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Preparation of products rich in resistant starch from maize starch by an enzymatic method

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ABSTRACT

We prepared resistant starch (RS) from maize starch using a method combining α-amylase and pullulanase. We optimized reaction conditions for α-amylase, including temperature (*T*), pH, reaction time (*t*), and amount of α-amylase. The highest formation of RS (58.87%) was obtained under the following conditions: temperature, 90 °C; pH, 5.5; time, 15 min; and amount of α-amylase, 4 μ/g. Scanning electron microscopy and differential scanning calorimetric analyses showed that maize starch underwent pasting treatment with α-amylase contained shorter amylase chains, and decreased steric hindrance among molecules compared with native maize starch. It is advantageous for amylopectin to be debranched by pullulanase, and the short amylose chains released from amylopectin can form double helices. Compared with native maize starch, maize RS showed increased crystallinity, and a larger, more compact laminiplantation structure. The increased density of the crystals greatly increases their resistance to starch-degrading enzymes. This is a promising method for preparing RS-rich products.

1. Introduction

Because of increasing health awareness and the growing demands of consumers for functional foods, the international food industry is investigating ways to produce innovative food products with health benefits (Lopez-Rubio, Gavara, & Lagaron, 2006). There has been considerable interest in developing carbohydrate products with lower glycemic impacts. Such products can improve control of obesity and diabetes and, subsequently, reduce the risk of cardiovascular disease (Aung et al., 2010; Brand-Miller, 2005; Brennan & Tudorica, 2003; Morita et al., 2005). Resistant starch (RS) has recently gained attention as a functional food ingredient because of its potential health benefits and functional properties in foods (Sajilata, Singhal, & Kulkarni, 2006). According to Englyst, Kingman, and Cummings (1992), RS is classified into four major groups; RS1, RS2, RS3, and RS4. RS1 is physically inaccessible starch, such as that located in plant tissue structures. RS2 is condensed and partially crystalline native (uncooked) starch granules. RS3 consists mainly of retrograded or recrystallized amylose (Berry, 1986; Ring, Gee, Whittam, Oxford, & Johnson, 1988), which forms in cooked products after cooling, e.g., in breads, corn flakes, or potatoes. RS4 can be produced by chemical modifications, such as conversion, substitution, or cross-linking. Such modifications prevent digestion of RS4 by blocking access to enzymes and by forming atypical linkages, e.g., $1 \rightarrow 2$, $1 \rightarrow 3$, $1 \rightarrow 4$, and $1 \rightarrow 6$.

Although it is not digested in the small intestine, RS may be fermented and disintegrated into short chain fatty acids (SCFA) and gases (Haralampu, 2000; Kritchevsky, 1995; Muir et al., 1995; Thompson, 2000) by the microflora in the large bowel. SCFA, particularly butyrate, have been implicated in promoting good colonic health and preventing the incidence of colo-rectal cancer (Champ, 2004; Jacobasch, Schmiedl, Kruschewski, & Schmehl, 1999). RS plays important physiological roles and has the potential to improve human health and lower the risk of many diet-related diseases. Compared with traditional insoluble fibers, RS has many advantageous features; it has a natural white color, a bland flavor, and has a better appearance and texture. When formulated into products, it has a less gritty mouthfeel, and it masks other flavors less than other typical insoluble fibers (Sajilata et al., 2006). The main use of RS has been as a functional ingredient in low-moisture food products (Yue & Waring, 1998), particularly in bakery products such as bread and muffins, and in breakfast cereals.

The RS yield of foods depends on the botanical source of the starch, the type of processing, the amylose/amylopectin ratio, physical form, the degree of gelatinization, and thermal, cooling, and storage conditions (Sievert & Pomeranz, 1989). Many food processes decrease or eliminate RS1 and RS2 but have the potential to generate RS3, and there are some security issues for RS4. RS3 is the form of RS most often used as a food ingredient. It can be obtained by hydrothermal treatments and retrogradation of starch derived from cereal grains, roots, tubers and legumes, such

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as wheat, corn, oat, rice, potato, tapioca, and mung bean. Among these starches, high amylose corn starch (HACS) is most often used for preparation of RS3 (Dimantov, Kesselman, & Shimoni, 2004; Fishman, Coffin, Unruh, & Ly, 1996; Herman & Remon, 1989; Sievert & Pomeranz, 1989). The currently accepted mechanism by which RS3 resists amylase digestion is that linear amylose segments align into condensed double helical structures after gelatinization (amylose retrogradation). This arrangement renders α-1,4 glucosidic linkages inaccessible to amylase.

Cyclic heating and cooling procedures, generally referred to as annealing procedures, are used to form RS3 (Thompson, 2000). Among the methods used to increase RS3 yield, acid hydrolysis of amylomaize starch (Lee, Mun, & Shin, 1997; Russell, Berry, & Greenwell, 1989; Vasanthan & Bhatty, 1998) and repeated freeze-thawings (Chung, Jeong, & Lima, 2003) are the most widely investigated. However, few studies have investigated the effects of enzymes on maize starch. Berry (1986) reported that debranching of potato amylopectin with pullulanase before heating and cooling cycles substantially increased the RS3 yield. In our previous work (Zhang & Jin, 2011), hydrolyzing maize starch with pullulanase (time, 32 h; pH, 5.0; temperature, 46 °C; pullulanase, 12 ASPU/g maize starch) effectively increased the RS3 yield (to 44.7%), However, retrogradation is affected by the amylose/amylopectin ratio and the length of amylase chains; thus, maize starch must be pressure-cooked in an autoclave at 121 °C for 1 h before debranching. In the present study, maize starch was subjected to an α-amylase pasting treatment to reduce the length of amylase chains. We optimized enzymatic reaction parameters of α-amylase to increase RS3 yield of the resulting starch powders. The results of this study may provide an alternative route for preparation of RS-rich products.

2. Materials and methods

2.1. Materials

Native maize starch was obtained from the Carbohydrate Research Center, Jiangnan University, China. Pullulanase was purchased from Genencor Bio-Products Co. Ltd. (Wuxi, China). The enzymatic activity is 400 ASPU/g determined by the reaction at 50 °C with pullulan (1.0%) buffered with sodium acetate (pH 5.0). α-Amylase and amyloglucosidase were the products of the Sigma–Aldrich Trading Co. Ltd. (Shanghai, China). The activity of α-amylase is 1400 μ/g and the amyloglucosidase activity is 100,000 μ/g. The enzymatic activity was determined by the reaction at 37 °C with soluble starch (1.0%) buffered with sodium acetate (pH 4.4) (Chen, Huang, Tang, Chen, & Zhang, 2011). All other chemicals and reagents were of reagent grade.

2.2. Preparation of resistant starch

Resistant starch was prepared as shown in Fig. 1. Maize starch (20%, w/v) was pregelatinized with distilled water by stirring for 20 min at 80 °C/pH 6.0. The starch paste was treated with thermostable α-amylase in various reaction conditions, then pullulanase was added. The mixture was incubated in a shaking water bath at 46 °C for 12 h, cooled at room temperature, and then stored at 4 °C overnight. The mixture was treated with a thermostable α-amylase (1%, w/w) and incubated in a boiling water bath (95 °C) for 45 min then centrifuged (4,000 × *g*, 15 min). The supernatant was discarded, and the residue suspended in 100% ethanol (1:4 residue:ethanol) and shaken for 15 min. The ethanol was removed by centrifugation (4000 × *g*, 15 min) and the above procedure was repeated twice more. The residue was dried overnight at 40 °C, ground in a centrifugal mill to pass through a 0.5 mm sieve, and kept in airtight containers at room temperature until analysis.

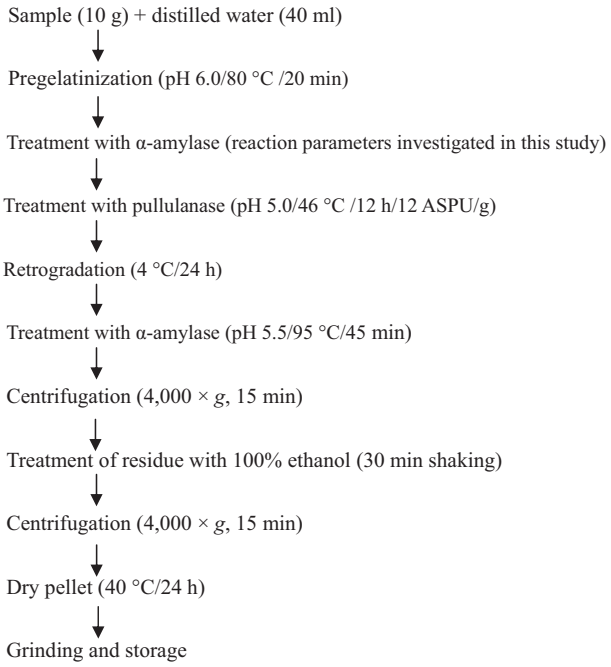

Fig. 1. Preparation of resistant starch.

2.3. Optimization of reaction conditions for α-amylase hydrolysis for maize starch

To determine the effects of hydrolysis conditions on formation of RS, the enzymatic reactions were carried out at various temperatures (70, 75, 80, 85, 90, 95 °C), pHs (4.5, 5.0, 5.5, 6.0, 6.5, 7.0), times (10, 15, 20, 25, 30, 35 min) and amount of α-amylase (0, 2, 4, 6, 8, 10 μ/g). The formation of RS produced in these conditions was determined.

2.4. Resistant starch determination

Resistant starch was determined according to the methods of Goni, Garcia-Diz, Manas, and Saura-Calixto (1996) with minor modifications. Each sample (10 mg) was dispersed with 5 ml 2 M KOH and stirred for 30 min at ambient temperature. The pH was adjusted to 4.5 with diluted HCl, then amyloglucosidase (0.05 ml) was added and the mixture was incubated at 60 °C for 35 min. The reaction was terminated by addition of 4 ml ethanol. Samples were centrifuged (3000 × *g*, 15 min) and the supernatants collected. The residue was resuspended in 10 ml distilled water and the centrifugation repeated. The supernatants were combined with the water washes and adjusted to 100 ml with distilled water. Total glucose was analyzed using GOD-POD reagent. The content of resistant starch was calculated as the product of free glucose released from hydrolysis of resistant starch by amyloglucosidase and a correction factor of 0.9, as follows: resistant starch = glucose × 0.9 (Escarpa, Gonzaĭlez, Morales, & Saura-Calixto, 1997).

$$\text{Yield of RS}\,(\%) = \frac{\text{Resistant starch weight}}{\text{Starch dry weight}} \times 100$$

2.5. Scanning electron microscopy (SEM)

The surface topography of product samples was observed by SEM using the method of Kim et al. (2008). A dry, finely ground

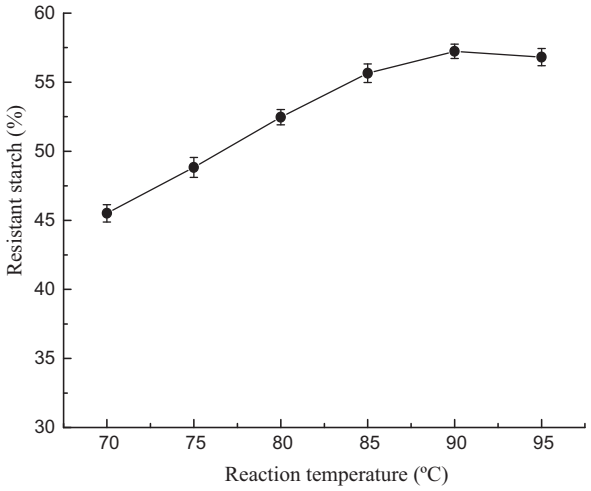

Fig. 2. Effect of temperature (70–95 °C) on resistant starch yield.

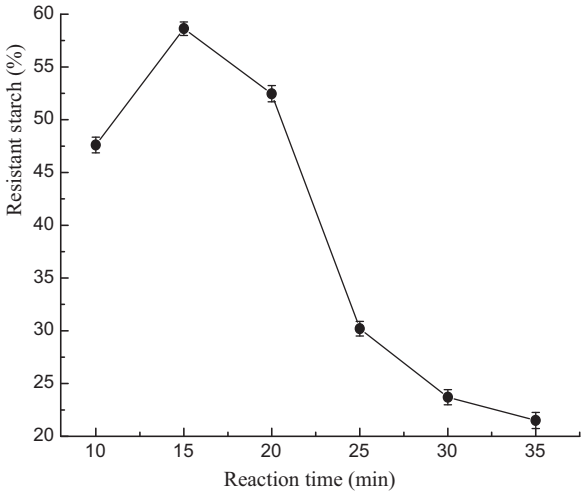

Fig. 4. Effect of time (10–35 min) on resistant starch yield.

sample was placed on double-sided Scotch tape, mounted on an aluminum specimen holder, and coated with a thin film of gold under vacuum. Samples were observed under a Jeol scanning electron microscope (JSM 5410LV, Jeol, Japan).

2.6. Differential scanning calorimetry (DSC)

The thermal properties of products produced via the enzymatic processes described above were investigated using a differential scanning calorimeter (DSC, PE Pyris 6, USA) as described by Chanvrier et al. (2007) with minor modifications. Indium was used as the calibration standard. Each product sample (approx. 2 mg) was placed in a stainless-steel pan, excess water was added (8 μl), and the container hermetically sealed. The scan was carried out immediately to minimize retrogradation. Samples were heated at 10 °C/min from 20 to 180 °C to observe the presence of any residual enthalpy gelatinization peak. Distilled water was used as a reference.

2.7. Data and statistical analysis

All experiments were performed in triplicate, and data shown were mean values. Statistical analysis was carried out using the data analysis tool pack of the software Origin 7.5.

3. Results and discussion

3.1. Effect of temperature on resistant starch yield

The effect of temperature on RS yield is shown in Fig. 2. The maximum RS yield was 57.24% at $T = 90$ °C. The RS yield increased markedly from 45.51% to 57.24% as the temperature was increased from 70 to 90 °C, but decreased at 95 °C, implying that 90 °C is the optimum temperature for α-amylase to hydrolyze starch. Retrogradation of starch is affected by the length of the amylose chains. Longer chains of amylose are hydrolyzed by α-amylase, which attacks the α-1,4 chemical bonds, whereas pullulanase shows greater activity towards short amylose chains. The shorter chains of amylose would facilitate molecular contact to form a crystalline structure. Therefore, the inhibition of α-amylase activity at higher temperatures resulted in decreased yield of RS.

3.2. Effect of pH on resistant starch yield

The pH affects the hydrolytic activity of α-amylase. Therefore, we investigated α-amylase activity at pHs ranging from 4.5 to 7.0. As shown in Fig. 3, the amount of RS produced in the reaction between maize starch and α-amylase increased as pH increased. The maximum RS yield (57.48%) was obtained at pH 5.5. The

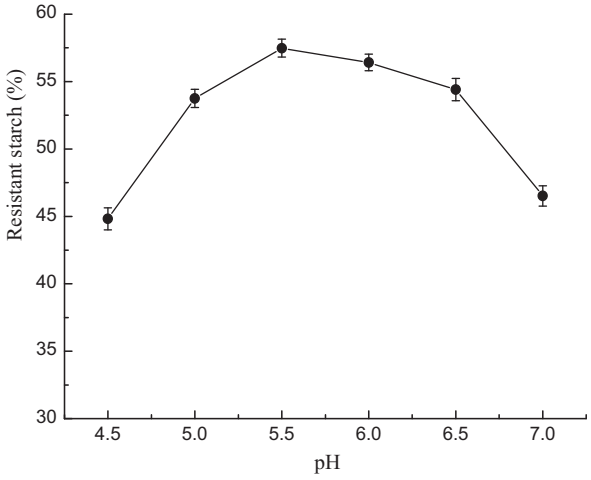

Fig. 3. Effect of pH (4.5–7.0) on resistant starch yield.

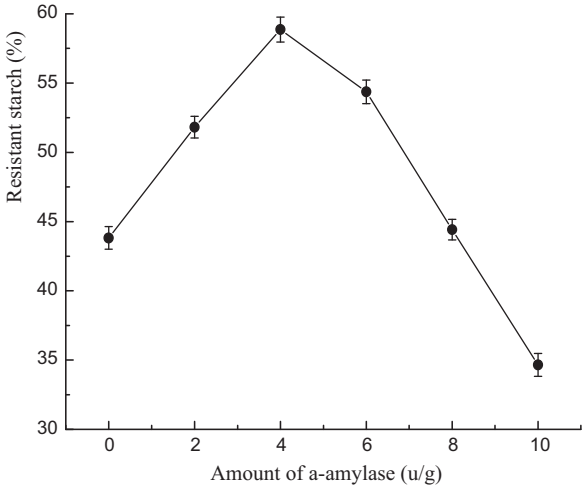

Fig. 5. Effect of α-amylase amount (0–10 μ/g) on resistant starch yield.

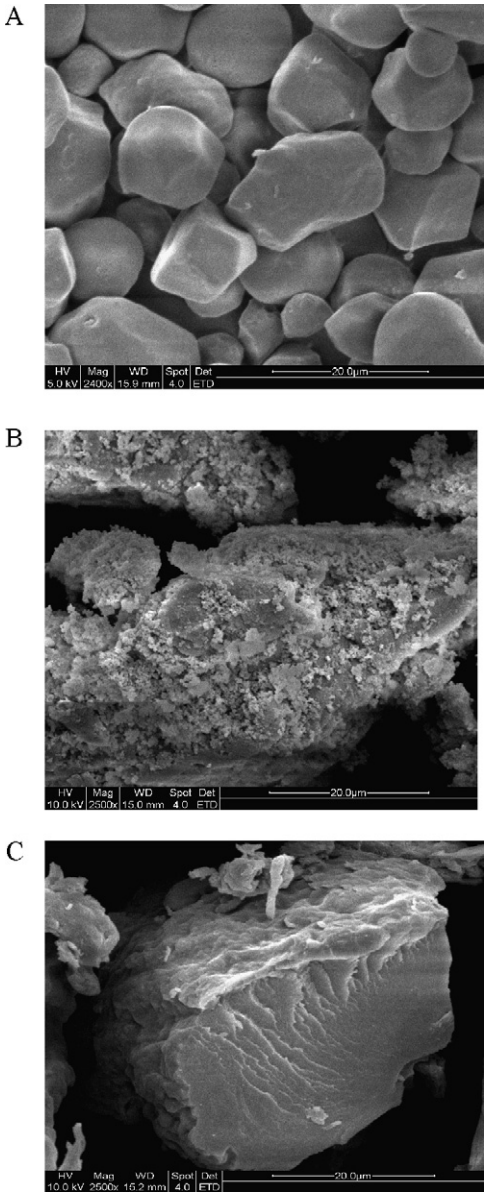

Fig. 6. Scanning electron micrographs of native (unprocessed) maize starch, control (without α-amylase), and resistant starch (C). (A) Native maize starch; (B) control (without α-amylase); (C) resistant starch (temperature, 90 °C; pH, 5.5; time, 15 min; and amount of α-amylase, 4 μ/g; RS = 58.87%).

amount of RS produced decreased as the pH increased higher than 5.5, reflecting a decrease in α-amylase activity at higher pH.

3.3. Effect of reaction time on resistant starch yield

The reaction time is one of the most important factors in the enzymatic production of RS. Given sufficient time, the amylose molecules could be completely degraded into glucoside micromolecules, which would prevent formation of RS. The RS yield increased gradually as the reaction time increased from 10 to 15 min (Fig. 4), reaching a maximum of 58.62% at 15 min. However, reaction times longer than 15 min resulted in a reduction in RS yield. A marked reduction from 52.46% to 32.40% was observed when the reaction time increased from 20 to 25 min, indicating that many of the amylose chains were hydrolyzed into glucoside micromolecules. At reaction times longer than 30 min, the RS yield

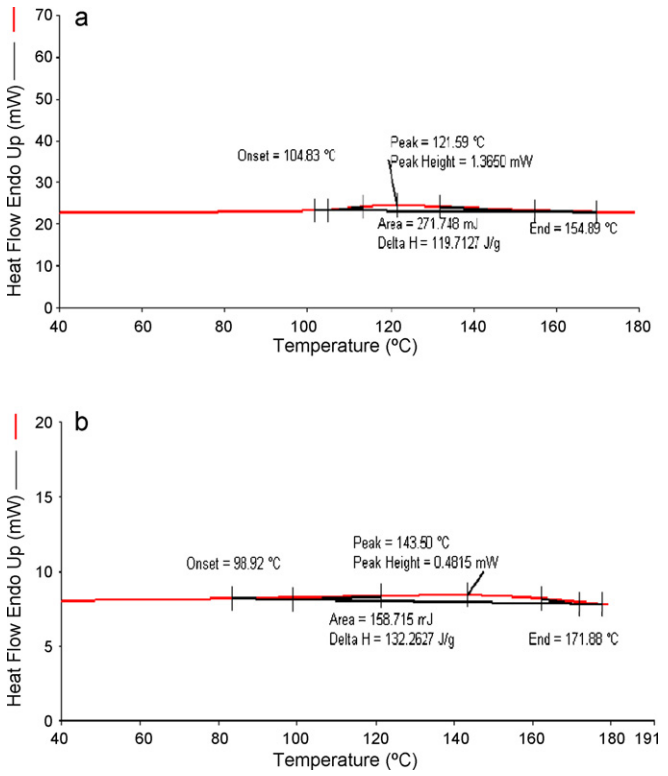

Fig. 7. DSC thermograms of native (unprocessed) maize starch samples (a) and resistant starch (b). (a) Native maize starch samples; (b) resistant starch (temperature, 90 °C; pH, 5.5; time, 15 min; and amount of α-amylase, 4 μ/g; RS = 58.87%).

decreased more slowly. This may have been because the hydrolysis reaction tends to balance due to the consumption of substrate.

3.4. Effect of α-amylase amount on resistant starch yield

We evaluated the effects of α-amylase amount (0–10 μ/g) on production of RS. As shown in Fig. 5, the maximum RS yield (58.87%) was obtained using 4 μ/g α-amylase. The production of RS gradually increased as the amount of α-amylase increased from 0 to 4 μ/g. However, further increases in α-amylase amount resulted in decreased RS production. This may be because greater amounts of α-amylase result in more rapid hydrolysis of maize starch. Therefore, the yield of RS may decrease at higher amount of α-amylase, because the glucoside micromolecules that are produced during the reaction will prevent crystallization of amylose when the mixture cools. These results indicated that the optimum amount of α-amylase under these conditions was 4 μ/g.

3.5. Scanning electron microscopy

Native (unprocessed) maize starch granules had smooth surfaces and irregular, polygonal shapes with diameters of approx. 20 mm (Fig. 6A), and no obvious defects or signs of damage on the surface. However, control starch granules (i.e., those processed without α-amylase) lost their granular shapes and adhered together to form a coarse, honeycomb-like, filamentous network structure (Fig. 6B). Morphological retrogradation occurred, and the irregularly shaped particles formed a white porous network. This continuous network structure was composed of amylose and amylopectin. Similar results have been reported by Jirapa, Anchanee, Onanong, & Kuakoon (2009). In contrast, resistant starch showed increased crystallinity, and formed a larger, more compact laminiplantation structure (Fig. 6C). This was attributed to retrogradation of more amylose chains, which resulted in reorganization of the

starch structure into a helical complex. This increased density of the crystal structure greatly increased its resistance to enzyme attack.

3.6. Differential scanning calorimetry (DSC)

The thermal transitions of RS and native maize starch were determined by DSC. The DSC thermograms of both RS and native starch showed an endothermic peak (Fig. 7), which was attributed to the melting of amylose double helices (Chung et al., 2003). The thermal transitions of RS showed wider temperature ranges and higher enthalpies than native maize starch. This probably reflects the presence of short amylose chains that were released from amylopectin by pullulanase hydrolysis. Such chains could be capable of forming double helices. Hydrolysis by α-amylase could produce shorter amylose chains, thereby increasing regional concentrations of starch chains, so that the amylose chains have more opportunities for physical associations. Acid hydrolysis decreases the length of amylose chains, thus increasing their mobility. This increases crystal formation, which results in a higher thermal transition peak temperature than that of native maize starch.

4. Conclusions

The resistant starch yield of foods depends on the botanical source of the starch and the processing conditions. In this study, we analyzed reaction conditions in the pasting process of maize starch, and found that the production of RS could be greatly increased by treatment with thermostable α-amylase before debranching with pullulanase. For the α-amylase reaction, we investigated the effects of various reaction conditions, including temperature (T), pH, time (t), and enzyme concentration, on production of RS. The optimum reaction conditions were as follows: temperature, 90 °C; pH, 5.5; time, 15 min; and amount of α-amylase, 4 μ/g. Under these conditions, we obtained an RS yield of 58.87% (w/w). One of the most important factors in the formation of RS is an increase in the amylose/amylopectin ratio. This could be because amylose chains form double helices, which reorganize starch structure. Another possibility might be that amylose chains with reduced length would become mobile, thus enhancing crystal formation. An increase in the density of the crystal structure greatly increases the resistance of starch to enzymes.

Acknowledgments

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