Improvement of thermo-resistance and quality of soybean oil by blending with cold-pressed oils using simplex lattice mixture design

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Abstract

Soybean oil is the most consumed oil worldwide due to its cheapness but presented a weak thermo-resistance compared to most other oils used. This study aims to improve the thermo-stability of soybean oil by blending with crude oils. For this, physicochemical and antioxidant parameters of each blended oil were assessed before and after thermal treatment at 170°C for 10h/day for 5 days. The results of binary blended oils indicated that soybean oil mixed with lentisk, sesame, and almond oils (70:30%) manifested the best thermo-stability parameters. The Simplex Lattice Mixture Design applied for these three selected oils indicated that the combination of soybean oil at the proportion of 70% with lentisk oil and sesame oil at proportions of 17.7% and 12.3% respectively was selected as the best blending for maximizing soybean oil properties. Fatty acids analysis by GC/FID technique showed that the level of the degradation of linoleic acid (ω6) and linolenic acid (ω3) after heat treatment was more important in soybean oil than in blended oil. This study demonstrated that the thermo-resistance and nutritional quality of soybean oil were improved by blending with sesame and lentisk oils.

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Abstract

Soybean oil is the most consumed oil worldwide due to its cheapness but presented a weak thermo-resistance compared to most other oils used. This study aims to improve the thermo-stability of soybean oil by blending with crude oils. For this, physicochemical and antioxidant parameters of each blended oil were assessed before and after thermal treatment at 170°C for 10h/day for 5 days. The results of binary blended oils indicated that soybean oil mixed with lentisk, sesame, and almond oils (70:30%) manifested the best thermo-stability parameters. The Simplex Lattice Mixture Design applied for these three selected oils indicated that the combination of soybean oil at the proportion of 70% with lentisk oil and sesame oil at proportions of 17.7% and 12.3% respectively was selected as the best blending for maximizing soybean oil properties. Fatty acids analysis by GC/FID technique showed that the level of the degradation of linoleic acid (ω6) and linolenic acid (ω3) after heat treatment was more important in soybean oil than in blended oil. This study demonstrated that the thermo-resistance and nutritional quality of soybean oil were improved by blending with sesame and lentisk oils.

Keywords: Soybean oil, Cold-pressed oil, Thermo-stability, Fatty acids composition, Simplex lattice mixture design

Introduction

The industrials are interested in different variety of oleaginous resources to provide a wide range of fats and oils with a variable range of properties. The obtained fats must simultaneously meet many expectations, like providing high nutritional quality, offering a required texture, and the resistance to cooking temperature [26].

The most important factors for edible oils in food technology are quality, stability, and nutritional characteristics; it is on this basis that oils are intended for many uses in the preparation of different food products [16]. Several external factors can influence the quality and stability of oils such as air (oxygen), light, and temperature that accelerate oxidation reactions which results in the production of unwanted flavors and odors associated to low weight volatiles molecular as aldehydes, ketones, and alcohols [3, 12].

The blending process of oils is the simplest and secure methods that can improves and create new specific products with desired textural and oxidative properties and achieving to desirable physical and chemical characteristics [16, 30]. Oil blending is a widely used practice in the edible oil industry to produce blended oils with enhanced stability and good nutritional and sensory properties [6]. In addition, it is an effective strategy to address consumer demands for oils containing only natural products [11]. Mixing different vegetable oils can change fatty acid composition and give higher levels of natural bioactive compounds [2] that could contribute as sources of important antioxidants related to the prevention of chronic diseases associated to oxidative stress, such as in cancer and coronary diseases [24].

In order to optimize the proportions of different compounds of the given mixture, the mixture design was used. This type of design can allow the establishment of the mathematical model used in the prediction of the response for any mixture or combination of the ingredients. The influence on the response of each component singly and in combination with other components can be easily obtained.

The quality of oils can be provided by some physicochemical tests. Peroxide value and specific extinction can be taken as a picture of the oxidative state, while the amount of acidity reveals some information about the quality of oil [22]. The iodine number provides information on the degree of unsaturation of lipids that related to oxidative stability and rancidity of oil [31]. Also, rancimat test, antioxidant activity, and the total phenolic compounds informed about the degree of resistance of the oil to oxidation. The study of all these parameters can guide to the selection of better oils and oils mixture.

Soybean oil is one of the most used oil in the world. The total world production of soybean is estimated at 333 million tons in 2019 [9]. The crude soybean oil is rich in protein with highly nutritious properties and it may protect against some cancers as breast and prostate, but cannot be used for cooking purpose due to its unpleasant smell [24, 35]. The refined soybean oil is widely used as all-purpose cooking oil with a good nutritive value owing to a high proportion of polyunsaturated fatty acid, which in contrast causes its high thermal degradation [30].

Some studies demonstrated that soybean oil blended with other crude oils improved its quality. The mixing of two or more of high-oleic oils produced blends with ameliorated nutritional and/or physicochemical properties. Some oil blends exhibited a high oxidative stability due to the presence of numerous minor components as natural antioxidants leading to reduce the free radical action particularly at frying temperature, that produce stable and economical viable frying oils [10, 27, 29]. Dhyani, et al. [8] reported that soybean oil blended with cold pressed oils (sesame, rice bran, sea camellia, peanut, and buckthorn oils) were more stable with a high oxidative stability and radical scavenging activity than soybean oil alone.

For this purpose, this study carried out to improve the thermo-resistance of soybean oil using the blending with crude oils. For this, the study of the individual (soybean and six crude oils: almond, lentisk, nigella, peanut, sesame, and wild-olive oils) was performed. After that, the binary blend of soybean oil with each other six oils (70:30) was investigated. Finally, the three selected best oils were used in the simplex lattice mixture design to optimize the best oils combination that improves soybean oil resistance to thermal treatment.

Materials and Methods

Reagents

Phenolphthalein, potassium hydroxide, potassium iodide, sodium thiosulfate, Wijs reagent, ABTS (2.2'-azino-bis-(3-ethylbenzthiazoline-6-ulfonic acid)), sodium carbonate, potassium persulfate, gallic acid, and Folin–Ciocalteu phenol reagent were from Sigma–Aldrich (Germany). Fatty Acid Methyl Esters (FAME) standards were purchased from Supelco (USA). All used solvents were at analytical grade and procured from Prolabo (France).

Vegetable oil samples

The sample of refined soybean oil was obtained from Cevital Company (Bejaia, Algeria) in PET bottles of 1 L. Six crude oils extracted from almond, lentisk, nigella, peanut, sesame, and wild-olive oils were used for blending with soybean oil. These oils were extracted with cold pressing by the Parapharmaceutical Products and Equipment Manufacturing Company (Bejaia, Algeria). All oils were transported to the laboratory taking into account protective measures against oxidation (low temperature, darkness and inert and closed containers). Once in the laboratory, they were stored in the refrigerator at 6°C until analyses.

Preparation of oils

In the first step, the studied oils (soybean, almond, lentisk, nigella, peanut, sesame, and wild-olive oils) were individually analyzed in order to determine the initial characteristics. In the second one, the soybean oil was mixed with each of the six crude oils at a ratio of 70%:30% (v/v). The obtained blend binary oils were analyzed before and after thermal treatment to select three best oils that improve thermo-oxidation resistance of soybean oil.

In the last step, the three best oils selected from the second steps were combined according to Simplex Lattice Mixture Design in order to assess the optimal oils combination (30%) that improves the thermo-resistance of soybean oil (70%). Each vertices of equilateral triangle represented the chosen oils (almond, lentisk or sesame oils) that varied from 0 to 1, the combination binary levels were 1/3, 2/3 and the ternary mixture were represented by the center of triangle with a combination of the three oils at a levels of 1/3, 1/3, and 1/3

[5]. The ten different oil combinations were indicated in Table 3. The analyzed variable or responses were physicochemical parameters (specific absorptivity, acidity, peroxide value, iodine value, and rancimat test), total phenolic content, and antioxidant activity. The responses were fitted as polynomial model according to the *Eq.* (1).

$$Y = b_1x_1 + b_2x_2 + b_3x_3 + b_{1,2}x_1x_2 + b_{1,3}x_1x_3 + b_{2,3}x_2x_3 \quad Eq.\ (1)$$

Where Y is the response; b_1 ,b_2 , and $b3$ are the linear terms of the equation, $b_{1,2}$, $b_{1,3}$, and$b_{2,3}$ are the interaction terms of the equation;x_1, x_2, andx_3 are the factors (sesame, lentisk and almond oils).

Thermal treatment

The oils obtained from soybean oil mixed with other crude oils as well as soybean oil alone (as control) was treated with heating. For mixed oils, 70g of soybean oil was put in a 120ml glass flask and 30g of each oil or combined crude oils were added. To ensure a good homogenization, oil mixtures were well shacked. All oil flasks were thermally treated with a temperature of 170°C for 10 hours during 5 cycles of heating.

Physicochemical analysis

The physicochemical parameters assessed were specific absorptivity at 232 and 270, acidity, peroxide value, and iodine values. The different analyses were performed on soybean oil (control), crude oils, binary blend oils, and oils of the simplex lattice mixture design.

Specific absorptivity

For specific absorptivity measurement, 2.5g of sample oil was adjusted to 25ml with cyclohexane and homogenized [15]. Absorbance was measured at 232 and 270nm in a spectrophotometer (Uvline 9400, Secomam, France). The specific absorptivity of oil was calculated using the following equation:

$$^{1\%}{}_{1cm}() = A()/(C\ x\ d) \quad Eq.\ (2)$$

Where $^{1\%}{}_{1\mathrm{cm}}(\lambda)$, specific absorptivity at wavelength of 232nm or 270nm; A(λ), absorbance at 232nm or 270nm; C, concentration of analyzed solution in g/100ml; d, width of spectrophotometer quartz cell (cm).

Acidity

Two grams of sample oil were weighted in an Erlenmeyer flask and 100ml of ethanol/chloroform (v/v) were added as well as 4 drops of phenolphthalein as colored indicator. The mixture was titrated with potassium hydroxide (KOH; 0.1N) until the appearance of persistent pink color [21]. The acidity (Ac) was expressed in percentage of oleic acid per 100g of oil using equation (3).

$$Ac\ (\%) = (V\ x\ N\ x\ 282.2)\ x\ 100\ /\ (w\ x\ 1000) \quad Eq.\ (3)$$

Where A_C (%), acidity in percentage; V , the volume of KOH solution used (ml); N : normality of KOH solution (0.1N); *282.2* , molecular weight of oleic acid (mol/L);w , weight of oil aliquot (g).

2.5.3. Peroxide value

The peroxide value of oils was measured according to Novidzro, et al. [28]. An aliquot of oil (2g) was weighed in an Erlenmeyer flask and 15 ml of acetic acid than 1 ml of saturated potassium iodide solution were added. After putting the cap, the flask was shaken for 1min and then stands for exactly 5min in obscurity. 75 ml of distilled water were added then the mixture was titrated with sodium thiosulfate solution (0.02 N) using starch solution as a color indicator. A blank test was simultaneously carried out containing all reagents in the exception of oil. The peroxide value (PV) was expressed in milliequivalents of active oxygen per kilogram of oil (meq O_2/kg oil) and calculated following equation 4.

$$PV = (VxNx1000)/w \quad Eq.\ (4)$$

Where PV , peroxide value (meq/kg); V , volume of sodium thiosulfate solution used (ml); N , concentration of thiosulfate solution (0.02 N); w , weight of oil aliquot (g).

Iodine value

A quantity of 0.15g of oil was dissolved with 15 ml of chloroform in an Erlenmeyer flask. Then a volume of 25ml of Wijs reagent was added. The mixture was stirred and placed in dark for1 hour. After that, 20 ml of potassium iodide solution (10%) and 150 ml of water were added to the previous mixture. The iodine released is titrated with sodium thiosulfate (0.1N) in the presence of starch as an indicator until the medium turns colorless. A blank test was carried out under the same conditions using all elements of the mixture in the exception of oil [23]. The iodine value was calculated following equation 5:

$IV = (V_0\text{-}Vs)\ x12.69/w$ *Eq. (5)*

Where IV , Iodine value (g I_2/100 g oil); V_0 , volume of sodium thiosulfate solution used (ml, 0.1N) for the blank; V_s , volume of sodium thiosulfate solution used (ml, 0.1N) for the oil; w , weight of oil aliquot (g); *12.69* , concentration conversion coefficient.

Rancimat test

The Rancimat method is an accelerated ageing test commonly using for assessment of oxidative stability of oils or fats. The oxidative stability of the investigated oil samples was tested using the Rancimat apparatus (CH 9100, Methrom, Switzerland). An aliquot of oil (3g) was subjected to a temperature of 100°C at an airflow rate of 10L/h. The measurement was based on the conductimetric detection of volatile acids. The results were expressed as the Induction Time (IT) in hours, that represents the duration needed for the decomposition of hydroperoxides produced by oil after oxidation [17].

Phenolic compounds extraction, quantification and antioxidant evaluation

The extraction of phenolic antioxidants was occurred following Gutfinger [13] method. Five milliliters of hexane were added to 1g of oil and then 5 ml of 60% methanol were added. The mixture was vortexed during 3min and then centrifuged (Nüve NF 200, Ankara, Turkey) at 4000rpt/5min. The lower methanolic phase was recovered and the hexanic layer was reextracted using 5 ml of 60% methanol following the same procedure. The two extractions were combined.

Total phenolic content was determined as described by Bachir bey, et al. [4]. Folin-Ciocalteu reagent (750µl) was added to methanolic extract (700µl) and of sodium carbonate (7%, 400µl) was added after 5min.The absorbance was recorded at 750nm after 1h of incubation. The results were expressed as mg gallic acid equivalent per 100 g of oil (mg GAE/100g oil).

The radical scavenging activity (RSA) was determined by ABTS radical (2.2'-azino-bis-(3-ethylbenzthiazoline-6-ulfonic acid)) as reported by Lu-Martínez, et al. [25]. 400µl of oil extract were mixed with 2ml of alcoholic solution of $ABTS^{*+}$. The absorbance was measured at 734nm after 15 min. The percentage of RSA was estimated by following equation:

$RSA\ (\%) = (A_c\text{-}A_s)\ x100/A_c$ *Eq. (6)*

Where A_c , absorbance of control; A_s , absorbance of sample.

Fatty acids analysis

Fatty acids of soybean oil and optimal blending oil before and after thermal treatment were identified and quantified by Gas Chromatography according to ISO 5508 [19].In the preliminary step fatty acid methyl esters (FAME) were prepared by methanolic boron triflouride (13-15%) following to ISO 5508 [19] procedure. Analysis was carried out by using a gas chromatograph (6890 Network GC System from Agilent Technologies, USA) equipped with a Flame Ionization Detector and split/splitless injector and fitted with a capillary column DB 23 Agilent 122-2362 (60 m × 0.25 mm internal diameter × 0.25 µm film thickness). The carrier gas was H_2 with a debit of one mL/min and a pressure of 14.84 psi. One microliter of the sample was injected in split mode with a ratio of 1:50. Initially, the oven temperature was maintained at 130 °C for one minute and afterward, increased from 130 °C to 170 °C at a rate of 6.5 °C/min, then from 170° to 215 °C at 2.75

°C/min and held at this temperature for 12 min; finally, it was quickly (40°C/min) increased to 230 °C and held isotherm for 3 min. Injector and detector temperatures were set at 250 °C and 270 °C, respectively.

Statistical analysis

The results were expressed as the mean ± standard deviation of three replicates. The statistical analysis was performed using ANOVA following LSD test (Least Significant Difference) by Statistica Software version 10.0 (Stat Soft, Inc.). The data of simplex lattice mixture design were analyzed using JMP software version 10 (Statistical Analysis System Inc., SAS). The checking of qualities of mathematical models was evaluated by ANOVA and coefficients of regression by Student-t test.

Results and discussion

In a first step, the results present the different physicochemical parameters, phenolic compounds and antioxidant activity of pure oils studied (almond, nigella, lentisk, peanut, sesame, wild-olive and soybean oils) in order to characterize each of them. The second step represents the results of mixed crude oils with soybean oil on binary blends effect before and after heat treatment, then the choice of the three first classified oils. In a third step, the results represent the various physicochemical parameters of the combination of three oils with soybean oil and selection of the best proportion from them using the Simplex Lattice Mixture Design method. Finally, fatty acid profiles obtained by GC/FID of the optimal blending oil before and after heating treatment were compared to soybean oil.

The physicochemical parameters allow describing the quality of oils and estimating their oxidation state. Specific absorptivity at specified wavelengths in the ultraviolet region is related to the formation in oil system of conjugated diene and products of primary oxidation (at 232nm) and conjugated triene as well as products of secondary oxidation (at 270nm). The higher extinction at 232nm indicates the more peroxidized oil. Likewise, the extinction at 270nm explains the increase of secondary oxidation products and reflects a low aptitude for preservation and thermal treatment [21, 34]. The acidity (Ac) represents the percentage of free fatty acids conventionally expressed as oleic acid [18]. The peroxide value (PV) represents the amount of active oxygen contained in a kilogram of product [28]. The iodine value measured the unsaturation degree of fats and oils and hence their sensitivity to oxidation [33].

Crude oils characteristics

The results of physicochemical parameters, total phenolic compounds and antioxidant activity, describing the quality of the different oils are represented in the table 1. It can be noticed that soybean oil represents significantly, the lowest values of oxidation parameters: specific extinction (K232 and K270), acidity and peroxide value which were in the standards due to the refining process applied to this oil. Among cold-pressed oils, almond, sesame, and lentisk oils significantly endowed the majority of the best characteristics comparing to wild-olive, nigella, and peanut oils.

The rancimat test measures the resistance of oil to oxidation under heated and oxygenated medium. From the results, lentisk oil stands out from others with an induction time of 26.83h, followed by nigella and sesame oils with similar values and then soybean oil. However, peanut and wild-olive oils presented the weakest induction times. The results of total phenolic compounds indicated that lentisk and nigella oils are significantly the richest, which in consequence manifested good antioxidant activity. Finally, it can also be noted that despite the improvement of certain parameters of soybean oil by the refining process, the phenolic content and antioxidant activity were lower than those of crude oils. For this, in order to improve the quality of soybean oil, each cold pressed oil was mixed at a percentage of 30% with soybean oil, and the different physicochemical parameters were analyzed before and after thermal treatment.

Binary blends of soybean oil with crude oils

Table 2 shows the results of physicochemical parameters of analyzed blend oils (30% each oil with 70% soybean oil) before and after thermal treatment as well as total phenolic compounds and antioxidant activity of untreated oils.

Before thermal treatment, the addition of cold-pressed oils to soybean oil produced a decrease of some physicochemical parameters (specific extinction, acidity, PV, and IV). This was due to the relatively low quality of these added crude oils. But the richness of these later in bioactive compounds significantly increased TPC and antioxidant activity of elaborated blended soybean oils.

After thermal treatment, all quality characteristics of blend oils and soybean oil were significantly reduced but some of the crude oils manifested a considerable amelioration of the oxidative stability of soybean oil. It can be noticed from the results that the soybean oil mixed with lentisk oil has better parameters than the control (soybean oil alone); with a significant reduction of both specific extinctions (K232 and K270) and acidity. Sesame oil was also an interesting one; it allowed reducing the formation incidence of primary oxidation compounds, manifested by a significant reduction of specific extinction at 270nm and peroxide value compared to the control. The determination of peroxide indicates on oxidation state of oils and reveals the current level of rancidity in unsaturated fats. Oils with peroxide values less than 5meq O_2/kg are considered to have an acceptable oxidation degree [20]. Soybean oil blended with almond oil demonstrated a low acidity and peroxide value than pure soybean oil. The blended oils prepared with lentisk or sesame oils were characterized with high iodine value compared to soybean oil indicating a good protection of double-bounds. Whereas soybean oil mixed with nigella, peanut or wild-olive oils presented equal or lower quality than the control.

The improvement of quality parameters of soybean oil mixed with some crude oils can be explained by enrichment with phenolic compounds that manifested by enhancing antioxidant activity then resistance to oxidation during heating treatment. For example, the use of lentisk oil in a binary system with soybean oil manifested a significant increase of TPC and antioxidant activity with 42 and 33%, respectively. This was due to the high TPC of lentisk crude oil (63.17mg GAE/100g) and manifested an antioxidant activity remarkably higher than that of soybean oil. According to the study of Karoui, et al. [20], the oil of*Pistacia lentiscus* seeds seemed to be a good source of antioxidant agents.

Numerous work demonstrated that physicochemical, nutritional and antioxidant characteristics of soybean oil were improved by blending with unrefined oils. Vidrih, et al. [37] found that unrefined oils have better and longer oxidative stability and more suitable for frying than refined oils. This indicates that their addition to soybean oil could enhance its resistance to oxidation. Indeed, the quality of soybean oil, measured by stability index and peroxide value, blended with sesame oil (60:40) has been strongly ameliorated. This is related to the presence in sesame oil antioxidants (sesamol, sesamol dimer, sesamolin, and tocopherols) that improved the thermo-oxidative stability of oil [7]. Furthermore, Hashempour-Baltork, et al. [16] showed that the peroxide value and radical scavenging activity of flaxseed oil were significantly improved by the addition of sesame or peanut oils. Hamed and Abo-Elwafa [14] showed that the blending of flax seed oil (containing a high content of polyunsaturated fatty acids making it very susceptible to oxidation) with other oils rich in natural antioxidants like*Nigella sativa* oil solved the problem and limits the oxidation. The blends oils formed with sea buckthorn, camellia, rice bran, sesame or peanut oils with soybean oil (at a level of 20%) demonstrated an interesting improvement of oxidative stability compared to soybean oil as control [24]. The authors suggested that these added oils could contribute as important sources of natural antioxidants. These later were recognized to protect oils from oxidation [1, 36, 38].

According to the statistical analysis of binary blend oils results obtained in this study, it can be retained that lentisk oil, followed by sesame oil and then almond oil improved the physicochemical parameters and antioxidants properties of soybean oil after thermal treatment. These three oils were selected and combined following the Simplex Lattice Mixture Design in order to find the best oils proportion that maximizes the oxidative stability of soybean oil.

Simplex Lattice Mixture Design

The results of the physicochemical parameters, TPC and antioxidant activity of different oil combinations of the simplex lattice design before thermal treatment were represented in table 3. It can be retained from this table that the analyzed parameters from the different combinations of the three oils with soybean oil were changed proportionally with percentages of crude oils in whole bled oil formed.

The results of physicochemical parameters obtained experimentally and calculated through mixture design were given in table 4. All quality characteristics of thermally treaded oils were significantly reduced comparatively to no treated oils (Table 3) that appears evident in which heating process induces oil oxidation. It can be observed from the results of heated oil that the combination of lentisk and sesame oils with soybean oil improved considerably all analyzed parameters (runs 7 and 8), but the introduction of almond oil reduce significantly characteristic of quality. From data of table 4, it can be noticed also that experimental and calculated results of different parameters were close which is supported by coefficient of determination that varied from 0.909 to 0.980 (Table 5). The variance analysis of blend oils models was presented in table 5. Fisher probability values of models abstained for all studied parameters were less than 0.05, indicating that elaborated models were significant and presented a high power of experimental prediction.

Effect of factors

The effects of the addition of lentisk, almond, and sesame oils on the thermo-resistance of soybean oil and their interactions on the response after thermal treatment were shown in Table 6. The three factors significantly influenced all analyzed parameters ($p<0.05$). The interaction that can be considered between oils was that of lentisk oil and sesame oil, that expressed a negative influence for K232, K270, acidity, and peroxide value and positive for iodine value. This indicated that the simultaneous increase of lentisk and sesame oils in blended oil induces a reduction in the incidence of oxidation and degradation and an increase in the protection of double bonds.

The mathematical models of analyzed parameters obtained from the mixture design of blend oil can be presented as the first order polynomial. The mathematical models taking into account the linear and interaction effects of responses and by considering the terms with significant influences can be presented as following equations *Eq.* (7 to 12):

$$Y_{K232} = 35.30x_1 + 41.09x_2 + 36.33x_3 - 51.45x_1x_3 \quad \text{Eq.(7)}$$

$$Y_{K720} = 5.25x_1 + 7.23x_2 + 5.10x_3 - 6.45x_1x_3 \quad \text{Eq.(8)}$$

$$Y_{\text{Acidity}} = 2.49x_1 + 2.92x_2 + 3.62x_3 - 5.39x_1x_3 \quad \text{Eq.(9)}$$

$$Y_{\text{PV}} = 6.57x_1 + 4.53x_2 + 5.08x_3 - 4.02x_1x_3 - 4.69x_1x_3 \quad \text{Eq.(10)}$$

$$Y_{\text{IV}} = 41.77x_1 + 33.52x_2 + 45.15x_3 - 199.67x_1x_3 \quad \text{Eq.(11)}$$

$$Y_{\text{IT}} = 18.09x_1 + 14.21x_2 + 16.14x_3 \quad \text{Eq.(12)}$$

Where Y is the response; x_1, Lentiskoil;x_2, Almond oil; x_3, Sesame oil; PV, Peroxide value; IV, Iodine value; IT, Induction time.

Experimental determination and validation of optimal combination

The different responses on blended oil can be illustrated by isoresponse plot (Fig. 1). In order to predict the optimal desirability taking into account the combined parameters, the profiler of prediction of JMP was used. This made it possible to estimate an optimal combination of these oils at percentages of 59% lentisk oil and 41% sesame oil without the addition of almond oil. Hence, the thermo-oxidative stability of soybean oil can be enhanced by blending 70g of this oil with 17.7 and 12.3g of lentisk and sesame oils, respectively. The predicted values of various physicochemical parameters were indicated in table 7. To confirm the optimal theoretical values, experimental validation was carried out. For this, the optimal mixture for blend oils was tested. The experimental result of each parameter obtained was very close to theoretical values and Student-t test no revealed any difference between actual and calculated values of studied variables (physicochemical traits) indicating the validity of the established mathematical models.

Effect of heat treatment on fatty acids composition of soybean oil vs optimal blend oils

Gas chromatography analysis of FAME derivatives revealed the presence of fifteen fatty acids in both soybean oil and optimal blend oil (70% soybean oil: 17.7% lentisk oil: 12.3% sesame oil). The results showed that blending of soybean oil with lentisk and sesame oils changed slightly the fatty acids composition. However, the obtained blend oil presented a significantly ($p<0.05$) high fraction of oleic acid and less level of linoleic acids than soybean oil alone (Table 8). The presence of the polyunsaturated fatty acids (PUFA) fraction at a low level in blending oil than soybean oil may explain its thermal stability that supported by the results of rancimat test. However, the results in table 8 showed that another factor was involved in the thermal stability of blending oil. It was noted that the level of the degradation of linoleic acid (ω6) and linolenic acid (ω3) was more important in soybean oil than in blended oil; 31% linoleic acid was degraded after heat treatment of soybean oil, but only 6.5% was lost in blended oil. Likewise, the level of thermal degradation of linolenic acid was 94% in soybean oil and only 45% in blended oil. The ratio ω6/ω3 after heating treatment was very low in blend oils (18.87) compared to soybean oil (118.18). It was established that a high ω6/ω3 ratio consumption promotes the pathogenesis of many diseases, including cardiovascular disorder, cancer, inflammatory, and autoimmune diseases due to the excess of linoleic acid (ω6) [32]. The antioxidants provided by lentisk and sesame oils seem to protect both linoleic and linolenic acids from thermal degradation.

Conclusion

In this study, we demonstrated that blending soybean oil at the proportion of 70% with lentisk oil and sesame oil at proportions of 17.7% and 12.3%, respectively, enhanced considerably the physicochemical characteristics, antioxidant properties, the thermo-stability of obtained blend oil, and fatty acids profile especially linoleic acid (ω6) and linolenic acid (ω3) (essentials fatty acids) and their ratio. The addition of these two crude oils to refined soybean oil had two consequences. On the one hand the decrease in the fraction of polyunsaturated fatty acids which are very susceptible to oxidation and on the other hand the increase in the level of antioxidants.

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Declaration of competing interest

The authors have no conflict of interest

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Figure Legend

Fig. 1. Ternary contour plots representing the effects of blended oils (lentisk, almond, and sesame oils) on physico-chemical parameters (K_{232}, K $_{270}$, acidity, and peroxide and iodine values) after thermal treatment.

Table 1 Physicochemical parameters, total phenolic compounds, and RSA of analyzed crude oils

Crude oils	K_{232}	K_{270}	Acidity	Peroxide value	Iodine value	Induction time	TPC	RSA
Almond	4.39±0.18^{e}	3.59±0.18^{c}	1.36±0.04^{e}	6.00±0.06^{d}	92.48±0.92^{c}	13.12±0.66^{d}	56.67±1.7^{c}	34.57±1
Lentisk	5.93±0.12^{c}	5.66±0.06^{a}	6.93±0.14^{d}	4.4±0.18^{e}	75.79±3.79^{d}	26.83±0.54^{a}	63.17±2.53^{a}	46.29±2
Nigella	11.27±0.11^{b}	5.64±0.23^{a}	8.26±0.08^{b}	9.58±0.29^{c}	81.47±2.44^{d}	16.04±0.48^{b}	63.16±2.53^{a}	50.43±1
Peanut	13.03±0.39^{a}	5.78±0.23^{a}	7.42±0.15^{c}	15.56±0.31^{a}	95.2±0.95^{c}	9.00 ±0.18^{e}	52.84±0.53^{d}	15.43±0
Sesame	5.47±0.16^{d}	4.43±0.18^{b}	1.38±0.03^{e}	4.53±0.18^{e}	106.08±5.3^{b}	15.7±0.16^{b}	60.71±0.61^{ab}	37.43±1
Wild-olive	5.68±0.28^{cd}	4.45±0.13^{b}	10.29±0.31^{a}	11.17±0.34^{b}	96.36±4.82^{c}	8.87±0.18^{e}	59.83±1.2^{b}	26.71±0
Soybean	3.17±0.13^{f}	1.23±0.01^{d}	0.23±0.01^{f}	1.33±0.07^{f}	124±2.48^{a}	15.00 ±0.15^{c}	42.36±1.69^{e}	30.29±0

K_{232} and K_{270}, ultraviolet specific extinctions at 232 and 270 nm; Acidity, expressed as equivalent oleic acid percentage; Peroxide value, expressed as meq O_2/kg oil; Iodine value, expressed as mg KOH/g oil; Induction time (hour); TPC, expressed as mg GAE/100g oil; RSA, $ABTS^+$ radical scavenging activity (%); for each column, results with different letters are statistically different (ANOVA-LSD, $p<0.05$, a>b>c>d>e>f).

Table 2 Physicochemical parameters of analyzed binary blend oils (30% crude oil with 70% soybean oil) before and after thermal treatment

Blend oil	Parameters before treatment	Parameters before treatment	Parameters before treat
	K_{232}	K_{270}	Acidity
Almond	3.51± 0.11^{d}	1.95± 0.06^{c}	0.56± 0.01^{e}
Lentisk	3.98± 0.08^{c}	2.56± 0.1^{a}	2.26± 0.09^{d}
Nigella	5.44± 0.27^{b}	2.57± 0.1^{a}	2.69± 0.13^{b}
Peanut	6.09± 0.06^{a}	2.56± 0.10^{a}	2.41± 0.02^{c}
Sesame	3.86± 0.15^{c}	2.14± 0.09^{b}	0.56± 0.01^{e}
Wild-olive	3.89± 0.16^{c}	2.17± 0.09^{b}	3.1± 0.09^{a}
Control (Soybean oil)	3.17± 0.13^{e}	1.23± 0.06^{d}	0.23± 0.01^{f}

K_{232} and K_{270}, ultraviolet specific extinctions at 232 and 270 nm; Acidity, expressed as equivalent oleic acid percentage; Peroxide value (PV), expressed as mill-equivalents oxygen/kg oil; Iodine value (IV), expressed as mg KOH/g oil; Induction time (hour); TPC expressed as mg GAE/100g oil; RSA, $ABTS^+$ radical scavenging activity (%); for each column, results with different letters are statistically different (ANOVA-LSD, $p<0.05$, a>b>c>d>e>f).

Table 3 Physicochemical and antioxidant parameters of simplex lattice design of blend oils before thermal treatment

	Variables	Variables	Variables	Parameters before treatment	Parameters before treatment	Parameters before treatment	Parameters before treatment	Parameters before treatment	Parameters before treatment
Run	**Lentisk oil (x_1)**	**Almond oil (x_2)**	**Sesame oil (x_3)**	**K_{232}**	**K_{270}**	**Acidity**	**PV**	**IV**	**TPC**
1	1	0	0	4.07	2.47	2.25	2.23	106.70	60.45
2	0.67	0.33	0	3.94	2.34	1.78	2.47	113.99	55.07
3	0.33	0.67	0	3.70	2.24	1.16	2.54	110.22	49.58
4	0	1	0	3.52	2.03	0.53	2.86	115.36	45.00
5	0	0.33	0.67	3.66	2.16	0.56	2.45	112.86	47.64
6	0.33	0.33	0.33	3.71	2.32	1.13	2.40	114.58	51.65
7	0.33	0	0.67	3.91	2.42	1.17	2.34	109.93	52.42
8	0.67	0	0.33	4.11	2.40	1.72	2.24	113.90	55.94
9	0	0	1	3.68	2.23	0.54	2.41	119.60	49.51
10	0	0.67	0.33	3.51	2.19	0.53	2.59	116.39	45.57

x_1, lentisk oil; x_2, almond oil; x_3, sesame oil; $K_{232}$and K_{270}, ultraviolet specific extinctions at 232 and 270 nm; Acidity, expressed as percentage equivalent of linoleic acid; Peroxide value (PV), expressed as meq O_2/kg oil; Iodine value (IV), expressed as mg KOH/g oil; TPC, expressed as mg GAE/100g oil; RSA, $ABTS^+$ radical scavenging activity (%).

Table 4 Physicochemical parameters of simplex lattice design of oils after thermal treatment and corresponding predicted values

Run	**Variables**	**Variables**	**Variables**	**Experimental**	**Experimental**	**Experimental**	**Experimental**	**Experimental**	**Experimental**	**Predicted**	**Predicted**	**Predicted**	**Pred**
	Lentisk oil (x_1)	**Almond oil (x_2)**	**Sesame oil (x_3)**	**K_{232}**	**K_{270}**	**Acidity**	**PV**	**IV**	**IT**	**K_{232}**	**K_{270}**	**Acidity**	**PV**
1	1	0	0	34.94	5	2.37	4	43	17.94	35.30	5.25	2.49	4.03
2	0.67	0.33	0	36.12	7.08	2.96	4.50	39.6	17.14	36.02	6.74	2.89	4.46
3	0.33	0.67	0	38.62	7.28	3.11	5.1	43.8	14.92	37.95	7.40	3.03	5.24
4	0	1	0	39.62	7.06	2.81	6.5	32.3	14.28	41.09	7.23	2.92	6.37
5	0	0.33	0.67	33.7	4.72	3.26	5.05	50.8	15.03	36.65	5.14	3.37	4.89
6	0.33	0.33	0.33	29.06	4.78	2.22	5	62.8	14.76	30.62	5.22	2.53	4.81
7	0.33	0	0.67	24.36	3.54	1.92	4.50	91.8	16.4	24.55	3.71	2.05	4.63
8	0.67	0	0.33	25.18	4.16	1.95	4.5	86	16.47	24.21	3.76	1.67	4.47
9	0	0	1	37.38	5.3	3.70	4.51	43	15.95	36.33	5.10	3.62	4.52
10	0	0.67	0.33	41.96	6.5	3.40	5.25	43	14.35	38.23	5.85	3.14	5.51

x_1, lentisk oil; x_2, almond oil; x_3, sesame oil; $K_{232}$and K_{270}, ultraviolet specific extinctions at 232 and 270 nm; Acidity, expressed as percentage equivalent of linoleic acid; Peroxide value (PV), expressed as meq O_2/kg oil; Iodine value (IV), expressed as mg KOH/g oil, Induction time (IT) expressed in hours.

Table 5 Adjustment and variance analysis of models

Variable	**Source**	**DF**	**Coefficient of determination**	**Sum of squares**	***F* Ratio**
K_{232}	Model	5	$R^2 = 0.909$	298.396	8.004

	Residues	4	Adj. R^2 = 0.796	29.824	Prob. > F
	Total model	9		328.220	0.0329*
K_{270}	Model	5	R^2 = 0.910	3.196	9.587
	Residues	4	Adj. R^2 = 0.797	0.317	Prob. > F
	Total model	9		3.513	0.0324*
Acidity	Model	5	R^2 = 0.909	0.091	7.9439
	Residues	4	Adj. R^2 = 0.794	0.009	Prob. > F
	Total model	9		0.100	0.0333*
Peroxide value	Model	5	R^2 = 0.925	3.859	9.897
	Residues	4	Adj. R^2 = 0.832	0.312	Prob. > F
	Total model	9		4.171	0.0227*
Iodine value	Model	5	R^2 = 0.980	3608.781	38.235
	Residues	4	Adj. R^2 = 0.954	75.508	Prob. > F
	Total model	9		3684.289	0.0018*
Induction time	Model	5	R^2 = 0.924	12.946	9.730
	Residues	4	Adj. R^2 = 0.829	1.064	Prob. > F
	Total model	9		14.011	0.0234*

K_{232} and K_{270}, ultraviolet specific extinctions at 232 and 270 nm; Acidity, expressed as equivalent oleic acid percentage; Peroxide value (PV), expressed as mill-equivalents oxygen/kg oil; Iodine value (IV), expressed as mg KOH/g oil; Induction time (hour); DF, Degrees of freedom; * indicates a significant value.

Table 6 Coefficients of mathematical equations of models and their statistical significance

Term	**K232** Estimation	**K232** Prob.>|t|	**K270** Estimation	**K270** Prob.>|t|	**Acidity** Estimation	**Acidity** Prob.>|t|	**Peroxide value** Estimation	**P** P
Lentisk oil	35.30	0.0002*	5.25	0.0006*	2.49	0.0007*	6.57	<
Almond oil	41.09	<0.0001*	7.23	0.0002*	2.92	0.0004*	4.53	<
Sesame oil	36.33	0.0001*	5.10	0.0006*	3.62	0.0002*	5.08	<
Lentisk oil*Almond oil	-5.45	0.6568	3.73	0.1836	1.13	0.3891	-4.02	0
Lentisk oil*Sesame oil	-51.45	0.0106*	-6.45	0.0498*	-5.39	0.0101*	-4.69	0
Almond oil*Sesame oil	-5.71	0.6422	-3.01	0.2648	-0.09	0.9425	-0.55	0

K_{232} and K_{270}, ultraviolet specific extinctions at 232 and 270 nm; Acidity, expressed as equivalent oleic acid percentage; Peroxide value (PV), expressed as mill-equivalents oxygen/kg oil; Iodine value (IV), expressed as mg KOH/g oil; Induction time (hour); * indicates a significant value.

Table 7 Optimal values of predicted and experimental parameters

Parameters	**Optimal**	**Optimal**
	Predicted	**Experimental**
Lentisk oil (%)	17.7	/
Sesame oil (%)	12.3	/
Almond oil (%)	0	/

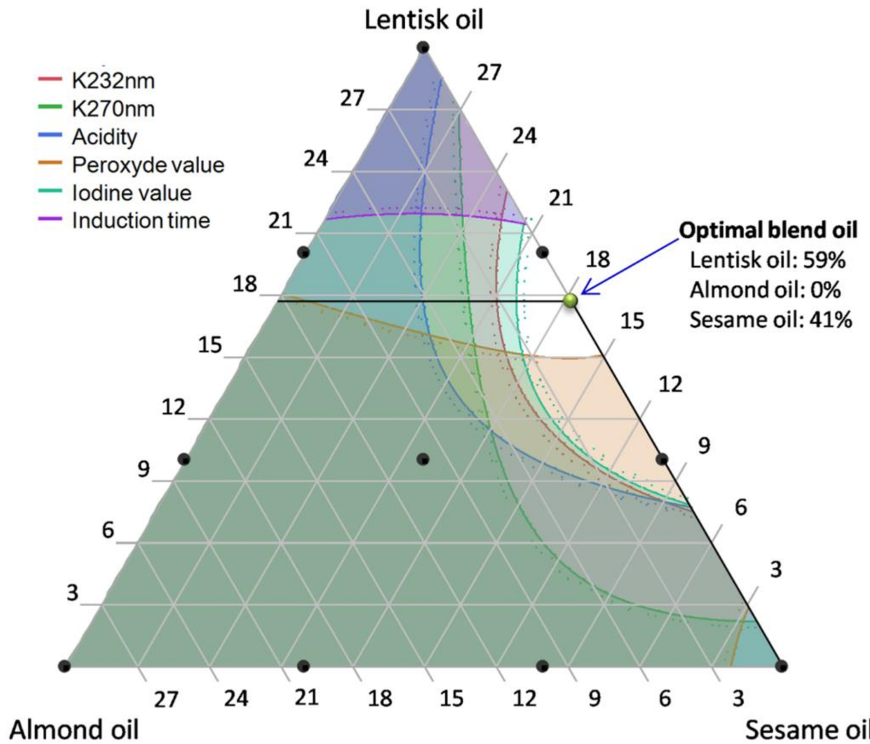
Parameters	**Optimal**	**Optimal**
K_{232}	23.24	25.12±2.45
K_{270}	3.52	4.22±0.38
Acidity	1.65	1.73±0.08
Peroxide value	4.53	4.48±0.13
Iodine value	91.64	86.64±3.95
Induction time	16.39	17.36±1.18

K_{232} and K_{270}, ultraviolet specific extinctions at 232 and 270 nm; Acidity, expressed as equivalent oleic acid percentage; Peroxide value (PV), expressed as mill-equivalents oxygen/kg oil; Iodine value (IV), expressed as mg KOH/g oil; Induction time (hour).

Table 8 Fatty acids composition (%)

Fatty acid	Chemical structure	Retention time (min)	Soybean oil (%)	Soybean oil after heating treatment (%)	Blended soybean oil (%)	Blended soybean oil after heating treatment (%)
Myristic acid	CI4:0	12.086	0.080±0.001^{b}	0.118±0.013^{a}	0.070±0.001^{c}	0.072±0.009^{c}
Palmitic acid	C16:0	14.175	10.770±0.038^{b}	13.008±1.581^{a}	12.619±1.220^{a}	13.074±1.120^{a}
Palmitoleic acid	C16:1	14.434	0.097±0.011^{d}	0.112±0.003^{c}	0.348±0.023^{a}	0.353±0.005^{a}
Margaric acid	C17:0	14.949	0.093±0.001^{b}	0.134±0.018^{a}	0.083±0.001^{c}	0.087±0.003^{c}
Margaroleic acid	C17:1	15.282	0.054±0.001^{b}	0.077±0.001^{a}	0.053±0.002^{b}	0.054±0.001^{b}
Stearic acid	C18:0	16.640	3.489±0.122^{b}	4.921±0.712^{a}	3.563±0.289^{b}	3.692±0.265^{b}
Oleic acid	C18:1*cis* ω9	17.061	24.198±0.656^{b}	29.089±0.895^{a}	29.927±0.156^{a}	30.526±0.235^{a}
Oleic acid	C18:1*cis* ω7	17.116	1.482±0.048^{b}	1.849±0.058^{a}	1.338±0.035^{c}	1.373±0.054^{c}
Linoleic acid	C18:2 ω6	17.851	51.531±0.581^{a}	35.603±0.848^{d}	45.971±0.247^{b}	43.205±0.563^{c}
Linolenic acid	C18:3 ω3	18.555	5.585±0.185^{a}	0.302±0.093^{d}	4.173±0.089^{b}	2.291±0.029^{c}
Arachidic acid	C20:0	19.184	0.380±0.010^{c}	0.735±0.104^{a}	0.391±0.012^{c}	0.511±0.096^{b}
Gadoleic acid	C20:1	19.617	0.234±0.004^{c}	0.529±0.001^{a}	0.220±0.006^{c}	0.269±0.001^{b}
Dihomolinoleic acid	C20:2	21.146	0.076±0.000^{a}	0.072±0.009^{b}	0.063±0.001^{c}	0.066±0.003^{c}
Behenic acid	C22:0	22.146	0.491±0.003^{b}	0.542±0.107^{a}	0.384±0.005^{d}	0.451±0.004^{c}
Lignoceric acid	C24:0	26.123	0.173±0.020^{c}	0.289±0.014^{a}	0.148±0.082^{d}	0.182±0.017^{b}
Total SFA			15.476	19.747	17.258	18.069
Total MUFA			26.065	31.656	31.886	32.575
Total PUFA			57.116	35.977	50.207	45.496

SFA, saturated fatty acids; MUFA; monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; for each row, results with different letters are statistically different (ANOVA-LSD, $p<0.05$, a>b>c>d)


Lentisk oil
Almond oil
Sesame oil
K232nm
K270nm
Acidity
Peroxyde value
Iodine value
Induction time
Optimal blend oil
Lentisk oil: 59%
Almond oil: 0%
Sesame oil: 41%
27
24
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