Optimization of Bitter Almond Oil (BAO) Extraction Conditions Using Natural Enzymes and Ultrasound Waves

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ABSTRACT: *Non-edible oils are typically applied for multiple uses, especially biofuel production. But, finding new methods that can maximize the extraction of vegetable oils is urgently needed. In this work, natural enzymes present in kiwi fruit and ginger along with the ultrasound waves method were used to extract Bitter Almond Oil (BAO). Important factors affecting extraction were optimized using response surface methodology (Box-Behnken design). Under optimum conditions, the maximum extracted oil with an average of 62.1 wt.% oil/seed was extracted from the bitter almond kernel, which was only 0.87% less than the predicted value by the equation. The optimum conditions were: pH=4.74, Kiwi-Ginger juice to almond of 11.3 mL/g (22.6 mL ethanol), incubation temperature of 52 °C, ultrasonic power of 257 watts, and ultrasonic time of 38 min. The results confirmed the validity of the model. The applied technique in this investigation is economically cost-effective and highly efficient which can be widely used in the edible oil industry due to the absence of toxic and hazardous substances.*

KEYWORDS: *Non-edible oil; Bitter almond; Enzymatic extraction; Ultrasound assisted extraction; Response Surface Methodology (RSM); Box-Behnken design.*

INTRODUCTION

As technology evolves, there is a growing need for energy consumption. Fossil fuel resources are declining, and it is almost impossible to offset them. In the last few decades, several renewable fuels have been invented that can replace fossil fuels in the future [1-3]. Biodiesel is one of these fuels. Biodiesel or alkyl ester is the result of the reaction of triglycerides with a light alcohol type. This reaction takes place in the presence of the catalyst in various ways [4]. The main cost of biodiesel production is related to raw materials (triglycerides), which comprise 70-80% of the total costs [5, 6]. Edible or non-edible oils, animal fats and waste edible oils are renewable sources of biodiesel fuel raw materials [7]. Many scientists are trying to apply non-edible or waste oils to produce this fuel because the global food crisis is inevitable and it is better not to use edible oils for biodiesel [8-10]. Some of the non-edible vegetable oils turned to account for this function are: Jatropha, Mahua, Neem, Rubber seed, Polanga, Yellow oleander, Croton megalocarpus, Calophyllum inophyllum, *Terminalia Catappa* Kernel Oil (TCKO) and so on [11-16]. Almonds are a kind of native fruit to the Middle East and South Asia. In principle, almonds are not considered

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true beans, but are of a variety of shafts, which consists of three parts: outer shell, hard inner crust, in which the seed (the brain) is covered within it. The highness of the almond tree is 4 to 10 m, and its diameter can reach more than 30 cm [17-19]. The largest almonds producing countries in the world are the United States and Spain. Iran is the fifth greatest producer of this crop, with 6% production. Almonds are generally classified into two classes: sweet almonds (with white blooms) and Bitter Almonds (BAs) (with pink blooms) [20, 21]. Bitter Almond Oil (BAO) is consumed for many functions, typically including the production of cosmetics [22].

Since the percentage of oil in the oilseeds is directly dependent on the extraction method and the cultivated area, this amount is not accurately reported but is reported as an interval [23, 24]. For almond seeds, this amount is reported to be about 40-50% by compression method. BA oil contains compounds such as hydrocyanide and benzaldehyde, which are placed in a bunch of non-edible seeds [20]. There are several methods for extracting oil from oilseeds. Some of them are Soxhlet extraction using n-hexane or ether [25, 26], supercritical CO_2 extraction [27], ultrasound-assisted extraction [28-30], microwave-assisted [31], cold pressing [32], and enzyme-assisted [33]. In solvent extraction, parameters such as solvent type, grain particle size, extraction time, and temperature affect oil extraction amount [34]. The studied works on BAO extraction with modern methods are limited. Meanwhile, *Liu et al.* used a salt (sodium bicarbonate solution) for better extraction. In this way, they obtained 90.9% of the oil in the seeds. The optimal conditions were as follows: solvent-to-sample ratio of 5:1, the concentration of sodium bicarbonate of 0.4 mol/L, extraction time 1h and extraction temperature of 84 °C [35]. In some cases, for the better oil extraction, several of the mentioned methods are used together [36]. Each of these methods has its own advantages and disadvantages. It should be deliberated that which method is economically more beneficial considering the type of the grain, obtained oil and consumption field. Among these techniques, enzymatic extraction method is utilized alone or in combination with other ways. Because of the use of enzymes as environmentally friendly agents at normal temperatures and pressures, and no need for special conditions so were employed as pretreatment oil extraction stage. The enzymes act as effective catalysts and by dissolving the seed cell wall, so interior compounds easily are released into the environment [37-39].

There are a number of fruits and vegetables that contain natural enzymes. Kiwi and ginger are among them. The kiwi has a high nutritional value. Contain vitamins, nutrients, minerals, and enzymes. Actinidine is a cysteine protease abundant in kiwi fruit. Proteases have an important position in the food and pharmaceutical industries. For domestic uses, kiwi juice or pulp is typically utilized to tenderize the meat, and actinidin in the kiwi seems to be efficacious [40-42]. Ginger has nutritional value, beneficial minerals, and enzymes that have long been used in the treatment and prevention of many diseases. Ginger moreover contains the natural Protease-type enzyme of Zingibain, which is remarkably effective in protein digestion [43, 44].

As many studies have shown the valuable effect of the presence of enzymes in maximizing oil extraction, one of the aims of this research is to use natural and inexpensive enzymes in the oil extraction phenomenon. In this investigation, a novel, low-cost, and high-efficiency approach for the extraction of BAO has been carried out. On the other hand, the results of other research always showed the combination of different methods in improving the result, so ultrasound waves are also used in this work. At various stages of work, key factors influencing oil extraction were specified. An experimental design was applied to determine the conditions for maximum oil extraction efficiency. In these conditions, the yield was obtained and the validity and reliability of the predictive model were evaluated.

EXPERIMENTAL SECTION

Materials

BAs (kernel average size 10were obtained from Zagros mountains in the west of Iran. The kiwi and ginger were freshly prepared from a local store. Citric acid (>99.5%) and ethanol (≥99.9%) were provided by Merck com. Distilled water and deionized water were employed during the procedures.

BAO extraction process

Later than separating the BA kernels from the shell, they were crushed to powder. The powder was passed through a sifter 60 mesh, so the maximum particle size was 250 microns and a very fine powder was obtained. The fine particles were then stored in a sealed container at 4 °C for the following use. After carefully separating the thick peel of the kiwi and ginger, a uniform paste was gained from equal weights of them by blender. 20 wt% of deionized water was added to the mixture and was stirred for 60 min (at 150 rpm). The mixture was then poured into the juicer to separate the liquid from the pulp. The obtained solution, as the source of the enzymes, was kept in a sealed container at 4 °C for later use.

After preparing the raw materials, 5 g of BA powder was poured into a vessel. The important variables and their definition range were as follows. The appropriate amount of Kiwi-Ginger juice (5-15 mL/g) was added to the BA powder. The citric acid (1 M) was consumed to regulate pH (3.5-7). The mixture was seamlessly blended and embedded in the shaker at the appropriate temperature (range of 30-60 ℃) for the enzyme-assisting extraction step. The time course of this step was considered fixed for all trials (4 h).

In the second step, oil extraction was done by assisting the ultrasound waves. An ultrasonic generator was operated for this object (UP400S- Hielscher, Germany). At this stage, exactly double the volume of the kiwi-ginger juice, ethanol was added to the gained mixture from the previous step. The mixture was stirred at ambient temperature at 150 rpm for 30 minutes. The post-homogenization mixture was used for exposure to ultrasound waves. The ultrasound homogenizer probe was inserted directly into the mixture. The used frequency was 24 kHz. The power used to extract from 150-300 watts was considered variable and the extraction time was also estimated to range 5-60 min. In this method, the mixing vessel of materials was set inside a larger container of water (as a bath to keep the temperature constant). After setting the appropriate conditions in terms of power and time required, the device turned on and the probe spread the waves inside the mixture.

Separation of extracted oil

At the end of oil extraction, a centrifuge was applied to isolate the oil (centrifuge condition: time 15 min, temperature 17 ℃ and speed 11,000 rpm). In the end, the mixture was divided into three almost separate layers. The top level of dissolved oil in ethanol was extracted by a micro-pipette. The intermediate layer made an emulsion of water and oil that was frozen at -20 ℃ for 12 hours after separation from the solid phase. It was subsequently placed at 30 °C for 2 hours and then was centrifuged in the same manner. Some of the oil that was combined with the water was also separated. After evaporating the ethanol in the oven at 80 °C, all oils were carefully weighed and the percentage of extraction efficiency was calculated using Equation (1):

$$\text{Oil extraction yield}(\%) = \frac{\text{Weight}_{\text{oil}}}{\text{Weight}_{\text{Bitter almond powder}}} \times 100 \qquad (1)$$

Experimental design

In this investigation, Design Expert 7.0.0 software (Stat-Ease, USA) was utilized to expedite the procedure and avoid wasting time. With this software, the best performance conditions are attained to achieve maximum efficiency (oil extraction). From the collection of Response Surface Methodology (RSM), the Box-Behnken design was chosen. The five considered variables were: pH, Kiwi-Ginger juice to almond (mL/g), incubation temperature (°C), ultrasonic power (watt), and ultrasonic time (min). The percentage oil yield was the response variable [45]. These independent variables are declared in Table 1 along with the intervals.

In this approach, each variable is considered in three levels (codes). The code of -1 corresponds to the beginning of the interval, zero is related to the midpoint of the interval, and +1 is recited to the endpoint of the desired interval. To measure the model flexibility or error caused by the trials, there are several experiments at midpoints for all variables (here, six-run). Finally, 46 tests were planned with the experimental answers provided in Table 2. The software fits the obtained data by applying multiple regression processes to obtain the most efficient quadratic model for the answer based on independent variables. The general form of the empirical equation is presented in Equation (2).

$$Y = \theta_0 + \sum_{i=1}^{n} \theta_i X_i + \sum_{i=1}^{n} \theta_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} \theta_{ij} X_i X_j \qquad (2)$$

Equation 2 consists of a constant value, and linear and quadratic terms. Y refers to the answer (oil extraction percentage here). The coefficients of the sentences are Offset (θ_0), Linear (θ_i), Quadratic (θ_{ii}), and Interactive (θ_{ij}). Moreover, X_i or X_j identifies the independent variable.

Table 1: Coded numerical variables and ranges.

Variables	Symbol	Ranges and Levels		
		-1	0	+1
pH	A	3.5	5.25	7
Kiwi-Ginger juice to almond (mL/g)	B	5	10	15
Incubation temperature (°C)	C	30	45	60
Ultrasonic power (watt)	D	150	225	300
Ultrasonic time (min)	E	5	32.5	60

The ANOVA test (analysis of variance) was typically utilized to confirm the confidence level of the obtained model.

Identification of fatty acids composition

An Agilent 6890N gas chromatography equipped with a capillary column (DB-1, 100m × 0.25 mm I.D., 0.5 μm film thickness) was utilized to analyze the varieties of fatty acids in BAO. Before the almond oil was injected into the system, the fatty acids were converted to methyl ester by the esterification reaction. The beginning capillary operation temperature was 50 °C (remained for 10 min). Then, the temperature was increased to 150 °C at 10 °C/min (stayed for 4 min). Afterward set upright to 200 °C at 2 °C/min, and increased to 230 °C at 0.5 °C/min. In the ultimate step, the temperature was elevated to 260 °C at 10 °C/min, and continued at 260 °C for about 50 min (up to the end). Further conditions were: injection volume of 1 μL; injector temperature of 280 °C; detector temperature of 240 °C. In addition, helium was applied as a carrier gas at a flow rate of 2 mL/min.

BAO characterization

According to the standards defined by the American Society for Testing and Materials (ASTM standard), significant properties of almond oil were carefully determined. These key features were: acid value, iodine value, specific gravity, moisture content, and saponification value. An Abbemat-3X00 refractometer (Anton Paar, Austria) was employed to measure of refractive index at 40 ºC. To avoid the reported error, all evaluations were carried out three times in separate samples and the values were reported as averages.

RESULTS AND DISCUSSION

ANOVA test results and experimental model

Based on the regression study of the responses from Table 2, the software obtained a quadratic polynomial model. This equation indicates how the percentage of oil extraction depends on the main variables. It should be mentioned that the independent variables are encoded within it. Here, Y refers to the oil extraction yield.

$$\begin{aligned} Y\ (\%) &= +61.87 + 0.75A + 0.55B + \\ &4.30C + 3.29D + 2.91E - 2.17AB + 0.26AC - \\ &0.69AD + 0.58AE - 1.86BC - 0.50BD - 0.34BE + \\ &0.19CD - 0.99CE - 0.35DE - 4.93A^2 - \\ &5.68B^2 - 4.62C^2 - 3.60D^2 - 4.48E^2 \end{aligned} \tag{3}$$

By maintaining the validity level of the equation, due to the fact that parameters with a *P-value* greater than 0.05 can be removed from the equation, the AC, BE, CD, and DE parameters can be removed from the equation and thus it becomes simpler, and can be rewritten as follows:

$$\begin{aligned} Y\ (\%) &= +61.87 + 0.75A + 0.55B + \\ &4.30C + 3.29D + 2.91E - 2.17AB - 0.69AD + \\ &0.58AE - 1.86BC - 0.50BD - 0.99CE - \\ &4.93A^2 - 5.68B^2 - 4.62C^2 - 3.60D^2 - 4.48E^2 \end{aligned} \tag{4}$$

Carefully with the coefficients of linear terms, it can be seen that the incubation temperature with the highest coefficient causes the greatest impact on the oil extraction efficiency. The order of importance of the factors represents as follows: incubation temperature, ultrasonic power, ultrasonic time, pH and Kiwi-Ginger juice to almond.

Table 2: BBD-matrix with responses.

Run No.	A: pH	B: Kiwi-Ginger juice to almond (mL/g)	C: Incubation temperature (°C)	D: Ultrasonic power (watt)	E: Ultrasonic time (min)	Oil Extraction Yield (%)
1	3.5	5	45	225	32.5	47.72
2	7	5	45	225	32.5	53.78
3	3.5	15	45	225	32.5	53.07
4	7	15	45	225	32.5	50.43
5	5.25	10	30	150	32.5	46.25
6	5.25	10	60	150	32.5	54.89
7	5.25	10	30	300	32.5	52.03
8	5.25	10	60	300	32.5	61.42
9	5.25	5	45	225	5	47.94
10	5.25	15	45	225	5	49.68
11	5.25	5	45	225	60	54.31
12	5.25	15	45	225	60	54.68
13	3.5	10	30	225	32.5	47.68
14	7	10	30	225	32.5	48.01
15	3.5	10	60	255	32.5	55.75
16	7	10	60	225	32.5	57.11
17	5.25	10	45	150	5	47.12
18	5.25	10	45	300	5	54.39
19	5.25	10	45	150	60	53.62
20	5.25	10	45	300	60	59.48
21	5.25	5	30	225	32.5	44.85
22	5.25	15	30	225	32.5	49.68
23	5.25	5	60	225	32.5	57.28
24	5.25	15	60	225	32.5	54.68
25	3.5	10	45	150	32.5	48.38
25	7	10	45	150	32.5	51.48
27	3.5	10	45	300	32.5	56.88
28	7	10	45	300	32.5	57.2
29	5.25	10	30	225	5	45.01
30	5.25	10	60	225	5	55.09
31	5.25	10	30	225	60	52.73
32	5.25	10	60	225	60	58.85
33	3.5	10	45	225	5	49.22
34	7	10	45	225	5	49.78
35	3.5	10	45	225	60	54.11
36	7	10	45	225	60	56.98
37	5.25	5	45	150	32.5	48.22
38	5.25	15	45	150	32.5	50.47
39	5.25	5	45	300	32.5	55.73
40	5.25	15	45	300	32.5	55.99
41	5.25	10	45	225	32.5	62.03
42	5.25	10	45	225	32.5	60.95
43	5.25	10	45	225	32.5	62.53
44	5.25	10	45	225	32.5	62.04
45	5.25	10	45	225	32.5	61.89
46	5.25	10	45	225	32.5	61.78

Table 3: ANOVA test results.

Source	Sum of Squares	df	Mean Square	F-Value	P-Value	
Model	1141.72	20	57.09	426.68	< 0.0001	significant
A: pH	8.94	1	8.94	66.82	< 0.0001	
B: Kiwi-Ginger juice to almond (mL/g)	4.90	1	4.90	36.59	< 0.0001	
C: Incubation temperature (°C)	296.10	1	296.10	2213.14	< 0.0001	
D: Ultrasonic power (watt)	173.51	1	173.51	1296.91	< 0.0001	
E: Ultrasonic time (min)	135.32	1	135.32	1011.39	< 0.0001	
AB	18.92	1	18.92	141.43	< 0.0001	
AC	0.27	1	0.27	1.98	0.1715	
AD	1.93	1	1.93	14.44	0.0008	
AE	1.33	1	1.33	9.97	0.0041	
BC	13.80	1	13.80	103.16	< 0.0001	
BD	0.99	1	0.99	7.40	0.0117	
BE	0.47	1	0.47	3.51	0.0728	
CD	0.14	1	0.14	1.05	0.3151	
CE	3.92	1	3.92	29.30	< 0.0001	
DE	0.50	1	0.50	3.71	0.0654	
A^2	211.92	1	211.92	1583.95	< 0.0001	
B^2	281.91	1	281.91	2107.12	< 0.0001	
C^2	186.03	1	186.03	1390.43	< 0.0001	
D^2	112.91	1	112.91	843.92	< 0.0001	
E^2	174.92	1	174.92	1307.38	< 0.0001	
Residual	3.34	25	0.13			
Lack of Fit	2.00	20	0.100	0.37	0.9487	not significant
Pure Error	1.34	5	0.27			
Cor Total	1145.07	45				
R^2: 0.9971 R^2_{adj}: 0.9947						

ANOVA test was applied to confirm the accuracy of the model performance. The results are reported in Table 3. One of the conditions of considerable significance for the model or parameters is the attending of a high F-value and a low p-value (< 0.05) [2, 46]. These two conditions include the model and all independent variables. Representing these causes, parameters of AC, BE, CD, and DE possesses smaller importance and can be excluded from the model. The other two most important parameters related to the model are R^2 and R^2_{adj}. If the value of these regression coefficients is higher than 0.75 and close to 1, indicates the strong validity of the model [7]. R^2=0.9971 shows that 99.7% of data are covered in the predictive model and does not include just 0.29% of the data, which is insignificant. In Fig. 1 (parity plot), a direct comparison is made between the obtained values from the experiment and the predictive model for oil extraction yield. The bisector line represents the equality of values, and the closest points to the line display the higher precision of the model.

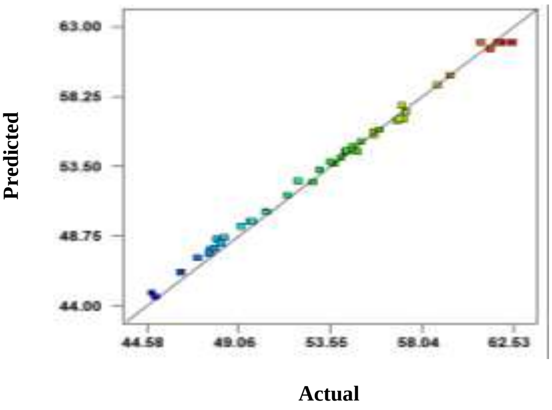

Fig. 1. Parity plot.

Interaction effect of independent variables on oil extraction efficiency

Using three-dimensional graphs, the simultaneous effect of two variables on the response is shown. Fig. 2 indicates the collection of these diagrams. In each graph, the two horizontal axes represent the independent variables and the vertical pivot shows the oil extraction efficiency. The variables represent the actual values. In each graph, three other variables are at the midpoint of the corresponding interval. In Fig. 2-a, the effect of the simultaneous change of pH and Kiwi-Ginger juice to almond, on the response is demonstrated. Obviously, with increasing variable amounts, the yields were enhanced by about pH 5.75 and Kiwi-Ginger juice to the almond ratio of 11 mL/g. As a result of these interactions, a maximum yield in this area appeared, and then yields decreased.

The interaction effect of altering the pH and Incubation temperature on the extraction efficiency is shown in Fig.2-b. As is obvious, the pH changes effects are similar to Fig. 2-a, but the temperature causes a more significant effect on efficiency. As the temperature increases, the extraction efficiency increases, which may be due to the softening of the seed tissues by the temperature and the release of the oil or the optimal temperature of enzyme activity. However, with increasing temperature by more than 52 °C, the yield decreased slightly, which may be related to the maximum temperature for the enzyme activity. In Fig. 2-c, similar to Fig. 2-b, a uniform trend is seen, but here the opposite variable of pH is ultrasonic power. As a result of wave vibrations, bubbles are formed that forcefully hit the cell wall. This will break down the oil cell wall and intensify extraction. Enhancing the ultrasonic power to 260 watts increases the process productivity and after that higher power slightly reduces efficiency. The consequence of the simultaneous interaction between pH and ultrasonic time on yield is exhibited in Fig. 2-d. In the process of extraction with the help of ultrasonic waves, like any other phenomenon, the time passing positively affects the efficiency. By expanding the time to about 37 min, oil extraction efficiency increases. Subsequently, the rise in extraction time did not cause many effects on the extraction increment but rather the extraction rate decreased somewhat. In Figs. 2-e to 2-j, nearly similar trends are observed with the previous four diagrams. The maximum points for the extraction yield are identified as the tip of the domed area in the graphs [45].

The best-operating conditions

As pointed out earlier, one of the consequences of using the design of experiments is to derive an empirical model to predict the answer founded on the available variables. On the other hand, following this model and analyzing the results, the software supplies the best performance conditions to achieve the maximum possible yield. These optimal conditions are pH=4.74, Kiwi-Ginger juice to almond of 11.28 mL/g (~11.3 so 22.6 mL ethanol), incubation temperature of 51.62 °C (~52 °C), ultrasonic power of 257 watts and ultrasonic time of 37.9 min (~38 min). Controlled by these conditions, oil extraction was measured three times. The average value for the yield was 62.1%, which was only 0.87% lower than the predicted value of the software (62.97%). These excellent results demonstrate the robustness of the model and its accuracy [45].

Research on the extraction of oil from different plant seeds has been done with various conditions. Therefore, a comprehensive comparison of the results obtained in this study can't be performed. But some aspects of it can be examined, like extraction time, type of used enzyme, the strength of waves, and so on. In a study conducted by *Hu et al.* [47], at best conditions, they used extra power for ultrasound and a more acidic environment for oil extraction. They examined the simultaneous effect of combining three extraction techniques (microwave, ultrasound, and enzymes) to obtain cherry kernel oil. The particle size of the seed powder was less than 0.425 mm. Employing the design of experiments, the optimal conditions for obtaining maximum oil extraction (83.85 ± 0.78%)

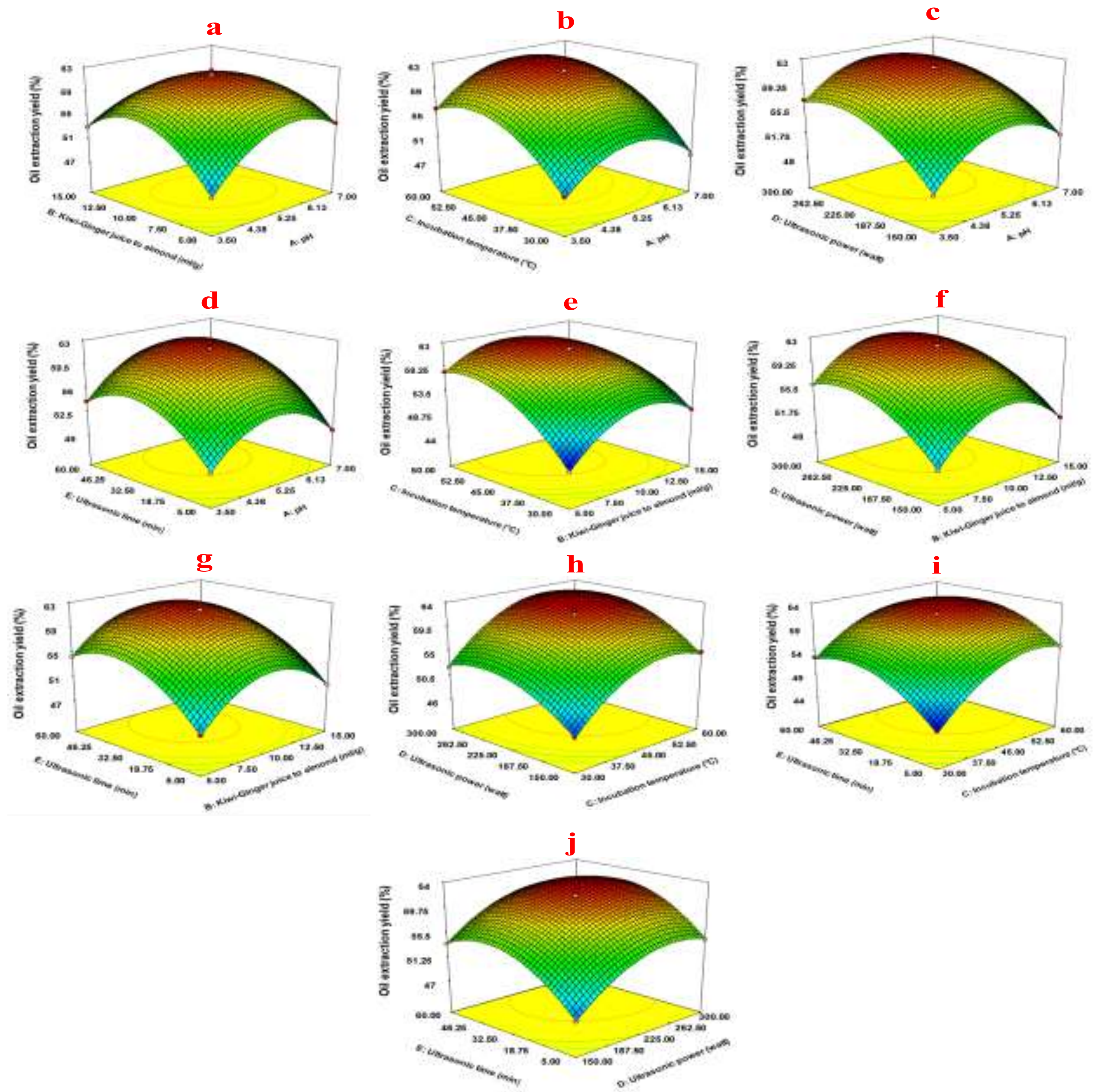

Fig. 2: Interaction effect of independent variables on oil extraction efficiency.

were: enzyme concentration of 2.7%, 560 W for ultrasonic power, extraction time of 38 min, microwave power of 323 W, extraction temperature of 40°C, and pH of 3.5. In another study, researchers showed that by consuming more time for the enzyme to function on oily cells, a significant percentage of extraction could be achieved in a medium with a neutral pH. *Liu et al.* [48] Investigated the extraction of oil from *Sapindus mukorossi* seed by combining two methods of adding enzymes and using ultrasound waves. They reached relatively good results (82.67% ± 2.21%) for oil extraction. The optimal conditions they achieved were: 4 wt.% of the enzyme, incubation time of 8 h, incubation temperature of 60 °C, pH of 7, water solid the ratio of 16 mL/g, shaking speed of 600 rpm, ultrasonic power of 240 W and ultrasonic time of 56 min. In another study in the same field, *Goula et al.* [49] surveyed pomegranate seed oil extraction by incorporating two methods using enzymes and ultrasound waves.

The maximum efficiency obtained by them was 15.33 g oil/100 g dry seeds in 2 h extraction. The extraction time and temperature were changed between 2-8 h and 35-55 °C respectively. The water/seeds ratio and enzyme concentration also varied between 2-6:1 mL/g and 2-4 wt.%.

As can be seen, each of the factors influencing oil extraction, if changed, affects the amount of extraction, but then, to maintain the percentage of yield, other conditions must be revised simultaneously.

Fatty acids profile of BAO

The quantity and type of fatty acids affect the standard of edible oil for diverse uses. In the case of non-edible oil as well, this information is extremely valuable for the produced fuel derived from it. Depending on the outcomes of gas chromatography, the bulk of BAO was constituted from seven types of fatty acids. A considerable percentage was unsaturated fatty acids. The kind and quantity of these fatty acids are myristic acids, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and arachidic acid. As indicated, almost 77.45% and 21.71% of the BAO are composed of unsaturated and saturated fatty acids respectively. Also, the 0.84% involves some other fatty acids that were unmentioned because of their low content. The varieties of identified fatty acids along with their quantities are classified in Table 4. Utilizing these details, the molecular weight of BAO was also measured and reported [15, 34, 50-53].

Table 4: Distribution of fatty acids in of BAO.

Property	Quantity (wt.%)
Myristic acid (C14:0)	0.914
Palmitic acid (C16:0)	11.8
Stearic acid (C18:0)	4.18
Oleic acid (C18:1)	51.24
Linoleic acid (C18:2)	25.15
Linolenic acid (C18:3)	1.06
Arachidic acid (C20:0)	4.82

Physical and chemical properties of BAO

These characteristics consist of: acid value, iodine value, specific gravity, moisture content, saponification value, and refractive index are presented in Table 5. It should be marked that the acidic value was 0.36 mg KOH/g. Since the acid number is less than 2 mg KOH/g, therefore, without the acid pre-treatment step, it can be employed as an oil source to produce biodiesel with any variety of catalysts. SEM images of BAs powder, before and after oil extraction, are displayed in Fig. 3. As shown in the figure, the appearance of the particles has changed due to the loss of oil [15, 45].

Table 5: Some physical and chemical properties of BAO.

Property	Quantity
Acid value (mg KOH/g)	0.36
Saponification value (mg/g)	193.32
Iodine value (g I_2/100 g)	82.25
Density at 15 ℃ (g/cm^3)	0.916
Viscosity at 40 ℃ (mm^2/s)	33.64
Refractive index	1.45
Moisture content (%)	0.12
Flash point (℃)	169
Pour point (℃)	-4
Cloud point (℃)	9

CONCLUSIONS

In this investigation, BAO was extracted utilizing an innovative method. Using natural enzymes (Kiwi and ginger) as a low-cost source of enzyme supply can be extremely effective in reducing extraction costs. On the other hand, the proper use of ultrasound waves raised the extraction rate considerably. Using the design of the experiments, the conditions for maximum extraction were investigated. These optimum conditions were: pH=4.74, Kiwi-Ginger juice to almond of 11.3 mL/g (so 22.6 mL ethanol), incubation temperature of 52 °C, ultrasonic power of 257 watts, and ultrasonic time of 38 min. Under these favorable conditions, an oil extraction yield of 62.1% was gained, which was just slightly different from 62.97%, the value estimated by the software. This proves the excellent accuracy and precision of the obtained experimental model for predicting BAO extraction. The extracted oil under optimal conditions was examined to investigate the composition of the fatty acids and some of the physical and chemical properties. The results showed that the following oils accounted for the highest to lowest weight percentages, respectively: Oleic acid

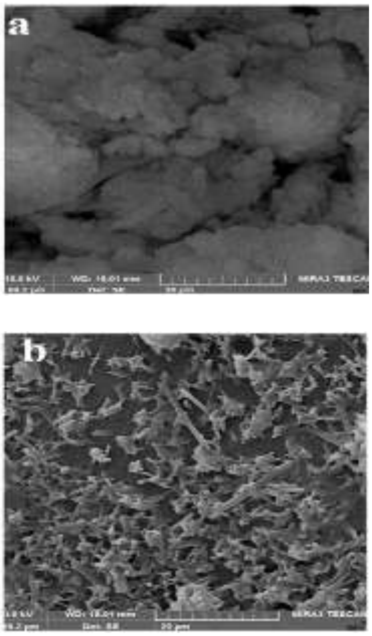

Fig. 3. ***SEM images of BA powder a) before oil extraction, b) after oil extraction.***

(C18:1, 51.24%), Linoleic acid (C18:2, 25.15%), Palmitic acid (C16:0, 11.8%), Arachidic acid (C20:0, 4.82%), Stearic acid (C18:0, 4.18%), Linolenic acid (C18:3, 1.06%) and Myristic acid (C14:0, 0.914%). Outstanding features of the obtained oil were its viscosity 33.64 (mm^2/s at 40 ℃) and low acid number (0.36 mg KOH/g), which makes it suitable as a raw material for biodiesel fuel. Also, the SEM images of oil seed powder showed that this new extraction method affected the oil cells well and the oil was excellently extracted.

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