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Effect of ageing process on rice physicochemical and digestion properties

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ABSTRACT

Three rice cultivars with different amylose contents were used for studying the effect of storage on rice structure and digestion behaviours. The amount of cell wall remnants of rice stored at 37°C increased over the storage. The pasting studies indicate that rice samples stored at 37°C demonstrated a consistent increase in time to peak viscosity (TTPV) of the RVA curve during the storage, suggesting a quick ageing progress. Ageing led to the decrease in the leaching of starch molecules during cooking, indicating the reduction of hydration and swelling of starch granules in aged rice grain. Digestion study shows that ageing process significantly reduced rice digestion kinetics both in rate and extent. Thus, it is assumed that ageing led to rice grain becoming more organized in structure which reduced starch granule disruption and molecules leaching, and subsequently affected the rice digestion properties. This study suggests that the changes in digestion behaviours of rice are highly associated with the changes in rice physicochemical properties occurred during storage and ageing is one of useful methods for manipulating rice digestion properties.

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KEYWORDS

Rice;
Storage;
Cell wall;
Pasting property;
Digestion.

INTRODUCTION

Rice is normally consumed as cooked rice and only a small amount of rice is used to make ingredients for processed foods. This pattern of usage results in the need to store rice over varying periods. It has been well documented that a number of changes in rice chemical and physical properties occurred during storage, which is usually termed rice ageing. Rice ageing commences before harvest and continues^[1,2]. The extensive studies indicate that ageing-induced changes occur in rice composition, pasting properties, thermal properties and texture^{[3-}

^{10]}. Attempts to explain the changes in rice functionality associated with ageing have focused on the properties of rice components, such as starch, protein, and lipids during storage^[4,11,12]. As with functionality, changes in those components were most apparent at an elevated storage temperature^[4]. For example, Chrastil and Zarins^[13] reported that the number of disulfide bridges increased and the lower molecular weight peptides decreased with increase of the higher molecular weight peptides during storage. Changes in fatty acid profiles and an increase of free fatty acids during storage have been noted as well^[14].

Recently, the interactions amongst rice components

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occurred during storage have attracted more interests^[15,16]. Previous study suggests that interactions between starch and non-starch components play an important role on rice properties during the ageing process^[9]. Nevertheless, the effect of aging process on rice property is complicated and the mechanism of rice ageing is still being investigated.

More recently, there has been a considerable interest in the possibility of improving diabetic control by altering the glycemic impact of the carbohydrates ingested. Several nutritional advantages can be anticipated with products of low glycemic index (GI) and such foods are usually associated with lower postprandial responses of glucose and insulin^[17,18]. In recent years, a considerable number of studies have focused on the importance of low GI products as a substrate for colonic fermentation^[19]. Briefly, these sorts of food products will provide substrate to the colonic microflora, thus promoting short-chain fatty acid production in the colon with potential health benefits^[20]. Our preliminary survey suggested that the people consuming aged rice would have a lower risk to have diabetes mellitus compared the community of consuming fresh rice (data not shown). However, to the best of our knowledge, the information regarding the effect of ageing process on rice digestion behaviour is very limited. Thus, this study extends our previous work of the effect of storage on rice physicochemical properties^[9,10] and exams the effect of ageing process on rice digestion property.

MATERIALS AND METHODS

Rice samples

Commercially grown samples of three Australian cultivars, Koshihikari and Doongara were selected in this study. Koshihikari is a medium rice grain with 18% amylose content; Keeyma, an aromatic rice grain with 26% amylose content; Doongara, a long rice grain with 28% amylose content. The three varieties were grown in the Murrumbidgee Irrigation Area (MIA) of New South Wales, Australia.

Rice storage

Previous studies suggest that storage at 37°C accelerated the ageing process, whilst storage at 4°C retarded the process and that samples stored at these

two temperatures provide a valid comparison of rice ageing [9,10,21,22]. Thus, in this paper, two storage temperatures, 4°C and 37°C were used for the studies of the rice ageing process.

Milled rice samples (10 kg) were placed in air-tight glass bottles, sealed and stored in the dark at 4°C and 37°C in thermostatically controlled incubators. Samples were withdrawn from the same bottle at the beginning of storage and at different intervals. Prior to analyses, rice grains were ground immediately after being withdrawn from the storage containers using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) and passed through a 0.5 mm sieve screen.

Cell wall remnants in rice grain

Rice flour (3.0 g; 12% moisture) was mixed with 25 mL of sodium acetate buffer (0.005 M, pH 6.5) and then 50 µL of thermostable α -amylase (Source: *Bacillus licheniformis*) was added. The mixture was incubated in a boiling water bath for 4 min to hydrolyse starch and cooled in an ice bath. The hydrolysate was centrifuged at 2095 \times g for 10 min and the supernatant was discarded. The residue was washed with 50 mL of distilled water, stirred and centrifuged. The washing procedure was repeated twice. The residue was combined with 20 mL of sodium acetate – acetic acid buffer (0.005 M, pH 5.0) and then 50 µL of glucoamylase and 20 µL of isoamylase were added. The mixture was incubated at 50°C for 20 h.

After incubation, the mixture was centrifuged at 2095 \times g for 10 min and the residue was collected and washed using 50 mL of distilled water and centrifuged. The washing and centrifugation procedures were repeated twice using fresh distilled water. The collected residue was then washed twice with ethanol followed by acetone. The residue was dried to constant weight in an oven at 105°C and the cell wall remnant was calculated and expressed as per cent of dry rice grain.

Rasting analysis - rapid viscoanalysis (RVA)

The pasting properties of the rice samples were determined with a RVA (Newport Scientific, Warriewood, NSW, Australia). Rice flour (2.8 g; 12% moisture) was slurried with distilled water (25 mL). The temperature profile involved an initial 10 s high-speed (960 rotations min⁻¹) stir that dispersed the sample prior to the

beginning of the measuring phase at 160 rotations min^{-1} . Temperature was held at 50°C for 1 min and then raised to 95°C in 3.75 min, held for 2.5 min, cooled to 50°C in 3.75 min, and held for 5 min.

Moreover, an “extending temperature profile” was also designed for further investigating the pasting properties of the rice samples.

Soluble starch profile after gelatinization

Rice flour was gelatinized using a RVA under a normal heating profile (see Section 2.5). After the RVA run, the paste in the RVA canister was instantly collected. 0.5 g of the rice pasting was mixed with 0.5 mL of 0.05 M sodium acetate buffer (pH 5.0), vigorously vortexed and centrifuged at 10,000 rpm for 10 min. The supernatant was used for measuring starch molecular profile using a size-exclusion high-performance liquid chromatography (SE-HPLC) system. The system comprised a Waters 2690 pump equipped with an autosampler and a differential refractive index detector (Waters, Model 410, Milford, MA). An Ultrahydrogel™ 250 column (Waters, 7.8 mm \times 300 mm), guard column (Phenomenex Inc., Australia) and detector were maintained at 37°C and injection was at 25°C. Sodium acetate – acetic acid buffer (0.05 M; pH 5.0) containing 0.02% sodium azide was used as mobile phase at a flow rate of 0.4 mL min^{-1} .

Rice cooking

Milled Rice grain (2.5 g, dry basis) was combined with 25 mL of distilled water in a quick-fit conical flask fitted with a glass stopper. The flask was immersed in a boiling water bath for 15 min after which it was placed in an ice bath for 5 min. After cooking, the rice was freeze dried, and was visualized using a Scanning Electron Microscopy (SEM).

Rice digestion *in vitro*

The digestion procedure of the cooked rice was described as followings: 0.5g of the cooked rice (on dried basis) was added into 25mL of pH 5.2 NaAc buffer (0.1 mol L^{-1}). Prior to the digestion, 0.55 ml of CaCl_2 (0.1 mol L^{-1}) was added into the mixture. The starch suspension was equilibrated at 37°C for 1 h with magnetic stirring, and then 0.15 mL of enzyme solution containing 2.3U of porcine pancreatin α -amylase and 24 U of amyloglucosidase was added. The digestion was carried out at 37°C with magnetic stirring, and 0.3

mL aliquots of hydrolysed solution were withdrawn at different time intervals. The aliquots were immediately put in a boiling water bath for 10 min to deactivate the enzymes. The glucose content in each digestion slurry was determined using the Megazyme glucose assay kit (GOPOD method, manufacture instruction). Analysis was performed in triplicates.

Scanning electron microscopy (SEM)

Visualisations of rice grain structure were performed on a Cambridge S360 Scanning Electron Microscope with an attached Oxford CT 1500 Cryotrans cold stage / coating unit. An aliquot of cooked rice digesta was centrifuged at 2095 $\times g_{av}$ for 10 min and the supernatant aspirated to waste and the resultant pellet washed using RO water and centrifuged again under the same conditions. The final pellet was air dried in a fume hood and glued onto a sample holder, plunged into liquid nitrogen slush (at -230C) and transferred immediately onto the SEM cold-stage. The specimens were warmed to a controlled -80°C to sublimate off ice crystals. Samples were then transferred into the cryotrons and coated with approximately 10 nanometres of pure gold. The coated specimens were then observed at -180°C on the cold-stage of the SEM.

Statistical analysis

Digestion rate of each rice sample is the measurement of the increase in glucose concentration in the system at each designated time. Digestion extent of cooked rice was calculated as the percentage of digested starch in the total starch at the designated incubation intervals. The amount of digested starch was calculated after conversion of released glucose into starch by use of factor 0.9. Initial velocity of amylase hydrolysis of the starch was expressed as the slope of digestion curve within the first 20 min incubation time. Experimental data were subjected to analysis of variance using one-way analysis of variance, and significant differences between fractions were determined using Duncan’s Multiple Range Test. The differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSIONS

Cell wall remnants

Milled rice grain is composed of endosperm cells

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varying in size and shape (Figure 1a, b). The cell walls are thin-walled and are packed with amyloplasts containing compound starch granules which are evenly distributed. After the digestion of starch by amylases, the residues were collected and determined as “cell wall remnants” (Figure 2). Doongara rice had the highest amount of cell wall remnants among the three cultivars (TABLE 1).

The effect of storage on the amount of cell wall remnants is listed in TABLE 1. In general, storage at 4°C did not affect the amount of cell wall remnants in the three cultivars. However, the amount of cell wall remnants increased when rice stored at 37°C. The measurement of cell wall remnants is of interest due to its important role for providing a structural framework with which the grain is organized and as a physical boundary

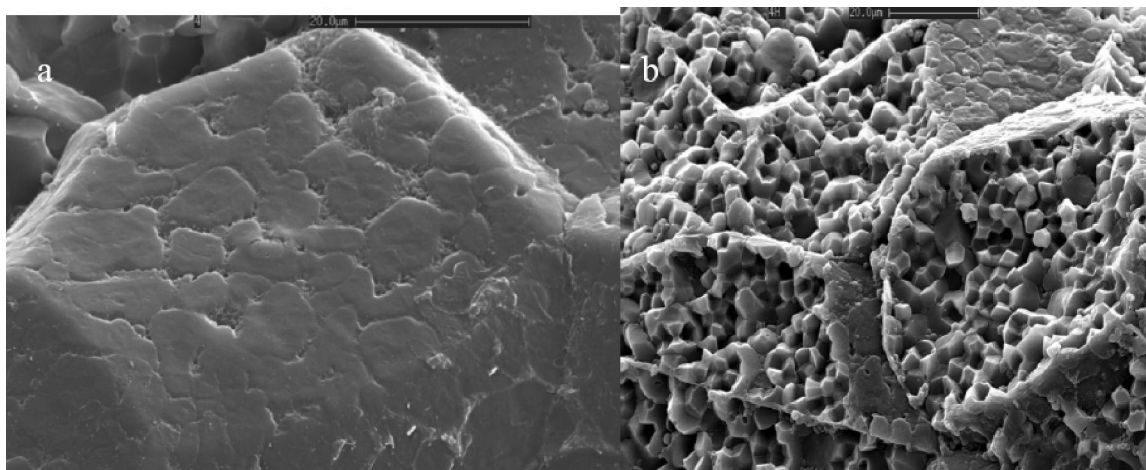


Figure 1 : Scanning electron micrograph showing cell wall structure inside the rice. Rice grain (var. Doongara) was stored for 16 months at 4°C with integrated cell wall (a) and at 37°C with broken cell wall (b).

TABLE 1 : Changes in the contents of cell wall remnants of rice grain following storage at 4°C and 37°C for 16 months.

Rice cultivars	Koshihikari		Doongara		Kyeema		
	4°C	37°C	4°C	37°C	4°C	37°C	
Storage temperature							
	Cell wall remnants (% , dry grain)*						
Storage time (months)	0	6.6±0.3	6.6±0.3	7.9±0.4	7.9±0.4	7.3±0.3	7.3±0.3
	4	6.6±0.3	7.2±0.2	8.0±0.4	8.7±0.2	7.3±0.3	7.9±0.4
	7	6.7±0.2	7.5±0.3	7.9±0.3	8.9±0.2	7.3±0.2	8.1±0.3
	10	6.7±0.3	7.5±0.2	7.9±0.1	9.1±0.1	7.5±0.2	8.3±0.2
	12	6.8±0.4	7.7±0.4	8.1±0.3	9.1±0.3	7.4±0.3	8.3±0.3
	16	6.7±0.2	7.8±0.3	8.1±0.3	9.0±0.2	7.5±0.2	8.5±0.3

to access by moisture and heating energy during starch gelatinization/cooking process^[23]. Cell walls bound together impart a degree of rigidity and are composed of cellulose and hemicelluloses and other minor non-starch components^[24]. In cell wall remnants, phenolic compounds exert a significant effect on the properties of the cell walls, which are mechanically strengthened^[25] by the cross-linkings of phenolic acids with polysaccharides and contribute to cell wall rigidity^[26]. The increase in the amount of cell wall remnants during storage at 37°C might be related to the formation of ferulate dimers^[27], which makes the cell wall remnant more “ligni-

fied” thereby reducing its solubility.

Pasting properties

Pasting property is considered as one of the most sensitive parameters for monitoring rice ageing process, which is normally measured using thermovisco-metry and particularly amylography^[1,2]. Among the RVA parameters, time to peak viscosity (TTPV) and breakdown are the most sensitive indices for evaluating rice ageing. The increase in TTPV of the aged rice (37°C storage) is one of the most notable impacts of ageing (Figure 3). There was no change in TTPV when the rice sample

stored at 4°C for 16 months, whereas TTPV increased significantly ($P < 0.01$) following storage at 37°C.

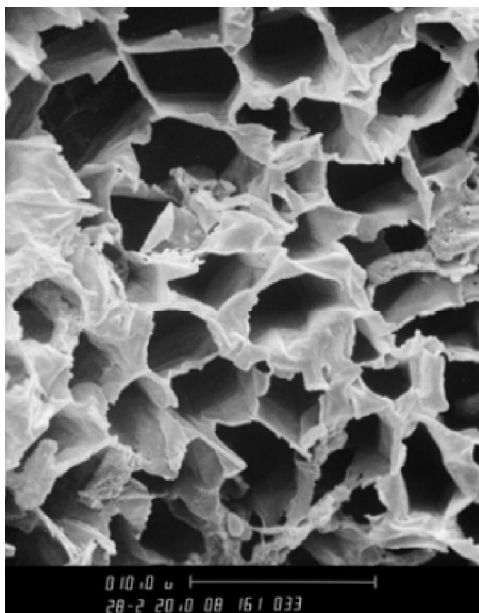


Figure 2 : Scanning electron micrograph showing cell wall remnants in rice after removing starch granules. Rice grain (var. Doongara) was stored at 37°C for 16 months.

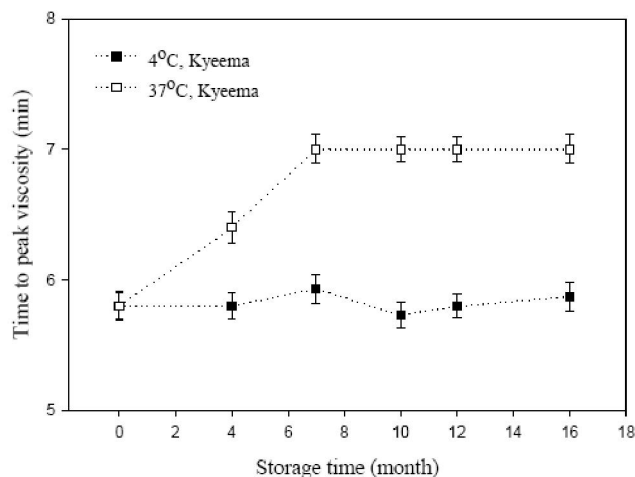


Figure 3. Changes in TTPV following storage for 16 months at 4°C and 37°C (var. Kyeema) as determined by RVA.

The changes in rice pasting properties are also investigated using an “extended heating profile” and represented in Figure 4. The difference in the viscosity profile between the two storage temperatures was seen when temperature raised from 50 to 90°C, suggesting that rice structure was highly strengthened and thus demonstrated higher resistance to be cooked for aged rice (37°C storage) than fresh rice (4°C storage). It is assumed that the more organized structure in rice grain formed during ageing would contribute to the signifi-

cant increase in the TTPV ($P < 0.001$) and other pasting parameters (Figure 3, 4)^[5,8]. Moreover, ageing-induced changes in the structure of lipids and proteins present on the starch granules in aged rice would also affect starch gelatinization^[28,29].

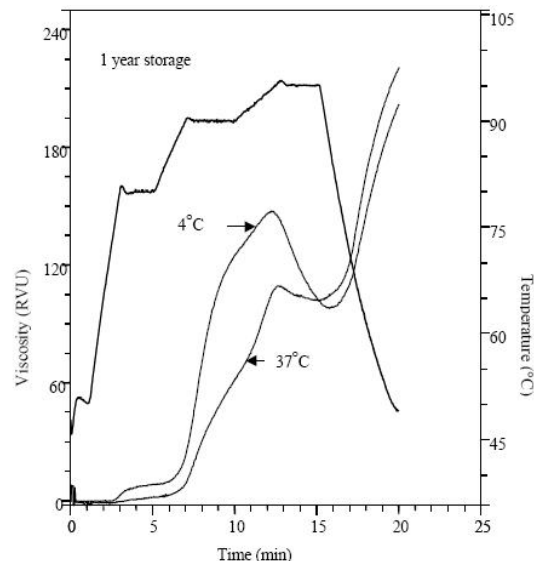


Figure 4 : Differences in RVA profile under an “extended heating profile” for the rice grains (var. Doongara) stored at 4°C and 37°C for 12 months.

Soluble starch molecular profile

After the RVA run under a normal temperature profile, the gelatinized rice sample was collected and the soluble starch molecular profile was analysed and presented in Figure 5a, b. The chromatographs suggest that storage at a higher temperature significantly reduced the leaching of starch molecules in total. This result was consistent with our previous study that that storage at 37°C led to a decrease in the amount of solid content in residual cooking water^[10]. This reduction indicates that the starch components became more difficult to be extruded from the rice grain during cooking^[30].

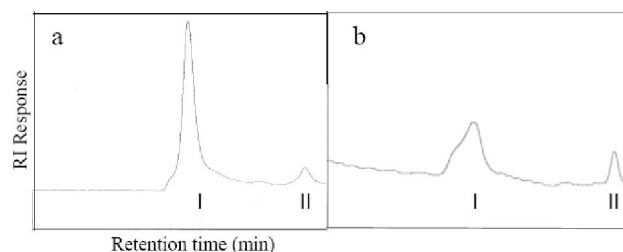


Figure 5 : Chromatographs of soluble starch molecules in rice cooking water. Rice grain (var. Koshihikari) was stored for 12 months at 4°C (a) and 37°C (b). Fraction I: amylopectin, Fraction II: amylose.

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Morphology of cooked rice

The morphology of cooked rice samples is presented in Figure 6. Fresh rice (4°C storage) had a smoother surface after cooking. In contrast, starch in the aged rice (storage at 37°C) is harder to be extruded because of its unique grain structure formed during storage, which would contribute to a coarser structure on the surface of its cooked rice particles. It is assumed that aged rice grain is more difficult to be hydrated, and thus its starch demonstrates a greater resistance to be hydrothermally disrupted compared to the rice stored at 4°C.

Digestion kinetics

The digestion curve of the cooked rice samples is

presented in Figure 7, which is the measurement of the increase in glucose concentration in the incubation system at designated intervals. The digestion curves for all the rice samples showed a biphasic pattern, i.e. a rapid rate at the initial hydrolysis stage followed by a progressively decreased rate thereafter (Figure 7). The overall digestion rate of the aged rice (37°C storage) was lower than that of the fresh rice (4°C storage). In comparison with the digestion curve pattern, the digestion of aged rice exhibited a lower initial velocity (expressed as the slope of digestion curve within the first 20 min incubation time) compared to the digestion of fresh rice. The changes in rice digestion property during storage might be related to the changes in rice physicochemical property as revealed above.

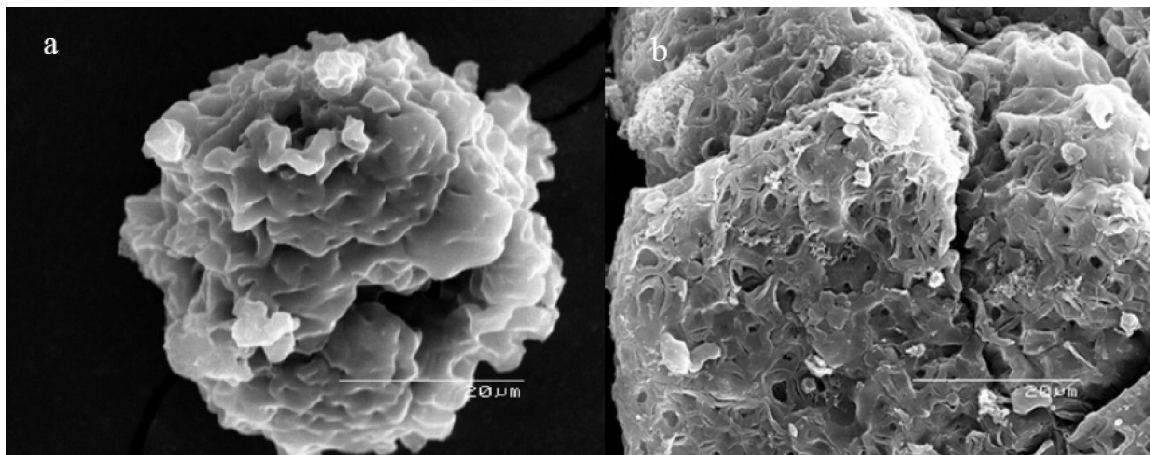


Figure 6 : Representative scanning electron micrographs showing the transverse structure of cooked rice grain (var. Doongara) following storage at (a) 4°C and (b) 37°C for 16 months.

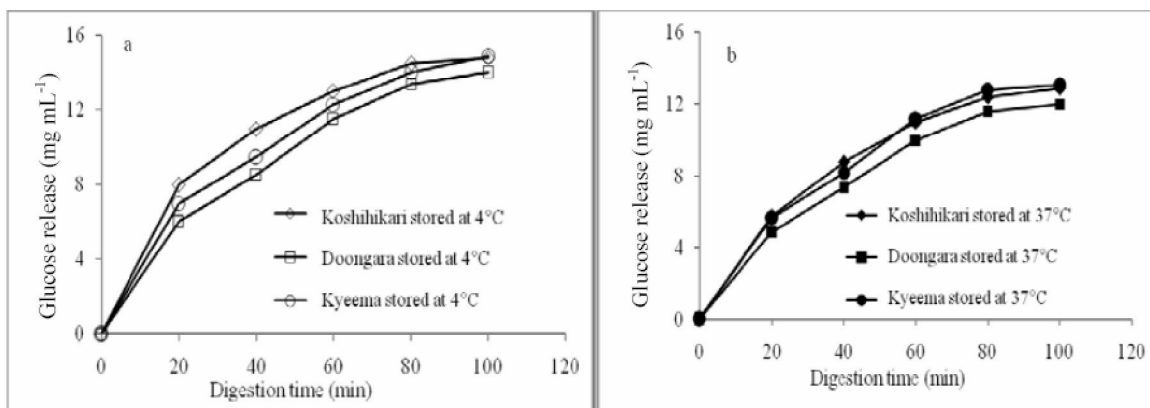


Figure 7 : The differences in digestion properties of three cooked rice cultivars after the rice grains were stored at 4°C (a) and 37°C (b) for 12 months.

Furthermore, the digestion extent was also measured, and the results showed that over 95% of the starch in rice stored at 4°C was digested after the

completion of the incubation, whereas the digestion of starch in the aged rice (stored at 37°C) was reduced to 79.3% for Doongara, 83.1% for Keermaand and

85.2% for Koshihikari, respectively. These significant difference ($P < 0.01$) in the digestion rate and extent between fresh (4°C storage) and aged (37°C storage) rice confirmed that ageing process altered rice digestion behaviours.

CONCLUSION

Milled rice grains are composed of endosperm cells which are thin-walled and packed with amyloplasts containing compound starch granules. Cell walls bound together contributing to rice grain in a good rigidity. Study showed that there was an increase in the amount of cell wall remnants during ageing, which might lead to a strengthen structure in the rice. Pasting studies suggest that the hydration and swelling of aged rice decreased and the starch granules became more resistant to be hydrothermally disrupted. Ageing process led to rice grain becoming more organised and thus limited the leaching of starch components. It is hypothesised that the reduction in the hydration-facilitated disruption of the starch granules in aged rice would be correlated to its lower digestion rate and extent.

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