Changes in the structural and physicochemical characterization of pea starch modified by Bacillus-produced α-amylase

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ABSTRACT: In this study, changes in structural and physicochemical properties of pea starch treated with Bacillus-produced α-amylase were determined. The results showed that enzymatically modified pea starch had lower amylose content and granule size but higher branching degree and relative crystallinity. After enzyme hydrolysis, the distribution of A and B1 chains slightly decreased, while the distribution of B2 and B3 chains increased lightly. Enzymatic hydrolysis preferentially occurred in the amorphous region and cannot change the crystalline structure of pea starch. Moreover, pea starch showed lower light transmittance, peak viscosity, breakdown viscosity, pasting temperature, shear viscosity, storage modulus and loss modulus, while the oil adsorption capacity and gelatinization enthalpy significantly increased with increasing α-amylase hydrolysis time. Correlation analysis indicated that α-amylase hydrolysis had different effects on different pea varieties. This research could provide ideas for exploring new applications for enzymatically modified pea starch in food industry.

Industrial relevance: This study found that Bacillus-produced α-amylase significantly changed the amylose content, granule size and viscosity of pea starch, which was helpful to further investigate the modified starch. This technology is expected to widen the applications of pea starch modified by Bacillus-produced α-amylase in food industry, for example as thickener, stabilizer and beverage, to improve the texture, stability and shelf-life of various food products.

Keywords: Pea starch; Bacillus-produced α-amylase; physicochemical properties; rheological properties

1. Introduction

Peas (*Pisum sativum* L.) are widely grown in temperate regions of the world and are the world's leading export crop, accounting for around 35-40% of the total trade in pulses (Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001). Peas are now gaining more and more recognition due to their richness in starch, protein, resistant starch, dietary fiber and minerals (Zhou, Ma, Yin, Hu, & Boye, 2019). Traditionally, pea is consumed as dried seeds or as a fresh vegetable, the dried seeds are mainly applied in soups and are increasingly being processed into value-added food ingredients (Ren, Setia, Warkentin, & Ai, 2020). Pea flour has unique aromatic profiles and desirable functional properties and is often processed through milling, making it suitable for use in a variety of foods, including baked goods, noodles and meat products (Gu et al., 2021). Pea starch, accounting for about 40-50% of the seed, is the most abundant carbohydrate in pea seeds. The amylose content of pea starch is in the range of 30-60% with varying varieties and is considered to be an important factor determining the pea quality (Hoover et al. 2010). Compared to potato, wheat and maize starch, pea starch is considered as an inexpensive source of starch (Zhou, Ma, Yin, Hu, & Boye, 2019). Industrially, starches obtained from maize, potato, cassava and wheat are almost exclusively natural and modified. Nowadays, starches isolated from peas are widely used in industry due to their unique properties such as extent of digestion, high resistant starch content as well as high elasticity of gels (Sun, Sun, & Xiong, 2014), making them suitable substitutes to modified starches in a range of products (Raphaelides & Georgiadis, 2008).

Native starches are not entirely applied in the food industry due to their poor water solubility and heat stability, which cannot meet the requirements of specific applications (Cui et al., 2023). Therefore, native starches are often modified by chemical, physical or enzymatic methods to improve their industrial applications. Compared to chemical and physical methods, the enzymatic approach has the advantages of simple operation, high reaction efficiency and high yield, which is conductive to meeting the requirements and suitability of food products. It has also been reported that enzymatic modification can effectively improve the utilization of the native starch (Chen et al., 2021). Alpha-amylase is a commonly used enzyme that usually cleaves the α-1, 4-glycosidic bond of amylose or amylopectin in the internal position to obtain a product with an α-configuration (Lin et al., 2016). Previous studies have shown that the modification of starch with α-amylase is effective in improving the structural and physicochemical properties of starch. Shariffa, Karim, Fazilah and Zaidul (2009) have reported that sweet potato starch was higher in amylose content, water solubility and swelling power after fungal-produced α-amylase hydrolysis. Similarly, corn starch hydrolyzed by α-amylase had higher amylose content, longer amylopectin chains along with smaller granules (Song et al., 2020). When treated with maltogenic α-amylase, the molecular weight of rice starch was significantly reduced, while significant increase was observed in the short-chain levels (DP < 13) (Wang et al., 2022). Compared to fungal sources of α-amylase, the Bacillus-produced α-amylase has greater thermal stability and pH tolerance (Martínez et al., 1997), making it more suitable for certain industrial applications, such as starch liquefaction or baking. Additionally, Bacillus

strains are less susceptible to contamination and are highly capable of producing thermostable α-amylase (Schwab et al., 2009). The modified cereal starches have unique physicochemical properties and specially applications. However, previous studies have mainly focused on the modification of potato and maize starch by α-amylase, while few reports have been studied on the modification of pea starch by Bacillus-produced α-amylase, especially regarding the changes in structural, physicochemical and rheological properties, which limits the application of modified pea starch in the food industry. Therefore, there is a need to explore potential applications of pea starch modification based on α-amylase produced by Bacillus.

In this study, three pea varieties with different amylose content were used to investigate the effects of Bacillus-produced α-amylase on the particle size distribution, amylopectin chain length distribution, branching degree, crystalline structure, short-ranged ordered structure, light transmittance, water and oil absorption capacity, pasting and thermal properties of the native pea starch. The changes in structural, physicochemical and rheological properties of the modified pea starch and its applications were then further explored by comparing with the native pea starch. The purpose of this research was to provide a theoretical basis for the development of the modified starch and widen the applications of pea starch modified by Bacillus-produced α-amylase in the food industry.

2. Materials and methods

2.1 Materials

Three pea varieties used in this study were Xiwan 1 (XW1), Xiwan 2 (XW2) and

Xiwan 3 (XW3) provided by the Minor Grains Laboratory of the Northwest A & F University. The XW1 and XW2 had wrinkled surfaces and were mainly used as grain varieties, while XW3 had smooth shape and was generally used for cooking. All varieties were planted in 2020 at the experimental farm in Guan village, Yangling, Shaanxi province. The thermostable Bacillus-produced alpha-amylase (Cas. No. 9000-90-2, 3700 U/g) was obtained from Beijing Solaibao Technology Co., LTD. All chemicals and reagents were of analytical grade.

2.2 Starch isolation

Pea seeds were ground into flour using a universal crusher and pea starch was isolated following the method of Raghunathan, Hoover, Waduge, Liu and Warkentin (2017) with some modifications. Firstly, the pea flour was mixed with distilled water (1:3, w/v) and the solution was then passed through a 100-mesh sieve. Next, the crude extract was obtained and left at 25 °C for 24 h and centrifuged at 4000 g for 10 min. The sediment was then repeatedly washed with distilled water until it turned white. Finally, the starch samples were dried at 40 °C for 24 h, ground and passed through an 80-mesh sieve. The main chemical composition of the native pea starch was summarized in Table S1.

2.3 Preparation of α-amylase-modified starch

Starch sample (30 g) was suspended in the 0.1 M sodium acetate buffer (pH = 6.0). Then, α-amylase (0.59 g) was added to the starch suspension, pre-incubated at 50 ℃ for 30 min, gently stirred and shaken at 50 ℃ and 100 rpm for 4, 8 and 16 h, respectively (All samples were prepared under the same condition). Next, sodium hydroxide

solution (2 M) was added to make enzymes inactivated until each solution had a pH of 10.8. The pH of each solution was then adjusted to 6.0 and the supernatant was removed and centrifuged at 4000 g for 10 min (repeated 3 times). Finally, the sediment was freeze-dried and the native and enzymatically modified starches were used for later analysis (Song et al., 2020).

2.4 Amylose content

The amylose content of pea starch was determined using the iodine colorimetric determination following the method of Gao et al. (2016), and the content was calculated from the blue value and measured in 3 replicates with the results expressed as a percentage.

2.5 Structural characteristics of pea starch

2.5.1 Granule size distribution

The granule size distribution of pea starch was measured using a laser diffraction particle size analyzer (Master 2000, Malvern, England). Starch samples were measured from 0.1 to 2000 um with the shading factor of 1.3330 and the shading range of 12-17%. The measurements were reported in terms of the volume distribution.

2.5.2 Chain length distribution of amylopectin

The amylopectin chain length distribution of pea starch was measured using high performance anion exchange chromatography equipped with pulsed amperometric detection (HPAEC-PAD) system (ICS 500+, Thermo Fisher Scientific, USA) according to the method of Yang et al. (2019). Pea starch (5 mg) was suspended in the ultrapure water (5 mL) and the solution was then heated in a boiling water bath for 60 min. Next,

the gelatinized sample (2.5 mL) was mixed with sodium acetate (125 μL), NaN_3 (5 μL) and isoamylase (5 μL) and left at 38 ℃ for 24 h to hydrolyze the α-1, 6 glycosidic bonds. After centrifugation at 12000 g for 5 min, the samples were passed through a 0.45 μm membrane filter and the supernatant was taken for testing. The mobile phase A was 200 mM NaOH, phase B was 200 mM NaOH / 200 mM NaAC, and the column temperature was 30 ℃. The chain length distribution was calculated based on the following formula: relative area = area / total area * 100%.

2.5.3 Branching degree analysis

The branching degree of pea starch was measured by nuclear magnetic resonance (NMR) spectroscopy following the method of Bai and Shi (2016). The sample (10 mg) and D6-DMSO (1 mL) were added into the EP tube, then heated at 80 ℃ and left for 12 h. After centrifugation at 12000 g for 10 min, the supernatant was added into the NMR tube for detection. The frequency was 500.23 MHz for ^1H, and the ^1H spectra were collected in 32 individual scans. The peak position of α-1, 4 bond was around 5.05 ppm, while α-1, 6 bond was near 4.71 ppm. The degree of branching (DB) was calculated as follows: DB = α-1, 6 / (α-1, 6 + α-1, 4).

2.5.4 X-ray diffraction (XRD)

The crystalline structure of pea starch was determined using an X-ray diffractometer (D/Max 2550 VB +/PC, Rigaku, Japan) through the method of Zhang et al. (2019). The samples were measured by the diffractometer at 40 kV and 100 mA in the diffraction angle (2θ) from 5° to 40° at a constant speed of 10 °/min. The relative crystallinity (%) was calculated from the ratio of crystallinity area to total diffraction

area.

2.5.5 Fourier transformed infrared spectrometry (FTIR) analysis

The short-range ordered structure of pea starch was performed using a FTIR spectrometer (7000, Varian, USA) equipped with an ATR single-reflectance cell containing a germanium crystal (45° incidence angle) following the method of Zhu et al. (2017).

2.6 Physicochemical properties of pea starch

2.6.1 Light transmittance

The light transmittance of pea starch was measured as described by Chao et al. (2015). The starch paste (1 g/mL) was heated in boiling water for 30 min. After gelatinization, the starch samples were cooled and placed at room temperature (25 °C) for 24 h. Then, a visible-light spectrophotometer (Lab Tech Ltd., Beijing, China) was used to determine the light transmittance at 650 nm with distilled water as a reference.

2.6.2 Absorption capacity of water and oil

The water absorption capacity and oil absorption capacity of pea starch was measured through the method of Song et al. (2020). The oil used in this study was edible peanut oil with room temperature. Absorption capacity (g/g) was calculated by dividing the weight of wet precipitate by the dry weight of the sample. The analysis was performed in triplicate.

2.6.3 Pasting properties

The pasting properties of pea starch were carried out using a Rapid Visco Analyzer (Perten, Stockholm, Sweden) according to the method of Gao et al. (2016). Briefly, the starch slurries were held at 50 °C for 1 min before heating from 50 to 95 °C at 12 °C/min.

Then, the samples were maintained at 95 °C for 2.5 min, cooled to 50 °C at a constant rate of 12 °C/min and finally held at 50 °C for 2 min. The peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BD), final viscosity (FV) and setback viscosity (SB) were observed from the pasting curve.

2.6.4 Thermal properties

The differential scanning calorimetry (DSC; Q2000, USA) was used to measure the thermal behavior of pea starch through our previous method reported by Gao et al. (2020). Briefly, starch sample and deionized water were mixed at a ratio of 1:3, and the solution was then added to the aluminum pan. Next, the pan was sealed hermetically and kept at 4 °C for 24 h before analysis. The samples were heated from 30 to 100 °C at a constant rate of 10 °C/min. An empty sealed aluminum pan was used as a reference.

2.6.5 Rheological properties

The TA instrument rheometer (DHR-1, USA), employed with a stainless parallel plate geometry (40 mm diameter) and a gap size of 1000 μm, was used to determine the rheological behaviors of pea starch. The starch suspension (8%, w/v) was gelatinized completely and then cooled to room temperature (25°C). Next, the starch sample was transferred to the parallel plate of rotary rheometer and covered by a circle of silicone oil to prevent moisture to evaporate. All the experiments were conducted at 25°C.

2.7 Statistical analysis

All experiments were conducted in triplicate. The results were analyzed using one-way ANOVA through the SPSS 17.0 software. The significant difference level among

199 the means was conducted using the Duncan's multiple range test ($P < 0.05$). All figures
200 were drawn using Origin Pro software (version 2021, USA).

201 **3. Results and discussion**

202 *3.1 Amylose content*

203 The amylose contents of the native and enzymatically modified pea starch were
204 summarized in Table 1. It was clearly shown that the amylose content of pea starch
205 ranged from 26.20 to 39.38%, and that was significantly reduced with increasing α-
206 amylase incubation time. It has been reported that the amylose content of legume starch
207 was between 27.35 and 35.21% (Zhou, Hoover, & Liu, 2004), which was slightly lower
208 than our findings. In addition, significant differences in amylose content were obtained
209 among the three pea varieties, with XW1 having the highest value, followed by XW2
210 and XW3, respectively. Compared to the corresponding native starch, the amylose
211 content of XW1, XW2 and XW3 after 16 h of enzymatic hydrolysis decreased by 12%,
212 14% and 14%, respectively. Song et al. (2020) have found that the amylose content of
213 corn starch was significantly reduced when treated with Bacillus-produced α-amylase,
214 which was consistent with the results of this study. It has been reported that the value
215 of amylose content is not only related to the amylose molecules, but also to the long
216 branches of amylopectin when enzymatic hydrolysis of starch (Shariffa, Karim, Fazilah,
217 & Zaidul, 2009). In addition, α-amylase has been shown to preferentially hydrolyze
218 amylose chains, which indicated that α-amylase preferentially acted on amylose of pea
219 starch, leading to the decrease of amylose content.

220 *3.2 Granule size distribution*

As can be seen from Fig. 1, there were significant differences in granule size distribution of the native and enzymatically modified pea starch among different pea varieties. It was clearly shown that all starch samples showed a unimodal curve of granule size distribution, but the corresponding particle size was different when the volume distribution reached the maximum value (Fig. 1 A-C). Normally, the particle size distribution is divided into A-granule (> 15 μm), B-granule (5-15 μm) and C-granule (< 5 μm) according to the finding of Bechtel, Zayas, Dempster, & Wilson (1993). In this study, the granule size distribution of pea starch was mainly A and B granules, ranging from 92.54 to 99.60% (A-granule) and from 0.40 to 7.46 (B-granule), respectively (Table 1). With the extension of α-amylase hydrolysis time, the distribution of A-granule significantly decreased while obvious increase was obtained in B-granule, meaning that prolonged enzyme hydrolysis could lead to the breakage of large starch granule, thus reducing the volume distribution of pea starch. Additionally, both the D [3,2] and D [4,3] significantly decreased with the increase of α-amylase hydrolysis time, ranging from 24.83 to 28.64 μm (D [3,2]) and from 27.11 to 31.08 μm (D [4,3]), respectively. These results of this study confirmed that α-amylase hydrolysis was effective in reducing granule size and probably be related to the rupture and damage of native starch granules due to hydrolysis (Zhao et al., 2018), which was consistent with the previous report by Jiang et al. (2017). In addition, the volume distribution of the native pea starch had different reaction to α-amylase hydrolysis. With the increase of α-amylase hydrolysis time, the volume distribution of all starch samples significantly increased, while the granule size of the corresponding pea starch was different when

the volume distribution peaked. This finding showed that the volume distribution of different pea varieties responded differently to α-amylase hydrolysis, indicating that α-amylase hydrolysis can change the volume distribution of pea starch, thereby resulting in variations in the physicochemical properties of starch granules. Differences in the response of different pea varieties to α-amylase may be related to the genotype.

3.3 Chain length distribution

Generally, the branch chains of amylopectin are classified into A-chains (DP 6-12), B1-chains (DP 13-24), B2-chains (25-36) and B3-chains (DP > 37) based on the degree of polymerization (DP) (Hanashiro, Abe, & Hizukuri, 1996). In this study, the highest content of B1-chains (52.78%, mean of the three varieties, below) were noticed of the native pea starch, followed by A-chains (19.20%) and B2-chains (15.45%), and a small amount of very long chains (12.56%) was also observed (Fig. 1 D-F). These results indicated that the B1-chains may play a major role in the relative properties of the native pea starch. In addition, the chain length distribution of the native pea starch firstly increased and then reduced with increasing DP values, with all starch samples having the highest peak at DP 13. After enzymatic hydrolysis, the chain length distribution of all samples still had a maximum value at DP 13, with the average branched chain length of enzymatically modified starch ranging from 21.86 to 21.96% (XW1), from 21.69 to 22.02% (XW2) and from 21.97 to 22.08% (XW3), respectively. The distribution of A-chains and B1-chains on pea starch significantly decreased with the prolongation of enzyme hydrolysis time, while slight increases were observed in the distribution of B2-chains and B3-chains (Table 1). These results may be related to the fact that the stearic

acid in the pea starch structure blocked the hydrolysis of the amylopectin chains (B2-chain and B3-chain). Moreover, the difference values among three pea varieties significantly differed at different hydrolysis time, with XW1 and XW2 showing the most significant effect after 4 h enzymatic modification while XW3 showed the most significant effect after 8 h enzymatic modification (Fig. 1 G-I). This result indicated that the Bacillus-produced α-amylase had different impacts on the pea varieties, which may be related to the variations in amylose content.

3.4 Branching degree analysis

The branching degree of pea starch was summarized in Table 2, and the peak intensities of ^{1}H NMR spectra were shown in Fig. S 1. All samples produced resonance signals from 4.2 to 5.6 ppm (Fig. S 1 A-C). For the ^{1}H NMR spectrum of pea starch, the signals at 5.05 and 4.71 ppm were assigned to α-1, 4 and α-1, 6-linked heterodimeric protons, respectively. These results showed that the DB of the native pea starch ranged from 0.76 to 1.05%, and that of the wrinkled pea was slightly higher than that of the smooth pea (Table 2). After enzyme hydrolysis, the signals of the enzymatically modified starch slightly changed at different chemical shift positions. In addition, the value of the DB was in the range of 1.83-3.66% with increasing α-amylase incubation time from 4 h to 16 h. Compared with the native pea starch, the DB of the enzymatically modified pea starch was much higher and was increased by 146.32% (XW1), 218.10% (XW2) and 342.11% (XW3), respectively. These results confirmed that the new branched chain was hydrolyzed from the α-1,4-glycosidic linkage in the native pea starch chain, and transformed into a new α-1,6-glycosidic linkage on other chains as

shown in Fig. S1. It has been reported that the DB of corn starch was increased with increasing time of 1,4-α-glucan branching enzyme action (Li et al., 2016), which was consistent with the finding of our study.

3.5 Crystalline structure

The XRD patterns of the native and enzymatically modified pea starch were presented in Fig. 2 A and the relative crystallinities were summarized in Table 2. Generally, cereal starches are divided into three main types based on their XRD spectra, including A-type, B-type and C-type (Cheetham & Tao, 1998). In this study, all starch samples exhibited a typical C-type diffraction pattern with strong peaks at around 15°, 17° and 23° (2θ) and a small peak at about 6° (2θ), indicating that the X-ray diffraction pattern of the native pea starch was not changed when treated with α-amylase. Similar phenomenon was observed on corn starch with A-type patterns treated with α-amylase (Song et al., 2020). In addition, the peak position of pea starch was also not affected after enzyme hydrolysis, which might be that the double-helical crystalline structure was not changed by Bacillus-produced α-amylase. For enzymatically modified pea starch, the peaks appeared to be sharper and higher than those of the native starch (Fig. 3 A), indicating that the enzyme hydrolysis of Bacillus-produced α-amylase mainly occurred in the amorphous region of the starch granules (Shariffa, Karim, Fazilah, & Zaidul, 2009). With the increase of α-amylase hydrolysis time, the relative crystallinity of pea starch significantly increased, and the relative crystallinity of enzymatically modified pea starch was significantly higher than that of the native pea starch, ranging from 28.36 to 32.14% (XW1), from 28.67 to 30.14% (XW2) and from 27.83 to 31.08%

(XW3), respectively (Table 2). Similar change trend was observed on corn starch treated with Bacillus-produced α-amylase (Song et al., 2020; Zhao et al., 2018), indicating that the crystalline structure of the C-type starch granule was more resistant to enzyme hydrolysis of bacillus-produced α-amylase than the amorphous structure. However, enzyme hydrolysis resulted in lower value of the relative crystallinity on wheat starch (Li, Li, & Guo, 2020), while no significant changes were founded in relative crystallinity on waxy maize starch and normal potato starch (Teng, Torsten, Wang, Li, & Jovin, 2016), meaning that the relative crystallinity of various starch sources responded differently to α-amylase hydrolysis. Amylopectin is the main crystalline component of starch granules, while amylose can weaken the crystalline structure of amylopectin (Zhang et al., 2019). The low relative crystallinity of starch may be attributed to a poorly organized crystalline structure (Zhou, Hoover, & Liu, 2004). In this study, the increased relative crystallinity indicated that the crystalline structure of pea starch became better when treated with α-amylase. Starch granules with higher amylose content have stronger resistance to enzyme hydrolysis. This explanation seemed reasonable, since the amylose content of pea starch was lower as the enzyme hydrolysis time increased in this study (Table 1), which can be used to explain the higher relative crystallinity degree of pea starch after α-amylase hydrolysis.

3.6 ATR-FTIR analysis

The ATR-FTIR method was used to measure the short-ranged ordered structure of the native and enzymatically modified pea starch, and the spectra of the starch samples were presented in Fig. 2 B. It was clear that the position of the characteristic absorption

peak of pea starch cannot be changed after α-amylase hydrolysis, indicating that the Bacillus-produced α-amylase hydrolysis neither changed the type of chemical group in the starch molecules nor produced a new one (Zhao et al., 2018). Normally, the absorbance intensity of starch at 1045, 1022 and 995 cm^{-1} varies with the change of its conformation, and these values can reflect the crystalline characteristics of the starch granules (Lopez-Baron et al., 2018). The ratio of 1045/1022 cm^{-1} (R_1) is used to determine the ordered degree, while the ratio of 1022/995 cm^{-1} (R_2) can be used as an indicator of the proportion of amorphous to ordered carbohydrate structure (Jiang et al., 2020). In this research, the R_1 ratio of pea starch ranged from 0.71 to 0.83 (XW1), from 0.79 to 0.96 (XW2) and from 0.80 to 0.93 (XW3), respectively (Table 2). With increasing α-amylase hydrolysis time, the R_1 ratio obviously increased and the R_1 ratio of enzymatically modified pea starch was much higher than that of the native starch. The higher crystalline fraction presented after enzymatic hydrolysis suggested that the mechanical damage caused by Bacillus-produced α-amylase mainly affected the amorphous region of pea starch granules rather than the crystalline region, and the amorphous region was more easily digested by Bacillus α-amylase during the incubation process (Chen & Zhang, 2012), which was similar to the findings for corn starch reported by Zhao et al., (2018). However, the R_2 ratio significantly decreased, and after 16 h of enzymatic modification, the ratio of R_2 decreased by 0.70% (XW1), 5.79% (XW2) and 2.40% (XW3), respectively, compared with the native starch (Table 2), which suggested that the helical alignment of pea starch can be changed by Bacillus-produced α-amylase hydrolysis. These results suggested that Bacillus-produced α-

amylase hydrolysis played a main role in increasing crystalline regions of pea starch, which could be explained by the increase of the relative crystallinity degree as shown in Table 2. In addition, significant differences in R_1 ratio were obtained among three pea varieties, with XW1 having a lower R_1 ratio than XW2 and XW3, but a higher R_2 ratio, indicating that XW1 was less ordered but had a higher helical alignment than other varieties and the differences among three pea starches might be attributed to the biological origin, the molecular structure of amylopectin, and the contents of amylose and amylopectin (Li & Zhu, 2017).

3.7 Light transmittance

The light transmittance of the native and enzymatically modified pea starch was presented in Table 2. Significant difference was observed between the winkled and smooth pea, and the native starch was in the order of XW1 (92.53%) > XW2 (91.74%) > XW3 (90.01%). After incubation with α-amylase, the light transmittance of all samples significantly decreased and ranged from 39.92 to 83.50%. Compared to the native starch, the light transmittance of pea starch after 16 h enzymatic hydrolysis was reduced by 50.74% for XW1, 28.77% for XW2 and 55.65% for XW3, respectively, indicating that α-amylase had the most significant effect on XW3, and this difference may be related to the genotype of different pea varieties. The light transmittance has been shown to be influenced by the amylose content, starch granule size and the ratio of amylose to amylopectin (Zhang et al., 2019). Wang, Tang, Fu, Huang and Zhang (2016) have concluded that the reduction in light transmittance of starch during retrogradation is due to the association of starch with each other through hydrogen bonds. In this study,

375 the lower value of the light transmittance of pea starch could be attributed to its lower
376 amylose content and lower distribution of A-granules (Table 1). Therefore, the modified
377 pea starch was not suitable for foods with high light transmittance requirements like
378 cold skin, cold noodles and crystal shrimp dumplings. In addition, we speculated that
379 the molecules of pea starch in this study were rearranged and amylose was reassembled
380 to form crystals after α-amylase hydrolysis, thus resulting in the reduction of the light
381 transmittance.

382 *3.8 Absorption capacities of water and oil*

383 The absorption capacities of water and oil of the native and enzymatically
384 modified pea starch were shown in Fig. S 2. The water absorption capacity of the native
385 pea starch ranged from 1.82 to 1.85 g/g, whereas that of the enzymatically modified
386 starch was increased by 8.90% for XW1, 12.64% for XW2 and 12.97% for XW3,
387 respectively. As for the oil absorption capacity, all the values significantly increased
388 with the increase of enzyme hydrolysis time, and that of XW1, XW2 and XW3 after 16
389 h enzyme hydrolysis was increased by 10.70%, 10.16% and 22.40%, respectively. Song
390 et al. (2020) have found that both the water-absorption capacity and oil-absorption
391 capacity of corn starch were obviously increased with increasing enzyme hydrolysis
392 time, which was similar to the findings of our study. It has been reported that the
393 increased water absorption capacity of starch was related to the small starch granule
394 size, which could explain the finding that there was more B-granules of pea starch after
395 Bacillus-produced α-amylase hydrolysis. These results showed that the water
396 absorption capacity and oil absorption capacity of the native pea starch could be

significantly affected by α-amylase hydrolysis, which was mainly due to the formation of a large number of pores and destruction of the amorphous region, making the granules expand more freely. The differences in the water absorption capacity and oil absorption capacity of pea starch might be related to the genotypes among three varieties.

3.9 Pasting properties

The pasting profiles of the native and enzymatically modified pea starch were presented in Fig. 3, and the corresponding parameters were summarized in Table 3. All starch samples showed a single peak curve, while there was significant variation between different pea varieties. Peak viscosity can reflect the swelling degree of starch granules (Gao et al., 2016). After enzyme hydrolysis, the PV significantly decreased and ranged from 90.00 to 3879.00 cP (XW1), from 362.00 to 3585.67 cP (XW2) and from 1635.00 to 2265.00 cP (XW3), respectively, which was consistent with the results on corn starch (Zhao et al., 2018). The finding of this study indicated that α-amylase hydrolysis can change the swelling degree of pea starch granules, thereby influencing the granule size and reducing the PV. The obvious decrease in the PV could be related to the hydrolysis of starch molecules, since small molecules provide lower viscosity than large molecules (Benavent-Gil & Rosell, 2017). As observed in 3.2, the A-granule distribution of pea starch significantly decreased with the time of enzymatic hydrolysis, which could be used to explain the decrease in the PV. Similar result was found in the TV, and the TV values of XW1, XW2 and XW3 were reduced by 99.65%, 99.42% and 92.51%, respectively, after 16 h of enzymatic hydrolysis compared with the native

starch. High breakdown viscosity indicates that the starch is less resistant to shear forces during heating (Kong, Zhu, Sui, & Bao, 2015). In this study, the BD of the pea starch treated with Bacillus-produced α-amylase was lower than that of native starch, which might be due to the weakened structure of granule caused by the mechanical forces of α-amylase hydrolysis. This finding indicated that enzymatic hydrolysis could enhance the resistance of pea starch to heating, which may be related to the fact that enzymatic hydrolysis preferentially occurs in the amorphous region as observed in 3.6. Similar result has been proved that α-amylase hydrolyzed corn starch granules with lower BD compared with the native starch (Dura, Blaszczak, & Rosell, 2014), while there was a slight increase of the BD on popcorn starch studied by Song et al. (2020), which was not consistent with our results, and the difference might be due to the various reactions of different starch sources to α-amylase hydrolysis. With the increase of α-amylase hydrolysis time, the FV also significantly reduced and ranged from 11.00 to 5554.67 cP, and obvious difference could be obtained among three pea varieties. Setback viscosity can reflect the retrogradation tendency of starch paste, the higher setback viscosity, the higher ability to retrograde (Zhu et al., 2017). When enzyme hydrolysis time reached to 16 h, the SB of pea starch was reduced by 99.93%, 99.82% and 98.48%, corresponding to XW1, XW2 and XW3, respectively. The low setback value at high enzyme hydrolysis time indicated that it was difficult for pea starch to retrograde after α-amylase hydrolysis. Pasting temperature refers to the temperature when the starch paste begins to rise (Zhu et al., 2017). Li, Li and Guo, (2020) have concluded that wheat starch possessed higher pasting temperature at higher enzyme hydrolysis time, while a

significant decrease was observed on pea starch as enzyme hydrolysis time increased, indicating that it was hard for the enzymatically modified pea starch to swell. The difference in pasting behaviors might be due to the variations in granule size distribution, amylose content and branch-chain length distribution of amylopectin (Yang et al., 2019).

3.10 Thermal properties

The DSC analysis was used to determine the gelatinization properties of the native and enzymatically modified pea starch. The thermograms were presented in Fig. 2 C and the thermal parameters were shown in Table 3. All starch samples displayed a single peak in the DSC thermogram, which was consistent with the XRD analysis, indicating that α-amylase hydrolysis cannot change the crystalline structure of pea starch, which confirmed the conclusion that α-amylase hydrolysis mainly occurred in the amorphous region of the native pea starch. The melting temperatures of starch crystals, including onset temperature, peak temperature and conclusion temperature, are indirectly controlled by the amorphous region around starch granules (Kalin, Plyushchev, Fedotov, Sevryukov, & Gol'tsev, 2002). In this study, the To was in the range of 55.33-58.42°C, the Tp ranged from 63.29 to 67.27°C, and the Tc ranged from 71.20 to 76.01°C. In addition, significant difference was observed among three pea varieties, XW3 showed higher gelatinization temperatures than XW1 and XW2, which might be due to the genotypes of different pea varieties. After α-amylase hydrolysis, it was shown that pea starch had higher values of the To, Tp and Tc, indicating that the gelatinization process was delayed when treated with α-amylase, which was similar to the findings of corn starch (Zhao et al., 2018). With increasing enzyme hydrolysis time, the gelatinization

463 temperatures of pea starch significantly increased, which indicated that the linear
464 dextrin produced during enzymatic hydrolysis of starch granules could be rearranged
465 into thermally stable crystalline structure. Higher gelatinization temperatures mean that
466 starches require higher cooking temperatures and longer cooking time. The results of
467 this study indicated that the cooking temperature and time of pea starch-based foods
468 were enhanced by α-amylase and heating during hydrolysis. The differences of
469 gelatinization temperatures at different enzyme hydrolysis time might be related to the
470 granule size distribution of starch, amylose content, and fine structure of amylopectin
471 (Park, Ibáez, Fang, & Shoemaker, 2007). Gelatinization enthalpy (ΔH) reflects the
472 quantity of crystallinity of the starch and is used to measure the loss of the double helical
473 structure and crystalline structure of the starch (Hoover & Ratnayake, 2002). In this
474 study, significant increases were obtained in the ΔH of pea starch with increasing
475 enzymatic hydrolysis time, ranging from 6.33 to 8.27 J/g for XW1, from 6.78 to 8.09
476 J/g for XW2 and from 7.66 to 8.17 J/g for XW3, respectively. The high value of the ΔH
477 on pea starch was attributed to the high relative crystallinity degree and low amylose
478 content after enzyme hydrolysis (Zhu et al., 2016), which was consistent with the results
479 of the XRD analysis and amylose content as shown in Table 1. These results indicated
480 that the interactions between the double helix structures of pea starch hydrolyzed by
481 Bacillus-produced α-amylase may be more extensive through hydrogen bonding to
482 form crystalline regions.

483 *3.11 Rheological properties*

484 The rheological properties of the native and enzymatically modified pea starch

were shown in Fig. 4. When the shear rate was about 90 s^{-1}, the shear viscosity of pea starch rapidly decreased and then tended to be stable with the increase of shear rate (Fig. 4 A, B and C). Compared with the native pea starch, the shear viscosity of enzymatically modified pea starch was lower and significantly decreased with increasing α-amylase incubation time. The phenomenon of increased shear rate and reduced shear viscosity indicated that pea starch can be regarded as a shear-thinning system, which was in accordance with the quinoa starch studied by Jiang et al. (2020). In addition, significant difference could be found between the wrinkled and smooth pea, and the shear-thinning phenomenon of XW3 was more obvious but lower than XW1 and XW2, which may be due to the differences in amylose content as reported in 3.1.

At present, frequency scanning method has been widely used to further understand the dynamic rheological behavior of starch. The storage modulus (G′) and loss modulus (G″) represent the solid-like and liquid-like properties of starch gels, respectively (Yuan, Sang, Wang, & Cui, 2018). In this study, the G′ and G″ of all starch samples stably increased at first and then rapidly increased to the maximum value with the increase of angular frequency, and there was no crossing both the G′ and G″. After the α-amylase hydrolysis, the G′ and G″ of all samples significantly decreased with the minimum value at 16 h, while the values of the G′ and G″ of the enzymatically modified pea starch were significantly lower than those of the native pea starch. In addition, the G′ was significantly higher than the corresponding G″ at different enzymatic time within the frequency scanning range (Fig. 4 D-F). These results indicated that the gel strength of pea starch could be weakened when treated by α-amylase, and the gel strength of the

native pea starch was higher than the enzymatically modified starch. It has been found that the rheological behaviors of starch depend on the interactions between the amylose chains and the swelling granules (Li et al., 2017). In this study, the amylose content and A-granule distribution of pea starch gradually decreased with increasing α-amylase incubation time, resulting in lower values of the G′ and G″.

3.12 Correlation analysis

The correlation analysis was used to explore the relationship between the α-amylase and physicochemical properties of pea starch (Fig. 5). It was clearly shown that the amylose content of pea starch after enzyme hydrolysis was positively correlated with the water absorption capacity and pasting parameters, but negatively correlated with the branching degree, relative crystallinity, oil absorption capacity and gelatinization enthalpy. The branching degree of pea starch was positively correlated with the relative crystallinity and gelatinization enthalpy, but negatively correlated with the pasting temperature. In addition, there were significant differences between the three pea varieties. For XW1 and XW2, the amylose content was positively correlated with the A-chains, but the oil absorption capacity was negatively correlated with the ratio of 1022/995 cm^{-1}. However, an opposite change trend was observed on the XW3. The differences might be related to the variability of genotypes between the wrinkled and smooth pea. These results suggested that the hydrolysis of α-amylase had a significant effect on the structural and physicochemical properties of pea starch and that different types of pea starch responded differently to α-amylase.

4. Conclusions

In this study, Bacillus-produced α-amylase was applied to modify pea starch isolated from three varieties to explore the changes in structural, physicochemical and rheological properties. With increasing enzyme hydrolysis time, the amylose content, amylopectin chain length distribution and water adsorption capacity significantly decreased, while branching degree and light transmittance obviously increased. Moreover, the crystalline structure was not changed but the relative crystallinity significantly increased after enzyme hydrolysis. Compared with the native pea starch, the viscosity, pasting temperature, shear viscosity, storage modulus and loss modulus of enzymatically modified starch all significantly decreased, while significant increases were found in gelatinization enthalpies. Overall, pea starch modified by Bacillus-produced α-amylase has a wide range of applications in the food industry, as it can improve the texture, stability and shelf-life of various food products. The results of this study could provide valuable information for the enzymatic modification of pea starch to design novel products with desirable structural and physicochemical properties.

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References

Bai, Y., & Shi, Y. (2016). Chemical structures in pyrodextrin determined by nuclear magnetic resonance spectroscopy. *Carbohydrate Polymers*, *151*, 426-433. https://doi.org/10.1016/j.carbpol.2016.05.058

Bechtel, D., Zayas, I., Dempster, R., & Wilson, J. (1993). Size distribution of starch granules isolated

552 from hard red winter and soft red winter wheats. *Cereal Chemistry*, *70*, 238-240.
553 Benavent-Gil, Y., & Rosell, C. (2017). Comparison of porous starches obtained from different enzyme
554 types and levels. *Carbohydrate Polymers*, *157*, 533-540.
555 https://doi.org/10.1016/j.carbpol.2016.10.047
556 Chao, G., Gao, J., Liu, R., Wang, L., Li, C., Wang, Y., Qu, Y., & Feng, B. (2015). Starch physicochemical
557 properties of waxy proso millet (*Panicum Miliaceum* L.). *Starch - Starke*, *66*,1005-1012.
558 https://doi.org/10.1002/star.201400018
559 Cheetham, N., & Tao, L. (1998). Variation in crystalline type with amylose content in maize starch
560 granules: an X-ray powder diffraction study. *Carbohydrate Polymers, 36*, 277-284.
561 https://doi.org/10.1016/S0144-8617(98)00007-1
562 Chen, G., & Zhang, B. (2012). Hydrolysis of granular corn starch with controlled pore size. *Journal of*
563 *Cereal Science*, *56*, 316-320. https://doi.org/10.1016/j.jcs.2012.01.011
564 Chen, L., Dai, Y., Hou, H., Wang, W., Ding, X., & Zhang, H. (2021). Effect of high pressure
565 microfluidization on the morphology, structure and rheology of sweet potato starch. *Food*
566 *Hydrocolloids*, *115*, 106606. https://doi.org/10.1016/j.foodhyd.2021.106606
567 Cui, Y., Han, X., Huang, X., Xie, W., Zhang, X., Zhang, Z., Yu, Q., Tao, L., Li, T., & Li, S. (2023).
568 Effects of different sources of β-glucan on pasting, gelation, and digestive properties of pea starch.
569 *Food Hydrocolloids, 135*, 108172. https://doi.org/10.1016/j.foodhyd.2022.108172
570 Dura, A., Blaszczak, W., & Rosell, C. (2014). Functionality of porous starch obtained by amylase or
571 amyloglucosidase treatments. *Carbohydrate Polymers*, *101*, 837-845.
572 https://doi.org/10.1016/j.carbpol.2013.10.013
573 Gao, L., Wang, H., Wan, C., Leng, J., Wang, P., Yang, P., Gao, X. & Gao, J. (2020). Structural, pasting
574 and thermal properties of common buckwheat (*Fagopyrum esculentum* Moench) starches affected
575 by molecular structure. *International Journal of Biological Macromolecules*, *156*, 120-126.
576 https://doi.org/10.1016/j.ijbiomac.2020.04.064
577 Gao, J., Kreft, I., Chao, G., Wang, Y., Liu, X., Wang, L., Wang, P., Gao, X., & Feng, B. (2016). Tartary
578 buckwheat (*Fagopyrum tataricum* Gaertn.) starch, a side product in functional food production, as a
579 potential source of retrograded starch. *Food Chemistry*, *190*, 552-558.
580 https://doi.org/10.1016/j.foodchem.2015.05.122

Gu, Z., Jiang, H., Zha, F., Frank, M., Rao, J., & Chen, B. (2021). Toward a comprehensive understanding of ultracentrifugal milling on the physicochemical properties and aromatic profile of yellow pea flour. *Food Chemistry*, *345*, 128760. https://doi.org/10.1016/j.foodchem.2020.128760

Hanashiro, I., Abe, J., & Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydrate Research*, *283*, 151-159. https://doi.org/10.1016/0008-6215(95)00408-4

Hoover, R., Hughes, T., Chung, H. J., & Liu, Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International*, *43*, 399-413. https://doi.org/10.1016/j.foodres.2009.09.001

Hoover, R., & Ratnayake, W. (2002). Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chemistry*, *78*, 489-498. https://doi.org/10.1016/S0308-8146(02)00163-2

Jiang, F., Du, C., Guo, Y., Fu, J., Jiang, W., & Du, S. (2020). Physicochemical and structural properties of starches isolated from quinoa varieties. *Food Hydrocolloids*, *101*, 105515. https://doi.org/10.1016/j.foodhyd.2019.105515

Jiang, S., Yu, Z., Hu, H., Lv, J., Wang, H., & Jiang, S. (2017). Adsorption of procyanidins onto chitosan-modified porous rice starch. *LWT-Food Science and Technology*, *84*, 10-17. https://doi.org/10.1016/j.lwt.2017.05.047

Kalin, B., Plyushchev, A., Fedotov, V., Sevryukov, O., & Gol'tsev, V. (2002). Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydrate Polymers*, *49*, 425-437. https://doi.org/10.1016/S0144-8617(01)00354-X

Kong, X., Zhu, P., Sui, Z., & Bao, J. (2015). Physicochemical properties of starches from diverse rice cultivars varying in apparent amylose content and gelatinisation temperature combinations. *Food Chemistry*, 172, 433-440. https://doi.org/10.1016/j.foodchem.2014.09.085

Li, G., & Zhu, F. (2017). Amylopectin molecular structure in relation to physicochemical properties of quinoa starch. *Carbohydrate Polymers*, *164*, 396-402. https://doi.org/10.1016/j.carbpol.2017.02.014

Li, H., Li, J., & Guo, L. (2020). Rheological and pasting characteristics of wheat starch modified with sequential triple enzymes. *Carbohydrate Polymers*, *230*, 115667. https://doi.org/10.1016/j.carbpol.2019.115667

Li, W., Li, C., Gu, Z., Qiu, Y., Cheng, L., Hong, Y., & Li, Z. (2016). Relationship between structure and retrogradation properties of corn starch treated with 1,4-α-glucan branching enzyme. *Food Hydrocolloids*, *52*, 868-875. https://doi.org/10.1016/j.foodhyd.2015.09.009

Li, Y., Li, C., Gu, Z., Hong, Y., Cheng, L., & Li, Z. (2017). Effect of modification with 1,4-α-glucan branching enzyme on the rheological properties of cassava starch. *International journal of Biological Macromolecules*, *103*, 630-639. https://doi.org/10.1016/j.ijbiomac.2017.05.045

Lin, L., Guo, D., Huang, J., Zhang, X., Zhang, L., & Wei, C. (2016). Molecular structure and enzymatic hydrolysis properties of starches from high-amylose maize inbred lines and their hybrids. *Food Hydrocolloids*, *58*, 246-254. https://doi.org/10.1016/j.foodhyd.2016.03.001

Lopez-Baron, N., Sagnelli, D., Blennow, A., Holse, M., Gao, J., Saaby, L., Mullertz, A., Jespersen, B., & Vasanthan, T. (2018). Hydrolysed pea proteins mitigate in vitro wheat starch digestibility. *Food Hydrocolloids*, *79*, 117-126. https://doi.org/10.1016/j.foodhyd.2017.12.009

Martínez, A., Ramírez, O., & Valle, F. (1997). Improvement of culture conditions to overproduce β-galactosidase from Escherichia coli in Bacillus subtilis. *Applied Microbiology Biotechnology*, *47*, 40-45. https://doi.org/10.1007/s002530050885

Park, I., Ibáez, A., Fang, Z., & Shoemaker, C. (2007). Gelatinization and pasting properties of waxy and non-waxy rice starches. *Starch - Starke*, *59*, 388-396. https://doi.org/10.1002/star.200600570

Raghunathan, R., Hoover, R., Waduge, R., Liu, Q., & Warkentin, T. (2017). Impact of molecular structure on the physicochemical properties of starches isolated from different field pea (*Pisum sativum* L.) cultivars grown in Saskatchewan, Canada. *Food Chemistry*, *221*, 1514-1521. https://doi.org/10.1016/j.foodchem.2016.10.142

Raphaelides, S., & Georgiadis, N. (2008). Effect of fatty acids on the rheological behaviour of pea starch dispersions during heating, *Food Research International*, *41*, 75-88. https://doi.org/10.1016/j.foodhyd.2006.09.005

Ratnayake, W., Hoover, R., Shahidi, F., Perera, C., & Jane, J. (2001). Composition, molecular structure, and physicochemical properties of starches from four field pea (*Pisum sativum* L.) cultivars. *Food Chemistry*, *74*, 189-202. https://doi.org/10.1016/S0308-8146(01)00124-8

Ren, Y., Setia, R., Warkentin, T., & Ai, Y. (2020). Functionality and starch digestibility of wrinkled and round pea flours of two different particle sizes. *Food Chemistry*, *336*, 127711.

https://doi.org/10.1016/j.foodchem.2020.127711

Schwab, K., Bader, J., Brokamp, C., Popović, M. K., Bajpai, R., & Berovič, M. (2009). Dual feeding strategy for the production of α-amylase by Bacillus caldolyticus using complex media. *New biotechnology*, *26*, 68-74. https://doi.org/10.1016/j.nbt.2009.04.005

Shariffa, Y., Karim, A., Fazilah, A., & Zaidul, I. (2009). Enzymatic hydrolysis of granular native and mildly heat-treated tapioca and sweet potato starches at sub-gelatinization temperature. *Food Hydrocolloids*, *23*, 434-440. https://doi.org/10.1016/j.foodhyd.2008.03.009

Song, Z., Zhong, Y., Tian, W., Zhang, C., Hansen, A., Blennow, A., Liang, W., & Guo, D. (2020). Structural and functional characterizations of α-amylase-treated porous popcorn starch. *Food Hydrocolloids*, *108*, 105606. https://doi.org/10.1016/j.foodhyd.2019.105606

Sun, Q., Sun, C., & Xiong, L. (2014). Functional and pasting properties of pea starch and peanut protein isolate blends. *Carbohydrate Polymers*. *101*, 1134-1139. https://doi.org/10.1016/j.carbpol.2013.10.064

Teng, A., Torsten, W., Wang, K., Li, M., & Jovin, H. (2016). Molecular rearrangement of waxy and normal maize starch granules during in *vitro* digestion. *Carbohydrate Polymers*, *139*, 10-19. https://doi.org/10.1016/j.carbpol.2015.11.062

Wang, C., Tang, C., Fu, X., Huang, Q., & Zhang, B. (2016). Granular size of potato starch affects structural properties, octenylsuccinic anhydride modification and flowability. *Food Chemistry*, *212*, 453-459. https://doi.org/10.1016/j.foodchem.2016.06.006

Wang, Y., Bai, Y., Ji, H., Dong, J., Li, X., Liu, J., & Jin, Z. (2022). Insights into rice starch degradation by maltogenic α-amylase: Effect of starch structure on its rheological properties. *Food Hydrocolloids*, *124*, 107289. https://doi.org/10.1016/j.foodhyd.2021.107289

Yang, Q., Zhang, W., Luo, Y., Li, J., Gao, J., Yang, P., Gao, X., & Feng, B. (2019). Comparison of structural and physicochemical properties of starches from five coarse grains. *Food Chemistry*, *288*, 283-290. https://doi.org/10.1016/j.foodchem.2019.02.134

Yuan, C., Sang, L., Wang, Y., & Cui, B. (2018). Influence of cyclodextrins on the gel properties of kappa-carrageenan. *Food Chemistry*, *266*, 545-550. https://doi.org/10.1016/j.foodchem.2018.06.060

Zhang, W., Yang, Q., Xia, M., Bai, W., Wang, P., Gao, X., Li, J., Feng, B., & Gao J. (2019). Effects of nitrogen level on the physicochemical properties of Tartary buckwheat (*Fagopyrum tataricum* (L.)

668 Gaertn.) starch. *International Journal of Biological Macromolecules*, *129*, 799-808.
669 https://doi.org/10.1016/j.ijbiomac.2019.02.018
670 Zhao, A., Yu, L., Yang, M., Wang, C., Wang, M., & Bai, X. (2018). Effects of the combination of freeze-
671 thawing and enzymatic hydrolysis on the microstructure and physicochemical properties of porous
672 corn starch. *Food Hydrocolloids*, *83*, 465-472. https://doi.org/10.1016/j.foodhyd.2018.04.041
673 Zhou, D., Ma, Z., Yin, X., Hu, X. & Boye, J. (2019). Structural characteristics and physicochemical
674 properties of field pea starch modified by physical, enzymatic, and acid treatments. *Food*
675 *Hydrocolloids*, *93*, 386-394. https://doi.org/10.1016/j.foodhyd.2019.02.048
676 Zhou, Y., Hoover, R., & Liu, Q. (2004). Relationship between a-amylase degradation and the structure
677 and physicochemical properties of legume starches. *Carbohydrate Polymers*, *57*, 299-317.
678 https://doi.org/10.1016/j.carbpol.2004.05.010
679 Zhu, D., Zhang, H., Guo, B., Xu, K., Dai, Q., Wei, C., Wei, H., Gao, H., Hu, Y., & Cui, P. (2016). Effect
680 of nitrogen management on the structure and physicochemical properties of rice starch. *Journal of*
681 *Agricultural & Food Chemistry*, *64*, 8019-8025. https://doi.org/10.1021/acs.jafc.6b03173
682 Zhu, D., Zhang, H., Guo, B., Xu, K., Dai, Q., Wei, C., Zhou, G., & Huo, Z. (2017). Effects of nitrogen
683 level on structure and physicochemical properties of rice starch. *Food Hydrocolloids*, *63*, 525-532.
684 https://doi.org/10.1016/j.foodhyd.2016.09.042

685 **Figure legends**

686 **Fig. 1** Granule size distribution and chain length distribution of amylopectin of the
687 native and enzymatically modified pea starch.

688 **Fig. 2** X-ray diffraction patterns, ATR-FTIR spectra and DSC thermograms of the
689 native and enzymatically modified pea starch.

690 **Fig. 3** RVA curves of the native and enzymatically modified pea starch.

691 **Fig. 4** Diagram of shear viscosity, storage modulus and loss modulus of frequency scan
692 of the native and enzymatically modified pea starch.

693 **Fig. 5** Correlations analysis of the structural and physicochemical properties of pea

694 starch. Am: amylose content; Bd: branching degree; Rc: relative crystallinity; Lt: light
695 transmittance; Wa: water absorption capacity; Oa: oil absorption capacity; PV: peak
696 viscosity; TV: trough viscosity; FV: final viscosity; SB: setback; BD: breakdown; PT:
697 pasting temperature; To: onset gelatinization temperature; Tp: peak gelatinization
698 temperature; Tc: conclusion gelatinization temperature; ΔH: gelatinization enthalpy.
699