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Indigestible Carbohydrate Contents and Physical Properties of Goami2 harvested at the Maximized Milling Quality

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Abstract Milling qualities and indigestible carbohydrate fractions (ICF) depending on harvesting time of Goami2 (G2), mutant of Ilpum (IP) rice, was examined. Fifty days after heading (DAH) maximized head rice milling quality (57.69%) and ICF content (5.09±0.36 g/100 g). ICF contents and physical properties of G2 and IP at 50 DAH were compared. ICF of G2 was three times higher than that of IP (1.61±0.09 g/100 g). Parboiling treatment increased ICF of G2 to 7.18±0.16 g/100 g. G2 showed lower water absorption index, which could lower pasting properties, but higher water solubility index, implying it contains more soluble components. Texture properties of G2 were different from those of IP, showing higher hardness, and lower adhesiveness and cohesiveness. Positive correlation was observed between ICF and hardness, but reverse correlation between ICF and cohesiveness.

Keywords: Goami2 rice, milling quality, indigestible carbohydrates, physical properties, pasting properties

Introduction

Rice, being one of the primary dietary sources of carbohydrates worldwide, is the major energy and nutritional sources. Nutritional quality, as well as physical properties and cooking/eating qualities of rice are very important for increasing rice consumption (1, 2). In recent years, demands have been increasing for rice with a wide range of value-added properties, such as enhanced nutrient, texture, aroma, color, size, and shape of rice kernels, as well as processed rice products with higher functional properties.

Goami2 (G2), identical to Suwon 464 rice variety, was developed by mutation breeding via *N*-methyl-*N*-nitrosourea (MNU) treatment to Ilpum (IP), also identical to Ilpumbyeo, high quality japonica rice. A previous study on G2 rice (3) revealed that G2 starch has a strikingly different structure from its mother variety of IP, reporting the starch granules of G2 were much smaller in size than those of IP, and that many of G2 starch were not separated from amyloplasts. These differences in the structural characteristics might contribute to the quite different properties observed in G2 rice compared to IP, such as poor gelatinization and hard texture of cooked rice. However, they suggested that, although G2 rice was not suitable for cooked rice, due to its higher contents of protein, lipid, amylose, and fiber, which could impart a significant health benefit, G2 could be an excellent candidate for other processed food products.

The physiological role of carbohydrates, particularly in the case of unavailable carbohydrates, which include dietary fiber and resistant starch, has attracted the attention of food producers. Enzyme-resistant starch has received considerable attention, because it has a reduced caloric content and is characterized by physiological effects that make it comparable to dietary fiber. Adequate provision of slowly digestible carbohydrates is related to the reduction of glycemic response, which could have beneficial implications in the management of diabetes (4). Björck and Asp (5) reported that resistant starch and slowly digestible starch resulted in low glycemic index in starch-based food products.

The objectives of this study were to determine the milling qualities and indigestible carbohydrate fractions (ICF) of G2 by harvesting time and to compare the ICF contents and physical properties of G2 rice, which were at the maximized milling qualities and ICF contents, with those of Ilpum, a mother variety of G2.

Materials and Methods

Rice sample preparation G2 rice was grown at the National Institute of Crop Science, RDA, Suwon, Korea during the 2004 growing season. G2 was harvested at 30, 40, 50, and 60 days after heading (DAH), then dried to 14-15% moisture contents. Rice paddies stored at 15°C were dehusked using a rice sheller (Model SY88-TH; Sangyong Ltd., Incheon, Korea) for brown rice, which was then milled into white rice using a rice mill (Model MC-90A; Toyo Co. Ltd., Tokyo, Japan). The milled rice was further ground into fine particles (100 mesh) using a grinding mill (Cyclotec™ 1093 sample mill; Foss Tecator Co., Hillerod, Denmark). For parboiled rice, rice paddies were soaked in warm water at 65.0±1.0°C for 5 hr, and autoclaved at 110±1.0°C (0.07 MPa) for 10 min (6). After autoclaving, samples were cooled at room temperature, and dried at 45.0±1.0°C until the moisture content of 12-14% was attained. IP was used as a control to compare the experimental results of G2.

Milling yields and milling quality Milling yields (%) was evaluated based on dehusking (brown rice) and milling

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(milled rice) recoveries by measuring the percent husk removed from the paddy rice and the percent bran removed from the brown rice, respectively. Milling quality was evaluated as the percentage of head rice yield based on general appearance, a subjective evaluation method performed by human eyes.

Indigestible carbohydrate fractions Indigestible carbohydrate fractions (ICF) was determined by measuring dietary fiber content in milled rice according to the AOAC official method 991.43, an enzymatic-gravimetric method using MES-Tris buffer. Analysis was conducted in triplicates using a dietary fiber extraction equipment (Fibertec™ System, 1023 Filtration Module; Foss Tecator Co., Hillerod, Denmark).

Water absorption index (WAI) and water solubility index (WSI) WAI and WSI were determined using the method of Anderson (7). The rice flour sample (2.5 g) was dispersed in 30 mL distilled water, followed by heating in a water bath at 20, 40, 60, and 80°C for 1 hr. The dispersion was rinsed into a tarred centrifuge tube made up to 40 mL, then centrifuged at 3000×g for 10 min. After draining the supernatant carefully, the hydrated residue was weighed for WAI determination. The drained supernatant was dried overnight at 70°C, then WSI was determined as the weight of dry solids in the supernatant expressed as a percentage of the sample weight.

$$\text{WAI (g/g)} = \frac{\text{Wt. of water uptake in hydrated residue}}{\text{Wt. of rice flour sample (db)}}$$

$$\text{WSI (\%)} = \frac{\text{Wt. of dissolved solids in supernatnat}}{\text{Wt. of rice flour sample (db)}} \times 100$$

Pasting properties analysis Paste viscosity was determined using a Rapid Visco™ Analyser Series 4 (Newport Scientific Pty. Ltd., Warriewood, Australia) in accordance with the AACC 61-02 method. The rice flour samples of milled and parboiled G2 and IP (3.0 g each) were placed in a clean RVA test canister, and 25 mL distilled water was carefully dispensed into the canister, to which a paddle was placed. For the initial 10 sec, high speed mix (960 rpm) was used to disperse the sample, followed by a moderate application of shear (160 rpm) for the remainder of 12.5 min test period. The test profiles were: equilibrating at 50°C for 1.0 min, ramping up to 95°C in 4.8 min, and holding at 95°C until 7.5 min. The temperature was linearly ramped down to 50°C at 11 min and held at 50°C until 12.5 min. The recorded viscosity parameters were peak viscosity, hot paste viscosity (or through), final viscosity, and their derived parameters of breakdown and setback. The onset temperature of starch gelatinization was also determined. All parameters were determined in arbitrary Rapid Visco Units (RVU), and the measurements were conducted in triplicates.

Texture profile analysis (TPA) The resulting rice flour gels from paste viscosity analysis were kept in a RVA canister, sealed with parafilm™, and held at 4°C for 24 hr (8, 9). TPA was carried out on a *TA-XT2* Texture Analyzer (Stable Micro System Ltd., Haslemere, England). A standard TPA operated by two-cycle program was used to compress the gels for a distance of 10 mm at 1.0 mm/sec using a 20 mm cylindrical probe with a flat end (contact area, 314 mm^2). Parameters recorded were hardness (maximum force, *g*), adhesiveness (negative force required to pull the compression plunger from the sample, *g·sec*), springiness (recovery in height by the sample during the time between the end of 1st cycle and the start of 2nd cycle, *mm*), cohesiveness (ratio of the positive force area during the second compression to that during the first compression), and gumminess (the quality being cohesive or sticky calculated by hardness multiplied by cohesiveness).

Statistical analysis Data were analyzed by a statistical analysis system version 8.01 (SAS Institute Inc., Cary, NC, USA). Significance of the differences between samples was evaluated using Duncan's multiple comparison tests at 5% significance level.

Results and Discussion

Studies on the proximate compositions of G2, a mutant rice of IP, revealed that G2 has significantly higher crude protein, lipid, fiber, and amylose contents than IP (3, 10). G2 rice can also be easily distinguished from IP (translucency in color) by its milky color (chalkiness/ opacity), which is frequently shown in waxy rice, and smaller grain size.

Milling quality and indigestible carbohydrate fractions in G2 ***Milling yields and milling quality*** Milling yields and quality of G2 harvested at 30, 40, 50, and 60 days after heading (DAH) were evaluated (Table 1). Maximum milling recovery was obtained at around 40 DAH (64.10 %), followed by 50 DAH (63.3%), whereas early and late harvestings (30 and 60 DAH) decreased the milling recovery. Maximum head rice yields were obtained at 50 DAH (91.12%), followed by 40 (85.18%), 60 (84.26%), and 30 DAH (80.25%), indicating the broken and damaged rice recoveries were the lowest at 50 DAH. Accordingly, head rice milling yield was calculated based on the milling recovery and head rice yields, and results showed that G2 harvested at 50 DAH (57.69%) had the highest head rice milling yield.

Milling qualities were analyzed by classifying head rice, broken rice, and damaged rice. Damaged rice included imperfect rice grains, white belly rice kernels, and green rice kernels. High head rice yield is one of the most important criteria for milled rice quality and is evaluated by the proportion of whole grains in milled rice. Although there have been recent advances in automating head rice evaluation, head rice evaluation by general appearance, which was a subjective evaluation performed by human eyes, is still frequently the first applied among the rice quality criteria (1).

Indigestible carbohydrate fractions in rice Indigestible carbohydrate fractions (ICF) measured by dietary fiber content in G2 harvested at 30, 40, 50, and 60 DAH are shown in Table 2. Significant mean difference ($p<0.05$) was observed in ICF contents depending on the harvest

Table 1. Milling yields and milling qualities of Goami2 by harvesting time

DAH$^{1)}$	Milling yields (%)		Milling qualities (%)			Head rice milling yields$^{2)}$ (%)
	dehusking recovery	milling recovery	head rice	broken rice	damaged rice	
30	73.56$^{c3)}$	62.31^{c}	80.25^{d}	16.40^{a}	3.45^{b}	49.97^{d}
40	76.38^{a}	64.10^{a}	85.18^{b}	9.86^{c}	5.11^{a}	54.55^{b}
50	76.40^{a}	63.33^{b}	91.12^{a}	7.32^{d}	1.62^{c}	57.69^{a}
60	75.56^{b}	61.84^{d}	84.26^{c}	14.49^{b}	1.47^{c}	52.09^{c}

$^{1)}$DAH means days afte heading.
$^{2)}$head rice milling yields was calculated by percentile of milling yields multiplied by head rice yields.
$^{3)}$Means with different letter in the same column are significant differently at p<0.05.

time. In spite of some variations in the mean values of ICF contents, Duncan's comparison indicated a significant mean difference (p<0.05) between early (before 50 DAH) and late stage (after 50 DAH) of G2 endosperm maturation. G2 harvested at the late stage maturation contained higher total dietary fiber (TF), ranging 4.98± 0.58 to 5.08±0.35 (g/100 g), compared to those harvested at the early stage, ranging from 4.04±0.22 to 4.09±0.06 (g/ 100 g). The highest TF (5.09±0.35 g/100 g) was found in G2 at 50 DAH. Correspondingly, the insoluble dietary fiber (IF) and soluble dietary fiber (SF) contents were the highest at 50 DAH. On the other hand, the IF contents were significantly higher than SF contents, indicating the major dietary fiber in G2 rice was insoluble dietary fiber.

The reported values of dietary fiber in milled rice have shown large variations depending on the rice varieties and/ or analysis methodologies. The national nutrient database (11) for standard reference reported by the United State Department of Agriculture (USDA) indicated 1.29 (g/100 g) of dietary fiber in white rice (long-grain, raw, enriched) and 1.68 (g/100 g) in parboiling rice (long-grain, parboiled, dry). A study evaluated the dietary fiber contents of 54 Korean vegetable foods, and reported that the total dietary fiber contents were 1.19% in highly polished rice (90%), 1.28% in less polished rice (70%), and 3.32% in brown rice (12).

Comparisons on indigestible carbohydrates and physical properties of Goami2 with Ilpum at 50 days after heading (DAH) ***Indigestible carbohydrate fractions (ICF) in milled and parboiled rice*:** Based on the findings that G2 at 50 DAH showed the highest head rice milling quality (Table 1) and ICF contents (Table 2), G2 and IP at 50 DAH were compared in terms of their ICF contents and physical properties.

Table 3 shows the comparison of ICF of raw and parboiled G2 and IP at 50 DAH. A significant mean difference (p<0.05) was found in TF between G2 and IP (5.09±0.35 vs. 1.61±0.09), approximately three times higher TF in G2 rice. In addition, a significant mean difference in TF between raw and parboiled G2 was observed, ranging from 5.09±0.36 to 7.18±0.16 (g/100 g), which might be due to heat treatment by parboiling process. However, no significant difference was observed between raw and parboiled IP (1.61±0.09 to 1.80±0.23). The results of IF, SF, and TF for G2 were obtained with positive values for all gravimetric corrections of ash, protein, and blank, but negative SF value for IP. A negative SF value was also shown in other studies, explaining that the measurement of samples with very low concentration might attribute to the negative value (13). Increased ICF in parboiled G2 could be accordance with the results of the formation of resistant starch in a processed grain-based product due to hydrothermal treatment (14, 15). Those studies reported that in hydrothermal process, applying heat to high-moisture rough rice, structure modifications, and/or rupture of starch molecules could occur, and these fragments could combine with other molecules, creating new compounds resistant to enzymatic digestion. Asp and Björck (16) reported that repeated autoclaving of wheat starch could generate up to 10% resistant starch measured by the enzymatic gravimetric dietary fiber method. They also noted that the

Table 2. Means of insoluble, soluble and total dietary fiber of milled Goami2 at different harvesting time

DAH$^{1)}$	Dietary fiber (g/100 g)$^{2)}$		
	Insoluble (IF)	Soluble (SF)	Total (TF)
30	4.08±0.05^{b}	0.01±0.00^{b}	4.09±0.06^{ab}
40	3.96±0.11^{b}	0.15±0.00^{b}	4.04±0.22^{b}
50	4.47±0.00^{a}	0.61±0.21^{a}	5.09±0.35^{a}
60	4.46±0.19^{a}	0.52±0.11^{a}	4.98±0.58^{ab}

$^{1)}$DAH means days after heading.
$^{2)}$Means with different letter in the same column are significantly different at p<0.05.

Table 3. Means of insoluble, soluble and total dietary fiber content of raw and parboiled Goami2 and Ilpum at 50 days after heading (DAH)

Rice variety	Dietary fiber (g/100 g)		
	Insoluble	Soluble	Total
Raw rice			
Goiami2	4.47±0.01$^{b1)}$	0.61±0.35^{a}	5.09±0.36^{b}
Ilpum	1.61±0.09^{c}	-	1.61±0.09^{c}
Parboiled rice			
Goami2	7.07±0.12^{a}	0.12±0.05^{a}	7.18±0.16^{a}
Ilpum	1.80±0.03^{c}	-	1.80±0.23^{c}

$^{1)}$Means with different letter in the same column are significantly different at p<0.05.

newly formed resistant starch appeared to be strongly associated with the amylose content. In particular, retrogradation of amylose was identified as the main mechanism for the formation of resistant starch. In addition, Sievert and Pomeranz (17) reported that amylose/amylopection ratio, physical form, degree of gelatinization, thermal treatments, cooling, and storage affected the formation of resistant starch contents.

Water absorption index (WAI) and water solubility index (WSI) WAI and WSI were examined as functions of temperature (Fig. 1 and 2, respectively). WAI of G2 was relatively lower than that of IP, showing slight increment over the temperature range of 25 to 80°C. This implies that hydration in G2 starch molecules could occur very slowly, resulting in poor swelling of the amorphous regions between crystallites, which, in turn, could affect its gelatinization quality (18). Weak swelling power due to lower water absorption could affect gelatinization of pasting and texture properties of G2 rice. On the other hand, WSI of G2 was significantly higher than that of IP at low temperatures, and then reached to almost the same level at high temperatures. The higher WSI of G2 at lower temperature suggested that G2 might have more soluble component available at low temperature. Upon considering the compositions of G2, the higher content of amylose and/or soluble dietary fiber contained in G2 rice might be attributable to the higher WSI observed. Increment of WSI with increasing temperature was also observed in a study that investigated the effect of elevated steeping temperature to swelling power and solubility of rice starch properties (19).

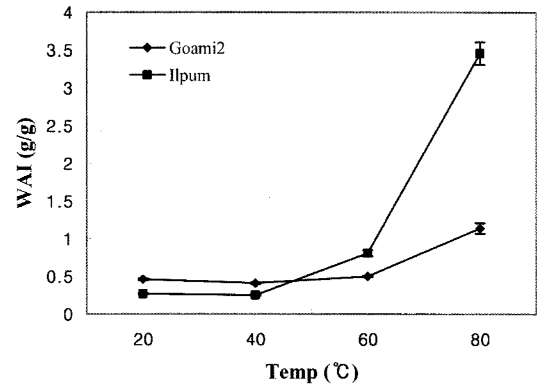

Fig. 1. Water absorption index (WAI) of Goami2 and Ilpum.

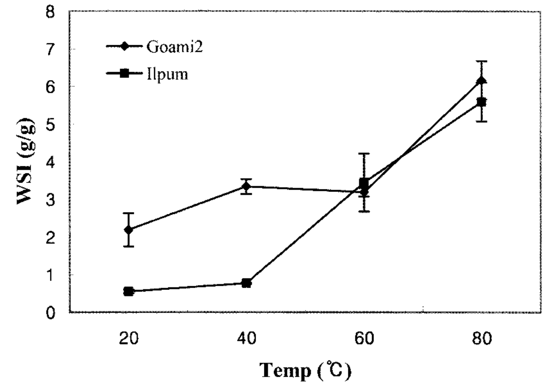

Fig. 2. Water solubility index (WSI) of Goami2 and Ilpum.

Pasting properties analysis A significant difference ($p<0.05$) was observed between the pasting properties of raw and parboiled G2 and IP (Table 4). At the onset of gelatinization, the initial pasting temperatures (PTEMP) of G2 and IP were 82.03±0.6 and 68.09±0.1, respectively. Parboiled G2 also showed higher initial pasting temperature than parboiled IP. Starches with higher gelatinization temperatures started water absorption and dissolution at a higher temperature and required longer cooking time than those with lower gelatinization temperatures (20). The values of all pasting parameters of G2 were lower than those of IP except the high value of setback (SB), which was often used to evaluate the retrogradation of starch (Table 4). Thus, higher SB of G2 could explain the hard gel consistency, implying G2 rice gel hardened faster during cooling (21). Harder gel consistency is associated with harder cooked rice, and this feature is particularly evident in high-amylose rice. A study reported that amylose retrograded quickly and was responsible for textural changes occurring in starch gels during the first few hours of cold storage (22). Thus, the higher amylose content of G2 rice could be attributable to the fast retrogradation. On the other hand, the paste viscosity of parboiled IP was relatively similar to that of raw IP rice; however, in the case of parboiled G2, the paste viscosity

Table 4. Paste viscosity properties of raw and parboiled Goami2 and Ilpum at 50 days after heading (DAH) (units: RVU)$^{2)}$

	Pasting properties$^{3)}$					
	PV	HV	FV	BD	SB	Ptemp
Raw rice						
Goami2	72.65±4.77$^{b1)}$	65.73±4.31^{b}	149.69±8.06^{b}	6.92±0.74^{b}	77.04±3.96^{a}	82.03±0.65^{a}
Ilpum	187.63±5.46^{a}	129.13±6.41^{a}	229.40±7.21^{a}	58.50±3.79^{a}	41.77±3.01^{b}	68.09±0.08^{b}
Parboiled rice						
Goami2	22.60±1.59^{b}	21.85±1.14^{b}	37.81±1.57^{b}	0.75±0.52^{b}	15.21±0.77^{b}	88.66±0.36^{a}
Ilpum	205.25±11.81^{a}	169.90±12.14^{a}	263.35±11.94^{a}	35.35±5.56^{a}	58.10±1.01^{a}	70.49±0.63^{b}

1) Means with different letter on the same column are significantly different at $p<0.05$.
2) RVU = Rapid Visco Units.
3) PV, peak viscosity; HV, hot paste viscosity; FV, final viscosity; BD, break down; SB, set back; Ptemp, initial paste temperature

was extremely low, reflecting unsuitability for application in RVA paste viscosity testing.

Gelatinization was affected by several factors including water content of the gel, amylose content, degree of crystallinity in the amylopectin fraction, and amylopectin chain length (23). Other factors that influenced starch gelatinization included placement and content of starch granule, which is associated with protein and lipid. To determine the pasting characteristics, the critical role of protein in rice kernel was studies, and results showed that protein has a negative correlation to the peak viscosity of starch paste, whereas a positive correlation to the pasting temperature (24). These results on the effect of protein to pasting properties reflected that high protein content of G2 could result in higher pasting temperature and lower peak viscosity compared to IP. Yoon and Kim (25) studied physicochemical properties of three rice cultivars depending on different milling degrees, reporting protein, fat, and ash contents linearly decreased with increasing degree of milling (DM), whereas peak viscosity linearly increased with increasing in DM.

Texture profile analysis (TPA) The gel texture of rice kept in the RVA canister as evaluated by TPA is shown in Table 5. A significant mean difference was found between raw G2 and IP. G2 rice was high in hardness but low in adhesiveness and cohesiveness, whereas the springiness was comparable to that of IP. The values of gumminess, which is defined as the gel quality being cohesiveness or sticky, were 43.15±0.65 for G2 and 44.46±3.01 for IP. The lower gumminess of G2, which was calculated by multiplying hardness by cohesiveness, was considered to be due to the significantly lower cohesiveness of G2. On the other hand, parboiling treatment to IP increased all texture properties. Unlike the parboiled IP, parboiled G2 showed extremely low hardness and adhesiveness, probably due to the poor pasting properties. Accordingly, the parboiled G2 resulted in very low gumminess. The relationship between indigestible carbohydrate fractions and texture properties was estimated, and the correlation coefficients relating the ICF to the texture analysis results are shown in Table 6. A strong positive correlation was observed between ICF and hardness ($r = 0.9684$, $p<0.05$), whereas a negative correlation between ICF and cohesiveness ($r=-0.9873$, $p<0.05$), indications that higher ICF in G2 rice resulted in increased hardness, but decreased cohesiveness. In addition, a strong negative correlation was observed between hardness and cohesiveness ($r = -0.9922$, $p<0.05$). The correlation between ICF and texture properties reflected that the higher hardness but lower cohesiveness of G2 could be attributable to the higher contents of indigestible carbohydrate fractions.

Ong and Blanshard (22) examined the starch components as the primary determinants of rice texture, and suggested that a higher amylose content and longer amylopectin chains provide a favorable environment for inter- or intra-molecular interactions of starch with other components in rice, such as protein, lipid, and non-starch polysaccharides (e.g. from the endosperm cell wall components). Other studies (26, 22) also reported that protein-starch interactions might be well-favored between the highly dispersed proteins associated with starch granules, restraining the

Table 5. Texture profile analysis (TPA) of Goami2 and Ilpum

Rice variety	TPA parameters				
	Hardness (g)	Adhesiveness (g·sec)	Springiness (mm)	Cohesiveness	Gumminess
Raw rice					
Goami2	129.15±5.39$^{a1)}$	-106.78±18.82^{b}	0.87±0.02^{a}	0.33±0.01^{b}	43.15±0.60^{a}
Ilpum	87.34±9.62^{b}	-143.26±5.99^{a}	0.88±0.00^{a}	0.51±0.02^{a}	44.46±3.01^{a}
Parboiled rice					
Goami2	21.59±3.49^{b}	-8.23±10.54^{b}	0.80±0.11^{a}	0.51±0.03^{a}	10.89±1.04^{b}
Ilpum	136.80±7.97^{a}	-161.53±37.14^{a}	0.93±0.03^{a}	0.56±0.02^{a}	75.96±7.02^{a}

1)Means with different letter on the same column are significantly different at $p<0.05$.

Table 6. Correlation coefficients relating total dietary fiber (TDF) to texture profile analysis (TPA) results

	Correlation coefficients					
	TDF	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess
TDF	1.00					
Hardness	0.9684$^{1)}$	1.00				
Adhesiveness	0.8092	0.7382	1.00			
Springiness	-0.3942	-0.3374	-0.8549	1.00		
Cohesiveness	-0.9873$^{1)}$	-0.9922$^{2)}$	-0.8094	0.4229	1.00	
Gumminess	-0.3933	-0.1542	-0.5621	0.4429	0.2659	1.00

1)Significant at $p<0.05$.
2)Significant at $p<0.01$.

swelling of the starch granules. The final textural properties of starch could well be dependent on the type of amylose-lipid complexes due to their different crystallization and melting temperatures (27, 28).

In conclusion, head rice milling yield (91.12%) of G2 rice was maximized at 50 DAH, followed by 40, 60, and 30 DAH. ICF of G2 was also maximized at 50 DAH (5.09 ±0.35 g/100 g), which was approximately three times higher than that of IP (1.61±0.09 g/100 g) at 50 DAH. G2 was characterized with higher WAI but lower WSI compared to IP. The rigid structure of G2 starch (3) could inhibit the easy entry of water into starch granules, resulting in very low WAI value. However, the higher WSI indicated that G2 rice could have more soluble components, such as higher amylose and/or soluble dietary fiber. The pasting properties showed that G2 had higher gelatinization temperature, implying water absorption and dissolution at a higher temperature and longer cooking time. In addition, G2 showed hard gel consistency and easy to retrograde compared to IP. Pasting properties increased in parboiled IP, but decreased significantly in parboiled G2, indicating that the parboiled G2 was not able to form gel-like semisolid due to pre-heat treatment. Based on the TPA, G2 rice gel was characterized by higher hardness with lower adhesiveness and cohesiveness. The compositions of G2, higher in protein, lipid, and amylose, could be responsible for the different texture of G2 as compared to that of IP. Parboiling treatment to G2 resulted in lower texture properties due probably to poor gel formation. The correlation analysis indicated a positive correlation between ICF and hardness, but a reverse correlation between ICF and cohesiveness.

Acknowledgments

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