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Short communication

Pasting investigation, SEM observation and the possible interaction study on rice starch–pullulan combination

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A B S T R A C T

The pasting properties of rice starch (RS) with high concentration (10%, w/w) were investigated in the presence or absence of pullulan (PUL) using a rapid visco-analyzer (RVA). Addition of pullulan resulted in the reduction of peak viscosity, trough viscosity, final viscosity, and setback value of RS. Furthermore, an interesting phenomenon, i.e. a small viscosity peak appeared in the RVA curves of RS–PUL mixtures, was observed. It indicated that addition of pullulan might suppress the gelatinization of starch granules by maintaining the integration of some granules. The scanning electron microscope (SEM) observation of samples suggested that starch granules could be wrapped by a thin membrane composed of pullulan and/or pullulan-amylose associations. The coating ability of pullulan and/or the possible molecular interactions between pullulan and amylose could be responsible for these results.

1. Introduction

Starch-hydrocolloid combinations have been extensively used in food industry [1] with the hope of modifying various characteristics of starch, such as pasting and rheological properties [2,3], retrogradation behaviors [4–6], and texture [7]. Taking the pasting properties for example, the addition of hydrocolloids in starch can modify the paste viscosity and overcome the shortcomings of native starch. A common result of starch-hydrocolloid combinations is an increase in paste viscosity [1], which indicated that hydrocolloids might be used as thickening agent and stabilizing agent in starch-based food. Meanwhile, some hydrocolloids, such as gum arabic and soybean-soluble polysaccharide, were reported to decrease paste viscosity [8]. Therefore, it is possible for us to regulate the pasting properties of starch-based food varying with products features and customer requirements.

Pullulan is an exocellular homo-polysaccharide produced by *Aureobasidium pullulans* [9] and possesses a linear structure of α-$(1 \rightarrow 6)$-linked maltotriose repeating units. Pullulan is widely used in food industry because of its unique characteristics, especially the perfect film forming ability [10].

As a polysaccharide hydrocolloid, pullulan might stand a good chance of modifying the properties of starch. Based on our previous study [2], pullulan was found to have the ability of retarding the swelling and gelatinization of rice starch (RS), and the possible interactions between starch and pullulan were hypothesized to be responsible for this action. However, the mechanism of this action is still unclear, and additional evidence for the hypothesis is demanded. Thus, a follow-up investigation was performed in the current study with the purpose of giving an interpretation about the effect of pullulan on the properties of RS.

2. Materials and methods

2.1. Materials

Rice starch with 23.30% amylose, and 11.82% moisture was obtained from Jiangsu Baby Corporation (Suqian, China). Pullulan was purchased from Hayashibara Biochemical Inc. (Shanghai, China).

2.2. Pasting properties

The rice starch (10%, w/v)-pullulan (0.1%, 0.3%, and 0.5%, w/v) mixtures (RS–PUL) were prepared as follows. The calculated amounts of pullulan powder were primarily dissolved thoroughly in distilled water with continuous stirring for 1 h, and then RS was

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Table 1
Pasting properties of rice starch with and without the addition of pullulan.[a]

PUL conc. (%)	Viscosity (cP)				
	Peak	Trough	Breakdown	Final	Setback
0.00	3481.33 ± 20.13a	2695.67 ± 11.59a	785.67 ± 9.29b	3892.67 ± 28.10a	1197.00 ± 16.52a
0.10	3465.67 ± 27.10a	2575.67 ± 22.12b	890.00 ± 5.00a	3649.00 ± 20.66b	1073.33 ± 7.77b
0.30	3064.00 ± 32.79b	2444.00 ± 22.72c	620.00 ± 12.29c	3428.00 ± 25.53c	984.00 ± 5.29c
0.50	2834.00 ± 33.05c	2300.67 ± 19.01d	533.33 ± 14.19d	3127.33 ± 21.39d	826.67 ± 5.03d

Different letters in the same column indicated significant differences ($p < 0.05$) by Duncan's test.
[a] Tests were performed in triplicate and the data were shown in mean ± standard deviation.

slurried in pullulan solutions at room temperature by magnetic stirring for 30 min. The prepared slurry (28 g) was transferred into an aluminum canister and stirred using a plastic paddle. Pasting properties of RS and RS–PUL mixtures were measured by a rapid visco-analyzer (RVA-4500, Newport Scientific Pty. Ltd., Australia) using standard 1 method. Briefly, the prepared slurry (28 g) was equilibrated at 50 °C for 60 s, heated to 95 °C within 260 s, and then held at 95 °C for 110 s. The hot paste was subsequently cooled to 50 °C within 260 s, and maintained at 50 °C for 90 s. Paddle speed was 960 rpm for the beginning 10 s to disperse the sample, and then the speed of paddle was set at 160 rpm during the measurement. Pure pullulan solution at a concentration of 0.50% was run under the same RVA procedure. Data on each RVA parameter were presented as means ± SD of triplicate.

2.3. Scanning electron microscope (SEM)

SEM was used to examine the changes on micromorphology during gelatinization with the addition of pullulan. The RS–PUL (with 0.00% and 0.50% PUL) paste obtained from RVA was subjected to cool, freeze, and freeze dehydration. Consequently, dried samples were stuck on a specimen holder and coated with gold palladium using a sputter coater. The microstructure of samples was observed at 1000× and 5000× resolution, respectively, with a scanning electron microscope (S-4800, Hitachi Science Systems, Ltd., Japan) operating at an accelerating voltage of 20 kV.

2.4. Statistical analysis

Statistical significance was assessed by one-way analysis of variance (ANOVA) using SPSS 20.0 (SPSS Inc., Chicago, USA) for windows program. The level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Pasting properties

Effect of pullulan on the viscosity parameters of RS was summarized in Table 1. The peak viscosity of RS decreased from 3481.33 ± 20.13 cP to 2834.00 ± 33.05 cP when the concentration of pullulan increased from 0.00% to 0.50%. Similarly, trough viscosity, final viscosity, and setback value of RS were all declined with the addition of pullulan, and the higher the concentration of pullulan added, the higher the decrements of these parameters.

The reduction of peak viscosity might be ascribed to the retarding effect of pullulan on the swelling and subsequent gelatinization of RS [2]. Pullulan might adsorb onto the surface of starch granules and coat around these granules during heating. However, the interaction between pullulan and amylose leached from the granules could not be excluded to interpret the lower viscosity, because pullulan possessed a starch-like structure of linkage α-D-glucan primarily consisting of maltotriose repeating units interconnected by α-(1 → 6) linkages [9]. The decrease in peak viscosity with the addition of pullulan was in agreement with the results of Funami et al. [8] who evaluated the effect of soybean-soluble polysaccharide and gum arabic on the gelatinization and retrogradation behaviors of wheat starch.

Breakdown value representing the differences between peak viscosity and trough viscosity could reflect the stability of hot paste. Obviously, pullulan could hamper the fragmentation of starch granules during heating for that the breakdown viscosity decreasing from 785.67 ± 9.29 to 533.33 ± 14.19 with the pullulan addition except in the level of 0.10%.

Setback value represented the differences between trough viscosity and final viscosity, and it is mainly resulted from the rearrangement of starch molecules (especially amylose) in the system during cooling. As shown in Table 1, setback value of RS markedly decreased ($p < 0.05$) with increasing of pullulan concentration. For instance, the setback viscosity of RS was reduced by 30.94% when 0.50% pullulan was introduced in the system.

Apart from these results discussed above, an interesting phenomenon was found (Fig. 1) in the RVA pasting curves. A small viscosity peak was observed when RS was pasted in pullulan solution. This phenomenon was not observed in either the RS alone system or the 5% RS–PUL mixtures [2]. Furthermore, this peak became more visible and appeared at a later time with the increasing of pullulan concentration. This indicated that there must be some interactions between starch granules and pullulan, and additional discussion for the phenomenon was given in Section 3.3.

3.2. Morphological structures of RS pastes with and without pullulan

The microstructures of RS pasted in the presence or absence of pullulan were displayed in Fig. 2. In the sample of RS alone (Fig. 2a_1 and a_2), only irregular laminate structures were observed, indicating the sufficient gelatinization and thorough disintegration of starch granules. However, in the RS–PUL mixture (Fig. 2b_1 and b_2), the presence of intact and swollen starch granules were observed. Additionally, it was possible to observe the membranous structure in the RS–PUL mixture, and some of them obviously adhered or even captured the granules, as shown at the arrow in Fig. 2b_1 and b_2. The SEM images circumstantiated that pullulan indeed wrapped up the surface of starch granules and consequently inhibited the swelling and the subsequent gelatinization of RS.

3.3. The possible mechanism behind the phenomenon

Based on these results from pasting curves and the SEM observations, a schematic diagram (Fig. 3) was illustrated with the purpose of explaining the possible interactions among starch granules, starch molecules, and pullulan. The swelling and gelatinization of RS in pullulan solution might be divided into five phases, as shown in Fig. 3.

In phase I, starch granules dispersed and swelled slightly in pullulan solution, this swelling was reversible on drying. In phase II, starch granules were heated in pullulan solution, and large mounts of water migrated into granules upon heating, resulting in the