

Antioxidant Activity of Peptides Obtained from Cotton Ground Oil-Cake Proteins

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Abstract: Antioxidant activity of the peptides derived from proteins of defatted cottonseed kernels and cotton ground oil-cake by their enzymatic hydrolysis with acidic (*Asp. niger*) and neutral proteinases (*Bac. amyloliquefaciens*) was studied. Antioxidant activity of the derived peptides depended on the used proteins and enzymes. The peptides derived by using of neutral proteinase possessed higher antioxidant activity, in comparison with the peptides derived by acidic proteinases.

Key words: Proteins, cotton ground oil-cake, hydrolysis, acid and neutral protease, peptides, antioxidant activity.

1. Introduction

Antioxidants are an important group of food additives that have the ability to protect against detrimental change of oxidizable nutrients and consequently they extend shelf-life of foods [1].

Various natural and synthetic preservatives and antioxidants are used for extending the storage period of end products. Great attention is also paid to the use of natural antioxidants based on polyphenols [2-5] and peptides derived from plant materials [6-8].

Some active peptide antioxidants and peptides that can utilize free radicals are identified in various hydrolysates of proteins such as ovalbumin [8], soybean protein [9], milk proteins such as α -laktalbumin and β -lactoglobulin [10, 11] etc.

One study that aims at production of antioxidant peptides [12] focuses on the effect of various enzyme preparations on the enzymatic hydrolysis of defatted peanut kernels. Hydrolysates obtained by esperase possess higher antioxidant than the ones, produced with neutrase, pepsin, protease A and protease N. Antioxidant activity is measured kinetically, using linoleic acid. The molecular weight of the peptide

derived with esperase ranged from 3 kDa to 5 kDa. Antioxidant activity was 3 times higher than that of ascorbic acid.

Cottonseed (*Gossypium*) is one of the important oilseed crops in Uzbekistan. The main by-product of the oil extraction process is cottonseed ground oil-cake, which has relatively high protein content of 35%-40%, making it an attractive and promising source of vegetable proteins. However, the presence of anti-nutritional compounds is a major drawback in the use of this bioresource as human food. Hence, it is usually used as an animal feed.

The aim of this work is a comparative study of the antioxidant activity of peptides derived from proteins of cotton ground oil-cake.

2. Materials and Methods

Neutral proteolytic enzyme preparation from *Bacillus amyloliquefaciens* (Neutrase, "Novozymes", Denmark) and acid proteolytic enzyme preparation from fungus—*Aspergillus niger* (Prolive PAC 30L "EnzymeBioProduct" Ltd Russia) were used. Proteins isolated from defatted cottonseed kernels and cotton ground oil-cake were used as substrate. Substrates were water-soluble and salt-soluble (10% NaCl).

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2.1 Enzymatic Hydrolysis of Proteins

1% solution of the corresponding protein in 0.1 M universal buffer, pH 4.2 (in the case of acid proteinase) and pH 7.0 (in the case of “neutrased”) were prepared and a 0.1% proteinase solution was added. The mixture was stirred and kept for some time in a thermostat at 30 °C, then 2 mL TCA (trichloroacetic acid) was added to 2 mL of the sample in order to stop enzymatic reaction. Then the settled solution was passed through a paper filter and to 1 mL of filtrate 5 mL of 0.5 M solution of sodium carbonate was added. While stirring, 1 mL of working solution of Folin was added. The intensity of the blue coloring was measured with a photoelectric colorimeter at 670 nm against the control sample in a 10 mm cuvette [13] (GOST 20264.2-85, 1985). The content of hydrolysis products (*P*) was determined by a calibration curve, obtained using tyrosine.

2.2 Sample Preparation

5 mL fractions of the reaction mixture were collected at 0, 1st, 2nd, 4th, 6th, and 8th hour. Sample fractions were heated in a water bath for 5-10 min to inactivate the enzyme and passed through a paper filter.

The effect of peptides in the samples on the rate of oxidation of (+)-catechin was determined in model system, as well.

2.3 Measurement of Rate Oxidation of (+)-catechin

(+)-Catechin (4 mM) was used as an internal standard for quantification. It was dissolved in acetate buffer (0.1 M, pH 4.2) containing ethanol (20%, v).

The rather high ethanol concentration was chosen in order to avoid microbial development during storage of the solutions. Ferrous chloride was added to give final iron concentrations of 10 mg/L. The content of hydrolysis products of protein in 10 mL incubation medium was 0.2 mL [14].

Browning of the solutions (40 °C) was estimated by measuring the increase in absorbance in a 10 mm cuvette at 440 nm using a KFK-2-YXL 4.2 photoelectric colorimeter (Russia).

3. Results and Discussion

It is known that the protein content of cotton seed core ranges between 25% and 38%. Thus, albumin and globulin is 90% of total amount of protein.

These proteins are hydrolyzed by acidic and neutral proteases with different rate.

Table 1 shows the comparison of hydrolysis rate of water-soluble and salt-soluble proteins isolated from defatted cottonseed kernels and cotton ground oil-cake.

The presented data show that the initial rate of hydrolysis of albumin and globulin of defatted cottonseed kernels with acidic proteinase was nearly the same. Enzymatic hydrolysis of albumin with neutral proteinase goes deeply than previous one and the hydrolysis rate was 0.4 mkmol/h, and for globulin it was 0.26 mkmol/h.

Heat treatment of cottonseed kernels promotes change of hydrolyzability of proteins. Thus, the rate of hydrolysis of albumin with acidic and neutral proteinase was decreased 8-9 times and it was 0.07 mkmol/h and 0.05 mkmol/h, respectively. Globulin

Table 1 The hydrolysis rate of proteins which extracted from defatted cottonseed kernels and cotton ground oil-cake (mkmol/h).

Enzyme	Protein source			
	Defatted cottonseed kernels		Cotton ground oil-cake	
	albumin	globulin	albumin	globulin
Acidic proteinase	0.58 ± 0.25	0.53 ± 0.023	0.4 ± 0.02	0.80 ± 0.03
Neutral proteinase	0.4 ± 0.02	0.26 ± 0.01	0.05 ± 0.003	0.62 ± 0.03

Composition of the reaction medium: protein content 1%, temperature 37 °C, pH-7.0 in the case of neutral proteinase and pH-2.8 in the case of acid proteinase.

Table 2 The effect of washing conditions of cotton ground oil-cake on hydrolysis of proteins.

The pH of water	The content of amino acids, mkmol/mL
3.5	0.7 ± 0.03
7.0	0.8 ± 0.03
9.0	1.2 ± 0.04

derived from cotton ground oil-cake became more hydrolyzable with acidic and neutral proteinases. The hydrolysis rate by acidic and neutral proteinases is 0.8 and 0.62 mkmol/h, respectively.

It should be noted, that at processing of cotton seeds according to conventional technology, there are many factors adversely affecting to catalytic properties of the enzymes.

We preliminary washed cotton ground oil-cake to remove accompanying substances with water of different pH values (Table 2).

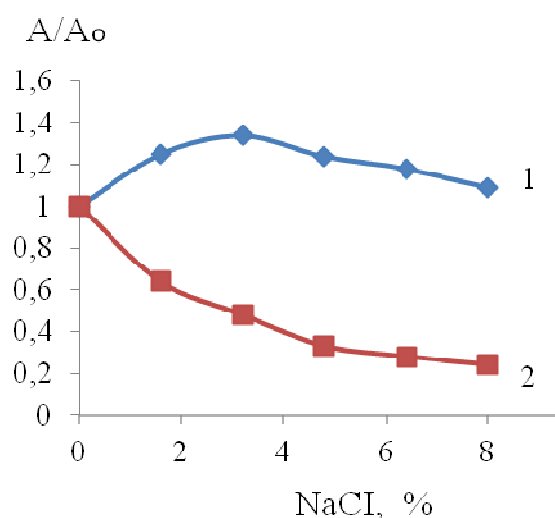
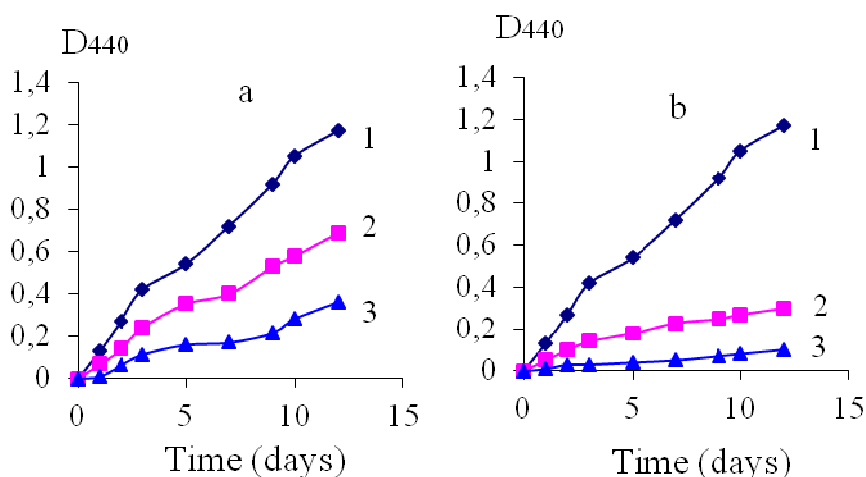
The data presented show that washing of cotton ground oil-cake with hot water with pH 9.0 gives the best results. In this case this hydrolyzability of protein is increased 1.4-1.7 times in comparison with water at pH 3.5 and pH 7.0.

Fig. 1 shows the effect of sodium chloride ions on the activity of acidic and neutral proteinases in the hydrolysis of cotton ground oil-cake proteins.

Sodium chloride ions have a positive effect on the

activity of acidic proteinases. The rate of hydrolysis of cotton ground oil-cake proteins at concentration of NaCl 3%, is increased by 1.3-1.4 times. At this concentration, neutral proteinase loses 50% of activity.

The products of hydrolysis of proteins isolated from cottonseed kernels and cotton ground oil-cake have different antioxidant properties. The antioxidant activity of peptides derived from cottonseed kernels and cotton ground oil-cake is shown in Fig. 2.


Fig. 1 The effect of sodium chloride on the activity of acidic (1) and neutral (2) proteinases in hydrolysis of cotton ground oil-cake proteins.

Fig. 2 The effect of peptides derived from globulin with acid and neutral proteases on the rate oxidation of (+)-catechin.

a—globulin from defatted cottonseed kernels; b—globulin from cotton ground oil-cake; 1—control; 2—peptides derived with acidic proteinase; 3—peptides derived with neutral protease.

The presented data show that the reduction of the oxidation rate (+)-catechin observed with all peptide samples. Significant difference can be seen in the case of peptides obtained by the globulin hydrolysis with various enzymes. The globulin hydrolysis products obtained with acidic proteinases have lower antioxidant activity and the (+)-catechin the oxidation rate in model systems decreases slightly (Fig. 2a, curve 2) as compared with control.

Peptides obtained by using neutral proteinase have the highest antioxidant activity (Fig. 2b, curve 3). The presented data show that reducing the oxidation rate of (+)-catechin in this case is much lower than if medium have peptides derived from globulin by using an acidic proteinase.

Thus, cotton ground oil-cake proteins can be used to obtain peptides with antioxidant properties.

4. Conclusion

Enzymatic hydrolysis of cottonseed proteins with acidic and neutral proteinases leads to obtaining peptides with different antioxidant properties. It was shown that antioxidant activity of peptides depends on a type of proteolytic enzyme. In the case of cotton ground oil-cake globulin, antioxidant activity of peptides, produced with neutral proteinases was by 2-3 times higher than the one of peptides derived with the acidic proteinase. In all cases, peptides, obtained by using acidic proteinase, reduced the oxidation rate of (+)-catechin a little.

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