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EFFECT OF AUTOCLAVING-COOLING ON THE PHYSICAL PROPERTIES, MICROSTRUCTURE AND STARCH HYDROLYSIS OF MILLED RICE

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ABSTRACT

The research aimed to determine the effect of autoclaving and cooling on physical properties, particularly texture and color of cooked rice grains, the microscopic images and starch hydrolysis. Autoclaving and cooling were applied on rice grains to slow down rice starch hydrolysis. Three rice varieties were selected based on amylose level (high, medium and low amylose content of rice varieties). Autoclaving-cooling was conducted by autoclaving rice grains in excess water at 120ºC for 15 minutes, followed by cooling the rice grains at temperature of 4ºC for 24 hours. The process of autoclaving and cooling were repeated for the treatments of two and three autoclaving-cooling cycles. Results showed that rice variety (based on amylose content) and number of autoclaving-cooling cycles had significant effect on the rate of starch hydrolysis, on the other hand, they had no significant effect on the texture and color of cooked rice. Microstructure images showed that the autoclaved-cooled rice grains displayed more condensed structure and a continuous network with irregular shape formed. The higher amylose rice variety and more autoclaving-cooling cycles applied on rice grains resulted in a lower rate of constant (k) of total dissolved solids content during starch hydrolysis. The condensed microstructure in a rice grain and a lower rate of starch hydrolysis might indicate the content of resistant starch in rice grains increased; therefore it would result in a slower glucose release during digestion.

1. Introduction

Rice contributes the largest amount of energy in daily energy requirement for most people in Indonesia. Rice is usually consumed as steamed rice grains. Starch is the major constituent in rice, and it can be easily broken down into glucose during digestion, thereby increasing blood sugar. There are some products that have been developed to overcome the difficulties for people with diabetics, such low glycemic indexed milk, and foods that are made of resistant starch, however there were not so many against the whole rice grains. Some manufacturers have developed analog rice by using ingredients with low glycemic indexed starch. However, it is difficult for the Indonesian people to change the main diet of steamed rice grains into analog rice. Accordingly, this study was designed to address the problem by changing the components of the starch in rice grains into starch retrogradation or resistant starch. Resistant starch is starch that is not digested by the digestive enzymes so there is no nutrition absorption by the intestine (Hassan et al., 2010; Yun et al., 2010; Ranawana et al., 2009). Re-arrangement of the structure in the starch fraction during processing which includes the process of gelatinization and crystallization can cause starch to retrograde (Hug-Iten et al., 2003). It is possible to produce high resistant starch in rice grains due to substantial amount of starch in rice grains. A few experimental works has been reported in increasing resistant starch in rice grains (Kim et al., 2006; Milasinovic et al., 2009; Widowati et al., 2010). Those papers mostly focused on the amount of the resistant starch produced through various methods such as parboiling, autoclaving–cooling, or the combination of autoclaving and hydrolysis by enzyme. It has no doubt that those methods successfully increased the amount of resistant starch in rice grains, however, none of those works report on the physical properties of autoclaved and cooled rice grains particularly the microscopic images, texture and color of the rice. In this study, the rice grains were processed by autoclaving-cooling method, and the product was called as "autoclaved-cooled" rice grains.

Rice consumers are increasingly selective in determining the type of rice for consumption. They not only concern about the nutritive aspect of cooked rice but also the physical properties such as texture and color of rice. Texture is one of physical properties that are important in judging the eating quality of cooked rice. The texture which could indicate hardness of rice could be measured by texture analyzer that was expressed as the energy needed to disintegrate the cooked rice and texture is affected by cohesiveness of rice grains. The harder of the texture of cooked rice was indicated by higher energy (gram force) to penetrate into rice grains.

One of the components that determine the eating quality is amylose content. Rice grains can be classified into three groups based on amylose level, namely rice with high amylose content (25-33%), medium (20-25%), and low (2-9%). Different rice varieties will result in different texture and color of cooked rice. Therefore this experimental work determined the physical characteristics (texture and color) of cooked autoclaved-cooled rice from some selected rice varieties differing in amylose content. Autoclaving-cooling was chosen as method to reduce the starch digestibility of rice grains due to its environmental friendly method. The repeating process of autoclaving-cooling which was so called as "cycle" could increase the amount of retrograded starch, and therefore it could slow down the rice starch digestibility. Starch digestibility could be indicated by starch hydrolysis during which starch was broken down into simpler carbohydrate.

Most previous studies investigated the effect of autoclaving-cooling on some properties of starches rather than on grains (Babu and Parimalavalli, 2013; Zhao and Lin, 2009; Kim et al., 2009). Therefore, this study was undertaken to investigate the effects of autoclaving-cooling cycles of rice grains differing in amylose content on the physical properties, microscopic images and rate of starch hydrolysis.

2. Materials and methods

2.1. Materials

The selected rice varieties were high amylose content of rice variety (IR 42 with the amylose content of 25.5%), medium amylose content of rice variety (Karawang with the amylose content of 11.3%), and low amylose content of rice variety (waxy rice with the amylose content of 1.4%). IR 42 and Karawang rice varieties were obtained from the Rice Central Production in South Sumatera, Indonesia. Waxy rice was purchased from local traditional market in Palembang city, South Sumatera. Enzyme used in the analysis of rate of starch hydrolysis was α-amylase solution (Sigma-Aldrich, A8220).

2.2.Autoclaving and cooling on rice

Each milled rice grain variety (IR 42, Karawang, waxy rice) was weighed as amount of 250 g and 750 mL of aquadest was added. It was cooked in a cooking pot until all of the aquadest was absorbed by the rice grains. The partial gelatinized rice grains were transferred into a one Liter-beaker glass, covered by aluminium foil and placed in a plastic bag and it was heated in an autoclave at the sterilizing temperature (121^{o}C) for 15 minutes. The completely gelatinized rice grains were taken out from autoclave and cooled for one hour at room temperature. The gelatinized grains were placed in a refrigerator at 4^{o}C for 24 hours. The rice grain that was processed up to this stage was called as one cycle of autoclaving-cooling. For two cycles of autoclaving-cooling, the gelatinized rice grains that had been refrigerated were then re-heated in the autoclave for 15 minutes (121°C), followed by cooling at a temperature of 4°C for 24 hours. The similar process was conducted for three cycles of autoclaving-cooling. The last step was drying the rice grains at 50 °C in a hot air oven until the moisture content of the rice grains reached approximately 8%. Each treatment was performed in triplicates.

2.3. Microscopic images, texture and color analysis

The microscopic images of internal structure of autoclaved-cooled rice grains were determined by using Scanning Electron Microscopy (SEM 5200 (JEOL) at 1000 magnifications.

Texture and color analysis were conducted on autoclaved-cooled rice grains that had been cooked in a rice cooker (ratio of rice and water = 1:3). The texture measurement was performed in individual cooked rice grain by applying trigger 0.9 g, distance 1 mm and speed 1mm/s.

Analysis of the texture of cooked rice was conducted by using texture analyzer (LFRA 1500 Texture Analyzer, Brookfield, USA) with flat end (cylinder type) stainless steel probe (probe specification was TA 39 in 2 mm of diameter, 20 mm in length). The color measurement of cooked rice was carried out by using a color reader (Konica Minolta CR-10) for lightness (*L*), chroma (*C*) and hue (*h*).

2.4.Starch hydrolysis by α-amylase (*in vitro*)

Starch hydrolysis was measured by measuring the total dissolved solids of rice grains during hydrolysis. Dried rice grains were ground and weighed for 2 g, and placed in a 25 mL of Beaker glass. An amount of 10 mL aquadest was added into sample and heated on a hot plate to gelatinize the sample. The gel was cooled at room temperature and measured for its initial total dissolved solids by using a refractometer (Atago, 0-85 Brix%). Enzyme solution of α-amylase (0.1 mL) was added into the gel solution and gently stirred with a magnetic stirrer. The total dissolved solids were periodically measured every 30 minutes to 120 minutes. The rate of starch hydrolysis by enzymatic process was analyzed by the Arrhenius equation.

2.5. Statistical Analysis

An analysis of variance (ANOVA) was performed on physical analysis of cooked rice grains, and the significant difference treatments were further analyzed by Tukey's test using the Statistical Analysis System. P values < 0.05 were considered significant. Rice variety was as factor A, and number of cycles of autoclaving and cooling was as factor B.

2.6. Rate of Starch Hydrolysis

The rate of starch hydrolysis during enzymatic process was modeled as:

$$-\frac{dC}{dt} = kC^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \quad (1)$$

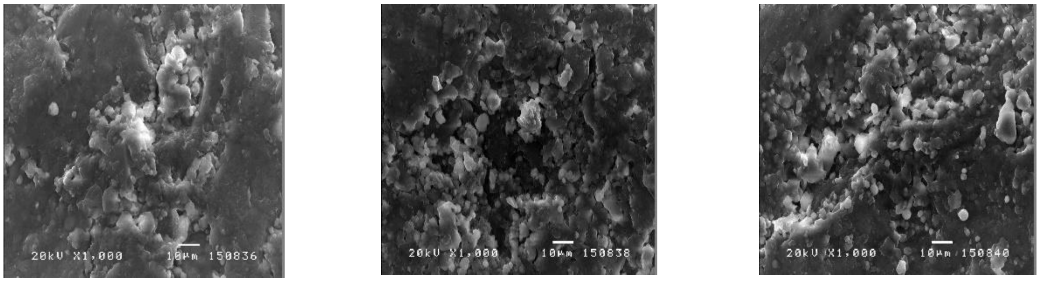
Where *C* is total dissolved solids (Brix%), *t* is hydrolysis period (minute), *k* is a pseudo-rate of constant, *n* is reaction order. The reaction order (*n*) in the starch hydrolysis was assumed to follow the first reaction order. If *n* is equal to one, equation (1) collapses to equation (2) (Labuza and Riboh, 1982).

$$-\frac{dC}{dt} = kC \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

3. Results and discussions

3.1. Microscopic Images

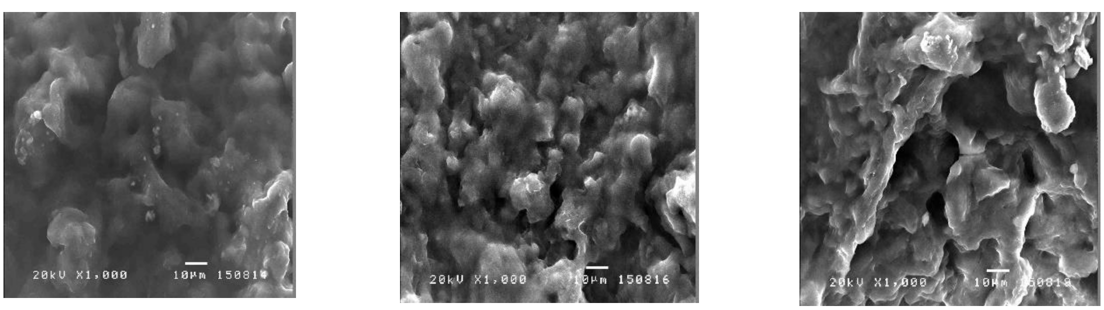
Microscopic images of rice were presented in Figures 1 and 2. Figure 1 exhibited granular appearances in the image, while Figure 2 clearly illustrated that the granular structure disappeared and a continuous network with irregular shape formed. The microstructure images displayed more condensed microstructure in the rice grain along with more cycles of autoclaving-cooling.



IR 42 rice Karawang rice Waxy rice

Figure 1. Microstructures of untreated IR 42, Karawang and waxy rice at magnification of 1000x under SEM.

Figure 1 shows that the untreated rice grain of IR 42 variety had a denser region compared to Karawang variety and waxy rice. It might be due to higher amount of amylose (linier fraction of starch) in IR 42 variety. During autoclaving and cooling process in a rice grain, amylose and amylopectin intensively interacted each other (Hoover, 2010). The re-arrangement of amylose and amylose fractions, amylose and amylopectin fraction during autoclaving-cooling resulted in a denser gelatinized starch region than the untreated rice grains. As shown in Figure 2, more cycles of autoclaving and cooling applied on a rice grain, a denser gelatinized starch region in rice grains. The re-arrangement of linier chains in amylose fraction was more readily compared to amylopectin; therefore the IR 42 rice variety that contained higher amylose content showed a dense gelatinized starch than others.



IR 42 – 1 cycle IR 42- 2 cycles IR 42- 3 cycles

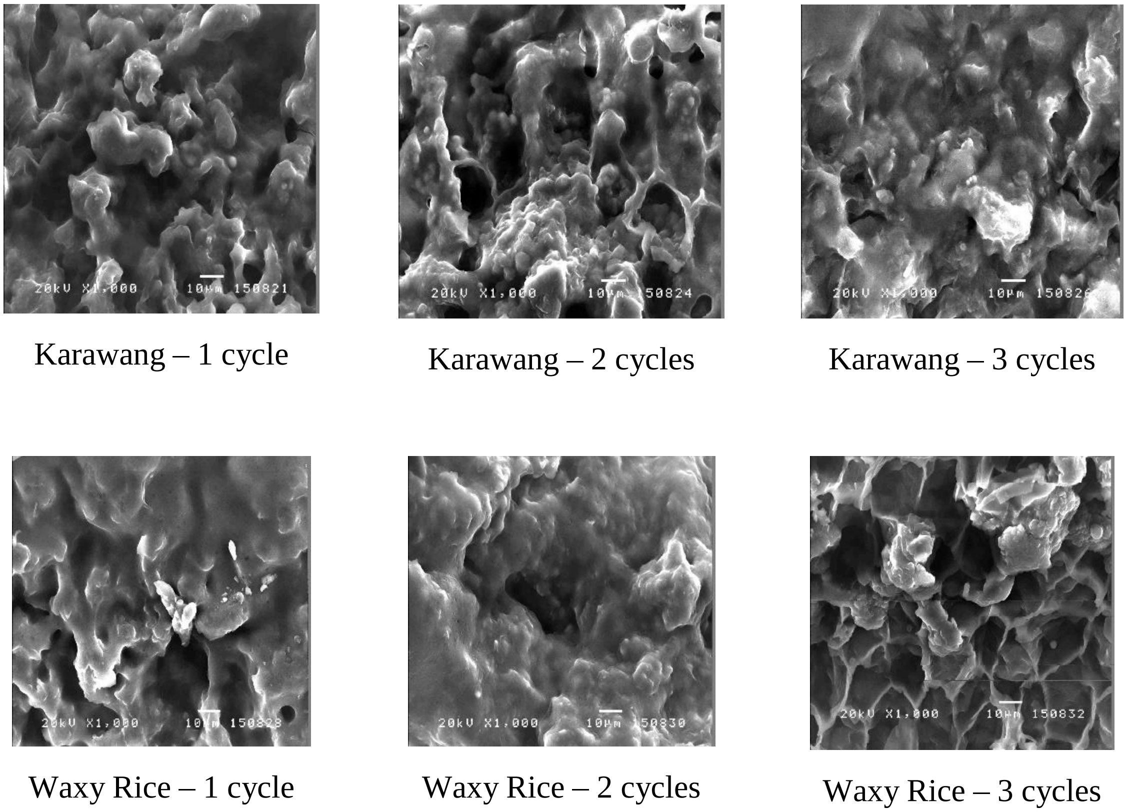


Figure 2. Microstructures of autoclaved and cooled rice at magnification of 1000x under scanning electron microscope.

3.2. Hardness

Analysis of variance showed that the rice variety (based on amylose content), number of autoclaving-cooling cycles and their interactions had significant effect ($P<0.05$) on the texture (hardness) of the cooked rice samples. Post Hoc comparison using Tukey's test were presented in Table 1 to 3.

Table 1. Pos Hoc comparison using Tukey's test on rice variety

Rice variety	Hardness (gf)
Waxy rice	$(12.41\pm0.36)^{a}$
Karawang	$(17.05\pm0.41)^{b}$
IR 42	$(22.16\pm0.39)^{c}$

Means with different superscript letters are significantly different ($P<0.05$) (Tukey's$_{0.05}$ = 0.15)

Table 2. Pos Hoc comparison using Tukey's test on number of cycles

Number of cycles	Hardness (gf)
1 cycle	$(16.00\pm0.15)^{a}$
2 cycles	$(17.33\pm0.52)^{b}$
3 cycles	$(18.30\pm0.49)^{c}$

Means with different superscript letters are significantly different ($P<0.05$) (Tukey's$_{0.05}$ = 0.15)

Table 3. Pos Hoc comparison using Tukey's test on interaction of rice variety and number of cycles

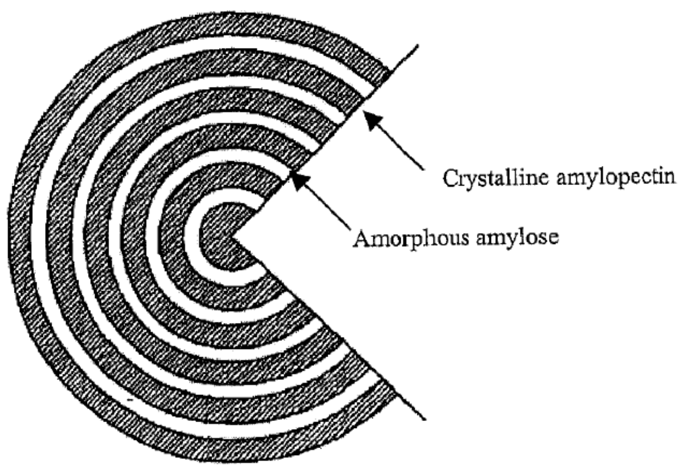
Rice variety	Number of autoclaving-cooling cycle		
	one cycle	two cycles	three cycles
IR42	$(20.36\pm0.12)^{f}$	$(22.09\pm0.61)^{g}$	$(24.04\pm0.45)^{h}$
Karawang	$(16.36\pm0.12)^{d}$	$(17.04\pm0.45)^{e}$	$(17.15\pm0.66)^{e}$

Waxy rice	(11.27±0.23)[a]	(12.87±0.49)[b]	(13.10+0.36)[c]

Means with different superscript letters are significantly different (P<0.05) (Tukey's$_{0.05}$ = 0.79)

Factors affecting the cooked rice have long been recognized, owing to extensive research in this area (Cameron and Wang, 2005; Asghar et al., 2012; Widjajaseputra, 2012; Han and Lim, 2009; Narkrugsa and Saeleaw, 2009). However, texture and color are quality attributes that mostly affect the consumers' preference of cooked rice. The texture of cooked rice in this research was measured as hardness. The hardness of the rice samples are recorded as peak load and final load in grams force per millimeter (gf/mm). Peak load value indicates the amount of grams force needed to disintegrate the rice sample, while the final load indicates the magnitude of gram force after the probe entered into the sample. Final value of peak load and load in all the samples were relatively equal, and therefore the values of the final load were reported in this experiment.

IR42 rice varieties contained the highest amylose content (25.5%) among the selected rice varieties; therefore, as expected, more retrograded starch occurred during cooling due to easier re-arrangement of amylose. Amylose exists within a starch granule as an entity that is largely separated from the amylopectin fraction. Amylose is located in bundles between amylopectin clusters, and randomly interdispersed among the clusters in both amorphous and crystalline regions. A schematic diagram of the structure of a starch granule, showing the amorphous amylose and crystalline layers of amylopectin is reproduced in Figure 3.



Source: Morrison et al. (1994)

Figure 3. A schematic model of a starch granule that shows amorphous amylose and crystalline amylopectin domains

The retrograded starch was clearly shown in the microstructure images by the dense gelatinized starch region. Unlike IR 42 and Karawang rice varieties, waxy rice only contained 1% of amylose content, therefore only slight retrograded starch occurred during cooling of gelatinized rice grains. As a consequence, the texture of cooked autoclaved-cooled rice grains of waxy rice was lower than the other two rice varieties. Amylose fractions are more readily retrograded than amylopectin fraction.

3.3. Color analysis

Color attributes included lightness, chroma and hue. The lowest lightness value

(69.37%) and chroma value (2.20%) were found in cooked rice of IR 42 variety (high amylose variety) that was processed by one cycle of autoclaving-cooling, on the other hand, the highest lightness value (77.80%) was found in medium amylose rice variety (Karawang). The highest chroma value (7.20%) was obtained in cooked waxy rice that was processed by three cycles of autoclaving-cooling. Hue showed the dominant color appeared on the surface of the cooked rice. The hue values ranged from 87.67 ^{o} to 129.47^o that has the spectrum of yellowish white. Lightness, chroma and hue values of all treatments were shown in Figures 4, 5 and 6, respectively.

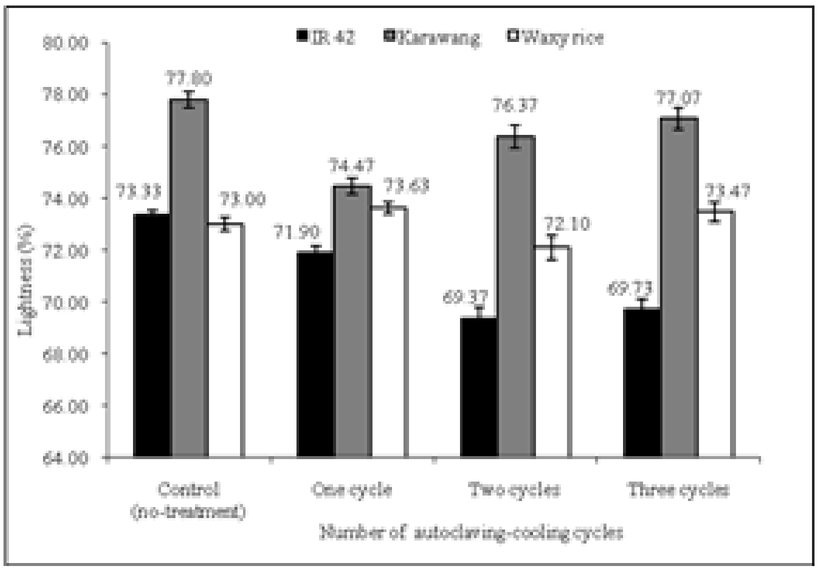


Figure 4. Lightness value (%) of cooked autoclaved– cooled rice grains

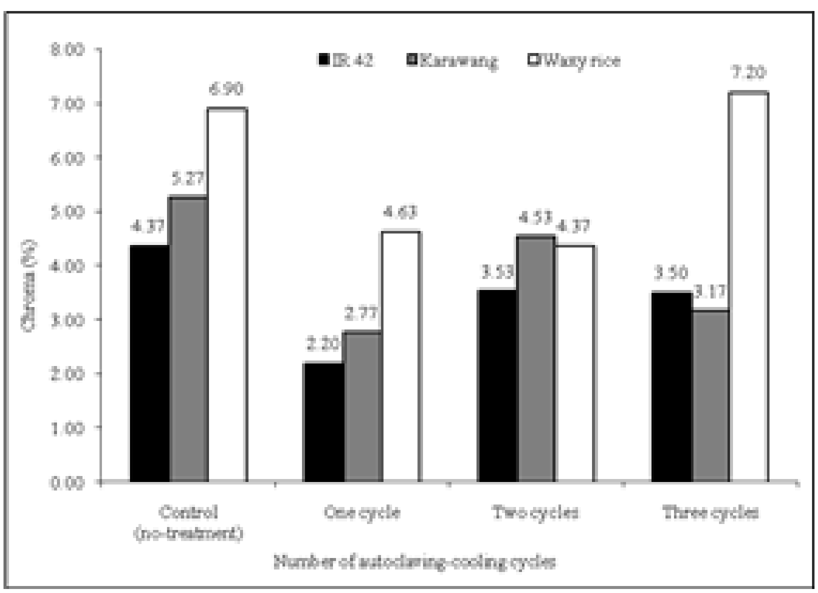


Figure 5. Chroma values (%) of cooked autoclaved–cooled

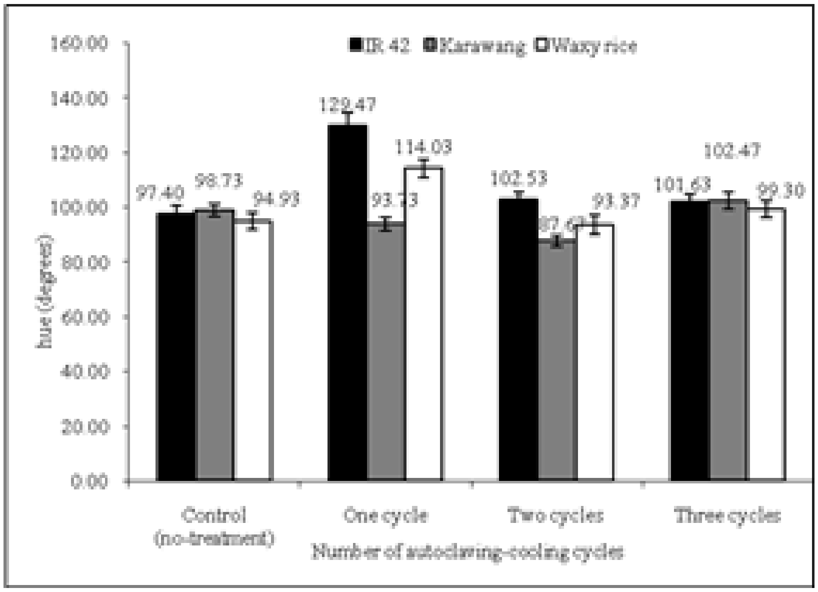


Figure 6. Hue value (º) of cooked autoclaved–cooled

No significant difference was observed on lightness, chroma and hue of cooked rice samples (P>0.05) for any of the variables. These insignificant differences (P>0.05) in the cooked rice sample gave possibilities of this product to be accepted by most rice consumers in respect of the similar appearance of color as the untreated cooked rice.

The rice grains used in the research were white milled rice in which all of the bran layers and germs were brushed off during milling and polishing. With all of the bran layers removed resulted in white endosperm of rice, therefore, there was no significant effect of rice amylose content and number of autoclaving-cooling cycles on the color of treated rice grains.

3.3. Rate of starch hydrolysis

Hydrolysis of starch would increase the amount of solids dissolve in the liquid, therefore the rate of starch hydrolysis in this experiment was based on the total dissolved solids. The rates of starch hydrolysis (*k*) of all samples are summarized in Table 4. The *k* values decreased along with the increase number of autoclaving-cooling cycles. It indicated that starch in rice had been depreciated by retrogradation.

Table 4. Rate of constant (*k*) of total dissolved solids during starch hydrolysis (according to first order reaction)

Treatments	Rate of starch hydrolysis (*k*) based on total dissolved solids content (Brix% minute^{-1})
Rice processed by one cycle of autoclaving-cooling	
IR 42	1.4×10^{-3} **c**
Karawang	2×10^{-3} **e**
Waxy Rice	2.94×10^{-2} **h**
Rice processed by two cycles of autoclaving-cooling	

IR 42	10^{-3} **b**
Karawang	1.8×10^{-3} **d**
Waxy Rice	2.87×10^{-2} **g**
Rice processed by three cycles of autoclaving-cooling	
IR 42	8×10^{-4} **a**
Karawang	8×10^{-4} **a**
Waxy Rice	2.28×10^{-2} **f**

Means with different superscript letters are significantly different (P<0.05)

The main component in the rice is starch that can exceed 85%. Yin and Cheng (1980) explained that starch dissolves in water when heated and can form crystalline structure of amylopectin, whilst amorphous amylose is damaged and form a gel. When the gel is cooled, the linear part of amylose and amylopectin linear part will condense and form a crystalline structure by way of mutual binding through hydrogen bonding. Temperatures between -8°C to 8°C can lead to the formation of the crystalline structure. As further stated by Kim et al. (2009) that starch was completely gelatinized and amylose could be leached out during autoclaving; whereas the crystalline regions (amylopectin) of clusters of branched amylopectin chains were broken down. The leached out amylose would re-arrange and lead to formation of retrograded starch or resistant starch. In other words, the retrograded starch formed a tightly packed starch and hardened the texture of rice grains. It resulted in a slower rate of starch hydrolysis. This finding is in agreement with Vatanasuchart et al. (2009) who stated that rice snacks made of low amylose rice had low resistant starch or retrograded starch.

Autoclaving and cooling of rice grains would be able to increase the amount of resistant starch; therefore autoclaving and cooling of rice grains could retard the rice starch to be hydrolyzed. The slow rate of starch hydrolysis was indicated by lower *k* values (Table 2). Higher value of *k* indicates that starch in rice sample could be rapidly hydrolyzed by amylase. Our findings are in agreement with Ozturk et al. (2009) and Pongjanta et al. (2009) who stated starch with higher content of amylose could be more readily formed resistant starch that that of lower content of amylose. As stated earlier that waxy rice contained the lowest amount of amylose content, and therefore, the *k* value of starch hydrolysis in waxy rice was the highest among the rice samples. The *k* value of starch hydrolysis decreased with increasing number of autoclaving-cooling cycles applied on rice samples which was due to the formation of more resistant starch with more cycles of autoclaving-cooling.

4. Conclusions

The rice variety and number of autoclaving-cooling cycles significantly affected the rate of starch hydrolysis but not on texture and color of cooked autoclave-cooled rice grains. The internal structure of autoclaved-cooled rice grains were more condensed rather than that of without treatment. The texture of cooked rice tend to be harder after experiencing more cycles of autoclaving-cooling particularly in rice grains with higher amylose content. The rate of starch hydrolysis (*k*) decreased with more autoclaving-cooling cycles. The lowest *k* value (rate of starch hydrolysis) was found in cooked rice with three autoclaving-cooling cycles of both high and medium amylose rice varieties.

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