RESEARCH ARTICLE

Effect of α-Amylase Degradation on Physicochemical Properties of Pre-High Hydrostatic Pressure-Treated Potato Starch

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Abstract

The effect of high hydrostatic pressure (HHP) on the susceptibility of potato starch (25%, w/v) suspended in water to degradation by exposure to bacterial α-amylase (0.02%, 0.04% and 0.06%, w/v) for 40 min at 25°C was investigated. Significant differences (p < 0.05) in the structure, morphology and physicochemical properties were observed. HHP-treated potato starch (PS) exposed to α-amylase (0.06%, w/v) showed a significantly greater degree of hydrolysis and amount of reducing sugar released compared to α-amylase at a concentration of 0.04% (w/v) or 0.02% (w/v). Native PS (NPS) granules have a spherical and elliptical form with a smooth surface, whereas the hydrolyzed NPS (hNPS) and hydrolyzed HHP-treated PS granules showed irregular and ruptured forms with several cracks and holes on the surface. Hydrolysis of HHP-treated PS by α-amylase could decrease the average granule size significantly (p < 0.05) from 29.43 to 20.03 μm. Swelling power decreased and solubility increased with increasing enzyme concentration and increasing pressure from 200–600 MPa, with the exception of the solubility of HHP-treated PS at 600 MPa (HHP600 PS). Fourier transform infrared spectroscopy (FTIR) showed extensive degradation of the starch in both the ordered and the amorphous structure, especially in hydrolyzed HHP600 PS. The B-type of hydrolyzed HHP600 PS with α-amylase at a concentration 0.06% (w/v) changed to a B+V type with an additional peak at 2θ = 19.36°. The HHP600 starch with 0.06% (w/v) α-amylase displayed the lowest value of T_o (onset temperature), T_c (conclusion temperature) and ΔH_gel (enthalpies of gelatinization). These results indicate the pre-HHP treatment of NPS leads to increased susceptibility of the granules to enzymatic degradation and eventually changes of both the amorphous and the crystalline structures.

Introduction

Potato (Solanum tuberosum) is the third most important food crop in the world after rice (Oryza sativa) and wheat (Triticum aestivum) in terms of human consumption [1]. China leads the world in potato production, and nearly a third of the world's potatoes are harvested in
Potato starch (PS) is used extensively in the food industry as a coating, blending, bulking and thickening agent. This wide application of starch takes advantage of its desirable flavor and colorless appearance of SP [3]. In terms of physical properties, PS is unique among commercially available starches (e.g. cereal type) and is used widely in a variety of food systems [4]. This uniqueness is due to the large granule size, purity, relatively long amylose and amylopectin chains, the existence of phosphate ester groups on amylopectin and the ability to form thick visco-elastic gels upon heating and subsequent cooling [5].

In general, native starches have an intrinsic imperfect nature, are insoluble in water and tend to retrograde and undergo syneresis, producing weak, cohesive, rubbery pastes when heated and undesirable gels when the pastes are cooled [6], which restricts their use in the food industry. Various modification processes, including physical, chemical, enzymatic and biotechnological, are used to improve the properties and functionality of starch, paying attention to imperfect intrinsic characteristics, water insolubility, tendency to retrograde and undergo syneresis to make it suitable for many food and industrial applications. Physical modification methods are, in general, safe, inexpensive and do not involve any chemical treatment potentially harmful to human health. HHP treatment provides the possibility of making some products with higher nutritional value, more desirable sensory properties and novel texture [7]. There is vast interest in the use of HHP for physical modification of starches, since HHP-gelatinized starch has different properties compared to heat-gelatinized starch [8]. The structure of starch can be altered by treatment with HHP [9]. Reversible hydration of the amorphous phase is followed by irreversible distortion of the crystalline region; the secondary and tertiary structures are broken but the covalent bonds are unaffected [10]. The extent of gelatinization achieved by treatment with HHP is highly dependent on the type of starch, water content, pressure level, temperature range and length of treatment time [11]. Homogenously partially gelatinized starch with a branched star-like structure is one of the characteristics of HHP-treated starch [12], which could be used as transport and delivery vehicles for substances of interest in the cosmetic, food and pharmaceutical industries. In addition, the use of enzymatic modification of starch gives high yields of more specific hydrolysis products and allows better control of the process and the nature of the end products [13]. This process achieves desired functional properties, which extends their range of applications in food and non-food industries, including the manufacture of pharmaceutical, paper and textile products [14]. However, no study of the effect of α-amylase treatment on the structure and physicochemical properties of HHP-treated PS has been reported.

The objectives of this study were to evaluate the effect of treatment with HHP (200, 400 and 600 MPa) on the susceptibility of PS to α-amylase hydrolysis and to determine the morphology, thermal properties and crystal properties of native, HHP-treated and hydrolyzed HHP-treated PS. This knowledge will provide a better understanding of modified PS as a functional agent in industry.

Materials and Methods
Materials
A Chinese potato cultivar (Qingshu no. 9) frequently used for industrial starch production was selected for this study. Qingshu no. 9 was grown in North Eastern China. Bacterial α-amylase (A4551; activity of 570 units mg⁻¹) was purchased from Sigma Chemical Co., Ltd. (Saint Louis, MO, USA). All other chemicals used in this study were of analytical grade.

Starch isolation
Fresh potatoes were washed, cut into small pieces, immersed in 0.1% (w/v) sodium bisulfite (1 kg of tuber in 1 L of solution) for 10 min and then blended in a domestic juice extractor for 3–4
The resulting slurry was passed through a fine muslin cloth to separate the cell debris as described by Peshin [15]. The suspension was filtered three times using a 150 mm mesh size sieve and left to settle overnight at room temperature. The precipitated starch was spread onto oven trays lined with aluminum foil and dried in a hot air dryer (DGG-9240B, Senxin Instruments, Shanghai, China) at 45°C for 24 h. The dried starch was finely ground for 30 s to get powder (FW100 high-speed universal hand mill; TaiSiTe Instrument, Tianjin, China), packed-in tightly covered polypropylene containers and stored at 4°C. The native PS (NPS) was composed of 93.00% (w/w) starch [16] method 996.11, consisting of 19.40% (w/w) amylose and 81.60% (w/w) amylopectin [17], 0.10% (w/w) protein [16] method 955.04, 0.25% (w/w) total lipids [16] method 960.39, 0.03% (w/w) phosphorus [18], 0.33% (w/w) ash[16] method 923.03 and 3.30% (w/w) moisture.

### Treatment with HHP

Treatment with HHP was done in a discontinuous HHP machine (HPP.L3-600/0.6, HuaTai SenMiao Biotechnology Co., Ltd., Tianjin, China) at a pressure of 600 MPa in a steel vessel of 0.6 L capacity (60 mm × 210 mm). A mixture of starch (25%, w/w) and water in a polymer pouch was placed into the vessel containing water as the pressure medium. The temperature inside the vessel was controlled at 25°C by a circulating thermostatically controlled water bath. In each experiment, pressure was increased at a rate of ~100 MPa min⁻¹ and was kept at 200, 400 or 600 MPa for 40 min, which were optimized by single-factor experiment (data not shown). After each treatment, the pressure was decreased to atmospheric pressure at ~100 MPa min⁻¹, the starch was decanted, freeze-dried and stored in a desiccator. Control starch (NPS) was treated at atmospheric pressure (0.1 MPa) at room temperature (~ 25°C).

### Starch hydrolysis

The starch samples were hydrolyzed with bacterial α-amylase, in duplicate, essentially as described by Franco and Ciacco [19] but with some modification. The starches (25%, w/w) were dispersed in sodium phosphate buffer (0.2 M pH 6.0) with 3 mL of different concentrations (0.02%, 0.04% and 0.06%, w/v) of bacterial α-amylase. Sodium azide (1 mL of 10%, w/v) was added to prevent microbial growth. The starch dispersions were incubated at 20°C for 48 h in an orbital shaker, of which the hydrolysis time were optimized by single-factor experiment (data not shown). Samples of the supernatant were removed at the end of each hydrolysis period and the amount of reducing sugars was measured using 3,5-dinitrosalicylic acid [20]. The hydrolyzed starch slurry were inactivated by adding 0.1 M HCl to achieve pH 3, stirred for 15 min, neutralized by the addition of 0.1 M NaOH and centrifuged at 2000 g for 15 min. The hydrolysis residue was washed with water and ethanol. The hydrolyzed starch obtained was air-dried in a forced-air oven at 50°C for 24 h. The percentage (dry weight basis) of hydrolysis was calculated as follows [21]:

\[
\text{Hydrolysis} = 100 \times \left( \frac{W_o - W_1}{W_0} \right) \%
\]

where \(W_0\) is the weight (dry basis) of untreated starch and \(W_1\) is the weight (dry basis) of hydrolyzed starch.

### Scanning electron microscopy (SEM)

The starch granules were observed using a scanning electron microscope (Hitachi S-3400N, Tokyo, Japan). The starch samples were sprinkled onto double-sided tape mounted on an SEM stub, coated with gold and placed into the SEM chamber. Photomicrographs were taken using an SEM apparatus at an accelerating voltage of 15 kV.
Particle size

Particle size distribution of the starch granules was measured with a laser particle size analyzer (BT-9300H, Dandong Bettersize™ Instruments Ltd., Dandong, China). The mean granule size of each starch sample was calculated as the mean volume diameter ($D_{50}$).

Differential scanning calorimetry (DSC)

Thermal properties of the starches were analyzed using a differential scanning calorimeter (Q200 TA Instruments, New Castle, DE) essentially as described by Kaur et al. [22] but with slight modification. A sample of starch (2–5 mg) was weighed into an aluminum pan and the appropriate volume of water was added (final mix 1:3, w/w). The pan was sealed hermetically and equilibrated at room temperature for 2 h before the analysis. The DSC instrument was calibrated using indium and an empty pan as the reference. Samples were heated at 10°C min$^{-1}$ over a temperature range of 30°C–120°C. Peak temperature ($T_p$); onset gelatinization temperature ($T_o$); conclusion temperature ($T_c$) and enthalpy of gelatinization ($\Delta H_{gel}$) were recorded. The gelatinization temperature range ($R$) was calculated as $2(T_p - T_o)$ [23].

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the native and hydrolyzed HHP-treated starches were obtained with an IR spectrophotometer (Tensor 37, Bruker Opticals Company, Germany). Starch samples were blended with KBr powder and pressed into thin films before the measurement.

X-ray diffraction (XRD)

The XRD patterns of native and hydrolyzed HHP-treated starches were collected with X’pert PRO (PANalytical B.V., Holland) according to the modified method described by Chang et al. [24]. The diffractometer was operated at 40 mA and 40 kV. Signals of the reflection angle $2\theta$ from $5^\circ$–$40^\circ$ at a scanning rate of 0.21° s$^{-1}$ were recorded.

Swelling power and solubility

Swelling power and solubility of the starches were determined essentially as described Crosbie [25] but with slight modification. A sample (1.0 ± 0.001 g, dry basis) was weighed into a 50 mL centrifuge tube and distilled water (30 mL) was added from a rapid dispensing pipette. The screw cap was fastened onto the tube immediately and the contents mixed in a vortex mixer; any delay at this stage caused the starch to form lumps. The tube was placed into a constant temperature water bath at 92.5°C ± 0.5°C for 30 min with mixing. The resulting slurry was cooled to room temperature and centrifuged at 2000 g for 15 min. The supernatant was decanted into an evaporating dish and dried at 105°C for 5 h. The dried supernatant and the residue were weighed. The solubility ($S$, %) and swelling power ($SP$, g g$^{-1}$, dry basis) were calculated as follows:

\[
S = \frac{\text{Weight of dried supernatant}}{\text{Weight of dry starch}} \times 100\%
\]

\[
SP = \frac{\text{Weight of sediment}}{\text{Weight of dry starch}} \times (100 - S)\%
\]

Statistical analysis

All experiments were done in triplicate and statistical analysis was done with SAS version 9.2 (SAS Institute, Inc., Cary, NC) to determine difference of means using Duncan’s multiple range test functions. Differences were considered to be statistically significant at $p < 0.05$. All data were expressed as mean ± standard deviation.
Results and Discussion
Enzymatic hydrolysis

During hydrolysis with α-amylase, the α-(1→4) linkages of the interior of polymeric starch chains were cleaved and produced simple sugars as further hydrolysis products of maltose, maltotriose and α-dextrins. The varying level of HHP influenced the action of the enzyme at different concentrations on granular starch (Fig 1 and Table 1). The reducing sugar contents and percentage of hydrolysis of hydrolyzed native potato starch (hNPS) with 0.02% (w/v) α-amylase had the lowest values, 15.64 mg mL⁻¹ and 16.53%, respectively. Hydrolyzed starch with HHP600 by 0.06% (w/v) α-amylase yielded the greatest amount of reducing sugar and the highest percentage of hydrolysis, 29.64 mg mL⁻¹ and 49.36%, respectively. Using an increased enzyme concentration with hNPS and HHP hydrolyzed starches increased the yield of reducing sugar and the percentage hydrolysis. This observation was based on an increased amount of glucose released as a result of the cleavage of α-(1→4) glycosidic bonds. In addition, owing to the effect of high pressure on the starch granule, some hydrogen bonds in the amorphous

Table 1. Percentage hydrolysis of starches subjected to α-amylase hydrolysis with different enzyme concentration after HHP treatment.

| HHP (MPa) | Enzyme (%) | Hydrolysis(%)a) |
|-----------|------------|-----------------|
| 0.1b)     | 0.02%      | 16.53±0.10g     |
|           | 0.04%      | 17.86±0.20fg    |
|           | 0.06%      | 20.73±1.80 fde  |
| 200       | 0.02%      | 17.80±2.10fg    |
|           | 0.04%      | 21.10±2.82 de   |
|           | 0.06%      | 27.46±0.10 c    |
| 400       | 0.02%      | 18.30±0.05 fge  |
|           | 0.04%      | 22.00±2.00d     |
|           | 0.06%      | 28.02±1.98 c    |
| 600       | 0.02%      | 28.80±2.00 c    |
|           | 0.04%      | 36.90±2.21b     |
|           | 0.06%      | 49.36±2.55a     |

a) Means in a column with same letters are not significantly different at P≤0.05
b) 0.1 MPa is atmospheric pressure implying starch was only hydrolyzed by enzyme hence called hNPS.
area were broken and the amorphous growth ring regions were hydrated; therefore, amorphous lamellae were formed in the crystalline domain [26] and hydrolysis of starch granules was increased with increasing levels of pressure and enzyme concentration. Furthermore, high pressure can change the ordered and amorphous structure in starch granules; for example, at 600 MPa starch granules were partially gelatinized, allowing easier accessibility for the enzyme to act on the starch granule. The increase in reducing sugar content might be due to the increased effects of enzyme and HHP treatment.

We found a significant difference between hNPS and HHP-treated hydrolyzed starch at all three concentrations of enzyme (Table 1). The outer structure of PS is more ordered in the outer regions [27], which might be the reason, at least in part, why the hydrolyzed native potato starch (hNPS) without pre-HHP treatment showed greater resistance to enzymatic hydrolysis compared to HHP-treated starch.

Microstructure and granule size distribution

The shape and granule size of various modified starches are given in Table 2 and Fig 2, respectively. Starches that were treated with HHP showed an increase in granule size with increasing pressure. Indeed, starch treated at 600 MPa had the greatest average diameter ($D_{50}$) (29.43 ±0.1 μm) and diameter range (1.45–130.37 μm). These findings could be attributed to the partial gelatinization of starch granules. PS, which has a B-type pattern and a greater resistance to pressure compared to other starches [28], was not gelatinized completely by HHP in the range 200–600 MPa. In addition, $D_{50}$ of the starch granules decreased with increase in enzyme concentration.
concentration. This could be attributed to the degradation of starch granules after enzyme digestion. For better understanding and monitoring the extent of hydrolysis, SEM photographs of native, HHP-treated, native hydrolyzed and hydrolyzed HHP600 potato starch are shown in Fig 2. NPS had a typical elliptical and spherical shape with a smooth granule surface without pores, cracks or fissures, consistent with the report by Kaur et al. [29]. After hydrolysis, starch granules showed degradation of the external parts caused by enzymatic digestion (Fig 2C, 2E and 2G). The extent of degradation increased with increased enzyme concentration. Treatment with HHP partially gelatinized the starch granules making them more susceptible to enzymatic attack compared to native forms (Fig 2D, 2F and 2H).
Table 3. Gelatinization parameters of native, native hydrolyzed and hydrolyzed HHP treated (200, 400, and 600 MPa) potato starches with different α-amylase concentration a.

| HHP (MPa) | Enzyme (%) | T₀ (°C) | Tᵢ (°C) | Tₚ (°C) | Tₑ (°C) | ΔH₆gel (J/g) | R (°C) |
|-----------|------------|---------|---------|---------|---------|-------------|-------|
| Nativeb) | /          | 57.09±0.01a | 60.40±0.05gh | 62.00±0.06a | 10.75±0.02a | 6.62±0.01k |
| 0.1c)     | 0.02%      | 57.66±0.05a | 60.86±0.02j | 61.41±0.02b | 10.47±0.06ab | 6.40±0.02l |
|           | 0.04%      | 57.79±0.10b | 60.33±0.06h | 60.58±0.10e | 10.16±0.09c | 4.73±0.05m  |
|           | 0.06%      | 58.37±0.06b | 60.97±0.02e | 59.11±0.05i | 10.92±0.06d | 4.84±0.02m  |
| 200       | 0.0%       | 54.03±0.01cde | 57.95±0.01i | 62.18±0.02a | 10.24±0.05cb | 7.84±0.01j |
|           | 0.02%      | 54.38±0.01c | 60.73±0.01f | 61.09±0.02e | 10.23±0.05c | 12.70±0.01g |
|           | 0.04%      | 53.74±0.05ef | 60.78±0.03f | 57.87±0.03k | 9.68±0.02e  | 14.80±0.05d |
|           | 0.06%      | 53.81±0.10ef | 60.45±0.10g | 58.71±0.10j | 7.70±0.10g  | 13.28±0.10f  |
| 400       | 0.0%       | 54.26±0.02cd | 57.59±0.02m | 59.34±0.02h | 10.14±0.05cd | 6.66±0.02k  |
|           | 0.02%      | 54.23±0.02cd | 60.09±0.02i | 58.94±0.02i | 9.54±0.05e  | 11.72±0.02h |
|           | 0.04%      | 53.90±0.06h | 61.21±0.05d | 60.61±0.05d | 9.07±0.09f  | 14.62±0.02e |
|           | 0.06%      | 53.20±0.10hg | 61.85±0.09c | 59.96±0.08f | 6.18±0.05h  | 17.30±0.06b |
| 600       | 0.0%       | 53.39±0.01hg | 58.51±0.03k | 59.63±0.02g | 6.11±0.02h  | 10.24±0.01i |
|           | 0.02%      | 53.60±0.10efg | 61.83±0.03c | 60.62±0.02d | 5.43±0.09i  | 16.64±0.01c |
|           | 0.04%      | 53.51±0.20fhg | 62.15±0.09b | 60.59±0.10d | 5.19±0.06j  | 17.28±0.09b |
|           | 0.06%      | 52.42±0.20i  | 63.19±0.06a | 61.11±0.10c | 4.41±0.30k  | 21.54±0.05a |

T₀, onset transition temperature; Tᵢ, peak transition temperature, Tₑ, conclusion transition temperature, R, gelatinization range 2(Tₚ- T₀), ΔH₆gel, enthalpy of gelatinization.

a) Means in a column with same letters are not significantly different at P≤0.05
b) Native potato starch (NPS)
c) 0.1 MPa is atmospheric pressure implying starch was only hydrolyzed by enzyme hence called hNPS.

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DSC analysis

The gelatinization parameters for native, HHP-treated, native hydrolyzed and hydrolyzed HHP-treated PS are given in Table 3. The onset temperature (T₀) ranged between 52.42°C (for hydrolyzed HHP600 PS with 0.06% (w/v) α-amylase) and 58.37°C (for hNPS with 0.06% (w/v) α-amylase). The peak temperature (Tₚ) ranged between 57.59°C (for PS HHP-treated at 400 MPa) and 63.19°C (for hydrolyzed HHP600 PS with 0.06% (w/v) α-amylase). Gelatinization enthalpies (ΔH₆gel) ranged between 4.41 J g⁻¹ (for hydrolyzed HHP600 PS with 0.06% (w/v) α-amylase) to 10.92 J g⁻¹ (for hNPS with 0.06% (w/v) α-amylase). Compared with NPS, a reduction was found in gelatinization temperatures and enthalpy with increasing pressure treatment, which was accordance with the results of lotus seed starch treated with HHP [30]. Starch could be gelatinized by HHP treatment when dispersed in the excess water. It was reported that when starch dispersed in the excess water the HHP-induced starch gelatinization had two stages, at the first stage, the changing of starch granule morphology could not be observed, but once the pressure up to a specific level the starch granule would beyond the endpoint of the first stage and reach the second stage in which starch granules will be disintegrated [31]. For hNPS, the transition temperatures (T₀ and Tₚ) and the enthalpies of gelatinization (ΔH₆gel) shifted to higher values with increasing enzyme concentration. Two events occur during endothermic transition First, gelatinization, primarily water molecules diffuse freely into the amorphous region of starch. Second, they penetrate the crystalline region [32]. Enzymatic treatment of NPS could have had more effect on the amorphous region compared to the crystalline region, thus delaying gelatinization, which requires a higher temperature to initiate the gelatinization process, leading to a lower gelatinization temperature range. Conversely, hydrolyzed HHP-
treated starch displayed different behavior. These results could be attributed to increased high pressure and enzyme concentration since the degree of crystallinity decreased. Treatment with HHP disrupted the organization of the starch granules, leading to easy accessibility of the crystalline region of the granule to water resulting in a lower gelatinization temperature. In addition, increasing pressure caused partial gelatinization, so the crystalline structure and molecular order had been lost. Similar results have been reported for waxy maize (Zea mays var. ceratina), maize (Zea mays var. indentata), rice (Oryza sativa), potato (Solanum tuberosum) starch and wheat (Triticum aestivum) starch [11].

The difference in R-value between the samples suggested a transformation in the crystalline regions of the starch granules [33]. Gunaratne & Hoover [34] suggested variability of the R-value for the various starch types might be owing to the differences in the strength of heterogeneous crystals.

Overall, the decrease in transition temperatures and enthalpies of gelatinization of the hydrolyzed HHP-treated samples demonstrated a reduction in the amorphous area of the granules, which was accompanied by a change in the crystalline regions following a cooperative process in the case of HHP, but led to more crystalline structure in the hNPS starches.

**FTIR analysis**

FTIR spectra of native, native hydrolyzed and hydrolyzed HHP$_{600}$ potato starch are presented in Fig 3. Spectral changes can be divided into band narrowing and changes in the absorption intensity of specific bands [35]. Band narrowing was caused by ordering of the polymers and a reduction in the number of conformations; change of band intensity resulted from change of specific conformations, such as long-range ordering and crystallinity [36]. The broad bands from 3100–3700 (cm$^{-1}$) represent complex vibrational stretches associated with intermolecular hydroxyl groups [37]. The native, hydrolyzed native and hydrolyzed HHP$_{600}$ PS with 0.02% 

![FTIR spectrum of native (NPS), hydrolyzed native potato starch (hNPS) and 600 MPa treated potato starches (PS) with 0.02%, 0.04% and 0.06% (w/v) α-amylase. a: 0.02% (w/v) α-amylase; b: 0.04% (w/v) α-amylase; c: 0.06% (w/v) α-amylase.](https://example.com/figure3.png)

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and 0.04% (w/v) α-amylase showed the same broad bands from 3100–3700 cm$^{-1}$ (Fig 3A and 3B), while hydrolyzed native and hydrolyzed HHP$_{600}$ PS with 0.06% (w/v) α-amylase showed a greater intensity (Fig 3C). The sharp bands at 2920–2928 cm$^{-1}$ were related to the CH$_2$ vibration [38], as shown in Fig 3C for the native hydrolyzed and hydrolyzed HHP-treated potato starch with 0.06% (w/v) α-amylase. This could be attributed to changes in conformation and crystallinity. The peak at ~1640 cm$^{-1}$ was a sign of firmly bound water present in the native, enzyme-treated and hydrolyzed HHP-treated starch under different conditions. Compared to native starch, peak height was increased but there was no significant change of peak width after hydrolysis and treatment with HHP. The absorption band at ~1458 cm$^{-1}$ was attributed to compactly bound water present in starch [39], of which significant changes were observed for all three treatment conditions compared to NPS. The intensity at 920–980 cm$^{-1}$ was increased but there was no significant change of intensity after hydrolysis and treatment with HHP. The band at ~850 cm$^{-1}$ was sensitive to changes of crystallinity [35]. Compared to NPS, the native hydrolyzed and hydrolyzed HHP-treated potato starches with 0.02% and 0.04% (w/v) α-amylase showed no significant change of peak position or intensity but exhibited significant changes in the fingerprint area. Peaks became sharper with increased enzyme concentration and some bands were absent or not pronounced in the NPS spectrum, suggesting significant changes in both the ordered and the amorphous structures of PS.

**XRD analysis**

The XRD pattern of NPS, native hydrolyzed and hydrolyzed HHP$_{600}$ PS are presented in Fig 4. They show a typical B-type X-ray diffraction pattern with strong peaks at 2θ of 5.7°, 17.1° and 22.5° [40], HHP-treated potato starch with 0.6% α-amylase was an exception, with an extra weak peak at 2θ = 19.28° characteristic of a V-type crystallite. The peaks of native hydrolyzed starches became sharper with increased enzyme concentration, indicating the amorphous parts of the starch granules had been disrupted [41]. The hydrolyzed HHP$_{600}$ starches, however, displayed different behavior. The intensity of the peaks was changed slightly owing to the loss of crystallinity during treatment with HHP [42]. The small peak at 5.6° disappeared, while the peaks at ~19° and ~15° became larger (Fig 4B and 4C). An additional peak at 2θ = 19.36° indicated the crystal of HHP$_{600}$ potato starch with 0.06% (w/v) α-amylase was in the transformation from B type to B+V type with a majority of B-type crystallite. It might be owing to an increased proportion of short amylose chains, which tend to form double helices [43]. In addition, α-amylase can penetrate the starch granule more efficiently and change the organized structure of granules pretreated with HHP compared to hydrolyzed NPS. These results indicated amylolysis occurs in both the amorphous and the crystalline regions of starch granules.

**Swelling power and solubility**

The swelling power and solubility of NPS, hydrolyzed NPS, HHP-treated and hydrolyzed HHP-treated PS are presented in Fig 5. A decrease of swelling power was observed for HHP-treated and hydrolyzed HHP-treated PS with different concentrations of enzyme (Fig 5A). Compared to NPS, the swelling power of HHP-treated PS was decreased from 16.93 to 15.35 g/g. After hydrolyzed by α-amylase, the swelling power of hydrolyzed NPS and hydrolyzed HHP-treated PS decreased significantly, among which the hydrolyzed HHP-treated PS with α-amylase at a concentration 0.06% (w/v) showed lowest swelling power, 8.47 g/g (P< 0.05). The results above might be due to that HHP and enzymatic hydrolysis could alter both the crystalline and the amorphous structure of starch granules. These results were in accord with the report by Tester et al. [44] focused on the effect of hydrolysis on the swelling behavior.
of starch granules. The solubility of native and hydrolyzed HHP-treated (200 and 400 MPa) samples increased with increasing α-amylase concentration. It is worth mentioning PS is more pressure-resistant compared to other starches [45] and pressures of less than 600 MPa did not have a significant effect on starch granules; hence, potato starch granules needed > 600 MPa pressure for complete gelatinization [46]. The action of α-amylase led to the conversion of amylose into low molecular mass components such as glucose and dextrin. And the characteristics of amylose and amylopectin were also changed, including the molecular mass, degree of branching, chain length and conformation. This is in accord with the study of hydrolysis and heat-treated starches at sub-gelatinization reported by Uthumporn et al. [41]. By contrast, the HHP_{600} sample hydrolyzed with different concentrations of enzyme showed decreased solubility compared to hydrolyzed HHP-treated (200 and 400 MPa) starches at different enzyme concentrations. It is likely high pressure induced formation of the amylose–lipid complex and inhibited amylose leaching, which led to decreased solubility [47].

**Conclusion**

This study demonstrated the effect of HHP and enzymatic hydrolysis with α-amylase at different levels of pressure and concentration of enzyme on various physicochemical properties of
potato starch. Pre-HHP treatment of starches at 600 MPa increased susceptibility to enzymatic hydrolysis with an increased amount of reducing sugar produced. Further, there was significant change of the $D_{50}$ value, thermal properties and crystalline structure pattern. Swelling power and solubility decreased with increasing level of HHP, owing, at least in part, to change in the intramolecular structure of starch. The hydrolyzed HHP-treated starch, however, showed decreased swelling power but increased solubility with increasing HHP level and enzyme concentration. By contrast, hydrolyzed starches HHP-treated at 200 and 400 MPa showed little or no difference in structural, morphological or functional properties compared to the native form, which showed that potato starch with B-type pattern are resistant to high pressure, especially below 600 MPa. The information obtained from this study suggests the possibility of modifying the physicochemical, functional and morphological properties of PS without heat treatment, obviating any undesirable side effects. This modification process could be practical in the food and related industries.

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Author Contributions

Conceived and designed the experiments: TM MZ LR HS. Performed the experiments: LR. Analyzed the data: TM MZ LR CW. Contributed reagents/materials/analysis tools: TM MZ LR CW. Wrote the paper: TM MZ LR HS.

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