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Preparation, Evaluation and Functional Properties of Gossypol-poor Cottonseed Protein Isolates

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ABSTRACT

*The glanded cottonseed (*Gossypol barbadense, *variety Ashmouni) was used in the present study. The location, distribution and properties of the most important constituents of the seeds, namely protein, oil and gossypol were examined.*

Four treatments were used to prepare gossypol-poor flour from the cottonseed. Treatment I depended on differential settling using fresh hexane in all different steps. The process was repeated to prepare different fractions of gossypol-poor flour. Treatment II was similar to I except that fresh hexane was used only in the first step and the produced oil-hexane miscella was used in the subsequent steps. Treatments III and IV depended on a combination of sieving (200 mesh sieve) and the differential settling process. For treatment III the residual flour was ground, sieved and used to prepare fractions of gossypol-poor flour using fresh hexane. The oil-hexane miscella was used in treatment IV.

The four treatments can be arranged in the following decreasing order according to the amount of edible protein obtained from 100 g of dehulled cottonseed: III > I > IV > II. Three protein isolates, namely A, B and D' were obtained from treatment III giving a recovery of 21·2% of edible flour from dehulled cottonseed, i.e. 66% of the original protein present in the Ashmouni cottonseed variety.

The protein isolates obtained were evaluated for nitrogen extractability as well as some chemical, nutritional and physicochemical properties. The

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results illustrate the possibility of using the prepared protein isolates as thickening and whipping agents, emulsifiers and extenders in different food products.

INTRODUCTION

In Egypt, cottonseed represents the most important field crop. Originally, it was considered as the most valuable non-food cash crop. As well as extracting the oil, successful use of its protein part may help in satisfying the ever-increasing nutritional needs of the population. Removal of the pigment glands is of great importance to the potential use of cottonseed proteins as human food. The cellular structure of cottonseed mainly contains three components of great interest in this study: pigment glands, aleurone grains and spherosomes. The cottonseed pigment glands are spherical in shape and about 100–400 μm in diameter (Gardner *et al.*, 1976). The seeds also contain aleurone grains (protein bodies) and spherosomes (lipid particles) which are embedded in the aleurone and bounded by limiting membranes. The aleurone grain dimensions have not been determined while the spherosomes are about 2 μm in diameter (Yatsu *et al.*, 1974).

The pigment gland is more than 12 times as large as the spongy mesophyll cells (aleurone grains) and this is one basis for their separation. The slow settling characteristics of the fine cellular tissue (aleurone) compared with the more rapid settling of the pigment glands and other large particles, such as the hulls, suggested separation using differential settling in a liquid medium (Vix *et al.*, 1949).

In this work four treatments, depending on differential settling alone or in combination with sieving, were utilized to prepare gossypol-poor cottonseed flours.

Three protein isolates were prepared from the gossypol-poor fractions of the most effective treatment. The protein isolates were evaluated for nitrogen extractability, chemical and nutritional properties and physico-chemical properties, i.e. emulsion capacity, viscosity, foam capacity, water- and oil-holding capacities and whipping capacity.

MATERIALS AND METHODS

Materials

The glanded cottonseed, *Gossypol barbadense*, variety Ashmouni, was obtained from the Field Crops Research Farm, Faculty of Agriculture, University of Alexandria, Egypt.

Methods

Electron microscopy

Small pieces of dehulled cottonseed meat (ca. 4 mm^3) were fixed and dehydrated as described by Meek (1976). The fixed, dehydrated pieces, were infiltrated as described by Hayat (1975). Thin sections of 30 μm were prepared using an ultramicrotome. The sections were stained in 2% uranyl acetate for 30 min and observed using a JEM-60 electron microscope.

Preparation of gossypol-poor cottonseed flours

Gossypol-poor cottonseed flours were prepared using a combination of the sieving and differential settling process as described by Gardner *et al.* (1973).

The seeds were dehulled manually and ground for 3 min. The samples were stored in Kilner-jars at 5°C until used. In the sieving treatments, the ground seeds were sieved through a 200 mesh sieve.

Four treatments were used to produce gossypol-poor flours. Treatments I and II depended on the differential settling process as shown in Fig. 1. Fresh hexane was used in the different steps of treatment I, while fresh hexane was used in the first step of treatment II and the produced oil-hexane miscella was used in the subsequent steps.

A slurry of ground cottonseed with hexane at a ratio 1:2 (w/v) was fed into the top of a glass column (40 × 5 cm) filled with hexane. The lower layer containing the pigment glands was collected, filtered, solvent evaporated, ground and used to prepare the next fraction. The upper layer which contained the gossypol-poor flour was collected, filtered, solvent evaporated at room temperature and then ground.

This sequence was repeated four times to produce the gossypol-poor fractions A, B, C and D; the differential settling process was repeated again on fractions B, C and D to prepare B′, C′ and D′ fractions.

Treatments III and IV depending on the combination of sieving and the differential settling process are shown in Fig. 2. Dehulled ground cottonseeds were first sieved through 200 mesh sieve to obtain fraction A. The residual was ground and used to prepare fraction B using differential settling. The layer containing the pigment glands was filtered, solvent evaporated, ground and sieved through a 200 mesh sieve to prepare fraction C. The residual was fed into the column to produce fractions D and E. The process was repeated on fractions D and E to prepare fractions D′ and E′.

Solubility at different pH values

Twenty-five grams of the defatted cottonseed flours were extracted using 500 ml H_2O at pH 12 for 1 h; clear extracts were obtained by centrifugation

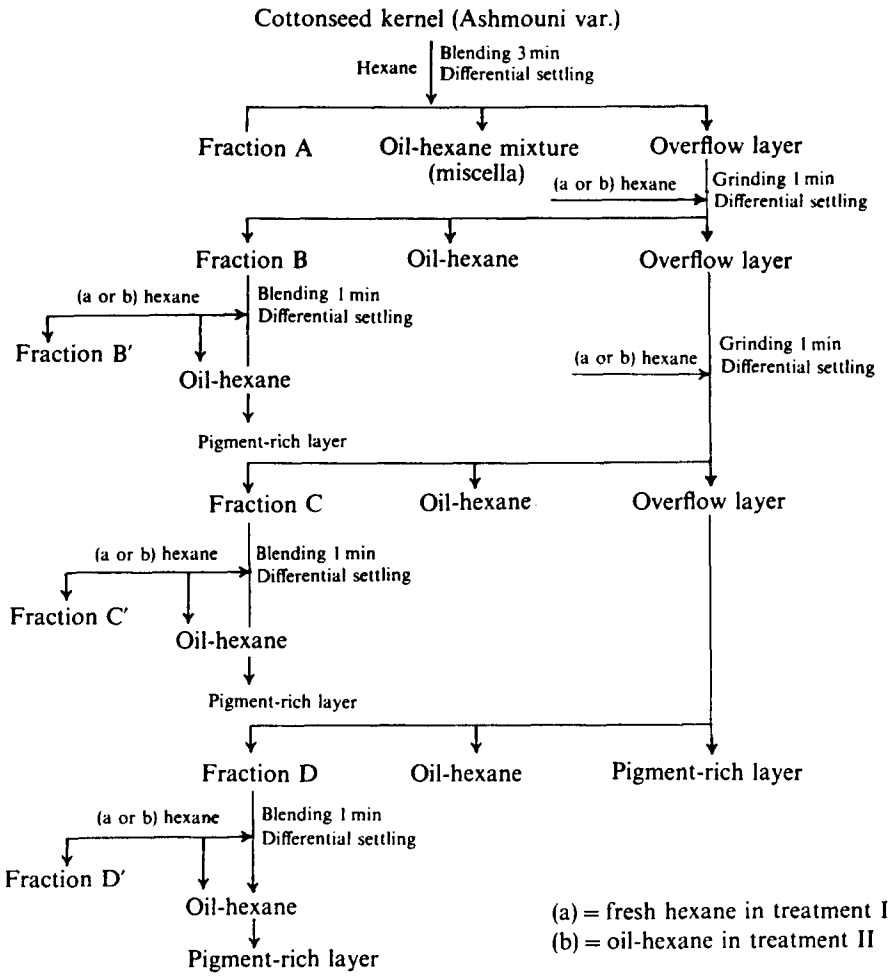

Fig. 1. Schematic diagram of differential settling process used for the preparation of gossypol-poor protein fractions

at 3500 rev/min for 30 min. Twenty-five ml aliquots of the extract were pipetted into 250 ml centrifugal tubes. The pH was adjusted in the range of 1–12 using 0·5 N HCl, the resulting precipitates were separated by centrifugation at 3500 rev/min for 30 min. The nitrogen present in the supernatants were determined by the micro-Kjeldahl method as described in AOAC (1975) and reported as a percentage of total soluble (or non-precipitated) nitrogen (Mattil, 1971).

Preparation of protein isolates

The protein isolates were prepared from the defatted fractions A, B and D' of gossypol-poor flours, obtained from treatment III as described by Müller *et al.* (1976).

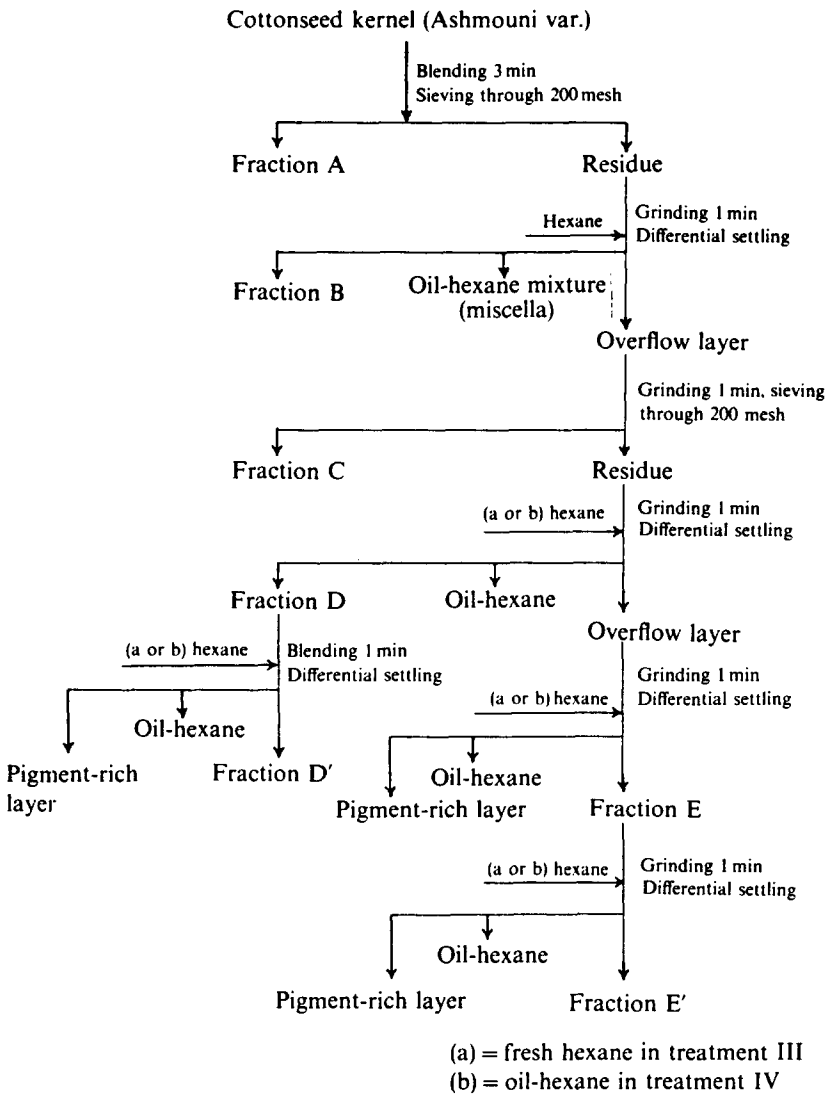

Fig. 2. Schematic diagram of combination of sieving and differential settling process used for the preparation of gossypol-poor protein fractions.

Analytical methods

Moisture, oil, free gossypol and total nitrogen were determined according to the methods of the AOCS (1966). The crude protein was calculated by multiplying the total nitrogen by the factor 6·25. Tryptophan content was determined by the spectrophotometric method of Spies & Chambers (1949).

Total and available lysine were determined according to the method of Carpenter *et al.* (1957).

Estimation of digestibility

Pepsin, pancreatin and pepsin followed by pancreatin digests were prepared by incubation of each protein isolate with the appropriate enzyme according to the method of Akeson & Stahman (1964). At the end of the incubation period, 7·5 ml of 1·6 M trichloroacetic acid were added. The soluble nitrogen in the supernatant was determined (AOCS, 1966) and percentage nitrogen digested was calculated with respect to total nitrogen in the sample.

Physicochemical methods

Foaming capacity and foaming stability were measured according to Lawhon & Cater (1971).

Whipping capacity

Whippability measurements were carried out as described by Lawhon *et al.* (1972) with the following modifications: (a) 3 g of the dried protein isolate were dispersed in 100 ml of 0·2 M citrate-phosphate buffer pH 7·0; (b) the suspension was whipped for 6 or 8 min.; (c) 75 g of sucrose were added to the suspension obtained in (a) then whipped for 6 min.; (d) whipping was carried out for 6 min., then 75 g of sucrose were added to the whip and the whipping resumed for an additional 2 min. The percentage volume increase was taken as a measure of whipping capacity. The viscosity of each whip was measured using a MacMichael viscometer (Fischer Scientific Co., USA).

The emulsion capacity of the cottonseed protein isolates was determined by the procedure described by McWatters & Cherry (1975). Water- and oil-holding capacities were determined by the method of Childs & Park (1976).

Heat-coagulated protein was measured according to Kramer & Kwee (1977) and expressed as a percentage of the crude protein.

RESULTS AND DISCUSSION

Cellular structure of cottonseed

The electron microscopic examination shows that the average diameter of the aleurone grains (protein bodies) ranged from 0·3 to 1 μm, while both spherosomes (lipid particles) and globoids (phytin storage sites) were 0·2 to 0·8 μm.

Light microscopy of thin sections prepared from cottonseed shows that the gossypol glands have a diameter ranging from 100 to 400 μm, a finding which agrees with that reported by Gardner *et al.* (1976).

TABLE 1
Main Constituents of Fractions Obtained by Differential Settling Process Using Fresh Hexane (Treatment I)

Gossypol-poor fraction	*Yield*[a] (%)	*Protein recovery of each fraction* (%)	*Main constituents of each fraction*[b] (%)		
			Protein	*Oil*	*Free gossypol*
A	34·0	47·8	45·2	18·5	0·061
B	13·9	20·5	47·2	11·9	0·105
C	7·9	12·1	49·0	7·00	0·275
D	5·4	11·2	50·7	5·50	0·420
B′	10·16	15·5	49·2	6·25	0·068
C′	4·50	7·50	50·5	3·50	0·180
D′	3·20	5·12	51·6	3·00	0·230
Residue	15·50	24·5	—	—	—

[a] = g obtained from 100 g dehulled cottonseed.
[b] = calculated on dry weight basis.

Preparation of gossypol-poor flours using the differential settling process

Table 1 shows the main constituents of the fractions obtained from treatment I using fresh hexane. The yield of the fractions obtained from 100 g of dehulled seeds gradually decreased in each subsequent stage while the percentage of protein in these fractions increased, reaching its maximum of 51·6% in fraction D′. The effect of this process on the removal of pigment glands shows a reduction of free gossypol in fraction A to 0·06% (the original being 0·86% in the raw seeds).

Fractions B′, C′ and D′ contain lesser amounts of free gossypol than fractions B, C and D. This is due to the repeated treatment with hexane. It can be concluded that both fractions A and B′ (recoveries of 47·8 and 15·5% protein) are suitable for human consumption as such; their contents of free gossypol are very close to the limits allowed by the FAO (1971), i.e. 0·06%. The other fractions may be mixed with other proteins to arrive at a free gossypol content close to or lower than the allowed limits.

Table 2 gives the results obtained from treatment II using the reused oil-hexane miscella. The data show that the use of oil-hexane miscella resulted in fractions with higher oil contents than those obtained with fresh hexane. The efficiency of oil-hexane miscella in the removal of the pigment glands from cottonseed was lower than that obtained with the fresh hexane used in treatment I. Fraction A only, with a recovery of 51·8% of total protein, was the only fraction which can be used as human food because of its low free

TABLE 2
Main Constituents of Fractions Obtained by Differential Settling Process with the Reuse of the Oil-hexane Miscella (Treatment II)

Gossypol-poor fraction	*Yield*[a] (%)	*Protein recovery of each fraction* (%)	*Main constituents of each fraction*[b] (%)		
			Protein	*Oil*	*Free gossypol*
A	36·4	51·8	46·0	18·2	0·063
B	11·9	17·0	46·3	14·3	0·145
C	6·6	9·60	48·0	11·9	0·410
D	7·1	10·8	48·9	8·45	0·800
B′	9·9	14·7	48·0	12·7	0·120
C′	4·9	7·45	49·2	6·50	0·305
D′	5·9	9·00	49·6	6·04	0·640
Residue	12·9	17·1	—	—	—

[a] = g obtained from 100 g dehulled cottonseed.
[b] = calculated on dry weight basis.

gossypol content. The low recovery figures and higher free gossypol contents of fractions D and D′ exclude them from human use. The other fractions B, C and C′ may be mixed with other proteins so as to obtain mixtures containing gossypol contents within the allowed limits.

Preparation of gossypol-poor flours using a combination of sieving and differential settling processes

A 200 mesh sieve (nominal sieve opening 75 μm) was utilized to remove the glands but allow the fine particles to pass. Table 3 gives the main constituents of the fractions obtained in treatment III using fresh hexane. In this treatment five fractions were obtained instead of four only in treatments I and II. It is clear from this Table that using a combination of sieving and differential settling gave the first fraction A with a significantly lower amount (0·05%) of free gossypol, i.e. lower than the limit set by FAO. Fraction B also has an amount of free gossypol close to that allowed by FAO and considerably lower than its corresponding fraction in treatment II. Treating fraction D with fresh hexane resulted in fraction D′ with a free gossypol content of 0·057%, making it suitable for human consumption. Each of the three fractions A, B and D′, totalling 65·9% of the original protein, has a free gossypol content lower than or very close to that allowed by FAO (1971) and hence these fractions can be used as human food. The other fractions have a low percentage recovery and high free gossypol contents.

TABLE 3
Main Constituents of Fractions obtained by Sieving and Differential Settling Process utilizing Fresh Hexane (Treatment III)

Gossypol-poor fraction	*Yield*[a] (%)	*Protein recovery of each fraction* (%)	*Main constituent of each fraction*[b] (%)		
			Protein	*Oil*	*Free gossypol*
A	8·9	8·80	32·5	34·3	0·050
B	28·9	42·0	46·8	17·7	0·066
C	6·3	9·00	46·4	16·0	0·180
D	11·8	17·9	49·0	12·5	0·110
E	8·1	12·7	50·7	7·0	0·280
D′	9·6	15·1	50·4	9·30	0·057
E′	6·2	10·1	52·3	5·60	0·140
Residue	12·0	15·0	—	—	—

[a] = g obtained from 100 g dehulled cottonseed.
[b] = calculated on dry weight basis.

Table 4 gives the main constituents of the fractions obtained in treatment IV using the reused oil-hexane miscella. Protein contents of the different fractions obtained were generally lower than those of treatment III. The two fractions A and B, totalling 56·8% of the original protein, have free gossypol contents of 0·06% and 0·063% respectively. These contents of gossypol permit the use of both fractions for human consumption through

TABLE 4
Main Constituents of Fractions obtained by Sieving and Differential Settling Process utilizing Oil-Hexane Miscella (Treatment IV)

Gossypol-poor fraction	*Yield*[a] (%)	*Protein recovery of each fraction* (%)	*Main constituents of each fraction*[b] (%)		
			Protein	*Oil*	*Free gossypol*
A	11·4	11·3	32·1	35·0	0·060
B	29·7	45·5	47·2	16·92	0·063
C	7·60	10·5	44·4	13·4	0·190
D	11·3	16·1	46·3	11·2	0·210
E	9·40	13·6	46·9	9·57	0·505
D′	7·80	11·2	47·6	8·10	0·160
E′	4·68	7·0	48·8	7·30	0·310
Residue	12·3	14·6	—	—	—

[a] = g obtained from 100 g dehulled cottonseed.
[b] = calculated on dry weight basis.

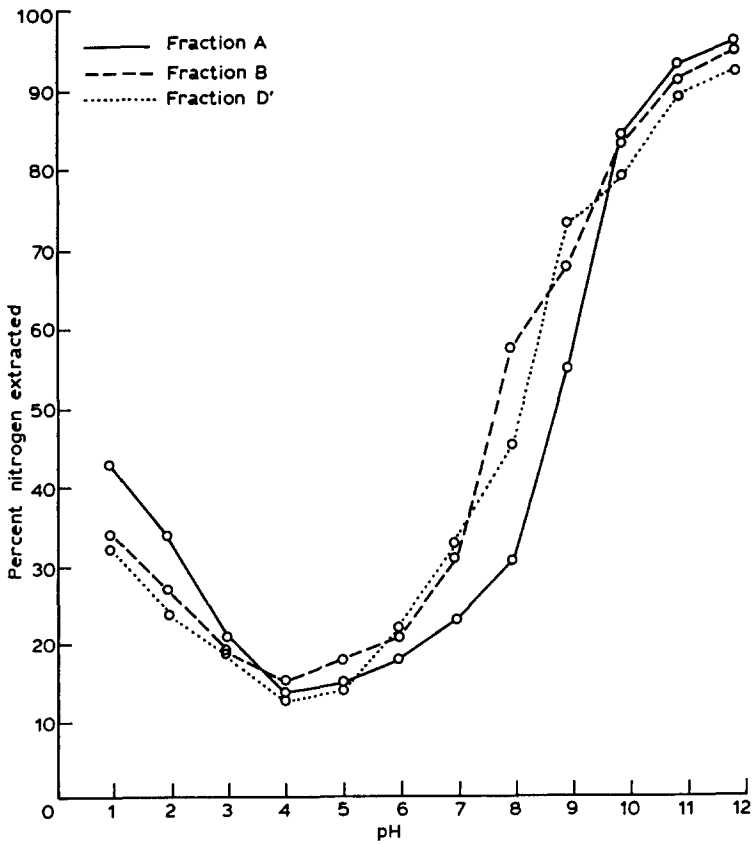

Fig. 3. Percentage nitrogen extracted from gossypol-poor fractions at various pH values.

direct use or enrichment. The other fractions obtained by this treatment have higher free gossypol contents and lower protein recoveries.

Combination of sieving and differential settling in treatment III can allow the use of fractions A, B and D' for edible purposes. These fractions represented 65·9% of the original protein and gave 21·3 g protein recovered from every 100 g of the dehulled cottonseed. These three fractions were utilized in further experiments.

Effect of pH on the extractability of nitrogenous components from gossypol-poor fractions

Figure 3 gives the percentage nitrogen extracted from fractions A, B and D' at pH values ranging between 1 and 12. The results are in agreement with those of Mattil (1971) and Crenwelge *et al.* (1974). Maximum extractability is on the alkaline side at pH 12 and minimum extractability is at pH 4. The three fractions do not differ significantly in nitrogen extractability. It may

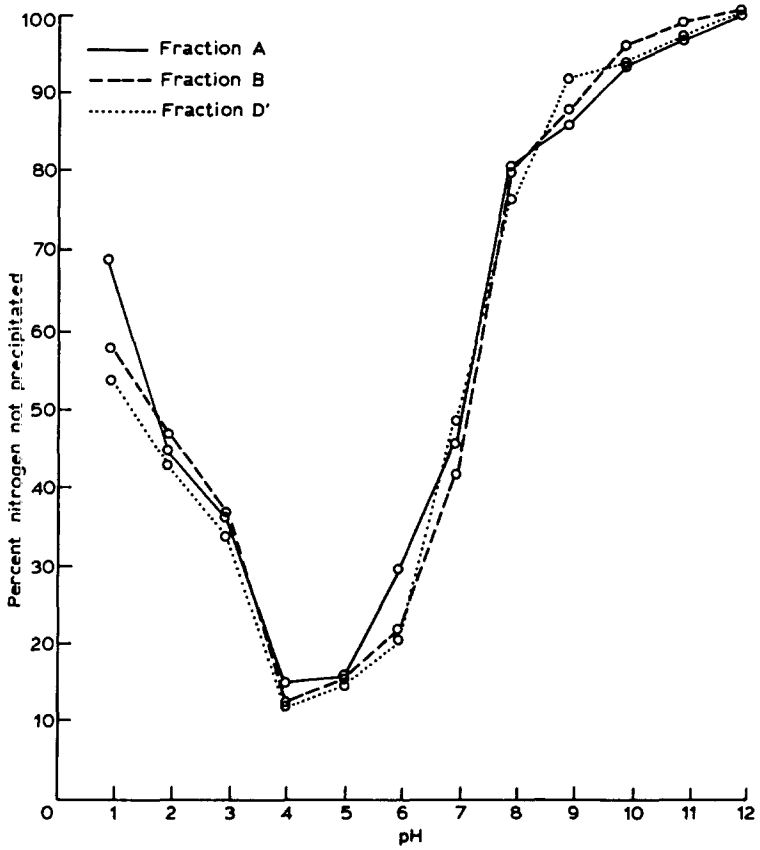

Fig. 4. Percentage nitrogen not precipitated from gossypol-poor fractions at various pH values.

be safe to conclude that the different treatments utilized to prepare these fractions had no effect on nitrogen extractability.

Effect of pH on precipitation

The percentage unprecipitated (soluble) nitrogen (N) at every pH value was determined. Results are given in Fig. 4, from which it can be noted that maximum solubility was in the range of pH 8–12 and maximum precipitation was in the range of 4–5. This is in agreement with the findings of Martinez *et al.* (1970) and Moharram *et al.* (1980).

Nutritional evaluation of gossypol-poor protein isolates

Table 5 gives tryptophan, total and available lysine contents of the gossypol-poor protein isolates. For tryptophan these contents compare favourably with those reported by Lawhorn *et al.* (1974) (1·5 g/16 g N) and by Cater *et al.* (1977) (1·4 g/16 g N). The same authors reported values for lysine

TABLE 5
Tryptophan, Total and Available Lysine Contents of Gossypol-poor Protein Isolates

Amino acid	*Amino acid content as g/16 g N*		
	Fraction (A)	*Fraction (B)*	*Fraction (D')*
Tryptophan	1·34	1·98	1·37
Total lysine	3·88	4·27	3·88
Available lysine	3·42	3·92	3·25
Available lysine/total lysine	0·86	0·92	8·81

in cottonseed flour of 4·0 to 4·2 g/16 g N. Not all lysine is available: the highest availability was in fraction B. The differences between the total and available lysine (which ranges between 0·05 and 0·19%) may be attributed to the reaction between gossypol and the protein (Lyman, 1961).

In-vitro digestibility of cottonseed gossypol-poor protein isolates

The results presented in Table 6 indicate that the digestibilities of the three protein isolates from the fractions are relatively high in most cases and especially for fraction A, which is higher than 90%. The differences in digestibility can be attributed to enzyme specificity, indicating different configurations for the different fractions.

Functional properties of cottonseed protein isolates

The three protein isolates under investigation in this study as well as egg albumin (BDH) were used to evaluate their physicochemical properties.

Foam capacity and stability

The results (Table 7) indicate that foam capacity (as ml of foam formed) of the prepared cottonseed isolates is better at pH 7 than at pH 4. It should be

TABLE 6
In-vitro Digestibility of Gossypol-poor Protein Isolates Compared with Casein

Protein Samples	*% Digestibility as a % of Casein Digestibility*		
	Pepsin	*Pancreatin*	*Pepsin followed by pancreatin*
Fraction (A)	92	94	90
Fraction (B)	89	87	91
Fraction (D')	84	89	89

TABLE 7
Foaming Capacity and Foam Stability of Gossypol-poor Cottonseed Protein Isolates and Albumin

Protein samples	*Foaming capacity (ml) in ½ min*						*Foam stability (ml)*							
							10 min				*2h*			
	pH 4			*pH 7*			*pH 4*		*pH 7*		*pH 4*		*pH 7*	
	Liquid (ml)	*Foam (ml)*	*Total volume*	*Liquid (ml)*	*Foam (ml)*	*Total volume*	*Foam (ml)*	*Decrease in foam volume (%)*	*Foam (ml)*	*Decrease foam volume (%)*	*Foam (ml)*	*Decrease in foam volume (%)*	*Foam (ml)*	*Decrease in foam volume (%)*
Fraction A	41	30	71	36	54	90	17	43·3	37·5	30·6	6	80·0	32	40·7
Fraction B	40	33	73	31	69	100	18	45·4	49·0	30·0	7	78·8	45	34·7
Fraction D′	45	26	71	35	58	93	14·5	44·2	43·0	25·9	11	57·6	39	32·8
Egg albumin	32	63	95	32	61	93	48·0	23·8	41·0	32·8	29	53·9	24	60·0

TABLE 8
Whipping Capacity and Viscosity of Gossypol-Poor Cottonseed Protein Isolates and Albumin

Protein samples	*Increase in volume[a] and viscosity[b] after whipping (%)*									
	6 min without sugar		*8 min without sugar*		*6 min without sugar + 2 min with sugar*		*6 min with sugar*		*8 min with sugar*	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Fraction A	80	3·9	85·5	3·6	96	10·3	105	11·5	107	11·8
Fraction B	114	4·3	125	4·2	112	12·7	120	13·1	130	13·6
Fraction D′	94	4·4	100	4·8	98	12·1	100	13·1	100	13·3
Egg albumin	72	4·7	80	4·5	96	13·9	102	14·3	108	14·4

[a] Whipping capacity as % volume increase.
[b] Viscosity of whip (cp).

noted here that pH 4–5 was found to be their precipitation range while their maximum solubility was at pH 11.

The foaming capacities of these isolates at pH 7 are equal to or, at times (especially that of fraction B), better than that of albumin. Lawhon & Cater (1971) reported values of 64 to 82 ml for glandless cottonseed flour while Satterlee *et al.* (1975) reported good foaming capacity for albumin at a protein level of 5% and pH 4–5. The same Table (7) gives the values of foam stability of these isolates and of albumin at pH 4 and 7 and after standing for 10 min and 2 h. From these results it can be seen that foam stability for the isolates at pH 4 was inferior to albumin. At 10 min the values of percentage decrease in foam volume at pH 7 for fractions A and B are comparable to that of albumin if not very slightly better. The same value for fraction D′ is lower than that of albumin showing a better foam stability than that of albumin. After 2 h at pH 7 all the cottonseed protein isolate foams are much more stable than that of albumin, especially fractions B and D′, though the stability, in general, is somewhat less at 2 h than at 10 min at pH 7. Lawhon & Cater (1971) reported that the foam stability values for four glandless cottonseed protein isolates were, after 10 min and 2 h at pH 4: 11·3 to 32·5 ml and 4·0 to 29·3 ml from an original 64 to 82 ml respectively. These values are much higher than those obtained in the present study in which isolates were utilized. It may be safe to say that the preparation processes involved in obtaining these gossypol-poor cottonseed isolates allowed foam stabilities and foam capacities at pH 7 comparable with or superior to those of egg albumin.

Whipping properties and viscosity

When the isolates' whipping capacity values are compared (Table 8), it can be observed that (in general) fraction A values were almost equal while the values of fraction B and D′ were superior to those of albumin. It can also be seen that the presence of sugar ameliorates the whipping capacity and that 8 min whipping gave slightly higher whipping capacity values than whipping for 6 min only.

The results for viscosity determinations are also given in Table 8. Lawhon *et al.* (1972) reported, for glandless cottonseed flour, viscosities of 11·8 cp after whipping for 6 min without sugar and 15·9 cp if whipping is continued after that in the presence of sugar for another 2 min. The results obtained in this study of the protein isolates are comparable with those of albumin. These results also indicate that the presence of sugar during whipping increases the viscosity though increasing whipping time from 6 to 8 min did not increase the viscosity. The addition of sugar increased the viscosities significantly.

TABLE 9
Water- and Oil-holding Capacities, Emulsion Capacity and Percentage Coagulated Protein of Gossypol-poor Cottonseed Protein Isolates and Albumin

Protein samples	*Water-holding capacity (ml/g)*	*Oil-holding capacity (ml/g)*	*Emulsion capacity (ml oil/100 mg) soluble protein)*	*Calculated protein (%)*
Fraction A	3·40	2·10	25·0	33·3
Fraction B	3·40	2·30	35·2	52·2
Fraction D′	3·25	2·27	27·5	36·6
Egg	3·27	2·30	27·5	33·3

Water- and oil-holding capacities and emulsion capacity

The oil- and water-holding capacities of the cottonseed isolates and albumin (Table 9) exhibit very little or insignificant differences. Childs & Park (1976) reported water- and oil-holding capacities for glandless cottonseed flour of 3·5 and 2·6 ml/g respectively. These values are in agreement with those found in this study for the gossypol-poor protein isolates. Thus the processes of the preparation of these isolates did not affect these properties.

The emulsion capacity values, however, exhibit a different picture. Fractions A and D′ and albumin have almost equal emulsion capacities. Fraction B protein isolates exhibited superior emulsion capacities.

Carpenter & Saffle (1965) stated that the differences exhibited in cottonseed protein isolate emulsification may be attributed to differences in the shape and charge produced in the protein during the different preparation processes. The differences in the results of emulsion capacity and those of water- and oil-holding capacities are yet to be explained.

The percentage coagulated protein for fraction B is much higher than for the corresponding values of all other protein isolates and albumin. Both high coagulable and high soluble proteins have their uses in different food products.

In conclusion the results obtained from this study showed:

1 The possibility of preparing edible gossypol-poor proteins for glanded cottonseed by using a combination of sieving and differential settling processes. The isolates can be used to enrich wheat and corn flours which are deficient in tryptophan and lysine (Altschul, 1958; Mossalem *et al.*, 1979).

2 The prepared gossypol-poor cottonseed protein isolates had good whipping capacities. Their foaming capacities allow their use in topping, chiffon mixes and confectionery products, especially at neutral pH. In emulsion capacity two fractions (A and D') were equal to albumin while the other isolate (fraction B) was even superior to albumin. Thus, all are good potential food emulsifiers. As to their viscosity, results of all isolates did not differ much from albumin. Thus these gossypol-poor cottonseed isolates can be used as thickeners.

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