Structural, pasting and sensory properties of rice from main and ratoon crops

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**ABSTRACT**

Ratooning was a practical approach to increase the grain yield of rice. In this research, main and ratoon crop rice were compared in quality attributes, including composition, starch morphology, starch crystalline structure, protein components, pasting property, texture, and sensory attributes of cooked rice. As compared to main crop rice, ratoon crop rice showed an increase in amylase content and a decrease in protein content. Both main and ratoon crop rice starches exhibited similar polyhedral shape and A-type crystalline structure, but the ratoon crop rice had a lower relative crystallinity. Globulin content of ratoon crop rice protein was higher than that of main crop rice protein. As compared to main crop rice flour, ratoon crop rice flour showed a decrease in pasting temperature, peak viscosity, and breakdown viscosity but an increase in final viscosity and setback viscosity. After cooking, ratoon crop rice had higher hardness, cohesiveness, gummyiness, and resilience than main crop rice. In sensory evaluation conducted with trained panelists, ratoon crop rice scored higher than main crop rice in overall acceptance.

**Introduction**

Rice (\textit{Oryza sativa} L.) is one of the most widely cultivated cereal crops, serving as a staple food source for nearly half of the world’s population.\textsuperscript{[1]} Rice grains can be directly cooked and can also be developed into various kinds of foods, such as rice noodles, steamed rice bread, and rice puffs.\textsuperscript{[2–4]} Rice is mainly cultivated in warm regions of the world, for example, Asia, Africa, and the Rice Belt of the United States.\textsuperscript{[5]}

Grain yields of cereal crops must be increased to meet the requirements of the growing population of the earth and to maintain global food security,\textsuperscript{[6]} for example, supplying cereal foods during the Covid-19 epidemic in 2020. There are several ways to enhance the rice grain yield. Breeding green varieties that require less resource input and have high-yield potential is an ideal strategy, which has achieved considerable success in recent decades.\textsuperscript{[7,8]} Crop management practices can also be optimized to enhance the production of rice.\textsuperscript{[9,10]} In recent years, there has been growing interest in using ratoon crops to produce rice grains.

Rice ratooning refers to the production of a second rice crop from the stubble after the harvest of the main crop.\textsuperscript{[11]} Postharvest ratooning turns out to be a practical approach for achieving increased production levels with limited labor input and could increase the total grain yield by 58.3%.\textsuperscript{[12]} Torres et al.\textsuperscript{[13]} obtained a rice grain yield of more than 3.5 t/ha from the ratoon crops. Compared with
traditional cropping mode, ratoon cropping of rice has higher efficiency in the use of irrigation water and lower emissions of greenhouse gases (i.e. methane and nitrous oxide), which pose a smaller environmental burden.\textsuperscript{[12,14]} The areas suitable for planting ratoon rice are those where the cumulative temperature is surplus for traditional single cropping rice but insufficient for double cropping rice.\textsuperscript{[15]} Many countries have cultivated ratoon rice, for example, Japan, the Philippines, Brazil, and China.\textsuperscript{[16]}

Main crop and ratoon crop differ in growth temperature conditions. For example, in Hubei, China, the grain-filling stage is in summer for main rice crop but is in fall for ratoon rice crop. Wang et al.\textsuperscript{[17]} reported that the change in atmospheric temperature during the grain-filling stage can affect the activities of granule-bound starch synthetase and starch branching enzyme of rice grains, resulting in the change of amylose content. Chun et al.\textsuperscript{[18]} found that increasing grain-filling temperature would decrease the amylose content of rice grains and increase the protein content. Liang et al.\textsuperscript{[19]} reported that the activities of glutamine synthetase, glutamate synthase, and glutamate pyruvate transaminase increased as grain-filling temperature increased, contributing to the increase of protein content in rice grains. However, there have been few studies reporting the differences of main and ratoon crops in rice quality. This research, therefore, was aimed at comparing the quality attributes of rice from these two crops. This research can provide reference for the government to make policy on popularization of rice-ratooning technique.

Materials and methods

Materials

Two varieties of Indica rice (Quanyou822 and Fengliangyouxiang1) were used in this research, which were coded as Qy and Flyx. For each variety, rough rice of main crop and ratoon crop grew in the same farm field in Honghu (Hubei, China). Rice seedlings were transplanted into the paddy field in April 2019. The main and ratoon crops were harvested in August 2019 and November 2019, respectively. Rice grains were dehulled (JLGJ 4.5 Rice huller, Taizhou Grain Meter Factory, Taizhou, China) and then milled (JNM-3 rice polishing machine, Sinograin Chengdu Storage Research Institute, Sichuan, China) for 50 s. For Qy variety, white rice samples of main and ratoon crops were coded as Qy-MC and Qy-RTC, respectively. For Flyx variety, white rice samples of main and ratoon crops were coded as Flyx-MC and Flyx-RTC, respectively. For all rice types, a portion of white rice was milled to flour using a JXFM110 hammer mill (Shanghai Jiading Grain and Oil Equipment Co., Ltd., Shanghai, China) for analysis of composition, starch structure, protein component, and pasting property. Chemicals were of analytical grade without otherwise stated.

Proximate analysis

The total starch content of rice was determined enzymatically (AACC1 method 76–13.01) using the total starch assay kit from Megazyme International Ireland Ltd. (Wicklow, Ireland). The amylose content of rice was determined according to the method of Lin et al.\textsuperscript{[20]} The total nitrogen content of rice was determined by AOAC 920.87 and was used to calculate protein content by a conversion factor of 6.25. The fat content of rice was determined according to ACC Method 30–20.01. The ash content of rice was determined according to AACC Method 08–01.01.

Morphology and crystalline structure analysis of starch

Starch was isolated from rice according to the method of Wang et al.\textsuperscript{[21]} The isolated starches were coated with gold in a vacuum evaporator, and then viewed by an S-3000 N scanning electron microscope (SEM, Hitachi High-Technologies Corp., Tokyo, Japan) at an accelerating voltage of 15 kV. The crystalline structures of starches were determined with an Xpert PRO diffractometer (PANalytical B.V., Almelo, The Netherlands), using a Cu Kα radiation source at λ of 0.154 nm.
(40 kW and 40 mA). X-ray diffraction (XRD) patterns with diffraction angle (2θ) range 3–40° were obtained at a scanning speed of 10° min⁻¹ and a scanning step of 0.033°.

**Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of rice protein**

SDS-PAGE was performed according to the method of Zhao et al. [22] with slight modification. Each sample (50 mg) was stirred in 1 mL of extraction buffer (0.01 M Tris–HCl, pH 6.8, including 10% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 0.1% (w/v) bromphenol blue). Samples were heated for 5 min at 100°C, and then centrifuged for 5 min at 8000 × g. Electrophoresis was conducted with 12% separating gel and 5% stacking gel. Sample volumes of 7 μL were loaded into each well and electrophoresis was performed at 120 V during the run. The gel was stained with 0.25% (w/v) Coomassie brilliant blue, and de-stained in 10% acetic acid.

**Determinations of protein components**

Contents of rice protein components (albumin, globulin, prolamin, and glutelin) were determined according to the method of Chandi and Sogi [23] with slight modification. Briefly, rice flours were defatted with n-hexane and then dried. Dried flours were extracted sequentially with distilled water, 5% NaCl, and 70% ethanol to extract albumin, globulin, and prolamin, respectively. After each extraction, one batch of the residue was dried in a freeze dryer. The protein content of residues was determined using Dumas combustion method (conversion factor 6.25), which was used to calculate the contents of four components in rice protein.

**Pasting property determination**

A Rapid Visco Analyzer (RVA, Newport Scientific Pty, Ltd., Warriewood, Australia) was used to determine the pasting properties of rice flour. Viscosity of rice flour suspensions (12%, w/w) was recorded using a programmed heating-cooling cycle. The suspensions were maintained at 50°C for 1 min, then heated to 95°C at a rate of 12°C/min, maintained at 95°C for 2.5 min, cooled to 50°C at a rate of 12°C/min, and then maintained at 50°C for 1.5 min.

**Texture analysis of cooked rice**

Rice was cooked according to the method of Pan et al. [24] Ten grams of rice were rinsed twice with distilled water. Water was added to the rice at a volume ratio of 1.3:1. After soaking at 25°C for 30 min, rice was cooked in a steamer for 40 min and simmered for 20 min. Textural parameters of the cooked rice were determined using a TA.XT2i Texture Analyzer (Stable Micro System, Surrey, UK). A two-cycle compression program was conducted at a compression ratio of 70% and a test speed of 1 mm/min.

**Sensory evaluation of cooked rice**

The sensory analyses of cooked rice were performed by 10 trained panelists according to the method of Pan et al. [24] Each panelist had an experience of greater than 2 years in evaluating a wide spectrum of usually cooked rice once every 2 weeks. The cooked rice samples were placed on white porcelain plates, which were coded with random three-digit numbers. The panelists tasted the cooked rice while the samples were hot. The panelists were instructed to clean their palates with water prior to testing a fresh sample. Score was as follows: 20 points for smell, 20 points for appearance, 30 points for palatability, 25 points for taste, and 5 points for cold rice texture. Smell was evaluated by sniffing the aroma of cooked rice before putting it into the mouth. Appearance were evaluated as the surface characteristics (color,
glossiness, and integrity) of cooked rice. Palatability was a comprehensive evaluation of hardness (the force needed to bite through the rice kernels at the first bite), stickiness (the degree of adhesion of rice to the molar teeth during chewing), and elasticity (the resilience force during chewing) of the sample. Taste was a comprehensive evaluation of sweetness and fragrance, and also the purity and durability of the taste. The texture of cold rice was evaluated by assessing the elasticity, stickiness, hardness, and looseness of the cold rice.

**Statistical analysis**

Data are averages of duplicate determinations. An analysis of variance with a significance level of 5% was conducted and Duncan’s multiple-range test was applied to determine differences between means using the commercial statistical package (SPSS, Inc, Chicago, IL, USA).

**Results and discussion**

**Composition**

Compositions of main and ratoon rice are shown in Table 1. The difference of Qy ratoon and main crop rice in starch content was not significant. However, for Flyx variety, starch content of the ratoon crop rice was significantly lower than that of the main crop rice. For both varieties, ratoon crop rice had a higher amylose content than main crop rice. It is widely accepted that the amylose content is one of the main factors determining the eating and processing properties of rice. The different amylose contents of rice of main and ratoon crops could be attributed to the different grain-filling temperatures of the two crops. The grain-filling stages of main and ratoon crops were in summer and fall, respectively. The higher temperature during grain-filling may have affected granule-bound starch synthetase and starch branching enzyme activities and contributed to the lower amylose content in main crop rice. As compared to main crop rice, ratoon crop rice exhibited a significant decrease in protein content. The higher protein content of main crop rice could also be attributed to the higher grain-filling temperature of the crop. Liang et al. reported that increase of grain-filling temperature can affect the activities of nitrogen metabolism-related enzymes (glutamine synthetase, glutamate synthase, glutamate pyruvate transaminase, aspartate aminotransferase, and aspartokinase), resulting in the increase in protein content of rice grains. There were some minor differences between main and ratoon crop rice in fat and ash contents.

**Morphology and crystalline structure of starch**

SEM images of starches isolated from main and ratoon rice are shown in Figure 1, and the XRD patterns of the starches are shown in Figure 2. For all four starches, granules exhibited typical rice starch morphology of polyhedral shape (Figure 1). This observation was in agreement with previous researches in rice starch. Starches of main and ratoon crop rice had a typical A-type crystalline structure, with characteristic peaks appearing at 2θ of 15.0°, 17.0°, 17.9°, and 23.0° (Figure 2). As compared with main crop rice starch, the relative crystallinity of ratoon crop rice starch was significantly decreased (P < .05). The relative crystallinities of starches of Qy and Flyx main crop

| Starch (%) | Amylose (%) | Protein (%) | Fat (%) | Ash (%) |
|------------|-------------|-------------|---------|---------|
| Qy-MC      | 81.48 ± 1.41a | 9.90 ± 0.05b | 8.35 ± 0.06b | 0.28 ± 0.01a | 0.30 ± 0.01a |
| Qy-RTC     | 80.38 ± 3.50b | 16.40 ± 0.08a | 7.86 ± 0.05a | 0.38 ± 0.02c | 0.29 ± 0.04a |
| Flyx-MC    | 81.55 ± 0.31b | 8.40 ± 0.09a | 8.46 ± 0.04b | 0.35 ± 0.01b | 0.30 ± 0.01a |
| Flyx-RTC   | 75.73 ± 1.37a | 14.87 ± 0.05a | 7.91 ± 0.05a | 0.28 ± 0.01a | 0.40 ± 0.01b |

Data were expressed as means ± SD. Means within a column that had the same letter were not significantly different (α = 0.05).
were 38.5% and 39.2%, respectively. However, for starches of ratoon crop rice, the relative crystallinities were reduced to 33.4% and 30.9%, respectively. Chung et al.\[31\] found that the relative crystallinity of rice starch was inversely correlated with amylose content. Cheetham and Tao\[32\] reported similar results in the research of maize starch. In starch granules, amylopectin is thought to be chiefly responsible for the crystalline organization of starch granules, while amylose is generally considered as an amorphous polymer.\[33\]

**Protein components**

SDS-PAGE patterns of proteins of main and ratoon crop rice are shown in Figure 3. Rice albumin has two abundant proteins with molecular weights (MW) of 16 and 25 kDa.\[34\] Rice globulin has polypeptide subunits with MW of 11, 13, 19–22, and 53–56 kDa, with 19–22 kDa subunit being predominant.\[35\] Rice prolamin is composed of three polypeptide groups having MW of 10, 13, and 16 kDa, with the 13 kDa prolamin being predominant.\[36\] Rice glutelin has three major polypeptide subunits with MW of about 30–40 (α-glutelin), 17–21 (β-glutelin), and 51–57 kDa (glutelin precursor).\[35,37\] Some bands of SDS-PAGE pattern might be subunit mixtures of different fractions with similar MW. In general, protein bands of main crop rice were darker than those of ratoon crop rice, implying the higher contents of total protein and protein components of the main crop rice. Liang et al.\[27\] reported that high temperature during grain-filling was beneficial to the accumulation of albumin, globulin, prolamin, and glutelin in rice grains and the enhancement of Asp metabolism might play an important role in the increase of protein components.

The percentages of four protein components (albumin, globulin, prolamin, and glutelin) in total proteins of main and ratoon crop rice are shown in Figure 4. For all rice samples, the most abundant protein component was glutelin, followed by globulin. This result was in consistent with Ju et al.\[38\]

![Figure 1. SEM images of starches isolated from main and ratoon crop rice. (a) Qy-MC; (b) Qy-RTC; (c) Flyx-MC; (d) Flyx-RTC.](image-url)
There were slight differences between main and ratoon crop rice proteins in proportions of four components, depending upon the rice variety. For both varieties, globulin content of ratoon crop rice protein was higher than that of main crop rice protein. Compared with the main crop rice protein, Flyx ratoon crop rice protein showed a significant increase in albumin content, but ratoon crop rice protein of Qy variety had a significant decrease in glutelin content and a significant increase in prolamin content. These results were generally consistent with the results of Liang et al.\cite{27} and Cao et al.\cite{37} Glutelin is the most abundant storage protein component in rice grains and its biosynthesis is sensitive to the grain-filling temperature. As grain-filling temperature changes, the change in glutelin accumulation contributes more to the alteration of total protein content compared with other three fractions.\cite{37} Compared with main rice crop, the ratoon rice crop had a decrease in grain-filling temperature. Though the decrease of grain-filling temperature would suppress the accumulation of all four protein components, the pronounced decrease of glutelin accumulation might lead to increased proportions of other three components in total protein.\cite{27}

**Pasting property of rice flour**

Pasting parameters of main and ratoon crop rice flours are shown in Table 2. For both varieties, pasting temperature (PT) of ratoon crop rice flour was lower than that of main crop rice, which might be due to the decreased relative crystallinity of ratoon crop starch. This result was in consistent with the researches of Chung et al.\cite{31} and Li et al.\cite{39} As compared to main crop rice flour, ratoon crop rice flour showed a decrease in peak viscosity (PV) and breakdown viscosity (BDV). This was consistent with the finding of Varavinit et al.\cite{40} and Guha and Ali.\cite{41} Increasing amylose content can restrict the swelling of starch granules during heating; at the same time, granules can resist disintegration induced by crowding and shearing.\cite{41} Final viscosity (FV) and setback viscosity (SBV) of ratoon crop rice flour
were higher than those of main crop rice flour. It is widely accepted that starch with higher amylose content is easier to retrograde.\textsuperscript{[42, 43]}

**Texture of cooked rice**

Texture attributes of cooked rice are shown in Table 3. After cooking, hardness of ratoon crop rice was higher than that of main crop rice. This result was in accordance with some previous researches. Sing et al.\textsuperscript{[44]} and Yu et al.\textsuperscript{[45]} found hardness of cooked rice was positively related to the amylose content. The harder texture of cooked rice with high amylose content can be ascribed to the retrogradation extent of starch.\textsuperscript{[46]} As discussed above, ratoon crop rice samples contained higher amounts of amylose and had higher SBV values than those of main crop rice samples, thus presenting higher hardness in texture after cooking. For both varieties, cooked product of ratoon crop rice had a lower adhesiveness than that of main crop rice; however, the differences between main and ratoon crop rice in springiness of cooked product were not significant. For other texture attributes, ratoon crop rice exhibited higher cohesiveness, gumminess, chewiness, and resilience in cooked products as compared to main crop rice.

**Sensory attributes of cooked rice**

Sensory attributes of cooked rice are shown in Table 4. As compared to main crop rice, ratoon crop rice showed significant increase in scores of smell, appearance, palatability, and taste after cooking. These results suggested that for freshly cooked rice, the sensory attributes of ratoon crop rice were more accepted by the panelists than those of main crop rice. In previous research, Yu et al.\textsuperscript{[47]} found that palatability of cooked rice was negatively correlated with the protein content. Main and ratoon crop rice did not have
Figure 4. Percentages of four protein components in total proteins of main and ratoon crop rice. For a specific protein component, means with the same letter were not significantly different (α = 0.05).

Table 2. RVA parameters of main and ratoon crop rice flours†.

| Protein Type | PT (°C)   | PV (cP) | TRV (cP) | BDV (cP) | FV (cP) | SBV (cP) |
|--------------|-----------|---------|----------|----------|---------|----------|
| Qy-MC        | 77.88 ± 0.04b | 3544 ± 149c | 1579 ± 103b | 1965 ± 47c | 2439 ± 90a | 859 ± 13b |
| Qy-RTC       | 70.80 ± 0.70ab | 2274 ± 4ab | 1407 ± 13a | 866 ± 18b | 2687 ± 15b | 1280 ± 1c |
| Flyx-MC      | 78.30 ± 0.64b | 3766 ± 47c | 1780 ± 56c | 1986 ± 8d | 2529 ± 53ab | 749 ± 3b |
| Flyx-RTC     | 71.25 ± 0.57a | 2474 ± 175b | 1830 ± 99c | 644 ± 76a | 3182 ± 157c | 1352 ± 58d |

†Data were expressed as means ± SD. Means within a column that had the same letter were not significantly different (α = 0.05). PT: pasting temperature; PV: peak viscosity; TRV: trough viscosity; BDV: breakdown viscosity; FV: final viscosity; SBV: setback viscosity.

Table 3. Texture attributes of main and ratoon crop rice after cooking†.

| Protein Type | Hardness | Adhesiveness | Springiness | Cohesiveness | Gumminess | Chewiness | Resilience |
|--------------|----------|--------------|-------------|--------------|-----------|-----------|------------|
| Qy-MC        | 289.1 ± 14.9b | -63.2 ± 8.5c | 0.83 ± 0.03a | 0.46 ± 0.02b | 134.0 ± 8.8b | 111.2 ± 9.0b | 0.18 ± 0.01a |
| Qy-RTC       | 435.9 ± 24.0d | -37.6 ± 2.8a | 0.88 ± 0.04a | 0.56 ± 0.02d | 242.8 ± 10.1d | 214.0 ± 18.0d | 0.23 ± 0.03b |
| Flyx-MC      | 202.2 ± 17.6b | -51.2 ± 7.4b | 0.81 ± 0.03b | 0.43 ± 0.01b | 87.6 ± 6.8b | 70.9 ± 7.6b | 0.15 ± 0.01b |
| Flyx-RTC     | 335.7 ± 15.6c | -33.2 ± 5.1a | 0.87 ± 0.08b | 0.59 ± 0.03b | 198.7 ± 9.4c | 172.8 ± 15.7c | 0.24 ± 0.03b |

†Data were expressed as means ± SD. Means within a column that had the same letter were not significantly different (α = 0.05).

Table 4. Sensory attributes of main and ratoon crop rice after cooking†.

| Protein Type | Smell | Appearance | Palatability | Taste | Cold rice texture | Overall acceptance |
|--------------|-------|------------|--------------|-------|------------------|--------------------|
| Qy-MC        | 13.9 ± 2.0a | 14.1 ± 1.5a | 20.1 ± 2.0abc | 18.4 ± 1.6a | 3.9 ± 0.7a | 70.4 ± 5.0a |
| Qy-RTC       | 16.5 ± 1.4b | 16.6 ± 1.4b | 22.5 ± 2.0c | 20.6 ± 2.1bc | 3.6 ± 0.5a | 79.8 ± 4.4b |
| Flyx-MC      | 14.4 ± 2.1a | 14.7 ± 1.5a | 18.8 ± 2.3a | 19.0 ± 1.8abc | 3.6 ± 0.7a | 70.5 ± 5.2a |
| Flyx-RTC     | 16.3 ± 1.7b | 16.1 ± 1.4b | 21.8 ± 2.4ac | 21.1 ± 2.6c | 4.0 ± 0.7a | 79.3 ± 5.8b |

†Data were expressed as means ± SD. Means within a column that had the same letter were not significantly different (α = 0.05).
significant difference in cold rice texture. For both varieties, the total sensory score of ratoon crop rice was higher than that of main crop rice. This result is in agreement with some previous researches. Lu and Zhu \cite{48} reported that rice with amylose content of 10–20% had the best sensory attributes. Xu et al.\cite{25} reported that the overall quality of cooked rice was positively correlated with amylose content (range 13.2–20.4%), while its correlation with protein content (range 6.2–9.1%) was negative.

**Conclusion**

Main and ratoon crop rice were different in many quality properties. Ratoon crop rice had higher amylose content but lower protein content than main crop rice. As compared to main crop rice starch, ratoon crop rice starch exhibited a decrease in relative crystallinity. Ratoon crop rice contained higher proportion of globulin in protein than main crop rice. As compared to main crop rice flour, ratoon crop rice flour showed decreased pasting temperature, peak viscosity, and breakdown viscosity but increased final viscosity and setback viscosity. After cooking, ratoon crop rice had greater hardness, cohesiveness, gumminess, and resilience than main crop rice. Ratoon crop scored higher than main crop rice in overall acceptance in sensory evaluation. These results can provide data reference for the government to popularize the ratooning technique.

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**Declaration of competing interest**

All authors declare no conflict of interest.

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