The functional properties evaluation of Abu-Grab extraction gossypol poor cotton seed protein

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Abstract

In this study the protein fractions were prepared indifferent setting method. This prepared gossypol poor cotton seed protein isolates had good whipping capacities allowed their use in topping chiffon mixes and confectionery products especially at neutral pH. In emulsion capacity two fraction (A and D) were equal to albumin while the other isolates (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 cotton seed isolates can be use as thickeners as well as in pharmaceutical preparation.

تقييم الخصائص الوظيفية لبروتين بذور القطن فقير الكوسيبول نوع ابو غريب

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الخلاصة

في هذه الدراسة حضر الجزء البروتيني من بذور القطن بطريقة استخلاص متعددة الترتيب، حيث جهزت عزلة بروتين بذور القطن الفقير بالكوسيبول. فاظهر سعة فصل عالية مثخن مما يسمح باستعماله في Topping Chiffon mixes وتكييف المنتجات خصوصاً (Fraction B) فكانت أعلى من الألبومين وبهذا أظهرت كل الأجزاء من عزلات البروتين فعل استحلاب غذائي جيد ولم تختلف لزوجه البروتين المعزول كثيراً عن الألبومين. هكذا يمكن أن يستخدم البروتين المعزول من بذور القطن الفقير بالكوسيبول كمثخن إضافة إلى المستحضرات الصيدلانية ومعالجة الخيوط الجراحية وزيادة اللين في الشبكات المستخدمه بالفتوق الجراحية.

Introduction

Cotton plant is one strategic plants of Iraq and some other countries. Cottonseeds have been used in including and biological chain since the ancient civilization. Ground whole seeds or pressed are used as a concentrate for animal ration rich in protein and oil. The biggest problem associated with cottonseed is the toxic pigment gossypol which must be removed before being eaten or used in pharmaceutical products (1,2). Reported that gossypium species contain 1.4 % to 3.4 % total gossypol comprises free and bound gossypol. Only the free gossypol is of concern with regard to toxicity bond gossypol is not toxic. Edible grade cotton seed flour should not contain more than 0.06 % free gossypol (3). The problem associated with protein isolation processes which produce liquid by-products are how to recover or properly precipitated these proteins (4). It is also reported that, the isoelectric point, the solubility of proteins is minimal, because protein are more soluble when combined with bases or acids than in neutral states, they also mentioned that addition of salts increases water solubility of most proteins at the

isoelectric point (5). Altschull (5) showed that proteins can be precipitated by heat, dialysis electro-dialysis, salts, acids, Bases, or organic chemicals (6).

Mattil (6) reported that lowest nitrogen solubility of cottonseed protein is at pH 4.0 (7), Beradi *et. al*. (7) reported that the isoelectric point for water-soluble proteins is at pH 4.0 and that of storage proteins is at pH 7.0 and that a combination of the two groups in the proportion found in the flour minimum solubility at pH 5.0 (8). El. Tinay *et al*. (8) reported that a protein coagulation of 96% could be obtained at pH 4.5 (9). Pant and Tulsiani (1969) reported that treatment of protein fractions with strong acids and bases decrease there nutritive value. This paper concentrates on factors affecting protein and evaluation of function properties of cotton seed protein (Abu-Grab type) obtain from different settling method, as a sutural material which can be used in animal surgery.

Materials and Methods

- **Analitical Methods:**

Moisture,Oil, total Nitrogen,were determined according to methods of the FAO(10).

The crude protein was calculated by multiplying the total nitrogen by the factor 6.25. Treptophan contents was determined by the spectrophotometric method Spies and Chanbers, (11). Total and available lysine were determined according to the method of Carpenter *et al*. (12).

- **Cotton seed protein digestibility estimation:**

Pepsin, Pencreatine and Pepsin followed by Pencreatine digests were prepared by incubation with each extracted protein will the appropriate enzyme according to the method of Akeson and Stahman (13). At the end of the incubation period (7.5 ml) of (1.6M) tricloroacetic acid were added the soluble nitrogen in the supernatant was determined (10) and percentage of digested nitrogen was calculated with respect to total nitrogen in the sample.

- **Physicochemical parameter:**

Foaming capacity and foaming stability were measured according to Iawhon and Carter (14).

- **Whipping capacity of cotton seed protein:**

Whippability measurements were carried out as described by Iawhon and Carter (15) with the following modifications:

a. (3 g) of the dried protein isolates were dispersed in 100 ml of 0.2M citrate phosphate buffer pH (7).
b. The suspension was whipped for (8) min.
c. (75 g) of sucrose were added to 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 viscometer (Mac Michel system macrco. Co. Italy.)

The cotton Seed protein emulsion capacity water-oil-holding capacities and heat-coagulated protein were preformed according to work described by several authors (16, 17, and 18).

Results

- **Nutritional evaluation of gossypol-poor protein isolates:**

The results of Tryptophan, Total and available lysine contents of the gossypol-poor protein isolates (A, B and D') as shown in table (1). The Tryptophan, Total lysine,

available lysine and available lysine, total lysine in Fraction (B) were significantly higher ($P<0.05$) than other fraction. Available lysine were significantly lower ($P<0.05$) than total lysine.

- **In-vitro digestibility of cotton seed gossypol – poor protein isolates:**

The results of the effect of the Pepsin, Pancreatin and Pepsin followed Pancriatin on certain Gossypol poor protein Fraction are shown in table (2). The digestibilities of the protein isolates were significantly higher($P<0.05$) as compared with Casein digestibility. The digestibility of fraction A was significantly higher than other fraction.

- **Foam capacity and stability:**

The results of foam capacity of Gossypol are shown in table (3). The best result of foam capacity at pH 7 and pH 4 as compared to egg Albumin. The foaming capacities of protein isolates was significantly ($P<0.05$) better than the albumin. Where as the result of foam stability at pH 4 and pH 7 after 10min and 2 hr showed the foaming capacity of protein isolates at pH 4 was significantly ($P<0.05$) inferior to albumin at each time (10min and 2hr).

- **Whipping properties and viscosity:**

The whipping capacity results are shown in table (4). There were no significant difference ($P>0.05$) as compared with eggs Albumin where as each fraction B and D' were higher than Albumin. Further more the presence of sugar ameliorates the whipping capacity at (8) min showed a significant difference ($P>0.05$) higher value than at 6min.

The viscosity indicate that the presence of sugar during whipping significantly ($P<0.05$) increases the viscosity though increasing whipping time from 6 to 8 min although no significant difference.

- **Water and oil holding capacities and emulation capacity:**

Table (5) showed water holding capacities of the cottonseed isolates and albumin, there were no significant ($P>0.05$) differences among them the emulsion capacity exhibits fraction B significant ($P<0.05$) superior emulsion capacities where as there were no significant among fraction A, D' and albumin.

Discussion

Table (1) gives tryptophan, total and available lysine contents of the gossypol-poor protein isolates for tryptophan these contents compare favorably with these reported by (19) (1.5g / 16g N) and by (20) (1.4g / 16 gN).

The same authors reported values for lysine in cotton seed flour of (4.2 g) / 16 g N . Not availability was faction B. the difference 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 (21).

The results presented in table (2) 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 then 90%. The difference in digestibility can be attributed to enzyme specificity indicating different configuration for the different fraction.

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

The results (Table 3) indicate that foam capacity (as ml of foam formed) of the prepared cottonseed isolates is better at pH 7 then pH 4 it should be 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 then that of albumin. Iawhon and cater (14) reported values of 64 ml to 82 for gland less cotton seed flour while (22) 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 5% and pH 4-5. The same table 7 and after standing for 10min and 12h from these result it can be seen that foam stability for the isolates at pH 4 was inferior to albumin. At 10min 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 2h at pH 7 at all the cotton seed protein isolate foams are much more stable then that of albumin, especially fractions B and D though the stability in general is some what less at 2h then at 10min at pH 7. (14) reported that the foam stability values for four gland less cotton seed protein isolates were, after 10min and 2h at pH 4: (11.3) to 32.5ml) and 4 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 by safe to say that the preparation processes involved in obtaining these gossypol – poor cotton seed isolates allowed foam stabilities and foam capacities at pH 7 comparable with or superior to these of egg albumin.

When the isolates whipping capacity values are compared (Table 4), 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 whipping capacity values than whipping for 6 min only.

The result of viscosity determination are also given in table (4) the (14) reported for glandless cotton seed 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 result 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 increase viscosity through increasing whipping time from 6 to 8 min did not increase the viscosity. The addition of sugar increased the viscosities significantly.

The water and oil holding capacities of the cotton seed isolates and albumin (Table 5) exhibit very little or no insignificant differences. (17) Reported water and oil holding capacities for cover 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 superior emulsion capacities.

The differences in the shape and charge produced in the protein during the different preparation processes (23). The differences in the results of emulsion capacity and those of water and oil holding capacities are yet to be explained.It was concluded from this study that high coagulable and soluble proteins may be applied in suture material in animal surgery and other operations.

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

Table (1) Tryptophan, total and available lysine contents of gossypol- poor protein Isolates

Amino Acid	Amino Acid content as g 116 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

Table (2) *In-vitro* digestibility of gossypol poor protein isolates compared with casein

Protein Samples			
Fractions	**Pepsin**	**Panceratin**	**Pepsin followed by Panceratin**
A	92	94	90
B	89	87	91
D'	84	89	89

Table (3) Foaming capacity and foam stability of gossypol- poor cotton seed protein isolates and Albumin

Protein samples	Foaming capacity (ml) in 1/2 min						Foam stability (ml) 10 min							
	pH4			pH7			pH4		pH7		pH4		pH7	
Fraction	**Liquid**	**Foam**	**Total Volume**	**Liquid**	**Foam**	**Total Volume**	**Foam**	**Decrease in Foam volume (%)**	**Foam**	**Decrease in Foam volume (%)**	**Foam**	**Decrease in Foam volume (%)**	**Foam**	**Decrease in Foam volume (%)**
A	41	30	71	36	54	90	17	43.3	37.5	30.6	6	80.0	32	40.7
B	40	33	73	31	69	100	18	45.4	49.0	30.0	7	78.8	45	34.7
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 (4) Whipping capacity and viscosity of Gossypol- poor cotton seed protein isolates and albumin

Protein samples	Increase in volume and viscosity a									
	6 min without sugar		8 min without sugar		8 min without sugar + 2 min without sugar		6 min without sugar		8 min without sugar	
	A	B	A	B	A	B	A	B	A	B
A	80	3.9	85.5	3.6	96	10.3	105	11.5	107	11.8
B	114	4.3	125	4.2	112	12.7	120	13.1	130	13.6
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

Table (5) water and holding capacities, emulsion capacity and percentage coagulated protein of gossypol – poor cotton protein isolates and albumin

Protein samples	Water holding capacity (ml/g)	Oil holding capacity (ml/g)	Emulsion capacity ml/100mg soluble protein	Calculated protein (%)
Fraction A	3.40	2.10	25.0	33.3
Fraction B	2.40	2.30	35.2	52.2
Fraction D	3.25	2.27	27.5	36.6
Egg	3.27	2.30	2.30	33.3

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