Determination of Gossypol Content in Cottonseed Oil and Cake by HPLC and GC/ms

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ABSTRACT

This study investigated gossypol content in cottonseed oil and cottonseed cake of Bt cottonseed (Seeni1) and non-Bt cottonseed (Barakat 90, Abdeen, Mixed) in oil and cake by difference methods. Among the all non-Bt cottonseed varieties Barakat (90) had shown higher amount of gossypol content in oil and cake (470 and 467) ppm (890 and 897) ppm by using HPLC and GC/MS respectively. Moreover, Bt cottonseed variety (Seeni1) had shown lower amount of gossypol content using both methods (320 and 326) ppm and (640 and 635) ppm by using HPLC and GC/MS respectively. From the present study, we observed that was no significant difference in gossypol content between Bt cottonseed and non-Bt cottonseed varieties. Bt cottonseed variety has lower amount of gossypol being of more economical importance to farmers having cotton as their cash crop.

INTRODUCTION

Cotton (*Gossypium* spp.) is an arborous plant from the Malvaceae family. It is one of the earliest plants that were cultivated by man and it has been used for over 4,000 years. It is primarily cultivated for fiber used in the textile industry and the oil from the cottonseed, (Blanco, 2008).

Cotton is the leading commercial crop for the production of natural fibres for the textile industry worldwide. In addition, cotton seeds are an excellent source of edible protein (23%) and oil (21%), are rich in unsaturated fatty acids and have the potential to feed half a billion people globally (wei Gao et al., 2019; Sunilkumar et al., 2006).

However, the potential of cotton as a food source is limited due to the toxicity of gossypol for humans and other monogastric animals (Zhang et al., 2007).

Cottonseed meal is a by-product of cotton that is used for animal feeding because it is rich in oil and proteins, (Ivana et al, 2014). Food and animal agricultural industries must manage cotton-derivative product levels to avoid toxicity; for example, only ruminant microflora can digest gossypol, and then only to a certain level, and cottonseed oil must be refined. Genetically engineered cotton plants that contain little gossypol in the seed may still contain the compound in the stems and leaves, (Kay, 2018).

Free gossypol is a toxic compound which naturally occurs in cottonseed and its derivates, affecting animal and possibly human health, (Alessandra et al., 2021).

Considering the importance of pigment glands for cotton, knowledge about their biogenesis as well as the secondary metabolites they accumulate is critical for the improvement of this important crop (Janga et al., 2019; Ma et al., 2016; Tian et al., 2018).

Genetically modified plants expressing Bacillus thuringiensis (Bt) is an insect-resistant transgenic crop designed to combat the bollworm. Bt cotton was created by genetically altering the cotton genome to express a microbial protein from the bacterium Bacillus thuringiensis, (Kranthi, 2012). The Bt cottonseed was the new raw material on Sudan in the year 2012 the area that were planted with Bt cotton by the Sudanese-Brazilian Partnership in Blue Nile was approximately 17,000 feddans and expected to rise up to 70,000 feddans next seasons, (Sharief, 2012).

There are many methods to determine gossypol such as spectrophotometry, the non-aqueous titrimetric method, gas chromatography and high-performance liquid chromatography but the chemical methods are not very specific as gossypol analogs may give positive values resulting into significant overestimation. Each of these methods can reflect the relative levels of gossypol. HPLC method is more accurate, effective ad specialized, (Jolanta etal., 2012).

MATERIALS AND METHODS

MATERIAL

The cottonseeds used as sample for the analysis were collected from the seed unit, Agricultural Research Corporation (ARC), Wad madani Gazira State, Sudan. The variety of cottonseed used is non-Bt cotton (Barakat 90, Abdeen, and Mixed) and Genetically Modified (GM) Cotton (Seeni1 (Bt)). The collected seeds were properly cleaned to remove farm residues and other impurities. All the cottonseed samples were manually crushed followed by grinding the samples to powder form. Then the extraction oil was done by soxhlet with n-hexane.

Experimental Section

Quantitative Analysis of Gossypol Content in Seed Cake and Oil by using Gas Chromatography-Ion Trap/ Mass Spectrophotometer (Gc/Ms)

A Saturn-3/Varian (Varian Instruments) ITMS associated with a Varian model 3400 CX GC equipped with a 30-m DB-5 capillary column (J&W Scientific, Folsom, CA), with an internal diameter of 0.25 mm and 0.25 µm film thickness was used Helium was used as carrier gas and gave a column head pressure of 12 p.s.i (1 p.s.i. = 6894.76 Pa) and an averge flux of 1 ml/min.

The temperature program for the GC column consisted of a 2-min hold at 60 ^{o}C, a 23.70 ^{o}C/min ramp to 250 ^{o}C, a total run time was 30.23 min. the injector, transfer line, and ion trap temperature were set at 260 ^{o}C, 270 ^{o}C and 170 ^{o}C, respectively.

The mass range used was m/z 65 to 400 with a scan time of 500 ms in the electron impact mode with electrons of 70 ev. The electron multiplier voltage was set as 1500 V, and the storage radio frequency was 1.1 MHz. the selected ion storage (SIS), which is a resonant ejection mode, was used. This technique consists of an application of multifrequency

waveforms imposed to the end-capelectodes so that undesired ions are ejected and characteristic ions are stored. For this purpose, a waveform was built in order to store gossypol ions at m/z 127, 152, and 181 eject unwanted ones

Quantitative Analysis of Gossypol Content in Seed Cake and Oil by using High Performance Liquid Chromatography (HPLC)

This method describes a procedure for the quantitative determination of gossypol content in cake and oil using HPLC.

The HPLC procedure optimized by Marquie and Borrely (1991) was used to analyze the gossypol in the seed cake and oil. After being-peeled, cut and weight, the seeds were ground and sieved (30 mesh).
A sample of ground seed (100 mg each) (5ml in case of oil) were hydrolyzed for 10 min in boiling water bath at 100 ◦C with 20 ml of glacial acetic acid. At the same time, two samples (1-2 mg) of standard gossypol were treated similarly.

The solutions were filtered through salinized glass wool into 50 ml volumetric flask. The residues were rinsed three times with 2 to 3 ml of water / acetonitrile (50:50; v/v) mixture, the recovered solutions were diluted up to 50 ml and homogenized carefully. The samples were then left at room temperature for 3 hr. before being filtered through a 0.20 μm nylon membrane (MSI).

The samples were directly analyzed on a Merck Hitachi L 6200 chromatograph (Hitachi) Ltd., Tokyo, Japan) equipped with a Merck Hitachi L4000 UV. The chromatographic signals were integrated on a Hewlett. Packard HP 1000 integrator (Hewlett Packard,USA).
Other analytical conditions were fixed as follows:

I. Column inertial: 5-μm ODS-3 from chrompack (The Netherlands); (100 × 3mm).
II. Mobile phase: acetonitrile/water (acidified to pH = 2.6 with phosphoric acid 88:12 (v/v) at flow rate of 0.5 ml/min.
III. UV detection at 272 nm.
IV. Duplicates of 20μl injections were made for all samples.

RESULTS and DISCUSSION

In this study, we compared between the amount of gossypol in cake and oil, and we observed that the amount of gossypol was less in oil for each variety than in cake.

From table (1) and (2) the range of concentration of gossypol in oil by Gc/Ms is (326 – 467) ppm and the range of concentration of gossypol in cake is (635 – 897) ppm. The range of concentration of gossypol in oil by HPLC is (320 – 470) ppm and the range of concentration in cake is (640 – 890) ppm.

Table (1): Gossypol Content in Oil and Cake Determination by Gc/Ms

	Oil		Cake	
	ppm	%	ppm	%
Seeni1	326	0.033	635	0.064
Barakat (90)	467	0.047	897	0.090
Abdeen	340	0.035	669	0.067
Mixed	428	0.043	849	0.085

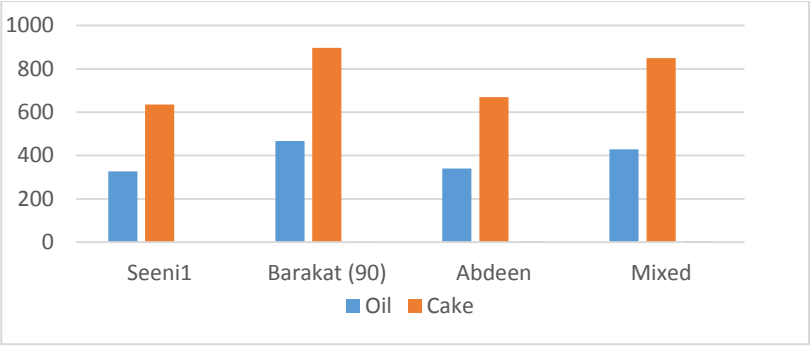

Figure (1): Gossypol Content in Oil and Cake Determination by Gc/Ms

Table (2): Gossypol Content in Oil and Cake Determination by HPLC

	Oil		Cake	
	ppm	%	Ppm	%
Seeni1	320	0.032	640	0.064
Barakat (90)	470	0.047	890	0.089
Abdeen	334	0.033	675	0.068
Mixed	425	0.043	856	0.086

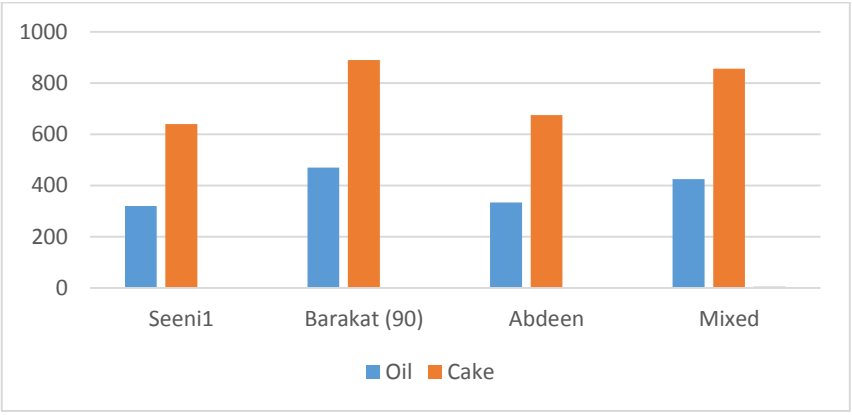

Figure (2): Gossypol Content in Oil and Cake Determination by Gc/Ms

Concentration of Gossypol in Oil

The comparison of concentration of gossypol in oil was determined by different methods (High Performance Liquid Chromatography (HPLC), and Gas Chromatography /Mass Spectrophotometer (GC/Ms), in variety samples the results are shown in table (3) and figure (3).

***Table (3): Concentration of Gossypol in Oil as (ppm and percentage) by Different Methods**

Variety	HPLC		GC/ Ms	
	ppm	Percentage	ppm	percentage
Seeni1	320	0.032%	326	0. 033%
Bara.	470	0.047%	467	0.047 %
Abd.	334	0.033%	340	0.034 %
Mixed	425	0.043%	428	0.043%

The highest value of concentration of gossypol in oil was found in Babakat (90) variety and the lowest value of concentration of gossypol was found in Seeni (1) variety. No signification difference between the concentrations of gossypol in oil estimated by both method.

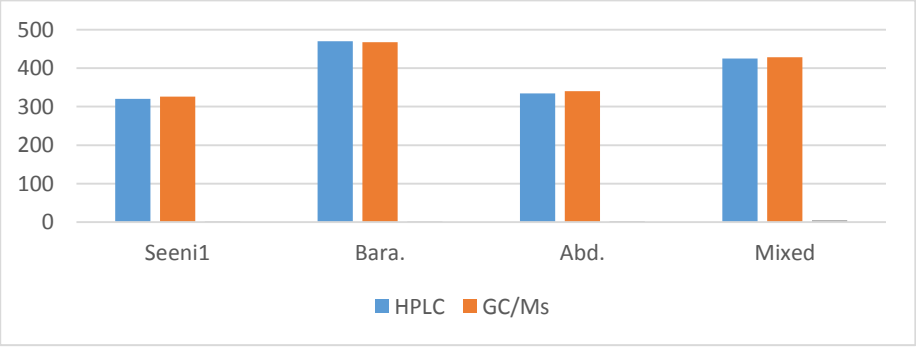

Figure (3): compression of Concentration of Gossypol in cake as (ppm) by different Methods

Concentration of Gossypol in Cake

The concentration of gossypol in cake determined by different methods (High Performance Liquid Chromatography (HPLC), and Gas Chromatography /Mass Spectrophotometer (GC/Ms) was compared in variety of samples, the results are shown in table (4) and figures (4).

Table (4.): Concentration of Gossypol in cake as (ppm and percentage) by different Methods

Variety	HPLC		GC/ Ms	
	ppm	percentage	ppm	percentage
Seeni(1)	640	0.064%	635	0.064 %
Bara.	890	0.089%	897	0.090 %
Abd.	675	0.068%	669	0.067 %
Mixed	856	0.086%	849	0.085%

The highest value of concentration of gossypol in cake was found in Babakat (90) variety determined by each methods, and the lowest value of concentration of gossypol was found in Seeni1 variety.

Gossypol exits in two different forms, free form and total form in cottonseeds. However, both the forms of gossypol can be toxic to animals, which limit the usefulness of cottonseed as animal feed. The free gossypol content in whole cottonseeds varies among the many cotton varieties (Alexander, 2008); gossypol concentrations range from 0.02 to 6.64%, (Price et al, 2003). Cottonseed may contain concentrations greater than 14,000 mg/kg of total gossypol and 7,000 mg/kg of free gossypol, (Alexander, 2008). However, after oil extraction from the seeds, up to 0.6% is available following solvent extraction, but approximately 0.06% is available, if the extraction process involves mechanical pressure and heat treatment (Nicholson, 2012). From (Jan et al., 2008) for data on levels of gossypol in whole or processed cottonseed little information could be collected from two Member Countries that showed a gossypol content ranging from 100 to 8416 mg/kg, but it was reported that whole cottonseed can contain more than 14,000 mg/kg of total and free gossypol (Jan et al., 2008).

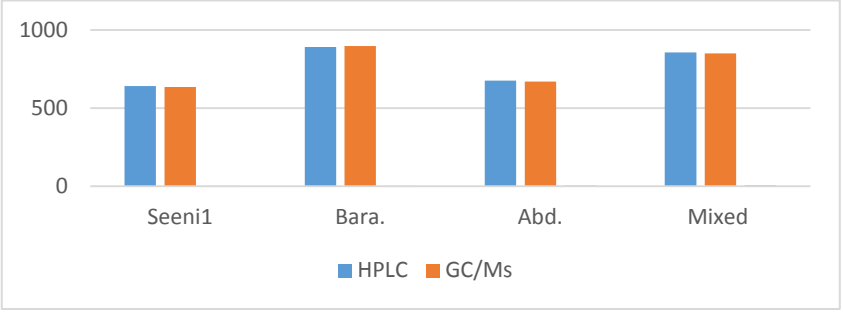

Figure (4): compression of Concentration of Gossypol in cake as (ppm) by different Methods.

Conclusion

We successfully determined the amount gossypol content in both Bt and Non-Bt cotton seed samples by HPLC and Gc/mc. Among non-Bt cottonseed varieties samples Barakat (90) has the highest gossypol content in both oil and cake. The Bt cottonseed variety Seeni1 has least amount of gossypol. We observed that the amount of gossypol was less in oil for each variety than in cake of these varieties. From the result, it is clear that the concentration of gossypol in oil and cake estimated by both methods (HPLC and GC/Ms methods) is approximately equal.

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