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Long- and short-range structural characteristics of pea starch modified by autoclaving, α-amylolysis, and pullulanase debranching

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ABSTRACT

Pea starch (S) was modified by autoclaving (A), α-amylolysis (E), and pullulanase debranching (P), the effect of pretreatments including autoclaving and α-amylolysis on the structural modifications to the pullulanase debranched starch was investigated. All processed pea starch was transformed from a C– to a B-type crystalline structure. The power law exponent (α) ranging from 1.85 to 2.64 suggested the existence of mass fractal structure. Compared with native starch, all treatments applied caused an enhanced short-range order which was reflected by the increased values of α, degree of double helix (DD), degree of order (DO), and double helix content based on SAXS, FTIR, and ^{13}CNMR observations. The processed starch sample of AS, and APS exhibited the highest DO, and α values, as well as the stronger absorption peak between 3000 and 3695 cm^{-1}on FT-IR spectrum. AEPS exhibited the significantly highest double helix content, indicating that the higher extent of degradation induced by the combined treatments of autoclaving, α-amylolysis, and pullulanase debranching would give the molecular chains a higher alignment opportunity for the evolution towards coil-to-helix transition. The results would be helpful for better understanding the structure-processing relationship and to provide theoretical foundation for the development of food ingredients with targeted functional properties.

1. Introduction

Starch, which is the dominant component in tuber- and cereal-based products, provides the primary glycemic carbohydrate source of energy for humans. It is well known that the multi-scale structure of starch is compose of a mixture of essentially linear amylose containing few long branches, and highly branched amylopectin with 5% branching points and a large quantity of short branches [1]. These chains of amylopectin correspond to the unbranched outmost chains (A), the branched inner chains (B) which are further classified as B1, B2, B3, and B4 chains, and the single chain (C) per molecule which contains a sole reducing residue [2]. The A and B1 chains (i.e., the outer parts of amylopectin branches) build up the double helices clusters, which further construct the crystalline lamellae. The B2, B3 and C chains (i.e., the internal parts of amylopectin) are located in the amorphous lamellar structure of starch granule [3].

Pullulanase (Pullulan 6 glucanohydrolase, EC 3.2.1.41), which is a debranching enzyme, can directly clave the α 1,6 glucosidic linkage, releasing a mixture of long and shorter linear unit glucans from their parent amylose and amylopectin [4]. The long linear chains obtained after debranching are derived from amylose having average chain lengths (CL) ranging from 35 to 550, whereas the short linear chains are a derivative from amylopectin with average CL between 20 and 30 [5]. Pullulanase debranching is considered as a safe and cost-effective method to facilitate the RS_3 formation which has been commonly used alone or together with autoclaving-cooling for the production of starch-based foods with promising health benefits. The mechanism involved is based on the generation of increased number of short amylose and amylopectin molecules with the appropriate chain lengths upon debranching which provides greater opportunities for molecular alignment and aggregation, and thereby the association of amylose into double helix. The debranching efficiency for hydrolyzing the α 1,6 glucosidic bonds by pullulanase is much lower for starch samples with long lateral chains. Therefore, pretreatments such as pre-heating, autoclaving, or α-amylolysis have been used prior to the application of pullulanase debranching [6–8], leaving the gelatinized and/or degraded starch molecules with reduced degree of polymerization and increased fluidity of substrate for better accessibility of the debranching

Abbreviations: NS, native starch; AS, autoclaved starch; PS, pullulanase debranched starch; EPS, enzymatic hydrolyzed starch by α-amylase; APS, autoclaved and pullulanase debranched starch; AEPS, autoclaved, enzymatic hydrolyzed and pullulanase debranched starch; XRD, X-ray diffraction; SAXS, small angle X-ray scattering; FT-IR, Fourier transform infrared spectroscopy; SEM, scanning electron microscopy; ^{13}C CP/MAS NMR, ^{13}C cross-polarization and magic angle spinning nuclear magnetic resonance; DO, degree of order; DD, degree of double helix; PPA, proportion of amorphous phase.

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enzyme. The application of pullulanase combined with α-amylase could lead to the co-hydrolysis of pea starch chains into reduced amylose chains with decreased steric hindrance among the molecules [7]. Autoclaving could modify the structural organization of crystalline and amorphous regions of native starch granules upon gelatinization, leading to an increased availability of amylose and amylopectin prior to pullulanase debranching. Moreover, the degradation and hydrolysis of starch long chains are also likely to take place during autoclaving [9]. A number of techniques have been frequently used for characterizing the molecular arrangement of starch during different processing treatments. X-ray diffraction (XRD) is used for detecting the long-range periodicity of starch by providing information of regularly repeating orientation of double helices. Small-angle X-ray scattering (SAXS), solid-state ^{13}C NMR, and Fourier transform infrared spectroscopy (FT-IR) are shown to be sensitive to changes in short-range structural characteristics including helicity, chain conformation, order along the chains, and double helix structure of starch granules [10].

Pea starch, which is considered as a cheap source of starch compared to wheat, potato and corn starches, has a wide application in food industry as thickening, blending, and coating agent in commercially processed foods [11]. Contrary to the traditional principle of processing, which is aiming to enhance the food digestibility, the current tendency of processing has been transformed to design palatable food products with reduced glycemic index and potential health benefits. Although numerous studies have been done on investigating the physicochemical and structural properties of debranched starch from different botanical origins, much less is known about the effect of pretreatments including autoclaving and α-amylase hydrolysis on the structural modifications to the pullulanase debranched starch. Such pre-processing treatments, is of great economic importance to the food industry. Therefore, the objective of the present study was to investigate the effect of autoclaving, α-amylolysis, and pullulanase debranching, as well as the combined treatments on the long- and short-range structural characteristics of pea starch. The results obtained are expected to provide useful information to better understand the structure-processing relationship and to help the food industry to design novel starch materials with precisely-controlled molecular structure.

2. Materials and methods

2.1. Materials

Native pea starch was supplied by Chengdongwang Food company™ (Chengdu, China). Pullulanase (E.C.3.2.1.41, ~1498 U/g), and pancreatic α-amylase (E.C. 3.2.1.1, ~50 U/mg) were purchased from Sigma Chemical Company (St. Louis, Missouri, USA). Chemicals and solvents used in this study were of analytical grade.

2.2. Methods

2.2.1. Sample preparation

Pea starch slurry (10%, w/v) was pre-gelatinized at 85 °C for 15 min. The autoclaved sample was prepared by pressure cooking the pre-gelatinized sample at 121 °C (0.1 MPa) for 20 min and is referred to as AS (autoclaved starch). The obtained paste was cooled to 58 °C and mixed with pullulanase. The mixture was then incubated with constant stirring and debranched at 58 °C for 12 h, and the processed sample is referred to as APS (autoclaved-pullulanase debranched starch). As a control, PS (pullulanase debranched starch) was prepared by directly debranching the gelatinized starch with pullulanase. The same pre-gelatinized pea starch was autoclaved, hydrolyzed by α-amylase, and then debranched by pullulanase which is referred to as AEPS (autoclaved-enzymatic hydrolyzed-pullulanase debranched starch). The above sample without the pretreatment of autoclaving is referred to as EPS (enzymatic hydrolyzed-pullulanase debranched starch). All processed starch samples were boiled for 10 min to inactivate the enzymes expect for AS. All obtained samples were then retrograded at 4 °C for 24 h, dried at 40 °C, and passed through a 149 μm mesh screen until further analysis.

2.2.2. X-ray diffraction (XRD)

The native and processed pea starches were scanned by an X-ray diffractometer (D8-Advance, Bruker Inc., Karlsruhe, Germany) equipped with an array detector (LYNXEYE DETECTOR) through the 2θ angular range of 4–40°. Traces were obtained by using a Cu-Kα radiation (λ = 1.5418 Å) at the operating conditions of 40 mA, 40 kV at the step size of 0.100 s for continuous scanning.

2.2.3. Small angle X-ray scattering (SAXS)

Small angle X-ray scattering measurement was carried out on a Nanostar SAXS (Bruker AXS GmbH, Karlsruhe, Germany) equipped with a Vantec-2000 2D detector following the method described by Ma, Yin, Hu, Li, Liu and Boye [12].

2.2.4. Fourier transform infrared spectroscopy (FT-IR)

The native and processed pea starch powder was ground with KBr in an agate mortar and dried. Samples were scanned from 400 cm^{-1} to 4000 cm^{-1} at a resolving power of 4 cm^{-1} using Fourier transform infrared spectroscopy (Tensor 27, Bruker Optics GmbH, Germany).

2.2.5. Solid-state ^{13}C nuclear magnetic resonance spectroscopy ($^{13}CNMR$)

The cross-polarization and magic angle spinning ^{3}C nuclear magnetic resonance experiment was performed using a solid-state ^{13}C CP/MAS NMR (AVANCE III 400 MHz WB spectrometer, Bruker Inc., Karlsruhe, Germany) following the method described in our previous study [13]. The double helix content and proportion of amorphous region (PPA) were calculated by using PeakFit software (version 4.12, SeaSolve Inc., Framingham, MA, USA),

2.2.6. Scanning electron microscopy (SEM)

The native and processed starch samples were uniformly distributed on the conductive adhesive which was attached to a circular aluminum stub and then were sputter-coated with gold by an ion sputtering instrument (BAL-TEC, SCD005) for 80 s. The morphological features were observed by a scanning electron microscopy (Quanta 200, FEI company, Oregon, USA) at an accelerating voltage of 20 kV.

2.3. Statistical analysis

Values given in tables are the average of three determinations. The analysis of variance (ANOVA) was evaluated using SPASS version 17.0 software (SPSS Inc., Chicago, Illinois, USA). Statistical differences between the average values were determined by Tukey's multiple comparison test at the 5% significance level.

3. Results and discussion

3.1. X-ray diffraction pattern and long-range crystallinity

The X-ray diffraction patterns of native and processed pea starch samples are shown in Fig. 1. The native pea starch exhibited strong diffraction peaks at 2θ = 15.42, 17.22, 18.15, 23.17° and weak reflections at 2θ = 5.87, 11.59, 26.73°, which were characterized as the typical C-type crystalline pattern. Regardless of the pretreatments applied, the crystal type of all processed pea starch was transformed to a type B structure, with strong reflection appeared at 2θ = 17.20°, and weak additional peaks at 2θ = 5.80, 15.50, 22.20°. The observation agreed with previous studies, which reported that the debranched starch and autoclaved starch tended to form the typical B-type crystalline polymorph [14–16]. The more intense and sharper peak at 2θ = 17.20° for APS, EPS, and AEPS compared with AS and PS signified that the combined treatments applied on pea starch in this study were in favor for