# Acidic Hexane Extraction of Oilseeds: Product Quality

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## ABSTRACT

Cottonseed was extracted with hexane and acidic hexane (hexane that contained 2-25% acetic acid) and properties of the resultant miscellae and meals were compared for each sample. Pigment contents were only slightly larger in acidic hexane miscellae. Settling rates of marc particles were much faster through acidic hexane miscellae. Nutritional analyses of the extracted cottonseed meals indicated that protein efficiency ratios, digestibilities, epsilon amino-free lysine values and free and total gossypol contents were essentially identical with each type of solvent. Aflatoxin contents of similarly treated, contaminated peanuts did not change. The results are discussed with regard to previous findings concerning the more thorough extractions of lipid from oilseeds with acidic hexane than with hexane alone.

## INTRODUCTION

In previous communications (1-3), we described advantages of solvent extraction of cottonseed and soybean with acidic hexane (azeotropic mixtures of hexane and acetic acid) compared to solvent extraction with hexane alone. With acidic hexane, a more thorough extraction of neutral oil and phospholipid and a more rapid separation rate of miscella from marc were obtained. Furthermore, within practical limits of acetic acid concentration, no adverse effects on protein solubility were observed. In this communication, we describe the effects of acidic hexane extraction on the pigment content of the miscella, on the settling rate of marc particles in miscella, on several nutritive properties of the resultant oil-free meal, and on the fate of aflatoxin in contaminated seed. These results are summarized with previous results and are discussed with regard to advantages of oilseed extraction with acidic hexane.

## EXPERIMENTAL PROCEDURES

Extracting solvents were industrial hexane (Skellysolve B) and industrial hexane containing 2 to 25% (v/v) glacial acetic acid.

Dehulled glanded and glandless cottonseed meats were dry-milled in an impact stud mill as described previously (1,3). Meals were prepared by solvent extraction with filtration through fritted glass Buchner funnels as described previously (1,3) or by tube differential settling (4-7).

The effect of contact time on the pigment content of miscella was determined by extracting 1 g of glanded cottonseed with 5 mL of hexane or acidic hexane with filtration (1,3). Absorbances at 360 nm of the miscella were determined after contact times of 0.5-3 hrs.

Settling behavior of glanded cottonseed particles was assessed by suspending 5.2 g of comminuted meats in 40 mL of hexane or acidic hexane. The tubes were photographed after several time intervals for observations of settling rates.

Analyses of free and total gossypol and EAF (epsilon amino-free) lysine were conducted by Barrow-Agee Laboratories, Memphis, TN, according to their protocol. Assays were performed on simulated liquid cyclone process (LCP) "overflow" of glanded cottonseed prepared by differential settlings (6,7). The "overflow" is defined as the solids that remain suspended after 18 min in an undisturbed 36 × 47 cm cylinder (500 mL) containing solvent with 12% solids. Solvents were hexane or acidic hexane.

Rat feeding tests were performed only on glandless meals, so that complications arising from pigments such as gossypol would be avoided. For certain tests, steam was passed through the meals for 5 min and the resultant meals were oven dried at 90 C. Protein efficiency ratios were determined as described by Derse (8). The assays utilized a 26-day feeding period with five weanling male Sprague-Dawley rats per test group. Table I shows the compositions of the diets. All diets contained 10% protein, the source of which was either casein (control) or one of the cottonseed meals.

Aflatoxin was determined by the procedure described by Pons (9). Aflatoxin-contaminated peanut meats, containing 445 ppb of aflatoxin, were extracted with hexane or acidic hexane and contents of aflatoxin in the meals, prepared by filtration (1,3), were then assessed by extraction and thin layer chromatography (9).

## RESULTS

Since acetic acid in hexane can rupture pigment glands of cottonseed (1), the extent of gland rupture upon extended contact of hexane-acetic acid with glanded cottonseed meats was determined. Figure 1 shows that although all concentrations of acetic acid in hexane increased the pigment content of each miscella somewhat, contact time of miscella with marc was not crucial except at concentrations above 6% acetic acid in hexane, and then only after an hour or more of contact.

Earlier, we reported that the separation of miscella from marc by filtration was about twice as fast in acidic hexane as that in hexane alone (1,3). Figure 2 shows that a faster separation of miscella from marc was also obtained by settling; the faster settling rate of marc particles is another demonstration of the rapid marc-miscella separation in

**TABLE I**

**Composition of Diets**

| Ingredients | Control (%) | Experimental (%) |
|---|---|---|
| Casein | 11.62 | – |
| Cottonseed | – | 17.86 |
| Corn oil | 8.00 | 7.91 |
| $H_2O$ | 4.02 | 3.75 |
| Mineral mixture[a] | 4.73 | 3.75 |
| Cellulose | 3.00 | 1.39 |
| Vitamin mixture[b] | 2.00 | 2.00 |
| Corn starch | 20.00 | 20.00 |
| Dextrose | 46.63 | 43.34 |

[a]Salt mixture VSP XIV fortified with $ZnSO_4 \cdot 7H_2O$ (548 mg/kg) and $CoCl_2 \cdot 6H_2O$ (23 mg/kg).

[b]Each kilogram of mixture contained the following vitamins, triturated in dextrose: 4.5 g vitamin A (200,000 units/g); 0.25 g vitamin D (400,000 units/g); 5.0 g alpha-tocopherol; 45.0 g ascorbic acid; 5.0 g inositol; 75.0 g choline chloride; 1.0 g riboflavin; 2.25 g menadione; 5.0 g *p*-aminobenzoic acid; 4.5 g niacin; 1.0 g pyridoxine·HCl; 1.0 g thiamine·HCl; 3.0 g Ca pantothenate; 20 μg biotin; 90 μg folic acid; 1.35 μg vitamin $B_{12}$ (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp.).

[1]ARS, USDA.

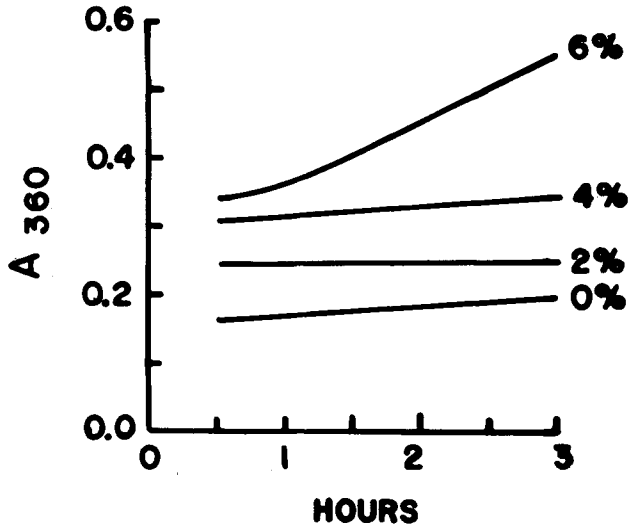


FIG. 1. Effects of solvent composition and contact time upon pigment content of miscella. Values on the right represent percentages of acetic acid in hexane; values on the ordinate are absorbances at 360 nm.

acidic hexane. The rapid settling is probably related to protonation of the particles by acetic acid.

Analyses of gossypol and EAF lysine contents were also performed on meals prepared with hexane and acidic hexane. Glanded cottonseed was used and the meals were prepared by differential settling (6-7). Analyses of each "overflow" yielded contents of free and total gossypol of 0.04% and 0.05%, respectively, for extractions with hexane, 2% acetic acid in hexane and 4% acetic acid in hexane, and 0.06% and 0.07%, respectively, for extraction with 6% acetic acid in hexane. EAF lysine ranged from 3.94-3.96 for all solvents. The results show that acidic hexane as an extraction vehicle had no influence on the contents of free and total gossypol and EAF lysine in cottonseed meal compared to that of hexane alone.

Since acidic hexane extracted cottonseed and soybean lipids more thoroughly than did hexane alone (1-3), it was desirable to determine the effect of acidic hexane on the nutritive properties of the residual, oil-free meal. Glandless cottonseed meals were prepared by filtration after extraction of meats with hexane or hexane-acetic acid (5% acetic acid, v/v). The resultant meals were either air-dried or treated with steam and oven-dried, and rat feeding tests were conducted. Steam treatment and oven drying removed all solvent odor regardless of the presence or absence of acetic acid in the solvent. Table II shows results of the feeding tests. As expected, the actual PER values of the four cottonseed meals were all significantly lower than the casein standard ($P>0.01$), but no significant difference existed among the four meals, all of which fell within a very narrow range (1.96-2.09). Thus, the extractive solvent and the steam treatment with oven drying had no influence on the PER value of cottonseed meal. Final body weights of the rats fed with cottonseed meals were slightly below the casein group but not significantly so. With the possible exception of the slightly lower nitrogen digestibility value for air-dried meal prepared with acidic hexane, the values for the test samples were only slightly below the value for casein. Thus, the meals were well digested, regardless of the solvent or steam treatment.

The fate of aflatoxin upon solvent extraction of aflatoxin-contaminated oilseeds with acidic hexane was also determined. Contaminated peanuts were extracted with hexane containing from 0 to 25% acetic acid (v/v) and the aflatoxin contents of the resultant meals were assessed.

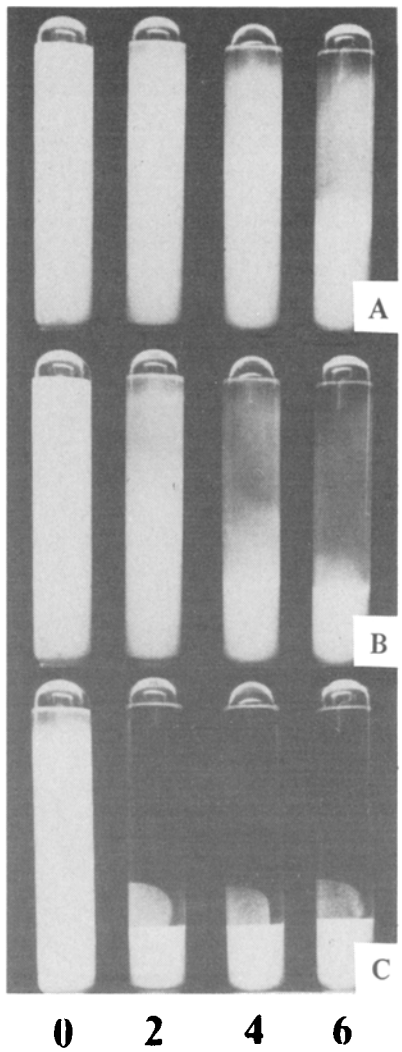


FIG. 2. Comminuted cottonseed particles in hexane and acidic hexane 13 min (A), 26 min (B) and 17 hr (C) after mixing by repeated inversion. In all figures, the numbers represent percentages of acetic acid in hexane, e.g., 2 = 2% acetic acid (v/v).

Results showed that mixtures of hexane and acetic acid, even at concentrations of acetic acid to 25%, had virtually no effect on the contents of aflatoxin $B_1$, $B_2$, $G_1$ or $G_2$. They were neither extracted from the meal nor destroyed (nor otherwise modified) by contact with the solvent. Therefore, other than extracting more neutral oil and phospholipid from oilseed without affecting protein properties (Ref. 1 and Table II), acidic hexane offered no advantages over hexane for solvent extraction of aflatoxin-contaminated oilseeds.

## DISCUSSION

From results presented in this report, plus those in previous communications (1-3), the use of acidic hexane as an extracting solvent compared to hexane alone offers several advantages. Aside from increased amounts of extracted neutral oil (phospholipid-free)—about 3% from cottonseed (1) and 6% from soybean (3)—acidic hexane extracted about 5-fold to 7-fold and 16-fold to 35-fold greater amounts of phospholipid from cottonseed and soybeans, respectively, than did hexane alone, as described in previous communications (1-3). Not only is isolated phospholipid a saleable item, its removal reduces bitter flavors in residual meal (10-12).

TABLE II

**Effects of Dietary Source of Protein, Solvent and Meal Treatment on Growth of Weanling Rats**

| Dietary source of protein[a] | Solvent | Meal treatment | Final body weights[b] | PER[c] | | Digestibility (%)[d] | |
|---|---|---|---|---|---|---|---|
| | | | | Actual | Corrected | Diet | Nitrogen |
| Casein | – | – | 167 ± 19 | 3.39 ± 0.16[f] | 2.50 | 95 | 93 |
| Cottonseed meal | Hexane | Air-dried | 155 ± 26 | 2.75 ± 0.22 | 2.03 | 94 | 88 |
| Cottonseed meal | Hexane | Steam-deodorized, oven-dried | 158 ± 17 | 2.83 ± 0.19 | 2.09 | 94 | 88 |
| Cottonseed meal | Acidic hexane[e] | Air-dried | 146 ± 8 | 2.66 ± 0.10 | 1.96 | 94 | 85 |
| Cottonseed meal | Acidic hexane[e] | Steam-deodorized, oven-dried | 146 ± 16 | 2.67 ± 0.13 | 1.97 | 94 | 88 |

[a]Diets contained 10% protein.

[b]Values are means in grams ± standard deviations of five rats per group. Mean initial weight was 56 g per rat and initial age was 21 days.

[c]Protein efficiency ratio (PER) = g of weight gain per g of protein intake. Values represent means in grams ± standard deviations.

[d]Digestibility (%) = 100 (g of feed intake – g of fecal weight)/g of feed intake.

[e]Acidic hexane is 5% acetic acid in hexane (v/v).

[f]Value is significantly greater than values for cottonseed meals ($p>0.01$).

The faster separations of marc from miscella in acidic hexane compared to hexane alone, whether by filtration (1-3) or settling (Fig. 2), indicate smaller energy requirements, greater volumes of material processed per unit time, and operational ease with acidic hexane. Other energy-conserving features are that acidic hexane extracted as much neutral lipid at room temperature as hexane extracted at 60 C (3) and that mixtures of hexane with acetic acid in the range studied here are azeotropic with boiling points lower than the boiling point of hexane (13,14).

The residual, oil-free meal prepared with acidic hexane did not differ from that prepared with hexane. Both protein solubility (1) and nutritive value (including PER, digestibility, and contents of EAF lysine and gossypol), were not altered significantly upon oil extraction with acidic hexane compared to hexane alone. Also, aflatoxin contents of contaminated meats were not affected. Acidic hexane produced no adverse effects, the residual meal remained nutritious (Table II). Treatment of cottonseed with acetic acid-containing solvents before extraction with methylene chloride also did not affect the protein properties and chemical score of the meal (15).

The odor of acetic acid that remained in acidic hexane-extracted meal was readily removed with steam. Such treatment had no effect on the nutritive value of the meal (Table II). Processing oilseed meals with steam to inactivate antinutritional factors and to texturize proteins might also remove residual odors of acetic acid.

Extraction of pigments or rupture of pigment glands in cottonseed meats by acidic hexane was relatively slight, judging from contents of free and total gossypol in meals. However, contact time of solvent with glands appeared an important consideration (Fig. 1).

The results indicated that extraction of oilseeds, even glanded cottonseed, with acidic hexane offers several advantages over extraction with hexane alone. Larger-scaled and pilot plant investigations are necessary to determine more fully whether use of acidic hexane is indeed advantageous on an industrial level. Our results show that it is technically and perhaps economically feasible.

ACKNOWLEDGMENTS

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