Extraction of Lipids From Cottonseed Tissue: I. Comparison of Hexane-Acetone-Water, its Nonaqueous Components and Chloroform-Methanol

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Abstract

Flaked cottonseed was extracted with chloroform-methanol-water, chloroform-methanol, hexane-acetone-water, hexane-acetone, hexane and acetone. Amounts of total material in the miscellae were greatest with chloroform-methanol-water and decreased to acetone in the order given above. The first three solvents extracted 6% more neutral oil and over 100% more lipophilic phosphorus than the latter three solvents. All solvents showed similar rates of extraction, each removed over 70% of extractables with the first of four passes.

Introduction

Mixtures of solvents greatly differing in polarity have been used for several years to extract tissue lipids. In 1914 Bloor (1) introduced a mixture of alcohol and ether that combined "the penetrating power of alcohol with the greater solvent power of ether." This medium was widely used in laboratories until 1951 when Folch et al. (2) introduced chloroform-methanol.

Industrial use of mixed solvents, particularly to extract oil from oilseeds, has been minimal. Although extractions of cottonseed with mixtures such as diethyl ether with dichloroethane (3) or hexane-acetone-water (4) have been reported, hexane is generally employed commercially. The resultant meal from extraction with hexane retains much gossypol but when meal of lower gossypol content is desired, extraction of cottonseed with hexane-acetone-water is advantageous (4). Since hexane-acetone-water is an efficient vehicle of lipid extraction (5), a comparison of this mixture to certain other solvents with respect to extraction of lipid from cottonseed was of interest.

Materials and Methods

Extracting media were: H, hexane; A, anhydrous acetone; HA, hexane-anhydrous acetone (31:20); HAW, hexane-acetone-water, which is HA containing 1.8% water; CM, chloroform-methanol (3:1); CMW, chloroform-methanol-water, which is CM containing 3% water. All amounts are in volumes.

Flakes of dehulled, glanded cottonseed (*Gossypium hirsutum*, L.), averaging 0.3 mm thickness, were dried over P_2O_5 in vacuo to constant weight. Two grams of flakes were mixed with 6 ml of extracting medium in a fritted glass Buchner funnel of medium porosity. After 5 min of continuous stirring, the miscella was collected by filtration with reduced pressure for 2 min. The marc was subjected to this treatment with fresh solvent three more times for a total of four extraction steps. Content of phosphorus and weight of the extracted material from each step and weight of the final marc were determined after solvents were removed in vacuo. In experiments where noted, miscellae from each extraction step were combined before removing the solvent and analyzing the residue.

Water-soluble materials were removed from extracted lipids by a modified procedure of Folch et al. (2). Residue from combined miscellae, after removal of solvent, was redissolved in 30 ml of CM contained in a beaker. The beaker was then submerged in running tap water, which was constantly being changed at a rate of 10 liters/hr for 64 hr with occasional swirling of the nonaqueous phase. The residue in the chloroform layer was weighed and analyzed for phosphorus after removal of chloroform in vacuo.

Amounts of neutral oil in the combined miscellae were determined by a modified method of Singleton et al. (6). This consisted of suspending the lipid from 2 g of tissue in 20 ml of hexane, stirring continuously for 20 min with activated BC clay (clay-lipid, 2:1 w/w), passing the filtrate through a column of reagent grade aluminum oxide powder previously heated at 110 C for several hours (aluminum oxide-lipid, 5:1 w/w), and weighing the oil after removal of hexane in vacuo. No phosphorus was detected in the resultant oil. When purified, refined neutral oil was taken through this procedure, essentially all was recovered.

Phosphorus was determined after digestion of material at 300 C with 10 N H_2SO_4 (usually 1.0 ml). A few drops of concentrated HNO_3 were added at 140 C and, when solid matter was digested, a few drops of concentrated $HClO_3$ were added at higher temperatures. Tubes were cooled before addition of acid. The digested sample was diluted with 1.0 ml of H_2O and held over steam for 10 min for hydrolysis of pyrophosphate. To obtain reproducible results of analyses, the final acidities of the digested samples had to be standardized. Each sample was neutralized with freshly prepared 10 N NaOH in the presence of 1 drop of 0.5% water-soluble phenol red as an indicator and then acidified with 0.5 ml of 10 N H_2SO_4. Content of phosphorus was then estimated colorimetrically (7).

Results

The ability of HAW to extract lipoidal material from cottonseed flakes was compared to those of H, A, HA, CM and CMW. Two grams of flakes were extracted four times, each pass with 6 ml of fresh solvent. Amounts of material in the marc and combined miscellae were determined gravimetrically after removal of solvents in vacuo. Results presented in Table I show that the mixture, HA, extracted more material than either H or A alone and that addition of water to HA or CM resulted in more material extracted than without water. A comparison of the amounts of total materials extracted is represented as CMW > CM > HAW > HA > H, A (Table I).

To determine whether the increase of materials extracted upon mixing H with A and upon addition of water to HA and CM resulted from an increase in amounts of neutral oil or of water-soluble ma-

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TABLE I
Fractionation of Cottonseed by Solvent Extraction[a]

Solvent	Total extractables,[b] mg	Residue,[c] mg	Recovery,[d] %
CMW	803	1242	101.7
CM	777	1235	100.3
HAW	757	1260	100.3
HA	721	1285	100.6
H	697	1290	99.1
A	696	1305	100.1

[a] Results are averages of six or more determinations.
[b] Total extractables are materials in miscellae from 2 g of cottonseed flakes.
[c] Residue is final marc.
[d] Recovery = 100 (total extractables + residue)/starting material.

terials, these components were quantitatively determined in the various combined miscellae. Results presented in Table II show that mixing H and A increased extraction of materials other than neutral oil. However, addition of water to HA increased the amount of neutral oil extracted by dry HA, which oil accounted for the increase of total extractables in the miscella (Table I). In contrast, addition of water to CM increased only the amount of materials other than neutral oil. In summary, CMW, CM and HAW extracted similar amounts of neutral oil, which amounts were greater than those extracted by HA, A and H. The small quantities of water-soluble materials in the miscellae (Table II) did not account for the increase in miscellar material that occurred by addition of water to CM or HA.

Since miscellae used in this study were produced by collecting filtrates from four passes of solvents through flaked cottonseed, it was of interest to determine the relative portions of material extracted in each pass. The results, presented in Table III, were similar for each solvent and show that 76% of the total extractable materials and 70% of the lipophilic phosphorus were present in the miscella produced by the first pass of solvent. Over 90% of the materials that were extracted in four passes of solvent were extracted in two passes. In summary, CMW, CM and HAW extracted more material than HA, H and A (Tables I and II), but relative rates of extraction were similar (Table III).

Discussion

Treatment of tissue with solvents to extract lipids necessarily involves: (a) ingress of solvent into the tissue, (b) dissolution of lipid by solvent, and (c) egress of the lipid solution out of the tissue. Since reserve oil of dry oilseeds is stored as intracellular,

TABLE II
Content of Neutral Oil, Water-Soluble Components and Lipoidal Phosphorus in Miscellar Material[a]

Solvent	Neutral Oil,[b] mg	Water-Soluble,[c] mg	Phosphorus μg
CMW	705	4	1325
CM	706	0	1057
HAW	704	4	1415
HA	664	0	518
H	668	..	286
A	656	..	373

[a] Results are averages of three or more determinations.
[b] After treatment of material in miscella by a modified method of Singleton et al. (6).
[c] After extraction of material in miscella with water according to Folch et al. (2).

TABLE III
Amount of Total Miscellar Material Present in Each Pass[a]

Pass	Total Extractables		Lipoidal Phosphorus	
	Per cent of total	accumulative %	Per cent of total	accumulative %
1	76.2	76.2	70.0	70.0
2	18.1	94.3	21.4	91.4
3	4.2	98.5	5.4	96.8
4	1.5	100.0	3.2	100.0

[a] Results are averages of at least three determinations for each of the six solvents.

hydrophobic particles (spherosomes) embedded in a dry, hydrophilic milieu (cytoplasm) (8,9), movement of lipophilic liquids through the tissue might be hindered by cell walls and cytoplasm. However, results of cytological examinations, which will be presented later, indicate that penetration of solvents was sufficiently thorough for contact of solvents with spherosomal lipid. Since lipid was readily soluble in the solvents used in our work, no differences in amounts of oil extracted from cottonseed were expected among the solvents. However, CMW, CM and HAW extracted over 6% more neutral oil than HA, H and A. Perhaps the three former solvents, which are more polar and more acidic than the latter, dislodged and dissolved oil that is tightly bound to nonlipid constituents, e.g., lipid associated inside intracellular, protein-rich aleurone grains (10).

Hexane is used commercially to extract oil from cottonseed. In this regard, our results show that use of HAW rather than H for extraction of unheated cottonseed would produce only about 5% more neutral oil but would almost double the amount of other components in the miscella. Lipophilic phosphorus in the miscella would increase fivefold under such conditions (Table II). Apparently, the costs (11) to use HAW and to clean the resultant oil are not commensurate to the value of greater oil yields. However, HAW-prepared meal might prove more acceptable in certain animal rations (12) since it would contain less gossypol (4) and aflatoxin, if present (13,14). The absence of heat treatments would reduce binding of gossypol and destruction of lysine in meal produced from glanded cottonseed (4).

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