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Structure and enzymatic resistivity of debranched high temperature–pressure treated high-amylose corn starch

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ABSTRACT

High-amylose corn starch (HACS) was treated with high temperature–pressure (HTP) treatment and pullulanase debranching. It was found that 24 h storage was favorable for resistant starch (RS) formation. Structure (granular morphology, fractal structure, lamellar structure, crystalline structure, weight-average molecular weight) and properties (swelling power, solubility, enzymatic resistivity) were evaluated for native starch and the samples with 24 h storage. By modification, the surface became loose and rough fragmented and the birefringence crosses disappeared. All samples displayed a B + V crystalline structure. The scattering objects of native starch at the higher scale level were more compact than those of modified starches, and the latter displayed a mass fractal structure which became more compact as debranching increased. The native starch contained RS2 and RS5, while the modified samples included RS3 and RS5. The higher amount of V-type crystals and the starch chains with smaller molecular weight could lead to form more RS. Interestingly, a surface fractal structure with D_{s2} was measured for the modified starches, leading to more RS, since some active sites of starch molecules were masked by the ordered-aggregations of molecular chains in the scattering objects. Furthermore, the more compact scattering objects with D_{m1} contributed to forming more RS.

1. Introduction

The increase in consumer demand for high quality food products has led to a growth in the use of new technologies and ingredients (Fuentes-Zaragoza et al., 2010). Resistant starch (RS), which is defined as the fraction of starch degradation that escapes digestion in the small intestine of healthy people (EURESTA, 1993) may be fermented by the colonic microorganism (Li et al., 2011), has gained attention as a functional food ingredient due to its potential health benefits and functional properties in foods (Sajilata et al., 2006). RS plays key physiological roles and has the potential to improve human health and lower the risk of many diet-related diseases (Zhang and Jin, 2011). Compared with traditional insoluble fibers, RS has many advantages, i.e. a natural white color, a bland flavor, and a better appearance and texture. RS products have a less gritty mouth feel, and RS masks other flavors less than other typical insoluble fibers (Sajilata et al., 2006).

It is concluded from previous studies (Brown et al., 1995; Englyst et al., 1992), RS is normally defined as four main types, i.e. RS1, starch trapped within whole plant cells and food matrices, RS2, starch granules from certain plants containing uncooked starch such as green banana starch or starch that was gelatinized poorly and hydrolyzed slowly by amylases such as high-amylose corn starches, RS3, RS formed from retrogradation of starch after cooking, and RS4, chemically modified starches such as ethers and esters. An additionally RS5 is formed from high amylose starches that require higher temperatures for gelatinization and are more susceptible to retrograde, which is actually an amylose–lipid complexed starch (Jiang et al., 2010). There are many factors that can affect the formation of RS in foods, such as 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 and Pomeranz, 1989).

While being heated at a temperature higher than a specific value with abundant water, starch granules undergo an irreversible swelling, and the crystalline structure in starch is destroyed (Atwell et al., 1988). This endothermic phenomenon is commonly called "gelatinization", which renders the molecules fully accessible to

Abbreviations: DC, degree of crystallinity; DP, degree of polymerization; DSC, differential scanning calorimetry; GPC, gel permeation chromatography; HACS, high-amylose corn starch; HTP, high temperature–pressure; MALS, multi-angle light scattering; MC, moisture content; PLM, polarization microscope; RS, resistant starch; S, solubility; SAXS, small-angle X-ray scattering; SEM, scanning electron microscopy; SP, swelling power; XRD, X-ray diffraction.

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digestive enzymes. Some sort of hydrated cooking operation is typical in the preparation of starchy foods for consumption, rendering the starch rapidly digestible (Haralampu, 2000). If these starch gels are then cooled, they can form starch crystals that are resistant to enzyme digestion. This form of 'retrograded' starch has been found in small quantities (approximately 5%) in foods such as "corn-flakes" or cooked and cooled potatoes, as used in a potato salad (Fuentes-Zaragoza et al., 2010). Furthermore, debranching would give chains more opportunities to align and aggregate to form perfectly crystalline structures, thereby leading to form more RS (Guraya et al., 2001). High-amylose corn starch (HACS) can be used for preparing RS3 (Dimantov et al., 2004; Sievert and Pomeranz, 1989). It is currently accepted that the mechanism by which RS3 resists amylase digestion is that linear amylose segments align into condensed double helical structures after gelatinization (amylose retrogradation), rendering α-1,4 glucosidic linkages inaccessible to amylase (Zhang and Jin, 2011). However, the RS content in cooked rice was determined by the degree of polymerization (DP) of the side chains of amylopectin (Shu et al., 2007). That is, the mechanism of RS formation in retrograded starch was not clarified. Moreover, studies on the relationship between the structure (especially the fractal structure) and RS formation of debranched high temperature–pressure (HTP) treated HACS are limited.

In this study, HACS was chosen as a starch base for modification, using a reactor to achieve a constant HTP condition. The effect of storage time on the formation of RS, besides, the aggregation structure (granular morphology, fractal structure, lamellar structure, and crystalline structure), weight-average molecular weight, and properties (swelling power, solubility, and RS content) for the native G50 starch and modified starches with 24 h storage, were investigated. The results would form the basis for further investigations on the mechanism of the formation of RS in HACS to widen the application of HACS in foods.

2. Materials and methods

2.1. Materials

High-amylose corn starch (G50) with amylose content ~50% from Penford (Australia) was used in this experimental work. The moisture content, determined by using a moisture analyzer (MA35, Sartorius Stedim Biotech GmbH, Germany), is 14.98%. Pullulanase (1130 ASPU/g) was obtained from Unikbio Biotech (Guangzhou) Ltd (Guangzhou, China).

2.2. High temperature–pressure (HTP) treatment and debranching

About 50 g dry starch was dispersed in 250 mL distilled water and cooked in a HTP reactor (PARR4545, PARR) at 110 °C and 10.3 MPa with stirring for 30 min. The gelatinized starch dispersion was divided into four batches and cooled to 60 °C. These samples were incubated with different amounts of Pullulanase (0, 140, 200 and 280 ASPU/g dry starch) for 2 h by stirring in a water bath, which were referred to as HTP-0, HTP-140, HTP-200, and HTP-280. The samples were heat-treated at 100 °C for 10 min to stop enzyme activity and stored at 4 °C for 0, 24, 48, 72, 96, 120 h. Finally, all samples were air dried at 60 °C for 48 h and ground for further analysis.

2.3. Resistant starch content

The RS content of each sample was determined using method 991.43, total dietary fiber (TDF), of the Association of Official Analytical Chemists (AOAC, 2000).

2.4. Characteristics of debranched HTP treated samples with 24 h storage

2.4.1. Scanning electron microscopy (SEM)

Granular morphology was studied using an EVO18 SEM (ZEISS, Germany) operated at 10.0 kV high voltages. Before examination in the microscope, the samples were coated with a thin gold film.

2.4.2. Small-angle X-ray scattering (SAXS)

SAXS experiments were performed using a SAXSess small-angle X-ray scattering system (Anton-Paar, Austria) equipped with a PW3830 X-ray generator (PANalytical), operated at 50 mA and 40 kV, using Cu Kα radiation with a wavelength of 0.1542 nm as X-ray source. Starch slurries with similar moisture content (MC, about 60%) were prepared for this experiment and equilibrated at 20 °C for 24 h before the analysis. The samples were filled into a sample cell and measured for 10 min. The data, recorded using an image plate, were collected by the IP Reader software with a Perkin Elmer storage phosphor system. All data were normalized, and the background intensity and smeared intensity were removed using SAXSquant 3.0 software for further analysis.

Fractal geometry has been used as a natural description for disordered objects possessing dilation symmetry, meaning that they look geometrically self-similar under transformation of scale such as changing the magnification of a microscope (Schaefer, 1989). The fractal structures can be characterized by the fractal dimension D, which is related to the scattering power-law equation:

$$I \sim q^{-\alpha} \tag{1}$$

where I is the SAXS intensity and α is an exponent, which can be used to calculate the value of D of the surface/mass fractal structure and can be obtained from the slope of the log–log SAXS graph. The relation between α and D follows as $D_s = 6 - \alpha$ ($3 < \alpha < 4$) representing a surface fractal, and $D_m = \alpha$ ($1 < \alpha < 3$), which is classified as a mass fractal. D_s can be seen as an indicator of the degree of smoothness, and the value of D_s is equal to 2 when the surface of the scattering objects is smooth. However, D_m is used to indicate the compactness (Suzuki et al., 1997). The scattering objects of surface fractal are more compact than those of mass fractal.

2.4.3. Microscopy

Polarized light microscopy was performed using a polarization microscope (PLM) (Axioskop 40 Pol/40A Pol, ZEISS, Oberkochen, Germany) equipped with a 35 mm SLA camera (Power Shot G5, Canon, Tokyo, Japan). The magnification was 500 (50 × 10). Each sample was dispersed as 10 mg starch in 1 mL of distilled water in glass vials. A drop of starch suspension was transferred onto a slide, covered by a cover slip. Polarized light was used for observation.

2.4.4. X-ray diffraction (XRD)

XRD analysis was performed with an Xpert PRO diffractometer (Panlytical, Netherlands), operated at 40 mA and 40 kV, using Cu Kα radiation with a wavelength of 0.1542 nm as X-ray source. The scanning of diffraction angle (2θ) was from 5° to 40° with a scanning speed of 10°/min and scanning step of 0.033°. The MC of each sample was about 10%. The relative crystallinity of each sample was calculated using the method of Hermans and Weidinger (1948).

2.4.5. Differential scanning calorimetry (DSC)

Thermal behaviors were studied using a Perkin–Elmer DSC Diamond-I with an internal coolant (Intercooler 1P) and nitrogen purge gas. A high-pressure stainless steel pan (PE NO. B0182901) with a gold-plated copper seal (PE NO. 042-191758) was used to