is a statistical tool that helps to optimize the process and find the optimal conditions for a given response. In this study, BBD was utilized to optimize the UAE system for extracting high-added value products from olive leaves. RSM was implemented using Design-Expert software version 12.0.1.0 from StatEase Inc., USA. The independent variables, along with their respective units and symbols, used in the UAE method are presented in Table 2. TPC findings were considered the dependent variables.

Table 2. Independent variables, units, symbol, and coded levels

Independent Variable	Units	Symbol	Coded levels		
			−1	**0**	**1**
Particle size	mm	A	0.5	1.25	2
Solid mass	g	B	0.1	0.3	0.5
Water content	(%, v/v)	C	20	50	80

3. Results and Discussion

3.1. Experimental plan, variance analysis and model fitting

Table 3 shows the experimental plan for UAE of bioactive substances from olive leaf extract using BBD of RSM, with 5 central points (1.25 mm particle size, 0.3 grams sample, and 50% water content in DES), and their corresponding TPC findings. As presented in Table 3, the TPC findings ranged from 9.02 to 71.19 mg-GAE per gram of dried matter (mg-GAE/g-DM).

Table 3. Experimental plan generated by BBD for UAE of olive leaf extract and TPC findings depending on independent parameters

Run	A: Particle size (mm)	B: Solid mass (g)	C: Water content (%, v/v)	TPC (mg-GAE/g-DM)
1	1.25	0.3	50	66.31 ± 0.002
2	1.25	0.3	50	71.19 ± 0.004
3	1.25	0.5	20	20.49 ± 0.004
4	1.25	0.3	50	66.89 ± 0.005
5	2	0.3	80	27.11 ± 0.002
6	1.25	0.3	50	68.21 ± 0.002
7	2	0.1	50	24.85 ± 0.003
8	1.25	0.5	80	31.65 ± 0.001
9	0.5	0.1	50	44.51 ± 0.003
10	1.25	0.1	20	22.02 ± 0.004
11	2	0.3	20	9.02 ± 0.005
12	2	0.5	50	19.74 ± 0.002
13	0.5	0.5	50	31.81 ± 0.002
14	1.25	0.1	80	36.36 ± 0.003
15	0.5	0.3	80	28.39 ± 0.001
16	1.25	0.3	50	67.09 ± 0.001
17	0.5	0.3	20	29.45 ± 0.002

* Data are given as the mean (n=3) ± standard deviation

Table 4 summarizes the analysis of variance (ANOVA) test results for TPC findings. The quadratic polynomial model (Eq. 1) proposed by Design-Expert software for TPC responses in terms of coded factors was determined to be statistically significant at $p<0.0001$, as shown in Table 4.

$$Y_{TPC} = 67.94 - 6.68\,A - 3.01\,B + 5.32\,C + 1.90\,AB + 4.79\,AC - 0.7950\,BC - 20.92\,A^2 - 16.79\,B^2 - 23.52\,C^2 \quad (1)$$

All of the selected independent variables (A, B and C) and their second power effects (A^2, B^2 and C^2) were found to have a statistically important effect based on their p-values ($p<0.05$). The second power effect of water content in DES (C^2) was found to be the most significant independent variable on TPC response, followed by the second power effect of particle size (A^2) and the second power effect of solid mass (B^2) ($p<0.0001$). It was followed by particle size (A), water content in DES (C), the interaction between particle size and water content in DES (AC) and solid mass (B) at $p<0.05$. As seen in Table 4, the interactions between the independent variables were not found to be statistically significant, except for the interaction between particle size and water content in DES (AC) ($p<0.05$).

Table 4 provides a comprehensive set of evaluation metrics that can be used to assess the goodness of fit of a model. These metrics include the lack of fit, coefficient of variance (C.V.), R^2, predicted R^2 and adjusted R^2 values. As seen in Table 4, a non-significant lack of fit value at $p>0.05$ is favorable because it indicates that the models fit the experimental data well. Additionally, the coefficient of determination values (R^2, adjusted R^2, and predicted R^2) are close to 1, which implies that the model can account for a substantial amount of the variation in the dependent variable.

Moreover, a coefficient of variation (C.V.) value less than 10% indicates a low degree of variation in the data relative to the mean, which suggests that the data is relatively consistent and reliable.

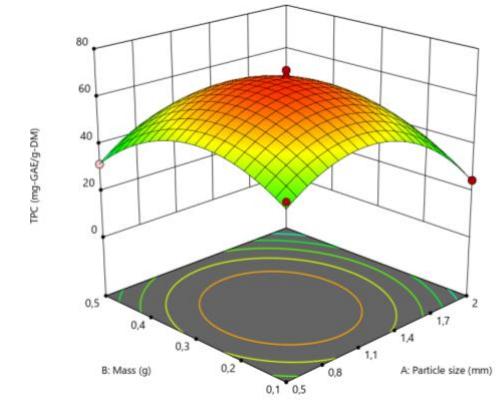
Table 4. Analysis of variance test results on TPC findings of olive leaf extracts obtained through UAE based on BBD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6737.64	9	748.63	98.19	< 0.0001	significant
A- Particle size	356.98	1	356.98	46.82	0.0002	
B- Solid mass	72.30	1	72.30	9.48	0.0178	
C- Water content	226.10	1	226.10	29.66	0.0010	
AB	14.40	1	14.40	1.89	0.2117	
AC	91.68	1	91.68	12.03	0.0104	
BC	2.53	1	2.53	0.3316	0.5828	
A^2	1843.43	1	1843.43	241.79	< 0.0001	
B^2	1186.47	1	1186.47	155.62	< 0.0001	
C^2	2329.52	1	2329.52	305.55	< 0.0001	
Residual	53.37	7	7.62			
Lack of Fit	38.25	3	12.75	3.37	0.1354	not significant
Pure Error	15.12	4	3.78			
Cor Total	6791.01	16				
C.V.: 7.06%		*R^2=0.9921*	*Adjusted R^2=0.9820*		*Predicted R^2=0.9064*	

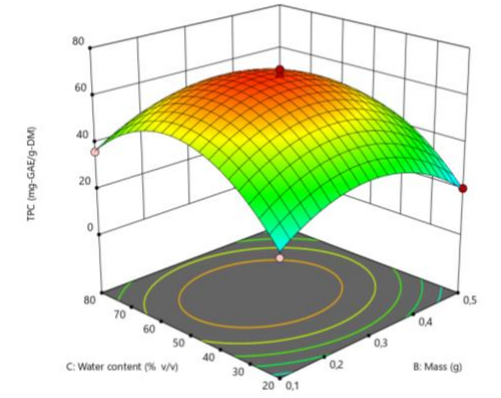
3.3. Effect of independent variables

Figure 2 shows 3D (three-dimensional) surface plots generated with Design-Expert software to visually depict how changes in independent variables affect the outcome (dependent variable). Figure 3 shows the Box-Behnken design cube with 17 experimental runs including 5 center points with 3 factors and 3 level.

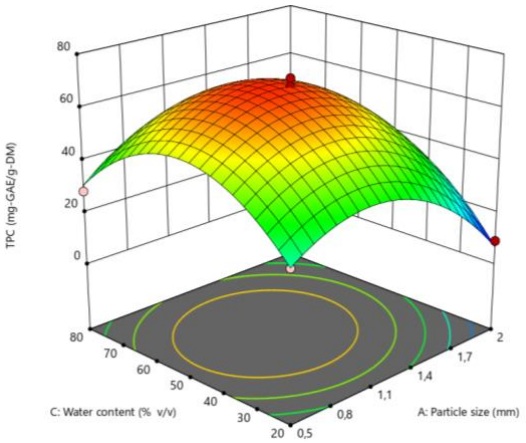
Figure 2a illustrates the impact of particle size on total phenolic content (TPC) yield. Initially, increasing the particle size resulted in higher yields. This situation may be attributed to the larger particle sizes can reduce agglomeration typically associated with smaller powders. However, after a certain point, increasing the particle size led to a decrease in TPC yield. This may be attributed to the lower surface area-to-volume ratio of larger particles, which could impede solvent penetration and extraction of phenolic compounds from the plant material [10]. Figure 2b indicates that the total phenolic content increases with an increase in solid mass until a certain point, after which it starts to decrease. After optimal point is reached, as the sample mass increases, the available surface area for solvent penetration and dissolution of phenolic compounds decreases. This results in reduced extraction efficiency and lower yields, since the solvent is unable to fully solubilize the target components. [12]. As shown in Figure 2c, initially, the addition of water to the solvent system increased the TPC yield due to a decrease in surface tension and viscosity, as well as an increase in polarity. However, after optimal concentration of water, the total phenolic yield decreased because excessive water concentration can have a negative impact on the interaction between DES and target compounds, leading to a reduction in extraction efficiency [13].



a



b



c

Figure 2. Effect of particle size to sample mass (**a**), sample mass to water content in DES (**b**), and particle size to water content in DES (**c**) on the total phenolic content

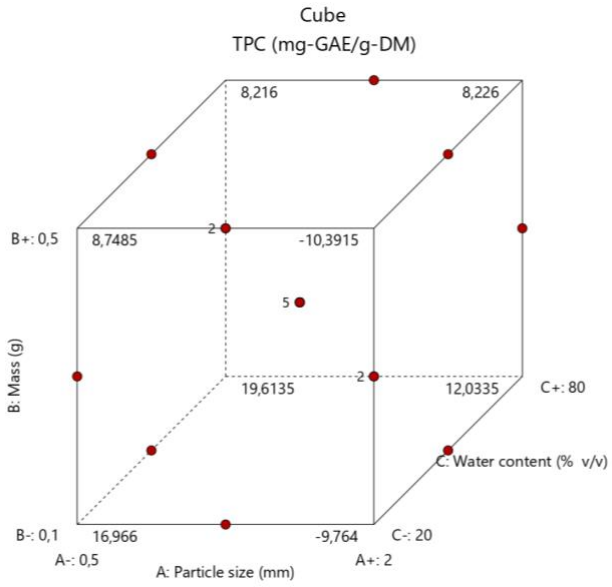


Figure 3. Box-Behnken design cube

3.3. Optimization study

The optimization study determined that the highest TPC yield (68.863 mg-GAE/g-DM) was achieved under the following optimum conditions: 1.135 mm particle size, 0.280 g sample mass, and 52.964% (v/v) water content in the DES system. The accuracy of the quadratic model equation based on TPC findings generated by RSM was confirmed with the validation study. Figure 4 illustrates the compatibility between the actual and estimated values.

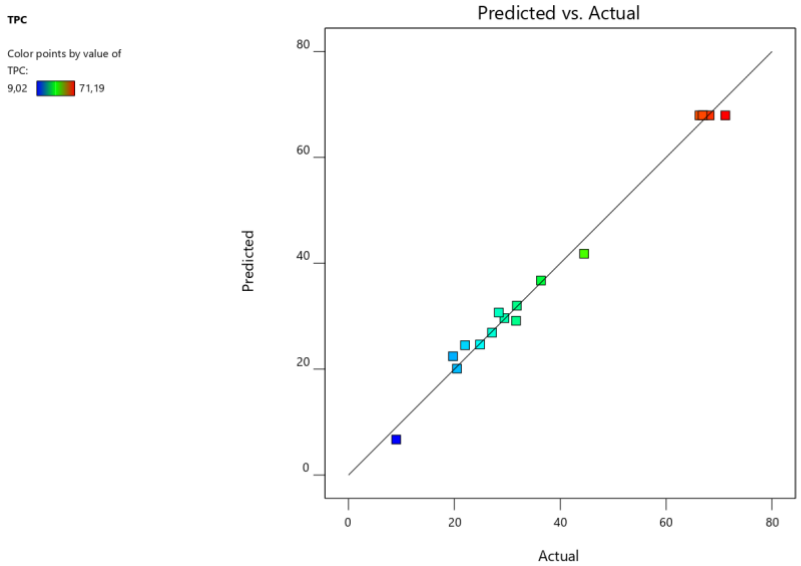


Figure 4. Comparison between actual and predicted TPC findings

3.3. Bioactive content of the extract

Table 5 reports the findings of two different tests used to evaluate the antioxidant activity of olive leaf extracts such as DPPH and ABTS assays, as well as the total flavonoid content (TFC). To better understand the antioxidant activity mechanism of the extracts, two different antioxidant activity tests, such as DPPH and ABTS assays, were performed. The results of these tests were found to be strongly correlated with each other, with a correlation coefficient of r>0.83. ABTS and DPPH assays showed a strong and positive correlation with TFC, with correlation coefficients of >0.84 and >0.81, respectively. These results suggest that the extracts have significant antioxidant potential, as indicated by the high correlation between TFC and both antioxidant activity tests. Additionaly, this relationship is thought to be due to the ability of flavonoids to act as effective scavengers of free radicals and other reactive species, thereby reducing oxidative stress and protecting cells from damage.

Table 5. Bioactive content of olive leaf extract

No	TFC (mg-CE/g-DM)	DPPH (mg-TEAC/g-DM)	ABTS (mg-TEAC/g-DM)
1	54.38 ± 0.002	19.61 ± 0.006	17.87 ± 0.001
2	55.69 ± 0.003	20.66 ± 0.005	18.52 ± 0.003
3	59.19 ± 0.004	14.07 ± 0.005	12.15 ± 0.003
4	61.15 ± 0.002	21.26 ± 0.006	18.61 ± 0.004
5	65.94 ± 0.001	20.08 ± 0.002	16.91 ± 0.004
6	60.11 ± 0.004	19.74 ± 0.004	18.30 ± 0.003
7	64.38 ± 0.002	22.25 ± 0.004	15.29 ± 0.003
8	59.31 ± 0.002	24.01 ± 0.003	17.66 ± 0.002
9	93.33 ± 0.003	34.83 ± 0.002	27.50 ± 0.002
10	58.68 ± 0.002	17.58 ± 0.002	14.64 ± 0.005
11	31.25 ± 0.005	8.99 ± 0.003	6.73 ± 0.003
12	51.46 ± 0.004	10.80 ± 0.004	10.36 ± 0.002
13	37.65 ± 0.002	14.93 ± 0.004	9.24 ± 0.002
14	111.19 ± 0.002	39.93 ± 0.006	28.82 ± 0.001
15	59.11 ± 0.001	22.38 ± 0.003	16.60 ± 0.001
16	58.06 ± 0.003	20.47 ± 0.003	18.53 ± 0.004
17	47.57 ± 0.003	19.41± 0.002	8.57 ± 0.005

* Data are given as the mean (n=3) ± standard deviation

3.4. Kinetic Analysis

Figure 4 shows the TPC variation over time at different amplitudes. The results revealed that after 75 minutes of ultrasound treatment at three different amplitudes (10%, 30%, and 50%), the TPC yield of the extraction process decreased significantly for all amplitudes after 45 minutes. Based on the findings, the optimal extraction conditions were identified to be an output amplitude of 30% and an extraction time of 45 minutes, and the extraction process was carried out under these conditions. Table 1 lists the kinetic models applied in the study, such as the pseudo-first-order model, pseudo-second-order model, film theory, and Peleg's model. The calculated kinetic parameters and correlation coefficients of determination (R^2) for these models using the kinetic data obtained from Figure 4 are presented in Table 6. Furthermore, the R^2 values obtained from the kinetic models suggest that the kinetic data is in agreement with the proposed kinetic equations (R^2 = 0.9081–0.9960). According to film theory, the extraction process occurs in two stages, with the fast extraction stage being followed by a slow diffusion stage, and the washing coefficient for optimum amplitude (30%) was found to be

higher than the slow extraction coefficient, as indicated in Table 6 [14]. As seen in Table 6, an increase in extraction rate constants was observed for all kinetic models as the amplitude was increased from 10% to 30%. This can be attributed to the enhanced cavitation and mechanical effects of ultrasound [15]. Furthermore, high amplitude levels in ultrasonication can also generate excessive heat, which can lead to thermal degradation of the phenolic compounds. Therefore, it is important to optimize the ultrasonication parameters, including the amplitude, to avoid excessive damage and ensure that the maximum phenolic content yield is achieved [16].

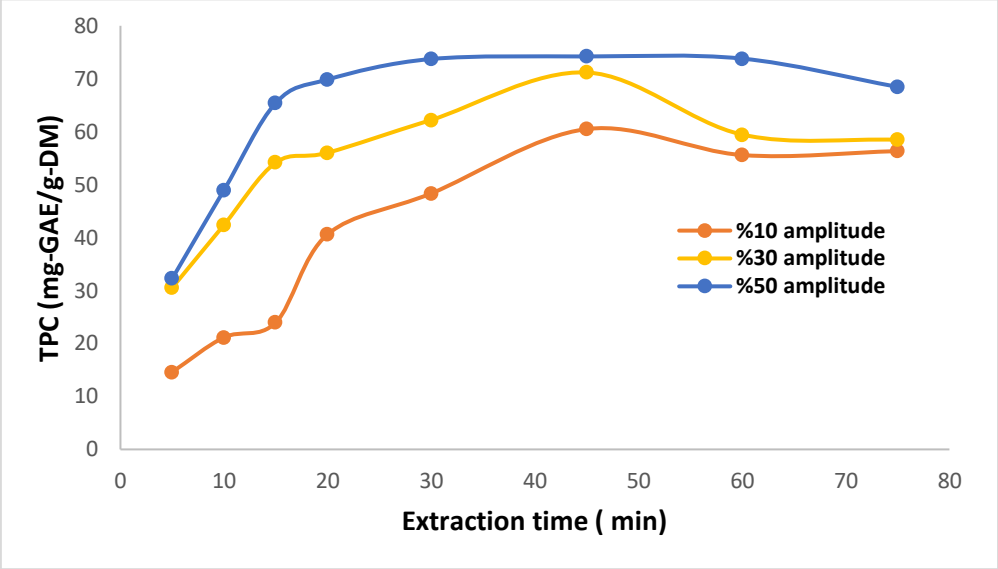


Figure 4. Effect of amplitude on TPC yield over time

Table 6. Analysis of kinetic model parameters for ultrasonic extraction of total phenolic compounds from olive leaves

Model	**Parameters**			
Pseudo first-order	**Output amplitude (%)**	**k_1 (min^{-1})**		**R^2**
	10	0.0528		0.9437
	30	0.0563		0.9854
	50	0.114		0.9960
Pseudo second-order	**Output amplitude (%)**	**k_2 (L mg^{-1} min-1)**		**R^2**
	10	$3.193*10^{-5}$		0.9081
	30	$2.063*10^{-4}$		0.9839
	50	$1.655*10^{-4}$		0.9918
Film-theory	**Output amplitude (%)**	**b**	**k_3 (min^{-1})**	**R^2**
	10	-	0.0528	0.9437
	30	0.1949	0.0563	0.9854
	50	-	0.114	0.9960
Peleg's model	**Output amplitude (%)**	**k_4 (min g mg^{-1})**	**k_5 (g mg^{-1})**	**R^2**
	10	0.2913	0.0136	0.9434
	30	0.0928	0.0138	0.9461

50	0.0944	0.0111	0.9455

4. Conclusions

In vitro analyses have revealed that olive leaf extract contains potent bioactive components. To extract these components from dried olive leaves, a method combining ultrasound-assisted extraction with a lactic acid-based deep eutectic solvent was successfully employed. The most influential factor on total phenolic content response was found to be the second power effect of water content in DES (C^2). The optimal parameters for the ultrasound-assisted extraction process were determined to be a particle size of 1.135 mm, a sample mass of 0.280 g, and a water content of 52.964% (v/v) in the lactic acid-based deep eutectic solvent system, according to the results of the optimization study. The statistical indicators from the analysis of variance suggest that the quadratic model equation used to relate to the total phenolic content findings was a good fit for the experimental data. The total flavonoid content has been found to exhibit a strong correlation with antioxidant activity tests, with a correlation coefficient greater than 0.80. This correlation is believed to be attributed to the capability of flavonoids to function as potent scavengers of free radicals and other reactive species and diminishing oxidative stress. The all kinetic models used in the study are appropriate for describing the kinetic behavior of the olive leaf extracts obtained by ultrasound-assisted extraction depending on the high correlation coefficients (R^2 >0.90). Additionally, the use of a green solvent in the optimization process may have important implications for sustainability and reducing the environmental impact of the extraction process. Overall, this method could provide a potential new approach for the extraction of bioactive compounds from olive leaves, which could have various applications in the food, pharmaceutical, and nutraceutical industries.

Conflict of Interest

The author declares that there is no conflict of interest in writing upon submission of the manuscript.

References

1. Yücel M, Şahin S (2022) An eco-friendly and sustainable system for monitoring the oleuropein-rich extract from olive tree (Olea europaea) leaves. Biomass Convers Biorefinery 12:47–60. https://doi.org/10.1007/S13399-021-01556-0/TABLES/12
2. Moudache M, Colon M, Nerín C, Zaidi F (2016) Phenolic content and antioxidant activity of olive by-products and antioxidant film containing olive leaf extract. Food Chem 212:521–527. https://doi.org/10.1016/J.FOODCHEM.2016.06.001
3. Boss A, Bishop KS, Marlow G, et al (2016) Evidence to Support the Anti-Cancer Effect of Olive Leaf Extract and Future Directions. Nutr 2016, Vol 8, Page 513 8:513. https://doi.org/10.3390/NU8080513
4. Xu DP, Li Y, Meng X, et al (2017) Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int J Mol Sci 2017, Vol 18, Page 96 18:96. https://doi.org/10.3390/IJMS18010096
5. Wen C, Zhang J, Zhang H, et al (2018) Advances in ultrasound assisted extraction of bioactive compounds from cash crops – A review. Ultrason Sonochem 48:538–549. https://doi.org/10.1016/J.ULTSONCH.2018.07.018
6. Yusoff IM, Mat Taher Z, Rahmat Z, Chua LS (2022) A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. Food Res Int 157:. https://doi.org/10.1016/J.FOODRES.2022.111268
7. Smith EL, Abbott AP, Ryder KS (2014) Deep Eutectic Solvents (DESs) and Their Applications. Chem Rev 114:11060–11082. https://doi.org/10.1021/cr300162p
8. Ling JKU, Hadinoto K (2022) Deep Eutectic Solvent as Green Solvent in Extraction of Biological Macromolecules: A Review. Int J Mol Sci 2022, Vol 23, Page 3381 23:3381. https://doi.org/10.3390/IJMS23063381
9. Esclapez MD, García-Pérez J V., Mulet A, Cárcel JA (2011) Ultrasound-Assisted Extraction of Natural

Products. Food Eng Rev 3:108–120. https://doi.org/10.1007/S12393-011-9036-6/METRICS
10. Albarri R, Şahin S (2022) A green method for the extraction of Moringa oleifera leaves: evaluation of several in vitro assays for bioactive properties. Biomass Convers Biorefinery 1:1–9. https://doi.org/10.1007/S13399-022-02690-Z/FIGURES/3
11. Yu J, Wang L, Walzem RL, et al (2005) Antioxidant activity of citrus limonoids, flavonoids, and coumarins. J Agric Food Chem 53:2009–2014. https://doi.org/10.1021/jf0484632
12. Şahin S, Samli R, Tan ASB, et al (2017) Solvent-Free Microwave-Assisted Extraction of Polyphenols from Olive Tree Leaves: Antioxidant and Antimicrobial Properties. Molecules 22:1056. https://doi.org/10.3390/molecules22071056
13. Peng X, Duan M-H, Yao X-H, et al (2016) Green extraction of five target phenolic acids from Lonicerae japonicae Flos with deep eutectic solvent. Sep Purif Technol 157:249–257. https://doi.org/10.1016/J.SEPPUR.2015.10.065
14. Segovia FJ, Corral-Pérez JJ, Almajano MP (2016) Avocado seed: Modeling extraction of bioactive compounds. Ind Crops Prod 85:213–220. https://doi.org/10.1016/J.INDCROP.2016.03.005
15. Goula AM (2013) Ultrasound-assisted extraction of pomegranate seed oil – Kinetic modeling. J Food Eng 117:492–498. https://doi.org/10.1016/J.JFOODENG.2012.10.009
16. Şahin S, Pekel AG, Toprakçı İ (2022) Sonication-assisted extraction of Hibiscus sabdariffa for the polyphenols recovery: application of a specially designed deep eutectic solvent. Biomass Convers Biorefinery 12:4959–4969. https://doi.org/10.1007/S13399-020-00837-4/FIGURES/3