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STUDY ON CHOLESTEROL LOWERING COMPOUNDS IN RED YEAST RICE PREPARED FROM THAI GLUTINOUS RICE

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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. Kor Kho 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 and the content of monacolins, the cholesterol lowering compounds, were carried out using high performance liquid chromatography (HPLC). The presence of the monacolins was confirmed by the retention time of the reference compounds and LC-MS. The results were compared to those obtained from the Chinese redyeast rice and Thai non-glutinous rice (*Oryza sativa* L. cv. Mali105). The chromatograms shows the presence of monacolin K acid form (MKA), compactin (P1) , monacolin M acid form (MMA), monacolin K (MK), monacolin M (MM) and dehydromonacolin K (DMK). A large peak of a compound with the molecular weight of 358 was also detected but cannot be identified. The amount of two important monacolins, compactin and monacolin K, were determined. It was found that the highest amount of compactin and monacolin K were 21.98 and 33.79 mg/g respectively when using Thai rice variety *Oryza sativa* L. cv. RD6 which was fermented without adding soybean milk.

Keyword: Glutinous rice, HMG-CoA reductase, Monacolins, *Monascus purpureus*, Red yeast rice.

Introduction

Red yeast rice or more precisely red mold rice is a product of rice fermentation using *Monascus purpureus*. It is used as a colorants in food industry. Red yeast rice is rich in many useful compounds such as monacolins, GABA, glucosamine, lecithin, flavonoids and dimerumic acid. These compounds are very important for health. The main useful compound is monacolin K which is a cholesterol-lowering agent and able to reduce risk of colon cancer cells (Hong, et al., 2008). Monacolin K decreases the amount of cholesterol by inhibition of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase). The key enzyme produces mevalonyl-CoA which is the important rate determining step to synthesize cholesterol (Hajjaj, et al., 2001; Su, et al., 2003). This, therefore, helps to decrease blood pressure (Li, et al., 2004; Wang, et al., 1997). In the United States, statin drugs with the same property as monacolin K were manufactured and used for lowering cholesterol with the trade name of lovastatin (the brand name is Mevacor) (Erdogrul and Azirak 2004; Journoud and Jones 2004). In China, it has been found that red yeast rice made from non-glutinous rice or normal rice gave the highest amount of monacolin K at 2.52 mg/g (Chen and Hu 2005). 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 favorably impact lipid profiles of hypercholesterolemic patients (Li, et al., 2005). Chinese red yeast rice is imported into Thailand to be used as a food colorant. The use of red yeast rice benefits Thai people, whose death is caused by hypertension and heart attacks. In Thailand 2 main kinds of rice are grown and consumed by most people in different part of the country. 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 without amylose (Insomphun, 2003). The difference in their main composition may affect the content of useful fermentation metabolites, especially monacolin K. Therefore, the study of useful compounds content of red yeast rice is one of the most interesting subjects. This research aims to prepare red yeast rice using glutinous rice abundantly found and consumed in every day life among northern Thai people with *M. purpureus* CMU001 (Chairote, et al., 2007). The final purpose is to find the best variety and condition which gives the highest content of monacolins

Materials and Methods

Materials

Commercial Chinese red yeast rice, available in local traditional shops was used to isolate *M. purpureus* CMU001 strain, and to compare with Thai red yeast rice. Non-glutinous rice, *Oryza sativa* L. cv. Mali105 and glutinous rice; *Oryza sativa* L. cv. Kam (Kam), *Oryza sativa* L. cv. Kor Kho 6 (RD6) and *Oryza sativa* L. cv. Sanpatong1 (SPT1) 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 and kept in the same condition.

Preparation of red yeast rice

Stepwise preparation of red yeast rice is carried out by firstly immersion of each cultivar in water for 6 h following by steaming for 20 min. After cooling, 50 g of steam rice was put in 250 ml flask and sterilized at 15 psi and 121 °C for 15 min. One week old precultured *M. purpureus* CMU001 was used as inoculum. 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 h to obtain dried red yeast rice. In case of non-glutinous rice (Mali105) which was used for comparison, the red yeast

rice preparation was done without immersion of rice grains in water before streaming. In order to study the effect of adding nitrogen compounds, addition of 1 ml of 0.25 g/ml soybean milk solution was done.

Sample extraction

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 using 3,000 rpm speed at 4 °C (Li, et al., 2004). The extraction was repeated three times and all of the extracts were mixed together and made up to 50 ml using 75% ethanol. Finally, the extract was filtered through a 0.2 µm membrane and kept in a vial before being analyzed.

Analysis of monacolins by HPLC and LC-MS

The extracts were analyzed for monacolins by HPLC and LC-MS. The chemical profiling procedure conducted on the HPLC (Agilent HP 1100) with a photodiode array detector was optimized by testing various system conditions. The symmetry and resolution were increased by lowering the pH value of elution. The results suggested a system composed of 0.1 % trifluoroacetic acid (TFA) and acetonitrile as an ideal system for the separation of the monacolin compounds. For the consideration of resolution, running time and solvent-saving, the column of Hypersil ODS (250 mm x 4.0 mm i.d., 5µm) was used. The chromatography was performed using a gradient of acetonitrile (eluent A) and 0.1 % TFA (eluent B). Linear gradient elution (1 ml/min) from 35 to 75 % A in 20 min and keeping at 75 % A from 20 to 28 min was applied. The total analysis time was 35 min, including column stabilization. The photo-diode array detector was set at 210-350 nm and the chromatogram was detected at 237 nm. The column temperature was set at 35 °C, and the injection volume was 10 µl (Li, et al., 2005). For LC-MS analysis, the apparatus used were Agilent HP 1100 and MSD VL model. The eluents used and injection volume were the same as the HPLC analysis only the flow rate that was set at 0.5 ml/min from 35 to 75% of solvent A in 30 min and kept at 75% of solvent A for 5 minutes. The total analysis time was 50 min, including column stabilization. The length of the column used was 125 mm instead of 250 mm.

Results and Discussion

Red yeast rice prepared from Thai glutinous rice. Red yeast rice samples prepared from Thai glutinous rice with or without soybean milk to act as amino acid source were compared with commercial Chinese red yeast rice and red yeast rice prepared from non-glutinous, *Oryza sativa* L. cv. Mali105. Red yeast rice prepared from *Oryza sativa* L. cv. RD6 with the addition of soy bean milk for 3 weeks of cultivation had darker red color. The shapes of red yeast rice grains made from glutinous rice, *Oryza sativa* L. cv. Kam, *Oryza sativa* L. cv. RD6 and *Oryza sativa* L. cv. SPT1, were different from *Oryza sativa* L. cv. Mali105 distinctly. The texture is not hard with a pleasant sweet odor and dark red in color. In contrast, the rice made from non-glutinous rice, *Oryza sativa* L. cv. Mali105 had broken bright red grains and stuck together.

Yield of red yeast rice

The yield of red yeast rice was evaluated as percentage yield. The percentage yields (% yield) were obtained by using the following equation.

$$\% \text{ yield} = \frac{\text{Weight of dried end product} \times 100}{\text{Weight of steamed rice used}}$$

The percentage yield of prepared red yeast rice after 2 and 3 weeks cultivation using *Oryza sativa* L. cv. Mali105, *Oryza sativa* L. cv. Kam, *Oryza sativa* L. cv. RD6 and *Oryza sativa* L. cv. SPT1 rice are shown in Table 1. All varieties gave red yeast rice production varying from pale red to darker red depending on the kind of rice used as shown in Figure 1

Table 1. Percentage yield of red yeast rice

Fermented rice	Without soybean milk		With soybean milk	
	2 weeks	3 weeks	2 weeks	3 weeks
	% yield	% yield	% yield	% yield
<i>Oryza sativa</i> L. cv. Mali105	16.39	12.37	25.22	13.69
<i>Oryza sativa</i> L. cv. Kam	28.50	22.56	22.92	19.81
<i>Oryza sativa</i> L. cv. RD6	18.41	16.02	15.24	14.10
<i>Oryza sativa</i> L. cv. SPT1	17.03	15.28	15.93	13.40

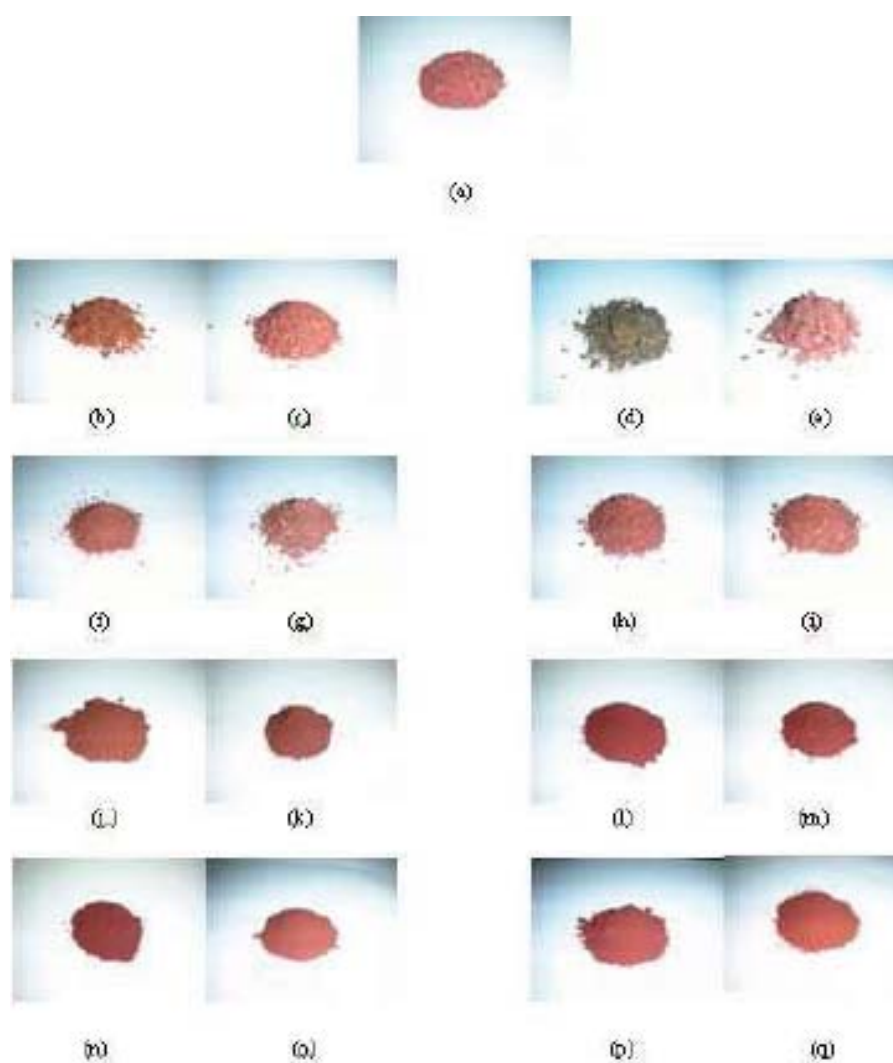


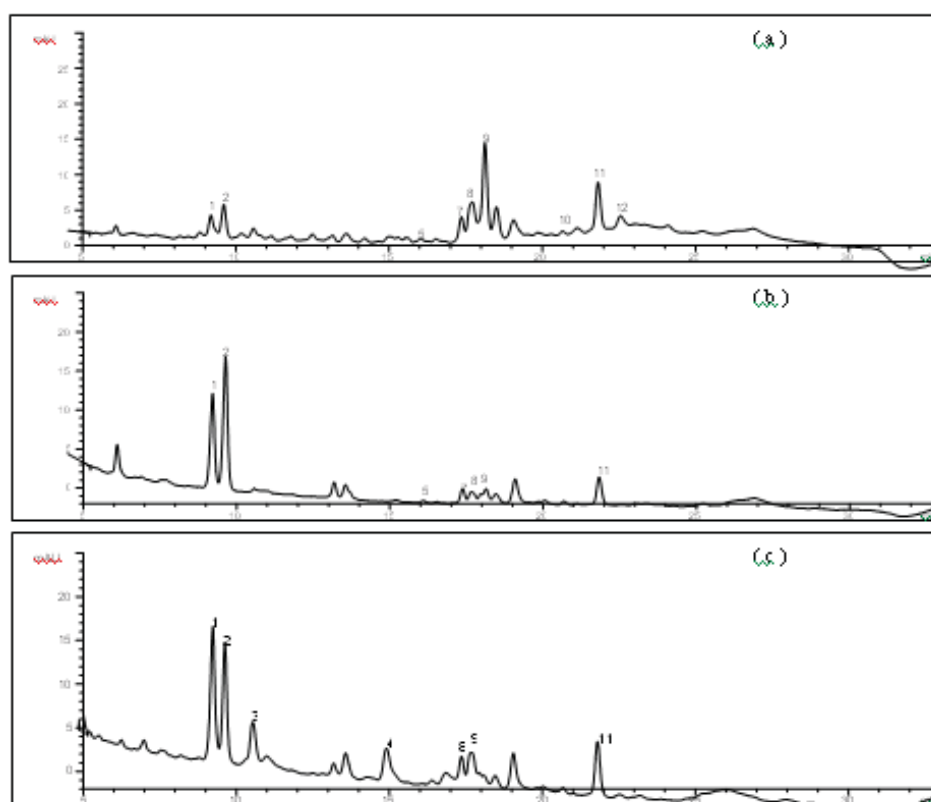
Figure 1. Product of red yeast rice

(a) Commercial Chinese red yeast rice (b), (f), (j), (n) Mali105, Kam, RD6 and SPT1, respectively, without soybean milk, 2 weeks (c), (g), (k), (o) Mali105, Kam, RD6 and SPT1, respectively, with soybeanmilk, 2weeks (d), (h), (l), (p) Mali105, Kam, RD6 and SPT1, respectively, without soybean milk, 3 weeks (e), (i), (m), (q) Mali105, Kam, RD6 and SPT1, respectively, with soybean milk, 3weeks

Monacolins in red yeast rice

An earlier report [4] shows the presence of 14 kinds of monacolins such as monacolin K (MK) or mevinolin, monacolin J (MJ), monacolin L (ML), monacolin M (MM), monacolin X (MX) and their hydroxyacid form, MK acid form (MKA), MJ acid form (MJA), ML acid form (MLA), MX acid form (MXA), MM acid form (MMA) as well as dehydromonacolin K (DMK), dihydromonacolin L (DML), compactin (P1), and 3 α -hydroxy-3,5-dihydromonacolin L (HDML).

The results obtained by detecting at 237 nm shows about 12 interesting peaks. The chromatograms of a 3 weeks period of fermentation with and without the addition of soybean milk using non-glutinous rice, *Oryza sativa* L. cv. Mali105, glutinous rice; *Oryza sativa* L. cv. Kam, *Oryza sativa* L. cv. RD6 and *Oryza sativa* L. cv. SPT1 are shown in Figure 2



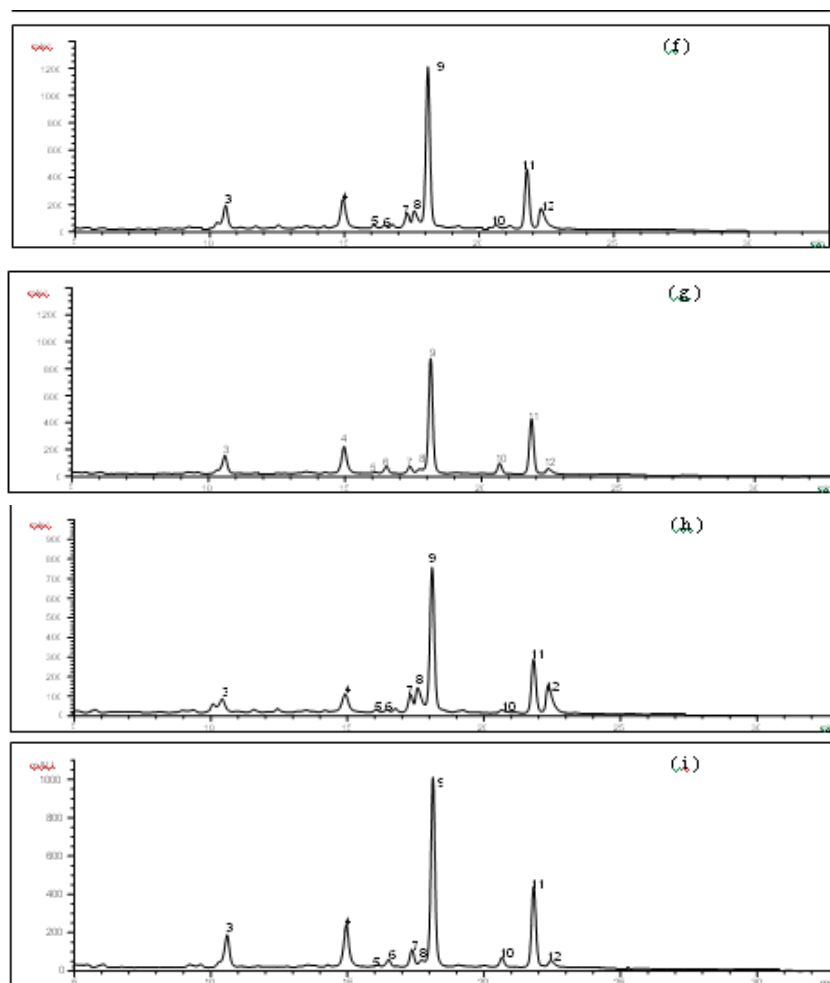


Figure 2. Chromatographic chemical profiling of monacolins in fermented red yeast rice from non-lutinous rice and glutinous rice.

(a) commercial Chinese red yeast rice (Comryr) (b) and (c) *Oryza sativa* L. cv. Mali105 without and with soybean milk (d) and (e) *Oryza sativa* L. cv. Kam without and with soybean milk (f) and (g) *Oryza sativa* L. cv. RD6 without and with soybean milk The chromatographic profile of commercial Chinese red yeast rice and Thai non-glutinous rice (*Oryza sativa* L. cv. Mali105) had common similarities while Thai glutinous rice varieties had almost the same profile. Thai glutinous rice seems to give more intense monacolins peaks than non-glutinous rice and commercial Chinese red yeast rice. The relative retention time, enrichment techniques and LC- MS were used to confirm the position of monacolins peaks. The peaks identified were based on the results obtained as shown in Table 2- 3 with their

Table 2. Data of chemical profiling of monacolins in fermented red yeast rice (peak area) from non- glutinous rice and glutinous rice without soybean milk for 3 weeks

Peak No	MW ^a	Ref. ^b	Compound	Com ryr ^c	<i>Oryza sativa</i> L. cv. Mahil05	<i>Oryza sativa</i> L. cv. Kam	<i>Oryza sativa</i> L. cv. RD6	<i>Oryza sativa</i> L. cv. SPT1
1	-	-	Unidentified	50.19 ±0.01	128.94 ±0.12	66.72 ±0.00	-	-
2	-	-	Unidentified	62.14 ±0.00	182.08 ±0.07	112.61 ±0.04	-	-
3	-	-	Unidentified	-	-	84.48 ±0.04	2528.99 ±0.13	1289.37 ±0.08
4	422	[12], [13]	Monacolin K Acid form (MKA)	-	-	81.61 ±0.02	4397.43 ±0.04	2457.56 ±0.29
5	390	[14], [15]	Compactin (P1)	39.49 ±0.03	3.02 ±0.08	4.90 ±0.03	536.09 ±0.01	376.07 ±0.11
6	-	-	Unidentified	-	-	-	536.79 ±0.01	331.89 ±0.03
7	424	[4]	Monacolin M acid form (MMA)	73.92 ±0.06	21.62 ±0.51	186.41 ±0.06	1792.46 ±0.01	1307.55 ±0.15
8	404	[16], [17]	Monacolin K (MK)	126.49 ±0.32	22.72 ±0.69	198.29 ±0.01	2138.68 ±0.01	2122.45 ±0.12
9	358	-	Unkwown	211.72 ±0.11	22.95 ±0.95	1030.99 ±0.12	14742.30 ±0.00	9654.09 ±0.02
10	406	[4]	Monacolin M (MM)	68.36 ±0.39	-	64.59 ±0.05	875.56 ±0.01	593.00 ±0.17
11	386	[18], [19]	Dehydromonacol in K (DMK)	233.89 ±0.55	45.54 ±0.03	773.51 ±0.09	5733.99 ±0.01	3650.18 ±0.03
12	-	-	Unidentified	209.32 ±0.73	-	244.95 ±0.02	3465.02 ±0.02	2890.51 ±0.22

Not detectable

a Molecular weight

b Reference

c commercial Chinese red yeast rice

Table 3. Data of chemical profiling of monacolins in fermented red yeast rice (peak area) from non- glutinous rice and glutinous rice with soybean milk for 3 weeks

Peak No	MW ^a	Ref. ^b	Compound	<i>Oryza sativa</i> L. cv. Mahil05	<i>Oryza sativa</i> L. cv. Kam	<i>Oryza sativa</i> L. cv. RD6	<i>Oryza sativa</i> L. cv. SPTI
1	-	-	Unidentified	174.56 ±0.01	191.00 ±0.05	-	-
2	-	-	Unidentified	148.35 ±0.00	163.41 ±0.03	-	-
3	-	-	Unidentified	82.57 ±0.02	163.93 ±0.01	2374.28 ±0.08	2837.37 ±0.03
4	422	[12], [13]	Monacolin K acid form (MKa)	75.92 ±0.00	212.76 ±0.04	3579.93 ±0.02	3947.22 ±0.04
5	390	[14], [15]	Compactin (P1)	-	5.68 ±0.01	325.70 ±0.00	403.80 ±0.27
6	-	-	Unidentified	-	55.68 ±0.01	940.37 ±0.01	783.07 ±0.01
7	424	[4]	Monacolin M Acid form (MMA)	45.55 ±0.00	276.07 ±0.00	1014.3 ±0.01	1489.96 ±0.01
8	404	[16], [17]	Monacolin K (MK)	70.69 ±0.00	80.71 ±0.03	595.71 ±0.00	667.89 ±0.00
9	358	-	Unkown	-	660.56 ±0.08	10336.9 ±0.00	11952.9 0 ±0.00
10	406	[4]	Monacolin M (MM)	-	85.13 ±0.12	1602.02 ±0.01	1355.48 ±153.45
11	386	[18], [19]	Dehy-dromonacolin K (DMK)	79.96 ±0.00	863.15 ±0.22	5369.92 ±0.01	5761.05 ±0.01
12	-	-	Unidentified	-	-	1266.94 ±0.01	1424.89 ±0.01

Not detectable

a Molecular weight

b Reference

The amount of compactin and monacolin K was determined using standard compounds as shown in Table 4. It was found that monacolin K content in red yeast rice from *Oryza sativa* L. cv. RD6 fermented without addition of soybean milk are higher with 33.79 mg/g dry weight. As for compactin, the same results were obtained with the higher amount in *Oryza sativa* L. cv. RD6 with 21.98 mg/g dry weight without the addition of soybean milk. Thai glutinous rice seems to give a satisfactory amount of monacolin K and compactin than both Thai non-glutinous rice and commercial Chinese red yeast rice. A report by Chen & Hu (2005) indicated that the highest amount of monacolin K was 2.42 mg/g in red yeast rice, which is less than in Thai glutinous red yeast rice (*Oryza sativa* L. cv. RD6). The study on red yeast rice made from Thai glutinous rice would be beneficial to improve its quality. Monacolin K and compactin could be obtained without adding soybean milk. The result does not correspond to the increase of the red color as when soybean milk is added. The addition of soybean milk darkened the color rather than increased monacolin K. The result may be explained that nitrogen source involve in the red color instead of monacolins synthesis (Pinthong, et al., 2004).

Table 4. Compactin, Monacolin K content in 3 weeks old red yeast rice

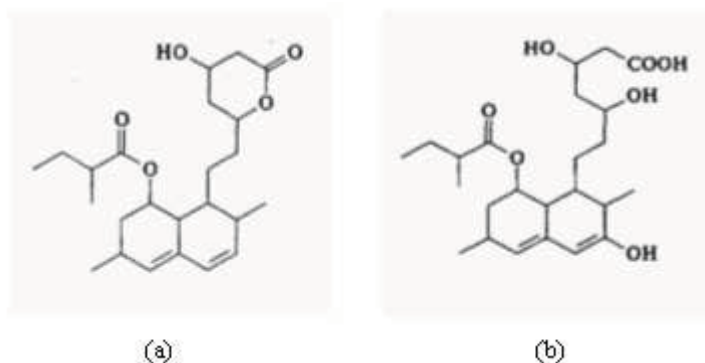
Kinds of red yeast rice	Compactin ^a (mg/g)	Monacolin ^a K (mg/g)	Compactin ^b (mg/g)	Monacolin ^b K (mg/g)
Comyr ^c	1.62 ± 0.00	2.00 ± 0.01	Not available	
<i>Oryza sativa</i> L. cv. Mali105	0.12 ± 0.00	0.36 ± 0.01	Not detectable	1.12 ± 0.00
<i>Oryza sativa</i> L. cv. Kam	0.20 ± 0.00	3.13 ± 0.00	0.23 ± 0.00	1.28 ± 0.00
<i>Oryza sativa</i> L. cv. RD6	21.98 ± 0.00	33.79 ± 0.00	13.35 ± 0.00	9.41 ± 0.00
<i>Oryza sativa</i> L. cv. SPT1	15.42 ± 0.01	33.54 ± 0.00	16.56 ± 2.47	10.55 ± 0.00

Not detectable

a Molecular weight

b Reference

c commercial Chinese red yeast rice

**Figure 3.** (a) Monacolin K in lactone form (b) Monacolin K in acid form

Another interesting compound is the peak number 9 where the molecular weight was found to be 358 by LC-MS. The structure of the compound may have something to do with monacolin K but with the loss of H₂O (dehydration) and two methyl groups. The compound seems to be related to monacolin K but with the loss of H₂O or γ-hydroxyl group. Dehydromonacolin K is also relevant. Monacolin K is not active in its natural form but will be active by hydrolysis of the lactone moiety to form γ-hydroxy acid (Figure 3). It is the hydroxy acid opened lactone structure which is active for inhibition of HMG CoA reductase (Alberts, et al., 1980; Trenin and Katruhha 1997). The γ-hydroxy acid contributed to a competitive inhibition of HMG-CoA reductase and the production of cholesterol. The loss of γ-hydroxyl group in the case of compound number 9 and dehydromonacolin K may decrease the inhibition efficiency. The study on the relevant of these compounds, which seem to be more abundant, in the presence of monacolin K or compactin is of interest for further study. If the increase of the compounds number 9 and dehydromonacolin K cause the decrease of Monacolin K and compactin, the optimum condition for the production of high concentration of monacolin K and compactin may be obtained by blocking the production of the compound number 9 and dehydromonacolin K. However, many kinds of fungi also produce mycotoxin (Pattanagul, et al., 2008; Lin, et al., 2008). Therefore, the determination of some mycotoxin such as citrinin should be carried out accurately. The amount of citrinin is not allowed more than 200 ng/g in Japan (Chen and Hu 2005). In China and the European Economic Community, the similar citrinin level is still under debate (Mandt, M. 1998).

Conclusion

Red yeast rice prepared from glutinous rice gave higher concentration of useful compounds. The result indicates the potency to used glutinous rice to prepare red yeast rice production for cholesterol lowering purpose. Further work should be the study on the content citrinin in red yeast rice prepared from glutinous rice

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