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SOLUBILITY OF THE PROTEINS AND PHYTATES OF COTTONSEED MEAL IN THE PRESENCE OF METAL IONS

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The solubility of the proteins and phytates of cotton seeds in the presence of metal ions (Ca^{2+}, Fe^{3+}, Zn^{2+}) at various pH values of the medium has been studied. It has been shown that complete precipitation of the protein by phytin from solution ($C_{protein}$) = 1.5%), sets in at a mass ratio of phytin to protein of 1:15, pH 1.5.

On the titration of an extract of phytin (C = 5.7 mg/ml) by Ca^{2+} ions, insoluble complexes are formed at pH 3.0-5.0, while with Zn^{2+} ions they are formed at pH 4.0-5.2. It is known that the mechanism of the interaction of proteins with phytates may vary and depends on the pH of the medium and also on the amount of metal ions and phytates in the system [1].

As has been shown for soybean proteins [2], phytins are not precipitated in the presence of Ca^{2+} and Zn^{2+} ions at pH values below 4.0, regardless of the Ca^{2+}:phytate ratio. The addition of phytates to solutions of a protein shifts the point of maximum precipitation into the region of low pH values [2, 3].

The problem of isolating proteins from various plant sources without such antinutritional components as phytates has not yet been solved, since the mechanism of the interaction of proteins with phytates is not clear. Fontaine et al. [4] have shown for the first time that the solubility of proteins (soybean, peanut, cottonseed) and phytates changes as a function of the pH of the medium. It was established experimentally that for all the proteins studied, a pH range exists (from 1.5 to 3.5) in which the solubility profiles of the proteins and the phytates do not coincide. These authors formulated the hypothesis that in this pH interval there is a formation of protein–phytate complexes which break down at the isoelectric point of the protein. It was later established that complexes of phytates with Ca^{2+} and Mg^{2+} ions are soluble in acid pH ranges (below 5.0) and are insoluble at pH values above 6.0 [5].

We have shown previously [3] how the solubility of cottonseed proteins changes in the presence of phytates.

Fontaine [4] determined regions of maximum solubility both for cottonseed proteins (pH 1.5-2.0 and 8.0-10.0) and for phytates (pH 5.0). On the basis of Fontaine's results, a technology has been developed for the isolation of protein from cottonseed meal by a two-stage extraction method with elimination of the phytates (pH 5.0) in the first stage [6]. By modeling the process of the one-stage (pH 1.5-2.0) extraction of protein from cottonseed meal by the method of turbidimetric titration, we have shown that the total precipitation of proteins by phytin from an extract ($C_{protein}$) = 1.5%) sets in at a mass ratio of phytin to protein of 1:15.

In the present paper we give information on the influence of metal ion on the extractability of the proteins from prepressed cottonseed meal (GOST [State Standard] 606-75) in the presence of phytates.

In a recent investigation of soybean proteins, the possibility has been shown of extracting the protein in the presence of phytates and of calcium ions [2]. In view of the fact that the phytate content of cotton seeds is several times higher than that of soybeans, we have undertaken an analogous investigation of the possibility of isolating proteins from cotton seeds in the presence of phytates.

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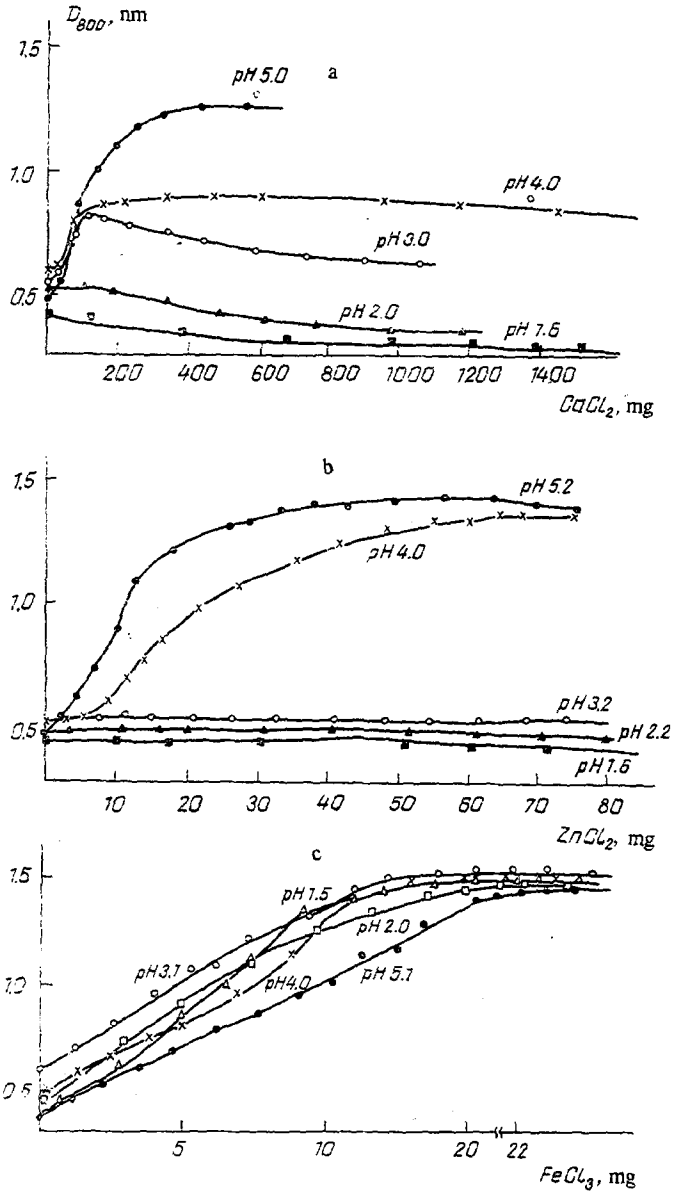

Fig. 1. Solubility of phytates from cottonseed meal at various pH values in the presence of calcium (a), zinc (b), and iron (c) ions.

It was necessary in the first place to study the solubility of phytates isolated from cottonseed meal in the presence of metal ions (Ca^{2+}, Zn^{2+}, and Fe^{3+}) at various pH values of the medium. The solubilities of all the samples were studied by turbidimetric titration. Titration was conducted in 15 ml of a phytate extract (C = 5.7 mg/ml). The solubility of the phytates was investigated in the pH range of 1.6-5.0, i.e., at those pH values where the maximum extractability of the proteins and phytates in two-stage extraction from cottonseed meal is observed [4].

At low pH values (1.6-2.0), the addition of calcium chloride to a solution of phytates (C = 5.7 mg/ml) even in a mass ratio of phytate to $CaCl_2$ of 1:15 did not lead to a worsening of its solubility. At an initial pH of 3.0, the maximum turbidity of the phytate solution on titration was observed at a mass ratio of phytate to $CaCl_2$ of 1:15, while at pH 4.0 it was 1:18, and at pH 5.0 it was 1:3.0 (Fig. 1a).

In the presence of zinc chloride, the maximum turbidity of solutions of phytates was observed on the addition of smaller amounts of the salt: at a mass ratio of phytate to $ZnCl_2$ of 3:1 at pH 5.2, and 2.6:1 at pH 4.0, while with a further lowering of the pH from 3.2 to 1.6 the addition of zinc chloride in any proportions did not lead to their precipitation (Fig. 1b).

TABLE 1. Yields of Protein and Phytin in the Presence of Calcium Ions

Experiment	Amount of $CaCl_2$ added, %	Yield of protein, %	Yield of phytin, %
1	0.05	0.2	1.7
2	0.1	0.3	2.0
3	0.2	0.4	1.9
4	0.5	0.6	2.3
5	0.8	1.0	2.4
6	1.0	1.0	2.1
7	1.5	1.0	2.5
8	1.5	0.6	-
9	Control*	10.0	5.5

*As control we used protein obtained by the two-stage extraction method, i.e., after the preliminary separation of the phytates at pH 5.0.

The titration of solutions of phytates in the presence of ferric chloride led to their precipitation at lower concentrations of metal ions: at a mass ratio of phytate to $FeCl_3$ of 5:1 at pH 5.1, 6.6:1 at pH 4.0, 7:1 at pH 3.1, and 7:1 at pH 1.5. As can be seen from the titration curves (Fig. 1c), the phytates were precipitated from solution at all the pH values studied, i.e., no formation of soluble complexes was observed.

The titration curves of solutions of the phytates in the presence of various concentrations of calcium ions showed that insoluble complexes of phytates with calcium ions were formed in the pH interval of 3.0-5.0, while in the case of zinc ions such complexes were formed at pH 4.0-5.0. Soluble complexes were formed at other pH values, regardless of the ratio of phytate to metal ion (see Fig. 1).

We did not succeed in selecting conditions for the isolation of protein in high yield and with a low phytate content by varying the conditions of isolating the protein from cottonseed meal in the presence of calcium ions while omitting the stage of eliminating phytates (5.0). We assumed that the addition of calcium ions at the moment when the protein and phytate are present in solution would lead predominantly to the binding of the phytates with the formation of insoluble complexes and the subsequent isolation of the protein in high yield. As experiment showed, the addition of calcium chloride in concentrations of from 0.05 to 1.5% did not lead to an increase in the yield of protein on one-stage extraction as compared with the control (<10%). Table 1 shows the change in the yield of protein and of phytin in the presence of calcium ions.

We used two variants of one-stage extraction: calcium chloride was added 1) after the extraction of the phytin, and 2) after the pH had been brought to 1.5-2.0, i.e., under the conditions for isolating the protein.

In the first case (Table 1, experiments 1-7), the calcium chloride was added after the extraction of the phytin at pH 5.0 for 30 min without separating the extracts from the pulp, and then the pH was lowered to 1.5-2.0 and the extracting of the protein was continued for another 30-40 min. The resulting extracts were separated from the pulp and stagewise precipitation of the protein at 4.5-5.0 and of the phytates at pH 8.0 was carried out. The nitrogen content of the samples precipitated at pH 4.5-5.0, i.e., under the conditions for the precipitation of the protein, was only 2.42% from which the amount of protein was calculated to be 15.1%. This shows that the point of precipitation of the phytates in the presence of Ca^{2+} and of the protein had shifted into the acid region. The yield of phytates in the presence of protein at pH 8.0 also fell sharply, to 1.7-2.5%.

In the second case (experiment 8), the addition of calcium chloride (1.5%) in the extraction of the protein (pH 1.5-2.0) without the preliminary extraction of phytates at pH 5.0 lowered the yield of protein to 0.6%. The yield of protein in the samples precipitated at pH 4.5-5.0, determined from their nitrogen content, showed that it is not possible to isolate protein in high yield from cottonseed meal in the presence of calcium ions without the preliminary elimination of the phytates. Conversely, from soybean flour which contains 3-4 times less phytates than cotton seeds it is possible to obtain protein with a high yield without the elimination of the phytates [2].

Thus, the solubility of phytates in the presence of the metal ions Ca^{2+}, Zn^{2+}, and Fe^{3+} has been studied at various pH values (1.6-5.0).

On the titration of a phytin extract (C = 5.7 mg/ml) by calcium ions, insoluble complexes are formed in the pH interval of 3.0-5.0, and with zinc ions in the pH interval of 4.0-5.2.

EXPERIMENTAL

Turbidimetric titration was carried out as described previously [3].

The concentration of protein in solution was determined by the micro biuret method, as in [7]. The total nitrogen content and the phosphorus content were determined by standard methods [8 and 9, respectively].

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