# Ethanol Vapor Deactivation of Gossypol in Cottonseed Meal[1]

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**ABSTRACT:** Most cottonseed cultivars contain gossypol, a polyphenolic antinutritional compound. "Free" gossypol is a physiologically active form of gossypol, which is toxic to young- and nonruminant animals. To utilize solvent-extracted cottonseed meal as a general feed, gossypol must be either removed or deactivated to a minimum level specified for each class of animal. Normally, deactivation is carried out prior to oil extraction; however, the desired level of deactivation is not always attained. A new supplemental method of deactivation has been found by using either ethanol or isopropanol vapors on solvent-extracted meal. In a bench-top set-up, ethanol vapor reduced free gossypol from 0.115 to 0.053%, and a further reduction to 0.026% has been observed with the addition of ferrous sulfate. The supplemental deactivation method can, in most cases, reduce free gossypol to significantly safer levels for feeding, thus increasing utility, and possibly demand, for cottonseed meal as a general animal feed protein source.
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**KEY WORDS:** Alcohol, cottonseed, deactivation, ethanol, feed, ferrous sulfate, gossypol, isopropanol, vapor.

Cotton is grown mainly for its fiber, cottonseed is a by-product of the crop. Ginned seed is fed directly to dairy cows to increase the butterfat content of milk or is extracted with hexane to produce an edible oil and a feed-grade meal. Although cottonseed meal is an economical protein source, it is currently used primarily in ruminant feeds, that is, for cattle, sheep, and goats. This restriction is necessary due to the fact that cottonseed generally contains from 0.5 to 2% of a polyphenolic binaphthyl aldehyde, better known as gossypol, which is toxic to monogastric animals. Gossypol is a highly active compound that, during processing with heat and moisture to produce an edible oil, binds to various seed components to become what is known as "bound" gossypol. The extent and type of this binding varies widely depending on the processing method. Bound gossypol is thought to be inactivated and relatively nontoxic. Gossypol that is not bound during processing is designated as "free" gossypol. It is toxic and seriously limits the use of extracted cottonseed meal in poultry, swine, or aquaculture feeds (1). Reliable removal or deactivation of free gossypol to low, tolerable levels in meals could eliminate most of the meal's present feed limitations and possibly increase the economic worth of cottonseed to both the farmer and the seed processor.

Many methods have been reported that remove or deactivate free gossypol prior to, during, and after oil removal by solvent extraction (2–4). Although a small amount of gossypol is removed during extraction, most of it is inactivated during preparation of the seed for extraction. At one time, the standard method of seed preparation included cooking and flaking. However, flaking usually resulted in extracted meals with free gossypol from 0.1–0.5%. Because of its many advantages over flaking alone, one of which lowers free gossypol content to 0.05–0.1%, the current, preferred method uses a combination of flaking and an expander. Expanders use heat, moisture, pressure, and a short treatment time of approximately 30 s to produce dense, porous collets from which oil is easily extracted. One possible problem with expanders is that, due to the somewhat mild processing conditions sometimes used and short treatment time, some of the bound gossypol may be only weakly bound and easily converted back to an active form in an animal's digestive system (5). The use of chemical additives to deactivate gossypol is not new. Over 25 different chemical methods have been proposed to deactivate gossypol (2). The most promising treatment appears to be the addition of iron sulfate to extracted meals. Extensive feeding studies in the early 1960s showed that its addition as ferrous sulfate resulted in extracted meals that were safe enough to be fed to poultry and swine (1,6). Apparently, iron reacts with gossypol on a mole per mole basis to produce a stable chelate that is inactive in an animal's gut. This study reports on a new supplementary chemical treatment that uses either 95% aqueous ethanol or 91% aqueous isopropanol vapors either alone, or in combination with ferrous sulfate, to reduce free gossypol in extracted meals to safer levels with possibly less chance of the bound gossypol reverting back to an active form in an animal's digestive system.

## EXPERIMENTAL PROCEDURES

*Materials.* A direct-solvent–extracted (flaked) and an expander-solvent–extracted cottonseed meal were obtained from two Mississippi valley oil mills. One expander-solvent–

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extracted meal was obtained from a Texas high plains oil mill. All meal samples were obtained from 1992 production and were remilled through a U.S. 10 screen. The solvents used in vapor treatments were azeotropic 92% (w/w) commercial-grade aqueous ethanol and 87.7% (w/w) aqueous isopropanol. The aqueous isopropanol was made by diluting reagent-grade isopropanol (99.9% w/w). ACS reagent-grade ferrous sulfate heptahydrate was used.

*Vapor treatment.* Figure 1 shows the lab-bench set-up used to vapor-treat the direct-solvent and two expander-processed, solvent-extracted meals. A 40-g ground sample was placed in a standard butt tube used in lipid analysis. The tube was jacketed and fitted with a 20-mesh sample-retaining screen. Hot water (82°C) was circulated through the jacket to keep either of the alcohol vapors from condensing on the meal during treatment. All samples were first heated in the butt tube for 20 min to bring them up to 82°C. After heating, the butt tube was connected to a 500-mL round-bottom boiling flask, filled with either 250 mL of boiling ethanol or boiling isopropanol and fitted with a rheostat-controlled heating mantle. The round-bottom flask also contained an angle neck, and a 1-m long, glass tubing safety vent was connected to the neck. Samples were treated in duplicate at these five different conditions: 2 h dry heat; 1 h dry heat plus 1 h alcohol vapor; 0.1% by weight ferrous sulfate heptahydrate mixed into sample plus 1 h dry heat; 0.1% ferrous sulfate heptahydrate mixed into sample plus 1 h dry heat plus 1 h alcohol vapor; and 1.0% ferrous sulfate heptahydrate mixed into sample plus 1 h dry heat plus 1 h alcohol vapor.

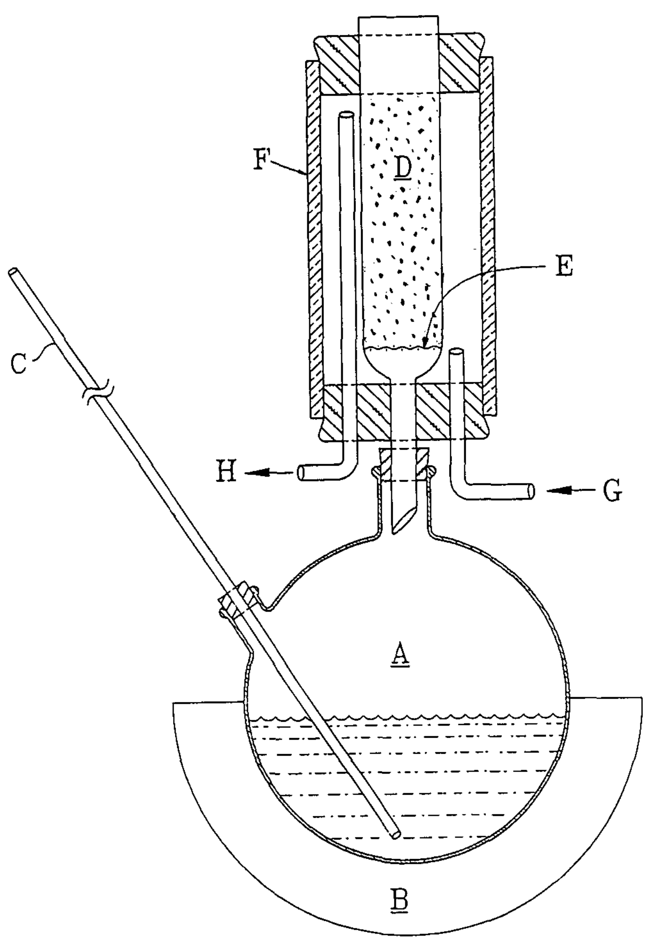


**FIG. 1.** Vapor treatment apparatus: A, boiling flask containing alcohol; B, heating mantle; C, safety vent tubing; D, butt tube containing cottonseed meal sample; E, sample-retaining screen; F, plexiglas jacket; G, jacket water inlet; and H, jacket water outlet.

*Chemical analyses.* Percent free gossypol was determined by AOCS method Ba 7-58 (7). All free gossypol data reported are means of duplicate test treatments, which showed insignificant differences from the coefficient of variation reported for the assay.

## RESULTS AND DISCUSSION

Table 1 shows the results of a one-hour vapor treatment. The three meals tested contained what could be considered large amounts of free gossypol for the respective processing methods. Plain heating alone reduced the percent of free gossypol very little. Treatment with heat and isopropanol vapor was not as effective as heat and ethanol vapors, which showed a little more than 50% reduction.

Because ferrous sulfate had been shown in the past to be effective in inactivating gossypol, we tested its effects in combination with ethanol vapor (Table 2). The vapor treatment was repeated, except that 0.1% ferrous sulfate was first mixed into a direct-solvent and an expander-processed meal. Heating alone resulted in about a 10% reduction in free gossypol, the addition of 0.1% ferrous sulfate alone produced about a 40% reduction, and ethanol vapor alone about a 50% reduction. The combination of ethanol and 0.1% ferrous sulfate reduced the free gossypol by up to 70%, and, by increasing the level of ferrous sulfate to 1%, a free-gossypol reduction of almost 80% was realized. Although the remaining levels of free gossypol are still slightly above the limits recommended for direct feeding of these meals to swine and poultry, it must be remembered that most extracted meals are not fed alone. They are normally used as part of a feed ration that contains vitamins, minerals, and other nutrients. The meals used in these tests purposely contained higher levels of free gossypol than the industry average for these types of products. High levels were evaluated as the worst-case scenario to determine the maximum effectiveness of the new method. Because direct solvent extraction of flakes has been essentially replaced by solvent extraction of expanded collets, it can be expected that meals from an efficiently run expander operation, once treated with either ethanol vapor or ferrous sulfate and ethanol vapor, could be used freely and safely as ideal ingredients for a large number of nonruminant animal feeds. It has been reported previously that when cottonseed meal treated with isopropanol was fed to poultry layers, it was a highly nutritive meal (8) and did not result in egg discoloration upon storage (9). Similar results would be expected with ethanol. Additional research is planned to determine if an alcohol treatment also results in minimal, if any,

**TABLE 1**
**Percent Free Gossypol in Heat-Treated, and, in Isopropanol (IPA) and Ethanol (EtOH) Vapor-Treated Cottonseed Meals**

| | | Treatment | | |
|---|---|---|---|---|
| Method of processing | Initial | Heat | Heat + IPA vapor | Heat + EtOH vapor |
| Direct solvent | 0.381 | 0.366 | 0.219 | 0.171 |
| Expander source "A" | 0.115 | 0.104 | 0.053 | 0.054 |
| Expander source "B" | 0.133 | 0.123 | 0.075 | 0.058 |

**TABLE 2**
**Percent Free Gossypol in Ferrous Sulfate-Treated, Heat-Treated, and EtOH Vapor-Treated Cottonseed Meals[a]**

| | | Treatment | | | | |
|---|---|---|---|---|---|---|
| Method of processing | Initial | Heat | 0.1% $FeSO_4$ + heat | Heat + EtOH vapor | 0.1% $FeSO_4$ + heat + EtOH vapor | 1.0% $FeSO_4$ + heat + EtOH vapor |
| Direct solvent | 0.381 | 0.345 | 0.239 | 0.171 | 0.144 | 0.092 |
| Expander, source "A" | 0.115 | 0.104 | 0.062 | 0.054 | 0.031 | 0.026 |

[a]See Table 1 for abbreviation.

reactivation of free gossypol during digestion in other animal species. The bench-top, batch process described here can be scaled-up fairly easily and added to an existing commercial cottonseed oil process. The scale-up could take many forms. Commercially extracted meal could be further processed in a vapor desolventizer unit, originally designed by the Dravo Corp. (Pittsburgh, PA), to produce soy flakes with high protein dispersibility index (10). The modified unit would consist of a horizontal cylindrical vessel into which the required amounts of meal and ferrous sulfate would be metered. With rotation, the meal and iron meal would be lifted through a stream of superheated ethanol. The vapors would be circulated by a blower through an external heater and back to the vessel. A second vessel would be needed to remove small amounts of residual solvent. EMI's (Des Plaines, IL) flash desolventization unit, which circulates superheated vapors cocurrently with meal in a loop tube arrangement, could also be adapted to this purpose (11); and, finally, fluidized bed technology, also with superheated ethanol vapor, could be utilized to perform the deactivation.

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