Antioxidant profile of Amaranthus paniculatus L. of the Pamyat of Kovas variety

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Abstract. The study of the composition and content of biologically active compounds in the leaves and inflorescences of the Pamyat of Kovas vegetable variety of the VNIISSOK selection revealed a difference in their accumulation in the leaves of the lower and upper layers. The maximum accumulation of biologically active substances was found in the leaves and inflorescences of the upper layer, exhibiting high antioxidant activity. In the studied extracts, antioxidant metabolites were found: amarantine, ascorbic acid, flavonoids, hydroxycinnamic acids, simple phenols and hydroxybenzoic acids, as well as pectins. The features typical for vegetable leaf crops were found: the maximum number of studied antioxidant metabolites accumulates in the leaves of the upper layer and amaranth inflorescences, while the decrease in the content of antioxidants is observed in aging leaves.

1. Introduction

Amaranth vegetable is a popular leafy vegetable plant, which is believed to be found in tropical South Asia and spread throughout tropical and temperate regions of the globe [1].

Amaranth vegetables are considered the most popular leafy vegetable crops for their protein, vitamin and mineral-rich leaves and stems in India, China, Indonesia and Africa. Amaranth vegetables are sown and harvested year-round in humid regions. Tender young seedlings sell in urban markets. In Greece, Japan and Western Europe, it is a popular green vegetable and it is used as a substitute for spinach (Spinacea oleracea) during hot summer season.

The nutritional value of amaranth leaves is high, they provide energy to human body, being a rich source of carbohydrates, protein, balanced for essential amino acids, vitamin K, folic acid, riboflavin, vitamins A, B6, C [2].

It is known that vegetables play an important role in the prevention of free radical diseases associated with aging, obesity, cancer and cardiovascular diseases. The healing effect of leafy
vegetable plants is associated with the presence of natural dietary antioxidants [3]. Therefore, it is important to understand which plant organs accumulate the maximum amount of antioxidants.

The purpose of the research is to study the composition and content of antioxidant metabolites in leaves, taking into account their age and inflorescences, in order to understand the patterns of changes in the number of major antioxidant metabolites in the process of growth and development of amaranth.

2. Methods and materials

The object of the research was fresh and dried leaves and inflorescences of amaranth Amaranthus paniculatus L., of Pamyati of Kovas varieties, of VNISSOK breeding (authors: P.F. Kononkov, V.K. Gins, M.S. Gins). Plants were grown in open ground in the experimental fields of the institute. The dried leaves were ground in a mill to a particle size of 1–2 mm. Fresh material was ground in a mortar. The extraction of the crushed leaves was carried out with distilled water at room temperature (water duty 1:10), followed by centrifugation at 6000 rpm. Next, the sediment was extracted with boiling 80% alcohol and the content of phenolic compounds was determined according to the procedure [4].

Amaranthine content was determined spectrometrically at a wave length of 538 nm. The content of the reduced form of ascorbic acid was determined by the iodometric method based on titration of ascorbic acid in colored extracts with potassium iodate in an acidic medium with the presence of potassium iodide, starch and sugar according to the modified Bertrand-Bieri method. The total content of antioxidants (TCA) was determined by amperometric method [5, 6]. The qualitative composition of the metabolites of aqueous extracts of leaves of the upper and lower layers of plants was analyzed by gas chromatography-mass spectrometry (GC / MS) on a JMS-Q1050GC chromatograph (JEOL Ltd, Japan) in accordance with the method proposed in the work [7]. The substances were identified by the retention parameters and mass spectra of the NIST-5 library of the National Institute of Standards and Technology (USA). The scanning range was 33–900 m/z.

3. Results

The analysis of the biochemical composition of the aqueous extract of the leaves of the lower and upper layers of the leaves of amaranth plant and its red-colored inflorescences revealed low molecular weight antioxidant metabolites: betacyanin (amaranthine), ascorbic acid, soluble monosaccharides, disaccharides and organic acids (Table 1).

Figure 1 shows the characteristic profiles of the aqueous fraction of extracts of photosynthetic leaves of the upper and lower layers. The figure clearly shows that in the leaves of the upper layer, the content of common components is several times higher than in the leaves of the lower layer.

| Group of compounds | Antioxidant Metabolites | Young leaves | Old leaves | Inflorescence |
|--------------------|------------------------|--------------|------------|--------------|
| Betacyanins        | Amaranitin, mg/g of wet weight | 0.40±0.02 | 0.21±0.01 | 2.1±0.1 |
| Organic acids      | Ascorbic acid, mg % of wet weight | 160±7 | 80±5 | 155±7 |
|                    | Oxalic acid, mg % of wet weight | 7.0±0.3 | 10.0±0.6 | - |
|                    | monosaccharides, disaccharides % absolute dry weight | 2.3±0.1 | 1.9±0.1 | 2.3±0.1 |
| Carbonhydrates     | Pectin, % of absolute dry weight | 6.1±0.3 | 5.7±0.3 | - |

Young leaves with not fully developed, as well as developed leaf blade (upper layer) were distinguished by a high content of the studied metabolites – antioxidants in comparison with aging leaves. Moreover, the level of ascorbic acid, pectin and the amount of free sugars in the inflorescences was comparable with their amount in the leaves of the upper layer. While in comparison with leaves, the content of amaranthine was significantly higher in inflorescences.
In amaranth inflorescences of the variety Pamyat of Kovas, a large amount of red-violet pigment amaranthine accumulates, which belongs to the group of secondary compounds – betalain pigments. Amarantin is a natural red dye with antioxidant activity. In addition to its coloring ability, amaranthine has a wide and diverse biological activity due to its antioxidant properties: antibacterial activity, inhibits the proliferation of cancer cells, prevents the appearance of malignant tumors and stimulates the growth of bifidobacteria and lactobacilli.

The extract from amaranth leaves has antidiabetic properties [2]. The mechanisms of the antioxidant activity of amaranthine are identified, reasoned by the fact that it is able to neutralize the superoxide oxygen radical, free radicals and chelate divalent ions of transition metal – iron, copper, etc. Biologically active compounds L-tyrosine and glucose are used as precursors of amaranthine: metabolites are antistress antioxidants.

Vitamin C is the most important antioxidant in human body, which supports the health of all cells. This compound protects body from several types of reactive oxygen species: both free radicals and peroxides [6]. In young leaves of amaranth, up to 160 mg % of ascorbic acid is heated, in old leaves its amount decreases to 120 mg % and below. In growing inflorescences, vitamin C up to 130 mg % is accumulated before flowering. L-ascorbic acid is a metabolite distributed in all organs of vegetable amaranth and a key antioxidant that, together with polyphenols, can interrupt the oxidative damage to cell components, indirectly initiating the natural defense of the cell by neutralizing free radicals [8].

The substances that can perform protective functions of cells include carbohydrates: low molecular weight sugars and pectin. The antioxidant effect of carbohydrates is reasoned by their ability to bind free radicals, including inactivating reactive oxygen species that occur during low temperature stress.
As it can be seen from Table 1, the sum of free sugars and the content of water-soluble pectin are higher in growing and actively photosynthetic amaranth leaves compared to aging and old ones. Moreover, the difference in the content of the sum of sugars and pectin between leaves and inflorescences is not as great as in the content of antioxidant amaranthine.

The extracts of the leaves of upper layer and amaranth inflorescences of the Pamyat of Kovas variety richer in antioxidants to some extent – phenolic photochemical substances, as simple phenols and hydroxybenzoic acids, hydroxycinnamic acids, flavonoids (Table 2) compared to the leaves of lower layer. While the content of condensed and polymeric compounds increases in old leaves, the amount of lignin also increases: 12.2 % versus 10.4 % in young leaves.

Table 2. Fractional composition of polyphenols in different leaves of amaranth

| Polyphenol fraction                      | Young leaves | Old leaves | Inflorescence |
|-----------------------------------------|--------------|------------|---------------|
| Simple phenols and hydroxybenzoic acids | 0.51±0.02    | 0.34±0.01  | 0.56±0.02     |
| Hydroxycinnamic acids                   | 0.14±0.01    | 0.10±0.01  | 0.14±0.01     |
| Flavonoids                              | 4.5±0.2      | 4.2±0.2    | 4.4±0.2       |
| Condensed and Polymer Polyphenols       | 0.55±0.02    | 0.89±0.04  | 0.81±0.04     |

Antioxidants, as a rule, have a positive effect in large doses. It is reasoned by the fact that the antioxidant molecule is inactivated by reaction with free radicals. In order for the antioxidant to remain in an active state, it is necessary for it to function in combination with reducing agents. In the amaranth extract, ascorbic acid is reduced by phenolic compounds and can function effectively without the loss of antioxidant activity. Therefore, the determination of the antioxidant activity of not only individual compounds but also of crude plant extracts is of interest in connection with the search for substances the antioxidant properties of which have not yet been studied.

The determination of the total content of antioxidants in amaranth leaves of different ages revealed features characteristic of leaf crops [6, 10], the maximum amount of antioxidants per 1 g of fresh weight accumulates in young growing leaves of 2.1 mg/g, while in aging leaves there is a decrease in antioxidant content up to 1.5 mg/g.

The extracts of the leaves and inflorescences of the amaranth of the vegetable variety of Pamyat of Kovas are rich in antioxidants, phytochemicals: betacyanins, ascorbic acid, phenolcarboxylic acids, monosaccharides, which represent a pharmacological complex of antioxidant metabolites. The studied extracts have biological and antioxidant activity and can be used for further study and search for new biologically active substances with the aim of the creation of preventive anti-stress products, dietary supplements and preparations from fresh leaves and powders from dried leaves as a source of gluten-free protein with a complete set of essential amino acids, vitamins and minerals to improve the digestive system, reduce hunger, and prevent obesity and calcium deficiency.

4. Conclusion

The leaves of the upper layer of Amaranthus paniculatus L. plants serve as a rich source of biologically active substances with antioxidant activity.

Aqueous extracts of amaranth leaves contain a pharmacological complex of antioxidant metabolites such as: amaranthine, ascorbic acid, flavonoids and phenolcarboxylic acids.

The peculiarities characteristic of vegetable leaf crops were found: the maximum number of studied antioxidant metabolites accumulates in the leaves of the upper layer and amaranth inflorescences.

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