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VARIATIONS IN PHYSICOCHEMICAL ATTRIBUTES OF SEED OIL AMONG DIFFERENT VARIETIES OF COTTON (*GOSSYPIUM HIRSUTUM* L.)

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Abstract

Variation in the physicochemical attributes of the seeds and the extracted seed oils from six varieties (CIM-496, N-121, Z-33, AA-802, Desi, and CIM-534) of cotton (*Gossypium hirsutum* L.) were appraised. The amount of oil and protein in the tested seeds varied from 15.06 to 18.35% and 20.42 and 27.03%, respectively revealing a significant (p<0.05) differences among varieties analyzed while the contents of fiber (20.65-21.31%), ash (3.46-4.64%) and moisture (6.36-8.44%) did not vary considerably.The physicochemical properties including density (24ºC) 0.9154-0.9207 mg/mL, refractive index (40°C) 1.4607-1.4632, iodine value 100.54-108.73 I/100g of oil, saponification value 180.39-190.28 (mg of KOH/g of oil), unsaponifiable matter 0.49-0.58%, free fatty acids content 0.71-1.24% %, and color 12.01-13.04 R +63.61-68.11Yof the extracted cottonseed oils (CSOs) indicated a slight variation among varieties selected. The oxidation parameters of CSOs, as assessed by estimation of conjugated dienes, conjugated trienes, peroxide value, *para*-anisidine and induction period (Rancimat, 20 L/ h, 120°C), were noted to be 2.32-2.61, 0.91-0.99, 1.81-1.98 (meq/ kg of oil), 2.00-2.31and 3.19-3.61 h, respectively. The tested CSOs mainly contained linoleic acid (48.96-50.46%), followed by palmitic acid (24.42-25.80%), oleic acid (17.81-23.15%) and stearic acid (2.49-2.81%). The contents of α (125.47-296.20), γ (269.23-326.21) and δ (2.23-5.47 mg/kg) tocopherols among CSOs varied significantly. In conclusion, some of the physicochemical parameters of the oils varied significantly (p<0.05) among varieties selected that might be attributed to the different genetic makeup of the cotton plants. The results of this study can be useful for the selection of an appropriate cotton variety in the specified area.

Key-words: Cottonseed oil yield, Analytical characterization, Iodine value, Oxidation status, Fatty acids, Tocopherols.

Introduction

Cotton, from the family *Malvaceae* and the genus *Gossypium*, has a number of species such as *G. hirsutum, G. barbadense, G. arboreum* and *G. herbaceum* of commercial importance and food value (Percival *et al*., 1999; Wrostlad, 2003). Many varieties of cotton have been developed with improved crop yield and productivity (Calhoun & Bowman, 1999). *Gossypium hirsutum* being a native to tropical and sub-tropical regions, is the most important fiber/food crop in the world. It is also considered as one of the best source of plant (vegetable) protein after soybean and the fifth prominent seed oil crop after soybean, palm, canola and sunflower (Sawan *et al*., 2006).

Cotton is regarded as one of the important conventional oilseed crops with potential to bridge the existing gap between the supply and domestic demand of vegetable oils (Sekhar & Rao, 2011). Cotton as a major crop covers about 2.5% of the world's cultivated lands and is also recognized to be a dual-purpose crop, used both for its natural fiber (Ashraf, 2002), as well as contributes to almost 4% of the world's vegetable oil production. In Pakistan, it contributes 65-70% in the local edible oil industry (Khan & Hassan, 2011).

Cotton seeds are directly or indirectly employed in human food and the livestock (Bertrand *et al*., 2005; Elangovan *et al*., 2006). The seeds are a good source of oil and protein (Saxena *et al*., 2011). Technically, CSO is suitable as ingredient for baked goods and cake icings and is being employed for domestic frying and preparation of margarine (butter substitute) and hydrogenated vegetable oil/vegetable ghee (Sekhar & Rao, 2011). CSO is a good source of an essential fatty acid (linoleic acid, C18:2) as well as contains reasonable amount of oleic acid (Brien *et al*., 2005). The oil has good oxidative stability due to its high antioxidant tocopherol contents and oleic acid (Brien *et al*., 2000; Sekhar & Rao, 2011). As far as the industrial uses of cotton seed oil are concerned, this oil is in use for preparation of alkyl resins for interior paints, special bio-lubricants and soft soaps (Saxena *et al*., 2011). The oil has also been explored as a feedstock for biodiesel production (Rashid *et al*., 2009).

The physicochemical properties and attributes of vegetable oils, in particular, the fatty acids profile, is an important factor in establishing their commercial uses and market value (Akbar *et al*., 2009; Dep *et al*., 2013). It is commonly accepted that the yield, chemical composition, nutritive and functional properties of plant seed oils are significantly affected by the genetic/varietal, agroclimatic and geographic factors (Bambawale *et al*., 2004; Figueiredo *et al*., 2008). Similarly, plant tolerance to drought is reported to affect the composition and quality of seed lipids. The soil salinity shows an adverse effect on the plant growth and yield but a cotton variety namely CIM-473 is quite resistant to the salts and hence can be recommended for cultivation in saline areas (Anjum *et al*., 2005).

Bahawalpur district is divided mainly into three regions such as the riverine areas along river Sutlej, the canal water irrigated plain areas and the Cholistan desert. The weather of the district Bahawalpur is quite hot and dry in summer but cold and dry in winter season. The usual rainfall is quite low because the district is situated at the tail end of monsoon area. There are three

types of soils relating to the above specified regions resulting in a unique topographical combination suitable for cultivation of various food and feed crops. Canal irrigated plain areas in the district of Bahawalpur are blessed for the production of wheat, sugarcane, sunflower, lentils, mustard/rapeseed with cotton as the major cash crop (Chaudhary, 2003).

It is expected that varietal variations might affect the seed oil yield and oil quality characteristics; hence it seems rather interesting to appraise these parameters of oil from different cotton varieties grown widely in the district of Bahawalpur. The present study, therefore, was designed with the prime objective to investigate the variations of the physicochemical characteristics among the oils extracted from selected varieties of cottonseeds cultivated in Bahawalpur district (Punjab), Pakistan.

Materials and Methods

Collection of samples: The seeds of six varieties of cotton (*Gossypium hirsutum* L.) namely CIM-496, N-121, Z-33, AA-802, Desi, and CIM-534 were procured/collected directly from agricultural fields of different areas of Bahawalpur district (harvested at 13BC, Yazman; Clay loam, Hasilpur; Loam, Khairpur; Loam), Punjab, Pakistan. The seeds/specimens were further identified and authenticated from the Department of Agriculture Bahawalpur, Pakistan. For each variety 2 kg seeds were obtained. After removing the impurities, representative seeds were taken for the estimation of moisture content and the remainder was dried, ground/crushed and stored in airtight polythene bags until used for extraction and further analyses.

Reagents, chemicals, and standards: Pure standards of selected tocopherols [DL-α-tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol], and esters of fatty acids and ascorbic acid were got from Sigma Chemical Co. (St. Louis, MO, USA). All analytical and/or HPLC grade reagents and chemicals were used from Merck (Darmstadt, Germany) or Sigma Aldrich (Busch, Switzerland) or Merck (Darmstadt, Germany).

Oil extraction: The ground cotton seed (50g) material, packed in a cellulose thimble, was placed in a glass Soxhelt extractor, fitted with 1.L round bottomed flask and a cold water condenser. The extraction was done with 0.5 L of *n*-hexane on a water bath. The excess extraction solvent was removed by distilling off under reduced pressure in a rotary evaporator (Eyela N-N Series, Rikakikai Co. Ltd., Tokyo, Japan) at 45^{o}C yielding crude cotton seed oil (CSO). The CSO was stored under refrigerator (4 ^{o}C), until used for further analyses. The defatted meal thus obtained (oil seed residue/meal) was ground in a mortar and pestle to uniform particle size and analyzed for proximate parameters.

Analysis of oilseed residues: The cottonseed residues (meals), for each of the tested varieties, after oil removal, were analyzed for contents of protein, fiber and ash. Protein content (percent N × 6.25) was estimated according to the Association of Official Analytical Chemists AOAC standard method 976.06 (AOAC, 1990) using a Kjeldahl apparatus. The fiber and ash contents were determined according to the ISO method 5983 (ISO, 1981) and ISO method 749 (ISO, 1977), respectively.

Analysis of extracted oils

Physical and chemical parameters of oils: Density, refractive index (RI), iodine value (IV), peroxide value (PV), free fatty acids (FFA), saponification value (SV) and unsaponifiable matter (USM) of the extracted CSOs were analyzed as per standard AOCS methods (AOCS, 1997). The color of oil was measured with a Tintometer in a 1-inch measuring cell. Specific extinctions in expressions of $\square_{1cm}^{1\%}$ were considered after measuring the absorbance of oil at 232 and 270 nm. A Hitachi U-2001spectrophotometer (Hitachi, Instruments Inc., Tokyo/Japan) was used for recording the absorbance. The CSO samples were mixed with *iso*-octane to take the absorbance readings and $\square_{1cm}^{1\%}$ were calculated according to the IUPAC method II D.23 (IUPAC, 1987). *Para*-anisidine value for the oils was also determined following IUPAC method (IUPAC, 1987).

Oxidative stability: An auto-matically controlled Metrohm Rancimat apparatus (Model 679) was used to record the induction period (IP) of CSOs. The operating temperature of Rancimat machine was maintained at 120 ± 0.1°C, and IP noted following the procedure described earlier (Anwar *et al*., 2003). Samples in duplicate (2.5 g) were weighed (placed) in glass reaction tubes and run concurrently. IP was noted automatically and was linked and corresponded to the break points of the curves plotted.

Fatty acids composition: The oil fatty acids (FAs) were converted into their respective FA methyl esters (FAMEs) via transmethylation according to IUPAC method 2.301 (IUPAC, 1987) and analyzed on a SHIMADZU gas chromatograph machine (model 17-A), fitted with a flame ionization detector/ FID. The separation of FAMEs was done on an SP-2330 (SUPELCO, INC., Supelco Park, Bellefonte, PA, 16823-0048 USA) methyl-lignocerate-coated (thickness of the coating/film 0.20 µm), polar capillary (open tubular) column (dimension 30 m x 0.32 mm). Pure (oxygen free) nitrogen (N_2) gas (flow rate 3 mL/ min) was used as a mobile phase/carrier. Of the other operating parameters, the preliminary temperature was set at 180^{o}C and increased by the rate of 5^{o}C/ min to a final temperature of 220^{o}C. The initial and final hold up temperature was kept to be 2 min and 10 min, respectively. The injector temperature was maintained at 230^{o}C while that of the detector at 240^{o}C. A sample volume of 1.0 µL was injected using split injection mode. $FAME_S$ were identified by matching/relating their relative and absolute retention times (RT) with those of pure and authentic standards from Sigma Chemical Co. (St Louis, MO, USA).The FA composition was expressed as a relative percentage of the total peak areas (TPAs in%).

Tocopherols content: Analysis of the tocopherol compounds (α, γ and δ) in CSOs was performed by HPLC following the procedure described earlier (Wrolstad, 2003). Samples were prepared by taking oil (0.1g) along with ascorbic acid (0.05 g) in a test tube (with Teflon cape) followed by addition of five milliliters of 90% ethanol and 0.5 mL of 80% aqueous (w/v) KOH solution in to the same tube. The contents of the tube were thoroughly mixed by vortexing on a vortex machine for 60 s. The tube was purged with nitrogen, capped and subjected to incubation at 70^{o}C for 30 minutes in a water bath with occasional vortexing.

After cooling the tube at room temperature, 3 mL deionized water and 5 mL *n*-hexane were added and the tube vortexed again for 30 s followed by centrifugation at (1,000× *g*) for 10 min. The top (hexane) layer was separated in another tube. The aqueous layer and the residue, left over, were re-extracted and the top hexane layer collected by exercising the same procedure. The hexane layers from both the extractions were pooled and then evaporated to dryness under nitrogen streaming.

One mL of the mobile phase was added to re-dissolve the extract obtained and then transferred to an HPLC sample vial. A 20-µLsample volume was injected into a Supelcosil C-18 column (250 × 4.6 mm; Supelco Inc.) and eluted with a mobile phase consisting of a binary mixture of acetonitrile: MeOH (65: 35 v/v) at a flow rate of 1.0 mL/ min. Detection of the tocopherols was performed at 295 nm whereas these were identified by comparing their retention times with those of authentic standard compounds. The tocopherols were quantified relative to peak areas of the standards used according to an external standard calibration procedure.

Statistical analysis: The samples were analyzed separately in triplicate. Data were reported as means ± SD (n=3×3). ANOVA (one way analysis of variance) was used to appraise the significant differences of the means ± SD (n=3×3) among the varieties, considering a level of significance to be less than 5% (p<0.05) by using statistical software ANOVA (Steel *et al*., 1997).

Results and Discussion

Physical and chemical parameters of oils: The data for the proximate analysis of selected varieties (CIM-496, N-121, Z-33, AA-802, Desi, and CIM-534) of cottonseed harvested from different areas of Bahawalpur are given in Table 1. The oil content from different varieties of cotton seed ranged from 15.06 to 18.35 %.The oil yield was highest (18.35%) in variety N-121 whereas lowest (15.06%) in Desi.

The present oil yield (18.35%) in variety N-121 was quite comparable with another cotton variety namely NIAB-III (18.60%) harvested from Faisalabad (Ahmad *et al*., 2007). The oil yield of presently tested cotton seed varieties was lower than the seed oil yield of cotton (*G. hirsutum)* variety SLH-279 cultivated in Peshawar, 30.15%; CIM-506; 29.10% and CIM-499; 27.52% (Khan *et al*., 2010). This variation in the seed oil yield from local cotton might be ascribed to the diversity in agro-climatic conditions as well as the genetic make-up of the cotton plants. Cotton seed oil yield is controlled by multiple genes and is strongly influenced by the environment factors (Ashokkumar & Ravikesavan, 2011). Seed oil contents in the present analysis were comparable to several other cotton species primarily G. *arboretum*; 14.4-18.7% and *G. hirsutum*; 15.8-20.2% (Sharma *et al*., 2009) but lower than *G. arboretum*; 22.89%, and higher than a wild specie *G. stocksii* of India (Gotmare *et al*., 2004).

The range of seed oil yield (15.06-18.35%) of the present work was in line to those of the oil contents of cottonseed grown in India; 15-20%, 19.50% (Bambawale *et al*., 2004; Dawodu, 2009), Nigeria; 15-20% (Dawodu, 2009; Sekhar & Rao, 2011), 15.0-24.0% (Brien & Wakelyn, 2005) and some other Asian and European countries 15.0-24% (Rossell & Pritchard, 1991). However, the present oil yield was lower than cottonseed varieties of Nigeria, 42% (Dawodu, 2009). Such variation in oil yield across countries might be mainly ascribed to the agro-climatic conditions of the regions in addition to genetic make-up of the cotton plants.

Analysis of the oilseed residues revealed a notable (p>0.05) variation for protein content (20.42 to 27.03%) but the amount of fiber (20.65-21.31%), ash (3.46-4.64%) and moisture (6.36-8.44%) varied slightly among the varieties tested. The average seed protein content (23.76%) of the tested cottonseed varieties was comparable to that reported in the literature, 22.31% (Mujahid *et al*., 2000) but was lower than that investigated earlier (34.0-36.2%) from Pakistan (Ahmad *et al*., 2007) as well as for some Nigerian cotton varieties, 37.4% (Ikurior & Fetuga, 1987), and 25.6-34.8% (Sharma *et al*., 2007). The present protein contents were higher than that reported earlier in some studies for cotton 15.40 19.40% (Deferne & Pate, 1996; Pritchard, 1991; Adelola & Ndudi, 2012). Protein contents in this analysis were also comparable with some conventional oilseeds such as linseed (24%), sesame (20-25%) (Dawodu, 2009), groundnut (27.5%) (Pritchard, 1991), hemp (23.00-26.50%) (Anwar *et al*., 2006) but was higher than palm (18%) (Pritchard, 1991) and *Bombax glabrum* seeds (10.23%) (Olaposi & Adunni, 2010). The present analysis proposes the cotton meal to be a good source of protein that could be used as energy source in poultry feed as well as for bio-fertilizer applications.

The cotton seed moisture contents were in the range of 6.36 to 8.44% (Table 1). The variety Desi has the maximum (8.44%) while N-121 the minimum (6.36%) value. The moisture content of the cottonseed in the present analysis was comparable to the cotton variety NIAB-III grown on a saline (7.1%) and a non-saline area (7.2%) of Punjab Pakistan (Ahmad *et al*., 2007) but it was lower than that given in the literature for cottonseeds (9.9%) (Pritchard, 1991). In this study, the moisture contents were generally low supporting that the seeds can be stored for a longer time in good condition. The fiber contents (20.65 to 21.31%) were higher than those of soybean (4.8%) and Palm (6.5%) (Pritchard, 1991). The data regarding ash content indicated the values to be varied from 3.46-4.46% among varieties tested.

Table 1. Proximate composition of different varieties of cottonseed from Bahawalpur district*.

Constituent (%)	Varieties					
	CIM-496	N-121	Z-33	AA-802	Desi	CIM-534
Oil	18.35 ± 0.89^{a}	16.63 ± 0.79^{bc}	17.63 ± 0.87^{bc}	16.02 ± 0.69^{cd}	15.06 ± 0.81^{d}	15.15 ± 0.82b^{c}
Moisture	7.21 ± 0.28^{bc}	6.36 ± 0.25^{d}	7.24 ± 0.25^{bc}	7.42 ± 0.24^{bc}	8.44 ± 0.3^{a}	6.44 ± 0.26^{d}
Fiber	20.65 ± 1.11^{a}	21.31± 0.97^{a}	21.06 ± 0.98^{a}	20.94 ± 1.21^{a}	21.21 ± 0.85^{a}	20.82 ± 1.21^{a}
Ash	4.47 ± 0.29^{a}	3.46 ± 0.30^{a}	4.64 ± 0.25^{a}	4.49 ± 0.22^{a}	4.49 ± 0.29^{a}	4.56 ± 0.31^{a}
Protein	24.11 ± 1.01^{a}	25.86 ± 0.81^{a}	22.92 ± 0.91^{a}	24.89 ± 0.95^{a}	20.42 ± 0.98^{a}	27.03 ± 0.97^{a}

*Values are means ± SD (n=3 × 3), calculated as percentage on dry seed weight basis. Mean values in the same row followed by the same superscript letters are not significantly different (p>0.05) among varieties selected.

Table 2. Physicochemical properties of different varieties of cottonseed oils from Bahawalpur*.

Constituents	Varieties					
	CIM-496	N-121	Z -33	AA-802	Desi	CIM-534
Refractive index (40°C)	1.4619±0.004^{a}	1.4621±0.003^{a}	1.4632±0.003^{a}	1.4615±0.005^{a}	1.4607±0.006^{a}	1.4617±0.004^{a}
Density 24°C (g mL^{-1})	0.9154±0.01^{a}	0 .9162±0.02^{a}	0.9191±0.01^{a}	0.9207±0.01^{a}	0.9181±0.02^{a}	0.9201±0.02^{a}
Saponification value (mg KOH/g oil)	187.44±3.24^{a}	185.53±3.14^{a}	187.88±2.61^{a}	189.33±2.88^{a}	180.39±3.88^{b}	190.28±2.11^{a}
Free fatty acid contents (% as oleic acid)	1.24±0.04^{a}	0.91±0..02^{a}	0.71±0.04^{a}	0.96±0.06^{a}	1.01±0.04^{a}	0.86±0.02^{a}
Iodine value (g I /100g oil)	103.14±4.14^{a}	105.87±4.18^{a}	100.54±3.17^{a}	102.74±5.06^{a}	108.73±3.61^{a}	104.94±5.25^{a}
Unsaponifiable matter (% w/w)	0.58±0.06^{a}	0.52±0.07^{a}	0.49±0.04^{a}	0.55±0.06^{a}	0.51±0.07^{a}	0.56±0.06^{a}
Color (1-in. cell)						
Red units	12.62R± 0.28^{a}	12.51R±0.27^{a}	12.01R±0.28^{a}	12.55R±0.25^{a}	13.04R±0.29^{a}	12.01R±0.31^{a}
Yellow units	68.11Y±1.21^{a}	68.01Y±1.31^{a}	63.61Y±1.12 ^{a}	65.21Y±1.11^{a}	67.91Y±1.31^{a}	65.11Y±1.20^{a}

*Values are means ± SD (n=3 × 3). Mean values in the same row followed by the same superscript letters are not significantly different (p>0.05) among varieties selected.

The physicochemical properties and quality-oriented attributes including those of density (24^{o}C), refractive index (40^{o}C), saponification number, free fatty acids, iodine value and unsaponifiable matter for the extracted CSO were found to be 0.9154-0.9207 mg/ mL, 1.4607-1.4632, 180.39-190.28 mg KOH/g oil, 0.71-1.24% (as oleic acid), 100.54-108.73g I/100g oil, and 0.49-0.58% (w/w), respectively (Table 2).

Desi cotton seed oil has maximum (108.73 g I/100g oil) while Z-33 has minimum iodine value (100.54g I/100g oil). The iodine value is an indicator for assessing the degree of oil unsaturation (Dawodu, 2009). The oil iodine values in the present work were within the range given in the literature 99-119 g of iodine/100 g of oil (Rossell & Pritchard, 1991) and genus G. *hirsuteum*as (101-113 g I/100g oil) (Sharma *et al.,* 2009) but these were lower than that reported in another study for cotton seed oil (119.78 g I/100g oil) (Warra *et al*., 2011). The values for refractive index (1.4607-1.4632) of the tested oils, measured at 40°C, were comparable to those reported in the literature for NIAB-III, 1.4643 (Ahmad *et al*., 2007) and Nigerian cotton, 1.458-1.466 seed oils (Olaposi & Adunni, 2010). The present data for density and refractive index were also in line with those of commonly used vegetable oils such as soybean, sunflower and canola (Rossell & Pritchard, 1991).

The saponification values of the tested CSOs are in line with those given in the literature for cotton seed oils and several other conventional seed oils (Rossell & Pritchard, 1991). The saponification value of variety CIM-534 (190.28 mg of KOH /g of oil) was highest while that of Desi (180.39) had the least value among others.

This analysis showed that Z-33 seed oil has minimum (0.49%) value of unsaponifiable matter while that of CIM- 496 has the maximum (0.58%) value. The average (0.53%) unsaponifiable matter of the tested CSOs in this analysis is noted to be in line with that reported in the literature (Rossell, & Pritchard, 1991).The unsaponifiable matter in oil is the quantity of those substances which could not be saponified with alkali and hence can be used to indicate and assess the magnitude of tocopherols, coloring pigments and other non-lipidic minor components (Rossell & Pritchard, 1991).

The free fatty acids (FFA), which reflect the extent of enzymatic or chemical hydrolytic products in oil, were varied over 0.71-1.24% as oleic acid. These were lowest in Z-33 while highest in CIM-496. These FFA values are comparable with most varieties of the cotton seed oils investigated in the literature (Sharma *et al*., 2009).

The color values for the tested CSOs were recorded to be 12.01-13.04 in terms of red units and 63.61-68.11 in terms of yellow units predicating no significant difference for colors within the varieties used. The intensity of the color of vegetable oils is mainly due to occurrence of coloring pigments, such as carotenoids and chlorophyll which have to be removed during oil bleaching. Vegetable oils with least color intensity are recognized to be more appealing from commercial view-point (Sadia *et al.*, 2009).

The investigated CSOs showed (displayed) good oxidation state in terms of measurement of related oxidation parameters (Table 3). The values of conjugated dienes (determined at 232 nm) for the tested oils were 2.32-2.61 and those of conjugated trienes (determined at 268 nm) 0.91-0.99. The peroxide value, which reflects hydro peroxide products in the oils and fats, of the oils tested is quite low in the range of 1.81-1.98 (meq /kg of oil). Induction period (IP) is directly linked with the oxidative stability of oils (Rossel, 1989). The average IP for oils of different cotton seed varieties of Bahawalpur was 3.40 h (range 3.19-3.61h). The minimum and maximum values were noted for Z-33 and CIM-534 variety oils, respectively. Overall, most of the physicochemical attributes given in Table 2 varied slightly among tested seed oils which may be linked and related to the fact that the varieties selected have been harvested under quite similar agro-climatic conditions.

Table 3. Oxidation state of different varieties of cottonseed oils from Bahawalpur district*.

Constituents	Varieties					
	CIM-496	N-121	Z-33	AA-802	Desi	CIM-534
Conjugated dienes $\square_{1\%}^{1cm}$ (λ 232)	2.52 ± 0.09^{b}	2.50 ± 0.09^{b}	2.61 ± 0.11^{a}	2.32 ± 0.07^{d}	2.53 ± 0.06^{b}	2.44 ± 0.08^{c}
Conjugated trienes $\square_{1\%}^{1cm}$ (λ 270)	0.96 ± 0.03^{a}	0.94 ± 0.02^{b}	0.99 ± 0.03^{a}	0.95 ± 0.04^{a}	0.97 ± 0.02^{a}	0.91 ± 0.03^{c}
Peroxide value (meq /kg)	1.88 ± 0.06^{b}	1.84 ± 0.03^{bc}	1.98 ± 0.04^{a}	1.85 ± 0.07^{bc}	1.92 ± 0.05^{a}	1.81 ± 0.06^{c}
p-Anisidine	2.15 ± 0.04^{bc}	2.14 ± 0.05^{bc}	$2.31 \pm 0.05_{a}$	2.10 ± 0.03^{bc}	2.19 ± 0.04^{bc}	2.00 ± 0.04^{c}
Induction period Rancimat method (h)	3.35 ± 0.13^{c}	3.51 ± 0.11^{ab}	3.19 ± 0.12^{d}	3.41 ± 0.15^{bc}	3.22 ± 0.14^{c}	3.61 ± 0.17^{a}

*Values are means ± SD (n=3 × 3). Mean values in the same row followed by the same superscript letters are not significantly different (p>0.05) among varieties selected.

Table 4. Fatty acid composition (g/100 g FA) of different varieties of cottonseed oils from Bahawalpur district*.

Fatty acids	Varieties					
	CIM- 496	N-121	Z-33	AA-802	Desi	CIM-534
$C_{16:0}$	24.75 ± 0.26^{bc}	25.8 ± 0.25^{a}	24.75 ± 0.27^{bc}	24.51 ± 0.23^{c}	24.86 ± 0.29^{bc}	24.42 ± 0.25^{c}
$C_{18:0}$	2.81 ± 0.07^{a}	2.49 ± 0.05^{a}	2.81 ± 0.04^{a}	2.54 ± 0.06^{a}	2.77 ± 0.05^{a}	2.51 ± 0.08^{a}
$C_{18:1}$	20.4 ± 0.23^{a}	19.30 ± 0.28^{c}	17.81 ± 0.24^{d}	20.46 ± 0.26^{bc}	21.37 ± 0.24^{bc}	23.15 ± 0.22^{a}
$C_{18:2}$	49.63 ± 0.83^{ab}	49.81 ± 0.97^{ab}	49.46 ± 0.91^{ab}	50.46 ± 0.85^{a}	48.96 ± 0.89^{c}	50.02 ± 0.81^{c}
Others	2.41 ± 0.11^{b}	2.54 ± 0.04^{b}	5.17 ± 0.06^{a}	2.03 ± 0.05^{b}	2.04 ± 0.04^{b}	0.91 ± 0.02^{c}
EFA	49.63	49.81	49.46	50.46	48.96	49.01
TSF	27.56	28.35	27.56	27.05	27.63	26.93
TUFA	70.03	69.11	67.27	70.92	70.33	73.17

*Values are means ± SD (n=3 × 3). Mean values in the same row followed by the same superscript letters are not significantly different (p>0.05) among varieties selected.

TSF: Total saturated fatty acids, EFA: Essential fatty acids, TUFA: Total unsaturated fatty acids, Others: Some unidentified fatty acids

Fatty acids composition of the tested CSOs was analyzed using GLC. It can be seen that linoleic, palmitic, and oleic acids are the major fatty acids. The content of the principal fatty acid *i.e.*, linoleic acid (C18:2) varied from 48.46 to 50.46%. The contents of palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (18:1) in the subject CSOs were 24.42-25.80%, 2.49-2.81% and 17.81-23.15%, respectively. The oils were found to contain a high level of unsaturation (69.11-73.17%). The fatty acids analysis generally shows a minor variation in the contents of fatty acids among varieties analyzed. Literature showed that cottonseed oil contained a variety of FA, with linoleic acid (C18:2) as principal component (Savanam & Bhaskara, 2011). The present contents of C16:0 (25.11%) and C18:0 (2.65%) were found to be comparable with that reported in the literature *i.e.,* 24.70 and 2.20%,

respectively (Savanam & Bhaskara, 2011). Moreover, the results of fatty acids composition in the present analysis of CSOs closely resembled with the fatty acids composition of cotton seed oil investigated by Ahmad *et al*., (2007). A high content of linoleic acid present in cottonseed oil can be useful towards regulation of membrane fluidity by stabilizing the lipid bilayer. Besides, it might be beneficial in reducing cholesterol in the blood stream and thus act as agonistic against atherosclerosis (Liu *et al*., 2002). However, a higher degree of unsaturation may prone oils to oxidative rancidity. It is understandable that thermal effect, due to heat or light, accelerates the oxidation and degradation of oils; hence highly unsaturated oils are not recommended for repeated frying purposes (Table 4).

The data for the quantification of tocopherols (α, γ, and δ) of CSOs of different cotton varieties is presented in Table 5. The content (mg/ kg) of α-tocopherol in the seed oils of cotton varieties investigated varied notably (p>0.05) from 125.47-296.20 mg/kg. The presently determined average α-tocopherol (210.84 mg/ kg) of cottonseed oils is low as compared for cottonseed oil, 429; soybean oil, 1021; rapeseed oil, 490 and maize oil, 1034 but was higher than that of palm oil, 18 (Rossell, 1991). The amount of δ tocopherol, which offers the strongest antioxidant potency than either α- and γ- tocopherols (Anwar *et al*., 2006), is noted to be 2.23-5.47 mg/ kg. The amount of γ tocopherol in the tested oils was found to be highest 269.23 (Z-33) to 326.21 (CIM-496) mg/kg among others. Within the varieties tested, the contents of total oil tocopherols varied between 403.4 and 627.8 mg/ kg. The high tocopherols content would be expected to contribute high oxidative stability to the cottonseed oil during storage and processing.

Table 5. Tocopherols content in different varieties of cottonseed oils from Bahawalpur district*.

Tocopherols (mg Kg^{-1})	Varieties					
	CIM- 496	**N-121**	**Z-33**	**AA-802**	**Desi**	**CIM-534**
α-Tocopherol	296.20 ± 8.00^{a}	125.47 ±8.00^{d}	142.50 ± 7.00^{c}	162.70 ± 6.00^{c}	242.23 ± 7.00^{ab}	174.50 ± 8.00^{c}
γ-Tocopherol	326.21 ±7.00^{a}	275.43 ± 6.00^{a}	269.23 ± 6.00^{a}	289.20 ± 6.00^{a}	320.40 ± 7.00^{a}	324.25 ± 7.00^{a}
δ-Tocopherol	5.47 ± 0.31^{a}	2.50 ± 0.22^{c}	2.70 ± 0.20^{c}	2.23 ± 0.22^{d}	4.50 ± 0.31^{b}	4.70 ± 0.20^{b}
Total	627.8	403.4	414.4	454.1	567.1	503.5

*Values are means ± SD (n=3 × 3). Mean values in the same row followed by the same superscript letters are not significantly different (p>0.05) among varieties selected.

Conclusions

The results of the present study revealed that most of the characteristics of cottonseed oils tested from Bahawalpur, Pakistan were quite identical with cotton seed oils of different countries. Regardless of the fact that some nutritionally important physicochemical attributes, for example, tocopehrols and fatty acids were considerably varied but most of the other characteristics of cotton seed oils were quite comparable among the varieties selected. The seed oil, extracted from cotton variety CIM-534, has the highest level of total unsaturated fatty acids while the seed oil of variety CIM-496 has highest concentration of tocopherols. The results of this study might be useful for selection and cultivation of an appropriate cotton variety in the specific areas with the purpose of gaining maximum nutritional benefits.

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