



Comparative Assessment on Physico-Chemical Properties and Mineralogical Analysis of Different Types of Cottonseed Available in Bangladesh

Research Article

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Abstract : Cottonseed is one of the most ancient sources of oil. In this work, a comparative study on the proximate properties and mineralogical content of cottonseeds, and physico-chemical characterization of cottonseed oils was performed using three types of cottonseed (CB-09, SR-05 and Shimultula) available in Bangladesh. The results of proximate parameters demonstrate that among the tested cottonseeds, moisture amount varied from 1.30 to 12.99%, ash 6.67 to 8.30%, protein 27.95 to 42.43%, crude fiber 19.53 to 36.45%, carbohydrates 3.45 to 18.21%, oil yield 9.08 to 14.93% and food energy of oil 267.96 to 315.17 calories g⁻¹. Physico-chemical properties such as color, appearance, solubility, specific gravity, refractive index, iodine value, saponification value, acid value and peroxide value were measured by standard procedure. The investigation of composition of fatty acids of oil pointed out that both unsaturated and saturated fatty acids are presented in oils. Among the fatty acids, oleic acid contributes the highest proportion in oils from both CB-09 (29.44%) and SR-05 (29.39%) while oil from Shimultula contains palmitic acid as highest proportion (18%). Other major constituents were stearic acid and oleic acid. Mineralogical analysis shows that cottonseeds of all three types were rich in major mineral elements and their compositions were similar which indicates that residue from cottonseeds can be used as feed for animals and fishes.

Keywords: *Comparative assessment • Cottonseed • Physico-chemical properties • Fatty acid • Mineral content*

1. Introduction

Cotton is the single most source of natural fiber and plays a key role in global economy as a cash crop (Mendoza *et al.*, 2016; Rojo-Gutiérrez *et al.*, 2020; Egbuta *et al.*, 2017; McCarty *et al.*, 2018). It functions as the backbone of fabric industries where nearly 80% of garment products are sourced from cotton (Mert *et al.*, 2015). Bangladesh ranked 40th position in the world among the 75 key cotton producing countries. Global production of cotton during 2018-19 was around 25.89 × 10⁶ tons where production of cotton in Bangladesh at the same period was 3 × 10⁴ tons (USDA, 2019). Around 150 kg cottonseed can be obtained from 100 kg of lint fiber

ginned from cotton as by-product (Yu *et al.*, 2012). It is the fifth most produced oilseed worldwide after sunflower, canola, palm and soybean reaching 44.3 million tons during the 2019-2020 (Sawan *et al.*, 2006). Oil content in different types of cottonseeds varies from 12 to 25% (Mert *et al.*, 2000). Oil yield percentage of cottonseed can be varied due to the genotype, ecological conditions of a particular area, cultivation process and storage conditions (Reddy and Aruna, 2009). Edible oil from cottonseeds is low in cholesterol with high protein content and more nutritious than soybean oil. It contains bioactive compounds such as antioxidants, including

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tocopherols, sterols, and flavonoids (Nix *et al.*, 2017; Mariod and Mattähus, 2011), as well as pigments [Tian *et al.*, 2016], which can be useful for the enrichment, fortification of flavor and stabilization of shelf life for food and medical applications. In addition, because of exclusive fatty acid composition, cottonseed oil is considered as special among other vegetable oils due to relatively high level of unsaturation and is believed to be a healthy vegetable oil. Cotton seed oil is used in various purposes such as in vegetable oil mixtures (Metinet *et al.*, 2003), in the production of margarine, as cooking and salad oil, mayonnaise, sauces, shortening and biofuel (Sekhar and Rao, 2011; Dowd *et al.*, 2010). The fine quality oil is used in cosmetics industry for example moisturizing lotions and bath soaps. The cakes produced as a by-product after oil extraction are used for feed production since it contains high protein (24%), high fat (20%), crude fiber (40%) and enriched in mineral content which are beneficial for animal and fish (Okonkwo and Okafor, 2016). Moreover, ashes from cottonseed and cotton-seed meal are often used as supplement in organic hydroponic solutions. Fertilizer from cottonseed meal is used for vegetable, roses or camellias gardens.

Composition of fatty acid obtained from cottonseed oil is one of the important indicator (Ping *et al.*, 2009) that determines oil quality. It is explained as naturally hydrogenated because its fatty acid usually consists of 70% unsaturated fatty acids, with 18% mono-unsaturated (oleic) and 52% poly-unsaturated (linoleic), and 26% saturated (mostly palmitic and stearic) acids. These properties improve the stability of oil for frying with no need for extra processing or the formation of *trans*-fatty acids (Sekhar *et al.*, 2011). It is usually acknowledged that the chemical content, functional properties, nutritional value and yield of plant seed oils are notably altered by the certain factors such as geographic, agroclimatic and genetic/variatal (Mert *et al.*, 2004).

Bangladesh primarily produces two types of cotton such as American Upland (*Gossypium hirsutum* L.) and Tree (*Gossypium arboretum* L.) cotton that stand for 95% and 5% of entire production, respectively (USDA, 2019). Upland cotton is mainly grown in the southwestern, central and northern areas while tree cotton is cultivated in the southeastern hill areas. These cottons are locally known as Karpastula. Another type of cotton that is obtained from tall tree commonly termed as cotton tree or red cotton tree (*Bombax ceiba* L.) locally called Shimultula tree is available in southeastern Asian countries including Bangladesh. Shimultula tree is not cultivated commercially as a source cotton in Bangladesh rather it grows naturally. In this study, cottonseeds from two varieties of American upland cotton such as CB-09 and SR-05, and locally origin Shimultula were selected for physico-chemical and mineralogical investigation.

Herein, this work aims to conduct a comparative study on the proximate characteristics of cottonseeds such as ash moisture content, ash content, crude seed oil yield, and physico-chemical properties of oil as well as mineral content in cottonseeds of three types of cotton seeds available in Bangladesh.

2 Materials and Methods

2.1 Chemicals and sample collection

All the chemicals used in this work were of Anal ar grade and purchased from Merck (Germany). Cotton seed of Shimultula was collected from New Market in Dhaka, and seeds of CB-09 and SR-05 were collected from Bangladesh Cotton Development Board in Dhaka, Bangladesh. Healthy disease free and mature fresh fruit samples of cottonseed were collected for analysis. The seeds external part was removed, rinsed thoroughly with running water and dried in oven at 105°C till constant weight. The dry seeds were grounded into fine particles using blender. All tests were carried out three times using dry sample and the analytical results presented are the average values.

2.2 Extraction of oil from cottonseeds

For the extraction of oil from cottonseeds, petroleum ether (b.p. 40–60°C) was used to extract oil from 100 g of powder using a soxhlet apparatus for 72hrs. In order to remove solvent, oil extract was filtered followed by vacuum distillation. The color of the extracted oil was chocolate maroon. In final stage, activated charcoal was used to filter the extracted oil which gave the light golden color of the oil. The oil content was calculated and preserved in a refrigerator for further analysis.

2.3 Proximate analysis

The proximate analysis of cottonseed was performed using authorized methods developed by Association of Official Analytical Chemistry (AOAC) [AOAC, 1990]. The equations used for calculating proximate parameters are given below:

Moisture content was calculated using the formula:

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where: W_1 = weight (g) of the sample before drying, W_2 = weight (g) of sample after drying.

Ash content percentage (%) was calculated using the following formula:

$$\text{Ash (\%)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

The total protein content was calculated using the following equation:

$$\text{Protein (\%)} = \frac{(A - B) \times N \times 1.4007}{w} = 6.25$$

Where: A = volume (ml) of 0.2 N HCl used to titrate the sample; B = volume (ml) of 0.2 N HCl used to titrate the blank; N = normality of HCl; W = weight (g) of sample; 14.007 = atomic mass of nitrogen; 6.25 = the protein nitrogen conversion factor.

The solvent was evaporated and the % of crude fat was estimated using the following equation:

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of Sample}} \times 100$$

The crude fiber content was calculated using the following equation:

$$\text{Crude fiber (\%)} = \frac{\text{Loss of weight onashing}}{\text{Weight of sample}} \times 100$$

The content of total carbohydrates was calculated by subtracting the sum of moisture, protein, fat, ash and crude fiber as described in AOAC.

2.4 Determination of the physicochemical characteristics of the oil

The physicochemical properties of the extracted oil were investigated as per cited standard methods [Kirk *et al.*, 1991; Anonymous, 2004].

The iodine value was calculated from the following equation:

$$\text{Iodine value} \left(\frac{\text{gl}_2}{100\text{g}} \right) = \frac{(S - B) M \times 12.69}{W}$$

Where: B = blank titrate (ml) value, S = sample titrate (ml) value, M = molarity of $\text{Na}_2\text{S}_2\text{O}_3$, w = sample weight (g) and 12.69 = conversion factor.

The saponification value was calculated using the following equation:

$$\text{Saponification Value (mg KOH)} = \frac{(B - S) \times M \times 56.1}{W}$$

Where: B = blank titrate value (ml); S = Sample titrate value (ml); M = molarity of HCl;

56.1 = the molecular weight of KOH; W = weight of sample (g).

Acid value was calculated using the following equation:

$$\text{Acid value (mg KOH g}^{-1}\text{)} = \frac{56.1 \times N \times V}{W}$$

Where: 56.1 = molecular weight of KOH; N = normality of KOH; V = volume (ml) of 0.1 N KOH; W = weight of sample.

The peroxide value was calculated as follows:

$$\text{Peroxide value (meqO}_2\text{kg}^{-1}\text{)} = \frac{(V_s - V_b) \times F \times 10}{w}$$

Where: V_s = Titration volume (ml); V_b = Blank level (0.00 ml); F = Factor of reagent (1.006); w = weight of sample (g).

2.5 Chemical analysis of fatty acids

Gas Chromatography technique (GC-FID) was used for the analysis of fatty acids (Shimadzu GC-2025, Detector: Flame ionization detector (FID), Column: capillary type (Capillary HP 5), packing material: 10% diethylene glycol succinate on 100-120 mesh, carrier gas: N_2 with flow rate 1 mL/min). The fatty acid methyl esters (FAMES) were prepared by complete esterification of oil using BF_3 -MeOH complex (Mariod, 2011; Kirk *et al.*, 1991). Standard FAMES were collected from Sigma chemical Co, St Louis, Mo, USA (Fatty acid methyl ester containing 11 standards such as caprylic acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, linoleic acid, oleic acid, stearic acid, arachidonic acid, behenic acid, lignoceric acid and arachidic acid) were used for the identification and quantification of the peaks. Each FAME in extracts was identified by comparing retention times.

2.6 Analysis of Mineral composition cottonseeds

Estimation of mineral content such as Mg, P, Zn, S, K, Ca, Cr, Mn, Fe, Ni, B, Pb, Cd etc. were carried out in the fresh seed powder on dry weight basis. Elemental analysis was conducted by using Atomic Absorption Spectroscopy (AAS) technique (Model: SpectrAA 55B, origin: Australia. Instrument type: wave length dispersive AAS (WDXRF) with SC and PC type detector, light source: X-ray tube anode Rh tube, fully automated). The values of macronutrients are expressed in percentage (g/100g) and micronutrients were expressed as mgKg^{-1} on dry weight basis.

3. Results and Discussion

3.1 Proximate analysis

The quality of vegetable oils and production of cotton varieties can be evaluated from analysis of their physical and chemical properties. The results of proximate analysis of cottonseeds such as percent of moisture, ash, acid soluble ash, organic matter, nitrogen, protein, crude fiber, carbohydrate and food energy content are presented in Table 1. Data shows that moisture content in seeds of CB-09 (1.3%) and SR-05 (1.4%) were very low and does not vary significantly. However, moisture content in Shimultula was significantly higher (12.99%). The difference in moisture contents may be due to genetic nature of different types of cotton. Maximum ash content was found in CB-09 (8.4%) which is very close to SR-05(8.3%). Ash content in Shimultula was considerably lower compared to two other varieties (6.67%). The results are in agreement with the previous findings (Rashid *et al.*,2009) who also observed significant difference in ash content among different cottonseeds. The variation of ash content in varieties might be due to varieties character. Protein content varied from 28.31 to

41.75% where maximum protein content was found in seeds of Shimultula and minimum content was recorded in CB-09 (28.31). It is noteworthy that protein content in both CB-09 and SR-05 is almost similar. Maximum carbohydrate content was noted in SR-05 (18.21%) and minimum carbohydrate content was recorded in Shimultula (3.45%). The carbohydrate content is correlated with many factors such as moisture content, ash content, crude protein and fiber content. The carbohydrate content shows significant difference and the results showed a good agreement with that has been reported by Mariod (Mariod *et al.*, 2011). The percentage of oil yield was found in the range of 9.08–14.93%. The oil content is a quantitative trait whose variability is conditioned with genetic difference between the varieties is reported by researchers (Anwar *et al.*, 2016). For example, Agarwal *et al.* (Agarwal *et al.*, 2003) noted 12.40 to 25.20% oil in diverse genotypes of upland cotton. Percentage of oil in cotton seeds of different types can vary due to storage period and genetic variety. Oil content decrease with passing of time may be due to lipid peroxidation which is considered as one of the major cause of seed deterioration in storage [Salam *et al.*, 2018]

3.2 Physical properties cottonseed oil

The comparative studies on physical properties of oils extracted from seeds of CB-09, SR-05 and Shimultula were conducted and the results are presented in Table 2. As illustrated in Table 2, physical properties of oils indicate that oils are homogeneous, nearly liquid and dark to yellowish colour with a characteristics odor having mild but not pungent taste. The intensity of the color of vegetable oils is linked with the presence of different pigments such as chlorophyll and carotenoids. The vegetable oils with low color values are better for edible and domestic applications. Other properties such as specific gravity (measured at 30°C) and refractive index values of the oils were found to be in the range between 0.898–0.901 and 1.4525–1.4859. Specific gravity and refractive index values are similar to the results of Zerihun and Berhe (Zerihun and Berhe, 2018). The solubility test results demonstrate that all samples were freely miscible with organic solvent such as chloroform, carbon tetrachloride, petroleum ether, *n*-hexane and alcohol but immiscible in water.

3.3 Chemical properties of oil

Oil quality, structural and stability information of fatty acid constituent can be obtained by chemical analysis such as iodine value, saponification value, acid value and peroxide value. These parameters are very important for the industrial uses as well as edible point of view. Chemical analysis was carried out using standard methods as described in materials and method section

and the results are illustrated in Table 3. The iodine value cottonseed oils ranged between 47.76 - 93.86 gI₂/100g where maximum value was found in the oil of CB-09 seeds and minimum value was recorded for Shimultula. Iodine value of CB-09 and SR-05 was very close to each other (93.86 and 89.82). These results pointed out that the oils obtained from CB-09 and SR-05 contains relatively high proportion of unsaturation compared to Shimultula. These values are similar to finding of Shah and his coworkers (Shah *et al.*, 2017). Extent of unsaturation of oil varies depending on genotype of cotton, climate, geographical factors and oil processing method. The saponification value of cottonseed oil was found in the range of 188.50–248.36 mgKOHg⁻¹ and it fall within the range of values obtained for some vegetable oil 188–235mgKOHg⁻¹ (Aremu *et al.*, 2006). Acid values of the oils were found to be in the range between 8.9–9.6 mgKOHg⁻¹. This implies that they contain low amount of fatty acids making them fit for edible purposes. One of the most common markers of edible oil quality in the production, storage and recovery process is peroxide value which indicates the extent of oxidation in the foodstuffs (Akubor *et al.*, 2008). Oils with a high degree of unsaturation are most susceptible to autoxidation. Peroxide values of cotton seed oil were ranged between 28.93–32.40 meqO₂kg⁻¹. These values showed that the oil had much free active oxygen enabling autoxidation of the oil (Jacobs, 2006).

3.4 Chemical Composition of fatty acids

Composition of fatty acid oil extracted from three types of cottonseeds was analysed by Gas Liquid Chromatography (GLC). Results of analysis are presented in Table4. Results substantiate that the most abundant fatty acid in oil extracted from CB-09 and SR-05 seed was oleic acid (29.44 and 29.39%). However, major constituent in oil from Shimultula was palmitic acid. The second most abundant constituent in all three types of oil was linoleic acid (13.11–15.73%). It can be concluded from the results that all three types of oil contains unsaturated fatty acid (oleic acid and linoleic acid) as major composition where as saturated fatty acids (palmitic acid and stearic acid) are presented at lesser extent. The oleic acid percentage was similar to those of corn oil (19–49%), grape seed oil (12–33%), mustard oil (20–22%) and soyabean oil (22–34%). The value of palmitic acid content was in agreement with that of olive oil (7.0–20%) [Jacobs *et al.*, 2006; Nollet, 2004]. Healthy fats are usually lower in saturated and higher in monounsaturated fatty acid content. Presence of high oleic acid in fatty oil helps to decrease the higher level of

total plasma cholesterol without reducing the high density lipoprotein (HDL) cholesterol level. Vegetable oils typically contain 6–15% saturated fatty acids [Tindal *et al.*, 2020] whereas cotton seed oil has 29.40% saturated fatty acids of the total oil, so it is slightly above in respect of the lower limit of the said percentage range due to existence of gossypol.

3.5 Mineral content in Cottonseed

Mineral and non-mineral nutrients are also the indicator of cottonseed quality for human and animal health that is using different products from cottonseeds. The beneficial effect of minerals to human health along with animals is reported in literature. For instance, an unbalanced

accumulation of micronutrients e.g. Fe, Zn may leads to our malnutrition (Lu *et al.*, 2008). Therefore, level of mineral content in the cottonseeds is critical. In this study both macronutrient and micronutrients in cottonseeds are investigated and results are presented in Table 5. Experimental results reveal that all types of cottonseeds investigated are enriched with both macro and micronutrients. These results demonstrate a good agreement with the findings of Bellaloui and coworkers [Bellaloui *et al.*, 2015].

For Macronutrients (Ca, Mg, S, K and S), results are expressed in percentage (%) whereas for other elements, unit is mg Kg⁻¹.

Table 1: Proximate analysis of cottonseeds.

Sample	Moisture (%)	Ash (%)	Protein (%)	Crude fiber (%)	Carbohydrates (%)	Oil yield (%)	Food energy (calories g ⁻¹)
CB-09	1.3	8.4	28.33	34.59	14.91	13.76	296.72
SR-05	1.4	8.3	27.95	36.45	18.21	9.08	267.96
Shimultula	12.99	6.67	42.43	19.53	3.45	14.93	315.17

Table 2: Physical properties of fatty oil extracted from cottonseeds.

Properties	CB-09	SR-05	Shimultula
Appearance	Oily	Like butter	Oily
Colour	Dark	Yellowish	Dark
Specific gravity (30°C)	0.901	0.898	0.899
Refractive index (30°C)	1.4525	1.4859	1.4525
Solubility	Soluble in chloroform, Carbon tetrachloride, <i>n</i> -hexane, petroleum ether and alcohol but insoluble in water	Soluble in chloroform, Carbon tetrachloride, <i>n</i> -hexane, petroleum ether, and alcohol but insoluble in water	Soluble in chloroform, Carbon tetrachloride, <i>n</i> -hexane, petroleum ether and alcohol but insoluble in water

Table 3: Chemical analysis of three types of cottonseed oil.

Types of cotton seed	Iodine value (gI ₂ /100g)	Saponification value (mgKOHg ⁻¹)	Acid value (mgKOHg ⁻¹)	Peroxide Value (meqO ₂ kg ⁻¹)
CB-09	93.86	205.38	9.5	28.93
SR-05	89.82	188.50	9.6	28.93
Shimultula	47.76	248.36	8.9	32.60

Table 4: Fatty acid composition of oil extracted from of different types of cottonseed (%).

Constituents (No. of carbon: No of double bond)	CB-09	SR-05	Shimultula
Palmitic acid (C16:0)	10.21	11.73	18.21
Stearic acid (C18:0)	8.99	9.90	-
Oleic acid (C18:1)	29.44	29.39	11.07
Linoleic acid (C18:2)	15.73	13.11	14.49

Table 5: Mineralogical content in different types of cottonseed.

Type	Ca	Mg	S	K	P	Zn	B	Mn	Fe	Ni	Pb	Cd
CB-09	1.76	0.93	0.66	0.76	0.48	65	42	16.70	161.1	4.1	2.10	1.20
SR-05	1.64	0.78	0.58	0.80	0.54	68	32	18.40	115.5	4.20	2.80	1.30
Shimultula	1.59	0.66	0.44	0.94	1.25	99	39	7.60	89.8	2.50	7.4	1.10

4. Conclusion

The results of proximate analysis, physico-chemical properties and elemental composition demonstrated that the investigated parameters of cottonseeds and oil for two varieties of American upland cotton (CB-09 and SR-05) are very close to each other while these parameters for Shimultula are significantly different from the results obtained for CB-09 and SR-05. Based on the chemical and composition analysis, it can be concluded that oils from both CB-09 and SR-05 can be used in toiletry and laundry soap in untreated condition. This oil can be used as edible oil after refining. The protein, carbohydrate and mineral content in cottonseeds of all three types confirm that the cottonseeds residue can be used as feed for animal and fish.

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