International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **4(12),** 34-39, December **(2015)** *Int. Res. J. Biological Sci.*

In Vitro Digestibility of Indonesian Cooked Rice Treated with Cooling-Reheating Process and Coconut Milk Addition

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Available online at: www.isca.in, www.isca.me
Received 22nd October 2015, revised 9th November 2015, accepted 27th November 2015

Abstract

One of the traditional cooked rice in Indonesia was made from a mixture of rice and coconut milk, but very limited exploration has been done on its characteristic. The aim of this study was to investigate the effect of cooling-reheating process and addition of coconut milk on the in vitro digestibility of Indonesian cooked rice. Nine rice varieties were analyzed for their amylose and resistant starch content. Among the varieties, the highest amylose content (23.69%) and resistant starch content (21.13%) were found in Setra ramos. On the other hand, Mentik susu had the lowest amylose content (15.22%) and resistant starch content (10.39%). Cooked rice treated with cooling-reheating process and addition of coconut milk contained higher resistant starch than freshly cooked rice. In vitro starch digestibility showed that the cooked rice with addition of coconut milk had the lowest hydrolysis index (31.14) and predicted glycemic index (56.80) compared to the other cooked rice samples.

Keywords: Rice, amylose, resistant starch, coconut milk, in vitro digestibility.

Introduction

Rice (*Oryza sativa* L.) is the staple food of Indonesian people. Usually rice was cooked from popular varieties such as Rojo lele and Pandan wangi since they provide excellent aroma and soft texture upon cooking. The texture parameter is very important to consumer acceptability. For this reason those two rice varieties are favorable for cooked rice.

It is well known that starch digestibility is varies among different starchy foods including rice starch, rice flour and cooked rice. Several factors have been reported affect the digestibility of cooked rice such as inherent rice characteristics and cooking methods. Rice characteristics, including variety and grain type[1], cultivation systems[2], surface organization, granular size and amylose content[3], amylose and amylopectin fine structures[4] are reported to affect the digestibility of cooked rice.

Processing method has been reported to affects the digestibility of starchy foods because it may affect both gelatinization and retro gradation. Gelatinization is a disruption process of native molecular orders of starch granules during thermal processing in the presence of water, whereas retro gradation is a changes process of gelatinized starch from an amorphous form to a crystalline form[5]. Gelatinization increases starch digestibility, whereas retro gradation reduces starch digestibility.

Starch digestibility has been reported to have correlation with resistant starch content. Starch digestibility decreased as resistant starch content increased[3]. Increasing resistant starch in cooked rice can be done in other ways, such as thermal cooking using rice cooker or oven microwave followed by cooling or freezing[6,7]. On the other hand, the cycled temperature condition of 4 and 30˚C could induce resistant starch and significantly reduced starch digestibility of waxy maize starch[8].

Traditional cooked rice in Indonesia was prepared as mixture of rice and coconut milk. It is well known that coconut milk has a unique taste and flavor. The components of coconut milk are fat, water, carbohydrate, protein and ash with the major components are water and fat[9]. The effect of coconut milk addition on cooked rice digestibility has not been reported.

In this study, nine Indonesian rice varieties were selected based on their amylose and resistant starch content. Then, the selected rice varieties were cooked with or without coconut milk treated with reheating and recooling. This study was aimed to investigate the effect of reheating-recooling and addition of coconut milk on the digestibility of Indonesian cooked rice.

Material and Methods

Materials: The rice samples were collected from a local market in Jogjakarta, Indonesia. The rice varieties that collected were Pandan wangi, Rojo lele, C4 raja, Setra ramos, Mentik wangi, Mentik susu, Genjahr ante, Jasmin and Inpari 23 Bantul. Three different form of lipid were used in this study i.e. coconut milk, coconut oil, and fatty acid mixtures as a model. The fatty acids mixtures contain 65% (w/w) lauric acid, 18% (w/w) caprylic acid and 17% (w/w) myristic acid. Coconut milk and coconut oil were purchased from a local market in Jogjakarta, Indonesia.

Preparation of cooked rice samples: A 100 g raw rice was cooked with a 1.6 fold addition (w/v) of water using home-style rice cooker (Maspion MRJ-108, power output 350 W, capacity 1 L; Maspion Co. Ltd., Indonesia) until automatic shutoff (about 20 min). The cooked rice was kept at room temperature for 15 min before cooling treatment. After that, the cooked rice sample was cooled in refrigerator (4˚C) for 12 h and reheated using microwave oven (Sharp R-240F, power output 800 W, Sharp Co. Ltd., Indonesia) for 90 sec. Freshly cooked rice was prepared without cooling and reheating treatment. The same procedure was done for cooked rice samples with addition of coconut milk or oil. The addition of coconut milk was 50 ml while the addition of the coconut oil was 2% of the volume of coconut milk.

Preparation of rice flour: Rice flour was prepared according to Yu et al.[10] with modification. Each of rice variety was milled and passed through 100 mesh sieve. The rice flour that obtained then stored in plastic bag and kept at 4˚C in a freezer before processed.

Preparation of rice starch: Rice starch was prepared according to Sodhi and Singh[11] with modification. About 20 g of rice flours were soaked in 200 ml 0.2% NaOH solution for 3 h and steeped at 20˚C overnight. The steep liquor was drained off and the slurry was then diluted to the original volume with 0.2% NaOH solution. The process was repeated four times until the supernatant become clear and gives a negative reaction to the Biuret test. The slurry was centrifuged at 3500 rpm and then the starch that obtained was dried in cabinet drier at 50˚C overnight. The starch was passed through 100 mesh sieve and stored in plastic bag at -4˚C in a freezer until being processed.

Preparation of rice starch or rice flour model with fatty acids mixture addition[12]: Five g of rice starch or rice flour was dissolved in 250 ml DMSO and heated to 90˚C for 30 min. The resulting clear DMSO solution was then added with a mixture of fatty acids and continuously heated for 60 min with vigorous stirring. Then, the mixture was cooled to 25˚C and centrifuged (3525 rpm, 20 min). The resulted precipitates were washed with 50% ethanol solution. The washing step was repeated three times. Then, the ethanol was evaporated and the pellets were resulted.

Amylose content analyses: The amylose content was analyzed according to AOAC method[13].

Resistant starch content analyses[14]: About 100 mg of ground samples were incubated with 0.2 ml of pepsin solution (1 g pepsin / 10 ml buffer KCl-HCl pH 1.5 (Sigma No. P7012). The first incubation was done at 40˚C for 60 min with constant shaking. Then, 1 ml of the α-amylase solution (40 mg α-amylase / 1 ml Tris-maleate buffer pH 6.9) (Sigma No. A-3176) was added to the solution. The second incubation was done at 37˚C for 16 h with constant shaking. The hydrolysates that obtained then centrifuged at 3500 rpm for 15 min and the residues from centrifugation were washed with 10 ml of distilled water. The centrifugation was repeated two times. Then, 3 ml distilled water was added to the residue. Three ml of 4 M KOH was added and kept for 30 min at room temperature. After 30 min, 5.5 ml of 2 M HCl and 3 ml of 0.4 M sodium acetate buffer pH 4.75 were added. Then, 80 µl of amyloglucosidase (Sigma No. A9913) was added and incubated at 60˚C for 45 min with constant shaking. After that, the centrifugation was done at 3500 rpm for 15 min and the residue was washed with 10 ml of distilled water. The centrifugation was repeated two times and the supernatant was combined with that obtained previously. The final volume was made up 25-1000 ml depending on resistant starch content. The glucose content was measured using a glucose oxidase-peroxidase kit. The resistant starch content was calculated as mg glucose × 0.9.

In vitro starch digestion rate, HI and predicted GI determination[15]: A 50 mg of ground food samples were incubated with 10 ml buffer KCl-HCl pH 1.5 and homogenized for 2 min. Pepsin solution (0,2 ml) (Sigma No. P7012) was added and incubated at 40˚C for 60 min. Then, the volume was made up to 25 ml with a Tris-maleate buffer solution pH 6.9. Then, 5 ml of Tris-maleate buffer solution containing 2.6 IU α-amylase (Sigma No. A3176) was added and incubated at 37˚C. The aliquot samples (0.1 ml) were taken from each tube every 30 min from 0 to 180 min and placed in a tube at 100˚C. Then, 1 ml of sodium acetate buffer solution pH 4.75 was added. After that, 30 µl of amyloglucosidase (Sigma No. A9913) was added at 60˚C for 45 min. The glucose content was measured using glucose oxidase-peroxidase kit. The digestible starch was calculated as mg glucose × 0.9. The in vitro starch digestion rate was expressed as the percentage of total starch hydrolysed at different times. The area under curve (AUC) was calculated using the first-order equation by Goniet al[15].

$$AUC = C\infty(t_f - t_o) - (C\infty / k)[1 - \exp[-k(t_f - t_o)]]$$

Where $C\infty$ corresponds t_o the concentration at equilibrium (t_{180}), t_f is the final time (180 min), t_o is the initial time (0 min) and the $k$is the kinetic constant. A hydrolysis index (HI) was calculated by comparison with the AUC of a reference food (fresh white bread). The predicted glycemic index (GI) was estimated by the equation

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

Statistical analyses: The data were subjected to analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($p < 0.05$) using SPSS software version 15.0 (SPSS Inc., Chicago, IL). Values expressed were means ± SD.

Results and Discussion

Amylose and resistant starch content of nine rice varieties: The amylose content of nine rice varieties ranged from 15.22 to 23.69% (table-1). Mentik susu had the lowest amylose content (15.22%), whereas Setra ramos had the highest amylose content (23.69%). The same trends were also observed in their resistant starch content. The resistant starch content was increased with the increasing levels of amylose. Setra ramos had the highest resistant starch content (21.13%), whereas Mentik susu showed lowest resistant starch content (10.39%). This result was consistent with previous studies on rice. Zhu et al.[3] observed that amylose content was positively correlated with their resistant starch content. Eerlingen and Delcour[16]reported that increasing of resistant starch content might be due to re-association of amylose chains in the double helices form that resists to the hydrolytic enzymes. Based on the amylose and resistant starch content, Setra ramos was selected for further study.

Table-1
Amylose content and resistant starch content from different rice varieties

Rice variety	Amylose content (%)	Resistant starch content (%)
Mentik susu	15.22 ± 0.64^{a}	10.39 ± 0.90^{a}
Inpari 23 Bantul	18.91 ± 0.23^{b}	11.92 ± 0.46^{b}
Jasmin	19.72 ± 0.66^{bc}	15.35 ± 0.96^{c}
Mentik wangi	20.14 ± 0.46^{c}	19.77 ± 0.72^{e}
Rojo lele	20.58 ± 0.50^{c}	17.02 ± 0.40^{d}
Genjah rante	22.07 ± 0.48^{d}	19.46 ± 0.43^{e}
Pandan wangi	22.43 ± 0.29^{d}	17.90 ± 0.45^{d}
C4 raja	22.62 ± 0.68^{d}	20.02 ± 0.48^{e}
Setra ramos	23.69 ± 0.56^{e}	21.13 ± 0.45^{f}

Means not sharing a common letter in a column are significantly different at $p < 0.05$

Cooked rice digestibility treated with cooling-reheating process and addition of coconut milk: Freshly cooked rice gave the lowest resistant starch content (1.25%) among the samples (table-2). The obtained results agreed with the previously studies. For example, Marsono and Topping[6] investigated the resistant starch content of Australian cooked rice and reported that the resistant starch content of freshly-rice cooker cooked was lower than refrigerated-rice cooker cooked and frozen-rice cooker cooked. On the other hand, our resistant starch content was much lower than previous study on freshly cooked rice. Patindol et al.[17] and Vatanasu chart et al.[18] reported that resistant starch content of freshly cooked rice was 4.3% and 7.1%, respectively. This discrepancy could be due to difference in sample rice varieties.

As can be seen in table-2, cooling and reheating processes gave the higher resistant starch content than freshly cooked rice. This result indicated that the higher resistant starch content was due to retro gradation at 4˚C during cooling and reheating process. These findings agreed with Frei et al.[19] who reported a higher resistant starch content due to the rice cooked samples that stored at 4˚C for 24 h. Furthermore, Park et al.[8] reported that the cycled temperature conditions at 4 and 30˚C gave a higher of resistant starch content. The resistant starch content was related to the intensity of crystalline form in retrograded starch gel, as reported by Chung et al.[20].

In contrast, cooling and reheating process did not increase resistant starch content of cooked rice with added coconut milk or coconut oil compared to freshly cooked rice with coconut milk or coconut oil addition (Table-2). This result indicated that cooling and reheating process were more effective to increase resistant starch content in cooked rice although the resistant starch content of cooked rice samples with coconut milk addition was still higher than other cooked rice samples. The increasing of resistant starch content due to retro gradation during cooling and reheating process might be correlated with the stability of ordered structure of crystalline amylose. Kawai et al.[21] stated that crystalline amylose has double helical order and affects the resistant starch content. In addition, crystalline amylose was more stable than amylose-lipid complex. On the other hand, Palav and Seetharaman[22]stated that formation of film polymer coating the granule surface of starch during microwave heating due to vibrational motion of the water molecules and rapid heating rate of microwave reduced enzyme susceptibility on starch. Furthermore, Fan et al.[23] reported that the molecular vibration due to rapid heating rate of microwave slightly influences the lamellar structure of rice starch.

Compared to all cooked rice samples, all rice starch or rice flour models showed higher resistant starch content (table-2). Resistant starch of the rice starch or rice flour models ranged from 21.13 to 27.02%. The highest resistant starch content (27.02%) was found in rice starch with fatty acids added. This result might be due to the complex formation of rice starch and fatty acids mixture. Study done by Zhou et al.[24] showed that the addition of stearic acid on rice starch significantly changed the properties of rice starch compared to linoleic acid.

Table-2
Resistant starch content of cooked rice samples and rice starch or rice flour models

Sample	Treatment	Resistant starch content (%)	k	Calculated HI	Predicted GI
Cooked rice	Freshly	1.25 ± 0.28[a]	0.016	69.18	77.69
	Reheated 1×	3.42 ± 0.25[c]	0.015	53.03	68.82
	Reheated 2×	4.40 ± 0.34[d]	0.015	51.28	67.86
Cooked rice with coconut milk	Freshly	5.35 ± 0.06[e]	0.014	40.03	61.69
	Reheated 1×	5.61 ± 0.10[e]	0.014	36.80	59.91
	Reheated 2×	5.64 ± 0.14[e]	0.013	31.14	56.80
Cooked rice with coconut oil	Freshly	1.61 ± 0.20[ab]	0.016	68.59	77.37
	Reheated 1×	1.90 ± 0.09[b]	0.015	57.06	71.03
	Reheated 2×	2.02 ± 0.02[b]	0.015	59.03	72.12
Rice starch with fatty acids	-	27.02 ± 0.65[h]	0.013	25.23	53.56
Rice flour with fatty acids	-	25.14 ± 0.12[g]	0.013	27.78	54.96
Rice starch	-	25.46 ± 0.52[g]	0.014	45.28	64.57
Rice flour	-	21.13 ± 0.45[f]	0.015	42.07	62.80

Means not sharing a common letter in a column are significantly different at $p < 0.05$

The in vitro starch hydrolysis rate of cooked rice samples at different time intervals were significantly higher than all rice starch or rice flour models (figure-1). The starch hydrolysis rate of cooked rice with added coconut milk was lower than cooked rice or cooked rice with coconut oil added. This result confirmed with their resistant starch content in Table-2 and indicated that the addition of coconut milk caused a decreased of the starch hydrolysis rate of cooked rice.

As can be seen in table-2, the reduction of predicted GI was observed in cooked rice with or without coconut milk addition treated with cooling and reheating process. Compare to cooked rice samples, addition of the coconut milk had the lowest HI and predicted GI. The lowest HI (31.14) and predicted GI (56.80) among the cooked rice samples was observed for the cooked rice sample with coconut milk addition treated with cooling and reheating two times. On the other hand, the highest HI (69.18) and predicted GI (77.69) were found in freshly cooked rice.Previous studies on the in vitro digestibilityof rice starch stated that predicted GI of freshly cooked rice samples ranged between 109 for the waxy cultivar and 68 for the high amylose cultivar[19]. In addition, storing the cooked samples at 4˚C for 24 h gave a reduction of HI and predicted GI for all rice varieties.

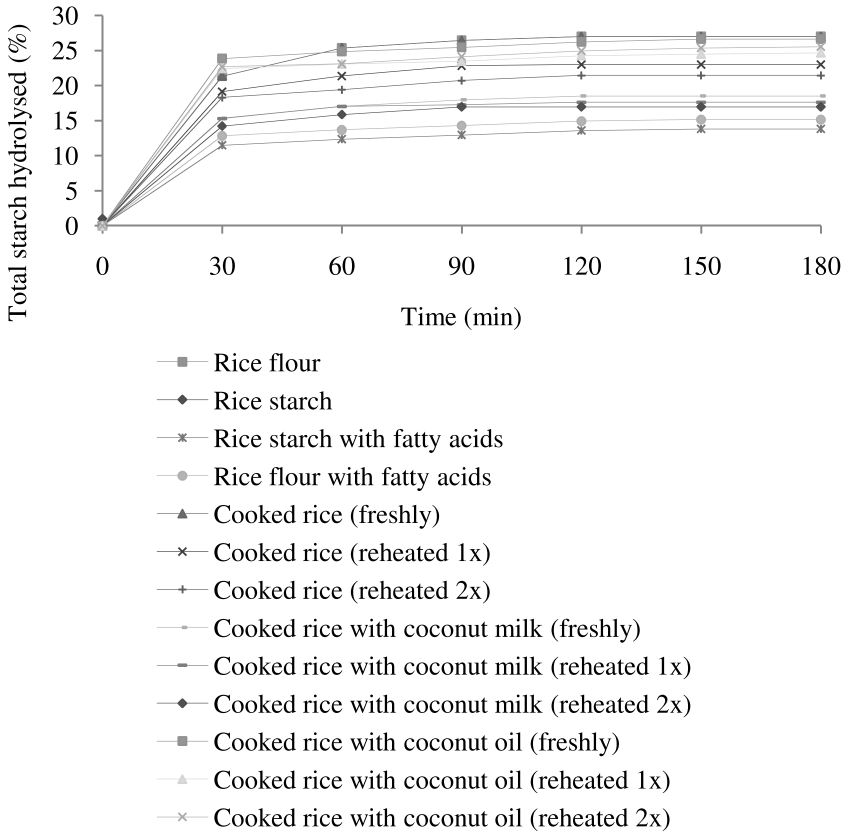
The rice starch with added fatty acids had the lowest HI (25.23) and predicted GI (53.56) among all samples due to resistance of amylose-lipid complex to the enzymatic hydrolysis (Table-2). These findings aggred with those obtained previously. Study done by Kawai et al.[21] showed that addition of different lipids to the starch decreased enzymatic-hydrolysis rates of starch and the highest of percentage of hydrolysis of starch with the addition of lipids was stearic acid, followed by palmitic acid, corn oil, oleic acid, soy lecithin, and lauric acid. On the other hand, Ai et al.[25] reported that the significant effect of fatty acids addition on the starch content hydrolyzed were found in lauric acid and oleic acid.

Conclusion

Cooling-reheating process and addition of coconut milk in rice cooking showed different effects on the digestibility rate of cooked rice. Cooling and reheating two times gave a higher resistant starch content of cooked rice. Adding coconut milk during rice cooking enhanced their resistant starch content and decreased the HI and predicted GI.

Abbreviation

AOAC, Association of Official Analytical Chemists.



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