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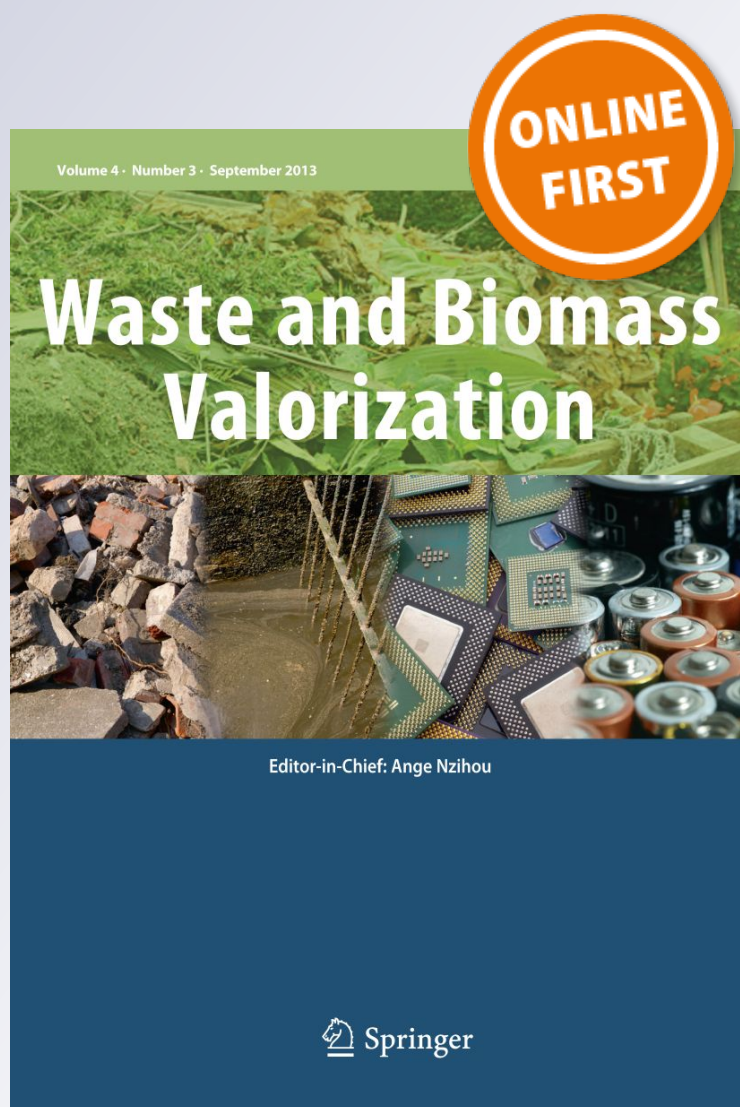
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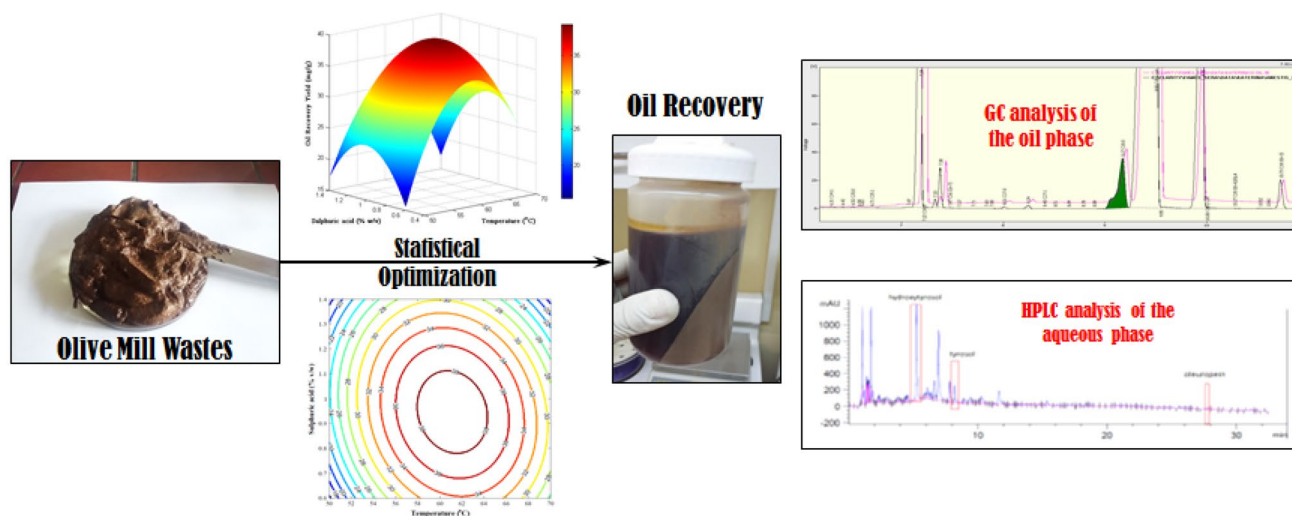
Pretreatment of Olive Mill Wastes for the Extraction of Residual Oil and High Added Value Compounds

Konstantinos Tzathas¹ · Evdokia Chrysagi¹ · Gerasimos Lyberatos¹ · Apostolos Vlyssides¹ · Anestis Vlysidis¹ Received: 7 February 2019 / Accepted: 17 June 2019
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Abstract

A novel extraction method for recovering the residual oil from olive mill wastes (OMW) from II-phase olive mills was developed in this study. The new acidification/hydrolysis method is based on the addition of sulphuric acid and ferrous sulphate heptahydrate. A range of water dilutions and extraction temperatures were tested. Experimental runs were carried out according to a central composite design taking into consideration all the most important factors that affect the extraction process such as the solid to liquid ratio (w/v), the addition of H₂SO₄ (% v/w), the addition of FeSO₄·7H₂O (% w/w) and temperature (°C). The optimum residual oil yield was 47.7 mg/g OMW dry basis (db) at 0.25 w/v solid to liquid ratio, 1.4% v/w H₂SO₄, 0.6% w/w FeSO₄·7H₂O and 70 °C. Moreover, the extraction kinetics of the residual oil from OMW were examined at the optimum conditions and were well fitted by using second order kinetics. Analysis of the residual oil showed similar fatty acid profile with the virgin olive oil. Finally, characterisation of the aqueous phase following the residual oil extraction process demonstrated a high content of phenolic compounds and especially tyrosol and hydroxytyrosol.

Graphic Abstract

**Keywords** Pretreatment of OMW · Recovery of residual oil · Phenolic compounds · Statistical optimisation✉ Anestis Vlysidis
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Statement of Novelty

Wastes from two-phase olive mills contain high added-value compounds such as residual oil and polyphenols. Small and middle size olive mills are difficult to undertake

large investments for processing their OMW. Currently, the predominant method for recovering the secondary oil from OMW is by solvent extraction. However, due to their high moisture content, a lot of energy is required for drying, making it economically not sustainable. In this study, a novel inexpensive acidification method to recover the residual oil from OMW is developed. The simplicity of our proposed extraction process allows the olive mill owners to reduce the capital investment to zero as they can use the existing equipment of their facilities such as mixers, centrifuges and decanters.

Introduction

The worldwide production of olive oil is estimated to be 2.6 million tons per year where 78% of this amount (approximately 2.03 million tons) is produced in EU with Spain, Italy and Greece being the highest olive oil producers. More than 95% of the world's olives production are harvested in the Mediterranean region [1]. Olive oil is obtained from olive fruit by mechanical means, whereas its production involves one of the following extraction processes: (1) discontinuous (press) extraction, (2) three-phase centrifugal extraction, or (3) two-phase centrifugal extraction [2]. The traditional olive pressing and the three-phase continuous systems produce three streams: olive oil which is the main product, olive cake (or kernel), which is the main side-product, and olive mill wastewater (OMWW), which is the main waste stream produced with an annual worldwide generation estimated between 10 and 30 million m³ [3]. The latter causes a number of serious environmental problems.

OMWW has a slightly acidic pH (range 4.8–6.8) and a red-to-black colour which results from both the olive fruit and the water added during olive oil processing. OMWW has a solid content ranging from 4.1 to 16.4%, depending on the amount of water added during the olive oil production process and contains high amounts of polyphenolic compounds such as hydroxytyrosol, tyrosol, and flavonoids [4–6]. OMWW is a hazardous effluent, since its high organic matter content and turbidity cause poor light penetration and poor oxygenation in water bodies when it is released uncontrolled, causing the development and augmentation of eutrophication phenomena [7]. OMWW may contain up to 150 g/L of total solids (TS), 170 g/L of biochemical oxygen demand (BOD₅) and 110 g/L of chemical oxygen demand (COD) [8]. Therefore, its detoxification and pretreatment is essential before managing this waste with conventional biological technologies.

The two-phase centrifugal system was introduced during the 1990s as an ecological solution that enables reduction of the volume of olive mill wastes [8]. This technology creates a semi-solid waste called “alpeorujo” in Spanish which

contains about 60% water and 3% oil [9]. In the continuous two-phase extraction process, water injection is performed only in the final vertical centrifugation step; therefore, the volume of liquid effluents is reduced by one-third on average in comparison with the three-phase system. On the other hand, a significant organic matter fraction remains in the solid waste, which has a higher moisture content than the three-phase pomace (60–80% instead of 40–55%) [10].

Different technologies have been proposed for treating two-phase OMW based on evaporation ponds, thermal concentration and phenolic components' extraction [11, 12] and its application to cultivations for enhancing the sorption of herbicides and insecticides [13, 14]. Another method for utilising this waste is composting in order to produce organic fertilizers and soil amendments. Composting can be economically and ecologically sound and may well represent an acceptable solution for disposing of the two-phase OMW [15]. Also anaerobic processes for the production of methane and hydrogen have been used [16–18]. During the last decades, research studies have been conducted on the recovery of valuable compounds from OMW such as phenolic compounds and residual oil. More specifically, in Spain, alpeorujo is usually processed in a centrifugation process called “repasso” in order to produce secondary oil and wet olive husk. The latter can be used, after drying it, as a solid biofuel [19].

In the case of Greece, the problem of two-phase OMW disposal has not been resolved yet and research into new technological procedures that permit its sustainable utilisation and treatment is necessary to be developed and applied. Currently, the predominant method of processing either the olive pomace from the III-phase olive mills or OMW from II-phase systems for recovering the secondary oil is solvent extraction. After drying the OMW, hexane is used as a solvent in order to extract the oil. The obtained mixture is then separated by evaporation to pomace oil and hexane. However, due to the high moisture content of OMW, a large amount of energy is required for drying, making the solvent extraction process economically non sustainable. On top of that, since olive mills in Greece are mainly small family or cooperative enterprises, large investments for processing the OMW such as the repasso process are not realistic [10].

In this study, a novel inexpensive method to recover the residual oil from OMW is developed. A statistical optimisation methodology based on design of experiments is employed to determine the optimum conditions to maximize the recovery yield of the residual oil. For that purpose, different parameters affecting the effectiveness of the proposed method have been evaluated in order to define the relevant factors and their interactions, as well as to simulate and optimize the whole system. Moreover, a kinetic study of the oil extraction process was conducted on the optimum conditions designated by the statistical model. Finally, the obtained aqueous solution that

is derived from the extraction process is analysed in terms of its phenolic content.

Materials and Methods

Olive Mill Waste (OMW)

OMW was obtained from a two-phase Olive Mill from the island of Thasos in Northern Greece. Around 300 kg of OMW were transferred to the Organic Chemical Technology Laboratory (NTUA) and stored at $-30\text{ }^{\circ}\text{C}$ for further use.

Extraction Experiments of Residual Oil from OMW

The experimental procedure for the extraction of residual oil from two-phase OMW involved two steps, an acidification step using sulphuric acid, water and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and a separation step using centrifugation. The acidification procedure was conducted by using 250 g of OMW which were placed in a 500 mL conical flask. Then sulphuric acid, water and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were added at various concentrations in each experiment indicated by the experimental design that was performed (see “Experimental Design” section). The created slurry was then stirred with a Teflon-coated magnetic stirrer, at 300 rpm and heated by a temperature-controlled hot plate (IKA, Model 9009300, Germany) at temperatures varying from 50 to $70\text{ }^{\circ}\text{C}$, for 1 h. After the acidification process, the extracts were centrifuged at 10,000 rpm for 10 min and left in funnels overnight for further separation of the three formed phases (an oil phase, an aqueous phase and a solid phase). Once separated, the oil (top) phase was collected, weighed and stored at $4\text{ }^{\circ}\text{C}$ for further analysis, while the aqueous (middle) phase was then filtered through a paper filter and a 0.45 mm syringe filter and stored at $4\text{ }^{\circ}\text{C}$ for further analysis.

Experimental Design

The extraction process of the residual oil of OMW was subjected to statistical optimization, selecting the four most important operational parameters, namely the solid to liquid ratio, the concentration of H_2SO_4 , the concentration of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and the temperature. A central composite design (CCD) was selected to evaluate the four optimization parameters. The selected ranges of the four factors was based on preliminary acidification experiments and their values are presented in Table 1. The following equation (Eq. 1) was used in order to code the four optimization parameters:

$$x_f = \frac{X_f - X_f^{CP}}{\Delta X_f} \quad (1)$$

Table 1 Ranges of the four factors for the CCD experiment

Level	Solid to liquid ratio (% w/v)	H_2SO_4 (% v/w)	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (% w/w)	Temperature ($^{\circ}\text{C}$)
-2	0.250	0.60	0.60	50
-1	0.333	0.80	0.80	55
0	0.417	1.00	1.00	60
1	0.500	1.20	1.20	65
2	0.583	1.40	1.40	70

where f is the optimization factor ($f = 1, \dots, 4$), x_f is the coded value, X_f is the real value, X_f^{CP} is the real value at the central point and ΔX_f is the step change value.

The experimental responses were predicted using Eq. 2:

$$Y_{RO} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_1 \beta_2 x_1 x_2 + \beta_1 \beta_3 x_1 x_3 + \beta_1 \beta_4 x_1 x_4 + \beta_2 \beta_3 x_2 x_3 + \beta_2 \beta_4 x_2 x_4 + \beta_3 \beta_4 x_3 x_4 + \beta_1 \beta_2 \beta_3 x_1 x_2 x_3 + \beta_1 \beta_2 \beta_4 x_1 x_2 x_4 + \beta_1 \beta_3 \beta_4 x_1 x_3 x_4 + \beta_2 \beta_3 \beta_4 x_2 x_3 x_4 + \beta_1 \beta_1 x_1^2 + \beta_2 \beta_2 x_2^2 + \beta_3 \beta_3 x_3^2 + \beta_4 \beta_4 x_4^2 \quad (2)$$

where Y_{RO} is the predicted response which in this case is the recovery yield of the residual oil in mg of residual oil per g of dried OMW, β_0 is the intercept element, $\beta_1, \beta_2, \beta_3$ and β_4 are the linear effects of the four optimization parameters, $\beta_1 \beta_2, \beta_1 \beta_3, \beta_1 \beta_4, \beta_2 \beta_3, \beta_2 \beta_4, \beta_3 \beta_4, \beta_1 \beta_2 \beta_3, \beta_1 \beta_2 \beta_4, \beta_1 \beta_3 \beta_4, \beta_2 \beta_3 \beta_4$ and $\beta_1 \beta_2 \beta_3 \beta_4$ are the linear interaction parameters and $\beta_1 \beta_1, \beta_2 \beta_2, \beta_3 \beta_3$ and $\beta_4 \beta_4$ are the quadratic terms for the four optimization parameters.

The optimization parameters were obtained by linear regression minimizing the difference between predicted and experimental values. Matlab software (R2007b) was used to estimate the coefficients in the CCD.

Extraction Kinetic Experiments

The kinetics of the extraction process were carried out at the optimal conditions indicated by the CCD. The amount of OMW used for these runs was 1000 g while the extraction process lasted for 120 min and samples of 30 mL each were taken every 10–30 min. All samples were centrifuged for 10 min at 10,000 rpm and saturated solution of sodium chloride was added at the oil phase up to 50 mL to increase the ionic strength. Then 50 mL of hexane were added to each sample and they were left in separating funnels. Afterwards, the residual oil of each sample was separated with the use of a rotary evaporator.

Analytical Procedures

Determination of Total Phenolic Content

Total phenolic content (TPC) was determined with the Folin–Ciocalteu reagent according to a procedure described by Singleton and Rossi (1965) [20]. Briefly, 0.50 mL of the diluted sample was reacted with 2.5 mL of 0.2 mol/L Folin–Ciocalteu reagent while steering for 2 min, and then 2 mL saturated sodium carbonate solution (about 75 g/L) was added into the reaction mixture followed by steering for 2 more minutes. The absorbance readings were taken at 765 nm after incubation at room temperature for 2 h. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of OMW.

Determination of Chemical Oxygen Demand

Chemical oxygen demand (COD) of the effluent was measured by the closed reflux, titrimetric method using 0.12 M FAS titrant (Standard Method 5220C). The sample was first diluted at 1:200 and placed in 16 × 100 mm culture tubes. After the addition of the digestion solution and the sulfuric acid reagent, the tubes were heated in a block digester for 2 h and titrated with a ferroin indicator. The results were calculated using the equation provided in the standard method as mg O₂/L.

HPLC–DAD Quantification

Analysis was performed on an Agilent 1290 Infinity II LC system with a diode-array detector (DAD) (Agilent Technologies, Santa Clara, CA, USA) and an Agilent C18 Proshell 120 column (4 μm, 4.6 × 100 mm) at a flow rate of 1 mL/min. The mobile phase consisted of 0.2% acetic acid in water (A) and acetonitrile (B). The following elution gradient was used: 2% B at 0 min up to 30% B at 40–45 min and back to 2% B at 45–50 min. Injection volume was 20 μL. DAD signals were recorded at a range of 210–360 nm.

All solvents used for chromatographic analyses were HPLC grade. Folin–Ciocalteu phenol reagent, acetonitrile HPLC grade, sulfuric acid, acetic acid and gallic acid were purchased from Sigma-Aldrich.

Determination of Oil Content and FAMES Analysis

The solvent extraction was conducted according to the ISO method 659:2009 with hexane as solvent using a Soxhlet type of extractor [21]. The OMW was initially dried and grinded and 10 g of the pretreated OMW was added in an extraction thimble which was placed inside the Soxhlet equipment. Approximately, 250 mL of hexane were added

to the heated flask. The extraction procedure lasted for 4 h to ensure that no more oil was further extracted. The hexane-oil mixture was then placed in an evaporator at 55 °C under vacuum conditions to separate the extracted oil from hexane. The extracted oil was then weighted and expressed as % w/w (grams of oil per gram of dry OMW).

The analysis of fatty acid composition of the residual oil was carried out through production of fatty acid methyl esters following a two-step reaction process. Initially, the fatty acids were gone through a transesterification step using sodium methoxide (MeONa) followed by an esterification step using methanol and HCl as acid catalyst [22]. The determination of FAMES was carried out by GC-FID using a Fisons 8130 equipped with a chrompack column (60 m × 0.32 mm) and using helium as carrier gas (2 mL/min).

Results

Characterization of OMW

OMW was initially analyzed for its moisture, COD, TPC and oil content. Its moisture content was 78% while its oil content was 10.2% dry basis. Its COD and TPC were determined by carrying out repeated extractions at the zero level of the CCD. The total COD and TPC were calculated by summing up the COD and TPC of each extraction, respectively. COD was equal to 89.2 g-O₂/L and TPC was equal to 10.0 g/L.



Fig. 1 Generation of the three phases after the acidification/hydrolysis and the centrifugation process (upper phase: residual oil; middle phase: aqueous phase rich in phenolic compounds and sugars; bottom phase: remaining solids)

In Fig. 1, the three formed phases after the first extraction and centrifugation process are shown. The top phase is the residual oil resulting from the acidification–extraction process, the middle phase is the aqueous phase containing water soluble substances such as phenolic compounds and reducing sugars, while the bottom phase is the solid phase. Residual oil has an existing market and can be sold to olive oil refineries. The aqueous phase resulting from the first extraction has a COD value of 30.2 g-O₂/L and TPC equal to 4.1 g/L. Phenolic compounds are soluble in water and hence, this wastewater requires further treatment either by an anaerobic digestion process to produce biogas or by extracting/oxidizing the phenolic compounds [23]. Finally, the solid phase can be used in a composting process to produce a soil amendment rich in nutrients. The solid phase after the centrifugation process has a moisture content of 55–65% and a decreased oil content of 1–2% dry matter. It has been studied from Chiodra et al. (2018) that absence of oils, fats and grease in OMW facilitates the composting

process [24]. Moreover, phenolic compounds can inhibit, severely, most of the biological processes, and hence, their extraction prior to composting will improve, in terms of productivity, a composting process that utilizes the solid phase from the oil extraction process [10, 23, 24].

Experimental Results and Model Predictions from the Extraction Process

The experimental results and model predictions from the CCD experiment are shown in Table 2. Maximum oil recovery is 43.7 mg/g db in run 17, where the solid to liquid ratio is at the highest level. Minimum oil recovery is 23.9 mg/g db and occurs in run 24, where temperature is at the lowest level. High oil recovery yields also take place at level zero (runs 25–28). For TPC, elevated extraction yields (> 40 mg/g) occur when the dilution is high (i.e. low solid to liquid ratio). The highest TPC (48.22 mg/g db) is at run 18 where the solid to liquid ratio is at –2 level.

Table 2 Experimental results and model predictions from the CCD

Run	Solid to liquid ratio (% w/v)	H ₂ SO ₄ (%v/w)	FeSO ₄ (% w/w)	Temperature (°C)	Oil yield (mg/g)	Predicted oil yield (mg/g)	TPC yield ^a (mg/g)
1	1	1	1	1	34.0	33.6	35.2
2	-1	1	1	1	25.7	23.9	41.2
3	1	-1	1	1	38.6	39.9	29.8
4	1	1	-1	1	38.5	37.3	31.7
5	1	1	1	-1	32.9	32.8	33.8
6	-1	-1	1	1	36.0	36.0	30.4
7	-1	1	-1	1	39.4	36.9	40.1
8	-1	1	1	-1	36.1	34.7	37.6
9	1	-1	-1	1	34.7	35.3	33.8
10	1	-1	1	-1	29.8	31.5	34.0
11	1	1	-1	-1	36.6	35.8	34.9
12	-1	-1	-1	1	33.0	32.3	33.6
13	-1	-1	1	-1	35.5	35.9	40.1
14	-1	1	-1	-1	25.2	23.1	40.9
15	1	-1	-1	-1	31.2	32.2	32.6
16	-1	-1	-1	-1	28.9	28.6	37.0
17	2	0	0	0	43.7	41.8	32.4
18	-2	0	0	0	31.8	35.0	48.2
19	0	2	0	0	25.5	29.8	36.4
20	0	-2	0	0	36.2	33.2	34.5
21	0	0	2	0	38.0	37.1	39.6
22	0	0	-2	0	33.2	35.4	35.9
23	0	0	0	2	27.3	28.8	36.6
24	0	0	0	-2	23.9	23.7	36.9
25	0	0	0	0	38.8	39.0	36.7
26	0	0	0	0	39.2	39.0	36.9
27	0	0	0	0	39.6	39.0	36.5
28	0	0	0	0	38.4	39.0	37.2

^aThe TPC values are for the generated aqueous phase

The implementation of the regression analysis computes the parameters of Eq. 2. The latter becomes:

$$\begin{aligned}
 Y_{OR} = & 39 + 1.679x_1 + 0.0863x_2 + 0.446x_3 + 1.271x_4 \\
 & + 0.919x_1x_2 - 0.781x_1x_3 + 0.431x_1x_4 - 1.444x_2x_3 \\
 & - 0.631x_2x_4 - 1.481x_3x_4 + 0.106x_1x_2x_3 \\
 & - 0.531x_1x_2x_4 + 2.044x_1x_3x_4 - 1.694x_2x_3x_4 \\
 & + 0.931x_1x_2x_3x_4 - 0.136x_1^2 \\
 & - 1.861x_2^2 - 0.674x_3^2 - 3.174x_4^2
 \end{aligned}$$

The parameters of Eq. 2 together with their *t*-ratio values and the ANOVA results are shown in Table 3. The significance of the model was checked by the *F*-test where the *F*(19,8) was higher than the tabulated value for 0.05 level of importance (*F*(19,8)=3.656 > tabulated *F*(19,8)_{0.05}=3.176). Figure 2 illustrates the experimental and predicted values of the oil recovery yield where *R*² is 0.90. The importance of the parameters was assessed by the student's *t*-distribution (see Table 3). According to the latter, the square of the Temperature ($\beta_4\beta_4$) has the strongest effect on the recovery of the oil yield followed by the square of sulphuric acid (β_2),

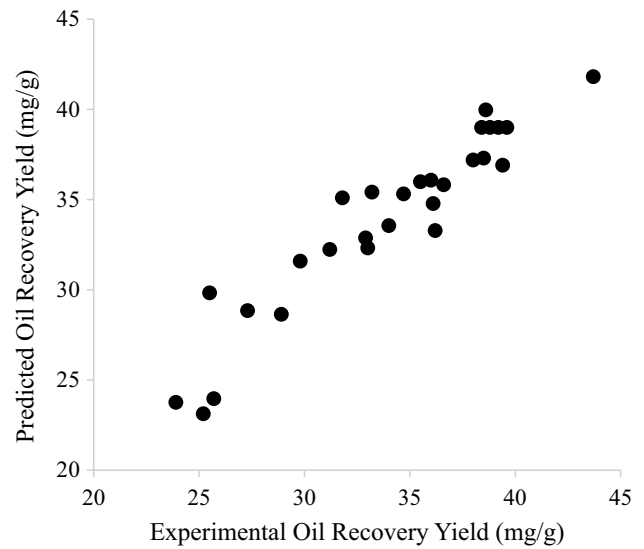


Fig. 2 Experimental and model predictions of the oil recovery yield by using Eq. 2

Table 3 ANOVA according to the CCD and estimation of parameter values for oil recovery yield (the significant parameters are in bold)

Source	Degrees of freedom	Sum of squares	Mean square	<i>F</i> -value	<i>P</i> > <i>F</i>
Model	19	653.59	34.40	3.656	0.033
Error	8	75.27	9.41		
Lack of fit	5	74.47	14.89	55.85	0.0037
Pure error	3	0.80	0.27		
Total	8	75.27			
Parameters	Estimate	Standard error	<i>t</i> Ratio	<i>Prob</i> > <i>t</i>	
Intercept	39.0	1.534	25.43	<0.0001*	
β_1	1.679	0.626	2.68	0.028*	
β_2	-0.863	0.626	-1.38	0.206	
β_3	0.446	0.626	0.71	0.497	
β_4	1.271	0.626	2.03	0.077	
$\beta_1 \beta_2$	0.919	0.767	1.20	0.265	
$\beta_1 \beta_3$	-0.781	0.769	-1.02	0.338	
$\beta_1 \beta_4$	0.431	0.767	0.56	0.589	
$\beta_2 \beta_3$	-1.444	0.767	-1.88	0.096	
$\beta_2 \beta_4$	-0.631	0.767	-0.82	0.434	
$\beta_3 \beta_4$	-1.481	0.767	-1.93	0.089	
$\beta_1 \beta_2 \beta_3$	0.106	0.767	0.14	0.893	
$\beta_1 \beta_2 \beta_4$	-0.531	0.767	-0.69	0.508	
$\beta_1 \beta_3 \beta_4$	2.044	0.767	2.67	0.029	
$\beta_2 \beta_3 \beta_4$	-1.694	0.767	-2.21	0.058	
$\beta_1 \beta_2 \beta_3 \beta_4$	0.931	0.767	1.21	0.259	
$\beta_1 \beta_1$	-0.137	0.626	-0.22	0.833	
$\beta_2 \beta_2$	-1.862	0.626	-2.97	0.018	
$\beta_3 \beta_3$	-0.674	0.626	-1.08	0.313	
$\beta_4 \beta_4$	-3.174	0.626	-5.07	0.001	

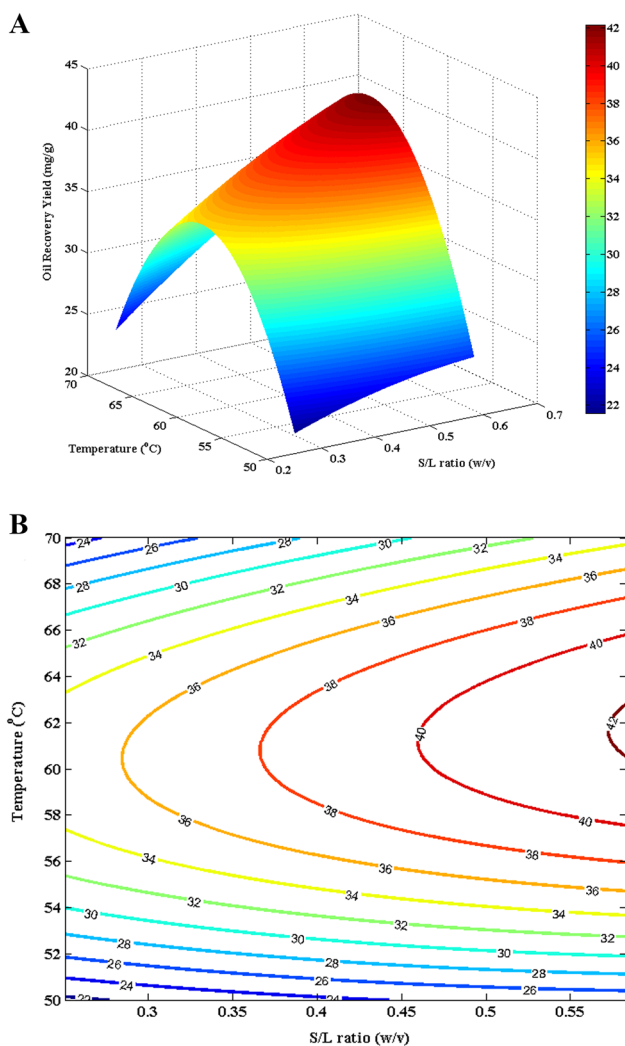


Fig. 3 Oil recovery yield with respect to X_1 (S/L ratio) and X_4 (temperature) in 3-D plot (a) and contour plot (b); the coded values for X_2 and X_3 are set to zero

the S/L ratio (β_1) and the linear effect of H_2SO_4 , $FeSO_4$ and Temperature ($\beta_2\beta_3\beta_4$).

The model predictions of Eq. 2 for the examined area are shown in Fig. 3 for X_1 , X_4 variables, in Fig. 4 for X_1 , X_2 variables and in Fig. 5 for X_2 , X_4 in the form of 3-D and contour plots. Due to the minor impact of X_3 ($FeSO_4 \cdot 7H_2O$) on the oil recovery yield, graphs with this variable have been omitted.

Validation of the model was carried out at optimum conditions where the coded values of X_1 , X_2 , X_3 and X_4 were -2 , 2 , -2 and 2 , respectively and the oil extraction yield is equal to 62.7 mg/g. The real values of the four parameters were 0.25 for the solid to liquid ratio (250 g of OMW and 1000 mL of water, 3.5 mL of sulfuric acid, 1.5 g of $FeSO_4 \cdot 7H_2O$ and 70 °C temperature. Experimental value on the optimum conditions gave an oil recovery yield of

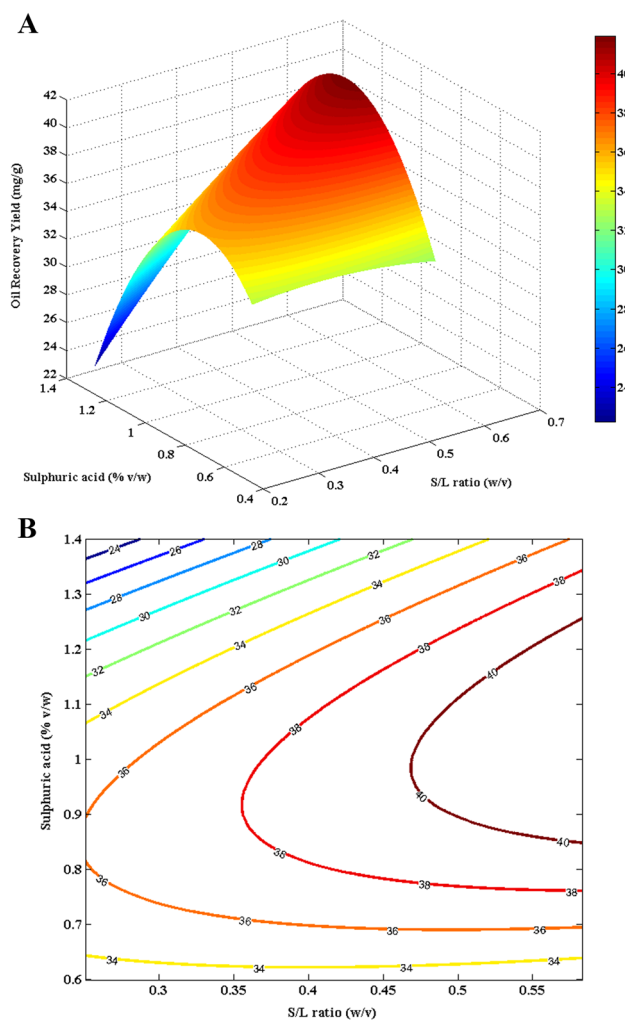


Fig. 4 Oil recovery yield with respect to X_1 (S/L ratio) and X_2 (sulphuric acid) in 3-D plot (a) and contour plot (b); the coded values for X_3 and X_4 are set to zero

47.7 mg/g. Although, this value is lower than the predicted one, it is significantly higher than the experimental values shown in Table 2. The TPC of the aqueous phase on the same extraction conditions was 48.2 mg/g OMW (db).

Extraction Kinetics on the Optimum Conditions

Once the optimum conditions for residual oil extraction were obtained and validated via an experimental run, a kinetic model was developed. Solid–liquid extraction can be considered as a reverse absorption process. Thus, the basic kinetic equations of absorption can be applied.

From the non-linear regression analysis of the results, it was found that the oil yield follows the equation:

$$C_t = -0.05t^2 + 1.023t + 10.979 \quad (3)$$

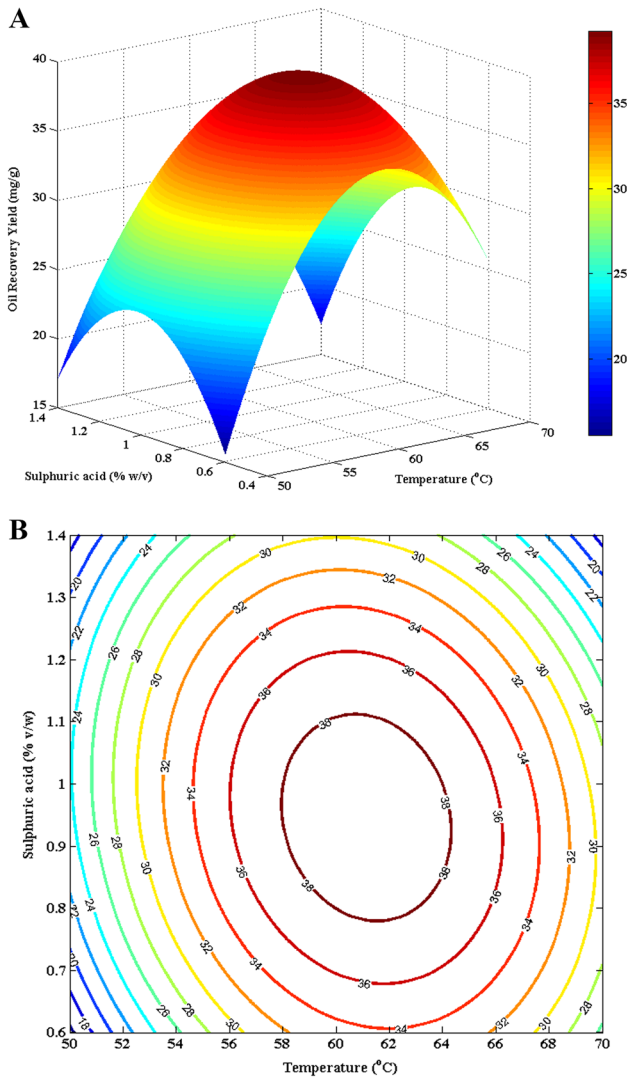


Fig. 5 Oil recovery yield with respect to X_2 (sulphuric acid) and X_4 (temperature) in 3-D plot (a) and contour plot (b); the coded values for X_1 and X_3 are set to zero

which is statistically significant with $p < 0.001$ and $R^2 = 0.98$.

As the second order kinetic model was found to be able to deliver the best fitting (results not shown), the following equation can be used:

$$\frac{dC_t}{dt} = k \times (C_e - C_t)^2 \quad (4)$$

where k is the extraction rate constant (L/g min), C_e is the oil concentration in the liquid extract (g/L) (extractability) and C_t the oil concentration (g/L) in the extraction liquid at extraction time t . By integrating Eq. 3 for $t=0$ to t and $C_t=0$ to C_t , the equation becomes:

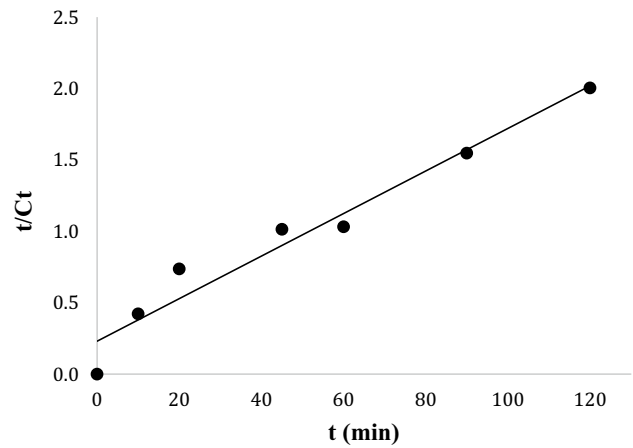


Fig. 6 Second order kinetic model showing the correlation of t/C_t with time

$$C_t = \frac{k \times t \times C_e^2}{1 + k \times t \times C_e} \quad (5)$$

The linearization of the above equation leads to Eq. 6:

$$\frac{t}{C_t} = \frac{1}{k \times C_e^2} + \frac{1}{C_e} = \frac{1}{h} + \frac{1}{C_e} \quad (6)$$

where h is the initial extraction rate (g/L min) and is equal to $h = k \times C_e^2$.

In Fig. 6 the correlation of t/C_t with time is shown to be linear, with $R^2=0.96$, confirming the predictive capability of the second order kinetic model.

From the linear least squares fitting, we were able to obtain the following parameters for Eq. 7:

$$\frac{t}{C_t} = 0.0149 \times t + 0.2305 \quad (7)$$

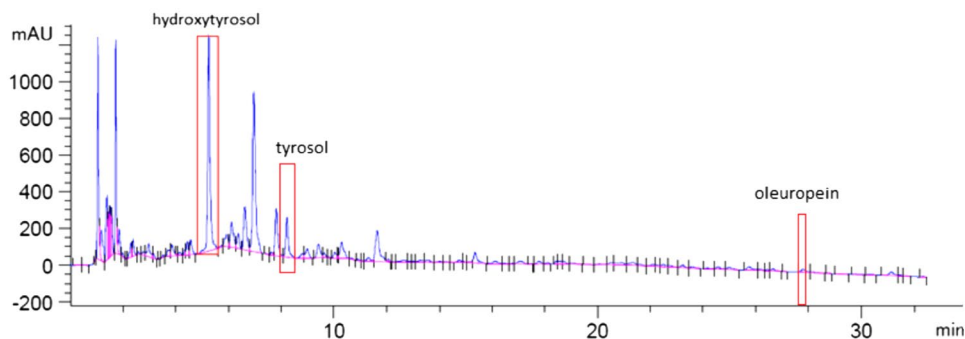
where $C_e = 67.114$ mg/mL, $h = 4.338$ mg/mL min and $k = 9.631 \times 10^{-4}$ mL/mg min.

Analysis of the Residual Oil

Table 4 presents the fatty acid profile of the residual oil recovered from OMW using the acidification/hydrolysis method with sulphuric acid on the optimum extraction conditions. We also compare our findings with an analysis of a virgin olive oil. The main fatty acid was oleic acid (Δ^9 C18:1) followed by palmitic acid (C16:0) and linoleic acid ($\Delta^9,12$ C18:2). There is also a small percentage of stearic acid (C18:0). Oleic acid was present at 72% while palmitic acid was at 10.9% and linoleic acid at 12.5%. The most important is that the fatty acid analysis of the residual oil from OMW is very similar with the one of virgin

Table 4 Fatty acid profile of residual oil of OMW and virgin olive oil

Substrate	Fatty acid methyl esters %						
	C16:0	Δ^9 C16:1	C18:0	Δ^9 C18:1	$\Delta^{9,12}$ C18:2	$\Delta^{9,12,15}$ C18:3	Others
Virgin olive oil	11.8	0.7	2.7	77.0	6.4	0.6	0.6
Oil recovered from OMW	10.9	0.6	2.5	72.0	12.5	0.7	0.7

Fig. 7 HPLC chromatogram from the analysis of the aqueous phase formed after the extraction process on the optimum olive oil extraction conditions

olive oil illustrating that this product is a high-value end product with existing market and increasing demand. The acidity of the residual oil on the optimum conditions designated by the experimental design was equal to 1.8.

Determination of the Phenolic Compounds Contained in the Aqueous Liquid Stream

HPLC was used for separating and measuring the most important phenolic compounds present in the aqueous phase generated after the extraction process. Figure 7 illustrates the chromatogram produced from the HPLC analysis on the optimum olive oil extraction conditions where distinct peaks of hydroxytyrosol (6.9 mg/g OMW db) and tyrosol (5.1 mg/g OMW db) are shown. Oleuropein is also present but in much lower amounts (<0.3 mg/g OMW db) as this phenolic compound is found mainly in olive leaf rather than olive fruit [25]. The aqueous fraction should be further treated and/or utilized before it can be disposed to conventional biological treatment methods. It can be used as feeding in an anaerobic digestion process [23, 26] or in a co-composting process with olive mill solid residues [10] or it can be further valorized by extracting its valuable phenolic compounds. The latter can be implemented by using a number of sequential processes such as ultra-filtration, ion-exchange resins and evaporation [27, 28].

Conclusions

In this study, a new acidification/hydrolysis process for extracting the residual oil from olive mill wastes from II-phase olive mills was presented. The extraction process was subject to

statistical optimisation implementing a CCD with four variables and three levels. Maximum experimental results showed that from 1 ton of OMW with 78% of moisture 10.5 kg of residual oil can be extracted. Moreover, the oil extraction kinetic can be simulated by using a second order kinetic model. Regarding the fatty acid composition, the residual oil has a similar profile with the virgin olive oil. Once extracted, the residual oil can be sold to olive oil refineries under a market price 1–2 €/kg [29], and hence, a middle size II-phase olive mill in Greece generating 3000 tons of OMW can have an income of 31,500–63,000 €/y. The simplicity of the extraction process allows the olive mill owners to reduce the capital investment to zero as they can use the existing equipment of their facilities such as mixers, centrifuges and decanters for extracting the residual oil. Finally, after the oil extraction process, the generated aqueous phase contains notable amounts of significant phenolic compounds such as hydroxytyrosol and tyrosol which are of marketable interest and may result, with the proper treatment which is subject of a future publication from our research group, in an additional revenue for olive mills.

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