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Existing cell wall fragments modify the thermal properties and hydrolysis of potato starch

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ABSTRACT

The effect of cell wall fragments on the thermal properties and hydrolysis of potato starch were investigated. The relative degree of crystallinity of purified starch and starch with cell wall fragments were 19.7% and 20.7%, respectively. The onset temperature (*To*) and peak temperature (*Tp*) of the starch with cell wall fragments were much higher than those of purified starch, whereas the $^{\triangle}H$ value of purified starch (9.5 ± 0.5 J/g) was much higher than that of the starch with cell wall fragments (7.6 ± 0.7 J/g). Moreover, slightly lower equilibrium hydrolysis of starch with cell wall fragments (78.8 ± 6.3%) and different morphologies changes between those two samples during heating were also observed. Our study demonstrated that it might be an alternative way to improve or extend the property of potato starch with the remaining of its cell wall fragments.

1. Introduction

Potato (*Solanum tuberosum* L.) has been widely planted and consumed around the world as an important source of carbohydrate in human diet (Singh, Kaur, & Moughan, 2012). However, processed potato also has a high content of rapid digested starch (RDS), and is classified as a food having an intermediate or a high glycemic index (GI). Thus, the long-term consumption of processed potato is considered a potential contributor to hyperglycemia, type II diabetes, and other chronic diseases (Ek, Brand-Miller, & Copeland, 2012). A number of studies have looked into ways of decreasing the GI of the potato and the results of such studies have demonstrated that the GI could be affected by multiple factors, including cultivars, maturity, starch structure, amylose content, and cooking methods (Nayak, De J. Berrios & Tang, 2014). Current studies aimed at reducing the GI of the potato are mainly focused on the cooking/processing methods or improving the amylose content through transgenic engineering (Tian, Chen, Chen, & Ye, 2016a). Few studies have examined whether the cell wall can affect the digestibility of potato starch. The cell wall, which is mainly comprised of cellulose and hemicellulose, is the second most important component of potato, followed only by starch (Bordoloi, Kaur, & Singh, 2012). In the raw potato, the cell wall forms a honeycomb structure in which small starch granules are embedded. The cell wall might impede the water swelling of starch during gelatinization or play as a barrier to reduce the contact between enzymes and starch during its digestion (Tian et al., 2018). Frost et al. (2016) analyzed the effect of composition and structure of tuber cell walls on the digestibility of potato and found that the higher amounts of RG-I galactan in potato cell walls could impedes access of digestive enzymes to potato starch effectively. However, whether the cell wall will affect the crystallinity degrees as well as the thermal properties of potato starch have not been well studied, thus a better understanding of the impact of the cell wall on the thermal properties and digestibility of potato is needed.

With the aim of making up the insufficiency of the current studies, the thermal properties and crystal forms of purified potato starch and potato starch with cell wall fragments were analyzed using a DSC and X-ray diffraction. An enzyme hydrolysis experiment was also undertaken, and the microstructural changes of starch granules and starch with cell walls, incubated in water at different temperatures (60 °C and 90 °C), were also examined by scanning electron microscopy (SEM).

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2. Material and methods

2.1. Materials and chemicals

Potato tubers (*Xiapodi sp*. of similar size and weight 200–230 g) were purchased from a local supermarket in Hangzhou, China. Porcine pancreatic α-amylase (900 U/mg) and invertase (Invertase, grade VII from baker's yeast, 401 U/mg solid) were purchased from Sigma-Aldrich Ltd. (St Louis, USA). Amyloglucosidase (3260 U/ml), total starch assay kit and glucose oxidaseperoxidase (GOPOD) kit were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Other chemicals and reagents were analytical grade and purchased from Aladdin (Shanghai, China).

2.2. Preparation of potato starch and starch with cell wall fragments

The purified potato starch was prepared according to Frost et al. (2016) with some modification. Briefly, the peeled potatoes were cut into thin slices and immersed in a solution containing 20 mM sodium bisulfite and 10 mM citric acid for 2 h at room temperature to avoid browning. Then, the slices were blended and suspended in 500 mL water and filtered with gauze. The starch milk was allowed to sediment for 1.5 h, after which the supernatant were decanted and the starch precipitate was re-suspended in 500 mL water for three-times or more, decanting the supernatant each time. Starch was recovered by vacuum filtration with repeated washes, immersed in liquid nitrogen and freeze-dried (−50 °C, pressure lower than 0.1 mBar) (FreeZone 6, Labconco, USA) for future analysis. Starch with cell wall fragments was prepared from potatoes that were peeled manually, cut into small cubes (1 cm^3) and immediately immersed in liquid nitrogen and freeze dried as mentioned above, the dried samples were ground using a commercial grinder (JYL-C022, Jiuyang, China), passed through a number 100 sieve and stored at −80 °C for future analysis.

2.3. Microscopy observation

One gram of purified potato starch, starch with cell wall fragments and intact potato tissues were mixed thoroughly with 25 mL of distilled water in a plastic tube respectively and maintained in a water bath at 60 °C or 90 °C for 20 min. The tubes were centrifuged at 1500 × *g* for 3 min and the supernatants were decanted and the precipitates were collected and freeze dried. Finally, the samples were coated with gold (SCD 050, Balzers, Liechtenstein) and their microstructures were observed with a SEM (TM-1000, HITACHI, Japan).

2.4. X-ray diffraction analysis

X-ray measurements were made with an X-ray diffractometer (X'Pert PRO, PANalytical B.V., Netherlands). X-ray diffraction patterns were acquired at room temperature under the following conditions: 40 kV and 20 mA for the Cu-Kα radiation source at a wavelength of 0.15418 nm; a scanning rate of 2°/min in the scattering range (2*θ*) of 5–40°. Diffraction data were analyzed with Jade software (Version 6.5, Material Date, Inc. Livermore, California, USA). The crystallinity (*Xc*) was calculated as the ratio of $Ac/(Ac + Aa)$, where *Ac* and *Aa* are the areas of crystalline and amorphous phases, respectively (Tian et al., 2016b).

2.5. Analysis of thermal properties

The thermal properties of the samples were analyzed with a Modulated Differential Scanning Calorimeter MDS1 instrument (Mettler, Toledo, Switzerland). Detailed procedures for DSC measurements and analysis, described by Tian et al. (2016b), were used with some modifications. Briefly, 10 mg freeze-dried starch sample was accurately weighed, mixed with 20 μL of deionized water and hermetically sealed in an aluminum pan. After equilibrating at room temperature (25 °C) for 24 h, the pan was heated from 20 °C to 100 °C at a rate of 10 °C/min. An empty pan was also used as a reference and the onset temperature (*To*), peak temperature (*Tp*), Endset (*Tc*) and melting enthalpy ($\triangle H$) were calculated using DSC software (Mettler, Toledo, Switzerland).

2.6. Hydrolysis analysis

The hydrolysis analysis was performed according to Gularte and Rosell (2011) with some modification. Briefly, certain samples were weighed, mixed with distilled water thoroughly and pre-incubated at 90 °C for 30 min; the solutions were cooled down and maintained at 37 °C in a water bath to analyze the hydrolysis of purified starch and starch with cell wall fragments. Porcine pancreatic α-amylase was added to obtain a final concentration of starch and α-amylase of 10 mg/mL and 0.33 U/mL, respectively. Five hundred microliters supernatant was collected at 5, 15, 30, 60, 90, 120, 150 min and diluted immediately in 2.5 mL of 95% ethanol to inactivate the enzyme. The mixture was centrifuged at 2000 × *g* for 10 min. Supernatant (0.1 mL) was recovered and incubated with 0.5 mL amyloglucosidase/invertase solution (37.5 mg invertase and 1 mL amyloglucosidase dissolved in 100 mL of 0.05 M potassium acetate) for 10 min at 37 °C to convert all the oligosaccharides and disaccharides produced during hydrolysis to glucose (Tamura, Singh, Kaur, & Ogawa, 2016). Glucose concentrations of the incubated mixtures were measured with a glucose oxidaseperoxidase (GOPOD) kit; the absorbance was measured with a spectrophotometer (UV-2550, Shimadzu, Japan) at 510 nm. And the kinetics of starch hydrolysis was modelled with a first-order equation (1) according to Goñi, Garcia-Alonso, and Saura-Calixto (1997), where *C* represents the hydrolysis percentage of starch at time *t*, C_∞ is the equilibrium concentration of hydrolyzed starch and *k* represents the kinetic constant.

$$C = C_\infty(1 - e^{-kt}) \quad (1)$$

2.7. Statistical analysis

All the experiments were carried out in triplicate, and the results reported as mean value ± standard deviation. One-way Analysis of Variance (ANOVA) was performed to determine significance between variables using an SPSS Program, version 20.0 (SPSS, IBM, US). Significant differences of means were determined by the Duncan's test at 95% ($p < 0.05$).

3. Results and discussion

3.1. Microstructure changes during heating

The microstructure of purified starch, starch with cell wall fragments and intact potato tissues are shown in Fig. 1. The potato starch granules showed oval shapes of various sizes (Fig. 1A). The samples of starch with cell wall fragments showed small starch granules aggregated together imbedded inside cell wall structures. Some of the cell walls were partly broken, possibly attributed to the grinding process, exposing some of the starch granules (Fig. 1B). In contrast to these microstructures, the intact potato tissues exhibited a compact cell structure with most of the small starch granules on the inside (Fig. 1C). These morphologies are consistent with previous reports (Blazek & Gilbert, 2010; Bordoloi et al., 2012).

When potato starch granules are heated in water at 60 °C, the granules swelled and fused together. Some small granules remained, indicating that the starch granules were only partly gelatinized (Fig. 1D). Similar changes were also observed in starch with cell wall fragments and intact potato tissues, but the sizes of the fused starch granules in those samples were much smaller (Fig. 1E and F). This

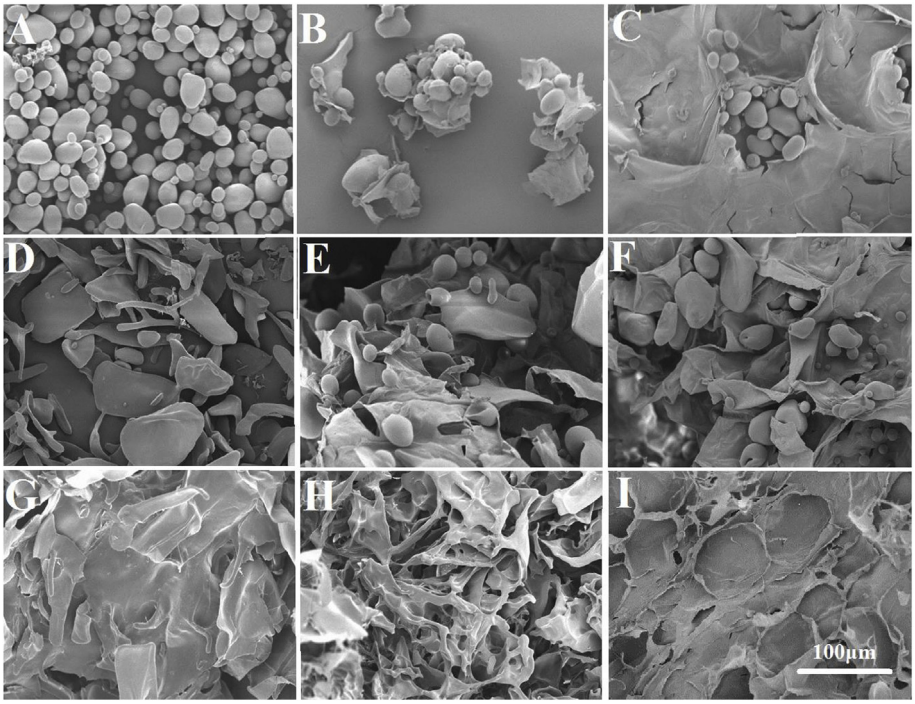

Fig. 1. Microstructure of potato starch. A, D, G: purified starch; B, E, H: starch with cell wall fragments; C, F, I: intact potato tissues; A, B, C: the natural purified starch, starch with cell wall fragments and intact potato tissues; D, E, F: incubated at 60 °C; G, H, I: incubated at 90 °C.

might be explained by that the cell walls act as barriers to prevent contact between the starch granules and water, limiting granules swelling, and resulting in a lower degree of gelatinization than observed for potato starch (Kim & Kim, 2015). However, when the temperature was increased to 90 °C, the highly ordered structures of the starch granules were fully disrupted and fused together and no independent granules could be observed (Fig. 1G). This phenomenon indicates that starch granules are fully gelatinized at 90 °C. The microstructures of starch with cell wall fragments that incubated at 90 °C were quite different from those of potato starch. The surface structures were complex and irregular (Fig. 1H), which might attribute to the fragments impeding the fusion of starch granules during starch gelatinization. The morphologies observed for the intact potato tissues are shown in Fig. 1I. After incubating at 90 °C, only partial breakdown of the cell wall fragments were observed and while swelling and gelatinization of the starch took place the cell structures were mainly maintained. Other researchers have reported similar morphologies in cooked potatoes (Bordoloi et al., 2012; Tian et al., 2016b).

3.2. X-ray diffraction differences

The X-ray diffraction patterns of purified starch and starch with cell wall fragments are described in Fig. 2. Both samples show expected peaks at 5.8°, 14.8°, 17° and 23-25° (2θ), confirming that potato starch is a typical B-type starch (Lorenz & Kulp, 1982). The degree of crystallinity was also calculated and the results indicate that the degree of crystallinity of starch with cell wall fragments of 20.7% was much higher than that of the purified starch (19.7%). These results are in accordance with those of Shin, Baik, and Kim (2015), who reported a higher degree of crystallinity in Atlantic and Superior potato starch with cell walls than that of the starch from those two potatoes and attributed those differences to the higher relative crystallinity of the wall components present in the samples. The cell wall fragments, which are mainly comprised of cellulose and hemicellulose, showed an intense peaks at 6.7°, 7.4°, 10.2°, and 16° 2θ (Boisset et al., 1999), and were accounted for the higher degree of crystallinity of samples.

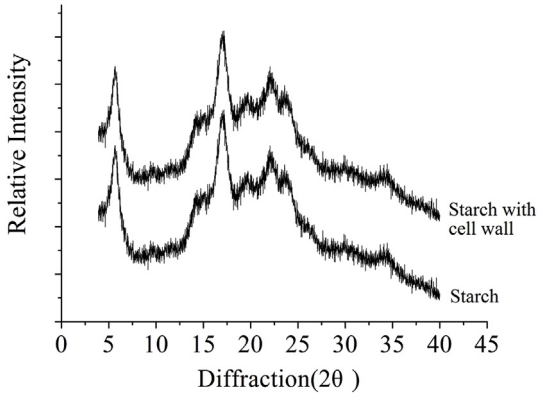

Fig. 2. X-Ray diffraction of potato starch and starch with cell wall fragments.

Table 1
Thermal properties of gelatinization of potato starch and starch with cell wall fragments.

Sample	*To*	*Tp*	*Tc*	ΔH (J/g)
Starch1*	58.10 ± 0.32^{b}	64.30 ± 0.27^{b}	70.00 ± 0.29^{a}	9.50 ± 0.51^{a}
Starch2	59.37 ± 0.45^{a}	65.84 ± 0.69^{a}	71.13 ± 0.96^{a}	7.90 ± 0.72^{b}

*Note: Starch1is the pure starch, Starch2 is starch with cell wall fragments; Different letters in each column represent significantly different at $p < 0.05$.

3.3. Thermal properties

DSC analysis was applied to investigate the thermal properties of purified starch and starch with cell wall fragments, and the results from the thermograms are presented in Table 1. Compared to purified starch, the onset temperature (*To*) and peak temperature (*Tp*) of the starch with cell wall fragments increased significantly ($p < 0.05$), whereas the conclusion temperature (*Tc*) showed no significant difference. The results are consistent with the morphology changes described in Section 3.1, suggesting that the starch granules were only partly gelatinized at

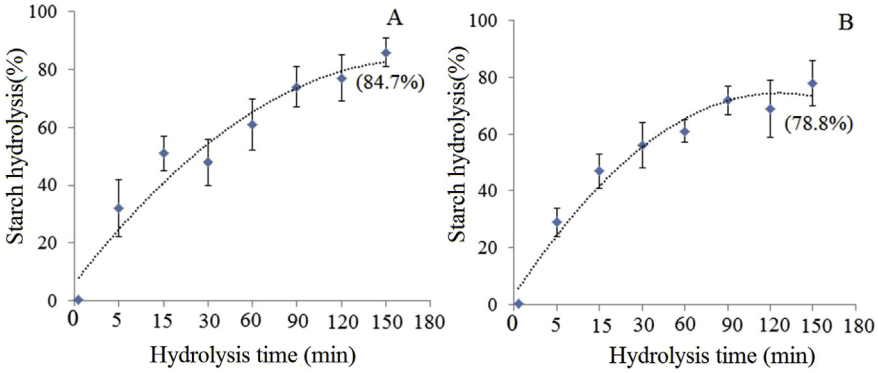

Fig. 3. Hydrolysis curves of potato starch hydrolyzed with porcine pancreatic α-amylase. A: purified starch; B: starch with cell wall fragments.

60 °C. The $\triangle H$ value was higher in the purified starch (9.5 ± 0.5 J/g) than that of the starch with cell wall fragments (7.6 ± 0.7 J/g). Similar results were also reported by Tester and Sommerville (2003), who studied the effects of non-starch polysaccharides on the extent of gelatinization, swelling and α-amylase hydrolysis of maize and wheat starches, and suggested that the non-starch polysaccharides profoundly modified starch gelatinization by prohibiting water access to amorphous parts of the granules.

3.4. Hydrolysis

Both the purified starch and starch with cell wall fragments were fully gelatinized and hydrolyzed with porcine pancreatic α-amylase to verify whether the cell walls affects the hydrolysis of the potato starch (Fig. 3). The equilibrium concentration of hydrolyzed starch in starch with cell wall fragments (78.8 ± 6.3%) were slightly lower than that of the purified starch (84.7 ± 5.5%), but this difference was not significant ($p > 0.05$). Similar trends were also observed for the kinetic constant, which were $15.22 \pm 1.56 \times 10^{-2}$ and $13.82 \pm 1.04 \times 10^{-2}$ (min^{-1}), respectively. Cell walls can play a role as physical barriers between the starch and enzymes and decrease the hydrolysis of starch (Frost et al., 2016). In previous studies, Dhital, Bhattarai, Gorham, and Gidley (2016), reported that intactness of cell walls structure controlled the *in vitro* digestion of starch in legumes. However, in the present study, the hydrolysis showed only slight decrease in starch with cell wall fragments, a possible explanation was that the integrity of cell walls were destroyed and these broken cell walls are an ineffective barrier between starch and enzymes. Similar results were also reported by Berg, Singh, Hardacre, and Boland (2012), who studied the role of cotyledon cell structure on the *in vitro* digestion of starch in navy beans and found that the navy beans showed little difference between milled bean flour and bean starch (85–90%).

4. Conclusions

The present study investigated the impact of cell wall fragments on characteristics of potato starch prepared from whole potato tissues and demonstrates that the presence of a cell wall fragments altered the thermal properties, degree of crystallinity and the morphology of potato starch during gelatinization. Furthermore, the cell wall fragments also slightly decrease the hydrolysis of potato starch. Our results indicate that retaining cell wall fragments might represent an alternative approach for improving or extending the properties of potato starch. Since the compositions and content of cell walls from different potato varieties are very similar (Frost et al., 2016), additional studies focused on the analysis of the cell wall structure and their effect on the potato starch properties and digestibility are warranted.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.foodhyd.2018.07.033.

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