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AMOUNTS OF PROTEIN AND OIL AND ACTIVITY OF THE TRYPSIN INHIBITOR IN DIFFERENT VARIETIES OF SOYBEAN

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Fifteen varieties of soybean have been investigated for the amounts of protein, oil, and trypsin inhibitor that they contain. A high thermal stability of the latter has been detected. Information is given on the kinetics of the inhibition of the amidase and proteinase activity of trypsin. It has been shown that the "trypsin inhibitor" consists of a mixture of six proteins.

Soybeans occupy an exceptional place among leguminous and cereal crops, since their protein is the only one out of all plant proteins that is close in food value to the proteins of animal origin [1]. It must be mentioned, however, that soybean seeds contain larger or smaller amounts of inhibitor proteins that are responsible in certain cases for reducing the assimilability of soybean protein by animals because of the inhibition of the proteolytic enzymes of the digestive tract and, in the first place, of trypsin and chymotrypsin [2-4]. Furthermore, it has been established that these inhibitor proteins are far from harmless. They not only suppress the growth of the animal but may also cause hypertrophy of the pancreas [5, 6]. Consequently, a real increase in the food value of soybeans may take place in two directions. The first is the freeing of the nutrient protein of the soybean from the accompanying inhibitor proteins in the process of preparing the beans, which is economically unfavorable, and the second is the isolation of new varieties of soybean with a reduced content of inhibitor proteins. In the latter case, these inhibitors become peculiar genetic markers that can be used both in the analysis of already existing genetic material of the soybean and in the process of isolating new varieties. In view of this, it must be stated that there is another important factor – proteins inhibiting proteolytic enzymes have an independent value, since a number of preparations used in medical practice have been created from them abroad. We have investigated 15 varieties of soybean grown in one of the regions where it is cultivated, Tadzhikistan, for their contents of oil and protein and have also made a comparative study of the activities of the inhibitor proteins in relation to trypsin. As can be seen from Table 1, the amounts of protein in different varieties of soybean range between 30.3 and 43.4%, and the amounts of oil between 14.3 and 28%. According to the figures in Table 1, the varieties of soybean investigated can be divided in relation to the protein and oil in the seeds into three groups: 1) the protein-rich, oil-rich varieties (1-5); 2) the protein-poor, oil-rich varieties (7-15); and 3) a protein-rich, oil-poor variety (6). The mean values of the ratios of the amounts of protein and oil in these groups are 1.87, 1.41, and 2.76, respectively, the sum of protein and oil ranging from 65.2 to 51.3%.

The specific activity of the trypsin inhibiters from the different varieties of soybean ranged from 17 to 135 units/ml of extract (determined in the presence of N-benzoyl-d,*l*-arginine p-nitroanilide (BAPA)) and from 1.95 to 11.5 units/ml of extract (determined with respect to casein). These results show the absence of a connection between an increased content of protein and oil and the specific inhibitor of the trypsin inhibitors isolated from soybean seeds.

It may be assumed that the difference in the activities of the trypsin inhibitors present in soybean protein depends on a genotypic feature of a particular variety. In view of this, the activity of the trypsin inhibitors may be a good test for selecting those varieties that would combine a high protein content with a low activity of the trypsin inhibitors. From this point of view, according to the figures in Table 1, the most promising varieties

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TABLE 1. Amounts of Protein and Oil and Specific Activity of the Trypsin Inhibitors in Soybean Seeds (1975-1976 Crop, Tadzhikistan)

Variety	Protein content, %	Oil content, %	Protein: oil ratio	Sum of protein + oil	Specific activity of the trypsin inhibitors			
					amidase		proteinase	
					per ml of solution	per mg of soybean protein	per ml of solution	per mg of soybean protein
Protein-rich, oil-rich								
1. Amurskaya-41	43.4	21.8	1.99	65.2	135.0	133.0	11.50	11.4
2. Imeretinskaya	41.0	21.4	1.90	62.4	17.2		1.95	
3. Kubanskaya-636	40.1	20.6	1.94	60.7	19.3		2.26	
4. Kubanskaya-276	39.9	23.9	1.67	63.8	21.5		2.42	
5. Khersonskaya-2	39.5	21.3	1.85	60.8	112.5		4.70	
Protein-rich, oil-poor								
6. Amurskaya-310	39.5	14.3	2.76	53.8	19.4		1.26	
Protein-poor, oil-rich								
7. Orzu*	38.3	23.0	1.66	61.3	49	50.6	3.60	3.7
8. Mao-shi-do	37.5	23.0	1.60	60.5	27.0		2.10	
9. Clark	36.8	27.8	1.32	64.6	73.2		6.35	
10. Chernovitskaya	34.7	25.0	1.38	59.7	25.3		2.76	
11. Komsomolka	33.9	26.5	1.28	60.4			—	
12. Khersonskaya-6	33.4	22.3	1.50	55.7	130.6		7.40	
13. Uzbekskaya-2	33.0	23.0	1.40	56.0	51.9	51.9	5.50	5.5
14. Ford	32.3	28.0	1.15	60.3	23.1		3.00	
15. Berunitsa-12	30.3	21.0	1.44	51.3	27.2		2.10	

*Orzu is a hybrid of Chernovitskaya-4 × Kormovaya-28.

of soybean under the conditions of Tadzhikistan are Imeretinskaya, Bel'tskaya-636, Kubanskaya-276, Amurskaya-310, Orzu, and Ford, in which the total of proteins and oil reaches 64.6%. Figure 1A shows the results of the inhibition of the amidase activity of trypsin by protein extracts of three varieties of soybean. The highest inhibiting activity was found in a protein extract of the variety Amurskaya-41. Complete inhibition of the amidase activity of trypsin by aqueous extracts of the varieties Amurskaya-41, Orzu, and Uzbekskaya-2 was achieved with amounts of 0.06, 0.165, and 0.156 ml, respectively. The dynamics of the inhibition of the proteinase activity of trypsin are shown in Fig. 1b. The amounts of extracts necessary for the complete inhibition of the trypsin activity were, in the same sequence of varieties, 0.11, 0.35, and 0.23 ml. The specific activity of the trypsin inhibitor in 1 ml of an aqueous extract of the variety Amurskaya-41 was almost three times greater than that for Orzu and Uzbekskaya-2 which, recalculated to a milligram of the protein of the variety Amurskaya-41, corresponds to 133 μg of trypsin (Table 1). It follows from this that although the protein content of variety Amurskaya exceeds that of variety Orzu by 4.1% and that of the variety Uzbekskaya-2 by 10.4%, nevertheless the high specific activity of the trypsin inhibitor considerably reduces its value. Consequently, some of the varieties given in Table 1 have an undoubted advantage over others such as Amurskaya-41 and Khersonskaya-2 and -6, since their proteins are assimilated considerably better.

It is known that the trypsin inhibitor proteins present in aqueous extracts that were mentioned above [7, 8] consist of mixtures of at least six individual protein fractions with different molecular weights. A similar pattern is observed in the electrophoresis of aqueous protein extracts of these three varieties. The electrophoretic spectrum showed identity of the number of protein components but variety differences was shown clearly in relation to the electrophoretic mobilities of the inhibitor proteins (Fig. 2). It may be assumed that the differences in the relative electrophoretic mobilities of the proteins and their inhibitory activities with respect to trypsin depend on a genotypic feature of the variety. In view of this, the activity of the trypsin inhibitor and the relative electrophoretic mobility of its protein fractions may be a good test for selecting varieties in breeding work.

We must also mention the high thermal stability of the inhibitor proteins. Thus, after the treatment of an aqueous extract containing trypsin inhibitor at 120 °C for an hour, 65-70% of its activity was retained (Fig. 1C). This indicated, that in the first place, not all the inhibitor proteins are heat-stable, and, in the second place that thermal treatment has no fundamental influence on the inactivation of the trypsin inhibitors.

Thus, in the evaluation of the food qualities of soybeans one must also take into account such an index as the inhibitor protein content.

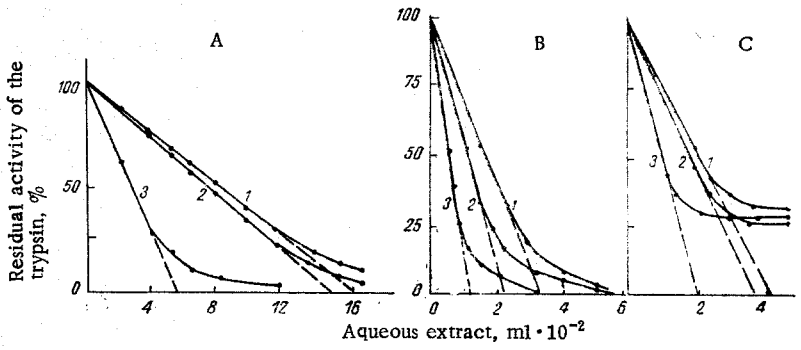

Fig. 1

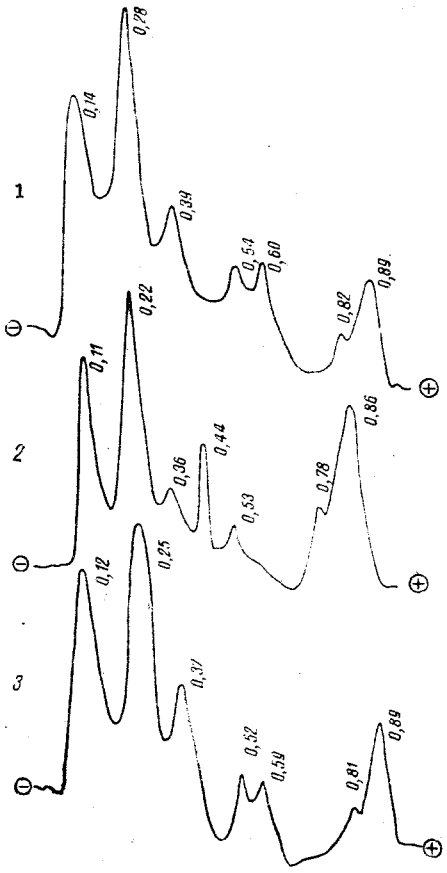

Fig. 2

Fig. 1. A. Inhibition of the amidase activity of trypsin (C = 20 mg) by an extract of the water-soluble proteins of the soybean (pH 8.2, temperature 25°C). B. Inhibition of the proteolytic activity of trypsin (C = 25 mg) by an aqueous extract of the proteins of the soybean (pH 7.8, temperature 40°C). C. Inhibition of the proteolytic activity of trypsin (C = 25 mg) by an aqueous extract of the proteins of the soybean after its thermal treatment at 120°C for 1 h. Soybean varieties: 1) Orzu; 2) Uzbekskaya-2; 3) Amurskaya-41.

Fig. 2. Densitograms of gel electrophoretograms (polyacrylamide gel, 7.5%, pH 8.9, 280 V, time 120 min, temperature 4°C). The figures attached to the densitograms denote the relative electrophoretic mobilities of the fractions in disk electrophoresis. The serial numbers attached to the curves denote the same varieties as in Fig. 1.

EXPERIMENTAL

The seeds of 15 varieties of soybean from the 1976-1977 crop were obtained in the variety section of the Crop Institute of the Ministry of Agriculture of the Tadzhik SSR. We used casein (kh.ch. ["chemically pure"]) and trypsin (both from Czechoslovakia), the trypsin having been twice recrystallized and having a specific activity with respect to BAPA (Hungary) of 8.1 μg/min and with respect to casein of 34.8 mg/h.

Preparation of the Soybean Flour. The beans were freed from husks and the cotyledons were ground and passed through a sieve with 0.1-mm apertures. The flour was defatted with hexane in a Soxhlet apparatus and was stored at 2-4°C.

Aqueous Extract of Trypsin Inhibitor. The flour (50 mg) was extracted with distilled water in a ratio of 1:200 in the cold for 1 h with stirring. The suspension was centrifuged in the cold at 6000 rpm for 30 min. The supernatant liquid obtained was dialyzed against distilled water for 10 h.

Determination of the Trypsin Inhibitor Activity. From $1 \cdot 10^{-2}$ to $1.6 \cdot 10^{-2}$ ml of aqueous soybean extract was added to 0.4 ml of a solution of trypsin (20 μg) and the volume was made up to 2 ml with standard buffer. The standard buffers used were 0.05 M tris, 0.02 M $CaCl_2$, pH 8.2, in the determination of amidase activity,

and tris-HCl, $1 \cdot 10^{-2}$ M $CaCl_2$, pH 8, in the determination of proteinase activity. The solution was incubated at 25°C for 5 min, and then 1 ml of BAPA solution was added and incubation was carried out for 10 min. The reaction was stopped by the addition of 0.5 ml of 30% CH_3COOH. The extinction of the sample was measured on a Spektrom-20 4 spectrophotometer at 405 nm against a blank (BAPA – CH_3COOH – aqueous extract – trypsin). By a graphical method [9] with the drawing of a tangent, the volume that could quantitatively inhibit 100% of the activity of trypsin was found (Fig. 1). The concentration of trypsin in a 0.001 N HCl solution was determined spectrophotometrically ($E_{280} = 0.65$). The amidase activity of the trypsin was found by the casein method of Kaverzneva [10]. Disk electrophoresis was performed by a known method [11] in 7.5% polyacrylamide gel at pH 8.9. The densitograms were recorded on a "Joyce" instrument (United Kingdom). The amounts of protein in the aqueous extracts were found by Lowry's method [12] and in the soybeans by the micro-Keldahl method.

SUMMARY

1. An investigation of 15 varieties of soybean for their contents of oil, protein, and trypsin inhibitors has shown that the amount of inhibitor in some varieties may reach a considerable figure, which reduces their food and fodder value.

2. A fairly high heat stability of the trypsin inhibitors has been found.

3. The amount of inhibitor protein in soybeans is an important index of their quality and must be taken into account in the selection of new varieties.

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