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Research Article

Physicochemical Properties of Rice Flour Suspension Treated by Ultrahigh Hydrostatic Pressure

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This study investigated the physicochemical properties of rice flour suspensions under ultrahigh hydrostatic pressure (UHP) treatment. Rice flour suspensions were subjected to 200, 400, and 600 MPa of pressure for 10 min, and heat treatment was used as a control. Proximate characteristics of different rice cultivar were analyzed to amylose, damage starch content, and particle size. Changes in physicochemical properties of rice flour suspensions according to UHP treatment were analyzed to microscopic structure, iodine reaction, α-amylase hydrolysis rate, and resistant starch content. Microscopic structural analyses showed that the structures of the rice flours were altered under both heat and 600 MPa treatment conditions. Water absorption rates were highest under heat treatment (467.53–554.85%), followed by 600 MPa treatment (269.55–334.57%). Iodine reaction values increased with increasing applied pressure. α-Amylase hydrolysis rates and resistant starch contents were highest under heat treatment and increased with increasing applied pressure. Based on these results, 600 MPa treatment of rice flour suspensions was shown to be comparable to heat treatment; as a result, the development of the new rice processing method with different physicochemical properties is expected from rice cultivars treated under UHP processing methods.

1. Introduction

Ultrahigh hydrostatic pressure (UHP) refers to pressure above the maximum pressure (110 MPa) found in nature. UHP treatment involves sealing a liquid or solid food into a flexible container and applying high pressure via water as a pressure transfer medium. Pressure applies equally to all parts of a sample, regardless of size, shape, or time. And pressurization leads to extinction of microorganisms, changes in enzymatic reaction rates, reversible and irreversible inactivation of enzymes, and gelation of proteins and starches [1].

Nonthermal processes are evolving as potential alternatives to thermal and chemical operations in food-processing methods. UHP is a typical nonthermal process for food preservation. Pressure is a quantifiable energy, like temperature is to heat; therefore, pressure can be used as a thermodynamic factor of changes in the state of a material. However, unlike heat treatment, covalent bond formation and breakage does not occur under high pressure treatment, which affects only noncovalent bonds, retaining nutrients at room temperature [2]. UHP technologies have been developed extensively for food processing and can address the needs of modern consumers who prefer minimally processed foods [3].

Rice (*Oryza sativa* L.) crop serves as a staple food of Korea, Asia, Africa, and Latin America and is an important grain consumed as more than half of the calories per day in

Asia. However, rice consumption has steadily decreased due to the emergence of alternative and various instant processed foods. Recently, rice varieties with improved functionalities and processabilities have been developed to promote rice consumption. To this end, it is therefore necessary to develop and materialize new properties and varieties of rice. Processed type rice, distinct from normal type rice, is developed for the industries of rice cakes, noodles, baking, and brewing [4]. Therefore, differences in starch structure and gelatinization properties between these two types of rice exist. Most rice is processed by gelatinization through heat treatment, but recently, methods of gelatinization through nonthermal treatments have been studied. Therefore, it is necessary to confirm the properties of rice cultivars undergoing nonthermal treatment gelatinization. It is reported that starch gelatinization occurs at applied pressures of more than 400 MPa [5].

Therefore, the aims of this study were to investigate the physicochemical properties of rice flour suspensions under UHP treatment and develop a correlation between the gelatinization and physicochemical properties of rice cultivars under this method.

2. Materials and Methods

2.1. Materials and Suspension Preparation. The rice used in this study was normal type cultivar *Samkwang* and processing type cultivars *Shingil* and *Hangaru*. The rice cultivars were provided by the Rural Development Administration (Jeonju, Jeollabuk-do, Republic of Korea). *Samkwang* is hard rice, with a polygonal starch structure. *Shingil* and *Hangaru* are soft rice, with round starch structures, like those of wheat starch. Corn starch (Tureban Co., Ltd., Goyang, Republic of Korea) was purchased from the market and used as a control. The three rice cultivars were dry milled using Air Mill (MCM-3, Nara machinery Co., LTD., Tokyo, Japan). The suspensions were composed of distilled water at concentrations of 15% [6].

2.2. Measurement of Amylose Content. Amylose content was evaluated using the method described by Juliano [7]. The rice flours (100 mg) were placed in volumetric flasks, and 1 mL of 95% ethanol and 9 mL of 1 M NaOH were added. The suspensions were heated in a boiling water bath for 10 min, followed by cooling to room temperature. The flasks were then filled to the 100 mL mark with distilled water. A 5 mL aliquot of each diluted sample was collected. Then, 1 mL of 1 N acetic acid and 2 mL of 2% iodine solution (0.2% w/v I^2 in 2% KI) were added, and the solutions were adjusted to 100 mL with distilled water. The samples were evaluated using a spectrophotometer at a wavelength of 620 nm. Different concentrations of amylose, free of amylopectin (Sigma-Aldrich, St. Louis, MO), were used to create a calibration curve.

2.3. Measurement of Damage Starch and Particle Size. Damaged starch content analyses were performed according to the AACC method [8] using a Megazyme kit (Megazyme, Wicklow, Ireland). To 100 mg of each rice flour sample, 50 U/mL of α-amylase (Megazyme, Wicklow, Ireland) was added. The mixtures were then reacted at 40°C for 10 min, followed by addition of 0.2% sulfuric acid. After centrifugation, amyloglucosidase (Megazyme, Wicklow, Ireland) was added to the supernatants to calculate the amounts of damaged starch present based on glucose contents generated after reaction at 40°C for 10 min.

The particle sizes of the rice flours were measured using a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). The rice flours were measured at a 0.03% concentration using ethanol as a dispersion solvent and expressed as an average particle size (μm). Also, particle RI was measured at 1.52 and dispersant RI at 1.36 conditions.

2.4. Ultrahigh Hydrostatic Pressure Treatment. UHP treatment was performed using an ultrahigh pressure instrument (nonstirred autoclave system; Ilshin Autoclave Inc., Daejeon, Republic of Korea). The suspensions were packed without air in polyethylene film (New Pack, Seoul, Republic of Korea) and treated under pressures of 200, 400, and 600 MPa and 25°C for 10 min. Heat treatment at 90°C for 30 min was used as a control.

2.5. Measurement of Microscopic Structure. Microscopic structural analyses were performed on the UHP treatment suspensions. The samples were dried using a freeze dryer, then mounted on a scanning electron microscope (SEM) stub with double-sided adhesive tape, and coated with gold. Scanning electron micrographs were obtained using an ULTRA PLUS instrument (Carl Zeiss AG, Oberkochen, Germany).

2.6. Measurement of Moisture Absorption. Moisture absorption was measured using a modified method by Medcalf and Gilles [9]. The ultrahigh pressure and heat treated samples (1 g) with distilled water (40 mL) were placed in 50 mL centrifuge tubes and centrifuged at 3,500 rpm for 30 min. The precipitates, obtained by centrifugation, were weighed to calculate moisture absorption as a ratio of sample weight prior to immersion.

2.7. Measurement of Iodine Reaction. Iodine reaction was measured using a modified method by Williams et al. [10]. To a 400 mg aliquot of each suspension, 0.5 N of KOH (10 mL) was added and decentralized. Then, distilled water was added to a final volume of 100 mL. To 10 mL aliquots of each diluted solution, 5 mL of 0.1 N HCl and 0.5 mL of iodine solution (0.2 g I^2 + 2.0 g KI/100 mL distilled water) were added and mixed. Distilled water was then added to a final volume of 50 mL. After standing at room temperature for 20 min, the absorbance of each solution was measured at 625 nm.

2.8. Measurement of α-Amylase Hydrolysis Rate. α-Amylase hydrolysis rates were assessed using a modified method by Xue et al. [11]. Approximately, 50 mg of each sample was diluted in 2 mL of 0.2 M phosphate buffer (pH 6.9). Then, 0.5 mL of amylase buffer, prepared by dissolving 60 mg of pancreatic α-amylase (30 U/mg; Sigma-Aldrich, St. Louis, MO) in 50 mL of 0.2 M phosphate buffer (pH 6.9), was added. The samples were incubated at 37°C for 2 h. Reducing sugars were estimated with 3,5-dinitrosalicylic acid reagent (Sigma-Aldrich, St. Louis, MO). After incubation, 4 mL of 3,5-dinitrosalicylic acid reagent was added to each sample. The samples were then heated at 100°C for 5 min and cooled to room temperature. To 300 μL of each sample, 900 μL of distilled water was added and mixed. Then, the samples were analyzed using a spectrophotometer at a wavelength of 550 nm. Different concentrations of maltose were used to generate a calibration curve, and α-amylase hydrolysis was expressed in terms of mg of maltose released/g of the sample.

2.9. Measurement of Resistant Starch Content. Resistant starch content analyses were performed using a Megazyme Resistant Starch Assay Kit (Megazyme, Wicklow, Ireland). Four milliliters of mixed α-amylase and amyloglucosidase (Megazyme, Wicklow, Ireland) solution was added to 0.1 g of each sample, and the mixtures were left in a water bath (KMC-12055W1; Vision Scientific, Daejeon, Republic of Korea) at 200 rpm and 37°C for 16 h. Thereafter, 4 mL of 99% ethanol (Daejung Chemicals & Metals Co., Ltd., Gyeonggi, Republic of Korea) was added to stop the enzymatic reaction. After centrifugation (3,000 rpm, 10 min), 2 mL of 2 M KOH was added to the precipitates, which were then stirred for 20 min in an ice water bath. Neutralization was performed by adding 1.2 M sodium acetate buffer (pH 3.8; Sigma-Aldrich, St. Louis, MO) and 0.1 mL of amyloglucosidase to the samples, followed by heating in a water bath at 50°C for 30 min. After centrifugation (3,000 rpm, 10 min), 3 mL of glucose oxidase/peroxidase reagent (Megazyme, Wicklow, Ireland) was added to 0.1 mL of each supernatant and reacted for 20 min in a 50°C water bath. Absorbances were measured at 510 nm to determine glucose contents which were converted to resistant starch contents.

2.10. Statistical Analyses. All analyses were performed in triplicate and expressed as mean ± SD. For statistical analyses, SPSS statistical software (Statistical Package for Social Science, Ver. 12.0; SPSS Inc., Chicago, IL) was used to calculate the mean and standard deviation of each treatment group, and Duncan's multiple range test was used to determine significance.

3. Results and Discussion

3.1. Amylose Content, Damaged Starch Content, and Particle Size of the Raw Rice Flours. The amylose contents of the rice flours depending on cultivars are shown in Table 1. The amylose content of corn starch was the highest, 27.80%, while the *Shingil* cultivar had a significantly higher content, 22.98%, than the other rice cultivars. These results are similar to those reported by Guo et al. [12], in which the amylose contents of rice and corn starch were approximately 20 and 28%, respectively. According to the Rural Development Administration, the amylose contents of *Samkwang*, *Shingil*, and *Hangaru* cultivars are 18.3, 23.4, and 19.2%, respectively, which are similar to the values obtained in this experiment. Damaged starch content was significantly high in the *Samkwang* cultivar, 5.86%, while corn starch had the lowest content, 0.49%. Damaged starch is a small particle that breaks off of starch granules during crushing. The *Samkwang* cultivar had a higher damaged starch content than the other cultivars because *Samkwang* is a hard rice, while *Shingil* and *Hangaru* are soft rice. According to the study by Choi et al. [13], rice with low hardness has a lower amount of damaged starch. The particle size of *Samkwang* was the largest, 56.50 μm, while corn starch was significantly smaller, 39.28 μm. Particle size and damaged starch content of rice flour are known to affect water binding forces and gelatinization [14, 15]. These differences in the raw rice flours depending on the amylose content, damaged starch content, and particle size of the different cultivar are believed to affect their quality properties of rice flour suspension according to heat or UHP treatment.

Table 1: Proximate compositions, amylose content, damaged starch content, and particle size of different rice flours.

Rice cultivars	Amylose (%)	Damaged starch (%)	Particle size (μm)
Samkwang	$18.95 \pm 0.15^{1)c}$	5.86 ± 0.42^{a}	56.50 ± 3.30^{a}
Shingil	22.98 ± 0.80^{b}	4.29 ± 0.33^{b}	46.10 ± 3.20^{b}
Hangaru	19.71 ± 0.68^{c}	3.79 ± 0.31^{b}	52.90 ± 3.70^{a}
Corn starch	27.80 ± 0.59^{a}	0.49 ± 0.08^{c}	39.25 ± 3.32^{c}

[1)]Values are mean ± SD. $n = 3$. [a–c]Different small letters in the column indicate a significant difference by Duncan's range test ($p < 0.05$) according to the sample.

3.2. Microscopic Structure. Scanning electron micrographs of the samples from the different treatment methods are shown in Figure 1. In the untreated samples, the *Samkwang* cultivar has a polygonal starch shape and the *Shingil* and *Hangaru* cultivars have round shapes, like those of wheat starch [16, 17]. When heat or UHP (600 MPa) treatments proceeded, it was confirmed that the structure of the starch collapsed and was irreversibly deformed. Choi et al. [18] showed that as starch becomes gelatinized, its structure unravels and collapses, such that its original structure cannot be observed. The deformations observed for the starch particles under pressure were similar to research trends presented by Li et al. [19] and Katopo et al. [20] for starch deformation under 600 MPa treatment.

3.3. Moisture Absorption. Moisture absorptions for the rice flours under heat and UHP treatments are shown in Figure 2. Moisture absorptions for the heat treatment samples were the highest, occurring in the following order: corn starch > *Samkwang* > *Hangaru* > *Shingil*, with values of 554.85, 519.45, 483.03, and 467.53%, respectively. Moisture absorption rate measured the amount of water penetrated into interior or adsorbed on the surface of starch particles, and these abilities enhanced with increasing the amorphous part,

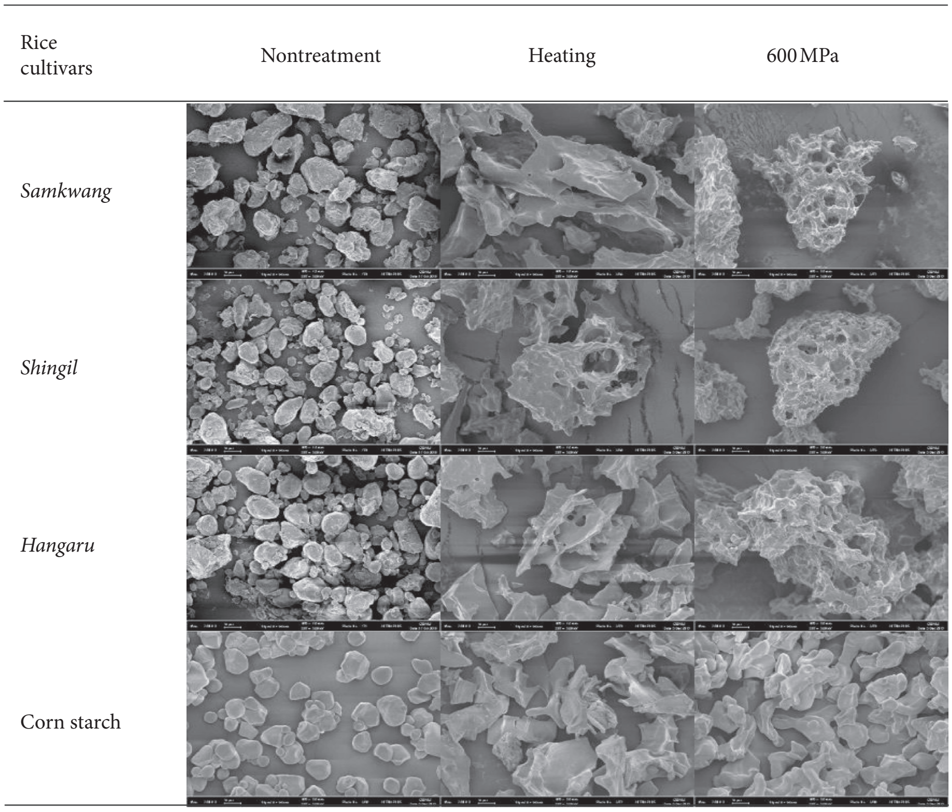

Figure 1: Scanning electron microscope of different rice flours by treatment methods (×200).

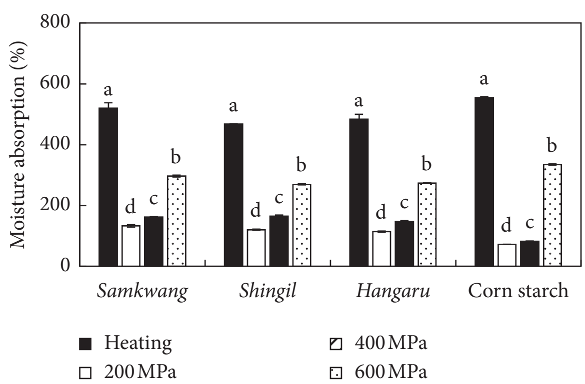

Figure 2: Moisture absorption of different rice flours by treatment methods. Values are mean ± SD. $n = 3$. Different small letters in the same items indicate a significant difference by Duncan's range test ($p < 0.05$) according to the treated method.

which is the branching part of amylopectin of the starch [5]. Therefore, *Samkwang* cultivar, which relatively has low amylose cultivar, was the highest value in moisture absorption rate. Overall, UHP treatment showed lower moisture absorption than heat treatment. The *Samkwang* cultivar had a significantly high value, 132.85%, while corn starch had a low value, 72.27%, under 200 MPa treatment. As the applied pressure increased, moisture absorption increased. Corn starch had a significantly high value, 334.57%, while *Shingil* had a low value, 269.55%, under 600 MPa treatment. Moisture absorption is expressed as the sum total of water absorbed to the amorphous portion of the particles, their surfaces, and interconnection between them. When starch is gelatinized via heating, water penetrates the starch infrastructure, forming hydrogen bonds with hydroxyl groups, causing the starch granules to expand [21]. Similarly, when pressure is applied to a starch suspension, water molecules penetrate the starch granules, form hydrogen bonds, and lead to particle expansion [6]. However, gelatinization via pressure has been reported to inhibit volume growth [22]. Therefore, moisture absorption is considered to be low.

3.4. Iodine Reaction. Results of the iodine reactions of the rice flours under heat and UHP treatments are shown in Figure 3. The absorbances of untreated samples were high, following the order: corn starch > *Shingil* > *Hangaru* > *Samkwang*, with values of 0.648, 0.357, 0.500, and 0.341, respectively. Upon heat treatment, the absorbances of the samples decreased. Comparatively, UHP treatment samples showed an increase at 200 MPa, then decreased with increasing pressure. *Shingil*, which has a high amylose content, had the highest absorbance values. In the case of heat treatment, the absorbance values decreased due to dissolution of the chain structures of amylose. In the UHP treatment, as the pressure increased, the absorbance tended to decrease. The decreased absorbance with increased applied pressure was due to amylose leaching from swollen starch molecules [23, 24]. Amylose binds to lipids to form an amylose-lipid complex, which seems to reduce iodine response [6].

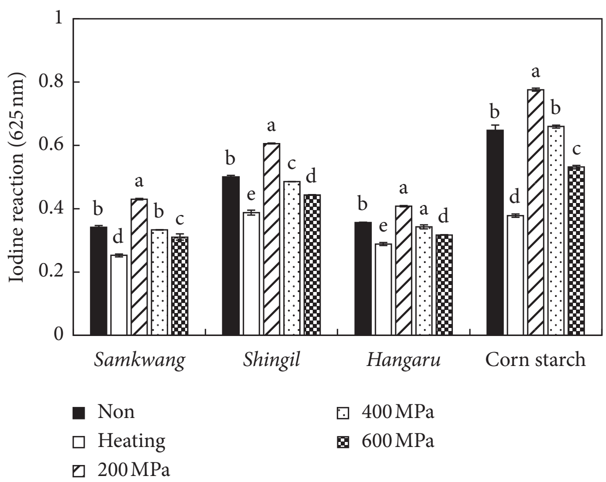

Figure 3: Iodine reaction (625 nm) of different rice flours by treatment methods. Values are mean ± SD. $n = 3$. Different small letters in the same items indicate a significant difference by Duncan's range test ($p < 0.05$) according to the treated method.

3.5. α-Amylase Hydrolysis Rate. The α-amylase hydrolysis rates of the rice flours under heat and UHP treatments are shown in Figure 4. The α-amylase hydrolysis rates of the untreated samples were 4.81–6.73%. Under heat treatment, hydrolysis rates increased with starch gelatinization. Corn starch had the highest rate, 55.76%, and new road cultivation had the lowest rate, 50.20%. Gelatinized starch is easily digested by starch degrading enzymes due to the complete release of starch molecules [12]. According to Selmi et al. [25], enzymatic hydrolysis of corn starch shows a higher hydrolysis rate under heat treatment compared to 600 MPa treatment. However, after a period of time, UHP treatment hydrolysis rates increase to heat treatment levels. Li et al. [26] also reported that hydrolysis of starch suspensions has a negative correlation to amylose content, which is thought to be due to increased enzymatic resistant through formation of amylose-lipid compounds [27].

3.6. Resistant Starch Content. The resistant starch contents of the rice flours under heat and UHP treatments are shown in Figure 5. Untreated samples had the highest resistant starch contents, following the order: corn starch > *Shingil* > *Hangaru* > *Samkwang*, with values of 2.08, 0.87, 0.86, and 0.74%, respectively. Heat treatment samples showed a significantly high value for corn starch, 3.19%, and a low value for *Hangaru* cultivar, 2.53%. However, there were no significant content differences among the rice cultivars. Among the UHP treated samples, the resistant starch contents increased with increasing pressure, with corn starch being the highest. Resistant starch content has a positive correlation to amylose content because degraded amylose consists of linear molecules of α-1,4-d-glucan [28]. After UHP treatment, it is reported that starch deforms to a type-B structure, reducing its sensitivity to enzymes [29]. According to Li et al. [30], the resistant starch content of corn starch immediately increases after high hydrostatic pressure treatment, which is similar to the results observed in this experiment.

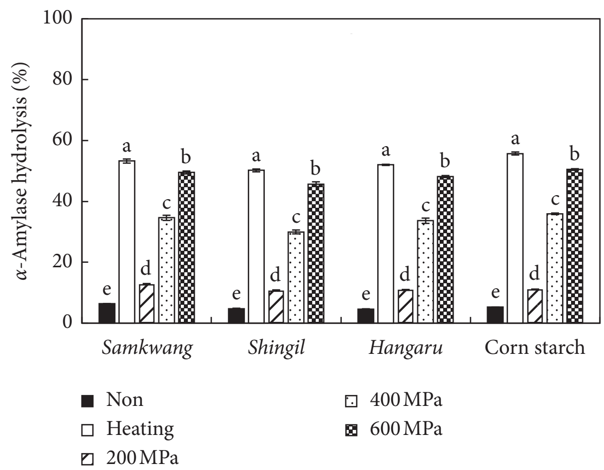

Figure 4: α-Amylase hydrolysis of different rice flours by treatment methods. Values are mean ± SD. $n = 3$. Different small letters in the same items indicate a significant difference by Duncan's range test ($p < 0.05$) according to the treated method.

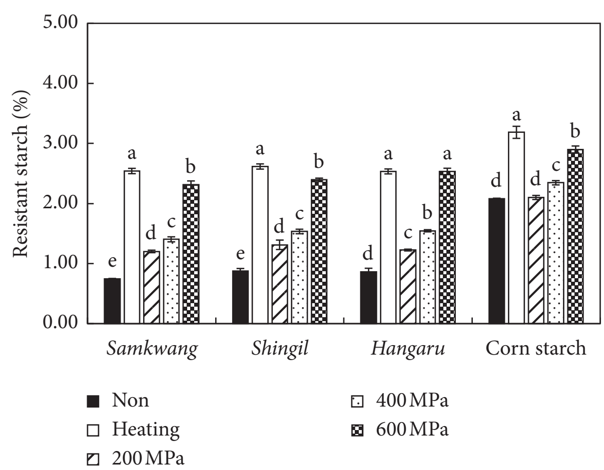

Figure 5: Resistant starch content of different rice flours by treatment methods. Values are mean ± SD. $n = 3$. Different small letters in the same items indicate a significant difference by Duncan's range test ($p < 0.05$) according to the treated method.

4. Conclusion

This study investigated the physicochemical properties of rice flour suspension according to cultivars and the food-processing method (heat and UPH treatment). Normal type (*Samkwang* cultivar) and processing type (*Shingil* and *Hangaru* cultivars) rice was shown to have different starch structures, and especially *Shingil* showed the highest amylose content (22.98%) with round shape of microscopic structure like those of wheat starch. A comparison of heat and UHP treatments of rice suspensions showed the moisture absorptions of heat treatment samples (467.53–554.85%) were higher than those of UHP treatment samples (72.27–334.57%). As the applied pressure was increased, iodine reaction values decreased below those of the untreated samples and hydrolysis rates of α-amylase increased to 45.69–50.48%, which were lower than those of the heat treatment samples (50.20–55.76%). The hydrolysis rate of α-amylase was lowest for the *Shingil* cultivar, which contained a high amylose content, while little difference between the *Samkwang* and *Hangaru* cultivars was observed. Resistant starch contents of the UHP treatment samples were also similar to those of the heat treatment samples (2.53–3.19%). Therefore, development of raw materials for new foods with different physicochemical properties is expected from rice cultivars via UHP processing.

Data Availability

No data were used to support the findings of this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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