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Preparation of resistant starch by hydrolysis of maize starch with pullulanase

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ABSTRACT

In this paper, high resistant starch content product was prepared by hydrolyzing of maize starch with pullulanase. The optimal hydrolyzing conditions were investigated and the optimum conditions were as follows: time, 32 h; pH, 5.0; temperature, 46 °C; amount of pullulanase, 12 ASPU/g. The product of resistant starch was obtained by pressure-cooking the resulting hydrolysate in an autoclave at 121 °C for 1 h, cooling at room temperature, storing at 4 °C overnight, autoclaving/cooling for 2 repetition cycle, drying an oven (105 °C) and finally grounding into fine particles (<150 μm). The content of resistant starch in the product was 44.7% (w/w). Results of experiments indicated that this was a promising way of preparation of product of high content of resistant starch.

1. Introduction

Starch is one of the most important ingredients in human diet. It contributes to about 60–70% of the total energy consumed, of which nearly 75% of the starch comes from cereals and pulses (Asp, 1995). It is a glucose polymer and has two distinct structural forms, amylase and amylopectin (Tharanathan & Tharanathan, 2001). The gelatinized starch may revert to a form that could be highly resistant to hydrolysis by α-amylaseand is called resistant starch (RS) during processing (Annison & Topping, 1994). RS has attracted great interest in amongst the nutritionists and food industry, due to its reduced levels of plasma glucose and insulin, increased faecal bulk, and short-chain fatty acid (SCFA) production through fermentation in the large intestine (Mun & Shin, 2006).

At present, RS is manufactured by a heating-cooling process and chemical modification, respectively (Mun & Shin, 2006). However, chemical modification may have safety problems. At the same time, RS content may be relatively low by heating-cooling process alone due to the structure of nature starch. Pullulanase (EC 3.2.1.41) hydrolyzes the α-1, 6-glucosidic linkages in starch and produces amylose and may increase RS content. Therefore, it is of our interesting to investigate the way for producing high RS content product by hydrolyzing of maize starch with pullulanase. The hydrolyzing conditions were optimized and the composition of the product was studied.

2. Materials and methods

2.1. Hydrolysis of maize starch

The pH of a slurry of maize starch at the concentration of 5% (w/v) was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5, respectively using 1 M HCl, instantly heated to 70 °C for gelatinization, and quickly reduced to 40 °C within 1 min. Pullulanase (Genencor International, Inc., L-1000, 10 ASPU/g) was added to the hydrolysate and allowed to further hydrolysis at 42, 44, 46, 48, 50 and 52 °C, respectively for 8, 16, 24, 32, 40 and 48 h, respectively.

2.2. Preparation of RS

RS was prepared according to the methods described by Sievert and Pomeranz (1989) with slight modification. The solution was pressure-cooked in an autoclave at 121 °C for 1 h, cooled at room temperature and then stored at 4 °C overnight. After two repetitions of the autoclaving/cooling cycle, the sample was dried in an oven (105 °C) and ground into fine particles (<150 μm).

2.3. Isolation of resistant starch

RS was isolated according to the method described by Wang, Jin, and Yuan (2007). One gram of the powders of RS sample was weighed and into a bioreactor containing 100 ml of distilled water, and the mixture was heated by circulating hot water from a water bath equipped with a pump. After the internal temperature of the bioreactor reached 95 °C, 0.5 ml of α-amylase (Termamyl 120 l) was added. After the slurry was incubated at the desired temperature for 30 min, the reaction mixture was cooled to room temperature and centrifuged at 5000 × *g* for 10 min. The resulting residue

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was suspended in 100 ml of phosphate buffer (0.08 M, pH 7.5) and treated with protease (0.5 ml) at 60 °C for 30 min. After the pH had been adjusted to 4.5 with diluted HCl, amyloglucosidase (0.5 ml) was added and the mixture was incubated at 60 °C for 30 min. The suspension was centrifuged at 5000 × *g* for 10 min. The insoluble residue was washed several times with distilled water. Finally, it was washed twice with 80% (v/v) ethanol and 95% (v/v) ethanol successively, and then dried at 40 °C overnight in a vacuum oven to get resistant starch.

2.4. Analytical methods

The pH of the solution was recorded using a digital pH meter (Model: PHS-3C, CD Instruments, China). Ash, moisture and protein content of the samples were determined as per standard methods (Anonymous, 1984). The resistant starch was analysed according to the methods of Goni, Garcia-Diz, Manas, and Saura-Calixto (1996). Sample (10 mg) was dispersed with 5 ml of 2 M KOH and stirred for 30 min at ambient temperature. After the pH had been adjusted to 4.5 with diluted HCl, amyloglucosidase (0.05 ml) was added and the mixture was incubated at 60 °C for 35 min. The released glucose was measured by the glucose oxidase method (Dahlqvist, 1964). The content of resistant starch was calculated as the product of free glucose from resistant starch hydrolysis with amyloglucosidase and a correction factor of 0.9 as follows: resistant starch = glucose × 0.9 (Escarpa, Gonzaĭlez, Morales, & Saura-Calixto, 1997).

3. Results and discussion

3.1. Effect of time on hydrolyzing

Effect of time on the hydrolyzing of maize starch with pullulanase were carried out and the results were shown in Fig. 1, there was a sharp increase within 16 h and a slow increase on 16–24 h in RS content. The maximum RS content (32.1 g/100 g) was achieved after 24 h. Therefore, the optimum hydrolyzing time was judged to be 24 h. Wu, Chen, Tong, Xu, and Jin (2009) observed that the optimum hydrolyzing time was 6 h when hydrolyzed of pullulan with pullulanase. This great difference may due to that pullulan, rather than maize starch, is the fittest substrate to pullulanase.

3.2. Effect of pH on hydrolyzing

The pH influences the activity of pullulanase and hydrolyzing of maize starch. Therefore, it was important to investigate the effects of different pH values ranging from 4.0 to 5.5 of the reaction mixture on maize starch hydrolyzation with pullulanase. The maximum of RS content of the hydrolysate (36.3 g/100 g) was achieved at a pH of 5.0 (Fig. 2). In other reports, optimal conditions for hydrolyzation with pullulanase were obtained at the pH of 5.0 (Roy, Messaoud, & Bejar, 2003; Wu et al., 2009), 5.9 (Kriegshauser & Liebl, 2000), 6.0 (Kuroiwa, Shoda, Ichikawa, Sato, & Mukataka, 2005) and 7.0 (Swamy & Seenayya, 1996). The different optimal pH values reported in the literature may be due to the differences in substrates and the source of pullulanase.

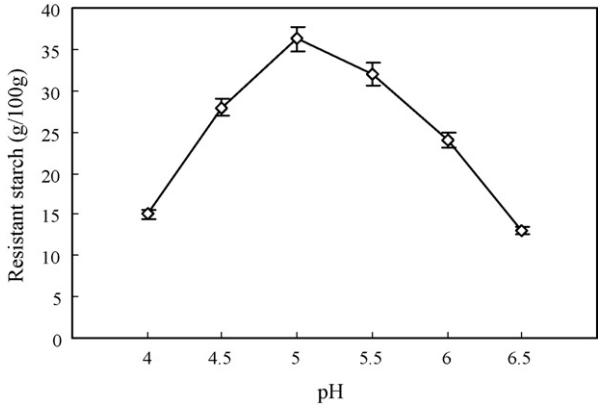

Fig. 2. Effect of pH on hydrolysis of maize starch (the results are from three replicate experiments).

3.3. Effect of temperature on hydrolyzing

The temperature plays a pivotal role in the activity of pullulanase and too high or too low temperature decrease the activity of it. So, it was necessary to study the effects of different temperature varying from 42 to 52 °C of the reaction mixture on hydrolyzing maize starch with pullulanase. The maximum of RS content of the hydrolysate (40.2 g/100 g) was achieved at a temperature of 46 °C (Fig. 3). In contrast, optimal temperature for hydrolyzation with pullulanase were obtained at 45 °C (Wu et al., 2009), 50 °C (Kuroiwa et al., 2005), 60 °C (Swamy & Seenayya, 1996), 75 °C (Kuriki, Park, & Imanaka, 1990; Messaoud, Ammar, Mellouli, & Bejar, 2002), and 90 °C (Badal, Saha, & Zeikus, 1989; Kriegshauser & Liebl, 2000). The different optimal temperature reported may also be due to the differences in substrates and the source of pullulanase.

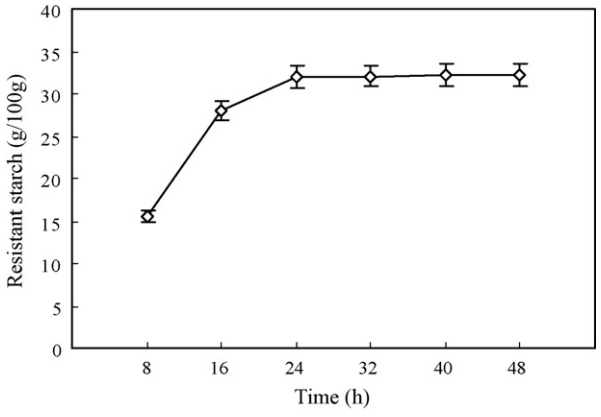

Fig. 1. Effect of time on hydrolysis of maize starch (the results are from three replicate experiments).

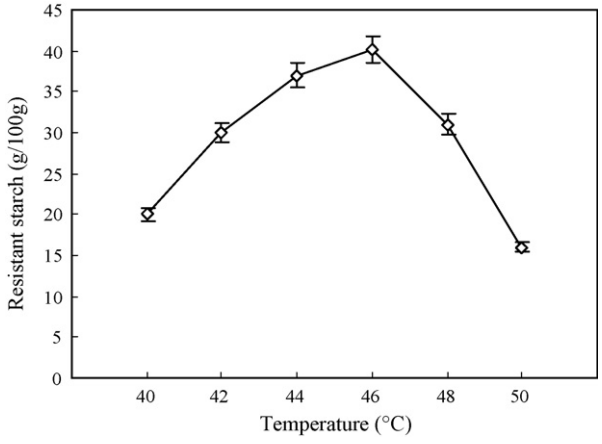

Fig. 3. Effect of temperature on hydrolysis of maize starch (the results are from three replicate experiments).

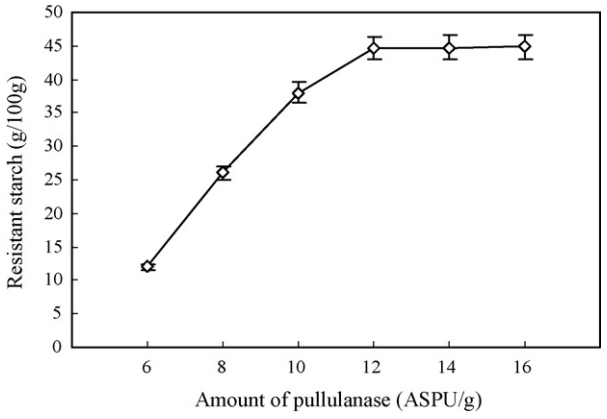

Fig. 4. Effect of the amount of enzyme on hydrolysis of maize starch (the results are from three replicate experiments).

3.4. Effect of amount of pullulanase on hydrolyzing

Fig. 4 shows the Effect of amount of pullulanase on hydrolyzing of maize starch. When the amount of pullulanase was lower than 12 ASPU/g, The RS content showed a steady increase as the level of pullulanase in the reaction mixture increased. The maximum RS content was observed at the level of 12 ASPU/g of pullulanase (44.7 g/100 g). Results of these experiments indicated that at this level of pullulanase, all maize starch was saturated by pullulanase. It is therefore that the optimum amount of pullulanase under this condition was judged to be 12 ASPU/g. In a previous report, optimum amount of pullulanase was 10 ASPU/g (Wu et al., 2009), probably due to the difference in substrate.

3.5. Characterization of the product

The sample of RS sample obtained was characterised. The ash, moisture, RS were 0.4% (w/w), 3.0% (w/w) and 44.7% (w/w), respectively.

4. Conclusions

RS can be prepared by hydrolyzing of maize starch with pullulanase. The optimum hydrolyzing conditions were as follows: time, 24 h; pH, 5.0; temperature, 46 °C; amount of pullulanase, 12 ASPU/g. Under these conditions, the maximum RS content was obtained. The hydrolysates were pressure-cooked in an autoclave at 121 °C for 1 h, cooled at room temperature and then stored at 4 °C overnight. After two repetitions of the autoclaving/cooling cycle, the sample was dried in an oven (105 °C) and ground into fine particles (<150 μm). The content of RS in the product was 16.5% (w/w).

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