Influence of Industrial Processing on Physiochemical Characteristics of Soybean Oil and Deodorizer Distillates

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Summary: The present study aimed to evaluate the impact of industrial processing (neutralization/degumming, bleaching, and deodorization) on physicochemical characteristics of soybean oil and soybean oil deodorizer distillate (SBO-DD) collected from two different industries. The substantial impact of processing was observed on all physicochemical parameters except the iodine value (IV) and saponification value (SV). Gas chromatography-flame ionization detector (GC-FID) and Gas chromatography-mass spectrometry (GC-MS) analytical techniques were used for the quantification of individual fatty acids, sterols, and 3-monochloropropane diol (3-MCPD) ester. Among the fatty acids, palmitic and linoleic acids were present at higher concentrations in all processing stages. Among sterols, β-sitosterol was found to be higher (25.65 µg/g) in crude soybean oil and reduced to 16.44 µg/g after processing till the final deodorization stage. 3-MCPD ester was developed during the neutralization/degumming process and further increased during bleaching and deodorization up to 315 µg/kg, respectively. SBO-DD was found to be a rich source of total and individual sterols as compared to crude or processed soybean oil. High free fatty acid (FFA) level in deodorizer distillate indicated that SBO-DD is a potential source of biodiesel production.

Keywords: Soybean oil; Processing; Physicochemical characteristics; Fatty acid profile; Sterols.

Introduction

Globally, soybean oil is the second most-edible oil after palm oil [1,2]. Protein and oil are the two important seed constituents that make soybean an important crop. It contains 40-44 % high-quality protein and 18-22 % oil. Soybean oil also contains appreciable amounts of health-beneficial fatty acids, i.e. α- linolenic acid (omega-3 acid), linoleic acid (omega-6 acid), and oleic acid (omega-9 acid) [3-5]. Quality and stability are the key features that decide the acceptance and value of vegetable oils in the market. There are some unwanted minor components such as phosphatides, free fatty acids (FFA), trace metals, color pigments, and odoriferous compounds that affect the stability of vegetable oil [6]. Oxidation reactions are involved in changes in the physical appearance and impact the stability of vegetable oils. The oxidation rate depends on the degree of unsaturation in oil and fats, and oxidation increased with increased double bonds in the fatty acids chain. During the primary oxidation, some low molecular weight oxygenated products are formed such as aldehydes, alcohols, FFA, and ketones; these compounds increase the rancidity of vegetable oils. Most of these undesirable components can be removed during refining steps [7]. Nowadays, food safety is the top priority of consumers, there are

many lethal substances found in edible oils such as polycyclic aromatic hydrocarbons (PAHs), *trans* fatty acids (TFAs) and 3-MCPD ester, which have been considered hazardous substances and have a great potential to contaminate the refined oils. Crude soybean oils are not utilized directly for cooking purposes, therefore refining of crude oil is necessary to remove undesirable components that cause the instability of the oil in foodstuffs [8]. There are two procedures widely used for refining crude soybean oil on an industrial scale, i.e., physical refining and chemical refining. The choice of refining depends on the level of FFAs in crude oil [9-12]. In chemical refining, four steps are involved, i.e. (degumming, neutralization, bleaching, and deodorization). Degumming is the first step of the refining, in this step phospholipids can be removed from the soybean oil. There are two types of phospholipids, i.e., hydratable and nonhydratable phospholipids. Hydratable phospholipids are removed by using hot water, the process is called water degumming, and nonhydratable phospholipids are removed by using the acid (usually phosphoric acid) this process is called acid degumming [13,14]. Neutralization is a second important process in which sodium hydroxide (caustic soda) is added to the crude oil to reduce

FFAs in soybean oil by the formation of sodium soap which is removed from the neutralized oil. Generally, degumming and neutralization steps are carried out together through a continuous process. Bleaching is usually the third step of refining, and 1-2% bleaching clay is used to remove the color pigments from the edible oil. However, more or less bleaching clay could be used depending on the intensity of the strength of the color of oils to be bleached [15]. The deodorization is the most important and final step of the refining process, in this step, mostly volatile compounds and FFAs, aldehydes, and ketones are removed [16].

Soybean oil deodorizer distillate (SBO-DD) collected as a by-product during the deodorization steps of edible oil industries. The deodorizer distillate of soybean oil contains valuable bioactive components such as fatty acids, tocopherols, and phytosterols. Phytosterols such as campesterol, stigmasterol, and β-sitosterol naturally present in the soybean oil. Phytosterols play a vital role in several areas, including health, pharmacy, cosmetics, synthesis of therapeutic steroids, and functional food [17]. β-sitosterol exhibits the most important anticancer properties and functions. It is also used as a medicine for lowering the cholesterol effect in preventing internal absorption [18].

Other reported studies are either related to the processing stages of the oil or deodorizer distillate. In contrast, the present study describes the impact of processing on soybean oil that how much health-beneficial components are decreased and the components which harm the health of consumers, such as 3-MCPD esters and quality of deodorizer distillate obtained during deodorization of the same oil. Therefore, the present study aimed to investigate the impact of each processing stage (neutralization/degumming, bleaching, and deodorization) on the physicochemical characteristics, fatty acid profile, sterol composition, and 3-MCPD ester.

Experimental

Reagent and sample collection

All chemicals and solvents used in the present study were obtained from E. Merck (Darmstadt, Germany), and fatty acid methyl ester (FAME) standards (GLC 481-B) were purchased from Nu Check-Prep, Inc. (Elysian, MN). 3-MCPD ester was purchased from (Pointe-Claire, Canada). The two sets of soybean oil, including crude oil,

neutralized/degummed oil, bleached oil, deodorized oil, and SBO-DD were collected from two different industries in Karachi, Pakistan. However, the origin of both sets of soybean oil was the same as these were extracted from the soybean seeds imported from America. The samples were kept at 4 °C until further analysis.

Physicochemical parameters

The physicochemical parameters such as moisture, phosphorus, color, FFA, PV, SV, IV, fatty acid profile, and sterol composition of both sets (I & II) of soybean oil sample and deodorizer distillates were determined by official AOCS methods.

Moisture content

The moisture content in soybean oil samples and deodorizer distillates sets was determined using the AOCS official method Ca 2c-25 [19]. About 5 g of soybean oil was put into the oven (Memmert, Schwabach, Germany) at 105 °C for 3 h. Moisture content was calculated by the difference in the mass of the sample before and after drying at oven temperature.

Phosphorus content

Phosphorous content was determined by ashing the sample in the presence of zinc oxide, followed by the spectrophotometric measurement of phosphorus as a blue phosphomolybdic acid complex according to the AOCS method Ca 12- 55 [19].

Color measurement

The color measurement of both sets of soybean oil and their deodorizer distillates was performed using the Tintometer color scale according to AOCS method Cc 13b- 45 [19].

Free fatty acid content

FFAs content in both sets of soybean oil and their deodorizer distillates were determined using the official AOCS method Aa 6-38 [19]. The oil sample was titrated against the standardized aqueous solution of sodium hydroxide in the presence of a phenolphthalein indicator.

Peroxide value

AOCS official method Cd 8-53 [19] was followed to determine PV of oil in which 2 g of the

sample was dissolved in a 15 mL mixture of chloroform and glacial acetic acid (3:2 V/V). The solution was titrated against a standardized solution of sodium thiosulphate (0.01N) using starch (1%) as an indicator.

Saponification value

The SV of soybean oil samples and deodorizer distillates was determined according to the AOCS method Cd 3-25 [19]. About 2 g each sample was put into 25 mL of 95 % of ethanolic potassium hydroxide. The mixture was refluxed for 60 min. The reflexed sample was placed in a 250 mL conical flask and titrated with a 0.5 N solution of hydrochloric acid using phenolphthalein as an indicator.

Iodine value

The IV of soybean oil samples and deodorizer distillates was determined according to the AOCS method Cd 1-25 [19]. The oil sample was dissolved in 15 mL carbon tetrachloride with (25 mL) Wij's reagent and (5%) potassium iodide solution. The solution was put in the dark for 30 min for the liberation iodine and titrated with 0.1N standard solution of sodium thiosulfate solution using starch as an indicator.

Determination of fatty acid composition by GC-FID

For the determination of fatty acids composition of soybean oil sets, fatty acids were chemically converted into respective volatile fatty acid methyl esters (FAMEs) according to IUPAC method 2.30 [20]. Around 1 g of oil sample was dissolved in 1 mL of 2N methanolic KOH for 10 min at room temperature. After the incubation, 7 mL of n-hexane was added to the reaction mixture, the mixture further homogenized, and then centrifuged at 4000 rpm. Volatile FAMEs were injected into GC, the analysis parameters and conditions were as described by Topkafa *et al.*, [21]. Agilent 7890 series gas chromatography was fitted with flame ionization detector (GC-FID) and HP-88 column (100 m, 0.25 mm, 0.25 mm, Agilent Technologies). About 1 mL FAMEs were injected in a split mode at a split ratio of 100:1. Hydrogen was used as a carrier gas, and the flow rate was set at 1.3 mL/min. The initial temperature programing of oven was set 50 °C for 10 min, and then increased 4 °C /min to final temperature 240 °C and final stay time was set 10 min. For the conformation of the FAMEs peaks was compared with the retention time of the authentic

standard samples. The peak area under each fatty acid compares with the peak relative to the total area of all fatty acids.

Determination of sterols

For the determination of sterols concentration, the standard solutions were prepared within chloroform at a 10 mg/mL concertation for plant sterols kept at -20 °C. Cholestenol was used as an internal standard (IS). The solution was vigorously shaken for two min and centrifuged for 5 min. After centrifugation, the solvent was evaporated with the stream of nitrogen gas. A mixture at a concentration of 10 mg/mL of these standards solutions with IS was silylated and injected into the GC following the chromatographic conditions described in the section on the fatty acid composition.

Determination of 3-MCPD esters

The 3-MCPD ester of soybean oil set I and II were analysed according to the reported study of Xie *et al.*, [22]. The transesterification reaction was carried out in terms of the release of the 3-MCPD from its esters. The extraction of 3-MCPD was carried out with ammonium sulphate and phenylboronic acid (PBA) for derivatization. For deuterated purpose (3-MCPD-d$_5$) was used as an IS for the quantification of 3-MCPD. Determination of 3-MCPD concentration, the extracted sample was injected into GC-MS. The quantification is based on the peak area ratio at (*m/z* 147) of 3-MCPD, related to IS at (*m/z* 150).

Statistical analysis

The identification and quantification of fatty acids and sterols in both soybean oil sets were collected from two different oil industries. The FAMEs result was performed by comparing the retention time of known FAMEs standards, and other physicochemical parameters were expressed as the mean ± standard deviation (x ± SD), x= is the mean of three replications.

Results and Discussion

Two sets of soybean oil were collected from two different edible oil processing industries. Fig.1 shows the representative samples containing the crude oil, neutralized/degummed oil, bleached oil, deodorized oil, and deodorizer distillate. From the visual appearance, the impact of the processing could be easily observed.

Table-1. Physicochemical characteristics of soybean oil and SBO-DD set-I and set-II

Parameters	Set	Crude	Neutralized/ degummed	Bleached	Deodorized	Deodorizer Distillate
Moisture Content (%)	I	0.42 ±0.01b	0.12±0.02c	0.09±0.01d	0.02±0.00e	0.81±0.02a
	II	0.45±0.02b	0.15±0.01c	0.08±0.02d	0.04±0.01e	0.78±0.21a
Phosphorus (ppm)	I	797±2.11a	223±1.32b	5.98±0.21c	0.51±0.01d	0.87±0.12d
	II	799±3.01a	227±2.01b	5.85±0.31c	0.53±0.02d	0.84±0.03d
Colour (Red, Yellow and Blue units)	I	R 3.2, Y 32	R 2.4, Y 24	R 0.6, Y 6.0	R 0.2, Y 2.0	R 17.6, Y 76, B 3.1
	II	R 3.4, Y 34	R 2.2, Y 22	R 0.5, Y 5.0	R 0.1, Y 1.0	R 17.8, Y 78, B 3.3
FFA (%, oleic acid)	I	1.12±0.01b	0.40±0.02c	0.37±0.01c	0.20±0.03d	43.16±1.21a
	II	1.14±0.02b	0.43±0.01c	0.38±0.02c	0.21±0.00d	43.21±2.31a
PV (meqO$_2$/kg)	I	1.54±0.01b	1.42±0.03b	1.33±0.01b	1.11±0.21bc	6.80±1.01a
	II	1.57±0.03b	1.39±0.01c	1.23±0.01c	1.08±0.04cd	6.78±0.31a
SV (mg KOH/g)	I	141±2.45bc	142±1.34b	137±1.21c	134±0.34d	152±2.34a
	II	143±3.10bc	144±2.54b	138±1.34c	136±1.12d	153±3.21a
IV (gL/100g)	I	133±1.34a	131±2.12b	130±1.23c	129±1.21d	109±1.23e
	II	134±2.13a	132±1.54b	131±2.13c	130±1.24d	110±2.41e

Values of physicochemical characteristics are average of triplicate analysis with the standard deviation (± SD) and small letters (a-e) are significantly different from each other ($p < 0.05$)

Fig.1:   Representative samples of crude, neutralized/degummed, bleached, deodorized and SBO-DD of set I & II.

The results of physicochemical parameters such as moisture content, phosphorus content, color, FFA, PV, SV, and IV are presented in Table-1.

Moisture

The moisture content plays a key role in the oxidative stability and shelf life of edible oils. It is a common observation that the storage life of the oil is affected by the level of moisture content. The moisture content of crude soybean oil in the set I and II were found to be 0.42 % and 0.45 %, respectively. In the neutralization/degumming stage, the moisture content of crude oil was decreased from 0.42 % to 0.12 % in the set I and 0.45 % to 0.15 % in set II. In the bleaching processing step, the level of moisture content further decreased as compared to the neutralization/degumming step, the moisture content in bleaching oil was found to be 0.09 % and 0.08 %, respectively. Deodorization is a final, and key step of

the processing carried out at a high temperature, that temperature cause the further reduction in moisture content as compared to the previous steps of refining (neutralization//degumming and bleaching), the moisture content in deodorized oil in the set I and set II was found to be 0.02 % and 0.04 %, respectively.

The SBO-DD-I and SBO-DD-II were collected from two different edible oil industries and investigated their physicochemical parameters before analysing fatty acid and sterols composition. The moisture content in SBO-DD-I and SBO-DD-II was found to be 0.81 % and 0.78 %, respectively. In the present study, the moisture content in SBO-DD set I and II was almost similar to the study reported by Benites *et al.*, [23].

Phosphorus content

The phosphorus content of crude soybean oil in the set I and II was 797 ppm and 799 ppm. Phosphorus contents were reduced in neutralized/degummed oil as compared to the crude oil. Phosphorus contents in neutralized/degummed oil sets I and II were found to be 223 ppm and 227 ppm, respectively. In the bleaching step, levels of phosphorus contents were further decreased in the set I and II and found to be 5.98 ppm and 5.85 ppm, respectively. In deodorized oil, the phosphorus content further decreased in the set I, and set II was found to be 0.51 ppm and 0.53 ppm, respectively. Phosphorus content in SBO-DD-I and SBO-DD-II was found to be 0.87 ppm and 0.84 ppm, respectively.

Color

The color of vegetable oils is an important parameter for determining their market value and quality index. The color of the oil is due to the presence of chlorophyll, carotenoids, and some other pigments naturally present in plant seeds. The basic colors of crude and processed soybean oil were determined using a Tintometer for differencing the colors such as red (R) and yellow (Y) units. The color of crude soybean oil in the set I and II were found to be 3.2 R, 32Y, and 3.4 R, 34Y, respectively. The color of crude oil is reduced at the neutralization/degumming stage from 3.2 R to 2.4 R, 32Y to 24Y, and 3.4 R to 2.2 R, 34Y to 22Y in the set I, and II, respectively. In the bleaching stage, red and yellow color units' intensity was further decreased because in this step, bleaching clay act as an adsorbent. In the bleaching stage, the color of soybean oil was found to 0.6 R, 6.0 Y in set I, and 0.5 R, 5.0Y in set II. In the deodorization stage, the color was further reduced and was found to be 0.2 R, 2.0 Y, and 0.1 R, 1.0 Y, respectively.

The color in both sets of SBO-DD was also studied. The results of the Lovibond Tintometer indicated the concentration of red, yellow, and blue color units was found to be 17.6 R, 76 Y, 3.1 B, and 17.8 R, 78 Y, 3.3B, respectively. Color units of soybean oil deodorizer distillate were not reported in previous studies [23] and [24].

Free fatty acid

For the good quality of edible oils, the FFA level should not exceed more than 0.1% [25]. During the long storage, the higher FFA may cause a bad odor and change in the taste and color of the oil [26]. In the present study, FFA of crude soybean oil set I and II was found to be 1.12 % and 1.14 %, respectively. In the neutralization/degumming, stage FFA level reduced as compared to the crude oil, and FFA was found to be in neutralized/degummed oil in both sets at 0.40 % and 0.43 %. After the neutralization/degumming stage, the FFA level was gradually decreased during bleaching and deodorization steps in both sets of soybean oil and was found to be 0.37 and 0.38 %; 0.20 and 0.21 %, respectively.

SBO-DD is the waste product of edible oil industries; it is collected during the deodorization step. A higher temperature is maintained to remove the volatile and unwanted components from the edible oils in this stage. In SBO-DD higher FFA level was found in both sets 43.16 and 43.21%. In the present study, FFA value in the set I and II was smaller as compared to the earlier reported by Benites *et al.*, (>53.15 %) [23], while a little higher was reported by Khatoon *et al.*, [24].

Peroxide value

PV is an important parameter of the quality and stability of oil and fats [27]. Crude oil is easily oxidized due to the presence of peroxides and hydroperoxides concentration. In the present study, PV of crude soybean oil in the set I and II were found to be 1.54 and 1.57 $meqO_2/kg$, respectively. In the neutralization/degumming stage, PV steadily reduced and was found to be 1.42 and 1.39 $meqO_2/kg$. In the bleaching stage, the PV was further decreased in both sets of soybean oil and found 1.33 and 1.23 $meqO_2/kg$, respectively. Deodorization is carried out at higher temperatures to remove the volatile and odoriferous compounds from the vegetable oils. In the deodorization stage, the PV was found to be 1.11 and 1.08 $meqO_2/kg$, respectively.

The PV in both sets of SBO-DD was found to be 6.80 and 6.78 $meqO_2/kg$, respectively. The PV of the current study was found smaller than the reported studies by Benites *et al.*, [23] and Khatoon *et al.*, [24].

Saponification value

SV in vegetable oils shows the average molecular mass of fatty acids or chain length. SV of crude oil in the set I and II were found to be 141 and 143 mg KOH/g, respectively. In the neutralization/degumming stage, SV was slightly increased may be due to alkali treatment (soap stock formation) and found to be 142 and 144 mg KOH/g, respectively. In bleaching and deodorization stages of

soybean oil set I and II, SV was decreased from 137 and 138 mg KOH/g to 134, 136 mg KOH/g, respectively.

The SV in SBO-DD of both sets was found to be 152 and 153 mg KOH/g. The SV in the current study was smaller than the reported studies by Benites *et al.*, [23]and Khatoon *et al.*, [24] 159 mg KOH/g and 166 mg KOH/g, respectively.

Iodine value

IV shows the degree of unsaturation in oil and fats. More C=C bonds in oil and fats indicate a higher IV. In the present study, slightly higher IV was found in soybean crude oil 133 and 134 g I_2/100 g. No substantial change in IV level was observed after neutralization/degumming, bleaching, and deodorization steps. The IV in the set I and II of soybean oil were found to be in these steps at 131- 132 g I_2/100 g, 130-131 g I_2/100 g, and 129 - 130 g I_2/100 g, respectively.

The IV of SBO-DD in the set I and II were found to be109 and 110 gI_2/100g. The IV in the present study was found to be relatively similar to the reported study of Benites *et al.*, [23].

Fatty acid profile

In the present study, two sets of soybean oil (crude oil, neutralized/degummed oil, bleached oil, deodorized oil, and deodorizer distillate) were examined to check the processing impact on the level of the fatty acids composition. Table-2 shows the fatty acid composition of processed soybean oil sets I and II. Fig. 2 shows the separation results of saturated and unsaturated fatty acids of soybean oil. There is no significant impact of processing observed on the level of fatty acid composition. Among the saturated fatty acids,

palmitic acid (C16:0) was present in higher concentrations in both sets including crude oil, neutralized/degummed oil, bleached oil, and deodorized oil 11.31-11.29 %, 11.0 -11.11 %, 10.97-10.96 %, and 10.77-10.81 %, respectively. Margaric acid (C17:0) and stearic acid (C18:0) were determined in low concentration from crude to deodorized oil. The relative percentage of (C17:0) was found in both sets at 0.06-0.07%, 0.05-0.04 %, 0.03-0.02 % and 0.03- 0.01 %, respectively. Stearic acid (C18:0) was found at 4.08-4.04 %, 4.06-4.05 %, 4.05-4.04 % and 4.04-4.03 %, respectively.

Unsaturated fatty acid (UFA) has significant importance for the biological and nutritional value of our daily diet. In the present study, C16:1, C18:1, C18:2 cis, C18:2-*trans,* and C18:3n3 were determined in both sets of soybean oil. Among the monounsaturated fatty acid, C18:1was found a higher concentration in crude oil to deodorized oil in the set I and II at 25.39-25.41%, 25.25-25.23%, 25.15-25.17%, and 25.12-25.11%, respectively.

The polyunsaturated fatty acids (PUFA), including C18:2 cis, C18:2-*trans,* and C18:3n3 was found in both sets of soybean oil. The higher percentage of C18:2 cis was found in the set I and II, their relative percentage was calculated at 50.22-50.23%, 50.16-50.13 %, 50.12-50.10 %, and 49.89-49.87 %, respectively. C18:2-*trans* was not detected in crude oil, but low concentration was observed in processed samples such as neutralized/degummed oil, bleached oil, and deodorized oil at 0.17-0.18 %, 0.98-0.99 %, and 1.88-1.91 %, respectively. The α-linolenic acid (C18:3n3) was found to be 6.80-6.81 %, 6.77-6.87 %, 6.61-6.63 %, and 5.52-5.98 %, respectively.
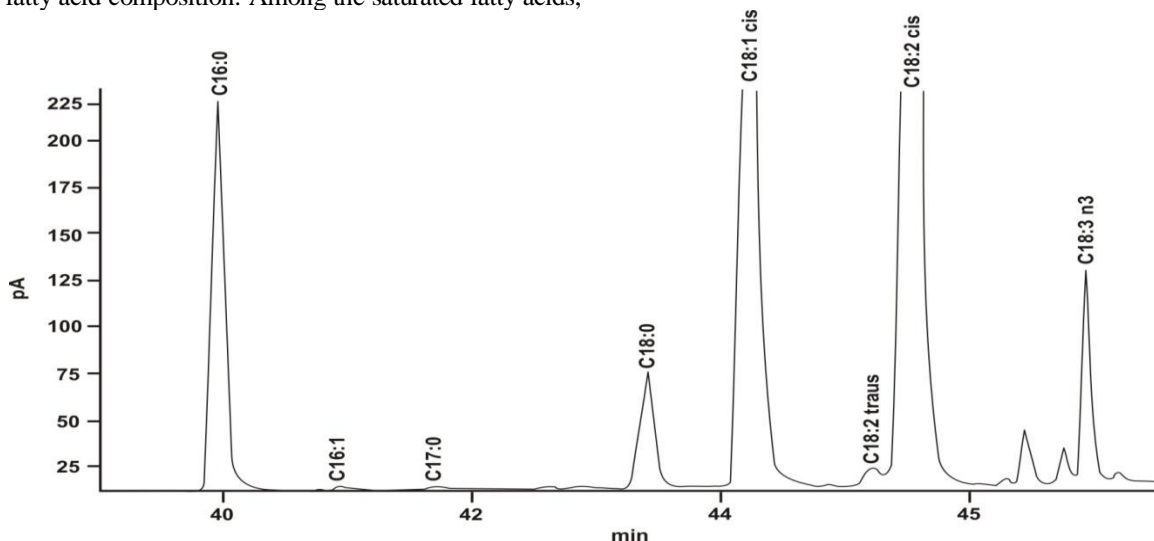

Fig. 2: GC-FID representative chromatogram of the fatty acid composition of soybean oil.

Table 2. Fatty composition of soybean oil and SBO-DD set-I and set-II

Fatty Acid (%)	Set	Crude	Neutralized/ Degummed	Bleached	Deodorized	Deodorizer Distillate
C16:0	I	11.31±1.21b	11.10±0.32b	10.97±1.11c	10.77±1.34c	22.11 ± 0.01a
	II	11.29±2.01b	11.11±1.21b	10.96±0.21c	10.81±0.51c	23.13 ± 0.02a
C17:0	I	0.06±0.01b	0.05±0.01b	0.04±0.002b	0.03±0.00c	0.34 ± 0.02a
	II	0.07±0.01b	0.04±0.00b	0.02±0.001bc	0.01±0.00c	0.33 ± 0.01a
C18:0	I	4.08±0.02a	4.06±0.21ab	4.05±0.11ab	4.04±0.13ab	2.21 ± 0.03b
	II	4.04±0.03ab	4.05±0.32a	4.04±0.21ab	4.03±0.42ab	2.32 ± 0.02ab
C16:1	I	2.14±0.02a	2.10±0.32ab	2.07±0.21ab	2.05±0.36b	nd
	II	2.15±0.34a	2.12±0.11ab	2.09±0.01ab	2.07±0.11ab	nd
C18:1 cis	I	25.39±1.23a	25.25±2.01ab	25.15±1.31ab	25.12±0.23ab	24.68 ± 0.03b
	II	25.41±2.11a	25.23±1.21ab	25.17±1.02ab	25.11±1.11ab	24.56 ± 0.02b
C18:2 cis	I	50.22±2.12a	50.16±3.40ab	50.12±2.01ab	49.89±2.13b	43.27 ± 1.04c
	II	50.23±3.11a	50.13±2.33ab	50.10±1.23ab	49.87±2.01b	43.25 ± 3.01c
C18:2 *trans*	I	Nd	0.17±0.01c	0.98±0.03b	1.88±0.06a	nd
	II	Nd	0.18±0.01c	0.99±0.02b	1.91±0.01a	nd
C18:3n3	I	6.80±0.04b	6.77±0.21b	6.61±0.43bc	5.52±0.01c	7.39 ± 0.02a
	II	6.81±0.01a	6.78±0.32ab	6.63±0.11ab	5.98±0.32c	6.41 ± 0.02b
Σ (SFA)	I	15.45±1.00b	15.21±0.23b	15.06±1.23b	14.84±1.23c	24.46±1.21a
	II	15.40±2.01b	15.20±1.01b	15.02±0.23b	14.85±0.45c	25.78±1.21a
Σ (UFA)	I	84.55±1.21ab	84.45±2.10b	84.93±1.13b	85.16±2.11a	75.34±2.13c
	II	84.60±2.11ab	84.44±1.11ab	84.98±3.14ab	85.15±1.13a	74.22±3.01c

Values of the fatty acids are average of triplicate analysis with the standard deviation (± SD) and small letters (a-d) are significantly different from each other (p < 0.05)
nd=not detected

The fatty acid composition of SBO-DD in the set I and II were also determined. Table-2 shows the fatty acid profile of SBO-DD of both sets contains the saturated and unsaturated fatty acids such as palmitic acid (C16:0), margaric acid (C17:0) and stearic acid (C18:0), palmitoleic acid (C16:1), oleic acid (C18:1-cis), linoleic acid (C18:2-cis, C18:2-*trans*) and α-linoleic acid (C18:3n3).

Among these, palmitic acid (C16:0) and linoleic acid (C18:2-cis) was found in higher concentrations in both sets of DD. In the set I and II, a higher concentration of palmitic acid, (C16:0) was found to be 22.11 and 23.13 %. While other minor saturated fatty acids, including margaric acid (C17:0) and stearic acid (C18:0) was found to be 0.34-0.33 % and 2.21-2.32 %, respectively. Among the monounsaturated fatty acids, a major fatty acid in the set I and II of soybean oil DD was found to be oleic acid (C18:1, cis) at the level of 24.68 and 24.56 %, respectively.

Among the polyunsaturated fatty acids (PUFAs), linoleic (C18:2 cis) and linolenic (C18:3n3) acids were found in both sets of soybean oil DD. The linoleic acid (C18:2 cis) was found in higher concentrations at 43.27 and 43.25 % in the set I and II, respectively. While remaining other minor PUFAs, including C18:3n3 were found to be 7.39 and 6.41%, respectively.

The average value of C18:1in both sets of SBO- DD found in the present study is higher than the reported study by Khatoon *et al.*, [24]. The level of C18:2 cis was found to be comparable to the reported

study by Khatoon *et al.*, [24], while lower than the reported study by Nandi [28].

Sterol composition

Table-3 represents the sterols composition of crude and processed soybean oil and its DD, while Fig. 3 is the representative GC-FID chromatogram of sterols. Among the sterols, β-sitosterol was found in higher concentrations in soybean crude oil (25.60 and 25.65 µg/g). During neutralization/degumming, bleaching, and deodorization processing steps, the level of β-sitosterol of sets I and II was reduced to 23.51-23.45 µg/g, 17.71-17.74 µg/g and 16.51-16.44µg/g, respectively. Stigmasterol was found to be10.41 to 7.59 µg/g during the initial processing step (neutralization/degumming) to final processing (deodorization). While remaining sterols, including avenasterol, campesterol, and cholestenol were found as 11.10 to 7.31µg/g, 2.80 to 1.13 µg/g, and 2.40 to 1.0 µg/g, respectively. All processing stages, including neutralization/degumming, bleaching, and deodorization were found to be responsible for the reduction of individual and total sterols. The average values of total sterols in crude, neutralized/degummed, bleached, and deodorized soybean oil of both sets were found to be 52.36, 37.68, 37.13, and 33.54 µg/g, respectively. Therefore, a decreasing trend in neutralization/degumming, bleaching, and deodorization processing steps was observed as 8.9, 60.83, and 9.52µg/g, respectively, on the input and output basis.

The unsaponifiable matter of SBO-DD is a rich source of phytosterols. Phytosterols contain many health benefits such as lowering blood cholesterol as well as anti-inflammatory, antibacterial, anti-ulcerative, and antitumor activities [29]. The sterol composition of

SBO-DD of set I and II are shown in Table-3. The β-sitosterol was the most abundant phytosterols in both sets of SBO-DD followed by campesterol, stigmasterol, avenasterol, and cholestenol. The deodorization process was found to be responsible for the maximum reduction of individual and total sterols. The average values of cholestenol, campesterol, stigmasterol, β -sitosterol, and avenasterol in both sets of SBO-DD were found to be 12544.5, 65162.5, 56021.5, 85235, and 36987.5 μg/g, respectively. Whereas the average amount of total sterols in both sets was found to be 255951 μg/g. In the present study, the average value of β-sitosterol was similar to the study reported by Sherazi *et al.*, [8], while higher than the reported study by Kasim *et al.*, [30]. If the origin of soybean, the method used for the analysis, and processing parameters are the same then almost all results are similar. In the present study, the average value of campesterol and stigmasterol is a little bit higher as compared to the study reported by Sherazi *et al.*, [8] and Kasim *et al.*, [30]. The results concluded that soybean oil is a rich source of health-beneficial sterols that could be used as a useful ingredient in pharmaceutical and cosmetic industries for the formulation of many daily used products.

Determination of 3-MCPD

The concentration of 3-MCPD ester in both sets of soybean oil was determined and shown in Table-3. In crude soybean oil, 3-MCPD was not detected in both sets of soybean oil, as shown in Fig 4. During the processing of soybean oil, 3-MCPD ester was developed in both sets. In the neutralized/degumming stage the concentration of 3-MCPD ester was found to be 20-18 μg/kg. In bleaching and deodorization stages, the concentration of 3-MCPD ester was further increased and found to be 122 and 315 μg/kg, respectively may due to the impact of acid-activated clay in bleaching and high temperature (>250 °C) in deodorization [31, 32]. The results of the present study for 3-MCPD were found to be lower as compared to the study reported by Xie *et al.*, [22], which may be due to the different processing conditions.
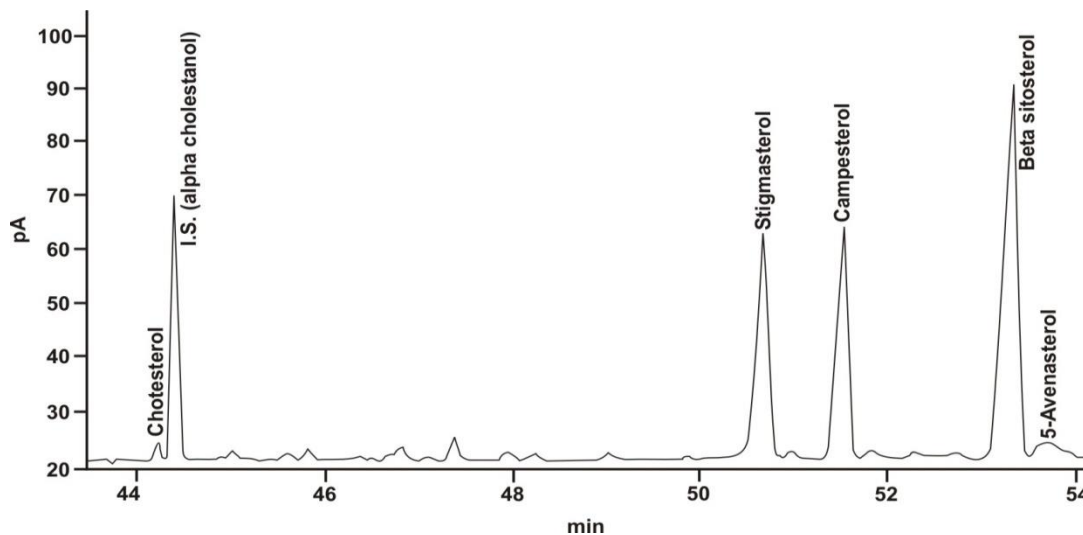

Fig. 3:   GC-FID representative chromatogram of sterol analysis of soybean oil.

Table-3: Sterols and 3-MCPD ester of soybean oil and SBO-DD set-I and set-II.

Sterols (μg/g)	Set	Crude	Neutralized/ degummed	Bleached	Deodorized	Deodorizer Distillate
Cholestenol	I	2.40±0.01b	2.37±0.02b	1.10±0.01c	1.01±0.001c	12543±4.45a
	II	2.41±0.11b	2.39±0.01b	1.13±0.02c	1.11±0.12c	12546±3.67a
Campesterol	I	2.80±0.31b	2.76±0.31b	1.56±0.11c	1.13±0.23c	65161±5.02a
	II	2.78±0.01b	2.78±0.22b	1.55±0.23c	1.12±0.01c	65164±4.21a
Stigmasterol	I	10.41±0.11b	9.31±0.02b	8.30±0.01b	7.59±0.23c	56021±5.21a
	II	10.43±1.21b	9.34±0.01b	8.33±0.21b	7.55±0.22c	56022±5.22a
β –Sitosterol	I	25.60±2.11b	23.51±1.21b	17.71±1.01c	16.51±2.11c	85234±7.23a
	II	25.65±1.12b	23.45±1.23b	17.74±2.01c	16.44±0.41c	85236±6.21a
Avenasterol	I	11.10±0.12b	9.70±0.14bc	8.40±0.54c	7.31±0.21cd	36987±2.86a
	II	11.14±0.34b	9.76±0.34bc	8.45±0.56c	7.33±0.21cd	36988±5.21a
Total	I	52.31±2.21b	47.65±1.20b	37.07±0.34bc	33.54±1.23bc	255946±4.23a
	II	52.41±3.11b	47.72±2.34bc	37.2±1.11c	33.55±0.47c	255956±5.34a
3-MCPD ester (μg/kg)	I	Nd	20±0.23c	122±3.21b	315±2.45a	Nd
	II	Nd	18±0.13c	119±2.10b	311±3.01c	Nd

Values of sterols are average of triplicate analysis with the standard deviation ($\pm$ SD) and small letters (a-d) are significantly different from each other ($p < 0.05$)
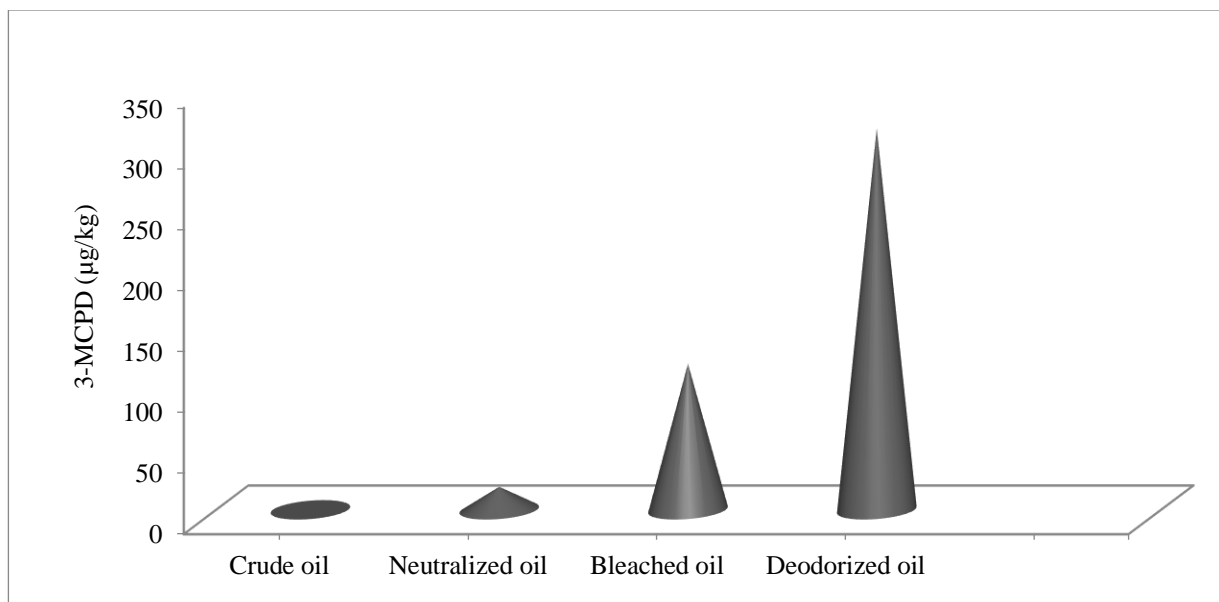
nd=not detected.

Fig. 4: Change of 3-MCPD ester concentration in processed soybean oil

Conclusion

Impacts of processing steps, including neutralization/degumming, bleaching, and deodorization on the physiochemical parameters, fatty acid profile, and phytosterols of soybean oil and its deodorizer distillate were evaluated. During industrial processing, physiochemical characteristics such as moisture, phosphorus, color, FFA, PV, and SV were decreased. A higher impact of processing was observed on the level of sterols of soybean oil as compared to the fatty acid composition. The concentration of 3-MCPD ester was increased in the bleaching and deodorization step of processing. The efficiency of each processing stage was found to be satisfactory as no parameter was above or below the standard values. Quality of crude soybean oil, refined, bleached, and deodorized soybean oil and SBO-DD of both sets was found to be comparable as no significant difference was observed in the physicochemical parameters, although these were collected from two different industries. Deodorizer distillate of soybean oil is a rich source of sterols and FFAs. The high FFA contents in deodorizer distillate samples also indicated that it could be used as a potential and cheap source for biodiesel production.

Acknowledgments

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