Gossypol Removal by Adsorption from Cottonseed Miscella

M.S. Kuk* and R. Tetlow

Southern Regional Research Center, ARS, USDA, New Orleans, Louisiana 70179

ABSTRACT: A variety of aluminas (acidic, basic, and neutral), silica, and synthetic magnesium silicates were screened to evaluate their potential use as adsorbents for reducing the content of gossypol and related compounds from hexane-based cottonseed miscella. Among the tested adsorbents, one type of silica and two types of magnesium silicate with a relatively high Brunauer-Emmet-Teller (BET) surface area (≥240 m^2/g) showed a superior adsorption capacity in reducing gossypol from hexane miscella. As a possible regenerating method for the spent adsorbents, washing with acetone was tested. The effect of pH in the washing solvent was also evaluated. Adsorbents that were regenerated by washing with acetone at pH values between 7 and 7.5 performed better than those at pH values between 6.5 and 7. The pH values of washing solvent were adjusted by using a small amount of caustic and 1 M HCl.

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KEY WORDS: Adsorption, cottonseed extraction, gossypol removal, hexane, mineral adsorbents, miscella, regeneration.

During cottonseed processing, gossypol must be removed to produce edible oil and animal feed. Gossypol is typically removed in the miscella refining step that uses caustics, thereby producing a by-product of cottonseed processing, soapstock (1). In the miscella refining step, other lipid group compounds, such as FFA and phospholipids (PL), are also removed along with gossypol from hexane miscella. Because of the high nutritional value of the FA, however, the soapstock is added back to the cottonseed meal. The amount of addition to animal feed is limited because of the gossypol toxicity (2).

If an alternative method to the caustic refining were available, thereby reducing the content of gossypol in soapstock, this alternative method could significantly enhance the commercial value and quality of cottonseed meal. In this study of alternative processing methods to caustic refining for the removal of gossypol from cottonseed miscella, a few types of commercially available adsorbents were screened to evaluate the adsorption potential. Subsequent tests of adsorbent regeneration were conducted. The results of the screening and regeneration tests are presented.

EXPERIMENTAL PROCEDURES

Nine commercially available adsorbents, chosen for their basic properties (Table 1), were selected for screening gossypol adsorption. In addition, the following commercial adsorbents showed only marginal adsorption capacity for gossypol and were excluded from further consideration: diatomaceous earth (J.T.Baker, Phillipsburg, NJ), Celite, and 3 and 4 Å molecular sieves (Fisher Scientific, Pittsburgh, PA).

The physical properties of adsorbents, such as pH and particle size distribution, were determined unless specified by the suppliers: pH values of adsorbents were determined by immersing adsorbents in deionized water, and the particle size distributions by the use of standard U.S. sieves. The BET surface area was determined for all the screened adsorbents (Table 1) by using a Micromeritics Gemini apparatus (Model 2375; Norcross, GA). For the four types of adsorbents (three Mg silicates and Silica #1) showing a higher adsorption capacity for gossypol, the adsorption pore size distribution and the area available for adsorption in the micropores were determined with the same equipment used for determining the BET surface area of adsorbents.

A hexane-based miscella from an industrial source, produced by extracting cottonseed collets with commercial-grade hexane, was used for the screening test. The chemical properties of the hexane miscella used in evaluating the adsorption performance test are as follows: cottonseed crude oil density (at room temperature after solvent removal by evaporation in a vacuum), 0.91 g/cc; amount of solvent, 47%; gossypol content in crude oil without solvent, 0.75% (3); FFA content in crude oil without solvent (reported as oleic acid), 4.4% (3); phosphorus content in crude oil without solvent, 845 ppm [determined by inductively coupled plasma (ICP) coupled with atomic emission spectroscopy using either ASTM D 5373/E 258 (4) or U.S Environmental Protection Agency method EPA-6010B (5)]; nitrogen content in crude oil without solvent, measured using ICP after acid digestion, 0.02%.

Adsorption screening test. The adsorption screening test was conducted at room temperature as follows: A predetermined amount of the selected adsorbent (either 5, 10, or 15 g) and hexane-miscella in the mass ratio of 1:2 were charged together in a 50 mL vial and shaken vigorously for 1 h, allowing the adsorbents and miscella to equilibrate, followed by laboratory centrifugation (3340 × *g*; Model P3411 centrifuge; International Clinical, Boston, MA). The resultant bottom layer was composed of adsorbents and retained miscella. The supernatant (filtrate) layer was recovered by decantation and passed through a medium-fast (Whatman No. 2) filter to remove any residual fines. The amount of filtrate was determined on the basis of the unit mass of the tested miscella. To ensure the complete

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*To whom correspondence should be addressed at SRRC, ARS, USDA, P.O. Box 19687, New Orleans, LA 70179. E-mail: mskuk@srrc.ars.usda.gov

TABLE 1
Properties of Adsorbents

Material	Surface area[a] (m^2/g)	pH[b]	Particle size (mesh)	LOD[c] (%)	Supplier[d]
Alumina, acid	106.9	4.2	70–230	0.5	J.T.Baker
Alumina, basic	143.7	9.8	60–325	1.0	Fisher
Alumina, neutral	105.7	7.6	7–200	0.5	J.T.Baker
Bentonite	72.1	10.1	70–200	5.9	Southern Clay
Mg silicate[e] #1	446.1	8.3	60–200	16.5	Dallas Group
Mg silicate[e] #2	418.6	8.4	400[f]	16.2	Dallas Group
Mg silicate[e] #3	240.7	9.0	400[f]	13.6	Dallas Group
Silica #1	282.5	6.9	60–200	3.0	J.T.Baker
Silica #2	107.7	8.0	70–230	5.0	EM Science

[a] BET surface area.
[b] Determined in-house unless vendor specified a value (for adsorbents immersed in deionized water).
[c] Loss on drying for 2 h at 101°C.
[d] Suppliers: J.T.Baker (Phillipsburg, NJ); Dallas Group of America (Whitehouse, NJ); EM Science (Brinkman; Cherry Hill, NJ); Fisher Scientific (Pittsburgh, PA).
[e] Mg content for Mg silicate #1, #2, and #3, was 8.8, 7.9, and 8.9%, respectively.
[f] 30% retainable.

removal of the residual hexane, the filtrate sample was dried at 45°C in a vacuum (200 mm Hg) until no further change in the sample mass was recorded. A gossypol analysis was performed for the filtrate samples from the screening test. A gossypol analysis was also conducted for the oil retained in the bottom layer. This oil was recovered by using a Soxhlet apparatus with hexane as described elsewhere (6). For the adsorbents that showed a high adsorption capacity for gossypol, the additional analysis was performed to determine the amount of phosphorus and FFA in the oil from the filtrate.

Regeneration of adsorbents. The adsorbents from the screening test were washed with either acetone or methyl ethyl ketone (MEK) to desorb gossypol. To evaluate the potential of washing with the solvents as the regeneration method, the same adsorption screening test was conducted on the regenerated adsorbents after they had been thoroughly washed and dried *in vacuo* (200 mm Hg). To determine the effect of pH in the regeneration method, the pH of the washing solvent was controlled in the vicinity of pH 7: either (i) at a pH between 6.5 and 7, or (ii) at a pH between pH 7 and 7.5. pH control in the washing solvent was achieved initially by washing with acetone containing 0.5 M sodium hydroxide (vol/vol = 7:3); the pH was later adjusted by neutralization with 1 M HCl (reagent grade NaOH and HCl; J.T.Baker).

All of the adsorption screening and regeneration tests were conducted in duplicate, and the experimental results were replicated with an acceptable experimental deviation (±5%).

RESULTS AND DISCUSSION

In the 1950s, Pominski and Pack (7) published data on the disappearance of gossypol that occurs at temperatures above 210°C while heating cottonseed soapstock with heating elements in a metallic (steel) chamber. Other early investigators (8) also reported that gossypol compounds in soapstock underwent numerous degradation reactions when soapstock boiled. Because of the complexity of the thermal degradation reactions involved with gossypol compounds in boiling soapstock, no further investigation followed on the characterization of the reaction products from this thermal degradation. The cottonseed processing industry has not pursued thermal degradation as a method for gossypol removal, primarily because of the high energy cost required to heat soapstock above 210°C.

Recently, Kuk and Bland (9) reported that gossypol complexes with phospholipids to form a group of total gossypol compounds, especially with the phosphatidylethanolamine (PE) of cottonseed. These researchers also showed that the gossypol–PE complexes underwent conversion reactions, producing free gossypol under acidic conditions at around pH 1. Because it is highly acidic (near pH 1), the conversion from total to free gossypol may occur in an animal's digestive tract following its consumption of cottonseed meal. Because free gossypol is more toxic than total gossypol (2), it is important to remove or reduce the gossypol content, especially prior to caustic refining, instead of producing soapstock with gossypol in it. In cottonseed meal, a higher amount of total gossypol is allowed than the free form of gossypol (2).

Adsorption with a variety of adsorbents and filter aids for the purpose of removing various color bodies such as lutein,

TABLE 2
Gossypol in Filtrate (supernatant layer) from the Adsorption Test with Fresh Adsorbents

Adsorbents	Collected filtrate[a] (%)	Gossypol[b] amount (g)
Alumina, acidic	5.5	0.40
Alumina, basic	5.3	0.25
Alumina, neutral	4.7	0.58
Bentonite	10.5	0.36
Magnesium silicate #1	2.7	0.03
Magnesium silicate #2	3.9	0.03
Magnesium silicate #3	4.2	0.07
Silica #1	2.2	0.16
Silica #2	7.6	0.32

[a] Based on the screening test, which used 10 g adsorbent and 20 g miscella.
[b] Gossypol (%) in cottonseed crude oil (without solvent).

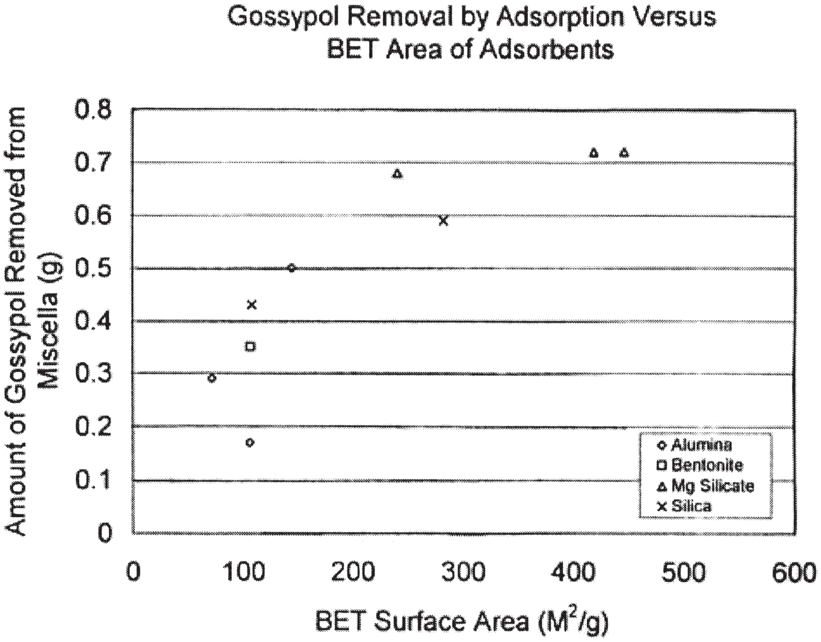

FIG. 1. Gossypol removal by adsorption vs. BET area of adsorbents.

chlorophyll, and other polar compounds in the refining steps of edible oil and the regenerating steps for oil from deep frying has been extensively investigated (10–15). Nonetheless, gossypol reduction by adsorption, especially with hexane-based miscella, has seldom been investigated. An adsorption study with ethanol-based miscella was reported (16). As suggested in the literature (10–16), adsorption of polar compounds and phospholipids from vegetable oils onto various adsorbents and filtering aids is achieved *via* complex mechanisms mostly beyond physical adsorption but by chemisorption. Although the amount of gossypol reduction by adsorption using ethanol-based miscella (16) was significant, it was not expected that a similar reduction of gossypol would be achieved with the hexane miscella because of differences in polarity, diffusivity, and viscosity of the two types of miscellas.

The materials that were screened for their ability to adsorb gossypol were selected on the basis of factors that may affect the adsorption, such as pH, BET surface area, and type of cations present in the selected adsorbents. After conducting the preliminary adsorption tests using an experimental protocol similar to that which would be used in the screening test, a variety of commercially available silica, alumina, and bleaching earth materials were excluded from the current investigation because of their poor gossypol adsorption capacity. The final nine types of adsorbents that were selected for further study are listed in Table 1.

The amount of gossypol remaining in the filtrates after the test with the virgin adsorbents is listed in Table 2. The amount of filtrate collected is based on the screening test, which used 10 g of adsorbent and 20 g of hexane miscella. The gossypol in the filtrate is the residual gossypol in the bulk phase of the hexane miscella after the gossypol adsorption was completed. Three types of Mg silicates (#1, #2, and #3) and one type of silica (#1) showed a significantly higher adsorption capacity. One of the selected adsorbents, bentonite, had a high gossypol adsorption capacity when tested with the ethanol miscella (16). According to the vendor's data, bentonite contains *ca.* 1.75% Mg. Considering the poor gossypol adsorption by bentonite, one can conclude that the high capacities for gossypol adsorption by synthetic Mg silicates and silica #1 are unrelated to the presence of Mg; Mg silicates #1, #2, and #3 contained 8 to 9% Mg (Table 1).

One can also conclude that neither the basic nor the acidic properties, as shown by pH values, of the fresh adsorbents

TABLE 3
Gossypol in the Oil Retained by Fresh Adsorbents in the Adsorption Test

Adsorbent	Oil recovered by Soxhlet extraction (g)	Gossypol[a] (%)
Mg silicate #1	7.4	0.05
Mg silicate #2	7.3	0.05
Mg silicate #3	2.4	0.05
Silica #1	6.6	0.22

[a] Gossypol (%) in crude oil (without solvent).

TABLE 4
Phosphorus and FFA in the Filtrate from the Adsorption Screen Test with Fresh Adsorbents

Adsorbent	Phosphorus (ppm)	FFA (%)
Mg silicate #1	8.5	1.5
Mg silicate #2	9.0	1.5
Mg silicate #3	25.0	2.1
Silica #1	10.0	2.0

TABLE 5
Gossypol in Filtrate from the Adsorption Test Using the Adsorbents Regenerated by Solvent Washing

Adsorbent	Washing solvent	Collected filtrate[a,b] amount (g)	Gossypol[a] (%)
Mg silicate #1	Acetone	7.4	0.22
	MEK	8.5	0.25
Mg silicate #2	Acetone	7.2	0.33
	MEK	13.3	0.38
Mg silicate #3	Acetone	9.5	0.35
	MEK	8.8	0.34
Silica #1	Acetone	6.8	0.24
	MEK	13.2	0.28

[a] Average value from duplicate determinations.
[b] 10 g adsorbents and 20 g miscella used in the test. MEK, methyl ethyl ketone.

TABLE 6
pH Effects in the Gossypol Adsorption for Adsorbents Regenerated by Acetone Washing

Adsorbent	Regenerated pH range	Collected filtrate amount (g)	Gossypol[a] (%)
Mg silicate #1	6.5–7.0	11.9	0.26
	7.0–7.5	8.2	0.22
Mg silicate #2	6.5–7.0	10.8	0.44
	7.0–7.5	7.7	0.15
Mg silicate #3	6.5–7.0	10.6	0.46
	7.0–7.5	8.9	0.25
Silica #1	6.5–7.0	3.6	0.25
	7.0–7.5	11.3	0.30

[a] Gossypol (%) in the crude oil of starting miscella was 0.75%.

played a significant role in gossypol adsorption. The basic alumina as well as bentonite, with pH values around 10, showed a poor adsorption capacity, although gossypol is known to react with caustics (1). The particle size of the adsorbents also has a negligible effect on gossypol removal. The amount of surface moisture retained by adsorbents at room temperature did not play a significant role in the adsorption performance. Mg silicates #1, #2, and #3 and Silica #1 had relatively higher amounts of surface water than the other adsorbents (see column headed LOD in Table 1).

The amount of gossypol in the filtrate (Table 2) demonstrated that Mg silicates and Silica #1 had a superior capacity for gossypol adsorption compared with the rest of the tested adsorbents. Both Mg silicates #1 and #2 were able to remove 96 wt% of the gossypol in the starting miscella by adsorption.

In Figure 1, there is a positive correlation between the amount of gossypol removed by the adsorbents and their BET surface areas. Not only did the four types of adsorbents with the higher gossypol adsorption capacity have a higher BET surface area, but they also had relatively larger surface areas of micropores, having diameters greater than 20 Å. The micropore surface areas (m^2/g) of Mg silicates #1, #2, #3, and Silica #1 were 135, 157, 187, and 32, respectively. The proportion of the surface area contained in micropores of bentonite, which showed a higher adsorption capacity than alumina (neutral and acidic type), was almost zero. Stokes' radii of gossypol and FA with M.W. near or below 1000 are on the order of 10–20 Å (17). Hence, it is reasonable to expect that adsorbents with a higher surface area within micropores, especially of pores having radii in the range of 20 Å, have a higher capacity for gossypol adsorption.

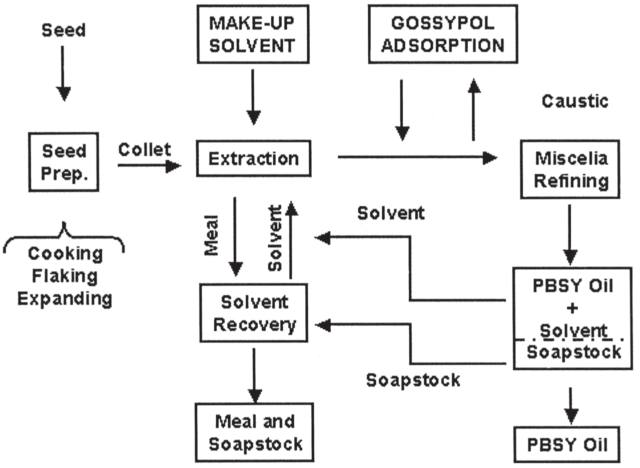

FIG. 2. Block diagram of cottonseed processing with inclusion of the unit operation of gossypol removal by adsorption. PBSY, prime bleachable summer yellow.

The amount of gossypol in the filtrate represents the gossypol concentration in equilibrium with the cottonseed crude oil and solvent retained by the micropores, macropores, and interstices of the adsorbents. The separation by centrifugation and recovery of the filtrate from the bottom layer was reasonably clean. After the centrifugal separation, the bottom layer, which mainly contained adsorbents to which gossypol was sorbed and retained cottonseed crude oil, was extracted with hexane using a Soxhlet apparatus. The gossypol retained by the adsorbents, which was recovered by Soxhlet extraction, may represent gossypol physically adsorbed in the pores and interstices of the adsorbent. On the other hand, gossypol that was chemically bound to the adsorbents may not have been completely removed by the Soxhlet extraction with hexane. A similar phenomenon regarding gossypol extraction with hexane from the collets of cottonseed was observed (6), i.e., some of gossypol chemically bound to the cottonseed protein was not extracted by hexane. The gossypol amount (wt%) in the oil retained in the adsorbents is presented in Table 3. From the gossypol amounts shown in Tables 2 and 3, one can conclude that Mg silicates have a relatively higher adsorption capacity than Silica #1.

Analysis of FFA and phosphorus was performed on the oil recovered from the filtrate of the four adsorbents having a higher capacity for gossypol adsorption (Table 4). The results indicate that phospholipids and FFA were competing with gossypol for the active adsorption sites in the four adsorbents. More than half of the FFA and more than 90% of phospholipids present in the hexane miscella were removed by adsorption. As competitive adsorption between FFA and phospholipids of vegetable oils has been reported (10–16), the results in Table 4 were not surprising.

Since gossypol dissolves well in low M.W. ketones (18), the regeneration of the adsorbent was attempted by washing with acetone or with MEK. As can be seen in Table 5, the adsorption performance of the adsorbents regenerated by acetone washing, which is represented by the residual gossypol in the filtrate, is slightly better than that by MEK. Although MEK has a larger amount of filtrate (i.e., smaller amount of the miscella retained by the adsorbents), acetone would be a better choice as the solvent of regeneration. Acetone used in the regeneration can easily be recovered by distillation and separated from hexane miscella.

To examine the role of pH in the washing solvent, the used adsorbents were washed with acetone containing a small amount of caustics and later with 1 M HCl. As shown in Table 6, generally, the regenerated adsorbent with pH values between 7 and 7.5 performed better in terms of the gossypol removal than acetone at pH values between 6.5 and 7. In particular, 80% of the gossypol in the starting miscella was removed from Mg silicate #2 washed with acetone having pH values between 7 and 7.5. The significant removal of phospholipids and FFA by the four types of adsorbents (Tables 4–6) indicates that the adsorptive route for removing gossypol may reduce or replace the role of caustic refining. A possible process block diagram with the incorporation of the adsorptive gossypol removal into cottonseed processing is given in Figure 2.

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