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Total phenolic, antioxidant activity and physic-chemical properties of waxy pigmented and non-pigmented rice in Cambodia

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Abstract

This study determined the physicochemical properties, total phenolic content, and antioxidant activity of waxy pigmented and non-pigmented rice varieties in Cambodia. The results showed that, waxy pigmented rice has 2.1% for amylose, 8.31% for crude protein, 2.67% crude fat, 0.77% crude fiber, 0.59 % crude ash, 7 mm for length, 1.8 mm for width, 16.57 g weight/1000 grain, 2047 $\mu g\ g^{-1}$ for total phenolic content, 12.54% for DPPH-2,2-diphenyl-1-picrylhydrazyl (DPPH) and 0.138% Copper reduction antioxidant capacity (CUPRAC) was higher compared to waxy non-pigmented rice but the 86.69% of starch and 11.7% of moisture content was lower compared to waxy non-pigmented rice. The results of total phenolic content and antioxidant activity is one way of utilizing the local drought resistant crops for nutritious and safe products in the promotion of food security in the country.

1. Introduction

In the mid-1990s food security was recognized as a significant concern, spanning a spectrum from the individual to the global level. However, access now involved sufficient food, indicating continuing concern with protein-energy malnutrition. But the definition was broadened to incorporate food safety and also nutritional balance, reflecting concerns about food composition and minor nutrient requirements for an active and healthy life. At present, food security does not only mean enough quantity of food to feed the population but exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life.

Domneub is a sweet rice or glutinous rice variety in Cambodia which has two types; the white waxy non-pigmented rice (*Domneubsor* in Khmer) and black waxy pigmented rice (*Domneobkhmau* in Khmer). *Domneubsor* is used throughout the country for production of rice cake, dessert, tape (saccharified rice), among others, while the *Domneubkhmau* is used for traditional medicine processing by fermentation, fish fermentation, tapekhmau (black saccharified rice). However, there is very limited information about the benefits and composition properties in both rice.

Pigmented or colored rice is characterized by its grain with red brown or dark purple color covering layers. Pigments, which are located in the aleurone layer of rice grain, have been reported as a mixture of anthocyanin compounds, which belong to the family of flavonoids (Yawadio *et al*., 2007). The phenolic compounds have been found as a major active component for antioxidation (Iqbal *et al*., 2000; Zhang *et al*., 2006; Yawadio *et al*., 2007; Tabart *et al*., 2009). Antioxidative activity of pigmented rice has been reported by Zhang *et al*. (2006); Nam *et al*. (2006) and Chung and Shin (2007). However, there is no specific information on the total phenolic content and the antioxidant activity of *Domneubsor* and *Domneubkhmau* grown in Cambodia.

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Thus, this study evaluated the physicochemical properties, total phenolic content and antioxidant activity of the waxy pigmented and non-pigmented Cambodian rice varieties.

2. Materials and methods

2.1 Materials

Waxy pigmented and non-pigmented rice varieties were purchased from Cambodia and transported to the Philippines for properties composition analysis. Waxy pigmented rice was obtained from Trapeang Ta Moung Village, Prey Sleok Commune, Treang District, Takeo Province known as *angkordomneubkhmao* or black sticky rice. The waxy non-pigmented rice was collected from Teamchas Village, Kompongsvay Commune and District, Kompong Thom Province, Cambodia locally known as *angkordomneubsor* or white sticky rice.

2.2 Amylose content (AC)

The amylose content of the rice samples was determined by Iodine-Amylose colorimetric assays for apparent AC method following the procedure of Juliano *et al*. (2012). Waxy pigmented and non-pigmented milled rice flour (100mg) were wetted with 1.0mL of 95% ethanol and swirled carefully to disperse clumps. The ethanol-wetted flour was dispersed in 1N NaOH (9.0mL) in a 100mL volumetric flask and allowed to stand overnight. The mixture was made up to 100mL with distilled water, mixed and a 5mL aliquot (0.09N NaOH) was placed in a 100mL volumetric flask with ≈ 50mL of distilled water. Then, 1.0mL of 0.9N NH4Cl was added, followed by 2mL of 0.15% iodine in 1.5% KI, and the solution was made up to volume with distilled water to obtain a stable deep-blue color with the least amount of interference from amylopectin (waxy starch produces a greenish tinge). The absorbance of the color was read at 620 nm within 20-60 minutes and its stability and pH were measured. AC was calculated from standard curves based on potato amylose V (Avebe) alone (0, 5, 10, 15, 25 and 35 mg/100mL) of 0.09N NaOH.

Rice varieties were classified into five groups according to their amylose content: waxy (0.0-2%), Very low amylose (2.1–9.9%), low amylose (10.0–17.0%), intermediate amylose (17.1-21.9%) and high amylose (≥22.1%) (Rice Chemistry and Quality Laboratory, Philippine Rice Research Institute, University of the Philippines Los Banos 2014).

2.3 Physicochemical analysis

The physicochemical properties of both the raw materials (waxy pigmented and non-pigmented rice) inrice wine resulting from traditional and multi-parallel fermentation using the developed starter culture were analyzed. For the raw materials, one hundred grains of each rice variety were taken randomly and the length and width were measured by using a micrometer. Representative samples of waxy pigmented and non-pigmented rice varieties (50g) were drawn randomly and thousand grain weights was recorded in grams/1000 kernel by counting grains and weighing on an electric balance described by AACC (2000). Protein content was determined using a Kjeldahl digestion system based on the Association of Official Analytical Chemists (AOAC) method (2000). Crude fat was determined by extraction with hexane for 6h using a Soxhlet apparatus. Ash content was determined by burning in a muffle furnace at 550°C for 3h according to the AACC (2000). The moisture content in each sample was determined by drying 4g sample in an air forced draft oven maintained at a temperature of 105±5°C according to the procedure described in AACC (2000) method No. 44-15A. For the determination of fiber content, the rice samples were digested with 1.25% H_2SO_4 followed by 1.25% NaOH solution and crude fiber content was determined according to AACC (2000) method No. 32-10.

2.4 Determination of total phenolic content

The total phenolic content was determined by Folin-Ciocalteu method according to the procedure of Teresa Escribano-Bailón *et al*. (2002). The sample was diluted with water and to 0.5 mL of the diluted samples, 0.5 mL of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich) and 0.5% Na2CO3 were added. After standing for 5 minutes, 5 mL distilled water were added. The absorbance readings were measured at 720 nm (Shimadzu UV-1601 spectrophotometer) with water plus reagent as blank sample. Total phenolic content was computed in a standard curve with gallic acid as reference phenol. The results were expressed as gallic acid equivalents (GAE mL^{-1}).

2.5 Determination of antioxidant activity (DPPH radical scavenging method)

The antioxidant activity of polyphenols is measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Ribeiro *et al*. (2008) with some modifications with synthetic antioxidants butylatedhydroxy-anisole (BHA) and butylatedhydroxy-toluene (BHT) as reference antioxidants.

Five (5) mL of 0.1mM DPPH were pipetted in test tubes and added with 100 µL of the standards and sample. The mixture was vortexed and allowed to stand for 20 minutes. The control consisted of 100 µL methanol and the reagent served as control. The absorbance was read at 517 nm with water to zero the instrument. The percentage of inhibition was expressed using the following equation:

$$\% \, Inhibition = \left[\frac{A_{control} - A_{Sample}}{A_{Control}}\right] x \, 100$$

2.6 Copper reduction antioxidant capacity (CUPRAC) test

Copper reduction antioxidant capacity (CUPRAC) test is a variant of FRAP test using Cu instead of Fe. The assay is based on the reduction of Cu^{+2} to Cu^{+1} by the combined action of all antioxidants (reducing agents) in a sample. The Cu^{+1} then forms a yellow colored complex with neocuproine (2,9-dimethyl-1,10-phenanthroline) which absorbs at 450 nm. Therefore, the higher the absorbance at 450 nm, the higher the reduction of copper by the antioxidants, the higher the antioxidant capacity, the better the health beneficial quality of foods. One (1) mL of $CuCl_2$, 1.0mL of NH_4AC, 1.0 mL of neocuproine, 0.5 mL of antioxidant and 0.6 mL of water were pipetted in test tubes. The concentration of antioxidants was prepared using different concentrations of 5, 10, 15, 20 and 25 µg $GAEml^{-1}$. The mixture was vortexed and allowed to stand for 30 minutes and then read at 450 against a blank sample consisted of water and the reagents (Alpinar *et al*., 2009).

3. Results and discussion

3.1 Physicochemical properties of the waxy pigmented and non-pigmented rice

Short-grained varieties of rice are considered best for sake (Japanese wine) manufacture and long-grained varieties are considered desirable. Types of rice-long grain, medium grain and short grain are based on the length: width ratio of kernels of rice grains that are unbroken (Matz, 1991). Table 1 shows the comparison of the physical and chemical properties between the rice varieties (Domneupkmau and Domneupsor) used in the study for production of rice wine. Both varieties fall under long-grained types. Their length ranges from 6 to 7 mm while the width is from 1.5 to 1.8 mm.

The amylose content of rice is one of the most important criteria of rice quality in terms of cooking and pasting properties (Adu-Kwarteng *et al*., 2003). The rice varieties tested were observed, of which non-pigmented rice can be classified as the variety with "very low amylose" content than waxy pigmented rice. According to the classification of Rice Chemistry and Quality Laboratory, Philippine Rice Research Institute, University of the Philippines Los Banos, waxy rice (0.02-2.0%) with very low amylose content (2.1-9.9%) are sticky after cooking. Thus, waxy pigmented and non-pigmented rice samples are classified as varieties with "very low amylose" content and "waxy". In addition, amylopectin predominates in waxy rice whereas amylose is much more predominant in non-waxy rice. Amylose is more soluble in water than amylopectin and their ratio influences cooked rice properties.

Waxy non-pigmented rice has high moisture content (12.64%) compared to waxy pigmented rice (11.7%). In general, except for starch and moisture content, all mean values were found higher in waxy pigmented than non-pigmented rice variety. Protein influences the nutritional quality of rice. In this study the protein content was appreciably high (6-8%) for both tested varieties. According to Nunokawa (1972), most rice varieties for sake making contain 7.0 to 9.0% crude protein.

Table 1. Physical and chemical properties of the waxy pigmented and non-pigmented rice.

Constituents	Waxy	
	Pigmented rice	Non-pigmented rice
Starch (%)	86.69	92.66
Amylose (%)	2.1	0.4
Crude protein (%)	8.31	6.05
Crude fat (%)	2.67	0.64
Crude fiber (%)	0.77	0.15
Crude ash (%)	0.59	0.46
Moisture content (%)	11.70	12.64
Length (mm)	7	6
Width (mm)	1.8	1.5
Weight/1000grain	16.57	14.54
Rice type	Long-grain	Long-grain
Total phenolic content (µg g^{-1})	2074	134

3.2 Total phenolic content in waxy pigmented and non-pigmented rice

The total phenolic content of the waxy pigmented and non-pigmented rice samples expressed in milligram of gallic acid equivalent (GAE 100^{-g}) dry sample is presented in Table 1. The total polyphenol content of the different types of waxy rice varies significantly from each other. Results revealed that the waxy pigmented rice exhibited a significantly higher total polyphenol content of 2074 µg GAE g^{-1} compared to that of the waxy non-pigmented rice of 134 µg GAE g^{-1} db. Waxy pigmented rice

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had higher total phenolic content than waxy non-pigmented rice. The waxy pigmented rice had higher phenolic content than the red and black rice varieties with mean phenolic contents of 470.1 and 1055.7 mg/100g, respectively, as reported by Goffman and Bergman (2004) and Shen *et al*. (2009). Polyphenols are the most effective antioxidative constituents in plant products consumed (Escribano-BailÓn *et al*., 2004).

3.3 Antioxidant activity in waxy pigmented and non-pigmented rice

DPPH radical scavenging activity. DPPH is a free radical that accepts an electron or hydrogen radical to form a more stable compound. DPPH assay is based on the decrease in purple color of the DPPH solution when the nitrogen atom in DPPH is reduced upon receiving a hydrogen atom from an antioxidant. The DPPH free radical scavenging effect expressed as percent inhibition of waxy pigmented and non-pigmented rice at various concentrations are shown in Table 2. The percentage of inhibition of the DPPH radical by the extracts of the waxy pigmented rice was observed to increase with increasing concentration which exhibited the highest percentage inhibition of 75.37%. However, the percentage of inhibition of the DPPH radical by the extracts of the waxy non-pigmented rice decreased with increasing concentration which is 6.72% in total phenolic concentration of 1.7 µg GAE g^{-1}.

Table 2. DPPH radical scavenging activity in waxy pigmented and non-pigmented rice.

Total phenolic concentration (µg GAE g^{-1})	DPPH inhibition (%)	
	Pigmented Rice	Non-pigmented Rice
1.7	12.54	6.72
5	22.99	Nd
10	39.02	Nd
15	52.09	Nd
20	65.16	Nd
25	75.37	Nd

3.4 Copper reducing activity

Copper reduction antioxidant capacity (CUPRAC) test is a variant of FRAP test using Cu instead of Fe. The assay is based on the reduction of Cu^{+2} to Cu^{+1} by the combined action of all antioxidants (reducing agents) in a sample. The Cu^{+1} then forms a yellow colored complex with neocuproine (2,9-dimethyl-1,10-phenanthroline) which absorbs at 450 nm. Therefore, the higher the absorbance at 450 nm, the higher the reduction of copper by the antioxidants; the higher the antioxidant capacity, the better the health beneficial quality of foods.

Copper, free and in phenanthroline complexes, has a lower redox potential than iron, so its reactions are more selective; sugars and citric acid, common interference with Ferric Reducing Ability of Plasma (FARP), are not oxidized in CUPRAC. At the same time, the low redox potential enhances redox cycling, so copper reduction may be an even more sensitive indicator of potential pro-oxidant antioxidants. In the present study, the percentage of copper reducing activity of the waxy pigmented and non-pigmented rice extracts was observed to increase with increasing concentration of the total phenolic concentration (µg GAE g^{-1}) the highest percentage of 0.754% and 0.377%, respectively (Table 3). However, the percentage of copper reducing activity was already not detected in the extracts of the waxy non-pigmented rice at concentration of 20 µg GAE mg^{-1}.

Consumption of waxy pigmented rice resulted in reduced atherosclerotic lesions (Ling *et al*., 2001), reduced oxidative stress and inflammatory effects (Xia *et al*., 2003). Antioxidant activity in white rice hull had already been reported by Lee *et al*. (2003). Pigmented rice is composed of high content of phenolic compounds (Clifford, 2000; Oki *et al*., 2002). They are distributed in the plant as secondary structure metabolite (Matinez-Valverde *et al*., 2000). Various benefits of the phenolic compounds are known to have various effects in human including oxidative damage of lipid, low density lipoproteins inhibiting platelet aggregation (Daniel *et al*., 1999), and reduced coronary heart disease and cancer risk (Matinez-Valverde *et al*., 2000). Fruits and vegetables are major dietary sources of phenolic compounds (Tian *et al*., 2004), however, waxy pigmented and non-pigmented rice have been found as an excellent source of the phenolic compounds.

Table 3. The copper reducing activity of waxy pigmented and non-pigmented rice.

Total phenolic concentration (µg GAEmg^{-1})	Copper reducing activity (Absorbance)*	
	Waxy Pigmented Rice	Waxy Non-pigmented Rice
5	0.138	0.081
10	0.310	0.191
15	0.446	0.308
20	0.613	0.377
25	0.754	Nd

*Higher values indicate higher copper reducing activity

4. Conclusion

Waxy pigmented rice has 2.1% for amylose, 8.31% for crude protein, 2.67% crude fat, 0.77% crude fiber, 0.59% crude ash, 7 mm for length, 1.8 mm for width, 16.57 g weight/1000 grain, 2047 µg g^{-1} for total phenolic content, 12.54% for DPPH and 0.138% CUPRAC compared to waxy non-pigmented rice but the 86.69% of starch and 11.7% of moisture content was lower compared to waxy non-pigmented rice. So, the waxy pigmented rice has higher TP, DPPH and CUPRAC then waxy pigmented rice.

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