Parameters Optimization of Ultrasound-Assisted Deodorization of Sheep Tail Fat Using Response Surface Technique

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ABSTRACT: *Sheep tail fat is a common frying oil in Iran due to its good flavor and stability. Deodorization is a high-temperature vacuum purification process for removing the volatile compounds from the edible fats and oils. In this paper, the effects of temperature, time, and ultrasound power on the quality attributes of sheep tail fat during the deodorization process were studied using response surface methodology. Variations of the acid value, peroxide value, iodine value, and saponification value, as well as extinction coefficient, were investigated. The best equations were created for the responses of acid value (R^2 = 0.9143, $p < 0.0001$), peroxide value (R^2 = 0.9862, $p < 0.0001$), iodine value (R^2 = 0.9670, $p < 0.0001$), refractive index (R^2 = 0.9816, $p < 0.0001$), saponification value (R^2 = 0.9345, $p < 0.0001$) and extinction coefficient (R^2 = 0.9562, $p < 0.0001$). Finally, the temperature of 200 ºC, the processing time of 80 min, and the ultrasound power of 307 W were recommended for the optimal conditions of sheep tail fat ultrasound-assisted deodorization.*

KEYWORDS: *Edible Fats; Extinction Coefficient; Free Fatty Acids; Peroxide Value; Ultrasound Power.*

INTRODUCTION

In the food industry, most animal fats are provided from the pig, cattle, and sheep tissues. Animal fats also provided from the milk fat. The amount of the saturated fatty acids in animal fats is higher than most of the vegetable oils [1]. In Iran, sheep tail fat is the most substantial part of the edible animal fats, slaughtering more than 10 million sheep per year and producing 50,000 tons of tail tissue. Therefore, investigation of the animal

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fats attributes as an alternative for the more expensive conventional fats and oils is essential [2].

Refining of the edible oils is performed for the separation of undesirable compounds and keeping the useful components and neutral oil [3]. In general, oil refining involves chemical and physical techniques. The physical method is different from the chemical one in Free Fatty Acids (FFAs) removing procedure. The physical refining is accomplished in three steps: degumming, bleaching, and deodorization [3]. During the deodorization, FFAs are removed at the high temperatures (220 – 270 ºC), and low pressures (2 – 5 mbar), under the presence of steam (0.5 – 2.0% w/w) as the stripping gas [4]. The physical refining is applied for some oils such as palm, palm kernel, coconut, sunflower, and corn oils, and animal fats. In this process the FFAs and volatile impurities are removed by steam refining, while the nonvolatile impurities are eliminated by degumming and clay treating [5]. Deodorization is usually the last and most critical refining step and has a significant effect on the final product quality attributes. Among the oil ingredients, the heavy FFAs are less volatile, so they are the last components that leaving the oil [5].

The frequencies of the high-intensity ultrasound are higher than the human hearing range (usually at 20 kHz). These waves have the ability to make cavitation in food solutions [6]. The success of this method mostly depends on the cavitation, mechanical, and thermal abilities which can bring about the break of cell walls, particle size reduction, and improved mass transfer across the particles [7]. Cavitation leads to some beneficial changes in food properties such as strong micro-turbulence and free radicals formation, which can be applied in food production [8]. Due to the rapid transfer of acoustic energy to the food product, ultrasound technology can be used to reduce the processing time and decrease energy consumption [6]. *Moulton* and *Mounts* (1990) developed an ultrasound-assisted procedure for minimizing the phospholipids in soybean oil. The evaluation of the odor and flavor indices indicated that the salad oil refined by the ultrasonic degumming was comparable with conventionally processed oil in quality and stability aspects [9]. In oil extraction, acoustic energy leads to a reduction in the processing time and an improvement in the extraction yield without notable changes in the fatty acids profile [10]. Satisfactory results were obtained when the ultrasound was used in the adsorption-based bleaching process of the olive oil [11]. Results of fat crystallization revealed that acoustic energy leads to faster crystallization and smaller crystal size [12]. In a study, red palm oil deodorization was performed at the different temperatures under the pressure of 2.6 kPa and a nitrogen flow rate of 20 L/h. Regarding the oil quality parameters, applying the temperature of 140 ºC for 1 h was recommended to achieve a neutralized deodorized palm oil with high carotenes retention [13].

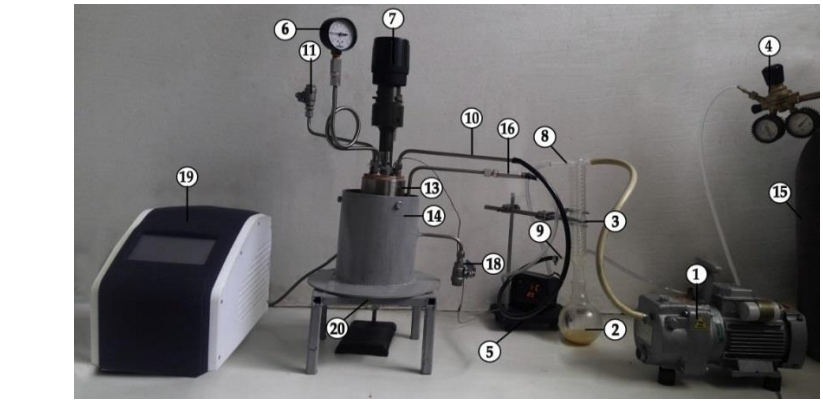
Response surface methodology (RSM) is a useful method for modeling between response variables and independent factors. This method is preferred because of the simplicity and high efficiency [14]. The RSM is a statistical technique that usually used for experiment design and finding the best conditions to reach the optimized results [15]. In a research study, the RSM was employed to study the effect of temperature and time on the deodorization parameters of kenaf seed oil. Variations of the FFAs, total color difference, para-Anisidine value, tocopherol, and tocotrienol contents were investigated during the process. The optimum conditions were achieved at the time of 1.5 h and the temperature of 220 ºC [16].

Regarding the importance of the edible oil deodorization in final product characteristics and the high consumption of lamb fat in the food industry in Iran, the present work was proposed to study the sheep tail fat deodorization parameters in an ultrasound-assisted system. For this purpose, the effects of the temperature, time, and ultrasound power on deodorized sheep tail fat quality attributes were investigated. The responses of the Acid Value (AV), Peroxide Value (PV), Iodine Value (IV), and Saponification Value (SV), as well as the Refractive Index (RI) and Extinction Coefficient (EC), were studied during the different deodorization conditions. The literature survey was indicated the lack of information on the application of ultrasound power on the deodorization of oils and fats.

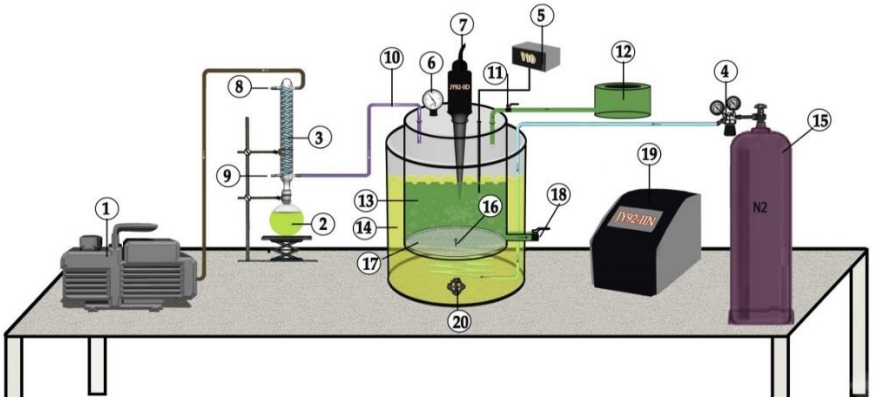
EXPERIMENTAL SECTION

Deodorizer Setup

The experimental setup used for deodorization of sheep tail fat was included the reaction tank, mineral oil tank, vacuum pump, ultrasonic homogenizer, condenser, collector, barometer, thermocouple, nitrogen gas tank, gas flow meter, One-way control valve, and heater. Nitrogen,



(a)



(b)

Fig. 1: (a) A photograph and (b) a schematic diagram of the deodorization system. (1.Vacuum pump, 2.Collector, 3.Condenser, 4.Gas flow meter, 5.Thermocouple, 6.Barometer, 7.Ultrasonic probe, 8.Water outlet, 9.Water inlet, 10.Gas outlet, 11.Oil inlet valve, 12.Animal oil storage, 13.Animal oil tank (main reactor), 14.Mineral oil tank, 15.Nitrogen gas cylinder, 16.One-way valve, 17.Porous plate, 18.Oil drain valve, 19.Ultrasonic homogenizer, 20.Heater).

as the stripping gas, passed through the oil for moving volatile compounds away from the liquid phase. The vacuum pump (SF 0006F, PUSH Co, Iran) evacuated non-condensable gases from the tank and passed them through the cooling condenser, where the condensed components were separated from the moving gas and transferred to the collector. The temperature controller (TC4S-24R, South Korea) controlled the temperature of the reaction tank according to the set program by turning the heater on and off. The pressure gauge was mounted on the tank to measure the pressure variations during the processing time (Fig. 1).

The reaction tank was a vertical cylindrical stainless steel (SS 316) vessel of 546.96 cm^3 capacity. The tank had a perforated feeder for uniform feeding the nitrogen to the oil. For reaching the high temperatures (up to 270 ℃) and preventing the burning of the animal fat, a heating bath was considered using a heater (2000 W) in the mineral oil surrounding the reaction tank. The nitrogen gas was also pre-heated by the oil bath (Fig. 1). The ultrasonic homogenizer (JY92-IID, SCIENT Co, Taiwan) was used to supply the different ultrasonic powers (22.05, 80, 165, 250 and 307.95 W) during the process. The ultrasound output power transferred into the media was measured calorimetrically according to the method described by *Ciğeroğlu et al.* [17] and relationship between the nominal and actual ultrasound power was obtained as the following equation:

$$AP = 0.89NP - 0.14 \quad (1)$$

Where AP and NP represent the actual and nominal ultrasound power (W), respectively.

Experimental procedure

The sheep tail was melted by heat treatment, and then rendered fat was filtered to remove any suspended burnt fat particles. After that, the samples were bleached by the surface adsorption using activated carbon, and stored in a glass container in the refrigerator (at −18±1 ℃) till use. For each experiment, the extracted sheep tail fat (about 300 mL) was imported into the reaction tank. The tests were directed at the different temperatures (Table 1) under a constant vacuum condition (20 mm Hg), which was supplied by the vacuum pump during the process. The nitrogen gas passed to the tank at a constant flow rate of 2±0.1 L min^{-1}. The ultrasonic homogenizer with the frequency of 24 kHz was applied during the experiments. After processing, the deodorized fat samples were cooled till 60 ℃ under vacuum conditions and removed from the reaction tank for physiochemical analysis.

Physiochemical analysis

Variations of the AV after deodorization process were measured according to the AOCS official method (Ca 5a-40) and expressed in mg KOH/ g fat [18]. The International Dairy Federal (IDF) standard (IDF 74:2006, ISO 3976) was used for the PV determination of sheep tail fat [19]. The EC (K232) was calculated based on the absorbance at 232 nm, with a UV-vis spectrophotometer (UNICO 2802, Unico Instrument Co, China) using a 1% (w/v) solution of oil in cyclohexane and a path length of 1 cm [20]. The IV was determined according to the AOAC procedure (method 920-158) [21]. The SV was measured using the AOAC procedure (method 920-160) [19]. The RI was measured using AOAC instruction (method 921-08) with a refractometer (2WAJ, Optika Co, Italy) [21].

Statistical analysis

Experiment design was performed based on the RSM using design-expert version 7.0 software (State-Ease, Minneapolis, USA). Central Composite Design (CCD) was employed for finding the optimum levels of the experimental factors, including the temperature, time, and ultrasound power. Five levels were considered for each factor. Coded and actual values for each factor are shown in Table 1.

Based on the CCD, 20 experimental runs were considered for the analysis. The responses were AV (mg KOH/g fat), PV (mEq O_2 / kg fat), EC (%), IV (100 × g I_2 / g fat), SV (mg KOH/g fat), and RI. Experimental data were analyzed and fitted into the empirical second-order polynomial model, as shown in the following equation:

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

Where Y represents the experimental responses, β_0, β_i, β_{ii}, and β_{ij} are regression coefficients in the intercept, linear, quadratic, and interaction terms, respectively; and X_1, X_2, and X_3 are the independent variables. The statistical significance of data was evaluated by the F-test analysis [22]. The correlation coefficient (R^2), reduced chi-square (χ^2) and Coot Mean Square Error (RMSE) criteria are employed to determine the deviation between the experimental and predicted data. The error function parameters were calculated using the following expressions [17, 23]:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} \left(F_{exp.i} - F_{pre.i}\right)^2}{N}} \quad (3)$$

$$R^2 = 1 - \left[\frac{\sum_{i=1}^{N} \left(F_{exp.i} - F_{pre.i}\right)^2}{\sum_{i=1}^{N} \left(\overline{F_{exp}} - F_{pre.i}\right)^2}\right] \quad (4)$$

$$\chi^2 = \frac{\sum_{i=1}^{N} \left(F_{exp.i} - F_{pre.i}\right)^2}{N - z} \quad (5)$$

where, $F_{exp.i}$ is the i^{th} measured data, $F_{pre.i}$ is the i^{th} predicted data, $\overline{F_{exp}}$ is the average of measured data, N and Z are the number of observations and number of model constants, respectively.

RESULTS AND DISCUSSION

The obtained results of dependent variables at experimental conditions are shown in Table 2. Investigation of the responses variations showed that usually the AV, PV, SV, and EC were decreased during the deodorization process, while the RI and IV had ascending trend. These results are in agreement with some previous studies [24-34]. Parameters optimization of kenaf seed oil deodorization showed that increasing of

Table 1: Coded and actual values of independent variables used for central composite design in the deodorization of sheep tail fat.

Symbol	Variables			Levels		
		-1.68	-1	0	+1	+1.68
X_1	Temperature (ºC)	119.55	140	170	200	220.45
X_2	Time (min)	9.55	30	60	90	110.45
X_3	Ultrasonic power (W)	22.05	80	165	250	307.95

Table 2: Central composite design and observed responses of deodorized sheep tail fat.

Run	Actual variable			AV (mg KOH g^{-1})	PV(mEq O_2 kg^{-1})	EC (%)	SV (mg KOH g^{-1})	IV(100 × g I_2 g^{-1})	RI
	X_3	X_2	X_1						
	Before deodorization			1.0599	1.0008	1.8771	197.398	36.108	1.4644
1	119.55	60.00	165.00	0.6617	0.8832	1.8649	196.715	37.446	1.4645
2	140.00	30.00	80.00	0.8232	0.9221	1.8766	195.877	36.920	1.4647
3	140.00	30.00	250.00	0.7219	0.8718	1.8700	195.296	37.401	1.4648
4	140.00	90.00	80.00	0.4694	0.7897	1.8657	194.704	37.823	1.4649
5	140.00	90.00	250.00	0.3011	0.6706	1.8542	193.953	38.285	1.4651
6	170.00	09.55	165.00	0.8845	0.7113	1.8794	195.806	37.167	1.4651
7	170.00	60.00	22.05	0.5131	0.6143	1.8670	194.288	38.635	1.4653
8	170.00	60.00	165.00	0.3621	0.4903	1.8606	193.927	39.066	1.4654
9	170.00	60.00	165.00	0.4312	0.5187	1.8634	193.784	39.085	1.4655
10	170.00	60.00	165.00	0.3730	0.4796	1.8576	193.242	39.166	1.4654
11	170.00	60.00	165.00	0.3051	0.5096	1.8562	193.547	39.523	1.4655
12	170.00	60.00	165.00	0.4601	0.5440	1.8621	193.177	39.463	1.4653
13	170.00	60.00	165.00	0.4059	0.5279	1.8535	193.917	39.126	1.4654
14	170.00	60.00	307.95	0.2034	0.4321	1.8421	192.851	39.186	1.4657
15	170.00	110.45	165.00	0.1487	0.2854	1.8377	192.005	39.552	1.4657
16	200.00	30.00	80.00	0.3825	0.49252	1.8439	192.262	38.301	1.4660
17	200.00	30.00	250.00	0.2304	0.45595	1.8377	191.635	38.896	1.4662
18	200.00	90.00	80.00	0.1303	0.15344	1.8325	191.553	39.884	1.4664
19	200.00	90.00	250.00	0.0413	7.2×10^{-5}	1.8128	191.005	40.354	1.4666
20	220.45	60.00	165.00	0.1511	0.15218	1.8225	191.547	39.374	1.4665

AV: acid value, PV: peroxide value, EC: Extinction coefficient, SV: saponification value, IV: iodine value, RI: refractive index

temperature and time leads to reduction of AV, PV, and para-Anisidine value of refined oil [16]. Using of physical techniques for palm oil refining decreased the FFA content and approved the qualitative features of the final product [24]. Further discussion about the physicochemical attributes of the deodorized oil under different conditions and comparison of these attributes with the results of other studies are presented separately in the following sections.

Analysis of variance (ANOVA) of the responses of AV, PV, RI, SV, IV, and EC are shown in Table 3. The probability (p) values of the models are less than 0.0001 for the responses of AV, PV, RI, SV, IV, and EC, which shows the significant effects of the independent variables on the responses. The R^2 values were in the range of 0.9143 to 0.9862, indicating a good correlation between independent and dependent variables.

The regression coefficients and p-values of the responses for the significant parameters of the first-order, second-order, and interactions between the independent parameters of temperature (X_1), time (X_2), and ultrasonic power (X_3) are represented in Table 4.

The error function parameters of experimental and predicted data are shown in Table 5. It can be found that for all of the parameters, the proposed models are suitable.

Analysis of AV

The AV is a vital quality character of edible oils and indicates the presence of FFAs that leads to undesirable flavors of oils. So, the FFA removal is considered as a quality improvement refining step during the oil processing [5]. The AV of the deodorized fat samples was measured after the processing (Table 2). It is clear that the AV decreased under the deodorization, and reduced from 1.0599 to 0.0413 mg KOH g^{-1} fat during the process. This finding is in accordance with the results of Widarta et al. [24], who reported that deodorization of neutralized red palm oil in a pilot plant scale reduced the FFAs to 0.13%, with 87.30% of carotene recovery [24]. Similarly, the results of chew et al. indicated that FFAs in kenaf seed oil were decreased during the deodorization [25]. In another study, the AV of tuna (*Thunnus albacares*) oil decreased from 1.96% to 0.3% during the refining and deodorization, representing a decrease of 85% [26].

Taking into account the significant ($p < 0.05$) terms, the results confirmed that there was a good relationship between the AV and independent variables in the deodorization of sheep tail fat. The obtained model is as follow:

$$AV = 1.9856 - 5.3403\times10^{-3}X_1 - 0.0134X_2 - 8.8863\times10^{-4}X_3 + 6.867\times10^{-5}X_2^2 \quad (6)$$

The results of ANOVA (Table 4) show that the first-order terms of temperature and time had highly significant ($p < 0.001$) effects on the AV reduction of the deodorized sheep tail fat. Furthermore, ultrasound power and the second-order term of time (X_2^2) caused significant effects ($p < 0.05$) on the AV variations during the deodorization process. Fig. 2 shows that the AV decreased with rising deodorization temperature. It is also exhibited a decreasing trend in the AV with time. Fig. 2 shows that increasing the ultrasonic power decreased the AV with a lower slope. Comparison of experimental and predicted AV are also given in Fig. 2(d). Clearly, the data are distributed around the straight line that confirms the goodness of fit.

Analysis of PV

Shelf stability of oils is ruled by their peroxide and para-anisidine values. The PV represents the primary products of the reaction between oxygen and unsaturated fatty acids [5]. The PV was measured after deodorization for each experiment. As shown in Table 2, deodorization reduced the PV of the oil from 1.0008 to the ultimate value of 7.2×10^{-5} mEq O_2 kg^{-1} fat. These findings are in agreement with the results of Ortega-Garcia et al., who reported that deodorization significantly (p <0.05) decreased the PV to 0.25 mEq O_2 kg^{-1} oil [27]. In another study, Zacchi and Eggers explained that hydroperoxide content was affected by the amount of operating independent variables, including temperature, time, and the rate of steam injection during the deodorization [28]. Riyadi et al. concluded that deodorization at the temperature of 150 °C, significantly reduced the PV of oil. In this study, a maximum PV reduction of 99.7% was obtained [13]. It is clear from Table 4 that temperature and time had highly significant ($p < 0.001$) effects on the PV of the deodorized sheep tail fat. Moreover, the ultrasonic power and the interaction of temperature and time showed a significant effect ($p < 0.05$) on the PV. Considering the significant ($p < 0.05$) factors, the best model for PV is given as below:

Table 3: Analysis of variance (ANOVA) of the AV, PV, RI, SV, IV, and EC.

Source	Sum of squares	Degree of freedom	Mean square	F-value	*p*-value
AV					
Model	0.81	9	0.20	30.59	< 0.0001
Residual	0.100	10	6.808×10^{-3}		
Lack of Fit	0.085	5	8.450×10^{-3}	2.79	0.1341
Pure Error	0.015	5	3.027×10^{-3}		
Correction total	0.92	19			
PV					
Model	1.15	9	0.13	79.49	< 0.0001
Residual	0.016	10	1.611×10^{-3}		
Lack of Fit	0.013	5	2.653×10^{-3}	4.67	0.0581
Pure Error	2.843×10^{-3}	5	5.686×10^{-4}		
Correction total	1.17	19			
EC					
Model	5.705×10^{-3}	9	6.339×10^{-4}	24.24	< 0.0001
Residual	2.615×10^{-4}	10	2.615×10^{-5}		
Lack of Fit	1.897×10^{-4}	5	3.794×10^{-5}	2.64	0.1548
Pure Error	7.175×10^{-5}	5	1.435×10^{-5}		
Correction total	5.967×10^{-3}	19			
RI					
Model	6.636×10^{-6}	9	7.373×10^{-7}	59.43	< 0.0001
Residual	1.241×10^{-7}	10	1.241×10^{-8}		
Lack of Fit	9.574×10^{-8}	5	1.915×10^{-8}	3.38	0.1038
Pure Error	2.833×10^{-8}	5	5.667×10^{-9}		
Correction total	6.760×10^{-6}	19			
SV					
Model	45.65	9	5.07	15.84	< 0.0001
Residual	3.20	10	0.32		
Lack of Fit	2.65	5	0.53	4.80	0.0551
Pure Error	0.55	5	0.11		
Correction total	48.86	19			
IV					
Model	16.83	9	1.87	32.60	< 0.0001
Residual	0.57	10	0.057		
Lack of Fit	0.37	5	0.074	1.83	0.2612
Pure Error	0.20	5	0.040		
Correction total	17.40	19			

P < 0.05 indicates statistical significance

AV: acid value, PV: peroxide value, EC: Extinction coefficient, SV: saponification value, IV: iodine value, RI: refractive index

Table 4: Estimated regression coefficients and p-values of the significant independent parameters on the responses.

Responses	Variables	Estimated Coefficients	p-value
AV	X_o	+1.98555	
	X_1	-5.34035×10^{-3}	< 0.0001
	X_2	−0.013408	< 0.0001
	X_3	-8.88631×10^{-4}	0.0038
	X_2^2	$+6.86699\times10^{-5}$	0.0109
PV	X_o	+1.63958	
	X_1	-4.40894×10^{-3}	< 0.0001
	X_2	$+6.39061\times10^{-3}$	< 0.0001
	X_3	-5.73584×10^{-4}	0.0003
	X_1X_2	-6.40797×10^{-5}	0.0012
	X_2^2	$+3.70988\times10^{-5}$	0.0069
SV	X_o	+204.91166	
	X_1	−0.053861	< 0.0001
	X_2	−0.025013	< 0.0001
	X_3	-4.24190×10^{-3}	0.0140
RI	X_o	+1.47	
	X_1	$+5.946\times10^{-4}$	< 0.0001
	X_2	$+1.691\times10^{-4}$	< 0.0001
	X_3	$+1.005\times10^{-4}$	0.0009
	X_1^2	$+5.244\times10^{-5}$	0.0375
	X_3^2	$+5.244\times10^{-5}$	0.0375
	X_1X_2	$+1.179\times10^{-4}$	0.0273
EC	X_o	+1.79383	
	X_1	$+1.63032\times10^{-3}$	< 0.0001
	X_2	-3.24957×10^{-4}	< 0.0001
	X_3	-7.39441×10^{-5}	0.0004
	X_1^2	-6.3110×10^{-6}	0.0007
IV	X_o	+23.69562	
	X_1	+0.12390	< 0.0001
	X_2	+0.0344	< 0.0001
	X_3	$+8.12696\times10^{-3}$	0.0100
	X_1X_2	$+1.7427\times10^{-4}$	0.0661
	X_1^2	-3.30241×10^{-4}	0.0010
	X_2^2	-3.59893×10^{-4}	0.0006
	X_3^2	-1.7090×10^{-5}	0.0540

AV: acid value, PV: peroxide value, EC: Extinction coefficient, SV: saponification value, IV: iodine value, RI: refractive index

Table 5: Descriptive performance indices of RSM models for dependent variables.

Responses	RMSE	R^2	χ^2
AV	0.00335	0.9143	1.18068×10^{-5}
PV	0.04395	0.9862	0.002034
IV	0.08287	0.9670	0.033223
SV	0.09076	0.9345	0.008672
RI	0.00104	0.9816	1.13758×10^{-6}
EC	0.00039	0.9562	1.65216×10^{-7}

AV: acid value, PV: peroxide value, EC: Extinction coefficient, SV: saponification value, IV: iodine value, RI: refractive index

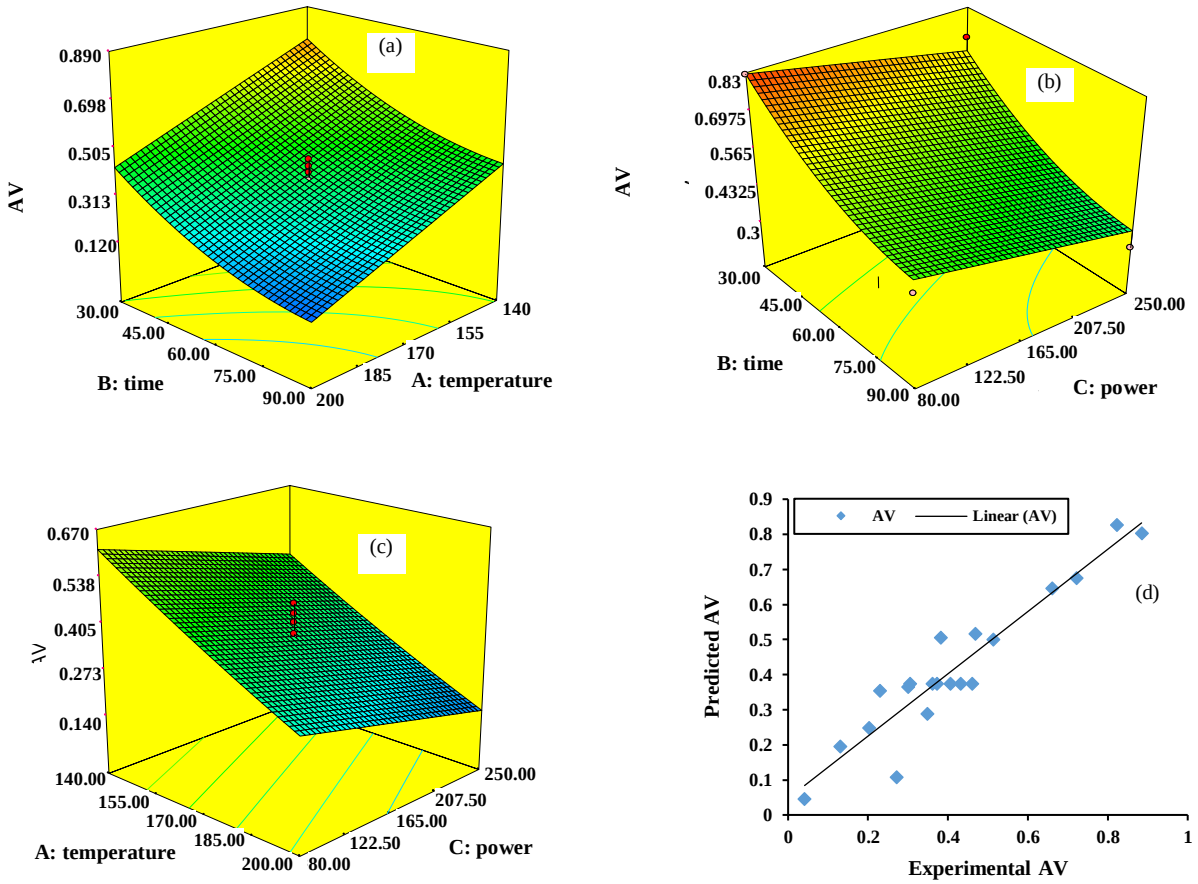


Fig. 2: The effects of different parameters on AV of sheep tail fat after deodorization (a, b, and c) and comparison of experimental and predicted AV of deodorized lamb fat at different conditions (d). (A: Temperature, B: Time, and C: Ultrasonic power).

$$PV = 1.63958 - 4.40894\times10^{-3}X_1 + 6.39061\times10^{-3}X_2 - 5.73584\times10^{-4}X_3 - 6.40797\times10^{-5}X_1X_2 + 3.70988\times10^{-5}X_2^2 \quad (7)$$

Comparison between the experimental data and obtained data from the RSM model are also given in Fig. 3(d). It is clear from Fig. 3 that the PV decreased with deodorization time, temperature, and ultrasonic power.

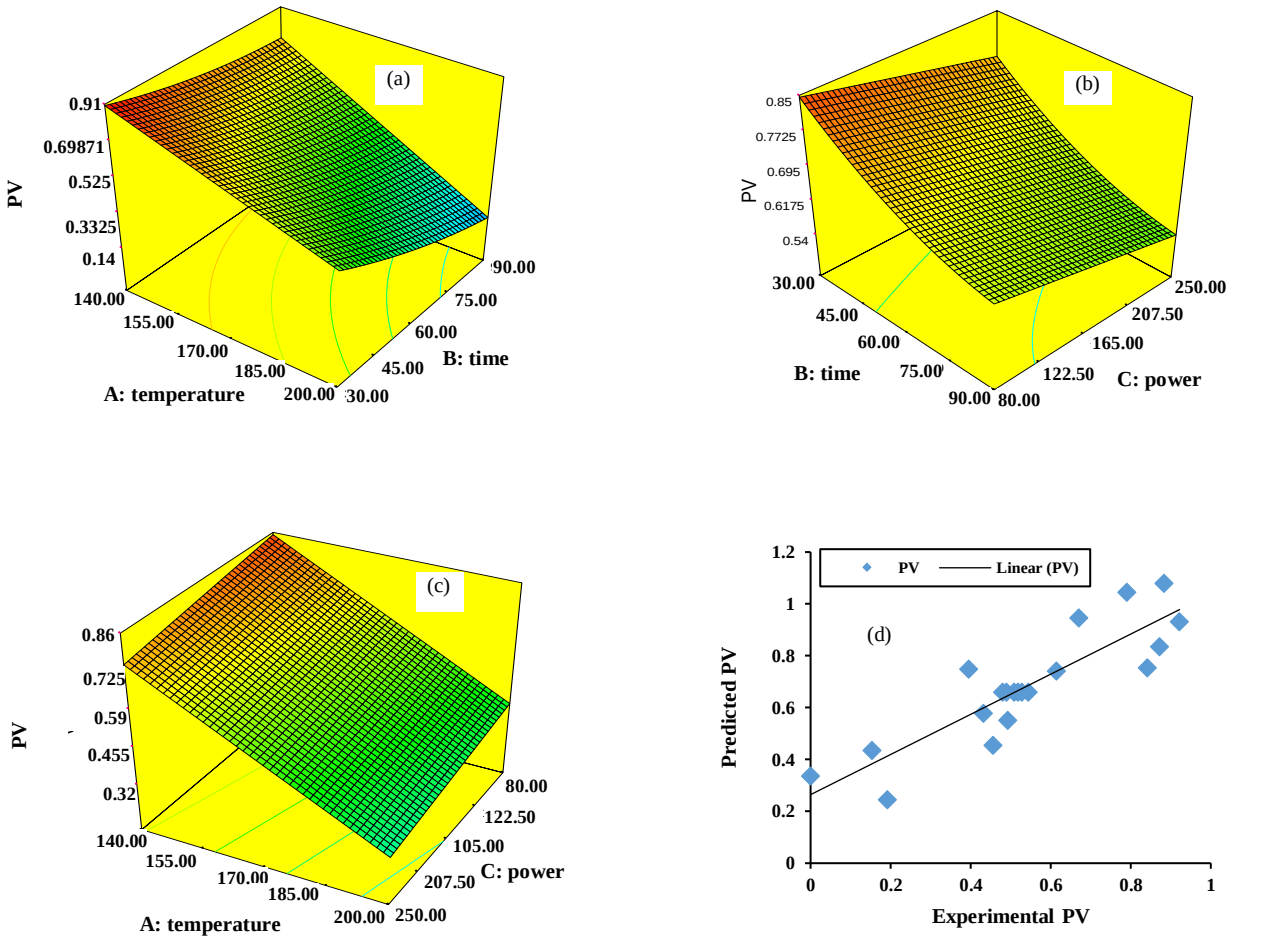


Fig. 3: The effects of different parameters on PV of sheep tail fat after deodorization (a, b, and c) and comparison of experimental and predicted PV of deodorized lamb fat at different conditions (d). (A: Temperature, B: Time, and C: Ultrasonic power).

The temperature was the most effective factor in the reduction of the PV during the deodorization process.

Analysis of RI

The RI in oils and fats indicates the fatty acids chain length and degree of unsaturation in the structure of triglycerides [1]. The RI increased from 1.4644 to 1.4666 after deodorization (Table 2). During the refining of tuna oil, the RI has increased from 1.478 to 1.480 [26]. The results are in agreement with the studies on the refining processes of Nile tilapia oil [29] and blue grenadier oil [30].

The generated response surface model based on the second-order polynomial equation is as below:

$$RI = 1.47 + 5.946\times10^{-4}X_1 + 1.691\times10^{-4}X_2 + 1.005\times10^{-4}X_3 + 5.244\times10^{-5}X_1^2 + 5.244\times10^{-5}X_3^2 + 1.179\times10^{-4}X_1X_2 \quad (8)$$

The statistical analysis (Table 4) indicates that the terms of temperature and time had highly significant ($p < 0.001$) effects on the RI of the deodorized sheep tail fat. Also, the ultrasonic power, the second-order terms of temperature and time, and the interaction between temperature and time had significant effects ($p < 0.05$) on the RI of deodorized fat. Fig. 4 shows that temperature was the most effective parameter on the RI, while variations of ultrasonic power had the lowest impact on the RI value. Pandurangan et al. revealed that a small increase in the RI could be occurred during the deodorization due to the

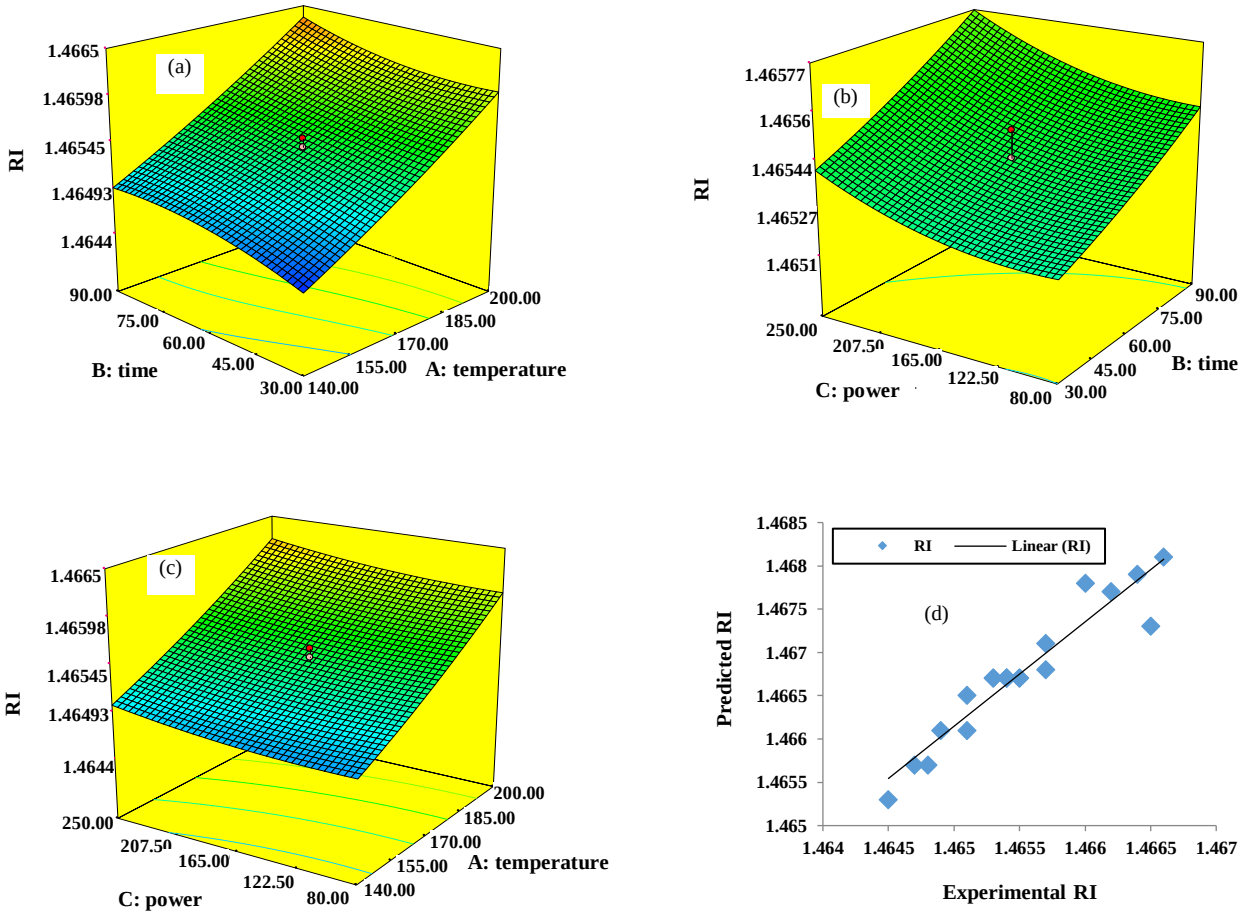


Fig. 4: The effects of different parameters on RI of sheep tail fat after deodorization (a, b, and c) and comparison of experimental and predicted RI of deodorized lamb fat at different temperatures. (A: Temperature, B: Time, and C: Ultrasonic power).

elimination of a part of the saturated fatty acids [31]. Comparison of the experimental data and RSM model data for RI are also given in Fig. 4(d). It is clear that there is a good agreement between the experimental and the predicted data.

Analysis of SV

The SV is related to the average molecular weight of triacylglycerols. A higher SV is an indicator of short-chain fatty acids in triacylglycerol molecules [32]. The SV of the deodorized products was measured for each treatment. The results show that the SV decreased from 197.398 to 191.005 mg KOH g^{-1} fat (Table 2). Considering the significant ($p < 0.05$) parameters, the achieved model that describes the relationship between the SV of final product and independent variables is given as below:

$$SV = 204.9117 - 0.0538X_1 - 0.0250X_2 - 4.2419 \times 10^{-3} X_3 \quad (9)$$

The *p*-values of ANOVA results revealed that the terms of temperature (X_1) and time (X_2) had highly significant ($p < 0.001$) effects on the SV of deodorized sheep tail fat. Meanwhile, the ultrasonic power (X_3) showed a significant ($p < 0.05$) effect. Fig. 5 illustrates that the SV of the deodorized sheep tail fat decreased with increasing the temperature and time. Besides, the ultrasonic power had a low effect on the SV of the deodorized fat. Fig. 5(d) shows the comparison of the experimental and predicted SV. Bounding the points around the straight line confirms that the obtained model satisfactorily predicts the experimental data.

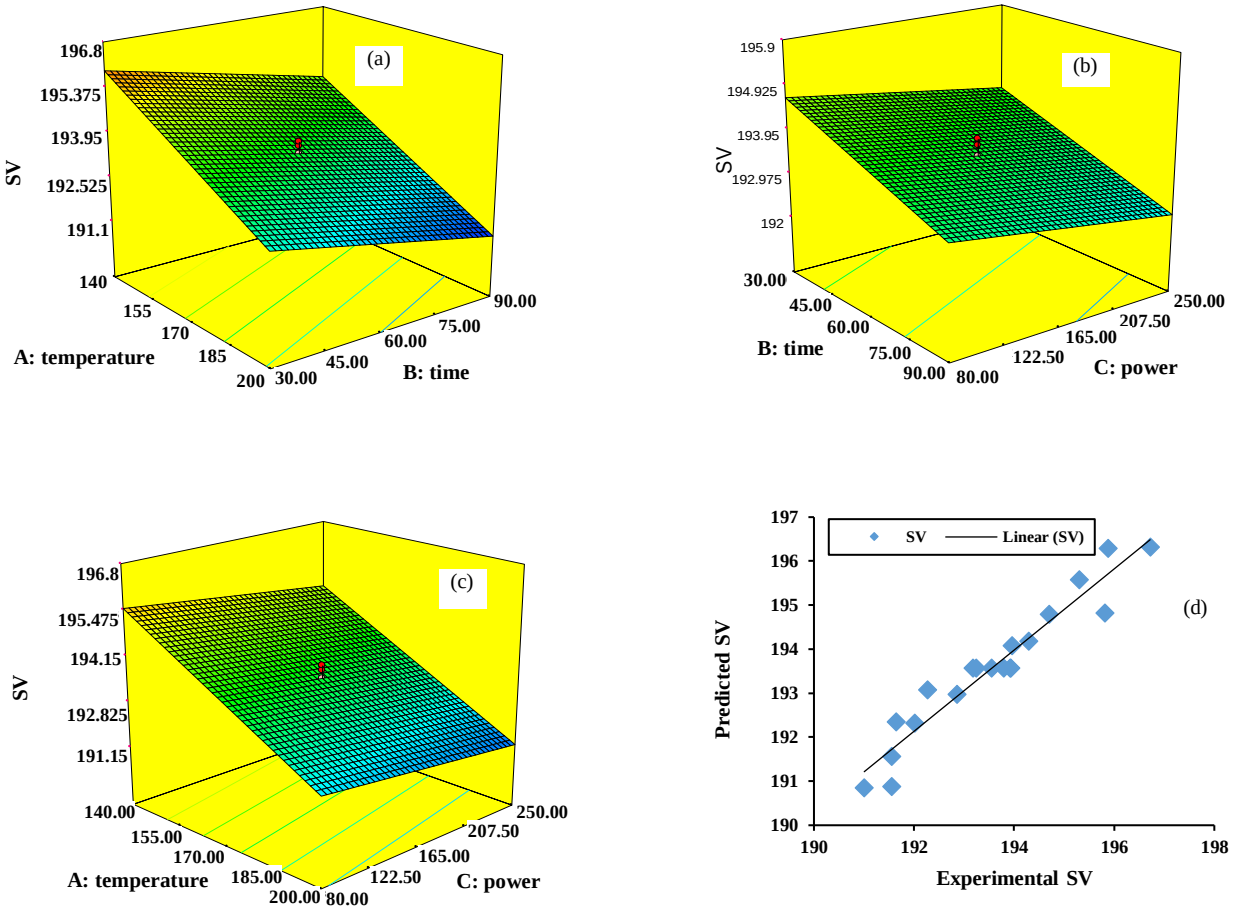


Fig. 5: The effects of different parameters on SV of sheep tail fat after deodorization (a, b, and c) and comparison of experimental and predicted SV of deodorized lamb fat at different conditions (d). (A: Temperature, B: Time, and C: Ultrasonic power).

Analysis of IV

The IV shows the amount of unsaturated fatty acids in triacylglycerol molecules. By adding a certain amount of iodine to the edible oils, the iodine reacts with the double bonds of fatty acids and leads to the saturation of this bonds. The residual iodine is titrated with sodium thiosulfate. On other words, the IV indicates the degree of unsaturation of fatty acids in triacylglycerols [33]. It is clear from Table 2 that the IV of the deodorized fats (40.354 g I_2 $(100g)^{-1}$ fat) was higher than crude fat (36.108 g I_2 $(100g)^{-1}$ fat). In case of red palm oil deodorization, the IV increased from 44 g I_2 $(100g)^{-1}$ in bleached oil to 57 g I_2 $(100g)^{-1}$ in the olein fraction [34]. A significant ($p < 0.05$) increment was observed between the IV of crude (27.12 g I_2 $(100g)^{-1}$ oil) and refined oil (50.40 g I_2 $(100g)^{-1}$ oil) during the deodorization of tuna oil [26].

The first-order term of temperature and time (X_1 and X_2) had highly significant ($p < 0.001$) effects on the IV of deodorized fat, while the first-order term of ultrasonic power and the second-order terms of temperature and time (X_3, X_1^2, and X_2^2) showed significant ($p < 0.05$) effects on the IV of the final product (Table 4). The response surface model for IV was generated based on the fitted second-order polynomial equation as below:

$$IV = 23.6956 + 0.1239X_1 + 0.0344X_2 + 8.12696\times10^{-3}X_3 + 1.7427\times10^{-4}X_1X_2 - 3.3024\times10^{-4}X_1^2 - 3.5989\times10^{-4}X_2^2 - 1.7090\times10^{-5}X_3^2 \quad (10)$$

Fig. 6 shows that the IV increased with rising the temperature, but the rate of its variation was higher at the lower temperatures. However, the IV increased with

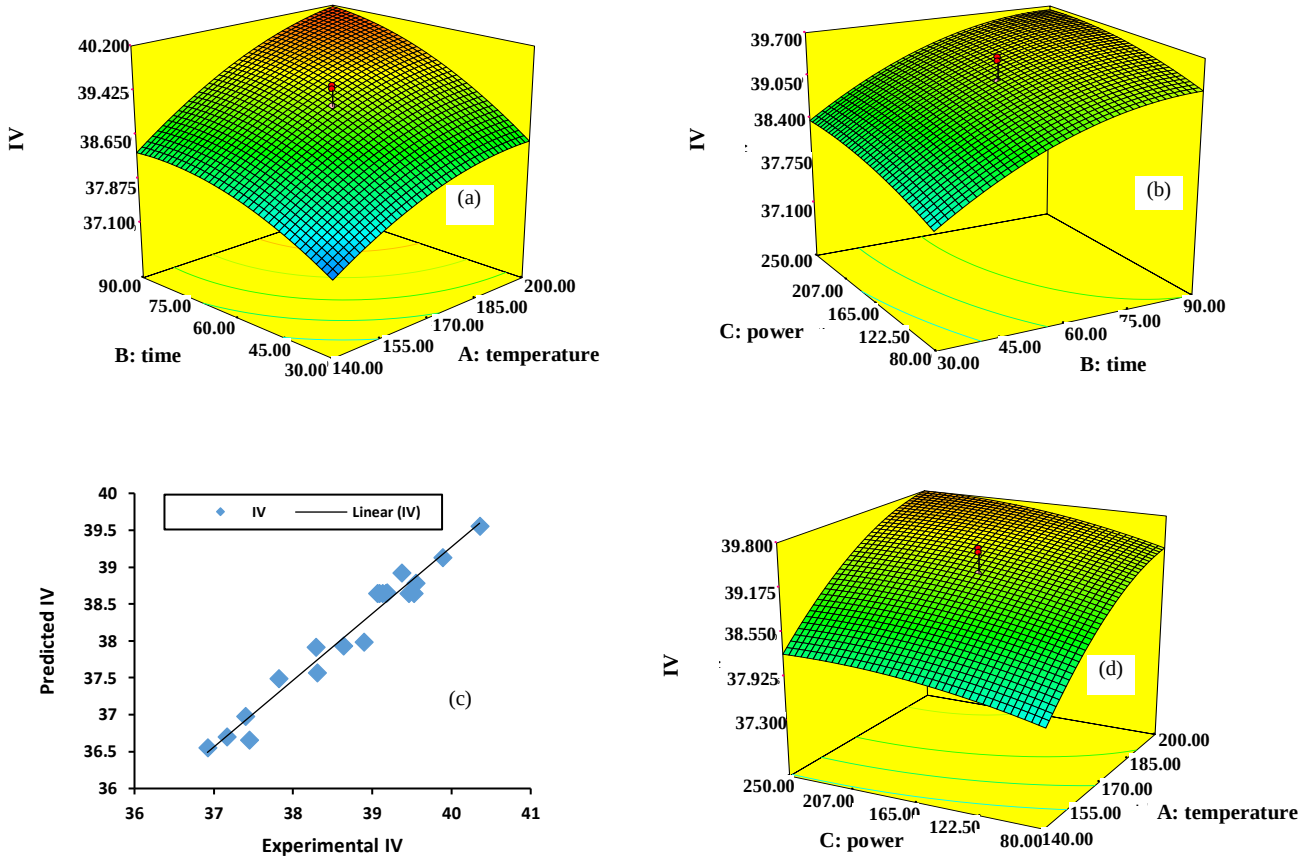


Fig. 6: The effects of different parameters on IV of sheep tail fat after deodorization (a, b, and c) and comparison of experimental and predicted IV of deodorized lamb fat at different conditions (d). (A: Temperature, B: Time, and C: Ultrasonic power).

the time, but as it is clear from Fig. 6, the effect of time on the IV variations was less than temperature. Fig. 6 also demonstrates that raising the ultrasonic power led to a slight increase in the IV. The comparison of experimental and predicted IV is shown in Fig. 6(d).

Analysis of EC

The measurement of EC at 232 nm (K232) is a spectrophotometric feature for detection of hydroperoxides (primary oxidation products) and conjugated dienes (intermediate oxidation products) as oxidative reactions products [1]. Table 2 shows that the EC of crude edible fat reduced from 1.8771 to 1.8128 during the deodorization. Taking into account the significant ($p < 0.05$) factors in Table 4, the obtained model for describing the relationship between the EC of deodorized sheep tail fat and independent variables is given below:

$$EC = 1.7938 + 1.6303\times10^{-3} X_1 - 3.2496\times10^{-4} X_2 - 7.3944\times10^{-5} X_3 - 6.3110\times10^{-6} X_1^2 \quad (11)$$

Fig. 7 shows that the EC of deodorized sheep tail fat was almost constant at lower temperatures while it dramatically decreased at higher temperatures. Similarly, EC decreased with increasing the deodorization time and ultrasonic power, but the temperature was the most effective factor on the EC variations. Experimental vs predicted data are illustrated in Fig. 7(d). It is clear that the data are bounded around the straight line that displays the suitability of the selected model.

Numerical optimization and model verification

Optimization was performed to determine the optimum values of the independent variables during the

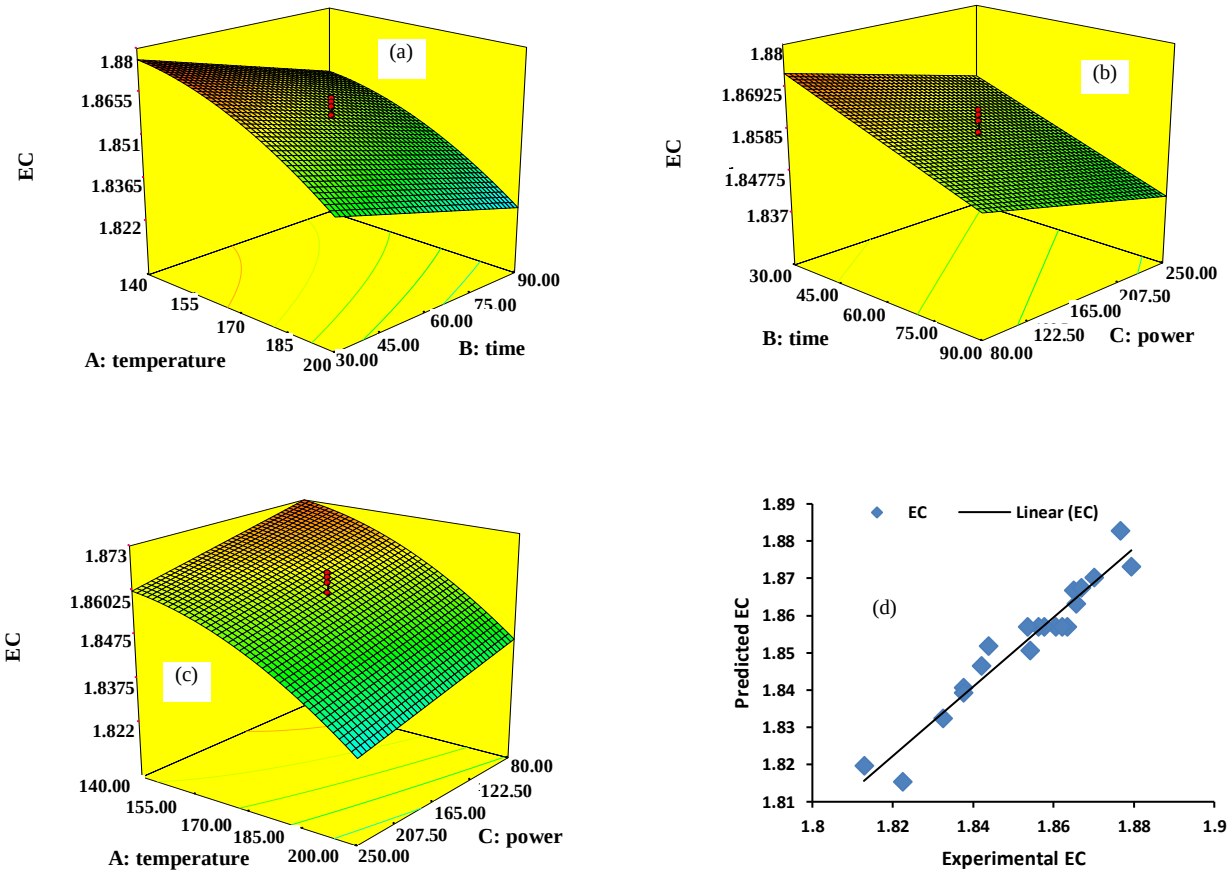


Fig. 7: The effects of different parameters on EC of sheep tail fat after deodorization (a, b, and c) and comparison of experimental and predicted EC of deodorized lamb fat at different conditions (d). (A: Temperature, B: Time, and C: Ultrasonic power).

deodorization of sheep tail fat through the RSM technique. The obtained optimized values were the temperature of 200 ºC, time of 80 min, and ultrasonic power of 307 W. To verify the amount of the AV, PV, IV, SV, RI, and EC at the optimum deodorization conditions, triplicate runs were considered for the experiments. Table 6 shows the results of crude fat, and experimental and predicted values of the responses at the optimized conditions of the temperature, time, and ultrasonic power. The differences between the experimental and predicted values of the responses were not noticeable which revealed that the models suitably predicted the variations of the responses. The achieved optimum factors are in the range of the optimum conditions attained by the previous studies for the other types of oils. In this way, some studies recommended deodorization at the temperature of 200 − 250 ºC and the time of 1 − 2 h under vacuum pressure conditions [24, 27, 35, 36].

Wei et al. (2015) reported that deodorization of tea seed oil at 100 ºC was not sufficient for volatile compounds evaporation, whereas rising the temperature above 150 ºC (150, 200, and 250 ºC) significantly increased the amount of the exhausted volatile compounds. They finally selected the temperature of 150 ºC at the pressure of 3 kPa as the optimum conditions for removing the undesirable odorous compounds and maintaining the bioactive materials of tea seed oil [37]. Separation of FFAs from soybean oil was carried out at the temperature range of 100 - 180 ºC, and the feed flow rate of the product was in the range of 1.5 - 23.0 g min^{-1}. The results indicated that the FFA removal and tocopherol recovery were 96.16% and 81.23%, respectively [38]. *Chew et al.* (2017) recommended the temperature of 220 ºC and the time of 1.5 h for refining and deodorization of kenaf seed oil. A refined kenaf seed oil with the para-Anisidine value of 6.67, 0.036% of FFAs,

Table 6: Characterization of crude oil and experimental and predicted values of the responses in sheep tail fat after deodorization under optimized conditions.

Responses	Crude	Experimental	Predicted
AV (mg KOH g^{-1} fat)	1.0599	0.0015±0.0003	0.0019
PV (mEq O_2 kg^{-1} fat)	1.001	0.035±0.005	0.060
IV (100 × g I_2 g^{-1} fat)	36.11	40.24±0.03	40.12
SV (mg KOH g^{-1} fat)	197.398	191.070±0.050	190.739
RI	1.4644	1.4700±0.0400	1.4666
EC (%)	1.8771	1.8200±0.2500	1.8172

AV: acid value, PV: peroxide value, EC: Extinction coefficient, SV: saponification value, IV: iodine value, RI: refractive index

0 meq/kg of PV, and 39.69 mg $(100g)^{-1}$ of tocopherols and tocotrienols content was achieved at the optimum conditions of refining [16].

CONCLUSIONS

In this study, the effects of deodorization temperature, time, and ultrasonic power on some quality attributes of sheep tail fat were investigated, and the optimized conditions of the processing parameters were determined using the response surface technique. The statistical analysis indicates that the regression models were statistically suitable with a significance level of $p < 0.0001$ for the responses of AV, PV, IV, and SV as well as the RI, and EC. The optimum conditions during the deodorization of sheep tail fat were the temperature of 200 ºC, the time of 80 min, and the ultrasonic power of 307 W. The obtained experimental data at the optimum conditions significantly agreed with the predicted values that confirm the adequacy and suitability of the introduced models. This study is useful for improving the sheep tail fat deodorization process and providing a product with proper nutritional and qualitative characteristics for use in the food and cosmetic industries.

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