Extraction of Lipids from Cottonseed Tissue: IV. Use of Hexane-Acetic Acid[1]

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ABSTRACT

Hexane and mixtures of hexane and 2-25% acetic acid (v/v) were used to prepare oil and protein from glanded cottonseed by solvent extraction. As the amount of acetic acid in the solvent increased, the amounts of total lipid, phospholipid, neutral oil, and gossypol in each miscella increased, but the amount of free fatty acids did not change significantly. However, the solubility of protein in 0.02 N NaOH decreased as the amount of acetic acid in the solvent used to prepare each meal increased. Other aspects of using acidified hexane are described.

INTRODUCTION

Storage oils of oilseeds are located in intracellular packets called spherosomes (1,2). These organelles are 1-2 μ in diameter, and each is coated with a limiting, half-unit membrane (3). Results from physicochemical characterizations of spherosomal membranes, which will be presented in a future communication, show that the membranes are labile in acidic, aqueous media. Therefore, determination of whether acidification of an oil-solvent would increase the yield of oil from cottonseed was of interest. Comminuted meats from glanded cottonseed were extracted with hexane and mixtures of hexane and 2-25% acetic acid (v/v). Contents of the miscellae and effects of solvents on the meals are reported in this communication.

EXPERIMENTAL PROCEDURES

Extracting media were industrial hexane (Skellysolve B) and industrial hexane containing from 2-25% (v/v) glacial acetic acid.

Dehulled glanded cottonseed meats were dry-milled in a sieveless Alpine American Contraplex (impact stud mill) with disc speeds at 9500 and 2500 rpm. The resultant particles were stored over P_2O_5 in vacuo. Samples of the dried particles were stirred continuously with extracting media in fritted glass Buchner funnels of medium porosity (6 ml solvent/2 g meats, 5 min contact), and resultant miscellae were collected by filtration with reduced pressure for 2 min (4). Each marc was reextracted twice with fresh solvent, and the three miscellae obtained from each sample were combined. Wt of the extracted lipids were determined after solvents were removed in vacuo.

Amounts of neutral oil in the miscellae were determined by a modification of methods described previously (4,5). In addition to treatments with activated clay and aluminum oxide (4,5), the oil was dissolved in acetone and filtered to remove a slightly yellow hue and a small amount of finely suspended matter that was soluble in hexane but not in the oil or acetone. This particulate matter was especially evidenced in miscellae produced with hexane containing high concentrations of acetic acid.

Contents of free fatty acids in miscellae were determined by titration according to the official methods of AOCS (6), except that the indicator nile blue was substituted for phenolphthalein to overcome the difficulty of observing red color changes in a reddish milieu.

Phosphorus was determined photometrically (7) after digestion of materials as described previously (4).

Contents of gossypol in miscellae were estimated from absorbancies at 360 nm, due to gossypol pigments (8), determined spectrophotometrically as described previously (9,10).

Solubility of protein in oil-free meals was determined as described below. After being dried in vacuo, 1 g meal was suspended by sonication for 15 sec in 30 ml H_2O containing 4 dps of Dow Corning Antifoam B (antifoaming

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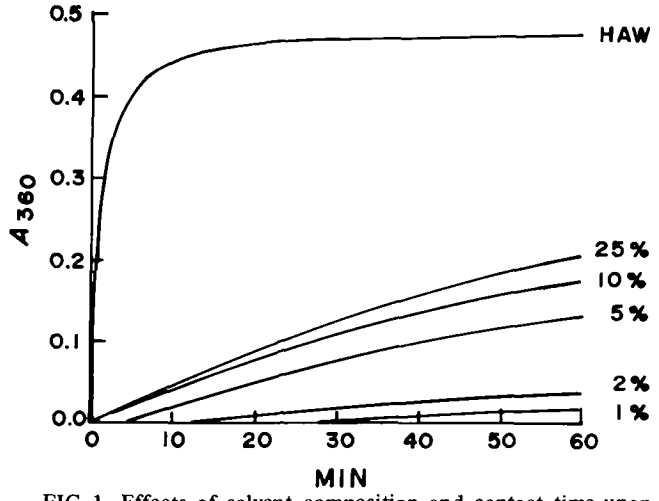

FIG. 1. Effects of solvent composition and contact time upon disruption of isolated pigment glands. Values on right ordinate represent percentages of acetic acid in hexane; HAW is hexane-acetone-water.

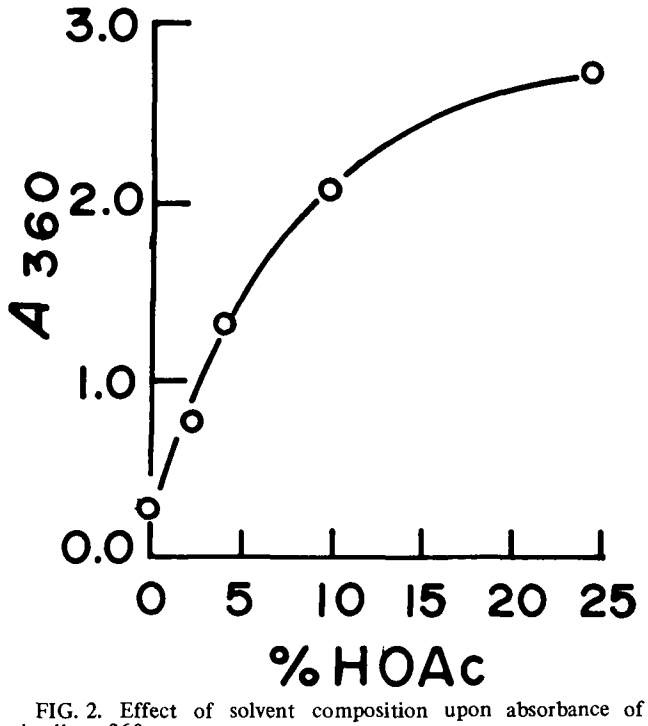

FIG. 2. Effect of solvent composition upon absorbance of miscella at 360 nm.

TABLE I

Components of Miscella from 2 g of Meats[a]

Percent of HOAc in hexane (v/v)	Total lipid[b] mg	Phosphorus mg	Gossypol mg	Free fatty acids, mg	Neutral oil, mg
0	686 ± 6.2	0.12	0.21	15.9 ± 0.8	612 ± 4.0
2	709 ± 2.2	0.37	0.63	19.0 ± 0.4	629 ± 0.6
4	716 ± 3.5	0.60	0.98	18.8 ± 0.4	631 ± 1.1
10	740 ± 3.0	1.05	1.57	17.0 ± 0.7	639 ± 4.1
25	753 ± 6.7	1.22	2.02	16.3 ± 0.7	632 ± 7.5

[a]Values represent means ± standard deviations from the means. No standard deviations are shown when the deviations were so small as to be essentially 0.

[b]Total lipid is all materials in miscella after evaporation of solvent.

agent). The mixture was adjusted to pH 7.0 with 1.0 N NaOH, 0.8 ml 1.0 N NaOH then was added, and the suspension was diluted to 40 ml with H_2O. The mixture was sonicated for 15 sec, continuously mixed by shaking for 1 hr, and then centrifuged at 18,000 x g for 10 min. Nitrogen content of the supernatant was determined by the procedure of Minari and Zilversmit (11).

Abilities of solvents to rupture pigment glands were estimated from amounts of pigments in miscellae obtained from isolated glands. Dried glands, prepared as described previously (10), were mixed with solvents (50 ml solvent/2 mg glands) and occasionally shaken. Absorbancies of miscellae at 360 nm were determined during 1 hr of contact. Hexane in the solvents was spectrograde quality. The content of acetic acid in the solvents had no effect upon the absorbance of purified gossypol at 360 nm.

RESULTS

Since glanded meats were used in this study, the property of acidified hexane to rupture pigment glands was examined. Isolated glands were treated with hexane-acetone-water, which readily disrupts pigment glands (10,12), and with mixtures of hexane and acetic acid. Absorbancies at 360 nm of miscellae during 1 hr of contact (Fig. 1) indicated that glands were slowly ruptured in hexane containing 5% or more acetic acid. However, even after 1 hr of contact, less than half of the glands were disrupted by hexane containing 25% acetic acid. In addition, the effect of acidified hexane upon glands in the dry-milled meats was examined. Absorbancies of miscellae obtained from extractions of these meats, shown in Figure 2, indicated that amounts of pigment in miscellae also increased as concentrations of acetic acid in hexane increased.

Abilities of mixtures of hexane and acetic acid to extract lipoidal materials from glanded cottonseed were compared to that of hexane. Dry-milled meats (2 g) were extracted 3 times with 6 ml fresh solvent, and the miscellae were combined. Amounts of miscellar components extracted by each solvent are presented in Table I. The results show that, as the amount of acetic acid in the hexane increased, the amounts of total lipid, phospholipid (phosphorus), gossypol, and neutral oil in each miscella increased. The amount of free fatty acids did not change significantly.

Effects of acidified hexane upon proteins of cottonseed were examined by determining acidities of extracted meals and solubilities of proteins in 0.02 N NaOH. Results presented in Table II indicated that acetic acid apparently was bound in the meals even after the meals had been dried in vacuo. Therefore, to determine comparatively solubilities of proteins of each meal in alkali, the pH of each meal had to be adjusted to an arbitrarily chosen standard value, in this case pH 7.0. Table II shows the milliequivalents of base required for this neutralization of each suspension of 1 g meal. Protein solubility of each neutralized meal in 0.02 N NaOH also is shown in Table II. Apparently, contact of cottonseed tissue with hexane containing at least 10% acetic acid rendered some of the protein insoluble in alkali.

Since the presence of acetic acid in hexane makes the solvent corrosive, effects of acidic hexane upon equipment materials were investigated. After being in continuous contact for 28 days with hexane containing 2% acetic acid, stainless steel, Teflon, and polyethylene were not affected, except for slight swelling of the plastics. However, soft steel, copper, and galvanized metal developed coatings. These observations indicate that use of acidic hexane would not be a problem in cottonseed processing if the proper materials were used for the processing equipment.

An unexpected result of using hexane-acetic acid mixtures was that drainage of the miscella through the marc was much more rapid when the solvent was acidic hexane than when it was hexane alone. Since separation of miscella and marc is often a limiting factor in many processes, use of acidic hexane might greatly increase the volume of material processed.

DISCUSSION

Hydrophilic cytoplasmic components tend to present barriers to movement of lipophilic liquids through oilseed tissues. Results that will be presented in a future publication indicate that, of these components, membranous

TABLE II

Properties of Meals from Extracted Meats

Percent of HOAc in hexane (v/v)[a]	pH of aqueous suspension[b]	mE of base for neutralization	Soluble protein/g meal	
			mg	Percent of 0
0	6.0	0.96	725	---
2	5.85	0.98	725	100
4	5.85	0.99	725	100
10	5.3	1.21	662	91.3
25	5.0	1.445	488	67.3

[a]Solvent used to prepare meal from meats.

[b]Dried meal (1 g) in 30 ml H_2O.

elements of the cytoplasm are labile in acidic aqueous solvents. Therefore, acidification of oil solvents, in this case hexane, improved extraction of lipids during processing of oilseeds, probably by increasing the permeability of membranes to solvents and lipid-solvent mixtures. Results of extracting comminuted cottonseed meats with mixtures of hexane-acetic acid showed that these mixtures extracted from 3 to over 4% more neutral oil than did hexane alone (Table I). Greater amounts of other lipoidal constituents also were obtained; but, depending upon time of contact and concentration of acetic acid, relatively few pigment glands were disrupted by the solvent-acid mixtures (Fig. 1). These features indicated that acidified hexane should be especially suitable for the liquid cyclone process (13,14) where glands are separated from other parts of tissue during extraction of oil.

Our results also indicated that ca. 4% acetic acid in hexane would be the desired upper limit of acidification. Above that amount, both the oil (Fig. 2) and the marc were colored, and solvent recovery became difficult. Furthermore, hexane-acetic acid mixtures containing low concentrations of acetic acid are azeotropic (15,16). This latter point is being investigated further.

Protein solubility decreased for meals prepared with hexane-acetic acid mixtures containing at least 10% acetic acid (Table II). Apparently, forces that maintained a protein structure conferring solubility in dilute alkali were disrupted by acetic acid. Of future interest are physicochemical characterizations of proteins in the meal and identification of the species of protein that is rendered insoluble.

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