RESEARCH OF THE PROCESS OF ENZYMATIC HYDROLYSIS OF MILK WHEY PROTEINS AND SOY PROTEINS

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The paper deals with studies of enzymatic proteolysis of whey protein concentrate (WPC) and isolated soy protein (ISP) with food enzymes for the further creation of products for special dietary purposes. It has been shown that the use of the discrete pulse energy input method, developed at the Institute of Engineering Thermophysics of the Ukraine National Academy of Sciences, is an effective way to restore and dissolve high-protein products in an aqueous environment. The method makes it possible to reduce the duration of their recovery several times and improve the unsolubility index for ISP by 25% and for WPC by 33%. The dependence of the degree of hydrolysis of ISP and WPC on the mass fraction of the enzymes Protamex, Protease C and ORBAproteo P-1200 was studied. It was determined that hydrolysis of proteins at a mass fraction of enzyme of 5% (of the protein mass), a mass fraction of protein in an aqueous solution of 7.3-8.1%, a temperature of 55-57°C and pH of 6.1-6.2 for 60 min allows to obtain a degree of hydrolysis for ISP 65-75% with Protamex, 65-68% with Protease C and 55-60% with ORBAproteo P-1200. Under the same conditions, the degree of hydrolysis of WPC was 60-65% with Protease C, 50-52% with Protamex and 45-48% with ORBAproteo P-1200.

Keywords: whey proteins, soy proteins, proteolysis, degree of hydrolysis

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Introduction

One of the powerful factor in preventive and therapeutic nutrition is protein support, which plays an extremely important role in restoring the body's strength. The results of numerous studies confirm that proteins exhibit particularly high efficiency in their hydrolyzed form – a mixture of peptides of different molecular weights and free amino acids obtained by enzymatic treatment. Due to their high nutritional and biological value, easy digestion and low incidence of allergic reactions, protein hydrolysates contribute to an accelerated period of rehabilitation in the complex therapy of a number of diseases that require enhanced protein nutrition, rapid regeneration of body proteins, and building muscle tissue (Chalamaiah et al., 2018). Protein hydrolysates are often included in a number of dietary products and products for special medical purposes, namely: enteral formulas, human milk formulas, protein supplements for sports nutrition, etc.

In products for special medical purposes, which may be the only source of nutrition for a long period, the protein component, as a rule, consists of fully or partially hydrolyzed whey proteins, which are the most complete and easily absorbed by a weakened body. They are most often consumed for protein-energy malnutrition, disorders of the gastrointestinal tract, metabolism, burns and allergic conditions (for example, Peptamen AF Neutral Dual, Switzerland (Zadak et al., 2009; Dullius et al., 2018).

Partially or extensively hydrolyzed whey proteins are included in hypoallergenic human milk replacers for infants and young children with allergies to milk proteins or soy proteins,for instance Pepti (Nutricia, the Netherlands), Humana HA 1 with LC PUFA (Humana, the Netherlands, etc.) (Fleddermann et al., 2024). The advisability of their consumption has been confirmed by numerous clinical studies (Rigo et al., 2019).

The popularity of an active lifestyle and sports throughout the world and in Ukraine has led to a growing demand for sports nutrition products – a special group of products and safe dietary supplements that complement the main diet and consist of concentrated nutrient complexes. Among them, high-protein mixtures are in particular demand, as well as gainers designed to accelerate the growth of muscle mass, containing 20-40% proteins, most often fast digesting whey proteins with varying degrees of hydrolysis, for example, Platinum Hydrowhy (Optimum nutrition, USA, etc.) (López-Martínez et al., 2022; Manninen, 2009).

On the Ukrainian market, most products containing hydrolyzed proteins are represented by foreign manufacturing corporations (Danon, Nutricia, Nestle, Optimum Nutrition, etc.). Ukrainian manufacturers offer whey protein concentrates (WPC), but in non-hydrolyzed form, for example: WPC–UF–65 (Tekhmolprom LLC, Gadyach), WPC–70 (CJSC «Buchach Cheese Factory», Buchach), etc. The product is offered as a sports nutrition supplement.

Varying the operating parameters of protein hydrolysis and the type of protease allows for targeted structural modification of proteins to the required degree of hydrolysis, improving the functional and technological properties of proteins and energy saving conditions as well (Tang, et al., 2023).

Whey and soy proteins and their mixtures are widely used as hydrolysates due to their balanced amino acid composition (with the exception of the deficiency of sulfur-containing amino acids in soy proteins) and high substrate specificity to the action of serine proteases. The proteolytic enzymes Alcalase, Protamex, Flavourzyme, Neutrase, pancreatin, trypsin, chymotrypsin, thermolysin, papain and bromelain are the most commonly used commercial enzyme preparations that break down protein into peptide fragments containing 2–20 amino acids (Islam et al., 2022; Avdieieva, et al. 2019).

To obtain hydrolyzed products in dry form, spray drying is often used, which helps reduce specific energy costs. Due to high rates of heat and moisture transfer and the occurance of most heat and mass transfer processes at wet-bulb temperature, dehydration conditions make it possible to obtain powder materials with improved structural and physicochemical properties. When spray drying, the thermal effect on proteins is minimal, which allows the biological value of proteins to be preserved.

After proteolysis, the viscosity of solutions of hydrolyzed proteins decreases. Thus, in (Murphy et al., 2015; Dermiki et al., 2020) it was shown that the viscosity of the infant formula, which contained β-lactoglobulin hydrolyzed by trypsin, was significantly lower (14.8 mPa·s) than the viscosity of mixtures with non-hydrolyzed and partially hydrolyzed protein – 48.6 mPa·s and 27.6 mPa·s, respectively. This makes it possible to use standard pumps of lower power when feeding a hydrolyzed protein solution to spray drying and thus further reduce energy costs.

In addition, as a result of the hydrolysis of β-lactoglobulin with trypsin, biologically active peptides are formed that can indirectly increase insulin secretion, which also confirms the advisability of using whey protein hydrolysates in special-purpose products (Fernández et al., 2013).

After hydrolysis of soy proteins with Protamex (Novozymes, Denmark), the solution of hydrolyzed proteins has fairly high solubility index (84%), emulsifying (95 m^2/g) and antioxidant activity (62%), foaming (94%) and fat-holding capacity (4.5 g/g). After hydrolysis with Alcalase and Protamex, the majority of soy protein hydrolysates contain low molecular weight peptides (150–700 Da) (Islam, Huang et al., 2022). However, soybean hydrolysates with a molecular weight of 2–4 kDa are also known to have a bitter taste (Myong et al., 2004), which can be reduced by microencapsulating mixtures such as WPC with sodium alginate (Ma et al., 2014) or maltodextrin with β-cyclodextrin (Yang et al. 2012).

In the conditions of increasing demand for domestic products with hydrolyzed proteins, it is important to continue research into the proteolytic effect of enzyme preparations and their complexes, including those produced domestically, in order to obtain high-quality protein hydrolysates with specified properties while observing the factors of energy efficiency and economic feasibility factors.

The purpose of the work is to determine the optimal energy-saving technological parameters for the enzymatic hydrolysis of whey and soy proteins.

Materials and Methods

Protein products:

- isolated soy protein (ISP) Supro 500E («Solae Europe, S.A.», USA), containing mass fractions: protein 90.0 %, fat 0.5 %, ash 5.5 %, moisture 4.0%, pH 6.9-7.6;
- whey protein concentrate WPC-80 («Agri-Mark Inc.», USA), containing mass fractions: protein 82.5%, fat 5.2%, ash 2.3%, carbohydrates 6.0%, moisture 4.0%.

Proteolytic enzymes:

- Protamex (Novozymes, Denmark) is a neutral protease obtained from the strain *Bacillus licheniformis* and *Bacillus amyloliquefaciens*, the temperature optimum is t = 35-60°C, the optimum pH is 5.5-7.5, enzyme activity is 1.5 AU/g;
- Protease C (Plant of microbiological synthesis preparations manufacture «Enzyme», Ladyzhyn, Ukraine) is fungal alkaline protease, temperature optimum is t = 30-70 °C at pH 8-10 (pH 5-11), enzyme activity is 50 000 units/g;
- ORBAproteo P–1200 (China) is enzyme Protease produced by the *Bacillus subtilis* strain, temperature optimum is 20-50 °C, pH 5-8, inactivation temperature – t = 60 °C, protease activity 3500 PU/g.

The degree of protein hydrolysis (DH) was determined by spectrophotometric method at wave λ=280 нм (Lazarus, 1973); DH is presented as a percentage of the total protein mass in the sample.

The insolubility indices of ISP and WPC were determined according to ISO 8156:2005 Dried milk and dried milk products – Determination of insolubility index.

Results and Discussion

One of the key technological stages, which subsequently has a significant impact on the rate and degree of protein proteolysis, the hydrolytic reaction, is the process of protein recovery in an aqueous environment to ensure maximum action of proteases. Albumins, which make up about half of whey proteins, due to the large number of polar groups on the surface of the molecules easily bind to water molecules, have a large hydration shell and, accordingly, solubility. Soy proteins, which are 85-95% globulins, are much less soluble in water. In addition, a number of other factors influence the solubility of proteins: pH of the medium, the amount of electrolytes present, temperature, method of obtaining proteins, etc.

One of the energy-saving methods for protein recovery is the discrete pulse energy input (DPEI) method, developed at the Institute of Engineering Thermophysics of the Ukraine National Academy of Sciences. In rotary pulsation apparatus (RPA), in which this method is implemented for the restoration of dispersed systems, there is an intensification of hydrodynamic and heat and mass transfer processes and a maximum reduction in overhead costs due to the acceleration of interphase heat and mass transfer and the rational use of energy in comparison with traditional devices and methods for recovery of milk powder (Dolinskyi et al., 2008). As a result of exposure to DPEI, the homogeneity of the reduced disperse system increases, an intensive reduction in the size of dispersed particles occurs and, accordingly, an increase in the surface area. This, in turn, makes it possible to improve the conditions of proteolysis and increase the activity of proteases.

The efficiency of recovery of ISP and WPC using the DPEI method can be indirectly judged by their solubility indicators. When ISP was reduced using the standard method, the insolubility index was 6.0 cm^3 of insoluble precipitate, while after DPEI treatment in RPA its value decreased by 25% and amounted to 4.5 cm^3. WPC with a insolubility index of 0.3 cm^3 under the same conditions dissolved almost completely and amounted to 0.1 cm^3 of insoluble sediment.

An important parameter influencing the rate of proteolysis is the mass fraction of the protease ($\omega_{protease}$) in the aqueous solution of the substrate. If the amount of enzyme is insufficient, proteolysis will last longer, which will accordingly increase energy consumption to maintain the temperature of the solution, and the depth of protein hydrolysis may be insufficient. As $\omega_{protease}$ increases, the DH may increase slightly, while the cost of the product will increase.

The dependences of the DH of ISP and WPC on the mass fraction of Protamex, Protease C and ORBAProteo P–1200 in the range $\omega_{protease}$=1–5% (by weight of protein), with the mass fraction of protein in water $\omega_{protein}$ =7.3% for WPC and $\omega_{protein}$=8.1% for ISP were experimentally studied. Protein hydrolysis was carried out in the optimal temperature range for these enzymes 55–57°C during 60 min. The pH of the initial aqueous solutions of proteins was 6.1 for WPC and 6.2 for ISP. As can be seen from Figure 1, in all samples, with an increase in $\omega_{protease}$, the degree of DH also increases, but to varying degrees depending on the type of substrate and enzyme.

As can be seen from the data in Figure 1a, the highest DH value for soy proteins was obtained under the action of Protamex, which exhibits a fairly high enzymatic specificity for soy proteins. At $\omega_{protease}$=1%, the DH of ISP was about 54–55% and reached DH=65–75% at $\omega_{protease}$=5%. The effect of the fungal protease Protease C under the same conditions on ISP is somewhat less: DH = 48% at $\omega_{protease}$=1% and DH=65–68% at $\omega_{protease}$=5%, which can be explained by the fact that hydrolysis was carried out at pH 6.2, which is somewhat below the optimal pH 8–10. When hydrolyzed with ORBAproteo P–1200, the accumulation of peptides and free amino acids occurred less; the degree of ISP hydrolysis at $\omega_{protease}$=5% after 60 min did not exceed DH = 55–60%.

The results of WPC proteolysis (Figure 1b) indicate that Protease C has the highest specificity for whey proteins among the selected enzymes: up to DH=60–65% with $\omega_{protease}$=5%. The effect of Protamex and ORBAproteo P–1200 was less, the degree of their hydrolysis was within the limits of DH=52% and DH=48%, respectively, with $\omega_{protease}$=5%.

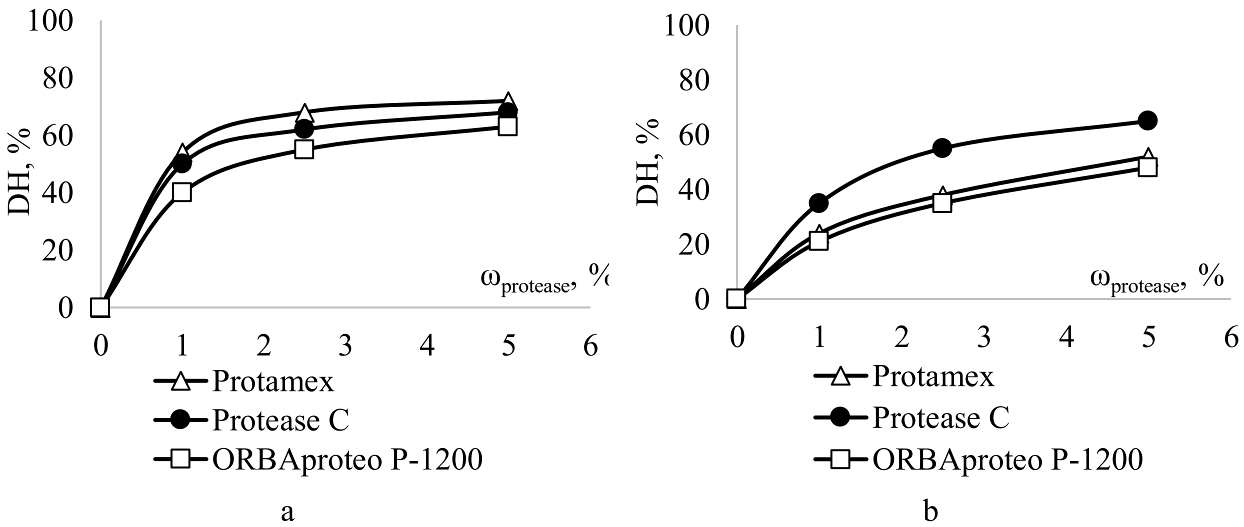

Figure 1. Dependences of DH of ISP(a) and WPC (b) from $\omega_{protease}$

Increasing $\omega_{protease}$ to 10% does not lead to a significant increase in the hydrolysis products of both ISP and WPC. The decrease in the rate of enzymatic reactions over time is due to the drop in the concentration of the substrate and the effect of the reverse inhibitory reaction, which can be significant as the concentration of the products of the enzymatic reaction increases.

Conclusions

The development of technologies for products containing proteins in hydrolyzed form remains a promising area in the domestic food industry.

The use of equipment developed at Institute of Thermal Engineering of Ukraine National Academy of Sciences allows to improve the technological properties of proteins during their recovery.

Rational energy-saving operating characteristics of the enzymatic hydrolysis of whey proteins and soy proteins have been determined. The results of experimental studies of the process of enzymatic hydrolysis of ISP and WPC with proteolytic enzymes Protamex, Protease C and ORBAproteo P–1200 showed the high specificity of the selected proteases to proteins.

Carrying out proteolysis at $\omega_{protein}$=7.3–8.1% (of protein weight), $\omega_{protease}$=5%, temperature t=55–57°C, pH 6.1–6.2 for 60 min allows ISP to be hydrolyzed to DH=65–75% with Protamex, DH=65–68% with Protease C and DH=55–60% with ORBAproteo P–1200.

Under the same conditions, the DH for WPC was DH=60–65% when hydrolyzed with Protease C, DH=50–52% when hydrolyzed with Protamex and DH=45–48% when hydrolyzed with ORBAproteo P–1200.

The introduction of more proteolytic enzymes is impractical both because of their high cost and from the point of view of technological conditions.

The obtained results can be used in the development of technologies for special or dietary products.

Conflict of interest

The authors state no conflict of interest.

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