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Physicochemical properties of germinated brown rice (*Oryza sativa* L.) starch

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Physicochemical properties of white rice (WR), brown rice (BR) and germinated brown rice (GBR) starches from a mixed variety of MR219 and MR220, commonly consumed Malaysian varieties, were compared in this study. The granular size of the starch particles, measured using scanning electron microscope (SEM), varied from 2 to 8 µm for all starches and appeared in polyhedral shapes. Amylose content in WR, BR and GBR as analysed using colorimetric method was found to be 25.77, 23.83 and 21.78%, respectively. The amylose content of GBR was significantly lower than that of WR or BR. Results also show that germination affected gelatinization and pasting properties. This study has profound implications for future studies on functional properties of GBR and may help us understand what role changes in physicochemical properties, brought about by germination, play in determining functional effects.

Key words: Starch characterization, amylase, gelatinization, germinated brown rice.

INTRODUCTION

Rice is one of the most important crops around the world. It is a staple food for a great majority of people making it widely cultivated in over 100 countries including Malaysia. The total paddy production area is about 154 million hectares and the annual production of rice is about 594 million metric tons globally. Rice accounts for over 22% of global energy intake. Asia is the major rice producer, wherein rice production accounts for about 92% of the world's total production (Ohtsubo et al., 2005). In Malaysia, total rice consumption annually is around 2 million metric tons accounting for close to 70% of its production (FAO, 2005).

White rice (WR) or polished rice is manufactured by eliminating the fiber-rich bran layer from unpolished rice, also known as brown rice (BR). BR contains more, nutritional components such as dietary fibers, phytic acid E and B vitamins and γ-aminobutyric acid (GABA) than WR due to the presence of outer bran layer being the main source for the nutritional elements (Ohtsubo et al., 2005). Although, BR is more nutritious than WR, its intake is somewhat limited by the chewy texture and reduced digestibility. This problem can be overcome by subjecting BR to partial germination, thus, producing germinated brown rice.

With an increasing realization of the role of diet in the pathogenesis of certain chronic diseases, functional diets have received a lot of attention for their promising role in the prevention of such diseases. In line with this, nowadays, consumption of germinated brown rice (GBR) is gaining appreciation among health conscious individuals due to its better nutritive and organoleptic value and textural properties than the BR even after

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Abbreviations: WR, White rice; BR, brown rice; GBR, germinated brown rice.

cooking. GBR is basically produced by soaking BR in water and allowing it to slightly germinate (Hagiwara et al., 2004). The outer bran layer becomes soft and more prone to water absorption, making it easier to cook.

Rice quality is mostly determined by its chemical, physical, cooking and also eating characteristics (Cameron et al., 2007). Additionally, starch, a major component of rice is composed of individual granules which consist mainly of two glucose homopolymers: amylose and amylopectin. Amylose is essentially a linear polymer, whereas amylopectin molecules are highly branched and together they have been shown to affect the properties of starch (Goddard et al., 1984; Morvarid et al., 1998). Germination has been widely reported to affect many constituents of BR (Komatsuzaki et al., 2007; Jongjareonrak et al., 2009; Ohtsubo et al., 2005) and some physicochemical properties (Charoenthaikij et al., 2009; Mohan et al., 2010). It is also known that different germination conditions will affect the properties of BR differently (Charoenthaikij et al., 2009) and even though these different GBR varieties are produced under different conditions; studies have reported consistently positive results in terms of functional properties from different countries (Hsu et al., 2008; Ito et al., 2005; Mamiya et al., 2004, 2007; Sakamoto et al., 2007) including Malaysia (Roohinejad et al., 2009). Because many functional properties of rice are believed to be closely related to physicochemical and other properties (Goddard et al., 1984; Miller et al., 1992; Hallfrisch and Behall, 2000; Morvarid et al., 1998), we felt it necessary to elucidate the properties of the commonly consumed Malaysian rice varieties. As far as we know, there is no earlier study report on investigating the effects of germination on the properties of the common Malaysian MR219 and MR220 BR varieties. Other parameters like the effects of germination on x-ray diffraction patterns and starch morphology studied here have also not been reported else where. This study will enable us understand what contribution germination makes to the physicochemical properties of these Malaysian BR varieties and eventually help us project whether or not physicochemical properties would play any role in determining the functional effects of the corresponding Malaysian GBR varieties. This is important since majority of Malaysian population is rice eating and WR which is commonly consumed has been linked with the development of especially type 2 diabetes mellitus (Nanri et al., 2010; Sun et al., 2010). We hope studies like these will pave way for more studies on GBR, so that the entire properties of GBR varieties will be unraveled. With a positive attitude towards GBR, the Malaysian authorities could benefit a lot from knowing about these physicochemical properties and follow up studies on functional properties of these GBR varieties, per chance they could make policies that would favor the consumption of GBR more than WR. Thus, in this study, the effects of germination on some physicochemical properties of GBR rice starches were evaluated and compared with WR and

BR.

MATERIALS AND METHODS

White and brown *indica* rice (mixed varieties of MR219 and MR220) of Malaysian origin was supplied by National Rice Berhad (BERNAS Sdn Bhd., Malaysia). Germinated brown rice was prepared at Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The amylose and amylopectin standards were obtained from Sigma (St. Louis, MO, USA). All reagents were of analytical grade, obtained from Merck (Darmstadt, Germany).

Starch isolation

Starch was isolated by alkaline extraction of the proteins as described by Sodhi and Singh (2003). In order to soften the endosperm, rice flours (100 g) were steeped in 1 L of aqueous sodium hydroxide (0.25% NaOH w/v) and allowed to settle for 24 h at 20°C. Part of the supernatant liquor (750 ml) was discarded and the remaining slurry was diluted to the original volume (1 L) with the NaOH solution. The mixture was shaken for 10 min and centrifuged at 4200 x g for 5 min in an analytical centrifuge (Model 6K15, Sigma laboratory centrifuges). Then, the supernatant and the slurry were once more separated, as described earlier. This procedure was repeated eight times, after which the remaining slurry was suspended in distilled water, passed through a nylon sieve and centrifuged for 5 min at 6600 x g. Finally, the sample was washed with distilled water until pH 7.0, dried at 35 to 38°C to get the treated starch.

Determination of amylose content

The amylose content was determined according to the micro method used by McGrance et al. (1998). The amylose content was calculated using a standard curve plotted from different mixtures of potato amylose and amylopectin containing 0, 10, 25, 50, 75 and 100% amylose.

Starch morphology

Granule characterization

The morphologies of WR, BR and GBR starches were observed under scanning electron microscopy (SEM) Leo 1455 VPSEM (UK). One drop of the starch powder was applied on an aluminium stub and the starch was coated with gold-palladium (60:40). An acceleration potential of 10 kV was used during micrography.

X-ray diffraction

The x-ray diffraction patterns were obtained from the x-ray diffractometer (PANalytical, Philips, The Netherlands) operating in a transmission mode with Ni filter Cu-K α radiation at 40 kV and 40 mA. The x-ray diffraction pattern was recorded at angles (2 θ) 10 to 40°. Degree of relative crystallinity was calculated by the method of Muljana et al. (2009) modified from Komiya and Nara (1986):

$$X_c = A_c/A_t$$

$$X_{rc} = X_c/X_n$$

Where, X_c is the degree of crystallinity of samples; A_c is the area of the crystalline reflections; A_t is the overall area; X_{rc} is the relative

crystallinity and X_n is the degree of crystallinity of native starch.

Swelling power and solubility

Starch slurries at 1% w/v (dry basis) were prepared in a centrifuge tube and heated for 30 min at 80°C with stirring after every 5 min. The samples were then cooled to room temperature before centrifuging at 8500 x *g* for 15 min. The supernatant was separated from the swollen granules and 5 ml of supernatant was dried in a stainless steel dish at 110°C over night. Swelling power and solubility (%) were calculated as follows (Abdel-Rahman et al., 2008):

% Soluble (on a dry basis) = Residue weight in (g) water weight in (g) / Aliquot volume (ml) Sample weight in (g) X 100

Swelling power(g/g)= weight of sedimented starch in (g)/ weight of sample in (g) (100- % soluble in (on a dry basis)

Thermal properties

Starch gelatinization was studied in a differential scanning calorimeter (Mettler Toledo DSC 823-E, Switzerland) under oxygen-free N₂ flow rate of 50 ml/min, using 1:3 (w/v) starch-water mixtures. The samples were hermetically sealed in a pre-weighed aluminum pan at room temperature and re-weighed in a microbalance. After sealing the pan and leaving it to equilibrate for about 1 h, the samples were heated from 30 to 110°C at the rate of 10°C /min. An empty pan was used as a reference. The temperatures of the characteristic transitions, onset temperature (T_o), peak temperature (T_p) and end temperature (T_e) were recorded and the temperature range (T_e-T_o, ΔT) was calculated. The enthalpy (ΔH_G) of the transition was expressed as mJ/g on a dry weight basis.

Pasting properties

The RVA paste viscosity was determined on a rapid visco analyser (Model RVA Series 4, Newport Scientific Pvt. Ltd., Warriewood, Australia) using the American Association of Cereal Chemists' Standard Method AACC 61-02 (1995). Three grams of the rice starches were weighed, to which 25 ml of distilled water was added in a clean test canisters. A paddle was placed in the canister and the blade was vigorously jogged up and down through the sample ten times. The slurry was dispersed at a rotation speed of 160 rpm until viscosity was sensed, followed by rotating the paddle at 960 rpm for 10 s. The idle temperature was set to 50°C and the following 12.5 min test profiles were run: (1) an initial temperature of 50°C was held for 1.0 min; (2) the temperature was linearly ramped up to 95°C until 4.8 min; (3) the temperature was held at 95°C until 7.5 min; (4) the temperature was then linearly ramped down to 50°C until 11 min; (5) finally, held at 50°C until 12.5 min. Starches paste viscosity characteristics were studied by three important parameters of the pasting curve: peak (first peak viscosity after gelatinization), hot paste (paste viscosity at the end of the 95°C holding period) and cool paste viscosity (paste viscosity at the end of the test). In addition, breakdown, setback and consistency were derived from peak minus hot paste viscosity values, cool paste viscosity minus peak viscosity values and cool paste viscosity minus hot paste viscosity values, respectively. All the viscosity parameters were measured in rapid visco units (RVU).

Statistical analysis

All measurements were carried out in triplicates and the data were

expressed as means ± standard deviations. One-way analysis of variance (ANOVA) using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used to assess the significance of the differences between means at $p < 0.05$.

RESULTS AND DISCUSSION

Determination of amylose content

During the analysis of amylose content, the formation of a helical complex between amylose and iodine results in the deep blue color of starch dispersions stained with iodine. This principle lies behind the quantification of amylose content. Figure 1 displays the composition of amylose in GBR, BR and WR isolated starches. GBR isolated starch showed the lowest amylose content (21.78%) among all, followed by BR isolated starch (23.83%) and WR (25.77%). The amylose content in GBR starch was significantly different ($p < 0.05$) from that of BR and WR starches. Previous research (Juliano, 1992) had categorized rice according to amylose content into four groups that is, waxy (0 to 5%), very low (5 to 12%), low (12 to 20%), intermediate (20 to 25%) and high (25 to 33%). However, commercially, rice is classified based on amylose content as either low (less than 20% amylose), medium (21 to 25% amylose) or high (25 to 33% amylose). So, based on amylose content in Malaysian cultivated rice (21.78 to 25.77%), we categorized the starches as having intermediate amylose content, using the criteria suggested by Juliano (1992). The significantly different ($p < 0.05$) amylose content of GBR starch when compared with BR starch indicated that the germination process can indeed reduce the amylose content in these Malaysian rice cultivar. The reduction in the level of amylose was likely as a result of the activation of amylase which is activated under chemical pretreatment of BR and anoxic conditions (Kaneko and Morohashi, 2003; Perata et al., 1993) similar to what we did. Similarly, Charoenthaikij et al. (2009) had reported that germination increased not only the activity of amylase but also increased reducing sugars in BR, supporting our findings of a lower amylose content in GBR when compared with BR. Similar findings have also been reported by other researchers (Jiamyanyuen and Ooraikul, 2008; Mohan et al., 2010). Amylose content plays a significant role in determining rice eating quality and it is used as a parameter for the texture of cooked rice (Suwannaporn et al., 2007) and so its reduction in BR will bring about a softer texture. Additionally, amylose content was reported to have a positive correlation with hardness and a negative correlation with stickiness (Juliano, 1992; Windham et al., 1997). This goes to say from our result that germination could actually reduce the hardness of BR, while it increases its stickiness, suggesting that GBR would be more palatable than BR taking into consideration its amylose content. Interestingly, amylase has been shown to have a negative

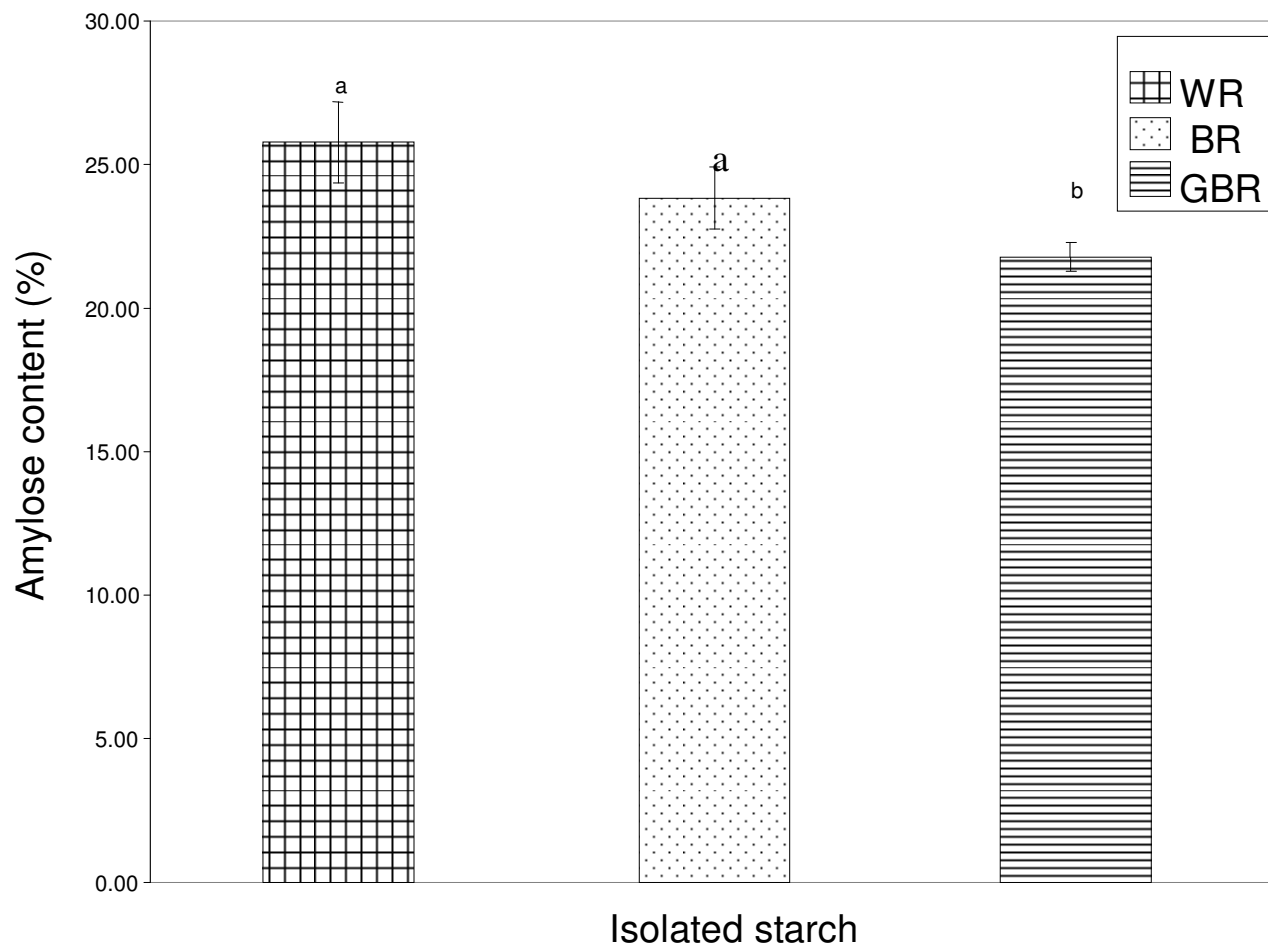


Figure 1. Amylose content of alkali-treated rice starches. Vertical bar represents \pm SD from the mean. Bars with same letter are not significantly different ($p > 0.05$).

correlation with glycemic index (GI) (Goddard et al., 1984; Miller et al., 1992) and though ours and other results (Charoenthaikij et al., 2009; Mohan et al., 2010) have shown that GBR possess low amylose content; it has also been proven to have low GI (Hsu et al., 2008; Ito et al., 2005). This is important information on GBR as a potential functional food for diabetics, since foods low in GI are good for this group of people. Additionally, this means that the better glucose lowering effect of GBR over BR is likely as a result of other bioactive compounds it contains and not determined by its amylose content.

Granule characterization

Figure 2 shows the images of isolated WR, BR and GBR viewed at 500 x g and 3000 x g magnification viewed under SEM (Leo 1455 VPSEM, UK). It is evident that WR, BR and GBR starch granules are mainly polyhedral in shapes. Their shapes and sizes are in average of 3 to 8 μ m and these results are in agreement with a previous study by Sodhi and Singh (2003). The smooth surface of

the granules of these starches indicated that the isolation treatment with 0.25% NaOH used in this study did not damage the starch granules. Thus, the shared morphological properties of GBR, BR and WR isolated starches indicated that the germination process did not affect the overall structure and size of rice starches.

Furthermore, it has been reported that the size of starch granule may affect its physicochemical properties, such as gelatinization, pasting, enzyme susceptibility, crystallinity and solubility (Lindeboom et al., 2004); however, our results do not conform to this. We found the granules of BR and GBR to be similar in morphology; though, some of the physicochemical properties studied were significantly different between the 2, meaning that the morphology might have played a very little role in determining the properties studied.

X-ray diffraction

Starch is semi-crystalline in nature and the level of crystallinity varies among plant types. Crystallinity of

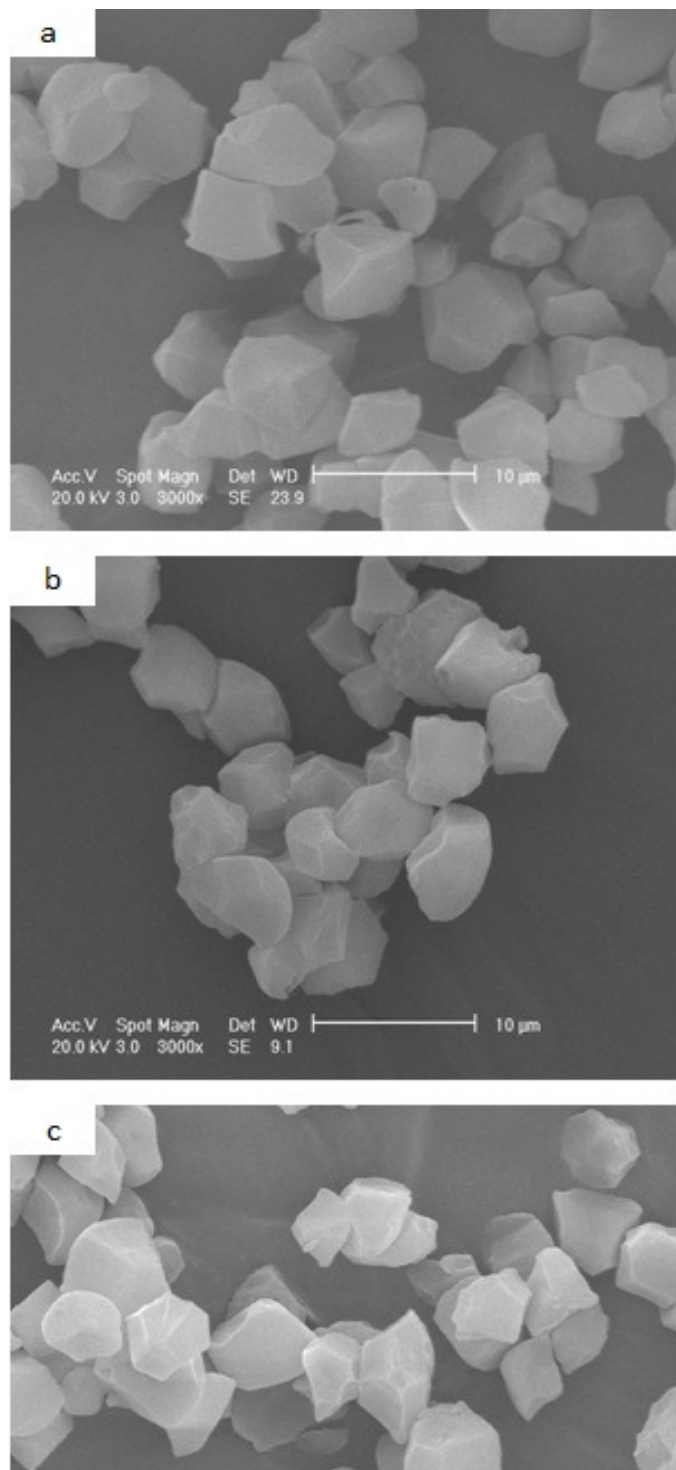


Figure 2. Rice starch granules observed under scanning electron microscope (a) white rice starch, (b) brown rice starch and (c) germinated brown rice starch.

starch granules is associated strongly with its amylopectin content, while the amylose is mainly represented in the amorphous region of the granules (Zobel, 1988a, b). The packaging of amylase and

amylopectin within the granules has been reported to vary among starches from different species (Singh et al., 2003). X-ray diffractometry has been widely used to detect and characterize the crystallinity pattern of starch granules (Hoover, 2001). The x-ray diffraction pattern of the three starches is shown in Figure 3. Native starch granules are known to exist in three crystalline structures which are A, B and C types based on x-ray diffractometry. All the three rice starches showed an A-type diffraction pattern characteristic of cereal grains like rice. The A-type diffraction pattern corresponds to a close packing of amylopectin double helices (Khunae et al., 2007). The major reflections or patterns of spots produced following exposure to a beam of x-rays, of WR starch were at 2θ 11.26, 15.09, 16.93, 17.93 and 22.97°. Those of BR were at 15.05, 16.91, 17.98, 20.26, 22.74 and 26.37°, while GBR had its major reflections at 14.98, 16.91, 17.90, 20.21 and 22.81° (Table 4). Relative crystallinities (RC), expressed in percentages, for the different rice starches are shown in Table 1. The %RC for the 1st, 2nd, 3rd, 4th and 5th peaks of WR was 19.91, 2.37, 20.85, 26.07 and 21.33% respectively. The corresponding values for BR were 18.56, 10.98, 23.86, 23.11 and 17.80%, while GBR had 20.16, 7.91, 24.90, 24.51 and 18.18% respectively.

These values were showed little variability, mainly at the 2nd to the 5th peaks during our analysis and even at these peaks the variability was more marked between WR on one hand and BR and GBR on the other, signifying that germination played no role in changing the RC of the starch of our BR variety. It is still possible however that the RC of other BR varieties would be affected by germination, since other properties we studied which were found to be significantly affected by germination of other BR varieties were not affected in our case.

Swelling power and solubility

The swelling powers of WR, BR and GBR isolated starches are presented in Figure 4. The ability of WR isolated starch to swell in the presence of excess water differed significantly from BR and GBR isolated starches. WR showed the lowest swelling power (1.30 ± 0.11 g/g) compared to BR (1.76 ± 0.28 g/g) and GBR (1.70 ± 0.08 g/g), with no difference between BR and GBR. Swelling is regulated by the degree of crystallinity of the starch granules and the swelling power is determined by the ability of starch granules to swell in the presence of excess water when heated. Generally speaking, swelling power of starches reflects the interactions between water molecules and starch chains in amorphous and crystalline domains, respectively (Ratnayake et al., 2002). In line with this, Lee and Osman (1991) reported that the swelling power of starch depends on the water holding capacity of glucose molecules as determined by

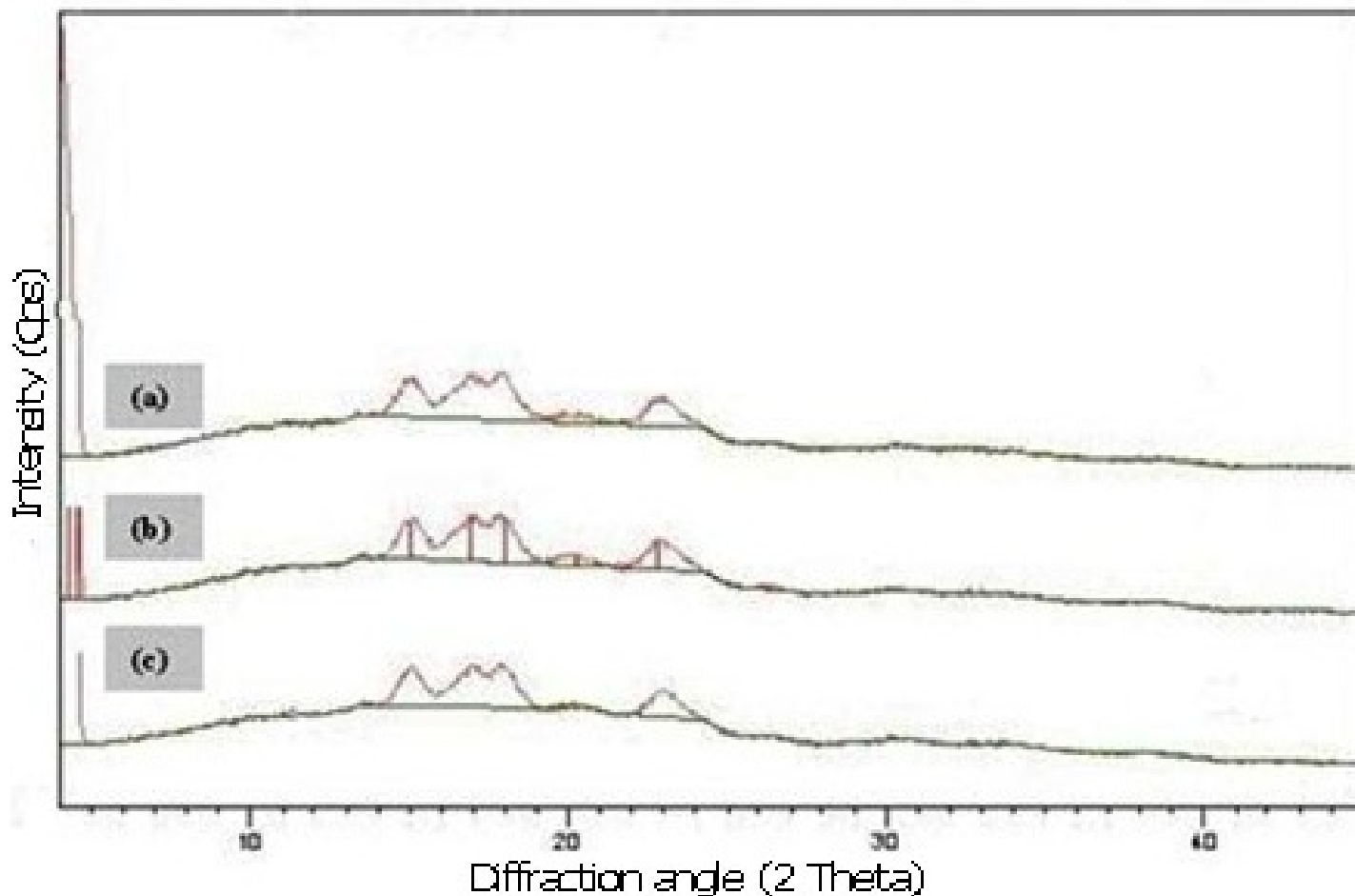
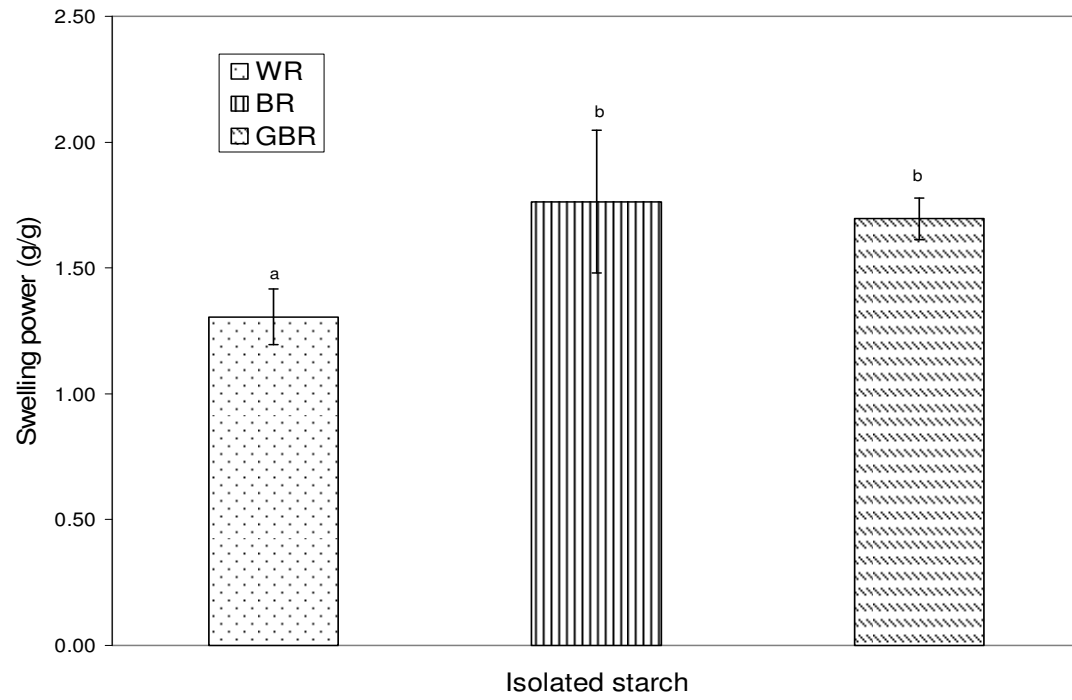


Figure 3. X-ray diffraction pattern of rice starches: (a) germinated brown rice starch, (b) brown rice, (c).white rice starch.

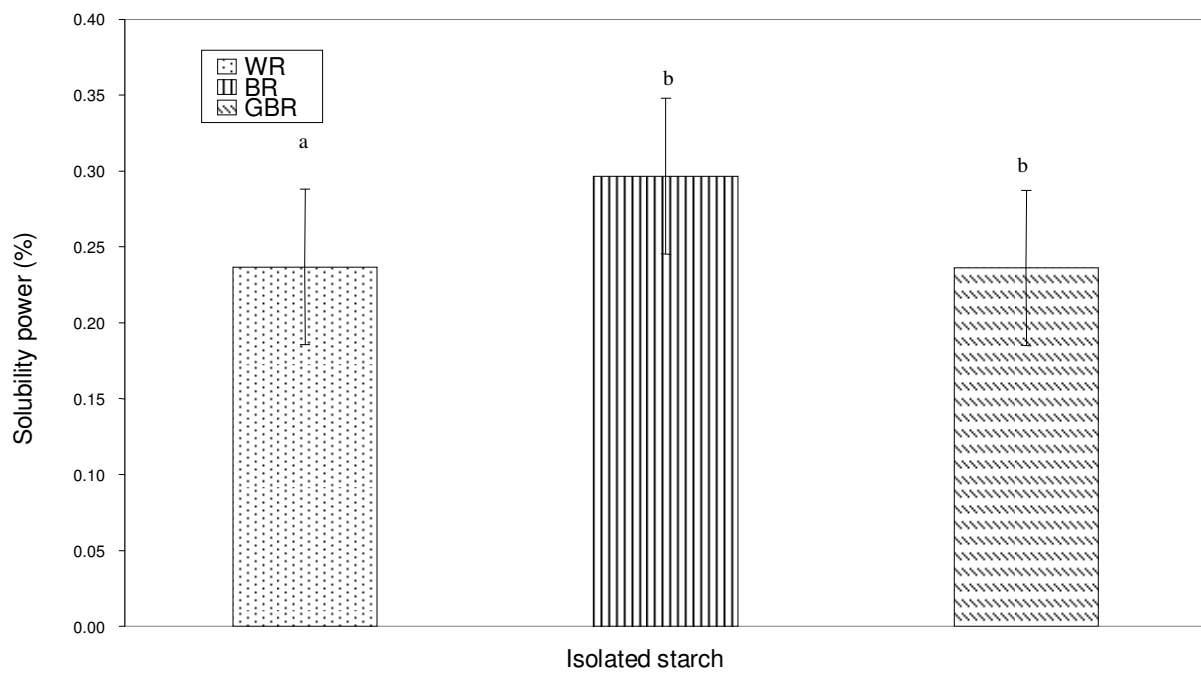
hydrogen bonding. Hydrogen bonds which stabilize the double helices in crystallites are lost during gelatinization and replaced with water. Thus, disruption of the crystalline structure weakens the bonding and allows an increase in granule swelling.

Our results however show that germination did not affect the swelling power of our BR and therefore, no relationship between amylose content and the swelling power could be drawn. Sandhya Rani and Bhattacharaya (1989) reported that starch granules with low amylose content are less rigid and swell more when heated as opposed to granules with high amylose content which are more rigid and swell less. However, Tester and Morrison (1990) proposed that amylopectin and not amylose is mainly responsible for the swelling behavior of starches, while amylose acts only as a diluent. In agreement with the previous study by Wang et al. (2008), our results reveal that there is a negative correlation between swelling power and amylose content only between WR and GBR, proving the effect of amylose content on swelling power of starches, though this is not to say that germination affected the relationship since the same cannot be said of BR and GBR.

Data from our solubility studies revealed no significant difference ($p > 0.05$) between WR, BR and GBR starches. Most previous researches have suggested a relationship between swelling power and solubility, but in contrast with the previous study by Wang et al. (2008), our results revealed no significant relationship between solubility and amylose content. Another study had related differences in solubility of starches with differences in amylose content, viscosity pattern and weakness of internal structure bonding due to negative charge of phosphate with the starch granules (Galliard and Bowler, 1987). The lack of relationship between amylose content and solubility from our study, therefore only means that even though germination affected amylose content, the difference in the amylose content was not reflected in the solubility of the different rice starches. On the other hand, Lii et al. (1996) had suggested a positive relationship in which high amylose content conferred high solubility, which was also not reflected by our results. From their study, what would be expected is that high amylose content will confer higher solubility but this was not seen in our study since the amylose contents of the different rice starches showed variability but the solubilities were



A



B

Figure 4. (a) Swelling power and (b) solubility of alkali-treated rice starch. Vertical bar represents \pm SD from the mean. Bars with same letter are not significantly different ($p > 0.05$).

not different.

Thermal properties

The pasting properties of WR, BR and GBR isolated starches are presented in Table 2. The pasting curves determined using RVA, measured the viscosity of starch suspension during the heating cycle. The pasting curves reflect what happens in starch granules and also the behavior of starch during cooking (Mishra and Rai, 2006). From our study, GBR was the first to swell and gelatinize due to water uptake at 81.60°C, followed by BR at 82.30°C and finally, WR at 83.10°C with a statistically significant difference between the 3 groups. WR starch had the lowest peak viscosity (283.79 RVU), while GBR starch showed the highest (328.04 RVU) with BR somewhere in the middle (324.09 RVU), which were all significantly different. Our study therefore suggests that germination increases peak viscosity, thereby confirming the negative relationship between amylose content and peak viscosity as reported by Noosuk et al. (2003); in their study, RVA peak viscosity was shown to have a negative correlation with amylose content, from which they suggested that high level of amylose restricts the swelling of granules, thereby reducing the peak viscosity. In contrast with our result and that of Noosuk et al. (2003), Charoenthaikij et al. (2009) as well as Mohan et al. (2010) reported that germination of BR resulted in a reduction of viscosity. Additionally, their results show a positive correlation between amylose content and viscosity, which contradict what Noosuk et al. (2003) and our results showed.

Setback, defined as the degree of re-association between the starch molecules, is the secondary increase in viscosity during cooling which eventually determines retrogradation of a starch. WR had the highest setback, while BR the lowest, with GBR in between. Setback for GBR was significantly higher than that of BR but not WR. This phenomenon is thought to be related to amylose content, length of amylose chain and dispersion of the amylose chain in starch polymers. Mishra and Rai (2006) also suggested that setback may largely be determined by degree of amylose polymerization. From our study, germination affected setback, though this cannot be related to the amylose content since the amylose content of GBR and WR was significantly different but their setback was not different. Similarly, breakdown of the different starches revealed that they were significantly different with the same pattern as viscosity. GBR had a higher breakdown than BR signifying also in this case that germination affected it. Mohan et al. (2010) had reported that germination of BR reduced setback and breakdown significantly, in contrast with our findings. These and other differences between our findings and those of others (Charoenthaikij et al., 2009; Mohan et al., 2010; Jiamyanyuen and Ooraikul, 2008) perhaps signify

that different BR varieties will possess different properties after germination. Another possibility is that the different germination conditions used was responsible; after all it had been proven that even the same BR variety when subjected to different germination conditions will have different properties (Jongjareonrak et al., 2009; Charoenthaikij et al., 2009; Roohinejad et al., 2009).

Transition temperature (T_o , T_p and T_c) and melting enthalpy (ΔH) of isolated WR, BR and GBR starches are shown in Table 3. There was a statistically significant difference ($p < 0.05$) in T_o , T_p , T_c and ΔH between WR, BR and GBR isolated starches. In WR, BR and GBR isolated starches, T_o , T_p and T_c ranged from 72.08 to 73.55°C, 75.46 to 76.85°C and 78.83 to 80.63°C, respectively. Gelatinization, which is a set of temperature dependent changes, is characterized by the irreversible disruption of molecular order depending on temperature and moisture. The granules resulting from such changes will have a larger size and an increased viscosity (Patel and Seetharaman, 2006). The gelatinization temperature and enthalpy of starches are dependent on the microstructure and degree of crystallinity within the granules, the granule size and ratio of amylose to amylopectin (Ahmad et al., 1999) and presence of phosphate esters (Yuan et al., 1993). A higher gelatinization temperature therefore, reflects a smaller size of the granules (Cowburn, 1989). The gelatinization temperature range of WR ($6.75 \pm 0.07^\circ\text{C}$) isolated starch from our study was significantly lower than those of BR and GBR. The temperature ranges for BR and GBR, $7.58 \pm 0.35^\circ\text{C}$ and $7.26 \pm 0.07^\circ\text{C}$ respectively were not different. Additionally, the absolute value for the ΔH of BR ($53.17 \pm 0.32 \text{ mJ}$) was higher than that of GBR ($52.39 \pm 0.17 \text{ mJ}$) signifying that germination also affected it. The ΔH of WR ($50.68 \pm 0.74 \text{ mJ}$) was significantly lower than BR and GBR. The endothermic enthalpies (ΔH_G) reflected the loss of double helical rather than the crystalline order (Cooke and Gidley, 1992). The endothermic transitions from WR and GBR isolated starches showed only a single transition; while BR isolated starch exhibited two relatively small separate endothermic transitions and a major endothermic transition. These transitions in BR isolated starch can be attributed to starch gelatinization and amylose-lipid complex disruption. As demonstrated by Liu et al. (2006), cereal starches such as rice starch showed a major first transition at a lower temperature attributable to starch gelatinization, followed by a second peak or shoulder at a higher temperature attributable to the disruption of an amylose-lipid complex. The occurrence of an amylose-lipid complex in starch has been demonstrated by Ahmad et al. (1999), which is seen in starches with a melting temperature outside 90 to 110°C. They further proposed that the amylose-lipid complex hastens the gelatinization temperature. Finally, our findings regarding low T_o , T_p , T_c and ΔH of the isolated starches are consistent with what Noda et al. (1996) postulated. The reason is

Table 2. Pasting properties of 10% (w/v) rice starches suspension.

Starch	Pasting temperature (°C)	*Rapid visco unit (RVU)		
		Peak viscosity	Breakdown	Setback
WR	^a 83.10 ± 0	^a 283.79 ± 1.54	^a 193.50 ± 0.63	^a 90.29 ± 2.21
BR	^b 82.30 ± 0	^b 324.09 ± 0.09	^b 199.75 ± 1.50	^b 86.67 ± 0.50
GBR	^c 81.60 ± 0	^c 328.04 ± 1.46	^c 204.67 ± 3.0	^a 88.71 ± 0.62

Means within a column with the same letter are not significantly different ($p > 0.05$). *Values are means ± SD ($n = 3$).

Table 3. Gelatinization property of rice starches.

Starch	*Gelatinization(°C)			
	Onset temperature (To)	Peak temperature (Tp)	Endset temperature (Tc)	Enthalpy ΔHG (mJ)
WR	^a 72.08 ± 0.21	^a 75.46 ± 0.19	^a 78.83 ± 0.14	^a 50.68 ± 0.74
BR	^b 73.55 ± 0.28	^b 76.85 ± 0.12	^b 80.63 ± 0.43	^b 53.17 ± 0.32
GBR	^c 72.50 ± 0.10	^c 76.07 ± 0.01	^c 79.76 ± 0.03	^c 52.39 ± 0.17

Values with the same superscript letter in the same column are not significantly different ($p > 0.05$). *Values are means ± SD ($n = 3$).

Table 4. Major reflections of starches based on x-ray diffraction patterns of germinated brown rice starch, brown rice and white rice starch.

Starch	Major reflection/diffraction angle (2 theta)
GBR	14.98, 16.91, 17.90, 20.21 and 22.81 °
BR	15.05, 16.91, 17.98, 20.26, 22.74 and 26.37 °
WR	11.26, 15.09, 16.93, 17.93 and 22.97 °

believed to be due to the molecular arrangement of the crystalline region corresponding to the distribution of amylopectin short chain (DP 6-11) and not the proportion of crystalline region corresponding to the ratio of amylose to amylopectin.

Finally, from the analysis thus far, we can assume that the changes in physicochemical properties of starch produced by germination of BR could contribute towards some yet-to-be discovered functional properties of GBR or the outcome of further studies will hopefully discover their role in bringing about some of its already documented functional properties.

Conclusion

This study has provided some insight into understanding the effects of germination on Malaysian rice starch (MR219 and MR220 varieties) with respect to amylose content, pasting, gelatinization, swelling, solubility and crystallinity properties. In this study, amylose content, gelatinization (To, Tp and Te) and pasting properties (temperature, viscosity, breakdown and setback) were affected by germination.

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REFERENCES

- Abdel-Rahman ESA, El-Fishawy FA, El-Geddawy MA, Kurz T, El-Rify MN (2008). Isolation and Physico-chemical Characterization of Mung Bean Starches. *Int. J. Food Eng.*, 4(1): 1.
- Ahmad FB, William PA, Doublier JL, Durand S, Buleon A (1999). Physicochemical characterization of sago starch. *Carbohydr. Polym.*, 38: 361-370.
- American Association of Cereal Chemistry (1995): *Approved Method of AACC*. St. Paul MN. 9th ed., Vol 1-2.
- Cameron DK, Wang Y-J, Moldenhauer KA (2007). Comparison of Starch Physicochemical Properties from Medium-Grain Rice Cultivars Grown in California and Arkansas. *Starch*. 59: 600-608.
- Charoenthaikij P, Jangchud K, Jangchud A, Piyachomkwan K, Tungtrakul P, Prinyawiwatkul W (2009). Germination conditions affect physicochemical properties of germinated brown rice flour. *J. Food Sci.*, 74(9): C658-C665.

- Cooke D, Gidley MJ (1992). Loss of crystalline and molecular order during starch gelatinization: origin of the enthalpic transition. *Carbohydr. Res.*, 227: 103-112.
- Cowburn P (1989). The role and function of starches in microwaveable formulation. In *Gums and stabilizers For Food Industries 5*; Philips G. O.; Wedlock D. J.; Williams P.A. (Eds.). IRL Press: Oxford, UK, pp. 79-88.
- Food and Agricultural organization (FAO) (2005). Proceedings of the FAO rice conference: Rice in global markets, 12-13 February, 2004, Rome. FAO commodities and trade proceedings, Rome, Italy. ISSN 1816-5303.
- Galliard T, Bowler P (1987). Morphology and composition of starch. In T. Galliard (Ed), *Starch properties and potential*. Wiley: Chichester, UK, pp 55-78.
- Goddard MS, Young G, Marcus R (1984). The effect of amylose content on insulin and glucose responses to ingested rice. *Am. J. Clin. Nutr.*, 39: 388-392.
- Hallfrisch J, Behall KM (2000). Mechanisms of the Effects of Grains on Insulin and Glucose Responses. *J. Am. Coll. Nutr.*, 19(90003): 320S-325S.
- Hagiwara H, Seki T, Ariga T (2004). The effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. *Biosci. Biotech. Biochem.*, 68: 444-447.
- Hoover R (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydr. Polym.*, 45: 253-267.
- Hsu T, Kise M, Wang M, Ito Y, Yang M, Aoto H, Yoshihara R, Yokoyama J, Kunii D, Yamamoto S (2008). Effects of pre-germinated brown rice on blood glucose and lipid levels in free-living patients with impaired fasting glucose or type 2 diabetes. *J. Nutr. Sci. Vitaminol.*, 54: 163-168.
- Ito Y, Aya M, Mitsuo K, Hiromichi A, Shigeru Y, Rie Y, Jyunichi Y (2005). Postprandial blood glucose and insulin responses to pre-germinated brown rice in healthy subjects. *J. Med. Invest.*, 52: 159-164.
- Jiamyanyuen S, Oraikul B (2008). The physico-chemical, eating and sensorial properties of germinated brown rice. *J. Food Agric. Environ.*, 6(2): 119-124.
- Jongjareonrak A, Sawaddiwong R, Benjakul S, Osako K, Tanaka M (2009). Effect of germination conditions on antioxidant activity of germinated brown rice. *Czech J. Food Sci.*, 27: S53.
- Juliano BO (1992). Structure, chemistry, and function of the rice grain and its fractions. *Cereal Food. World.* 37: 772-779.
- Kaneko Y, Morohashi Y (2003). The effect of sodium hypochlorite treatment on the development of α -amylase activity in mung bean cotyledons. *Plant Sci.*, 164(2): 287-292.
- Khunae P, Tran T, Sirivongpaisal P (2007). Effect of heat-moisture treatment on structural and thermal properties of rice starches differing in amylose content. *Starch*, 59: 593-599.
- Komatsuzaki N, Tsukahara K, Toyoshima H, Suzuki T, Shimizu N, Kimura T (2007). Effect of soaking and gaseous treatment on GABA content in germinated brown rice *J. Food Eng.*, 78(2): 556-560.
- Komiya T, Nara S (1986). Changes in crystallinity and gelatinization phenomena of potato starch by acid treatment. *Starch*, 38: 9-13.
- Lee YE, Osman EM (1991). Correlation of morphological changes of rice starch granules with rheological properties during heating in excess water. *J. Kor. Agric. Chem. Soc.*, 34: 379-385.
- Lii CY, Tsai ML, Tseng KH (1996). Effect of amylose content on the rheological properties of rice starch. *Cereal Chem.*, 73: 415-420.
- Lindeboom N, Chang PR, Tyler RT (2004). Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. *Starch*, 56: 89-99.
- Liu Q, Donner E, Yin Y, Huang RL, Fan MZ (2006). The physicochemical properties and *in vitro* digestibility of selected cereals, tubers and legumes grown in China. *Food Chemistry*. 99: 470-477.
- Mamiya T, Asanuma T, Kise M, Ito Y, Mizukuchi A, Aoto H, Ukai M (2004). Effects of Pre-Germinated Brown Rice on β -Amyloid Protein-Induced Learning and Memory Deficits in Mice. *Biol. Pharm. Bull.*, 27: 1041-1045.
- Mamiya T, Kise M, Morikawa K, Aoto H, Uka M, Noda Y (2007). Effects of pre-germinated brown rice on depression-like behavior in mice. *Pharmacol. Biochem. Be*, 86: 62-67.
- McGrance SJ, Cornell HJ, Rix CJ (1998). A simple and rapid colorimetric method for the determination of amylose in starch products. *Starch*, 50: 158-163.
- Miller JB, Pang E, Bramall L (1992). Rice: a high or low glycemic index food?. *Am. J. Clin. Nutr.*, 56: 1034-1036.
- Mishra S, Rai T (2006). Morphology and functional properties of corn, potato and tapioca starches. *Food Hydrocolloid*, 20: 557-566.
- Mohan BH, Malleshi NG, Koseki T (2010). Physico-chemical characteristics and non-starch polysaccharide contents of Indica and Japonica brown rice and their malts. *LWT - Food Sci. Technol.*, 43(5): 784-791.
- Morvarid K, Salwa WR, Martine C, Jing L, Josette B, Françoise B, Gérard S (1998). Dietary Amylose-Amylopectin Starch Content Affects Glucose and Lipid Metabolism in Adipocytes of Normal and Diabetic Rats. *J. Nutr.*, 128(1): 35-43.
- Muljana H, Picchioni F, Heeres HJ, Janssen LPBM (2009). Supercritical carbon dioxide (scCO₂) induced gelatinization of potato starch. *Carbohydr. Polym.*, 78: 511-519.
- Nanri A, Mizoue T, Noda M, Takahashi Y, Kato M, Inoue M, Tsugane S, Japan Public Health Center-based Prospective Study Group (2010). Rice intake and type 2 diabetes in Japanese men and women: the Japan Public Health Center-based Prospective Study. *Am. J. Clin. Nutr.*, 92(6): 1468-1477.
- Noda T, Takahata Y, Sato T, Ikoma T, Mochida H (1996). Physicochemical properties of starches from purple and orange fleshed sweet potato roots at two levels of fertilizer. *Starch*, 48: 395-399.
- Noosuk P, Hill SE, Pradipasena P, Mitchell JR (2003). Structure-viscosity relationships for Thai rice starches. *Starch*, 55: 337-344.
- Ohtsubo K, Suzuki K, Yasui Y, Kasumi T (2005). Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. *J. Food Compos. Anal.*, 18: 303-316.
- Patel BK, Seetharaman K (2006). Effect of heating rate on starch granule morphology and size. *Carbohydr. Polym.*, 65: 381-385.
- Perata P, Geshi N, Yamaguchi J, Akazawa T (1993). Effect of anoxia on the induction of α -amylase in cereal seeds. *Planta*, 191(3): 402-408.
- Ratnayake WS, Hoover R, Warkentin T (2002). Pea starch: composition, structure and properties – A review. *Starch*, 54: 217-234.
- Roohinejad S, Omidzadeh A, Mirhosseini H, Saari N, Mustafa S, Yusof RM, Hussin ASM, Hamid A, Abd Manap MY (2009). Effect of pre-germination time of brown rice on serum cholesterol levels of hypercholesterolaemic rats. *J. Sci. Food Agric.*, 90: 245-251.
- Sakamoto S, Hayashi T, Hayashi K, Murai F, Hori M, Kimoto K, Murakami K (2007). Pre-germinated brown rice could enhance maternal mental health and immunity during lactation. *Eur. J. Nutr.*, 46: 391-6.
- Sandhya Rani MR, Bhattacharya KR (1989). Rheology of rice-flour pastes: Effect of variety, concentration, and temperature and time of cooking. *J. Texture Stud.*, 20: 127-137.
- Singh N, Singh J, Kaur L, Sodhi NS, Gill BS (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chem.*, 81: 219-231.
- Sodhi NS, Singh N (2003). Morphological, thermal, and rheological properties of starches separated from rice cultivars grown in India. *Food Chem.*, 80: 99-108.
- Sun Q, Donna S, Rob MVD, Michelle DH, Vasanti SM, Walter CW, Frank BH (2010). White Rice, Brown Rice, and Risk of Type 2 Diabetes in US Men and Women. *Arch. Intern. Med.*, 170(11): 961-969.
- Suwannaporn P, Pitiphunpong S, Champangern S (2007). Classification of rice Amylose content by discriminant analysis of physicochemical properties. *Starch*, 59: 171-177.
- Tester RF, Morrison WR (1990). Swelling and gelatinization of cereal starches. I. Effects of Amylopectin, Amylose, and Lipids. *Cereal Chem.*, 67: 551-557.
- Wang L, Yin Z, Zhang Y, Xie B, Sun Z (2008). Morphological, Physicochemical and Textural Properties of Starch Separated from Chinese water chestnut. *Starch*, 60: 181-191.
- Windham WR, Lyon BG, Champagne ET, Barton FE, Webb BD,

- McClung AM, Moldenhauer KA, Linscombe S, McKenzie KS (1997). Prediction of cooked rice texture quality using near-infrared reflectance analysis of whole-grain milled samples. *Cereal Chem.*, 74: 626-632.
- Yuan RC, Thompson DB, Boyer CD (1993). Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three wax-containing genotypes in two inbred lines. *Cereal Chem.*, 70: 81-89.
- Zobel HF (1988a). Starch crystal transformations and their industrial importance. *Starch*, 40: 1-7.
- Zobel HF (1988b). Molecules to granules: A comprehensive starch review. *Starch*, 40: 44-50.