Role of Solvents and Extraction Techniques on the Recovery of Phenolic Compounds from Olive Pomace

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Abstract

Olive pomace (OP) is a valuable by-product of the olive oil industry. It is considered a low-cost source of bioactive compounds including polyphenols which have remarkable antioxidant activities and are widely recognized for their beneficial properties in human health. This study aimed to investigate the role of different solvents and extraction techniques in the recovery of phenolic compounds with high antioxidant activity from OP. The recovery of phenolic compounds from defatted OP obtained from the three-phase extraction process of the olive mill was performed using different extraction solvents (water, ethanol, methanol, hydro alcoholic mixtures, and natural deep eutectic solvents). Soxhlet, Microwave, and Ultrasound-Assisted Extraction (UAE) techniques were used to optimize bioactive compound recovery. The response surface methodology (RSM) was employed to evaluate the impact of three independent variables of UAE (sonication amplitude, extraction time, and solvent concentration) on total phenolic content (TPC), total flavonoids content (TFC), and antioxidant activity (DPPH assay). The results demonstrated that the optimal conditions for the recovery of phenolic compounds from OP were achieved using UAE techniques with ethanol:water (80:20), 80% sonication amplitude, and 20 minutes of sonication extraction. The highest level of TPC, TFC, and total antioxidant activity was obtained after extraction with UAE using 80% ethanol (104 mg GAE/g OP, 48 mg QE/g OP, and 93.5%, respectively).

Keywords: antioxidant activity, bioactive compounds, extraction techniques, sonication amplitude, total flavonoids content.

1. Introduction

The demand for olive oil has expanded production because of its beneficial health properties, including antioxidant, anti-atherogenic, anti-inflammatory, anti-aging, anti-tumor, anti-viral, anti-cancer, and immune modulator activities (Centrone et al., 2021, IOC 2022). About 97% of the worldwide production of olive oil and table olives is provided by Mediterranean countries. Olive oil production plays a significant role in the agricultural sector of the Mediterranean region, reaching 2,760,000t during the crop year 2022/23 (FAO 2023).

Large amounts of waste and by-products such as olive mill wastewater (OMW), olive pomace (OP), leaves, and stones with an environmental impact in short periods of time are generated by the olive oil industry (Katsinas et al., 2021). OMWW is characterized by a high content of organic matter, a high percentage of suspended solids and fats, an acidic pH, high conductivity due to its high salt content, and colored waters due to phenolic compounds (García-Pastor et al., 2023, Hadidi et al., 2021). Discharges of processing biomass, particularly liquid effluent, cause toxicity, contamination, and pollution, leading to a relevant environmental problem with a complicated technological, economic, and social solution (Markhali 2021).

Olive pomace is the major by-product of semisolid mass derived from the separation of olive oils from olive malaxation paste using traditional and classic pressure systems or modern continuous centrifugation systems (Zhao et al., 2023, Pantziaros et al., 2021). Olive pomace is composed of a lignocellulosic matrix, phenolic compounds, uronic acids, and oily residues (Ferhat et al., 2017) provides a rich source of natural antioxidants (Pagnanelli et al., 2010). It is considered a great low-cost source of bioactive compounds, such as antioxidants, fatty acids, and polyphenols that show remarkable antioxidant properties and can be utilized to develop new ingredients or products (Nunes et al., 2021, Gullón et al., 2020).

The utilization of natural additives recovered from olive oil processing by-products aligns with current health concerns and environmental awareness, which have been guiding consumer behaviour over the last few years (Gómez-Cruz et al., 2021). This movement can address the consumer trend toward additive-free products or those with natural ingredients, which also presents a challenge for food and beverage companies (de Carvalho et al., 2015).

Different approaches to recovering bioactive compounds from OP involve several extraction methodologies, such as conventional and green extractions, including solvent extraction using ethanol, methanol, dimethyl sulfoxide, and hexane or hydroalcoholic mixtures as solvents (Pikuli et al., 2023). The extraction of bioactive compounds from natural sources is the fundamental step to obtaining natural

antioxidants. Most of these conventional volatile organic solvents are often toxic to humans and the environment (Mir-Cerda et al., 2023).

The usage of natural deep eutectic solvents (NADES) is considered a sustainable alternative emerging as a promising new class of unconventional and environmentally friendly ionic solvents for phenolic compound recovery (Cannavacciuolo et al., 2023). NADES are formed of natural metabolites such as sugars, alcohols, organic acids, amino acids, and amines (Dai et al., 2015).

Depending on the nature of the material and the target compounds to be recovered, several methods have been applied to extract phenolic compounds from OP using different extraction solvents (Rodríguez et al., 2022). Conventional and Soxhlet extraction methods have been widely used to recover bioactive compounds with high antioxidant and antiradical effects (Stramarkou et al., 2023). Recently, extraction techniques in the food industry have included new technologies such as microwave-assisted extraction, pressurized liquid extraction, supercritical fluid extraction, solid-phase extraction, and ultrasound-assisted extraction (Ronca et al., 2024).

Ultrasound-assisted extraction (UAE) is considered an easy-to-use, relatively affordable, low-cost extraction that prevents the degradation of thermolabile compounds and protects the environment using small volumes of solvents (De Luna et al., 2020). The recovery rate of phenolic compounds from the OP is improved thanks to the application of the UAE and selective microwave dielectric heating during MAE (Milani et al., 2020). Using innovative techniques such as UAE and MAE provides several advantages compared to conventional extraction, showing better selectivity, reduction extraction time, and lower toxic organic solvent use (Shen et al., 2023). Meanwhile, those techniques are active not only as a laboratory tool but also for agri-food industries (Cádiz-Gurrea et al., 2019).

The present work aimed to study the role of different extraction techniques and solvents in the recovery rate of phenolic compounds with high antioxidant activity from olive pomace. Additionally, the main focus was optimization of the extraction conditions that maximized phenolic compound recovery from olive pomace generated from the Kalinjoti cultivar, an autochthonous olive cultivar most abundant in the South of Albania. Therefore, UAE and MAE extraction techniques were performed on OP using different extraction solvents to optimize bioactive compound recovery. On the other hand, after selecting the best extraction techniques, the effect of ultrasound-assisted extraction conditions on the total phenol content and antioxidant activity was studied. The response surface methodology (RSM) was employed to evaluate the impact of independent variables of UAE (sonication amplitude, extraction time, and solvent concentration) on total phenolic content (TPC), total flavonoids content (TFC), and

antioxidant activity (DPPH assay) due to their ability to determine the interaction among the process variables.

2. Materials and Methods

2.1. Sampling

Olive pomace samples were obtained in the 2023-2024 olive crop year. The pomace was collected directly from the three-phase centrifugal extraction process of the olive mill operating in the Vlora region. The variety of the processed olives was 'Kalinjoti" one of the most sprout autochthonous olive cultivars in the Southern part of Albania (Thomaj & Panajoti, 2003).

OP with an initial moisture content of 52-67% was dried at 40- 45^{0}C for 48 h in a tray dryer to prevent the degradation of phenolic compounds. The dried and deffated OP with a final moisture content of 4.5% was then finely ground using a mill flour with an average particle diameter of about 1mm and stored in dark place at room temperature (Pikuli & Devolli, 2024).

2.2 Extraction of Phenolic Compounds

Different extraction techniques, such as Soxhlet, Microwave, and Ultrasound-Assisted Extraction, were employed to recover the phenolic compounds from OP. Solid-liquid extraction methods using various solvents, including water, methanol, ethanol, hydro-alcoholic mixtures, hexane, and natural deep eutectic solvents, were used to optimize the extraction process of bioactive compounds from OP. The extraction procedure of phenolic compounds from OP was performed using the analytical methodology described by Chanioti et al. (2021) with some modifications. Ultrasound-assisted extraction of bioactive compounds from OP was carried out in an ultrasonic bath equipped with a probe, model Cole-Parmer 8893 (47 kHz, 230 W), using 10 g of OP dissolved in 50 mL of each solvent. Additionally, UAE was performed at room temperature in different sonication times (5 to 60 minutes) by applying different sonication amplitudes (30% to 100%) and two different hydro-alcoholic concentrations (ethanol: water) 80:20, and 50:50. Obtained extracts were filtered using 0.45 μm Millipore syringe filters and stored in the refrigerator for further analysis.

2.3 Design of Extraction Experiments

The response surface methodology (RSM) was employed to optimize the extraction parameters of OP. Hence, a Central Composition Design (CCD) was applied to identify the relationship between response functions and independent variables (Böhmer-Maas et al., 2020).

Furthermore, to determine the conditions that optimize the extraction process of phenolic compounds from OP, the impact of three independent variables (sonication time, sonication amplitude, and concentrations solvents) on the recovery rate of total phenolic content (TPC), total flavonoids content (TFC), and total antioxidant activity (DPPH assay) were evaluated. Twenty-one experiments were conducted, including three replicates. TPC, TFC, and TAA were selected as the dependent variables for a combination of the independent variables.

2.4 Analytical Methods

All chemicals and reagents were of the analytical grade, and doubly distilled water was used. Phenolic extracts were analysed at room temperature to prevent thermal degradation of the phenolic compounds, and the results were expressed as mean values ± standard deviation.

2.4.1 Total phenolic Content of Olive Pomace

The total phenolic content (TPC) of OP was evaluated by UV-vis spectrophotometry at a maximum wavelength of λ = 750 nm using the Folin-Ciocalteu redox assay as described by Pikuli & Devolli (2024). A standard calibration curve was constructed previously with known concentrations of gallic acid, ranging between 0 to120 $mg.L^{-1}$ (R^2 = 0.9996), and the results were expressed in milligrams of gallic acid equivalents (GAE) per gram of dried OP.

2.4.2 Total Flavonoid Content of Olive Pomace

The total flavonoid content (TFC) in phenolic extracts was determined using the aluminum chloride colorimetric method described by Saoudi et al., (2021) with some modifications. The absorbance was measured at 510 nm, and the results were expressed in milligrams of quercetin equivalents per gram of dried OP (mg QE/g OP).

2.4.3 Total Antioxidant Activity

According to Ballesteros et al. (2014), the total antioxidant activity of phenolic extracts was determined using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay. The calibration curve was previously built using standard antioxidant trolox with known concentrations ranging from 0 to 1000 µM (R^2 = 0.9937). The reduction of absorbance was measured using a UV-vis spectrophotometer (Biochrom Libra S22 model) at wavelength 515 nm against methanol as blank. The results of total antioxidant activity were expressed as a percentage of inhibition total of DPPH (% TAA) calculation based on the formula below:

$$\%TAA = \frac{A_0 - A_c}{A_0} \times 100$$

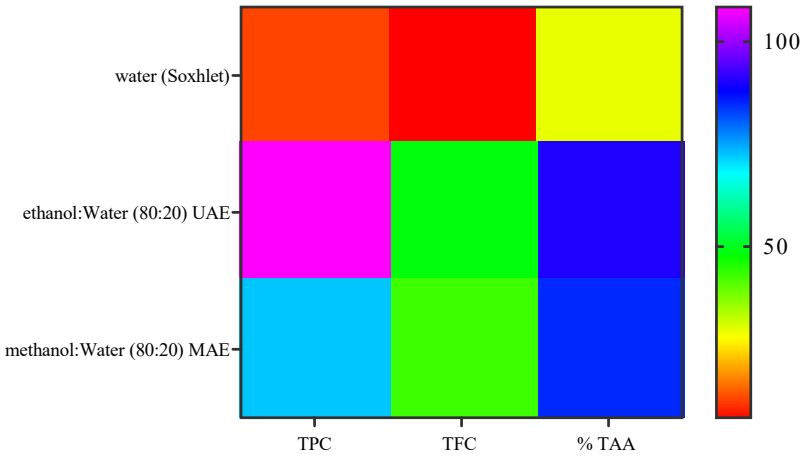
where A_0 was the absorbance of DPPH measured at the initial time, whereas A_c was the absorbance of the sample after a 40-minute rest in a dark place.

3. Results and Discussions

The optimization of the extraction process of bioactive compounds from OP was based on three dependent Parameters: Total Phenolic Content, Total Flavonoid Content, And Total Antioxidant Activity, Evaluated Using DPPH Radical Scavenging Activity.

Meanwhile, In This Study, The Optimization Object Corresponds To The Simultaneous Maximization Of The Process Parameters TPC, TFC, And TAA To Solvent Types And Extraction Techniques Of Phenolic Compounds From OP. The Obtained Results Of This Process Are Shown In Figure 1.

Figure 1. Analytical Results of phenolic extract recovered from olive pomace by UAE, MAE, and Soxhlet extraction techniques using water and ethanol: water (80:20) as extraction solvents.



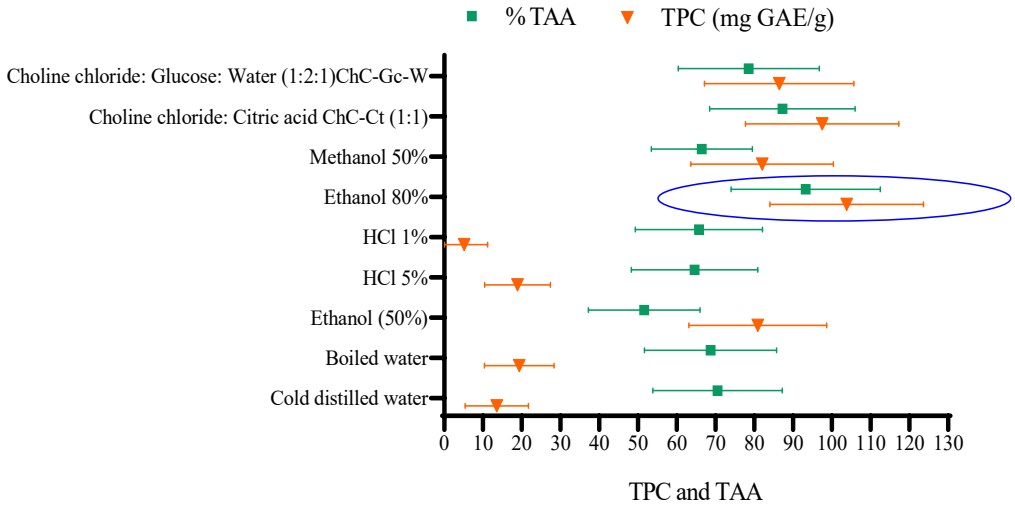
It was noted that the highest amounts of TPC, TFC, and TAA were obtained using the UAE extraction technique and ethanol:water (80:20) as an extraction solvent. MAE technique using ethanol:water (80:20) shows a moderate recovery rate of phenolic compounds from OP samples. The total phenolic content and total antioxidant activity (108.4 mg GAE/g d OP and 90.67 %) obtained in the present study by UAE using ethanol:water (80:20) as solvent agree in a similar range with the values reported by Cabrera et al., (2024) and Zhao et al., (2022).

Figure 2 shows the impact of extraction solvents on the recovery rate of phenolic compounds of OP by UAE techniques. Besides water (cold, boiled, and acidified with

HCl) and hidroalcoholic solvents, natural deep eutectic solvents (NADES) such as Choline chloride:Citric acid ChC-Ct (1:1) and Choline chloride:Glucose:Water ChC-Gc-W(1:2:1) were used.

The highest levels of TPC and TAA were obtained from ethanol:water (80:20), followed by ChC-Ct and ChC-Gc-W. Several studies have reported the importance of NADES usage on the extraction rate of phenolic compounds from different food matrices, mostly from OP (Cabrera et al., 2024, Bertolo et al., 2021, Bubalo et al., 2016). Meanwhile, the lowest level of TPC was obtained using acidified water and cold water (4.85 mg GAE/g dOP and 12.88 mg GAE/g dOP, respectively). These results have reported a similar range of TPC and TAA in OP to other studies conducted by Pikuli and Devolli, (2024), and Zhao et al., (2022).

Figure 2. Role of extraction solvents on the recovery of phenolic compounds (TPC and TAA) of OP by UAE techniques.



Obtained data of the experimental designs were analysed using RSM and ANOVA. The response surface methodology (RSM) was employed to study the impact of ultrasound extraction conditions (sonication amplitude, sonication time, and solvent concentration) and their interaction on the three dependent process variables. Analysis of variance (ANOVA) was carried out using the software SPPS 25 with a significant level ($p < 0.05$), and the results are presented in Tab. 1.

To optimize the extraction process of phenolic compounds from OP with UAE technique, a mixture of ethanol:water was selected as the extraction solvent due to

previous results of this study showing a higher rate of phenolic compounds recovery with hydro-alcoholic than other solvents.

As observed from Tab. 1, the TPC, TFC, and TAA levels in all experiments ranged from 11.84 to 104.38 mg GAE/g d OP, 6.09 to 49.52 mg QE/g d OP, and 22.33 to 93.33%, respectively.

Extraction experiments of OP using the UAE technique were conducted with varying mixtures of ethanol and water (80:20 and 50:50), sonication amplitudes (30%, 60%, 70%, 80%, and 100%), and sonication times (5, 20, and 60 minutes), as presented in Tab. 1. To determine the model's validity, the predicted and experimental values of TPC, TFC, and TAA were compared.

According to Tab. 1, the highest level of TPC, TFC, and TAA was obtained in the case of extraction conditions (60 min sonication, 100 % sonication amplitude, ethanol 80%). Meanwhile, the lowest level of extraction parameters was observed when ethanol concentration 50%, time sonication of 5 minutes, and 30% sonication amplitude was applied.

The increase of sonication time and amplitude favoured the extraction process of phenolic compounds with high antioxidant activity (Tab. 1). Moreover, the usage of ethanol:water (80:20) has noticeably improved the extraction process of polyphenols from OP.

Table 1. Coded and actual values for TPC, TFC, and TAA

Sonication time (minute)	Sonication amplitude (30-100%)	Solvent concentration (ethanol:water)	TPC ±Std	TPC predicted	TFC ±Std	TFC predicted	TAA ±Std	TAA predicted
-1 (5)	-2 (30)	0 (50)	11.84 ±1.78	42.88	6.09 ±1.01	12.57	22.33 ±1.24	20.42
-1 (5)	-2 (30)	+1 (80)	30.46 ±2.69	52.80	12.76 ±0.49	20.55	24.46 ±0.86	14.64
-1 (5)	-1 (60)	+1 (80)	32.98 ±1.98	60.53	11.09 ±1.42	21.88	28.34 ±1.82	27.49
0 (20)	-2 (30)	0 (50)	17.17 ±4.10	52.80	8.53 ±2.02	20.55	27.34 ±2.87	36.83
0 (20)	-1 (60)	+1 (80)	74.04 ±4.58	60.53	22.12 ±1.82	21.88	43.22 ±2.78	49.68
0 (20)	+1 (80)	+1 (80)	84.74 ±3.91	95.81	35.00 ±1.76	40.51	54.65 ±2.31	63.83
+1 (60)	-1 (60)	0 (50)	59.98 ±15.50	52.80	18.68 ±7.34	20.55	44.29 ±16.88	59.02

+1 (60)	-1 (60)	+1 (80)	78.56 ±14.30	60.53	31.16 ±5.42	21.88	66.77 ±23.88	71.87
+1 (60)	0 (70)	0 (50)	78.31 ±3.22	52.80	30.86 ±1.83	20.55	61.73 ±2.05	59.02
+1 (60)	0 (70)	+1 (80)	98.38 ±4.26	78.17	32.88 ±2.36	31.20	87.90 ±3.73	78.95
+1 (60)	+1 (80)	0 (50)	65.43 ±14.80	52.80	25.14 ±6.79	20.55	59.76 ±13.68	59.02
+1 (60)	+1 (80)	+1 (80)	104.18 ±4.13	95.81	45.75 ±0.61	40.51	93.33 ±3.46	86.02
+1 (60)	+2 (100)	0 (50)	84.10 ±2.67	52.80	33.98 ±1.89	20.55	72.10 ±14.36	59.02
+1 (60)	+2 (100)	+1 (80)	104.38 ±6.98	113.46	49.52 ±2.25	49.83	92.72 ±6.34	93.10

Regression equations (Eq. 1 to Eq. 3) are provided for the responses, taking into account the significant terms.

$$TPC = 52.80361111 + 25.36642125*X3 + 17.64359223*X9 \quad (1)$$

$$TFC = 20.54638889 + 10.65341694*X3 + 9.31511327*X9 \quad (2)$$

$$TAA = 36.8303397 + 22.18932059*X1 + 19.92902335*X3 + 7.07521232*X9 \quad (3)$$

X1:sonication time, *X2*: sonication amplitude, *X3*:solvent concentration, *X4*: sonication time^2, *X5*: sonication amplitude^2, *X6*: solvent concentration^2, *X7*:sonication time x sonication amplitude, *X8*: sonication time x solvent concentration, *X9*: sonication amplitude x solvent concentration.

2D response surface plots illustrated the relationship between the independent variables and the response variables. The influence of solvent concentration, sonication amplitude, and sonication time on the different studied responses (TPC, TFC, and TAA) are presented in Figure 3 and Figure 4.

Figure 3. Surface response of TPC and TFC of extracts of OP by UAE

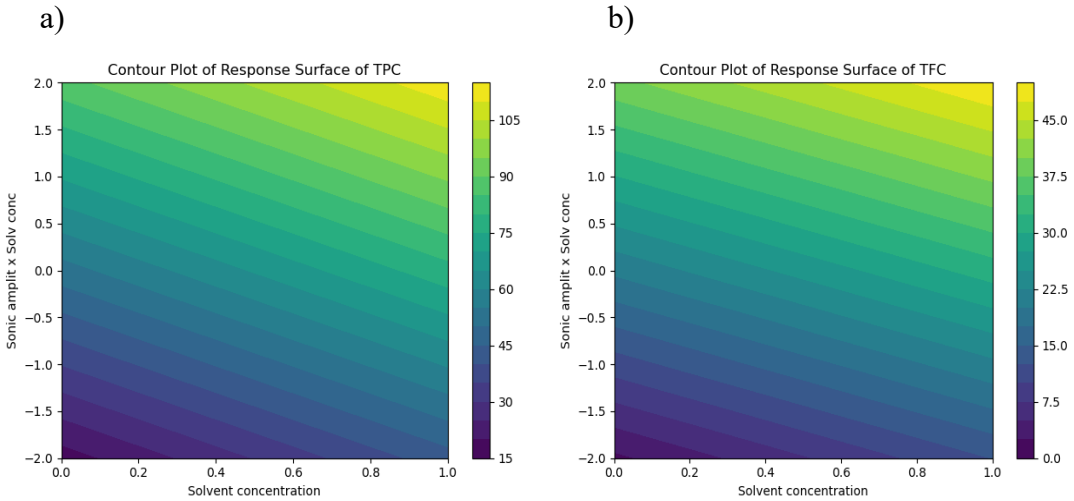
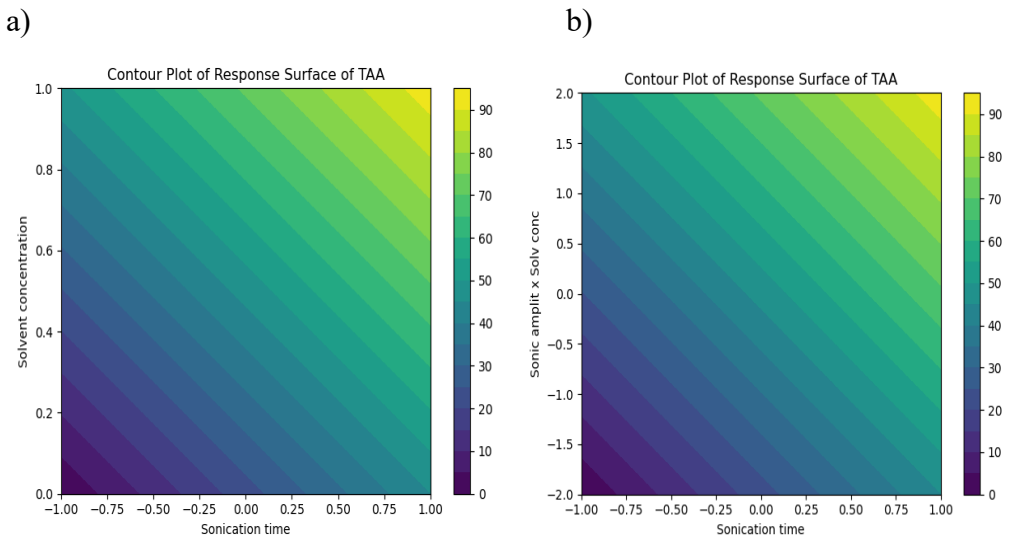
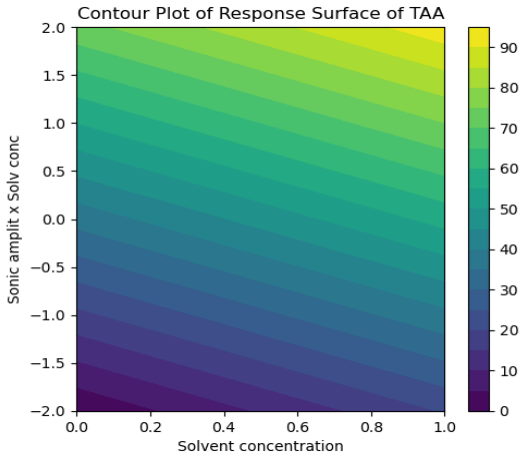


Figure 4. Surface response of TAA of extracts of OP by UAE



c)



Figures 3 (a) and (b) show that the highest levels of TPC and TFC in extracts were obtained (the yellow colour) at higher solvent concentration and sonication amplitude. As it can be seen from Figures 4 (a) (b), and (c), the higher level of TAA (the darkest yellow colour) was observed at the lower levels of the sonication time and amplitude variable.

Although the findings of this study support the notion that longer extraction times are not always the most effective, as demonstrated by De Bruno et al. (2018) are not in the same alignment with those of Soufi (2023), who found that a shorter extraction time of 2 minutes was sufficient to extract the highest amount of phenolic compounds and exhibit the highest antioxidant activity.

The experimental results indicated that the three factors had a linear effect on the total phenolic content, total flavonoid content, and total antioxidant activity of phenolic extracts recovered from OP. Those findings are in agreement with those reported by other studies that evaluated the efficacy of ultrasound extraction conditions of phenolic compounds from olive pomace (Cabrera et al., 2024, Soufi et al., 2023, Böhmer-Maas et al., 2020).

4. Conclusions

This research evaluated the role of different extraction techniques and extraction solvents on the recovery rate of phenolic compounds with high antioxidant activity from olive pomace. The ultrasound extraction technique has demonstrated a high extraction efficiency of phenolic compounds compared to soxhlet and microwave-assisted extraction.

Obtained results revealed that the interaction between solvent concentration, sonication time, and sonication amplitude promoted a positive and significant effect on the extraction of phenolic compounds with UAE techniques. The increase of the extraction time and solvent concentration (ethanol: water 80:20) promoted a higher recovery rate of phenolic compounds from OP.

The mathematical models that describe the relationships between the different tested operating conditions of UAE and the response variables of the extraction process (TPC, TFC, and TAA) were employed. The application of those models provided a satisfactory recovery rate of phenolic compounds from olive pomace using the UAE technique with 80% ethanol, 20 minutes of sonication, and 80% sonication amplitude.

Based on the extracts' parameters (TPC, TFC, and TAA) of OP, further research should be carried out to study the application of bioactive compounds in food formulation.

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