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Determination of Free Gossypol in Iranian Cottonseed and Cottonseed Cake

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ABSTRACT

Free gossypol was determined, using AOCS method Ba 7-58, in 11 varieties of cottonseed cultivated in Iran and six samples of cottonseed cake from three oil plants in (A) Pars, (B) Margarin and (C) Varamin. The mean value for free gossypol in normal glanded cottonseed samples ($n = 8$) was 1·14%. For the native cottonseed variety (Arya) 0·53% free gossypol was obtained, which was significantly lower than the normal glanded samples. The value for the hybridized sample (Sahel × GL_4) was very low (only 0·11%). No gossypol was detected in the glandless cottonseed variety (Coker 100 A). The average amount of free gossypol in cottonseed cake samples from oil plants (A) and (B) was 0·026% and in (C) it was 0·16%.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the main agricultural crops of Iran; its annual production in the year 1986/87 was 358 856 tonnes grown on 187 936 ha. This resulted in the production of *c.* 100 000 tonnes of cotton fibre, from which about 20 000 tonnes was exported to West European countries (COOI, 1987).

The main cotton producing provinces of Iran include Mazandaran (69%), Khorasan (15%), Fars (5%) and East Azarbayejan (5%), contributing to production of 94% of total cotton in the agriculture year 1986/87 (COOI, 1987).

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TABLE 1
Cotton (Boll) Production in Iran during 23 August 1986 to 22 August 1987 (COOI, 1987)

Variety	*Production (tonnes)*	*Main production province*
Sahel 53	235 000	Mazandaran
Varamin 51	76 966	Khorasan, E. Azarbayejan
Native	29 290	Khorasan, Fars
Hopicala	17 000	Fars
Total	358 856	

Table 1 shows the annual production of cotton varieties in Iran, with their main cultivation areas.

After the cotton fibres are removed, what remains is the cottonseed which is the most prevalent source of vegetable oil in Iran. After oil extraction, the remaining high-protein cottonseed cake is used as animal feed. Annual production of cottonseed was reported to be *c.* 145 000 tonnes in the year 1986/87, producing *c.* 20 000 tonnes cottonseed oil and *c.* 75 000 tonnes of cottonseed cake (IORDC, 1987).

Normal glanded cottonseed varieties contain gossypol, a yellow–green polyphenolic compound, toxic to man and monogastric animals. The use of cottonseed meal or cake as a feed for nonruminants (poultry) requires the determination and restriction of gossypol in these products (Berardi & Goldblatt, 1980).

The Institute of Standards and Industrial Research of Iran (ISIRI) has set maximum guidelines of 450 ppm (or 0·045%) free gossypol (major physiologically active form) in cottonseed flour used for human consumption (ISIRI, 1983*a*). The maximum recommended value of the Protein Advisory Group of WHO/FAO/UNICEF is 0·06% free gossypol or 1·2% total gossypol (Milner, 1965).

AOCS Official Methods have been approved for gossypol analysis in cottonseed and its products (ISIRI, 1975; 1983*a*). ISIRI'S guidelines for maximum free gossypol levels in poultry diets (when cottonseed meal is used) are 100 ppm for broilers and 20 ppm for laying hens (ISIRI, 1983*b*).

Different methods have been reported for the determination of gossypol, including spectrophotometry, paper chromatography and GLC (Pons, 1977). However, AOCS Official Methods (spectrophotometry) still have worldwide application for gossypol analysis in normal glanded cottonseed and its products, and modifications have been suggested to improve their sensitivities (Fisher *et al.*, 1987).

The objective of this study was to determine free gossypol in cottonseed varieties cultivated, commercially or experimentally, in Iran. Gossypol content of cottonseed cake from different oil plants was also measured.

MATERIALS AND METHODS

Samples

Eleven samples of cottonseed planted commercially or experimentally in Iran were collected from the Seed and Plant Improvement Center, Varamin (a small town near Tehran). Main cottonseed samples were kept in the sacks, each sack being representative of a different field. About 500 g samples were taken from different parts of each sack, put in plastic bags and kept in the freezer (−30°C) until analysed.

Six samples of cottonseed cake were obtained from three different oil plants, i.e. one sample from Pars (A), three samples from Margarin (B), and two samples from Varamin (C), in which oil extraction had been carried out by screw pressing. These samples were also stored at −30°C until analysed.

Instrumentation

A Varian Tectron Spectrophotometer model 635 with 1 cm cell was used.

Chemicals

All chemicals used were of general reagent grade (GR) and supplied by the Merck Company agency in Iran. Standard gossypol acetic acid (100% purity) was kindly donated by AOCS.

Procedure

Sample preparation
Lint and hulls were removed from cottonseed samples and meats were ground using a laboratory mill. Cottonseed cakes were ground, using a laboratory mill.

Gossypol analysis
The AOCS procedure was used for extraction and determination of free gossypol in the samples of cottonseed and cottonseed cake (AOCS, 1987). For each analysis a sample of 0·5 g cottonseed and 1 g cottonseed cake was used. Extraction of free gossypol was carried out in duplicate, and each extract was analysed three times.

Purity of standard

The purity of standard gossypol acetic acid was checked (Hoffpauir *et al.*, 1960) and its absorption spectrum in cyclohexane was recorded in the UV region. The absorption was measured at 358 nm. Different concentrations of this standard were used to draw the standard graph. Thus an average factor was obtained to calculate the gossypol content of the samples.

RESULTS AND DISCUSSION

The absorbance of the standard gossypol-acetic acid (0·02 g/litre in cyclohexane) at 358 nm was 0·72 and its absorptivity was found to be 36. This was in the range recommended by AOCS, i.e. $358 \pm 0{\cdot}2$ (AOCS, 1987). The absorption maxima of the standard were at wavelengths 236, 286 and 358 nm, which corresponded well with those of pure gossypol-acetic acid (Hoffpauir *et al.*, 1960). This confirmed the purity of the standard gossypol acetic acid, which was used for calibration.

The calibration graph (absorbance vs concentration) of standard gossypol-acetic acid by the AOCS colorimetric method (with aniline reagent) was linear. There was a positive and perfect correlation between absorbance (Y) and concentration (X) of free gossypol. The results are summarized in Table 2. An average factor (0·32) was used to calculate the free gossypol content of the samples.

TABLE 2
Results of Calibration of Standard Gossypol-Acetic Acid in AOCS Method Ba 7–58

Number	*Free gossypol concentration (mg/25 ml)*	*Corrected absorbance at 440 nm*	*Factor*
1	0·025	0·08	0·312 5
2	0·050	0·16	0·312 5
3	0·075	0·24	0·312 5
4	0·100	0·31	0·322 6
5	0·125	0·39	0·320 5
6	0·175	0·54	0·324 0
7	0·200	0·61	0·327 9
8	0·250	0·76	0·328 9

Average factor: $0{\cdot}320\,2 \pm 0{\cdot}002\,5$[a]
Calculated regression line equation: $\bar{y}_X = 0{\cdot}095 + 3{\cdot}014X$
Correlation coefficient: $r = +0{\cdot}99$

[a] Mean ± SEM.

TABLE 3
Percentage Free Gossypol in Iranian Cottonseed Varieties

Variety	*Free gossypol (%)*
Sahel 53	1·15
Varamin 51	1·12
Hopicala	1·06
Acala C-1517	1·22
Deltapine 16	1·25
Coker 312	1·31
$C_{12}C_{11}$	1·01
Todla	0·98
Normal glanded	$1·14 \pm 0·056$[a]
Arya (native)	0·53
Sahel × GL_4 (hybridized)	0·12
Coker 100A (glandless)	ND[b]

[a] Mean ± SEM.
[b] Not detectable.

As Table 3 shows, in the normal glanded Iranian cottonseed varieties, the mean value for free gossypol was 1·14%, ranging from 0·98 to 1·31%. In the native variety (Arya), free gossypol content was lower and, on average, 0·53%. The hybridized cottonseed sample (Sahel × GL_4) contained even less, i.e. 0·12% free gossypol and the glandless variety (Coker 100A) had no measurable gossypol (AOCS method). Raw cottonseed kernels normally contain from 0·6 to 2% free gossypol (Lusas & Jividen, 1987).

Although the native variety has a lower free gossypol content, compared to normal glanded varieties, it has the disadvantage of poor quality fibre. On the other hand, Sahel × GL_4 (hybridized), which is very low in gossypol content, has a good fibre quality and yield, and it is promising for commercial production. Coker 100A (glandless) is less resistant to insects and has a poor fibre quality and yield.

The average free gossypol content of cottonseed cake samples from two oil plants (A) and (B) was 0·026%, which was in the normal range reported for the screw pressing method, i.e. 0·02–0·05% (Berardi & Goldblatt, 1980), but in the third (C) it was 0·16% and out of range (Table 4). The oil content of the cakes was also determined. It was found that, on average, cakes from (A), (B) and (C) contained 5, 4 and 10% oil, respectively (dry weight basis), which confirms the unfavourable conditions of cottonseed processing in oil plant (C).

None of the cottonseed cake samples was suitable for direct use as poultry feed since the free gossypol content was higher than the maximum

TABLE 4
Percentage Free Gossypol in Iranian Cottonseed Cake

Oil plant	*(A)*	*(B)*	*(C)*
Free gossypol (%)	0·029	0·023 ± 0·000 7[a]	0·16 ± 0·05[a]

[a] Mean ± SEM.

recommended value for broilers and laying hens in Iran (ISIRI, 1983*b*). It is suggested that the gossypol content of cottonseed cake, or meal produced in Iran, should be determined before their utilization for non-ruminants. For lowering the free gossypol level, addition of iron salts ($FeSO_4$) which bind the gossypol in feeds and render it biologically inactive, is recommended.

As far as we know, there has been no serious research on the utilization of high protein cottonseed flour in human nutrition in Iran. We suggest that some research work be directed to the production of glandless cottonseed or glanded cottonseed varieties with low gossypol content and also to the use of low-cost processes, applicable in Iran, for removal or reduction of gossypol in glanded varieties. Research is also needed on the preparation and production of low-gossypol cottonseed flour, protein concentrates and isolates, and fortification of some food products such as bread and biscuit with them.

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