Effect of high hydrostatic pressure and retrogradation treatments on structural and physicochemical properties of waxy wheat starch

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Abstract

In this study, the effects of high hydrostatic pressure and retrogradation (HHPR) treatments on *in vitro* digestibility, structural and physicochemical properties of waxy wheat starch were investigated. The waxy wheat starch slurries (10%, w/v) were treated with high hydrostatic pressures of 300, 400, 500, 600 MPa at 20 °C for 30 min, respectively, and then retrograded at 4 °C for 4 d. The results indicated that the content of slowly digestible starch (SDS) in HHPR-treated starch samples increased with increasing pressure level, and it reached the maximum (31.12%) at 600 MPa. HHPR treatment decreased the gelatinization temperatures, the gelatinization enthalpy, the relative crystallinity and the peak viscosity of the starch samples. Moreover, HHPR treatment destroyed the surface and interior structures of starch granules. These results suggest that the *in vitro* digestibility, physicochemical, and structural properties of waxy wheat starch are effectively modified by HHPR.

Keywords: High hydrostatic pressure; Retrogradation; Digestibility; Structure; Physicochemical property; Waxy wheat starch

1. Introduction

Starch is the most abundant reserve carbohydrate in plants, and it is found in many different plant organs, including seeds, fruits, tubers and roots. Starch has been widely used in food, paper, textile, chemical and pharmaceutical industries, not only because it is a cheap and natural material but also because of the ease with which its physicochemical properties can be altered through chemical or enzymatic modification and/or physical treatment (Jobling, 2004). Proper rate of glucose release and absorption from digesting starch may play an important role in human health by maintaining proper blood glucose levels (Zhang, Ao, & Hamaker, 2008). According to the rate and extent of digestion *in vitro*, starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst & Hudson, 1996). SDS is the starch fraction that is digested completely in the small intestine at a lower rate than RDS, SDS tends to provide a sustained supply of glucose with a low glycemic index (GI) that may contribute to the control and prevention of various hyperglycaemia-related diseases (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996). Besides, SDS may be beneficial to maintaining body weight when it is used as a raw material in the production of foodstuffs (Jenkins et al., 2002). Hence, foods containing higher amount of SDS are regarded as functional foods with low GI (Han, Ao, Janaswamy, Jane, Chandrasekaran, & Hamaker, 2006; Zhang & Hamaker, 2009), and SDS has attracted more attention in recent years.

Recently, a number of methods have been applied to prepare SDS from various sources of starch. These methods include physical treatment (Xie, Hu, Jin, Xu, & Chen, 2014; Zeng, Ma, Kong, Gao, & Yu, 2015), citric acid treatment (Shin et al., 2007), and enzyme modification

(Shi, Zhang, Yu, Wang, Gilbert, & Gao, 2014). Among these methods, physical treatment, including thermal and non-thermal procedures, is safer and easily accessible than other methods. As a non-thermal technology, high pressure, such as ultra-high pressure (UHP) and high hydrostatic pressure (HHP), is gaining increasingly popularity in food industry at present. High pressure treatment is better beyond thermal treatment due to that this technology can make it possible to produce foods with novel textures and it is better to retain the nutritional and functional ingredients in the processing (Stolt, Oinonen, & Autio, 2001; Vallons & Arendt, 2009). Previous study indicated that high pressure could be beneficial to the application of starch in food products, such as being fat substitute in low-energy foods (Blaszcak, Valverde, & Fornal, 2005b). The effects of high pressure treatment on the structural and physicochemical properties of starches from various botanical sources have been investigated in recent years (Blaszcak et al., 2005b; Guo, Zeng, Lu, Zhou, Zheng, & Zheng, 2015; Li, Bai, Mousaa, Zhang, & Shen, 2012). Blaszcak et al. (2005b) showed that high pressure treatment at 600 MPa for 2 and 3 min resulted in a decrease in gelatinization temperatures (T_o , T_p , T_c) and gelatinization enthalpy of potato starch. Li et al. (2012) indicated that the treatment of rice starch with HHP under 600 MPa for 30 min resulted in a complete loss of birefringence and a gel-like appearance, and a decrease in breakdown and setback viscosities. Guo et al. (2015) also found that UHP-treated lotus seed starch showed a decrease in breakdown and setback viscosity. However, few studies regarding the effect of high pressure especially HHP treatment on *in vitro* digestibility and structural characteristics of waxy wheat starches have been reported.

Our previous studies have revealed the impacts of repeated retrogradation and

temperature-cycled retrogradation treatments on the structural characteristics and *in vitro* digestibility of waxy wheat starch (Hu, Huang, Mei, Jin, Xu, & Chen, 2015; Hu, Xie, Jin, Xu, & Chen, 2014), however, the effect of high hydrostatic pressure and retrogradation (HHPR) treatments on the structural characteristics of waxy wheat starch has not been reported. Therefore, the aims of the present study were to investigate the effect of HHPR treatment on *in vitro* digestibility, structural and physicochemical properties of waxy wheat starch.

2. Materials and methods

2.1 Materials

The waxy wheat was supplied by Yangzhou Academy of Agricultural Sciences, China. The enzymes used in the determination of starch digestibility were α -amylase type VI-B from porcine pancreas (EC 3.2.1.1, A3176, Sigma, St. Louis, MO. USA) and amyloglucosidase (EC 3.2.1.3, Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China). Other chemical reagents were of analytical grade (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China).

2.2 Isolation of waxy wheat starch

Starch was isolated from waxy wheat based on our previously described method (Hu et al., 2014). Briefly, waxy wheat flour was fully milled and dissolved in 0.03 M NaOH (1:3, w/w). After stirring at 1200 rpm and room temperature for 1.5 h, the soft top layer was discarded, and the remaining slurry was centrifuged at 3500 rpm for 5 min (TDL-40B, Anke, Shanghai, China), the upper greyish layer was discarded. The sediment was washed with distilled water and centrifuged (3500 rpm for 5 min) for several times till it was white. Subsequently, the white sediment was neutralized with 1 M HCl, centrifuged, and then the obtained residue was

starch. The residue was air-dried, milled (Model LG-04, Baixin Yaoji Corporation, Ruian, China) and passed through a sieve with 100 μ m openings.

2.3 High hydrostatic pressure and retrogradation (HHPR) treatments

Waxy wheat starch slurries (10%, w/v) were equilibrated at room temperature for 6 h, and then were vacuum-packed (Aixun Package Equipment Corporation, Qingdao, China) in polyethylene bags (10 cm $\times$ 15 cm). The starch samples were treated with HHP of 300, 400, 500, 600 MPa at room temperature (20 $^{\circ}$ C) for 30 min (HPP.L1-600/3, Huatai Senmiao Biological Engineering Corporation, Tianjin, China), respectively, and then retrograded at 4 $^{\circ}$ C for 4 d, and the obtained starch samples were designated as HHPR-300, HHPR-400, HHPR-500 and HHPR-600, respectively. The starch samples were air-dried in the oven (DHG-9101-2SA, Sanfa Scientific Instrument, Co., Ltd, Shanghai, China) at 45 $^{\circ}$ C for 24 h and milled to pass through a 100-mesh sieve.

2.4 Determination of *in vitro* digestibility of starch

Determination of *in vitro* digestibility of starch was performed according to our previous method (Hu et al., 2014), which was on the basis of the method described by Englyst, Kingman, & Cummings (1992) with a slight modification. In brief, 200 mg starch sample was dissolved in phosphate buffer (15 ml, pH 5.2) containing seven glass balls (10 mm diameter), and then equilibrated at 37 $^{\circ}$ C for 5 min. Subsequently, they were hydrolyzed by 5 ml mixed enzyme solution (porcine pancreatic α -amylase, 290 U/ml; amyloglucosidase, 15 U/ml, where U is defined as the amount of enzyme that liberates 1.0 mg glucose from starch in 1 min at pH 5.2 and 37 $^{\circ}$ C) in a water bath shaker at 37 $^{\circ}$ C. At time intervals of 20 and 120 min, aliquots (0.5 ml) of hydrolyzed solution were taken and mixed with absolute ethanol (4 ml) to

deactivate the enzyme, and then centrifuged at 2000 rpm for 10 min. The glucose content in the supernatant was measured by 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959). The percentage of hydrolyzed starch was calculated by multiplying the glucose content by a factor of 0.9. Each sample was determined in triplicate.

The percentages of RDS, SDS and RS fractions in starch samples were calculated by the following formulae:

$$\text{RDS (\%)} = [(G_{20} - \text{FG}) / \text{TS}] \times 0.9 \times 100$$

$$\text{SDS (\%)} = [(G_{120} - G_{20}) / \text{TS}] \times 0.9 \times 100$$

$$\text{RS (\%)} = [(\text{TS} - \text{RDS} - \text{SDS}) / \text{TS}] \times 100$$

where, G_{20} and G_{120} are the amounts of glucose released within 20 and 120 min of hydrolysis, respectively, and FG is the amount of free glucose in starch and TS was total starch weight.

2.5 Differential scanning calorimetry (DSC)

Thermal characteristics of starch samples were measured using DSC (Q200, TA, New Castle, DE, USA) (Hu et al., 2014). Approximately 3 mg starch sample was mixed with 6 μl deionized water and hermetically sealed in an aluminium pan, equilibrated at 4 °C for 24 h. The condition of the experiment was as follows: the starch sample was equilibrated at 20 °C for 10 min and then heated from 20 to 85 °C at a rate of 8 °C/min. An empty aluminium pan was regarded as the reference. The transition temperatures (T_o , T_p and T_c) and gelatinization enthalpy (ΔH) were analyzed and calculated using TA Universal Analysis Software (TA Instruments, New Castle, DE, USA). Experiments were conducted in triplicate.

2.6 X-ray diffraction (XRD)

The X-ray diffraction patterns of native and HHPR-treated starch samples were analyzed

by an X-ray diffractometer (D/MAX 2500V, Rigaku Corporation, Japan) operating at 40 kV and 40 mA with Cu K α radiation. The starch samples were dried at 40 °C for 6 h, and then scanned through 2θ range from 3° to 30° at room temperature. The degree of relative crystallinity was calculated by MDI-Jade 6.0 software (Material Date, Inc. Livermore, California, USA) in terms of the method of Nara and Komiya (1983).

2.7 Pasting properties

The pasting properties of native and HHP-treated starch samples were analyzed by a Rapid Visco Analyzer (RVA-4500, Perten Instruments, Sweden). The test method was on the basis of AACC method 76-21.01 (AACC, 1999) with a slight modification. Starch slurries (6% w/w, 25g of total weight) were equilibrated at 50 °C for 1 min, heated to 95 °C at a rate of 5 °C/min. Then they were held at 95 °C for 150 s, cooled to 50 °C at 5 °C/min, and held at 50 °C for 2 min. The rotating speed of paddle was 960 rpm for the first 10 s and then reduced and kept at 160 rpm throughout the remainder of the experiment.

2.8 Scanning electron microscopy (SEM)

The starch samples were sputter-coated with a thin film of gold under vacuum and then the microstructures of starch samples were observed using the field-emission scanning electron microscopy (SU8020, Hitachi Int., Japan) at an accelerating voltage of 1.0 kV within 50.0 μ m.

2.9 Statistical analysis

Results are expressed as the mean $\pm$ standard deviation of triplicate experiments. Data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS 18.0 Statistical Software Program (SPSS Incorporated, Chicago). A value of P < 0.05 was considered statistically significant.

3. Results and discussion

3.1 *In vitro* digestibility

After waxy wheat starch slurry samples were treated with high hydrostatic pressure of 300, 400, 500, and 600 MPa at 20 °C for 30 min and then retrograded at 4 °C for 4 d, the contents of RDS, SDS and RS fractions in HHPR-treated starch samples were determined, and the results are presented in Fig. 1.

As shown in Fig. 1, with the increase in the pressure level, the contents of RDS and SDS of starch samples increased while the RS content decreased, which indicated that higher level of HHPR treatment destroyed the structure of starch granules and broke the double helix, thus resulted in the decrease in the percentage of RS fraction (Fig. 1). When the hydrostatic pressure level reached the maximum value (600 MPa) of the machine, the content of SDS fraction was the highest (31.12%). Hu, Xu, Jin, Tian, Bai, & Xie (2011) reported that high hydrostatic pressure (HHP) treatment almost broke waxy rice starch granules completely. Moreover, HHP treatment could promote the distribution of water molecules into the crystalline region to disrupt the helical form of the amylopectin chains at a high enough pressure level (Li et al., 2012), which might benefit the formation of imperfect crystallites (Tian, Li, Zhao, Xu, & Jin, 2014).

3.2 Thermal properties

The transition temperatures (T_o , T_p , and T_c), the gelatinization temperature range (T_c-T_o) and the gelatinization enthalpy (ΔH) of native and HHPR-treated starch samples under different conditions are summarized in Table 1. As shown in Table 1, T_o values of HHPR-300

and HHPR-400 starch samples were similar to that of native starch, while the T_o values of HHPR-500 and HHPR-600 starch samples were significantly lower than that of native starch. It might be due to that the lower hydrostatic pressure treatment had relatively less effect on starch granules compared with the higher hydrostatic pressure treatment, which also resulted in lower content of RDS in HHPR-300 and HHPR-400 starch samples compared with HHPR-500 and HHPR-600 starch samples. The decrease in T_o suggested that double helices in the crystalline and non-crystalline regions were destroyed during the higher hydrostatic pressure treatment. The weakening of the starch granules by high hydrostatic pressure treatment led to early rupture of the amylopectin double helices (Adebowale & Lawal, 2003). Moreover, T_c - T_o values of higher hydrostatic pressure treated starch samples (HHPR-500 and HHPR-600 starch samples) were much higher than those of native and lower hydrostatic pressure treated starch samples. Besides, the increase in the pressure level led to the decrease in ΔH , which was due to the disruption of double helices, and it was related to the contents of SDS and RS (Chung, Liu, & Hoover, 2009; Shin, Kim, Lee, & Moon, 2005).

3.3 X-ray diffraction and relative crystallinity

X-ray diffractograms and relative crystallinity of HHPR-treated starch samples are presented in Fig. 2. In our previous study, native waxy wheat starch displayed typical A-type with an unresolved peak at 17-18° and individual peaks at 15° and 23°, and the relative crystallinity was 37.03% (Hu et al., 2014). Compared with the native starch, the starch samples treated with HHPR at 300 and 400 MPa also displayed A-type pattern because of the reflection intensities at 2 θ values of 15°, 17°, 17.9° and 23°, but the relative crystallinity significantly decreased to 33.47% and 31.83%, respectively. Moreover, the X-ray diffraction

pattern of HHPR-500 starch sample showed a faint A-type, and compared with the native starch, the relative crystallinity dramatically reduced to 25.11%. A previous study showed that A-type starches are the most sensitive to pressure treatment (Li et al., 2012), and HHP treatment of 500 MPa resulted in the destruction of A-type crystalline structure and a significant decrease in the relative crystallinity of corn starch (Hibi, Matsumoto, & Hagiwara, 1993). However, the diffraction pattern of starch sample treated with 600 MPa was A+B-type (2θ values of 15° , 17° , 17.9° and 22°) and the relative crystallinity was 16.93%. Hibi et al. (1993) reported that after treatment with 500 MPa for 20 and 60 min, the A-type X-ray diffraction pattern of corn starch tended to change into a faint B-type pattern. Waxy wheat starch was A-type crystalline starch and mainly consisted of amylopectin chains with a large percentage of scattered branching structures. Moreover, water was the necessary component in the crystalline structures. It was more possible to rearrange double helices and facilitate the combination of starch molecules and water molecules, which could transform the crystalline pattern from A-type to B-type. High hydrostatic pressure treatment resulted in the disruption of waxy wheat starch granules and the change in the granule structure to different extent.

3.4 Pasting properties

The peak viscosity (PV) of native starch was the highest, and the PV value of HHPR-treated starch samples decreased with the increase in HHP level (Fig. 3), indicating that the interactions between starch chains slowed down and the hydrogen bonds between chains decreased. HHP treatment can destroy the stability of starch granules and weaken resistance to shear; in addition, high pressure has been shown to affect high molecular weight polymers including crystallinity melting, amorphous hydrating and even gelatinizing of starch

granules (Blaszczyk et al., 2005b). Previous studies indicated that compared with the native starch, starch samples treated with HHP of 300-600 MPa had relatively lower PV and final viscosity (FV) values (Liu, Zhang, Shen, Hu, & Wu, 2012), which was consistent with our results. In the present study, we also found that the pasting temperatures of waxy wheat starch samples were positively correlated with the gelatinization peak temperatures (T_p). This could be attributed to the structural changes in starch granules during HHPR treatment. The pasting temperatures of native, HHPR-300 and HHPR-400 starch samples were similar and T_p values of them were similar as well. Moreover, Jane et al. (1999) reported that pasting properties of botanical starches were related to its amylopectin branch chain-length distribution. In this study, HHPR-600 starch sample exhibited the highest pasting temperature (70.2 °C) among all treated starch samples, and compared with the native starch. Srichuwong and Jane (2007) reported that starch consisting of amylopectin with more short chains (DP 6-12) displayed a lower pasting temperature, which was consistent with the result of the present study. Due to more stable and flexibly distributed double helices formed by the short branch chains in the crystalline and amorphous regions of HHPR-600 starch sample, thus resulted in its higher pasting temperature. Generally, breakdown (BD) reflected the disintegration of the gelatinized and swollen starch granules (Hermansson & Svegmark, 1996), and it was associated with the melting of starch crystals or the increase in viscosity, but not the end of structural changes in the starch suspensions. So BD value may be regarded as the endpoint of gelatinization, its variation trend was corresponded to that of T_c . In the present study, the BD value of HHPR-treated starch samples, except HHPR-300 sample, decreased with the increase in HHP level, but they were lower than that of native starch, meanwhile, their T_c values exhibited

similar varying trend. Moreover, starch granules in suspension swelled with heating, and the viscosity of the suspension varied with the starch concentration. The BD values of HHPR-treated starch samples were lower than that of the native starch, which may be associated with retrogradation treatment.

3.5 Morphological characteristics

Native and HHPR-treated starch samples were examined by SEM to observe and confirm changes in surface morphology of starch granules (Fig. 4). The changes in the morphology of starch granules were always related to the interactions between crystalline and amorphous regions in starch. Compared with the native starch granules, the granules of HHPR-300 starch sample remained the intact structure, but became tighter and rougher with few wrinkles on the surface, while HHPR-400 starch granules packed tighter with slight melting on the surface of granules than HHPR-300 starch granules. Besides, the majority of HHPR-300 and HHPR-400 starch granules retained granular shape of native starch to some extent. Higher level of HHP treatment could lead to stronger interactions between starch molecules, which contributed to dramatic destruction of starch granules integrity with the presence of cavities, fissures, and holes on the surface and gel-like network in the interior section (Blaszczyk, Fornal, Valverde, & Garrido, 2005a; Blaszczyk et al., 2005b). The granules of HHPR-500 starch sample displayed irreversible loss, and viscous gel-like regions were present among the granule boundaries, while the granular structure of HHPR-600 starch sample was dramatically destroyed and the leaf-like shape was appeared. The gel-like structure might result from the hydration of the amorphous phase and/or the melting of the crystalline structures (Rubens & Heremans, 2000). These observations were similar to the previous studies on HHP or high

pressure-treated rice starch (Deng et al., 2014; Li et al., 2012), wheat starch (Liu, Selomulyo, & Zhou, 2008), potato starch (Blaszcak et al., 2005b; Liu et al., 2008), waxy corn starch (Liu et al., 2012), barley starch (Stolt et al., 2001), and sorghum starch (Vallons & Arendt, 2009). In the present study, the retrogradation treatment after HHP treatment was inhibited to some extent, which exhibited a little change in morphological properties. Differences in the surface morphology of HHPR-treated starch granules varied with the pressure level, and it suggested that HHP treatment destroyed the surface structure of waxy wheat starch granules to different extents.

4. Conclusions

In vitro digestibility, structural, and physicochemical characteristics of native and HHPR-treated waxy wheat starch samples were investigated. HHPR treatment led to an increase in SDS content, and SDS content reached the maximum (31.12%) at 600 MPa. Moreover, the gelatinization enthalpy of HHPR-treated starch samples decreased gradually with the increase in pressure levels. Compared with native starch, HHPR treatment resulted in the decrease in the relative crystallinity and the peak viscosity of starch samples. In addition, HHPR treatment destroyed the surface and interior structure of waxy wheat starch granules. These results showed that HHPR-induced structural changes in waxy wheat starch significantly affected the digestibility and properties.

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Captions of Figures

Fig. 1 The content of RDS, SDS, and RS fractions in HHPR-treated starch samples.

RDS, SDS, RS indicate rapidly digestible starch, slowly digestible starch and resistant starch, respectively. HHPR-300, HHPR-400, HHPR-500, and HHPR-600 indicate that starch samples are treated with high hydrostatic pressures of 300, 400, 500, and 600 MPa at 20 °C for 30 min, respectively, and then retrograded at 4 °C for 4 d.

^{a-e} Bars with different letters indicate significant difference ($P < 0.05$).

Fig. 2 X-ray diffraction patterns of native and HHPR-treated starch samples.

NS indicates native starch. HHPR-300, HHPR-400, HHPR-500, and HHPR-600 indicate that starch samples are treated with high hydrostatic pressures of 300, 400, 500, and 600 MPa at 20 °C for 30 min, respectively, and then retrograded at 4 °C for 4 d.

Fig. 3 Pasting profiles of native and HHPR-treated starch samples.

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Fig. 4 The morphological characteristics of native and HHPR-treated starch samples.

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Table 1 Thermal properties of native and HHPR-treated starch samples

Sample	T_o (°C)	T_p (°C)	T_c (°C)	T_c-T_o (°C)	ΔH (J/g)
NS	61.17±0.24 ^a	64.87±0.20 ^b	71.19±0.43 ^c	10.02±0.53 ^e	13.48±0.68 ^a
HHPR-300	60.86±0.11 ^a	64.50±0.03 ^c	74.03±0.49 ^a	13.17±0.60 ^c	8.81±0.24 ^b
HHPR-400	61.36±0.02 ^a	65.27±0.09 ^a	72.73±0.78 ^b	11.37±0.77 ^d	6.44±0.15 ^c
HHPR-500	45.51±0.80 ^b	55.47±0.36 ^d	65.46±0.15 ^d	19.96±0.94 ^a	3.09±0.14 ^d
HHPR-600	45.34±0.16 ^b	53.70±0.14 ^e	62.57±0.24 ^e	17.23±0.09 ^b	2.81±0.10 ^d

NS indicates native starch. HHPR indicates high hydrostatic pressure and retrogradation. HHPR-300, HHPR-400, HHPR-500, and HHPR-600 indicate that starch samples are treated with high hydrostatic pressures of 300, 400, 500, and 600 MPa at 20 °C for 30 min, respectively, and then retrograded at 4 °C for 4 d.

T_o , onset temperature; T_p , peak temperature; T_c , conclusion temperature; T_c-T_o , gelatinization temperature range; ΔH , gelatinization enthalpy.

Mean of three measurements $\pm$ standard deviation.

The values with different superscripts within a column are significantly different ($P < 0.05$).

Fig. 1 The content of RDS, SDS, and RS fractions in HHPR-treated starch samples.

NS indicates native starch. RDS, SDS, RS indicate rapidly digestible starch, slowly digestible starch and resistant starch, respectively. HHPR-300, HHPR-400, HHPR-500, and HHPR-600 indicate that starch samples are treated with high hydrostatic pressures of 300, 400, 500, and 600 MPa at 20 °C for 30 min, respectively, and then retrograded at 4 °C for 4 d.

^{a-e} Bars with different letters indicate significant difference ($P < 0.05$).

Fig. 2 X-ray diffraction patterns of native and HHPR-treated starch samples.

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Fig. 3 Pasting profiles of native and HHPR-treated starch samples.

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Fig. 4 The morphological characteristics of native and HHPR-treated starch samples.

NS indicates native starch. HHPR-300, HHPR-400, HHPR-500, and HHPR-600 indicate that starch samples are treated with high hydrostatic pressures of 300, 400, 500, and 600 MPa at 20 °C for 30 min, respectively, and then retrograded at 4 °C for 4 d.