Quality Evaluation of Canola Oils and Deodorizer Distillate during Industrial Processing

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Summary: Aim of present study was to evaluate quality of three collected sets of canola oil containing crude oil, neutralized oil, bleached oil, deodorized oil and canola oil deodorizer distillate (DD) form three different edible oil processing industries. Physiochemical properties such as moisture, color, free fatty acid (FFA), acid value (AV), peroxide value (PV), p-Anisidine value (AV), total oxidative (totox) value, saponification value (SV), iodine value (IV), unsaponifiable matter and soap content were evaluated. The results of the present study indicated that each stage of processing has different impact on the determined quality parameters. Overall processing was well controlled and final product i.e. refined, bleached and deodorized (RBD) canola oil was found to be fit for human consumption. Only soap contents should be further controlled during neutralization process to avoid extra processing time and losses in the bleaching process. High FFA contents in DD samples indicated that it could be used as a potential and cheap source for biodiesel production.

Keywords: Canola oil, Physiochemical characteristics, Deodorization, Deodorizer distillate.

Introduction

Worldwide canola seed is also known as Brassica *napus*. Canola oil is the third most consumed vegetable oil throughout the world after palm and soybean oil [1]. Initially, Canada has introduced canola oil in 1974. Canola oil is extracted from the seeds of *Brassica napa* L. At present, most of cultivated rapeseed varieties contain low amount of erucic acid and glucosinolates, while high level of oleic acid. Through plant breeding, many varieties of canola seeds have been developed which contain high oleic and stearic as well as lauric acids but low content of linoleic acid. High stearic and lauric acids containing canola oils are not used as cooking oils but mainly used in the confectionary coating, whipped toppings, baked foodstuffs and for coffee creamer.

The canola council of Canada propagated the canola oil as safe and healthy for cooking oil. According to American Dietetic Association, and American Heart Association, it has unique benefits for coronary heart diseases and is acknowledged by many health professional bodies [2]. Triglycerides are the main component of canola oil but it is also composed of other minor components such as monoacylglycerols, diacylglycerol, FFAs, phospholipids, polyphenols, tocopherols, triterpene alcohols, tocotrienols, squalene, chlorophylls, carotenes, gums, waxes, oxidation products, trace metals such as iron, copper and sulphur, flavor compounds and sometimes pesticide residues [3].

Oil extracted from the canola seed is in crude form. Therefore, refining of crude oil is necessary in order to remove unwanted minor compounds that lead to instability of the oil in foodstuffs [4]. There are two procedures in practice for the refining of vegetable oils i.e. physical refining and chemical refining. During the physical refining process mainly FFAs are removed by the process of distillation whereas in chemical process FFAs are removed through alkali neutralization process. This is main difference between the physical and chemical refining. Chemical refining is mostly used when oil contain FFAs lower than 3% [5-9].

In chemical refining process, four main steps are involved; first is degumming, second is neutralization, third is bleaching and the last step is deodorization which are almost common for all vegetables oils. Degumming is usually initial step for those oils which contain phospholipids such as soybean oil. Phospholipids may be hydratable or non-hydratable. Therefore, two different types of degumming process are carried out in edible oil processing industries i.e. water degumming and acid degumming. In order to remove hydratable phospholipids, water degumming is performed and to remove non-hydratable phospholipids acid degumming is used with the help of phosphoric acid [10-11]. In neutralization process generally sodium hydroxide (caustic soda) is added in the crude oil to reduce the quantity of FFA in the oil, by the formation of sodium

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soaps in oil. Addition of the appropriate amount of NaOH to crude oil is based on the amount of FFAs present in the oil [12-14].

Mainly color pigments such as chlorophyll and carotenoids are removed in the bleaching process [3]. The last stage of refining is deodorization. This stage enhances the quality of edible oil by decreasing the amount of FFA existing in the oil. At this stage volatile compounds that produce unfavorable taste, flavor, color and odor in the oil are also eliminate [15]. The byproduct DD is obtained during the last step of refining that is the deodorization process [16]. It is the complex mixture of many health beneficial constituents such as tocopherols and tocotrienols [17]. These are the natural antioxidants communally called as vitamin E. These components are important because they impede the oxidation reaction and prevent material from oxidative damage. These are generally applicable used in cosmetic and pharmaceutical products [18].

The aim of present study was to evaluate the quality of crude, neutralized, bleached and deodorized oils before and after processing as well as DD collected form edible oil processing industry. To check the quality of neutralized, bleached and deodorized oil is very necessary which is directly related to the health of consumers. Also achieved results could be significant data base for other researcher and by-product such as DD could be explored for useful applications.

Experimental

Reagents and sample collection

All chemicals, reagents and solvents used were from E. Merck (Darmstadt, Germany).

Three sets of canola oil samples were collected from different edible oil industries located in Karachi and Hyderabad, Pakistan. The samples of canola oil were kept in refrigerator at 4ºC for further analysis.

Physiochemical Parameters

AOCS official methods were used for the analysis of all physiochemical parameters such as moisture content, color, FFA, AV, PV, AV, Totox, SV, unsaponifiable matter and soap content of the canola oil samples.

Moisture

For the determination of moisture content in canola oil, AOCS method Ca 2c-25 was used [19]. Canola oil sample was weighed (15 g), placed in Petri dish and heated in oven (Memmert, Schwabach, Germany) for 30 min at 130 ºC.

Phosphorus

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

Color

To check the color of all collected canola oil samples and DD, Official AOCS method Cc 13b-45 was used [19]. This method determines color by comparison with glass slides of known color characteristics. Sample was placed in a 25.4 mm and 127 mm cell for crude and refined oil, respectively using Lovinond Tintometer. The color of the sample was determined by achieving the best possible match with the standard color slide.

FFA

The quantity of FFA in all collected canola oil samples and DD was determined by using official AOCS method Aa 6-38 [19]. About 56 g of canola oil sample was placed in 250 mL conical flask and then added neutral alcohol 50 mL. Then the mixture was shaken and heated at hot plate and added few drops of phenolphthalein indicator and titrated with 0.1 M NaOH until the pink color disappearance.

Acid value

Official AOCS method Cd 3d-63 [19] was used for the determination of AV of all collected canola oil samples and DD. In a 250 mL of conical flask, added about 20 g of canola oil sample or 0.1 g of DD sample and then added 125 mL of neutralized solvent mixture of equal parts by volume of isopropyl alcohol and toluene which contain 2 mL of phenolphthalein indicator. Shaken the test portion vigorously while titrating with 0.1 M KOH until the permanent pink color was appeared. Similarly, the blank titration was carried out by using 125 mL of the neutralized solvent mixture.

Peroxide value

Official AOCS method Cd 8-53 [19] was used for the determination of PV of all collected canola oil samples and DD. In a 250 mL of conical flask, added about 2 g of canola oil sample in it and then added 15 mL (3:2 v/v) mixture of glacial acetic acid and chloroform. Added few drops of 1% starch as an

indicator to detect the end point in the mixture and then titrated with 0.1 N standardized sodium thiosulphate.

p-Anisidine value

Official AOCS method Cd 8-53 [19] was used for the determination of p-AV of all collected canola oil samples and DD. About 2 g of oil sample were weighed into 25 mL volumetric flask and diluted with isooctane. Absorbance of sample solution was measured by using cuvette at 350 nm with spectrophotometer, then 5 mL of oil solution were taken in test tube and 1 mL of p-anisidine solution was added. Absorbance of oil p-anisidine solution was measured at 350 nm after exactly 10 min.

Totox value

Total oxidation value of the all collected canola oil samples and DD was determined by sum of peroxide value and p-anisidine value [20].

Saponification value

Official AOCS method Cd 3-25 [19] was used for the determination of SV of all collected canola oil samples and DD. In 50 mL of round bottom flask, added about 2 g of canola oil sample and added 25 mL of 95% ethanolic potassium hydroxide. The mixture was refluxed for 30 min. The refluxed mixture was placed in 250 mL conical flask and added few drops of phenolphthalein indicator and titrated with 0.5 N hydrochloric (HCl).

Iodine value

Official AOCS method Cd 1-25 [19] was used for the determination of IV in all collected canola oil samples and DD**.** The presence of unsaturation in the oil sample can be determined by finding the IV of oil. Sample of canola oil was placed in conical flask and added 15 mL of carbon tetrachloride, 25 mL of Wij's reagent. And then solution was kept in dark for 30 min. After 30 min, added (20 mL) solution of potassium iodide (10%) followed by 100 mL of distilled water and titrated against 0.1 N standard sodium thiosulphate solutions using starch (1 %) as an indicator.

Unsaponifiable Matter

Official AOCS method Ca 6a-40 [19], was used to determine the unsaponifiable matter of all collected canola oil samples and DD. Unsaponifiable matter are those substances which frequently found dissolved in fats and oils, which cannot be saponified by the usual caustic treatment, but are soluble in ordinary fats and oils solvents. About 5 g of well mixed test sample was taken in 50 mL round bottom flask and added 30 mL of 95% ethanol and 5 mL of 50% KOH solution. The solution gently boiled and steadily under reflux for 1 h. After reflux, the solution was transferred in the extraction cylinder and washed with 40 mL of 95% ethanol, 40 mL distilled water and 5 mL of petroleum ether. Removed out upper layer and combined in a 500 mL separating funnel. The extraction repeated six times using 50 mL portions of petroleum ether each time with vigorous shaking. The combined extracts washed in the separatory funnel three times, using 25 mL portions of 10% ethanol shaking vigorously and drained out the aqueous layer after each extraction. The washing practice continued with 10% ethanol solution until the wash solution no longer gives pink color.

Soap content

The soap content of canola oil samples was determined using AOCS official Method Cc 17-95 [19]. Soap content determines the alkalinity of test sample as sodium oleate. About 40 g of the sample was taken in a test tube and added 1 mL of water, warmed in a water bath and shaken vigorously. Added 50 mL of the test solution (contain 0.5 mL of bromophenol indicator solution in 100 mL of aqueous acetone solution and titrated with 0.01 M HCl until the test solution is just yellow in color) and shaken the solution until two layers formed. The solution titrated with 0.01M HCl until the yellow color of the upper layer remains permanent.

Statistical analysis

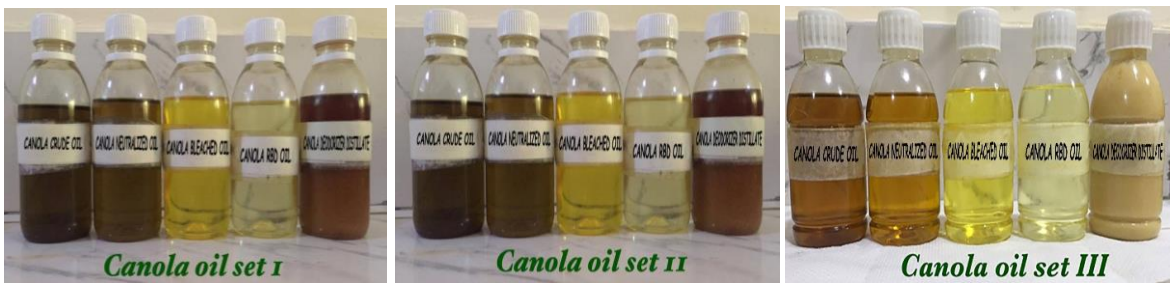
Three sets of samples were collected from three different edible oil industries. Each set contain five samples i.e. crude canola oil, neutralized canola oil, bleached canola oil, deodorized canola oil and canola oil DD. Each parameter was analyzed in triplicate (n=3×5×3). The results were evaluated statistically using OriginPro 8 and presented as mean with the standard deviation (SD).

Results and Discussion

Physiochemical properties of deodorizer distillate of canola oil

Three sets of canola oil containing crude oil, neutralized oil, bleached oil, deodorized oil and DD were collected from three different edible oil processing industries as shown in Fig. 1.

All collected canola oil samples were analyzed for their physiochemical characteristics. The results of physiochemical parameters such as moisture content, color, FFA, AV, PV, AV, Totox, SV, IV, unsaponifiable matter and soap content determined in crude, neutralized, bleached and deodorized oils are presented in Table-1.



(a) **(b)** **(c)**

Fig. 1: Samples of Canola oil (a) set I (b) set II and (c) set III.

Table-1: Physiochemical properties of crude and industrially processed canola oil set I, II and III.

Parameter	Canola Oil Samples				
	Set	Crude (a)	Neutralized (b)	Bleached ©	Deodorized (d)
Moisture (%)	1	0.82±0.04	0.14±0.02	0.13±0.01	0.02±0.05
	2	(0.92±0.06)	(0.13±0.04)	(0.12±0.02)	(0.08±0.07)
	3	[0.03±0.00]	[0.03±0.00]	[0.03±0.00]	[0.02±0.00]
Mean		0.59±0.03	$0.1±0.02^{ba}$	$0.09±0.01^{cb}$	$0.04±0.04^{dc}$
Phosphorus (mg/kg)	1	500±16.6	152±6.03	2.9±0.08	1.6±0.06
	2	(482±19.8)	(183±7.12)	(2.4±0.10)	(1.5±0.05)
	3	[492±17.5]	[252±9.42]	[5.8±0.26]	[3.3±0.16]
Mean		491±17.96	$196±7.52^{ba}$	$3.7±0.15^{cb}$	$2.1±0.09^{dc}$
Color Red units	1	3.5± 0.65 R	3.0±0.52 R	1.0±0.78 R	0.1±0.68 R
	2	(3.6±0.63 R)	(3.0±0.54 R)	1.1±0.74 R	0.1±0.66 R
	3	[4.2±0.42 R]	[3.5±0.24 R]	3.0±0.01 R	1.0±0.00 R
Mean		3.8±0.57	$3.2±0.43R^{ba}$	$1.7±0.51R^{cb}$	$0.4±0.45R^{dc}$
Yellow units	1	36±0.66 Y	30±0.54 Y	10±0.76 Y	1.0± 0.67 Y
	2	(36±0.67 Y)	(30±0.56 Y)	(10±0.71Y)	(1.0± 0.65 Y)
	3	42±0.14 Y	[35±0.34 Y]	[30±0.33 Y]	[14±0.44 Y]
Mean		38±0.49	$31.7±0.47Y^{ba}$	$16.7±0.6Y^{cb}$	$5.3±0.59Y^{dc}$
Blue units	1	0.5±0.02 B	0.1±0.01 B		
	2	(0.4±0.02 B)	(0.3±0.02 B)	nd	nd
	3	[1.1±0.01 B]	[0.1±0.00 B]		
		0.76±0.02	$0.17±0.01B^{ba}$		
FFA (%)	1	1.25±0.07	0.12±0.10	0.18±0.04	0.08±0.10
	2	(1.12±0.05)	(0.13±0.02)	(0.14±0.06)	(0.07±0.04)
	3	[1.9±0.02]	[0.7±0.04]	[0.9±0.04]	[0.1±0.02]
Mean		1.42±0.04	$0.32±0.05^{ba}$	$0.41±0.05^{cb}$	$0.08±0.05^{dc}$
Acid value (mg KOH/g)	1	2.50±0.01	0.24±0.02	0.36±0.01	0.16±0.00
	2	(2.24±0.02)	(0.26±0.00)	(0.28±0.01)	(0.14±0.00)
	3	3.81±0.04	[1.48±0.02]	[1.82±0.01]	[0.20±0.00]
Mean		2.85±0.02	$0.66±0.01^{ba}$	$0.82±0.01^{cb}$	$0.17±0.00^{dc}$
PV ($meqO_2/kg$)	1	7.56±1.12	2.54±1.04	2.21±0.18	1.53±0.07
	2	(7.54±1.14)	(1.53±1.08)	(1.04±0.24)	(0.25±0.05)
	3	[4.5±0.12]	[2.42±0.14]	[1.94±0.04]	[1.25±0.01]
Mean		6.53±0.79	$2.16±0.75^{ba}$	$1.73±0.15^{cb}$	$1.01±0.04^{dc}$
p-Anisidine value	1	3.29±1.03	2.43±1.01	2.01±0.00	1.65±0.02
	2	(3.14±1.09)	(2.36±0.73)	(2.00±0.01)	(1.47±0.01)
	3	[2.96±0.03]	[2.34±0.01]	[2.16±0.02]	[1.92±0.01]
Mean		3.13±0.75	$2.38±0.58^{ba}$	$2.06±0.01^{cb}$	$1.68±0.01^{dc}$
Totox	1	18.41±3.07	7.51±1.02	6.43±1.08	4.71±1.04
	2	(18.22±3.08)	(5.42±1.04)	(4.08±1.02)	(1.97±1.00)
	3	[11.96±2.06]	[7.20±1.22]	[6.04±0.15]	[4.42±1.02]
Mean		16.19±2.74	$6.71±1.09^{ba}$	$5.52±0.75^{cb}$	$3.7±1.02^{dc}$
SV (mgKOH/g)	1	182.64±2.42	186.53±2.45	186.02±2.19	186.72±2.19
	2	(182.24±2.38)	(186.32±2.35)	(185.86±2.15)	(186.14±2.17)
	3	[182.82±2.38]	186.42±2.35	[186.40±2.35]	[185.65±2.15]
Mean		182.57±2.39	$186.42±2.38^{ba}$	$186.09±2.23^{cb}$	$186.17±2.17^{dc}$
IV ($gI_2/100g$)	1	98.92±1.06	98.13±1.03	97.29±1.03	96.44±1.03
	2	(99.96±1.19)	(98.94±1.13)	(98.13±1.03)	(98.29±1.13)
	3	[99.42±1.19]	[98.14±1.13]	[98.06 ±1.13]	[97.29 ±1.03]
Mean		99.43±1.15	$98.40±1.09^{ba}$	$97.83±1.06^{cb}$	$97.34±1.06^{dc}$
Unsaponifiable Matter (%)	1	1.36±0.09	1.96±0.10	1.56±0.05	1.32±0.07
	2	(1.34±0.08)	(1.95±0.07)	(1.54±0.08)	(1.32±0.02)
	3	[1.45±0.04]	[1.63±0.07]	[1.54±0.07]	[1.32±0.08]
Mean		1.38±0.07	$1.85±0.08^{ba}$	$1.55±0.07^{cb}$	$1.32±0.06^{dc}$
Soap content (ppm)	1		91.01±1.34		
	2	nd	(91.03±1.28)	nd	nd
	3		[91.32±0.14]		
Mean			91.12±0.92		

Values without any brackets belong to Set 1, while ( ) and [ ] refer to data for sets II and III, respectively nd, detected, a, crude oil; b, neutralized oil, c, bleached oil; d, deodorized oil

The value provided in the Table 1 are the mean values of triplicate analysis with standard deviation. Means followed by different superscripts in the same column differ significantly (Tukey's test at 0.05 p-level the population are significantly different).

Moisture

It is well known fact that oil free from moisture has advantage in terms of oxidative stability, since higher the moisture content lower the storability and suitability of oil preservation for a longer period [21]. Moisture content of crude canola oil in set I, II and III were found at 0.82 %, 0.92 % and 0.03 %, respectively. In one of the study, Przybylski *et al.,* [22] has reported moisture content of crude canola oil was <0.3 %. The current values of moisture content of set I and II in crude canola oil were higher and set III was lower than the reported study. Difference of moisture present in samples of three different industries may be due to the different conditions which were used for the extraction of oil especially during the mechanical extraction. During neutralization, moisture in crude canola oil was decreased from 0.82 % to 0.14 % in set I and 0.92 % to 0.13 % in sets II but level of moisture was remained same as 0.03 % in set III. In the bleaching stage, moisture content was further reduced in set I and II but remain same in sets III as neutralized oil. Results of moisture in neutralized and bleached oil of set I, II and III clearly indicated that it should be controlled in the crude oil during extraction of process from canola seed to prevent quality deterioration and economical processing. During deodorization process, moisture contents were further decreased to 0.02 %, 0.08 % and 0.02 % in sets I, II and III, respectively. Overall impact of processing from crude to deodorize was found to be 97.5 % in set I, 91.3 % in set II and 33.3 % in set III.

Phosphorus

The phosphorus contents in crude canola oil play adverse impact during refining process. In neutralization process, phosphorus content (in the form of phosphatides) may cause greater loss of neutral oil. Furthermore, if it is not controlled during degumming neutralization process then their presence leads to oil discoloration during bleaching and deodorization process. Phosphorus content of crude canola oil in set I, II and III were found to be 500 mg/kg, 482 mg/kg and 493 mg/kg, respectively. Przybylski *et al.,* [22] has reported phosphorus content of crude canola oil in between 300-500 mg/kg. The level of phosphorus content in set I, II and III was found to be comparable to the reported study. From neutralization to deodorization, phosphorus contents were decreased from 152 to 1.64 mg/kg, 482 to 1.51 mg/kg and 492 to 3.3 mg/kg in sets I, II and III, respectively. While in reported study, phosphorus content in the deodorized oil was found to be less than 2 mg/kg [22]. Overall impact of processing on the reduction of phosphorus from crude to deodorized oil was found to be 99.7 mg/kg, 99.7 mg/kg and 99.3 mg/kg in set I, II and III, respectively.

Color

Chlorophyll, carotenoids and some other pigments are responsible for the color of oil. The Tintometer is often used to differentiate the color of oil mostly in terms of red (R), yellow (Y) and Blue (B) units [19]. Color of crude canola oil was measured in 1 inch cell and the color of bleached, neutralized, and deodorized oil and its deodorizer distillate were measured in 5.25 inch cell. The color of crude canola oil in set I, II and III was found to be 3.5R, 36 Y, 0.5B in set I 3.6 R, 36 Y, 0.4B in set II and 4.2R, 42 Y, 1.1B in set III. Significant reduction of crude canola oil color was observed in the stage of neutralization from 3.5 to 3.0R , 36 to 30Y and 0.5 to 0.1B in set I, whereas 3.6 to 3.0 R , 36 to 30 Y and 0.4 to 0.3B in set II and similarly 4.2 to 3.5R , 42 to 35Y and 1.1 to 0.1B in set III. In the bleaching stage, color of canola oil was reduced to 1.0R and 10Y in set I, while 1.1R and 10Y in set II, similarly 3.0R and 30Y in set III. In the deodorizer process the color in both sets of bleached oil was further reduced to 0.1R and 10Y in both sets I and II, while in set III 1.0R and 14Y. Overall reduction of red color and yellow color in set I, set II and set III from neutralization to deodorization was found to be 97.1 % R and 97.2 Y %,; 97.2 % R and 97.2 %Y; and 76.19 % R and 66.6 %Y, respectively.

Free fatty acid

FFAs are produced by the hydrolysis of oils and fats. In terms of oil quality, the FFA value of oil is an important qualitative parameter. After processing, oils and fats contain very small level of FFA which may increase during transportation and upon long storage. The FFA of crude canola oil of set I, II and III were found 1.25%, 1.12% and 1.9%, respectively, which is almost comparable to the reported study, Przybylski *et al.,* [22]. During different processing stages, FFA of canola oil was reduced gradually. After the neutralization process the FFA of neutralized canola oil were reduced in all sets from 1.25 % to 0.12 %, 1.12 % to 0.13 % and 1.9 % to 0.7 % in set I, II and III, respectively. During bleaching, FFA was increased from 0.12 to 0.18 %, 0.13 to 0.14 % and 0.7 % to 0.9 % in set I, II and III, respectively. Increase of FFA in all three sets is due to the acidic nature of bleaching clay which was used for the bleaching process. After last stage of processing (deodorization), FFA in the deodorized canola oil was found at 0.08%, 0.07% and 0.3% in set I, II and III, respectively. In the reported study by Przybylski *et al.,* [22], FFA in deodorized canola oil was 0.03 % which is lower than the values of present study. Overall reduction of FFA in whole processing from neutralization to deodorization was found to be 93.6 % 93.8 % and 94.7 % in set I, II and III, respectively.

Acid Value

The AV is the number of milligrams of potassium hydroxide necessary to neutralize the free fatty acid in 1 g of test sample with the test sample that contain virtually no free acids other than fatty acids., The acid value may be directly converted by means of a suitable factor to present FFA. In present study, the AV was increased initially than decreased during refining while slightly increase in bleaching process. The AV of crude canola oil of set I, II and III were found at 2.50, 2.24 and 3.81 mg KOH/g, respectively. These values of set I and II were lower and set III were higher than the previous study (3.22 mg KOH/g) reported by Farhoosh *et al.,* [23]. During refining process of canola oil, AV was reduced in the neutralization stage. When crude canola oil reacted with alkali, the AV of crude canola oil decreased from 2.50 mgKOH/g to 0.24 mg KOH/g in set I, 2.24 mg KOH/g to 0.26 mg KOH/g in set II and 3.81 to 1.48 mgKOH/g in set III. The AV of neutralized canola oil determined in this study was higher than the reported for neutralized canola oil 0.05 mgKOH/g by Farhoosh *et al.,* [23]. In bleached canola oil, the AV were slightly increased in all sets and found at 0.36 mgKOH/g, 0.28 mgKOH/g and 1.82 mg KOH/g in set I, II and set III, respectively. The current values were relatively higher to the reported AV 0.10 mg KOH/g Farhoosh *et al.,* [23]. During deodorization, AV of deodorized oil was obtained 0.16 mg KOH/g, 0.14 mg KOH/g and 0.20 mg KOH/g in set I, set II and set III, respectively. In comparison to the current study, the AV in deodorized canola oil reported was almost similar (0.14 mg KOH/g). During refining processes the overall impact of AV from crude to deodorization process was found to be 93.6 % 93.75 % and 94.7 % in set I, II and III, respectively.

Peroxide Value

The PV is a measure of the amount of peroxides and hydro peroxides formed in the oils and fats as a result of oxidation. The PV determination is most commonly used for the measurement of oxidative rancidity of oils and fats [24]. In present study, PV was increased initially than decreased during refining. The PV of crude canola oil of set I, II and III were measured as 7.56 meqO_2/kg, 7.54 meqO_2/kg and 4.51 meqO_2/kg, respectively. These values were higher than the previous reported study (1.94 meqO_2/kg) by Farhoosh *et al.,* [23]. During refining process of canola oil, PV was reduced in the neutralization stage. When crude canola oil reacted with alkali, the PV of crude canola oil decreased from 7.56 meqO_2/kg to 2.54 meqO_2/kg in set I, 7.54 meqO_2/kg to 1.53 meqO_2/kg in set II and 4.51 meqO_2/kg to 2.42 meqO_2/kg in set III. The PV of neutralized canola oil determined in this study was almost similar to the PV reported for neutralized canola oil (1.94 meqO_2/kg) by Farhoosh *et al.,* [23]. Similar trend was observed in bleached canola oil, the PV were reduced in all sets and found at 0.18 meqO_2/kg, 1.04 meqO_2/kg and 1.94 meqO_2/kg in set I, II and set III, respectively. The current values were almost similar to the reported PV (1.27 meqO_2/kg) except PV of set I Farhoosh *et al.,* [23]. In the last stage of processing, the PV of deodorized oil were obtained 0.08 meqO_2/kg, 0.25 meqO_2/kg and 1.25 meqO_2/kg in set I, II and set III, respectively. In comparison to the current study, the higher values for PV in deodorized canola oil reported (1.78 meqO_2/kg). In current study reverse trend was observed for PV during deodorization which may be due to the improper setting of deodorization parameters or leakage of the vacuum. During refining processes the overall impact of PV from crude to deodorization process for sets I, II and III was found to be 80.1 % 96.7 % and 72.22 %.

p-Anisidine value

In oil or fat, p-Anisidine value (p-AV) test is used to evaluate the secondary oxidation, which is generally related to the concentration of aldehydes and ketones, and is therefore capable to articulate the oxidation "history" of oils and fats. In current study, the p-AV was studied during each refining stage from crude to deodorized oil. The p-AV of crude canola oil of set I, II and III were found to be 3.29, 3.14, and 2.96, respectively. These values were found to be comparable with the study reported by (Przybylski *et al.,* [22]. During refining process p-AV was decrease from crude to neutralized oil. In the neutralized, p-AV of all sets was found 2.43, 2.36 and 2.34 in set I, II and III, respectively. In bleached oil, the p-AV was decreased from 2.43 to 2.01 in set I, 2.36 to 2.00 in set II and 2.34 to 2.16 in set III. In the last stage of refining, p-AV was decreased in deodorized oil to 1.65, 1.47 and 1.92 in set I, II and III, respectively as reported (<2) by Przybylski *et al.,* [22]. During refining processes the overall impact of p-AV from crude to deodorization process for sets I, II and III was found to be 49.8 %, 53.18 % and 35.14 %, respectively.

Totox value

Totox value is the sum of peroxide and anisidne value. The totox value was examined from crude to deodorized oil. The totox value of crude, neutralized, bleached and deodorized canola oils of set I, II and III were found to be in decreasing trend as 18.41, 7.51 6.43 and 4.71; 18.22, 5.42, 4.08 and 1.97; 11.96, 7.20, 6.04 and 4.42 in set I, set II and set III, respectively. To best of our knowledge, these values were not reported in any study. During refining

processes the overall impact of totox values from crude to deodorization process for sets I, II and III set were found to be 74.17 %, 89.19 % and 63.04 %, respectively.

Saponification Value

SV is an indication of the size or nature of fatty acid chains esterified to glycerol and gives a measure of the average length of the fatty acid chain that makes up a fat. SV of crude canola oil of set I, II and III were observed 182.64 mg KOH/g, 182.24 mg KOH/g and 182.82 mg KOH/g, respectively. Slight increase of SV was observed from neutralized to deodorized canola oil. In the neutralized oil, SV was found as186.53 mg KOH/g in set I, 186.32 mgKOH/g in set II and 186.42 mgKOH/g in set III. After the neutralization process, the SV of bleached and deodorized stages of canola oil were observed almost similar in all sets. If we see the overall effect of industrial processing from crude to deodorization process, the SV of set I, II and III was found to be 2.2%, 2.1% and 1.552 %, respectively.

Iodine value

IV is used for determination of amount of unsaturation in fatty acids. More C=C bonds are present in oil and fat indicates the higher IV. In current study, during the refining process of canola oil no any significant change of IV was observed in all stages of refining. There was only slight change was noticed from crude to deodorization process of canola oil. In crude, neutralized, bleached and deodorization stages, the IV of canola oil was observed 98.92 gI_2/100g, 98.13 gI_2/100g, 97.29 gI_2/100g, 96.44 gI_2/100g in set I, 99.96 gI_2/100g, 98.94 gI_2/100g, 98.13 gI_2/100g, 98.29 gI_2/100g in set II and 99.42 gI_2/100g, 98.14 gI_2/100g, 98.06 gI_2/100g and 97.29 gI_2/100g in set III. The overall effect of processing from crude to deodorized oil was found to be 2.5 %, 1.7 % and 2.14 %, respectively.

Unsaponifiable Matter

Substances that can't be saponified by the conventional caustic treatment but are soluble in ordinary fat and oil are called unsaponifiable matters. During industrial processing of canola oil, the unsaponifiable matter was increased from crude to neutralized oil during neutralization process and then decreased in the bleaching and deodorization stages. Unsaponifiable matter of canola oil was observed as 1.36 %, 1.34 % and 1.45 %; 1.96 %, 1.95 % and 1.63 %; 1.56 %, 1.54 % and 1.54 % for crude oil, neutralized oil and bleached oil in set I, II and III, respectively. Whereas, the unsaponifiable matter of deodorized canola oil was found to be same as 1.32 %. The overall effect of processing of unsaponifiable matter from crude oil to deodorized oil was found to be 2.9% in set I, 1.5 % in set II and 8.97 % in set III.

Soap content

Metal salt of fatty acids in vegetable oil is known as soap content. Stability and shelf life of the oil can be affected by the soap content, higher the soap content lower is the shelf life [22]. Soap contents were only found in neutralized canola oil at almost equal level of around 91 ppm in the samples of set I, set II and set III. In the current study, no soap content was found in bleached and deodorized canola oil. In the neutralization stage the soap content was found because in this stage of sodium hydroxide is used to reduce the amount of FFA present in the oil which leads to the formation of sodium salt of fatty acids.

Physiochemical properties of deodorizer distillate of Canola oil

Moisture

The moisture content in DD of canola oil of all sets was found in the range of 0.04-0.36 % with an average value of moisture content 0.23 %. The value of moisture content in DD of canola oil obtained in current study were higher than the reported values 0.08 % and 0.02 % by Naz *et al.,* [13] and Ramamurthi and McCurdy [25], respectively.

Phosphorus

Phosphorus content was not detected in canola oil DD samples of all sets.

Color

The color in DD of all sets of canola oil was found in the range of 11.1-14.2 R, 71-72 Y and 0.1-2.0 B with an average color value of 12.1 R, 71.3 Y and 1.0 B. The higher values of color in DD are possibly due to the presence of heat sensitive coloring pigments which stripped off during heating in the deodorization stage and greater concentration of FFA. Przybylski *et al.,* [22] reported lower color values (< 1.5R, 10Y) than present study.

Free Fatty acid

The FFA in DD of three sets of canola oil was found in the range of 52.43-63.45 % with an average value of 58.37 %. The FFA value obtained in current study was relatively similar to the earlier reported value for canola oil DD (54.38 %) by Naz *et al.,* [13]. In other

studies, slightly lower FFA values of DD were reported by Güçlü-Üstündağ *et al.,* [26] and Martins *et al.,* [27] i.e. 50.39 % and 49.80 %, respectively.

Acid Value

The AV in DD of all sets of canola oil was found in the range of 104.86 – 126.92 mgKOH/g with an average value of 116.74 mgKOH/g. In comparison to the reported values (157.6 mg KOH/g) by Ramamurthi and McCurdy [24], the current value of AV of DD samples was found to be lower. Variation may be due to the difference in the parameters of deodorization such as temperature, vacuum, retention time and amount as well as quality of open steam.

Peroxide Value

The PV in DD of all sets of canola oil was found in the range of 5.92-7.52 meqO_2/kg with an average value of 6.76 meqO_2/kg. The PV found in current study was almost similar to the values (6.95 meqO_2/kg) reported by Naz *et al.,* [13].

p-Anisidine Value

The p-AV in DD of all sets of canola oil was found in the range of 5.32-6.42 with an average value of 5.77. These values are not reported in any study.

Totox Value

The totox in DD of all sets of canola oil was found in the range of 17.07- 21.42 with an average value of 57.84. These values are also not reported in any earlier study.

Saponification Value

The SV in DD of all sets of canola oil was found in the range of 161.27-165.46 mg KOH/g with an average value of 163.76 mg KOH/g. The SV found in current study was almost similar to the earlier reported values 164.24 mg KOH/g and 163.1 mgKOH/g, by Naz *et al.,* [13] and Ramamurthi and McCurdy [25], respectively.

Iodine Value

The IV in DD of all sets of canola oil was found to be similar with an average value of 99.9 gI_2/100g. The IV found in current was comparable to the reported study 99.83 gI_2/100g Naz *et al.*,[13], while comparatively higher than those reported value 74.3 ± 0.5 gI_2/100g by Ramamurthi and McCurdy [25].

Unsaponifiable matter

The unsaponifiable matter in DD of all sets of canola oil was found in the range of 17.12- 19.35 % with an average value of 18.23 %. In comparison to the reported values (14.6%), the current value of unsaponifiable matter of DD was found higher as reported by Ramamurthi and McCurdy [25].

Soap content

In all sets of canola oil DD samples, soap content was not detected in our present study and also not reported before in any study.

Table-2: Physiochemical properties of canola oil deodorizer distillate of set I, II and III.

Parameters	Set I	Set II	Set III	Average
Moisture (%)	**0.28±0.01** ^{dd2dd1}	**0.36±0.01** ^{dd2dd3}	**0.04±0.00** ^{dd3dd1}	**0. 23±0.01**
Phosphorus (mg/kg)	**nd**	**Nd**	**nd**	**nd**
Color	**14.1 ±0.03** ^{dd2dd1}	**11.1±0.08** ^{dd2dd3}	**14.2±0.07** ^{dd3dd1}	**12.1±0.06**
Red units	**71 ±0.62** ^{dd2dd1}	**71 ±0.92** ^{dd2dd3}	**72±0.04** ^{dd3dd1}	**71.3 ±0.53**
Yellow units	**0.1±0.00** ^{dd2dd1}	**2.0±0.02** ^{dd2dd3}	**1.0±0.00** ^{dd3dd1}	**1.03±0.01**
Blue units				
FFA (%)	**59.22±0.07** ^{dd2dd1}	**52.43±0.30** ^{dd2dd3}	**63.45±0.12** ^{dd3dd1}	**58.37±0.16**
Acid value (mg KOH/g)	**118.44±0.32** ^{dd2dd1}	**104.86±0.20** ^{dd2dd3}	**126.92±0.04** ^{dd3dd1}	**116.74±0.19**
PV (meqO_2/kg)	**6.84±0.11** ^{dd2dd1}	**5.92±0.02** ^{dd2dd3}	**7.52±0.05** ^{dd3dd1}	**6.76±0.06**
p-Anisidine value	**5.67±0.03** ^{dd2dd1}	**5.23±0.42** ^{dd2dd3}	**6.42±0.34** ^{dd3dd1}	**5.77±0.26**
Totox	**19.35±0.05** ^{dd2dd1}	**17.07±0.93** ^{dd2dd3}	**21.42±0.43** ^{dd3dd1}	**57.84±0.047**
SV (mgKOH/g)	**165.46±0.53** ^{dd2dd1}	**161.27±0.89** ^{dd2dd3}	**164.54±0.90** ^{dd3dd1}	**163.76±0.77**
IV (gI_2/100g)	**99.89±0.14** ^{dd2dd1}	**99.89±0.09** ^{dd2dd3}	**99.97±0.10** ^{dd3dd1}	**99.9±0.11**
Unsaponifiable Matter (%)	**17.12±0.01** ^{dd2dd1}	**18.24±0.02** ^{dd2dd3}	**19.35±0.02** ^{dd3dd1}	**18.23±0.02**
Soap content (%)	**nd**	**Nd**	**nd**	**nd**

nd, not detected

dd1=set I, dd2=set II and dd3= set III.

The value provided in the Table 2 are the mean values of triplicate analysis with standard deviation. Means followed by different superscripts in the same column differ significantly (Tukey's test at 0.05 p-level the population are significantly different).

Table-3: Comparison of physiochemical properties of DD with reported studies.

Parameters	Present study	Naz *et al.*, [12]	Güçlü-Üstündağ and Temelli [25]	Martins *et al.*, [26	Ramamurthi and McCurdy [24]
Moisture (%)	0. 23±0.01	0.08±0.01	nd	nd	0.02+ 0.01
Phosphorus (mg/kg)	nd	nd	nd	nd	nd
Color					
Red units	12.1±0.06				
Yellow units	71.3 ±0.53				
Blue units	1.03±0.01	nd	nd	nd	nd
FFA (%)	58.37±0.16	54.38 ±0.32	50.39 ±1.13	49.80 ± 1.2	nd
Acid value (mg KOH/	116.74±0.19	- nd	nd	nd	157.6 ±0.1
PV (meqO_2/kg)	6.76±0.06	6.95±0.23	nd	nd	nd
Anisidine value	5.77±0.26	-	nd	nd	nd
Totox	57.84±0.04	-	nd	nd	nd
SV (mgKOH/g)	163.76±0.77	164.24±0.89	nd	nd	163.1 ±1.0
IV (gI_2/100g)	99.9±0.11	99.83±0.09	nd	nd	74.3 ± 0.5
Unsaponifiable Matter (%)	18.23±0.02	14.6 ± 0.1	nd	nd	nd
Soap content (%)	nd	nd	nd	nd	nd

nd, not detected

The value provided in the Table 3 are the mean values of triplicate analysis.

Conclusion

Impacts of processing stages such as neutralization, bleaching and deodorization on the physiochemical parameters of canola oil and its deodorizer distillate were evaluated and compared with the reported studies. During industrial processing the moisture content, color, PV, p-AV, Totox value and IV were decreased while FFA and AV were increased during bleaching process may be due to the use of acidic bleaching clay. SV and unsaponifiable matter were increased in neutralization on the basis of input crude oil and output neutralized oil, while in bleaching process opposite trend was observed. Soap contents were developed in neutralization process which was not totally removed in this process. However, soap contents were controlled in bleaching process. In case of DD higher values of color, FFA, PV and unsaponifiable matter were observed in comparison to canola oil. It can be concluded that industrial processing removed unwanted components and improved the quality of canola oil and found to be suitable for human consumption. Due to high content of FFA, DD could be used for biodiesel production and high unsaponifiable matter in deodorizer distillate indicates that it can be utilized as a natural source of bioactive components in food, cosmetic and pharmaceutical industries.

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