JOURNAL OF NORTHEASTERN UNIVERSITY

Volume 25 Issue 04, 2022 ISSN: 1005-3026 https://dbdxxb.cn/ Original Research Paper

ISOLATION AND CHARACTERIZATION OF PROTEINS FROM GREEN AND BLACK RADISH

Abdullaev Shavkat Vohidovich
dr. chem. Sciences, Professor, Namangan State University, Uzbekistan, Namangan E-mail: abdullayev sh v@mail.ru

Mamatkulova Surayyo Abdusamatovna
Lecturer, Fergana State University, Uzbekistan, Ferghana E-mail: indijon4@gmail. com

Dekhkanov Rahmatullo Sultanovich
to chem. Sciences, Associate Professor, Namangan State University, Uzbekistan, Namangan
E-mail: @mail.ru

Ubaidullo Ulmasbekovich
Student of Fergana State University, Republic of Uzbekistan, Ferghana

ANNOTATION

The main objective of the training program in Higher Educational Institutions (HEI) is the release of comprehensively developed and independently thinking specialists. It is expedient and relevant in the educational process of the university to study the nature and properties of cultivated plants, their composition and use in the national economy. To date, a large theoretical and experimental material has been accumulated in domestic and world science and practice, indicating the high efficiency of the use of amino acids in medicine. The aim of the study is to isolate the protein and analyze its amino acid composition from cultivated plants of Uzbekistan green and black rare.

Green radish is the root crop of the plant of the same name, one of the varieties of radish, a popular agricultural crop known as garden radish. It is distinguished by a light green color and a more intense bitter taste of the pulp. 100 grams of green radish contains about 32 kcal.

The chemical composition of green radish is characterized by a significant content of proteins, carbohydrates, mono and disaccharides, fiber, vitamin C, ash, macro (potassium, calcium, magnesium, sodium, phosphorus) and microelements (iron, cobalt, fluorine).

Black radish is the root crop of the herbaceous plant of the same name, one of the varieties of radish. In addition to the black color, this vegetable differs from them in the most bitter taste, which becomes much softer after heat treatment. 100 grams of black radish contains about 32 kcal.

Black radish has a rich chemical composition. Its roots contain sugars, raphanol, phytoncides, the enzyme lysozyme, an antibacterial agent that destroys cell walls, bacteria: cellulose, there are many vitamins: ascorbic acid, beta-carotene, vitamins C, E, and PP, a lot of potassium, there is calcium, magnesium , iron, phosphorus, purine bases, choline, sulfurous essential oil. In addition, the chemical composition of black radish roots is characterized by a high content

Submitted: 03/12/2022 **Accepted**: 24/12/2022

of proteins, carbohydrates, fiber, vitamin C, macro (potassium, calcium, magnesium, sodium, phosphorus) and microelements (iron, cobalt, iodine).

It is known that black radish in terms of chemical composition is a whole complex of vitamins, minerals and other biologically active substances, which causes this vegetable to have a lot of useful properties along with a burning taste. Its use has a bactericidal, anti-inflammatory and immune stimulating effect on the human body, which helps prevent the occurrence and development of colds. In addition, black radish stimulates the secret function of the gastrointestinal tract, which improves appetite, and with it the work of the entire digestive system as a whole. Another useful property of this vegetable is due to the presence of a significant amount of potassium in its composition, due to which its use contributes to the normalization of heart rate and blood pressure.

The object of our research was the proteins of green and black radish. The roots of green and black radish were finely chopped and dried at 500C in an oven for four hours until complete dehydration. Dry crushed green radish root 17.5 grams and black radish 19.0 grams were extracted with 0.2N sodium hydroxide on a magnetic stirrer for 1 hour with constant stirring at a ratio of 1:10. After extraction, the obtained extract was centrifuged for 20 minutes at 3000 rpm. The supernatant (transparent supernatant solution) was subjected to ammonium sulphate precipitation with dry ammonium sulphate at a ratio per 100 ml. supernatant 53 gr. salt. The resulting suspension was centrifuged for 30 minutes at 6000 rpm. The resulting protein precipitate was dissolved in a minimum volume of 0.2N sodium hydroxide and dialyzed in running water for 24 hours in cellophane bags. The desalted protein solution obtained after dialysis was freeze-dried at a temperature of –35°C under high vacuum. Freeze-dried protein was used to determine the quantitative amino acid composition and determine the content of lysozyme by thin layer chromatography.

The quantitative content of the protein in the solution after extraction with 0.2N sodium hydroxide in the supernatant was carried out using the Kalkar spectrophotometric method. [1] Spectrophotometric protein quantification is based on the ability of aromatic amino acids (tryptophan and tyrosine) to absorb ultraviolet color with an absorption maximum at 280 nm. Thus, by measuring the optical density at this wavelength, one can judge the amount of protein present in the test solution. The presence of nucleic acids and nucleotides interferes with the determination of a protein by this method. Their influence can be eliminated by measuring the optical density not only at 280 nm, but also at 260 nm, and then calculating the true protein content. Conventionally, it is considered that at a protein concentration in a solution equal to 1 mg / ml, the value of optical density at 280 nm 1 when using a cuvette with a layer thickness of 10 mm. The reference solution is the solution used in the extraction process, in our case 0.2N sodium hydroxide (NaOH). The concentration of the protein under study in the solution should be from 0.05 to 2 mg/ml, if the solution is diluted above and the dilution is taken into account in the calculations. The content of protein X (mg/ml) was calculated using the Kalkar formula:

$$X=1.45XD280 -0.74xD260$$

Where D280 is the spectrophotometer reading at a wavelength of 280 nm;

D260 - spectrophotometer reading at a wavelength of 260 nm;
X-protein content in 1 ml. protein solution
Protein solutions were measured on an SF-46 spectrophotometer. In the process of isolation and purification of the protein according to the above scheme, a sample of the protein solution (4 ml) was taken at the stage from the supernatant after extraction with 0.2 N sodium hydroxide and centrifugation, before precipitation of the protein with ammonium sulfate.

Continuing the study of freeze-dried green and black radish proteins, the task was to determine their quantitative amino acid composition. For this, acid hydrolysis of an accurate sample of 5.7 N hydrochloric acid was carried out - 10 mg of each protein for 24 hours at a temperature of 110ºC under vacuum conditions in heat-resistant ampoules [3]. The resulting hydrolysates were analyzed.

HPLC analysis of PTC-derivatives of amino acids. Synthesis of FTC (phenylthiocarbomayl) derivatives of amino acids was carried out according to the method of Steven A., Cohen Daviel [4].

Identification of FTC-amino acids is carried out on an Agilent Technologies 1200 chromatograph on a 75x4.6 mm Discovery HS C18 column. Solution A: 0.14 M CH3COONa + 0.05% TEA pH 6.4, B: CH3CN. Flow rate 1.2 ml/min, absorbance 269nm. Gradient %B/min: 1-6%/0-2.5min; 6-30%/2.51-40min; 30-60%/40.1-45min; 60-60%/45.1-50min; 60-0%/50.1-55min.

Name of amino acids	green radish	black radish
	concentration (mg/g)	
Aspartic Acid Asp	13,8019	14,0564
Glutamic acid Glu	22,9217	78,8502
Serene Ser	9,9403	10,9404
Glycine Gly	77,7432	18,9338
Asparagine Asn	0	0
Glutamine Gln	0	0
Cysteine Cys	25,8128	25,0991
Threonine* Thr	16,1820	34,2095
Arginine Arg	16,0463	4,4914
Alanine Ala	7,4721	3,5494

Proline Pro	0	5,5861
Tyrosine Tyr	6,6553	28,1526
Valine* Val	11,3502	16,0866
Methionine* Met	13,9991	9,0541
Isoleucine* Ile	11,6902	14,3218
Leucine* Leu	17,8374	21,2896
Histidine* His	15,9146	15,7544
Tryptophan Trp	0	0
Phenylalanine* Phe	112,2356	35,2893
Lysine HCl* Lys	13,9550	22,9175
Total	393,5586	358,5822

* essential amino acids

As can be seen from the results obtained, the amino acid composition of the proteins of two types of radish contains the entire set of essential amino acids - threonine, valine, methionine, isoleucine, leucine, lysine, phenylalanine, histidine.

Balanced in essential amino acids. The composition is dominated by glutamic acid, aspartic acid, argenine, lyucine, lysine.

Taking into account the reading of the spectrophotometer at a wavelength of 280 nm and 260 nm, the following calculations were made for green X1 and black radish X2. In this case, the calculations were carried out at a 20-fold dilution of protein solutions.

$$X_1=1,45 \times D_{280} - 0,74 \times D_{260}$$

$$X_1=1,45 \times 0,298 - 0,74 \times 0,446$$

$$X_1=0,42 - 0,43 = 0,09 \times 20=1,8 \times 80=144\text{мг}$$

Which is 0.82% protein content in 17.5 gr. green rare

$$X_2=1,45 \times 0,431 - 0,74 \times 0,698$$

$$X_2=0,62 - 0,52=0,1 \times 20=2 \times 100= 200\ \text{мг},$$

What is 1.05% protein content in 19 gr. black radish

From the results obtained on the protein content in green and black radish, we can conclude that 1 kg of dried green radish contains 8.23 g. squirrel. In the case of black radish, 1 kg contains 10.5 gr. squirrel.

From the literature data, it is known that green and black radish contain lysozyme, an antibacterial agent, in their composition. The next stage of our research was the qualitative

determination of the content of lysozyme in two types of radish by thin layer chromatography in the presence of a lysozyme standard. Upward chromatography was carried out on Silufol plates in the system normal butanol, acetic acid, pyridine, water (15:3:10:12). The chromatogram was developed with a 1% solution of ninhydrin in acetone. As can be seen from the obtained chromatogram, after development with ninhydrin in acetone, lysozyme was found in the proteins of the studied root crops.

References:

1. G.A. Kochetov. A practical guide to enzymology. Publishing house "Higher school"., Moscow., 1980., S.259

2. Mashkovsky M.D., Babayan E.A., Oboymakova A.N. State Pharmacopoeia of the USSR. Volume 2, GF XI. p.31. Publishing house "Medicine", Moscow, 1989.

3. T. Daveny, Ya. Gergey. Amino acids, peptides and proteins. Publishing house "Mir". Moscow. 1976. p.355

4 Steven A.C. Amino Acid analisis Utilizing Phenylisothiocyanate Derivatives/ D.J. Strydom // Anal., Biochem.- 1988.-174.-1-16.