OBTAINING ANTIOXIDANT PEPTIDES AND SUGARY SUBSTANCES FROM AMARANTH SEEDS

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ABSTRACT
With a view to the effective use of biopolymers of amaranth seeds, the processes for producing peptides with antioxidant properties from amaranth proteins and sugary substances from have been studied starch using proteolytic and amylolitic enzymes. It has shown that it is possible to produce peptides with various antioxidant properties, as well as sugary substances from starch, from amaranth proteins under the action of neutral proteinases. On the results, a principal scheme for the integrated treatment of Amaranth seeds was established.

Key words: amaranth, protein, starch, hydrolysis, enzyme, amylase, proteinase, peptides, sugar substances.

In recent years considerable attention is paid to the processing of amaranth seeds to produce biologically active substances [1]. This is due to that the composition of the amaranth seed is a raw material rich in biologically active substances, proteins, starch, vitamins (vitamins A, B1, B2 and C) and minerals compared to other cereals. A separate place on the amino acid composition is its protein composition. Amaranth proteins have a more balanced amino acid composition and therefore a higher biological value than most cereals [2].
Amaranth seed oil is of particular importance because of its biological properties. Amaranth seed oil consists of 46% Omega-6 fatty acids, 5% Omega-3 and 23% Omega-9. However, it contains 8-10% squalene with antioxidant and bactericidal properties [3].
In recent years, a great deal of attention has been paid to the processing of Amaranth seeds to produce amaranth oil and biologically active substances. Amaranth seeds contain 6-7% fat, which is mainly extracted by cold pressing and extraction. High pressure in the production of amarant oil during compression causes heating of the raw material and thus lowers the biological value of the components of the amaranth seeds.
The purpose of this study was to study the influence of hydrolytic enzymes on the production of peptides with antioxidant properties and the sugary matter from starch.

EXPERIMENTAL PART.

2. Materials and methods

2.1. Materials.

The work used the seeds of the «Kharkov», «Giant» and «Golden Giant» varieties of amarant grown in the Khorezm region of Uzbekistan.
The proteolytic enzymes used were the neutrase (Novozymes (Denmark) from Bacillus myloliquefaciens) and the sour proteinase Prolive PAC 30L (Enzymeproduct bioltd, Russia) from the fungus Aspergillus niger.
The amylolic enzymes used were α-amylase (Amilek 3T, LLC «Expo Tex», Russia) and β-amylase (Diazim4, LLC «Expo Tex», Russia).

Water-soluble proteins (albumines) extracted from amarant seeds under laboratory conditions, proteins soluble in 10% Nacl solution (globules), proteins soluble in 80% alcohol (prolamines), and proteins soluble in 0.2% Naoh solution (gluten).

2.2. Enzymatic hydrolysis of proteins.

Prepared 1% solution of the corresponding protein of 0.1 M universal buffer (pH 7.0 for neutral proteinase and pH 2.5 for acidic proteinase), added 0.1% neutrase. Mixture mixed and held for a certain time in the thermostat at a temperature of 30^0C, after which a sample of 2 ml was taken, 2 ml of TCC (trichloroacetic acid CCl_3COOH) was added to stop the enzymatic reaction. The solvents were then filtered through a paper filter and sampled 1 ml of leachate, adding 5 ml 0.5 M of sodium carbon dioxide solution. By mixing, 1 ml of Folin's working solution was added. Slightly laid-off solutions have a blue colour, the intensity of which was determined Photocolorimeter (at 670 nm wavelength) versus test sample in thick Ditches 10 mm [4]. The content of the products of hydrolysis (P) was determined by a calibration curve based on tyrosine.

2.3. Preparation of a sample of peptides.

During enzymatic hydrolysis of proteins for a certain time, the reaction mixture was selected 5 ml each, the samples were heated in a water bath and held for 5-10 min. to inactivate the enzyme. Then filtered through a paper filter and studied their antioxidant activity of hydrolysis products.

2.4. Determination of the antioxidant properties of peptides.

The antioxidant activity of peptides was estimated by the rate of oxidation (+)-catechine by iron ions at high temperatures in the presence and absence of peptides. The reaction medium of 0.1 M acetate buffer pH 4.2 contained 4 mM (+)-catechin, 20% ethanol, and 10 mg/l Feci3. The peptide content was 10 ml. The reaction medium was 0.2 ml. The reaction mixture was incubated in a thermostat at a temperature of 45oC for 15 days. Every day the optical density of the solution was measured on the photoelectric colorimeter at a wavelength of 440 nm [5]. The reduction of oxidation rate (+)-catechin was evaluated on the basis of the results.

2.5. Saccharification of starch of amaranth seeds.

After the proteolytic treatment of 200 g. of amaranth flour, the resultant products were separated by filtration and the residue was twice rinsed with water. Then 500 ml. distilled water added to the draught and the pH value of the medium was increased to 5.6 by hydrochloric acid. The mixture was then placed in a heat-resistant glass and added 0.3 ml of enzyme solution α-amylase (Amilek 3T). When mixed, the mixing temperature was raised to 85 °C and held at this temperature 30 min. They then lowered the temperature of the mixture to 50-53 °C and added 0.2 ml of the enzyme β-amylase (Diazim X4). At this temperature, they held for 30 minutes. The saccharated liquid produced was separated by filtration and measured in refractometric methods the dry matter content of the solution, and the reducing substances according to the method [6].

RESULTS AND DISCUSSIONS THEREON

Peptide antioxidants have recently become of great interest among numerous antioxidants of various origins used in the food industry. [7]

Many researchers have shown that peptide antioxidants can be obtained by enzymatic hydrolysis of various proteins. For example, some active peptide antioxidants and peptides recovering free radicals have been identified in various protein hydrolysates such as egg albumin [8], soy protein [9], and milk proteins such as α-lactathalbumin and β- lactoglobulin [10] etc.

One of the main advantages is that using different enzymes from the same protein source can produce peptides with different properties.

Amaranth seeds are also valuable raw materials for the production of various biologically active substances [3]. Some approaches to address the processing of Amaranth seeds using hydrolytic enzymes are outlined below.

Production of antioxidant peptides from amaranth seed proteins.

Water-soluble proteins (albumines), 3-10% soluble proteins (globulins), 60-80% alcohol-resistant proteins (prolamines) and 0.2% alkaline-soluble proteins are found in amaranth seeds. The protein composition of some cereal seeds is shown in (table 1).

Table 1: Cereal seed protein components (percentage of dry matter)

Corn crop	Total protein relative to dry substance, %	Including			
		Albumins	Globulins	Prolamins	Glutelins
Wheat	12-18	4	8	40	48
Barley	8-14	28	22	32	18
Corn	9-14	0,5	20	40	30
Amaranth	9-11	19	38	13	21

The table shows that amaranth seeds also contain all proteins, as do other cereals. The concentrations of albumin, globulin, prolamine and glutelin in amaranth seeds are 19, 38, 13 and 19 percent, respectively.

These proteins are hydrolyzed at different speeds with proteolytic enzymes. (Table 2) shows the activity of proteolytic enzymes obtained from different sources.

Table 2: Activity of various enzymes

№	enzyme	Relative activity of the enzyme, unit/g.	pH optimum
1	Acidic proteinase (Asp. oeryzae)	170	2,2-2,5
2	Neuralproteinase (Bacillus amyloliquefaciens)	860	6,7-7,0

The table shows that the activity of neutral proteinase is 5 times that of acidic proteinase and is 860 unit/g.

Other amaranth seed proteins are hydrolyzed at different speeds by the specified enzymes. The results are presented in (table 3).

Table 3: Rate of hydrolysis of proteins isolated from amaranth seeds by acidic and neutral proteins

enzyme	Rate of protein hydrolysis, mMol/hour			
	Albumins	Globulins	Prolamins	Glutelins
Acidic proteinase	0,1±0,01	0,3±0,02	0,4±0,02	0,80±0,03
Neuralproteinase	0,4±0,02	1,2±0,06	0,5±0,03	0,62±0,03

From the data presented, it can be seen that of the amaranth seed proteins, glutelins are hydrolyzed at the highest rate with acidic and neutral proteinase. The rate of hydrolysis of the

specified proteins is
0.8 and 0.62 µmol/h. Albumins are the slowest and globulins. The rate of hydrolysis of the above-mentioned proteins with acidic proteinase is less than 4 times that of the neutral proteinase. The hydrolysis of prolamine and gluteline by neutral proteinase did not differ sharply from acidic proteinase.
In further studies, we studied the antioxidant activity of the peptides produced. The antioxidant properties of the peptides obtained from the Amaranth seed proteins, presented in figure 1.

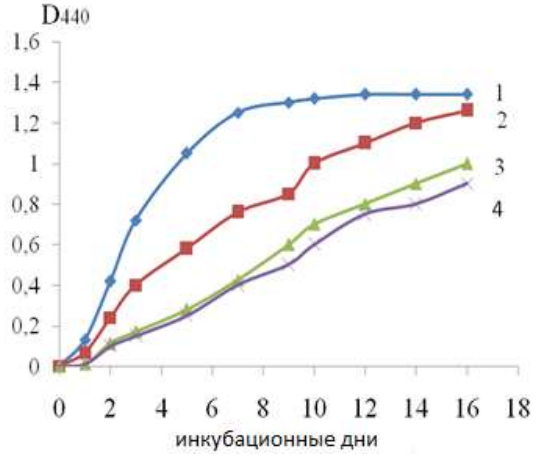
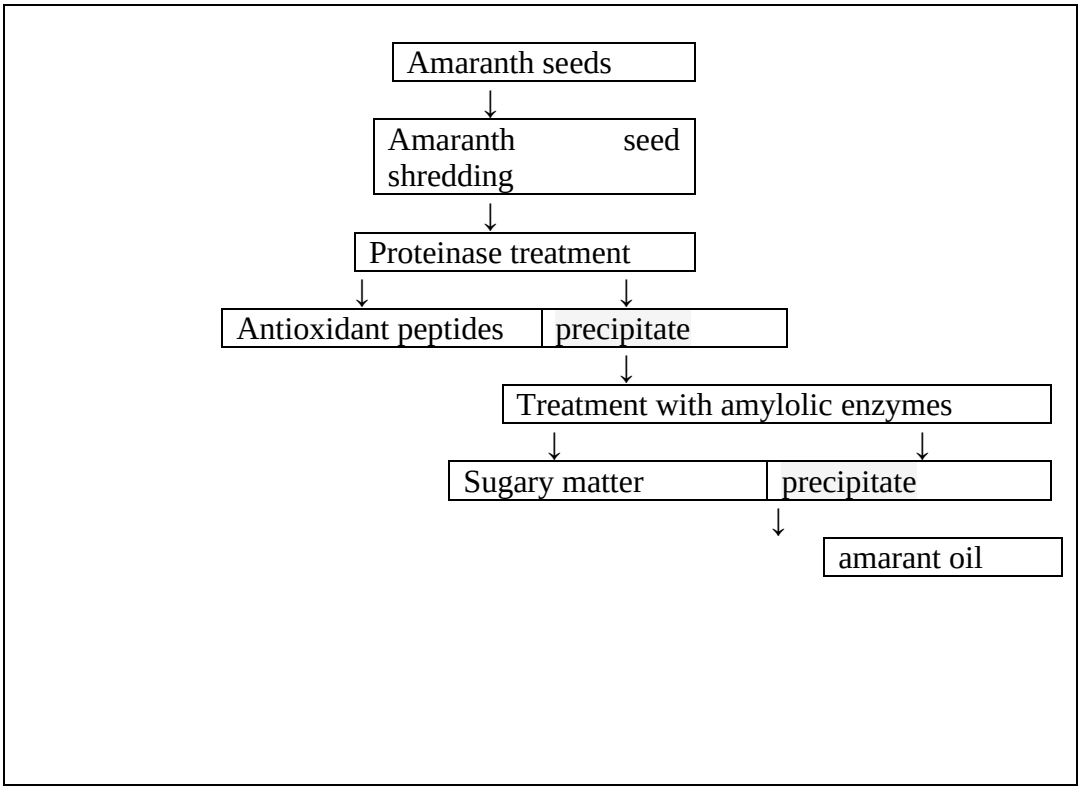


Figure 1. Influence of peptides on the rate of oxidation (+)-catechine in the presence of peptides produced by neutral proteinase.
1-check, 2-4 - in the presence of peptides derived from albumin, globulin and glutelin, respectively.
The presented data show that the optical density of the solution as a result of oxidation (+) - catechin at 45 °C for 10 days in the presence of iron ions is 1.3 units (Fig. 1, curve 1). When peptides (+) are added, the rate of oxidation of catechines decreases. In particular, the peptides produced from globulin and glutelin slow down the process, and the optical density of the solution is only 0.5-0.6 units. Thus, based on the proteins of the amaranth seeds, it is possible to produce peptides with antioxidant properties.
In the next part of our study, we analyzed the process of saccharification of the remaining starch after the hydrolysis of the proteins of the amaranth seeds.
Production of sugary substances from the starch of amarant seeds. After enzymatic removal of the Amarant seed proteins, 60-65% of the starch remains in the sediment. For their effective use, the processes of turning starch into sugary substances by the action of amylolic enzymes were investigated. Microbial enzymes were used as an enzyme. To do this, distilled water was added to the sediment after washing with water in a ratio of 1:2.5. Then, continuous mixing was added 0.3 ml α-amylase and started heating at 1o C per minute. Heated up to 85°C and kept at a temperature of 30 minutes. After complete starch hydrolysis, the mixture was cooled to a temperature of 50-53°C, and added β-amylase was kept at this temperature for 30 minutes. The saccharinated solution was then filtered. The chemical analysis of the sugared solution obtained is given in (table 4)
table 4: Coated Amarant Starch Juice

Enzyme to be used	Volume of solution, ml	Dry matter content, %	Reinstating sugars (relative to dry matter),%
after treatment with α-amylase	480	22	27

after treatment α- and β- amylase	470	23	52

On the basis of the results, the following Amaranth seed processing principle scheme is recommended.



Thus, the enzymatic treatment of amaranth seeds in a certain sequence could be obtain from peptide proteins with antioxidant properties and from starch sugars. Waste is solid residues containing lipids from which lipids could be extract by extraction.

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