Aqueous Acetone Extraction of Cottonseed[1]

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Abstract

Extraction of cottonseed flakes with acetone containing 25–30% water removes essentially all of the gossypol, most of the free fatty acids, about half the raffinose, and negligible quantities of neutral oil and protein. After drying and re-flaking of the aqueous acetone extracted marc, the oil may be removed either by hexane extraction or pressing to produce light-colored meals exceptionally low in gossypol pigments, high in protein and available lysine content. Crude oils are light-colored, contain negligible amounts of gossypol, are high in neutral oil content, and refine and bleach to a prime color value. The process is effective for the removal of such toxic mold metabolites as aflatoxins from mold-damaged seed.

Introduction

GOSSYPOL PIGMENTS, present in cottonseed in amounts ranging from about 0.4 to 1.5% of the weight of the kernel (1), have long presented unique problems in the processing of this oilseed. These native gossypol pigments have adverse physiological effects on nonruminants (2), and their presence in crude cottonseed oil leads to rapid color-fixation reactions that result in dark-colored refined and bleached oils (3–6). In most present commercial cottonseed processes these undesirable effects are minimized by various types of wet-cooking procedures designed to react, or "bind," gossypol pigments with the protein and other meal constituents prior to oil extraction (2–4,6). Although this serves to decrease the physiological activity of gossypol pigments and to reduce extraction of gossypol into the oil, more recent observations have demonstrated that wet cooking of cottonseed adversely affects protein value, primarily because of the reaction of gossypol with the epsilon amino group of lysine, the limiting amino acid of cottonseed (2,7–10).

Worldwide interest in oilseeds as protein-rich supplements in human diets has recently focused attention on cottonseed as a potentially valuable protein supplement in areas where serious protein and caloric deficiencies exist (11–14). Although the role of gossypol in human nutrition has not been completely defined (13,15), the known physiological activity of gossypol and its derivatives suggests that protein-rich cottonseed concentrates of optimum quality should ideally contain low levels of gossypol and high levels of epsilon amino free lysine.

In addition to these considerations, potential processing methods for oilseeds should also be evaluated in terms of their efficiency for removing such toxic mold metabolites as aflatoxins, which can result from the invasion of agricultural products by certain strains of comomn molds such as *Aspergillus flavus* during unfavorable conditions of harvesting and storage (16,17). Such considerations have been recommended by UNICEF-FAO (18).

An ideal solution to the preparation of cottonseed meal concentrates and oils of optimum quality involves selective extraction of such undesirable seed constituents as gossypol and aflatoxins, under conditions favorable to retention of maximum protein value and oil content, followed by extraction of the oil under equally favorable conditions. This approach utilizes selective extraction of gossypol pigments with aqueous acetone, a solvent in which triglycerides are essentially insoluble, followed by oil removal either by hexane extraction or mechanical pressing. Although the utility of acetone-water mixtures for the selective extraction of gossypol pigments (19,20,21) and aflatoxins (22) from cottonseed has been demonstrated, the present communication is a report on the conditions required for the preparation of meals and oils of optimum quality.

Materials and Methods

Seed

One lot each of cottonseed of high and low free fatty acid content, and two lots of mold-damaged seed containing 25 and 535 ppb (μg/kg) of aflatoxin B_1 respectively, were used. Seeds were mechanically decorticated, screened, and aspirated to remove hulls; the hull-free kernels passed through smooth rolls to provide flakes of 0.008 to 0.010-in. thickness for extraction studies.

Analytical Methods

Moisture, lipids, total nitrogen, free and total gossypol, neutral oil, free fatty acids, refining and bleaching tests were determined by appropriate methods of the American Oil Chemists' Society (23). Epsilon amino free lysine was determined by the procedure of Rao et al. (24), aflatoxins by the method of Pons and Goldblatt (22), and total sugars by the Munson-Walker procedure (25). "Bound," or non-

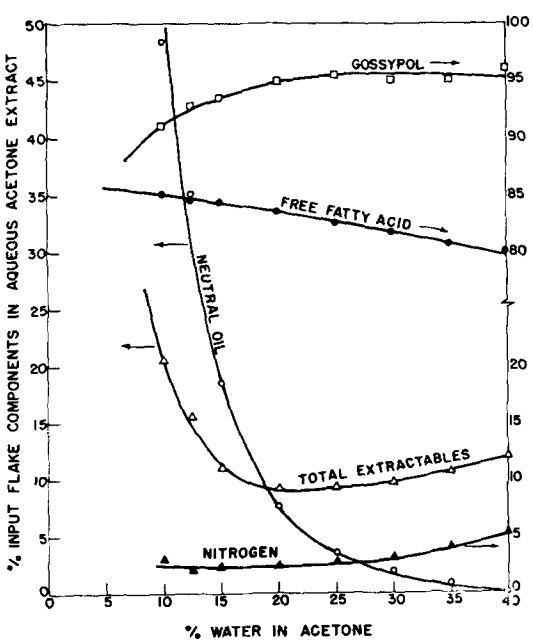

FIG. 1. Effect of acetone:water ratio on distribution of flake components in aqueous acetone extract. Conditions: temp. 30C; solv:flakes ratio 4:1; time, 30 min; washes, 2(2 parts solvent:1 part flakes per wash).

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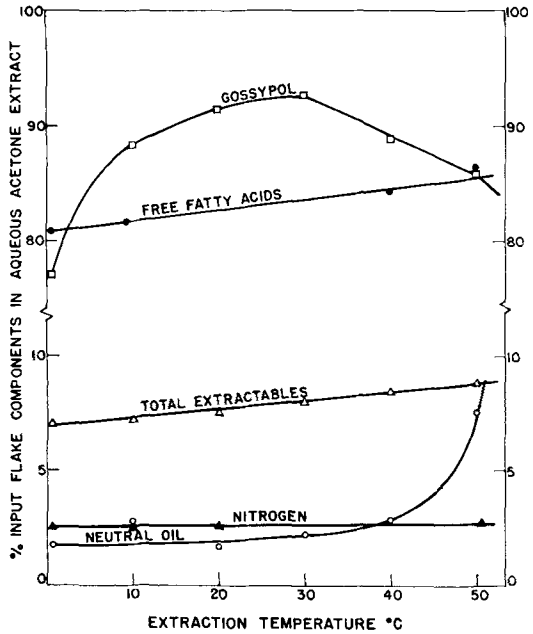

FIG. 2. Effect of extraction temperature on distribution of flake components in aqueous acetone extract. Conditions: acetone:water (70:30 w/w); time, 30 min; solv:flakes ratio, 2:1; washes 2.

acetone extractable gossypol was calculated from the difference between total and free gossypol analyses.

Product Yields

Aqueous acetone and hexane extracts (miscellas) were desolventized under vacuum in an all-glass rotary evaporator at 90C. Yields of crude aqueous acetone extracts, crude oils, aqueous acetone-extracted flakes (marc), and hexane-extracted or pressed meals were calculated in terms of grams of moisture and volatile free material per 100 grams of moisture-free input flakes. Product yields ranged from 98.2 to 100.5% and averaged 99.7%. The recovery and distribution of the input flake components in the products were calculated from observed weight yields and chemical analysis of the appropriate product. Inasmuch as the desolventized aqueous acetone extracts were not amenable to chemical analysis, the chemical composition of this fraction was estimated by the difference on the basis of the yield and composition of input flakes, the aqueous acetone-extracted flakes (marc), hexane-extracted or pressed meals, and crude oils.

Extraction Process

The influence of process variables was studied in small-scale extractions in which 100 g of flaked meats and the desired quantity of aqueous acetone, prepared from commercial-grade acetone and water on a weight basis, were weighed into an extraction vessel, equipped for constant temperature extraction, and stirred, usually at 30C, for the desired extraction time. The mixture was decanted onto a Buchner funnel and filtered under reduced pressure. The extraction vessel was rinsed with the desired number of displacement washes, using a weight ratio of two parts of solvent to one part of flakes per wash. Each wash was successively decanted onto the marc on the filter. Solvent damp marcs were then re-extracted with hexane under similar conditions to remove the oil. A larger-scale, simulated practical process was also conducted, as described later.

Extraction Variables

Acetone:Water Ratio

The amount of water in the acetone was the major variable that influenced the extraction of flake constituents. When the water content of the acetone was varied from 10 to 40% (other variables remained constant), minimum extraction of flake constituents occurred with acetone containing 20–30% of water (Fig. 1). Decreasing the water content below 20% markedly increased the extraction of flake components, primarily because of a greater extraction of neutral oil. Conversely, increasing the water content of acetone above 20% also increased the total amount of extracted material; the increase was primarily attributable to the removal of water-soluble carbohydrates. Maximum extraction of gossypol, on the order of 96%, occurred with 25–30% water in acetone. Notable is the fact that the extraction of free fatty acids from the input flakes was high, 80–85%, at all water:acetone ratios whereas the extraction of neutral oil markedly decreased as water in acetone was increased in the range of 25–40%. Extraction of protein, as measured by total nitrogen, was low and relatively unaffected at all solvent ratios. Wet marcs from extractions with 30%, or less, water in acetone were readily filtered, but those from higher water concentrations tended to be sticky and difficult to filter.

Extraction Temperature. In experiments in which the extraction temperature was varied from 1C to 50C (Fig. 2), maximum gossypol removal occurred at 25–30C. Low-temperature extraction yielded meals of relatively high free gossypol content, and those at temperatures above 30C were characterized by higher "bound" gossypol content, indicating the reaction of gossypol with protein. Extraction temperatures above 35C also increased neutral oil removal. In general, extraction temperature did not signifi-

TABLE I
Yield and Distribution of Flake Components from Aqueous Acetone-Hexane Extraction Under Optimum Conditions

Product	Product yields	Gossypol		Input flake components				
		Free	Total	Total N	Neutral oil	FFA	Total sugars	EAF Lysine
		As g per 100 g moist.-free flakes[c]						
Flakes	100	1.17	1.22	6.37	35.8	0.89	5.16	4.0[d]
Extract[a]	5.9	.....	1.13	0.16	0.61	0.68	2.28	....
Meal	58.7	0.01	0.08	6.21	0.31	.....	2.88	4.0[d]
Oil[b]	35.5	.....	0.01	0.002	34.9	0.22	.....	....
		% Distribution of flake components						
In extract	.....	.....	97.3	2.5	2.0	76.4	44.2	....
In meal	.....	.....	1.9	97.5	0.9	.....	55.8	100
In oil	.....	.....	0.8	.....	97.1	23.6	.....	....

[a] Crude aqueous acetone extract.
[b] By hexane extraction of aqueous acetone-extracted flakes.
[c] Average of five experiments.
[d] Calc. as g per 16 g N.

TABLE II
Average Composition of Products from Aqueous Acetone-Hexane Extraction

Property (Aver. of 5 experiments) analyses on d.b.	Product		
	Aqueous acetone extract	Meal	Crude oil
Free gossypol, %	.....	0.020	.....
Total gossypol, %	19.5	0.090	0.028
Total N, %	2.87	10.53	0.01
Protein, %	.....	65.8	.....
EAF. Lysine g/16g N	....	4.0	.....
Crude lipids, %	20.3	0.10	.....
Neutral oil, %	10.3	.....	99.2
F.F.A., %	11.7	.....	0.56
Total sugars, %	38.6	4.91	.....

cantly influence the extraction of free fatty acids, total solids, or total nitrogen.

Other Variables

Varying extraction time from 10–60 min (other variables remained constant) had little effect on the extraction of total solids, nitrogen, neutral oil, or free fatty acids. However maximum gossypol removal required a 30-min extraction.

Varying the solvent:flakes ratio from 1.7:1 to 4:1, or the number of on-filter displacement washes from 0 to 4, had analogous effects. In each instance, as the total amount of solvent contacting the flakes, either as slurry solvent or wash solvent, was increased, there was a small but gradual increase of 1–2% in the amount of total solids, gossypol, and neutral oil that was extracted. Total nitrogen and free fatty acids were not affected. From these studies a solvent:flakes ratio of 2:1, the minimum required to produce slurries with free liquid, and two on-filter displacement washes were selected as optimum.

Based on the above experimental results, the following optimum conditions were selected as those consistent with the maximum removal of such undesirable constituents as gossypol, and the minimum removal of protein or neutral oil for the aqueous acetone extraction of cottonseed:water in acetone 25–30% by weight; temperature 30C; time, 30 min; solvent:flakes ratio 2:1; displacement washes, 2.

Yields and Distribution

Average data for yields of products, and the calculated distribution of initial flake components in the products from five experiments conducted under the optimum conditions outlined above, are shown in Table I. It may be noted that 97% of the gossypol, 76% of the free fatty acids, about half of the total sugars, and some 2% of the total nitrogen and neutral oil in the input flakes were removed by aqueous acetone extraction. Nitrogen attributable to phospholipids and probably nucleic acids is responsible for the major portion of the aqueous acetone-soluble nitrogen. The data indicate that the 6% of the original flake solids removed during aqueous acetone extraction is primarily gossypol, crude lipids, free fatty acids, and raffinose, which is the principal sugar of cottonseed (26).

The average composition of the aqueous acetone extracts, the meals, and the oils from the five experiments are set forth in Table II.

The meals were light in color and were characterized by low free and total gossypol content, also by exceptionally high protein and epsilon amino free lysine. The crude oils were unique inasmuch as they were light-colored, had low gossypol content, and were high in neutral oil.

The desolventized aqueous acetone extract was an extremely viscous dark-colored material containing about 20% gossypol, 20% lipids, and some 39% total sugars. This fraction might serve as a convenient source of gossypol, as a raw material for the recovery of industrial or feed-grade fatty acids, or as a feed additive in ruminant rations where gossypol is not a serious problem.

Aqueous Acetone-Hexane

Meals and oils prepared in the experiments outlined in Tables I and II were all produced by hexane extraction of solvent damp aqueous acetone-extracted marc under conditions not suited to practical applications. In subsequent experiments it was found that drying of the marc prior to hexane extraction yielded hard random-sized lumps, which were not readily extractable by hexane. However, when the dried aqueous acetone extracted marc was pulverized to pass a 0.25-in. mesh screen and then passed through smooth rolls to provide reformed flakes of 0.008 to 0.010-in. thickness, the oil was readily removed by hexane extraction, producing light-colored meals with less than 1% residual oil. In larger-scale experiments 7–8 kg of cottonseed flakes were extracted with aqueous acetone under the optimum conditions previously outlined, the marc was then air-dried, ground, and reflaked prior to oil extraction with hexane. For comparative purposes a lot of the same raw cottonseed flakes was extracted in a single direct extraction with hexane. Miscellas from aqueous acetone-hexane and direct hexane-extracted oils were steam-stripped under vacuum at 80–90C. From the properties of the extracted meals (Table III) it is apparent that aqueous acetone-hexane extracted meal was much lower in gossypol content, residual lipids, and total sugars, and significantly higher in protein content, as compared with the direct hexane-extracted meal. Available lysine was equally high for both types of extraction.

The crude oil from aqueous acetone-hexane extraction (Table IV) was lower in gossypol content and free fatty acids and higher in neutral oil content than was the direct hexane-extracted oil. The Lovibond red value of the crude aqueous acetone-hexane extracted oil, 7.5, was exceptionally low for a crude oil.

TABLE III.
Comparison of Meals from Aqueous Acetone-Hexane and Direct Hexane Extraction of Cottonseed Flakes

Extraction methods	Material	Composition, Moisture-Free Basis						
		Gossypol		Nitrogen		Lipids	Total sugars	EAF. Lysine
		Free	Total	Total	Protein			
		%	%	%	%	%	%	g/16gN
None	Flakes	1.21	1.26	6.43	40.2	36.0	4.84	3.9
AA[a]	Marc	0.022	0.15	6.85	42.8	37.3	3.22	4.1
AA-Hexane[b]	Meal	0.045	0.22	10.83	67.7	0.9	3.08	4.0
Direct hexane[c]	Meal	1.40	1.56	9.84	61.5	1.5	7.36	4.0

[a] Acetone:water, 70:30 w/w; solv:flakes ratio, 2:1; time, 30 min; temp. 30C.
[b] Air-dried AA marc ground, re-formed into flakes (0.008–0.010 in.), and re-extracted with hexane.
[c] Original flakes extracted with hexane only.

TABLE IV
Properties of Oils Prepared by Aqueous Acetone-Hexane and Direct Hexane Extraction of Cottonseed Flakes

Property	Type of Crude Oil	
	Aqueous acetone -hexane	Direct hexane
Total gossypol, %	0.012	0.14
Neutral oil, %	98.7	97.0
Free fatty acids, %	0.50	1.20
Refining loss-14° Be, %	7.8	6.0
Crude oil color-Lovibond Red[a]	7.5	33+
Refined oil color, Lovibond Red[b]	4.2	8.4
Bleached oil color, Lovibond Red[b]	2.4	6.1

[a] Using 1-in. tube.
[b] Using 5.25-in. tube.

Moreover the crude oil refined and bleached to acceptably low-color values when subjected to standard cup refining and bleaching tests. It should be emphasized that the crude aqueous acetone-hexane oil was an unusual oil, and the application of standard cup refining tests is not an ideal refining test for such oils.

Aqueous Acetone-Pressing

Aqueous acetone extraction, followed by mechanical pressing for oil removal, may also be utilized to prepare superior meals and oils. Flaked meats were extracted with aqueous acetone under the optimum conditions previously outlined, dried, and re-flaked; the oil was removed by pressing with a laboratory model Carver press for 1–1.5 hr at a pressure of about 2,000 psi. One lot of extracted flakes was pressed at 25C and one lot at 90–95C. The properties of the products are shown in Table V. As expected, the oil content of the press cake is a function of press temperature. Both meals were light in color, low in gossypol content, and high in protein and available lysine. The meals had a bland, slightly nutlike taste when ground. Both of the crude oils were light-colored, low in gossypol, and high in neutral oil. Aqueous acetone extraction, followed by mechanical pressing, offers an opportunity to produce a high protein-high oil meal containing negligible gossypol. Such meals may have use in supplementing both protein and caloric deficiencies in human nutrition (14).

Extraction of Mold-Damaged Seed

Practical procedures for removing such toxic mold metabolites as aflatoxins from mold-damaged cottonseed would allow utilization of valuable oilseeds which might otherwise be unsuitable for the preparation of protein supplements for feed uses. The aqueous acetone-hexane process was evaluated for this purpose by using a lot of experimental mold-damaged cottonseed that contained a high level of aflatoxin B_1. The seeds were not uniformly contaminated with aflatoxins as analyses of subsamples yielded aflatoxin B_1 values ranging from 300 to 600 ppb (μg/kg). For comparative purposes a portion of the seed was also extracted with hexane in a single-stage extraction. The chemical properties of the original flakes and the resulting products are shown in Table VI. Flakes prepared from the mold-damaged seed were high in free fatty acids, protein, and epsilon amino free lysine. They were low in neutral oil and total sugars and contained a considerable amount of bound gossypol. In conformity with previous results, both meals and oils from these badly deteriorated seed were much improved in quality as compared with the direct hexane-extracted meal and oil. It is also noteworthy that the aqueous acetone-hexane process was highly effective in removing aflatoxins from the flakes, and the aqueous acetone-hexane extracted crude oil was devoid of aflatoxin B_1. Although aflatoxin B_1 at a level of 26 ppb of B_1 was detected in the aqueous acetone-hexane extracted meal, calculations on the per cent distribution of flake components indicated that 97% of the aflatoxin B_1 content of the flakes was removed in the aqueous acetone extraction and only 3% remained in the meal after subsequent hexane extraction. In contrast to these results, the direct hexane-extracted meal was high in aflatoxin B_1 content, 519 ppb, and the crude oil contained 24 ppb of B_1. Distribution calculations showed that, in direct single-stage hexane extraction, 98% of the original aflatoxin B_1 content of the flakes remained in the hexane-extracted meal; some 2% was removed in the crude oil. In other experiments, by using mold-damaged seed that contained 25 ppb of aflatoxin B_1, aqueous acetone-hexane extraction produced meal and oil free of detectable aflatoxin B_1 (< 1 ppb).

These results were achieved by use of the previously outlined optimum conditions for processing prime quality seed by aqueous acetone-hexane extraction. In subsequent experiments, by the use of three successive aqueous acetone extractions at solvent:flake ratios of 4:1 and the increasing of on-filter displacement washes to 4, the aflatoxin B_1 content of the meals was reduced to levels below about 1 ppb of aflatoxin B_1. However this does involve higher losses of flake solids and neutral oil.

Process Potential

Extraction of prime flaked cottonseed meats with aqueous acetone yields a marc which contains essentially all of the protein, epsilon amino free lysine, and neutral oil of the original flakes but from which essentially all of the gossypol, about half of the total sugars (raffinose), and more than 75% of the free fatty acids have been removed. After drying and reflaking, the marc can be extracted with hexane for recovery of the oil, yielding a very light-colored meal low in free and total gossypol, low in total sugars (raffinose), but exceptionally high in protein content. The epsilon amino free lysine is essentially equivalent

TABLE V
Composition of Meals and Oils by Aqueous Acetone-Hydraulic Pressing

	Composition, Moisture-Free Basis							
	Gossypol		Nitrogen		Lipids			EAF Lysine
	Free	Total	Total	Protein	Total	FFA	Neutral oil	
	%	%	%	%	%	%	%	g/16gN
Flakes	1.22	1.24	6.91	43.2	36.9	1.17[c]	97.1	4.0
Cold-Press meal[a]	0.016	0.12	8.16	51.0	25.6	0.59[c]	98.7	3.9
Hot-Press meal[b]	0.013	0.14	9.83	61.4	10.3	0.89[c]	99.1	3.9
Cold-Press oil[a]	.....	0.012	.....	.....	.....	0.13	99.4	....
Hot-Press oil[b]	.....	0.010	.....	.....	.....	0.14	99.6	....

[a] 1 hr at 25C, 2,000 psi.
[b] 1 hr at 90–95C, 2,000 psi.
[c] FFA of petroleum ether-extracted lipids.

TABLE VI

Composition of Products from Extraction of Mold-Damaged Cottonseed

Composition, Moist.-Free Basis	Type of Extraction				
	Original flakes	AA-Hexane[a]		Direct hexane	
		Meal	Crude oil	Meal	Crude oil
Yield, %[b]	100	60.0	25.2	68.0	32.0
Free gossypol, %	0.85	0.052	.....	1.33	.....
Total gossypol, %	0.98	0.24	0.080	1.53	0.14
Total N, %	5.97	9.60	.....	8.53	.....
Protein (N × 6.25), %	37.3	60.0	.....	53.3	.....
FFA, %	7.8	.....	7.4	.....	21.1
Neutral oil, %	25.7	0.4	91.5	0.7	76.1
Total sugars, %	2.42	1.90	.....	3.85	.....
EAF Lysine, g/16g N	3.9	4.0	.....	4.0	.....
Aflatoxin B_1, ppb	535[c]	26	None	519	24

[a] Acetone:water, 70:30 (w/w); solvent:flakes ratio, 2:1; on-filter washes, 2; time 30 min; temp. 30C. Data are aver. of three experiments.
[b] Yield on basis of input flakes, 14.8% of flake solids removed in aqueous acetone extract.
[c] Calculated from yields and analysis of aqueous acetone extract, meal, and crude oil for aflatoxin B_1 content.

to that of the original flakes. Alternatively the oil may be removed by mechanical pressing to yield meals of either medium or high fat content, low in gossypol, and high in protein and epsilon free amino lysine content.

Crude oils produced by either process are exceptionally light in color, low in gossypol and free fatty acids, and high in neutral oil. The crude oils refine and bleach to prime color values and should be free of color reversion problems associated with gossypol pigments in crude oils.

Meals produced by this process should be suitable for unrestricted use in the diets of the most gossypol-sensitive nonruminant animals and would be expected to find application as high-quality protein supplements in human nutrition.

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