

## Physicochemical Characteristics of Bt (Seeni-1) Vs. Local Hamid Cultivar Cotton Seed Oils

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### Abstract

An investigation on physicochemical characteristics of Bt (Seeni-1) vs local Hamid cultivar (cv) cottonseed oils (CSO) was conducted. Protein in Seeni-1 seed was relatively higher than Hamid cv seed. Oil content, ash and fibre of Hamid cv were relatively higher. Ash and oil content in black (chemical delinting) and white (mechanical delinting) seed were relatively higher in Hamid cv. There were no differences between the specific gravity (sp.gr.), refractive index (R.I.) and moisture content of both oils. Free fatty acids (FFA) and iodine value (IV) in Seeni-1 were relatively higher. Saturated fatty acids (SFAs) in Hamid cv oil proved to be more than Seeni-1 oil [automatically the USFA should be higher in Seeni-1]. Phosphorus content in Seeni-1 oil was lower than that of Hamid cv, whereas there was no significant difference in the peroxide value (PV).

**Keywords:** Seeni-1 cotton; Hamid cv cotton; Cottonseed oil; Physicochemical Characteristics, Bt cotton, Sudan

### Introduction

Cotton (*Gossypium* spp) is one of the most important hard currency earning crops produced in Sudan [1]; on the other hand, cotton is one of the main exports of several countries. Cotton is a perennial crop, but cultivated as annual [2]. World cotton production has been characterized by large fluctuations. According to the International Cotton Advisory Committee (ICAC), the world production level was at 26.1 million tons in 2007 - 2008, while it has decreased to 22 million tons in 2009 - 2010 [3]. During the seventies up to the late eighties in Sudan cotton alone contributed between 45 and 65% of the total foreign currency earning, in other words, contributed to Gross Domestic Product (GDP) [4]. The Agricultural Research Corporation (ARC) of the Sudan, since its establishment (1912), worked on the development of new cotton varieties. Cotton breeder aims to produce high yielding varieties and cultivars, excellent fibre quality, drought-tolerance, bacterial, fungal and viral diseases resistance, resistance or tolerance to some sucking and biting insects pests, reducing or eliminating gossypol and cyclopropanoid fatty acids, producing a high level of oil and protein, and increase oleic acid, while reducing the saturated fatty acid (SFAs) fraction. Gossypol was detected in Sudanese cottonseed oil (CSO) using Fourier transform infrared (FTIR) spectroscopy by Mirghani and Che Man [5]. Whereas, Mahgoub [6] reported that the main reasons that led to depression in the Sudanese cotton market are, among other factors related to the farmers and the economics of the crop, changes in the Sudanese cotton varieties and the introduction of GMO Chinese and other varieties, e.g. Bt cotton (Seeni-12 and Seeni-2, Indian), and RR varieties, instead of the familiar traditional varieties (Hamid, Burhan, Acala, etc.). These varieties are not favourable to the international cotton market, compared to the traditional varieties and cultivars.

The expansion of the world population has led to increasing the demand for agriculture products, resulting in pressure on the natural environment and lack of food. These have led to a search for other alternatives and more nutritional requirements. Within such circumstances, the interest in genetic modification (GM) technology has been revitalized. GM is a technology used to improve the nutritional

value of crops. In Sudan, in the early 1990s, diseases, resistance to pesticides and high production cost, led to decline in the cultivated cotton areas and the cotton yield quantitatively and qualitatively. The GM *Bacillus thuringiensis* (Bt) cotton was recently introduced to the country to control the African Bollworm (*Helicoverpa armigera*) infestation, but the GM crop faced severe attacks by the public, some scientists and politicians. The fate of Bt proteins in the environment, their direct and indirect impact on natural enemies and non-target organisms, the frequency with which pollen from Bt plants fertilizes other plants and horizontal gene transfer in which the plant genes may move into other organisms [7] are few of some of the concerns that must be thoroughly investigated.

Currently, two cultivars are released by the National Variety Release Committee (NVRC) of the the ARC. Thousands of feddans (F = 4200m<sup>2</sup>) are now grown in almost all of the known agricultural schemes of the country and almost substituted the classical cotton varieties. The main concern was about the use of the oil produced from the seeds, in addition to incorporating the seed cake in the animal feed, in addition to using the crop residues in the field for grazing. . The initial use of biotech cotton (also called Bt Cotton) started in 1996. Bt is short for *Bacillus thuringiensis* discovered by the Japanese in 1901 (Kranthi, 2012).

Genetic engineering (GE) is the process of inserting foreign genes into the chromosomes of a host plant's cells, to develop traits that do not naturally occur in the plant [8]. GE is used to incorporate useful traits, such as resistance to insects and fungal pathogens (to reduce reliance on pesticides), withstand specific herbicide application (e.g. RR cotton; better weed management) or environmental conditions (e.g. water-logging), to improve crop quality (nutritional value), for bioremediation (phytoremediation) and biomolecule production (molecular farming for medicinal products). Bt plants are crops that have been genetically engineered to produce the toxic crystalline protein (endotoxin) of *B. thuringiensis*. Bt cotton is a GM cotton crop that expresses that insecticidal protein to cater for the African bollworm (Kranthi, 2012).

Bt refers to *B. thuringiensis* soil-borne bacteria produced insecticidal toxin (endotoxin) specific to Lepidoptera; used for crop production either in the traditional form of bio-pesticides or in the modern form of genetically engineered [9]. Bt cotton variety named "Seeni-1" developed by Shandong Cotton Research Center (SCRC), released by the National Variety Release Committee (NVRC) in March 2012 and approved by the Biosafety Authority for commercial production in June 2012. In 2013, the total growing cotton area in Sudan was 69,132 ha, 89% of it was biotech [10].

Vegetable oils extracted from plants are derived from the seed [11]. Oils are made up of triglycerides, three molecules of FAs joined to a glycerol molecule [12]. The variation in FA composition is associated with both genetic and environmental factors [13,14]. These oils have the main function in food product and act as carriers of fat-soluble vitamins (A, D, E, and K). Also, the oil provides an essential acid, which is responsible for growth [15]. CSO is among the most unsaturated edible oils [16,17].

### Objectives

The objectives of this study were to compare the physicochemical characteristics of Bt (Seeni-1) versus the traditional variety (Hamid cv) CSO.

### Materials and Methods

#### Preparation of samples

BBt cotton seeds (var. Senni-1), which contain Cry1A gene was provided by the Sudan Gezira Board (SGB), Barakat, Sudan. Traditional cotton seeds Hamid cv obtained from the ARC, Wad Medani, Sudan. Seeds of both cultivars were mechanically delinted at Zhongtian International Industrial Co. Ltd., Gezira, Wad Medani, Sudan.

### Oil extraction

The oil (CSO) was extracted mechanically using Zx10 expeller in the National Oilseed Processing Research Institute (NOPRI) pilot plant, University of Gezira, Wad Medani, Sudan.

### Ash content

Ash content was determined according to AOCS official methods Ba 5a-49 (2003), using the following equation:

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

### Moisture content

The moisture content was determined by the vacuum oven method, according to AOCS official method Ba, 2a-38 (2003), using the following equation:

$$\text{Moisture content \%} = \frac{(A - B)}{(A - C)} \times 100$$

Where: A, B and C are the mass of the Petri dish with the sample (g), Petri dish with the dried sample (g) and Petri dish (g), respectively.

### Oil content

Oil content was determined according to AOCS official method Am, 2-93 (2000). The oil content was calculated using the following equation:

$$\text{Oil content \%} = \frac{\text{loss in weight}}{\text{initial sample weight}} \times 100$$

### Refractive index (RI)

RI was carried out according to AOCS method Cs 7.25 (1993) using an Abbe bench refractometer (model AIGO, DV, Y 120D) with the temperature maintained at 20°C.

### Specific gravity (sp.gr.)

Specific gravity was determined according to the AOAC method (1999) using a pycnometer:

$$\text{Specific gravity} = \frac{\text{weight of oil}}{\text{weight of water}}$$

### Crude protein (CP)

The Kjeldahl method according to AOAC (1990) was used for the determination of CP as follows:

$$\text{Nitrogen \%} = \frac{[(S - B)(N) \times 1.4007]}{g \text{ of sample}}$$

$$\text{Protein \%} = \text{Nitrogen\%} \times 6.25$$

Where: S = HCl titration for sample, B = HCl titration for blank, N = Normality for HCl.

### Free fatty acids (FFA)

The FFA was determined according to the AOCS official method Ca 5a-40 (2009). The FFA was calculated using the following equation:

$$FFA \% = \frac{(S)(N)}{(A)} \times 28.2$$

Where: S is the volume of the titrant (ml), N is the normality of KOH solution and A is the mass of the oil sample (g).

### Iodine value (IV)

The IV was determined based on the AOCS Method Cd 1d-92 (AOCS, 2009). The IV (g I<sub>2</sub>/100g of oil) was calculated using the following equation:

$$IV = \frac{(B - S)(N)}{(A)} \times 12.69$$

Where: B is the volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> required by the blank (ml), S is the volume of (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) required by the sample (ml), N is the normality of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (N) and A is the weight of the oil sample (g).

### Peroxide value (PV)

The PV was determined according to AOCS official method Cs, 13b-45 reapproved (1999). The peroxide value, PV (mill-equivalent peroxide/kg sample) was calculated using the following equation:

$$PV = \frac{(S - B)(N)}{(A)} \times 1000$$

Where S is the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> titration of the oil sample (ml), B is the Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> titration of the blank (ml), N is the normality of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and A is the weight of the oil sample (g).

### Phosphorus (P)

P was determined by using double beam spectrophotometric (model-UN-150-20) according to AOCS method Ca 12-55 (1993), using the following equation:

$$P \% = \frac{10*(A-B)}{W}$$

Where A is P- content of sample liquoring mg, B is P- content of blank aliquot in mg and W is mass of sample in g.

### Methyl esters (MEs)

The PORIM Official Test Method (1995) was used to prepare MEs from the FAs. The sample was melted and homogenized. Approx. 50 mg (about 3 drops) of the sample was placed in a 2 ml vial. To this, 0.95 ml n-hexane was added using a graduated pipette, the vial capped and the mixture was shaken to dissolve the oil. The cap was removed and 0.05 ml NaOH was added using a glass pipette. The cap was quickly replaced and the vial was shaken vigorously for 5 sec in a vortex mixer. The clear mixture turned turbid as sodium glyceroxide was precipitated. After 5 min or more, the clear upper layer of the ME was pipetted off for analysis.

**Fatty acid (FA) composition**

The fatty acid composition was determined by GC (Shimadzu 2010) under the following condition: column: InertCap FFAP, carrier gas: helium, flow rate: 35 ml/min, column temperature: 190°C, injector temperature: 210°C, detector temperature: 210°C, detector: FID detector.

**Statistical analysis**

All the experiments were conducted in triplicates under identical conditions. Data generated were subjected to statistical analysis IBM SPSS statistic Version 20, using ANOVA (RCD) design. The results are presented as mean ± SD and P values ≥ 0.05 were considered statistically insignificant.

**Results and Discussion**

Table (1) presents the physicochemical characteristics of Seeni-1 and Hamid cv oils. The protein content and moisture content in Seeni-1 seed (35.7%) was slightly higher than that of Hamid cv seed (34%), whereas ash and fibre contents were relatively higher in Hamid cv seed. Hamid cv seed contained 2.68% ash and 2.23% for Seeni-1, i.e. < 6.9% reportedS by Baileys [18]. Ashing is the first step in the preparation of a sample for specific elemental analysis.

		<b>Seeni-1 (mean ±SD)</b>	<b>Hamid cv (mean ±SD)</b>
Protein (%)		35.7 <sup>a</sup> ± 0.4	34.00 <sup>b</sup> ± 1.0
Fiber (%)		18.0 <sup>a</sup> ± 0.4	19.00 <sup>a</sup> ± 0.6
Ash (%)		2.23 <sup>a</sup> ± 0.3	2.68 <sup>a</sup> ± 0.5
Moisture %	Seed	7.18 <sup>a</sup> ± 0.5	6.63 <sup>a</sup> ± 0.1
	Oil	0.17 <sup>a</sup> ± 0.2	0.19 <sup>a</sup> ± 0.2
Oil Content %		21 <sup>a</sup> ± 0.5 <sup>(BS)</sup>	26.03 <sup>b</sup> ± 0.2 <sup>(BS)</sup>
		15.80 <sup>a</sup> ± 0.1 <sup>(WS)</sup>	17.61 <sup>b</sup> ± 0.3 <sup>(WS)</sup>
RI		1.46 <sup>a</sup> ± 1.0	1.46 <sup>a</sup> ± 0.7
Specific Gravity		0.91 <sup>a</sup> ± 0.4	0.92 <sup>a</sup> ± 0.7
FFA %		2.00 <sup>a</sup> ± 0.02	1.20 <sup>b</sup> ± 0.03
IV (w/w)%		113.10 <sup>a</sup> ± 0.6	112.40 <sup>a</sup> ± 0.5
PV (meq /Kg)		4.12 <sup>a</sup> ± 0.1	4.31 <sup>a</sup> ± 1.1
Phosphorus (ppm)		194.51 <sup>a</sup> ± 0.5	268.50 <sup>b</sup> ± 0.6

*Abbreviations: black seed (BS); white seed (WS); results are the mean of three replicates ± SD.*

**Table 1:** Physicochemical characteristics of Seeni-1 and Hamid cv oils.

*Note: Means followed with the same subscript letter(s) <sup>(a&b)</sup> in the same raw are not significantly different, however, different subscripts letter in the raw are significantly different at (p ≤ 0.05).*

*Abbreviations: Oil content of black seed (BS); Oil content of white seed (WS); results are the mean of three replicates ± SD.*

The values for fibre were 19% (Hamid) and 18% (Seeni-1). The moisture content of Seeni-1 and Hamid cv seeds were 7.18 and 6.63%, respectively. That is in line with the < 9% reported by Baileys [18]. Moreover, Simpson [19] reported that the moisture- content of the cottonseed should not be > 9%. The high moisture content in stored seeds causes lipases activation and hydrolysis is initiated, which increases the FFA and, therefore, decrease the quality of oilseeds [18]. The moisture content in Seeni-1 and Hamid cv oils were 0.17 and

0.19%, respectively. These values of both cultivars oils agreed with Baileys [18], which reported the range of moisture content is 0.1 to 0.2%. The RI of Seeni-1 was 1.466 and Hamid cv oils was 1.460, i.e. fall within the standard range reported by the Sudanese Standards and Metrology Organization (SSMO).

Hamid cv seed showed relatively higher oil content than Seeni-1 (Table 1), the oil -content of both varieties black seeds (chemically delinted) was 21 % and 26.6% for Seeni-1 and Hamid cv seeds, respectively. However, the oil content of the white seeds was 15.8% and 17.6%, following the same order of cultivars. Babiker [20], using GM cottonseed, cultivar CNCO2 and reported 15.53% oil content. The sp. gr. was found to be 0.91 and 0.92 for Seeni-1 and Hamid cv oils, respectively. The acceptable range of SSMO is 0.92 - 0.93, whereas Hamm *et al.* [21] stated a range of 0.91 - 0.93.

FFA is an important indicator of quality during all stages of oil processing. FFA%, reported by SSMO is supposed to be < 2.0%, whereas Gupta [22] reported a range of 0.3 - 3%. The FFA in both oils agreed with the standard range (Table 1). These values were 2.0% for Seeni-1 and 1.2% for Hamid cv oil. I.V. in Seeni-1 (113.1%) and Hamid cv oils (112.4%) proved to be within the standard range of 99.0 - 119% of SSMO. The high I.V. percentages indicate that crude CSO has a great number of unsaturated bonds. Peroxides are formed by the reaction between oxygen and the unsaturated FA. Peroxides are converted rapidly to aldehydes and ketones, which have strong undesirable flavour and odour. PV reported by SSMO is supposed to be < 15.0, whereas the PV for Seeni-and Hamid cv oils were 4.06 and 4.27, respectively (Table 1). The content of phosphorus (P) in Seeni-1 and Hamid cv were 194.5 and 268.5 ppm, respectively (Table 1). The P-content, according to Hamm., *et al.* [21] is 100 - 400 ppm, however, the SSMO standard is 100 ppm. The data showed that there is a significant difference in oil content between Seeni-1 and Hamid cv oils. The difference in FFA is highly significant ( $p \leq 0.05$ ). Moreover, there was no significant difference in PV and moisture content.

FAs composition (%) of Seeni-1 and Hamid cv oils is presented in table (2). The saturated FAs in Seeni-1 and Hamid cv oils are 26.4 and 28.45%, respectively. The value reported by NCPA [23] is 27.4%. SFAs palmitic and stearic content in Hamid cv oil were 26.66 and 0.98, respectively; they were higher than Seeni-1 oil (25.39% and 0.17%, respectively); NCPA [23] values are 24.4% and 2.2%, following the same order. The stearic acid proved to be relatively lower in Seeni-1 and Hamid cv oils than NCPA [23]. PUSFAs were 70.14% for Senni-1 and 69.7% for Hamid cv oils, whereas NCPA value is 55.3%. Linoleic acid is the major PUSFA found in CSO. The content of Linoleic acid in Hamid cv oil was 53.28%, relatively >Seeni-1 oil (52.55%), whereas the level of NCPA is 55.0%; i.e. >Seeni-1 and Hamid cv oils. The MUSFAs in Seeni-1 oil 17.3% and 16.35% in Hamid cv oil, were lower when compared to NCPA, which is 17.6. MUSFAs oleic FA values of Seeni-1 and Hamid cv oils (16.54 and 15.56, respectively), were relatively lower than the value reported by NCPA, which is 17.2%.

Fatty acids	Seeni-1 (%) (mean±SD)	Hamid cv (%) (mean±SD)	NCPA (2016) (%)
Myristic (14:0)	0.83 ± 0.10	0.87 ± 0.3	0.80
Palmitic (16:0)	25.39 ± 1.2	26.66 ± 1.40	24.40
Palmitoleic (16:1)	0.85 ± 0.07	0.79 ± 0.08	0.40
Stearic (18:0)	0.17 ± 0.10	0.98 ± 0.20	2.20
Oleic (18:1)	16.54 ± 1.50	15.56 ± 1.6	17.2
Linoleic (18:2)	52.55 ± 2.40	53.28 ± 1.9	55.00
Linolenic (18:3)	0.19 ± 0.03	0.16 ± 0.00	0.30
Arachidonic	0.16 ± 0.00	0.13 ± 0.02	-
Saturated (SFAs)	26.4	28.45	27.40
Monounsaturated (MUSFAs)	17.4	16.30	17.60
Polyunsaturated (PUSFAs)	52.74	53.40	55.30

**Table 2:** Fatty acids composition (%) of Seeni-1 and Hamid cv cottonseed oil.

Abbreviations: NCPA: National Cottonseed Products Associations (2016); Results are average of three replicates ± SD.

There is a significant difference at ( $P \leq 0.05$ ) in SFAs between the two oils and NCPA [23] as a standard, whereas the difference of MUS-FAs between both oils was highly significant. Moreover, the difference of PUSFAs of Seeni-1 vs. Hamid cv oils was highly significant at ( $p \leq 0.05$ ). The result of SFAs, MSFAs and PUSFAs of the two oils were in line with NCPA [23], within the health guidelines acceptable limits. A diet that has a high intake of linoleic and linolenic acids increases the HDL level (high-density lipoproteins-cholesterol) and decreases the LDL level (low-density lipoproteins- cholesterol)[24]. However, the SFAs nutritionally are undesirable, because the high intake of oleic acid decreases LDL level, but do not affect HDL [25].

### Conclusion

This study succeeded to extend in the determination of the Preliminary data of some physicochemical characteristics of Senni-1 and Hamid cv seeds oil. There is no significant difference in fibre, ash, moisture, R.I., sp. gr., I.V, and P.V. However, there is a significant difference in crude protein, oil content, FFAs and phosphorus between the two cultivars. Moreover, the SFAs in Hamid cv are relatively higher than Seeni-1.

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