ISOLATION OF PROTEINS FROM COTTONSEED MEAL

II. THE SALT METHOD

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UDC 664.38+582.796

The present paper gives the results of an investigation of the amino acid composition and assimilable lysine of the proteins obtained in the aqueous, salt, and alkali extraction of cottonseed meal. A comparison is made with respect to these indices and to the amounts of free and bound gossypol of the corresponding proteins from industrial meal and from defatted flour from cotton seeds.

The yield of extractable proteins from the meal is less than the yield of proteins from the flour. However, there is no proportionality for different protein fractions: the extractability of the "water-soluble proteins" and of the salt-soluble proteins from meal is only half that from flour (Table 1), and the extractability of the alkali-soluble proteins from the meal is higher, which leads to a fall in the quality of the protein (% of assimilable lysine).

In a comparison of the amino acid composition of the corresponding protein fractions, it can be seen that only the salt-soluble fraction of the meal has the same composition as the salt-soluble fraction of the flour. At the same time, only this fraction of the proteins of the meal is characterized by a high value of the EAA index and of the amount of assimilable lysine. The water-soluble and alkali-soluble proteins lower these indices sharply in the process of the industrial treatment of cotton seeds for the production of oil.

In agreement with the results given above, the method of salt extraction has been made the basis of the extraction of protein from industrial meal. The optimum conditions were as follows: extraction of the meal with 10% NaCl, pH 8.5 (1:10), heat denaturation of the proteins at 85°C for 15 min, desalting by dialysis against water, and precipitation of the proteins at pH 4.5. In the preparation of protein in large amounts, the stage of heat denaturation can be omitted and dialysis replaced by 15-fold dilution. The yield of protein from the meal amounts to 16% and from the flour 32%, the nitrogen contents being 15.8% and 16.5%, respectively.

The figures in Table 1 show that the protein obtained by this method is characterized by the required balance of essential amino acids (38%), a high nitrogen content (15.8%), and satisfactory values of the EAA index (the EAA index of egg protein was taken as 100%) and of assimilable lysine. No free gossypol was found in the protein, and the amount of bound gossypol was far lower than the permissible norm for edible flour from cotton seeds [1].

It must also be mentioned that the total proteins obtained from the meal is scarcely inferior to the protein from defatted cottonseed flour obtained by the salt method of extracting proteins in spite of the substantial differences in the amino acid composition of the various protein fractions (water-soluble, alkali-soluble).

EXPERIMENTAL

Isolation of the Protein Fractions. Cottonseed flour (or factory meal sieved through a 0.5-nm sieve) (50 g), which had been defatted with aqueous acetone (85%) and acetone, was extracted twice with water in a ratio of 1:5 for 3 h. The extracts were centrifuged at 3000 rpm for 15 min. The water-soluble proteins

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 626-628, September-October, 1974. Original article submitted May 4, 1973.

TABLE 1. Characteristics of the Proteins of Cottonseed Flour (I) and Meal (II)

Index, %	Proteins						Total protein	
	water-soluble		salt-soluble		alkali-soluble			
	I	II	I	II	I	II	I	II
Yield	16,0	6,6	25,0	13,0	3,0	7,5	32,0	16,0
Ash content	12,9	27,6	4,1	4,9	1,6	4,2	2,7	0,7
Moisture content	8,2	9,7	8,2	7,5	4,6	6,3	7,5	7,5
Nitrogen	9,9	4,7	15,4	15,9	11,3	12,4	16,5	15,8
Amino acid composition:								
lysine	4,5	2,1	3,2	3,1	3,0	2,7	4,2	3,0
histidine	1,5	0,7	3,1	3,2	2,1	2,1	3,1	3,2
arginine	9,8	6,1	10,0	12,5	4,8	9,6	11,1	11,5
aspartic acid	5,6	3,3	9,8	9,9	7,2	7,2	9,9	8,7
threonine	2,0	1,4	2,9	3,2	4,3	2,2	3,4	3,0
serine	2,6	2,9	5,4	4,6	5,6	3,5	5,9	5,1
glutamic acid	22,0	6,7	21,2	23,3	8,5	18,7	22,0	21,8
proline	7,0	1,8	1,4	0,7	0,4	5,8	5,0	4,0
glycine	2,8	1,1	3,9	4,1	1,7	3,2	6,7	3,8
alanine	2,4	3,3	3,6	3,9	3,6	2,9	4,3	3,9
cysteine 1/2	1,5	1,9	1,2	1,1	1,1	1,7	2,3	1,2
valine	2,4	3,1	5,5	5,7	4,0	3,5	4,1	4,0
methionine	1,5	0,5	1,4	1,4	1,8	1,5	1,6	1,0
isoleucine	1,8	5,3	3,5	3,6	3,0	2,5	4,2	3,5
leucine	2,8	3,0	6,3	6,4	6,6	4,6	7,0	5,9
tyrosine	3,1	1,1	3,7	4,3	3,7	3,1	4,4	3,2
phenylalanine	2,6	6,7	7,0	5,3	4,1	4,9	7,8	6,5
tryptophan	0,9	1,9	1,1	2,4	2,0	0,2	1,3	0,6
Total amino acids	73,7	52,9	94,2	99,4	68,1	80,1	108,7	94,9
Crude protein, N × 6.25	62,0	29,4	96,2	99,5	70,5	77,4	103,0	100,0
Assimilable lysine	3,4	1,1	2,2	2,1	2,5	1,4	2,9	2,1
EAA index	37,5	52,4	67,7	70,2	56,0	62,0	75,5	73,6
Bound gossypol	0	0	0,02	0,02	0,02	0,8	0,1	0,1

were precipitated from the combined centrifugates by acetone (3 : 1) and were dehydrated with acetone. The salt-soluble proteins were extracted from the precipitate with 10% NaCl, with centrifuging at 3000 rpm for 15 min. The supernatants were dialyzed against water, and the precipitate that formed on dialysis was separated off by centrifuging and was dehydrated with acetone (salt-soluble fraction). The alkali-soluble fraction was obtained by the action of 0.2% NaOH on the residue after salt extraction and the proteins were precipitated by acidifying the extract with hydrochloric acid to pH 4.7. The suspension was centrifuged, and the precipitate was dehydrated with acetone.

Production of Protein. The flour or meal (100 g) was extracted twice with 10% NaCl at pH 8.5 in ratios of 1 : 5 and 1 : 3, and the solution was separated by centrifuging at 3000 rpm for 15 min. The proteins were precipitated from the clarified extracts by dialysis with subsequent acidification by hydrochloric acid to pH 4.5 [3]. Replacement of the dialysis stage by 15-fold dilution with water did not affect the quality and composition of the protein.

Amino Acid Composition. A 5-7-mg sample of the protein was hydrolyzed in 6 N HCl at 145°C for 4 h [4], and the amounts of amino acids in the hydrolyzate were determined on an AAA-881 amino acid analyzer. To determine the amount of tryptophan, a 0.01-0.02% solution of the protein in a 6 M solution of guanidine chloride at pH 6.5 was prepared, and the UV spectrum was taken on a EPS-3T spectrophotometer in the 220-340-nm region. The amount of tryptophan was calculated from the optical densities at 280 and 294 nm [5].

Assimilable lysine was determined by dinitrophenylation with the separation of the DNP derivatives of the amino acids in a thin layer [6].

Gossypol (total and combined) was determined by the p-anisidine method, decreasing the sample weights and volumes fivefold [7].

The industrial meal was obtained from the Andizhan oils and fats combine by the prepressing-extraction method on an HD-1250.

SUMMARY

On the basis of the results of a study of the amino acid compositions of various protein fractions from cottonseed flour and meal it has been established that the fraction of salt-soluble proteins (globulins) does not change its composition and properties in the processing of cotton seeds. The yield of protein from the industrial meal using the salt-extraction method is 16%.

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