Study of the chemical composition of the amaranth plant by the method of chromotography

Dildora Saidkhodzhaeva[1*], *Abdusattor* Choriyev[1], *Rano* Akramova[2], *Asilbek* Yulchiev[2], and *Shuxrat* Tukhtaev[2]

[1]Tashkent State Technical University, Tashkent, Uzbekistan
[2]Tashkent Chemical-Technological Institute, Tashkent, Uzbekistan

Abstract. The article is devoted to the study of the chemical composition of amaranth on the territory of the republic. The chemical composition of the amaranth plant has been studied. A study was carried out to determine the chemical composition of amaranth by chromatography. The method for determination of flavonoids is given.

1 Introduction

Analysis of literature on the topic. Amaranth is an annual plant belonging to the family (amaranth). Well adapted to the climatic conditions of Uzbekistan for growth. Drought-resistant, loves heat and light, self-pollinating and resistant to various diseases, quickly and easily adapts to a new place.

The height of the stem exceeds 2-2.5 meters. The upper part ends with a complex spike-shaped inflorescence (straight or hanging panicle). Forage or vegetable varieties have a long growing season, and amaranth seeds are small (about 1.4 mm), bright, black, pink, yellow or green. The optimum temperature for growth is 20-30 degrees, so the best growing season is sown from mid-May. Full flowering occurs after 110 days. Seeds ripen in mid-September, can be removed.

Currently, there are thousands of amaranth species, and in Uzbekistan it is cultivated mainly as an ornamental plant.

Amaranth is a thermophilic plant. The plant also grows well in brackish and mountainous areas. Depending on environmental conditions, amaranth can be harvested several times during the growing season [1,2]. Currently, research is underway around the world to develop effective technologies for the industrial processing of amaranth seeds in various ways. The use of amaranth seed treatment in various products is determined by researchers through experimentation. At present, it is important to increase the nutritional and biological value of food products [3]. Until now, many scientists of the world have studied the chemical composition of the amaranth plant and conducted their own research. In particular, scientists Venskutonis and Kraujalis (Kanus University of Technology, Lithuania) showed that amaranth seed proteins and their composition consist of albumins, globulins, glutelin's and

* Corresponding author: dildorasaidxodjayeva0@gmail.com

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

prolamins. Japanese scientists have found that ripe amaranth seeds contain B vitamins, and as a result of research, 100 grams of seeds contain riboflavin (B2), niacin (B3), pyridoxine (B6), folic and pontatenic acids.

In the course of our scientific work, we have chosen the type of amaranth plant that is cultivated in America. 1. Green-red amaranth microgreen. 2. Amaranth red garnet tricolor.

It is also used in cooking in different countries. Amaranth tricolor, leaves and stems in Japan can be eaten as a salad. In Africa, it is usually cooked as a leafy vegetable [4-7].

Sowed with seeds of 2 different types of tricolor amaranth and microgreens on the lands of TDTU, tricolor on the lands of Nazarbek of the Zangiata district of the Tashkent region and microgreens at the end of May, fertilized the land with mineral fertilizers. . Our seeds germinate well at an air temperature of 20-25 0C for the germination of our seeds. Since the air temperature was good, our seeds germinated in 1 week. We observed the growth process and carried out experimental work to determine the amount of B vitamins in the leaves of amaranth plants, the amount of protein, the amount of essential oils, the amount of flavonoids to test the 1-month-old leaves of the sprouted plant.

Amaranth seeds germinated 7 days

Amaranth seeds germinated 20 days

Fig. 1. Growth rate of amaranth plants.

2 Methods

Thiamine hydrochloride, pyridoxine hydrochloride, nicotinamide, riboflavin mononucleotide and ascorbic acid. Registration is carried out by the VEJX method.

Chromatography conditions: Equipment: liquid chromatograph Shimadzu HPLS-10VP with secondary analog UV detector with variable wavelength, gradient

pump and thermostatic column; Column: Hypersil ODS, 150 mm x 4.6 mm with analogue; Mobile phase: linear gradient.

Table 1. Chromatography conditions.

Time (min)	**0**	**4**	**8**	**12**	**13**	**20**
% A	90	90	70	70	90	90
% B	10	10	30	30	10	10

Mobile phase A: 1.0 g (accurately weighed) sodium 1-hexanesulfonate placed in a 1000 ml volumetric flask, add 500 ml of water purified, 10 ml acetic acid (min. 99%), pH of the resulting solution dilute to 3.0 with triethylamine, then dilute the volume of the solution with water to the mark and mix.

Mobile phase B: HPLC methanol.

Flow rate: 1.0 ml/min;

Detection: UV, 270 nm;

Injection volume: 20 µl;

Column temperature: 35°C.

Vitamin B1 standard solution (Standard 1). About 20 mg.

(accurate weight) powder RSO thiamine hydrochloride (ND 42-10173-06, ND 42-14058-06, BP, USP, Eur.Ph, CP) are placed in a 20 ml volumetric flask, dissolved in 15 ml of purified water and the volume of the solution is adjusted with the same solvent to the mark.

Vitamin B2 standard solution (Standard 2). About 6.0 mg

(accurately weighed) RSO riboflavin powder (ND 42-11641-05, ND 42-14222-06, BP, USP, Eur.Ph, SR) is placed in a 100 ml volumetric flask, 80-90 ml of purified water is added and heated for water bath at a temperature of (80-85) ° C until complete dissolution. The resulting solution is cooled to room temperature, the volume of the solution is adjusted to the mark with purified water and mixed thoroughly.

Vitamin B6 standard solution (Standard solution 3). About 20 mg

(accurately weighed) PSO powder of pyridoxine hydrochloride (ND 42-9472-06, ND 42-14723-07, BP, USP, Eur.Ph, SR) is placed in a 20 ml volumetric flask, dissolved in 15 ml of purified water and the volume is adjusted the same solution solvent to the mark.

A mixture of standard solutions of vitamins B1 B2, B6, nicotinamide and vitamin C. About 7.5 mg (accurately weighed) of nicotinamide RSO powder and about 50 mg (accurately weighed) of ascorbic acid RSO powder are placed in a 50 ml volumetric flask and dissolved in 20 ml of purified water. In the same flask, add 1.0 ml of standard vitamin B solution, (Standard solution 1), 15 ml vitamin B2 standard solution (Standard solution 2) and 5.0 ml of vitamin B6 standard solution (Standard solution 3), mix thoroughly and bring the volume of the solution with purified water to the mark (solution 4).

Test solution. Approximately 685 mg (accurately weighed) powder, ground tablets are placed in a 50 ml volumetric flask, 30 ml of water are added purified and heated in a water bath at a temperature of (70-80) ° C for 15 minutes. The flask was then cooled to room temperature, dilute the volume of the solution with purified water to the mark and mix thoroughly.

The resulting solution is centrifuged at 7000 rpm for 10 minutes.

The top layer is decanted and filtered through a membrane filter.

"Millipore" with a pore diameter of 0.45 microns.

A. Verification of the suitability of the chromatographic system.

20 µl of solvent and 5 times 20 µl of standards solution (solution 4) are injected into the chromatographic system brought into equilibrium.

Procedure: Introduce alternately 20 µl of solutions of a mixture of standard substances and the test sample into the chromatograph, obtaining at least 5 chromatograms for each solution.

Quantitation. The content of the sum of flavonoids in terms of quercetin in percent (X) must be at least 0.01%.

Preparation of solutions.

Standard sample solution (CO) of quercetin. About 0.005 g (accurately weighed) of CO of quercetin, previously dried at a temperature of (130-135)° C for 2 hours to a constant weight, is placed in a volumetric flask with a capacity of 25 ml, dissolved in 96% alcohol, the volume of the solution is adjusted to the mark with the same solvent and mix (solution A).

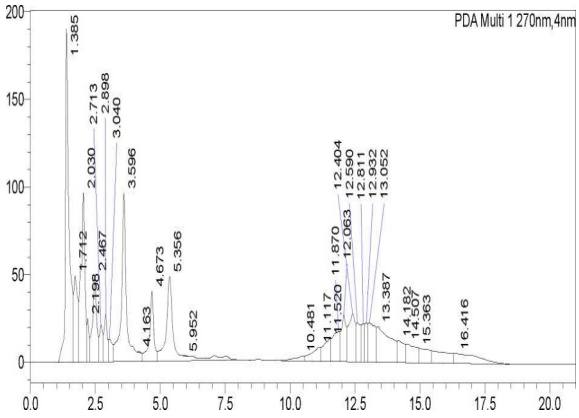
The shelf life of the solution is 30 days.

1.0 ml of solution A is placed in a volumetric flask with a capacity of 10 ml, 2.0 ml of aluminum chloride solution of 5% in 70% alcohol, 1.0 ml of acetic acid diluted 5%, 2.0 ml of 96% alcohol are added, the volume is adjusted with alcohol 70% to the mark and mix (solution B).

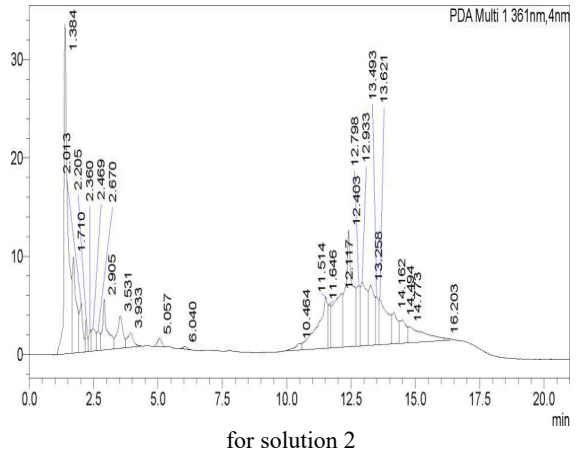
1.0 ml of the extract is placed in a volumetric flask with a capacity of 10 ml, 2.0 ml of aluminum chloride solution of 5% in 70% alcohol, 2.0 ml of 96% alcohol, 1.0 ml of acetic acid diluted 5% are added, the volume of the solution is adjusted with alcohol 70 % to the mark, mix and filter through a paper filter (test solution).

After 30 minutes, the optical density of the test solution is measured on a spectrophotometer at a wavelength of 425 ± 5 nm in a cuvette with a layer thickness of 10 mm relative to the reference solution.

As a reference solution, a solution consisting of 1.0 ml of the extract, 2.0 ml of 96% alcohol, 1.0 ml of diluted 5% acetic acid and brought to the mark with 70% alcohol in a volumetric flask with a capacity of 10 ml is used.



for solution 1



for solution 2

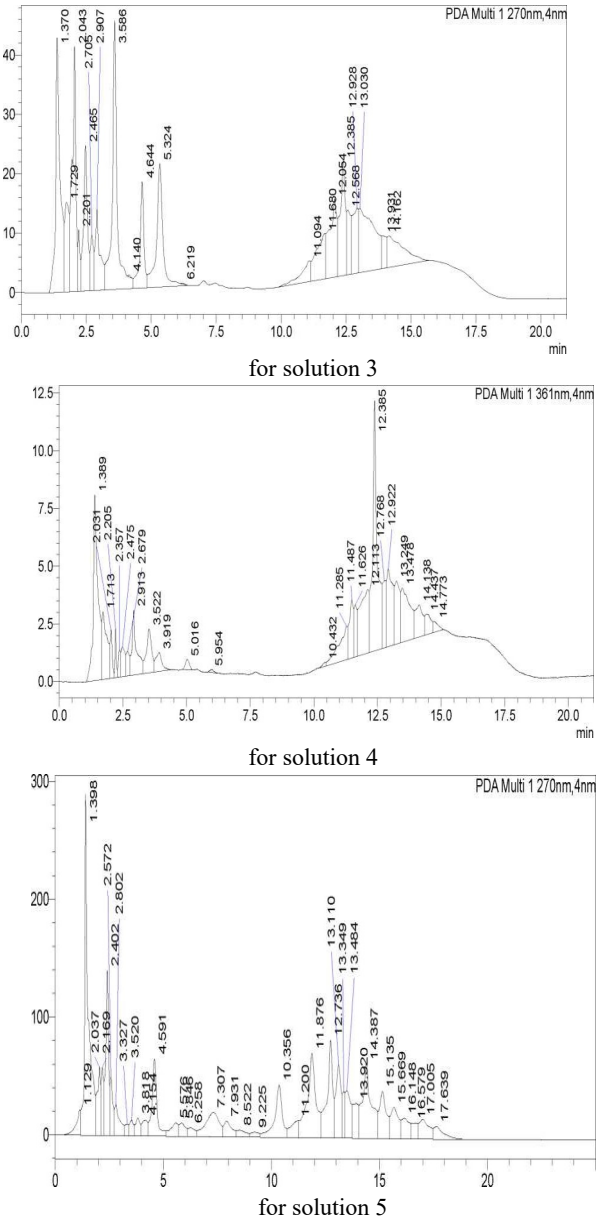


Fig. 2. Chromatogram for every 5 solutions.

At the same time, under the same conditions, the optical density of the BSO solution of quercetin is measured relative to the reference solution. As a reference solution, use a solution consisting of 1.0 ml of quercetin CO solution A, 1.0 ml of acetic acid diluted 5% and brought to the mark with alcohol 70% in a volumetric flask with a capacity of 10 ml.

3 Results and discussion

Amaranth surpasses traditional crops in terms of nutrient content, especially proteins, fats and vitamins. Its proteins are characterized by an optimal ratio of essential amino acids,

including lysine, which is the first limiting essential amino acid from wheat and rye grain proteins [8,10,11].

The amount of B vitamins and protein in the leaves of 2 varieties of amaranth is shown below (Tables 2 and 3).

Table 2. The amount of B vitamins and protein in 100 g of leaf (amaranth red microgreen).

№	Name of indicators	Experimental results; gr/ml	
		10gr-100ml	10gr-200ml
1.	Amount of extract in water Vitamin S Vitamin B1 Vitamin B2 Vitamin B6 Vitamin B12 Vitamin PP	 1.382mg/ml 0.187mg/ml 0.720mg/ml 0.661mg/ml 0.135mg/ml 0.283mg/ml	 0.937mg/ml 0.078mg/ml 0.397mg/ml 0.296mg/ml 0.005mg/ml 0.021mg/ml
2.	Quantitative determination of alcohol extract: - mass fraction of flavonoids in terms of quercetin	2.708%	1.538%
3.	Mass fraction of protein	24.4%	
4.	Mass fraction of essential oils	not recognized	

Table 3. The content of vitamins and protein in 100 g of leaves (amaranth red pomegranate tricolor).

Name of indicators	Experimental results; gr/ml	
	10gr-100ml	10gr-200ml
Amount of extract in water Vitamin B1 Vitamin B2 Vitamin B6 Vitamin B12	 0.14mg/ml 0.12mg/ml 0.1mg/ml -	 - 0.05mg/ml 0.06mg/ml -
Quantitative determination of alcohol extract: - mass fraction of flavonoids in terms of quercetin	3.14%	1.93%
Mass fraction of protein	20.77%	
Mass fraction of essential oils	not recognized	

The results of the experiment show that the composition of amaranth leaves grown from 2 different types of amaranth seeds brought from the USA, localized in the climatic conditions of Uzbekistan, contains more protein than the amount of amaranth grown in the USA.

4 Conclusion

So, if we compare the chemical composition of red amaranth microgreens, red garnet tricolor amaranth plants growing locally on the territory of our republic, with the chemical composition of the leaves of the gultochoroz plant growing in our country, based on the results of experiments, the amount of protein and vitamins it was found that the leaves of our amaranth seedlings imported from the USA and planted locally are higher.

References

1. V. I. Dergausov, *Amaranth is a promising crop* (Oils and fats, 2006)

2. P. F. Konokov et al, *Amaranth - a promising culture of the XXI century* (Ed. Center Academy, M., 1999)
3. T. H. Gamel, J. P. Linssen, Reent Progress in Medicinal Plants **15**, 347-361 (2006)
4. M. Sala, S. Berardi, P. Bondioli, Riv. ital. Sostanze Grasse **75(11)**, 503-506 (1998)
5. P. Kalac, J. Moudry, Composition and nutritional value of amaranth seeds Czech. J. Food Sci. **18(5)**, (2000)
6. A. V. Zheleznov, Chemistry and life **6**, 56 (2005)
7. V. I. Derenzhi, Bakery products **3**, 13-15 (2001)
8. G. P. Karpilenko, K. V. Traore, *Scientific and technical achievable and before experience in the bakery industry* (M., 1995)
9. V. N. Zelenkov, N. P. Zaksas, *Chemical and mineral composition of various parts* of amaranth Proc. of 3rd International Scientific and Production. conf. "Introduction of non-traditional and rare agricultural plants" (Penza), 18-19 (2000)
10. G. I. Vysochina, Chemistry of plant raw materials **2(1)**, 5-14 (2013)
11. D. Saidkhodzhaeva, A. Choriyev, Uzmu khabarlari **3/1/1**, 398-400 (2022)