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INVESTIGATION OF THE PROTEIN COMPOSITION OF THE SEEDS OF *Citrullus vulgaris*

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The total protein content amounts to 60% of the defatted flour or 14-15% of the weight of watermelon seeds. Water-, salt-, alcohol-, and alkali-soluble fractions of the proteins have been isolated and their percentage amounts in the seeds have been determined. More than half the total protein (53.3%) consists of globulins — the reserve proteins of the seeds. The seeds contain about 30% of lipids, which form a valuable component of them and may serve as an additional source of nutrient plant oil. Using the method of amino acid scales a comparative analysis has been made of the biological value of the watermelon seed proteins with standard proteins.

We have studied the protein composition of watermelon seeds and have determined their amino acid composition and the amount of essential amino acids in the protein fractions in order to evaluate the nutrient value of the seeds.

In the process of grinding and defatting the seeds it was found that 23-35% of them formed the defatted flour, 28-30% being lipids, and 45-49% seed coat, which agrees with literature information [1].

A determination of total nitrogen (9.6%) showed that the protein content of the flour was 60% [2]. By extraction and isolation, using known methods [3, 4], we obtained various protein fractions — water-soluble (albumins), salt-soluble (globulins), alcohol-soluble (prolamines) and alkali-soluble (glutelins):

Protein fractions	Amount, %,	
	in the flour	in the total protein
Albumins	6	10
Globulins	32	53.3
Prolamines	13.3	22.2
Glutelins	2	3.3
Unextractable protein	6.7	11.1
Nonprotein fraction	40	

The globulin fraction amounted to almost one third of the flour (32%), or 53.3% of the total protein. As can be seen from the figures given above, about 90% of the total protein was well extracted by aqueous solutions, and only about 11% of the proteins formed part of the more complex proteides, such as glyco- and lipoproteins, etc., strongly bound to cell structures.

The amino acid compositions of the total protein, the protein fractions, and the unextractable part of the flour are given in Table 1.

The total amount of essential amino acids was 41.7% of all the amino acids of the total protein, and in the albumin fraction it was 33.9%, in the globulin fraction 38.8%, in the prolamine fraction 46.2%, in the glutelin fraction 39.4%, and in the unextractable protein fraction 45.8%. Of the essential amino acids the most deficient for the animal organism were lysine and methionine. In a comparative analysis the prolamine fraction must be noted as the richest in lysine. This fraction was also enriched with histidine. All the methionine was found in the albumin fraction.

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TABLE 1. Amino Acid Compositions of the Proteins of Watermelon Seeds

Amino acid	Amount, % of the total					
	total protein	albumin	globulin	prolamine	glutelin	unextr. protein
Aspartic acid	5.62	8.65	11.92	3.85	10.48	7.41
Threonine	3.00	3.77	3.85	–	4.68	4.00
Serine	4.10	4.21	4.23	3.85	5.24	5.47
Glutamic acid	18.86	2.97	24.23	15.39	22.28	1.29
Proline	6.04	2.77	5.38	–	7.68	9.45
Alanine	5.38	4.66	6.15	7.70	5.80	5.00
Valine	5.75	4.21	5.38	3.84	6.55	5.00
Methionine	Tr.	0.89	–	–	–	–
Isoleucine	6.00	3.99	5.00	3.85	5.06	5.47
Leucine	9.15	6.87	8.46	3.85	8.80	9.45
Tyrosine	7.25	3.55	3.85	19.23	3.37	5.00
Phenylalanine	13.67	4.00	6.54	–	6.37	7.41
Histidine	4.12	3.10	3.08	11.54	3.56	8.00
Lysine	6.00	7.76	6.53	23.08	4.30	6.47
Glycine	5.00	9.09	5.39	3.85	5.80	9.00

TABLE 2. Comparison of the Amino Acid Scores of Watermelon Juice and Seeds with a Standard

Index	Watermelon juice	Watermelon seeds	Hens' eggs
Water, %	89.0	14.0	74.0
Protein, %	0.7	13.9	12.7
Recalculation factor	6.25	6.25	6.25
Essential amino acids,	169	6025	5243
including:			
valine	10	799	772
isoleucine	20	834	597
leucine	18	1272	1081
lysine	64	834	903
methionine	6	Tr.	424
threonine	28	417	610
tryptophan	7	–	204
phenylalanine	16	1900	652
Inessential amino acids,	583	6996	7348
including:			
alanine	34	748	710
arginine	18	–	787
aspartic acid	342	781	1229
histidine	8	573	340
glycine	29	695	416
glutamic acid	95	2621	1773
proline	20	–	396
serine	23	570	928
tyrosine	12	1008	476
cystine	2	–	293
total amount of amino acids	752	13052	12591
Limiting amino acid, score, %	Met. + cys. – 33; leu. – 37	Met. + cys.	None

By using the method of the amino acid scale, from the amino acid composition of the total protein it is possible to determine the biological value of the watermelon seed proteins in comparison with the analogous indices obtained by the use of standard proteins, as which we took whole hens' egg protein or cows' milk protein (Table 2) [5].

In the watermelon seed proteins the amounts of certain essential amino acids, such as valine, leucine, isoleucine, and phenylalanine, exceeded the amounts of these amino acids in the standard hens' egg protein. The amounts of inessential amino acids such as alanine, histidine, glycine, glutamic acid and tyrosine, were also higher in watermelon seeds than in hens' eggs. But the proteins of watermelon seeds were very poor in such essential amino acids as arginine and proline.

It is obvious that in order to use watermelon seed flour for the purpose of enriching food products with proteins its own protein content must be balanced with those amino acids the score of which is less than 100.

EXPERIMENTAL

Production of Watermelon Seed Flour. Watermelon seeds were ground and defatted with acetone in the cold (0-4°C), being treated with fresh solvent 3 times. The flour was filtered off with suction on a Büchner funnel and was washed with fresh acetone and dried. The ground seeds were ground further and passed through a fine sieve. Further defatting was carried out until the acetone was completely colorless. The flour was filtered off with suction, washed on the funnel, and dried in the air. After grinding and acetone defatting, 125 g of watermelon seeds yielded 29 g (23.2%) of defatted watermelon seed flour, 60 g (48%) of seed coat, and 36 g (28.8%) of lipids.

The defatted flour was used for determining total nitrogen and the amino acid composition, and also for isolating individual protein fractions such as the water-, salt-, alcohol-, and alkali-soluble proteins by standard methods [3, 4].

Isolation of Individual Protein Fractions. Isolation of the Albumins and Globulins. To isolate the water- and salt-soluble proteins, 15 g of the flour was homogenized with 150 ml of a 10% solution of NaCl. The homogenizate was stirred for 1 h and was then centrifuged at 6000 rpm for 1 h at 2-4°C. The extraction of the proteins was repeated another 6 times, by when the salt-soluble proteins had been extracted completely. The extract was dialyzed against distilled water with three changes. The precipitate of globulins that deposited was separated off by centrifugation at 3000 rpm for 20 min at 2-4°C. The solution of albumins and a suspension of the globulins in water were lyophilized. The yields from 15 g of flour were 0.9 g (10% on the total proteins) for the albumins and 4.8 g (53.3%) for the globulins.

Isolation of the Prolamines. The residual flour after extraction with sodium chloride was washed with distilled water and treated with 100 ml of 80% ethanol to extract the alcohol-soluble fraction of proteins. After being shaken for an hour, the suspension was centrifuged at 6000 rpm for 30 min at 2-4°C. The treatment with 80% ethanol was repeated another 5 times. The aqueous alcoholic extracts were combined and were evaporated under vacuum in a rotary evaporator at 30°C. The solution was acidified to a weakly acid pH, and acetone was added to give 90% saturation. The white amorphous precipitate that deposited was centrifuged off at 6000 rpm for 20 min at 2-4°C and it was washed by suspension in acetone, alcohol, and ether and dried under vacuum. The yield of prolamines was 2.0 g (22.2% on the total proteins).

Isolation of the Glutelins. The residual flour after the separation of the alcohol-soluble fraction of proteins was treated with 100 ml of 0.05 M NaOH solution, and the mixture was shaken for 1 h. Then it was centrifuged at 6000 rpm for 30 min at 2-4°C. This treatment was repeated another 6 times, by when the alkali-soluble proteins had been extracted completely. The alkaline solution was neutralized by the addition of 50% trichloroacetic acid follwed by the addition of more trichloroacetic acid to bring its concentration to 5%. The precipitate was separated off by centrifugation, washed with acetone, ethanol, and ether, and dried under vacuum. The yield of glutelins from the flour was 0.3 g (3.3% of the total proteins).

The residue after the separation of all the protein fractions was suspended in acetone, centrifuged off, and washed again with acetone, alcohol, and ether, and dried under vacuum. It amounted to 40% of the flour.

Hydrolysis of the Proteins. Weighed samples of the lyophilized proteins were transferred with 6 N HCl into tubes, which were frozen and sealed under vacuum, and hydrolysis was performed at 105°C for 24 h. After hydrolysis, the contents of the tubes were transferred to flasks and the tubes were rinsed three times with distilled water. The rinsing waters were added to the hydrolysate, which was then concentrated in a rotary evaporator at 40°C, with the addition of water and re-evaporation until the HCl had been completely eliminated.

The amino acid compositions of the proteins were determined on a model T-339 amino acid analyzer (Czechoslovakia) using a standard mixture of amino acids as internal reference material.

Determination of Tryptophan [3]. A 40-50 mg weighed sample of the protein was dissolved in 5 ml of 0.2 N NaOH. The reagent for determining tryptophan was prepared in the following way: to 8 ml of 23.7 N H_2SO_4 was added 1 ml of 2 N H_2SO_4 containing 30 mg per ml of *p*-dimethylaminobenzaldehyde, and the mixture was cooled to 25°C. To this mixture was added 1 ml of the protein solution and the contents of the test-tube were mixed and left for 12 h. Then 0.1 ml of a 0.045% solution of $NaNO_2$ was added to the test-tube, and after 30 h the optical density of the solution was measured in a spectrophotometer at 600 nm. For a control, water was used in place of the protein solution. Standard solutions of tryptophan were prepared similarly, and, after their optical densities had been determined, a calibration graph was plotted. The tryptophan concentrations in the samples studied were found from this graph.

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