Ethanol Extraction of Oil, Gossypol and Aflatoxin from Cottonseed[1]

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Commercial processing of cottonseed requires hexane to extract and recover edible oil. Gossypol and aflatoxin are not removed from extracted meals. A bench-top extraction process with 95% (vol/vol) aqueous ethanol (EtOH) solvent has been developed that extracts all three of the above materials with a much less volatile solvent. In this process, cottonseed is pretreated and extracted with ambient 95% EtOH to remove gossypol and then extracted with hot 95% EtOH to extract oil and aflatoxin. Membranes and adsorption columns are used to purify the various extract streams, so that they can be recycled directly. A representative extracted meal contained a total gossypol content of 0.47% (a 70% reduction) and 3 ppb aflatoxin (a 95% reduction). Residual oil content was approximately 2%. Although the process is technically feasible, it is presently not economical unless a mill has a continual, serious aflatoxin contamination problem. However, if a plant cannot meet the hexane emission standards under the Clean Air Act of 1990, this process could provide a safer solvent that may expand the use and increase the value of cottonseed meal as a feed for nonruminants.

KEY WORDS: Adsorption, aflatoxin, cottonseed, economics, ethanol, extraction, gossypol, membrane, oil, reverse osmosis.

Solvent extraction was first patented in England in 1856 and had become established as a batch-type operation in Europe by about 1870. The first large-scale, continuous extraction plant in the United States was started up in 1934, in which 100 tons of soybeans could be extracted per day with a hexane-type solvent (1). The long delay was due to the lack of the technical development of an economical continuous process and the use of inferior solvents, which produced low-grade feed meals. Although many other solvents have been evaluated over the intervening years (1–3), hexane remains the solvent of choice. However, when dealing with cottonseed, a hexane extraction cannot remove two important antinutrients, gossypol and aflatoxin, from the meal.

Gossypol is a polyphenolic compound that is contained in small discrete glands, which are distributed throughout the cottonseed kernel. In its free form, gossypol is toxic to monogastric animals and limits most of the cottonseed products to ruminant feeding (4). Before extracted oil and meal are used, it is desirable to remove or deactivate "free" or physiologically active gossypol. Gossypol can be removed from crude oil by refining, but in meals it must be deactivated, usually by reacting it with protein.

Under certain conditions, cottonseed can sometimes become moldy and contaminated with a carcinogenic mold metabolite, aflatoxin. Although not a problem in oil, this contaminant severely limits the use of meals as a feed source (5,6).

With the passage of the Clean Air Act Amendment of November 15, 1990, *n*-hexane is now considered a hazardous air pollutant, and control of its emission level in extraction plants will probably call for the purchase and installation of additional control equipment. Due to these problems with hexane, the cottonseed oil extraction industry is seeking a safer alternate solvent that also removes gossypol and aflatoxin to acceptable levels. An extensive survey indicated that the 95% ethanol and 91% isopropanol azeotropes had the most promise (2,3). The use of alcohols to extract oilseeds is not new. Japanese workers, among others, reported on the use of aqueous ethanol (EtOH) to extract soybeans as early as 1932 (7). However, none of the processes were ever commercialized for any significant period of time. Our bench-top research centered on EtOH primarily because of its generally recognized as safe (GRAS) rating. Early research resulted in a process with 95% EtOH to extract cottonseed oil, and the resulting alcoholic miscella was easily caustic-refined to produce acceptable oil (8–10). However, the process did not extract gossypol. Our most recent research indicates that oil, at least 50% of total gossypol, and at least 90% of the aflatoxin can be extracted in the two-step extraction process shown in Figure 1.

Materials and methods. Aflatoxin-contaminated samples were 1989-crop, mill-run cottonseed meats obtained from an Arizona oil mill. Uncontaminated samples were 1990-crop, mill-run cottonseed meats obtained from a Mississippi valley oil mill. The extraction solvent was USP 190-proof ethanol (U.S. Industrial Chemicals, Louisville, KY). Official AOCS methods (11) were used for aflatoxin B_1 (Aa 8-83) and free (Ba 7-58) and total gossypol (Ba 8-78) analyses. Residual oils were determined by extraction with petroleum ether in a Soxhlet apparatus. All analyses reported were carried out in duplicate.

RESULTS AND DISCUSSION

Pretreatment. Earlier research showed that high extraction temperatures, near the boiling point of 95% EtOH, are needed to extract oil and aflatoxin. However, high EtOH temperatures were found to cause gossypol to bind to protein, significantly reducing gossypol's solubility (8). Based upon these results, it appears that the first step in any process to extract all three materials is to extract a major portion of the gossypol first. To extract gossypol, gossypol-containing pigment glands must initially be ruptured in a pretreatment operation (Fig. 1). Although other gland rupture methods exist [i.e., use of dilute alcohol (12)], in this process, gland rupture was accomplished by first moisturizing meats to approximately 14% and equilibrating overnight. Moisturizing serves two purposes, (i) it softens glands, as shown by Boatner (13), increasing their ability to be ruptured during a 0.229-mm flaking operation in Ferrel-Ross (Bluffton, IN) pilot plant rolls and (ii) the large wet flakes, when dried on trays to approximately 3% moisture with 71°C air in a Proctor-Schwartz (Horsham, PA) forced-draft oven, develop "body," which minimizes fines development during extraction. Drying temperature was found to be critical.

Temperatures above approximately 82°C, common in conventional processing, result in extensive binding of "freed" gossypol to protein (14). If extensive binding is allowed to occur, gossypol cannot be leached out by EtOH in the next processing step. Drying to approximately 3%

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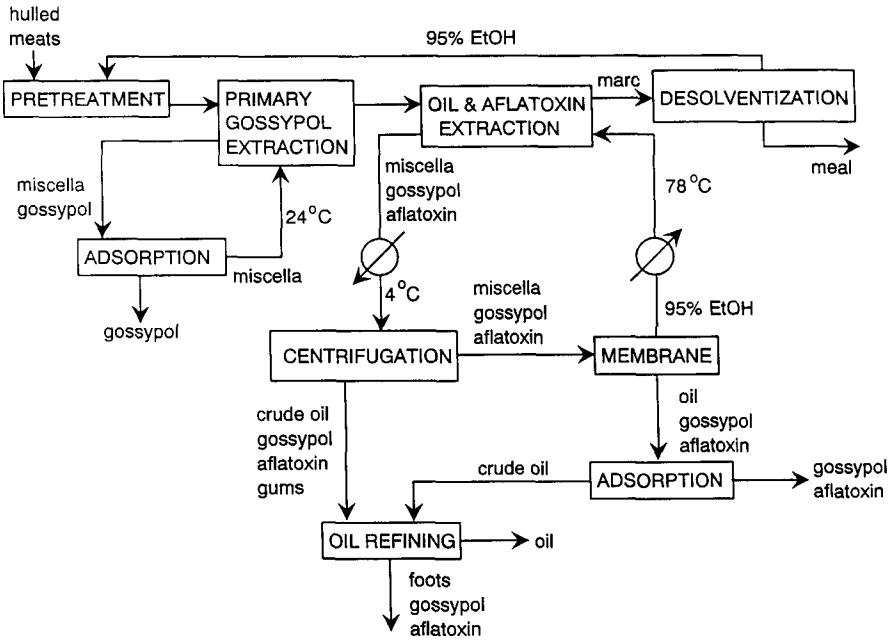

FIG. 1. Flow sheet for aqueous ethanol extraction of oil, gossypol and aflatoxin from cottonseed.

moisture was necessary, in our earlier research, to prevent 95% EtOH from absorbing moisture, which would significantly reduce its already limited extraction ability (9,14). Earlier work also showed that flakes lower than 2.5% moisture can absorb water and increase the concentration of EtOH (15). However, drying to this level is close to the "bound moisture" zone, which means that excessive energy may be needed to remove any small amount of remaining moisture. Although absolute EtOH is significantly better in solvent power than 95% EtOH (16), its use would involve significant extra costs for removing essentially all of the free moisture contained in cottonseed feed. And, either a three-component azeotropic distillation installation or the use of molecular sieves to remove the additional water above the 95% azeotropic concentration, obtained from a normal distillation recovery system, would be needed. As a last step in the pretreatment, the flakes are soaked in EtOH. In the bench-top process, soaking was carried out in a bench-top extractor described earlier (8). The extractor was charged with 500 g of dried flakes and 1200 g of pure 95% EtOH at room temperature and allowed to soak for at least 3 h. Dried cottonseed flakes swell when in contact with 95% EtOH. This swelling significantly reduces the mass diffusion rate of gossypol from ruptured glands into EtOH and necessitates a prolonged soak if the goal of a 50% reduction in total gossypol in extracted meal is to be reached. The use of pure 95% EtOH at 22–25°C in the soak extracts about 3–4% of the available 28–29% oil in the flakes.

Primary gossypol extraction. After soaking, the flakes were extracted, or a better description would be "washed," with room-temperature ethanol to remove gossypol and to minimize gossypol binding and oil extraction. Washing was carried out with three 1000-g batches of 22–25°C, 95% EtOH miscella. Each wash was recirculated for 20 min, and a 1-min drain period was used. After washing, the total gossypol content was reduced from approximately 1.6–1.7% (moisture and oil-free basis) in the original meats to approximately 0.6–0.8% in the washed flakes, depending on how well the pigment glands were ruptured during flaking. Because the miscella was saturated with oil, no additional oil was extracted during the washing.

Oil and aflatoxin extraction. The flakes were next extracted with hot, 78°C, recycled ethanol in a second-stage extraction to remove oil, aflatoxin and some additional gossypol. Four 20-min extractions, with recirculation, were performed on the room-temperature extracted flakes with 1000 g each of 78°C EtOH recycled miscella. Samples were then desolventized overnight in air, followed by 1 h in a forced-draft oven at 101°C. Residual lipids in desolventized meal samples from various runs varied from 0.96 to 2.7%, depending on the thickness of the flakes and their final moisture level after drying. Thick flakes (300–330 mm), or flakes with high moisture (≥4%), yielded higher residual lipids. Total gossypol varied from 0.45 to .70%, again depending upon the flake thickness and moisture content of the flakes and on the total gossypol content of the starting meat samples, which varied from 1.56 to 1.76%. Although it is desirable to reduce total gossypol content to below 0.5%, to date, no one has reported doing so with pure alcohol, probably due to the fact that even a low-temperature ethanol extraction results in some chemical binding of gossypol to protein (8). However, Hron *et al.* (17,18) found that, by extracting with ethanol acidified with either phosphoric or citric acid, bound gossypol is hydrolyzed and total gossypol content of the extracted meal can be significantly reduced to about 0.03%. Free gossypol in meals extracted with pure 95% EtOH, as expected, was consistently low and varied from 0.007 to 0.011%. Aflatoxin B_1 was reduced from 20–45 ppb in starting meat samples to 2–3 ppb in extracted meals. The

marc exiting this second extraction step contains about 60% volatiles, whereas a hexane process normally produces a marc with about 40% volatiles. The high level of volatiles could be reduced farther by pressing if necessary to lessen the volatilization load on the desolventizer. Sullivan *et al.* (19) pointed out that, with pressing, the total energy consumed in flake desolventization in an alcohol process is significantly less than the total energy used in the miscella evaporation and desolventization steps of a hexane process. However, it is also known that expander collets in a hexane process exit the extractor with roughly 20% volatiles. Because of this, future research will include investigating the use of an expander to produce collets rather than flakes in the pretreatment step. If successful, the use of collets should significantly reduce marc volatiles in the desolventization step and, because collets are denser but more porous than flakes, they should also increase extractor capacity. Because only small bench-top quantities of marc were produced, no data or specific method of desolventization was investigated. However, desolventization procedures reported by Karnofsky (20) and Baker and Sullivan (21) for aqueous isopropanol/soy should be applicable to aqueous EtOH/cottonseed. EtOH evaporated during desolventization can be reactified, if necessary, and recycled back to the pretreatment stage.

Miscella streams. Figure 1 shows that the various miscella streams produced are handled in a series of innovative processing steps. Room-temperature miscella from the primary gossypol extractor was first filtered to remove any solid fines and then passed through a 80–200 mesh, neutral-alumina adsorption column as described by Kuk *et al.* (22,23). The column reduces gossypol content from approximately 1000 to about 1–2 ppm, and the miscella, containing an equilibrium amount of crude oil, about 5 to 6%, is recycled back to the first-stage extractor. The presence of phosphatides and polysaccharides did not affect the gossypol adsorption capacities of the column. Adsorbed gossypol is eluted by washing the column with acetone, followed by methyl ethyl ketone. After drying, the column is treated with sodium hypochlorite to complete regeneration (23). The wash solvents are combined and recovered by evaporation, leaving a crude gossypol fraction. Although refined gossypol has many possible uses (24), it is presently selling for over $140,000/lb. Consequently, there is no real demand for it. However, a 600-ton-per-day cottonseed processing mill with an alcohol extraction process could theoretically recover over a ton of gossypol per day, which would result in a significant reduction in gossypol's sale price and possibly in various new demands for what is presently a waste product.

Returning to the flow sheet, rich miscella from the second-stage extraction, containing 10–12% oil (10), gossypol and aflatoxin, was cooled to approximately 4°C and then phase-separated by a centrifuge into a concentrated oil-gum fraction with about 15% EtOH and a lean miscella fraction containing about 3 1/2% oil and some gossypol and aflatoxin. Chill separation of the oil fraction in an alcohol process is a large energy-saving step when compared to miscella evaporation required in hexane processing (25). After passing through a series of reverse-osmosis aromatic-polyamide membrane separators (26), the lean miscella permeate exits with an oil content <1% and an aflatoxin content of 0 to 3 ppb. The miscella is then reheated to 78°C and recycled back to the extractor as solvent feed. This is another critical processing step because permeate oil and aflatoxin contents affect the minimum solvent-to-flake ratio necessary to give the desired residual oil content of 1% or less and less than 10 ppb aflatoxin in extracted meal. Under ideal conditions, miscella permeate that contains an oil content of less than 1% will yield a solvent-to-flake ratio of approximately 4:1 (10). The fraction retained by the membrane contains oil, gossypol and aflatoxin and is passed through an alumina adsorber to remove gossypol and some aflatoxin and a montmorillonite adsorber where aflatoxin is almost totally removed (27). Again, aflatoxin can be removed and the column regenerated by washing with acetone and sodium hypochlorite (5 vol%). The filtrate from the adsorber, consisting mostly of crude oil, is combined with the crude oil and gum fraction from the centrifuge and sent to an oil refining operation. Here, the combined stream is reacted with caustic and centrifuged to produce a once-refined oil and foots. Earlier work (8) showed that caustic refining of alcoholic miscella resulted in a refining loss of 1.5% compared to 2.2% for a hexane-extracted control. Refined oil from alcoholic miscella had a color of 35 yellow (Y) and 4.3 red (R) and a bleached color of 13Y and 1.3R.

Economic analysis. Updating an earlier cost analysis for an EtOH extraction of cottonseed oil (28) to include extraction of gossypol and oil, it is estimated that it will cost roughly 7 million dollars in capital cost to retrofit a plant that processes 600 tons of cottonseed per day (Table 1). An isopropyl alcohol-based process would cost slightly less. Presently, the dollar value for meal with reduced total gossypol of approximately 0.5% and free gossypol below 0.04% may be higher but cannot be determined. However, we do know that, by reducing aflatoxin to below 10 ppb, we should be able to recover the approximate $30 per ton penalty presently assessed meals that exceed this level. Figure 2 gives an idea on investment payback time. It is based on a 600 ton-per-day EtOH plant with a 7 million dollar capital cost investment for retrofit and operating costs of roughly 1.7 million dollars per year. The estimated costs for items used in the calculation of net present worth are as follows: capital investment (6,871,040); increased sales income (6,300,000); expenses (1,657,985); operating income (4,642,015); depreciation (687,104); gross profit (3,954,911); taxes (1,384,219); net profit (2,570,692); cash flow (3,257,796). As shown in the top graph of Figure 2A, if 100% of the seed processed by this plant contains over 20 ppb aflatoxin contamination, it will take a little over two years to recover the investment. If 75% contaminated (Fig. 2B), it will take almost four years, and for 50% contamination (Fig. 2C), it will take almost ten years. The reason that it takes longer to recoup the investment at lower levels of aflatoxin contamination is that, at these lower levels, the process is being "wasted" on clean seed. It is obvious from Figure 2 that, if there is no aflatoxin contamination, an investment in an alcohol process to extract all three components will never be recovered unless an increase in meal sale price (due to reduced gossypol content) can be quantified. There are other intangible benefits of alcohol extraction, such as safety, for which there is no dollar value. Economic analysis of a similar, aqueous isopropyl alcohol extraction process showed comparative results, except that payback times were slightly shorter. The results of bench-top

TABLE 1

Consolidated Costs in Dollars for a 600-Ton/Day Plant Operating 300 Days/Year[a]

	Drying		Extraction		Chill separation		Membrane separation and adsorption		Desolventizing	
	EtOH	IPA	EtOH	IPA	EtOH	IPA	EtOH	IPA	EtOH	IPA
Capital costs										
Equipment + building	1,816,730	1,816,730			2,054,310	2,054,310	3,000,000	2,400,000		
Operating costs										
Solvents			139,531	150,935						
Labor							78,516	78,516		
Laboratory							11,777	11,777		
Maintenance	99,920	99,920					165,000	132.000		
Supplies							363,333	290,666		
Electricity	91,847	91,848			141,799	85,080	165,240	132,192		
Cooling water					10,940	6,564				
Steam	129,000	80,625							261,078	210,463
Summary of operating costs	320,767	272,393	139,531	150,935	152,739	91,644	783,866	645,151	261,078	210,463

	EtOH	IPA
Total capital costs	6,871,040	6,271,040
Total operating costs/year	1,657,981	1,370,586

[a]EtOH, aqueous alcohol; IPA, isopropanol.

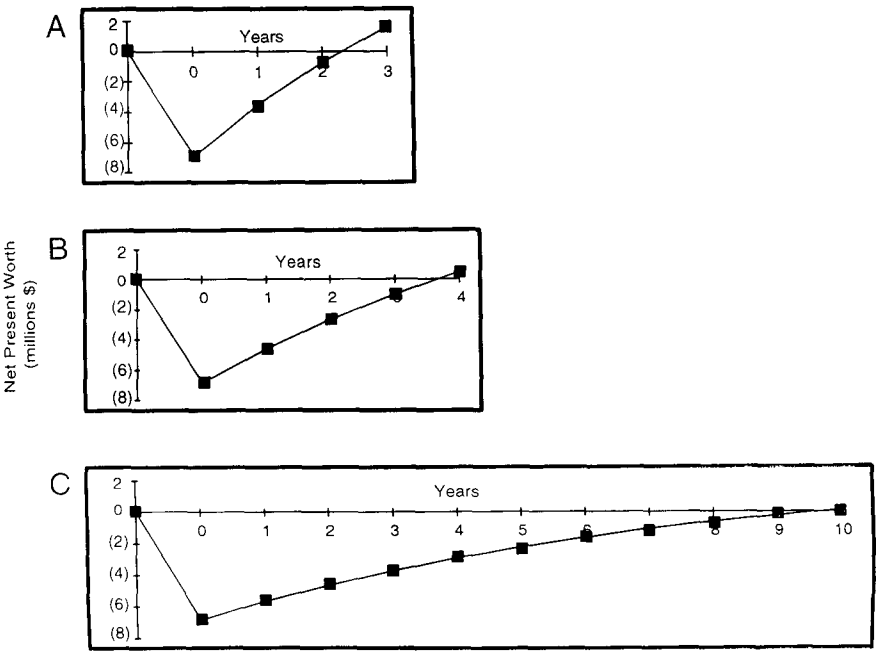

FIG. 2. Comparison of the net present worth for different levels of aflatoxin contamination in seed. A, 100%; B, 75% and C, 50%. Basis: increased profit of $35/ton meal and 15% rate of return.

research show that it is technically feasible to extract and recover oil, gossypol and aflatoxin from cottonseed with aqueous 95% EtOH as a solvent. Economically, it appears to be feasible for a mill with a continual, serious aflatoxin contamination problem to retrofit to an alcohol solvent. However, in every other case, because of the monetary effects for reduced gossypol in meal, the effect of future federal or state regulations under the 1990 Clean Air Act or even value of safety cannot be quantified presently, it would not be economically feasible to substitute EtOH for hexane in the extraction of oil, gossypol and aflatoxin from cottonseed. Nevertheless, future world conditions, as well as continued technological developments in cottonseed processing and gossypol research, could reverse this situation.

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