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Effect of ultra-high pressure treatment on quality of glutinous rice flours prepared with various water contents

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Abstract

In the present work, the glutinous rice flours prepared using ultra-high pressure (UHP) treatment at 200 MPa (100 – 1,000 MPa range) were compared with the glutinous rice flour prepared using the conventional wet process. Results indicated that the protein and ash contents of the UHP-prepared glutinous rice flour were higher than those of the traditionally prepared glutinous rice flour. Low field nuclear magnetic resonance (LF-NMR) analysis demonstrated that the application of UHP treatment facilitated the penetration of water into the inner structure of glutinous rice granules, resulting in a more uniform distribution of water within the rice. The brightness of glutinous rice flour obtained through the wet process was initially higher compared to that of glutinous rice flour treated with UHP. However, the brightness of the wet-processed flour gradually increased as the water content increased. The wet process resulted in glutinous rice flour with the lowest content of damaged starch, whereas the degree of starch granule damage in glutinous rice flour prepared by UHP decreased gradually as the water content increased. Scanning electron microscopy (SEM) revealed that at 200 MPa, UHP-damaged starch with a moisture content of 32% exhibited similar characteristics to that of the wet process, with relatively regular and uniform particle morphology. No additional peaks or chemical groups were detected in the FTIR analysis. The present work could have the potential to offer novel approaches for the production of glutinous rice flour.

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Introduction

The yield of glutinous rice in the overall rice production in China may be relatively low; however, it holds significant importance in people's daily lives (Zhou *et al*., 2013). Traditional Chinese festive foods frequently incorporate various glutinous rice products, such as *zongzi* (stuffed glutinous rice wrapped in bamboo leaves) during the Dragon Boat Festival, *nian gao* (rice cake) during the Spring Festival, and *ba bao fan* (eight treasure rice) and *yuanxiao* (glutinous rice ball / sweet dumpling) during the Lantern Festival (Ren *et al*., 2018; Wang *et al*., 2019).

In recent years, there has been an increasing application range for glutinous rice, including a wide variety of glutinous rice snack foods such as glutinous rice cakes, puffed glutinous rice cakes, and instant glutinous rice flour (Chen *et al*., 2017; Zhang *et al*., 2021). The introduction of quick-frozen foods, particularly the large-scale industrial production of quick-frozen dumplings, has led to extensive production and utilisation of glutinous rice (Chen *et al*., 2023). Glutinous rice is processed into glutinous rice flour, which serves as a raw material in various aspects of food processing, aligning with the development of the modern food industry, and contributing to the expansion of the application range

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of glutinous rice flour. Currently, the glutinous rice flour available in the market is produced using the water-grinding method, which has the drawback of wasting protein resources, and excessive water discharge.

The emergence of UHP technology offers a new possibility for the preparation of glutinous rice flour. UHP technology involves sealing food in a high-pressure container, and applying high pressure (100 – 1,000 MPa) to the materials using water or advanced hydraulic oil as the medium (Wang, 2013). Liu *et al*. (2014) primarily investigated the impact of UHP on the physicochemical properties of glutinous corn and cassava starches. They discovered that glutinous corn starch readily underwent gelatinisation under UHP. Stute *et al*. (1996) mentioned in their study that different types of starch exhibited varying levels of tolerance to UHP. Type A starch was the least resistant to high pressure, while Type B starch was the most resistant, and Type C fell in between. Glutinous rice belongs to the class A crystal form, and is primarily composed of branched starch, making it susceptible to gelatinisation under UHP.

Currently, there is limited research on the preparation of glutinous rice flour using UHP, domestically and internationally. Therefore, in order to improve product quality, and protect the environment, the present work conducted experiments with different water content levels (20, 25, 30, 32, and 35%) in glutinous rice. By comparing the quality of glutinous rice flour prepared using UHP treatment with that of the traditional wet process, the present work aimed to establish a theoretical basis for producing glutinous rice noodles using UHP.

Materials and methods

Materials

Songxiang paddy rice (a japonica rice cultivar) was obtained from Yihai Jiali Food Co. Ltd. The rice had 17% amylose content. Prior to use, foreign substances were removed by 10-mesh standard test sieves.

Sample preparation

Glutinous rice with varying water contents (20, 25, 30, 32, and 35%) was prepared after removing broken particles and impurities. The samples were then vacuum-packed in high-density polyethylene bags with distilled water, maintaining a water-to-glutinous rice ratio of 1:1. These packed samples were placed in a cylindrical loading container, and subjected to UHP treatment at ambient temperature for 30 min, and 200 MPa. Subsequently, the glutinous rice was dried in an oven at 40°C until the final moisture content of the sample reached approximately 14%. The dried samples were then ground into flour, and passed through 100-mesh sieves (Zhao *et al*., 2017). Finally, the processed samples were sealed in a bag, and stored at 4°C in a refrigerator for further analyses.

At the same time, the control group consisted of glutinous rice flour prepared using the traditional wet-process. The production steps for this control group were as follows: the glutinous rice was washed three times with distilled water, the washed rice was soaked for 12 h, the soaked rice was ground into a slurry using a slurry grinder, the rice slurry was filtered, and the filter cake was crushed, dried at 40°C until the moisture content reached approximately 14%, passed through a 100-mesh sieve, and the residue left on the sieve was considered the finished product.

Chemical analysis

Moisture (AACCI 44-15.02), ash (AACCI 08-01.01), and protein (AACCI 46-30.01) contents were determined by international approved methods.

Low-field nuclear magnetic resonance (LF-NMR)

The proton relaxation of rice grains after subjecting them to 200 MPa UHP treatment was investigated using LF-NMR. Ten grains of equal size were placed in a 10 mm nuclear magnetic tube. The instrument's temperature was kept constant at 32°C during the measurement. The Carr-Purcell-Meiboom-Gill (CPMG) sequence was employed to analyse the water distribution in the rice grains.

Colour

The colour of the glutinous rice flours treated at 200 MPa and subjected to grinding was determined using a Chroma Meter (CR 400/410, Konica Minolta, Japan). Colour difference analysis provides three indicators: L*, a*, and b*. L* represents brightness, a* represents red-green, and b* represents yellow-blue.

Damaged starch (DS)

The content of DS was determined using approved methods, specifically the alpha amylase method (GB/T 9826-2008).

Microstructure

The glutinous rice flour was stored in an electro-thermostatic blast oven at 40°C to be dried until it reached a solid state. The dried flours were then attached to a specimen holder using an aluminium plate, and coated with gold in a vacuum evaporator. The cross-sections were subsequently observed using SEM (S-4800, Hitachi, Japan) operated at an accelerating voltage of 3.0 kV.

Fourier-transform infrared spectroscopy

The glutinous rice flour was prepared using a 200-mesh screen and 200 MPa in the wet process. After preparation, the samples were mixed with KBr, and compressed into tablets for further analyses. Spectra were collected at a resolution of 4 cm^{-1}, and an average of 15 scans per sample (Zhao *et al.*, 2017).

Statistical analysis

All experiments were conducted in triplicate, and standard errors were determined for each experiment (Zhao *et al*., 2017). Data processing and analysis were performed using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA).

Results and discussion

Basic components analyses

The basic component analyses were conducted on glutinous rice flours obtained using 200 MPa and glutinous rice flour obtained through the grinding method. Table 1 shows that the moisture content of glutinous rice flour is influenced by the processing methods and other factors (Zhang *et al*., 2020). The moisture content of glutinous rice flour prepared using the wet-process was 14.31%, and significantly higher than that of flour subjected to 200 MPa.

The water contents of glutinous rice flours prepared at 200 MPa increased as the water content increased. However, there was no significant difference between the measured water contents of 32 and 35%, both of which were lower than the moisture content of wet-process glutinous rice flour. Therefore, it can be concluded that the moisture content of glutinous rice flour prepared using UHP is influenced by the moisture content of the glutinous rice and the treatment method (Ngamnikom and Songsermpong, 2011).

Table 1 shows that the ash content of glutinous rice flour treated with 200 MPa was higher compared to that of glutinous rice flour processed using the wet method. The wet process involved soaking treatment, which resulted in more effective removal of dust particles, leading to the lowest ash content. The ash content remained high even when the water and wet grinding treatment were continued at a moisture content of 20% and 200 MPa. However, as the moisture content increased, the ash content gradually decreased, although it still remained higher than that of wet-process glutinous rice flour (Zhao *et al*., 2017; Lin *et al*., 2021). This could have been due to the possibility that even when the raw materials were mixed with water during the preparation of glutinous rice flour at 200 MPa, the dust particles were not thoroughly washed off.

Table 1 shows that the protein content reached its peak at 32% when subjected to 200 MPa. The primary factor contributing to the loss of protein is likely the washing and soaking procedures employed during processing. Glutinous rice contains four types of protein: gluten, gliadin, globulin, and albumin. Albumin, being water-soluble, is particularly susceptible to loss during washing and soaking. Consequently, the protein content of wet-process glutinous rice flour was lower compared to that of glutinous rice flour processed at 200 MPa.

Table 1. Basic components of glutinous rice flours prepared at 200 MPa pressure and wet-process glutinous rice flour.

Method	Water	Ash	Protein
Wet-process	14.31 ± 0.01^a	0.25 ± 0.02^a	6.86 ± 0.02^d
20% - 200 MPa	10.2 ± 0.02^e	0.7 ± 0.01^b	7.2 ± 0.01^b
25% - 200 MPa	10.33 ± 0.01^d	0.66 ± 0.03^c	7.23 ± 0.02^b
30% - 200 MPa	11.3 ± 0.02^c	0.6 ± 0.01^d	7.21 ± 0.01^b
32% - 200 MPa	12.52 ± 0.01^b	0.53 ± 0.02^e	7.31 ± 0.02^a
35% - 200 MPa	12.62 ± 0.02^b	0.52 ± 0.01^f	7.16 ± 0.01^c

Means followed by different lowercase superscripts in similar column are significantly different ($p < 0.05$).

Water distribution

The proton relaxation times of glutinous rice and glutinous rice under 200 MPa high-pressure treatment are depicted in Figures 1A and 1B, respectively. The distribution of water is expressed in terms of transverse relaxation time (T2). The T2 value exhibited a negative correlation with water binding degree, and a positive correlation with water activity.

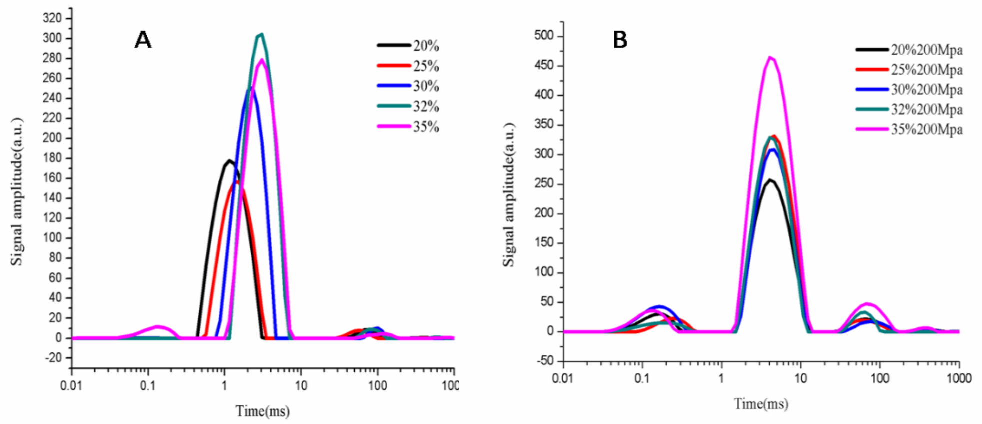

Figure 1. T2 relaxation time of glutinous rice **(A)** and glutinous rice with 200 MPa ultra-high-pressure treatment **(B)**.

Figure 1A illustrates the proton relaxation time of glutinous rice. As the moisture content increased, the overall trend of the image shifted to the right. This could have been due to the continuous infiltration of water into the glutinous rice granules with the increase in water content. Consequently, the relaxation peak increased. Notably, the peak intensity significantly increased in samples with moisture content above 30%, compared to those with 20 and 25% moisture contents. Additionally, the relaxation time primarily shifted from 0.1 - 1 to 1 - 10 ms. This shift indicates a transition from tightly bound to weakly bound water.

Figure 1B presents the proton relaxation time of glutinous rice under 200 MPa high-pressure treatment. The relaxation peak intensity was higher compared to that of glutinous rice. This suggested a higher content of tightly bound water, weakly bound water, and free water. The range of variation in water content was similar to that of glutinous rice. The increase in relaxation peak could have been due to the promotion of even water distribution into glutinous rice particles by the high-pressure treatment. The increase in free water content might have been due to the saturation of hydrogen bonds. When hydrogen bonds reach saturation, the remaining water cannot form new hydrogen bonds, and exists in the form of free water. The increase in tightly bound water might have been due to the interaction between water and materials, such as large molecules like starch and protein, as well as the orientation of hydrogen bonds. The hydroxyl group of starch molecules exhibits a high degree of hydration with water molecules, leading to an increase in its content (Peng *et al*., 2017; Li *et al*., 2018).

Colour

Table 2 shows that the change in colour of wet-process and 200 MPa pressure glutinous rice flour was primarily characterised by a decrease in brightness L* value, and an increase in yellow b* value. The numerical values indicated that wet-process glutinous rice flour was whiter, suggesting that the milling process significantly affected the colour and lustre of the flour. Wet-process glutinous rice flour exhibited greater brightness compared to high-pressure prepared glutinous rice flour. This is because the wet glutinous rice flour underwent continuous grinding with water during the preparation process, which might have resulted in the loss of water-soluble proteins, vitamins, and pigments (Chen *et al*., 2023). Consequently, wet-process glutinous rice flour appeared whiter than high-pressure prepared glutinous rice flour. The b* value of UHP treated glutinous rice flour was higher than that of wet glutinous rice flour. This difference might have been due to oxidation-reduction and Maillard reactions during mechanical milling preparation. As a result, the brightness of 200 MPa high pressure prepared glutinous rice flour was lower than that of the wet process (Asmeda *et al*., 2016).

Table 2. Colour difference of glutinous rice flours prepared at 200 MPa pressure and wet-process glutinous rice flour.

Samples	L*	a*	b*
Wet-process	96.34 ± 0.02^a	-0.37 ± 0.01^a	3.56 ± 0.03^d
20% - 200 MPa	95.31 ± 0.01^b	-0.41 ± 0.01^c	6.32 ± 0.03^a
25% - 200 MPa	95.17 ± 0.01^b	-0.40 ± 0.01^b	6.28 ± 0.01^b
30% - 200 MPa	94.52 ± 0.02^d	-0.41 ± 0.01^c	6.33 ± 0.02^a
32% - 200 MPa	95.05 ± 0.03^c	-0.42 ± 0.02^d	6.30 ± 0.03^b
35% - 200 MPa	95.21 ± 0.01^b	-0.42 ± 0.02^d	6.24 ± 0.03^c

Means followed by different lowercase superscripts in similar column are significantly different ($p < 0.05$).

Damaged starch

Damaged starch has high-water absorption capacity, and amylase easily reacts with damaged starch, thereby affecting the processing quality of glutinous rice flour. Figure 2 provides a visual representation of the difference in broken starch content between glutinous rice flour prepared using the wet-process and 200 MPa UHP methods, indicating that the powder processing method affected the content of broken starch. Figure 2 also demonstrates that the damage rate of wet-process glutinous rice flour is 3.1%. Furthermore, with an increase in water content, the damage rate of glutinous rice flour after 200 MPa UHP treatment decreased from 6.6 to 3.5%. This suggested that the broken content of wet-prepared glutinous rice flour was lower than that of 200 MPa UHP-prepared glutinous rice flour.

During the production of glutinous rice flour, the outer cell wall of starch is partially damaged due to thermal and mechanical friction, resulting in the damage of starch particles (Ma *et al.*, 2016; Xu *et al.*, 2018). The wet-process method of producing glutinous rice flour involves continuous grinding of the sample with water, with a large amount of water added. This method significantly reduces the contact and friction with the instrument, thereby minimising the damage rate (Tong *et al.*, 2017). In the process of preparing glutinous rice flour with 200 MPa pressure assistance, a relatively small amount of water is added initially. As a result of high mechanical heat production during the grinding process, the content of damaged starch also increases. From Figure 2, it can be observed that at a moisture content of 35%, the damaged starch content obtained under 200 MPa UHP treatment is the lowest and closest to that obtained by the wet-process method. This may be because the 200 MPa UHP treatment allowed sufficient water to enter the glutinous rice, thus softening the rice particles, and facilitating grinding. Additionally, UHP may disrupt the hydrogen bonds, thereby reducing the amount of damaged starch.

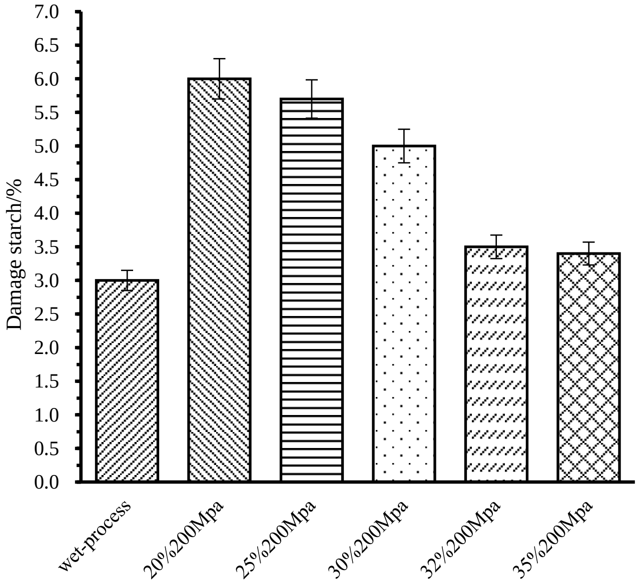

Figure 2. Damaged starch content of glutinous rice flours.

SEM

The morphology of damaged starches after wet grinding and UHP treatment is depicted in Figures 3A - 3F. Compared to glutinous rice flour prepared under 200 MPa UHP treatment, the glutinous rice flour particles obtained through the wet process exhibited a uniform size distribution, regular shape, and distinct edges and corners. Under 200 MPa, the water content was below 30%, resulting in irregularly shaped and inconsistently sized glutinous rice flour particles. However, when the water content exceeded 30%, the size and shape of the glutinous rice flour particles became relatively regular and uniform, aligning with the content of broken starch obtained previously.

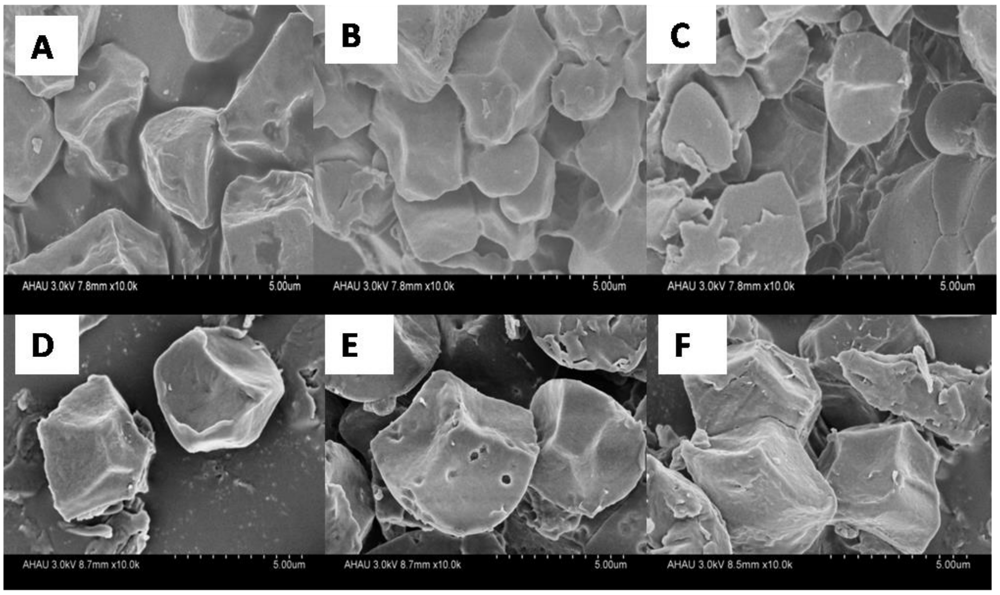

Figure 3. SEM of glutinous rice flours after wet grinding and UHP treatment: **(A)**: wet grinding; **(B)**: 20% moisture content; **(C)**: 25% moisture content; **(D)**: 30% moisture content; **(E)**: 32% moisture content; and **(F)**: 35% moisture content.

Fourier-transform infrared spectroscopy

Figure 4 shows the infrared spectra of glutinous rice flour that was prepared using various milling processes. It can be observed from Figure 4 that the position and shape of the characteristic peak in the wet-process glutinous rice flour samples and the processed glutinous rice flour samples do not exhibit significant differences. Furthermore, no new characteristic peak or group was observed, suggesting that the UHP treatment was a physical method.

There were three typical absorption peaks observed in glutinous rice starch, specifically at 3000 - 4000 cm^{-1}, 3300 - 2700 cm^{-1}, and 1640 cm^{-1}, which corresponded to OH, CH, and CH_2, respectively. The absorption peak at 2930 cm^{-1} corresponded to C-H stretching vibration, while the peak at 3000 - 4000 cm^{-1} corresponded to intermolecular hydrogen bonding. The O-H stretching vibration peak near 1600 cm^{-1} is attributed to the bending vibration of water molecules, which varies with moisture content. The absorption peak around 1030 cm^{-1} is caused by C-O stretching vibration and C-C skeletal vibrational bending. Protein characteristic absorption peaks were observed at 1650 and 1540 cm^{-1}. The characteristic absorption peaks were acyl I (1580 - 1720 cm^{-1}) and amide II (1580 - 1720 cm^{-1}), as both wet and UHP preparations of glutinous rice flour contained starch and protein. Figure 4 shows that the O-H vibration peak of wet-process glutinous rice flour is at 3385 cm^{-1}, while that of high-pressure glutinous rice flour is at 3410 cm^{-1}. This difference might have been due to the higher protein content in UHP-prepared glutinous rice flour, as the excessive presence of protein weakens the hydrogen bonds between starch molecules.

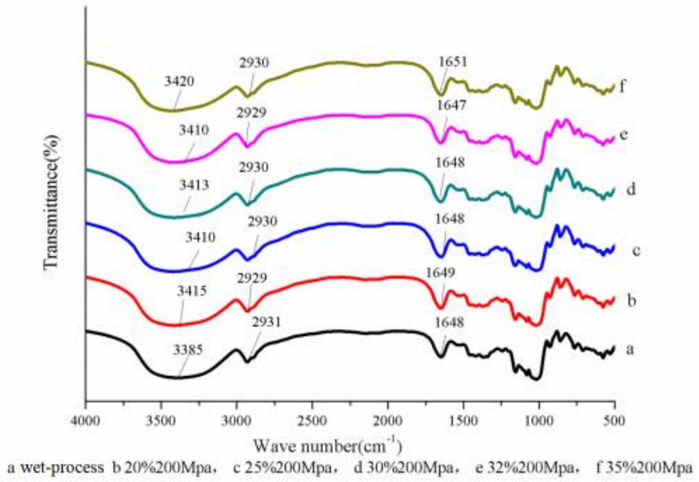

Figure 4. Infrared spectrum of glutinous rice flours.

Conclusion

The results obtained from low-field nuclear magnetic resonance demonstrated that subjecting the sample to 200 MPa UHP treatment led to an increase in the intensity of the relaxation peak. This treatment also resulted in higher levels of tightly bound water, weakly bound water, and free water, indicating that UHP treatment would facilitate the penetration of water into glutinous rice particles, resulting in a more uniform distribution of water. The analysis of broken starch content revealed a gradual decrease in broken starch content as the moisture content increased from 20 to 35%, with the lowest broken starch content observed in glutinous rice flour treated with 200 MPa at 35%. Additionally, particle size analysis showed that D10, D50, and D90 continuously decreased as the water content increased under 200 MPa UHP treatment. When comparing the composition of glutinous rice flour, it was found that the protein, crude starch, and ash contents were higher in glutinous rice flour prepared under 200 MPa UHP treatment compared to wet processing. The brightness of wet-processed glutinous rice flour was higher than that of UHP-treated flour, while the b* value of glutinous rice flour treated with 200 MPa UHP was higher than that of wet-processed flour. Examination with SEM revealed that starch granule damage decreased with increasing water content in glutinous rice flour treated with 200 MPa UHP. FTIR analysis showed that the O-H stretching vibration peak of wet glutinous rice flour was at 3395 cm^{-1}, whereas that of UHP-treated flour was around 3420 cm^{-1}, indicating a higher protein content in UHP-treated flour, and suggesting better nutrient retention.

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