Optimal preparation for gluten-free rice bread

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Abstract In this study, we aimed to elucidate the optimal conditions for baking gluten-free rice bread while considering the powder characteristics of 10 rice flour varieties by optimizing the quantity of water to be added and examining the effects of particle size, protein content, amylose content, and degree of starch damage in rice flour.

Rice breads were prepared with varying quantities of water, and the range of quantity of water that allowed baking varied depending on the variety of rice flour used. We determined that high protein content in rice flour allowed a wide range of quantity of water that can be added to be suitable for bread baking; however, concurrent with this are that the compressive stress of the bread increases as well. Moreover, we examined the effect of ingredient composition on the optimal quantity of water added to ensure the swelling of the rice flour bread and found that the quantity of water that can be added ranged from 15- to 17-fold when the quantity of water to be added was based on the protein content in rice flour. These results indicate that when preparing gluten-free rice bread with a large specific volume, the quantity of water to be added can be determined using the protein content in rice flour as an indicator. Additionally, we found that when the protein content in rice flour is not known, the quantity of water to be added can be adjusted to achieve 10 to 20 Pa loss modulus G’’ of the batter.

Keywords rice flour, gluten-free, bread, powder properties, rupture properties

1. Introduction

Among developed countries, Japan has one of the lowest rates in food self-sufficiency [1]; the improvement of such rates is an urgent issue that must be addressed. As Japan’s self-sufficiency rate for rice, which is the main crop in Japan, is high, the expansion of rice consumption is expected to improve food self-sufficiency rates.

Meanwhile, there has been an increase in food allergies in recent years and, concurrently, an increase in demand for nonallergenic food. The three major allergenic foods are eggs, milk, and wheat [2], and in particular, there is a need for improvement of bread production such that allergic individuals can consume bread with peace of mind. As wheat is the main ingredient of bread and Japan imports most of its wheat supply, the use of rice flour as a substitute to wheat can expand the utility of rice [3–6] and improve our food self-sufficiency.

The consumption of rice flour bread has increased in recent years owing to the government policy “Food Action Japan,” which intends to improve food self-sufficiency rates; however, most rice flour breads available today are intended for expanding rice consumption rather than addressing food allergy problems [4, 5]. Wheat flour in most products has been replaced by rice flour [7], or rice flour has been supplemented with gluten [8–15], and there are only a few bread products completely made with rice flour [16–22]. This is because rice flour bread has lesser capacity to rise and shorter preservation periods than wheat flour bread, as rice flour does not contain gluten.

Despite these disadvantages, the development of gluten-free rice bread that does not contain any of the three major food allergens, namely egg, milk, and wheat, has been advanced [23–27]. Through the course of development, the optimal water content and the rising capacity of bread were reported to vary depending on the rice flour used. As such, preparation conditions must be optimized according to the rice flour to be used. In a study wherein bread was made using rice flour with different powder characteristics, the degree of starch damage in rice flour was stated to be supposedly low [10]. The gelatinized rice flour and rice were reported to improve the texture of rice bread [19, 20, 27–32]. In another study, thickening polysaccharides were
reported to improve baking quality [28]. Moreover, there have been studies in which the effects of enzymes on bread were examined [33, 34]; however, these studies reported repeated trial and error using various rice flours rather than the optimal processing conditions for making bread using rice flour. We are yet to establish a scientific basis for the optimal preparation of rice bread regardless of the rice flour variety used.

Therefore, in this study, we examined the optimal conditions for rice bread preparation according to the powder characteristics of 10 rice flour varieties such that gluten-free rice bread can be prepared regardless of the rice flour variety to be used, by optimizing quantity of water added and examining the effects of particle size, protein content, amylose content, and degree of starch damage in rice flour on baking quality.

2. Method

(1) Materials

Ten rice flour varieties were used: A (Kitoku Shinryo Co., Ltd.), B (Gunma Flour Milling Co., Ltd.), C (Powder Techno Corporation), D (Namisato Co., Ltd.), E (Hinomoto Cereals Flour Milling Co., Ltd.), F (Mitake Food Manufacturing Co., Ltd.), G (Ibaraki Agricultural Research Center), H (Fukumori Bread Research Institute, Ltd.), I (Namisato Corporation), J (Hinomoto Cereals Flour Milling Co., Ltd.).

Rice flour, dry yeast (S. I. Lesaffre (France)), granulated sugar (Mitsui Sugar Co., Ltd.), table salt (The Salt Industry Center of Japan) and olive oil (Japanese Consumers’ Cooperative Union) were used as ingredients of bread.

(2) Ingredient analysis

Water content in rice flour was measured using a halogen moisture analyzer (MB45, Mettler-Toledo International Inc.). For the analysis, 3.0 g of the sample was heated at 105°C, and the weight at which the rate of change in weight becomes 1 mg/60 s or lower was considered the constant weight.

We requested the Food Analysis Technology Center Sunatec to analyze the protein and amylose contents in rice flour.

(3) Measurement of starch damage

The degree of starch damage in each rice flour variety was measured using a starch damage measurement kit (Biocon Ltd., Japan). First, 100 ± 10 mg rice flour in a microtube was weighed, and 0.1 mL fungal α-amylase (1,000 Ceralpha U/mL) was added. The mixture was vigorously mixed with a touch mixer and incubated for 10 min at 40°C to allow the enzymatic reaction to occur. Thereafter, 5 mL of 0.2% (v/v) dilute sulfuric acid was added to terminate the reaction, and the mixture was centrifuged for 5 min at 1000 × g. To 0.1 mL supernatant, 0.1 mL amyloglucosidase (200 U/mL) was added, and the mixture was incubated for 20 min at 40°C to allow degradation. The absorption of the mixture at 510 nm was measured, and the quantity of glucose generated was determined; this was used to calculate the degree of starch damage [10].

(4) Measurement of particle size distribution

The particle size distribution of rice flour was measured using a laser diffraction particle size analyzer (LMS-2000e, Seishin Enterprise Co., Ltd.) at dry mode [34].

(5) Electron microscopy

Platinum-palladium was vapor-deposited on rice flour using an ion sputter (E-10130, Hitachi, Ltd.), and the rice flour was observed using a desktop scanning electron microscope (JCM-600, Jeol Ltd.) at an accelerating voltage of 15 kV and magnification of 3,000.

(6) Analysis of Gelatinization characteristics

The Gelatinization characteristics of rice flour were measured using Rapid Visco Analyzer (RVA-30, Newport Scientific, Inc.). Measurement samples were prepared by mixing the samples with distilled water to a sample concentration of 8% by anhydrous conversion and to a total weight of 26 g. The samples were spun at 160 rpm and 50°C for 1 min; the temperature was increased to 95°C over 7 min, and the samples were incubated at 95°C for 5 min. Afterward, the temperature was decreased to 50°C over 7 min, and a viscosity-time curve was generated from the 20-min observation to determine the temperature at which viscosity starts to increase, as well as to determine the peak viscosity, peak temperature, breakdown, set back, and final viscosity [35].

(7) Preparation of bread samples

The basic composition of each rice flour dough was 8 g granulated sugar, 2 g table salt, 2.7 g dry yeast, and 3 g olive oil per 100 g rice flour. The amount of water added ranged from 50 g to 170 g. Each dough was prepared by adding rice flour, granulated sugar, and table salt into a bowl at 25°C and was mixed; distilled water was added, and the dough was mixed using a spatula until the texture was consistent. The dough was then mixed using a mixer (Aikoh KENMIX Chef Mixer KM-800, Aicohsha MFG. Co., Ltd.) at Setting 1 (rotation of 256 rpm, revolution of 77 rpm) for 1 min, followed by mixing at Setting 3 (rotation of 386 rpm, revolution of 116 rpm) for 4 min; olive oil was then added, and the dough was mixed for 3 min. Thereafter, yeast was added, and the dough was mixed for 2 min.
Finally, 50-g portions of the resulting dough were dispensed onto silicone muffin molds (with an upper diameter of 7 cm and a lower diameter of 5.2 cm) and placed for 20 min in an oven (Combination Range GMO-81300, Tega Sanyo Industry Co., Ltd.) that was preheated to 38°C for primary fermentation. As we observed that the fermentation conditions in the preliminary experiments varied according to environmental conditions (season, humidity, temperature etc.), we adjusted fermentation durations according to when the dough rose over the top of the silicone muffin molds. Subsequently, the doughs were mixed at a speed of 2 rotations/s, punched, allowed 10 min of secondary fermentation, and baked for 14 min at 230°C. The baked breads were stored, covered with a net, and left at room temperature (25°C) until measurements.

(8) Measurement of the specific volume of bread

The weight of breads after cooling for 2 h at 25°C was measured; the apparent volumes were measured using the rapeseed method [36], and specific volumes were calculated.

(9) Analysis of the mechanical properties of bread

A cuboid (W: 20 mm × D: 20 mm × H: 15 mm) cutout of the crumb of the bread was used as a measurement sample for compression tests performed using a creep meter (Rheoner RE-3305S, Yamaden Co., Ltd.). By using an acrylic resin disk-shaped plunger with a diameter of 4 cm, the sample was compressed to 80% of its height at a compression rate of 6 cm/min. We determined the compressive stress at 50% compression and the apparent elastic modulus from the obtained stress-strain curve [22]. The measurements were taken at 25°C.

(10) Viscoelasticity of rice flour batter

Viscoelasticity of rice flour batter was measured using a Rheolograph-Sol (S-1C, Toyoseiki Co., Ltd.). Measurements were taken by imparting a sinusoidal vibration with a frequency of 2.5 Hz and amplitude of ±125 μm to determine the storage modulus G’ and the loss modulus G” [37]. The measurements were taken at 25°C.

3. Results and Discussion

(1) Powder characteristics of rice flour

1) Ingredient composition

Table 1 shows the average particle size and results of ingredient analysis for each variety of rice flour. In the rice flour varieties used, the average particle size varied from 47 to 96 μm, the water content from 10.1% to 13.8%, protein content from 5.2% to 7.0%, and amylose content from 16.7% to 19.8%. The degree of starch damage tended to vary from variety to variety of rice flour, ranging between 2.2% and 14.6%. The jet-milled rice flour varieties A, C, D, E, G, and H showed varying degree of starch damage from 2.2% to 12.7%, with dry types showing higher degree of starch damage than the wet types.

Arisaka et al. considered that the degree of starch damage in the rice flour was related to the physical strength of the milled rice and its internal microstructure, and that the low physical strength of the rice flour, which has a high water content, facilitated the milling process and resulted in less damaged starch grains in the wet milling [38]. In our results, the degree of starch damage for wet type was lower than that for dry type, which may be due to this.

In contrast, the roll-milled rice flour varieties I and J
showed a near-constant degree of starch damage between 7.4% to 8.4%.

2) Particle size distribution

Fig. 1 shows the electron microscopy images and particle size distribution of rice flours with different characteristics. The electron microscope images revealed that particle size and shape vary depending on the type of rice flour. Rice flour varieties with relatively even particle size distribution and those with varying particle sizes were found. In rice flour A, the particle size distribution had a single peak at approximately 50 μm with symmetrical distributions to the left and right. Furthermore, rice flour B had two peaks of distribution at 10 μm and 100 μm, and rice flour F had two peaks at 25 μm and 100 μm.

3) Gelatinization characteristics

Table 2 shows the gelatinization characteristics of rice flour varieties determined by using Rapid Visco Analyzer. Gelatinization characteristics varied significantly depending on the rice flour variety; the maximum viscosity was lowest in rice flour C and highest in rice flour A showing viscosities of approximately 116 mPa·s and 203 mPa·s, respectively, indicating a difference of a little less than twofold. The breakdown was highest in rice flour J and lowest in rice flour D having viscosities of approximately 104 mPa·s and

![Fig. 1 SEM micrograph and particle size distributions of rice flours.](image-url)

**Table 2** Pasting characteristics of rice flour by RVA

<table>
<thead>
<tr>
<th>Rice flour</th>
<th>Maximum viscosity [mPa·s]</th>
<th>Break down [mPa·s]</th>
<th>Final viscosity [mPa·s]</th>
<th>Set back [mPa·s]</th>
<th>Peak time [min]</th>
<th>Pasting time [min]</th>
<th>Pasting temperature [°C]</th>
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<tr>
<td>A</td>
<td>202.8</td>
<td>98.8</td>
<td>210.7</td>
<td>106.7</td>
<td>9.9</td>
<td>3.3</td>
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<tr>
<td>B</td>
<td>171.0</td>
<td>95.6</td>
<td>156.8</td>
<td>81.3</td>
<td>9.7</td>
<td>3.7</td>
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<td>C</td>
<td>116.2</td>
<td>51.4</td>
<td>128.1</td>
<td>63.3</td>
<td>9.8</td>
<td>4.1</td>
<td>68.4</td>
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<tr>
<td>D</td>
<td>157.3</td>
<td>37.5</td>
<td>250.8</td>
<td>130.9</td>
<td>9.9</td>
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<td>E</td>
<td>183.0</td>
<td>100.3</td>
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<td>9.8</td>
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<td>F</td>
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<td>95.9</td>
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<td>6.5</td>
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<td>63.5</td>
<td>171.0</td>
<td>102.9</td>
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<td>3.5</td>
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<td>152.0</td>
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<td>52.1</td>
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38 mPa·s, respectively, indicating a difference of approximately 2.8-fold. The final viscosity was approximately 128 mPa·s in rice flour C and approximately 251 mPa·s in rice flour D, with approximately a twofold difference. The lowest setback was approximately 64 mPa·s in rice flour F and highest in rice flour D with approximately 131 mPa·s; these have an approximately twofold difference. The peak time was between 9.3 to 9.9 min, indicating that there was almost no difference based on the rice flour. The initial gelatinization temperature was lowest in rice flour A at approximately 64°C, indicating a significant difference in the initial gelatinization temperature according to rice flour varieties. Our results indicate that rice flour A had the highest maximum viscosity and final viscosity, was easily gelatinized, and readily expressed viscosity. On the contrary, rice flour C had the lowest maximum viscosity, setback, and final viscosity among all varieties, making it the rice flour with the least viscosity.

(2) Baking quality of gluten-free bread made using rice flour

1) Optimal quantity of water added for each rice flour variety and range of quantity of water added to allow baking

Fig. 2 shows the cross-sectional photographs of bread made using rice flour B and varying quantities of water added. At 70 g quantity of water added, there were no voids, and the bread did not appear bread-like. However, as the cellular structure formed at 75 g quantity of water added, we considered 75 g to be the minimum quantity of water added that can be added. When water content was further increased, the crumb of the bread formed vertical folds at 130 g quantity of water added and did not appear bread-like. As such, the maximum quantity of water added was considered to be 120 g. As the swelling rate was at maximum when the proportion of water added was 100 g, we considered this proportion as the optimal quantity of water added. The range from the minimum to maximum quantity of water added was regarded as the range of water content that can be added to allow tolerable baking.

Fig. 3 shows the optimal quantity of water added for each rice flour variety and the range of quantity of water that allowed baking. Moreover, while the range of quantity of water added to allow baking was narrow for rice flour D, i.e., from 70 g to 100 g, some varieties, including rice flour H (60 g to 160 g), had a wide range of quantity of water added that allowed baking. In rice flour F, bread baking was not possible when the quantity of water added was 110 g, owing to the formation of folds in the inner layers of the bread. These results
indicated that without strict control of the quantity of water added, swelling cannot be achieved in rice flour D, whereas bread baking can be achieved in rice flour H even if the water content deviated slightly from the optimal quantity of water added.

2) Relationship between the various properties of rice flour and optimal quantity of water added and the viscoelasticity of the dough with optimal quantity of water added

To determine which properties of rice flour affected the optimal quantity of water added, we examined the relationship between powder characteristics and optimal quantity of water added for the 10 varieties of rice flour. As shown in Fig. 4, the optimal quantity of water added that made bread baking possible was affected by the degree of starch damage in rice flour, and the optimal quantity of water added increased as higher degree of starch damage was achieved. Furthermore, the results indicate that the optimal quantity of water added is not markedly affected by the average particle size, variability in particle size (standard deviation of particle size), protein content, and amylose content in rice flour.

Fig. 5 shows the relationship between the powder characteristics of rice flour and the loss modulus of dough with optimal quantity of water added. No relationship between loss modulus $G''$ and average particle size, variability of particle size (standard deviation of particle size), protein content, amylose content, and degree of starch damage was identified. This indicated that the viscoelasticity of the dough is affected largely by the quantity of water added rather than the powder characteristics of the rice flour.

3) Specific volume and 50% compression stress of bread with optimal quantity of water added

Table 3 shows the water content, specific volume, and physical property parameters of bread made using different rice flour varieties in optimal quantity of water added conditions. The water content in bread when the optimal quantity of water added ranged from approximately 43% to 54%, due to differences in the quantity of water added to each sample.

The minimum and maximum specific volumes of bread were 1.85 and 2.51, respectively, and tended to vary depending on the variety of rice flour. According to a report on bread made using rice flour produced using different milling methods by Yoza et al., the specific volume of rice flour bread ranged between 1.98 to 3.82 when 20% gluten was added [10], and the bread prepared in our study was almost equivalent in specific volume as that in the past report, despite the bread in the present study being gluten-free. Additionally, despite the low degree of starch damage and low batter viscosity prior to gelatinization in rice flour varieties A, B, and E, a high specific volume of the resulting breads was achieved.

In addition, the 50% compression stress were as low as approximately $6 \times 10^3$ Pa and as high as approximately $15 \times 10^3$ Pa, indicating that 50% compression stress can differ by 2.5-fold depending on the rice flour variety used.
Although it is conceivable that the hardness of bread varied because the optimal quantity of water added varied according to sample, low 50% compression stress was observed in the bread produced using rice flour varieties A, E, and H; however, the water content in these breads was not significantly different from that in breads made using other rice flour varieties. We surmised that this is because these breads had relatively large specific volumes and contained large amounts of air internally. On the contrary, the bread made using the rice flour varieties G was determined to be significantly harder than the breads made using other rice flour varieties, despite not having markedly different water content and specific volume than other breads; these two varieties of rice flour had higher protein content than other rice flour varieties, thereby affecting the hardness of the solid phase when compressed. We suggest that protein content made the final bread product hard.

4) Relationship between the various properties of rice flour and the specific volume of bread

The specific volume of bread is reportedly affected by the powder characteristics of rice flour, and in particular, the swelling rate of bread tends to be high when the degree of starch damage is low [10]. Fig. 6 shows the relationship between the powder characteristics of rice flour and the specific volume of bread. The results clearly indicated that the specific volume of rice flour bread was affected by the amylose content and that specific volume tended to be larger as the amylose content in rice flour became higher. In this study, we showed that the specific volume of bread was not significantly affected by degree of starch damage in rice flour, average particle size, variability in particle size (standard deviation of particle size), or protein content. The lack of correlation between the average particle size of rice flour and specific volume of bread was consistent with the results reported by Yoza et al. [10].

5) Protein content in rice flour and range of quantity of water added that allow baking

Rice flour varieties with narrow ranges of quantity of water that can be added to allow rice flour bread baking and other varieties with wide ranges of the quantity of water that can added are shown in Fig. 3. To determine the differences in the ease of making bread among rice flour varieties, we examined the protein content in each rice flour

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<th>Table 3 Characteristics of rice flour breads</th>
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variety. In wheat flour, the network structure of gluten plays an important role in bread formation. We surmised that even though rice flour does not contain gluten, proteins may play a key role.

The relationship between the protein content in rice flour and the range of quantity of water added to allow bread baking is shown in Fig. 7. The range of quantity of water added that made bread baking possible was determined by subtracting the minimum quantity of water that can be added from the maximum quantity of water that can be added. The rice flour varieties examined in our study were largely classified into two types based on ranges of quantity of water added at approximately 6% of protein content in rice flour. In other words, the range of quantity of water added to allow bread baking was small when the protein content in rice flour was below 6%, and this range was large when the protein content in rice flour was above 6%, thereby allowing bread baking in the latter case.

6) Protein content and loss modulus $G''$ of batter with optimal condition of water added.

As the range of quantity of water that can be added to make baking possible varies depending on the protein content in rice flour, we calculated the protein content in the batter made using optimal quantity of water added. In addition, Fig. 8 shows the results of loss modulus $G''$ of bread made using optimal quantity of water added; these results indicated that for each variety of rice flour, the protein concentration in batter was approximately 3%. Furthermore, the loss modulus $G''$ of the batter when optimal quantity of water added was used ranged from 10 to 20 Pa except for rice flour F and H. These results indicate that if the protein content in rice flour is known and a certain quantity of water added such that protein content in batter will be approximately 3%, it would be possible to prepare the optimal bread using such rice flour. Moreover, we determined that when the protein content in rice flour is not known, adding water such that the batter would have loss modulus $G''$ of 10 to 20 Pa will allow the preparation of a near-optimal bread. However, as the rice flours F and H had
low protein concentration in their resulting batter, we surmised that the gelatinization of starch was inhibited by protein; further studies are needed in the future.

7) Effect of ingredient composition on the optimal quantity of water added for rice bread swelling

To examine the effect of ingredient composition on the optimal quantity of water added, we plotted the water content in batter applied based on the degree of starch damage (X-axis) against the specific volume of bread (Y-axis), as shown on the left panel of Fig. 9. On the right panel is a plot of the water content in batter applied based on protein content against the specific volume of bread. When applied based on the degree of starch damage, the quantity of water that can be added varied by 10- to 50-fold depending on the variety of rice flour; however, when applied based on protein content, the quantity of water that can be added varied by 15- to 17-fold. In a study, it was reported that rice flour with only a small degree of starch damage should be used to prepare rice bread [8]; however, we found that the optimal quantity of water to be added is affected more by protein content than the degree of starch damage in rice flour and that the dough should be prepared with quantity of water added that is approximately 15- to 17-fold the protein content.

8) Relationship between protein content in rice flour and rupture property of bread

To determine the effect of the protein content in rice flour on the quality of the bread, we examined the relationship of the protein content in rice flour with the compressive stress of bread at 50% compression (Fig. 10). A high protein content in rice flour was correlated with high com-
The protein content in rice flour is not known. To adjust the loss modulus of the batter to 10 to 20 Pa if the protein content in rice flour is known, or a quantity of water that would be 15- to 17-fold the protein content of water is used; however, we found that low protein content of flour with high protein content allowed easier bread baking as it widened the range of quantity of water that can be added for making bread baking possible is narrow and compressive stress, which resulted in bread that is hard and chewy.

Aoki et al, studied the properties of rice flour bread and rice flour characteristics, in which no correlation was found between the protein content of rice flour and the hardness of the bread [39]. In this report, the protein content ranged from 5.0 to 11.4 in a wide range, whereas the protein was almost gluten, which suggests that a correlation was not found.

4. Conclusion

We elucidated the optimal preparation conditions for baking rice bread using a variety of rice flours and identified that it is important to consider protein content in rice flour.

We found that the range of quantity of water that can be added for making bread baking possible is narrow and makes baking difficult when rice flour with low protein content is used; however, we found that low protein content enables the production of soft bread. In contrast, using rice flour with high protein content allowed easier bread baking as it widens the range of quantity of water that can be added to achieve baked breads.

We found that the optimal conditions for baking gluten-free rice bread include the addition of an optimal quantity of water of approximately 15- to 17-fold the protein content in rice flour when known, or a quantity of water that would adjust the loss modulus of the batter to 10 to 20 Pa if the protein content in rice flour is not known.

As such, to prepare gluten-free rice flour bread, the quantity of water added should be determined using protein content in rice flour as an indicator.

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Conflict of interest There is no conflict of interest in this study.

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