The quality of rice wine influenced by the crystal structure of rice starch

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Abstract Normal rice wine (NRW) and waxy rice wine (WRW) were fermented to study the relationship between the structure of starch as well as the taste and texture of rice wine. The total starch content of NRW decreased to 21.2%, and that of WRW decreased to 15.6%. The water-soluble sugar content of NRW increased to 169.3, and that of WRW increased to 194.4 mg/g. The ethanol content of NRW increased to 6.5%, and that of WRW increased to 8.9%. These changes indicated that WRW exhibited higher quality than NRW. Sweetness was negatively correlated with total starch content and positively correlated with ethanol content. Starch molecules were degraded by enzymolysis, thereby enhancing crystallinity. The size of starch particle was negatively correlated with crystallinity, whereas the chewiness and gumminess of vinasse (fermented rice grains) were positively correlated with the size of starch particle and negatively correlated with crystallinity. The higher degrees of chewiness and gumminess of vinasse render the texture of WRW superior to that of NRW. The results indicated that WRW is superior to NRW in taste and texture because of the difference in starch structure.

Keywords Rice wine · Starch · Texture · Crystallinity · Short-range molecular order

Introduction

Rice is a staple food of more than half the global population and constitutes 35–75% of daily caloric intake for most Asians. Rice is rich in starch (70–80%), protein, fat, vitamins, and minerals and can provide comprehensive nutrition. Starch comprises two types of molecules: amylose and amylopectin. Amylose, a relatively linear 1,4- α -D-glucose, is an unbranched helical structure with few long branches. Amylopectin, a group of highly branched glucose polymers, is mainly 1,4- α -D-glucose containing short numerous branches (glycosidic linkages are α -1,6) (Syahariza et al. 2013; Zhu et al. 2017). Amylopectin is insoluble in water; when heated and gelatinized, loosened molecule chains result in high viscosity and contribute to a glutinous taste. Rice can be classified by species (*indica* rice, *japonica* rice, waxy rice, and brown rice), levels of processing, morphology, structure, and quality (Wang et al. 2017). The amylose contents of *indica* rice, normal rice, and waxy rice are approximately 25%, 18%, and 0–2%, respectively (Asaoka et al. 1985).

Sweet rice wine has existed for more than 3000 years. It is a traditional Chinese drink with low alcohol content, fermented from steamed rice mixed with rice wine *koji* (Shen et al. 2012). Rice wine *koji* is a mixture of yeast and filamentous fungus (*Aspergillus oryzae*) used in Chinese and other East Asian countries to ferment alcoholic beverages. During fermentation, the fungus in *koji* is cultured on steamed rice, and enzymes such as α -amylase, glucoamylase, protease, and lipase are biosynthesized. Starch saccharification and protein decomposition are catalyzed

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by enzymes, and alcohol fermentation is facilitated by yeast (Yoshizaki et al. 2010). Therefore, fermented rice wine is sweet and alcoholic without the acidic taste.

Previous studies on rice wine have focused on optimal fermentation conditions (Jiao et al. 2017), the effect of yeast on nutrient components (Yang et al. 2017), the digestibility of and structural changes in waxy rice starch (Zhang et al. 2016), and the functions of the main components in rice wine (Meng et al. 2016); however, the relationship between the starch structure and quality of rice wine remains unclear. Thus, the current study aims to evaluate the effect of starch structure on the textural profile and chemical composition of rice wine with regard to the taste quality (starch, ethanol, water-soluble sugars) of rice wine during fermentation to provide a basis for the selection of raw materials for rice wine production.

Materials and methods

Materials

Two different rice species were selected based on an extensive survey on amylose content. Normal rice (*japonica* rice) and waxy rice (*Oryza sativa* L. var. glutinosa Matsum.) were purchased from a supermarket in Beijing, China. Rice wine *koji* (a mixture of yeasts and fungi) was rice leaven produced by Angel Company (Yichang, Hubei, China). All other chemicals were of analytical grade and supplied by Beijing Chemicals Corporation (Beijing, China).

Preparation of rice wine

Normal rice and waxy rice were soaked separately in cold water for 24 h. The wet rice was then steamed for 30 min until well done. After cooling to room temperature, 1 kg cooked rice, 500 g distilled water, and 5 g rice wine *koji* were mixed well and fermented by one-stage fermentation at 28 °C for 4 days. Each day, 100 g fermented normal rice wine (NRW) or waxy rice wine (WRW) was removed for analysis and labeled as NRW0, NRW24, NRW48, NRW72, NRW96 and WRW0, WRW24, WRW48, WRW72, WRW96. After the 4-day fermentation, the final product was a mixture of solid rice grains and free-run liquid wine with a ratio of approximately 2:1 (w/w). All rice wine samples were homogenized and used for analysis of amylase activity, chemical compounds, and sensory evaluation. The fermented rice grains (vinasse) obtained by separating out liquid wine were used for textural profile analysis (TPA) and starch extraction.

Analysis of amylase activity

The activity of α/β -amylase was determined using the 3,5-dinitrosalicylic acid assay described by Gusakov et al. (2011). The activity of $\alpha + \beta$ was measured, and the mixture was heated at $70\text{ °C} \pm 0.5\text{ °C}$ for 15 min to deactivate β -amylase and determine α -amylase activity. One unit of enzymatic activity was defined as 1 mg maltose produced per min. The activity of glucoamylase in the *koji* and rice wine samples was assayed using Association of Official Analytical Chemists (AOAC) Official Method 994.09 (AOAC 2001). One unit of glucoamylase activity was defined as 1 mg glucose released per hour.

Analysis of chemical compounds and sensory evaluation of degree of sweetness

The moisture in rice wine was determined using AOAC Official Method 2001.12 (AOAC 2001). Ethanol contents in NRW and WRW were measured in accordance with AOAC Official Method 992.29 (AOAC 2001). The water-soluble sugar content in rice wine was determined using the anthrone reagent method (Grandy et al. 2000). Total starch content was determined using AOAC Official Method 996.11 (AOAC 2001) and recorded based on dry weight (d.w.).

For starch extraction, the vinasse samples were added to 0.2% (w/v) sodium hydroxide at a ratio of 1:2. The mixture was homogenized and then held for 12 h before being passed through an 80-mesh sieve. The slurry was washed with distilled water and centrifuged at 4000 g for 5 min; subsequently, the supernatant was discarded. The sediment was washed with distilled water and centrifuged 3–4 times. The obtained wet starch samples were dried at 40 °C for 48 h, ground into powder, sealed in plastic bags, and stored at 4 °C. The resultant starch samples were used to analyze amylose content and physicochemical properties. Amylose content was determined using the Amylose/Amylopectin Assay Kit K-AMYL 07/11 (Megazyme International, Wicklow, Ireland), expressed as % of total starch.

The sweetness of the rice wine samples was analyzed by sensory evaluation. Sucrose solutions at different concentrations were prepared as a criterion. NRW and WRW samples of different concentrations (0.2%, 0.125%, 0.1%, 0.05%, 0.04%, 0.033%, and 0.025%) were tasted and compared by sensory evaluation panelists (three male and three female graduate students studying food science) using the criterion. The degree of sweetness of rice wine was calculated as the ratio of the concentration of sucrose solution to the concentration of rice wine at the same degree of sweetness:

Sweetness degree =

$$\frac{\text{concentration of standard sugar solution}}{\text{concentration of rice wine}}$$

Textural profile analysis

Each vinasse sample (10 g) was filled in a 100 mL cup. TPA was conducted using a TVT 6700 texture analyzer (Perten Instruments, North Ryde BC, NSW, Australia) under the following conditions: height of the samples, 10.0 mm; reserved height, 3.0 mm; compression ratio, 30.0 mm; post-test speed, 1.0 mm/s; test speed, 1.0 mm/s; retractive speed, 10.0 mm/s; time of residence, 2.0 s; trigger force, 5.0 g; frequency of data acquisition, 200 pps; and test probe, p/25.

Particle size distribution (PSD) of starch

Starch granule size was determined using an S3500 laser particle size analyzer (Malvern Instruments, Malvern, UK) in the wet-cell mode. Approximately 0.2 g starch was suspended in 0.8 mL distilled water, and the suspension was added to the reservoir until a shading rate of 15–20% was achieved.

Starch crystallinity

The crystal structure of the starch samples was identified using an X-ray diffractometer (Bruker D8 ADVANCE, Germany). The operating conditions were as follows: 40.0 kV, 40.0 mA, and a scanning speed of 2°/min through the 2 θ range of 5°–45° at a step size of 0.02°. The slit system was DS/RS/SS = 1°/0.3 mm/1. The degree of crystallinity was calculated as the percentage of the peak area to the total diffraction area.

Fourier transform infrared spectroscopy analysis

The starch samples and KBr were mixed, ground, pressed to transparent ingots, and scanned in the spectral range of 4000–400 cm⁻¹ by using a DTGS detector with a Fourier transform infrared spectrometer (Tensor 27, Bruker Corporation). The baseline was revised using OriginPro 8.0 to obtain the final spectrum. The ratio of spectral absorption peaks at 1047 and 1022 cm⁻¹ was used to characterize the short-range molecular order of the starch structure (Wei et al. 2011).

Statistical analysis

All experiments were conducted in triplicate. SPSS Statistics 22.0 (IBM Corporation, Armonk, NY, USA) was employed to analyze the correlations and significance of the parameters.

Results and discussion

Amylase activity in NRW and WRW during fermentation

The amylases in rice wine come from *A. oryzae* in *koji*, which were used during starch liquefaction and saccharification (Dung et al. 2006). The activities of α -amylase, β -amylase, and glucoamylase in *koji* were 1.1, 20.7, and 156.0 U/g, respectively. Variations in α/β -amylase and glucoamylase activities of NRW and WRW during fermentation are presented in Fig. 1. The α -amylase activity in NRW and WRW increased from 0 to 48 h before declining gradually, whereas the β -amylase activity increased within 24 h and then remained at the same level. The glucoamylase activity in NRW and WRW increased from 12 to 32–38 U/g within 96 h during fermentation. The increase was higher than that in α/β -amylase activity, which was consistent with previous studies (Xiao et al. 2006). α/β -Amylase activity was higher in WRW than in NRW during fermentation. Glucoamylase activity is considered a key factor in starch saccharification and increase in rice wine during fermentation. However, glucoamylase activity could be inhibited by the increased alcohol concentration (Zhang et al. 2016). A mathematical model of the complete reaction system was developed to potentially

Fig. 1 Variation in amylase activity in rice wine during fermentation. NRW: normal rice wine; WRW: waxy rice wine

explain the synergy between amylase and glucoamylase. The first stage in starch hydrolysis is liquefaction catalyzed by α/β -amylase, and the second is saccharification catalyzed mainly by glucoamylase (Presečki et al. 2013). α -Amylase cleaves internal α -1,4-glycosidic linkages in starch to produce glucose, maltose, or dextrin; whereas β -amylase acts on the α -1,4-glycosidic linkages to separate maltose from the nonreducing ends. Glucoamylase cuts α -1,4- and α -1,6-glycosidic linkages to release glucose from the nonreducing ends of starch (Xiao et al. 2006).

Chemical compositions of NRW and WRW during fermentation

The moisture content of raw normal rice and waxy rice was approximately 12%. Total starch contents were 75.0% and 73.4% (d.w.), and amylose comprised 18.5% and 1.7% of starch in raw normal rice and waxy rice, respectively. The moisture contents of the steamed rice and waxy rice after cooking were 31.1% and 37.0%, respectively.

The total starch contents in NRW and WRW decreased to 21.2% and 15.6% (d.w.), respectively, after rice wine fermentation (Fig. 2). The starch in rice wine was rapidly liquefied and saccharified by amylase, and the total starch content decreased quickly during fermentation (Presečki et al. 2013). The amylose ratio of the NRW starch ranged from 20.4 to 27.9%, and that of the WRW starch ranged from 2.9 to 8.7%. The amylose contents in both the NRW and WRW starches increased within 48 h of fermentation, potentially because of the faster formation of amylose by the debranching of amylopectin than that by the hydrolysis of amylose. Amylose content subsequently decreased because amylose hydrolysis became the main catalysis.

Fig. 2 Variation in starch and amylose contents during fermentation. NRW: normal rice wine; WRW: waxy rice wine. Amylose content means % of total starch

Fig. 3 Variation in sweetness, water-soluble sugar content, and ethanol content during fermentation. NRW: normal rice wine; WRW: waxy rice wine

The sweetness of WRW and NRW increased rapidly during fermentation with an increase in water-soluble sugar content (Fig. 3). Water-soluble sugar mainly includes monosaccharides, disaccharides, and short-chain saccharides, which are soluble in water and mostly have a sweet taste. The water-soluble sugar contents of NRW and WRW increased to 169.3 and 194.4 mg/g, respectively, at the end of fermentation, similar to previous research by Dung et al. (2006). Amylase activity was higher in WRW than in NRW; thus, the content of water-soluble sugar and the sweetness of WRW were expected to exceed those of NRW. Waxy rice starch has more short chains and fewer long chains, compared with normal rice starch (Zhou et al. 2015), which is easily debranched by amylase to produce short chains and generates a higher amount of water-soluble sugar in WRW, enhancing its sweetness and palatability.

Glucose was transformed to ethanol by the yeast strains during rice wine fermentation. The ethanol contents of NRW and WRW increased to 6.5% and 8.9%, respectively, at the end of fermentation. In the early stage of fermentation, starch was hydrolyzed to monosaccharide, disaccharide, and short-chain saccharides, and yeast was propagated simultaneously. After 24 h, ethanol was produced by yeast fermentation. The ethanol content of WRW increased more quickly than that of NRW owing to the larger number of fermentable saccharides in WRW (Zhao et al. 2009).

Textural properties of vinasse in NRW and WRW during fermentation

Statistically significant differences in hardness, adhesiveness, stickiness, chewiness, gumminess, stringiness, and springiness were observed between steamed rice and waxy rice (WRW0 and NRW0) (Table 1). The hardness,

Table 1 Textural properties of vinasse separated from rice wine at different stages of fermentation

	Hardness (g)	Springiness	Cohesiveness	Adhesiveness (J)	Chewiness (g)	Gumminess (g)	Stickiness (g)	Stringiness (mm)
NRW0	1707 ± 153b	0.72 ± 0.07a	0.44 ± 0.03a	484.95 ± 9.48b	553.7 ± 14.0b	758 ± 45b	− 158.33 ± 31.26b	2.23 ± 0.21c
NRW24	201 ± 28e	0.23 ± 1.10e	0.32 ± 0.06a	131.19 ± 17.66c	14.3 ± 4.5g	61 ± 13d	− 40.33 ± 1.73cd	3.72 ± 0.02a
NRW48	185 ± 18e	0.56 ± 0.14bc	0.42 ± 0.09a	39.02 ± 6.82e	24.0 ± 17.9f	76 ± 11d	− 28.67 ± 2.51d	3.71 ± 0.04a
NRW72	193 ± 11e	0.42 ± 0.02cd	0.34 ± 0.13a	43.32 ± 7.41e	26.3 ± 6.5fg	63 ± 9d	− 24.33 ± 7.60d	3.66 ± 0.05a
NRW96	220 ± 6e	0.55 ± 0.01bc	0.43 ± 0.02a	87.01 ± 11.86d	56.0 ± 6.1e	91 ± 5d	− 31.00 ± 2.23cd	3.68 ± 0.04a
WRW0	3775 ± 228a	0.42 ± 0.04cd	0.36 ± 0.08ab	719.75 ± 71.12a	639.3 ± 37.0a	1504 ± 125a	− 242.33 ± 18.55a	3.11 ± 0.10b
WRW24	598 ± 15c	0.29 ± 0.01de	0.30 ± 0.01b	155.04 ± 20.65c	53.0 ± 3.5ef	189 ± 7c	− 51.67 ± 5.69c	3.61 ± 0.01a
WRW48	676 ± 46cd	0.37 ± 0.05de	0.39 ± 0.03ab	50.89 ± 16.77de	79.0 ± 13.7de	236 ± 10c	− 33.67 ± 3.19cd	3.61 ± 0.01a
WRW72	545 ± 16d	0.39 ± 0.04d	0.40 ± 0.02ab	50.26 ± 5.69de	112.3 ± 13.7c	180 ± 21c	− 26.00 ± 9.50d	3.65 ± 0.01a
WRW96	591 ± 11d	0.67 ± 0.19ab	0.42 ± 0.17ab	57.22 ± 5.08de	91.0 ± 21.4cd	196 ± 8c	− 31.33 ± 2.21cd	3.66 ± 0.01a

NRW normal rice wine, WRW waxy rice wine; 0, 24, 48, 72 and 96 is the fermentation time (h). Mean ± SD is calculated from triplicated measurements. Values with different letters in the same column are significantly different at $p < 0.05$

adhesiveness, chewiness, gumminess, and stickiness in waxy rice were greater, compared with normal rice, indicating a harder and stickier texture in waxy rice than in normal rice (Pal et al. 2016). Normal rice exhibited high springiness, suggesting that high-amylose rice is more resistant to swelling, resulting in increased elasticity and reduced viscosity (Li et al. 2016; Kohyama et al. 2004; Kaur et al. 2016).

The gumminess, stringiness, stickiness, and adhesiveness of vinasse in WRW and NRW decreased during fermentation, but the cohesiveness remained unchanged. Meanwhile, the springiness of vinasse in WRW and NRW decreased initially and then increased slightly, along with the variation in amylose content (Zhou et al. 2015). The hardness, adhesiveness, chewiness, gumminess, and stickiness of vinasse in NRW and WRW decreased significantly ($p < 0.01$) during fermentation, whereas stringiness increased significantly ($p < 0.01$). Glycosidic linkages were cleaved by α/β -amylase and glucoamylase to shorten the starch chains, further reducing molecular polymerization and weakening the interaction between starch granules, which could result in decreased stickiness, gumminess, hardness, and adhesiveness. Declines in gumminess and chewiness fostered tender, delicate, and mild characteristics in both WRW and NRW, while significant decreases in adhesiveness and stickiness contributed to the loose and soft texture of vinasse. The hardness, gumminess, and chewiness of vinasse in WRW were higher than those in NRW throughout the fermentation, providing a chewy texture and a palatable taste.

Regarding correlations among all the textural properties of rice wine, chewiness ($r = 0.994$), gumminess ($r = 0.999$), and adhesiveness ($r = 0.992$) exhibited significantly positive correlations with hardness, whereas stickiness and stringiness were negatively correlated with hardness ($r = -0.995$ and -0.999 , respectively). Significant negative correlations were found between chewiness and stickiness ($r = -0.998$) and stringiness ($r = -0.997$). Gumminess was positively correlated with hardness ($r = 1.000$) and chewiness ($r = 0.999$).

Particle size distribution of NRW and WRW starch granules during fermentation

The distribution plot of starch granule size was significantly different across fermentation stages (Table 2). The average particle size of NRW starch at different fermentation stages was 27.0–86.3 μm , and that of WRW was 22.3–165.1 μm . The median diameter $d(0.5)$, $d(0.1)$, $d(0.9)$, $d(4,3)$, $d(3,2)$, peak volume (%), and particle size at maximum frequency decreased during fermentation, whereas the specific surface area increased. The functional properties and digestibility of starch were influenced by particle

Table 2 Particle size distribution of waxy rice and normal rice starch of fermentation

Fermentation period	d(0.5) (μm)	d(0.9) (μm)	d(0.1) (μm)	d(4,3) (μm)	d(3,2) (μm)	Peak volume (%)	SSA (m ² /g)	PS-MF (μm)
NRW0	73.2 ± 3.6b	171.6 ± 12b	19.8 ± 1.1a	86.3 ± 4.3b	40.4 ± 3.2a	7.4 ± 0.5a	0.1 ± 0.01f	91.2 ± 6.3b
NRW24	21.3 ± 1cd	56.6 ± 3.9f	5.7 ± 0.3c	27 ± 1.6e	13.2 ± 0.7b	6.3 ± 0.3b	0.5 ± 0.01d	30.2 ± 1.8d
NRW48	23.1 ± 1.1c	84.7 ± 5.9de	5 ± 0.3cd	35.4 ± 2.1d	12.7 ± 0.7bc	4.6 ± 0.2d	0.5 ± 0.01d	52.5 ± 3.1c
NRW72	15 ± 0.7de	69.8 ± 4.8ef	3.9 ± 0.2de	27.6 ± 1.3e	9.5 ± 0.5cd	4.4 ± 0.2ed	0.6 ± 0.01c	7.6 ± 0.4f
NRW96	10.8 ± 0.5e	77.3 ± 5.4e	3.5 ± 0.1e	27.1 ± 1.6e	8 ± 0.4d	5.3 ± 0.3c	0.7 ± 0.01b	7.6 ± 0.4f
WRW0	138.5 ± 9.6a	359.1 ± 32.3a	15.1 ± 0.9b	165.1 ± 9.9a	39.1 ± 2.3a	6.5 ± 0.3b	0.2 ± 0.01e	275.4 ± 22a
WRW24	14 ± 0.7de	52.8 ± 3.6f	4.4 ± 0.2d	22.3 ± 1.3e	9.9 ± 0.5cd	5.2 ± 0.2cd	0.6 ± 0.01c	11.5 ± 0.6e
WRW48	24 ± 1.2c	125.3 ± 6.2c	4.8 ± 0.2cd	47.5 ± 2.8c	12.6 ± 0.7bc	4.1 ± 0.2e	0.5 ± 0.01d	104.7 ± 6.2b
WRW72	18.9 ± 0.9d	92.9 ± 6.5d	4.2 ± 0.2de	36.1 ± 2.1cd	10.7 ± 0.6c	4 ± 0.2e	0.6 ± 0.01c	8.7 ± 0.4ef
WRW96	11.6 ± 0.5e	70.2 ± 6.3e	3.3 ± 0.1e	26.6 ± 1.3e	8 ± 0.4d	4.8 ± 0.2d	0.8 ± 0.02a	7.6 ± 0.6f

NRW normal rice wine, WRW waxy rice wine; 0, 24, 48, 72 and 96 is the fermentation time (h); d(0.5) means median diameter, indicating that 50% particle size is below this value; d(0.9) means 10% particle size exceeds this value; d(0.1) means 90% particle size exceeds this value; d(4,3) means volume mean particle size; d(3,2) means average surface area particle size; SSA: specific surface area; PS-MF: particle size at maximum frequency. Mean ± SD is calculated from triplicated measurements. Values with different letters in the same column are significantly different at $p < 0.05$

size (de la Hera et al. 2013). For normal and waxy barley, the average polymerization of amylopectin decreased with a reduction in particle size, whereas amylose content was unrelated to particle size (Tang et al. 2001). The amylopectin content of WRW during fermentation was higher than that of NRW, corresponding to a higher value for d(4,3). Tang et al. (2002) showed that the small granules of barley starch indicated high swelling power and susceptibility to enzymes, leading to an increase in the hydrolysis rate of starch as the grain size decreased. Comparison between d(0.9) and d(0.5) of starch in WRW and NRW revealed that waxy rice starch is damaged faster than normal rice, indicating a more thorough hydrolysis from a larger initial size with more hydrolysates produced in WRW.

Crystalline structure of NRW and WRW starch during fermentation

Both the NRW and WRW starches exhibited strong diffraction peaks at 15°, 17°, 18°, 20°, and 23° (2θ) with an unresolved double peak at 17° and 18° (2θ), creating an A-type polymorph (Zhou et al. 2015; Qiao et al. 2016; Luo and Shi 2012). The starch granule structure is described as amorphous with a semi-crystalline growth distribution. The amorphous region of starch contains amylose and likely less ordered amylopectin, and the semi-crystalline region of starch is ascribed to double helices formed by amylopectin branches (Vandeputte et al. 2003); however, the amorphous/semi-crystalline ratio seems to be unsolved (Singh et al. 2006). The crystallinity of starch is mainly associated with amylose content (Yu et al. 2012) and the

length distribution of amylopectin branch chains (Gomand et al. 2010). The cooked rice starch was only partly gelatinized owing to low moisture content (approximately 35%) in wet rice before steaming; accordingly, the crystal structure was retained. After fermentation, WRW and NRW starches still exhibited A-type crystallinity (Zhang et al. 2016). The crystallinity of WRW0 (22.3%) was higher than that of NRW0 (20.8%) because waxy rice contains more amylopectin, compared with normal rice (Table 3). Amylose, which forms the amorphous region, was hydrolyzed more quickly than amylopectin, resulting in greater crystallinity during fermentation. The crystallinity of NRW96 starch and WRW96 starch increased to 29.4% and 27.3%, respectively, after fermentation for 4 days. In a previous study, the crystallinity of starch increased during the early stage (6 days) of waxy rice fermentation but decreased gradually as fermentation progressed to 60 days (Zhang et al. 2016). The amylose that debranched from amylopectin exerted no significant influence on crystallinity because despite the increase in amylose content in rice wine, crystallinity still increased.

Short-range molecular order of NRW and WRW starch during fermentation

Fourier transform infrared spectroscopy was applied to analyze the ordered structure of starch granules. The absorption peak at 1047 cm⁻¹ can be attributed to the crystalline region of the starch molecule, representing a short-range molecular ordered structure; the characteristic peak of the amorphous region was located at 1022 cm⁻¹ (van Soest et al. 1995). The short-range molecular order of

Table 3 Crystallinity and short-range molecular order of starch at different fermentation stages in rice wine

Fermentation period	Crystallinity (%)	IR ratio of 1047/1022 cm^{-1}
NRW0	20.8 $\pm$ 1.1f	1.0043 $\pm$ 0.0045b
NRW24	24.8 $\pm$ 1.3d	1.0019 $\pm$ 0.0028b
NRW48	25.5 $\pm$ 1.2c	1.0000 $\pm$ 0.0011b
NRW72	25.9 $\pm$ 1.2c	1.0075 $\pm$ 0.0018ab
NRW96	29.4 $\pm$ 1.4a	1.0103 $\pm$ 0.0052a
WRW0	22.3 $\pm$ 0.9e	1.0099 $\pm$ 0.0044a
WRW24	25.7 $\pm$ 1.1c	1.0038 $\pm$ 0.0023b
WRW48	25.8 $\pm$ 1.2c	1.0000 $\pm$ 0.0019b
WRW72	27.3 $\pm$ 1.3b	1.0008 $\pm$ 0.0021b
WRW96	27.3 $\pm$ 1.1b	1.0100 $\pm$ 0.0048a

NRW normal rice wine, WRW waxy rice wine; 0, 24, 48, 72 and 96 is the fermentation time (h). Mean $\pm$ SD is calculated from triplicated measurements. Values with different letters in the same column are significantly different at $p < 0.05$

starch ($1047/1022 \text{ cm}^{-1}$) in NRW and WRW first decreased and then increased during fermentation (Table 3). During gelatinization, the ordered structure was gradually broken down into amorphous structural components. The highly branched starch molecule in WRW was higher than that in NRW in the short-range molecular order. The relative degree of order of starch decreased progressively in the early stage of fermentation, indicating that amylopectin was debranched into short chains, thereby damaging the ordered parts. After fermentation for 48 h, the infrared absorption peak at 1047 cm^{-1} represents an increase in double helices in several crystallites, illustrating that irregular cluster structures were degraded by enzymes and further increased the proportion of the internal ordered structure. No apparent change was observed in the ordered structure on the outer regions of the starch granules during enzymolysis, which could be attributed to the high resistance of the highly ordered structure of starches to enzymatic hydrolysis (Sevenou et al. 2002) and the formation of a polymer network folded and crystallized by amylose chains (Singh et al. 2007).

Correlation analysis

Rice starch was degraded into dextrins and maltose and then into glucose by amylase in *koji*, showing a significantly negative correlation between water-soluble sugar and total starch content ($r = -0.993$). Sweetness was positively correlated with water-soluble sugar content ($r = 0.842$) and negatively correlated with total starch ($r = -0.815$). Owing to the short chains debranched during hydrolyzation, amylopectin plays an important role in sweetness, contributing to a sweeter taste in WRW than in NRW. However, water-soluble sugar and amylose exhibited no significant correlation because of amylose degradation and amylopectin debranching. Amylose in the

amorphous region decomposed faster than amylopectin, resulting in an increase in crystallinity; thus, crystallinity was negatively correlated with total starch content ($r = -0.940$) and positively correlated with water-soluble sugar ($r = 0.938$) and sweetness ($r = 0.941$).

Glucose was continuously fermented to ethanol by yeast strains as water-soluble sugar was produced, reflecting a significant positive correlation between ethanol content and sweetness ($r = 0.978$) during fermentation. Ethanol and short-range molecular order were also significantly positively correlated ($r = 0.915$) owing to amylose decomposition. Median particle size $d(0.5)$ was positively correlated with total starch degradation ($r = 0.947$) and negatively correlated with water-soluble sugar ($r = -0.906$) and crystallinity ($r = -0.897$).

Chewiness, gumminess, and adhesiveness exhibited significant positive correlations with $d(0.5)$ ($r = 0.994$, 0.998 , and 0.979 , respectively) and negative correlations with crystallinity ($r = -0.823$, -0.870 , and -0.868 , respectively); meanwhile, stickiness and stringiness were each negatively correlated with $d(0.5)$ ($r = -0.988$ and -0.995 , respectively) and positively correlated with crystallinity ($r = -0.878$ and -0.899 , respectively). Textural properties were also influenced by chemical compounds; for example, adhesiveness was negatively correlated with water-soluble sugar ($r = -0.922$).

Conclusion

The total starch contents of NRW and WRW decreased as α/β -amylase and glucoamylase activities increased during fermentation. The relative crystallinity of starch was negatively correlated with total starch and positively correlated with water-soluble sugar and sweetness. After fermentation, the water-soluble sugar contents in WRW and NRW

were 194.4 and 169.3 mg/g, respectively, and ethanol contents were 8.9% and 6.5%, respectively, indicating that the quality of taste of WRW was superior to that of NRW. The hardness, adhesiveness, chewiness, gumminess, and stickiness of vinasse in NRW and WRW markedly decreased after fermentation. The gumminess and chewiness of vinasse in WRW were higher than those in NRW because of higher amylopectin content, suggesting that the palatability of WRW was higher than that of NRW.

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