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Comparative study of modeling the stability improvement of sunflower oil with olive leaf extract

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Abstract−Commercially available sunflower oil was enriched in polyphenols by adding olive leaf extract. After extracting the dried and ground olive leaves with the assistance of homogenizer, total phenolic content (TPC) and oleuropein concentration of the extract were determined. The dried extract was partially dissolved into the sunflower oil to increase the quality and shelf-life of the oil enriched by the substances in the plants by means of solid-liquid extraction method. A face central composite design (FCCD) through response surface methodology (RSM) was used to investigate the effects of enrichment conditions (extract content, time and mixing speed) on the responses, TPC and oleuropein concentration of the enriched sunflower oil as well as to design of experiments, to model and to optimize the process. The enriched sunflower oil obtained at optimum conditions was evaluated in terms of its TPC, oleuropein, total carotenoid content (TCC), antioxidant activity (AA), peroxide value (PV) and induction time (IT), depending on those of the crude oil. Furthermore, artificial neural networks (ANN) were also employed to compare the predicted results of RSM.

Keywords: Edible Oils, Natural Antioxidants, Mathematical Modeling, Response Surface Methodology, Artificial Neural Networks

INTRODUCTION

Sunflower oil, olive oil, palm oil, soybean oil, canola oil, corn oil, peanut oil and other vegetable oils are varieties of edible oils as well as animal-based oils like butter and lard. Sunflower oil is one of the most consumed oils (8.6 million tons per year) accounting for a significant fraction of world-wide edible oil production [1]. It is mostly produced in areas including the Russian Federation, Ukraine, Argentina and Turkey [2].

The consumption of vegetable oils has increased dramatically in the past century [3]. However, lipid oxidation is the most qualitative problem in food products containing fat such as vegetable oils. This situation leads to great economic loss in the food industry. Antioxidants are added to foods containing fats to prevent unpleasantness and formation of toxic components resulting from lipid oxidation. Recently, natural antioxidants have been studied intensively as a result of the limitations in their use due to the understanding of the toxic effects of synthetic antioxidants. Therefore, enrichment of edible oils with natural antioxidants is of great interest. In this study, olive tree (*Olea europaea*) leaf was used as a potential antioxidant additive since it is a cheap, renewable and abundant source of polyphenols [4]. As far as is known, the first application of direct olive leaf into the edible oil (olive oil) was by Japón-Luján et al. [5]. Ultrasound assisted extraction was used for solid-liquid extraction to dissolve the leaves into the oil. Achat et al. also utilized the same method to improve the nutritional value of olive oil by enriching it in phenolic compounds from olive leaves [6]. Salta et al. evaluated oils (olive oil, sunflower oil and palm oil) enriched with olive leaf extract for their oxidative stability and antioxidant capacity [7]. On the other hand, Paiva-Martins et al. used liquid-liquid extraction to enrich the refined olive oil to achieve the same quality of virgin olive oil before solid-liquid studies [8]. With respect to sunflower oil, except for a few studies, there is a lack of literature on the enrichment with olive leaves [7,9]. Olive leaf extracted by microwave to enhance the antioxidant activity of sunflower oil was also used in some studies [9,10]. In a similar study, olive leaf extracts obtained by pressure, supercritical and solvent-extraction were added to increase the oxidative stability of vegetable oils including sunflower oil [11]. Medina et al. used phenolic antioxidants from olive materials into several edible oils in addition to sunflower oil to assess the quality parameters by enriching with an electrical stirrer [12].

One of the most consumed edible oils, sunflower oil, has not been enriched in order to enhance its oxidative stability by olive leaf extract before. The quality parameters such as total phenolic and carotenoid contents, oleuropein (major individual phenolic compound of olive leaf), antioxidant activity, and peroxide value were also assesed as well as oxidative stability. Finally, it has been the first time that the experimental data was modelled by both RSM and ANN.

MATERIAL AND METHODS

1. Materials

Samples of the olive leaf were provided by Özgün Olive, Olive

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Oil Co's relevant departments. The leaves were harvested from Ayvalik in Aegean of Turkey. Sunflower oil was purchased from a local market.

Ethanol, methanol, and hexane were provided from Merck and were of >99.5%, >99.8% and >99% mass purity, respectively. Redistilled water was used in all experiments. Folin-Ciocalteu reagent, sodium carbonate, gallic acid and 2,2′-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich. Oleuropein standard was obtained from Extrasynthese (Genay, France). 18 mΩ deionized water from Human Power I water purification system was used to prepare mixtures and analyses.

2. Extraction of Olive Leaves

500 mg of dried and ground leaf samples were extracted three times with 30 mL of ethanol solution (%10, v/v) by blending in a homogenizer (IKA T25, ULTRA-TURRAX) at 4,000 rpm and 45 sec (three times at 10 s intervals). The mixture was centrifuged (Nüve, CN 180) at 5,000 ×g for 25 min. After the solvent was evaporated, the dried extracts were kept in the dark and stored at −20 °C.

3. Enrichment of Sunflower Oil

The dried extract was partially dissolved into the sunflower oil by means of solid-liquid extraction method. Olive leaf extracts were added to sunflower oil at concentrations of 1,000-1,500 ppm, and mixed vigorously at several conditions (4,000-10,000 rpm and 30-90 sec) by means of homogenizer.

4. Extraction of Polyphenols from the Enriched Sunflower Oil

2.5 mL hexane was added into 1 g of olive oil, and extracted with methanol twice at 7,000 rpm for 60 sec. The extracts were combined, washed with hexane and then filtered through a 0.45 μm syringe filter and stored at −20 °C until analysis for the biochemical measurements.

5. Determination of Total Phenolic Content

The concentration of total phenols in the extracts was measured by UV-spectrophotometry (PG Instruments, T60/Leicestershire, England), based on calorimetric oxidation/reduction reaction. The total phenolic content was determined according to the Folin-Ciocalteu method at a wavelength of 765 nm by following the procedure of [13].

6. Determination of Antioxidant Capacity by ABTS Assay

Free radical scavenging activity of the extracts by ABTS assay was done according to the modified method of [14]. After addition of 150 μL of sample solution to 2,850 μL of diluted ABTS solution, absorbance was measured at 10th minute at 734 nm against a blank sample without ABTS.

7. HPLC Analysis

Analyses were conducted on an Agilent 1260 chromatographic system (Agilent, Waldbronn, USA) equipped with quaternary pump, a degasser, manual injector and a diode-array-detector (DAD). Agilent Eclipse Plus C18 RRHD 18 column (3.0 mm×5.0 mm id, 1.8 μm particle size) was used to separate the extracts. The column temperature was maintained at 40 °C with a gradient elution of (A) 0.1% formic acid in H_2O and (B) 0.1% formic acid in acetonitrile. A gradient program was written according to the following profile: 0-14 min 0% B, 14-14.2 min 40% B, 14.2-17.2 min 100% B, 17.4-20 min 0% B. Injection volume was 20 μL and the detection wavelength was set at 276 nm.

Table 1. Values of the independent variables and their coded forms with their symbols employed in RSM for optimization of enrichment

Independent variables	Units	Symbol of the variables	Coded levels		
			−1	0	1
Mixing speed	rpm	X_1	4000	7000	10000
Time	sec	X_2	30	60	90
Solid mass	ppm	X_3	1000	1250	1500

8. Rancimat Method

The stability of sunflower oil before and after enrichment was evaluated by Rancimat method at 130 °C in a Rancimat 892 apparatus (Metrohm). The oxidation process was monitored upon 3 g oil sample at air velocity 20 L/h. The stability of the oils was expressed as the induction time (IT).

9. Peroxide Value

The peroxide value in mg oxygen equivalent per kg of oil (meq-O_2/kg-oil) was determined by titration of 0.1 N KI saturated solutions of the oil with 0.1 N $Na_2S_2O_3$ and starch as indicator [12].

10. Determination of Carotenoid

Total carotenoid content of the oil was determined by following the slightly modified method of [15] at 450 nm. The carotenoid content was calculated by using the calibration curve, obtained using known concentration of β-carotene dissolved in hexane.

11. Response Surface Methodology

Response surface methodology was employed for design of experiments, modeling and optimization of the enrichment conditions of sunflower oil with olive leaf extract. A three factor with three levels, face central composite design (FCCD) of RSM was selected to explore the effect of variables on the response (Table 1). Total phenolic content (Y_1) and oleuropein (Y_2) were the responses, respectively. Mixing speed (X_1), time (X_2) and solid mass (X_3) were independent variables, selected based on the preliminary experiments. To apply the FCCD, Design-Expert 9.0.6 software (Statease, Minneapolis, MN, USA) was used. Twenty experiments were conducted with six replications at the center values to evaluate the pure error sum of squares. The quadratic model proposed was shown with the following equation:

$$Y = \beta_0 + \Sigma_{i=1}^{n}\beta_i X_i + \Sigma_{i<j}^{n}\beta_i X_i X_j + \Sigma_{i=1}^{n}\beta_{ii} X_i^2 + \cdots \quad (1)$$

where Y is the response, β_0 is the constant coefficient often described as intercept, X_i (i=1-3) is the non-coded variable, β_i is the linear, and β_{ii} is the quadratic, and β_{ij} (i and j=3) is the second-order interaction coefficients [16].

12. Artificial Neural Networks

Artificial neural network was also utilized to model the experimental results. ANN is a method designed to model the way in which the brain performs a particular task or function of interest [17]. It represents a technology that is rooted in many disciplines such as mathematics, statistics, computer science, chemistry, physics, neurosciences and engineering. ANN has been applied successfully to processes concerning food such as beef [18], bean [19], wheat [20,21], sunflower [22] and olive [23-26].

In this study, a feed-forward multilayer ANN structure with back-propagation learning algorithm was used for estimating the responses

for each combination of the independent variables. ANN has a built-in capability to adopt synaptic weights to changes in the surrounding environment. Therefore, ANN structure is used with different numbers of hidden layer neurons to obtain the maximum convergence between the estimated and the observed results. The decision on the hidden neurons was taken depending on previous studies in the literature because of the lack of a definite method. 10, 20 and 30 were the numbers of hidden neurons chosen by the simulation program through MATLAB for the estimation. The other characteristics of the ANN system can be mentioned as follows: 70%, 15% and 15% input data were used for training, validation and test, respectively. The training algorithm was Levenberg-Marquardt algorithm, designed to approach second-order training speed without having to compute the Hessian matrix [27].

The process of training an ANN involves tuning the values of the weights and biases of the network to optimize network performance. The default performance function for feedforward networks is the mean square error (mse); mse is the average squared error between the network outputs a and the target outputs t, which is defined as follows:

$$F = mse = \frac{1}{N}\sum_{i=1}^{N}(e_i)^2 = \frac{1}{N}\sum_{i=1}^{N}(t_i - \alpha_i)^2 \quad (2)$$

One iteration of the training algorithm using mse function can be written as:

$$x_{k+1} = x_k - \alpha_k g_k \quad (3)$$

where

x_k is a vector of current weights and biases,
g_k is the current gradient,
α_k is the learning rate.

13. Statistical Analysis

Each experimental study was repeated three times followed by three spectrophotometric and chromatographic analyses from each sample. To determine the interaction between the process parameters and the responses, analysis of variance (ANOVA) test was applied by means of Design-Expert program.

RESULTS AND DISCUSSION

The enrichment process was conducted by means of solid-liquid extraction, in which solid olive leaf extract was partially dissolved into the sunflower oil.

Table 2. FCCD of the independent variables (X_1, X_2, X_3) and experimental results for the TPC and oleuropein*

Run number	Independent variables			Responses	
	X_1 (rpm)	X_2 (sec)	X_3 (ppm)	TPC (ppm)	Oleuropein (ppm)
1	10000	30	1500	139.22±0.01	23.92±0.33
2	4000	30	1500	87.48±0.01	18.55±0.31
3	7000	30	1250	92.75±0.02	20.72±0.41
4	4000	30	1000	84.37±0.01	12.65±0.10
5	7000	60	1500	88.34±0.00	14.57±0.35
6	7000	60	1000	85.07±0.00	21.47±0.09
7	10000	90	1500	124.57±0.02	24.72±0.01
8	7000	90	1250	85.65±0.02	13.60±0.20
9	7000	60	1250	101.12±0.02	12.12±0.22
10	10000	30	1000	150.68±0.02	24.12±0.33
11	4000	60	1250	80.71±0.00	11.68±0.30
12	7000	60	1250	90.82±0.01	15.33±0.29
13	10000	60	1250	139.44±0.01	23.00±0.35
14	7000	60	1250	91.23±0.00	11.99±0.38
15	7000	60	1250	90.74±0.02	12.01±0.40
16	4000	90	1500	72.89±0.00	11.89±0.09
17	10000	90	1000	105.27±0.01	18.17±0.36
18	7000	60	1250	100.54±0.01	12.37±0.22
19	4000	90	1000	99.12±0.00	13.09±0.21
20	7000	60	1250	101.04±0.01	12.01±0.31

*Data are expressed as the mean (n=9)±S.D.

Table 3. ANOVA for the quadratic equations of Design Expert 9.0.6 for the enrichment of TPC in sunflower oil

Source	Sum of squares	df	Mean square	F value	P-value Prob>F
Model	7859.98	9	873.33	10.47	0.0005
X_1-speed	5522.50	1	5522.50	66.21	<0.0001
X_2-time	448.90	1	448.90	5.38	0.0428
X_3-mass	16.90	1	16.90	0.20	0.6622
X_1X_2	450.00	1	450.00	5.40	0.0426
X_1X_3	128.00	1	128.00	1.53	0.2437
X_2X_3	9.095E-013	1	9.095E-013	1.090E-014	1.0000
X_1^2	1001.70	1	1001.70	12.01	0.0061
X_2^2	10.08	1	10.08	0.12	0.7353
X_3^2	42.14	1	42.14	0.51	0.4934
Residual	834.05	10	83.40		
Lack of fit	671.22	5	134.24	4.12	0.0731
Pure error	162.83	5	32.57		
Cor total	8694.03	19			

Table 4. ANOVA for the quadratic equations of Design Expert 9.0.6 for the enrichment of oleuropein in sunflower oil

Source	Sum of squares	df	Mean square	F value	P-value Prob>F
Model	339.02	9	37.67	3.29	0.0385
X_1-speed	212.19	1	212.19	18.56	0.0015
X_2-time	34.13	1	34.13	2.99	0.1147
X_3-mass	1.72	1	1.72	0.15	0.7062
X_1X_2	0.14	1	0.14	0.012	0.9135
X_1X_3	0.33	1	0.33	0.029	0.8680
X_2X_3	0.016	1	0.016	1.425E-003	0.9706
X_1^2	6.04	1	6.04	0.53	0.4839
X_2^2	4.64	1	4.64	0.41	0.5385
X_3^2	12.87	1	12.87	1.13	0.3136
Residual	114.34	10	11.43		
Lack of fit	105.56	5	21.11	12.03	0.0082
Pure error	8.78	5	1.76		
Cor total	453.36	19			

1. Modeling of the Process by RSM

Table 2 shows the randomly selected experimental runs by Design Expert 9.0.6 software. ANOVA results for the quadratic equations of Design Expert 9.0.6 for the TPC and oleuropein responses are given in Tables 3 and 4, respectively. The models derived for TPC and oleuropein were found significant ($p<0.05$) to display the relationship between the response and independent variables. As seen in Tables 3 and 4, mixing speed was found as the most significant (at the level of $p<0.05$) variable on the enrichment of both TPC and oleuropein in sunflower oil.

The quadratic models for TPC (Y_1) and oleuropein (Y_2) were derived as given in the equations below:

$$Y_1=93.48-23.5X_1-6.72X_2-1.3X_3-7.5X_1X_2+4.0X_1X_3 +1.676E-0.14X_1X_3+19.09X_1^2-1.91X_2^2-3.91X_3^2 \quad (4)$$

$$Y_2=13.93+4.61X_1-1.85X_2+0.41X_3+0.13X_1X_2+0.2X_1X_3 -0.045X_2X_3+1.48X_1^2+1.3X_2^2+2.16X_3^2 \quad (5)$$

The ANOVA results also showed that the experimental data had correlation coefficients (R^2) of 0.9041 and 0.7478 with the calculated models, accounting for the 90.41 and 74.78% of the results, respectively. The independent and dependent variables were tested for lack of fit on the quadratic response surface models. The first model derived for TPC had a non-significant lack of fit value ($p>0.05$), showing that the model accurately fits the data [28]. However, the lack of fit value of the other model calculated for oleuropein was found significant ($p<0.05$). The same results were also observed by various studies [29-32]. In [31] it is asserted that a model with a significant lack of fit could still be used when large amounts of data were included in the analysis. On the other hand,

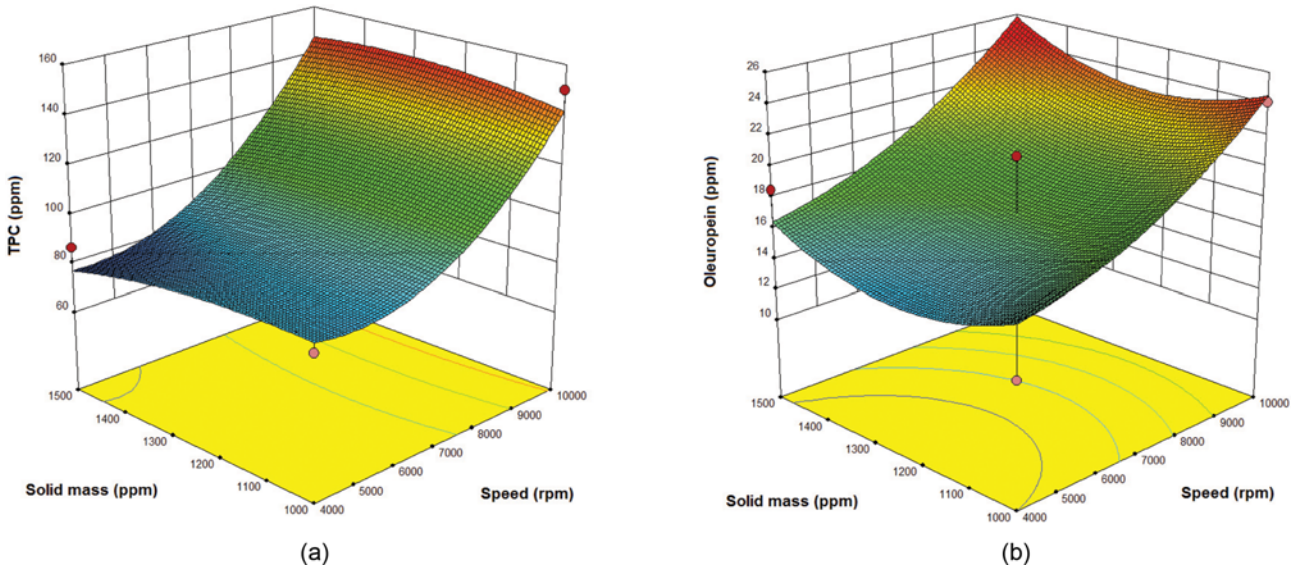

Fig. 1. Response surface plots for the TPC (a) and oleuropein (b) of enriched sunflower oil as a function of solid mass to mixing speed (time= 30 sec).

Adequate Precision measures the signal to noise ratio. Both the predicted models had ratios greater than 4, which is desirable in order to indicate adequate model discrimination. Therefore, the models can be utilized for navigation the design space [32].

To explore the potential relationships between the the variables, three-dimensional (3D) response surface plots (Figs. 1 to 3) were constructed according to the quadratic equations 4 and 5. As seen in Fig. 1, enrichments by TPC and oleuropein were both increased with mixing speed of homogenizer. Diffusion of the target compounds into the oil is expected to be increased with stirring [33]. Moreover, agitation also contributes to the favorable extraction with its additional energy and promoting the homogeneity [34,35].

As for extract quantity, oleuropein yield had a tendency to decrease until a point, then it started to increase. This is in agreement with the results of previous studies [36,37]. The study [36] observed a 35.8% reduction in TPC when extracting phenolic antioxidants from peanut skins in the mass of the skins from 1.5 to 3.5 g showed almost 22 and 13% decrease in the TPC and flavonoid yields of mandarin leaf exracts obtained by microwave-assisted extraction. This is mostly attributable to the surface area. Increasing the sample mass decreases the surface area, which is unfavorable for the penetration of the sample into the oil.

Time had a negative effect on the enrichment of sunflower oil for both variables (Fig. 2). This result is most probably due to the degradation of the components caused by overexposure to the heating of the long mixing time [38]. This might be explained by the

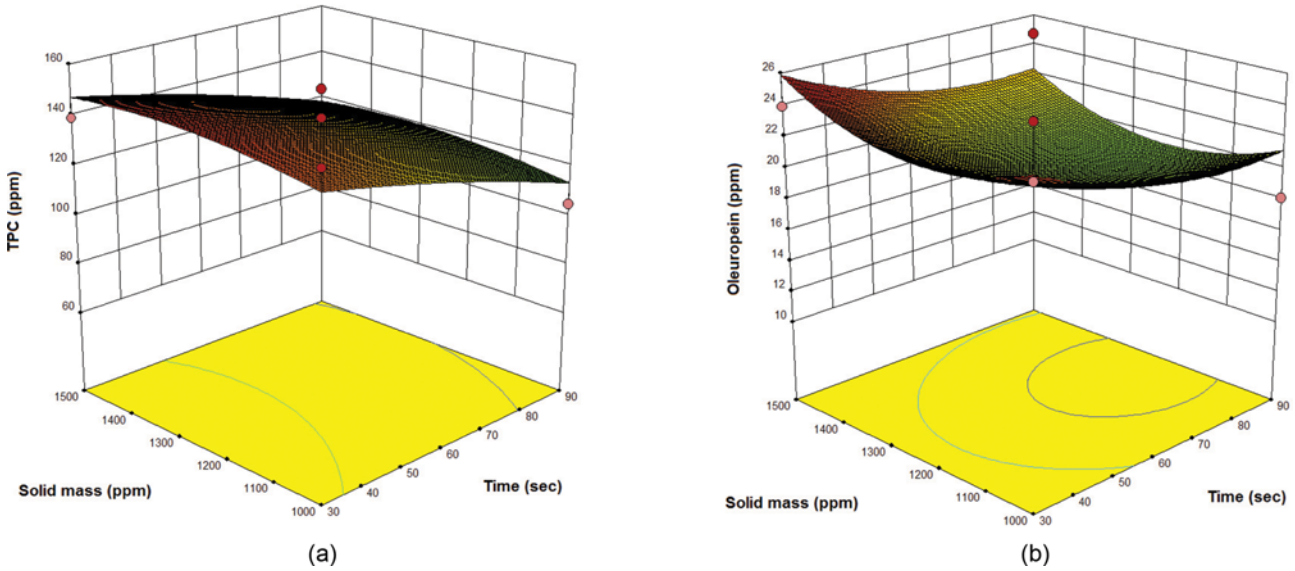

Fig. 2. Response surface plots for the TPC (a) and oleuropein (b) of enriched sunflower oil as a function of solid mass to mixing time (mixing speed=10,000 rpm).

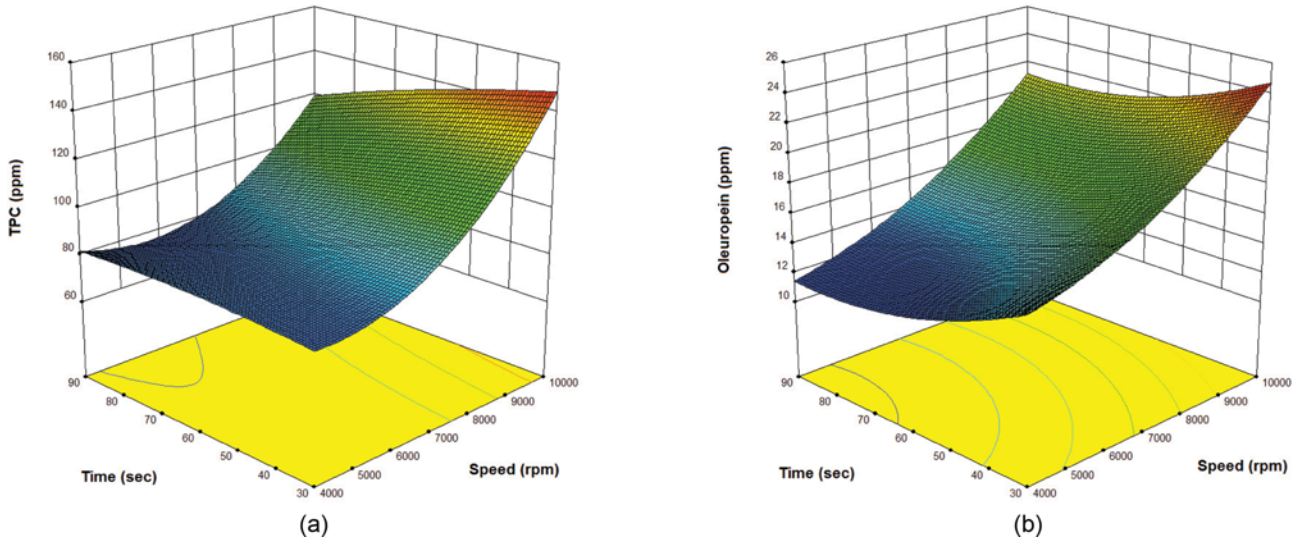

Fig. 3. Response surface plots for the TPC (a) and oleuropein (b) of enriched sunflower oil as a function of mixing time to speed (solid mass= 1,435 ppm).

fact that the major phenolic compound, oleuropein and the other polyphenols of the olive leaf, are not thermally stable. Expectedly, mixing speed indicated an increase in the oil enriched by TPC and oleuropein, while extraction time gave rise to a drop under a constant solid mass (Fig. 3).

2. Modeling of the Process by ANN

The results estimated for TPC and oleuropein with 10, 20 and 30 hidden neurons in the hidden layer of the ANN structure and

Table 5. The TPC values obtained with experimental study and predicted with ANN

Run no.	Experimental TPC (ppm)	Predicted TPC (ppm) with 10 hidden neurons	Predicted TPC (ppm) with 20 hidden neurons	Predicted TPC (ppm) with 30 hidden neurons
1	139.22	139.14	138.82	140.67
2	87.48	86.92	101.04	84.21
3	92.75	92.09	91.06	95.33
4	84.37	82.35	75.28	81.02
5	88.34	87.93	85.18	94.53
6	85.07	84.92	84.79	76.30
7	124.57	123.75	123.59	129.12
8	85.65	82.76	76.14	92.74
9	101.12	101.24	100.80	84.13
10	150.68	150.34	149.15	147.19
11	80.71	82.08	72.44	71.10
12	90.82	88.01	92.99	101.54
13	139.44	138.73	137.20	136.36
14	91.23	90.96	85.02	75.65
15	90.74	89.90	84.39	75.55
16	72.89	72.22	81.73	98.00
17	105.27	104.88	105.04	111.09
18	100.54	98.91	97.69	84.51
19	99.12	98.63	94.35	91.53
20	101.04	100.72	101.46	83.85

Table 6. The oleuropein values obtained with experimental study and predicted with ANN

Run no.	Experimental oleuropein (ppm)	Predicted oleuropein (ppm) with 10 hidden neurons	Predicted oleuropein (ppm) with 20 hidden neurons	Predicted oleuropein (ppm) with 30 hidden neurons
1	23.92	23.93	23.58	24.95
2	18.55	20.22	22.91	23.39
3	20.72	19.85	21.41	21.47
4	12.65	12.64	14.89	12.59
5	14.57	14.57	14.46	24.40
6	21.47	21.14	20.98	20.02
7	24.72	24.70	23.79	23.78
8	13.6	13.70	13.76	10.43
9	12.12	13.40	12.24	12.73
10	24.12	25.01	22.91	13.55
11	11.68	11.44	22.84	12.12
12	15.33	14.84	12.24	12.73
13	23.04	23.14	24.66	22.94
14	11.99	13.40	12.24	12.73
15	12.01	12.40	12.94	12.77
16	11.89	11.57	10.57	11.24
17	18.17	18.58	18.30	18.40
18	12.37	13.40	12.24	12.73
19	13.09	13.02	16.05	13.34
20	23.92	23.58	23.93	24.95

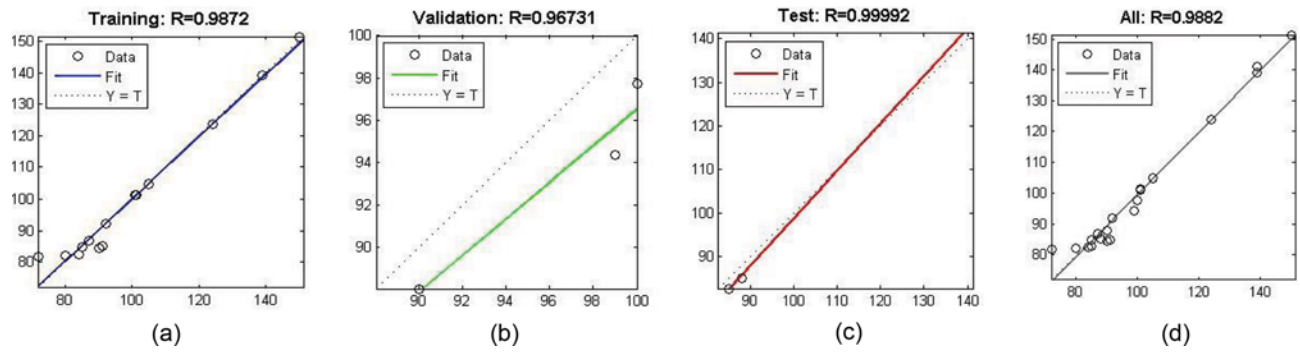

Fig. 4. The regression coefficients of (a) training phase (b) validation phase (c) test phase (d) average for all phases in the ANN structure (with 10 neurons in the hidden layer) for estimating TPC.

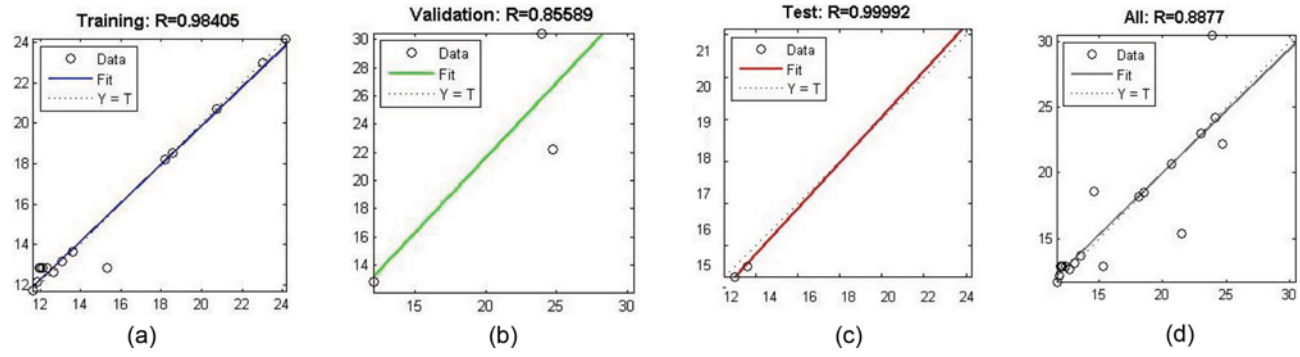

Fig. 5. The regression coefficients of (a) training phase (b) validation phase (c) test phase (d) average for all phases in the ANN structure (with 10 neurons in the hidden layer) for estimating oleuropein.

Table 7. Changes of sunflower oil stability parameters before and after their enrichment with the olive leaf extract at the optimum conditions (with 1,435 ppm of olive leaf extract at 10,000 rpm for 30 sec)

	TPC (ppm)	Oleuropein (ppm)	AA (mg-TEAC/g-oil)	TCC (mg-β carotene/kg-oil)	PV (meq-O_2/kg-oil)	IT (h)
Untreated sunflower oil	30±0.09	n.d.*	n.d.	n.d.	9.11±0.48	1.48±0.06
Treated sunflower oil	146.45±0.03	23.12±0.31	0.6357±0.11	4.4331±0.04	5.74±0.26	1.94±0.13

*n.d.: not detected

the error rates are given by Tables 5 and 6. Generally, the least errors values are the ones of 10 neuron ANN structure. As can be easily seen from these tables, the best ANN configuration to predict TPC and oleuropein involved a hidden layer with ten neurons. The graphs of regression coefficients for training, validation, test phases and average of all phases of ANN models with ten hidden neurons are illustrated by Figs. 4 and 5.

Additionally, it can be concluded that the response quantities estimated with ANN show the same tendency about increasing/decreasing balance with the experimental results. ANN modeling showed there is a direct proportion between mixing speed-TPC and mixing speed-oleuropein couples, inversely proportion between time-TPC couple, but the relationship between solid mass-TPC, time-oleuropein and solid mass-oleuropein couples cannot be determined. ANN model results of this study accounts for >95% estimation accuracy for both TPC and oleuropein in all cases of the experimental study.

3. Effect of Natural Antioxidant on the Sunflower Oil Quality

10,000 rpm of mixing speed, 30 sec of time and 1,435 ppm of olive leaf extract should be employed as optimal operating conditions in order to enrich the greatest TPC (148.19 ppm) and oleuropein (24.72 ppm) in sunflower oil according to the RSM calculations. Table 7 is the comparative results of quality parameters such as TPC, oleuropein, AA, TTC, PV and IT between the improved and the crude sunflower oils.

Total phenolic content of the enriched oil increased five-times over the crude sunflower oil (Table 7). Oleuropein was not detected in sunflower oil before enrichment process. Representative HPLC chromatograms for the olive leaf extract and enriched oil are displayed in Fig. 6 [6] put 150,000 ppm olive leaf into the olive oil and attained 50.7 and 111 mg oleuropein per gram of oil by ultrasound and conventional methods, respectively [7] added olive leaf extract having 1,680 ppm oleuropein into the sunflower oil and got 74.9 mg oleuropein per liter of oil [5] used 100,000 ppm olive

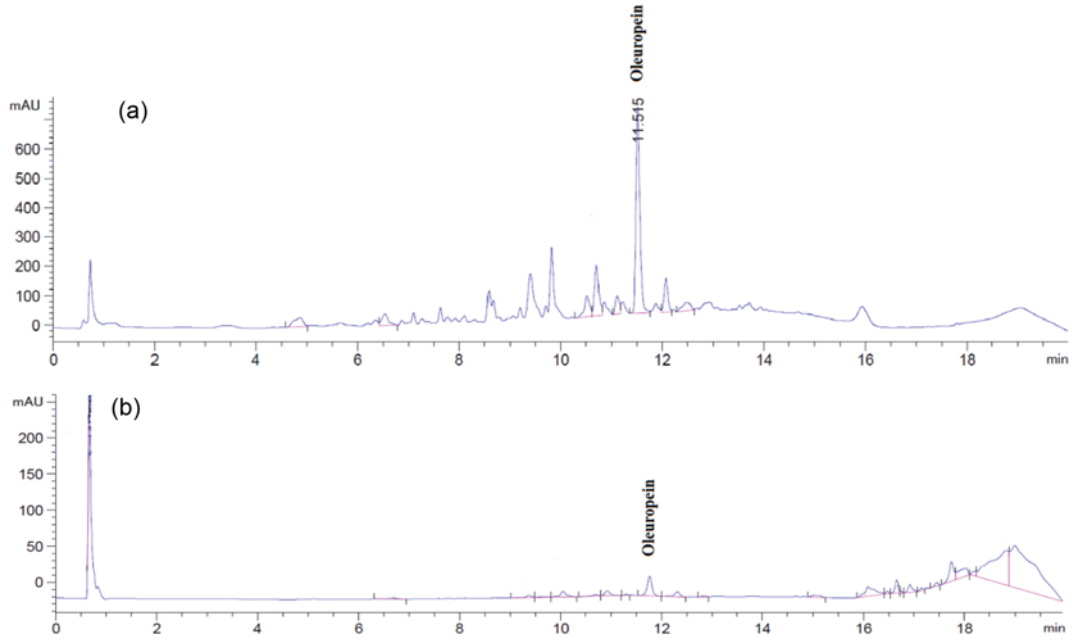

Fig. 6. HPLC chromatograms of (a) olive leaf extract and (b) sunflower oil enriched by olive leaf extract.

leaf to increase the quality of the sunflower oil, and obtained 10.21 mg oleuropein per liter of oil. However, our extract had 272 ppm oleuropein (Fig. 6(b)) depending on the method, cultivar, harvesting time etc. Therefore, our oleuropein enrichment in the sunflower oil is acceptable because only 1,435 ppm extract was applied in the samples.

The antioxidant activity of the enriched sunflower oil was found almost ten-times higher than that of the crude oil (10.34% versus 1.91%). Carotenoid was not detected in the pure sunflower oil before being treated with the extract. Peroxide value of the enriched oil decreased to almost half compared to the untreated oil.

The stability of the oil is defined with a parameter known as induction time (IT), which is required to produce a sudden increase in conductivity, due to the formation of volatile acids [39]. Fig. 7(a) and (b) show the induction times of treated and untreated oils measured at 130 °C through Rancimat method. Addition of 0.14% olive leaf extract gave rise to increase of the stability of the oil (≈24%). [11] also achieved almost the same rise in induction time by adding hydro alcoholic olive leaf extract into sunflower oil. This result complied with that of [40]. After adding 120 ppm TPC into the sunflower oil, they increased the stability of oil by nearly 20%.

CONCLUSIONS

Olive leaf extract rich in natural antioxidants has been applied for improving the quality of sunflower oil. Addition of 0.14% natural antioxidant has been proven to increase the stability of the oil (≈24%) as well as substantially increasing its added-value with high quality parameters. As supported by results obtained in both methods, olive leaf extract has proved that its phenolic content and especially major compound, oleuropein significantly contribute to the antioxidant capacity of the extract. Two different types of modeling method (RSM and ANN) have been applied for pre-

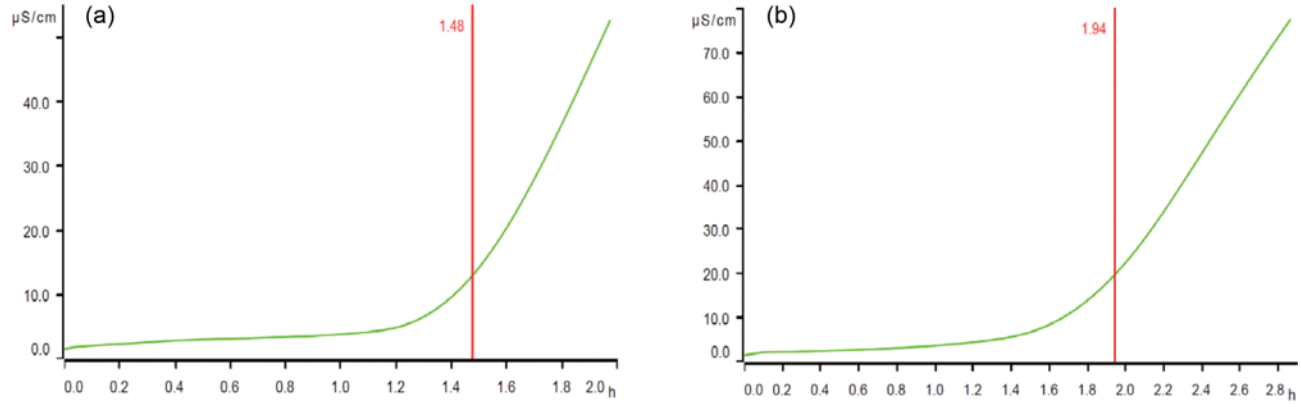

Fig. 7. Oxidative stability curves of (a) pure sunflower oil (b) sunflower oil enriched by olive leaf extract.

diction of TPC and oleuropein content of oil. Consequently, RSM showed higher deviation than ANN for modeling the stability improvement of sunflower oil. This study will contribute to food and food related industries with an inexpensive, easy to set-up, time saving and environmentally friendly process with natural additives.

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