Effects of Enzyme Treatments and Drying Methods on Gelatinization and Retrogradation of Instant Rice Porridge

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Instant rice porridge produced through cooking, drying and a series of other processes possesses inferior sensory quality which is related to the gelatinization and retrogradation of rice starch. The effects of different enzyme treatments including α-amylase, β-amylase and neutral protease and different drying methods including hot air drying, freeze drying and freeze-hot air drying on gelatinization and retrogradation of instant rice porridge were investigated in this work utilizing rapid viscosity analyzer (RVA), scanning electron microscopy (SEM) and X-ray diffraction (XRD). As for enzyme treatments, rice porridge treated by α-amylase demonstrated the lowest viscosity value and the greatest porosity which suggested that α-amylase exceeded other two enzymes in maintaining gelatinization, inhibiting retrogradation and facilitating rehydration of instant rice porridge. For different drying methods, it was found that freeze drying was most advantageous to maintain gelatinization with the lowest viscosity and the greatest porosity. This study is especially helpful to gain initial insight into the development of instant rice porridge processing technology.

Keywords: enzyme treatments, drying methods, instant rice porridge, gelatinization, retrogradation

Introduction

With the accelerating pace of modern life, people's demand for convenience foods has become more and more pressing. Instant rice porridge is a kind of convenience foods which is made of rice through cooking, drying and a series of other processes. At present, a few instant rice porridge products are available on the market, but one major problem still exists, namely, the inferior sensory quality compared with traditional freshly cooked rice porridge. The inferior sensory quality of instant rice porridge includes long rehydration time and hard texture which affects consumer acceptability and blocks the promotion of instant rice porridge.

The sensory quality of instant rice porridge is related to the gelatinization and retrogradation of rice starch, which is a major component of rice, accounting for more than 70% of the total weight. Gelatinized rice starch tends to retrograde during the drying and cooling process (Miles et al., 1985), thus affects the rehydration of instant rice porridge.

Literature can be reviewed concerning the effect of enzyme treatments and drying methods on the gelatinization, retrogradation and rehydration of starch. Sarikaya et al. (2000) examined the degradation abilities of α-amylase and β-amylases on raw starch granules from various botanical sources (potato, sweet potato, wheat, rice and corn), and suggested that the rice granules were the best substrate for enzymic hydrolysis by α and β-amylases and the α-amylase was more efficient than the β-amylases. Besides, Yao and Ding (2000) treated rice with β-amylase and neutral protease and slowed the speed of retrogradation of cooked rice. Afterwards, Gujral (2004) reported that chapatis prepared from rice flour showed lower retrogradation and the undesirable textural changes during storage can be delayed by the incorporation of α-amylase. More recent studies by Li et al. (2010) indicated that after α-amylase hydrolysis, a cavum was formed in the center of the starch granules extracted from Chinese yam, which may allow easy rehydration. However, the application of enzyme treatments in instant rice porridge preparing to inhibit rice starch retrogradation has not been extensively studied. Hence, in this experiment, α-amylase, β-amylases and neutral protease were used with their effects on gelatinization and retrogradation compared.

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Malumba et al. (2008) demonstrated that high-drying temperatures applied during the corn drying process reduced their swelling capacities, their water binding capacities and their water solubility index after gelatinization. Moreover, Prasert and Suwannaporn (2009) suggested that higher drying temperatures caused increases in hardness and chewiness of instant rice. In contrast, Rewthong et al. (2010) suggested that the texture of freeze-dried instant rice was not different from that of the air-dried one after rehydration. Besides drying, other processing conditions also affect the gelatinization and retrogradation of rice starch. Since the processes in preparing instant rice and instant rice porridge are quite different, the effects of drying methods on the rehydration of instant rice porridge may be different from that of instant rice. Drying process varies from single step drying to multi-step drying, from low temperature to high temperature. Nevertheless, researches on effects of different drying methods on the rehydration of instant rice porridge are quite few. Therefore, hot air drying, freeze drying and freeze-hot air drying were investigated in this experiment, and their effects on the gelatinization and retrogradation of instant rice porridge were compared.

Currently, many advanced technologies have been applied to study the gelatinization and retrogradation properties of starch. Rapid viscosity analyzer (RVA) was used to figure out the starch gelatinization curve (Torleya et al., 2004; Furnami et al., 2005; Satrapai and Suphantharika, 2007). X-ray diffraction (XRD) techniques were widely used to determine the degree of crystallinity of starch granules (Derycke et al., 2005; Zhou et al., 2008). Scanning electron microscopy (SEM) was used for the observation of starch granules during gelatinization and retrogradation (Ratnayake and Jackson, 2007; Prasert and Suwannaporn, 2009). However, since retrogradation is a complex process affected by many factors, it is unlikely that any single method would be able to give a complete picture of the retrogradation properties of starch gels at both the macroscopic and molecular levels (Karim et al., 2000). Hence, the objective of this work is to study the effect of different enzyme treatments and drying methods on gelatinization and retrogradation properties of instant rice porridge utilizing rapid viscosity analyzer (RVA), scanning electron microscopy (SEM) and X-ray diffraction (XRD). This study is especially helpful to control gelatinization state of starch and to gain initial insight into the development of the processing technology of instant rice porridge.

Materials and Methods

Materials The rice selected here was late rice produced by Arowana Corporation. The process of instant rice porridge was as follows:

Raw rice → Roasting → Cooking in boiling water → Heating with slow fire → Immersing → Steaming → Dispersing in cold water → Draining and Dispersing → Drying → Instant rice porridge

Specific parameters were as follows: rice was roasted with slow fire for 15 min; and cooked for 2 min in boiling water in a ratio of 1:4 (w/v); then heated with slow fire for 10 min; then soaked into water (1:4, w/v) at 50°C for 15 min. The rice was drained, and steamed at normal pressure for 15 min; then washed with cold water quickly. After draining, the rice was sprayed with dispersion agent (composed of 10% ethanol, 5% sucrose fatty acid ester and 85% distilled water) by 3 g/100g rice; then dried at 80°C for 150 min.

Based on the above processing parameters, to compare effects of α-amylase, β-amylase and neutral protease, the three enzymes were dissolved into the immersing water respectively. In order to investigate the effects of different drying methods, the rice was also dried by hot air drying, freeze drying and freeze-hot air drying respectively with other processing parameters unchanged.

α-amylase treatments α-amylase (E.C.3.2.1.1, ≥ 3700 U/g), was purchased from the Solarbio Corporation (Beijing, China). In the experiment, after draining, gelatinized rice was soaked into α-amylase solution at 50°C for 30 min, and α-amylase was added by 100 U/100 g rice.

β-amylase treatments β-amylase (E.C.3.2.1.2, 100000 U/g), was purchased from the Solarbio Corporation (Beijing, China). In the experiment, after draining, gelatinized rice was soaked into β-amylase solution at 50°C for 60 min, and β-amylase was added by 15000 U/100 g rice.

Neutral protease treatments Neutral protease (E.C.3.4.23.6, ≥ 60000 U/g), was purchased from the Solarbio Corporation (Beijing, China). In the experiment, after draining, gelatinized rice was soaked into neutral protease solution at 50°C for 60 min, and neutral protease was added by 10000 U/100 g rice.

Hot air drying Gelatinized rice was placed evenly on a metal screen sieve with a thickness of 5 mm, and then dried with 80°C hot air for 150 min.

Freeze drying Gelatinized rice was placed evenly on a metal screen sieve with a thickness of 5 mm, later freeze-dried quickly at −20°C, and then dried at −50°C, 90 Pa vacuum for 12 h.

Freeze-hot air drying Gelatinized rice was frozen at −20°C, then dried for 5 min at 160°C. The above two steps were conducted alternately 3 times, then the sample was dried at 80°C for 2 h.

Sample preparation The rice porridge samples obtained by different enzyme treatments and drying methods and raw rice were frozen in liquid nitrogen and dried with freeze
Results and Discussion

Enzyme treatments During the water losing and cooling process, the re-crystallization of amylase and the exterior short chain of amylpectin led to the retrogradation of rice starch (Takahiro et al., 2003; Yasunori et al., 2002). Therefore, to modify the molecular structure of rice starch with enzyme treatments was of importance to inhibit rice starch retrogradation.

Rapid visco-analysis The pasting properties of instant rice porridges were analyzed using a rapid viscosity analyzer (RVA) (Model Super-3, Newport Scientific Pvt Ltd., Australia) according to Karim et al. (2007) and Bao (2008) with some modifications. Instant rice porridge samples and raw rice samples were pasted according to an inherent thermal program in the apparatus, namely ‘Standard 1’. The agitation speed was fixed at 900 rpm for the first 10 s to ensure the uniformity of the sample, and then at 160 rpm throughout the rest of measurement. The samples were (1) held at 50°C for 1 min (2) heated from 50°C to 95°C at a constant rate of 12°C/min (3) held at 95°C for 2.5 min (4) cooled to 50°C at a constant rate of 12°C/min, then (5) held at 50°C for 2 min. The total time was 13 min. RVA characteristics, including peak viscosity (maximum viscosity during heating to and holding at 95°C), trough viscosity (minimum viscosity that occurs after the peak viscosity) and final viscosity (viscosity at the end of the test profile) were determined from RVA curves.

X-ray diffraction analysis X-ray diffraction patterns were obtained using a diffractometer (D8 Focus, Bruker AXS GmbH, Germany) according to Kim et al. (1997). Instant rice porridge samples and raw rice samples were spread evenly to obtain a smooth surface and then placed in sample holder for testing. The diffractometer was operated under following condition: 40 kV and 40 mA with Cu-Kα radiation at a wavelength of 0.154 nm at a step size of 0.02° 2θ per second. The scanning range of X-ray diffraction was at the diffraction angle (2θ) of 5 ~ 40°.

Scanning electron microscopy The microstructures of instant rice porridge samples were observed by scanning electron microscopy (SEM) (XL-30 TMP, Phillips, Netherlands). The samples were prepared by rupturing a rice at the center and sticking it to a slide. Observation of the samples was performed along the cross section after being girt by ion sputtering device (Prasert and Suwannaporn, 2009; Wójtowicz and Mościcki, 2009).

Table 1. Rapid visco-analysis (RVA) parameters of instant rice porridges produced by different enzyme treatments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pasting Temperature (°C)</th>
<th>Peak viscosity (cP)</th>
<th>Trough viscosity (cP)</th>
<th>Final viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw rice</td>
<td>85.6</td>
<td>1672</td>
<td>1285</td>
<td>2600</td>
</tr>
<tr>
<td>α-amylase</td>
<td>62.4</td>
<td>364</td>
<td>338</td>
<td>396</td>
</tr>
<tr>
<td>β-amylase</td>
<td>62.9</td>
<td>1935</td>
<td>1905</td>
<td>3278</td>
</tr>
<tr>
<td>Neutral protease</td>
<td>63.1</td>
<td>1779</td>
<td>1695</td>
<td>3199</td>
</tr>
</tbody>
</table>

Fig. 1. Rapid visco-analysis (RVA) curves of instant rice porridges produced by different enzymes.
The microscopic structures of instant rice porridge samples treated by α-amylase, β-amylase and neutral protease are illustrated in Fig. 3 with that of raw rice as a reference. According to Fig. 3, treated by α-amylase, a large hollow was formed in the central part of rice with spaces and cracks in the surface, which allows easy rehydration. The reason was that α-amylase resolved rice starch into small fragments and destroyed the dense structure of rice. Treated by β-amylase, hollows were also formed in the central part of rice, but the surrounding structure was dense and seamless, which makes it hard for rehydration, in that β-amylase could not break down α-1,6-glycosidic bond or bypass the branch points to act on α-1,4 glycosidic bond and the rice starch was not completely resolved. Treated by neutral protease, although the surface was destroyed due to the dissolution of proteins, the overall structure of rice was still dense, which makes it hard for the rehydration of rice. The microscopic structures corresponded well with the RVA results.

Compared the independent evidences derived from rapid viscosity analyzer, X-ray diffraction and scanning electron microscopy, a comprehensive conclusion was made that α-amylase exceeded β-amylase and neutral protease in maintaining the gelatinization state of instant rice porridge. There was a close correlation between retrogradation and the degree of polymerization (DP) of starch, and the retrogradation of starch largely depended on the retrogradation of amylopectin (Zhou et al., 2008). For the retrogradation of amylopectin, Ring et al. (1987) suggested that the association of amylopectin chains involves a crystallization process during which the double helix was formed, and the associated regions contained branched fragments, the individual chains of which had a average DP of 15. According to Hizukuri (1986), amylopectin crystallises according to a cluster model in which one amylopectin molecule spans several clusters and the branches can be labelled A, B or C. The A chains are the outer chains linked to an inner B chain. The B chains are linked to other B chains or the C chain. There is only one C chain per amylopectin molecule. Both A chains and B chains could join into crystallization as short chains. The more the short chains joined, the more crystals formed, which allowed easy accumulation of crytals, resulting in high crystallinity. Meanwhile, according to Gidley and Bulpin (1987), the DP had to be more than 10 to form a double helix structure between A chain and B chain. α-amylase treatment resolved rice starch into maltooligosaccharides, disaccharide, glucose and some other segments (Apar and özbek, 2005), which probably decreased the degree of polymerization of rice starch, shortened the chain length, destroyed the crystal structure, thus might lead to the inhibition of rice starch retrogradation.
Drying methods Drying is an essential process of instant rice porridge, during which gelatinized rice starch tends to retrograde and harden due to water loss and cooling. Thus the rehydration of instant rice porridge is affected leading to loss in sensory quality. Therefore, the selection of drying methods is of importance to improve the quality of instant rice porridge.

The RVA curves of instant rice porridge samples treated by the three different drying methods are illustrated in Fig. 4 with that of raw rice as a reference, and further analysis on the RVA parameters is illustrated in Table 2. According to Fig. 4, the RVA curves show that hot air drying leads to a higher peak viscosity compared to freeze-drying, freeze-hot air drying, and raw rice. The holding time at the peak viscosity is also longer for hot air drying. The peak viscosity of freeze-drying is lower than that of hot air drying but higher than that of freeze-hot air drying. Freeze-hot air drying results in a lower peak viscosity compared to hot air drying and freeze-drying, indicating a potential improvement in the quality of instant rice porridge.
X-ray diffraction patterns of instant rice porridge samples treated by different drying methods are illustrated in Fig. 5 with that of raw rice as a reference. The X-ray diffraction pattern of raw rice showed the A pattern which disappeared in instant rice porridges. Instant rice porridges showed the V-type pattern, or the diffraction peaks at $2\theta = 13^\circ$ and $20^\circ$, which indicated that the crystalline structure of the rice starch granules was destroyed during the drying process (Ratnayake and Jackson, 2007). This result agrees well with Miyoshi (2002), Prasert and Suwannaporn (2009), and Shih et al. (2007).

The microscopic structures of instant rice porridge samples treated by different drying methods are illustrated in Fig. 6 with that of raw rice as a reference. The X-ray diffraction pattern of raw rice showed the A pattern which disappeared in instant rice porridges. Instant rice porridges showed the V-type pattern, or the diffraction peaks at $2\theta = 13^\circ$ and $20^\circ$, which indicated that the crystalline structure of the rice starch granules was destroyed during the drying process (Ratnayake and Jackson, 2007). This result agrees well with Miyoshi (2002), Prasert and Suwannaporn (2009), and Shih et al. (2007).

The microscopic structures of instant rice porridge samples treated by different drying methods are illustrated in Fig. 6 with that of raw rice as a reference. According to Fig. 6, a large hollow was formed in the central part of rice obtained by hot air drying, whereas the exterior structure was dense and seamless, which resulted from the sudden expansion of water vapor in rice granule. By hot air drying, moisture was removed from the surface of the rice grains faster than from the interior, thus the surface became harder than the center (Prasert and Suwannaporn, 2009), which blocked water vapor leaching. However, this case hardening phenomenon makes it harder for rehydration. Rice obtained by freeze drying displayed a sponge-like structure with many cracks and pores, due to the sublimation of water on vacuum conditions. By freeze drying, moisture was removed at a constant low speed from both the surface and the interior of the rice grains. Therefore, instead of case hardening, many cracks and pores occurred, which allows easy rehydration. The structure of rice obtained by freeze-hot air drying was fluffier than that of hot air drying and denser than that of freeze drying, thus the rehydration was between that of other two drying methods. The microscopic structures correspond well with the RVA results.

Compared the independent evidences derived from rapid viscosity analyzer, X-ray diffraction and scanning electron microscopy, a comprehensive conclusion was made that freeze drying exceeded freeze-hot air drying and hot air drying in maintaining the gelatinization state of instant rice porridge. There was a close correlation between starch retrogradation and the temperature (Attanasio et al., 2004). The

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pasting Temperature (℃)</th>
<th>Peak viscosity (cP)</th>
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<th>Final viscosity (cP)</th>
</tr>
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<tbody>
<tr>
<td>Raw rice</td>
<td>85.6</td>
<td>1672</td>
<td>1285</td>
<td>2600</td>
</tr>
<tr>
<td>Hot air drying</td>
<td>78.4</td>
<td>1345</td>
<td>1124</td>
<td>3139</td>
</tr>
<tr>
<td>Freeze drying</td>
<td>76.1</td>
<td>733</td>
<td>634</td>
<td>1675</td>
</tr>
<tr>
<td>Freeze-hot air drying</td>
<td>76.7</td>
<td>883</td>
<td>751</td>
<td>1745</td>
</tr>
</tbody>
</table>

Table 2. Rapid visco-analysis (RVA) parameters of instant rice porridges produced by different drying methods.
starch retrogradation speed was low at 24°C or above, while high at 0 ~ 4°C, and can be inhibited at 0 ~ −10°C (Luangmalawat et al., 2008). The temperature was as low as −50°C during freeze drying, at which the rice starch froze rapidly and maintained the state of α before it was orderly arranged into microcrystal. Furthermore, vacuum sublimated the water effectively, thus the retrogradation of rice was inhibited and the rehydration of instant rice porridge was improved.

**Fig. 6.** Scanning electron microscopy (SEM) photos of instant porridges congee produced by different drying methods: a-hot air drying, b-magnifying a, c-freeze drying, d-magnifying c, e-freeze-hot air drying, f-magnifying e, g-raw rice.

**Conclusion**

The effects of different enzyme treatments and drying methods on gelatinization and retrogradation of instant rice porridge were investigated. As for different enzyme treatments, α-amylase exceeded other two enzymes in maintaining gelatinization, inhibiting retrogradation and facilitating rehydration of instant rice porridge. Of the three drying methods, freeze drying exceeded other two drying methods in maintaining gelatinization, inhibiting retrogradation and
facilitating rehydration of instant rice porridge. It is therefore recommended that α-amylase treatment and freeze drying should be conducted in instant rice porridge processing to pursue a shorter soaking time and better sensory quality.

References


