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Resistant starch levels and *in vitro* starch digestibility of selected cooked Philippine brown and milled rices varying in apparent amylose content and glycemic index

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ABSTRACT

Resistant starch (RS) content, starch digestibility, and hydrolysis index (HI) were analyzed *in vitro* for four selected Philippine rice varieties varying in apparent amylose content (AC) and glycemic index (GI), in cooked brown and milled rice forms. Starch digestibility curves were studied in relation to AC and reported GI values. Brown and milled rices of Improved *Malagkit Sungsong* 2 (IMS2), NSIC Rc160, IR64, and PSB Rc10 were cooked on separate beakers placed in automatic electric rice cookers and based on pre-determined water:rice ratios. RS levels of cooked milled rices ranged from 0.15 to 0.99% (mean = 0.45%). Their corresponding cooked brown rices had RS contents ranging from 0.24 to 1.61% (mean = 1.05%), with PSB Rc10 having the highest levels in both forms. HI ranged from 59.3 to 102.2%, with the highest noted for the waxy rice, IMS2, while corresponding brown rices had significantly lower HI spanning 49.2–66.9%. Previously reported GI values of these varieties were positively correlated with HI and estimated GI in this study. RS and non-resistant starch levels, and HI were highly correlated with AC. *In vitro* starch digestibility studies, as related to AC and GI, may be useful in screening for rice grain and nutritional properties aimed at developing new varieties with desirable quality and enhanced nutritional and functional properties.

1. Introduction

In the Philippines, milled rice is considered the main staple food and the primary source of dietary carbohydrates among Filipinos, followed by white corn that is usually consumed in some corn-producing regions of the country (Juliano, 2010). Caloric energy from the diet comes from the consumption and digestion of endosperm starch in these cereals, which makes up more than 80–90% of the milled rice grain (Juliano & Tuaño, 2019). Consumption of meals having whole grains, including brown rice, has been increasing nowadays due to the promotion and education campaigns about their functionalities and health-promoting properties, especially in helping combat lifestyle-related diseases such as cardiovascular diseases (CVDs), diabetes, different types of cancer, and some disorders of the gastrointestinal tract (Deepa, Singh, & Naidu, 2010). Starch, the most significant source of caloric energy by populations in different parts of the world, has been categorized in terms of their digestibility and behavior in the gastrointestinal tract. The main types of starch in terms of degree and rate of digestibility are the rapidly digestible starch or RDS, slowly digestible starch or SDS, and resistant starch or RS (Englyst, Kingman, & Cummings, 1992). RDS is the fraction of starch that yields a fast glucose release upon digestion and absorption subsequently giving an immediate rise in the blood glucose level of consumers after ingestion of certain carbohydrate foods. On the other hand, SDS confers complete digestion in the small intestine at a significantly lower rate than RDS, thereby causing a slow release of glucose from hydrolyzed starch and a gradual increase in blood glucose level upon digestion. Lastly, RS comprises the starch fraction, in combination with its hydrolysis products, that proceeds directly to the colon for fermentation, as these escape enzymatic digestion in the small intestine (Sajilata, Singhal, & Kulkarni, 2006). Several human studies have shown that RS has a lot of health benefits in relation to preventive approaches towards having high risks to metabolic syndrome and non-communicable lifestyle-related diseases. Some health-promoting benefits of RS include improving insulin sensitivity of peripheral cells, consistent lowering of blood glucose levels to normal

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range, reducing appetite thereby helping in weight loss and management of obesity, and other various benefits related to food digestion and colon health (Lunn & Buttriss, 2007; Nugent, 2005; Sajilata et al., 2006). It has also been reported to enhance colonic health as the beneficial microorganisms in the large intestine may feed on RS leading to high levels of short-chain fatty acids produced by these microorganisms, including but not limited to, butyrate, propionate, and acetate. In addition, RS can also regulate the high release of glucose from starchy foods thereby assisting in weight control for the obese people (Annison & Topping, 1994; Liu & Xu, 2008; Nugent, 2005; Sajilata et al., 2006). With the many significant health benefits of RS in humans, it has been used to add value in various food products such as noodles, pasta, baked products, energy bars, RS-enriched flours and starches, among others (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010).

Other than resistant starch levels, another important measure to describe starch digestibility of food is through the glycemic index (GI). GI is the physiological determinant of how abrupt a carbohydrate food or food product can release glucose in the blood. It is expressed as percentage of the incremental area under the curve (IAUC) of glucose response after a meal consumed by human subjects or volunteers relative to the IAUC of glucose response after ingestion of a reference food, usually white bread or glucose drink (Jenkins et al., & Goff, 1981; Wolever et al., 1990). It is widely accepted that low GI foods are beneficial to humans similar to resistant starch-rich foods. However, GI determination is costly and invasive since GI experiments involve human subjects scheduled to be fed for days or weeks with the test foods under study, at pre-determined amounts, followed by repeated blood extraction at certain time intervals and blood glucose response determination. Also, the conditions of these human subjects can be variable, thus, giving variable blood glucose responses (Frei, Siddhuraju, & Becker, 2003; Wolever et al., 1990). In order to eliminate these sources of variability in starch digestibility data, *in vitro* determination of starch hydrolysis index (HI) can be employed, of which data may be correlated with GI and expressed as estimated GI (EGI) (Frei et al., 2003; Goñi, Garcia-Alonso, & Saura-Calixto, 1997). When *in vitro* starch digestibility and HI of starch-rich foods can properly estimate GI, high throughput HI and EGI assays as well as easy clustering of a large number of food samples, including rice varieties, may be done in lesser time, in a non-invasive manner, and at relatively affordable cost.

Studying carbohydrates, specifically digestible and resistant starch of food crops like rice, have led to establishing their implications on some societal concerns on health, nutrition, and lifestyle-related diseases such as obesity and diabetes. Hu, Pan, Malik, and Sun (2012) reported a significantly elevated risk of type 2 diabetes among Asians associated with higher intake of white rice. In an *in vivo* GI study conducted among 9–10 healthy Filipino volunteers, it has been shown that Philippine milled rices varied in terms of GI in relation to apparent amylose content (AC), dietary fiber and protein contents of the grain. Intermediate-AC milled rices (18–25% AC), which are predominant in the Philippine rice breeding program (Juliano, 2010; Tuaño, 2013), in farmers' fields (Tuaño, Perez, Padolina, & Juliano, 2015), and in local markets (Tuaño, Regalado, & Juliano, 2016), commonly have medium GI. Milled rice with high AC (AC $>$ 25%) tended to have medium-to-high GI while those with low AC ($<$18%) had consistently high GI, provided the grain protein content is in the normal range (i.e. around 5–7%) (Juliano, 2010; Trinidad et al., 2013). Dietary fiber in brown rice is relatively higher than in milled rice and contributes to variations in the GI values of Philippine rices. Significant lowering of GI has been observed when brown rice was consumed in place of milled rice of the same variety and AC, especially for waxy and low-AC rices. When intermediate- and high-AC brown rices were consumed in place of their counterpart milled rices, the reduction in GI was lower than the two lower-AC rice types indicating that AC is the main determinant of GI among Philippine rices, followed by dietary fiber content, both having negative correlation with GI (Alhambra et al., 2019; Barcellano, 2015; Trinidad et al., 2013, 2014). However, considering that the physicochemical and cooking properties of brown rice differ greatly with those of milled rice, including but not limited to, cooked grain length–width ratio, gelatinization properties, swelling power, pasting viscosity, cooking time, gruel solid loss, and water uptake ratio (Wu et al., 2018), all affecting to certain degree the cooked rice sensory quality and consumer acceptability, there has been no report yet to date on the resistant starch levels and starch hydrolysis index of cooked brown rices analyzed side-by-side with their corresponding milled rices of the same variety, attempting to determine the extent of intact bran components' effect (including dietary fiber) on cooked brown rice starch digestibility. The present study intended to analyze the *in vitro* starch digestibility and hydrolysis index of four Philippine rice cultivars varying in apparent amylose content, in cooked milled rice and brown rice forms. Resistant starch content of these varieties were also determined along with other significant grain physicochemical properties and proximate composition, in an attempt to determine the relationship among these parameters, starch digestibility properties, and *in vivo* GI as previously reported (Trinidad et al., 2013, 2014). The present study involved the same rice samples and cooking method, as used in the feeding trials conducted in the *in vivo* GI experiment of Trinidad et al. (2014), to ensure consistency in terms of raw and cooked grain properties of the samples under investigation. To the best of our knowledge, this is the first report on *in vitro* resistant starch content and starch digestibility of cooked Philippine rice that utilized the same set of brown and milled rice samples previously subjected to a properly designed *in vivo* GI experiment among healthy Filipino human subjects, the same cooking method, and pre-determined water-rice ratios as employed in the said rice GI study.

2. Materials and methods

2.1. Rice samples and sample preparation

Four rice varieties with known *in vivo* GI data as previously reported by Trinidad et al. (2014) were used in this study. Aged rough rice samples of Improved *Malagkit Sungsong* 2 (IMS2), NSIC Rc160, IR64, and PSB Rc10 varieties were obtained from either the 2012 dry season or 2012 wet season crop at the Philippine Rice Research Institute (PhilRice) Los Baños.

Five hundred grams (500 g) of rough rice samples were dehulled to obtain brown rice using THU-35 rubber-roll type dehuller (Satake Corp., Japan). One hundred grams (100 g) of the resulting brown rice were passed through a McGill Miller No. 2 (Grainman Mfg. Inc., USA) to obtain milled rice. The remaining brown rice samples were kept in polytethylene plastic bags and stored in a freezer until further use in cooked rice texture and starch digestibility experiments. Five grams (5 g) of milled rice were ground to pass a 60-mesh sieve using a cyclone mill and stored in a freezer until further use for apparent amylose content (AC) analysis and pasting viscosity measurements. A portion of milled rice flour was further passed through a 100-mesh sieve to obtain finer flour samples for gel consistency analysis. Whole milled rice grains were used for alkali spreading value and Instron cooked rice hardness measurements.

For resistant starch assay and *in vitro* starch digestibility experiments, brown and milled rice grains were cooked in beakers with different water:rice ratios as employed in the study of Trinidad et al. (2014) and Barcellano (2015) to obtain the same cooked rice hardness as verified using Instron 3343 model (Instron, Norwood, MA, USA) with an Ottawa Texture Measuring System (OTMS) cell (Ottawa Texture Measuring System, ON, Canada). Twenty grams (20 g) milled or brown rice sample and the corresponding amount of distilled water as described by Barcellano (2015) were mixed in a 150-mL beaker. Washing was done by decanting the wash water and replacing it with

the same amount of distilled water to keep the initial volume constant at the onset of rice cooking. For cooking brown rice, sample was washed similarly and pre-soaked in a certain amount of distilled water for 30 min before cooking. The beaker containing the rice sample and distilled water was placed in an automatic electric rice cooker (Toshiba, Tokyo Shibaura Electric Co., Japan) containing 200 mL of distilled water in the outer pot, cooked for 20 min, and allowed to stand for 10 min without removing the cover. Afterwards, the beaker was then taken out of the rice cooker, covered with aluminum foil, placed in a sealed polyethylene plastic container, and allowed to stand for 1 h at room temperature prior to Instron hardness analysis.

2.2. Resistant, digestible, and total starch contents determination

Hydrolysis of digestible or non-resistant starch (non-RS). Determination of resistant, digestible, and total starch contents employed the use of Megazyme Resistant Starch Assay Kit (Megazyme Ltd., Wicklow, Ireland), with slight modifications. One hundred milligrams (100 mg) sample of cooked rice was weighed directly into a 15-mL polyethylene centrifuge tube ensuring that the sample was at the bottom of the tube. Exactly 4 mL of enzyme solution containing 10 mg/mL pancreatic α-amylase and 3 U/mL amyloglucosidase (AMG) were added. The mixture was mixed using a vortex mixer and was incubated at 37°C for 16 h with constant shaking in a shaking water bath set at medium speed (200 strokes/min). After incubation, 4 mL absolute ethanol was added to the mixture and then mixed using a vortex mixer. The uncapped tube was centrifuged at 1500*g* for 10 min. The supernatant was decanted and the pellet was resuspended in 2 mL 50% ethanol with vigorous mixing on a vortex mixer. Then, 6 mL 50% ethanol was added and the mixture was centrifuged at 1500*g* for 10 min. The supernatant was again decanted and the pellet was resuspended again in 2 mL 50% ethanol followed by vigorous mixing on a vortex mixer, then, recentrifuged at 1500*g* for 10 min. The supernatant was again decanted and the tube was inverted on absorbent paper towel to drain excess liquid. All supernatants were pooled and collected in a 100-mL volumetric flask for total starch determination.

Measurement of resistant starch (RS). The resulting pellet from the hydrolysis of digestible starch was used for resistant starch content analysis. Around 2 mL of 2 M potassium hydroxide (KOH) was added to the tube containing the pellet. The mixture was stirred vigorously for 20 min in an ice-cold water bath placed over a magnetic stirrer with magnetic bar to dissolve the pellet, ensuring that the mixture was vigorously stirred as the KOH solution was added to avoid formation of clumps. Addition of 8 mL 1.2 M sodium acetate (NaOAc) buffer (pH 3.8) was done with constant stirring using a magnetic stirrer, followed immediately by adding 0.1 mL AMG (3300 U/mL). The mixture was mixed well on a magnetic stirrer with magnetic bar and was incubated in a water bath set at 50°C for 30 min with intermittent mixing using a vortex mixer at 5 min intervals. The mixture was centrifuged at 1500*g* for 10 min and volume of the supernatant was measured. A 0.1-mL aliquot was transferred into a test tube, added with 3 mL glucose oxidase-peroxidase (GOPOD) reagent, mixed and incubated at 50°C for 20 min. Absorbance of the colored solution was read at 510 nm against a reagent blank using UV Mini 1240 spectrophotometer (Shimadzu Corp., Japan). The reagent blank solution was prepared by mixing 0.1 mL of 0.1 M NaOAc buffer (pH 4.5) and 3 mL GOPOD reagent. Glucose standards were prepared (in quadruplicate) by mixing 0.1 mL glucose (1–10 mg/mL each) and 3 mL GOPOD reagent, then incubated at 50°C for 20 min. The absorbance of each standard solution was measured at 510 nm against the reagent blank. RS levels were calculated following the method of Englyst et al. (1992) as described in the Megazyme Resistant Starch Assay Kit (Megazyme, Ltd., Wicklow, Ireland).

Measurement of digestible or non-resistant starch (non-RS). The stored pooled supernatants for each sample were diluted to volume in 100-mL volumetric flask with 100 mM NaOAc buffer (pH 4.5). A 0.1-mL aliquot of the solution was incubated with 10 μL of AMG (300 U/mL) in 100 mM sodium maleate buffer (pH 6.0) for 20 min at 50°C. Exactly 3 mL of GOPOD reagent was added and the mixture was incubated for 20 min at 50°C. The absorbance of the colored solution was measured at 510 nm against the reagent blank using UV Mini 1240 spectrophotometer. Non-RS levels were calculated following the method of Englyst et al. (1992) as described in the Megazyme Resistant Starch Assay Kit (Megazyme, Ltd., Wicklow, Ireland).

Measurement of total starch (TS). The total starch (TS) content of each cooked rice sample was calculated as the sum of the resistant and non-resistant starch contents obtained and was verified using the anthrone method. TS content using RS and non-RS data was calculated as follows:

$$\text{Total starch}(\%) = \text{Resistant starch}(\%) + \text{Non-resistant starch}(\%) \quad (1)$$

2.3. In vitro starch hydrolysis index and estimated glycemic index determination

Around 80 mg of the cooked rice sample was placed in a 15-mL polyethylene centrifuge conical tube, mixed and ground with 10 mL hydrochloric acid-potassium chloride (HCl-KCl) buffer (pH 1.5) and 0.2 mL of pepsin solution (1 g pepsin in 10 mL HCl-KCl buffer [pH 1.5]) using a glass stirring rod, followed by incubation in a shaking water bath set at 40°C for 1 h with constant medium-speed shaking. The volume of the mixture was adjusted to 20 mL with Tris-maleate buffer (pH 6.9). Five milliliters (5 mL) of α-amylase solution (40 mg α-amylase per mL Tris-maleate buffer [pH 6.9]) was added. The mixture was incubated in a water bath at 37°C with constant medium-speed shaking, then, 1 mL aliquot was collected from the incubated sample every 30 min within 3 h of incubation. The collected aliquot was placed in a centrifuge tube, boiled at 100°C, and was shaken for 5 min to inactivate the enzymes, followed by rapid cooling in a refrigerator and stored until the end of the incubation time prior to analysis. Then, around 400 μL of 0.4 M NaOAc buffer (pH 4.75) and 30 μL of amyloglucosidase (AMG) enzyme solution (300 U/mL) were added to each aliquot to hydrolyze the digested starch into free glucose. Afterwards, the mixture was incubated in a water bath at 50°C for 30 min. The mixture was then treated with 3 mL GOPOD reagent and further incubated at 50°C for 20 min. Absorbance of each solution was measured at 510 nm using UV Mini 1240 spectrophotometer (Shimadzu Corp., Japan) against the reagent blank and the amount of starch present in each mixture was determined using the following equation:

$$\%\ \text{Starch} = \Delta A \times F \times \left(\frac{100}{0.1}\right) \times \left(\frac{1}{1000}\right) \times \left(\frac{100}{W}\right) \times \left(\frac{162}{180}\right) \quad (2)$$

where, ΔA = averaged absorbances read against the reagent blank
F = 100 μg of glucose divided by the GOPOD absorbance obtained for this solution
W = weight of test portion of starch analyzed

The rate of starch digestion was expressed as % starch hydrolyzed, relative to total starch content, determined at various time points (i.e. at 0, 30, 60, 90, 120, 150, and 180 min) following the equation below:

$$\%\ \text{Starch hydrolyzed} = \left(\frac{\%\ \text{starch at certain time point}}{\text{total starch}\ (\%)}\right) \times 100 \quad (3)$$

The data points for % starch hydrolyzed were plotted against corresponding time points (in min) starting at 0 min and the area under the curve (AUC) for each cooked rice sample was determined using the trapezoid method via Microsoft Excel. The *in vitro* starch hydrolysis index (HI) was calculated as % of total glucose released from the hydrolyzed cooked rice samples from 0 to 180 min relative to that released from hydrolyzed white bread analyzed similarly. HI was expressed in % as shown below:

$$\mathrm{HI}(\%) = \left(\frac{\mathrm{AUC}_{\mathrm{sample}}}{\mathrm{AUC}_{\mathrm{white\ bread}}}\right) \times 100 \quad (4)$$

The estimated GI (EGI) of the cooked brown and milled rice samples was estimated according to the proposed equation of Goñi et al. (1997) and Frei et al. (2003) below, utilizing HI data from the *in vitro* starch digestibility experiments:

$$\mathrm{EGI} = 39.71 + (0.549 \times \mathrm{HI}) \quad (5)$$

2.4. Physicochemical properties and proximate composition determination

Apparent amylose content (AC) analysis of milled rice samples was based on Juliano et al. (2012), with slight modifications based on Tuaño et al. (2014) and AC classification ranges based on Tuaño et al. (2015). Exactly 100 mg of 60-mesh milled rice flour was placed in a 100-mL volumetric flask and wetted with 1 mL of 95% ethanol. The mixture was swirled to disperse the rice samples followed by adding 9 mL of 1 N sodium hydroxide (NaOH) and the solution was allowed to stand for 16 h. After standing, the solution was diluted to 100 mL with distilled water. An aliquot of 5 mL was transferred into a 100 mL volumetric flask containing approximately 50 mL of distilled water. One milliliter (1 mL) of 0.9 N ammonium chloride (NH_4Cl) was added, followed by 2 mL of iodine (I_2) solution (0.15% I_2 in 1.5% potassium iodide [KI]), then diluted to 100 mL with distilled water. Absorbance was read at 620 nm within 1 h using UV Mini 1240 spectrophotometer (Shimadzu Corp., Japan). The absorbance for the waxy rice sample, IMS2, was determined at 620 nm after 2 h of standing (Tuaño et al., 2014). AC was calculated using a standard curve generated from a set of standard amylose solutions (all analyzed in triplicate). Alkali spreading value (ASV) was determined to classify the rice samples into gelatinization temperature (GT) types. Triplicate six whole milled grains were soaked in 1.7% KOH for 23 h. Degree of grain disintegration was scored and GT type was verified using differential scanning calorimetry (DSC) following the procedure of Nakamura, Sato, and Juliano (2006) and the proposed GT ranges for Philippines rices were used (Tuaño et al., 2014). Gelatinization endotherm curves were obtained using DSC-6100 (Seiko Instruments, Chiba, Japan) on 3-mg rice starch samples added with 9 μL distilled water in an aluminum sample holder. Heating rate was set at 3°C/min from 10°C to 120°C. Mean relative SD was below 1% for all samples analyzed in triplicate. Pasting viscosity was analyzed using a Rapid Visco Analyser (RVA) TecMaster model (Newport Scientific, Sydney, Australia) following the AACC standard method for milled rice flour (AACC, 2000). Around 3 g milled rice flour (60-mesh) was dispersed in 25 mL distilled water in an aluminum canister fit for the RVA TecMaster. Sample was heated for 1 min at 50°C following fast stirring (10 s at 960 rpm), then heated at the rate of 12°C/min to reach 95°C. Sample cooking was maintained for 2.5 min at 95°C, and then cooled to 50°C at the same temperature ramp rate. Total RVA running condition lasted for 12.5 min. RVA peak viscosity (RVA Peak), final viscosity at 95°C (or trough viscosity; TV), and final viscosity at 50°C (FV) were recorded using the Thermoclyne for Windows Software. Breakdown viscosity (RVA BD; RVA Peak – FV); setback viscosity (RVA SB; FV – RVA Peak); and consistency (RVA CON; FV – TV) were calculated and presented as mean values of triplicate determinations in Rapid Visco Units (RVU) (Tuaño et al., 2011). Mean relative SD was below 1% for all samples analyzed. Milled rice flour (100-mesh) was used to determine gel consistency (GC) following Cagampang, Perez, and Juliano (1973) method. Exactly 100 mg sample (triplicate) was wetted with 2 mL 0.2 N KOH with thymol blue as dye in 13 mm × 100 mm culture tubes followed by reflux heating, cooling to room temperature, and cooling in an ice bath for 1 h prior to scoring. The length of the gel was measured and samples were classified in terms of GC as follows: soft GC 61–100 mm; medium GC 41–60 mm; and hard GC 26–40 mm (Juliano, 2010). Cooked brown or milled rice sample (17 g) was used for cooked rice hardness determination via Instron 3343 model with Ottawa Texture Measuring System (OTMS) cell. Cooked rice sample was transferred into the 10-cm^2 OTMS cell having a perforated base (i.e. having 24 holes, 5 mm in diameter each), packed, and extruded at a speed of 10 cm/min. Cooked rice hardness was recorded using the Bluehill Software for Windows and expressed as mean Instron hardness in kg/cm^2 (Tuaño et al., 2011). Mean relative SD was below 1% for all cooked rice samples analyzed for Instron hardness in triplicate. Cooked brown and milled rice samples were also subjected to proximate analysis following the standard protocols described in the Association of Official Analytical Chemists (AOAC, 2005) and American Association of Cereal Chemists (AACC, 2000) approved methods of analysis. Moisture, crude protein, crude fat, crude fiber, and crude ash were analyzed in triplicate. Nitrogen-free extract representing total carbohydrate content was calculated by difference using the proximate analysis data obtained for each sample.

2.5. Statistical analysis

All data were subjected to statistical analysis using CropStat for Windows Version 7.2 employing balanced analysis of variance (ANOVA) under completely randomized design at 95% confidence level. Mean comparisons were done using the Least Significant Difference (LSD) test at 5% probability level and significant correlations were analyzed using Pearson correlation analysis. All data were presented as mean ± SD, unless otherwise specified.

3. Results

3.1. Resistant, non-resistant, and total starch levels of cooked Philippine rices

Resistant starch (RS) levels of cooked milled rice samples were generally lower than their counterpart brown rices, with the high-AC variety, PSB Rc10, having the highest RS content while the lowest RS content was noted for the waxy sample, Improved *Malagkit Sungsong* 2 (IMS2), with RS levels of 0.99% and 0.15%, respectively (Table 1). RS obtained from cooked milled rice samples ranged from 0.15% to 0.99% with mean RS content of 0.45% while for the cooked brown rice samples, RS values ranged from 0.24% to 1.61% with a mean of 1.05%. RS contents of cooked milled rices varied significantly across AC types. Similarly, cooked brown rice RS levels showed significant variations across AC types except for IR64 and PSB Rc10, having no significant difference in RS levels, but both were significantly higher than the RS levels of cooked brown rice of NSIC Rc160 and IMS2. PSB Rc10 tended to have the highest RS content and IMS2 tended to have the least, in both brown and milled rice forms. All cooked milled rices had comparable RS with those of cooked brown rice of IMS2 and NSIC Rc160. Generally, an increasing trend in RS content was observed relative to increasing apparent amylose content (AC) (Tables 1 and 2). Non-resistant starch (non-RS) contents of cooked milled rices were higher than 30% except for PSB Rc10. IMS2 had significantly higher digestible starch (non-RS) than the rest of the cooked milled rice samples and showed a 13.7% difference with that of PSB Rc10 while intermediate-AC IR64 and low-AC NSIC Rc160 cooked milled rices had statistically similar non-RS levels. In contrast, cooked brown rice of PSB Rc10 and IR64 had comparable non-RS contents while IMS2 and NSIC Rc160 had statistically similar amounts, significantly higher than the two varieties with higher AC. Notably, cooked NSIC Rc160 brown rice tended to have relatively higher amount of non-resistant starch than cooked IMS2 brown rice, but difference did not reach statistical significance (data not shown). Total starch (TS) content showed the same trend as that observed for digestible or non-

Table 1
Resistant starch (RS), non-resistant starch (Non-RS), total starch (TS) contents, *in vitro* starch hydrolysis index (HI), and estimated glycemic index (EGI) of selected cooked Philippine milled and brown rices differing in apparent amylose content (AC), and at 61–74% moisture.

Rice variety	AC type	RS (%)	Non-RS (%)	TS (%)[#]	HI (%)	EGI
Milled rice						
IMS2[*]	Waxy	0.15 ± 0.08^{d}	39.7 ± 3.3^{a}	40.0^{a}	102.2 ± 3.6^{a}	95.8 ± 2.0^{a}
NSIC Rc160	Low	0.26 ± 0.04^{c}	34.7 ± 1.8^{b}	35.0^{b}	85.4 ± 2.9^{b}	86.6 ± 1.6^{b}
IR64	Intermediate	0.41 ± 0.10^{b}	32.4 ± 2.1^{b}	32.8^{b}	67.3 ± 5.6^{c}	76.6 ± 3.1^{c}
PSB Rc10	High	0.99 ± 0.21^{a}	26.0 ± 1.6^{c}	27.0^{c}	59.3 ± 4.1^{d}	72.3 ± 2.3^{d}
Brown rice						
IMS2[*]	Waxy	0.24 ± 0.03^{d}	25.2 ± 2.6^{a}	25.4^{a}	66.9 ± 2.2^{a}	76.4 ± 1.2^{a}
NSIC Rc160	Low	0.87 ± 0.17^{c}	27.2 ± 2.0^{a}	28.0^{a}	61.1 ± 2.4^{b}	73.2 ± 1.3^{b}
IR64	Intermediate	1.49 ± 0.44^{a}	20.8 ± 2.9^{b}	22.3^{b}	56.3 ± 1.1^{c}	70.6 ± 0.6^{c}
PSB Rc10	High	1.61 ± 0.08^{a}	20.4 ± 2.7^{b}	22.0^{b}	49.2 ± 2.0^{d}	66.7 ± 1.1^{d}

Means within a column (for a particular form of cooked rice) followed by same letters are not significantly different by Least Significant Difference (LSD) test at $\alpha = 0.05$.
[#] TS – total starch content (%) = resistant starch (RS) + non-resistant starch (Non-RS) of cooked milled or brown rice at 61–74% moisture.
[*] IMS2 – Improved *Malagkit Sungsong* 2.

Table 2
Apparent amylose content (AC), alkali spreading value (ASV), gelatinization temperature (GT), gel consistency (GC) and Rapid Visco Analyser (RVA) pasting viscosity[#] of selected Philippine rices with varying AC.

Rice variety	AC (%)[$]	AC Type	ASV[*]	GT (°C)[*]	GC (mm)[+]	RVA Peak	RVA BD	RVA SB	RVA CON
IMS2[**]	1.7 ± 0.2^{a}	W	6.0^{a} L	68.2^{b} L	84 ± 4^{a} S	178^{c}	73^{b}	−41^{c}	32^{c}
NSIC Rc160	13.3 ± 0.2^{b}	L	6.2^{a} L	64.4^{c} L	79 ± 3^{a} S	256^{b}	74^{b}	21^{b}	95^{b}
IR64	17.6 ± 0.1^{c}	I	4.0^{c} I	73.9^{a} I	50 ± 0^{b} M	236^{b}	87^{a}	22^{b}	109^{a}
PSB Rc10	24.0 ± 0.5^{d}	H	4.9^{b} I	73.7^{a} I	28 ± 2^{c} H	270^{a}	73^{b}	166^{a}	93^{b}

Means within a column (for a particular form of cooked rice) followed by same letters are not significantly different by Least Significant Difference (LSD) test at $\alpha = 0.05$.
[#] RVA pasting viscosity expressed in Rapid Visco Units (RVU) classified as: peak viscosity (RVA Peak), breakdown viscosity (RVA BD), setback viscosity (RVA SB), and consistency (RVA CON). Mean relative SD was statistically negligible below 1% for all samples analyzed in triplicate (AACC, 2000).
[$] AC types are classified as: High (H) > 22%; Intermediate (I) 17–22%; Low (L) 10–17%; Very Low (VL) 2–10%; and Waxy (W) 0–2% (Juliano et al., 2012; Tuaño et al., 2015).
[*] ASV is used to classify milled rice according to GT types as: Low (L) 6–7; High (H) 3–5 for waxy and low-AC rices; and Low (L) 6–7; Intermediate (I) 4–5; High (H) 2–3 (Tuaño et al., 2014) and results were verified with actual GT (°C) via differential scanning calorimtery (DSC) of triplicate samples. Relative SD was statistically negligible below 1% for all samples analyzed. Classification based on GT via DSC: High (H) > 74°C; Intermediate (I) 70–74°C; Low (L) <70°C (Juliano, 2010).
[+] GC classification: Hard (H) 26–40 mm; Medium (M) 41–60 mm; Soft (S) 61–100 mm (Juliano, 2010).
[**] IMS2 – Improved *Malagkit Sungsong* 2.

resistant starch content since RS levels for all rice samples in this study were generally lower than 2% (Table 1).

3.2. *In vitro* starch digestibility patterns of cooked Philippine rices

In vitro starch digestibility patterns are represented by *in vitro* starch hydrolysis curves obtained from measuring free glucose released within 3 h of enzymatic digestion of cooked rice (using α-amylase and amyloglucosidase) at 30 min intervals as shown in Figs. 1 and 2. *In vitro* starch hydrolysis index (HI) of cooked brown and milled rices were calculated based on the area under the curve (AUC) of each sample's starch hydrolysis curve relative to that of white bread used as standard reference food in this study (Table 1; Figs. 1 and 2). Cooked milled rice of the waxy variety IMS2 had the highest % starch hydrolyzed and was very close to the digestibility pattern of the reference food, white bread, at all time points (Fig. 1A) while the starch digestion curve of cooked low-AC milled rice (NSIC Rc160) was just beneath those of white bread and IMS2, with an average difference of 20% starch hydrolyzed at all time points. The pattern of starch digestion of cooked rice for these two varieties with lower AC than IR64 and PSB Rc10 were similar to that of white bread, which had an abrupt rise in % starch hydrolyzed measured as free glucose released at 30 min and leveled off with time until 180 min. Cooked milled rice of IR64 (intermediate AC) had a unique starch digestibility pattern showing that the plateau-forming part of the curve started at 90 min with a steady rise until 180 min at a relatively low extent. A steep slope corresponding to the release of free glucose from starch digestion was also observed from 0 min to 60 min (Fig. 1A). PSB Rc10 cooked milled rice had the same pattern of % starch hydrolyzed as compared to those of IMS2 and NSIC Rc160 during the 3-h digestion but the curve tended to slightly rise at 180 min, in contrast to that of NSIC Rc160, which had a gradual steady drop in % starch hydrolyzed starting at 120 min until 180 min (Fig. 1A). The average difference in % starch hydrolyzed of IR64 and PSB Rc10 was 30% and 40%, respectively, when compared to white bread. All cooked milled rice samples except IR64 had reached equilibrium in terms of % starch hydrolyzed after 60 min. The amount of starch hydrolyzed at the end of the 3-h enzymatic digestion of cooked rice was highest for IMS2, followed by NSIC Rc160, IR64, and lastly, PSB Rc10. IMS2 had comparable % starch hydrolyzed with the reference food, white bread, at the end of the *in vitro* starch hydrolysis experiments (Fig. 1A).

Similar trend of *in vitro* starch digestibility patterns and amount of starch hydrolyzed (in %) at the end of the 3-h digestion was observed for the cooked brown rice samples for all the varieties: IMS2 > NSIC Rc160 > IR64 > PSB Rc10 (Fig. 1B). However, the average differences in % starch hydrolyzed were very small and were not more than 10% among the samples. In general, the % starch hydrolyzed at all time points were significantly lower than that of white bread and the difference ranged from 30% to 50%. There was no significant difference between cooked brown rice of NSIC Rc160 and IR64, in terms

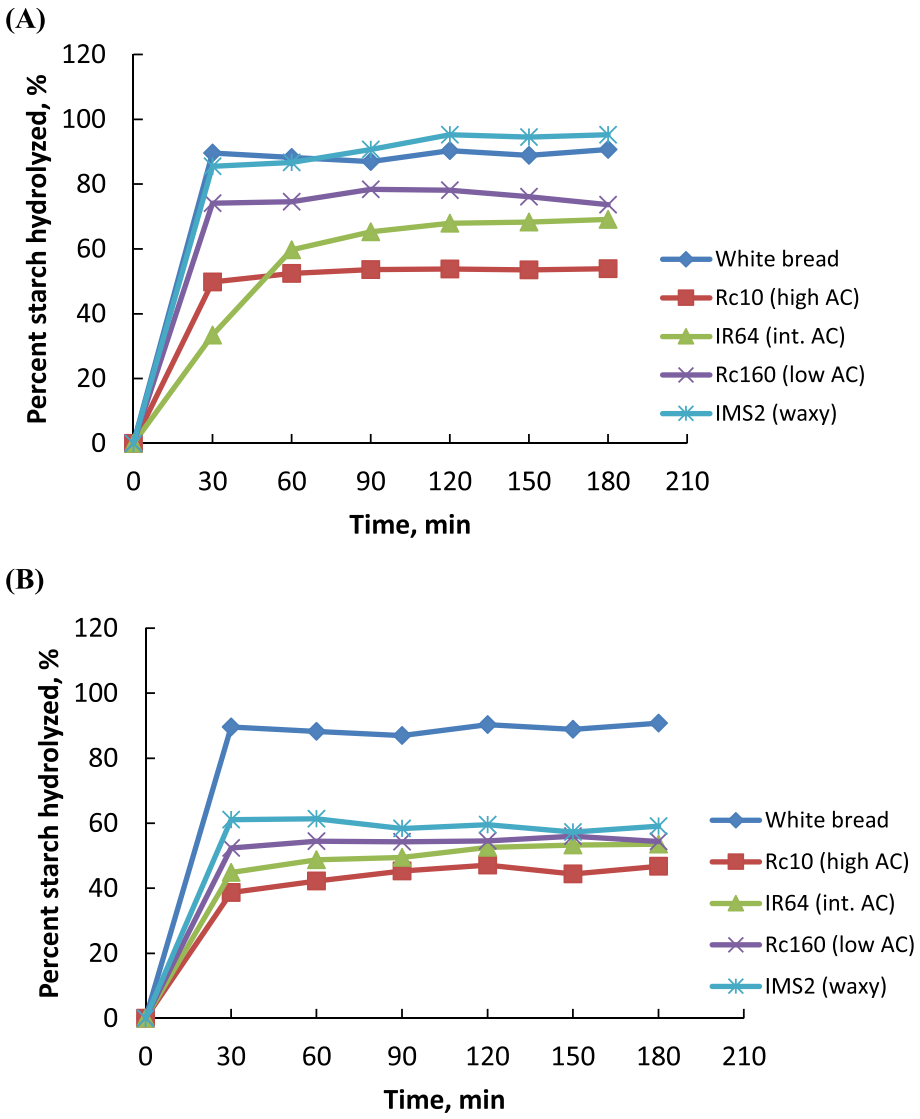

Fig. 1. *In vitro* starch digestibility curves of selected cooked Philippine (A) milled and (B) brown rices varying in apparent amylose content (AC). Rc10 – PSB Rc10; int. AC – intermediate AC; Rc160 – NSIC Rc160; IMS2 – Improved *Malagkit Sungsong* 2.

of % starch hydrolyzed at the end of the 3-h enzymatic digestion, while cooked PSB Rc10 brown rice remained to have significantly lower values and cooked IMS2 brown rice had significantly higher % starch hydrolyzed as compared to all the brown rice samples in this study (data not shown). Notably, the starch digestibility curve for the cooked brown rice of IR64 showed similar plateau-forming pattern starting at 30 min until 180 min with the rest of the brown rice and milled rice samples (Fig. 1B), in contrast to its counterpart cooked milled rice sample (Fig. 1A).

Comparison of superimposed starch digestibility curves of cooked brown and milled rice for each rice variety is shown in Fig. 2. For all the four varieties, the kinetics of starch hydrolysis of cooked milled rice was significantly higher than their cooked brown rice counterparts at $\alpha = 0.05$. When the difference in mean % starch hydrolyzed between brown rice and milled rice was calculated per variety, it was noted that the highest difference was for the waxy sample IMS2 followed by NSIC Rc160 (low AC), then IR64 (intermediate AC), and the lowest mean difference was noted for PSB Rc10 (high AC). Despite the very low mean difference in % total starch hydrolyzed for PSB Rc10, the amount of starch hydrolyzed for PSB Rc10, at each time interval, remained to be significantly different between cooked brown rice and cooked milled rice, as similarly observed for the other three rice varieties (Fig. 2).

3.3. *In vitro* starch hydrolysis index and estimated glycemic index

The *in vitro* starch hydrolysis index (HI) and estimated glycemic index (EGI) of all cooked brown rice and milled rice samples are shown in Table 1. For the cooked milled rices, the highest HI and EGI was noted for IMS2, followed by NSIC Rc160, then IR64, and the lowest was recorded for PSB Rc10. Cooked Philippine milled rices had HI ranging from 49% to 102% with a mean HI of 68% while EGI ranged from 66 to 96 with a mean EGI of 77 (Table 1). Significant differences in HI and EGI were observed among all cooked rice samples, regardless of AC type, in each category – brown rice and milled rice forms. Increasing trend in HI and EGI was also observed in each rice form relative to decreasing AC, with the cooked waxy rice IMS2 having the highest HI and EGI, and lowest AC (Tables 1 and 2). Interestingly, the HI of cooked PSB Rc10 brown rice was lower than 50% and the corresponding EGI was lower than 70 while IMS2 had over 100% HI and an EGI of 96 (Table 1). All cooked brown rice samples had EGI of not more than 80 with only PSB Rc10 having EGI less than 70.

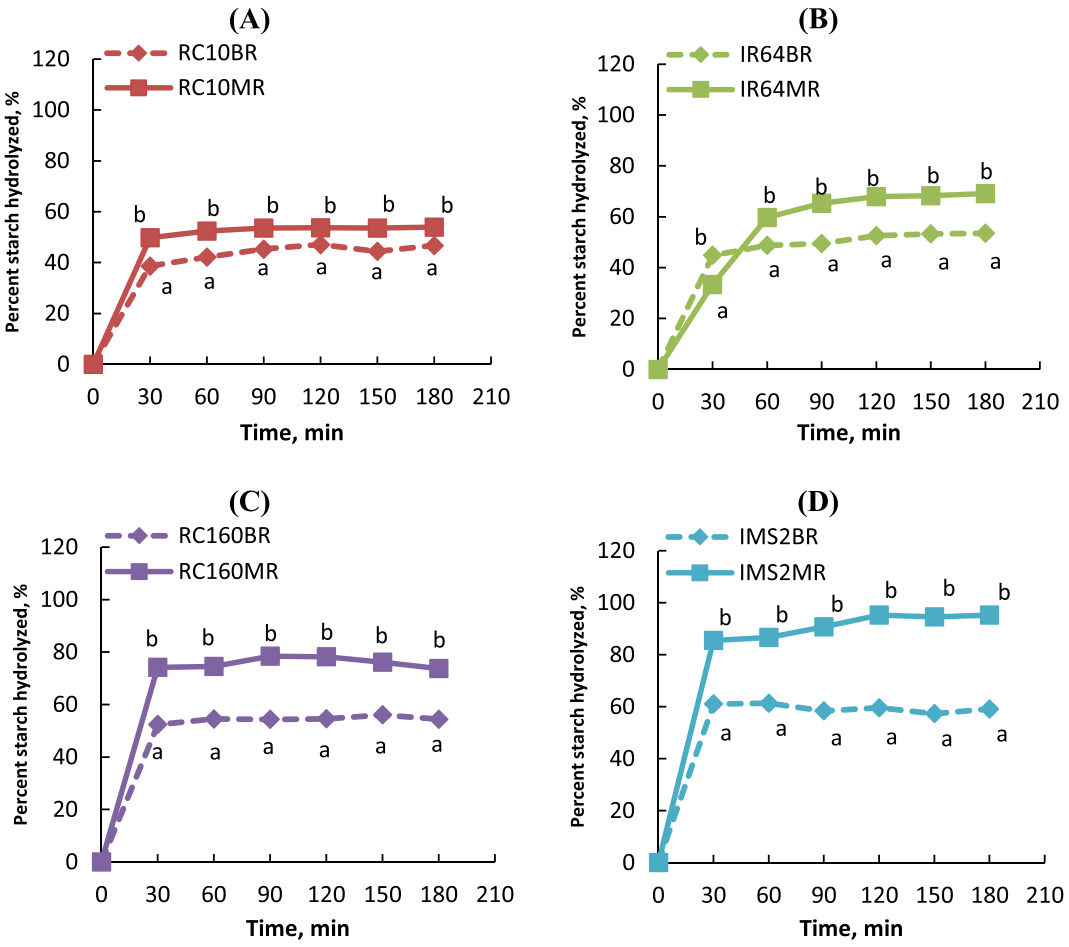

Fig. 2. Comparative *in vitro* starch hydrolysis curves of Philippine brown rice (BR) and milled rice (MR) per variety and apparent amylose content (AC) type. High AC PSB Rc10 (A); Intermediate AC IR64 (B); Low AC NSIC Rc160 (C); and Waxy Improved *Malagkit Sungsong* 2 (IMS2) (D). Data points within the hydrolysis curves followed by the same letter (for each variety) are not significantly different by Least Significant Difference (LSD) test at $\alpha = 0.05$.

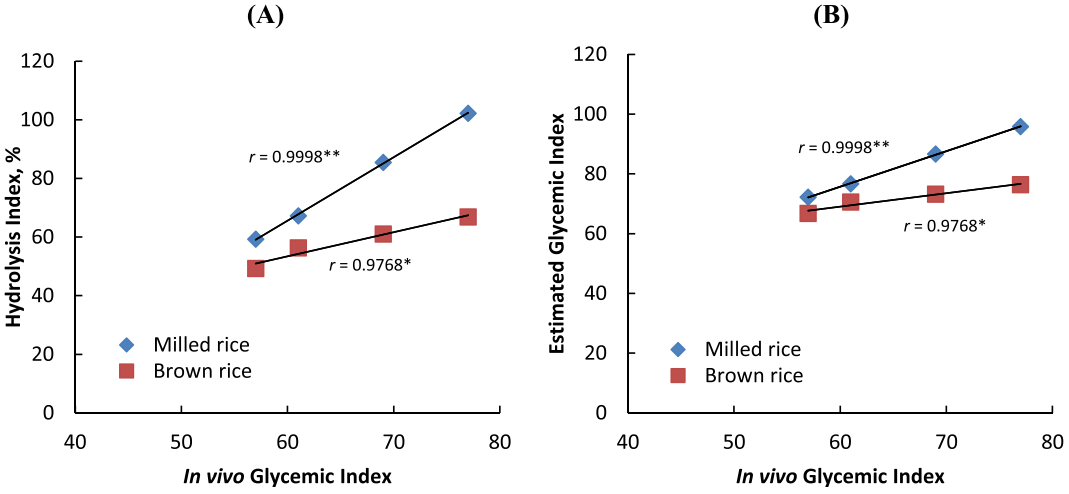

Fig. 3. Correlation between *in vivo* GI values of selected cooked Philippine brown and milled rices (Trinidad et al., 2014) and *in vitro* starch hydrolysis index (A); and estimated glycemic index (B). **Significant at $\alpha = 0.01$; and *significant at $\alpha = 0.05$.

Table 3
Proximate composition of cooked Philippine brown and milled rice samples differing in apparent amylose content (AC).

Rice variety	AC type	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	CHO (%)[#]	Crude ash (%)
Milled rice							
IMS2[*]	Waxy	64.3 ± 0.9^{c}	6.8 ± 0.6^{b}	5.7 ± 0.2^{a}	1.3 ± 0.6^{a}	21.9^{a}	0.1 ± 0.0^{b}
NSIC Rc160	Low	64.9 ± 0.2^{c}	8.7 ± 0.7^{a}	4.7 ± 0.8^{b}	1.3 ± 0.6^{a}	20.5^{ab}	0.6 ± 0.1^{a}
IR64	Intermediate	68.9 ± 0.2^{b}	8.9 ± 0.3^{a}	1.2 ± 0.1^{c}	1.0 ± 0.0^{a}	19.3^{b}	0.2 ± 0.1^{b}
PSB Rc10	High	73.7 ± 0.0^{a}	7.4 ± 0.2^{b}	1.8 ± 0.6^{c}	2.0 ± 0.6^{a}	15.5^{c}	0.4 ± 0.1^{a}
Brown rice							
IMS2[*]	Waxy	58.3 ± 0.9^{d}	6.3 ± 0.2^{c}	1.7 ± 0.4^{a}	2.0 ± 1.0^{b}	33.6^{a}	1.3 ± 0.1^{b}
NSIC Rc160	Low	61.1 ± 0.3^{c}	8.5 ± 0.3^{a}	0.9 ± 0.5^{b}	1.7 ± 0.6^{b}	28.9^{b}	1.2 ± 0.1^{b}
IR64	Intermediate	63.0 ± 0.5^{b}	8.7 ± 0.3^{a}	0.9 ± 0.0^{b}	3.0 ± 1.7^{a}	27.2^{b}	1.7 ± 0.1^{a}
PSB Rc10	High	69.6 ± 0.5^{a}	7.2 ± 0.3^{b}	1.6 ± 0.4^{a}	3.7 ± 0.6^{a}	21.2^{c}	1.6 ± 0.2^{a}

Means within a column (for a particular form of cooked rice) followed by same letters are not significantly different by Least Significant Difference (LSD) test at $\alpha = 0.05$.
[#] CHO (%) – total carbohydrates (%); calculated as nitrogen free extract (NFE) by difference.
[*] IMS2 – Improved *Malagkit Sungsong* 2.

Cooked NSIC Rc160 milled rice had comparable HI and EGI values. Cooked milled rice samples of IR64 and PSB Rc10 showed comparable EGI with cooked brown rice of IMS2, NSIC Rc160, and IR64 (Table 1).

3.4. In vitro starch digestibility in relation to rice grain quality and composition

Physicochemical properties and proximate composition of the rice samples in this study are presented in Tables 2 and 3. AC types of the Philippine rices used here were verified by the actual AC data via amylose-iodine colorimetry in ammonium buffered medium and showed significant differences across AC types (Table 2). GT types were also confirmed through the alkali spreading value (ASV) of each sample and the actual GT values obtained via differential scanning calorimetry (DSC). Degree of grain disintegration of IR64 and PSB Rc10 milled rice after soaking in 1.7% KOH for 23-h resulted in significantly different ASV, 4.0 and 4.9, respectively, but these ASV data still corresponded to the intermediate GT class for intermediate- and high-AC rices based on the proposed ranges for GT of Philippine rice (Tuaño et al., 2014) and based on the peak temperature of gelatinization of ~74°C (Juliano, 2010) (Table 2). On the other hand, IMS2 and NSIC Rc160 had statistically similar ASV and both were classified as low-GT rices but DSC GT data showed significantly higher gelatinization peak temperature for IMS2 (68.2°C) than NSIC Rc160 (64.4°C). Gel consistency (GC) of IMS2 and NSIC Rc 160 milled rices was soft and comparable while high-AC PSB Rc10 had the shortest cooled gel length and was classified as hard GC. The intermediate-AC rice IR64 had significantly higher GC than PSB Rc10 and was noted to be medium GC. Pasting properties via Rapid Visco Analyser (RVA) did not show significant variations among non-waxy rices in terms of RVA peak viscosity (RVA Peak), though the waxy rice IMS2 had significantly lower RVA Peak than the three non-waxy rices (Table 2). RVA breakdown viscosity (RVA BD) was similar for IMS2, NSIC Rc160, and surprisingly, PSB Rc10, while only IR64 had significantly higher RVA BD. Only IMS2 had a negative RVA setback viscosity (RVA SB) among the four rice samples while PSB Rc10 had the highest RVA SB, as expected for high-AC rices. NSIC Rc160 and IR64 had comparable RVA SB, significantly different from the two varieties earlier mentioned. Lastly, RVA consistency (RVA CON) was noted to be highest for IR64, followed by comparable RVA CON values for PSB Rc10 and NSIC Rc160 while IMS2 had the lowest RVA CON, as expected for typical waxy rices (Table 2). Instron hardness of all cooked rice samples subjected to *in vitro* starch hydrolysis experiments in this study, regardless of AC, was within the range of 1.2–1.3 kg/cm^2 following the water:rice ratios and cooking procedure employed by Trinidad et al. (2014) and Barcellano (2015) and periodically monitored using the Instron 3343 (data not shown).

Moisture content (MC) of the cooked rice samples ranged from 61% to 74% with a mean MC of 66%, with PSB Rc10 having the highest moisture among all the samples, in both brown and milled rice forms, as expected for high-AC rices. Only cooked brown rices had significant differences in terms of moisture content across the four AC types while cooked IMS2 and NSIC Rc160 milled rices had statistically similar MC. Generally, cooked milled rices had relatively higher MC than cooked brown rices. Notably, crude protein content was observed to be over 8% for NSIC Rc160 and IR64 cooked rice samples and below 7% for cooked IMS2, in both brown and milled rice forms. Crude fat for most of the samples was below 2% except for cooked IMS2 and NSIC Rc160 milled rices. Cooked milled rice for all the four varieties showed no significant variation in terms of crude fiber content but cooked brown rice of IR64 and PSB Rc10 had the highest crude fiber levels, significantly higher than those of cooked IMS2 and NSIC Rc160 milled rice. No unambiguous trend in crude fiber level was observed relative to AC for all the samples. Similarly, no clear trend in crude ash content was noted for all samples across all AC types but all cooked brown rices were observed to have significantly higher ash contents than their counterpart cooked milled rices, all greater than 1% and ranging from 1.2% to 1.7%, while cooked milled rice samples had lower than 1% crude ash ranging from 0.1% to 0.6% (Table 3).

Among the rice grain components determined via proximate analysis, only MC was found to have significant correlation with resistant starch content among all the rice samples when analyzed separately as brown rice and milled rice. However, correlation did not reach statistical significance when all eight data points were treated as a set (data not shown). MC had significantly positive relationship with RS among cooked milled rices ($r = 0.9731$*) and it tended to correlate positively with RS among cooked brown rices but not statistically significant ($r = 0.8620^{ns}$). Similar observation was noted for AC in relation to RS and non-RS levels. Interestingly, but for milled rices alone, AC tended to positively correlate with RS ($r = 0.8613^{ns}$), while for brown rices alone, AC had a significant positive correlation with RS ($r = 0.9706$*). Digestible or non-resistant starch content had no significant correlation with AC among the four cooked brown rice samples but showed a significant negative correlation with AC among cooked milled rice samples ($r = -0.9726$*) at $\alpha = 0.05$. Correlation among other *in vitro* starch digestibility parameters such as RS content and HI with AC, EGI, and *in vivo* GI data are presented in Fig. 3. Results showed that when treated separately as brown rice and milled rice groups, HI had a significant negative correlation with AC. The same was observed with RS as shown by its negative correlation with HI, though of moderate statistical significance, using all eight data points treated as a set, regardless of form (brown ro milled) and AC ($r = -0.7874$*). HI values would be of vital relevance in screening rice breeding lines and rice-based food products in the food industry,

for potential health benefits and improved nutritional value, when a significantly high correlation with *in vivo* GI data, determined from human studies, can be established. Fig. 3 shows that HI and the previously reported *in vivo* GI of the same set of cooked brown and milled rices (Trinidad et al., 2014) were highly and significantly positively correlated. Similarly, the EGI, calculated based on the obtained HI data, correlated positively well with the reported *in vivo* GI, for both the brown rice and milled rice groups in this study, each having cooked rice samples varying in AC, MC, and RS levels (Fig. 3).

4. Discussion

4.1. Resistant starch and grain properties of cooked Philippine rices

Rice in the Philippine breeding program, in local retail markets and supermarkets, in the International Rice Research Institute (IRRI) and the Philippine Rice Research Institute (PhilRice) gene banks, and in farmers' fields vary widely in terms of apparent amylose content (AC) and gelatinization temperature (GT). Intermediate-AC rice having intermediate GT has been found to be predominant among Philippine traditional and modern rice varieties and preferred by most Filipino consumers (Juliano, Perez, & Resurreccion, 2009; Juliano, 2010; Tuaño, 2013; Tuaño et al., 2015, 2016). Glycemic index (GI) of Philippine milled rice with varying AC and GT has been studied in 2013 parallel to a research project on short-term satiety of milled rice, both involving healthy Filipino volunteers (Felix, Trinidad, Tuaño, & Juliano, 2013; Trinidad et al., 2013). From these studies, AC and dietary fiber content had been reported to significantly affect the GI of cooked Philippine milled rices, i.e. as AC and/or dietary fiber level increase, GI decreases. In terms of AC, cooked milled rice of intermediate-AC varieties such as IR64, PSB Rc18, and PSB Rc12, had medium GI, regardless of GT, while the low-AC variety *Sinandomeng* had high GI and the high-AC variety PSB Rc10 had low GI (Trinidad et al., 2013). Interestingly, high protein content (around 9% of raw milled rice at 12–14% moisture) also affected GI as shown by the unexpectedly medium GI obtained for the high protein-IMS2 sample (GI = 63) as compared to the usually high GI of cooked IMS2 milled rice (GI = 94) with normal protein content (4–6%) (Trinidad et al., 2013, 2014). These previously reported GI values for cooked Philippine brown and milled rices were used as basis for this study in an attempt to correlate *in vivo* GI data with *in vitro* starch hydrolysis parameters such as resistant starch (RS), non-resistant (non-RS) or digestible starch, hydrolysis index (HI), and estimated GI (EGI) using the same set of rice samples, water:rice ratios, and cooking method previously reported (Trinidad et al., 2014; Barcellano, 2015).

Resistant starch is the sum of the starch fraction and its digestion products that is not hydrolyzed by digestive enzymes in the small intestine and proceeds to the large intestine for fermentation by the beneficial colonic microflora (Fuentes-Zaragoza et al., 2010; Nugent, 2005). Most of the rice RS and HI data available in the literature were determined on raw rice (Englyst et al., 1992; Frei et al., 2003; Goñi, García-Diz, Mañas, & Saura-Calixto, 1996), some used rice samples cooked using various cooking procedures (Alhambra et al., 2019; Chiu and Stewart, 2013; Deepa et al., 2010), and a few used both raw and cooked rice (Eggum, Juliano, Perez, & Acedo, 1993). The use of pre-determined water:rice ratio in cooking rice samples intended for GI and digestibility studies has been identified vital in order to maximize the effect of cooked rice grain components such as amylose and dietary fiber on varietal differences in GI and starch digestibility. Also, this may minimize the effect of rapid resistant starch formation due to retrogradation as well as the differences in the doneness and texture of cooked rice brought by single water:rice ratio used in cooked rice texture discrimination tests employed in several rice breeding programs. Use of single water rice:ratio in rice cooking may differentiate varieties in terms of cooked rice texture which may in turn affect the mastication and palatability perception of human subjects towards the rice samples under study and may be potential confounders in GI feeding trials where the ultimate goal is to determine variability in GI relative to AC and other grain properties (Felix et al., 2013; Juliano, 2010; Trinidad et al., 2014). Similarly, this study also aimed to determine the effect of AC and other grain properties on the RS content, HI, and other starch digestibility parameters of cooked Philippine brown and milled rices. *In vitro* starch digestibility patterns were compared among the different varieties and relative to the standard reference food, white bread. Results showed that RS level was significantly positively correlated with AC of brown and milled rice samples, when statistically treated as separate groups in terms of rice form (Tables 1 and 2). Rice with higher AC tended to have higher RS content than that with lower AC and generally, waxy rice tended to have very low amount of RS when cooked, in both brown and milled rice forms. Increase in RS levels of cooked starchy foods has been shown to be partly due to the presence of amylose capable of undergoing rapid retrogradation and amylose chain reassociation leading to increased formation of resistant starch 3 (RS3) – initially digestible starch converted into resistant starch via retrogradation, which is less susceptible to α-amylase digestion (Haralampu, 2000; Lunn and Buttriss, 2007; Sajilata et al., 2006). Cooked Philippine milled rice of high-AC varieties with intermediate-to-high GT, such as PSB Rc10 and NSIC Rc222, tended to be RS3-rich due to the high amylose levels in the endosperm, as compared to varieties with lower AC and GT, comprised mainly of gelatinized starch granules having high amounts of readily hydrolyzable amylopectin chains (Hsein-Chih, Wu, & Sarko, 1978; Zhu, Liu, Wilson, Gu, & Shi, 2011). The amylopectin chain-length distributions and amylopectin chain ratios (ACR) of the Philippine rices in this study have been previously characterized and reported as follows: waxy rice IMS2 (ACR = 0.246) and low-AC variety NSIC Rc160 (ACR = 0.249) both had S-type amylopectin while intermediate-AC rice IR64 (ACR = 0.173) and high-AC variety PSB Rc10 (ACR = 0.172) both had L-type amylopectin (Tuaño et al., 2014; Tuaño, 2013). The fine structure and distribution of amylopectin chains of the Philippine rice varieties used in this study were actually similar in waxy-to-low-AC and in intermediate-to-high AC pairs mentioned, hence, the observed variations in RS levels and starch hydrolysis index in this sample set may be largely attributed to AC differences and possibly to long-chain amylopectin (LCA) content as in the case of the intermediate- and high-AC samples. ACR and GT effects on starch retrogradation, RS, and starch digestibility varietal differences may be fully described using a more diverse pool of rice genotypes varying in gelatinization properties but having similar AC and LCA. In addition, long glucan chains of amylose and long-chain amylopectin have been reported to retrograde and reassociate faster and more effectively than the short outer chains of amylopectin, thus, rendering cooked high-AC cultivars more resistant to enzymatic digestion of their endosperm starch (Alhambra et al., 2019; Gallant, Bouchet, Buleon, & Perez, 1992; Hsein-Chih, Wu, & Sarko, 1978). Furthermore, present results showed that the RS content of brown rice for all the four varieties in this study, regardless of AC type, was significantly higher compared to their milled rice counterparts. This may be due to the inhibition of α-amylase by certain bran components and the inaccessibility of the gelatinized starch granule towards the hydrolytic action of α-amylase possibly indicating some sort of physical and chemical barrier effects of the intact bran layer, thus, causing slower and lesser degree of digestibility of cooked brown rice starch than that of milled rice. The presence of the bran in cooked brown rice grains has two possible effects: 1) the bran serves as an obstruction against the entry of water into the starch granules preventing the granule's immediate disruption and the slowed access of hydrolytic enzymes; and 2) the bran, particularly the aleurone layer which is rich in phytic acid (Juliano & Tuaño, 2019), may slow down starch digestion by enzyme inhibition or substrate and/or cofactor chelation. Phytic acid can interact with α-amylase and amyloglucosidase as well as with

the starch substrate through its phosphate moieties. It may also chelate calcium ions in the aqueous digestion medium, thereby reducing the hydrolytic activity of α-amylase (Eggum et al., 1993; Panlasigui & Thompson, 2006; Yoon, Thompson, & Jenkins, 1983) and this warrants further mechanistic studies using a diverse set of rice cultivars. With these, the rate and degree of starch hydrolysis decrease in cooked brown rice samples as evidenced by the high RS and low HI of cooked brown rices in this study (Table 1).

4.2. In vitro starch digestibility patterns, hydrolysis Index, and glycemic index of cooked Philippine rices

Zhu et al. (2011) has shown that long amylopectin chains in intermediate-AC rice starch have the ability to resist enzymatic hydrolysis due to their ability to form more stable double helices even prior to amylopectin staling, similar to amylose double helices that can rapidly retrograde. We have reported that intermediate-to-high-AC Philippine *indica* rices contain significant amounts of long-chain amylopectin (LCA) as compared to low-AC and waxy rices (Juliano et al., 2012; Tuaño et al., 2011, 2014). This may also support the higher RS contents and lower HI results for cooked rice of PSB Rc10 and IR64 obtained in this study, as compared to the RS levels and HI values for cooked rice of NSIC Rc160 and IMS2, in both brown and milled forms. In addition, low GT-rices are more susceptible to α-amylase digestion due to relative ease of disruption of the gelatinized starch granules and their short-chain amylopectin double helices (Alhambra et al., 2019; Zhu et al., 2011). Eggum et al. (1993) determined the RS content of raw and cooked milled rices varying in AC using *in vitro* assay and *in vivo* experiments using rat subjects and generally, cooked milled non-waxy rices reportedly had higher RS levels than the corresponding raw rice samples, indicating the effect of cooking and rapid amylose retrogradation of freshly cooked rice on RS content. Chiu and Stewart (2013) analyzed four white rice varieties cooked in different ways − via pressure cooker, oven, and rice cooker. RS content of these freshly cooked milled rice samples via rice cooker method ranged from 0.38% to 1.08% comparable to those obtained in this study using the beaker-in-rice cooker method (Table 1).

Frei et al. (2003) observed that in most of the milled rice samples analyzed for *in vitro* starch hydrolysis, the amount of starch hydrolyzed (measured as free glucose) equilibrated starting at 60 min and continued until the end of the 3-h enzymatic digestion. In contrast, results of the present study have shown that most, if not all, of the cooked rice samples had the onset of plateau-formation in the starch digestibility curves at 30 min and continued onwards to 180 min (Figs. 1 and 2). These contrasting observations may be due to the different cooking method, water:rice ratio, and cooking time employed in the previous study as compared to the present one. Nonetheless, our results were actually comparable with those of Frei et al. (2003) in terms of the average % starch hydrolyzed previously reported for milled waxy, low-, intermediate-, and high-AC traditional Philippine rices, i.e. around 81%, 72%, 35% and 30%, respectively.

For all cooked milled rice samples in this study, the volume expansion of rice after cooking was significantly higher than those of the corresponding cooked brown rices (data not shown). Greater volume expansion corresponds to easier hydration of endosperm starch and faster disruption of starch granules, hence, higher accessibility of starch granules to enzymatic hydrolysis (Panlasigui & Thompson, 2006). The extent of the effect of volume expansion on starch digestibility and HI was observed to be highest for the waxy variety, IMS2, where the presence of intact bran layer in cooked IMS2 brown rice grains resulted in reduced volume expansion upon cooking (data not shown). This resulted in more than 25% decrease in % starch hydrolyzed (Fig. 2D) of cooked IMS2 brown rice and nearly 15% difference in digestible starch level as compared to cooked IMS2 milled rice. A large significant lowering in HI from 102% to 66% was also observed (Table 1). The higher amount of RS in cooked brown rices, across all AC types, as compared to their milled rice counterparts, may also be similarly explained as above. The same observations were noted for the differences in the GI of cooked brown and milled rices for each rice variety as reported by Trinidad et al. (2014).

Estimated glycemic index (EGI) of cooked brown and milled rice samples in this study was not in absolute agreement with the *in vivo* GI values for all the samples analyzed, however, a significant positive correlation was noted between the two starch digestibility parameters (Fig. 3B). Reported GI for the cooked rice samples used in this study were: IMS2 brown rice: 77, milled rice: 94; NSIC Rc160 brown rice: 69, milled rice 85; IR64 brown rice: 61, milled rice: 69; and PSB Rc10 brown rice: 57, milled rice: 59 (Trinidad et al., 2014). Only the EGI for cooked IMS2 and NSIC Rc160 milled rices, (96 and 87, respectively) were close to the previously reported GI values (Trinidad et al., 2014), while the rest showed relatively higher EGI as compared to *in vivo* GI (Table 1). Nonetheless, both HI and EGI showed significantly high positive correlations with *in vivo* GI in this set of cooked Philippine rices differing in AC. Also, RS content and AC had significant positive correlations with HI in this sample set.

5. Conclusions

The *in vitro* starch digestibility parameters reported here utilizing selected Philippine brown and milled rice varieties differing in apparent amylose content may be useful in setting up a relatively faster, non-invasive, and less expensive protocol of screening elite rice breeding lines and rice-based food products for starch hydrolysis properties as compared to *in vivo* experiments involving laboratory animals or human subjects. Varietal differences in terms of resistant starch level, *in vitro* starch hydrolysis index, and starch digestibility have been shown to be mainly affected by milled rice AC and cooked rice moisture content, and to correlate significantly with AC and *in vivo* GI. But the effect of GT and other rice grain amylopectin properties also warrants further studies using a larger and more diverse set of cooked rice samples. The *in vitro* starch digestibility indices studied here may complement with existing methods for rice grain quality evaluation such as AC, GT, and GC, focusing more on the nutritional quality of cooked rice. The cooked rice digestibility properties described here may also be further enhanced and explored for their potential use in the characterization of Philippine rice varieties and other locally available starch-rich foods for value-adding purposes and for nutrition- and health-related uses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

APPT, ECGB, and MSR conceptualized and designed the study. ECGB and APPT performed the experiments. APPT, ECGB, and MSR analyzed and interpreted the results. APPT and ECGB wrote the paper. All authors have read and approved the final manuscript prior to submission.

References

[AACC] American Association of Cereal Chemists (2000). Approved Methods of the AACC. St. Paul, MN, USA: American Association of Cereal Chemists.

Alhambra, C. M., de Guzman, M. K., Dhital, S., Bonto, A. P., Dizon, E. I., Israel, K. A. C., ... Sreenivasulu, N. (2019). Long glucan chains reduce *in vitro* starch digestibility of freshly cooked and retrograded milled rice. *Journal of Cereal Science, 86*, 108–116.

Annison, G., & Topping, D. L. (1994). Nutritional role of resistant starch: Chemical structure vs physiological function. *Annual Review of Nutrition, 14*, 297–320.

[AOAC] Association of Official Analytical Chemists. (2005). AOAC Official Methods of Analysis. 18th ed. Arlington, VA, USA: Association of Official Analytical Chemists.

Barcellano, E. C. G. (2015). Resistant starch content and in vitro starch hydrolysis index of selected Philippine brown and milled rices differing in apparent amylose content. BSc Thesis, University of the Philippines Los Baños, Philippines.

Cagampang, G. B., Perez, C. M., & Juliano, B. O. (1973). A gel consistency test for eating quality of rice. *Journal of the Science of Food and Agriculture, 24*, 1589–1594.

Chiu, Y. T., & Stewart, M. L. (2013). Effect of variety and cooking method on resistant starch content of white rice and subsequent postprandial glucose response and appetite in humans. *Asia Pacific Journal of Clinical Nutrition, 22*, 372–379.

Deepa, G., Singh, V., & Naidu, K. A. (2010). A comparative study on starch digestibility, glycemic index and resistant starch of pigmented ('Njavara' and 'Jyothi') and a non-pigmented ('IR64') rice varieties. *Journal of Food Science and Technology, 47*, 644–649.

Eggum, B. O., Juliano, B. O., Perez, C. M., & Acedo, E. F. (1993). The resistant starch, undigestible energy, and undigestible protein contents of raw and cooked milled rice. *Journal of Cereal Science, 18*, 159–170.

Englyst, H., Kingman, S., & Cummings, J. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*, 33–50.

Felix, A. D. R., Trinidad, T. P., Tuaño, A. P. P., & Juliano, B. O. (2013). Short-term satiety of cooked Philippine rices of varying apparent amylose content and glycemic index. *Philippine Agricultural Scientist, 96*, 46–53.

Frei, M., Siddhuraju, P., & Becker, K. (2003). Studies on the *in vitro* starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. *Food Chemistry, 83*, 395–402.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M. J., Sánchez-Zapata, E., & Pérez-Álvarez, J. A. (2010). Resistant starch as functional ingredient: A review. *Food Research International, 43*, 931–942.

Gallant, D., Bouchet, B., Buleon, A., & Perez, S. (1992). Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition, 46*, S3–S16.

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*, 427–437.

Goñi, I., García-Diz, L., Mañas, E., & Saura-Calixto, F. (1996). Analysis of resistant starch: A method for foods and food products. *Food Chemistry, 56*, 445–449.

Haralampu, S. G. (2000). Resistant starch–a review of the physical properties and biological impact of RS3. *Carbohydrate Polymers, 41*, 285–292.

Hsein-Chih Wu, H., & Sarko, A. (1978). The double-helical molecular structure of crystalline B-amylose. *Carbohydrate Research, 61*, 7–25.

Hu, E. A., Pan, A., Malik V., Sun, Q. (2012). White rice consumption and risk of type 2 diabetes: Meta-analysis and systematic review. The British Medical Journal, 344: e1454. DOI:10.1136/bmj.e1454.

Jenkins, D., Wolever, T., Taylor, R., Fielden, H., Baldwin, J., Bowling, A., Newman, H.C., Jenkins, A., Goff, D.V. (1981). Glycemic index of foods: A physiological basis for carbohydrate exchange. American Journal of Clinical Nutrition, 34, 362-366.

Juliano, B. O., & Tuaño, A. P. P. (2019). Gross Structure and Composition of the Rice Grain. In J. S. Bao (Ed.), Rice Chemistry and Technology (4th ed) (pp. 31-53). St. Paul, MN, USA: American Association of Cereal Chemists International, Elsevier Inc.

Juliano, B. O., Perez, C. M., & Resurreccion, A. P. (2009). Apparent amylose content and gelatinization temperature types of Philippine rice accessions in the IRRI gene bank. *Philippine Agricultural Scientist, 92*, 106–109.

Juliano, B. O., Tuaño, A. P. P., Monteroso, D. N., Aoki, N., Mestres, C., Duldulao, J. B. A., & Bergonio, K. B. (2012). Replacement of acetate with ammonium buffer to determine apparent amylose content of milled rice. *Cereal Foods World, 57*, 14–19.

Juliano, B. O. (2010). Grain Quality of Philippine Rice. Muñoz, Nueva Ecija, Philippines: Philippine Rice Research Institute.

Liu, R., & Xu, G. (2008). Effects of resistant starch on colonic preneoplastic aberrant crypt foci in rats. *Food and Chemical Toxicology, 46*, 2672–2679.

Lunn, J., & Buttriss, J. (2007). Carbohydrates and dietary fibers. *Nutrition Bulletin, 32*, 21–64.

Nakamura, Y., Sato, A., & Juliano, B. O. (2006). Short-chain-length distribution in debranched rice starches differing in gelatinization temperature or cook rice hardness. *Starch/Stärke, 58*, 155–160.

Nugent, A. P. (2005). Health properties of resistant starch. *Nutrition Bulletin, 30*, 27–54.

Panlasigui, L. N., & Thompson, L. U. (2006). Blood glucose lowering effects of brown rice in normal and diabetic subjects. *International Journal of Food Sciences and Nutrition, 57*, 151–158.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch - A review. *Comprehensive Reviews in Food Science and Food Safety, 5*, 1–17.

Trinidad, T. P., Mallilin, A. C., Sagum, R. S., Felix, A. D. R., Tuano, A. P. P., & Juliano, B. O. (2014). Relative effect of apparent amylose content on the glycemic index of milled and brown rice. *Philippine Agricultural Scientist, 97*, 415–418.

Trinidad, T. P., Mallillin, A. C., Encabo, R. R., Sagum, R. S., Felix, A. D. R., & Juliano, B. O. (2013). The effect of apparent amylose content and dietary fibre on the glycemic response of different varieties of cooked milled and brown rice. *International Journal of Food Sciences and Nutrition, 64*, 89–93.

Tuaño, A. P. P., Aoki, N., Fujita, N., Oitome, N. F., Merca, F. E., & Juliano, B. O. (2014). Grain and starch properties of waxy and low-apparent amylose Philippine rices and of NSIC Rc222. *Philippine Agricultural Scientist, 97*, 329–339.

Tuaño, A. P. P., Perez, L. M., Padolina, T. F., & Juliano, B. O. (2015). Survey of grain quality of Philippine farmers' specialty rices. *Philippine Agricultural Scientist, 98*, 446–456.

Tuaño, A. P. P., Regalado, M. J. C., & Juliano, B. O. (2016). Grain quality of rice in selected retail stores and supermarkets in the Philippines. *International Journal of Philippine Science and Technology, 9*, 15–22.

Tuaño, A. P. P., Umemoto, T., Aoki, N., Nakamura, Y., Sawada, T., & Juliano, B. O. (2011). Grain quality and properties of starch and amylopectin of intermediate- and low-amylose *indica* rices. *Philippine Agricultural Scientist, 94*, 140–148.

Tuaño, A. P. P. (2013). Physicochemical characterization and starch fractionation of selected Philippine rices with atypical grain quality. MSc Thesis, University of the Philippines Los Baños, Philippines.

Wolever, T. M. S., Jenkins, D. J. A., Vuksan, V., Josse, R. G., Wong, G. S., & Jenkins, A. L. (1990). Glycemic index of foods in individual subjects. *Diabetes Care, 13*, 126–132.

Wu, K., Gunaratne, A., Gan, R., Bao, J., Corke, H., & Jiang, F. (2018). Relationships between cooking properties and physicochemical properties of brown and white rice. *Starch/Stärke, 70*, 1700167. https://doi.org/10.1002/star.201700167.

Yoon, J. H., Thompson, L. U., Jenkins, D. J. A. (1983). The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. American Journal of Clinical Nutrition, 38, 835-842.

Zhu, L.-J., Liu, Q.-Q., Wilson, J. D., Gu, M.-H., & Shi, Y.-C. (2011). Digestibility and physicochemical properties of rice (*Oryza sativa* L.) flours and starches differing in amylose content. *Carbohydrate Polymers, 86*, 1751–1759.