Removal of Cyclopropenoid Fatty Acids from Cottonseed Meals by Solvent Extraction[1]

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Abstract

Direct solvent-extraction procedures were explored for their effectiveness in removing the residual levels of cyclopropenoid fatty acids from commercial cottonseed meals. Of the seven solvent systems screened, a simple stepwise extraction with an acetone/hexane/water azeotrope was found suitable for the removal of up to 88% of the original CPA content of the meal.

Introduction

VARIOUS APPROACHES have been applied to the removal or inactivation of the cyclopropenoid fatty acids (CPA) present in cottonseed in order to improve the quality and the utilization of the processed meal. An excellent review of the cyclopropenoid components in cottonseed and cottonseed oils and the biological effects of these unique fatty acids has been prepared by Phelps et al. (1).

This study was directed toward the removal of the cyclopropenoid fatty acid moieties in commercially available cottonseed meals by solvent-extraction procedures which may be adaptable as an adjunct to presently employed commercial processing methods (2). Several solvent-extraction procedures have been reported to be useful for the removal of cyclopropenoid fatty acids from decorticated cottonseed flakes (3–5) as well as preserving the nutrient properties of the resulting meal (6).

In studies involving the exhaustive extraction of cottonseed meats with *n*-hexane, Bailey et al. (5) noted that the cyclopropenoid components in the meats did not appear to be readily accessible to this solvent, suggesting that the cyclopropenoid components may be in specific areas of the seed which are less accessible. This could involve binding to phospholipid structures which are not readily removed by neutral lipid solvents. Consequently the present study involved the screening of seven different solvent systems in order to evaluate their relative effectiveness in removing the cyclopropenoid moieties in cottonseed meals as based on the levels of CPA remaining in the meal.

Experimental Procedures

Equipment

Cottonseed meals representing two types of commerically processed meals were used in various phases of this study: direct solvent-extracted (DSE) and screw-pressed (SP). For the small bench-scale experiments, standard laboratory glassware and equipment were utilized with little or no modification. For the large-scale (30-lb) extractions, an 11-gal upright cylindrical tank with an airtight cover and a ½-in. drain spout was used. The drain spout was set in the side of the tank on a level with the bottom and fitted with a stopcock valve. To prevent the meal from escaping through the drain, a filter consisting of a fine-meshed screen was supported by a layer of ⅝-in. diameter marbles, which were placed in the bottom of the tank to support the meal above the top of the drain opening.

Technical-grade solvents were used except for the analytical experiments, in which reagent-grade solvents were substituted to avoid possible interference from trace impurities. The solvents used were hexane, benzene, methanol, petroleum ether (30 to 60C), and the azeotrope systems of benzene-methanol (62/38), hexane-methanol (74/26), and acetone/hexane/water azeotrope (42/56.5/1.5).

Analytical Methods

Appropriate methods of the American Oil Chemists' Society (7) were employed for the determination of moisture, lipids, total nitrogen, free and total gossypol, and crude fiber in both original and extracted meals. Epsilon amino free lysine was estimated by the procedure of Rao et al. (8), and residual CPA in the meals by the method proposed by Levi et al. (9).

Solvent-Extraction Studies

During preliminary experiments the various solvents were screened for their relative efficiency by using a standard Soxhlet extractor, fitted with a Friedrichs condenser. Double-walled extraction thimbles (33 × 80 mm) were filled with 30 g of meal, and the reflux rate was adjusted so that one syphon pass (about 15 ml) was made every 4 min. The extraction was continued until a total of 300 ml of solvent had been passed over the meal. The thimbles were then allowed to drain, and the extracts were analyzed for their CPA content. Table I summarizes the results of this screening procedure. Although both benzene and the benzene/methanol azeotrope exhibited high extraction efficiencies for the removal of CPA, they also removed a relatively large amount of meal solids and were not deemed to be suitable for practical applications.

Although exhaustive Soxhlet extraction with hexane was efficient for removing residual CPA (Table I), this solvent was found to be considerably less efficient

TABLE I

Comparison of Relative Efficiency of Various Solvents[a]

Solvent	Crude extract % of Meal	CPA Removed	
		ppm	% Efficiency[b]
Methanol	5.6	42	56
Hexane	2.6	52	69
Hexane-methanol azeotrope	3.0	45	60
Benzene	3.8	57	75
Benzene-methanol azeotrope	5.3	57	75
Acetone-hexane-water	3.5	54	71

[a] Soxhlet extraction; 7:1/solvent:meal ratio.

[b] Based on 75.6 ppm average CPA content found for this batch of direct solvent-extracted meal (9).

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[2] ARS, USDA.

TABLE II

Comparison of Relative Efficiency of Extraction Techniques[a]

Method	Crude extract % of Meal	CPA Removed ppm	CPA Removed % Efficiency[b]
Soxhlet, 2 hr at 47C	3.5	48	63
Soxhlet, 4 hr at 47C	3.8	47	62
Soxhlet, 6 hr at 47C	3.8	46	61
Room temperature with continuous dripping	3.3	45	60
Room temperature with 30-min soaking intervals	3.5	48	63
Room temperature, 1 hr agitation	3.4	63	83

[a] AHW single-step extractions.
[b] Based on 75.6 ppm average CPA control found for this batch of solvent-extracted meal (9).

than the acetone/hexane/water azeotrope (AHW) for the rapid removal of CPA in simple batchwise extractions. Consequently the AHW solvent was selected for further extraction studies.

The initial screening of solvents was followed by a series of experiments designed to determine the relative efficiencies of a number of extraction techniques with the AHW azeotrope. During the Soxhlet extractions the temperature of the meal rapidly rose to close to the reflux temperature of the solvent. To avoid this rise in temperature, the Friedrichs condenser was replaced by a dropping funnel containing the 300 ml of solvent. The solvent was allowed to drop onto the meal at a rate comparable with that used during the regular Soxhlet extraction. In another variation of this method, the extraction thimble was filled with solvent and the meal was allowed to soak for 30 min. Sufficient solvent was then added to induce siphoning, and the thimble was drained. The process was repeated until a total of 300 ml of solvent had been passed over the meal. The effect of simply soaking the meal, with or without agitation, in the solvent for various periods of time was also studied. The results of these experiments are summarized in Table II.

During the early experiments, no single-step extraction procedure, at least under the mild conditions employed, completely eliminated all the residual CPA from the meal. A series of stepwise extractions was therefore carried out to study the relationship between the solvent-to-meal ratios and the relative number of steps required to remove substantially all the CPA from the meal. The effect of the length of time the solvent was in contact with the meal during each step was also investigated. For this purpose 30 g of meal

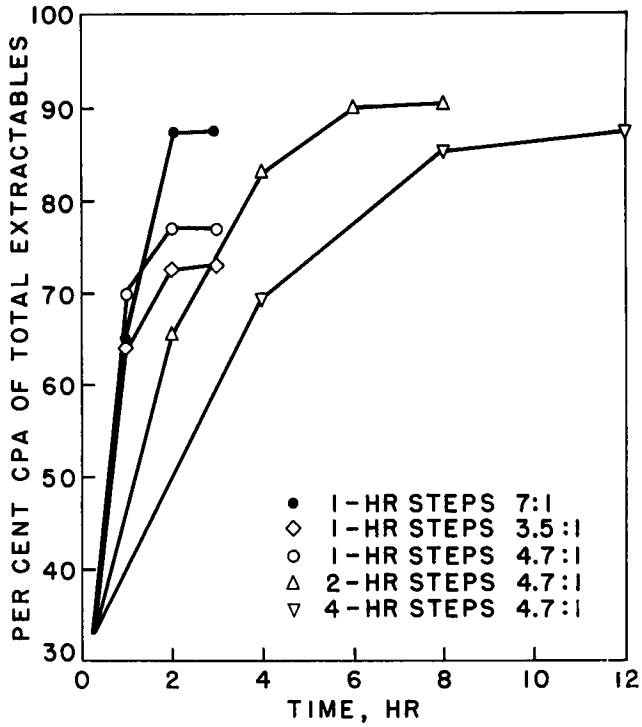

FIG. 1. Effect of solvent:meal ratio and extraction time on stepwise extraction with AHW solvent.

was weighed into a 500-ml, round-bottom, one-necked flask, and the appropriate amount of AHW solvent was added. The flask was attached, without suction, to a rotary evaporator by a spring clip and rotated to impart a tumbling action to the meal and thus provide mild agitation. This technique was used in preference to stirrers because the relatively high speeds required with bladed stirrers to attain efficient dispersion of the meal caused an excessive amount of comminution of the meal and rendered subsequent decantation or filtration difficult. The meal was agitated for the desired length of time, after which the solvent was removed by decantation and the meal was weighed to determine the amount of solvent hold-up and to calculate material balances.

The results of these studies are presented in Table III and in Fig. 1 and 2. As shown in Table III and Fig. 1, there appears to be little direct correlation between either the solvent ratios or the contact times in terms of the amount of CPA extracted in each step. This is demonstrated in Fig. 2, where the pro-

TABLE III

Comparison of Solvent:Meal Ratio and Number of Steps Required to Remove CPA from Meal[a]

Time of extr.; solvent ratio[b]	Extraction 1 Removed[c] ppm	Extraction 1 Removed[c] % of Total	Extraction 1 Left[d] ppm	Extraction 2 Removed[c] ppm	Extraction 2 Removed[c] % of Total	Extraction 2 Left[d] ppm	Extraction 3 Removed[c] ppm	Extraction 3 Removed[c] % of Total	Extraction 3 Left[d] ppm	Extraction 4 Removed[c] ppm	Extraction 4 Removed[c] % of Total	Extraction 4 Left ppm	CPA Removed ppm	Total CPA Removed[e] %
1 Hr; 7.1:1 (300 ml)	49.2	65.0	26.4	12.7	16.8	13.7	T	+	13.7	—	—	13.7	61.9	81.8
1 Hr; 4.7:1 (200 ml)	52.8	70.0	22.8	13.0	17.2	9.8	—	—	9.8	—	—	9.8	65.8	87.2
1 Hr; 3.5:1 (150 ml)	48.5	64.2	27.1	6.4	8.5	20.7	T	+	20.7	—	—	20.7	54.9	72.7
2 Hr; 4.7:1 (200 ml)	49.8	66.0	25.8	12.9	17.1	12.9	5.5	7.3	7.4	0.7	0.9	6.7	68.9	91.3
4 Hr; 4.7:1 (200 ml)	52.6	69.6	23.0	13.0	17.2	10.0	1.0	1.3	9.0	T	—	9.0	66.6	88
1-Hr Soxhlet[f] intervals	56.0	74.0	2.2	2.2	2.9	17.4	4.3	5.7	13.1	—	—	13.1	62.5	83

[a] Room-temperature, agitated extraction with AHW.
[b] Weight ratio of solvent to meal.
[c] Additional CPA removed in each step; T = trace.
[d] Difference between total CPA and amount removed.
[e] Total CPA found for this meal (75.6 ppm) (9).
[f] Sample dried between extraction steps.

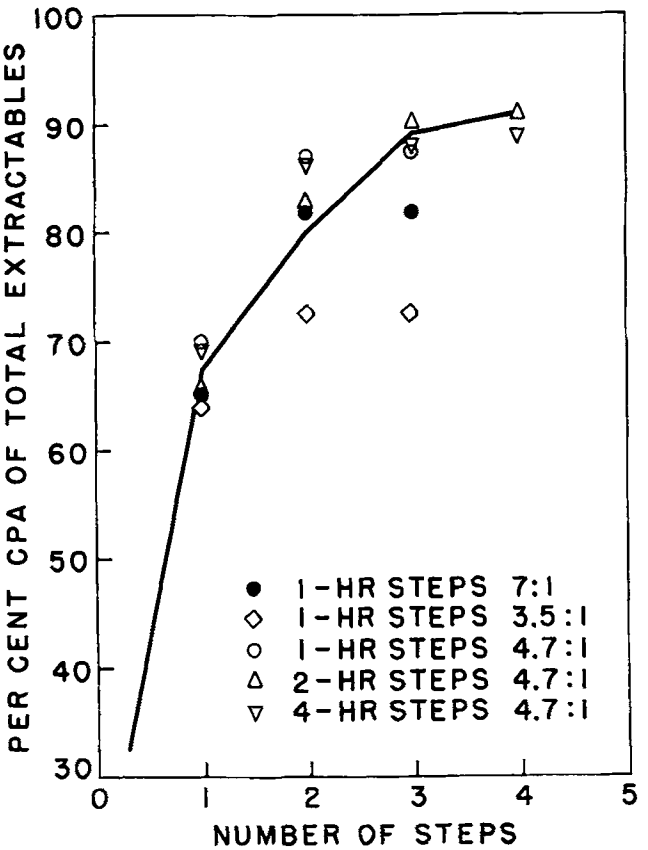

FIG. 2. Effect of number of steps on stepwise extraction with AHW solvent.

portion of CPA removed is plotted against the number of extraction steps.

For large-batch extractions, 30 lb of meal were weighed into the tank described above and covered with a wire mesh to prevent excessive mixing when the solvent was added. An equal weight of AHW solvent (19.3 liters) was then poured on the meal while the drain was open to permit the air to escape from the bottom and thus prevent serious channeling of solvent in the meal. The stopcock was closed after the solvent began to drain and was left closed until the air bubbles stopped rising. The tank also was shaken several times in order to help remove pockets of entrapped air. The stopcock was then opened, and the solvent was allowed to drain by gravity flow.

When the solvent stopped running freely, an air-tight cover was clamped on the tank so that the vapor pressure of the solvent above the meal would help force the remaining liquid to drain off the meal. This procedure was carried out as many times as necessary, depending on the degree of extraction and/or final CPA level desired.

The time required for each solvent pass varied from 30 to 60 min, depending on the packing of the meal; the shorter time was obtained for the first pass, and succeeding passes required successively longer periods. The SP meal, which appeared to be much finer than the DSE meal, required nearly 60 min per pass.

The effluent solvent was collected from each step, concentrated, and analyzed in order to monitor the removal of the CPA. When the amount of CPA remaining on the meal (based on CPA found in the extracts) was considered to be at the desired level, the meal was removed from the tank and spread on several large trays to air-dry for a period of at least 24 hr. The dried, extracted meals were then analyzed for CPA, moisture, fat, total nitrogen, epsilon amino free lysine, free and total gossypol, and crude fiber (Table IV).

Discussion

Data obtained from these solvent-extraction studies demonstrate that a reasonably efficient removal of CPA from cottonseed meal can be obtained by a mild extraction procedure. For example, in a single 1-hr AHW extraction, up to 70% of the original CPA content can be removed; if this is followed by a second extraction, approximately 87% of the total CPA content of the meal can be removed. With successive 2-hr extractions more than 90% of the CPA can be removed. This double-extraction technique would lower the CPA value of most commercial direct solvent-extracted meals to a level of about 5–10 ppm (Table III). On repeated extraction, proportionally lower values can be obtained; however such repeated extraction methods probably represent an unrealistic approach. Actually under the mild extraction conditions employed in these studies, subsequent feeding studies have indicated a pronounced improvement in egg quality when these meals were fed to laying hens (10).

Also of interest is the fact that the removal of CPA from the meal appeared to bear little relationship to a variation of two-fold differences in solvent-to-meal ratios and that slightly lower efficiencies were observed for the 2- and 4-hr extractions than for the 1-hr extraction. The first observation may be partially explained on the basis that the residual levels of CPA being measured are removed with the available "free oil" or unbound lipid components present in the meal.

Since these residual oil levels are of such a low magnitude (1–3 wt %), excessive volumes of solvent may be no more efficient than the minimum volume necessary for removal. This can be borne out by the relatively constant quantity of total extractable material removed (3.55 wt %) at a 7.1 to 1 solvent-to-meal ratio as compared with the quantity (3.50 wt %) removed by a 4.7 to 1 ratio for the same time-interval (1 hr). Apparently the latter ratio is approaching the minimum solvent volume since a ratio

TABLE IV
Analyses of Original and AHW Solvent-Extracted Commercial Cottonseed Meals

Meal	CPA ppm	Moisture %	Fat %	Crude fiber %	Total nitrogen %	Epsilon amino free lysine g/16 g of nitrogen	Gossypol %	
							Free	Total
Direct solvent-extracted meal								
3a. Original	70	7.46	2.78	11.9	7.05	3.94	0.18	0.88
3b. Extracted 2x	8	6.84	0.52	13.1	6.69	3.50	0.11	0.82
3c. Extracted 3x	5	8.60	0.55	10.6	7.01	3.44	0.13	0.84
Screw-pressed								
4a. Original	170	6.92	3.48	12.3	7.23	2.53	0.05	1.07
4b. Extracted 2x	29	7.54	0.81	12.9	6.86	2.60	0.04	0.86
4c. Extracted 3x	3	8.36	(?)	13.0	6.92	2.50	0.05	1.03

of 3.5 to 1 yielded a somewhat lower extractable lipid level of 3.20 wt %.

To eliminate the possibility that the lowering of the CPA level, because of the possible presence of free fatty acids, was not a result of interference of free fatty acids with the analytical procedure, a series of runs was carried out whereby known levels of free cottonseed fatty acids were introduced at various steps throughout the analytical procedure. In this study a refined cottonseed product of known CPA content was employed, and there was no indication of any change in CPA value.

On the basis of these preliminary data it would appear that a simple percolation type of solvent extraction, employing the AHW solvent system, could be used as an adjunct to present commercial cottonseed processing methods. Although such an approach would represent added cost, the resultant meal should have unrestricted use in diets for laying hens (10).

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