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PROTEIN HYDROLYSATES OF CASTOR BEAN SEED AND COTTONSEED MEALS

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The processes involved in the hydrolysis of the proteins of the toxic seed meals of the castor-oil plant and the cotton plant with hydrochloric acid have been studied. The optimum conditions of hydrolysis, at which the yield of desired product amounts to about 40%, have been established. The use of activated carbon and the cation-exchange resin KU-2×8 have enabled a hydrolysate containing 80—85% of amino acids to be obtained.

The shortage of protein for the production of full-value balanced feeds for agricultural animals is becoming a problem of using nontraditional sources of raw materials. These include oil cakes and meals obtained in the processing of the seeds of the castor-oil plant and the cotton plant.

It is known that the seeds of the castor-oil plant and the cotton plant contain 40—45% of proteins. Current methods of extraction permit 30—35% of proteins to be extracted from them and far less from industrial meals [1—3]. Here the main task is the maximum extraction of proteins and also their freeing from antinutritional substances, such as the toxic protein ricin, the allergens of the castor-oil plant, and the gossypol of cotton seeds [4—6].

One of the methods of detoxifying meals is the production of acid hydrolysates. Hydrolysis carried out with hydrochloric acid has a number of advantages over alkaline hydrolysis, thanks to the absence of racemization of amino acids and the comparative rapidity of the process. In addition, it is possible to avoid a demineralization stage by eliminating the acid under vacuum. In the hydrolysis process the proteins are cleaved into individual amino acids and peptides. The efficiency of the process is determined by the increase in amine nitrogen. This index is a measure of the splitting of protein.

The task of the present work was a study of the acid hydrolysis of castor bean seed and cotton seed meals at various temperatures and concentrations of hydrochloric acid. Factory meals were ground and passed through a No. 2 sieve. Hydrolysis was conducted in sealed tubes, using 3 and 6 N HCl at 60, 85, and 105°C. Samples were taken after 2, 4, 6, 8, 10, and 24 h from the beginning of hydrolysis, and their amine nitrogen contents were determined by the method of Matveev et al. [7].

For the quantitative evaluation of amine nitrogen we used a calibration graph of the formol titration of glycine solution with known concentrations, from which it followed that 1 ml of 0.1 N NaOH was consumed in the titration of 0.0015 g of nitrogen. Knowing the consumption of alkali we calculated the nitrogen content in the light of its maximum amount as calculated from the amino acid composition. For this, we calculated the nitrogen content from the molecular mass of each amino acid and then summed the result. The nitrogen calculated in this way represents the maximum amounts of amine nitrogen (4.25 and 6%) and is used in calculations based on formol titration. The ratio of amine nitrogen to total protein nitrogen, expressed as a percentage, is an index of the depth of hydrolysis of the meal proteins (Tables 1 and 2). As can be seen from the tables, hydrolysis took place most intensively in the first 10 h, after which the depth of hydrolysis amounted to 92% for the castor bean seed meal and to 82% for the cottonseed meal, which shows fairly complete hydrolysis under these conditions. In the following hours a slower rise in this index was observed, and the highest degree of splitting was reached after 24 h. At lower temperatures and acid concentrations, the depth of hydrolysis was less (Fig. 1). The results obtained permit us to consider as the optimum condition for the hydrolysis of the castor-oil plant and cotton plant proteins the use of 6 N HCl at 105°C for 10—12 h.

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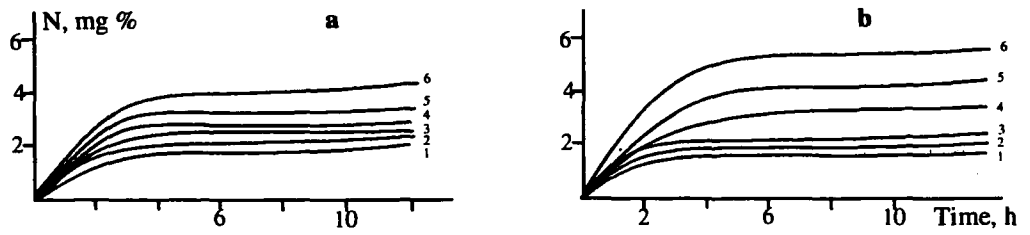

Fig. 1. Dynamics of the accumulation of amine nitrogen in hydrolysates of cottonseed meal (*a*) and castor bean seed meal (*b*) under various hydrolysis regimes:
1) 3 N HCl, 60°C; *2*) 6 N HCl, 60°C; *3*) 3 N HCl, 85°C; *4*) 6 N HCl, 85°C; *5*) 3 N HCl, 105°C; 6) 6 N HCl, 105°C.

TABLE 1. Dependence of the Accumulation of Amine Nitrogen in a Hydrolysate of Castor Bean Seed Meal on the Concentration of Acid and the Temperature and Time of Hydrolysis

Time, h	60°C						85°C						105°C					
	NaOH, ml		N-am., %		Depth of hydr., %		NaOH, ml		N-am., %		Depth of hydr., %		NaOH, ml		N-am., %		Depth of hydr., %	
	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N
2	1.3	2.8	0.5	1.1	12.0	26.0	2.6	3.3	1.0	1.4	23.5	33.0	4.0	5.3	1.5	2.0	35.3	47.0
4	2.7	3.3	1.0	1.3	23.5	31.0	3.9	4.7	1.5	2.0	35.5	47.0	5.4	6.6	2.0	2.5	47.0	58.8
6	2.9	4.2	1.1	1.6	26.0	37.6	4.6	5.4	1.7	2.2	40.0	52.0	6.4	8.6	2.4	3.3	56.5	77.6
8	3.3	4.7	1.3	1.8	31.0	42.3	5.3	6.0	2.0	2.5	47.3	59.0	8.0	9.3	3.0	3.5	71.0	82.0
10	4.0	5.3	1.5	2.0	35.3	47.0	5.4	6.7	2.0	2.8	47.7	66.0	8.7	10.3	3.2	3.9	75.0	91.8
24	5.2	6.0	2.0	2.3	47.0	54.0	6.3	7.2	2.4	3.0	56.0	71.0	9.4	11.3	3.5	4.3	82.0	100

TABLE 2. Dependence of the Accumulation of Amine Nitrogen in a Hydrolysate of Cottonseed Meal on the Concentration of Acid and the Temperature and Time of Hydrolysis

Time, h	60°C						85°C						105°C					
	NaOH, ml		N-am., %		Depth of hydr., %		NaOH, ml		N-am., %		Depth of hydr., %		NaOH, ml		N-am., %		Depth of hydr., %	
	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N	3N	6N
2	2.7	3.3	1.2	1.4	20.0	23.3	3.3	4.0	1.3	1.5	21.1	25.0	5.0	5.4	1.8	2.0	30.3	33.3
4	4.0	4.7	1.6	2.2	26.6	36.7	5.3	6.7	2.0	2.6	33.3	43.3	8.0	8.1	2.9	3.0	48.3	50.8
6	5.2	5.3	2.0	2.3	33.3	38.3	6.7	7.3	2.5	2.9	41.7	48.3	8.7	10.3	3.2	3.9	53.3	65.0
8	5.7	6.6	2.1	2.6	35.0	43.3	7.4	8.7	2.8	3.4	46.7	56.7	9.2	11.3	3.4	4.2	56.7	70.0
10	6.7	6.8	2.3	2.7	38.3	45.0	8.0	9.0	3.0	3.5	50.0	58.3	10.7	13.2	4.0	4.9	66.7	82.0
24	7.0	7.2	2.6	2.8	43.3	46.7	8.7	9.4	3.3	3.7	55.0	61.7	13.0	16.0	4.8	6.0	80.0	100

We then carried out a preparative work-up of the hydrolysates in accordance with the optimum conditions found. One part of the hydrolysates obtained was clarified with activated carbon and another was freed from carbohydrates and other nonnitrogenous components on a column with the cation-exchange resin KU-2×8 and was evaporated in a rotary evaporator. The amounts of amino acids, carbohydrates [8], and gossypol [9] in the hydrolysates were determined (Table 3). As can be seen from the table, the total amount of amino acids in the meal hydrolysates was 40—50%, and the amount of carbohydrates 8—10%.

As a result of purification the level of amino acids rose to 60—80%. A study of the amino acid composition showed the presence of practically all the amino acids present in the meal proteins, with the exception of tyrosine and phenylalanine, the low level of which was due to their absorption on the activated carbon. The use of the cation-exchange resin permitted the gossypol, carbohydrates, and other nonnitrogenous compounds to be eliminated, and it also prevented a loss of aromatic amino acids.

Thus, the hydrolysis of castor bean seed and cottonseed proteins has been studied. It has been established that it is desirable to perform hydrolysis in 6 N HCl at 105°C for 10—12 h. The use of activated carbon and the cation-exchange resin KU-2×8 to purify the hydrolysates enables a product containing a total of 63—82% of amino acids to be obtained. The yield amounts to 30—35%, which is considerably higher than in the known methods of extracting proteins from the meals studied.

TABLE 3. Characteristics of the Castor Bean Seed and the Cottonseed Proteins

Amino acid	Castor bean seed meal				Cottonseed meal			
	Initial meal, %	Protein nitrogen, %	Purification		Initial meal, %	Protein nitrogen, %	Purification	
			Act. C	KU-2×8			Act. C	KU-2×8
Asp	4.90	0.40	7.15	6.30	6.07	0.64	7.34	8.25
Thr	1.34	0.14	2.80	3.20	2.01	0.24	2.52	2.82
Ser	2.71	0.30	3.50	5.80	2.64	0.36	3.50	4.00
Glu	9.41	0.73	13.10	17.77	13.16	1.26	18.42	18.27
Pro	1.63	0.20	3.21	3.52	2.20	0.32	3.20	4.50
Gly	2.13	0.21	4.50	5.85	2.40	0.49	3.52	4.32
Ala	2.15	031	4.33	5.58	2.26	0.36	3.11	4.00
Val	2.22	0.26	3.41	4.18	2.16	0.26	3.32	3.98
Met	0.62	0.08	1.00	1.10	0.60	0.06	0.64	0.80
Ile	1.75	0.21	2.18	2.49	1.61	0.17	1.92	2.81
Leu	2.50	0.25	5.12	6.24	3.31	0.36	4.32	5.83
Tyr	1.31	0.20	0.68	1.92	1.79	0.39	0.80	2.10
Phe	1.51	0.21	0.58	2.27	2.95	0.26	0.96	2.90
His	0.84	0.09	2.46	2.95	1.46	0.14	2.16	4.00
Lys	1.30	0.08	2.40	2.57	2.05	0.22	3.04	3.18
Arg	4.14	0.33	5.51	10.26	5.64	0.41	6.12	9.97
Total amino acid content, %	40.90	4.25	63.68	82.69	52.11	6.00	63.09	81.73
Carbohydrates, %	8.60		0.60	0.09	9.60		0.50	0.10
Gossypol, %					1.34		0.11	Tr.
Yield of hydrolysate, % on the meal	40.00		30.00	35.00	45.00		30.00	35.0

EXPERIMENTAL

Determination of the Amino Acid Composition. The meals were ground and passed through a No. 2 sieve. Weighed samples of the meals were hydrolyzed in sealed tubes with 6 N HCl at 105—110°C for 24 h. Amino acid compositions were determined on an amino acid analyzer by a standard program.

Determination of Amine Nitrogen. The dynamics of the accumulation of amine nitrogen under isothermal conditions was studied at 60, 85, and 105°C with hydrochloric acid concentrations of 3 and 6 N by the method of Matveev et al. [7]. For the first two temperatures the reaction mixtures (25 g of meal in 100 ml of HCl) were prepared in 250-ml round-bottomed flasks with ground-in stoppers, and hydrolysis was conducted in a thermostat. Samples (4 ml each) were taken 2, 4, 6, 8, and 10 h from the beginning of the process and, after they had been cooled to room temperature, their nitrogen contents were determined by titration.

The dynamics of the process at 105°C was studied in the following way. Weighed samples of the meals (1 g in 4 ml of HCl) were introduced into tubes which were then sealed and placed in a thermostat. After the lapse of a predetermined time, they

were removed and cooled, and their amine nitrogen contents were determined.

To determine the maximum amine nitrogen content achievable under these conditions, hydrolysis was continued for 24 h. Amine nitrogen was determined in the following way. An aliquot (4 ml) of the suspension was centrifuged, and 1 ml was taken for titration. After the addition of a few drops of phenolphthalein, the hydrolysate was neutralized to the appearance of a coloration (pH 6.8—7.2 at room temperature). The solution was treated with 1 ml of a 30—40% solution of formaldehyde, whereupon the coloration disappeared. Titration was conducted with 0.1 N NaOH until the coloration reappeared (pH 8.8—9.5).

For quantitative calculations we used the results on the consumption of alkali in the titration of a glycine solution (1 ml per 0.0080 g of glycine or 0.0015 g of nitrogen). To determine the dynamics of the accumulation of amine nitrogen, the consumption of alkali in the titration of a 24-h hydrolysate (11.3 ml) was taken as the maximum. The other results were calculated from their ratio to the maximum consumption of alkali.

Determination of Carbohydrates. The carbohydrate contents of the hydrolysate were calculated by a published method [8]. To prepare the orcin reagent we dissolved 0.2 g of orcin in 5 ml of distilled water. A 20-mg sample of the hydrolysate was dissolved in 0.5 ml of water, and 0.5 ml of the orcin solution and, with cooling, 4 ml of conc. H_2SO_4 were added. The mixture was incubated in a water bath at 80°C for 1 min and was then cooled, and its absorption at 105 nm was measured on an SF-26 spectrophotometer.

The carbohydrate content was determined from a calibration graph obtained under the same conditions for a standard solution of glucose (0.2 mg of glucose gives an absorption of 1.0 at 505 nm). An absorption of 0.6 corresponds to 0.6% of carbohydrates in the castor-oil seed meal hydrolysate.

Determination of Total Gossypol by the Aniline Method.

Samples of the cottonseed meal and hydrolysate (2 g in each case) were introduced into 25-ml flasks with ground-in stoppers, 2 ml of 72% alcohol was added to each, and the mixture was left at room temperature for 10 min. Then 2 ml of freshly distilled aniline was added and the solution was left in a water bath at 60°C for 45 min. After this, it was cooled, 15 ml of chloroform was added, and the resulting mixture was shaken for 15 min and was filtered into a 25-ml flask and made up to the mark. Then 2 ml of the filtrate was transferred to a 25-ml flask and was diluted to the mark with chloroform, and the optical density at 470 nm was measured on a SF-26 spectrophotometer. The gossypol content was found from a calibration graph obtained by the method described above for gossypol of known concentration [9]. (An absorption of 0.31 corresponds to 0.087 g of gossypol). The gossypol content of the meal was 1.34%, and of the hydrolysate 0.11%.

Ion-exchange Chromatography on the Cation-exchange Resin KU-2×8. KU-2×8 Resin (200 g) was washed with distilled water on a Büchner funnel and was suspended and kept in 1 N NaOH for 30 min. Then it was washed with water to the neutral pH and was suspended and kept in 1 N HCl for 30 min and was again washed with water to the neutral pH.

A 3 × 50-cm column was filled with the cation-exchange resin KU-2×8, and the hydrolysate obtained from 100 g of meal was added. After elution with three volumes of water to eliminate neutral nonitrogenous substances, the amino acids and peptides were eluted with 1% ammonia, and the ninhydrin-positive fraction was collected. It was then evaporated in a rotary evaporator and its amino acid composition was determined. To reveal the ninhydrin-positive substances, the eluate was deposited on chromatographic paper, and, after drying, the spots were revealed with a 0.3% solution of ninhydrin in acetone at 80—100°C for 5—10 min. The amino acids gave a violet coloration.

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