Journal of Food and Nutrition Research, 2019, Vol. 7, No. 3, 231-236
Available online at http://pubs.sciepub.com/jfnr/7/3/8
Published by Science and Education Publishing
DOI:10.12691/jfnr-7-3-8

Effect of Preparation Method on Chemical Property of Different Thai Rice Variety

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Received January 15, 2019; Revised February 20, 2019; Accepted March 19, 2019

Abstract Improving benefits and reducing risk of staple food consumption are of interest among researchers nowadays. Rice is the major staple foods consumed in Asia. It has been reported that rice consumption has a positive association with the risk of chronic diseases. The effects of rice variety and preparation process on chemical characteristics of rice were investigated in the current study. Three Thai rice varieties, Khao Dok Mali 105 (KDML 105), Sao Hai (SH) and Riceberry (RB), underwent parboiling or non-parboiling as well as polishing or non-polishing prior to chemical property analysis. It was found that parboiling process possessed greater content of mineral as indicated by ash content as well as fiber and total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity when compared to non-parboiling treatments, whereas the reduction in amylose and TAC content, GI value and starch digestibility were observed in this sample. On the other hand, polishing process led to reduction in ash, amylose, fiber, TPC and TAC content and DPPH values. GI value and starch digestibility of non-parboiled rice were lowest in SH, RB and KDML 105, respectively. However, with parboiling treatment, GI values among rice varieties were not significantly different. It can be concluded that natural composition of each rice variety is different and it affects human health. However, preparation process can alter the chemical property of rice as well as influence health benefit of rice.

Keywords: *gac, glycemic index, parboil, rice*

Cite This Article: Cahyuning Isnaini, Pattavara Pathomrungsiyounggul, and Nattaya Konsue, "Effect of Preparation Method on Chemical Property of Different Thai Rice Variety." *Journal of Food and Nutrition Research*, vol. 7, no. 3 (2019): 231-236. doi: 10.12691/jfnr-7-3-8.

1. Introduction

Rice is the staple food that consumed more than half of population in the world. For this reason, the balance of benefit-risk of rice consumption is imperative. It has been reported that rice consumption exhibits a positive association with the risk of many chronic diseases such as type 2 diabetes and coronary heart disease [1]. Glutinous rice usually has high glycemic index (GI) implying that it causes rapidly increase in blood sugar level hence high in diabetes risk. High amylose content rice, on the other hand, has low GI value due to the formation of complexes between amylose and lipids during cooking and, therefore, less accessible to enzymatic digestion which results in a slower rate of digestion [2]

Thai rice is in the second rank of global rice exports. Among Thai rice, Khao Dok Mali 105 (KDML 105) is mostly consumed in Thailand because of the flavor and aroma [3]. The term Khao Dok Mali is given because of the white color and good aroma of this rice similar to jasmine flower. KDML105 is a non-glutinous rice and amylose content is around 12-17% [4]. Riceberry (RB) rice is low amylose pigmented rice. It is a new variety that cross-bred between varieties of low amylose content and intensive purple color. After being introduced to the market, it has gained popularity due to its unique cooking qualities and fragrance of whole grain with distinctive color and delicious aftertaste while the high antioxidant content was also established. Sao Hai (SH) is high amylose white rice that is good for health. The rice texture is hard and crumbly, good for serving with curry or soup. SH is relatively high amylose rice compared to KDML and RB with 21.8% amylose [5]. Despite its high amylose content, it has less popularity in Thailand compare to low amylose rice.

Parboiling process is involved the partial boiling of rice paddy following with drying and milling [6]. During boiling, the gelatinization occurs only in the outer layers of aleurone part and seals the grain hence the head rice yield improved. Normally, the GI of parboiled rice is lower than normal rice whereas bioactive compounds increased [7]. On the other hand, it can give adverse effects such as reducing stickiness and enhancing brown color and rancidity in rice.

Brown rice consists of bran layer which is rich in dietary fiber, vitamins, minerals, and other health-related components when compared to the white rice [8]. The bran layer is a major source of phenolic compound in the rice grain, polishing removes this part making the grain

less healthy [9]. The vast majority of research has focused on chronic disease prevention and control, whereby brown rice and rice bran have been shown to decrease risk of type 2 diabetes, regulate lipid metabolism, control metabolic syndrome and cardiovascular disease, and exhibit anti-cancer [10-12]. The objective of this research was to compare the effects of different rice processing namely parboiling and polishing on chemical properties of three major rice varieties in Thailand.

2. Materials and Methods

2.1. Sample Preparation

KDML 105, SH and RB paddy were obtained from Phrae Rice Research Center, Thailand. Paddy samples were grown in the same crop in the same year. Each variety was prepared with or without polishing and parboiling. Preparation of parboiled rice was carried out by soaking the paddy in water at 60°C for 1 h. The soaked paddy was steamed at 15 Psi for 10 min. Steamed paddy was tempered for 24 h prior to drying at 45°C for 24 h (until moisture content was below 14%) using hot air oven. The dried paddy was stored for 1 week prior to the milling process was conducted for 7-10 cycles (brown rice) following with polishing (white rice) using milling and polishing machine (Mitsubishi Electric type SC-KR).

2.2. Moisture Content Analysis

Moisture content (MC) analysis was conducted according to AOAC (2000) [13]. Sample (2-3 g) was put in pre-dried moisture can and placed in a hot air oven at 105±2°C for 16-18 h. Moisture content and dry matter were calculated.

2.3. Ash Content Analysis

The sample (2 g) was added to well-incinerated crucibles, burned in the hot plate for 30 min and followed with ashed in a muffle furnace at 550°C for 6 h [13].

2.4. Total Dietary Fiber Content (TDF) Analysis

Water- and fat-free samples (1 g) was added with 20 mL sodium phosphate buffer pH 6.0 and mixed. The mixture was included with 0.1 mL enzyme alpha-amylase (Thermamyl 120L, Sigma Aldrich) prior to incubation for 15 min in a shaking water bath at 100°C. HCl was added to reach pH 1.5. To the mixture, 1 mL pepsin was included, and continued incubation in a shaking water bath at 40°C for 60 min, then the pH was adjusted to 6.8 with NaOH. Thereafter, 100 mg pancreatin was added and re-incubated 40°C for 60 min. The pH was adjusted to 4.5 with HCl and filtered with Whatman paper number 4. The filtrate was washed twice with 10 mL distilled water, 10 mL ethanol 90% and 10 mL acetone, respectively and then dried at 105°C until constant weight (overnight) and the initial weight was recorded. Sample with Whatman paper was heated on a hot plate and put in a muffle furnace at 525°C for 6 h and weighed. The filtrate was used to analyze soluble dietary fiber. The distilled water was added to the filtrate until the volume reached 100 mL, then added 400 mL of warm 95% ethanol (60°C) and allowed precipitation for one night. This solution was filtered using Whatman paper number 4 and washed twice with 10 mL distilled water, 10 mL 90% ethanol and 10 mL acetone, respectively. Then the residue in Whatman paper was dried at 105°C until constant weight (overnight) and record the initial weight. Sample with Whatman paper was heated on a hot plate and put in a muffle furnace at 525°C for approximately 6 h and weighed. Finally, TDF was calculated from the summation of insoluble and soluble dietary fiber content [13].

2.5. *In vitro* Starch Digestibility Analysis

In vitro starch digestibility analysis was conducted according to Englyst et al. (1992) [14] with some modifications. Rice flour suspension (1% wt/vol in distilled water) was heated in a water bath for 30 min until it reached 90°C and then cooled. The suspension (2 mL) was added with 3 mL of distilled water and 5 ml of 0.1 M Na-phosphate buffer pH 7.0. The mixture was incubated in a water bath at 37°C for 15 min and 5 mL of alpha-amylase solution was added and re-incubated at 37°C for 15 min. One mL of the mixture was added with 2 mL dinitrosalicylic acid reagent, and the mixture was heated in a water bath at 100°C for 10 min. After cooling, the mixture was diluted with 10 mL of distilled water. Absorbance was measured using a spectrophotometer with the wavelength of 520 nm. Maltose levels of the reaction mixture were calculated using a standard curve of pure maltose. The digestibility of starch samples was calculated as a percentage relative to pure starch as follows:

$$\text{Digestibility}=\frac{\begin{pmatrix}\text{Maltose levels of sample}\\ \text{after the enzyme reaction}\end{pmatrix}\text{x}100}{\begin{pmatrix}\text{Maltose levels of pure starch}\\ \text{after the enzyme reaction}\end{pmatrix}}.$$

2.6. Amylose Content Analysis

A modified method was applied to analyze the amylose content [2]. Rice powder, 100 mg, was mixed with 1 mL of 95% ethanol and 9 mL of 1 M NaOH prior to incubation for 24 h at room temperature. Distilled water was added to reach the final volume of 100 mL. One mL of 1 M acetic acid and 2 mL iodine solution were added to 5 mL of sample. Distilled water was added to make up the final volume of 100 mL. The mixture was stirred and allowed to stand for 20 min before absorbance was measured at 620 nm utilizing UV-Spectrophotometer. The amylose content of the rice samples was calculated using a standard curve plotted from the absorbance of amylose standards.

2.7. Glycemic Index Analysis

Glycemic index (GI) analysis was conducted according to method described by [15]. Cooked rice sample was prepared and 50 mg of sample was added to the test tube. Pepsin (1 g) was added to 100 mL HCl-KCl (pH 1.5) buffer and 0.2 mL of the mixture was included to the test tube. The test tube was incubated at 40°C for 1 h in a shaking water bath. Tris-maleate buffer containing 2.6 UI

α-amylase (5 mL) was added to sample. The samples were continued to incubation at 37°C in a shaking water bath. Aliquot sample (1 mL) was taken every 30 min (from 0 to 3 h) and then placed in shaking water bath at 100°C for 5 min to inactivate the enzyme and refrigerated until the end of the incubation time. 3 mL of 0.4 M sodium acetate buffer pH 4.75 was added to each sample following with 60 µL of amyloglucosidase. The sample was incubated in the shaking water bath for 45 min at 60°C. Distilled water was added to reach 100 mL. The aliquot was taken (0.5 mL) and incubated with 3 mL of glucose GOD-POD (Glucose oxidase and Peroxidase) kit. Read absorbance using UV/VIS spectrophotometer at the wavelength of 505 nm. Percentage of glucose at 0, 30, 60, 90, 120, 150, and 180 min was counted as glucose digestion rate. The curve among the time interval was established and the area below the curve was calculated as area of hydrolysis curve (AHC). The AHC of sample and standard of white bread was calculated as the hydrolysis index (HI). Finally, the GI was calculated according to the equation of GI = 39.71 + (0.549 * HI).

2.8. Total Phenolic Content Analysis

Total phenolic content (TPC) of extracts was determined by Folin Ciocalteu method as described previously [16] with some modifications. Samples were extracted according to Walter (2013) [16] by adding 3 mL methanol to 1 g sample and placed in shaking water bath 60°C for 20 min. After centrifugation at 10,000 *x*g for 10 min, the supernatant was kept in freezer until analysis. Briefly, 100 µL of extract was mixed with 750 µL of fresh Folin-Ciocalteu reagent and incubated at room temperature for 5 min. After that, 750 µL of 6% (w/v) sodium carbonate was added to the mixtures and allowed to completely react for 60 min in the dark. The absorbance was read by spectrophotometer at 756 nm. The phenolic content was expressed as mg of gallic acid equivalent per g dry sample (mg gallic acid equivalent (GAE)/g)..

2.9. DPPH Radical Scavenging Analysis

The DPPH assay was conducted using Trolox as standard. The sample was extracted as described above. The extract (50 µL) was mixed with 1.95 mL 60 µM DPPH solution and left in the dark place for 30 min at room temperature. Absorbance was recorded at 517 nm using methanol as blank. The radical scavenging activity was expressed as mmol Trolox equivalent (TE)/g dry weight sample [17].

2.10. Anthocyanin Content Analysis

One mL of extract was added with potassium chloride buffer, pH 1 or sodium acetate buffer pH 4.5 to reach 10 mL. The mixture was vortexed and absorbance was read at 510 nm and 700 nm, respectively [18]. The calculation is shown below:

$$\text{Anthocyanin } (\text{mg/g}) = A \times MW \times DF \times 1000/26{,}900$$

A = (A510 – A700)pH1 - (A510-A700)pH4.5
MW = Molecular weight of anthocyanin (449.2)
DF= Dilution factor

2.11. Statistical Analysis

All the data were expressed as mean values. The standard deviations were analyzed and analysis of variance using SPSS for Window version 16 was carried out. Duncan's multiple range test was used to identify the difference among means at $p<0.05$.

3. Results and Discussion

3.1. Effect on Starch Properties

Moisture content of all samples was not significantly different and was on its safe range of dry grain (≤14%) [19] (Table 1). Ash content was higher in parboiled samples in all rice varieties. This could be due to parboiling process allows the nutrient in the rice husk to trap in the rice grain surface and pores. The result was in agreement with previous study [20] that parboiling process enhanced ash content of long grain rice up to 18%. According to Chukwu and Oseh (2009) [21] parboiling process led to superior amount of iron, zinc, and calcium compared to control sample. Moreover, non-polished or brown rice had higher ash content than white rice due to high mineral content in its bran layer. Satter (2014) [22] also found that bran is a rich vitamin and mineral source such as phosphorus, potassium, iron, copper, zinc and vitamin B. Accordingly, Sangeetha (2017) [23] reported that polishing process led to a decrease of ash content more than 40%. Among the variety, SH had the highest ash content whereas that in KDML 105 was the lowest. This could happen due to different mineral content in each rice variety.

It is apparent that amylose content of the rice grain was lower in the parboiled polished rice in all varieties. Parboiling process reduces amylose content as has been reported by Yenrina (2017) [24]. During parboiling, swelling of the rice grain occurs as a result of diffusion of water into the porous of rice grain at certain temperature and the starch becomes gelatinized, hydrogen bonds between amylose and amylopectin are disrupted [24]. Amylose content reduced because it has short chain and easy to be dissolved and leached out in water [25,26]. Moreover, it is believed that the lower in amylose content is because of alteration of its structure conformation [24] and bindings of amylose with lipid during parboiling process which creates resistant starch (RS) type 5 [27]. For these reasons, GI value of parboiled rice was still lower than non-parboiled rice even though the amylose content was lower (Table 1). Concerning the effect of polishing on amylose content it has also been reported that polishing led to reduction of the amylose content due to the elimination of the rice bran [28]. Among rice varieties, SH showed the highest amylose content as expected since it is a high amylose rice compared to other varieties inferring that without any treatment SH is the healthiest rice variety. It is noteworthy that parboiling process is successively reduced GI value of the low amylose rice varieties, KDML 105 and RB.

Table 1. Starch properties of rice samples treated by different preparation process

Rice	Parboiling Process	Polishing Process	Moisture Content (%)	Ash Content (%)	Amylose Content (%)	Total Dietary Fiber (%)	Glycemic Index (%)	Starch Digestibility (%)
KDML	No	White	13.06±0.03^{a}	0.61±0.03^{h}	13.67±1.13^{cde}	4.24±1.37^{d}	72.51±4.22^{a}	78.89±0.67^{a}
KDML	No	Brown	13.22±0.08^{a}	1.57±0.03^{fg}	17.21±1.40^{c}	6.19±0.55^{bcd}	58.42±4.22^{b}	60.70±1.60^{cde}
KDML	Yes	White	12.11±0.04^{c}	1.95±0.03^{de}	12.39±0.57^{e}	7.43±0.00^{ab}	56.64±2.43^{bc}	73.89±6.13^{ab}
KDML	Yes	Brown	11.95±0.03^{c}	2.41±0.20^{b}	15.24±2.21^{cde}	8.17±1.03^{ab}	55.90±5.22^{bc}	55.98±2.40^{de}
RB	No	White	12.65±0.16^{b}	0.60±0.13^{h}	13.09±2.84^{e}	3.73±0.13^{d}	68.19±1.18^{a}	73.04±6.00^{abc}
RB	No	Brown	12.47±0.08^{b}	1.50±0.03^{g}	16.86±0.92^{cd}	8.40±0.35^{ab}	52.61±1.25^{bc}	52.12±5.20^{def}
RB	Yes	White	12.05±0.17^{c}	1.36±0.18^{g}	12.39±0.64^{e}	6.85±1.15^{abc}	54.82±1.85^{bc}	59.94±5.33^{de}
RB	Yes	Brown	11.94±0.05^{c}	2.26±0.01^{bc}	13.32±0.89^{de}	8.44±1.10^{ab}	51.72±0.99^{bc}	39.21±10.93^{f}
SH	No	White	11.18±0.00^{d}	0.58±0.09^{h}	24.36±0.69^{ab}	4.45±0.30^{cd}	58.73±2.24^{b}	64.94±0.93^{bcd}
SH	No	Brown	10.92±0.06^{e}	1.77±0.02^{de}	27.41±0.18^{a}	4.93±0.39^{cd}	57.69±0.76^{bc}	50.61±7.33^{ef}
SH	Yes	White	11.03±0.07^{de}	2.14±0.11^{cd}	22.52±1.00^{b}	7.70±2.34^{ab}	56.29±1.87^{bc}	49.95±4.53^{ef}
SH	Yes	Brown	11.06±0.04^{de}	2.80±0.06^{a}	23.30±2.78^{b}	9.06±1.11^{a}	55.06±1.07^{bc}	48.16±5.46^{ef}

Considering TDF value, it was found that non-polishing and parboiling process led to higher TDF values in all rice varieties (Table 1). The bran layer of rice is a rich source of dietary fiber and has been used as an additive in foods [29]. Furthermore, parboiling process alters structure conformation of starch and leads to a stronger resistant to enzymes digestion or becoming a dietary fiber [24]. Immigration of TDF from the husk, production of resistant starch type 3 and type 5 by gelatinization, retrogradation, and binding with other component are the mechanism of TDF induction by parboiling. Aside from the structure conformation, the parboiling process also increases TDF content by the fiber immigration from husk layer to the rice grain. The result indicated that pigmented rice has higher TDF than non-pigmented rice as previously reported by Shao (2014) [30]. RB had higher TDF than KDML 105 and SH in all processing. KDML 105 had the lowest TDF which led to higher starch digestibility and GI value. This means that KDML 105 is the easiest to be digested. Brown rice samples in all varieties had higher TDF than white rice according to the presence of bran layer (Table 1).

3.2. Effect on Glycemic Index (GI) and *in vitro* Starch Digestibility

Table 1 shows GI values of rice samples. It is important to note that the GI values are inversely proportional to amylose content [31]. Considering rice variety, GI of SH rice was lower than RB and KDML 105, respectively. It is believed to be due to the high amylose content of SH rice. The GI value of normal consumed rice has been reported and the value of KDML 105 was 70.3 and RB was 62 [32]. In current study, the low GI value was observed in parboiled rice. In case of the KDML 105 and RB varieties, polishing caused significant lower value of GI in the samples without parboiling process whereas that of parboiled samples, the GI values were not significant different and were lower than the non-parboiled samples. Furthermore, in SH rice, the preparation process did not significantly alter GI values. These findings indicate that preparation process plays important role in disease prevention.

In vitro starch digestibility indicates how much the starch content can be digested by □-amylase enzyme from saliva and pancreas gland. Similar to GI values, the starch digestibility was lower in parboiled samples. Moreover, brown parboiled rice had lower starch digestibility than white parboiled rice. This infers that parboiled brown rice is harder to digest or need longer time to digest [30]. In brown rice, this could happen due to increase of the dietary fiber content whereas parboiling causes change in the structure of starch, diffusion and adhesion of rice bran and husk component [33]. Retrogradation that occurs after parboiling led to changes in the amorphous state of starch granules to a crystalline state. This crystallized starch form can resist enzymatic degradation in the small intestine for up to three hours and spontaneously lower the concentration of digestible starch in cooked rice [34]. B-type starch has a structure of double helices with hexagonal unit cell which is harder to be digested compared to A-type starch that has monocyclic unit cell [35]. It has been reported that retrogradation occurred after parboiling led to construction of B-type starch granules [36]. KDML 105 was the most digestible rice variety compared to SH and RB while non-parboiled RB showed high percentage of starch digestibility. SH had low digestible and high in resistant starch due to the native resistant starch type 1 (RS1) and type 2 (RS2) in SH cultivar. The rate of digestion depends on granule size, the amylose-amylopectin ratio, starch protein interaction, amylose-lipid complexes, and level of resistant starch. Soaking and steaming during parboiling process resulted in dispersion of fiber in embryo and aleurone layer into the endosperm. This condition causes lowering of the starch digestibility as has been reported that fiber content in the rice led to the lower digestibility of the starch [24].

Table 2. Antioxidant content and antioxidant activity of rice samples treated with different preparation process

Rice	Parboiling Process	PolishingProcess	Anthocyanin (mg/g)	TPC (mg GAE/g)	DPPH (mmol TE/g)
KDML	No	White	ND	0.36 ± 0.01^{g}	2.32 ± 0.00^{g}
KDML	No	Brown	ND	0.99 ± 0.00^{cde}	2.49 ± 0.03^{cd}
KDML	Yes	White	ND	0.81 ± 0.22^{def}	2.44 ± 0.03^{de}
KDML	Yes	Brown	ND	1.31 ± 0.23^{bc}	2.55 ± 0.06^{bc}
RB	No	White	0.03 ± 0.02^{c}	0.43 ± 0.08^{fg}	2.25 ± 0.02^{h}
RB	No	Brown	0.24 ± 0.05^{b}	1.56 ± 0.5^{ab}	2.48 ± 0.02^{d}
RB	Yes	White	0.05 ± 0.02^{c}	1.32 ± 0.16^{bc}	2.41 ± 0.00^{ef}
RB	Yes	Brown	0.60 ± 0.03^{a}	1.93 ± 0.16^{a}	2.55 ± 0.04^{bc}
SH	No	White	ND	0.29 ± 0.05^{g}	2.37 ± 0.01^{fg}
SH	No	Brown	ND	1.04 ± 0.00^{cd}	2.68 ± 0.03^{a}
SH	Yes	White	ND	0.57 ± 0.07^{efg}	2.45 ± 0.02^{de}
SH	Yes	Brown	ND	1.58 ± 0.00^{ab}	2.61 ± 0.01^{b}

Note: ND = Not detectable.

3.3. Effect on Antioxidant Content and Activity

Table 2 shows that TAC in RB was higher in non-polished samples and the effect was more pronounce in parboiling treatment. This result confirms that rice bran is the source of anthocyanin in RB as has been reported previously [37]. Similarly, the highest TPC content was also observed in RB. Considering processing, the brown rice had superior content of TPC than white rice both in pigmented and non-pigmented rice. As found in the work reported by [38] that polishing reduced significantly the content of phenolic content. According to Shao (2014) [30], DPPH value and TPC content in rice were found at the highest level in the bran layer followed by embryo, whole grain, and endosperm, respectively. Non-parboiled and parboiled white SH had lower TPC than KDML 105 but those in brown SH with or without parboiling process were higher than KDML 105 in the same treatment. It was discernible that parboiling increased TPC content. This is because parboiling process enhances absorption of bioactive compounds from the husk, embryo, and bran layer to the rice grain [39]. Antioxidant activity determined using DPPH assay showed that DPPH values were similar in all treatments which ranged between 2.33-2.68 mmol TE/g. However, the highest antioxidant activity was found in non-parboiled brown SH and the lowest valued obtained in non-parboiled white RB. These results imply that the bioactive compound content in rice can be reduced from milling and parboiling process because the bran was erased during milling and also leaching out of the compounds occurred during the parboiling process. High DPPH value was found in parboiled brown process for each variety but the highest DPPH was found in non-parboiled brown SH rice. This finding is also similar with Muntana and Prasong (2010) [40] who found that the highest antioxidant activity was observed in non-pigmented Thai rice compared to pigmented rice.

4. Conclusion

Rice variety and processing influenced chemical characteristics of rice. Parboiled brown RB showed high ash content which represented high mineral content. This rice also showed the highest dietary fiber, antioxidants, anthocyanin and TPC content and the lowest starch digestibility which supported its low glycemic index. This infers that parboiled brown RB is advantageous to diabetic consumers as well as beneficial for reducing disease risk to all consumers.

Acknowledgements

Authors would like to thank Mae Fah Luang University for financial support and technical assistance.

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