



Research Article

Physicochemical Properties, *In vitro* Starch Digestibility and Estimated Glycemic Index Following the Accelerated Aging of Freshly Harvested Rice

^{1,2}Tanwirul Millati, ²Yudi Pranoto, ²Tyas Utami and ³Nursigit Bintoro

¹Department of Agro-Industrial Technology, Faculty of Agriculture, University of Mangkurat, Jl. A. Yani Banjarbaru, South Kalimantan 707142, Indonesia

²Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia

³Department of Agricultural Engineering, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia

Abstract

Background and Objective: Cooked freshly harvested rice has high starch digestibility and a high glycemic index. This study aimed to examine the effect of the temperature and duration of accelerated aging on the physicochemical properties of freshly harvested rice, including *in vitro* starch digestibility and the estimated glycemic index of cooked rice. **Materials and Methods:** The rice used was freshly harvested IR 64 variety with $27.36 \pm 0.73\%$ moisture content. The accelerated aging process was conducted at various temperatures and durations, namely, room temperature (24-30°C), 40, 50, or 60°C and 2, 4 or 6 days. The parameters quantified were free fatty acid levels, amylose content, thermal and pasting properties, starch digestibility and estimated glycemic index of cooked rice. **Results:** Free fatty acids, amylose-lipid complexes and thermal properties increase during accelerated aging treatment. The pasting properties increased at 40°C, while at 60°C, there was a slight decrease in all properties except for the pasting temperature, which increased. The starch digestibility and estimated glycemic index decreased and the lowest values were obtained from accelerated aging at 40°C for 4 days, which was not significantly different from 60°C for 4 days, with estimated glycemic index values of 68.61 and 69.82, respectively. **Conclusion:** The accelerated aging of freshly harvested rice reduced starch digestibility and the estimated glycemic index of cooked rice. The best temperature and time for reducing the starch digestibility and changing the estimated glycemic index of cooked rice from the high to medium category were 40°C and 4 days.

Key words: Accelerated aging, freshly harvested rice, glycemic index, physicochemical properties, starch digestibility

Received:

Accepted:

Published:

Citation: Tanwirul Millati, Yudi Pranoto, Tyas Utami and Nursigit Bintoro, 2019. Physicochemical properties, *In vitro* starch digestibility and estimated glycemic index following the accelerated aging of freshly harvested rice. Pak. J. Nutr., CC: CC-CC.

Corresponding Author: Yudi Pranoto, Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia

Copyright: © 2019 Tanwirul Millati *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aging is a natural and spontaneous phenomenon that occurs during the storage of paddy, rough rice, brown rice or white rice¹. Rice undergoes several changes during the aging process that modify the cooking, processing, eating and nutritional qualities of rice and affect the commercial value of rice². Tananuwong and Malila³ reported that the aging process can be determined by evaluating physicochemical properties, including thermal and pasting properties. Changes in the physicochemical properties of rice involve enzymatic and non-enzymatic reactions in starch, proteins and lipids in rice kernels, including the hydrolysis of starch and lipids by amylase and lipase, respectively⁴, the formation of disulfide bridges in oryzenin⁵ and formation of amylose-lipid complexes⁶.

Natural aging takes a long time, approximately 4-6 months and requires plenty of space to store the rice. This increases operating costs, vulnerability to insect damage, as well as increases exposure to microorganisms and rodents during storage⁷. Therefore, rice aging needs to be accelerated to reduce the adverse effects of aging. Aging may be accelerated by treating the grain with dry or wet heat⁸. The storage conditions that most influence the aging process are time and temperature^{9,10} and higher temperature and moisture content yield more changes⁴.

Some previous studies have shown that the thermal properties of rice flour are influenced by temperature and that storage at higher temperatures will result in higher gelatinization temperatures³. The viscosity of rice paste increased dramatically after the storage of milled rice and these changes depended on storage temperature and time¹. The peak viscosity increased over 4 months of storage and the change in viscosity was greater at higher storage temperatures¹¹. Regarding heat treatment, Jaisut *et al.*¹² stated that accelerated the aging of jasmine brown rice by a high-temperature fluidization technique that decreased the digestion rate of rice starch and the glycemic index.

Freshly harvested rice generally has high moisture content (25±5%). High moisture content is expected to accelerate the enzymatic and non-enzymatic reactions in the starches, proteins and lipids of the rice kernel during storage, especially when combined with high temperatures. In this study, accelerated aging was performed by storing freshly harvested rice at various temperatures and lengths of storage. This study aims to determine the effects of various temperatures and times of accelerated aging treatment on the physicochemical properties of rice flour, including *in vitro* starch digestibility and the estimated glycemic index of cooked rice.

MATERIALS AND METHODS

Material: Freshly harvested rice IR 64 variety with a moisture content of 27.36±0.73% was obtained from local farmers in Senoboyo, Sleman, Yogyakarta, Indonesia. The enzymes used for starch digestibility analysis were pepsin (EC 3.4.23.1, Merck Inc. Germany), α -amylase (Sigma A9913, Sigma Aldrich Inc. US), amyloglucosidase (Sigma A7095, Sigma Aldrich, Denmark) and glucose oxidase-peroxidase kit (Glucose GOD FS-DiaSys Diagnostic System GmbH Germany). All other chemical reagents used in this research were analytical grade.

Accelerated aging: Freshly harvested rice weighed 1200±100 g was put in a plastic bag, tied and incubated at the various temperatures, including room temperature (24-30°C), 40, 50 and 60°C, for 10 days. Each temperature treatment consisted of 15 bags and every two days, 3 bags (samples) were removed for observations. The samples were dried in a cabinet dryer (Shimizu Scientific Instrument MFG CO, LTD model PSN-150, Japan) at 50°C for 8 h, followed by tempering at room temperature (24-30°C) for 12-15 h. Then, the grain was milled (Rapsco, Houston US) into milled rice. As a control, freshly harvested rice that was directly dried without accelerated aging was used. Next, the milled rice was separated by a grading machine (Rapsco, Houston US) to obtain head rice, broken rice and rice groats. The head rice was then used to analyze the chemical composition and the cooked rice starch digestibility value.

Preparation of rice flour: Rice flour was prepared according to Yu *et al.*¹³ with modification. Each of the head rice samples was milled and passed through a 60 mesh sieve. The resulting rice flour was then stored in the plastic bag and kept at 4°C in a refrigerator before analysis.

Free fatty acids (FFA) in rice flour: The FFA contents of rice flours were determined using the AOAC method (1996) with several modifications. Rice flour (10 g) and 25 mL of 95% alcohol were put into an Erlenmeyer flask, closed, heated until boiling and shaken hard. Then the flask was cooled, 3 drops of 1% phenolphthalein indicator was added and with the sample was titrated with 0.05N KOH until a constant pink color was observed for one min. The FFA was calculated using the following equation¹⁴:

$$\text{FFA\% (as palmitate)} = \frac{(\text{mL KOH}) \times (\text{N KOH}) \times 240}{\text{sample weight} \times 1000} \times 100\%$$

Amylose content of rice flour: Amylose content was determined by the iodine binding method according to Chrastil¹⁵. This method could be used to determine total amylose, free amylose and amylose-lipid complex content. Rice flour (20 mg) and 5 mL of 85% methanol were put into the test tube, heated at 60°C for 30 min and occasionally mixed to extract the lipids. The sample was centrifuged and the supernatant liquid was removed. The addition of 85% methanol, heating and centrifugation was repeated 3 times. Then, 4 mL distilled water and 2 mL of 0.4 N NaOH was added to the rice flour residue and the mixture was heated at 100°C for 30 min with occasional mixing. An aliquot (0.1 mL) was added to 5 mL of 0.05% trichloroacetic acid in a separate test tube, vortexed and then 0.05 mL of iodine solution (1.27 g I₂ and 3.0 g KI L⁻¹) was added and mixed immediately. The absorbance was read at 620 nm after 30 min at 25°C against a H₂O blank.

To calculate amylose content, a standard curve was made using amylose corn starch with amylose content from 0-75%. Total amylose and free amylose values were obtained from iodine binding analysis with or without lipid extraction with methanol, while the amylose-lipid complex was calculated as the difference between total amylose and free amylose.

Characterization of samples with high contents of amylose-lipid complexes: The samples used for characterization were 4 freshly harvested rice samples from the accelerated aging treatment that had an increased content of amylose-lipid complexes compared to control rice. The thermal and pasting properties of the rice flour as well as the *In vitro* digestibility and estimated glycemic index of cooked rice were characterized for each sample.

Thermal properties: Thermal analysis was conducted using differential scanning calorimetry (DSC) (Shimadzu DSC-60 Plus). Rice flour (3.0 mg) and 10 µL distilled water were placed into an aluminum pan and then closed tightly. The sample pan was heated from 40-150°C at a rate of 10°C min⁻¹. The main parameters of the DSC profile were expressed as onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and transition enthalpy (ΔH).

Pasting properties: The pasting property of rice flour was determined using a Rapid Visco Analyzer (RVA) (Newport Scientific RVA-S4). Rice flour (3.0 g in dry weight) was weighed in an RVA canister and then 25 g distilled water was added and mixed thoroughly. The RVA analysis included the heating process phase and cooling with constant stirring (160 rpm). In

the heating phase, the solution of starch was heated from 50-95°C at a rate of 12°C min⁻¹ and then held at 95°C for 5 min. After the heating phase was complete, the starch paste was passed to the cooling phase and the temperature was decreased from 95-50°C at a rate of 12°C min⁻¹ and then held at 50°C for 2 min. The pasting temperature (PT), peak viscosity (PV), breakdown (BD), final viscosity (FV) and setback (SB) were recorded.

***In vitro* starch digestibility, hydrolysis index and estimated glycemic index:** Milled rice starch digestion kinetics were determined according to the method proposed by Goni *et al.*¹⁶ Rice (50 mg) and 5 mL distilled water were prepared in 50 mL conical tubes and cooked in boiling water for 30 min. Subsequently, 10 mL HCl-KCl buffer at pH 1.5 was added and the sample was homogenized for 2 min. Then, 0.2 mL of a solution containing 1 g pepsin in 10 mL HCl-KCl buffer at pH 1.5 was added to the sample. The sample was incubated in a shaking water bath at 40°C for 60 min. After that, the volume was adjusted to 25 mL by adding 15 mL of tris-maleate buffer (pH 6.9). To start starch hydrolysis, 5 mL of tris-maleate buffer containing 2.6 IU of α-amylase was added to the sample. The samples were then placed in a shaking water bath at 37°C. An aliquot (0.1 mL) was taken from each tube at 30 min intervals from 0-3 h into an Eppendorf tube and boiled in water for 5 min to inactivate the α-amylase. Then, 1 mL of 0.4 M sodium acetate buffer at pH 4.75 and 30 µL of amyloglucosidase were added to hydrolyze the solubilized starch. The sample was then incubated at 60°C for 45 min. Finally, the glucose concentration was measured using the glucose oxidase-peroxidase kit. The rate of starch digestion was expressed as a percentage of the total starch hydrolyzed at different times (30, 60, 90, 120, 150 and 180 min).

A non-linear model was established to describe the kinetics of starch hydrolysis. The first order equation was $C = C_{\infty} (1 - e^{-kt})$, where C is the percentage of starch hydrolyzed at time t, C_∞ is the percentage of starch hydrolyzed after 180 min, k is the kinetic constant (min⁻¹) and t is the time (min). The parameters C_∞ and k were estimated based on the data obtained from the *In vitro* hydrolysis procedure. The area under the hydrolysis curve (AUC) was calculated using the following equation:

$$AUC = C_{\infty} (t_f - t_0) - (C_{\infty}/k) (1 - \exp(-k(t_f - t_0)))$$

where, t_f is the final time (180 min) and t₀ is the initial time (0 min). The hydrolysis index (HI) was defined as the AUC of each treatment divided by the AUC of white bread. Goni *et al.*¹⁶ suggested that the hydrolysis index is a good

indicator of the glycemic response. The glycemic index (GI) of cooked rice was thus estimated using the following equation:

$$GI = 39.71 + (0.549HI)$$

Statistical analysis: All tests were performed at least in triplicate. Data were analyzed with two-way analysis of variance using SPSS 20.0 for Windows. The difference in means was determined by Duncan's Multiple Range Test ($p < 0.05$).

RESULTS AND DISCUSSION

Free fatty acid content: The FFA increased during the accelerated aging treatments and the changes were greater at higher storage temperatures. Previous studies showed that the increased levels of FFA during storage were influenced by the temperature¹¹; however, according to Dhaliwal *et al.*⁴ the lipase activity of rice is influenced by water content and lipase activity decreases at a lower moisture content. The FFA content ranged from 0.57-1.21%, as presented in Table 1. The increased FFA during rice storage involved the hydrolysis of lipids by lipase to produce free fatty acids. The FFA could then form complexes with amylose or be oxidized to hydroperoxides and other secondary products¹¹.

Amylose content: The amylose content in this study was divided into total amylose, free amylose and amylose-lipid complex. Total and free amylose values were obtained from iodine binding with and without lipid extraction by hot methanol, respectively, whereas amylose-lipid complexes were calculated as the difference between the total amylose and free amylose.

The total amylose, free amylose and amylose-lipid complex contents were relatively constant with accelerating

aging at room temperature but at 40, 50 and 60°C, the total and free amylose contents decreased, while the level of amylose-lipid complexes increased. The total amylose, free amylose and amylose-lipid complex contents in milled rice were 28.97-30.63, 19.53-23.24 and 6.48-9.67%, respectively, as shown in Table 1.

Total and free amylose decreased during storage due to amylolytic enzyme activity. Dhaliwal *et al.*⁴ reported that endogenous amylase remains active during rice storage with a moisture content of 12-20.6%, with higher moisture content, the enzyme activity was greater, although this activity tends to decrease with time. An increase in amylose-lipid complexes occurred due to the formation of the complexes through the interaction between the helical chains of amylose with FFA that were already present in rice and derived from the hydrolysis of lipid. Amylose-lipid complexes could naturally occur in starch or be created during starch gelatinization in the presence of lipids¹⁷.

Based on Table 1, the highest content of amylose-lipid complexes was observed with the accelerated aging of rough rice at 40°C for 4 days, which was not significantly different from accelerated aging at 40°C for 6 days or 60°C for 4 and 6 days. Thus, these four rice samples were used to characterize the rice produced by the accelerated aging treatment.

Characterization of rice samples with high amylose-lipid complex content

Thermal properties: The values of T_o , T_p and T_c increased with the accelerated aging of freshly harvested grain and were significantly different from the control, as shown in Table 2. This indicates that there was a change in the structure of starch granules due to the formation of new crystals with higher thermal stability. This result was believed to be related to the endogenous amylase activity, which was relatively high due to the accelerated aging treatment administered to the

Table 1: Changes of FFA, amylose total, free amylose and amylose-lipid complex during accelerated aging of freshly harvested rice

| Temperature (°C) | Time (days) | FFA (db%) | Amylose total (db%) | Free amylose (db%) | Amylose-lipid complex (% db) |
|------------------|-------------|-------------------------|--------------------------|---------------------------|------------------------------|
| Control | 0 | 0.68±0.04 ^{bc} | 29.72±0.46 ^{ab} | 23.24±0.24 ^e | 6.48±0.47 ^{ab} |
| Room Temperature | 2 | 0.67±0.0 ^{bc} | 29.04±0.79 ^a | 22.44±0.24 ^{cde} | 6.59±0.63 ^{abc} |
| | 4 | 0.60±0.03 ^a | 30.32±0.15 ^b | 22.60±0.54 ^{de} | 7.72±0.60 ^{de} |
| | 6 | 0.58±0.0 ^a | 30.17±0.22 ^b | 22.87±0.12 ^{de} | 7.30±0.20 ^{bcd} |
| 40°C | 2 | 0.57±0.04 ^a | 30.63±0.46 ^b | 22.29±0.78 ^{cd} | 8.37±0.52 ^{ef} |
| | 4 | 0.65±0.02 ^b | 29.19±1.19 ^{ab} | 19.53±0.45 ^a | 9.67±0.82 ^g |
| | 6 | 0.72±0.02 ^c | 29.38±0.37 ^{ab} | 20.27±0.16 ^{ab} | 9.12±0.49 ^{fg} |
| 50°C | 2 | 0.81±0.03 ^d | 28.97±0.36 ^a | 22.90±0.50 ^{de} | 6.07±0.45 ^a |
| | 4 | 1.00±0.05 ^f | 29.63±0.08 ^{ab} | 21.76±0.30 ^c | 7.87±0.36 ^{de} |
| | 6 | 0.90±0.04 ^e | 29.63±0.13 ^{ab} | 22.16±0.67 ^{cd} | 7.7±0.57 ^{cde} |
| 60°C | 2 | 1.07±0.07 ^g | 30.08±0.81 ^b | 22.18±0.86 ^{cd} | 7.90±0.65 ^{de} |
| | 4 | 1.06±0.05 ^g | 29.34±0.35 ^{ab} | 20.13±0.07 ^{ab} | 9.21±0.31 ^g |
| | 6 | 1.21±0.02 ^h | 29.67±0.09 ^{ab} | 20.39±0.02 ^b | 9.28±0.06 ^g |

Different superscripts in the column mean that the mean values are significantly different at $p < 0.05$

Table 2: Changes in thermal properties during accelerated aging of freshly harvested rice

| Treatments | T _o (°C) | T _p (°C) | T _c (°C) | ΔH (J/g) |
|--------------|--------------------------|--------------------------|--------------------------|------------------------|
| Control | 72.60±1.11 ^a | 77.43±0.71 ^a | 81.76±0.70 ^a | 6.17±0.14 ^b |
| 40°C, 4 days | 74.63±0.74 ^{bc} | 79.52±0.28 ^{bc} | 84.51±0.05 ^b | 7.00±0.46 ^c |
| 40°C, 6 days | 74.01±0.91 ^b | 79.06±0.89 ^b | 84.24±0.56 ^b | 4.44±0.41 ^a |
| 60°C, 4 days | 75.12±0.21 ^{bc} | 79.68±0.11 ^{bc} | 84.95±0.50 ^{bc} | 7.54±0.25 ^d |
| 60°C, 6 days | 75.85±1.34 ^c | 80.02±0.18 ^c | 85.89±1.53 ^c | 8.55±0.11 ^e |

Different superscripts in the column mean that the mean values are significantly different at p<0.05

Table 3: Changes of pasting properties during accelerated aging of freshly harvested rice

| Treatments | PT (°C) | PV (cP) | BD (cP) | FV (cP) | SB (cP) |
|--------------|-------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Control | 75.98±0.17 ^a | 4036.50±9.19 ^c | 876.50±2.12 ^c | 4456.00±9.90 ^b | 1295.93±2.93 ^{bc} |
| 40°C, 4 days | 77.12±0.30 ^b | 5056.00±19.80 ^e | 1188.50±41.72 ^e | 5809.50±23.33 ^e | 1974.50±7.78 ^d |
| 40°C, 6 days | 76.76±0.20 ^b | 4677.50±12.02 ^d | 1151.00±2.83 ^d | 5126.00±12.73 ^d | 1331.70±76.02 ^c |
| 60°C, 4 days | 80.81±0.21 ^c | 3881.00±9.90 ^b | 635.00±1.41 ^b | 4525.00±11.31 ^c | 1278.50±3.54 ^b |
| 60°C, 6 days | 86.02±0.61 ^d | 3186.00±22.63 ^a | 491.50±3.54 ^a | 3893.50±27.58 ^a | 1199.00±8.49 ^a |

PT: Pasting temperature, PV: Peak viscosity, BD: Breakdown, FV: Final viscosity, SB: Setback, Different superscripts in the column mean that the mean values are significantly different at p<0.05

freshly harvested rice with high moisture content (27.36%). Awazuha *et al.*¹⁸ stated that the optimal temperature for the amylase activity of rice was 40-60°C. Amylase hydrolyzes 1,4 α glycosidic bonds in the amylose chain and the amylopectin branched chain produces short and straight chains that interact to form a double helix and new crystallites with varying stability¹⁹. Chung *et al.*²⁰ reported that the increases in T_o, T_p and T_c were related to the interactions between amylose-amylose and amylose-amylopectin as well as the formation of the amylose-lipid complex. Inter-chain interactions also result in an increase in ΔH, as shown in Table 2.

Gelatinization enthalpy (ΔH) shows the loss of the double helix order and is strongly influenced by amylose content, the chain length of amylopectin and amylose-lipid complex²⁰. Cooke and Gidley²¹ reported that the higher the temperature and the greater the total energy required for gelatinization, the stronger the crystalline structure of starch granules. Rice flour from the accelerated aging treatment showed an increase in ΔH compared to the control, except for the accelerated aging treatment at 40°C for 6 days, which showed a decrease. Miyoshi²² stated the greater gelatinization enthalpy at higher temperatures was due to the formation of amylose-lipid complexes after treatment, while decreasing ΔH indicates that crystalline amylopectin has been degraded during treatment. The decrease in ΔH indicates that some of the double helix present in the crystalline and in the non-crystalline regions of the granule may have been disrupted and that the proportion of short chains with DP 6-12^{20,23} increased because of the hydrolysis of the amylopectin side chain by amylase during the accelerated aging treatment. These chains were too short to form a stable double helix, so less energy was required to break them down and melt the double helix during gelatinization²⁰.

Pasting properties: The pasting properties of rice flour obtained from RVA were a measure of starch viscosity during the heating cycle, which reflects the molecular events that occur in starch granules. Table 3 shows the effect of temperature and length of accelerated aging on the pasting properties of rice flour. Pasting temperature (PT) is the initial temperature of the occurrence of gelatinization, which is characterized by an increase in the viscosity of the flour suspension. The PT of rice flour increases with increasing temperature and time of accelerated aging. This indicates that there has been a change in the molecular structure and crystallinity of starch so that starch granules have a higher resistance to swelling. There was a possibility of the association between amylose molecules and/or amylose molecules with a linear form of amylopectin branch, resulting in a more compact structure. The high temperature is needed by starch granules to reach maximum swelling²⁴.

The PV, BD, SB and FV of rice flours that underwent accelerated aging were higher than those of the control at 40°C, while at 60°C these values were lower in the accelerated aging group than in the control. The increase in pasting properties in the accelerated aging group at 40°C was probably caused by rice protein, especially disulfide bonds. Previous studies have suggested that the pasting properties of rice during storage are mainly due to changes in the structure of oryzenin, which is the main protein in rice^{25,26}. Chrastil⁵ reported that during the storage of milled rice at 4 or 40°C for 12 months, there was an increase in the average molecular weight of oryzenin and cystine, while the amount of cysteine in oryzenin decreased because the cysteine was oxidized to form disulfide bonds. The presence of disulfide bonds may reduce the fragility of swollen starch granules²⁷, so that during heating, the starch granules can absorb more water, swell larger, gelatinize at a lower temperature and

provide higher viscosity before being disrupted³. When starch granules are disrupted, amylose molecules come out of the granules and dissolve in the water during the heating process. This effect results in high BD and SB values. BD describes the ease of starch granule disruption upon heating after the maximum swelling at the peak viscosity. A high BD reflects low stability of the starch granules and more broken granules when heated. SB is a measure of the recrystallization or retrogradation of gelatinized starches during the cooling period. Retrogradation is a phenomenon where soluble starch molecules and amylopectin linear fractions are reassociated with hydrogen bonds²⁸. The high SB value indicates a high tendency for starch retrogradation to occur.

The decrease in PV, BD, SB and FV in rice flour resulting from accelerated aging at 60°C indicates that starch granules were more resistant to swelling, increasing the temperature required for gelatinization and the time required to achieve maximum viscosity. This possibility was related to the formation of amylose-lipid complexes (FFA) that occur during heating in RVA. Accelerated aging at 60°C shows a higher level of FFA than accelerated aging at 40°C, so there was a great opportunity to form complexes with amylose upon heating. Derycke *et al.*²⁹ stated that heating rice flour in the presence of water can damage the crystallinity of amylopectin, which can encourage the formation of amylose-lipid complexes. The increased crystalline matrix order and the formation of amylose-lipid complexes can reduce granular swelling capacity and increase pasting stability during heating, resulting in decreased PV, BD and SB³⁰. The amylose-lipid complex changes the rheological properties of the paste, increases the gelatinization temperature, retards retrogradation and changes the product texture^{31,32}.

***In vitro* starch digestibility and estimated glycemic index of cooked rice:**

The curve of the *in vitro* starch digestibility of cooked rice following the accelerated aging of freshly harvested rice are shown in Fig. 1. The hydrolysis of cooked rice starch increased with increasing digestion time. All cooked rice samples showed less than 30% digestibility after 180 min. This digestibility value was lower than that reported

by Goni *et al.*¹⁶, where the digestibility value of cooked rice starch was approximately 50% after 180 min. All rice resulting from accelerated aging showed a decrease in the starch hydrolysis rate compared to the controls. During accelerated aging, interactions occur between the starch chain and hydrogen bonds, such as amylose-amylose and/or amylose-amylopectin and amylose-lipid complex formation, which causes makes starch hydrolysis by the enzyme more difficult²⁰. The kinetic constants (k), HI (hydrolysis index) and e-GI (estimated glycemic index) are shown in Table 4. The rate of starch digestion indicated by HI and e-GI represents the digestibility of cooked rice starch in relation to the starch digestibility of reference foods, such as white bread³³. The kinetic constant of cooked rice starch varied from 0.055-0.067 higher than that reported by Goni *et al.*¹⁶ but lower than the kinetic constants reported by Rattanamechaikul *et al.*³². The HI and e-GI of cooked rice starch ranged from 52.64-65.54 and 68.61-75.69, respectively. The results indicate that the accelerated aging treatment of freshly harvested grain can be used to reduce the HI and e-GI values. Analysis of variance shows that HI and e-GI are affected by the temperature and length of accelerated aging treatment. The lowest value of HI and e-GI was observed in the freshly harvested rice treated

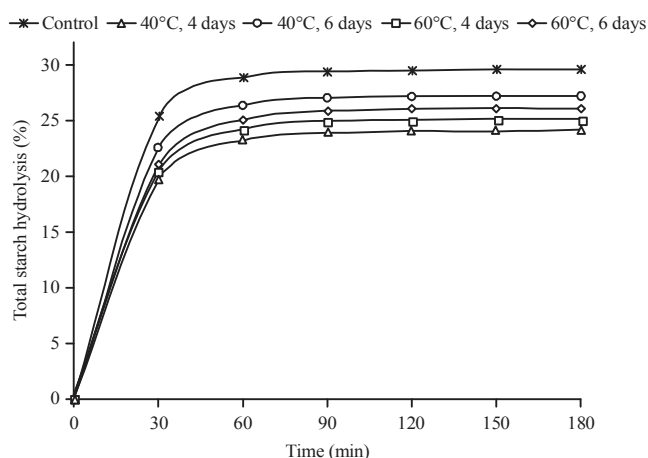


Fig. 1: *In vitro* starch hydrolysis rate of cooked rice obtained during accelerated aging of freshly harvested rice

Table 4: The kinetic constants (k), hydrolysis index (HI) and estimated glycemic index (e-GI) of cooked rice obtained from selected accelerated aging of freshly harvested rice

| Treatments | k (min ⁻¹) | HI | e-GI |
|--------------|------------------------|--------------------------|--------------------------|
| Control | 0.067±0.007 | 65.54±1.39 ^d | 75.69±0.77 ^d |
| 40°C, 4 days | 0.058±0.010 | 52.64±1.48 ^a | 68.61±0.81 ^a |
| 40°C, 6 days | 0.060±0.009 | 59.65±2.88 ^c | 72.46±1.58 ^c |
| 60°C, 4 days | 0.057±0.007 | 54.85±0.69 ^{ab} | 69.82±0.38 ^{ab} |
| 60°C, 6 days | 0.055±0.005 | 56.77±0.80 ^{bc} | 70.88±0.43 ^{bc} |

Different superscripts in the column mean that the mean values are significantly different at p<0.05

with accelerated aging treatment of 40°C for 4 days, which was not significantly different from the values resulting from treatment with 60°C for 4 days. A longer length of accelerated aging treatment (6 days) caused an increase of HI and e-GI, although the value was still lower than the control. This result was thought to be due to the continued activity of amylase, which hydrolyzes starch, producing amylose molecules with very short chains that form weak crystals and are easily hydrolyzed.

The decrease of HI and e-GI with accelerated aging at 40°C is mainly caused by the retrogradation of starch, which occurs after the rice is cooked, while at a temperature of 60°C, due to the formation of the amylose-lipid complex. The retrogradation of amylose is a more rapid process that occurs immediately while cooling but amylopectin requires longer time³⁴. Starch retrogradation could cause the increased resistance of amylose and amylopectin molecules to enzymatic hydrolysis¹⁹ and can reduce HI and e-GI³⁵. Jaisut *et al.*¹² stated that the reduction in GI was due to the formation of amylose-lipid complexes during the accelerated aging treatment. Amylose-lipid complexes are hydrolyzed and absorbed in the digestive tract at the same level as free amylose but in a slightly slower manner³⁶. The classification of food glycemic index by Foster-Powell *et al.*³⁷ divided it into three classes: low (GI <55), medium (GI 56-69), and high (GI > 70). According to this classification, accelerating the aging of harvested rice at temperatures of 40 and 60°C for 4 days decreases the value of e-GI rice from the high to the medium category. This study has shown that the accelerated aging of freshly harvested rice can be used to reduce the glycemic index of rice; thus, consuming this rice has advantageous health aspects. However, further research is needed to determine the nutritional value and consumer acceptance of the rice produced from accelerated aging.

CONCLUSION

The accelerated aging of freshly harvested rice at various temperatures and times can change the physicochemical properties, starch digestibility and e-GI of rice. After treatment, FFA and amylose-lipid complex levels increased, while total amylose and free amylose levels decreased slightly. The thermal properties of rice flour increased, while changes in the pasting properties depended on the temperature. The accelerated aging treatment at 40°C showed an increase in PT, PV, BD and SB, while at 60°C, PT increased and PV, BD and SB decreased. The increase in SB showed that the retrogradation tendency of rice starch increased. In addition, accelerated aging can reduce the starch digestibility and e-GI of cooked

rice. The temperature and length of accelerated aging that could reduce starch and e-GI digestibility from the high category to the medium category was 40 and 60°C for 4 days. The decrease in starch digestibility and e-GI after rice cooking was related to the retrogradation of starch and the formation of amylose-lipid complexes during accelerated aging, cooking and after cooking, although this still requires further research.

SIGNIFICANCE STATEMENT

This study found that the accelerated aging treatment (temperature and time) of freshly harvested rice can be used to reduce the digestibility of starch and the glycemic index of cooked rice. This research is useful for ensuring that rice is safe to consume, especially for individuals with chronic diseases, such as obesity and diabetes. Thus, a new theory regarding the method of accelerating the aging of rice and its relation to starch digestibility and glycemic index may be obtained.

ACKNOWLEDGMENT

The authors gratefully acknowledge to the Directorate General of Higher Education, Ministry of Research, Technology and Higher Education, Republic of Indonesia, for supporting the Doctoral Scholarship where the project was implemented.

REFERENCES

1. Zhou, Z., K. Robards, S. Helliwell and C. Blanchard, 2002. Ageing of stored rice: Changes in chemical and physical attributes. *J. Cereal Sci.*, 35: 65-78.
2. Mod, R.R. and R.L. Ory, 1986. Minor Components of Rice: Changes during Storage. In: *Handbook of Food and Beverage Stability: Chemical, Biochemical, Microbiological and Nutritional Aspects*, Charalambous, G. (Ed.). Academic Press Inc., New York, USA., ISBN: 978-0-12-169070-0, pp: 489-516.
3. Tananuwong, K. and Y. Malila, 2011. Changes in physicochemical properties of organic hulled rice during storage under different conditions. *Food Chem.*, 125: 179-185.
4. Dhaliwal, Y.S., K.S. Sekhon and H.P.S. Nagi, 1991. Enzymatic activities and rheological properties of stored rice. *Cereal Chem.*, 68: 18-21.
5. Chrastil, J., 1990. Protein-starch interactions in rice grains. Influence of storage on oryzenin and starch. *J. Agric. Food Chem.*, 38: 1804-1809.
6. Juliano, B.O., 1985. Criteria and Tests for Rice Grain Qualities. In: *Rice: Chemistry and Technology*, Juliano, B.O. (Ed.). 2nd Edn., American Association of Cereal Chemists, St. Paul, MN., USA., ISBN-13: 9780913250419, pp: 443-524.

7. Soponronnarit, S., M. Chiawwet, S. Prachayawarakorn, P. Tungtrakul and C. Taechapairoj, 2008. Comparative study of physicochemical properties of accelerated and naturally aged rice. *J. Food Eng.*, 85: 268-276.
8. Juliano, B., 1979. The Chemical Basis of Rice Grain Quality. In: *Chemical Aspects of Rice Grain Quality*, Brady, N.C. (Ed.). International Rice Research Institute, Los Banos, Philippines, ISBN-13: 9789711040109, pp: 69-90.
9. Keawpeng, I. and K. Venkatachalam, 2015. Effect of aging on changes in rice physical qualities. *Int. Food Res. J.*, 22: 2180-2187.
10. Parnsakhorn, S. and A. Noomhorm, 2012. Effects of storage temperature on physical and chemical properties of brown rice, parboiled brown rice and parboiled paddy. *Thai J. Agric. Sci.*, 45: 221-231.
11. Park, C.E., Y.S. Kim, K.J. Park and B.K. Kim, 2012. Changes in physicochemical characteristics of rice during storage at different temperatures. *J. Stored Prod. Res.*, 48: 25-29.
12. Jaisut, D., S. Prachayawarakorn, W. Varanyanon, P. Tungtrakul and S. Soponronnarit, 2009. Accelerated aging of jasmine brown rice by high-temperature fluidization technique. *Food Res. Int.*, 42: 674-681.
13. Yu, S., Y. Ma, L. Menager and D.W. Sun, 2012. Physicochemical properties of starch and flour from different rice cultivars. *Food Bioprocess Technol.*, 5: 626-637.
14. Bai, S.H., I. Darby, T. Nevenimo, G. Hannet and D. Hannet *et al.*, 2017. Effects of roasting on kernel peroxide value, free fatty acid, fatty acid composition and crude protein content. *PLoS ONE*, Vol. 12. 10.1371/journal.pone.0184279
15. Chrastil, J., 1987. Improved colorimetric determination of amylose in starches or flours. *Carbohydr. Res.*, 159: 154-158.
16. Goni, I., A. Garcia-Alonso and F. Saura-Calixto, 1997. A starch hydrolysis procedure to estimate glycemic index. *Nutr. Res.*, 17: 427-437.
17. Tang, M.C. and L. Copeland, 2007. Analysis of complexes between lipids and wheat starch. *Carbohydr. Polym.*, 67: 80-85.
18. Awazuhara, M., A. Nakagawa, J. Yamaguchi, T. Fujiwara and H. Hayashi *et al.*, 2000. Distribution and characterization of enzymes causing starch degradation in rice (*Oryza sativa* Cv. Koshihikari). *J. Agric. Food Chem.*, 48: 245-252.
19. Zhou, Y., S. Meng, D. Chen, X. Zhu and H. Yuan, 2014. Structure characterization and hypoglycemic effects of dual modified resistant starch from indica rice starch. *Carbohydr. Polym.*, 103: 81-86.
20. Chung, H.J., R. Hoover and Q. Liu, 2009. The impact of single and dual hydrothermal modifications on the molecular structure and physicochemical properties of normal corn starch. *Int. J. Biol. Macromol.*, 44: 203-210.
21. Cooke, D. and M.J. Gidley, 1992. Loss of crystalline and molecular order during starch gelatinization: Origin of the enthalpic transition. *Carbohydr. Res.*, 227: 103-112.
22. Miyoshi, E., 2002. Effects of heat-moisture treatment and lipids on gelatinization and retrogradation of maize and potato starches. *Cereal Chem.*, 79: 72-77.
23. Gunaratne, A. and R. Hoover, 2002. Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydr. Polym.*, 49: 425-437.
24. Pranoto, Y., Rahmayuni, Haryadi and S.K. Rakshit, 2014. Physicochemical properties of heat moisture treated sweet potato starches of selected Indonesian varieties. *Int. Food Res. J.*, 21: 2031-2038.
25. Martin, M. and M.A. Fitzgerald, 2002. Proteins in rice grains influence cooking properties! *J. Cereal Sci.*, 36: 285-294.
26. Zhou, Z., K. Robards, S. Helliwell, C. Blanchard and G. Baxterb, 2003. Rice ageing. I. Effect of changes in protein on starch behaviour. *Starch*, 55: 162-169.
27. Hamaker, B.R. and V.K. Griffin, 1993. Effect of disulfide bond-containing protein on rice starch gelatinization and pasting. *Cereal Chem.*, 70: 377-380.
28. Da Rosa Zavareze, E. and A.R.G. Dias, 2011. Impact of heat-moisture treatment and annealing in starches: A review. *Carbohydr. Polym.*, 83: 317-328.
29. Derycke, V., G.E. Vandeputte, R. Vermeylen, W. De Man, B. Goderis, M.H.J. Koch and J.A. Delcour, 2005. Starch gelatinization and amylose-lipid interactions during rice parboiling investigated by temperature resolved wide angle X-ray scattering and differential scanning calorimetry. *J. Cereal Sci.*, 42: 334-343.
30. Pukkahuta, C., B. Suwannawat, S. Shobsngob and S. Varavinit, 2008. Comparative study of pasting and thermal transition characteristics of osmotic pressure and heat-moisture treated corn starch. *Carbohydr. Polym.*, 72: 527-536.
31. Blazek, J., E.P. Gilbert and L. Copeland, 2011. Effects of monoglycerides on pasting properties of wheat starch after repeated heating and cooling. *J. Cereal Sci.*, 54: 151-159.
32. Rattanamechaiskul, C., S. Soponronnarit and S. Prachayawarakorn, 2014. Glycemic response to brown rice treated by different drying media. *J. Food Eng.*, 122: 48-55.
33. Kim, H.J. and P.J. White, 2012. *In vitro* digestion rate and estimated glycemic index of oat flours from typical and high β -glucan oat lines. *J. Agric. Food Chem.*, 60: 5237-5242.
34. Deepa, G., V. Singh and K.A. Naidu, 2010. A comparative study on starch digestibility, glycemic index and resistant starch of pigmented ('Njavara' and 'Jyothi') and a non-pigmented ('IR 64') rice varieties. *J. Food Sci. Technol.*, 47: 644-649.
35. Hu, P., H. Zhao, Z. Duan, Z. Linlin and D. Wu, 2004. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *J. Cereal Sci.*, 40: 231-237.
36. Holm, J., I. Bjorck, S. Ostrowska, A.C. Eliasson, N.G. Asp, K. Larsson and I. Lundquist, 1983. Digestibility of amylose-lipid complexes *in-vitro* and *in-vivo*. *Starch*, 35: 294-297.
37. Foster-Powell, K., S.H.A. Holt and J.C. Brand-Miller, 2002. International table of glycemic index and glycemic load values. *Am. J. Clin. Nutr.*, 76: 5-56.