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Received for review November 24, 1986. Accepted August 24, 1987. Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others that may also be suitable.

Total Gossypol Content of Glandless Cottonseed

Gordon S. Fisher,* Arlen W. Frank, and John P. Cherry[1]

This paper reports the presence of total gossypol (TG) in gland-free cottonseed kernels. Thinly sliced kernels were examined with a microscope to assure complete absence of glands and analyzed by a modification of the official AOCS method, which can detect less than 1 ppm TG. Although sound, gland-free kernels from five varieties contained much less than 1 ppm of TG, those from each of nine varieties averaged 2–7 ppm TG, with over 10 ppm in one sample. All varieties meet the National Cottonseed Products Association's standard for Grade AAA seed (i.e., not to exceed 10 ppm TG) if glanded seeds are rigorously excluded. On the basis of a 1.25-g sample of cottonseed, 10 glands would contribute about 1 ppm of TG. Moldy and discolored kernels, in which no glands were visible, contained more TG than normal kernels.

Cottonseed is a plant protein product that can be used in foods to improve nutritional and functional properties (Lusas and Jividen, 1987). However, traditional varieties contain about 1% gossypol, a sesquiterpenoid phenolic aldehyde, and related compounds (Boatner, 1948). These compounds are toxic to monogastric animals (Berardi and Goldblatt, 1969), which restricts the use of cottonseed in feeds and foods. They can be deactivated (bound) by condensation with amino groups in the seed, but this reduces available lysine and the bound gossypol causes discoloration in foods (Blouin et al., 1981). Free and bound aldehydes are determined as a group by Method Ba 8-78 of the American Oil Chemists' Society (1979) and reported as total gossypol (TG).

Kernels of traditional (glanded) varieties of cottonseed contain intercellular structures, called pigment glands or simply glands, which are deposition sites for gossypol and related pigments. In fully glanded seed, these amber to dark red glands, which are 100–400 μm in diameter, are distributed throughout the cotyledons and periphery of the axis (Boatner, 1948) and are clearly visible against the light background color of the seed. Since the pioneering work of McMichael (1960), cottonseed varieties that do not contain glands, along with some that are partially glanded, have been developed by breeders throughout the cotton belt. Recently, Lusas and Jividen (1987) published a review, with extensive references, covering the development of glandless cottonseed and its use in foods.

Three grades for glandless cottonseed, based on maximum TG allowed, have been established by the National Cottonseed Products Association (1985), which regulates sale of cottonseed. It has been assumed that glandless cottonseed will not contain any TG; therefore, if TG is found in any sample labeled glandless, it must be contaminated with glanded seed (Phelps, 1977). However, during development of a method for determination of TG at parts per million levels (Fisher et al., 1987) we found TG in samples of glandless cottonseed that had been carefully inspected for presence of glands. This paper presents data on TG content per gland and number of glands per partially glanded kernel, which are needed to assess the possibility that the TG found in these samples came from contamination with partially glanded kernels. Results of TG analyses of kernels from 15 varieties of cottonseed, 1–3 varieties from each of 9 sources scattered from Mississippi to California, which confirm the presence of TG in some gland-free kernels, are also reported.

Southern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, New Orleans, Louisiana 70124.
[1]Present address: Eastern Regional Center, Philadelphia, PA 19118.

MATERIALS AND METHODS

Cottonseed. Most of the glandless cottonseed used in this study were left over from other projects and had been stored in the dark at room temperature when not in use. Most were fuzzy seed, but a few were acid delinted. The total of 15 varieties included experimental as well as commercial glandless varieties and 2 partially glanded (low-gossypol) varieties.

Sample Preparation. Seeds as received were carefully dehulled by hand, and kernels that were obviously glanded, rotten, or discolored were removed. Partially glanded kernels were removed as soon as glands were detected, i.e. as whole kernels or during slicing if glands were clearly visible without magnification. Then each kernel was sliced into 8–10 slices (0.5–1.0-mm thick) with a clean razor blade, and the slices were examined with substage lighting under a 10× microscope (Olympus Model SZ III Zoomstereo). Only sliced kernels that were completely free from glands were used for analysis. For each analysis a separate sample was drawn from the lot. For determination of the sensitivity of the method in terms of number of glands that could be detected, portions of very thin slices of partially glanded kernels, in which glands could be counted, were used.

Method. Details of the method have been published elsewhere (Fisher et al., 1987). Briefly, 1–1.25-g (w) samples of sliced kernels were weighed into 15 × 150 mm screw-capped culture tubes and mashed with a glass rod. Extraction reagent (2.5 mL) consisting of 2 parts of 3-aminopropanol, 10 parts of acetic acid, and 88 parts of dimethylformamide was added to each sample and to a blank tube. Capped tubes were heated for 30 min at 100 °C and then cooled to room temperature. Hexane (4 mL) and 2-propanol (6 mL) were added to each tube. After thorough mixing of the contents, the tubes were centrifuged briefly. A 5- and a 4-mL aliquot of each clear extract were transferred to clean culture tubes. One-half volume of aqueous 1% thiourea solution was added to each aliquot. Tubes were shaken and then centrifuged to produce two layers. The upper layers, which contain lipids that interfere with determination of TG, were discarded. After addition of 0.5 mL of aniline to the sample (5 mL) aliquots, all tubes were heated to 100 °C for 30 min and then cooled for 1 h. Hexane (1 mL for samples and 0.8 mL for blanks) and 2 M citric acid (2.5 mL for samples and 2 mL for blanks) were added to each tube. Tubes were shaken and then centrifuged. Polar interfering components remain in the aqueous layer. Upper (hexane) layers were transferred to spectrophotometer cells, which were tightly stoppered. Absorbances at 418, 438, and 458 nm were measured for samples (S_{nm}), sample blanks (R_{nm}), and aniline blank (B_{nm}), with the reagent blank, without aniline, in the reference cell. Calculation of total gossypol concentration (ppm) is given by $40.7A_{438}/w$, where $A_{438} = S_{438} - R_{438} - FB_{438}$ and $F = [1.06(S_{418} - R_{438}) - S_{458} + R_{458}]/(1.06B_{418} - B_{458})$.

RESULTS AND DISCUSSION

In order to determine the level of contamination of gland-free kernels with glands that would be required to account for the 4–8 ppm TG found in two glandless varieties in the previous study (Fisher et al., 1987), TG content per gland was estimated by analyzing fragments of partially glanded kernels. Results are expressed in terms (ppm) of TG that would be found in a 1.25-g sample of kernels containing the number of glands observed in the fragment (Table I). The correlation between number of glands and TG is 0.998 with a regression line slope of 0.11 ppm per gland. Hence, in order to explain the 8.4 and 8.5 ppm TG found in duplicates of one variety (Fisher et al., 1987), about 80 glands per sample must have been overlooked.

Table I. Total Gossypol Content of Glands

no.	A_{438}[a]	ppm[b]	no.	A_{438}[a]	ppm[b]
1	0.0034	0.11	25	0.0609	1.98
5	0.0101	0.33	100	0.3533	11.0
10	0.0234	0.76			

[a] Corrected absorbance at 438 nm. [b] For a 1.25-g sample.

Table II. Partially Glanded Cottonseed Kernels

kernel	wt, g	TG[a] content, ppm: kernel	TG[a] content, ppm: sample[b]	glands/kernel[c]
1	0.088[d]	16	1.1	10
2	0.060	39	1.8	17
3	0.089	75	5.4	49
4	0.067	194	10.4	95
5	0.064	443	23	206
6	0.067	894	48	436
7	0.108	1400	121	1100

[a] Total gossypol. [b] Calculated for one such kernel in a 1.25-g sample. [c] One gland = 0.11 ppm in a 1.25-g sample. [d] For this low level of glanding, three kernels with this average weight were used.

The degree of glanding of partially glanded seed is highly variable (Table II). Kernel 7 represents a level of glanding that can be detected by careful inspection of an intact kernel without magnification. The others appeared to be glandless until sliced. After slicing, glands could be seen in kernels 5 and 6 without magnification. Glands were readily visible in the kernels 1–3, after slicing, with substage lighting at 10× magnification. Increased magnification did not reveal any abnormally small glands in these kernels. As noted in footnote *d*, the first entry in Table II is a composite of three kernels. These kernels had the lowest degree of glanding found in over 1000 kernels examined in the course of this work. Although there is no way to prove that lower levels of glanding were not overlooked, the sharp contrast between the dark, well-defined glands and the creamy, translucent appearance of the rest of the tissue and the ease with which ca. 10 glands could be seen gave us confidence that a careful observer would seldom fail to detect even 3 or 4 glands per kernel. This level of glanding would have to be overlooked in 3 of 20 kernels to account for 1 ppm of TG or in all 20 to account for 8 ppm of TG. Hence, we conclude that the TG contents reported by Fisher et al. (1987) represent extraglandular TG, not contamination.

In order to determine whether presence of extraglandular TG was limited to the two varieties reported previously, replicates of gland-free kernels from 15 varieties of cottonseed were analyzed. Varieties from the southeastern, southwestern, and western regions of the cotton belt were included. Although, as shown in Table III, no more than traces of TG were found in gland-free kernels of three varieties of glandless cottonseed, the average TG content of gland-free kernels from nine varieties ranged from 2 to 7 ppm. Hence, TG can be bred out of cottonseed, but absence of glands does not assure elimination of TG. Like degree of glanding (Lee et al., 1968), extraglandular TG content is quite variable even within a variety. No effort was made to achieve random sampling, so it is entirely possible that several seeds in a given sample came from the same plant or even the same boll. This is especially true of fuzzy seed, which are hard to mix. Even though elimination of glands does not ensure absence of TG, extra glandular TG could not prevent any of the the varieties reported in Table III from meeting the requirements for Grade AAA seed (National Cottonseed Products

Table III. Total Gossypol Content (ppm) of Glandless Cottonseed

variety	replicate 1	2	3	4	5	av
1-1[a]	0.0	0.0	0.0	0.0	0.0	0.0
2-2	0.0	tr[b]	0.0	0.0		0.0
3-3	0.0	tr	tr	tr	0.0[c]	0.0
4-2	0.0	0.7	0.0	0.0		0.2
5-4	0.7	0.0	0.0			0.2
6-5[d]	0.8	0.9				0.8
7-6	0.9	2.4	3.7	1.5		2.3
8-7	0.0	2.0	7.8	0.0		2.4
9-8	5.5	tr	0.0[e]	9.3	0.7	2.6
10-6	0.0	7.3	8.1	1.2	1.5	3.6
11-7	2.7	2.6	8.6	6.1	2.6	4.5
12-7	3.9	4.0	4.0	5.9		4.5
13-4	3.7	5.5	5.9			5.0
14-9	4.0	3.8	6.3	10.7		6.2
15-6	8.8	8.7	8.8	3.1		7.4

[a] First number is variety; second is source. [b] Trace. Corrected absorbance ratios were satisfactory, but A_{438} was less than 0.02. [c] Four other replicates also gave 0.0 TG. [d] These were roasted kernels. [e] One other replicate also gave 0.0 TG.

Association, 1985). However, commercial production of Grade AAA glandless cottonseed from varieties that contain relatively high concentrations of extraglandular TG may be difficult. Variety 15 could tolerate only about one-fourth as much contamination with glanded seed as varieities 1–5 could tolerate. A fat-free cottonseed flour, prepared in this laboratory from hand-sorted whole kernels graded AAA (6 ppm). Another, prepared from commercial glandless seed, graded A (175 ppm TG). We found about 3% of partially glanded kernels containing ca. 0.4% TG in a sample of the seed used to prepare this flour. A third flour, prepared from glanded kernels deglanded by the liquid cyclone process (Vix et al., 1971), also graded A (345 ppm TG).

In addition to glanded kernels, abnormal kernels that are present in most lots of seed need to be removed in order to get Grade AAA products. One type of abnormal kernel is dry and brittle, with an overall gray color. These, which typically contained about 70 ppm TG, might be eliminated during standard delinting and dehulling. Removal of others, which were amber-colored and contained about 30 ppm TG, would require sorting after dehulling. The high extraglandular TG content of these seeds may be explained by the observation of Halloin and Bell (1979) that extraglandular terpenoid aldehydes related to gossypol form in diseased seed.

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Received for review October 30, 1986. Revised manuscript received August 7, 1987. Accepted October 26, 1987. Presented in part at the 186th National Meeting of the American Chemical Society, Washington, DC, 1983.