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Functional properties of the enzyme-modified protein from oat bran

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ABSTRACT

The protein concentrate was prepared from oat bran pre-treated with amyloglucosidase. The influence of amyloglucosidase on the efficiency of protein extraction and the functional properties of the resulting protein products were studied in comparison to the protein concentrate obtained by the conventional alkaline method. Samples, pre-treated with the enzyme, had a higher protein content (83.8%), control – 66.7%. Significant changes in the functionality of the protein product are associated with the hydrolysis of carbohydrates. Solubility, water retention and foaming capacity of product pre-treated with amyloglucosidase are higher. However, the oil-retaining capacity and stability of the foam of protein products of different methods of production did not possess a single trend.

1. Introduction

The growing population of the world constantly dictates the need to develop new technologies for the production of protein bases, using alternative sources. Traditionally, products of animal origin are considered. However, attention should also be paid to crops, among which oat have significant advantages. Oats contain 20% protein, more than 23% of interchangeable and essential amino acids, a number of vitamins, many mineral elements, carbohydrates (Liu, Guan, Zhu, & Sun, 2008; Topping & Clifton, 2001). Oat protein has good nutritional value and functional properties (Klose & Arendt, 2012; Størsrud, Hulthen, & Lenner, 2003; Thompson, 2003). The high nutritional value of oats is also due to its soluble fiber, unsaturated fatty acids, antioxidants.

The most commonly used method for extracting protein from oats and other grains is the alkaline method (Fernández-Quintela, Macarulla, del Barrio, & Martinez, 1997; Ma, 1983). However, high alkaline environment, as a rule, leads to a decrease in nutritional value of proteins. Even under natural conditions, oat proteins have low functional properties, which can be increased, for example, by enzymatic hydrolysis. There are works that studied the influence of succinylation and deamidation (Jiang et al., 2015; Mirmoghtadaie, Kadivar, & Shahedi, 2009), hydrolysis of proteins (Ercili-Cura et al., 2015; Nieto-Nieto, Wang, Ozimek, & Chen, 2014; Scheuplein, Mizutani, & Yamaguchi, 2007) or carbohydrates (Jodayree, Smith, & Tsopmo, 2012; Scheuplein et al., 2007) on the functional properties of oat proteins. It is believed that proteolysis is controlled by qualitative, and hydrolysis of carbohydrates by quantitative characteristics of isolated proteins. Carbohydrases pre-treatment leads to the breakdown of the polysaccharide membrane of the cell wall and the release of proteins during extraction (Alrahmany & Tsopmo, 2012; Alrahmany, Avis, & Tsopmo, 2013). Jodayree et al. (2012) obtained a significant increase in the yield of protein upon hydrolysis of oat flours with amyloglucosidase; and determined their antioxidant activities but not other properties. The purpose of our work is to assess the effect on the functional properties of the protein product of amyloglucosidase pre-modified oat bran.

2. Materials and methods

2.1. Materials and chemicals

The study used oat bran (LLC “Sibirskaya Kletchatka”, Russia), amyloglucosidase (EC 3.2.1.3), with glucoamylase activity of 5500–6200 units/g (Diazim X4, Genencor International BVBA, Belgium). All other chemicals were analytical or better grade.

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2.2. Sample preparation

Oat bran was ground with an electric meat grinder and sieved through a mesh, 4.0 mm. Oat bran powder was degreased by stirring with hexane in a ratio of 1:3 (w/v) bran-on-solvent, for 1 h at room temperature. The suspension was filtered using a Buchner funnel; the solids were washed with hexane and air dried until a skimmed off-fat product with 10% moisture.

2.3. Protein extraction

The suspension (ratio of flour and solvent (w/v) 1:10) was adjusted to pH 11.5 with a 1 M NaOH solution immediately, and then incubated for 60 min at 55 °C under stirring conditions of 150 rpm. The resulting suspension was centrifuged at 4000 × *g* for 30 min, and the supernatant was used to extract the protein.

Suspensions were adjusted to pH 4.2 with a 10% HCl solution immediately, and then incubated for 30 min. The resulting suspension was centrifuged at 4000 × *g* for 30 min, after centrifugation, the protein content was determined by the Dumas method. The resulting protein product contained 66.67% protein.

2.4. Enzymatic pretreatment

Fat-free (paragraph 2.2) oat bran weighing 20 g, was mixed with 200 ml of distilled water in a ratio of 1:10 (w/v) and mixed until a homogeneous suspension. The pH of the suspension was adjusted to 4.2, and then the enzyme was added 0.0029 g. Solutions containing enzymes were incubated in a water bath mounted in a thermostatic mixer at 150 rpm for 3 h at a temperature of 55 °C. At the end of the incubation, the pH of all solutions was adjusted to 4.2 with a 10% solution of HCl immediately and incubated for another 30 min. The samples were cooled and centrifuged at 4000 × *g*, 30 min at 4 °C to produce protein isolates, each supernatant was collected and centrifuged at 4000 × *g* for 40 min at 4 °C. The resulting protein isolates were washed with water and centrifuged for 30 min at 1100 × *g*. The protein content was determined by the Dumas method.

2.5. Functional properties

2.5.1. Solubility

The solubility of the samples was evaluated by the nitrogen solubility index (NSI) according to the procedure described by Ponnampalam, Goulet, Amiot, and Brisson (1987), with a minor modification. 100 mg of the sample was dissolved in 15 ml of distilled water and the pH of the system was adjusted to the desired value (7.5–8.5) either with 0.1 M HCl or NaOH. The suspensions were stirred in a magnetic stirrer for 30 min at room temperature and analyzed for nitrogen content and soluble nitrogen for each pH value.

2.5.2. Water-holding capacity (WHC)

500 mg of the sample was dissolved in 20 ml of distilled water by a vortex mixer for 30 s in graduated centrifuge tubes. After standing for 6 h at room temperature, the dispersions were centrifuged at 2000 × *g* for 30 min. The volume of supernatant was determined and WHC was expressed in ml of water absorbed per 1 g of protein samples.

2.5.3. Oil-holding capacity (OHC)

5 ml of corn oil was added to 500 mg of the sample, mixed for 30 s with a vortex mixer in graduated centrifuge tubes. The oil dispersions were centrifuged at 2000 × *g* for 30 min. The volume of the supernatant was measured and the OHC was expressed in ml of oil absorbed by 1 g of protein sample.

2.5.4. Foaming properties

Foaming ability of protein samples was determined by the method of Fernandez and Macarulla (1997), with a slight modification. Portions of the solution of 40 ml (20 mg/ml) with different pH levels (3, 5, 7 and 9) were thoroughly mixed with a homogenizer at a speed of 10,000 rpm for 3 min. Foaming ability (FA) is calculated as a percentage increase in the volume of protein dispersion when mixed. Foam stability (FS) was evaluated as the remaining percentage of foam remaining after 30 min and 60 min.

2.6. Statistical analysis

Each experiment was repeated three times and data were expressed as means ± standard deviation. Data processing was carried out by standard methods of mathematical statistics. Homogeneity of the sampling effects was checked using the Student's *t*-test. Differences between means were considered significant when the confidence interval is smaller than 5% ($P \leq 0.05$). All statistics were completed with Statistica 10.0 (StatSoft Inc., 2007, USA).

3. Results and discussion

It is assumed (Alrahmany & Tsopmo, 2012; Alrahmany et al., 2013) that the presence of glycoprotein membranes and a large number of fibers limit the release of cereal proteins. Based on the work. Jodayree et al. (2012) amyloglucosidase with a concentration of 8 U/g was used as the enzyme pre-treatment. The results are shown in Table 1. Control (a sample from bran without an enzyme pre-treatment) had a protein content of 66.7%. Samples treated with amyloglucosidase had a protein content of 83.8%. The result is comparable to the results of the authors who obtained a protein yield of 82.0% (Jodayree et al., 2012).

Fig. 1 shows dependence of solubility of protein products from oat bran obtained by two extraction methods. In general, the values of the solubility parameters corresponding to the sample pre-treated with amyloglucosidase are greater for all pH values than for the control sample, however, the general trend of solubility of the protein product for the hydrolyzed samples was not observed. The control sample of a protein product has the polymodal distribution of solubility values with maxima for pH values of 3 and 8. For the protein product pre-treated with amyloglucosidase, a clear optimum for solubility is achieved for pH 5–6 and approximately equal to 50%. Since protein solubility is a fundamental property that determines the possibility of using a protein product in the food industry, a product with greater solubility corresponds to a larger range of applications. In our case, the hydrolyzed sample had the best characteristics of the protein product, the highest solubility was more than 4 times higher than the solubility of the control sample. In work of Guan, Yao, Chen, Shan, and Zhang (2007), in which protein hydrolysis by proteases was studied, the highest solubility corresponded to hydrolyzed samples for pH from 3 to 8, the largest deviation corresponded to pH = 5 and differed by almost 9 times. However, at a pH of 9, no significant differences between the control and hydrolyzed samples were observed and the solubility was no more than 20%. Also, insignificant heat treatment was indicated as a factor in increasing the solubility of oat bran proteins. Unlike enzymatic hydrolysis by proteases, pre-treatment of oat flakes by carbohydrases

Table 1
Effect of enzymatic hydrolysis with amyloglucosidase on protein extraction from oat bran.

Sample	pH	Concentration (unit/g defatted bran)	Protein content (%) of isolates
Control (no enzyme)	4.2	0	66.7[a]
Hydrolysate (amyloglucsidase)	5.5	8	83.8[b]

The data are expressed as mean ± standard deviation ($n = 3$). Values followed by different letter in a column indicate significant differences between groups ($P < 0.05$).