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GOSSYPOL LEVELS IN COTTONSEED OIL AND COTTONSEED CAKE PRODUCED IN ETHIOPIA

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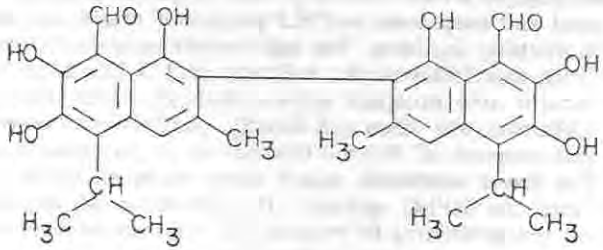
ABSTRACT. Using an HPLC method, capable of detecting as low as 5 μg/ml gossypol it was established that there is no detectable free gossypol in cottonseed oil prepared for human consumption. Most press cake samples analyzed also do not show the presence of gossypol. The decorticated cottonseed and unrefined oil samples were found to contain gossypol in the range 0.14 - 0.63 and 0.02 - 0.08%, respectively.

INTRODUCTION

The cotton plant which belongs to the genus *Gossypium* (family Malvaceae) is the major natural source of textiles. Furthermore the seeds which are by-products in fibre production are important sources of edible oil, protein and animal feed. About 82 kg of seed is produced for each 45 kg of fibre (1). Nearly 15% of the oil produced in the world today originates from cottonseed (2), thus placing it next to soya, which is the number one source of edible oil. In Ethiopia 50% of the total edible oil produced comes from cottonseed. The press cake also serves as animal feed particularly in urban areas. The utilization of cottonseed products in human nutrition is limited by the presence of gossypol, a phenolic constituent, which is toxic to non-ruminants and humans (3). The Protein Advisory Group of the United Nations sets the limits of gossypol in cottonseed-derived foodstuffs at 1.2% total gossypol and 0.06% free gossypol (4).



(I)

The aldehyde group of gossypol 1 is capable of condensing with the free amino groups of proteins to yield the bound form (4, 5). It has been established that gossypol is biologically active in its free form (6).

Different methods like colorimetery, non-aqueous titration, polarography, paper, thin layer and gas chromatography and recently HPLC have been used for the analysis of gossypol (7, 8). The HPLC method has been found to be most sensitive capable of detecting as low as 5 ng/ml of gossypol in human plasma (7). The objective of this study was to determine the level, if any, of free gossypol in edible oil and cottonseed cake produced by selected factories in Ethiopia.

In this study a modified procedure of that described in reference 8 was followed which resulted in better resolution with higher sensitivity. Instead of eluting with MeOH:H_2O:$CHCl_3$ (70:30:40) and detecting at 254 nm (8), methanol containing a few drops of phosphoric acid was employed as the mobile phase and monitored at 233 nm.

MATERIALS AND METHODS

Samples. Samples were obtained from two factories in Addis Ababa where mechanical pressing is used and one in Mojo which uses solvent extraction to obtain oil. The major production steps for edible oil are as shown in Scheme 1. Six replicates of each sample were used for the HPLC analyses.

HPLC system. Reversed phase HPLC using a 250 x 4 nm I.D. stainless steel column packed with Lichrosorb RP-18, 5 μm, and eluting with methanol (Riedel de Haen, chemically pure and redistilled) plus a few drops of phosphoric acid at a flow rate of 0.8 ml/min was used. Phosphoric acid was added to prevent tailing of the peaks. The solvent delivery system comprised two LKB 2150 HPLC pumps, a 2152 LC controller and a 7152 rheodyne injector. Detection was by UV absorbance at 233 nm on the LKB 2151 variable wavelength monitor with 2220 recording integrator and scanning over the range 197-370 nm on the 2140 rapid spectral detector interfaced with an NCR-personal computer-6. UV spectra were obtained using the LKB 2140 rapid spectral detector.

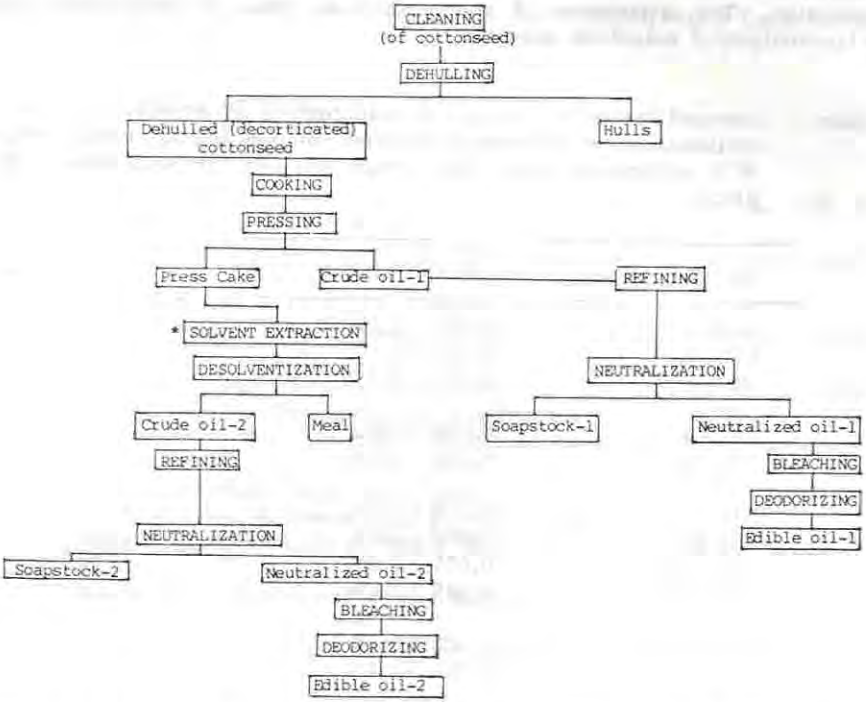
Calibration curve. A calibration curve employing the concentration range of 0.005 - 0.2 mg of gossypol-acetic acid per ml of methanol vs peak area was constructed. Absorbance was measured at 233 nm.

Sample preparation for HPLC. 300 mg each of ground, decorticated cottonseed, press cake, meal and soapstock, and 8.5 g each of crude and refined oil samples were soaked in acetone for 16 h. The solid particles were filtered and the solvent evaporated. This was followed by addition of 1% HOAc in chloroform to get the gossypol-acetic acid complex after which the chloroform was evaporated. The residue obtained was then repeatedly partitioned between methanol (25 ml) and a total amount of 75 ml hexane so as to remove the oil and fatty substances. The lower methanol layers were made up to 25 ml and 5 μl taken for injection into the HPLC system. The precision of the extraction method was checked by the efficiency of recovery of a known amount of gossypol-acetic acid added to 5 separate oil samples. The recovery was 95%.

The results of the HPLC analyses for the quantity of gossypol-acetic acid were then converted to the equivalent percent of gossypol by weight in the sample analyzed.

The acetone extracts of each test sample were subjected to TLC analysis and colour tests as described in references 9 and 10, resepectively.

Scheme 1. Major steps in edible oil production.



* The processing of oil after this step is carried out in separate solvent extraction plants. The solvent extraction method helps in further recovery of oil in the press cake which contains *ca.* 14% oil.

RESULTS AND DISCUSSION

Analysis of the samples obtained from refined oil showed in all cases the absence of gossypol, at the detection limit of methods used.

The decorticated cottonseed was found to contain 0.14% - 0.63% gossypol. As expected crude oil-1 had a higher amount of gossypol than crude oil-2 samples which are obtained after double extraction (see Table 1).

In the case of the cake samples, four of the meal samples which were of the pressed and solvent extracted type (see Scheme 1) consistently showed the absence of free gossypol, but two out of the six pressed-only cottonseed cake samples showed the presence of free gossypol in the HPLC (Table 1) and TLC analyses while the remaining four did not. HPLC tracings of the latter four samples sometimes showed a peak, though small, with a retention time close to that of gossypol. However it was confirmed that this spurious peak was not due to gossypol by checking its UV spectrum directly with the aid of the photodiode array detector. This was further confirmed by the negative TLC and colour tests with which it is possible to detect upto 5 µg/ml and 2.5 µg/ml of gossypol-acetic acid in solution, respectively.

The HPLC system has the advantage of providing more accurate results than the spectrophometric method where higher values are usually obtained due

to the interference of constituents other than gossypol (5, 8). One should however note the fact that in the HPLC method poor resolution may lead to erroneous results, and one should therefore develop optimum conditions to obtain maximum resolution. The appearance of poorly resolved peaks is particularly observed in the analysis of soapstock samples.

Table 1. Gossypol levels (X ± %S.D.: X = average % by weight, n = 6) in various cottonseed and cottonseed-derived samples (decorticated cottonseed, DCS, cottonseed cake, CSC, crude oil, CO, and soap stock, SS) using HPLC

Sample	Gossypol level (X ± %S.D.)
DCS-1	0.501 ± 2.2%
DCS-2	0.138 ± 2.1%
DCS-3	0.631 ± 2.1%
CSC-9	0.028 ± 2.0%
CSC-10	0.036 ± 2.1%
CO-1-1	0.073 ± 2.4%
CO-1-2	0.064 ± 2.0%
CO-1-3	0.075 ± 2.0%
CO-1-4	0.060 ± 2.5%
CO-2-1	0.023 ± 2.3%
CO-2-2	0.018 ± 2.1%
SS-1-1	0.118 ± 2.2%
SS-1-2	0.161 ± 2.1%

In conclusion, this preliminary study indicates the absence of gossypol in edible oil prepared from cottonseed.

Free gossypol was detected only in two press cake samples. The absence of detectable free gossypol in most of the press cake samples may be due to the fact that the cooking process facilitates the binding of gossypol with free amino acids liberated in the press cakes. Processing conditions such as variations in the temperature and duration of cooking, or even storage and starting material used may account for the presence of gossypol in the two samples.

TLC analysis and colour test of cottonseed cake samples soaked in 0.5% oxalic acid in acetone for 48 h in order to release the bound gossypol (11) showed that press cake samples including those in which no free gossypol was detected contain gossypol, apparently existing in the bound form.

On the other hand crude oil samples produced by expression of decorticated cottonseed as well as by solvent extraction of the press cake were found to contain gossypol. Crude oil, derived from press cakes by solvent extraction contain upto 0.02% gossypol. This observation can be accounted for by the release of gossypol from its bound form during the extraction process. Subsequent purification steps that convert the crude oil to refined oil remove and or alter the gossypol, thus rendering the edible oil free from gossypol.

ACKNOWLEDGMENTS

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