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Effect of Refining Process on the Quality Characteristics of Soybean and Cotton seed Oils

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A B S T R A C T

The objective of this research was to examine changes in physiochemical properties, oxidation indices, phenolic compounds and fatty acid composition during refining of cottonseed oil and soybean oil. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Rancimat method were used for determination of antioxidant capacities. The results obtained showed that specific gravity have decreased after refining, while refining process does not have a significant effect on the refractive index. Correspondingly, there was reduction in the chemical properties such as acid value, peroxide value, thiobarbituric acid, saponification value and unsaponifiable matter. The refining had a positive effect on the oxidative stability of the oil. Refined cottonseed oil showed relatively higher antioxidant activity by both DPPH and Rancimat methods than refined soybean oil. This study also revealed that the refining process caused approximately 48.8% and 50% decrease of total phenolic contents in soybean and cottonseed oils, respectively. There was an increase in the total monounsaturated fatty acids coupled with a significant decrease in the saturated fatty acids in the oil samples after refining process. The loss of bioactive compounds from crude oils is inevitable during the conventional refining processes. Therefore innovations in these oils processing are necessary to produce commercial oil with high antioxidant content.

Introduction

Vegetable oils are an important part of the human diets world-wide and their production by extraction processes greatly increased in the last century due to higher request and consumption, the increase in world population and the progressive availability of more efficient process technologies and equipment.

Edible oils are widely used by food industry due to their nutritional properties and their influence on the smell and taste of food products (Zulkurnain *et al.,* 2012). During the industrial production of vegetable oils and fats by crushing or solvent extraction, various kinds of minor constituents such as dirt, moisture, gums, waxes, color pigments, flavoring substances, phosphates, carbohydrates, protein, pesticide residues, odoriferous materials, trace metals, free fatty acids and the possible residual content of the solvent used for the extraction process are incorporated into triglycerides and may cause the oil to be darkened, foamed, smoked, precipitated, develop off-flavours, decrease thermal and oxidative stability, and increase oil loss (Ghazani *et al.,* 2013). The term crude

oil is assigned to oil that is not processed after being extracted from the animal or plant material. In order to become suitable for human consumption, crude oils are generally refined to remove the majority of these unwanted components to produce a stable product with desired color and a pleasant taste, with the least possible damages on desirable components and with the least possible loss of oil (Suliman *et al.,* 2013). In general, refined oil is clear, odorless, and resistant to rancidity

The traditional refining process of crude vegetable oils generally includes the steps of degumming, neutralization, bleaching and deodorization. However, during these processes, a large amounts of the micronutrients and antioxidants such as, polyphenols, tocopherols, sterols, carotenoids are lost, which thus reduce substantially the nutritional value and quality of vegetable oils (Szydłowska-Czerniak, 2013).

Cottonseed and soybean oils are among the most unsaturated oils, others being safflower, corn, rapeseed and sunflower seed oils. They are economically important because of their high qualities and quantities of oil. Soybean oil one of the most consumed polyunsaturated vegetable oil worldwide. Alone or in mixture, soybean oil is commonly used for frying as well as for cooking. Soybean oil lie in a group of vegetable oils containing substantial amounts of linolenic acid (C18:3), which is responsible for the development of an off-flavour problem known as flavour reversion (Malheiro *et al.,* 2013). Cottonseed oil has a ratio of 2: 1 of polyunsaturated to saturated fatty acids and generally consists of 65-70% unsaturated fatty acids including 18-24% monounsaturated (oleic) and 42-52% polyunsaturated (linoleic) and 26-35% saturated (palmitic and stearic). Although, specifications for refined oils may differ slightly from those of crude oils due to the

prefix added before the biological source of the oil. These refining processes a times modify the chemical properties and constituents of these oils to the point of which could be detrimental to human health hence this work geared towards evaluating the effect of the refining processes on the physicochemical properties, oxidation indices, antioxidant activity and phenolic compounds of soybean and cottonseed oils as well as their fatty acid profile.

Materials and Methods

Plant material

Crude and refined soybean and cottonseed oils were obtained from Seilla factory for oils and fat production, Elwatania Factory for oils and soap, Fayoum Governorate, Egypt.
Physicochemical properties of crude and refined oils

Physical constants

Specific gravity of the samples was measured using a 25 mL picnometer and a Carl zeiss refractometer was used for measuring the refractive index of the oils (A.O.A.C., 2005). The flow time of the studied oils was measured as an index of viscosity using an Ostwald viscometer for measuring the efflux time in second required to discharge fixed 5 ml oil at a specified temperature (40°C). Lovibond equipment was used for determination of color.

Chemical constants

The iodine value, saponification value, unsaponifiable matter, acid value, and peroxide value were determined according to standard IUPAC methods for the analysis of oils and fats (Dieffenbacher and Pocklington, 1987). The ester value was obtained by subtracting the acid value from the

saponification value. Thiobarbituric acid (TBA) value as an index of fat oxidation was determined directly on the samples as described by (Egan *et al*., 1981). All experiments were conducted in triplicate.

Evaluation of Antioxidant Activity

Determination of DPPH· radical scavenging capacity

The method consisted of spectrophotometric measurement of the acuity of the color change in solution depending on the amount of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The reaction was commenced by mixing 3.9 mL of the methanolic extract with 0.1 mL of DPPH˙(0.012 g/100 mL). The absorbance at λ_{max} 517 nm (UV–visspectronic 2000) was checked at 0, 0.5 and every 0.5 min until the reaction reached a steady state (Suja *et al.,* 2005). The activity of the extract in scavenging DPPH˙ was calculated as follows:

$$\% \text{ DPPH}^\bullet \text{ scavenging} = \left[\frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \right] \times 100$$

Induction period by Rancimat (stability test)

The induction periods, as the oxidative stability index, of the tested samples were measured by an automated Rancimat (Metrohm Ltd. CH-9100 Herisau, Switzeland, model 679), comprises of the control unit and the wet section containing 6 reaction vessels, according to the method described by Mendez *et al.* (1996).

Determination of total phenolic content (TPC)

The total phenolic content was determined spectrophotometrically using the Folin–Ciocalteu reagent, according to the procedure described previously by Orwa and Ansam (2104). 10g of oils was dissolved in 50 mL of hexane and extracted three times with 80% aqueous methanol.The extract was then made up to 100 mL with water and left to stand overnight. A 1 mL aliquot of the extract was mixed with 5ml Folin-Ciocalteus phenol reagent. The mixture was shaken well and let to stand for 5 minutes. Then 1 mL of saturated sodium carbonate (Na_2Co_3) was added and after 1 hour at room temperature the absorbance at 725 nm was measured. Gallic acid was used as standard curve and the results were expressed as mg Gallic acid / 100 g of oil.

Identification of Unsaponifable matter fraction in oils by Gas Chromatography (GC)

The unsaponifiable fractions were collected and taken to dryness under vacuum. The residue was analyzed using the gas chromatograph HP 5890 (Hewlett Packard) equipped with the MS detector (MSD 5970), EI, 70 ev and fitted with a capillarycolumn DB-1701 (12 m x 0.18 mm x 0.4 mm; J&W Scientific). The column temperature was programmed from 260 to 300 °C while injection temperature was set at 280 °C. Helium was the carrier gas at a flow rate of 0.7 cm^3/min. Identification of peaks was based on the retention time of standard substances and MS spectra. Analyses were run in duplicate. Calculations of percent composition of dimethyl hydrocarbons and dimethylsterol fractions were based on the peak area.

Analysis of fatty acid composition

Preparation of Methyl Esters

Methyl esters of fatty acids were prepared in accordance to the method of Morrison and Smith (1964) with some modification. For sample 100 mg adding 1mL BF$_3$/methanol (14%) and 1 mL hexane. The tube is vortexed and placed under nitrogen for 60 min at 100 °C. Esters of fatty acids were then extracted by adding 1 mL of hexane and washing with 2 mL of distilled water. After the centrifugation step (4500 rpm, 10 min, 20 ° C.), the supernatant is recovered in vials and then injected into the GC column.

Chromatographic analysis of the fatty acid methyl esters

Methyl esters were analyzed by GC-type CG-2010 Plus, Shimadzu, equipped with a flameionization detector and a capillary column of 60m length, 0.25 mm internal diameter, the thickness of the film is 0.20 microns. The oven temperature was 200°C. The detector and the injector are at a temperature of 250°C. The samples were separated on the column using helium as the carrier gas with a flow rate of 0.8 mL / min. The sample is injectedin split mode. The temperature program used in the analysis is to keep the unit at 120°C for 2 min and then climb to 180°C for 2 min and keep the sample at 220°C for 25min. The peak integration is done on thesoftware GC solution (Shimadzu). Peak identification of fatty acids on the chromatogram is made using standard fatty acids (Restek, Food industry FAME Mix - methylene chloride 30 mg/mL).

Results and Discussion

Physicochemical properties of the crude and refined oils

Specific gravity is the heaviness of a substance compared to that of water and it is expressed without units. There was a slightly decrease in the specific gravity of soybean and cottonseed oils during the refining process (Table 1). According to Aluyor *et al.* (2009), a slight decrease in specific gravity induced by chemical refining may be because of the removal of some polar compounds, gums and some coloring components from the oil by alkali refining, which affected the weight of the oil after refining. These results are in agreement with those mentioned by Guner *et al.,* (2006) who reported that the specific gravity of different refining oils varies with their molecular weights which are affected by refining process involved.

The Refractive Index (RI) is a parameter that relates to molecular weight, fatty acid chain length, degree of unsaturation and degree of conjugation. It is generalized that the refractive indices of oils increases with increase in the number of double bonds. With increase in temperature, the refractive indices of oils decrease. The refractive indices can also be influenced by oxidative damage of the oil. The RI obtained for all oil samples appeared to be approximately the same (Table 1). Hence, refining process does not have a significant effect on the RI of soybean and cottonseed oils. These findings were supported by previous reports of Dalia *et al.* (2015), who reported that the refractive index was stable throughout the refining. The slightly decrease from 1.4696 ± 0.002 to 1.4685 ± 0.0021 for soybean oil and from 1.4722 ± 0.001 to 1.4690 ± 0.0035 for cottonseed oil after refining, may be due to the continuous removal of impurities during the refining process. According to Pearson (1991), the amount of impurities that are contained in the oil affects the degree of reflection caused by a ray of light during refractive index determination. Ogunsina *et al.* (2014) reported a refractive index of 1.47, almost similar to that found in this study.

Viscosity of oil is a measure of the oil's resistance to shear. High viscosity implies a high resistance to flow while a low viscosity indicates a low resistance to flow. The results in Table 1 show that there was an increasing in flow time of soybean oil during the refining process, while a slight decrease in flow time from crude cottonseed oil to refined cottonseed oil was observed. Results also showed that the flow time is high in cottonseed oil as compared to soybean oil. It explains that the viscosity and density decreases with an increase in unsaturation and increases with high saturation and polymerization (Kim *et al*., 2010).

Carotenoids and chlorophyll are the main color pigments found in vegetable oils. Oxidation of these pigments or reaction of oxidized triglycerides with carotenoids may cause oil darkening (Chewa *et al.,* 2016). Moreover, there are many unwanted compounds like FFA, waxes, and phospholipids in oil might promote oxidation and result to undesirable odor and color in the final product. Therefore, one of the concerns in oil refining process is to reduce these color compounds in the crude oils. Neutralization, bleaching and deodorization are steps that neutralize FFA, reduce the yellow pigments and reduced the red pigment, respectively (Wei *et al.,* 2015). As shown in Table 1 the refined oils had a clear with a light golden color compared to those of crude oils. This is due to the effect of refining process on undesirable components.

The quantity of free fatty acids (FFAs), usually referred as ''the acid value'', is an important determinant of oil quality and has extensively been used as a traditional criterion for classifying olive oil into various commercial grades (Gharby *et al.,* 2015). FFA determination is particularly important for industrial purposes since FFA can modify the organoleptic or physicochemical properties of oil. FFA can act as pro-oxidants in vegetable oils and result in an undesirable taste and flavor (Ghazani *et al.,* 2013). Thus, one of the main objectives of oil refining is to remove FFA from crude oil. As shown in Table 1, the quantity of free fatty acids of crude cottonseed oil were significantly higher than those of the crude soybean oil. This may be due to the fact that the cottonseed oil had been exposed to less suitable conditions of storage and/or a strong enzymatic hydrolysis of cottonseeds during harvesting, handling or oil extraction (Gharby *et al.,* 2014). The acid value of the crude soybean oil (1.81±0.11 mg KOH/g oil) was almost similar to that reported by Reza *et al.* (2009) in crude soybean oil (2.09 mg KOH/g oil). The FFA of soybean and cottonseed oils was significantly reduced during the oil refining process with a removal of 91.5% and 97.5% of FFA in the crude oils, respectively. It has been reported that there is some decrease in the FFA content after the bleaching and degumming steps, while its major change is observed after the neutralization step, when they are saponified with sodium hydroxide and separated in soap stock . Deodorization is a vacuum-steam distillation at an elevated temperature during which, FFA and volatile odoriferous components can be eliminated in order to produce bland and odorless oil (Reza *et al.,* 2009; Onyema and Ibe 2016). These values in this study are within the ranged specified for edible oils as given by FAO/WHO (2010).

Peroxide value (PV) is used as a measure of the extent to which rancidity reactions have occurred during storage and could be used as an indication of the quality and stability of fats and oils. PV reflected the formation of hydroperoxides that caused by the primary oxidation. It may break down into non volatile secondary product, which decreases the quality of oil. The amount of oxidation products in the soybean and cottonseed oils changed after refining process (Table 1).

After the refining process, the PV decreased from 12.995±0.58 and 3.81±0.23 meq O_2/kg to 4.75±0.26 and 1.955±0.05 meq O_2/kg in soybean and cottonseed oils, respectively. The peroxide value of refined soybean oil (4.75 meq O_2/kg) in this study was almost similar to that reported by Ortega-García *et al.* (2006) in safflower oil (3.56 meq O_2/kg) and Noel *et al.* (2016) in sesame oil (3.44 meq O_2/kg). The refining has a positive effect on the oxidative stability of the oil. According to Kreps *et al.* (2014), the refining process removes 79.2% of hydroperoxides from crude oil. Nzikou *et al.* (2009) and Anwar *et al.* (2007) reported peroxide values of 1.67 and 1.27 meq/kg of soybean oil, respectively; these values are lower than those obtained in the present study. PV is an index of rancidity, thus the high PV of soybean oil indicated their poor resistance to peroxidation during storage. Shahidi and Zhong (2005) and Nyam *et al.* (2013) noted that the increase in peroxide value might be caused by low temperatures during the last phase of ripening in seeds or by the use of high temperatures in the process of oil extraction or due to the accumulation of hydroperoxides as a result of free radical attacking the unsaturated fatty acids. The results also revealed that cottonseed oil stability to oxidation is relatively better than soybean oil. This is because cottonseed oil includes high level of linoleic acid (18:2n -6) content but soybean oil is rich in α-linolenic acid (18:3n -3) in addition to linoleic acid (Table 4). These results indicate that oils with high linolenic contents have low oxidative stabilities. These values in the present study are in line with the standard specified by FAO/WHO (2010) for fresh edible oil, which is below 10 meq O_2/kg.

The saponification value (SV) describes the average molecular weight of the fatty acids in the lipids. Table 1 shows the SV of both the crude and refined oil samples under investigation. Slight decrease in saponification values could be noticed after the refining process for cottonseed oil, while soybean oil exhibited a little change with regards to the refining of the oil. These results are in agreement with those of Onyema and Ibe (2016) who noted that the saponification value decreased with the progression of the oils from crude to refined state. The decrease is understandable with the purification of the oil as the percentage free fatty acids and other fatty components with high molecular weight in the oil being reduced during refining (Chawla, 2003).

The ester value is calculated by subtracting the acid value of oil from the saponification value of the corresponding oils. It is found that in Table 1 the ester values showed a pattern in good agreement with that of the SV.

The determination of iodine is useful as a measure of the degree of unsaturation of the oils and to give some idea of their oxidative stability. High iodine-value oil contains a greater number of double bonds than low iodine-value oil and has usually a reduced oxidative stability. The changes of iodine value (IV) of the soybean and cottonseed oils are presented in Table 1. The iodine value obtained for soybean and cottonseed oils has increased during the refining process; this was an indication that the degree of unsaturation increases progressively as a result of refining. These findings are not supported by previous reports of Dalia *et al.* (2015), who reported that the iodine content was stable throughout the refining.

Unsaponifiable matter (USM) contributes to the stability of edible oil and fatty food through their antioxidant activity. The unsaponifiable matter of the crude and refined oils is showed in Table (1). The unsaponifiable matter decreased from 1.13% to 0.84% for crude and refined cottonseed oil respectively and 0.86% to 0.43% for crude

and refined soybean oil respectively after refining, because virtually all the refining processes lead to reduction in the unsaponifiable matter. Our results are in discordance with those obtained by Onyema and Ibe (2016) who found that the unsaponifiable matter in soybean oil increased after refining.

The oxidative state of any vegetable oil can be evaluated from the combined analysis of its peroxide value and thiobarbituric acid (TBA) which indicates the presence of primary and secondary oxidation products, respectively (Gharby *et al.,* 2014).

As shown in Fig. 1, the contents of TBA decreased also during the refining process from 0.076 to 0.056 malonaldehyde absorbance/kg for crude and refined soybean oil respectively and 0.998 to 0.043 malonaldehyde absorbance/kg for crude and refined cottonseed oil respectively. In general, the refined oils of the present study contain relatively low absorbance value which indicates a slow production of secondary oxidative products thereby greater stability in terms of the secondary oxidation.

Evaluation of antioxidant activity of oil samples

DPPH radical-scavenging activity

In the present study, influence of the refining processes on antioxidant activity of soybean and cottonseed oils have been evaluated by DPPH. DPPH$^{\bullet}$ is considered to be a model of a stable lipophilic radical with deep violet colour. Antioxidants react with DPPH$^{\bullet}$, reducing the number of DPPH$^{\bullet}$ free radicals equal to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH$^{\bullet}$. The scavenging activity of soybean and cottonseed oils against DPPH$^{\bullet}$

was concentration-dependent (Fig. 2). Refined cottonseed oil showed relatively high antioxidant activity than refined soybean oil at all the concentrations; this is might be due to the considerably higher level of unsaponifiable matter in refined cottonseed oil. The refined oils were considerably less effective radical-scavengers than the crude oils, indicating that antioxidants or active compounds could be removed during refining process.

Rancimat method

The susceptibility of crude and refined oils to oxidation was measured by the well-established Rancimat method and expressed as induction period (Gharby *et al.,* 2015). The Rancimat method is an accelerated aging test and a commonly used procedure in the food industry for the examination of the oxidative stability of edible oils and prediction of their shelf life. Rancimat results, due to the formation of secondary reaction products (Table 2), showed a pattern in good agreement with that of DPPH methods. As can be seen in Table 2, induction period of crude soybean oil was (24.6), while it was (21.18) for crude cottonseed oil.

The high oxidative stability of the crude oils may be due to their higher level of natural antioxidants, phenolic compounds and phosphatides that have synergistic effects on antioxidants (Taylor, 2005). On the other hand, the induction period of refined soybean and cottonseed oils were 1.90 and 2.30, respectively. These reductions in oxidative stability after refining process might be due to degumming and neutralization steps which remove these active compounds. Barriuso *et al.,* (2013) reported that phospholipid content of soybean oil reduced from 1-3% to essentially zero after degumming and alkali refining steps.

Total phenolic content

Total phenolic contents (TPC) were determined by using Folin-Ciocalteu reagent (FCR). Folin-Ciocalteu reagent reacts nonspecifically with phenolic compounds as it can be reduced by a number of non phenolic compounds. The amount of total phenolics determined in crude and refined oils is shown in Table 2. It is notable that the refining process caused approximately 48.8% and 50% decrease of TPC in soybean and cottonseed oils, respectively. For comparison, in the work of Aleksandra *et al.,* (2011), the refining process caused approximately from 26% to 55% decrease of TPC in palm oil. This is might due to the unstable of phenolic compounds during the oil refining process with heat treatment applied. Szydłowska-Czerniak *et al.,* (2008) observed that the highest losses of TPC occurred in the neutralization step of the rapeseed and soybean oil refining (40% and 64%, respectively). However, the amounts of total phenolic compounds in the refined cottonseed oil were about 3.5 times lower than the refined soybean oil. Refined cottonseed oil showed relatively high antioxidant activity by both DPPH and Rancimat methods than refined soybean oil. This is because antioxidant activity could not be predicted on the basis of the TPC only due to the synergism of polyphenolic compound with one another or with other components present in the sample with antioxidant potential that may contribute to the overall observed antioxidant activity (Ordonez *et al.,* 2005). These findings are in close agreement with previous findings of Yu *et al.* (2002) who reported that there is no correlation between the content of these main antioxidant compounds and radical-scavenging capacity.

Minor compounds of crude and refined oils

Soybean and cottonseed oils, like the majority of crude vegetable oils, contain a variety of minor components which generally constitute the unsaponifiable matter. The unsaponifiable matters present in vegetable oils and fats are usually composed of sterols, fatty alcohols, tocopherols, triterpene alcohols, and hydrocarbon (squalene) which have individual biological importance. Some of the above constituents may exert a pronounced effect on stability of the oils and their nutritional properties. Table 3 shows the changes in phytosterol, fatty alcohols, tocopherol, triterpene alcohols and hydrocarbon contents during the refining process.

In this study, Y-Sitosterols was the most abundant phytosterol in crude soybean and cottonseed oils, followed by campesterol, which agreed with the previous study of Chew *et al.* (2015). Phytosterols are encouraged to include in the human diet as their ability to lower blood cholesterol and phytosterols possess antibacterial, anti-inflammatory, antifungal, and antitumoral activities (Garoufi *et al.,* 2014). Owing to their role in the protection against oxidative deterioration of polyunsaturated fatty acids in plant material, tocopherols in seed oil are extremely important. Tocopherols have inhibitory effect on LDL oxidation and they have several nutritional benefits. Small amounts of hydrocarbons and triterpene alcohols were present in the refined oils. The whole refining process had removed 100% of the total phytosterol and tocopherols contents in both soybean and cottonseed oils. This could be explained that during industrial processing of oils and fats, sterols undergo dehydration, resulting in the formation of sterenes. Sterenes are formed during bleaching due to acid catalyzed, dehydration on the surface of the acid-activated bleaching earth or due to dehydration promoted by heat during the deodorization process (EL-Mallah *et al.*, 2011). During neutralization, the phytosterols are transferred by liquid-liquid

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partitioning to soap stock (Karabulut*et al.,* 2005). These results are in agreement with the findings of Rossi *et al.,* (2007) who reported the tocopherols are generally themselves first oxidized and are quickly decomposed by oxidation reactions or during the refining process and will therefore be present in a small proportion or essentially zero. On the other hand, our result was different from the previous studies, which showed a reduction in total tocopherol contents during bleaching, neutralization, and deodorization, and this is might due to their possible adsorption on bleaching earth, the tocopherol are unstable in the presence of oxygen and strong alkali condition, and the tocopherol might distillate under deodorizing conditions respectively (Suliman *et al.,* 2013).

Effect of refining on fatty acids composition of soybean and cottonseed oils

The fatty acids composition is an essential indicator of the nutritional value of the oil. Fatty acids from the crude and refined oils under study were identified and quantified using gas chromatography analyses. The fatty acid profile of crude and refined oils of soybean and cottonseed is presented in Table 4. In general, the oils exhibited a fatty acid profile with linoleic acid (C18:2n6) as the most prevalent fatty acid. The high percentage of linoleic acid recommended that these oils could be used as good sources of essential fatty acid due to their ability to reduce serum cholesterol and LDL level (Nyam *et al.,* 2009).

Table.1 Physicochemical properties of crude and refined soybean and cottonseed oilsa

Parameter	Crude soybean oil	Refined soybean oil	Crude cottonseed oil	Refined cottonseed oil
Specific gravity	0.9192±0.002	0.9167±0.004	0.9385±0.0030	0.9067±0.003
Refractive Index (RI)	1.4696±0.002	1.4685±0.0021	1.4722±0.001	1.4690±0.0035
Flow Time (Sec.)	4.885±0.007	6.250±0.042	7.815±0.22	7.290±0.099
Color	8 /35	1.3 /18	12 /35	8.5 /35
Acid value (A.V) (mg KOH / g oil)	1.81±0.11	0.145±0.007	6.11±0.10	0.135±0.01
Peroxide value (P. V) (meq O_2 / kg oil)	12.995±0.58	4.75±0.26	3.81±0.23	1.955±0.05
Saponification value (mg /g oil)	172.99±0.91	171.11±0.72	180.78±0.01	168.075±2.91
Ester value (mg / g oil)	171.18±2.46	170.97±0.73	174.67±0.11	167.94±2.91
Iodine value (IV) (g I_2 / 100 g of oil)	110.985±1.62	117.01±0.311	113.75±1.41	127.635±1.12
Unsaponifiable matter (%)	0.86±0.005	0.43±0.002	1.13±0.01	0.84±0.012

*Each value represents mean ± SD of three replicates.

Table.2 Oxidative stability and total phenolic content of oil samples

Sample	Oxidative stability (Induction Period / hr.)	Total phenolic content[a] (mg Gallic acid / 100 g of oil)
Crude soybean oil	24.60	64.37±0.54
Refined soybean oil	1.90	32.94± 0.92
Crude cottonseed oil	21.18	19.73± 0.27
Refined cottonseed oil	2.30	9.87±0.18

[a] Each value represents mean ± SD of three replicates

Table.3 Identification of unsaponifiable matters of crude and refined oils using GC-MS analysis.

Components Identified	Crude cottonseed oil	Refined cottonseed oil	Crude soybean oil	Refined soybean oil
Phytosterols				
Campesterol	2.550	nd	2.601	nd
Stigmasterol	nd	nd	2.328	nd
Y-Sitosterols	31.965	nd	7.853	nd
Pyracholestrobine	nd	nd	0.650	nd
Hydrocarbon				
Hexadecanoic acid	1.262	nd	2.546	nd
Heptacosan	nd	1.185	nd	1.780
Hexacosane	nd	nd	nd	2.290
Octadecanoic acid	nd	1.232	6.832	nd
1-Nonadecene	1.547	nd	3.955	nd
1-docosene	1.552	nd	0.524	nd
Pentatriacontene	1.262	nd	nd	0.726
Tochopherols	0.947	nd	nd	nd
Phenols				
Epoxiconazole	nd	nd	2.776	nd
Triterpene alcohols				
1-Tetracosanol	nd	nd	0.087	nd
Octadecanal	1.182	1.182	nd	0.598
Octatriacontanol	1.142	nd	nd	nd
Hexsadecanol	1.480	nd	nd	nd
1-Dodecanol	1.102	nd	nd	nd

nd: not detected

Table.4 Fatty acid composition (%) of crude and refined soybean and cottonseed oils

Compounds	Crude cottonseed oil	Refined cottonseed oil	Crude soybean oil	Refined soybean oil
C14 Myristic	0.66	0.06	0.08	0.08
C16 Palmitic	24.55	6.46	10.69	8.38
C16:1 Palmitoleic	0.66	0.07	4.37	4.42
C17 Margaric	0.19	nd	0.08	nd
C18 Stearic	0.21	3.46	4.12	4.12
C18:1 n9 cis Oleic	19.05	24.39	21.64	28.53
C18:1 n9 t Elaidic	2.10	nd	nd	nd
C18:2 n6 cis Linoleic	51.54	64.30	41.08	37.14
C18:3 n3 Linolenic	0.19	0.33	15.92	12.73
C20 arachidic	0.21	nd	0.03	0.14
C20:1 n-9 Gondoic	0.12	0.07	1.49	1.86
C22 Behenic acid	0.17	0.56	0.27	0.15
Unknown compound	0.34	0.31	0.25	2.45
Total SFA	26.00	10.54	15.26	12.87
Total unsaturated	73.66	89.15	84.49	84.68
Total MUFA	21.93	24.52	27.49	34.82
Total PUFA	51.73	64.63	57.00	49.86
SFA /UnSFA	0.35	0.12	0.18	0.15

[a] Results are expressed as percentage of the total fatty acids.
SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, UnSFA: unsaturated fatty acids, nd: not detected

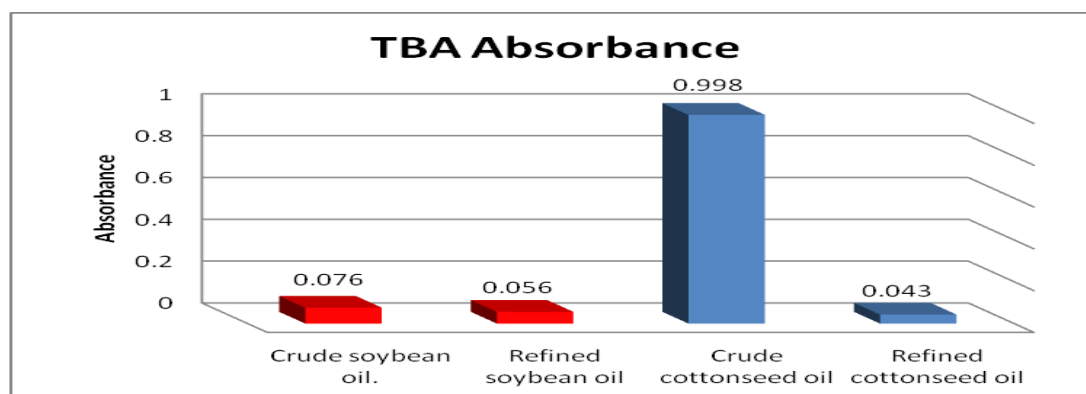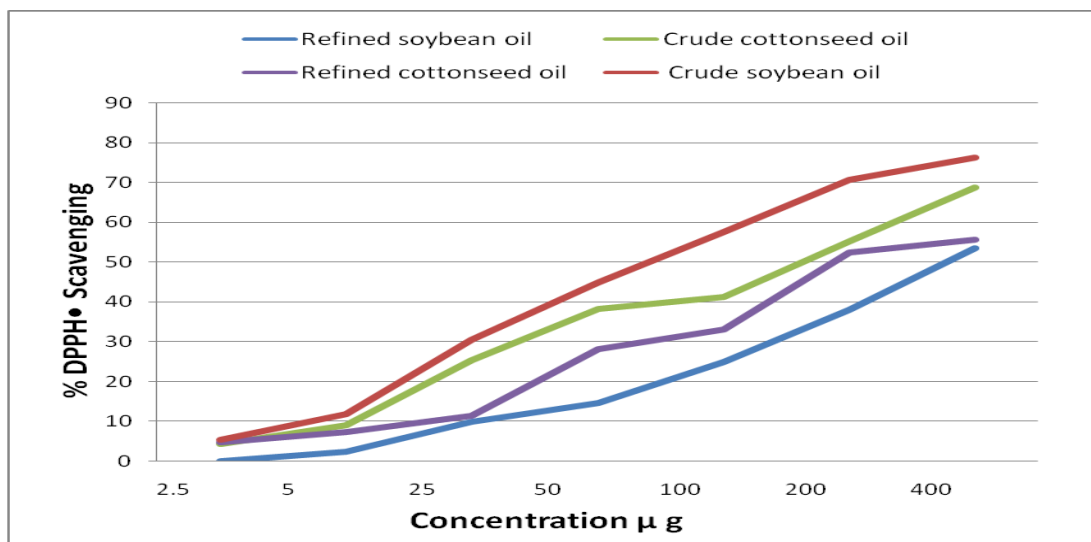**Fig.1** TBA value (as absorbance at 517 nm) of oils

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Fig.2 Scavenging activity of crude and refined soybean and cotton seed oils at different concentrations.

Previous studies showed that the refining process did not have a significant effect on the fatty acid composition of hazelnut oil and groundnut oil (Aluyor *et al.,* 2009). The results obtained by us do not support these claim, which a significant increase in the total monounsaturated fatty acids (MUFA) coupled with a significant decrease in the saturated fatty acids in the oil samples after the refining process were shown in the present work. Budiadnyani *et al.,* (2015) were reported that the saturated fatty acids in by product fish oil of tuna meal processing were decreased also during the refining process from 36.8% to 31.9%. The oils under study are highly unsaturated, with total polyunsaturated fatty acids (PUFA) ranged between 49.86% (refined soybean oil) and 64.63% (refined cottonseed oil). There was a slight decrease in linolenic acid (C18:3n3) in refined soybean oil compared to crude oil, while the oleic acid contents for both soybean and cottonseed oils have increased during the refining process.

Moderate amounts of palmitic acid were present in the refined soybean and cottonseed oils. A slight decrease in linolenic acid might be due to the oxidation at a higher temperature during the refining process caused to the increase in oleic acid (Bachari-Saleh *et al.,* 2013). The presence of high content of oleic acid in the refined oil samples make them desirable in terms of nutrition and high stability to act as cooking and frying oil. Besides that, a moderate level of palmitic acid is needed for the production of margarine, shortening and other fat products (Coetzee *et al.,* 2008). The ratio of saturated fatty acids to unsaturated fatty acids in refined oil samples was found 0.12 and 0.15, which clearly indicated the high amount of unsaturated fatty acids and can be positively considered from the nutritional point of view

In conclusion, refining is a necessary process to make a vegetable oil suitable for human consumption. In the present study, the impact of refining process on the physicochemical properties, antioxidant activity and bioactive compounds of soybean and cottonseed oils as well as their fatty acid profile were studied. Evidently, refining processes have more significant changes to chemical properties of the vegetable oils investigated than the physical parameter. Basically, the change is seen to be a general reduction in the chemical

properties is encouraged as they lead to the improvement on the quality of the refined oil. However, physical properties enjoy little or no effect while overlooking colour with significant reduction. While commending this, the decrease in the unsaponifiable matter is highly unacceptable to some extent though it increases refining losses, its overall importance cannot be overemphasized. Hence, methods for isolation of these unsaponifiable matter should be devised and attached to the refining system to reintroduce them to the oil while limiting their refining effects due to its importance (stability, nutrition, medicine) which cannot be neglected.

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