Acidic Ethanol Extraction of Cottonseed[1]

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Ethanol (EtOH) is being evaluated as an alternate solvent to hexane for the extraction of glanded cottonseed. Hot EtOH, needed for efficient oil and aflatoxin extraction, binds gossypol to protein. However, this binding can be minimized by acidifying aqueous EtOH with a tribasic acid, such as phosphoric or citric. While this solvent extracts oil and gossypol, it does not affect EtOH's ability to extract aflatoxin. The defatted cottonseed meals produced from this process contained 0.03% total gossypol (which is lower than meal prepared by most other processes) and the aflatoxin content was reduced from 69 to 2.9 ppb. These are preliminary results and additional research is needed to determine commercial feasibility. The removal of essentially all gossypol from an extracted meal has the potential to expand the use of cottonseed meal as a feed, increasing its value to both the cotton farmer and the seed processor.

KEY WORDS: Acid, aflatoxin, citric, cottonseed, ethanol, extraction, gossypol, oil, phosphoric.

Traditionally grown glanded cottonseed contains a phenolic pigment, gossypol, which is toxic to monogastric animals and young ruminants. While some whole cottonseed is fed to mature ruminants, most of it is solvent extracted with hexane to produce an edible oil and a high protein meal, which both contain gossypol. Before the oil and meal can be used, free gossypol (FG), which is physiologically active, either must be removed or deactivated. Gossypol and other contaminants are removed from oil by refining. FG is usually deactivated by heating moist meats or flakes to react most of the FG with the free amino groups of proteins to form bound gossypol (BG). BG is insoluble in hexane and is essentially physiologically inactive. The sum of FG and BG values is referred to as total gossypol (TG).

Unfavorable growing, harvesting or storage conditions can cause cottonseed to mold (*Aspergillus flavus* and or *A. parasiticus*) and become contaminated with the fungal metabolite aflatoxin, a carcinogen. Aflatoxin is insoluble in hexane and, therefore, it is desirable to find a solvent that can remove it so that residual levels do not exceed 20 ppb in extracted meals. High levels of residual gossypol and aflatoxin severely limit the use of cottonseed meal as a general feed source and significantly reduce the meal's cash value.

Ethanol (EtOH) is a solvent that, depending on temperature and moisture content, can extract oil, gossypol and aflatoxin. However, to extract oil and aflatoxin, EtOH must be close to its boiling point (78°C) which results in chemical binding of FG to protein. This binding produces BG, which is insoluble in EtOH. Removal of all three requires a two-step extraction process in which FG is first removed with ambient temperature EtOH, and then oil and aflatoxin are extracted with hot EtOH. A single-solvent extraction process that removes oil, gossypol and aflatoxin, leaving a higher valued protein meal, would be desirable. This study describes such a process (1).

[1]Presented in part at the 40th Oilseed Processing Clinic, March 4, 1991, New Orleans, LA.

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EXPERIMENTAL PROCEDURES

Materials. Aflatoxin-contaminated samples were 1989 crop, mill-run cottonseed meats obtained from an Arizona oil mill. Uncontaminated samples were 1989 crop, mill-run cottonseed meats obtained from a Mississippi valley oil mill. The solvent solutions used in extraction were comprised of industrial-grade hexane, 95% aqueous EtOH and 0.1 and 0.4 M citric acid (anhydrous, ACS grade), and 0.1 and 0.34 M phosphoric acid (85.7%, ACS grade) solutions in 95% (vol/vol) aqueous EtOH (USP).

Extraction process. Full-fat cottonseed meats, with and without aflatoxin, containing about 8% moisture, were flaked with flaking rolls set at 0.203 mm (0.008 in.) gap. A 300-g portion of flakes and 800 g of one of five different solvent solutions were placed in a jacketed, stainless steel, cylindrical extractor 15.2 cm in diameter × 15.2 cm deep (6 in. × 6 in.) fitted with a 12-mesh stainless steel screen at the bottom. Hot (79°C) water was circulated through the jacket. Solvent was recirculated through the flakes at a rate of 1 L/min for 10 min. Miscella (*ca.* 300 g) was drained from the extractor for 1 min and the flakes were re-extracted under the same conditions with 600-g portions of fresh solvent solution. After the seventh percolation extraction, each extraction being of about 10-min duration, the spent flakes were washed with 600 and then 50 g of pure 95% EtOH to remove any free or adsorbed acidic EtOH. Spent flakes were allowed to air-dry at room temperature overnight, and then were oven-dried at 101°C for 1 h. They were ground to pass a 20-mesh screen and analyzed for residual lipids, FG, TG, nitrogen and aflatoxin B_1. Extractions of the same lots of flakes were made with 95% EtOH without acid for comparison. Also as controls, samples were Soxhlet-extracted with non-acidified hexane or 95% EtOH for 4 h to provide exhaustively extracted samples for comparison. The spent flakes were desolventized and ground as above.

Chemical analyses. Because of the extremely low levels of gossypol obtained, the high-performance liquid chromatography (HPLC) method of Hron *et al.* (2) was used for their determination. Residual lipids were determined by using petroleum ether in a Soxtec apparatus. Nitrogen and aflatoxin were determined by AOCS methods Aa 5-38 and Aa 8-83, respectively (3).

RESULTS AND DISCUSSION

Table 1 shows the results of using EtOH with and without acid to extract oil and TG from cottonseed flakes. All four of the acidic ethanol solutions gave extracted meals with large reductions in TG when compared to control extractions with either hexane or 95% EtOH alone. Hexane extraction resulted in a decrease in FG, but extracted little TG. Although pure 95% EtOH reduced FG to 800 ppm, most of this reduction, as discussed earlier, was due to

TABLE 1

Effect of Acidic Ethanol Percolation Extraction on Residual Oil and Gossypol in Cottonseed Flakes

Solvent	Residual lipids (%)	Total gossypol[a] (%)	Free gossypol[a] (%)
Full-fat flakes	26.3	1.65	1.62
Hexane	0.2	1.46	0.56
Ethanol	0.8	1.08	0.08
0.1 M citric	1.1	0.40	0.02
0.4 M citric	1.1	0.09	0.007
0.1 M phosphoric	0.5	0.27	0.02
0.34 M phosphoric	1.4	0.03	0.005

[a]Moisture- and oil-free basis.

binding of FG to protein to produce BG, and not to extraction of gossypol. EtOH solvents containing citric acid were not as efficient in extracting TG as phosphoric acid-containing solvents. The largest removal of TG was obtained with a solvent containing 0.34 M phosphoric acid. In a single step, this solvent removed over 98% of TG to produce a material containing just 300 ppm TG. Residual oil (1.4%) in the product was slightly higher than the desired level of 1%. This probably could be improved by varying either pretreatment or processing conditions.

Table 2 is a replicate of Table 1, except that the meats were obtained from a different growing area and contained aflatoxin. Acid-containing EtOH again removed significant amounts of TG, *i.e.,* up to 95%. It also was observed that acid addition apparently had no effect on ethanol's ability to extract aflatoxin, with over 95% being removed by both nonacidified EtOH and the acid-containing ethanols. However, high molar levels (.4 and .34) of citric and phosphoric acid in EtOH appeared to give high residual oil in their respective meals, with the highest residual oil again being in the 0.34 M phosphoric acid-ethanol solvent-extracted meal. The high residual oils are probably due to the acids increasing the polarity of the solvent mixtures, thereby reducing their solvency for neutral oil.

TABLE 2

Effect of Acidic Ethanol Percolation Extraction on Oil, Gossypol and Aflatoxin in Cottonseed Flakes

Solvent	Residual lipids (%)	ppb B_1 Aflatoxin[a]	Total gossypol[a] (%)	Free gossypol[a] (%)
Full-fat flakes	26.2	69	1.47	1.22
Hexane	0.2	37.5	1.24	0.84
Ethanol	0.9	3.6	0.80	0.01
0.1 M citric	0.9	3.8	0.22	0.01
0.4 M citric	2.3	3.4	0.09	0.06
0.1 M phosphoric	1.1	3.7	0.08	0.01
0.34 M phosphoric	5.1	2.9	0.05	0.004

[a]Moisture- and oil-free basis.

TABLE 3

Residual Lipids and Gossypol in EtOH Soxhlet-Extracted Cottonseed Flakes and in Flakes Re-Extracted with Citric and Phosphoric Acids in EtOH[a]

Solvent	Residual lipids (%)	Total gossypol[b] (%)	Free gossypol[b] (%)
Soxhlet-extracted meal	6.5	1.40	0.02
0.4 M citric	0.3 ± 0.1	0.88 ± 0.01	0.12 ± 0.01
0.34 M phosphoric	0.2 ± 0.1	0.52 ± 0.05	0.09 ± 0.01

[a]Analyses are means of duplicate extractions.

[b]Moisture- and oil-free basis.

Table 3 shows the ability of acidic EtOH to extract BG. The gossypol was bound by Soxhlet-extracting full-fat flakes for 4 h with 95% EtOH. The flakes were then re-extracted with three batches of acidic EtOH solvents. Although re-extraction with acidic EtOH resulted in a large reduction of BG, residual BG was much higher than if full-fat flakes were just extracted three times with acidic EtOH. However, these results suggest a possible cottonseed extraction process in which cottonseed oil containing little gossypol could be extracted first with 95% EtOH and, if desired, gossypol could then be extracted separately with acidic EtOH. FG residuals were higher in the acid-treated samples, probably because only three extractions and no 95% EtOH wash were used.

A gossypol material balance run on meal and miscellas collected after a seven-stage extraction of flakes with 0.34 M phosphoric in EtOH showed a 99% recovery. This validated the HPLC method used in determining gossypol, as well as the ability of acidic EtOH to extract gossypol. Nitrogen content of acid EtOH- and hexane-extracted meals showed insignificant variation ranging from 7.13–7.32%.

Although oil was not recovered from any miscella samples or test refined, it is anticipated that refining loss could be minimized by initially separating gossypol from miscella or oil-rich fractions by reverse osmosis or adsorption techniques developed by Kuk *et al.* (4,5).

ACKNOWLEDGMENT

The authors acknowledge the technical assistance of Hector Huerta in extractions.

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[Received November 5, 1991; accepted June 17, 1992]