COTTON-PLANT PROTEIN ISOLATES

T. S. Yunusov and M. T. Turakhozhaev

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The seeds of the cotton plant are an important source of food protein. The presence of gossypol in the seeds limits the use of protein isolates as additives to food products. The use of salt and alkaline solutions for extracting the proteins leads to the formation of isolates with relatively high gossypol constants. Extraction in an acid medium permits the presence of the toxin to be excluded. In this process, a number of problems arise which are connected with the presence of a large amount of phytin (about 5%) in the seeds of the cotton plant. The latter affects both the yield of food protein on extraction and functional properties. A method is proposed for eliminating traces of phytin from an acid isolate. A substantial influence of phytin on the properties of the proteins has been observed in those cases where the latter is strongly bound to the proteins at acid pH values.

The high amount of protein (about 30%) in the seeds of the cotton plant and its relatively high quality in relation to essential amino acids makes the cotton plant an important source of food protein. Consequently, the attention of a large number of workers has been devoted to it, as to a whole series of other plant sources of protein. A number of methods of obtaining isolates from cotton seeds have been proposed which are based on the extraction of the protein in alkaline or acid media or in salt media at neutral pH values. Each method has its advantages and disadvantages, which have led research workers to seek new methods of obtaining protein isolates from the seeds of the cotton plant. The main requirements for these is the production of food protein without the toxic pigment gossypol.

Such methods of eliminating gossypol as the cyclone method [1] and extraction with organic solvents [2] have not come into wide use for a number of reasons [3]. The use of alkaline extraction or extraction with 5-10% solutions of sodium chloride to obtain isolates is limited primarily by the high concentration of gossypol in the proteins obtained. The production of cotton protein isolates in an acid medium is of great advantage in this connection. However, here a number of problems arise that are connected with the presence of phytin, the amount of which in the seeds reaches 5%. The interaction of phytic acid with proteins has been studied, for the case of soybean proteins, for 30 years already [4-6]. It has been shown that phytic acid is strongly bound to the protein at lysine, arginine, histidine residues [5]. Phytin changes the properties of the protein, and this must be taken into account in the production of a protein isolate with improved functional properties. Methods have been proposed for eliminating traces of phytin from proteins by dialysis in an acid medium against calcium ions [5], by dialysis in an acid medium, by treatment of the protein solution with ion-exchange resin, and by precipitation in an alkaline medium [4].

To eliminate phytin from cotton-plant isolates we have used treatment with a 2% solution of hydrochloric acid [7]. Quantitatively the main proteins of cotton seeds, as for many other plants, are the globulins. Consequently, changes in the properties of proteins isolated in the presence of phytin was studied in comparison with a native globulin fraction.

In Fig. 1A, curve 1 shows the marked decrease in the solubility of the native globulins with an increase in the concentration of HCl, which becomes zero at a concentration of 2%. Under these conditions the bond of the phytic acid with the protein is broken, and the former passes into solution and can be separated by filtration. Protein isolates were obtained by extraction in an acid medium from an industrial sample of meal that had been processed by a scheme of prepressing with subsequent extraction by an organic solvent. For comparison we used a native globulin fraction obtained from cotton seed flour defatted under mild conditions. The latter possesses the properties characteristic for the native globulins, which permits the changes in the proteins in the process of preparation and production of the industrial meal to be taken into account. The solubility of

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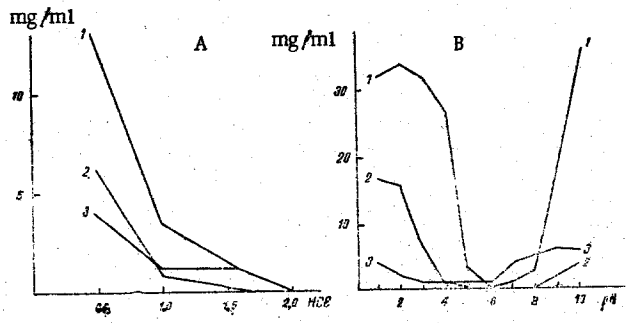

Fig. 1. Dependence of the solubility of the native globulin (1), of a protein isolate obtained by acid extraction (3), and of a fraction (2) obtained by treating the isolate (3) with hydrochloric acid solutions on the concentration of hydrochloric acid (A), and dependence of the solubility of various protein isolates from the cotton plant on the pH (B).

the protein isolate, obtained by our previous method [7] with preliminary extraction of the phytin (Fig. 1A, curve 3), fell considerably with an increase in the concentration of hydrochloric acid and was zero at a concentration of 2%. The nature of the solubility curve (Fig. 1A) shows the identity with respect to protein composition of the isolate obtained by extraction in an acid medium and the native globulin fraction, although this was appreciably denatured in the process of treating the seeds and also in the preparation of the isolate itself. Analysis of the protein isolates 1 and 3 (see Fig. 1A) showed the presence of phytin in both fractions (2.7 and 2.8%, respectively). The elimination of the phytin from fraction 3 (fraction 2) improved its solubility, even though the solubility in 2% acid was again equal to zero. The concentration of phytin in fraction 2 was 0.5%. The different amounts of phytin determined the nature of the solubility of fractions 2 and 3 at various pH values (Fig. 1B). It must be mentioned that fraction 2 was denatured to a greater degree ($h_1/h_2 = 0.44$, see the Experimental part) than fraction 3 ($h_1/h_2 = 0.48$), but both fractions differed considerably from fraction 1 ($h_1/h_2 = 0.62$).

It is impossible to refer to the direct influence of phytin on the solubility of the protein isolates in view of the solubilities of fraction 1 (2.7% of phytin) and 2 (0.5% of phytin) (Fig. 1B). The solubility is apparently affected to a large degree by the irreversible change in the conformation of the proteins under severe conditions of treating cotton seeds, and also by the formation of various protein complexes the presence of which sharply impairs the functional properties of the protein isolates. Treatment with 2% HCl solution changed the form of the solubility curve of fraction 1 (Fig. 1B) and approximated it to the curve of fraction 2. It must also be borne in mind that the similar concentrations of phytin in fractions 1 and 3 affected their solubilities differently. In the case of fraction 3, the solubility deteriorated sharply (as compared with fraction 2), while in fraction 1 the presence of phytin had practically no effect. Furthermore, the solubilities of fractions 3 and 2 at pH 1.0 were higher than at pH 2, while the solubility of fraction 1 fell under the same conditions. Phytin strongly bound to the proteins in an acid medium apparently has a substantial influence on their properties. The formation of various complexes of phytin with the proteins of cotton seeds and their use for obtaining food proteins with improved functional properties will be considered in subsequent publications.

EXPERIMENTAL

Fraction 1. A suspension of 10 g of defatted cottonseed flour in 100 ml of 10% sodium chloride solution, pH 7.4, was centrifuged at 6000 rpm for 10 min. The supernatant liquid was dialyzed against tap water for two days. The dialysate was centrifuged at 3000 rpm and +4°C for 30 min. The resulting precipitate was washed three times with distilled water (+4°C). The total native globulin fraction was freeze-dried.

Fraction 3. The phytin, the water-soluble carbohydrates, and the native pigments were extracted from a comminuted industrial meal containing 15% of husks. The proteins were obtained from the moist insoluble residue as described previously [7]. The proteins were freeze-dried.

Fraction 2. This was obtained by washing fraction 3 twice with 2% HCl solution. The solid matter was separated off by centrifugation at 8000 rpm, +25°C, for 10 min and was dissolved in distilled water, and the solution was neutralized to pH 6. The precipitate was deposited was separated off by centrifugation and was freeze-dried.

Solubility was studied by stirring a solution of the protein at the appropriate pH value or concentration of acid for 15 min. Then the suspension was centrifuged at 8000 rpm for 10 min. An aliquot was taken from the supernatant liquid and its protein content was determined by the biuret method.

The degrees of denaturation of the separated fractions were determined by a polarographic method on a LP-7 polarograph [8].

Phytin contents were determined as described by Tevekelov [9].

SUMMARY

1. It has been shown that protein isolates of the cotton plant obtained by extraction in an acid medium contain mainly globulins.

2. It has been established that the presence of phytin, which is strongly bound to proteins in an acid medium, affects the properties of the globulins.

LITERATURE CITED

1. US Patent No. 3,615,657 (1971).
2. US Patent No. 3,124,461 (1964).
3. E. W. Lucas, J. T. Jawhon, K. C. Rhee, and R. G. Simmons, J. Am. Oil Chemists' Soc., 58, No. 7, 581 (1981).
4. A. K. Smith and J. J. Rackis, J. Am. Chem.,Soc., 79, 633 (1957).
5. K. Okubo, D. V. Myers, and G. A. Jacobucci, Cereal Chem., 53, 513 (1976).
6. R. Barre, J. E. Courtois, P. Delrien, and R. Peries, Ann. Pharm. Fr., 12, 601 (1954).
7. T. S. Yunusov, M. T. Turakhozhaev, E. G. Yadgarov, and T. T. Shakirov, Khim. Prir. Soedin., 473 (1981).
8. S. I. Asatov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 800 (1980).
9. D. Tevekelov, Izv. Inst. Khranene Bolg. Akad. Nauk 7, 21 (1968).

ONE-STAGE SYNTHESIS OF INDANONES IN THE BENZIMIDAZOLIN-2-ONE SERIES

Ch. Sh. Kadyrov and S. S. Khalikov

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The possibility has been shown of a one-stage synthesis of 5,6-ureylene-3-methylindan-1-one and 6,7-ureylene-3,5-dimethylindan-1-one by condensing the corresponding benzimidazolin-2-ones with γ-butyrolactone or crotonic acid in the presence of aluminum chloride. The reaction was performed at a ratio of the reactants benzimidazolin-2-one (5-methylbenzimidazolin-2-one):γ-butyrolactone (crotonic acid):$AlCl_3$ = 1:1:6. Several derivatives (oximes, semicarbazones, substituted hydrazones) have been obtained that confirm the ketonic structures of the indanones synthesized. The plant growth inhibiting and fungicidal activities of the compounds synthesized have been studied.

Recently, various natural indanones possessing biological activity have been isolated from plant objects [1]. At the same time, polycyclic indanones exhibit the properties of plant growth inhibitors [2], and such ketone derivatives as semicarbazones, thiosemicarbazones, and hydrazones possess fungicidal activity [3]. We have previously synthesized 5,6-ureylene-3-methylindan-1-one (V) and 6,7-ureylene-3,5-dimethylindan-1-one (VI) by the cyclization of the corresponding 5-(2)carboxy-1-methylethyl)- and 5-(2-carboxy-1-methylethyl)-6-methylbenzimidazolin-2-ones (III) and (IV) in concentrated sulfuric acid [4].

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