RESEARCH ARTICLE

Structural characterizations and in vitro digestibility of acid-treated wrinkled and smooth pea starch (*Pisum sativum* L.)

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In this study, the molecular structure of acid-treated wrinkled and smooth pea starch residues was investigated, and the in vitro digestibility of the residues with 2.2 M HCl at 35°C for different time periods (1, 3, 5, 8, and 15 days) was assessed. After acid treatment, the amounts of rapidly digestible and slowly digestible starches increased, whereas the amount of resistant starch decreased. The granular appearance of the two pea starches was destroyed and small fractions formed aggregates. The changes in the ratio of absorbance at 1047 cm^{-1} to that at 1022 cm^{-1}, the intensities of major peaks, relative crystallinity, and thermodynamic parameters from DSC were observed during acid hydrolysis. These properties of wrinkled pea starches were significantly different from those of smooth pea starches. The crystallinity of acid-hydrolyzed starches increased slightly with increased acid-treatment time. From the entire hydrolysis process, the B polymorph of acid treated pea starch has a higher hydrolysis than that of A polymorph. A reduced tendency in chain-length distribution occurred with the degradation of chains in amylose and amylopectin. These results demonstrated that the slow digestion and resistance properties of wrinkled and smooth pea starches were affected by both the structure of the amorphous and crystalline regions of starch granules.

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1 Introduction

Starch is an important polysaccharide, being widely used in many food products as a major source of dietary carbohydrates and also in various nonfood applications [1]. The primary sources of starch include traditional staple food materials such as cereals, roots, seeds and tubers, all of which provide metabolic energy for human and animals [2].

Starch consists basically of two types of macromolecules: amylose, with a few long-chain branches and relatively low molecular weight, and amylopectin, a highly branched molecule with much higher molecular weight. The structure of starch granules includes an alternating arrangement of amorphous and crystalline lamellae from the hilum toward the surface of the granules, which is a growth ring with a semicrystalline layered structure [2]. Starch can be divided into A-, B- and C-type crystalline polymorphs according to the geometrical characteristics of the unit cell, the double-helix bulk density and the number of different bound water

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Abbreviations: CLD, chain-length distribution; **DSC,** differential scanning calorimetry; **RDS,** rapidly digestible starch; **RS,** resistant starch; **SDS,** slowly digestible starch; **SEC,** size-exclusion chromatography; **SEM,** scanning electron microscopy; **SP,** smooth pea starch; T_c, conclusion temperature; T_o, onset temperature; T_p, peak temperature; **WP,** wrinkled pea starch; **XRD,** X-ray diffraction; $\triangle H$, gelatinization enthalpy

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contents in the crystal structure. The multi-level structural characteristics of starch are closely linked to its physiochemical and nutritional properties, playing a dominant role in controlling the applications of starch in food and industrial products [3].

As an important source of starch, peas are produced in almost all countries all over the world. It is widely grown as a cool season grain legume that provides a good source of dietary protein and energy for humans and livestock [4]. The starch and protein contents of the grains range between 30–50% and 20–25%, respectively, of dry matter. In the species *P. sativum* L., two different seed phenotypes exist, namely, smooth (with a smooth seed surface) and wrinkled pea (wrinkled seed surface) [5]. The two types are genetically different and produce characteristic starches with different granular morphologies, structural and functional characteristics. In China, smooth pea is a commonly seen commercial legume type, and the structure and characteristics of its starch have been well studied [6]. Wrinkled pea, on the other hand, is a wild (uncultivated) variety found mainly in China. There is very limited information known about its starch structure and properties to date, and thus this merits further studies.

Acid modification is a very useful method in understanding the inner structure of starch granules and is also a commonly used method in preparing modified starches [7]. It could change the morphological structure, crystalline structure, gelatinization properties involving transition temperatures and gelatinization enthalpy of starch [8], producing modified starches with various structural and functional characteristics for different applications. Digestibility is an important nutritional characteristic of native and modified starches. Although rapidly digestible starch is favored for quickly providing energy and relieving hunger, slowing down starch digestion rate is of increasing importance because over-nutrition has become a significant issue. Therefore, analyzing the digestion properties of wrinkled pea starch could provide guidance for its application in food industry, and understanding its digestibility changes during acid hydrolysis may provide a tool in producing starches with slower digestion rates.

This study uses acid to partially hydrolyze pea starches to various degrees, and monitors the structure and property changes in starch over the hydrolysis process. This is to reveal the detailed inner granular and molecular structures of wrinkled pea starch and its functional and nutritional properties, as compared with smooth pea starch. We also aim to see if wrinkled pea starch offers any nutritional (digestibility) advantage compared to smooth pea starch. Understanding how acid hydrolysis changes starch digestibility may help in producing starches with improved digestion properties. Further, obtaining data in closely related varieties with slight but significantly different structural parameters may lead to improved understanding of the mechanistic reasons underlying structure-property relations.

2 Materials and methods

2.1 Materials

Soomth pea starch was purchased from Yantai Dongfang Protein Science and Technology Co., Ltd., China. Wrinkled pea starch was obtained from farmers in Dingxi, Gansu Province. Pancreatin from porcine pancreas (P7545) and amyloglucosidase from *Aspergillus niger* (A7095) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA). The glucose oxidase-peroxidase (GOPOD) assay kit was from Megazyme International Ireland Ltd. (Co. Wicklow, Ireland). Chemicals and solvents were of analytical grade.

2.2 Starch isolation and preparation of acid-modified starch

Starch was isolated using the method of Beta et al. [9]. Wrinkled peas (1000 g) were steeped in 2000 mL of water at room temperature for 24 h. The steeped peas were washed and ground with an equal volume of water. The slurry was filtered through a 200-mesh screen. The material remaining on the sieve was rinsed twice with deionized water. The filtrate was subsequently washed several times with NaOH (0.2% w/v) until the gray, top protein-rich layer was removed. The starch was washed with water to remove residual NaOH and dried for 24 h at 45°C. Native smooth and wrinkled pea starch contain 0.46 and 0.37% protein, 0.15 and 0.13% fat, and 0.16 and 0.14% ash, respectively.

The isolated starch was then modified by acid following the method of Wang et al. [10] with modifications. Native starch (10 g, dry basis) was hydrolyzed by suspending it in 200 mL of 2.2 M HCl solution at 35°C for 1, 3, 5, 8, and 15 days (denoted WP-1d to WP-15d for WP and SP-1d to SP-15d for SP) with stirring. The suspension was then washed several times with deionized water until the pH became 7. The collected starch was dried in an oven at 45°C (air stream) for 1 day, and was then ground into powder and passed through a 100 mesh sieve waiting for analysis.

2.3 In vitro digestion with pancreatin and amyloglucosidase

In vitro digestion was carried out following the method described by Englyst et al. [11] with modifications using pancreatin and amyloglucosidase. Pepsin digestion was omitted because the protein content in the starch samples was in negligible trace amounts. In sodium acetate buffer (7.5 mL, 0.1 M, pH 5.0), amyloglucosidase (0.75 mL, 300 U/mL) and pancreatin (2.25 g, 8 × USP) were mixed to prepare the enzyme solution. Starch (300 mg) with sodium acetate buffer (10 mL, 0.1 M, pH 5.0) was incubated at 37°C for 10 min, and then mixed with 0.75 mL of enzyme solution. The following enzyme digestion procedure refers to the method in our

previous paper [12]. Starch samples was enzymatically hydrolyzed at 37°C with shaking at 150 rpm. At 20 and 120 min, an aliquot of hydrolyzed solution (0.5 mL) was collected and added immediately to 20 mL of ethanol (95%). to deactivate the enzymes. After centrifugation (1500*g*, 10 min), the GOPOD assay kit was used to determine the glucose content. The percentage of hydrolyzed starch was calculated by multiplying the glucose content by a factor of 0.9. The values of RDS, SDS and RS were obtained by combining the values of G20 (glucose released at 20 min), G120 (glucose released at 120 min), FG (free glucose), and TS (total starch) using the following formulas:

$$\mathrm{RDS}(\%) = 90^{*}(\mathrm{G20} - \mathrm{FG})/\mathrm{TS}$$

$$\mathrm{SDS}(\%) = 90^{*}(\mathrm{G120} - \mathrm{G20})/\mathrm{TS}$$

$$\mathrm{RS}(\%) = 100 - \mathrm{RDS}\% - \mathrm{SDS}\%$$

2.4 Scanning electron microscopy (SEM)

The photograph of starch sample was obtained using scanning electron microscopy (EVO 18, Zeiss, Germany) at an accelerating potential of 20 kV. Starch samples (1–3 mg) were attached to a circular aluminum stub by double-sided adhesive tape, and was then coated with 20 nm of gold under vacuum.

2.5 Wide-angle X-ray diffraction patterns (XRD)

A D/Max-2200 X-ray diffractometer (Rigaku Denki Co., Tokyo, Japan) was used with Cu Kα radiation at 44 kV and 26 mA. Before analysis, starch samples were equilibrated in a sealed desiccator with water at room temperature for 12 h. The diffractogram scan was run between 4° and 35° (2θ) at a rate of 5°/min. The relative crystallinity was estimated from the ratio of the crystalline area to the total diffractogram area. The method for obtaining the fraction of B polymorph is in the Supporting Information.

2.6 Differential scanning calorimetry (DSC)

Thermal properties were measured using a PerkinElmer DSC (DSC8000, Norwalk, CT, USA) using Pyris thermal analysis software (PerkinElmer). Measurement procedures were as in our previous work [13].

2.7 Chain-length distribution (CLD) of starch samples using size-exclusion chromatography (SEC)

Starch samples were debranched using isoamylase in an acetate buffer solution (0.1 mL, 0.1 M, pH 3.5) and freeze-dried overnight using a previously described method [14, 15]. After drying, the starch was dissolved overnight in DMSO/0.5% wt LiBr solution in an 80°C thermomixer (Eppendorf, Hamburg, Germany) with shaking at 350 rpm. Insoluble components was removed from the starch solution by centrifugation. The SEC weight distributions were obtained using an Agilent 1100 Series SEC (Agilent Technologies, Waldbronn, Germany) equipped with an isocratic pump, a series of separation columns (GRAM precolumn, GRAM 30 and 1000 analytical columns, Polymer Standard Services, Mainz, Germany) and a refractive index detector (RID; ShimadzuRID-10A, Shimadzu Corp., Japan) as described elsewhere [16, 17]. The mobile phase was DMSO/LiBr solution, which was filtered through a 0.45 μm hydrophilic Teflon membrane filter before using (Millipore, Billerica, MA, USA). Pullulan standards with peak molecular weights ranging from 342 to 2.35×10^6 were used for calibration to convert SEC elution volume to molecular size (hydrodynamic volume, V_h, or equivalently hydrodynamic radius, R_h) using the Mark–Houwink equation [18]. Details are given in the Supporting Information.

2.8 Statistical analysis

Mean values were obtained from triplicate experiments. The differences between the mean values of multiple groups were analyzed by SPSS 17.0 and Origin 8.0 for one-way analysis of variance (ANOVA) with Duncan's multiple range tests. ANOVA data with a $p < 0.05$ were classified as statistically significant.

3 Results and discussion

3.1 In vitro digestion of starch samples with pancreatin and amyloglucosidase

The contents of rapidly digested starch, slowly digested starch and resistant starch are summarized in Table 1. Native WP (uncooked) starch had a much higher RS content (85%) compared with native SP starch (61%), whereas it showed significantly lower RDS and SDS contents (8 and 7%) than native SP starch (13 and 23%). It is generally agreed that RS has a range of nutritional benefits to humans, such as helping in weight management, aiding in preventing in metabolic diseases, especially diabetes, reducing the risk of acquiring colorectal cancer etc. [19]. From this point of view, WP is a more nutritious food source than SP.

Acid-treated WP and SP starches generally showed decreased resistant starch contents and increased rapidly digestible starch and slowly digestible starch contents, compared with the parent native starches. During the in vitro digestion process, the RS content decreased with acid hydrolysis from 1 to 15 days, accompanied by a corresponding increase in RDS. However, SDS content initially decreased

Table 1. Percentages (%, w/w, dry weight) of RDS, SDS, and RS[a]

Samples	RDS (%)	SDS (%)	RS (%)
WP	7.91 ± 0.53 b	7.40 ± 0.28 b	84.69 ± 0.81 h
WP-1d	7.29 ± 1.01 i	4.82 ± 0.65 a	87.88 ± 1.67 i
WP-3d	17.53 ± 0.29 d	16.94 ± 0.37 e	65.53 ± 0.66 f
WP-5d	35.28 ± 0.33 f	36.41 ± 1.12 k	28.30 ± 1.46 a
WP-8d	44.80 ± 0.57 h	27.82 ± 0.76 i	27.38 ± 0.19 a
WP-15d	45.92 ± 1.46 h	27.71 ± 0.38 i	26.37 ± 1.84 a
SP	16.28 ± 0.98 d	23.04 ± 0.66 g	60.68 ± 1.64 e
SP-1d	13.34 ± 0.78 c	9.77 ± 0.43 c	76.89 ± 0.35 g
SP-3d	24.20 ± 0.36 e	25.92 ± 0.29 h	49.89 ± 0.66 d
SP-5d	37.20 ± 0.47 g	30.55 ± 0.78 j	32.25 ± 1.25 b
SP-8d	44.72 ± 0.56 h	18.55 ± 0.98 f	36.73 ± 1.54 c
SP-15d	53.89 ± 0.84 i	13.11 ± 0.41 d	33.00 ± 1.25 b

Mean values were obtained from duplicate measurements.
a Values with a different letter in the same column are significantly different ($p < 0.05$).

after 1 day acid hydrolysis, and then increased with increasing hydrolysis time in the two pea starches. Disruption of the granular structure from acid hydrolysis, plus an increase in the effective surface area for amylase binding and hydrolysis (Fig. 1) as observed by SEM and described elsewhere, might be the reasons for the increased RDS in acid-etched starch residues [2]. However, there was a parallel change between SDS and relative crystallinity (Tables 1 and 2), which coincided with the degree of crystallinity and low susceptibility to amylolytic degradation. Therefore, SDS in this species may be related to the interplay between the crystalline layers and amorphous layers within a semicrystalline ring [20].

3.2 Scanning electron microscopy (SEM) of starch samples

Figure 1 gives SEM micrographs of native and acid-modified starches. Native WP and SP starches showed large elliptic and oval granules and small spherical granules. The surfaces of starch granules were smooth and had no fissure or rupture. Some of the larger starch granules had deep indentations and grooves (Fig. 1A1, A2). After 1–3 days of hydrolysis, WP starch granules showed a few fragments on the smooth surface (Fig. 1B1, C1). Some starch granules were shrunken, with degradation of the interior of the granules. After being acid-hydrolyzed for a longer time (5 days for WP and 3 days for SP), some granules disintegrated with extensive degradation of the inner structure (Fig. 1C2 and D1). Soomth pea starch granules were destroyed more easily than wrinkled pea starch granules, meaning that SP starch granules are probably more densely packed than WP starch granules. After 8 days of acid hydrolysis, granules of both SP and WP starches were extensively disrupted. No intact starch granules were

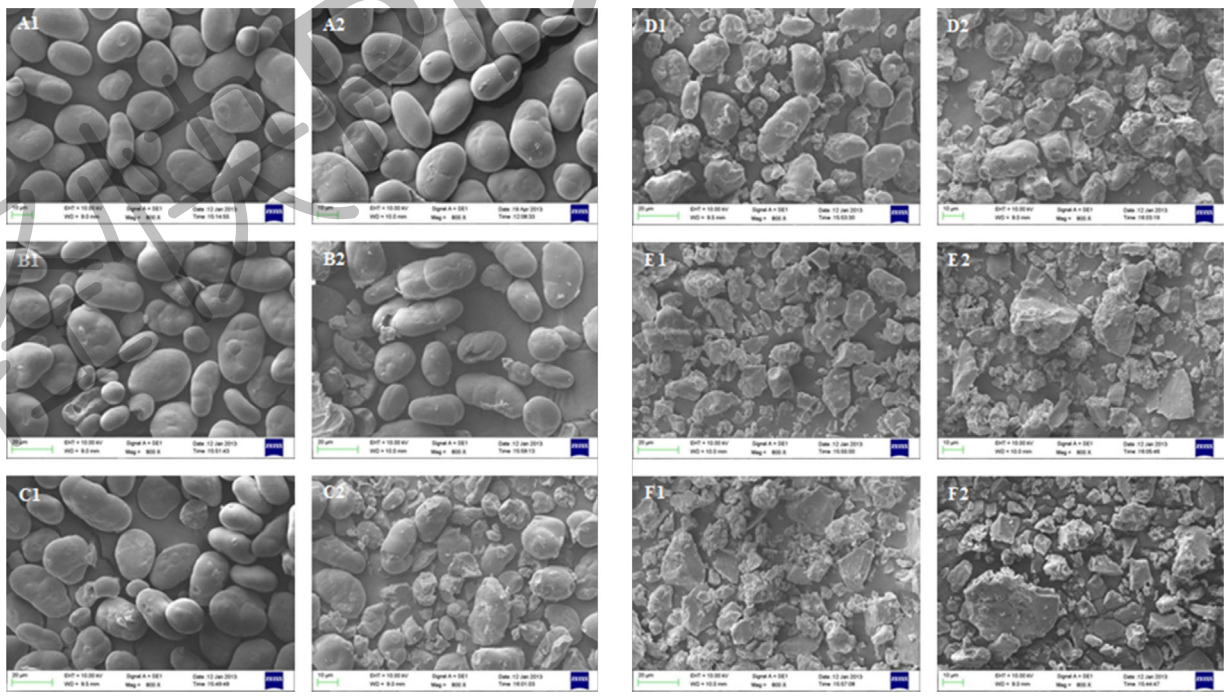

Figure 1. Scanning electron micrographs (×800) of native and acid-treated starch samples: (A1) Native wrinkled pea starch (WP), (B1) WP-1d, (C1) WP-3d, (D1) WP-5d, (E1) WP-8d, (F1) WP-15d; (A2) Native smooth pea starch (SP), (B2) SP-1d, (C2) SP-3d, (D2) SP-5d, (E2) SP-8d, (F2) SP-15d.

Table 2. The relative crystallinity, B polymorph, and thermal properties of native and acid-treated starches[a]

Samples	Relative crystallinity (%)	B polymorph (%)	T_o (°C)	T_p (°C)	T_c (° C)	T_c–T_o (°C)	ΔH (J/g)
WP	30.7 ± 0.3 b	28.1 ± 0.6 c	68.84 ± 0.06 b	73.57 ± 0.00 a	79.00 ± 0.03 a	10.16	11.10 ± 0.44 b
YP-1d[b]	32.0 ± 0.3 c	31.9 ± 0.7 d	72.85 ± 0.02 c	80.20 ± 0.00 c	87.39 ± 0.04 c	14.54	12.95 ± 0.29 c
WP-3d[b]	38.4 ± 0.1 e	38.4 ± 0.5 f	74.25 ± 0.05 d	90.32 ± 0.03 d	96.80 ± 0.23 d	22.55	14.99 ± 0.85 e
WP-5d[b]	43.1 ± 0.2 j	47.9 ± 1.1 h	77.95 ± 0.11 fg	95.10 ± 0.25 g	124.99 ± 0.68 h	47.04	16.80 ± 0.08 g
WP-8d[b]	42.7 ± 0.2 j	37.5 ± 0.4 f	78.35 ± 0.08 g	100.20 ± 0.00 j	132.49 ± 0.02 j	54.14	16.98 ± 0.45 g
WP-15d[b]	40.9 ± 0.3 h	21.8 ± 0.5 a	89.44 ± 0.01 i	97.38 ± 0.00 i	143.18 ± 0.02 k	53.74	16.63 ± 0.03 g
SP	29.2 ± 0.2 a	36.9 ± 1.4 e	66.54 ± 0.52 a	74.37 ± 0.43 b	81.61 ± 0.24 b	15.07	8.83 ± 0.64 a
SP-1d[c]	33.9 ± 0.2 d	32.3 ± 0.3 d	74.30 ± 0.08 d	80.39 ± 0.00 c	106.59 ± 0.09 e	32.29	13.94 ± 0.08 d
SP-3d[c]	39.9 ± 0.3 g	38.0 ± 0.2 f	76.79 ± 0.02 e	92.14 ± 0.00 e	116.77 ± 0.01 f	39.98	15.53 ± 0.06 ef
SP-5d[c]	41.9 ± 0.1 i	41.0 ± 0.8 g	77.31 ± 1.79 ef	96.33 ± 0.80 h	120.39 ± 1.30 g	43.08	15.85 ± 0.68 f
SP-8d[c]	39.4 ± 0.5 f	41.7 ± 0.5 g	77.68 ± 0.12 efg	97.23 ± 0.02 i	124.77 ± 0.08 h	47.09	15.51 ± 0.04 ef
SP-15d[c]	39.1 ± 0.4 f	23.0 ± 0.4 b	85.40 ± 0.08 h	94.57 ± 0.06 f	126.60 ± 0.13 i	41.20	15.35 ± 0.20 ef

Mean values were obtained from duplicate measurements.
a Values with a different letter in the same column are significantly different ($p < 0.05$).
b Wrinkled pea starch with acid-hydrolysis treatment for 1, 3, 5, 8, and 15 days.
c Smooth pea starch with acid-hydrolysis treatment for 1, 3, 5, 8, and 15 days.

observed and the fragments were stuck together (Fig. 1E1, E2, F1, and F2). This shows that acid corrosion firstly occurred in the inner part of the starch granules and then the outer part, which confirms that amorphous region is located in the interior of starch granules [21]. Bogracheva et al. [21] suggested that the gelatinization behavior of C-type granules begins from the hilum area and then spreads quickly through the central part of the granules. The outer parts of the granule showed slow disruption. This is in agreement with the conclusion by Wang et al. [22] for C-type Chinese yam starch granules, who reported that the starch granules changed from spherical to cakey in shape at the first stage of acid hydrolysis, and fractured at the later phase. Thus at the early stage, the inner region of starch granules tend to be easily hydrolyzed, whereas the surface structure of starch granules is sustained.

3.3 X-ray diffraction of starch samples

The X-ray diffraction patterns of acid-treated starches and their native counterparts are shown in Fig. 2, with the crystallinity values given in Table 2. For native WP and SP, the diffraction patterns were mainly composed of five characteristic peaks at $2\theta = 5.65°$, 11.5°, 15.4°, 17.6°, and 23.6°. These are indicative of C-type starch diffraction, which is a mixture of A- and B-type crystallinity. The peak/shoulder at 17.9°, a characteristic of only the A-type polymorph [21, 23], showed increased intensity over time, suggesting that more A-type crystalline structure was produced during acid hydrolysis. The intensity of the peak at 5.75°, which is characteristic of only the B-type polymorph, reduced over time. The results of C-type *Rhizoma Dioscorea* [24] and *Dioscorea rhizoma* starch [10] also showed that B-type polymorphs in the C-type starch granule were degraded faster than A-type under acid hydrolysis. After acid hydrolysis for 1–8 days, the proportions of B polymorph in the C-type pea starch granule were all higher than that of their counterpart native starches. This means that at this earlier stage, the hydrolysis of the A polymorph was faster than that of the B polymorph. At the later stage (8–15 days), the proportions of B polymorph sharply declined, and were lower than that of their counterpart native starches. At this stage, the hydrolysis of B polymorph accelerated. The proportions of B polymorph in WP and SP treated with acid were lower than that of their counterpart native starches by 6.3 and 13.9%, respectively. Finally, the B polymorph of acid treated pea starch has a higher hydrolysis rate than A polymorph, over the entire hydrolysis process.

After being hydrolyzed by acid, WP starches generally had higher values of relative crystallinity than SP. The crystallinity of acid-hydrolyzed starches increased slightly with increased acid-treatment time. The differences in the crystallinity (Table 2) between native starch and the acid-treated starch samples is attributed to the difference in the amount of crystalline regions, which is influenced by the amylose content and amylopectin CLD [20]. In general, the amorphous regions are the first to be hydrolyzed, followed by the crystalline regions [25]. After being hydrolyzed by acid over a long time (1–5 days), our starches showed a significant increase in crystallinity with time, possibly because more extensive hydrolysis of the amorphous layers and background leads to a relatively higher proportion of the crystalline layers, and the discrete starch chains in the amorphous layers allow rearrangement among cleaved chains to form a more perfect crystalline structure. After 5 days, the crystallinity of acid-hydrolyzed starches showed a slight decrease over time. This is probably because, by this stage, the hydrolysis of amorphous regions has become slow compared with the previous stage,

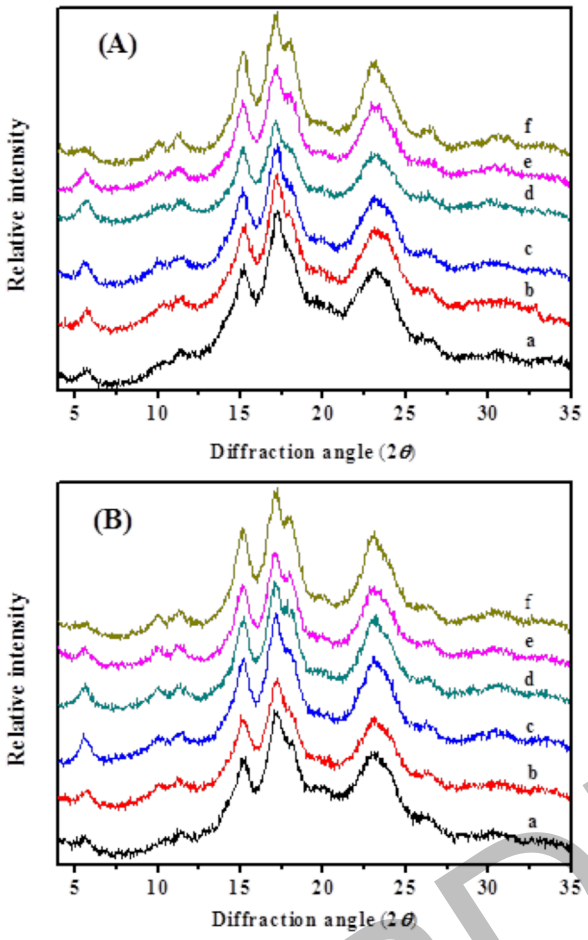

Figure 2. X-ray diffraction patterns of native and acid-treated starches: (A): (a) WP, (b) WP-1d, (c) WP-3d, (d) WP-5d, (e) WP-8d, (f) WP-15d; (B): (a) SP, (b) SP-1d, (c) SP-3d, (d) SP-5d, (e) SP-8d, (f) SP-15d.

and further hydrolysis mainly happens at the crystalline region, which occurs more slowly.

To account for the slower hydrolysis rate of the crystalline parts of the starch granule, two hypotheses have been proposed. First, starch chains in starch crystallites pack densely, which does not readily allow the penetration of H_3O^+ into the regions. Second, acid hydrolysis of a glucosidic bond may require a change in the conformation (from chair to half chair) of the D-glucopyranosyl unit [26].

3.4 Differential scanning calorimetry

Differential scanning calorimetry (DSC) provides additional information about the change in starch crystallinity to that from X-ray diffraction, which detects long-range periodic structures in the starch granules [27]. Table 2 gives the values of T_o, T_p, T_c gelatinization transition temperature range ($T_c - T_o$) and ΔH of WP and SP starches after acid hydrolysis. After being treated by acid, both WP and SP showed increased T_o and T_p values compared to their native parents. T_p provides a measure of crystallite quality. The increase in T_p suggests that acid-hydrolyzed starch has a more ordered and stable structure that requires a higher melting temperature after hydrolysis [28, 29]. The ($T_c - T_o$) of wrinkled and smooth pea starches increased significantly after being acid-hydrolyzed. The increase in ($T_c - T_o$) could be due to the formation of a broader range of ordered arrays, and a more independent melting of individual crystallites and double helices subsequent to hydrolysis of the amorphous phase [30]. The value of ($T_c - T_o$) can be interpreted as a measure of the homogeneity of crystalline and noncrystalline conformations, whereas ΔH reflects the amount of order, that is, the enthalpy of melting of crystallites and double helices [31]. After 15 days acid treatment, the enthalpy of WP increased more compared with that of SP. This indicates that there is a higher proportion of the molecular order structure of defective crystallites in acid-treated WP starches, resulting in a greater increase in the relative crystallinity and consequently increased gelatinization temperature and enthalpy. This is in agreement with the crystallinity results.

3.5 Chain-length distribution (CLD) of starch samples

The CLDs of native WP and SP starches and acid-treated counterparts are presented as the SEC weight distributions w (log R_h) in Fig. 3; all CLDs are normalized to the same height of the first peak. Typical CLDs are observed for all starch samples, with the usual three peaks corresponding to single-lamellar amylopectin (first peak), trans-lamellar amylopectin (second peak), and amylose chains (third region). The dividing point of amylose and amylopectin was set at the minimum of each CLD. Amylose content was obtained from the CLD of each sample by calculating the ratio of the area under the amylose distribution curve to the area under the whole starch CLD curve. The amylose content of SP and WP obtained were 31.1 and 30.8%, respectively. After treating starch with acid for 1 day, most amylose chains were hydrolyzed to short chains with DP <400, and they were further hydrolyzed to even shorter chains with DP <100 after 3 days. The second amylopectin peak disappeared (were hydrolyzed to shorter chains) after being acid-treated for 3 days. The maximum in the first amylopectin peak was slightly shifted from DP 17 to 14 after hydrolysis. These results show that amylose chains are more prone to acid hydrolysis than amylopectin chains. This might be because amylose chains are mainly in the amorphous region of starch granules, where chains are less tightly packed; therefore it is easier for acid to access amylose branches. Amylopectin chains, on the other hand, are mostly in the crystalline region, where branches are tightly packed. Thus it takes longer for acid to access amylopectin chains. The crystalline regions of starch are formed by clusters of short chains of amylopectin and the intercrystalline regions are the amorphous regions. Thus, the longer chains linking several clusters in amylopectin are actually in the amorphous region;

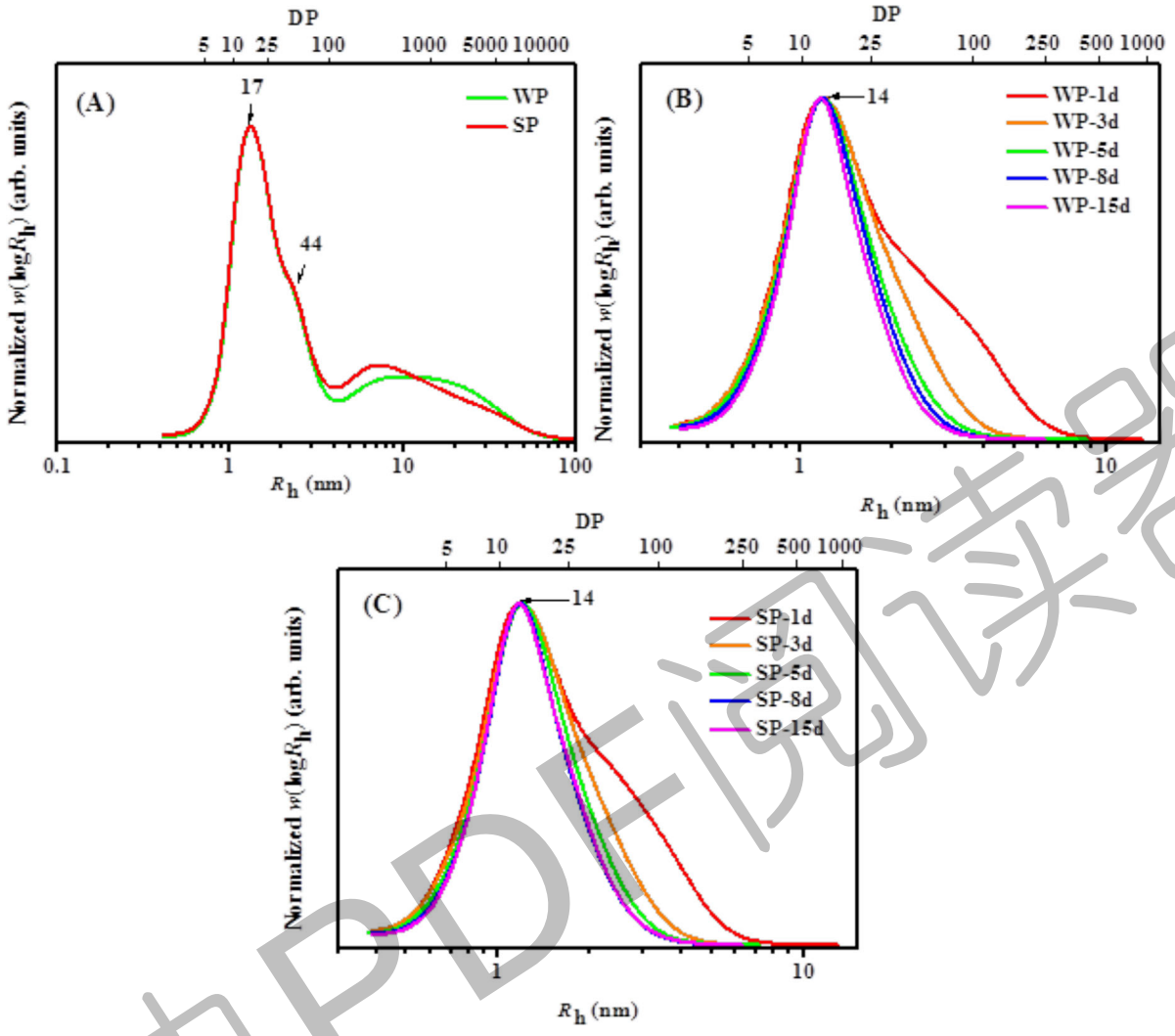

Figure 3. CLDs of native and acid-treated starches as SEC weight distribution: (A) Native wrinkled pea starch (WP) and smooth pea starch (SP), (B) Wrinkled pea starch with acid-hydrolysis treatment for 1, 3, 5, 8, and 15 days, (C) Smooth pea starch with acid-hydrolysis treatment for 1, 3, 5, 8, and 15 days. Numbers above the curves are degree of polymerization (DP) values.

therefore, they are hydrolyzed into shorter chains by acid in preference to shorter chains.

These results suggest that degradation of the amorphous regions involves hydrolysis of amylose and amylopectin branch points. Compared to the native starches, higher amounts of shorter amylopectin chains were produced during starch hydrolysis. As the hydrolysis time increased (from 1 to 15 days), more amylopectin was degraded into molecules with lower DPs; the reduction in amylopectin average chain length became slower over time. After 1 and 3 days acid hydrolysis, the WP amylopectin chains were longer than those of SP. After 5 days of acid hydrolysis, the CLDs of both starches did not show further change. This suggests that the molecular structure of WP starch is more compact than that of SP. Ratnayake et al. [4] also indicated starch chains within the amorphous and crystalline domains of WP starch are more closely associated than in SP. However, the changes in the CLDs of WP and SP starches showed the same trend, consistent with the two starches following the same acid-hydrolysis mechanism.

4 Conclusions

The changes in morphological, crystalline and molecular structure properties and in vitro digestibility were evaluated for C-type wrinkled and smooth pea starches after acid hydrolysis. Acid hydrolysis starts from the hilum of the starch granule (amorphous areas) and then proceeds to the outer crystalline parts. The amorphous regions are mainly located in the core of the starch granules, while the crystalline areas mainly exist on the surface of the starch

granules. With the increase of hydrolysis time, more amylopectin molecules were degraded into molecules with lower DPs, and the rate of amylopectin degradation became slower. Compared to SP starch, native WP starch has a higher proportion of RS, suggesting its nutritional advantage over SP. In vitro digestion results showed that long-time acid hydrolysis could increase the SDS content of both pea starches, although it decreases RS content. These phenomena resulted from changes in the crystalline structure and chain length distributions over acid hydrolysis. Similar to RS, it has been reported that SDS could reduce diet-related diseases, and thus the current study may provide an effective method in producing starches from pea with slow digestion rates.

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