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Improving the properties of fodder potato protein concentrate by enzymatic hydrolysis

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ABSTRACT

Protein hydrolysates of profitable properties were prepared from the fodder potato protein concentrate. The hydrolysis process was performed with the use of commercial available enzyme (Alcalase) over a 2 and 4 h incubation period. Chemical and amino acid composition as well as functional properties of resultant hydrolysates were determined. A 2 h long process occurred profitable to obtain preparations of well balanced amino acid composition as well as proved functional properties. The industrial preparation, modified within proteolytic enzyme, totally soluble (average 98%), was characterised by fivefold higher oil holding capacity (average 5.4 cm^3/g) and much better foam capacity (more than 150%) as compared to the material underwent modification (13.00%, 2.1 cm^3/g and 5.33%, respectively). Presented results suggested potential use of fodder potato protein not destined directly for food purposes as the suitable product for preparations characterised by high nutritive value and functional properties.

1. Introduction

In the process of potato starch production, large amounts of potato juice are produced. They can reach even to 7 m^3/tonne per tuber, which depends on the processing technology. As far as the waste water is concerned, it is a complex mixture of several components, where about 20–25% of dry matter is protein. In starch industries, the most common method applied to produce the protein preparations is thermal precipitation (in excess of 90 °C). It involves decreasing the pH as well as decanting and drying the concentrate (Pastuszewska, Tuśnio, Taciak, & Mazurczyk, 2009). Sometimes, in larger industries the latter process is preceded by initial concentration taking place with the help of reverse osmosis. Thermally obtained potato protein concentrates, refined as the by-products, are characterised by the high content of total protein and essential amino acids as well as by the light colour. They also contain low amounts of ash, low solubility along with the water and oil absorption which depends on the coagulation method. The above mentioned method limits the usage of potato protein concentrates in human foods (Løkra & Strætkvern, 2009). Moreover, in the effect of deterioration of the protein preparations' functional properties, they are hardly digestible to humans. However, since 2002 according to the Commission Decision, the protein preparations have been authorised as novel food products, which enabled to improve their functional properties (Commission Decision, 2002).

The demand to improve the industrially proceeded protein food products is connected with the change of their functional properties. Among many methods, the one that has been applied in this case was thermal coagulation with the increased ionic strength (Knorr, 1980), different pH and heat treatments (van Koningsveld et al., 2001). The membrane techniques were also used, such as ultrafiltration by membranes retaining the substances of appropriate MW and by chromatography (Zwijnenberg et al., 2002). The above methods impaired such useful functional properties found in the food industry as protein solubility and water or oil holding capacities.

The enzymatic hydrolysis, or peptide bond hydrolysis, is one of the most successful form of protein modification. It is often used to yield smaller protein products with the use of more uniform molecular size. Due to a decrease of the size along with the increase of the ionizable groups of α-NH_4^+ and α-COO^-, the resulting peptides are more water soluble and more amenable to further modification. To add to this, owing to a careful control of the hydrolysis process at well defined stages, it is possible to produce hydrolysates with different degrees of hydrolysis and different functional properties. There are several factors which can affect the physical and chemical properties of resultant hydrolysates.

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Among them, there is a choice of substrate, the protease enzyme employed in the process and the degree of hydrolysis (Adler-Nissen, 1986).

During the enzymatic modifications such various proteases as Alcalase (Zhao et al., 2012), Flavourzyme (Clemente et al., 1999), Pepsine (Viera, Martin, Saker-Sampaiao, Omar, & Gonsalves, 1995), Trypsine (Zhao et al., 2012) and Papaine (Hoyle & Merritt, 1994) have been applied to prepare protein hydrolysates. When it comes to food protein hydrolysis, Alcalase is considered to be the most important proteolytic enzyme, obtained through fermentation using *Bacillus licheniformis* strains.

Furthermore, it shows high thermostability (optimum temperature is 60 °C) and wide range of pH tolerance (pH 5.0–11.0), especially when compared to neutral or acidic enzymes (Klompong, Benjakul, Kantacnote, & Shiahidi, 2007).

The effectiveness of hydrolisation process is indicated by the degree of hydrolysis (DH) which is defined as the percent of cut peptides bonding. In addition, it expresses the quantity of free amino acids groups followed by hydrolysate and it depends on the nature of substrate, enzymes as well as reaction conditions. This parameter is closely correlated to the functional properties of resultant hydrolysates and it expresses solely how far the hydrolysis reaction has proceeded. Being an important factor, the time of the hydrolysis reaction of proteins depends on the course of the process and the properties of obtained hydrolysates. The majority of the hydrolysis reactions found in the scientific literature are conducted for no longer than 2 h (Galla, Karakala, Akula, & Pamidighantam, 2012; Klompong et al., 2007). Moreover, in the study presented by Vioque et al. (1999) the process of hydrolysing the rapeseed protein preparations with Alcalase confirms that the rate of hydrolysis is very fast initially. It reaches a steady state after 20–30 min. On the one hand, the hydrolysates obtained in this way are characterised by a high degree of hydrolysis and good functional properties, mainly water solubility and foaming properties. However, a very high degree of hydrolysis can have enormously negative effects on the functional properties (Klompong et al., 2007). In addition, a prolonged enzymatic hydrolysis can contribute to obtaining preparations containing easily digestible amino acids which will result in the lack of ability to indicate unfavourable organoleptic features. For instance, it will not be characterised by a bitter taste which implies the presence of peptides containing hydrophobic amino acid residues, such as leucine, proline, phenyloalanine, tyrosine, isoleucine and tryptophan (FitzGerald & O'Cuinn, 2006).

In the available literature there can be found information concerning enzymatic modification of potato proteins. Pihlanto, Akkanen, and Korhonen (2008) observed that potato proteins are a promising source of the production of bioactive compounds. They are also appropriate/suitable in order to develop functional foods which have a positive impact on cardiovascular health. Going further, studies carried by Cheng, Xiong, and Chen (2010) indicated that potato protein hydrolysates produced by Alcalase hydrolysis improved the oxidation stability of soybean O/W emulsion. Basing on these effects, Wang and Xiong (2005) incorporated potato protein hydrolysates obtained in such a way into cooked beef patties in order to change the antioxidant activity. Nevertheless, there is no information in the literature concerning the application of fodder potato protein concentrate which is produced in the starch industry from potato juice. It serves as the best material to obtain the following preparations of foodstuffs properties: an easy hydration and such favourable functional properties as water or oil holding capacities.

The hydrolysates produced as a result of a prolonged process can indicate detrimental functional properties. Therefore, the preparations which possess profitable amino acid composition together with optimal functional properties are to be further analysed. In this case, the use of the fodder protein preparation broadens its spectrum since it is an easily accessible and relatively inexpensive material. The aim of the present research is to study the effects of the prolonged fodder potato protein hydrolysis with the use of Alcalase enzyme as well as change unprofitable functional properties of the preparation which are usually used for animal feeding.

2. Materials and methods

2.1. Materials

The fodder potato protein concentrate (PPC) was provided by the starch factory in Łomża, Poland, in 2011. Alcalase (a bacterial endoproteinase from a strain of *Bacillus licheniformis*) with specific activity of 2.4 AU/g, was granted by Novozyme A/S, Poland Corp. and stored at 5 °C until it was used for the hydrolysis experiment. All chemicals and or reagents used in this work were of analytical grade.

2.2. Preparation of potato protein hydrolysates

Potato protein preparation (10 g in 100 ml water) was incubated with Alcalase for 2 (preparation PPC-Alc2) and 4 (preparation PPC-Alc4) hours at 50 °C and pH 8.5. Alcalase was added to the substrate based on its enzyme activity (2.4 AU/g protein). The extraction was conducted in a reaction vessel, equipped with a stirrer, thermometer, and pH electrode. After incubation, the enzyme was inactivated by heating at 80 °C for 15 min. The resultant slurry was directly cooled to the room temperature and centrifuged at 4300*g* for 10 min (Rotofix 32A by *Hettich*). The supernatant was collected and freeze dried. The lyophilizates were stored in a fridge for further analyses. The process used in the preparation of potato protein hydrolysates outlines Fig. 1.

2.3. Estimation of the degree of hydrolysis

The degree of hydrolysis, defined as the percentage of peptide bonds cleaved, was measured by determination of free amino groups by reaction with trinitrobenzenesulfonic acid (TNBS) according to Adler-Nissen (1986). Total number of amino groups were determined in a sample 100% of hydrolysed by acid hydrolysis at 110 °C for 24 h.

2.4. Proximate analysis

In order to determine the moisture content, approximately 2 g of a sample was placed into a pre-weighed vessel. Samples were dried at 105 °C until a constant weight was reached (AOAC, 2005). Total protein content was established with the use of Kjeldahl method based on AOAC (2005). As far as nitrogen to protein conversion is concerned, the factor 6.25 was used.

2.5. Amino acid analysis

The composition of amino acid was determined after 24 h hydrolysis which was achieved by means of high-performance liquid chromatography, with the use of 6 N HCl and the temperature at 110 °C. The hydrolysed amino acids were established with the use of AAA-400, *INGOS*, Czech Republic. When it comes to a detector, a two wave length (440 and 570 nm) was employed. The column length which was packed with ion exchanger Ostion LG ANB, INGOS was 350 × 3.7 mm height, whereas the column temperature was maintained at 40–70 °C and the detector at 121 °C. The prepared samples were analysed using the ninhydrine method (Spackman, Stein, & Moore, 1958). Finally, amino acid composition