The Effect of Xylanase on the Buckwheat Starch Hydrolysis

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Abstract. With the growing demand for alternative dairy products, there is growing interest in plant-based fermented foods. The plant base should be a favorable environment for lactic acid bacteria, that is, contain easily digestible carbohydrates. Therefore, a necessary stage in the production of a fermented product from plant materials is the preliminary hydrolysis of starch. When obtaining a base from buckwheat flour, an increased viscosity of the mixture of flour and water is observed. Non-starchy polysaccharides (hemicellulose and cellulose) increase the viscosity of the product. Xylanase enzymes can be used to hydrolyze non-starch carbohydrates. In the experiment, the xylanase enzyme in various doses was added to a mixture of buckwheat flour and water at 40 °C. The amylolytic enzyme was simultaneously added. The sample without xylanase was the control. Studies have shown that the introduction of xylanase into a mixture of buckwheat flour and water leads to a decrease in the viscosity of the mixture compared to the control by 38.21% and 25.69%, depending on the amount of xylanase. The xylanase enzyme can be used to lower the initial viscosity of the mixture and to maximize the efficiency of the buckwheat starch hydrolysis process.

1. Introduction

Currently, the demand for functional products is growing. With the growing interest of the population in functional food products, the problem of finding alternative food sources arose. This problem is relevant in the dairy market, since the majority of functional products are made from milk [1]. However, there are groups of people who cannot consume dairy products because they suffer from lactose, milk protein and gluten intolerance. Also, vegetarians try to avoid animal products. The production of food from plant materials such as oats, soybeans, rice and almonds could solve these problems [2]. In addition, buckwheat is a promising solution. Buckwheat is an extremely healthy and popular cereal in Russia. It contains balanced protein, healthy unsaturated fats, and is rich in vitamin P and minerals [3].

In the production of non-dairy products based on buckwheat, carbohydrates play a significant role. Buckwheat contains up to 70% carbohydrates, of which up to 50% is starch, although the quantitative composition of buckwheat carbohydrates depends on weather conditions, the geographical area of cultivation and the variety [4, 5]. Starch is the main carbohydrate in buckwheat. Starch consists of amyllose and amylopectin, the structural unit of which is glucose. Amylose is a linear polysaccharide in which glucose residues are linked by α-1,4-glycosidic bonds. In amylopectin, glucose molecules are also linked by α-1,4-glycosidic bonds, but there are branch points connected by an α-1,6-glycosidic bond [6].
In addition to starch, buckwheat contains a high level of non-starchy carbohydrates, also called dietary fiber (up to 14%) [7]. Non-starchy polysaccharides include cellulose, hemicellulose, pentosans, and gum. The quantitative composition of non-starchy polysaccharides in buckwheat is as follows: in husk – 35.55% of cellulose, in whole grains – 1.28%, and in crushed - the minimum amount of cellulose – 0.78%. During the technological process, parts of the outer layers of the grains containing a lot of cellulose are removed. Thus, there is 20% more cellulose in unroasted buckwheat than in fried buckwheat. The husk of the grain has the highest level of hemicellulose – 14.40%. Buckwheat processing waste contains 13.66% of hemicellulose. Whole buckwheat grain contains 1.29% hemicellulose, and crushed – 1.17%. The level of hemicellulose in buckwheat during roasting increases by 205% [8]. In 100 g of the edible part of buckwheat, hemicellulose is about 3.7 g [9]. The structure of non-starchy polysaccharides is different from that of starch. Cellulose is the main component of the plant cell wall and one of the most common linear polymers. Cellulose chains are formed by α, D-glucopyranose linked by β-1,4-glycosidic bonds [10]. The structural unit of cellulose is cellobiose, which, in turn, is formed by two glucose residues. Cellulose is insoluble in water and is not broken down by digestive enzymes.

Hemicellulose is a polysaccharide of the plant cell wall and, unlike cellulose, has an amorphous structure with possible branching. Hemicelluloses may contain not only glucose but also pyranoses and furanoses, represented by xylose, mannose, arabinose, and galacturonic acid. Xylose is the second most common monomer after glucose in hemicelluloses. There are four groups of hemicelluloses: xylans, xyloglucans, glucomannans, and β-glucans. Xylan is a branched polysaccharide, and its main chain is formed by xylose residues connected by β-1,4 bonds. The side chains of xylan are composed of various sugars, including arabinose, glucose, galactose, rhamnose, glucuronic and galacturonic acids. Xyloglucans are similar in structure to cellulose, but they contain side chains consisting of xylose. Glucomannans are branched polysaccharides formed by mannose and glucose linked by β-1,4 bonds [11]. β-Glucan is an example of a hemicellulose formed by hexoses, namely glucose. In β-glucan, glucose residues are linked by both β-1,4 and β-1,3 bonds [12]. Hemicelluloses formed by pentosans contain xyloses, arabinose, and uronic acids [11].

These compounds can form complexes with proteins, which during hydrolysis may lead to a significant increase in the viscosity of the buckwheat flour-water mixture [13]. Also, non-starch polysaccharides reduce the yield of plant-based drinks and their shelf-life, promote excessive fobbing and increase the foam retention of products. These compounds can have a negative effect on the enzymatic hydrolysis of grain [9]. When obtaining plant-based milk for further fermentation, enzymatic hydrolysis plays a key role. During enzymatic hydrolysis, starch grains swell and become more susceptible to the amylolytic enzymes. As a result, low molecular weight soluble dextrins with a low content of mono- and disaccharides are produced [14].

Usually, starch hydrolysis is carried out with amylolytic enzymes (α-amylase, β-amylase, glucoamylase) to produce glucose. Hydrolysis of starch to glucose is carried out with previously mentioned enzymes by a sequential splitting of starch with the formation of several intermediate products. First, starch is hydrolyzed to dextrins, then dextrins to maltose, and eventually maltose is hydrolyzed to glucose [15]. However, non-starchy polysaccharides are very hydrophilic and form a viscous mixture when finely ground buckwheat flour is mixed with water. The increased viscosity of this mixture prevents the hydration of starch and slows down hydrolysis, which results in a decrease in the yield of the finished product [16]. Amylolytic enzymes do not break down non-starchy polysaccharides. It is proposed to use hemicellulases and cellulolytic enzymes to decrease the initial viscosity of the mixture.

Fungi of the genus Trichoderma are the producers of enzymes that break down non-starch polysaccharides. These enzymes are widely used for the processing of plant materials and in biotechnological processes, for example, in the production of beer, alcohol, starch, and forage [17]. These enzymes accelerate the hydrolysis of non-starchy carbohydrates of plant materials to glucose and pentoses.
Also, the product yield increases because the glucose content in the mixture increases. Additionally, xylanases and glucanases reduce the product's viscosity and improve its rheological parameters [18].

The aim of the work was to determine the effect of the xylanase enzyme on the change in the viscosity of the buckwheat flour-water mixture (in ratio 1:3) during enzymatic hydrolysis.

2. Materials and methods
To determine the effect of the xylanase enzyme on the change in the viscosity of the mixture of buckwheat flour and water, enzymatic hydrolysis of carbohydrates was carried out. A mixture of water and buckwheat flour in a ratio of 1:3 (100 g of buckwheat flour and 300 ml of water) was prepared in a bioreactor with a stirrer at a temperature of 40 °C. α-Amylase AmyloLux with the activity of 2000 U/ml and xylanase Distizym GL with the activity of 720 U/ml were added into this mixture. Enzymes were prepared according to the manufacturer's recommendations as follows. 1 ml of the enzyme was diluted in water at 40 °C in a ratio of 1:20. Diluted enzymes were added at the rate of 0.5 activity units per 1g of starch for AmiloLux and 1 or 1.5 activity units per 1g of raw material for Distizym GL. The control sample (control) had only α-amylase. Sample 1 had α-amylase and 1 activity unit per 1g of the raw material of Distizym GL. Sample 2 contained α-amylase and 1.5 activity units per 1g of raw material for Distizym GL. A mixture of buckwheat flour, water, and enzymes was placed in a water bath with a temperature of 40 °C and left without stirring for 30 minutes for the enzymatic hydrolysis, as the obtained mixture was viscous for a stirrer to agitate. After 30 minutes, the viscosity of the mixture was measured using a Fungilab S.A. Visco Basic Plus viscometer with an R3 spindle at 20 rpm. After that, the stirrer in the bioreactor was turned on for constant stirring. In a water bath, the temperature was raised to 50 °C to heat the mixture, and then the viscosity was measured. Lastly, the viscosity was measured at temperatures of 60, 70, 80, and 90 °C. Buckwheat flour was obtained on an industrial mill from buckwheat groats grown in the Altai Territory.

The enzyme AmiloLux produced by OOO PO Sibbiopharm (Russia) is a thermostable α-amylase that catalyzes the hydrolysis of α-1,4-glycosidic bonds of starch and thereby reduces the viscosity of the mixture. This enzyme hydrolyzes starch into soluble low molecular weight dextrins with a low content of mono- and disaccharides (glucose and maltose).

The enzyme Distizym GL manufactured by Erbslöh Geisenheim GmbH (Germany) is an enzyme obtained from the strain of Trichoderma reesei fungi. The enzyme contains thermostable hemicellulases: endo 1,4-β-D-mannanases, 1,4-β-D-xylanases, 1,3-β-D-xylanases, exo 1,4-β-D-xylosidases and thermostable β-glucanase (endo 1,3 (4) -β-D- glucanase and 1,4-β-glucanase). Distizym GL enzyme is designed for the further viscosity decrease of the mixture, as it breaks down pentosans and β-glucans during the saccharification of the wort.

3. Results and discussion
The change in viscosity, measured on the viscometer with the spindle R3 at 20 rpm, in a mixture of buckwheat flour, water, and enzymes after holding for 30 minutes, is presented in table 1.

As can be seen from the table, samples of a mixture of buckwheat flour and water had a maximum viscosity at the beginning of hydrolysis, and then the viscosity decreased with increasing temperature. The minimum viscosity values were obtained at a temperature of 90 °C.

After holding for 30 minutes without stirring, the viscosity values in samples 1 and 2 allowed agitation with the stirrer built into the bioreactor. However, the control sample required an additional holding time during 10-15 minutes at 40°C before turning on the stirrer.
Table 1. Viscosity of the mixture of buckwheat flour and water.

| Temperature, °C | Sample | Viscosity, mPa·s |
|----------------|--------|-----------------|
| 40             | Control| 4320.4          |
|                | 1      | 2669.4          |
|                | 2      | 3210.3          |
| 50             | Control| 2468.6          |
|                | 1      | 2328.2          |
|                | 2      | 2341.4          |
| 60             | Control| 1180.1          |
|                | 1      | 1107.0          |
|                | 2      | 707.2           |
| 70             | Control| 737.2           |
|                | 1      | 755.0           |
|                | 2      | 399.8           |
| 80             | Control| 460.1           |
|                | 1      | 542.0           |
|                | 2      | 334.2           |
| 90             | Control| 416.2           |
|                | 1      | 405.3           |
|                | 2      | 266.7           |

The addition of the xylanolytic enzyme Distizym GL reduced the initial value of the viscosity by 38.21% when the enzyme was added at the rate of 1 activity unit per 1g of raw material and by 25.69% with the addition of 1.5 activity units per 1g of raw material. With the increasing temperature, the viscosity decreased faster in sample 2, which had a large amount of xylanase.

4. Conclusion
In the production of alternative products from plant materials, the problem of an excessive viscosity of the flour-water mixture arises. The increased viscosity prevents the effective hydrolysis of starch. The addition of the xylanolytic enzyme DistizymGL into a mixture of buckwheat flour and water, followed by holding for 30 minutes at a temperature of 40 °C leads to a decrease in the viscosity of the mixture compared to the control by 38.21% when the enzyme is added at the rate of 1 activity unit per 1g of raw material and by 25.69% with the addition of 1.5 activity unit per 1g of raw material. The use of the xylanase enzyme provides a decrease in the initial viscosity of the mixture and allows to achieve the highest efficiency of the hydrolysis of buckwheat starch by amylolytic enzymes.

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