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Outcome of Refining on the Physicochemical Properties of Cottonseed Oil

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Abstract

The influence of refining processes on the physicochemical properties including fatty acid composition of cottonseed oil (CSO) was studied. Physicochemical properties were determined by standard AOCS and IUPAC methods. The obtained results of physical parameters showed that neutralization, bleaching and deodorization processes on crude CSO significantly reduced the moisture content, color, freezing point and smoke point, while slightly decrease in refractive index was observed. Similarly, in the case of chemical properties free fatty acids, saponification value and peroxide value were reduced from 1.7 to 0.15%, 175 to 173 mg KOH/g oil and 3.4 to 1.2 mEq O_2/Kg oil, respectively. Refining processes did not showed significant effect on the fatty acid composition and iodine value. As, soap contents generated in the chemical neutralization step, therefore soap content were decreased from 61.0 to 15.5 ppm in bleaching and deodorization steps. Moreover, this work suggests that overall deodorization process has greater impact on physicochemical properties to improve the quality and stability of cottonseed oil.

Keywords: Cottonseed oil, Physiochemical parameters, Fatty acid composition.

Introduction

Cotton is a main and largest cultivated crop of Pakistan. Cottonseed oil is a by-product of cottonseed and a good source of edible oil. The seed of cotton contains approximately 15-20% oil depends on the species and the quality of seed [1]. Cottonseed oil is usually utilized in cooking and considered to be nutritious and healthful. Due to its unique fatty acid composition cottonseed oil is also used in some other industrial applications such as paints, special biolubricants and soft soaps [2]. The content of the physicochemical properties of cottonseed oil varies with cottonseed cultivar, climatic conditions, crop year, processing methods, and storage conditions [3]. Physicochemical properties, fatty acid composition and oxidative stability determine the nutritional value of cottonseed oil [4, 5]. Crude cottonseed oil is unsuitable for use in most of the food applications without refining, because of its dark color, high free fatty acid content, and objectionable flavor and odor.

Undesirable materials such as free fatty acid, peroxides and color pigments have negative influence on physicochemical properties, sensory characteristics and storage stability of the oil. Usually, the unwanted materials are removed by chemical or physical refining processes to maintain vegetable oil quality [6]. In chemical refining, three important processes are carried out during refining such as neutralization, bleaching, and deodorization. Most of the free fatty acids are removed during alkali neutralization process. Whereas, in physical refining free fatty acids in oils are removed during deodorization process [7]. Although chemical and physical refining processes are useful to maintain the quality of oil, but these

processes also remove some nutritionally valuable components form the oil as well [8].

This research work is aimed to investigate the influence of industrial chemical refining steps such as, neutralization, bleaching and deodorization and on some physicochemical properties of cottonseed oil. To the best of our knowledge no any work has been reported so far on the physicochemical properties of cottonseed oil during industrial refining.

Materials and Methods

Reagents and oil samples collection

All the chemicals and reagents used in the present work were purchased from E-Merck (Dermastd, Germany). Cottonseed oil samples (crude, neutralized, bleached and deodorized) were collected directly from the processing line from commercial oil factory located at Hyderabad, Pakistan. These representative samples were stored at 4˚C in dark glass containers and purged with nitrogen gas after filling to minimize oxidation of the samples.

Physicochemical parameters

Physicochemical properties such as moisture, color, freezing point ^{o}C, smoke point ^{o}C, refractive index (40°C), free fatty acids (FFA), saponification value, iodine value, peroxide value and fatty acids composition of industrially processed cottonseed oils were measured according to the American Oil Chemists Society (AOCS) methods [9]..

Moisture

Moisture content in the industrially processed cottonseed oil was checked by oven method at 105^{o}C ± 1^{o}C for 1 hour using Moisture Analyzer MX-50 (SHS) Super Hybrid Sensor by applying AOCS method Da 2a-48 [9].

Color

The color of industrially processed cottonseed oil was determined in terms of Lovibond units using AOCS method Cc 13a-43 [9]. Before checking of color, glass cell (1 inch and 5 ¼ inch) was washed, cleaned and dried followed by the measuring of color using Lovibond Tintometer (Model F). The color was matched by sliding the red, yellow and blue color of the Lovibond Tintometer glasses until a perfect match was obtained while observing through the eye piece (pin hole).

Freezing and smoke point

For freezing point approximately 50g of oil sample was taken and kept in the upper portion of refrigerator for 30 minutes at 2 to 3°C using AOCS method Cc 9a-47 [9]. Whereas, smoke point was observed using AOCS method Cc 9a-48 [9]. Briefly 50 mL of oil was kept on heating mental at above 175 °C for 1 hour.

Refractive index

The refractive index is the ratio of the speed of light in a vacuum to the speed of light in the substance. The index of refraction of cottonseed oil was carried out at 40°C±1 according to AOCS method Cc-7-25 [9] using Refractometer.

Free fatty acid

Free fatty acid in industrially processed cottonseed oil was determined by titration method using AOCS method Ca 5a-40 [9]. Briefly oil was dissolved in warm neutralized ethanol and shaken vigorously. The mixture was titrated with 0.1N sodium hydroxide in the presence of phenolphthalein indicator.

Saponification value

Approximately 2 g of oil was weighed in a round bottom flask and added 25 mL of alcoholic potassium hydroxide. The material was refluxed at water bath for one hour until reaction completed. After cooling of mixture, 1 mL of phenolphthalein indicator was added to it and titrated with 0.5 N of hydrochloric acid until discoloration of the pink color. Similarly, a blank test was also carried out in same manner except the presence of oil using AOCS method Da 16-48 [9].

Iodine value

It is expressed as the amount in grams of iodine absorbed by 100 gram of oil. Approximately, 0.1g oil was dissolved into carbon tetrachloride (7.5 mL) followed by Wijis reagent (12.5 mL) and inserted the stopper. The solution was shaken gently and placed in the dark for 1 hour. After that added fresh solution of potassium iodide 7.5 mL and poured 75 mL water and then added few drops of starch indicator. Liberated iodine from sample mixture was then titrated with 0.1 N standard sodium thiosulphate solution until the blue color disappears at end point. Similarly, a blank test was also carried out in same manner excluding the presence of oil as reported in the method, AOCS Cd Ib-87 [9].

Peroxide value

Approximately 2g of oil was weighed in conical flask and added 10 mL chloroform and stirred. After that 15mL of glacial acetic acid and 0.5 mL of potassium iodide was added and shaken well for 1 minute and kept the solution in dark for 5 minute. Flask was removed from the dark and added 75 mL water along with 2 to 3 drops of starch indicator. The mixture was titrated with 0.01 N sodium thiosulphate. Similarly, a blank test was also carried out in same manner without the presence of oil using AOCS method Cd 8- 53 [9].

Soap content

Approximately 10 mL of CSO was poured in conical flask, added 10 mL acetone and 3 drops of bromophenol indicator. The solution mixture was titrated against 0.01N hydrochloric acid till reddish green color changed to yellow color. The soap content was calculated by the formula as reported in AOCS Cc 17-95 method [9].

Fatty acid composition analysis

Fatty acid methyl esters (FAMEs) of CSO were prepared according to IUPAC standard method 2.301 [10]. Analysis of FAMEs of CSO were carried out on the gas chromatography instrument coupled with mass selective detector (GC-MS) model 6890 N from Agilent Technology. The ChemStation 6890 Scale Mode software was used for the chromatographic peak analysis. A capillary column HP-5MS (5% phenyl methylsiloxane) (30 m x 0.25 mm ID x 0.25 μm film thickness) was used for the separation of fatty acids. The initial oven temperature was 150°C; it was held for 2 min then raised to 230°C with ramp rate of 4°C/min. Helium was used as the carrier gas with a flow rate of 0.8 mL/min. Temperature was set at 240°C for injector and 260°C for detector temperature. 1 μL each sample was injected in the split mode ratio (50:1). All analysis was performed in triplicate.

Statistical analysis

The cottonseed oil samples were analyzed in triplicate. Data were reported as means ± Standard deviation (n=3×3).

Results and Discussion

Physical parameters of industrially processed cottonseed oil

The physical parameters of crude, neutralized, bleached and deodorized CSO samples collected from the edible oil industry located at Hyderabad, Pakistan were analyzed. Results of moisture, color, freezing point, smoke point and refractive index of crude CSO and after each processing stage are shown in (Table 1).

Table 1. **Physical properties of crude and industrially processed cottonseed oils.**

Parameters	Crude CSO	Neutralized CSO	Bleached CSO	Deodorized CSO
Moisture (%)	0.35±0.01	0.24±0.06	0.05±0.09	0.02±0.04
Color	12.2±0.62 R	3.0±0.78 R	2.1±0.52 R	1.5±0.33 R
Red Units, Yellow Units	65.0±0.66 Y	30.0±0.75 Y	21.0±0.49 Y	15.0±0.37 Y
Freezing point (°C)	3.0±0.14°C	2.5±0.56°C	2.2±0.76°C	2.1±0.79°C
Smoke point (°C)	210.0±0.88°C	212.0±0.99°C	215.0±1.09°C	220.0±0.96°C
Refractive Index (40°C)	1.4742±0.0010	1.4743±0.0013	1.4744±0.0015	1.4746±0.0017

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. During neutralization, moisture in crude cottonseed oil was decreased from 0.35 to 0.24% with the level of 31.43%. In the stage of bleaching, moisture was further reduced from 0.24 to 0.05% by the level of 79.2%. While deodorization process decreased the moisture level of bleached oil from 0.05 to 0.02% with 60% removal efficiency. Overall performance of neutralization, bleaching and deodorization for the reduction of moisture level was found to be 14.86, 40.54 and 44.59%, respectively.

Color

Chlorophyll, carotenoids and some other pigments are responsible for the color of the oil. The tintometer is often used to differentiate the color of oil in terms of red (R) and yellow (Y) units. Color of crude oil is usually measured in 1 inch cell whereas color of bleached and deodorized is evaluated in 5.25 inch cell. Alkali neutralization significantly reduced color from 12.2 to 3.0 R and 65 to 30 Y, which indicated that 75.40% and 53.84% loss of oil color. In bleaching stage, color was reduced from 3.0 to 2.1 R and 30 to 21 Y, which shows 82.78% and 67.69% color removal during bleaching. While in deodorization process the oil color further reduced from 2.1 to 1.5 R and 21 to 15 Y, which illustrated 87.70% and 76.92% reduction in color. Overall outcomes of industrial process on neutralization, bleaching and deodorization was found to be 30.66% R, 27.13% Y, 33.66% R, 34.10% Y and 35.66 %R, 38.76% Y, respectively. The color intensity of vegetable oils, mostly removed during bleaching and deodorization process. Vegetable oils with light color intensity are recognized to be more engaging from commercial view-point [11].

Freezing point

Waxes and triglycerides with saturated fatty acids usually cause sediment formation and resist flowing oil easily. Therefore freezing point is a key indicator to measure it. During cottonseed oil refining, it was observed that in neutralization, bleaching and deodorization stages freezing point of crude oil was decreased from 3.0 to 2.5 ^{o}C (16.67%), 2.5 to 2.2 ^{o}C (12%) and 2.2 to 2.1 ^{o}C 4.54%, correspondingly. Overall input of neutralization, bleaching and deodorization was found to be 0.28, 46.92 and 52.79%, respectively, which clearly shows that deodorization has major effect on freezing point.

Smoke point

Simply it can be defined as the temperature at which oil generates continuous thin stream of smoke when heated. Smoke point of oil characterizes appropriateness for frying purpose. During industrial refining process, it was noticed that smoke point of crude oil was decreased from 210 to 212 ^{o}C for neutralization, 212 to 215 ^{o}C for bleaching and 215 to 220 ^{o}C for deodorization stages. Overall input of neutralization, bleaching and deodorization was found to be 11.74, 29.41 and 58.83%, respectively.

Refractive index

The refractive index depends on the triglyceride and fatty acid composition of oil and fat. During analysis it was observed that refining has negligible effect on all stages. Although small variation was noticed from crude to neutralization and bleaching to deodorization as 1.4742 to 1.4743, 1.4744 to 1.4746, respectively. Overall effect on refractive index in terms of percentage was observed in the order of 1.36, 2.65 and 5.51%, respectively for neutralization, bleaching and deodorization.

Chemical parameters of industrially processed cottonseed oil

Chemical parameters are very important for the quality characteristics of edible oil. These parameters are largely essential for the edible point of view as well as industrial uses. Different chemical properties of CSO were checked during the course of refining process as shown in Table 2. The parameters include free fatty acid, saponification value, iodine value, peroxide value and soap content.

***Table 2*. Chemical properties of crude and industrially processed cottonseed oils.**

Parameters	Crude CSO	Neutralized CSO	Bleached CSO	Deodorized CSO
Free Fatty Acids (%)	1.70±0.53	0.28±0.06	0.25±0.03	0.15±0.07
Saponification Value (mg KOH/g of oil)	175.0±0.87	175.2±1.30	174.0±0.75	173.0±0.71
Iodine Value (gI_2/100g of oil)	98.0±0.95	98.5±0.69	97.9±0.77	97.2±0.88
Peroxide Value (mEq O_2 /kg of oil)	3.4±0.97	3.3±0.62	2.2 ±0.61	1.2±0.48
Soap Content (ppm)	-	61.0±0.90	31.0±0.83	15.5±0.79

Free fatty acids (FFA)

The fatty acids which are not chemically bound to glycerol molecules are known as FFA. This parameter is very important indicator to evaluate the edibility of oils. FFA negatively affects the odor, taste, and oxidative stability of oil. Usually, FFA contents in edible oil are removed or decreased by chemical and/or physical refining [12]. During different refining stages, noticeable change in the reduction of FFA was observed in neutralization [13], bleaching and deodorization stages. FFA in crude cottonseed oil was decreased from 1.70 to 0.28% (83.52%) in neutralization stage, 2.8 to 0.25% (10.71%) in bleaching stage and 0.25 to 0.15% (40%) in deodorization stage. If we look at the overall impact on FFA during refining, it can be suggested that all stages has almost equal share for neutralization, bleaching and deodorization 32.12, 32.80 and 35.06 %, respectively.

Saponification value (SV)

The SV depends on the type of fatty acids present in the oil. This value is also important for the soap production point of view. During industrial processing the minor effect on SV was observed from neutralization to deodorization stages. Slight increase of SV in crude to neutralized oil 175 to 175.2 mg KOH/g was observed. Whereas decreasing trend of SV was observed in bleaching and deodorization stages 175.2 to174 mg KOH/g and 174 to 173 mg KOH/g, respectively. Overall impact of refining was found to be 6.04%, 31.31% and 62.65%, respectively for neutralization, bleaching and deodorization.

Iodine value (IV)

IV expresses the degree of unsaturation of oil or fat. It is determined by measuring the amount of iodine reacts with a natural or processed fat under prescribed conditions. It is a well known fact that higher iodine numbers indicates highly unsaturated oil, while reverse is true for least unsaturated oil. The IV classifies the oil as drying and non-drying oils. During refining no any significant change observed in all stages. There was slight change in IV noticed from neutralization to deodorization 98 to 98.5 gI_2/100g, 98.5 to 97.9 gI_2/100g and 97.9 to 97.2 gI_2/100g. Overall input of neutralization, bleaching and deodorization was found to be 35.52, 7.12 and 57.36 %, respectively.

Peroxide value (PV)

The extent of fat or oil oxidation is measured by the amount of peroxides present. Peroxide are the primary compounds formed during the oxidation of unsaturated fatty acids, which may react further to form the compounds that can cause rancidity [14, 15]. During neutralization process no any significant effect observed on PV. On the other hand as compared to neutralization, bleaching [16], and deodorization stages showed somehow positive effect on PV [17]. In the stage of bleaching to deodorization, PV was reduced from 3.3 to 1.2 mEq O_2 /Kg. The overall impact during refining on the removal of PV showed following order 2.85, 34.28 and 62.85%, respectively in neutralization, bleaching and deodorization.

Soap content

Salt of fatty acids in vegetable oil is known as soap content. Stability and shelf life of the oil depends on the soap content, higher the soap content lower the shelf life. Soap content of crude oil was increased from 0.0 to 61% in the neutralization stage. On the other hand declining trend of soap content was observed during

bleaching (61 to 31%) and deodorization (31 to 15.5%) stages. The efficiency of processing on soap content showed following trend 61%, 49.18% and 50 %, respectively for neutralization, bleaching and deodorization. Overall contribution of each refining stage neutralization, bleaching and deodorization was 33.01, 26.61 and 40.36 %, respectively.

Fatty acid composition (FAC)

FAC composition of CSO samples of each refining stages are shown in (Table 3). Six different types of fatty acids were detected as shown in crude CSO (Fig. 1). The FAC of present study was consistent with those previously reported by IUPAC method 2.301 [10] for cottonseed oil. The total saturated fatty acids (SFA) and unsaturated fatty acids (UFA) accounted for CSO as 29.42 and 70.58 %, respectively in the last stage of refining. Linoleic acid (C18:2) showed the highest concentration in cottonseed oil followed by the oleic acid (C18:1), myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0). Poly unsaturated fatty acids (PUFA) are important fatty acids in edible oil, and also more susceptible to oxidative rancidity [18, 19]. From the results of fatty acids, it appeared that refining treatment processes has no important effect [20], except for the slight decrease in the relative percentage of some fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2).

Table 3. **Fatty acid composition of crude and industrially processed cottonseed oil.**

Fatty Acids (%)	Crude CSO	Neutralized CSO	Bleached CSO	Deodorized CSO
Myristic (C14:0)	0.57±0.01	0.54±0.03	0.52±0.08	0.51±0.09
Palmitic acid (C16:0)	26.18±0.59	26.17±0.67	26.16±0.55	26.15±0.45
Palmitoleic acid (C16:1)	0.31±0.04	0.30 ±0.03	0.26±0.05	0.25±0.06
Stearic acid (C18:0)	2.88±0.78	2.80±0.67	2.77±0.61	2.76±0.99
Oleic acid (C18:1)	26.42±0.58	26.33±0.66	26.33±0.88	26.32±0.56
Linoleic acid (C18:2)	43.64±0.12	43.86 ±0.59	43.96±0.89	44.01±0.88
Total Saturated Fatty Acids	29.63	29.51	29.45	29.42
Total Unsaturated Fatty Acids	70.37	70.49	70.55	70.58

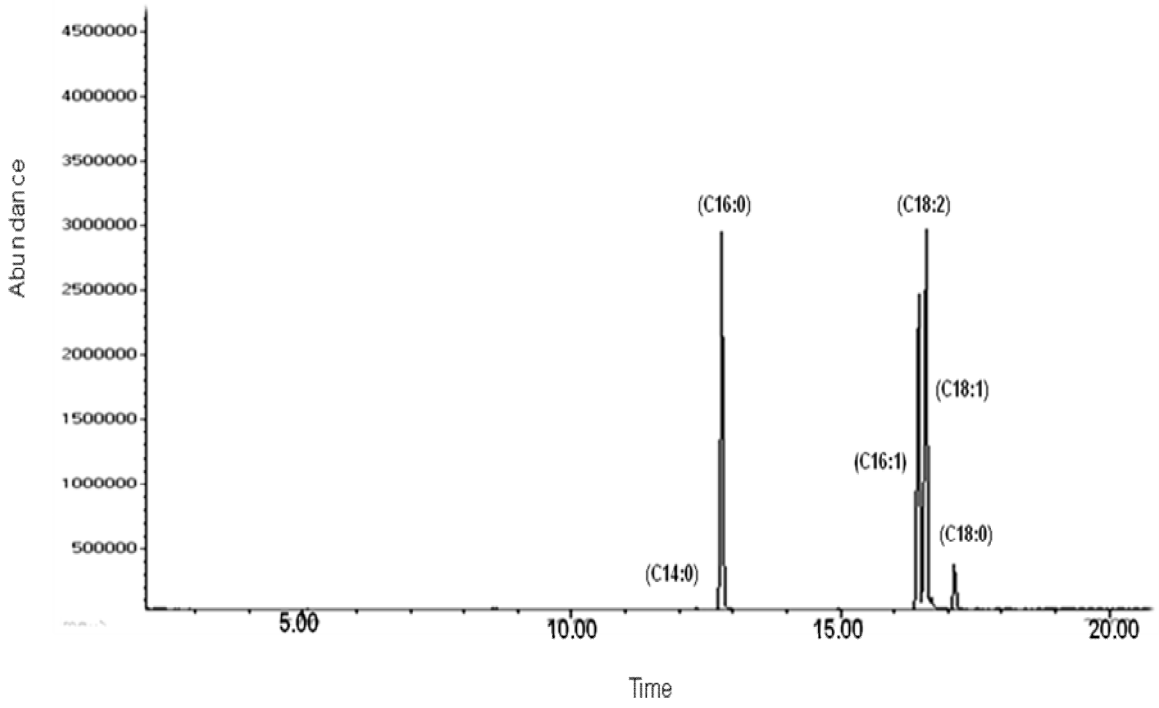

Figure 1. **GC-MS fatty acid chromatogram of cottonseed oil**

Conclusion

The main impact of overall refining and individual steps i.e. neutralization, bleaching and deodorization was found to be in the reduction of color, moisture, soap content, free fatty acid, and peroxide value. However, minor effect was noticed on freezing point, smoke point, refractive index, saponification value and iodine value. Similarly, neutralization, bleaching and deodorization showed negligible effect on the fatty acid composition of the crude cottonseed oil. It can be concluded that refining processes reduced unwanted materials to safe level for the edible application and improved the quality of oil.

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