USE OF THE PLASTEIN REACTION FOR THE MODIFICATION OF COTTON-PLANT PROTEINS

N. L. Ovchinnikova, M. A. Kuchenkova, and P. Kh. Yuldashev

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Pronase hydrolysates have been obtained from cottonseed meal which were then used as a substrate for the performance of the plastein reaction. The possibility has been shown of introducing deficient amino acids – lysine methionine, threonine, and tryptophan – by means of this reaction.

One of the possible methods for expanding the raw materials basis of the food industry is the utilization of nontraditional sources of protein after they have been freed from undesirable components and enriched with deficient amino acids. In this connection, the application of the plastein reaction to proteins of plant origin is of interest [1].

The wide use of cottonseed proteins in food is held back by the presence of a toxic component – gossypol – and also by a deficiency of such essential amino acids as lysine, threonine, methionine, and tryptophan.

Our task was to obtain from cottonseed meal a protein concentrate balanced in relation to essential amino acids and free from gossypol. With this aim, the meal was extracted with a 0.27% solution of ammonia and, as described previously [2], the total alkaline protein fraction was isolated. The latter* and also the meal itself, directly, without the isolation

TABLE 1. Chemical Characteristics of Pronase Hydrolysates of Cottonseed Meal

Amino acid	Protein hydrolysate			Meal hydrolysate		
	before chromatogr. on polyamide	peak I after chromatogr. on polyamide	peak II after chromatog. on polyamide	before chromatogr. on polyamide	peak I after chromatogr. on polyamide	peak II after chromatogr. on polyamide
Aspartic acid	4,8	7,03	1,89	4,27	7.14	5.17
Threonine	5.1	2,6	0,83	1.4	2,66	1.88
Serine	1,9	3.24	0,98	0,917	2,6	1,3
Glutamic acid	9,15	19,4	5,08	12,4	15.0	10,3
Proline	1,58	4.02	1,81	1,365	—	1.6
Lysine	1,5	2.57	0,86	1.916	2,6	2,45
Alanine	1,73	3,8	0.87	2.185	3,2	2,3
Valine	2.6	5,0	1.5	2 43	3,8	3,1
Methionine	—	0,74	0,25	0,37	—	0,65
Isoleucine	1,5	2,9	0,85	1,3	2.26	1,67
Leucine	2,1	6,29	1,82	2.6	4,7	3 5
Tyrosine	0,78	3,46	1,18	0,5	—	0,45
Phenylalanine	2,87	5.6	3,38	2,185	2,5	2,07
Histidine	—	—	—	—	4,4	2,27
Lysine	2,04	3,45	1,78	7,1	7,3	7,3
Arginine	3,24	8,2	7,1	1,96	3,96	1,64
E_i, %	37,3	78,12	32,2	42,9	62,12	47,6
Yield, %	33	65	20	30	35	30
N, %	14,35	18 24	15,3	9,74	14,5	10,9
Gossypol, total, %	0,7—0,9	0	0	0,6—0,8	0	—
Gossypol, free, %	0,62	0	0	0,2	0,012	—

*Below, in the text we shall call this hydrolysate the protein hydrolysate, in contrast to the meal hydrolysate.

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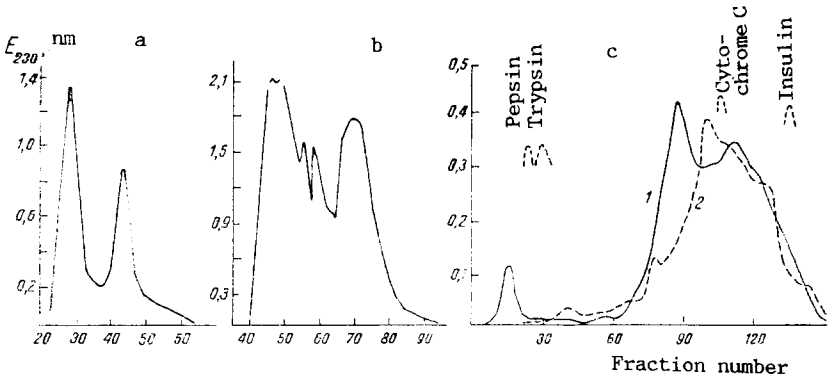

Fig. 1. Chromatography of pronase hydrolysates of the protein (a) and of the meal (b) on polyamide, and gel filtration of the pronase hydrolysates of the protein (1) and the meal (2) on Sephadex G-50.

of proteins from it, were then subjected to enzymatic hydrolysis with pronase E in 0.2 M ammonium bicarbonate for 45 h. Then the pronase hydrolysates were purified by chromatography on Woelm polyamide (Pharma) and Kapron [polycaproamide] (Fig. 1a, b).

The chemical characteristics of the pronase hydrolysates and their purified fractions were obtained (Table 1).

To evaluate the molecular-weight distribution of the products of pronase hydrolysis, the hydrolysates were chromatographed in 10% NaCl on a column of Sephadex G-50 (medium) that had previously been calibrated with standard proteins (pepsin, trypsin, cytochrome C, insulin) (Fig. 1c).

As a result of the pronase hydrolysis both of the protein and of the meal, in the main, mixtures of peptides with mean molecular weights of 1000-1500 (9- to 12-membered peptides) were formed which were then used as the substrates for the plastein reaction. The latter was performed by adding to the purified pronase hydrolysate, separately, the ethyl esters of L-Trp, DL-Met, L-Thr, and N^{ε}-Ac-L-Lys in a ratio of 10:1. In each case, the plastein reaction was performed in a 0.01 M solution of L-cysteine under the action of papain at an enzyme-substrate ratio of 1:30 (pH 6.0, 37°C, for 48 h). After the completion of the reaction, the incubation mixture was diluted with a tenfold excess of 0.1 M NaOH in 50% ethanol and was kept at room temperature for 6 h. Then it was neutralized with dilute HCl, which led to the precipitation of the plastein. After centrifugation this was freeze-dried.

The characteristics of the plasteins obtained are given in Table 2.

Thus, as the result of the plastein reaction, it was possible to obtain from cottonseed meal protein concentrates (plasteins) with increased amounts of the deficient amino acids: Lys by a factor of 1.7 (1.44), Met 5.6 (4), Thr 3.7 (4), Trp 1.8 (1.5).

EXPERIMENTAL

The isolation of the protein from the cottonseed meal was performed in accordance with [2]. The enzymatic hydrolysis of the protein and the meal were carried out with pronase E (Merck) in a 0.2 M solution of ammonium bicarbonate, pH 7.4-7.5 (the pH was brought to the required value by the addition of 0.1 N HCl) at an enzyme–substrate ratio of 1:100 (1:50 in the case of the meal) at a temperature of 37°C for 45 h. After the end of hydrolysis, the reaction mixture was centrifuged, acidified with 1 N HCl to pH 6.0, and freeze-dried. The yield of the protein hydrolysate was 33% and that of the meal hydrolysate 30%.

Purification of the Hydrolysates. The chromatography of 850 mg of the pronase hydrolysate was performed on a column (5 × 50 cm) of Woelm polyamide (Pharma) or Kapron, with elution by distilled water. The solvent was regenerated with 5% NH_4OH followed by washing with distilled water to neutrality. The yield of the protein fraction in the case of the protein hydrolysate was 85%, and in the case of the meal hydrolysate 65%. Amino acid compositions were determined on an LKB 4101 amino acid analyzer (Sweden) after the hydrolysis of the purified hydrolysates (5.7 N HCl, 110°C, 24 h).

TABLE 2. Chemical Characteristics of the Products of the Plastein Reaction

Amino acid	Pancreatic hydrolysate of the protein (the meal)*	Control**	L-Trp Plastein	DL-Met Plastein	L-Thr Plastein	N^{ε}-Ac-L-Lys Plastein
Aspartic acid	6,7 (6,2)	6,1 (6,2)	5,7 (6,1)	5,5 (5,8)	6,5 (5,5)	5,5 (5,9)
Threonine	2,5 (2,3)	2,13 (2,5)	2,3 (2,4)	1,9 (2,1)	9,2 (9,2)	2,2 (2,0)
Serine	3,1 (2,0)	2,9 (2,1)	2,5 (1,9)	2,5 (2,0)	2,8 (1,9)	3,1 (1,5)
Glutamic acid	18,5 (13,1)	10,3 (14,3)	10,1 (15,0)	9,8 (14,5)	11,5 (14,1)	11,1 (15,3)
Proline	3,0 (1,5)	2,9 (2,5)	2,5 (2,1)	2,0 (1,4)	2,8 (1,0)	3,0 (1,4)
Glycine	2,3 (2,5)	2,5 (2,05)	2,2 (2,21)	1,7 (2,1)	2,2 (1,9)	2,1 (2,3)
Alanine	3,5 (2,8)	3,4 (2,6)	3,5 (2,5)	3,2 (2,4)	3,5 (2,2)	3,7 (2,4)
Valine	5,0 (3,7)	6,1 (4,0)	5,7 (4,15)	5,9 (4,2)	6,3 (4,5)	7,0 (5,0)
Methionine	0,5 (0,5)	1,1 (1,1)	1,0 (0,8)	2,8 (2,00)	1,0 (1,2)	1,5 (1,5)
Isoleucine	2,7 (2,1)	3,0 (2,2)	3,5 (2,05)	4,1 (2,15)	3,4 (2,3)	3,5 (2,0)
Leucine	6,2 (4,1)	6,7 (4,0)	6,5 (4,3)	6,3 (4,22)	6,7 (4,5)	7,0 (4,0)
Tyrosine	3,5 (0 5)	4,1 (1,1)	4,0 (0,8)	4,0 (1,3)	3,9 (1,2)	4,0 (0,8)
Phenylalanine	5,6 (2,7)	5,4 (2,45)	5,7 (2,5)	5,6 (2,4)	5,5 (2,34)	5,7 (2,9)
Trytophan	—	—	1,8 (1,5)	—	—	—
Histidine	(3,5)	1,1 (3,2)	0,95 (3,15)	1,3 (3,0)	1,0 (2,5)	0,8 (3,7)
Lysine	3,3 (7,0)	2,9 (6,8)	3,25 (6,5)	3,0 (7,1)	3,2 (6,5)	5,8 (10,1)
Arginine	8,2 (2,6)	8,0 (2,45)	7,7 (2,5)	7,9 (2,6)	8,1 (3,1)	7,9 (3,0)
E_i, %	74,7 (57,1)	68,6 (59,6)	67,1 (61,3)	67,9 (60,02)	80,6 (60 64)	76,9 (65,4)
Yield, %	28,5 (33)	26,1 (2,70)	30,5 (32,1)	25,4 (24,5)	25,1 (27,3)	20,4 (19,8)
Molecular weight‡	1000—1500	2500	3000	2700	2700	2400

*In the table and below, the figures for the meal hydrolysate are given in parentheses.
†The control used was the product obtained under the conditions of the plastein reaction but without the addition of the amino acid esters.
‡The molecular weights of the plasteins were determined by gel chromatography on Sephadex G-50.

Soluble nitrogen was determined by Dumas combustion. Total gossypol was determined by Smith's method [3] and free gossypol by Adams' method [4].

Esterification of the Amino Acids. The ethyl esters of L-Tryp, DL-Met, and L-Thr were obtained as described by Boissonas et al. [5].

The purity of the preparations obtained was checked by thin layer chromatography on Silufol in the butanol–acetic acid–water (4:1:5) system. The ethyl ester of N^{ε}-Ac-L-Lys was obtained by the method of Neuberger and Sanger [6], mp 249-253°C (decomp.).

The plastein reaction was performed in a 0.01 M solution of L-cysteine with the addition to the substrate mixture (5 g of purified pronase hydrolysate and 0.5 g one of the ethyl esters of the amino acids mentioned above in the form of their hydrochloride) of papain (Reakhim) (0.183 g) at pH 6.0, temperature 37°C, for 48 h. After the completion of the reaction, a tenfold excess of 0.1 M NaOH in 50% ethanol was added and the mixture was kept at room temperature for 6 h. Then it was neutralized with 1 N HCl. The precipitate that deposited was centrifuged off and was freeze-dried. The yield of L-Trp plastein was 30.5% (32.1%), of DL-met plastein 25.4% (24.5%), of L-Thr plastein 25.1% (27.3%), and of N^{ε}-Ac-L-Lys plastein 20.4% (19.8%).

The molecular weights of the products of pronase hydrolysis and also of the products of the plastein reaction were determined by gel filtration on a column (2.5 × 75 cm) of Sephadex G-50 (medium) in the presence of standard proteins (pepsin, mol. wt. 35,000; trypsin, mol. wt. 24,000; cytochrome C, mol. wt. 12,400; and insulin, mol. wt. 5730). Elution was performed with 10% NaCl.

SUMMARY

The possibility has been shown of enriching cottonseed proteins with deficient amino acids with the aid of the plastein reaction.

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