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STUDY OF THE PEPTIDE ENZYMATIC HYDROLYSATES OF WHEY PROTEIN CONCENTRATE COW'S MILK TO DEVELOP FOODS FOR TOURISM AND RECREATION

The optimal conditions for pretreatment protein substrates (mixture of cow's milk whey proteins) and their hydrolysis by proteases of various classes for the target peptide fractions were determined.

Were obtained by enzymatic hydrolysis of the whey protein of cow's milk peptide fraction; qualitative and quantitative composition of the peptides were defined. Alcalase (a serine protease) in comparison with papain and neutrase (metal and cysteine protease) provides the most efficient hydrolysis of the major protein allergens whey: β-lactoglobulin, α-lactoalbumin. BSA removal is achieved by ultrafiltration or pre-heat treatment of the substrate. Optimum conditions for obtaining whey protein hydrolyzate is a mixture of pre-heat treatment of the substrates at 80°C for 10 min (pH 8.0), and subsequent cleavage of alkalas duration 120 min. At the same time there is a reduction in antigenicity of 20–25 times. It was established the absence in heat-treated hydrolysates bivalent antigenic determinants. Assessed the sensitizing effect of whey protein concentrate and milk enzymatic hydrolyzate.

Key words: tourism and recreation, whey protein concentrate, enzymatic hydrolyzate, peptides, antigenicity.

Introduction. The Republic of Belarus attaches great importance to the development of tourism and recreation activities. One of the most important aspects of tourism and recreational activities is a balanced and rational food which should include dairy products. On the basis of milk and milk products a variety of dietary and specialized food products were developed, including those who want to improve their health [1].

It was determined during the last two decades that milk proteins are source of biologically active peptides [2, 3]. Milk proteins also modify a wide range of physiological reactions [4].

However, native milk proteins have significant antigenicity related to their tertiary structure, that leads to allergization of the body, primarily children. It also represents a risk factor for the development of diseases such as atopic dermatitis and bronchial asthma [5].

In this regard, the task of reducing the allergenicity of milk proteins is of current interest.

Various technological processes of treating milk such as heat, high pressure, enzymatic hydrolysis and others can modify the allergenic action of food products [6, 7].

We have studied the possibility of reducing the antigenic properties of milk proteins of whey protein concentrate (WPC) by a combination of heat treatment and proteolysis.

Main part. Basic protein allergens were used as a source of WPC.

For the hydrolysis protein substrates were applied, such as β-lactoglobulin (β-LG), α-lactoalbumin (α-LA), bovine serum albumin (BSA) and serine protease (alcalase, KF 3.4.21.62, protease from *Bacillus licheniformis*, activity 2.64 E/g), aspartic protease (papain, KF 3.4.22.2 isolated from *Papaya latex*, activity 20 E/mg); metalloprotease (neutrase, KF 3.4.24.28, protease from *Bacillus amyloliquefaciens*, activity of 0.9 E/g) produced by Sigma (USA); WPC obtained by ultrafiltration method (WPC-UV-80, TU BY 100377914.550–2008).

The method presented in work [8] served as a base for electrophoretic separation of milk proteins and their enzymatic hydrolysates.

The study of the molecular weight distribution of peptides was carried out with the help of Bruker Microflex (Bruker, USA). The analysis was performed in the range between 1–66 kDa.

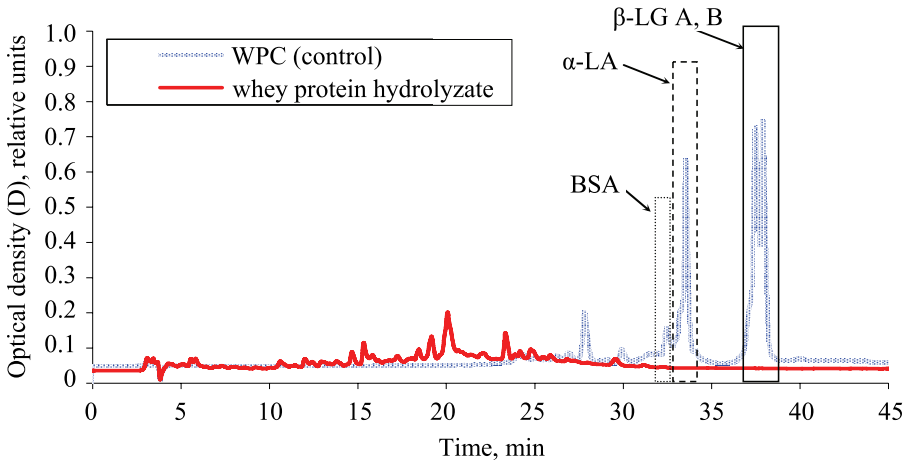
Diagramming and mathematical processing of the results was carried out using computer programs Microsoft Office Excel 2003 (Microsoft Corporation, USA) [9]. The significance of differences between samples of the experimental data were determined using the U Mann – Whitney test at the significance level of $p < 0.05$ [10, 11].

Results and discussion

Experimental data on the properties of the basic substrate of whey proteins during hydrolysis by different proteases such as alcalase, papain and natrosol were obtained.

Papain (KF 3.4.22.2) is a hydrolytic enzyme of the cysteine class of proteases, extracted from the green papaya (*Carica papaya*).

It was determined that about 60% β-LG and only 30% α-LA split on the intermediate peptides at the end of the enzymatic process, whereas BSA remains in all the samples of hydrolysates.



HPLC profile of enzymatic hydrolysate of milk whey proteins:
β-LG A, B are β-LG genetic variants

Neutrase (KF 3.4.24.28) is a proteolytic enzyme of the metalloprotease class, where producer is *Bacillus amyloliueaiens*. We present a method of producing serum protein hydrolysate based on the use of neutrase. According to the results of the electrophoretic analysis of the native whey proteins in 2 hours after proteolysis about 80% β-LG, 30% α-LA split into intermediate products, whereas BSA is resistant to hydrolysis by neutrase. In this regard, the efficiency of proteolysis of whey proteins by natrosol decreases in the series β-LG → α-LA → BSA. The number of fractions with $mr \leq 10$ kDa in the given hydrolysate is (48.0 ± 1.9)%.

Alcalase (serine endopeptidase, KF 3.4.21.62, or subtilisin), is a proteolytic enzyme obtained from *Bacillus subtilis*. It has been shown that practically all β-LG and α-LA undergo hydrolysis (90 min), and in 120 min after enzymatic reaction only trace amounts of whey proteins and intact BSA were found.

The degree of proteolysis of BSA does not change significantly, which indicates the low efficiency of hydrolysis of this substrate by alcalase. Analysis of the peptide composition of the WPC hydrolysate by alcalase showed the formation of discrete peptide fractions with $mr < 6.5$ kDa, which is significantly reduced after 90 and 120 min of proteolysis. In addition, the proportion of hydrolyzed fractions with $mr \leq 10$ kDa reaches (93.0 ± 0.6)%.

Modification of the WPC hydrolysis by alcalase with pre-heating of the samples up to 80°C for 10 min at pH 8.0 allowed us to obtain the hydrolysate with an almost complete proteolysis of β-LG, α-LA and BSA on intermediate peptides (Figure).

The study of the peptide profile obtained by high-efficiency liquid chromatography allowed us to determine that it does not contain whey proteins (Figure).

Conclusion. System of enzymatic hydrolysis of WPC using alcalase and samples preheating was developed allowing to obtain hydrolyzate with almost complete degradability of serum protein of BSA, α-lactoalbumin and β-lactoglobulin, which are extremely allergenic proteins. The experimental studies showed a significant reduction in the antigenicity of the obtained hydrolyzate, which can be further used to develop new functional hypoallergenic and health food products.

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