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Protein Isolates with Reduced Gossypol Content from Screw-Pressed Cottonseed Meal

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Gossypol was removed from commercial cottonseed meal by successive extractions with organic solvents. 1-Butanol hydrochloride was found the most suitable solvent, reducing the free gossypol content to the safe level of 0.0104% after three extractions. Protein isolates, with a protein content of 85%, were produced from raw and butanol-treated meals with yields of 50–80% based on extracted proteins. The isolates were light colored and had bland flavor. The butanol treatment influenced the protein isolation yield, lowering protein extractability, as confirmed by chromatographic analysis, but did not affect significantly the functional properties of the proteins.

INTRODUCTION

Although cottonseed meal is a source of good quality protein (Martinez et al., 1970a), its utilization in human nutrition is limited by the presence of a toxic substance, gossypol (Berardi and Goldblatt, 1980). Gossypol has adverse physiological effects on nonruminants (Smith, 1970), and the interaction between proteins and gossypol during the processing of cottonseed has a deleterious effect on protein quality (Damaty and Hudson, 1979). According to FDA regulations, free gossypol content of edible grade cottonseed products should not exceed 0.045% (450 ppm).

Great efforts have been devoted to devise practical methods for reducing the meal's gossypol content. The optimizing of the screw-press process by controlling the operating conditions leads to gossypol binding (Gizinos and Farmaki, 1985; Lusas and Jividen, 1987). In the case of the straight pressing, high pressure is applied and the nutritional value of meal is reduced because of protein denaturation.

Other approaches have also been made to the gossypol problem. One is the development of glandless varieties of cottonseed in which the pigment glands have been genetically depleted (Martinez et al., 1970b). A second is the development of process treatments that remove intact pigment glands without adversely affecting the protein fraction, namely air classification (Kadan et al., 1979) and liquid cyclone process (LCP) (Vix et al., 1971; Gardner et al., 1976). A third employs the removal of gossypol by solvent extraction of cottonseed meal (Altschul et al., 1958).

Many solvent extraction methods have been proposed by several workers, and a number of solvents and azeotropes have been used effectively.

Aqueous acetone was used by Vaccarino (1961), by Pons and Eaves (1967), and, in combination with anhydrous acetone at two stages, by Damaty and Hudson (1975). Furthermore, acetone–cyclohexane with or without water (Lawhon and Rao, 1967) and acetone–hexane–water azeotropes (King and Frampton, 1960) were used for extraction. Although acetone extraction produced cottonseed meal with low free gossypol content, it caused an objectionable flavor and odor of the meal (Alyevand et al., 1967).

Acidic butanol treatment of cottonseed meal (Canella and Sodini, 1977) could remove gossypol without altering the protein quality, but the free gossypol level of the products was higher than the level permitted in cottonseed products for food use. Dichloromethane could allow a larger reduction of free gossypol content (Cherry and Gray, 1981) with no adverse effect on the quality of the meal. 2-Propanol (or aqueous 2-propanol and 2-propanol–hexane mixture) was found to be an efficient solvent for extracting free gossypol (Rahma and Rao, 1984). Ethyl alcohol (aqueous or ethyl alcohol–hexane mixture) was also used in detoxification methods of cottonseed products (Liu et al., 1981; Hron and Koltun, 1984).

In our country cottonseed meal is produced almost exclusively by straight screw-pressing after prolonged and drastic heat treatment, and the subsequent meal has a free gossypol content of 0.03–0.3% and low quality (Gizinos and Farmaki, 1985).

The objective of this work was to find the most suitable solvent among 1-butanol, 2-propanol, and dichloromethane for the reduction of gossypol content from screw-pressed cottonseed meal, to produce low free gossypol protein isolates, and to study the effect of the solvent treatment on the quality of these products.

MATERIALS AND METHODS

Materials. The raw material was a commercial screw-pressed cottonseed cake, obtained from a local plant. Before crushing, the ground seed was subjected to a prolonged treatment with directly applied steam without prior dehulling and delintering. After pressing, the cake was dried at 120 °C.

Cottonseed cake was ground in a blender mixer and defatted with hexane in a Soxhlet apparatus for 8 h. The defatted material was dried at room temperature, ground in a laboratory mill, and screened through a 1-mm sieve; the passing fraction was used as starting cottonseed meal in our experiments.

Analytical Methods. The cottonseed meal and the protein isolates were analyzed for their proximate composition including moisture, ash, oil, and crude fiber contents with the AOAC (1984) methods. Protein content was determined according to the macro-Kjeldahl method (%N × 6.25). Total sugars were measured according to the phenol–sulfuric acid method (Dubois et al., 1956) using glucose as standard. Free gossypol and total gossypol were determined according to AOCS (1986) Official Methods Ba 7-58 and Ba 8-78, respectively.

Color measurements of the protein isolates were made with a Lovibond Tintometer Model E (The Tintometer Ltd., Salisbury, England) and of the solvent extraction filtrates with a Lovibond AOCS Tintometer (The Tintometer Ltd.).

Solvent Extraction of Gossypol. Defatted cottonseed meal (20 g) was extracted with one of the following solvents: 1-butanol–0.005 N HCl (92:8 v/v), 2-propanol–0.005 N HCl (92:8 v/v), or dichloromethane. Extractions were carried out at 25 °C for 30

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min at a meal-to-solvent ratio of 1:10 with stirring. The pH of the slurry was adjusted to 4.5 with 0.5 N HCl and was kept constant during the extraction, except in the case of dichloromethane, where no pH adjustment was made. At the end of the treatment the suspension was filtered through Whatman No. 3 paper under vacuum. The filtrate color was measured, and the residue was dried at 40 °C under vacuum. Free gossypol and total gossypol of treated meal were determined, and the extraction was repeated under the same conditions until the gossypol content of the meal reached the permitted level.

A larger scale experiment (100 g of meal in 1 L of solvent) with the best solvent system selected was also conducted. The solid material resulting from this treatment was used in protein isolate preparations.

Protein Isolate Preparation. Samples used for preparation of protein isolates include (i) meal without solvent extraction for gossypol removal and (ii) meal treated with the solvent which gave the highest gossypol removal.

The preparation of protein isolates was carried out as follows: Cottonseed meal (20 g) was extracted with the extracting solutions at a meal-to-solution ratio of 1:20 (w/v) at 40 °C for 30 min with constant stirring. The protein was extracted with 0.1 N NaOH at pH 12 and with 0.5% Na_2SO_3 (w/v) at pH 11.5. During the extraction the pH of the suspension was kept constant by adjusting with 0.5 N HCl or 0.5 N NaOH. The suspension was centrifuged at 3800 rpm for 10 min and filtered through Whatman No. 3 paper. The extract was acidified with 0.5 N HCl until the isoelectric value was attained. The isoelectric point (p*I*) was determined as the pH value of maximal precipitation and was found to be 4.37. Protein isolates were prepared after centrifugation of the suspension at 3800 rpm for 10 min, washing the precipitate with 50% 2-propanol, and freeze-drying the washed precipitate. The solid fraction remaining after protein extraction was dried at 60 °C under vacuum and was used for protein determination.

The results presented are the mean values of three trials.

Water Absorption Capacity. Water absorption capacity (WAC) was determined according to a modified method of Rahma and Rao (1983): 6 mL of distilled water was added to 0.25 g of sample in a weighed 10-mL glass centrifuge tube. The tube was agitated on a Vortex mixer for 2 min and then centrifuged for 20 min at 3000 rpm. The supernatant was decanted and discarded. The tube was weighed after the removal of the adhering drops of water. WAC is expressed as the weight of water bound per 100 g of sample.

Oil Absorption Capacity. Oil absorption capacity (OAC) was determined according to a modified method of Lin et al. (1974): 3 mL of refined corn oil was added to 0.5 g of sample in a graduated 10-mL glass centrifuge tube. The tube was agitated on a Vortex mixer for 1 min, left for 30 min, and centrifuged for 20 min at 3000 rpm; the volume of free oil was read. OAC is expressed as the volume of oil absorbed per 100 g of sample.

Soy protein isolate ISP 660 (BIOTREK S.A., Athens, Greece) was taken as reference for WAC and OAC measurements.

Electrofocusing and Chromatographic Separation. Isoelectrofocusing (Pharmacia, 1982a) was carried out with Phast System (Pharmacia LKB Biotechnology, Uppsala, Sweden) using PhastGel IEF 3-9 media (homogeneous polyacrylamide gels containing Pharmalyte carrier ampholytes). For detecting proteins the Coomassie staining technique was used (PhastGel Blue R).

Chromatographic analysis was carried out on a Waters 650 advanced protein purification system (Millipore, Milford, MA) with a Chromatofocusing kit (Pharmacia LKB Biotechnology). A column (1 × 10 cm) packed with Polybuffer exchanger PBE 94, equilibrated with starting buffer (0.025 M imidazole hydrochloride, pH 7) was used and eluted with Polybuffer 74 hydrochloride adjusted to pH 4 (Pharmacia, 1982b). Monitoring of protein content was done by a single-path monitor UV-1 (Pharmacia LKB Biotechnology) at 280 nm. Sample collection was carried out with a programmable fraction collector FRAC-300 (Pharmacia LKB Biotechnology) adjusted to collect samples in separate tubes every 1 min. The pH of each sample was measured by a pH-meter equipped with a small-size electrode.

The samples of NaOH and Na_2SO_3 extracts, used for chromatographic analysis, contained 1.3–2.8 mg of protein/mL of extract. The samples of the isolate proteins were dissolved in 0.015 N NaOH at a concentration 0.3–0.8 mg of protein/mL. The protein content was determined according to the method of Lowry et al. (1951).

For rapid desalting and buffer exchange of the samples Pharmacia PD-10 columns were used which contained Sephadex G-25 medium in distilled water.

Table I. Proximate Composition of the Cottonseed Cake, Meal, and 1-Butanol-Treated Meal (Percent, Dry Basis)

	cake	meal	treated meal
moisture	6.4	5.8	6.1
protein	26.8	32.1	32.1
oil	1.1	0.8	0.9
ash	4.9	5.6	5.4
crude fiber	34.6	27.3	28.7
total sugars	5.1	5.3	4.1

RESULTS AND DISCUSSION

The proximate compositions of the cottonseed cake and of the meal used as starting material in experiments are shown in Table I. The raw cake had high crude fiber content because of the presence of hulls and linters. The protein content of raw material was not very high (26.8% on a dry basis), and for this reason an enrichment by screening was tried, so that the starting material had a protein content of 32.1%.

For the gossypol removal from cottonseed meal the extracting procedure was based on the method of Canella and Sodini (1977). The major modifications were in the meal-to-solvent ratio and in the extraction time, which were 1:10 and 30 min instead of 1:20 and 15 min, respectively. This selection of the extraction conditions was made on the assumption that the same reduction of gossypol content could be attained by a lower number of extractions. In addition to 1-butanol, 2-propanol and dichloromethane were also used as extracting media. All extractions were conducted at the isoelectric pH (4.37) of proteins to minimize dissolution of the proteins in the solvents.

Table II shows how the free and total gossypol contents were lowered with each one of the three solvents. 1-Butanol was the most effective extracting solvent for free gossypol removal, which amounted to 80% by two extractions. These results were in good agreement with and in some cases even better than those found in the literature for similar procedures (Canella and Sodini, 1977). The gossypol extraction by 2-propanol was sufficient enough, while dichloromethane would not be suggested as it removed only 33% of free gossypol after four successive extractions. In addition to the large reduction of free gossypol, solvent extraction reduced markedly the bound gossypol content. It must be also noted that a larger proportion of the total gossypol was removed during the first extraction; on the contrary, for the free gossypol, each extraction removed almost equal proportions.

The results were in accordance with the color of the filtrates from the successive extractions with the three different solvents as shown in Table II. The protein content of the meal after the last extraction showed no significant difference and was 33.8%, 30.1% and 31.6% for 1-butanol, 2-propanol, and dichloromethane, respectively. The color of the butanol-extracted meal was slightly lighter than that of the meal extracted by the other solvents.

On the basis of the obtained results 1-butanol was selected to produce cottonseed meal for use in protein isolate preparation. Although an acceptable free gossypol level was achieved by two extractions with 1-butanol (Table II), one additional extraction step was added for greater

Table II. Effect of Solvent Extractions on the Gossypol Content of Cottonseed Meal (Dry Basis)

solvent	free gossypol, %		total gossypol, %		Lovibond color of filtrate
	content	removal	content	removal	
none	0.0971		1.2905		
1-butanol					
first extraction	0.0455	53.1	1.0131	21.5	10Y 0.3R
second extraction	0.0190	80.4	0.9422	27.0	4Y
2-propanol					
first extraction	0.0457	52.9	0.9698	24.9	5Y
second extraction	0.0260	73.2	0.9136	29.2	2Y
third extraction	0.0137	85.9	0.8321	35.5	1Y
dichloromethane					
first extraction	0.0732	24.6	0.9534	26.1	5Y 2.1R
second extraction	0.0686	29.4	0.9177	28.9	1Y 0.1R
third extraction	0.0679	30.1	0.8654	32.9	<1Y 0.1R
fourth extraction	0.0649	33.2	0.8345	35.3	

Table III. Effect of 1-Butanol Extractions (Larger Scale) on the Gossypol Content of Cottonseed Meal (Dry Basis)

1-butanol	free gossypol, %		total gossypol, %		Lovibond color of filtrate
	content	removal	content	removal	
first extraction	0.0456	53.0	0.9623	25.4	7Y 0.1R
second extraction	0.0189	80.5	0.8979	30.4	2Y
third extraction	0.0104	89.3	0.7586	41.2	<1Y

Table IV. Protein Yield and Protein Content of Produced Isolates from Treated and Untreated Cottonseed Meals (Dry Basis)

extracting solution	protein extraction, %	total protein recovery,[a] %	isolate: protein content, %	isolate: % of total wt
treated meal				
Na_2SO_3	44.3	47.8	86.0	7.6
NaOH	39.7	71.7	85.3	10.5
untreated meal				
Na_2SO_3	52.8	63.7	83.3	12.9
NaOH	54.2	81.8	83.4	16.7

[a] Total protein recovery is based on the protein content of the extract.

Table V. Proximate Composition of Protein Isolates Produced from Treated and Untreated Cottonseed Meals (Percent, Dry Basis)

	treated meal	untreated meal
protein	85.5	83.4
ash	1.5	1.1
total sugars	0.9	0.8
free gossypol	0.007	0.053
total gossypol	0.515	1.059

Table VI. Color, Water Absorption Capacity, and Oil Adsorption Capacity of Cottonseed Protein Isolates

isolate	Lovibond color of isolates	WAC,[a] g of H_2O/100 g of sample	OAC,[a] mL of oil/100 g of sample
treated meal			
Na_2SO_3	1.3Y 1.5R 1.1B	486.5 ± 29.0	472.7 ± 55.2
NaOH	1.7Y 2.4R 1.5B	395.3 ± 20.2	557.0 ± 48.4
untreated meal			
Na_2SO_3	2.0Y 1.6R 1.0B	353.5 ± 24.8	464.0 ± 46.6
NaOH	1.9Y 2.5R 1.0B	393.1 ± 6.6	532.3 ± 26.6
soy ISP 660	0.4Y	626.2 ± 39.8	197.8 ± 30.1

[a] Mean value ± standard deviation (N = 5).

safety in human nutrition and for usage in feed formulations for nonruminants (Smith, 1970). The results of the three successive extractions conducted in larger scale are presented in Table III. After the third extraction, the free gossypol content of the treated meal (0.0104%) was reduced to well below the 0.045% guideline. Total sugar content was also reduced, and the protein content of the meal was 32.1% (Table I).

To study the effect of butanol extraction on the quality of proteins, treated as well as untreated meals were used to prepare protein isolates. The experiments were based on extracting the proteins in alkaline solutions and precipitating them at the isoelectric point (p*I*) (Berardi et al., 1969; Yazicioglu et al., 1981). The extraction conditions were selected because they resulted in optimal protein extractability in previous experiments (Liadakis, 1990). The pH values used were higher than those found in the literature (Berardi et al., 1969; El Tinay et al., 1980; Yazicioglu et al., 1981), but they extracted greater amount of protein without affecting seriously the color of the isolates. The comparison of the results in Table IV between treated and untreated meals showed that the percent protein extraction as well as the total recovery of proteins was higher for untreated meal. This may be attributed to alteration of the protein properties, especially of the p*I*, by the butanol treatment because gossypol interacts with proteins, modifying their properties (King and Lamkin, 1977). However, the isolate from treated meal had a slight increase in protein content. The protein extraction yield as well as the protein recovery and content of the isolates was comparable with those found in the literature (Berardi et al., 1969; Damaty and Hudson, 1979; El Tinay et al., 1980). Of the two extracting solutions, 0.1 N NaOH showed greater total yield than 0.5% Na_2SO_3. A typical analysis of the resulting protein products (Table V) shows that the isolates contained about 85% protein. It should also be noted that the gossypol content was further reduced and this reduction was more pronounced in the case of the isolates from treated meal, as it was confirmed by the spectra of cottonseed proteins which did not show any peak at 390 nm (King and Lamkin, 1977). After protein extraction of the meal remains a byproduct (about 75% of total weight) with 20% proteins which can be further utilized in animal feed.

The color and the functional properties of isolates play major roles in determining their acceptability as food ingredients. Table VI presents data about the color and water/oil absorption capacities of the isolates compared with soy protein isolate. The isolates from treated and untreated meals showed no considerable differences in color. In all cases the produced isolates were light-colored. Washing with 50% 2-propanol probably contributes to this, because the alcohol is able to break H-bonds between polyphenols and proteins (Gheyasuddin et al., 1970). Na_2SO_3 produced isolates of lighter color from both meals. It is known that most of the dark brown pigments are extracted in alkali-soluble isolates (Choi et al., 1982). In addition, Na_2SO_3 on acidification by HCl is broken down into SO_2, which may bleach undesirable colors and inhibits

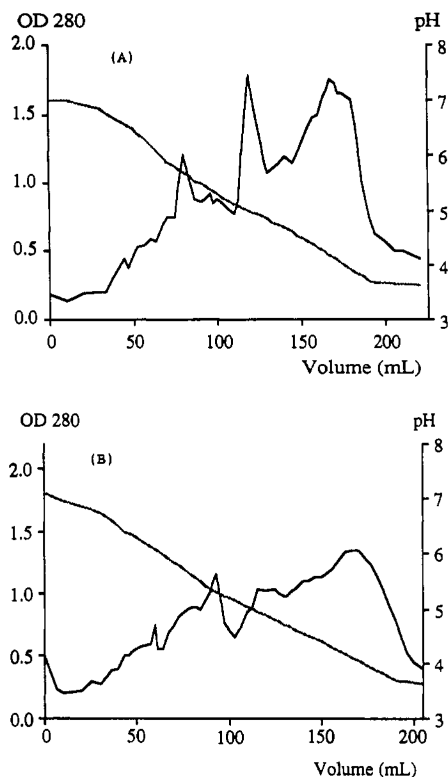

Figure 1. Fractionation of NaOH-extracted proteins from untreated cottonseed meal (A) and NaOH-extracted proteins from treated cottonseed meal (B). Column: PBE 94. Bed height: 10 cm. Sample: 1 mL containing 2.83 (A) or 1.33 (B) mg of protein. Elution conditions: start buffer 0.025 M imidazole hydrochloride, pH 7.4; elution buffer 0.0075 mmol/pH unit/mL Polybuffer 74, pH 4. Flow rate: 3 cm h^{-1}.

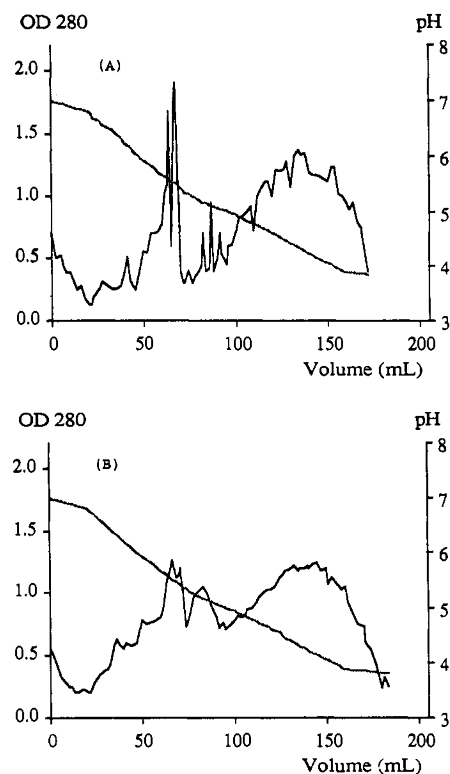

Figure 2. Fractionation of NaOH-soluble isolated proteins from untreated cottonseed meal extracted with NaOH (A) and from treated cottonseed meal extracted with NaOH (B). Column: PBE 94. Bed height: 10 cm. Sample: 1 mL containing 0.78 (A) or 0.40 (B) mg of protein. Elution conditions: start buffer 0.025 M imidazole hydrochloride, pH 7.4; elution buffer 0.0075 mmol/pH unit/mL Polybuffer 74, pH 4. Flow rate: 3 cm h^{-1}.

the browning reaction (Gheyasuddin et al., 1970). Data in Table VI also show that gossypol removal by 1-butanol did not affect significantly the WAC and OAC of the isolates. On the other hand, the results of the WAC and OAC were differentiated from soy protein isolate.

Further work was carried out to study the effect of butanol treatment on the extracted and precipitated protein fractions. So electrophoretic and chromatographic analyses of the NaOH- and Na_2SO_3-extracted proteins and of the NaOH-soluble isolate proteins were conducted. The isoelectric focusing method was used to select the best pH interval for p*I* measurements and to predict optimal separation conditions for chromatographic separation. The isoelectric focusing showed that various protein fractions existed in the pH range 3.5–7. Chromatofocusing separation was conducted to find the pH range of the main protein fractions of samples. In all patterns, one major protein fraction was present at pH around 4.2 and two minor ones not distinctly separated at pH around 5.2 and 5.8. This confirmed our previous experiments regarding the optimal pH of protein precipitation. The chromatographic patterns on PBE 94 gel columns when NaOH was used for protein extraction are presented in Figures 1 and 2. Comparison of parts A and B of Figure 1 indicated that the butanol treatment affects protein extractability, resulting in solubilization of fewer protein fractions. This agrees with the extractability resulted from our experiments and can be attributed, as has already been mentioned, to the alteration of protein properties, especially of the p*I*. Small differences were found between the NaOH- and Na_2SO_3-extracted proteins, which showed that both solutions extracted the same protein fractions. As can be seen by comparing Figures 1 and 2, the main protein fractions of the extracts were recovered in the precipitates. The analysis of the dissolved in NaOH isolates did not indicate differences in the precipitated proteins of produced isolates. This was expected since the pH of the protein precipitation was the same for all experiments and, subsequently, almost the same protein fractions were isolated at that pH value.

CONCLUSIONS

Using 1-butanol–HCl, we succeeded in reducing the free gossypol content of screw-pressed cottonseed meal to safe levels for human consumption, with only a few extractions. Protein isolates of high protein concentration (85%) and at high yields (81% based on NaOH-soluble proteins) were produced from cottonseed meal. Results obtained with butanol-treated meal allow us to conclude that it is possible to produce cottonseed isolates with acceptable properties and with free gossypol content lowered to an allowable level without damaging the water and oil absorption capacities.

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