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### Regulatory effect of porcine plasma protein hydrolysates on pasting and gelatinization action of corn starch

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#### ABSTRACT

The objective of this study was to investigate the regulatory effect of porcine plasma protein hydrolysates (PPPH) on the physicochemical, pasting, and gelatinization properties of corn starch (CS). The results showed that the solubility of CS markedly increased, whereas swelling power and gel penetration force decreased with increased PPPH concentration (P < 0.05). Compared with native CS, PPPH significantly lowered peak viscosity, minimum viscosity, final viscosity, and total setback, whereas it increased breakdown and pasting temperature in rapid visco analyzer (RVA) measurement (P < 0.05) and obviously enhanced the gelatinization temperature as determined in differential scanning calorimetry (DSC) (P < 0.05). Confocal laser scanning microscopy (CLSM) showed that PPPH surrounded the starch granules at room temperature ( $25 \,^\circ$ C) and then formed a network with swollen starch granules during gelatinization. Atomic force microscopy (AFM) images indicated that the blocklet sizes of gelatinized CS-PPPH mixtures were smaller and more uniform than native CS. The results proved that pasting and gelatinization abilities of CS can be effectively influenced by adding PPPH.

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#### 1. Introduction

Starch is an economical, abundant, biodegradable, and low-cost renewable carbohydrate polymer with unique physical and chemical characteristics. Native starches contribute greatly to the texture properties of many foods and are widely used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, and water retention agent [1]. Corn is a major crop, and corn starch (CS) is one of the most valuable ingredients of corn, accounting for 80% of total starch production [2]. Generally, the application of native CS has had some shortcomings, such as lack of water solubility, low viscosity, low heating stability, and high tendency toward syneresis and retrogradation [3], leading to damage to texture and taste in boiled and baked foods. However, the conjugation of non-starchy substances with starch is an important method to improve starch functionality. Some substances, such as proteins [4], polysaccharides [5], salts [6], and amino acids [7–9], could markedly influence the pasting and gelatinization profiles of starch by altering the viscosity, pasting temperature, swelling

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http://dx.doi.org/10.1016/j.ijbiomac.2015.10.026 0141-8130/© 2015 Published by Elsevier B.V. power, solubility, and syneresis. Therefore, it is important and essential to find an appropriate substance to coexist with CS to regulate the pasting and gelatinization behavior of CS to obtain the required physical properties.

In recent years, enzymatic hydrolysis modifications of protein have been proposed for its special nutritional and biological properties. Porcine blood is a valuable protein source from which bioactive peptides can be produced. In our previous work, we mainly focused on the antioxidant properties of porcine plasma protein hydrolysates (PPPH) [10]. Enzymatic hydrolysis also reduced the molecular size of the porcine plasma protein, and exposed ionizable amino and carboxyl groups, which increases the hydrophilicity of PPPH [11]. Moreover, Ribotta and Rosell [12] showed that the soy protein hydrolysates obviously influenced the rheological and pasting parameters of corn starch and cassava starch. Goel et al. [1] also found that incorporation of casein hydrolysates into corn starch pastes could obviously modify their viscosity and rheological profiles. It is well known that protein hydrolysates or peptides are typical charged materials, leading to some changes in the thermal stability of starch granules through electrostatic interaction [13].

Despite the studies on the effects of amino acids on the pasting behavior of starch by some researchers, there is little information about the impact of the protein hydrolysates on the pasting behavior and gelatinization of starch, and the mechanisms between starch and protein hydrolysate interactions also remain unknown.

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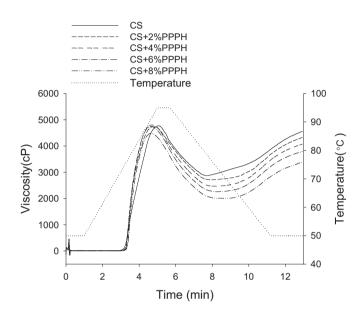
#### -Table 1

Effect of different concentrations of porcine plasma protein hydrolysates (PPPH) on the solubility and swelling power of corn starch (CS) at different temperatures.

	Solubility (%)					Swelling power (g/g)		
	60 ° C	70 °C	80°C	90°C	60 ° C	70°C	80°C	90 ° C
CS	$2.27\pm0.45^{\text{Db}}$	$2.53\pm0.29^{\text{Db}}$	$3.93\pm0.45^{\text{Ea}}$	$4.63\pm0.32^{\text{Ea}}$	$13.05\pm0.27^{\text{Ad}}$	$16.73 \pm 0.38^{Ac}$	$19.39 \pm 0.55^{Ab}$	$22.51 \pm 0.63^{Aa}$
CS + 2% PPPH <sup>x</sup>	$3.53 \pm 0.31^{Cc}$	$3.93 \pm 0.21^{Cc}$	$6.27\pm0.32^{\text{Db}}$	$7.43\pm0.25^{\text{Da}}$	$11.37 \pm 0.17^{Bd}$	$14.89 \pm 0.25^{Bc}$	$17.46 \pm 0.42^{\text{Bb}}$	$20.48 \pm 0.38^{Aa}$
CS+4% PPPH	$4.63\pm0.21^{Bc}$	$5.30 \pm 0.46^{Bc}$	$7.93 \pm 0.38^{\text{Cb}}$	$10.80 \pm 0.40^{Ca}$	$9.85 \pm 0.20^{Cd}$	$12.42 \pm 0.36^{Cc}$	$14.52 \pm 0.38^{\text{Cb}}$	$17.29 \pm 0.50^{Ba}$
CS+6% PPPH	$5.10 \pm 0.20^{Bc}$	$5.67 \pm 0.31^{Bc}$	$9.77 \pm 0.67^{Bb}$	$12.63 \pm 0.61^{Ba}$	$8.89\pm0.34^{\text{Dd}}$	$10.35 \pm 0.40^{Dc}$	$12.05 \pm 0.49^{\text{Db}}$	$14.72 \pm 0.41^{Ca}$
CS + 8% PPPH	$6.67\pm0.15^{\text{Ab}}$	$6.97\pm0.15^{Ab}$	$13.67\pm0.42^{\text{Aa}}$	$14.27\pm0.47^{\text{Aa}}$	$7.03\pm0.20^{\text{Ed}}$	$9.12\pm0.28^{\text{Ec}}$	$10.06\pm0.41^{\text{Eb}}$	$11.45 \pm 0.36^{\text{Da}}$

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Values are given as the means  $\pm$  SD from triplicate determinations; <sup>A-E</sup> means in the same column with different letters differ significantly (*P* < 0.05); <sup>a-d</sup> means in the same row for the same index with different letters differ significantly (*P* < 0.05); <sup>x</sup> means CS mixed with 2, 4, 6, and 8% PPPH at dry starch weight basis.



**Fig. 1.** Effect of different concentrations of porcine plasma protein hydrolysates (PPPH) on pasting time and viscosity behavior of corn starch (CS) suspension. The temperature profile is represented by the dotted line. CS mixed with 2, 4, 6, and 8% PPPH at dry starch weight basis.

In addition, many protein hydrolysates have been used in foods to improve their nutritional values and texture characteristics [14]. Hence, the objective of this study was to investigate the regulatory effect of PPPH on the physicochemical, pasting and gelatinization properties of CS. Moreover, the possible action mechanism between the polypeptide and CS was also proposed with the aid of confocal laser scanning microscopy (CLSM) and atomic force microscope (AFM).

#### 2. Materials and methods

#### 2.1. Samples and materials

CS was purchased from a local market (Da-Cheng Co., Ltd, Changchun, Jilin, China). Porcine plasma protein was obtained from Beidahuang Meat Corporation (Harbin, Heilongjiang, China), and the dry porcine plasma protein powder contained 85% protein and 13% moisture. Alcalase 2.4L ( $6 \times 10^4$  U/g) was obtained from Novozymes (Bagsvaerd, Denmark). All other chemicals and reagents used were analytical grade.

#### 2.2. Preparation of PPPH

Preparation of PPPH was performed using the method of Liu et al. [10]. Porcine plasma protein solution (40 mg protein/mL) was heated in a water bath for 5 min at 95 °C and then hydrolyzed with alcalase at 55 °C for 5 h. The enzyme to substrate ratio (E/S) was

2:100 (g/g). The pH of the porcine plasma protein solution was adjusted to the optimal values for Alcalase (pH 8.0) before hydrolysis was initiated, and it was readjusted to the optimal value every 15 min during hydrolysis with 1 M NaOH. After hydrolysis, the pH of the solution was brought to 7.0, and the solution was then heated to 95 °C for 5 min to inactivate the enzyme. The degree of hydrolysis (DH) of PPPH was 17.6%. The hydrolysates were desalted and then freeze-dried (LGJ-1 Freeze-Dryer, Shanghai, China), and the lyophilized hydrolysates were stored at 4 °C until use.

#### 2.3. Solubility and swelling power

Solubility and swelling power were determined according to the method of Steeneken and Woortman [15] with slight modification. Native CS (0.5 g, dry basis) was suspended in centrifuge tubes with 25 mL of distilled water, and then 2, 4, 6, and 8% PPPH at dry starch weight basis was added to the starch slurry and subsequently heated at 60, 70, 80, and 90 °C, respectively, for 30 min with manual stirring. Then, centrifuge tubes were immediately placed in cold water and left to cool at room temperature (20 °C). After that, the suspension was centrifuged at 4000 × g for 15 min at 20 °C, and then the supernatant was poured into to culture dishes and dried at 105 °C for 12 h to constant weight (Ms). Sediment paste was accurately weighed (Mp) and dried at 105 °C for 12 h to constant weight (Md). Solubility and swelling power were calculated using the following equation:

Solubility (%) = 
$$\frac{Ms}{0.5} \times 100$$

Swelling Power(g/g) =  $\frac{Mp}{Md}$ 

#### 2.4. Pasting properties

The pasting properties of CS and PPPH mixtures were evaluated using the rapid visco analyzer (RVA-4, Newport Scientific, Warriewood, Australia). Native CS (3.5 g, dry basis) was suspended in aluminum RVA sample canisters with 25 mL of distilled water, and 2, 4, 6, and 8% PPPH at dry starch weight basis was then added to the starch slurry. A programmed heating and cooling cycle was used in which the samples were held at 50 °C for 1 min and then heated to 95 °C at 12.2 °C/min and held for 2.5 min at 95 °C. It was then cooled to 50 °C (cooling rate of 11.8 °C/min) and maintained for 2 min. The parameters of the RVA measurement included peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), and pasting temperature (PT). Total setback (TSB) and breakdown (BD) were calculated using the formulas TSB = FV – MV and BD = PV – MV. All samples were analyzed in triplicate.

#### 2.5. Differential scanning calorimetry (DSC)

Thermal analysis experiments were performed on a PE Pyris 6-DSC thermal analyzer (Perkin-Elmer Optoelectronics, Fremont, CA,

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