Research Article

Pasting Properties of Various Waxy Rice Flours: Effect of \(\alpha\)-Amylase Activity, Protein, and Amylopectin

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Waxy rice has a long history of being cultivated and consumed in China. In this study, the effect of different factors including \(\alpha\)-amylase activity, protein, and amylopectin structure on the pasting properties of four waxy rice varieties were investigated. Rice flours were divided into four groups (Vietnam indica (VI), Jiangxi indica (JI), Anhui japonica (AJ), and Dongbei japonica (DJ) group) and treated with AgNO\textsubscript{3} solution, DL-dithiothreitol (DTT), or protease (\(n = 3\)). Results suggested that both \(\alpha\)-amylase activity and protein significantly decrease the pasting viscosity of waxy rice flours. Chain length distribution of amylopectin as measured by high performance ion exchange chromatography (HPAEC-PAD) showed that starch with a higher ratio of short chain leads to a higher pasting viscosity. X-Ray diffractograms showed that the crystal type of all the four varieties of rice starches were characteristic A-type. Relative crystallinity of each rice starch was further calculated, and higher crystallization resulted in a higher viscosity. Our study would provide a fundamental knowledge of the relationship between different factors and waxy starch pasting properties, as well as be a reference for controlling the quality of waxy rice starch-based food products.

1. Introduction

Waxy (or glutinous) rice (\textit{Oryza sativa} L.) has a long history of being cultivated and consumed in China. Traditional Chinese waxy rice-based foods include waxy rice ball, sweet green rice ball, waxy rice cake, nuomici, zongzi, etc. [1] while in the modern food industry, waxy rice is usually processed into flour before usage and consumption. The physicochemical properties of the flours especially pasting properties guarantee the appealing texture of its food products.

Many factors could influence the pasting properties of waxy rice flour, such as the endogenous \(\alpha\)-amylase activity, proteins, and amylopectin structure [2]. There is an agreement that endogenous \(\alpha\)-amylase strongly impacts the pasting properties of grain flours [3, 4], and the in-activation of \(\alpha\)-amylase by adding silver nitrate (AgNO\textsubscript{3}) would enhance their viscosities [4]. However, there were controversial opinions for proteins. Some reported that protein increased the pasting viscosity of rice flour [5, 6], while others only discovered a weak correlation between proteins and rice flour. Thus, more evidence should be documented in this area. Most starches consist of 20–30% amylose and 70–80% amylopectin, ratios vary with the botanical sources [7]. Comparing with that, waxy rice starch contains almost 100% of amylopectin that makes it crucial in its pasting characteristics [8–10] and therefore could serve as a good model to study the relationship between amylopectin structure and its pasting properties [2]. The molecular structure of amylopectin has the same basic structure as amylose, while considerably contains more \(\alpha\)-(1,6)-branches and shorter chains that contribute to a three-dimensional complex structure. The chain-length distributions were reported to be a main controller of its pasting properties [11]. Although there are a number of studies regarding the relationship between amylopectin chain-length distribution and pasting...
properties, conflicting results were obtained. Some studies reported that higher proportion of shorter chain (DP 6–12) and lower proportion of long chain (DP > 37) indicating a higher peak pasting viscosity [12–14], while others found out that peak viscosity is positively correlated with the amount of amylopectin medium and long chains. In this aspect, more studies should be focused on chain-length distribution and pasting viscosity in the future.

To our knowledge, the effect of α-amylase activity, protein, and amylopectin structure were often investigated separately in most studies, regarding the relationship between waxy rice starch and its pasting properties. The present work investigated the effect of three factors at the same time, with emphasis on the underlying reason for the difference of pasting properties among different cultivars from North and South China. In addition, the present study was the first to compare the pasting viscosities among the four varieties simultaneously. Thus, our study would not only provide a fundamental knowledge of the association between various factors and waxy starch pasting properties but also be a reference for quality control of waxy rice starch-based food to meet the desired texture.

2. Materials and Methods

2.1. Materials. Four representative high production waxy rice cultivars from North or South China were selected: Vietnam indica (VI); Jiangxi indica (JI); Anhui japonica (AJ); and Dongbei japonica (DJ). Rice samples were provided by Henan Huangguo Grains Industry Co., Ltd. (Henan, China). All reagents and chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) or Sigma-Aldrich (Bornem, Belgium) unless specified otherwise.

2.2. Preparation of Waxy Rice Flour. Waxy rice flour was prepared by a wet-milling method according to the method suggested by Tong et al. [15]. Briefly, rice samples were cleaned and washed three times to remove impurities and then soaked in distilled water at room temperature for 4 h before put into a colloid mill (JM-L50, Shanghai Nuoni Light Industrial Machinery Co. Ltd, Shanghai). The rice slurry was then vacuum filtered and dried in an air-dry oven at 40°C for 12 h, and then centrifuged at 1580 g for 15 min. The dark yellow supernatant was scraped from the sediment and discarded, and then the sediment was washed with distilled water and adjusted to pH 7.0 with 1 M HCl before being centrifuged for 15 min. The procedure was repeated twice until the sediment was pure white and then lyophilized to dryness to obtain high purity rice starch.

2.3. Composition Analyses of Waxy Rice Flour. Starch content was determined by PRC National Standard (GB 5009.9-2016) and measured by a Futura continuous flow analyzer (AMS Alliance, France). Crude protein content was estimated by determination of nitrogen (N) content using a Vario EL element analyzer (Elementar, German), and then, N values were converted to protein content by N × 5.95. Crude ash, crude lipid, and moisture content were determined by Chinese Standard GB/T5009.4-2010, GB/T5009.6-2003 and GB/T5009.3-2010, respectively. All the components were converted to dry base contents based on water content.

2.4. Rice Starch Isolation. Waxy rice starch was isolated by an alkaline method according to the study by Mu et al. [16] with slight modifications. The rice flour was soaked in 0.085 mol/L NaOH solution with a material to liquid ratio of 1:5 g/ml, stirred at 40°C for 4 h, and then centrifuged at 1580 × g for 15 min. The dark yellow supernatant was scraped from the sediment and discarded, and then the sediment was washed with distilled water and adjusted to pH 7.0 with 1 M HCl before being centrifuged for 15 min. The procedure was repeated twice until the sediment was pure white and then lyophilized to dryness to obtain high purity rice starch.

2.5. Determination of α-Amylase Activity. A colorimetric method as described by Li [17] was hired to determine α-amylase activity. Maltose was used for the standard curve, and 3,5-dinitrosalicylic acid (DNS) was used as a coloring agent. OD values were measured at 540 nm. The α-amylase activity of each waxy rice flours was calculated according to OD values and the maltose standard curve and expressed as micrograms per Gram per minute (mg/(g-min)).

2.6. Rapid Viscosity Analysis (RVA). The pasting properties of the selected four rice flours with and without 0.085 g/L AgNO₃ or 0.77 g/L DL-dithiothreitol (DTT) solution or protease were tested in triplicate with a Rapid Visco Analyzer (RVA) (Newport Scientific, Narrabeen, Australia), using std1 temperature programming according to ICC standard No. 162 and AACC66-21. In addition, pasting properties of rice starches were also tested.

2.7. Differential Scanning Calorimetry. The thermal properties of waxy rice flours and starch were measured by the differential scanning calorimetry (DSC) method using DSC 8500 (Perkin-Elmer Inc., CT, USA). Three milligram waxy rice flour or starch powder (on a dry basis) was accurately weighed and placed into a DSC aluminum sample pan. Distilled water was added to obtain the emulsion with a powder/water ratio of 1:3 (w/w). The DSC pans were sealed, equilibrated for 24 h, and then heated from 20 to 100°C at a rate of 10°C/min. The gas flow rate of nitrogen was 40 mL/min. An empty pan was used as a reference.

2.8. X-Ray Diffraction. X-Ray diffractograms were obtained with a D/Max-2200 X-ray diffractometer (Rigaku Denki Co., Tokyo, Japan) with Cu Ka radiation at 40 kV and 50 mA. The diffractograms were scanned between 5° and 60° (2θ) at a rate of 1.2°/min. Relative crystallinity was estimated by the ratio of the peak areas to the total diffractogram area as described by Chen et al. [18].

2.9. Chain-Length Distribution of Amylopectin. The branch chain-length distribution of amylopectin was measured by high-performance ion exchange chromatography (HPAEC)
equipped with a pulse amperometric detector (PAD) as described by Blennow et al [19]. Starch samples were gelatinized and then debranched by isoamylase. The debranched amylopectin solution was filtered through a PVDF membrane filter (0.45 μm) before being injected into the HPAEC-PAD system. Eluents A (0.5 M sodium hydroxide), B (0.5 M sodium acetate), and C (distilled water) were operated at a flow rate of 1 mL/min. The separation gradient of eluents was as follows: 0 min, 20% A, 10% B, and 70% C; 0–40 min, linear gradient to 20% A and 80% B.

2.10. Statistical Analysis. Each experiment was done in triplicate. Results were expressed as average ± standard deviation (SD). Analysis of one-way ANOVA with Duncan’s multiple range test and Pearson’s correlation were performed using SPSS software (version 19.0, Statistical Package for the Social Sciences Inc., Chicago, USA). A value of p < 0.05 was considered to be statistically significant.

3. Results and Discussion

3.1. Composition of Rice Flours. Total starch, protein, lipid, moisture, and ash contents of the four varieties from North and South China are listed in Table 1. Moisture content was higher in japonica rice than that in indica rice. Japonica rice contains more starch than indica, especially Dongbei japonica (DJ). The protein and starch contents of the four varieties were around 7% and 90%, respectively, which were in accordance with other findings [20, 21]. The moisture content of japonica rice was significant higher than that of indica rice (Table 1), which is in line with the findings of Ayabe et al. [22]. Both protein and lipid contents were the highest in Jiangxi indica (JI) among the four cultivars. Ash content in DJ was the highest followed by JI. These results indicate that the compositions of the rice may varies with their varieties and growing environment [23].

3.2. Gelatinization Temperatures of Waxy Rice Flour and Starches. The gelatinization temperatures (Tg) as determined by DSC measurement are shown in Table 2. The Tg value for each sample was in the range of 55–75°C, which was in accordance with other findings [24]. The gelatinization temperatures for the same variety treated by distilled water, AgNO3, or DTT seemed not changed according to Table 2, indicating that α-amylase or protein would not affect its Tg values. In the meantime, the Tg values of starches from the four varieties were lower than that of their flours, which could be explained by their different starch contents (88–91% in the flours) [24]. Besides, a certain amount of the starch might be damaged during the extraction process that lead to a lower Tg value [25]. Moreover, as shown in Table 2, the indica rice exhibits significant higher Tg values in their flours and starches than those of japonica rice because of the different branch chain-length distributions in their amylopectin structures (data are listed in Table 3 and discussed in section 3.3). Higher amount of short chain (DP6-12) and lower amount of long chain (DP > 37) would resulted in a lower gelatinization temperature [12].

3.3. Effect of α-Amylase on Pasting Properties of Waxy Rice Flours. AgNO3 could serve as an inhibitor for α-amylase to verify the presence of endogenous amylase activity in flours and characterize the pasting properties of flours without enzyme interference [2]. In this study, the pasting properties of different waxy rice flours treated with AgNO3 were tested, and the flours treated with distilled water were used as the control. Results are shown in Figure 1(a) and are summarized in Table 2. In the meantime, α-amylase activity was measured and results are shown in Table 1.

The peak, trough, and final viscosity of all the four varieties showed an increase with AgNO3 treatment when compared with water treatment (Figure 1(a) and Table 2), suggesting that the endogenous α-amylase of the four varieties decreased their viscosities. Meanwhile, the largest increasement of viscosity was showed in AJ (Figure 1(a)), which was found to possess the highest α-amylase activity among the four varieties (Table 1), indicating that higher activity of α-amylase may contribute to lower viscosity of AJ. This result was in accordance with the findings of Zhu et al. [2], Noda et al. [4], and Cheng et al. [26], since α-amylase catalyzes the endoα-hydrolisis of α-1, 4 glucan bonds and plays a major role during the degradation of native starch granules. Waxy rice flours seem to be sensitive to α-amylase breakdown, even a few internal cleavages of amylopectin would lead to a reduction in molecular weight and result in a lower viscosity [3]. The apparent difference in viscosities observed in the flours with and without enzyme inactivation underscores the effect of amylase activity on the waxy rice flour pasting profile.

To investigate the relationship between α-amylase activity, composition contents of flours, and pasting viscosities, correlation coefficients were further calculated. Significant inverse correlation was observed between α-amylase activity and pasting viscosities including peak (r = −0.958), trough (r = −0.967), and final (r = −0.957) viscosities at p < 0.05, which confirmed the effect of α-amylase activity on the pasting viscosity of waxy rice flours. However, no significant correlation was found between composition contents and pasting viscosities, indicating that the compositions of the waxy rice flour seemed to be not associated with its gelatinization characteristics.

3.4. Effect of Protein on Pasting Properties of Waxy Rice Flours. To investigate the effect of protein on pasting properties of waxy rice flours, DTT or protease was used in our study. Results are shown in Figure 1(b). For DTT treatment, all the flours showed a decrease in their viscosities, among which the peak viscosity of JI decreased most, followed by AJ and DJ. The starch granules break down easily in the absence of rigidity caused by disulfide-bound protein polymers that surround the native granules. DTT could cleave disulfide bonds between cysteines in the proteins and diminish the carbohydrate binding activity of starch with proteins, leading to an increase of starch to be released from the proteins. The released starch would correspondently get hydrolyzed by the endogenous α-amylase that further resulted in the decrease of viscosity in waxy rice flours. According to Figure 1(b), the decrease of viscosities varied in the four varieties, indicate that other than
protein, amylase activities, or other factors may play an additional role. The present study did not find obvious associations between α-amylase activities and the varied decrease of viscosities in the DTT treated flours (Table 2 and Figure 1(b)), which seemed not in consistence with the findings of Zhu et al. [2]. As they reported that α-amylase activity positively influences the decrease of viscosity in the DTT treatment, to clear out the influence of amylase activity and further study the effect of proteins on flours pasting properties, the flours were treated with and without protease in the presence of AgNO₃; results are shown in Figure 2. The pasting viscosities were increased in the four varieties in the protease-treated group (Figure 2 left) when compared with the non-protease-treated group (Figure 2 right), indicating that protein had an inhibitory effect on the pasting properties of waxy rice flour. DTT or protease, either by disulfide bond disruption or protein removal, was reported to pasting properties of waxy rice flour. DTT or protease, either by disulfide bond disruption or protein removal, was reported to pasting properties of waxy rice flour. 

| Table 1: Composition and α-amylase activities of waxy rice flours*. |
|---------------------|---------------------|------------------|-------------------|-------------------|-------------------|
| Rice cultivar       | Moisture content%   | Starch%          | Protein%          | Lipid%            | Ash%              | α-Amylase activity |
| VI                  | 12.11±0.12          | 88.24±0.49b      | 6.99±0.00b        | 0.89±0.04b        | 0.27±0.02b        | 11.3±0.11          |
| JJ                  | 11.26±0.08          | 89.64±0.43b      | 7.66±0.01c        | 1.22±0.08c        | 0.68±0.03c        | 10.9±0.2b          |
| AJ                  | 13.67±0.10          | 88.26±0.38a      | 7.52±0.00c        | 0.76±0.10a        | 0.42±0.02b        | 12.8±0.3d          |
| DJ                  | 14.64±0.06c         | 90.84±0.17c      | 6.43±0.00a        | 0.87±0.07b        | 0.75±0.02d        | 9.7±0.2a           |

*Values are mean ± standard deviation (n = 3). Means that do not share the same letter in a column are significantly different at p < 0.05. VI: Vietnam indica; JJ: Jiangxi indica; AJ: Anhui japonica; DJ: Dongbei japonica.

| Table 2: Pasting properties gelatinization temperatures of waxy rice flours and starches*. |
|---------------------|---------------------|---------------------|---------------------|
| Rice variety       | Peak Trough Final Setback | Gelatinization temperature (Tₚ/°C) |
| VI                  | 1503±82b 959±65b 746±76b 151±12b | 73.25±0.24b |
| JJ                  | 1400±88b 536±35b 685±35b 149±15b | 74.00±0.33b |
| AJ                  | 994±62b 218±23b 293±89a 75±6a | 66.23±0.12b |
| DJ                  | 2203±89c 769±78c 940±56c 171±13b | 67.45±0.21a |

*Values are mean ± standard deviation (n = 3). Means that do not share the same letter in a column are significantly different at p < 0.05. VI: Vietnam indica; JJ: Jiangxi indica; AJ: Anhui japonica; DJ: Dongbei japonica.

| Table 3: Branch chain-length distributions and the relative crystallinities of amyllopectin in waxy rice starches*. |
|---------------------|---------------------|---------------------|---------------------|
| Rice variety       | The relative crystallinity (%) | Chain length distribution of amyllopectin (%) |
| VI                  | 35.13               | DP6–12 29.37±0.35b 50.61±0.08a 11.58±0.16c 8.08±0.28b |
| JJ                  | 34.52               | DP13–24 27.60±0.23a 50.22±0.03a 12.12±0.29d 8.23±0.09b |
| AJ                  | 37.54               | DP25–36 30.38±0.33c 52.39±0.25b 9.20±0.09a 8.03±0.00b |
| DJ                  | 38.92               | DP > 37 31.68±0.41d 52.05±0.21b 10.66±0.05b 7.44±0.15a |

*Values are mean ± standard deviation (n = 3). Means that do not share the same letter in a column are significantly different at p < 0.05. VI: Vietnam indica; JJ: Jiangxi indica; AJ: Anhui japonica; DJ: Dongbei japonica.
Figure 1: Pasting curves of waxy rice flours treated with (a) distilled water or AgNO₃ and (b) distilled water or DTT. VI: Vietnam indica; JI: Jiangxi indica; AJ: Anhui japonica; DJ: Dongbei japonica.
Figure 2: Pasting curves of AgNO$_3$-treated waxy rice flours with or without protease. Vietnam indica (VI); Jiangxi indica (JI); Anhui japonica (AJ); Dongbei japonica (DJ).

Figure 3: Continued.
possessed the highest peak viscosity, followed by AJ. The peak viscosity was significantly higher in japonica than indica rice ($p > 0.05$). Although not statistically significant, the trough viscosity was still higher in japonica than indica rice. For final viscosities and setback, JI was the highest while VI was the lowest. The difference of pasting properties among various waxy rice starches might due to the difference in their structures [12, 27–29], such as their branch chain length and the degree of crystallinity in the amyllopectin. In our study, the branch chain-length distribution of amyllopectin was measured by high-performance ion exchange chromatography (HPAEC); meanwhile, the degree of crystallinity was estimated by the ratio of the peak areas to the total diffractogram area as described by Chen et al. [18].

The X-ray diffraction patterns are presented in Figure 3, and the relative crystallinity of the four waxy rice starches is summarized in Table 3. The waxy rice starches displayed the typical A-type polymorphic form with an unresolved peak at 17° and 18° and individual peaks at 15°, 20°, and 23° in the X-ray diffractograms (Figure 3). The relative crystallinity (Table 3) calculated from X-ray diffraction pattern ranged from 34.5% (JI) to 38.9% (DJ), which was slightly lower than that previously reported [30, 31]. This might due to different cultivars of waxy rice were used. Despite that, the amyllopectin chain-length distribution also had an impact on relative crystallinity [32]. According to Table 3, japonica rice possessed a higher relative crystallinity than indica rice. The relative crystallinity of DJ was the highest, meanwhile that of JI was the lowest. The relative crystallinity of each starches increased with the amounts of DP 6–12 (Table 3), because short chains (DP < 10) in amyllopectin resulted in a decrease in the stability of double helix that leads to a lower value of relative crystallinity [30]. Moreover, the relative amounts of DP 13–24 increased with relative crystallinity confirmed that the semicrystalline nature of starch was ascribed to amyllopectin double helices in the crystalline lamellae of the starch granule structure, which was in line with the studies of G. E. Vanderputte et al. [32]. A higher degree of crystallization in waxy starch probably reflects more closely packed crystal structures [3] which may lead to higher viscosities (Table 2).

The branch chain-length distribution of amyllopectin as measured by HPAEC-PAD are shown in Figure 3. The lengths of the amyllopectin chains were reported to have a major impact on rice starch properties [33]. The chain of amyllopectin was classified into A chain (DP 6–12), B1 chain (DP 13–24), B2 chain (DP 25–36), and B3+ chain (DP > 37) according to previous research studies [32–34]. The pattern of area percentages of amyllopectin chains showed a bimodal distribution; DPs of the four varieties of waxy rice starches ranged from 6 to 80 (Figure 3). All the four varieties displayed two distinct groups with DP 6–33 and DP 34–62 in the chromatogram were observed. Specifically, there were very few short chains of DP6 and a gradual increase in chains of DP 7–9, with a maximum peak at DP 12 and a shoulder at DP 15 in the DP 6–33 group, meanwhile the DP 34–62 group showed a peak at DP 40. These results were in accordance with previous research that A-type starches had its first peak at DP 12–14 and second peak at DP
41–51 [7]. Among the four varieties, JI possessed the lowest proportion of DP 6–12 chains (27.60 ± 0.23%), indicating that there were fewer short chain A chains in JI starch. DJ possessed the largest DP 6–12 (A chains) and lowest DP > 37 (B3 + chains) proportions, respectively, suggesting the average chain length of DJ was shorter. These results were in accordance with the findings that indica rice possessed more long chains (DP > 37) than japonica rice [13]. Waxy rice starch with a higher proportion of shorter chain (DP 6–12) and lower proportion of long chain (DP > 37), for example DJ, indicate a higher peak pasting viscosity (Tables 2 and 4) [12–14].

4. Conclusions

In conclusion, the present study showed that the pasting viscosities of the four waxy rice flours could be increased by adding AgNO₃, while decreased by their own protein. In addition, the crystal type of the four varieties of rice starches is characteristic A-type. Higher degree of crystallization with a more short chain in the amyllopectin structure would lead to higher viscosities. Taken together, the rice protein, α-amylase activity, and amyllopectin structure were the major causes of the different pasting properties among various varieties of waxy rice flours. Our study would provide a fundamental knowledge of the mechanism for the discrepancy of different pasting properties of waxy rice flour, transferring this fundamental knowledge to food production by controlling the viscosity of the starch from waxy rice flour-based food products to maintain an appealing texture and meet the requirements of consumers.

Data Availability

All the data regarding this study are presented in this article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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