Red Yeast Rice Prepared from Thai Glutinous Rice and the Antioxidant Activities

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ABSTRACT

Red yeast rice which is a product of solid fermentation was prepared from several kinds of Thai glutinous rice (Oryza sativa L.) cv. Korkor 6 (RD6), Kam (Kam) and Sanpatong1 (SPT1). Monascus purpureus CMU001 isolated from available Chinese red yeast rice was used as the fermentation starter. The analysis for the presence of antioxidant activity in red yeast rice was carried out by studying antioxidant capacity and β-carotene bleaching method (BCB). For the study on antioxidant activities of each kind of red yeast rice prepared, the ethanol extract from ground red yeast rice was used. Red yeast rice prepared from Oryza sativa L. cv. RD6 with the addition of soy bean milk for 3 weeks of cultivation had the darker red color. The shapes and the texture of the red yeast rice grains of glutinous rice appeared to be better. The highest antioxidant activity was obtained in red yeast rice of 3 weeks old from SPT1 and RD6 with the addition of soybean milk. The antioxidant capacity and the IC_{50} of BCB methods were found to be 0.53 mg gallic acid equivalent (GAE) / ml and 0.09 mg/ml for both RD6 and SPT1 respectively. This result is concurrent to the production of darker red pigments.

Keywords: glutinous rice, antioxidant, monascus purpureus, red yeast rice.

1. INTRODUCTION

Red yeast rice, or more precisely, Angkak Rice is a Chinese product of rice fermented by using Monascus purpureus. Red yeast rice has been used in Chinese cuisine and medicinal food to promote blood circulation for centuries. In other Asian countries, red yeast rice is a dietary staple and is used to make rice wine, as a flavoring agent, and to preserve the flavor and color of fish and meat. The medicinal properties of red yeast rice are that it favorably impacts lipid profiles of hypercholesterolemic patients [1, 2], and it is used as a colorant in the food industry. Many secondary metabolites are produced in red yeast rice. Pigments such as yellow pigment (ankaflavin & monascin), orange pigment (monascorubrin & rubropunctatin) and red pigment (monascorubramine & rubropunctamine) are mainly present. Antihypercholesterolemic agent; monacolin K, hypertensive agent; gamma-aminobutyric acid.
(GABA) and antioxidants are also comprised [3, 4]. These compounds are very important for health and as the component of health foods or functional foods.

In Thailand 2 main kinds of rice are grown and consumed by most of the people in different area. They are non-glutinous rice and glutinous rice. The amylopectin content in glutinous rice is higher (95 %) than in non-glutinous rice (70-90 %). The latter contains about 10-30% of amylose. Some varieties of glutinous rice have a very small amount of amylose or contain no amylose [5]. The difference in their main composition may affect the content of useful compounds in fermented products and the properties of those products, especially with antioxidant activity.

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage may lead to cancer. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals may cause. Examples of antioxidants include carotenoids; β-carotene [6], phenolic compound; gallic acid [7], flavonoids; quercetin [8], alkaloids; capsaicin [9], hydroxytoluene BHT (synthetic antioxidant) and other substance [10, 11]. Therefore, the study on the antioxidant activity of red yeast rice is very relevant.

This work aims to prepare red yeast rice from glutinous rice which is abundantly found and consumed in everyday life among Northern Thai people using M. purpureus CMU001 [12] isolated from available commercial Chinese red yeast rice. The main purpose is to compare the antioxidant activity of red yeast rice prepared from glutinous rice by using two different methods, namely, antioxidant capacity and β-carotene bleaching method [13 - 16].

2. MATERIALS AND METHODS

2.1 Materials

Commercial Chinese red yeast rice, available in local traditional shops was used to isolate M. purpureus CMU001 strain [12]. Non-glutinous rice, Oryza sativa L. cv. Mali105 and glutinous rice; Oryza sativa L. cv. Kam (Kam), Oryza sativa L. cv. Korkor 6 (RD6) and Oryza sativa L. cv. Sanpatong1 (SPT1) which are abundantly available in the north of Thailand, were used to prepare red yeast rice. These rice samples were purchased from the same rice supplier and the same batch of processing was used and the rice was kept under the same conditions.

2.2 Preparation of Red Yeast Rice

Stepwise preparation of red yeast rice was carried out by firstly soaking each cultivar in water for 6 hours followed by steaming for 20 min. After cooling, 50 g of steam rice was put in 250 ml flask and was sterilized at 15 psi and 121 °C for 15 min. One week old precultured M. purpureus CMU001 was used for inoculation. The inoculated rice was incubated at 30 °C for 2 and 3 weeks. The end-product was dried in the oven at 65 °C for 6 hours to obtain dried red yeast rice. In case of non-glutinous rice (Mali105) which was used for comparison, the rice was cooked by steam-cooking. In order to study the effect of adding nitrogen containing nutrients, the red yeast rice samples with the addition of 1 ml of 0.25 g/ml soybean milk solution before cultivation were also prepared.

2.3 Preparation of the Extracts and Determination of Antioxidant Properties

2.3.1 Extracts Preparation

An extraction of the sample was carried out using 0.5 g of ground rice. It was put into a 20 ml centrifugal tube. 10 ml of 75% HPLC grade ethanol was added and it was degassed in an ultrasonic bath for 60 min. The
supernatant was collected after centrifugation at 3,000 rpm and 4 °C [17].

The extraction was repeated three times and all of the extracts were combined and made up to 50 ml using 75% ethanol.

2.3.2 Antioxidant Capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extracts and subsequent formation of a green phosphate/Mo (V) complex at acidic pH [18]. The extracts were combined with the reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 725 nm [19]. The total antioxidant capacity was expressed based on gallic acid equivalents.

Preparation of standard calibration curve for total antioxidant capacity was carried out using standard gallic acid. 0.2 mg/ml gallic acid solution was prepared and diluted to give the different concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg/ml). The tubes were incubated at 95 °C for 90 min. After cooling the absorbance was measured at 725 nm.

To determine the total antioxidant capacity of the extracts, the sample of 0.1 ml was placed in a test tube and made up to 1 ml with distilled water.

2.3.3 β-carotene Bleaching Method

Compared to Standard BHT (Butylated hydroxytoluene) Method

The antioxidant activity of red yeast rice was determined according to the method of Jayaprakasha et al. [20]. In short, 0.2 ml β-carotene emulsion (0.2 mg/ml in chloroform) was pipetted into a round-bottom flask containing 20 μl of linoleic acid and 200 μl of Tween-20. Chloroform was removed from the resulting mixture using nitrogen gas, immediately followed by the addition of 100 ml distilled water and finally mixed well. Aliquots (5 ml) of the emulsion were pipetted into different test tubes containing 0.2 ml of ethanol extracts at different concentrations. BHT(0.05 mg in 1 ml methanol) was used as a comparative standard. A control containing 0.2 ml of methanol and 5 ml of the above emulsion was also prepared. The tubes were placed at 50°C in a water bath. Absorbance was taken at initial time (t=0) at 470 nm using UV-VIS spectrophotometer. Measurement of absorbance was continued until the color of β-carotene disappeared in the control (t=120 min). A mixture prepared as above without β-carotene served as a blank [15, 16].

Inhibition of β-carotene bleaching activity of the samples were determined. The percent inhibition activity was calculated using the following equation. 

\[ \text{Inhibition percentage for β-carotene oxidation} = \left[ 1 - \frac{A_0 - A_t}{A_0} \right] \times 100 \] 

where $A_0$ and $A_t$ are the absorbance values measured at the initial time of incubation for samples and control, while $A_0'$ and $A_t'$ are the absorbance in the samples and control at t minutes. The relationship between percent inhibitions of β-carotene oxidation versus concentration of the samples were plotted to find the half-inhibition concentration (IC₅₀) values.

3. RESULTS AND DISCUSSION

3.1 Red Yeast Rice Prepared from Thai Glutinous Rice

Red yeast rice samples prepared from Thai glutinous rice with or without soybean milk as a nitrogen source were compared with commercial Chinese red yeast rice and red yeast rice prepared from non-glutinous rice, *Oryza sativa* L. cv. Mali105. Red yeast rice prepared from *Oryza sativa* L. cv. RD6 with the addition of soybean milk and cultivated for 3 weeks had darker red color. The shapes of red yeast rice grains made from glutinous rice, *Oryza sativa* L. cv. Kam, *Oryza sativa* L. cv.
cv. RD6 and *Oryza sativa* L. cv. SPT1 were distinctly different from *Oryza sativa* L. cv. Mali105. The texture was not hard, the odor was pleasant with a sweet odor and the color was dark red. In contrast, the rice made from non-glutinous rice, *Oryza sativa* L. cv. Mali105 contained broken bright red grains and was stuck together. The Results are shown in Figure 1.

**Figure 1.** Product of red yeast rice.

(a) Commercial Chinese red yeast rice (Comryr)
(b), (f), (j), (n) Mali105, Kam, RD6 and SPT1, respectively, without soybean milk at 2 weeks.
(c), (g), (k), (o) Mali105, Kam, RD6 and SPT1, respectively, with soybean milk at 2 weeks
(d), (h), (l), (p) Mali105, Kam, RD6 and SPT1, respectively, without soybean milk at 3 weeks
(e), (i), (m), (q) Mali105, Kam, RD6 and SPT1, respectively, with soybean milk at 3 weeks
3.2 Antioxidant Activity in Red Yeast Rice

3.2.1 Total Antioxidant Capacity

In order to compare between each red yeast rice sample prepared using different condition, the total antioxidant capacity is the selected method. The kinds of antioxidants in red yeast rice have rarely been reported therefore the specific method for the specific group of compounds cannot be used. Total antioxidant capacity may cover the activity of most of the antioxidants possibly present in red yeast rice such as flavonoids, polyphenols, carotenoids, alkaloids, vitamins, etc.

The total antioxidant capacity results of red yeast rice were determined by the same method as described previously. The total antioxidant capacity in red yeast rice reported in Table 1 showed higher amounts of antioxidant capacity when glutinous rice was used except purple glutinous rice (Kam) which gave the lower amount in spite of being glutinous rice. The addition of soybean milk increased the antioxidant activity. For comparison of the cultivation time in Table 1, 3 weeks of cultivation increased the total antioxidant capacity for glutinous rice while the decrease was found in the case of non-glutinous and purple glutinous rice.

Regarding the increase of the total antioxidant capacity, the period of growth can be used for explanation. In the first balanced phase or trophophase the rate of uptake and utilization of nutrients is maximal. Secondary metabolites or some antioxidants are rarely produced in this phase. When the nutrients are depleted and become limited, the growth rate slows and the storage phase begins. It is in this phase that the synthesis of secondary metabolites begins [21]. In the case of glutinous rice (RD6 and SPT1) and the addition of soybean milk, the growth may reach the second storage phase faster. When the cultivation time is longer the production of secondary metabolite or antioxidants may accumulate more. The decrease in the case of Mali105 and Kam when cultivation time was 3 weeks, may have been due to the degradation of the metabolites.

3.2.2 \( \beta \)-carotene Bleaching Method (BCB)

The production of free radicals in human caused by lipid peroxidation reaction is very interesting. The antioxidants that can reduce such free radicals can be determined by \( \beta \)-carotene bleaching method. In this method, unsaturated fatty acid is used to give lipid

<table>
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<th>Fermented Rice</th>
<th>Without soybean milk</th>
<th>With soybean milk</th>
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<tr>
<td></td>
<td>GAE (( \mu g/ml ))</td>
<td>GAE (( \mu g/ml ))</td>
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<td>----------------</td>
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<tr>
<td>2 weeks</td>
<td>2 weeks</td>
<td>3 weeks</td>
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<tr>
<td>Comryr'</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Mali105</td>
<td>0.17</td>
<td>0.26</td>
</tr>
<tr>
<td>Kam</td>
<td>0.15</td>
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<tr>
<td>RD6</td>
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<td>0.50</td>
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<tr>
<td>SPT1</td>
<td>0.36</td>
<td>0.48</td>
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Comryr' Commercial Chinese red yeast rice.
GAE= Gallic acid equivalent.

Table 1. Total antioxidant capacity in red yeast rice (GAE(\( \mu g/ml \))).
peroxidated free radicals. The radicals can react and decolorize β-carotene. In the presence of antioxidants β-carotene bleaching will not occur. By the way, anti lipid peroxidation free radical can be monitored by measuring the degree of decolorization.

The results in Table 2 show that, in order to obtain IC\textsubscript{50}, red yeast rice was prepared from glutinous rice, in which RD6 and SPT1 were more effective. The IC\textsubscript{50} of RD6 and SPT1 extract was lower indicating that they gave higher potency levels. The addition of soybean milk during cultivation shows improved activity for glutinous rice. The result is concurrent to the antioxidant capacity in Table 1.

The rapid use of starch in reaching the second phase of growth in the case of glutinous rice seems to enhance the production of fungal secondary metabolites. The production of darker red pigment was concurrent to the increase of antioxidant potency. Many secondary metabolites are produced by fungi, among these are compounds derived from polyketides. The possible compounds are pigments and phenolic compounds most of which have antioxidant activity. Aniya et al.\cite{22} reported one of the secondary metabolites as an antioxidant of the mold, \textit{Monascus anka} is a form of dimeremic acid. Dimeremic acid inhibited NADPH-and iron (II)-dependent lipid peroxidation of rat liver microsomes at 20 and 200 μM, respectively \cite{23}.

To support the results obtained, the determination of the content of antioxidants such as carotenoids, phenolic compounds, flavonoids, alkaloids and polyketide derivatives should be done.

In conclusion, the highest antioxidant activity was obtained in red yeast rice of 3 weeks old from SPT1 and RD6 with the addition of soybean milk. This result is concurrent to the production of red pigments.

4. ACKNOWLEDGEMENTS

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<th>Fermented Rice</th>
<th>IC\textsubscript{50} (mg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Without soybean milk</td>
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<tr>
<td></td>
<td>2 weeks</td>
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<tr>
<td>BHT</td>
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</tr>
<tr>
<td>Comryr*</td>
<td>Not determined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mali105</td>
<td>0.23</td>
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<tr>
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<td>0.17</td>
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<td>RD6</td>
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<td>0.09</td>
</tr>
<tr>
<td>SPT1</td>
<td>0.12</td>
<td>0.09</td>
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Comryr* Commercial Chinese red yeast rice.
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REFERENCES


