Short Communications

PREPARATION OF RESISTANT STARCH BY DUAL MODIFIED METHOD FROM INDIKA RICE STARCH

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[Received August 29, 2015; Accepted October10, 2015]

[Communicated by Guest Editor Prof. Zhao-Jun Wei]

ABSTRACT: Resistant starch (RS) is defined as the part of the total starch that resists hydrolysis and absorption in the small intestine. RS has been proved to be beneficial to human health. Biochemical modification is also an effective way to prepare RS. In this study, we prepared RS from Indica rice starch using thermal treatment and dual-enzymatic (α-amylase and pullulanase) modification. Hydrolysis by increasing concentrations of α-amylase (0, 2, 4, 6, 8 and 10 U×g-1) was evaluated at increasing temperatures from 50 to 100°C, and pH between 4.5 and 7.0 for 10 to 60 min. The optimal conditions for hydrolysis was 6 U×g-1 α-amylase at 80 °C for 40 min with pH adjusted to 5.5. Under these conditions, the RS yield was 45.3%. Moreover, the RS product obtained under these conditions exhibited a lower hydrolysis rate than other starch samples.

KEY WORDS: Indica rice, resistant starch, dual-enzymatic modification, hydrolyzing condition, hydrolysis rate

INTRODUCTION

Starch as a natural high molecular compound that is the most important source of carbohydrates and the primary source of energy. Even though, starches have long been considered fully digestible and absorbent by our body. Recent studies have suggested that some starch could not be hydrolyzed even in the small intestine. Englyst et al. (2008) first defined this partial of starch as a resistant starch (RS). RS is characterized by a small molecule that made of 20-25 glucose residues. (linear polysaccharides that are connected by hydrogen bonds). Byrnes et al. (1995) found that RS can reduce the fat content in blood and liver. RS can also effectively control the symptoms from high cholesterol (Berry 1986). RS plays an important role in gastrointestinal motility and may provide a new strategy for treating colon cancer (Emmanuel and Roy 2007).

At present, thermal and chemical (Englyst, et al., 2008) methods are two common ways for treating RS. Previous study has showed that acid-alcohol treatment with acids has shown higher granular recovery. The method also allows controls of starch degradation with minor changes to the molecule at a time. (Kriegshauser and Liebl, 2000). In addition, Zhang and Jin (2011) found that hydrolysis of maize starch by pullulanase can enhance RS level to 44.7%. The majority of relevant studies have been focusing on RS that derived from potato, wheat, maize and sorghum sources. Given that low-priced Indica rice contains high levels of RS and China is the largest rice producing and consuming country in the world, Indica rice is potentially an even better source of RS in comparison to those listed above. The objectives of this study were to enhance RS production from Indica rice starch using a dual-enzymatic modification method, to estimate the effects of temperature, pH, hydrolysis time and α-amylase dose on the RS production during hydrolyzing process, and to study in vitro hydrolysis rate of the RS yielded from Indica rice.

MATERIALS AND METHODS

Materials

Indica rice starch was obtained from Golden Agriculture Biotech Co., Ltd. (Jiangxi, China). Pullulanase, heat-stable
α-amylase and amyloglucosidase were purchased from Sigma-Aldrich (Shanghai) Trading Co, Ltd.

Preparation of RS using different methods

Indica rice resistant starch (IR-RS) was prepared according to the methods described by Xu et al (2012) and with some dual-enzymatic modification (DM-RS). The gelatinized starch samples were generated by adding 40 mL acetate buffer to 10 g of Indica rice starch and with 40 mL of distilled water (0.1M, pH 5.5) and placed in a water bath at an 80 °C water bath for 30 min with consistent agitation. Then the gelatinized starch samples, added with 6 U×g^-1 α-amylase, were hydrolyzed at 80 °C for 40 min with continuous shaking. Afterwards, the starch samples were adjusted to pH 4.5 by acetate buffer (0.1 mM) and debranched by pullulanase at 46 °C for 12 h using 4 U×g^-1 dose of enzyme. The samples were removed from the water bath, cooled to room temperature and retrograded by storage at 4 °C for 12 h. The retrograded starch samples were dried at 50 °C for 24 h and milled (200 mesh) for later analyses. RS using single-enzymatic modification method (SM-RS) was prepared using the same method as DM-RS (Section B) without α-amylase treatment (using pullulanase only). RS using thermal treatment (TT-RS) was prepared without enzymatic modification. Indica rice starch suspensions (10 g, dry weight, 25% w/v) were gelatinized in a water bath in an 80 °C water bath for 30 min. After gelatinization, the samples were boiled for 90 min, followed by retrogradation (4°C, 12 h), dehydration (50°C, 24 h) and grind for analyses.

Effects of temperature, pH, hydrolysis time and α-amylase dose on the RS generation yield

Hydrolysis temperature, pH, hydrolysis time and α-amylase dose were considered to be the main factors affecting hydrolyzing process. We tested RS yield in response to the changes to the four factors listed above and the RS yield was calculated by the following formula:

\[
\text{RS yield (\%)} = \frac{\text{Glucose content} \times 0.9}{\text{Starch weight}} \times 100\%
\]

\[
\text{RS determination}
\]

RS was determined following Xu et al (2012) with a slight modification. RS (0.5 g) mixed with distilled water (10 mL) was digested with α-amylase (pH 5.8, 30 min, 100 °C). The reaction mixture was then cooled to room temperature for 30 min and centrifuged at 8000 g for 10 min. The pellet was collected and washed twice using distilled water. Each residue was then resuspended in 6 mL of a 2 M KOH solution and shaking for 30 min at room temperature. The pH of the sample was adjusted to 4.4, followed by adding an excessive amount of amyloglucosidase. Subsequently, the mixture was placed at a 60°C water bath for 45 min. The samples were then centrifuged (8000g, 10 min) and the supernatants were collected. The pellets were resuspended in 20 mL of distilled water and the centrifugation was repeated. All the supernatants were collected and adjusted volume to 100 mL in a 100-mL volumetric flask with distilled water. The reducing sugar contents were determined using the DNS method.

Hydrolysis rates of the starch samples

Each sample (5 g) was mixed with distilled water (45 mL), pregelatinized at 80 °C for 30 min. Following this, α-amylase was added and the mixture was placed in an 85°C water bath for 2, 6, 12, 24 and 36 h, and the reducing sugar contents in the solutions were determined using DNS method. All experiments were carried out in triplicates.

Statistical analyses

Origin version 7.5 (Origin Lab Corporation, USA) and SPSS version 16.0 software was used for statistical analyses of the results. All experimental data were expressed as means ± SD.

RESULTS AND DISCUSSION

Effects of different factors on hydrolyzing process

The effect of temperature on hydrolyzing process is shown in Fig. 1a. RS yield increased with the increase of temperature and RS generation started decreasing when the temperature was over 80°C. The highest production of RS is 42.25%, which was seen at 80 °C. The finding of 80 °C as the optimal temperature for RS formation can be explained by 1). Even though high temperature in hydrolysis can promote the degradation and retrogradation of starch to cause formation of RS, extremely high temperature (> 80°C), on the other hand, leads to excessive starch degradation, which can negatively affect formation of RS. 2). Extremely low or high temperatures can gradually decrease alpha-amylase activity, which in turn results in low RS generation. The effect of pH on hydrolyzing process is shown in Fig. 1b. It can be observed that pH significantly affects the RS yield. The RS yield increased with pH ranging 4.5-5.5 and decreased with pH ranging 5.5-7.0. The RS yield peaked at pH 5.5 (43.9%).

The effect of hydrolysis time on hydrolyzing process is depicted in Fig. 1c. Hydrolysis time also strongly affect the production of RS in our study. We found that RS generation kept increasing upto 40 min of reaction time and started decreasing onwards as indicated in fig. 1d. The reason of 40 min being the optimal hydrolysis time was determined by alpha-amylase activity. Longer than 40 min of reaction time can cause alpha-amylase to hydrolyze alpha-(1,4) linkages excessively. Excessive hydrolysis will block RS formation in the retrogradation process.

Fig. 1d showed the effect of α-amylase doses on hydrolyzing process in RS production. The RS generation showed a linear relationship with the increase of alpha-amylase doses. The peak generation of RS (41.5%) was
obtained when 6 U/g alpha-amylase was used. Interestingly, an excessive amount of alpha-amylase (> 6 U/g) resulted in a reduction on RS generation. Zhang and Jin (2011) have demonstrated that unwanted hydrolysis of starch occurs when higher doses of alpha-amylase were introduced to the reaction and this is very likely to be responsible to the decreased RS generation similar to what excessive hydrolysis time would do.

In conclusion, the optimal conditions of hydrolyzing process in generation of RS is the temperature of 80°C, pH 5.5, hydrolysis time of 40 min and 6 U×g⁻¹ of α-amylase.

**RS levels of different productions**

RS levels of native starch, TT-RS, SM-RS and DM-RS are shown in Fig. 2. It is shown in Fig. 2 that RS levels of DM-RS samples (42.28%) prepared using dual-enzymatic (α-amylase and pullulanase) modification method is significantly higher (p<0.01) than SM-RS and TT-RS. This indicates that the dual-enzymatic modification is an efficient way to enhance RS levels from starch ingredients. In enzymatic hydrolysis processes, pullulanase and α-amylase break α-(1, 6) linkages and α-(1, 4) linkages in starch molecule chains, which in turn generate more short length starch chains and increases amylose levels. Eventually, RS is formed from these amyloses by recrystallization during retrogradation process (incubation at 4 °C for 12 h).

**Hydrolysis rates of the RS samples**

The hydrolysis rates of the four types of starch samples are summarized in Table 1. Hydrolytic rates of the starch samples were determined in triplicates (n=3). Native starch samples were presented as controls. As shown in Table 1, the hydrolysis...
rates of all samples increased with the increase of hydrolysis time. It is worth noting that the hydrolysis rates of DMT starch were significantly lower than those of the other starch samples. This observation can be explained by the high RS contents of DMT starch. The starch molecules were hydrolyzed to short and straight chains by the dual enzymatic treatment with α-amylase and pullulanase. These short chains were then linked together by hydrogen bonds into a tight and double-helical structure. The new crystalline structures of starch molecule from hydrolytic process have reduced binding sites for amylase, which in turn are resistant to further hydrolysis by amylase. This also explains the anti-digestive potential of RS and amylose.

CONCLUSION

Our study demonstrated that the dual modification treatment is an efficient method for the preparation of Indica rice RS. Hydrolysis temperature, length of reaction, pH, and α-amylase dose all significantly influence the production of RS. The highest RS generation was 45.3% using optimized hydrolysis conditions consisting of 6 Ug-1 of α-amylase and a 40 min long incubation at 80 °C and pH 5.5. In addition, the RS level of DM-RS was significantly (p<0.01) higher than those of native starch, TT-RS, and SM-RS samples and DM-RS showed the strongest anti-digestive activity. The results of our study are indicative for efficiently producing starch ingredients rich in RS and our study promotes the use of RS generated from Indica rice as a healthy food resource.

ACKNOWLEDGEMENTS

We would like to thank the National College Students Innovation Training Program (Sanction NO. 201210359058) and the Department of Science and Technology and Anhui Province Natural Science Funds (Sanction NO. 1208085MC56) for their financial support for the research.

REFERENCES


TABLE 1. Hydrolysis rates of the starch samples. aHydrolysis rate (%) = (Reducing sugar contents) × 0.9/Sample dry weight ×100%; b p<0.01 compared to TT-RS

<table>
<thead>
<tr>
<th>Samples</th>
<th>Hydrolyzing rates a</th>
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<tbody>
<tr>
<td></td>
<td>2 h</td>
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<tr>
<td>Native starch</td>
<td>25.7±0.907</td>
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<tr>
<td>TT-RS</td>
<td>12.2±1.54</td>
</tr>
<tr>
<td>SM-RS</td>
<td>6.55±0.929</td>
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<tr>
<td>DM-RS</td>
<td>3.17±1.02</td>
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</tbody>
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