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Improvement of Monacolin K and Minimizing of Citrinin Content in Korkor 6 (RD 6) Red Yeast Rice

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Abstract**—**A strain of *Monascus purpureus* CMU001 was used to prepare red yeast rice from Thai glutinous rice Korkor 6 (RD 6). Adding of different amounts of histidine (156, 312, 625 and 1250 mg in 100 g of rice grains)) under aerobic and air limitation (air-lock) condition were used in solid fermentation. Determination of the yield as well as monacolin K content was done. Citrinin content was also determined in order to confirm the safety use of prepared red yeast rice. It was found that under air-lock condition with 1250 mg of histidine addition gave the highest yield of 37.40 g of dried red yeast rice prepared from 100 g of rice. Highest 5.72 mg content of monacolin K was obtained under air-lock condition with 312 mg histidine addition. In the other hand, citrinin content was found to be less than 24462 ng/g of all dried red yeast rice samples under the experimental methods used in this work.

Keywords**—**Citrinin, Glutinous rice, Monacolin K, Red yeast rice.

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I. Introduction

RED yeast rice or more precisely red mold rice that has been used in Chinese cuisine and medicinal food to promote blood circulation for centuries is a product of rice fermented by using *Monascus purpureus*. 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 hypercholesterolemia [1], [2]. Chinese red yeast rice is imported into Thailand to be used as a food colorant and flavoring agent.

In Thailand two main kinds of rice are grown and consumed by most people in different areas. 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 [3]. The different in their main composition may affect the content of useful compounds in fermentation products.

The preparation of red yeast rice from Thai glutinous rice using *Monascus purpureus* CMU001 has already been studied [4]-[6]. Various Thai glutinous rice as well as normal non-glutinous rice varieties were used for the studies while solid fermentation with and without addition of soybean milk were done. The presence of monacolins was confirmed and the amount of monacolins content was determined. The determination of the quantity of citrinin which is a mycotoxin damaging the human kidney was also carried out. The comparative results on the content of both monacolins and citrinin present in different fermentation conditions red yeast rice were obtained. In spite of the highest amount of monacolin K (mevalonin) obtained using Thai glutinous rice, Sanpatong 1 (SPT1), some compounds seemed to be more dominant among the monacolins. These compounds may have some inter-conversion with monacolin K making the decrease of monacolin K content. Thai glutinous rice, Korkor 6(RD 6) is another variety of interest due to its popularity and availability.

Improvement of red yeast rice manufacturing process, especially, to find an appropriate condition in order to increase moncolin K and decrease citrinin is more useful. High monacolin K and low citrinin content makes red yeast rice to be a product of more valuable. The consumption will be more profitable and safe. The use of Thai glutinous rice to produce red yeast rice instead of importing from China is, economically, the way to be sustainably self-supported.

In this work, the improvement of red yeast rice production was done using Korkor 6(RD 6) Thai glutinous rice which is the most popular variety. The variation of histidine addition and aerobic condition were studied. High performance liquid chromatography was used for the analysis. We expected to have a product with high content of monacolin K and low content of citrinin in order to meet high quality red yeast rice and generally recognition as safe.

II. Materials and Methods

A. Materials

Thai glutinous rice, *Oryza sativa* L. cv. Korkor 6 (RD 6), used for preparing red yeast rice was purchased from a local rice supplier in Chiang Mai province, Thailand. *Monascus purpureus* CMU001 isolated from commercial Chinese red yeast rice was kindly provided from the laboratory of micro-biotechnology, Chiang Mai University, Thailand, with best regards from Professor Saisamon Lumyong.

B. Methods

1. Preparation of Red Yeast Rice

Inoculation and cultivation of isolated *M. purpureus* CMU001 were done using Thai glutinous rice *Oryza sativa* L.

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cv. Korkor 6(RD 6). Various conditions with 50 g ste amed rice grains and 4 pieces of 4 cm^2 of 7 days culture inoculation were used. The solid fermentation was do ne in 2 50 ml Erlenmeyer flask with or without airlock cap. Different amount of histidine were also added into each flask. All of the samples were labeled as shown in Table I.

TABLE I
RED YEAST RICE SAMPLES WITH DIFFERENT CONDITIONS

Samples	Conditions		Histidine (mg)
	Normal (without Air-lock)	Air limitation (with Air-lock)	
1. AN	√	-	-
2. AN -AL	-	√	-
3. AN^1	√	-	156
4. AN^1- AL	-	√	156
5. AN^2	√	-	312
6. AN^2-AL	-	√	312
7. AN^3	√	-	625
8. AN^3 -AL	-	√	625
9. AN^4	√	-	1250
10. AN^4-AL	-	√	1250

Glutinous rice grains (RD 6) were immersed in water for 6 hours followed by steaming for 20 minutes. After cooling, 50 g of steam rice was put in 250 ml flask and sterilized at 15 psi and 121°C for 15 minutes. One week old pre-cultured *M. purpureus* CMU001 was used as inoculums. Each sample was prepared with or without an add ition of 1 mL histidine solution to make different concentration as shown in Table I. The air limited samples were prepared by usin g airlock cap after 10 days of fermentation. The inoculated rice was incubated at 30°C for 3 weeks. The product was dried in the oven at 6 5°C for 6 hours to obtain dried c onstant weight red yeast rice.

2. Preparation of Red Yeast Rice Extracts

In order to determine the monacolin K and citrinin content, extraction was carried out using 0.5 g of ground red yeast rice. Micro extraction was done in 1.5 mL micro centrifuge tube using 1 mL of 75% ethanol and sonication extraction. After 10000 rpm centrifugation, the supernatant was co llected, evaporated and made to appropriated amount by adding HPLC mobile phase solution.

3. Determination of Monacolin K and Citrinin Content

HPLC analysis was done at 238 nm and 1mL/min. flow rate of 70:30 v/v acetonitrile: trifluoroacetic acid or mobile phase for monacolin K. In case of citrinin, methanol (0.1%): phosphoric acid (80: 20 v/v) was used as a mobile phase.

The presence of monacolins or citrini n in the extracts was confirmed by HP LC (Agilent HP 1100) with Inertsil ODS -3 column (5 µm 4.6x150 mm 6BI85146) and photodiode array detector. The chromatography was performed using acetonitrile: trifluoroacetic acid (TFA) (70: 30 v/v) or methanol (0.1%): ph osphoric acid (80: 2 0 v/v) as a s olvent, depending on the determination of monacolin or citrinin.

III. RESULTS AND DISCUSSION

A. Preparation of Red Yeast Rice

Different weight of final products was obtained as shown in Table II. Red to red brown colored red yeast rice product s were obtained as shown in Fig. 1.

TABLE II
WEIGHTS AND PERCENTAGE YIELD OF DRIED RED YEAST RICE

Samples	Histidine(mg)	Weight(g)		%Yield
		Red yeast rice	Dried red yeast rice	
1.AN	-	100	31.57	31.57
2.AN -AL	-	100	24.91	24.91
3.AN^1	156	100	32.56	32.56
4.AN^1-AL	156	100	31.31	31.31
5.AN^2	312	100	28.06	28.06
6.AN^2 - AL	312	100	30.54	30.54
7.AN^3	625	100	27.72	27.72
8.AN^3 - AL	625	100	32.90	32.90
9.AN^4	1250	100	34.88	34.88
10. AN^4-AL	1250	100	37.40	37.40

AN without histidine AN – AL without histidine

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AN^1 histidine 156 mg | AN^1- AL histidine 156 mg

AN^2 histidine 312 mg | AN^2 –AL histidine 312mg

AN^3 histidine 625 mg | AN^3 –AL histidine 625 mg

AN^4 histidine 1250 mg | AN^4 –AL histidine 1250 mg

Fig. 1 Red yeast rice products with different condition
AN = without histidine without air-lock, AN-AL without histidine with air-lock
AN^1 = with 156 mg histidine without air-lock, AN^1-AL with 152 mg histidine with air-lock
AN^2 = with 312 mg histidine without air-lock, AN^2-AL with 312 mg histidine with air-lock
AN^3 = with 625 mg histidine without air-lock, AN^3-AL with 625 mg histidine with air-lock
AN^4 = with 1250 mg histidine without air-lock, AN^4-AL with 1250 mg histidine with air-lock

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Considering the result in Table II, normal or air-lock condition did not much affect the percentage yield of the products. In case of addition of histidine, air-lock condition gave a little decrease without adding or adding low amount of histidine whereas adding more histidine increased the percentage yield. When adding the proper amount of histidine, the cell mass increased notably. Histidine addition indicated its requirement as an important nutrient for growth.

B. Determination of Monacolin K Content

Ethanol extracts were used for the determination of monacolin K. The results indicated different amount of monacolin K in each product as shown in Table III. The amount of monacolin K seems to be higher when preparing under air-lock condition. Addition of histidine caused increasing of monacolin K but decreased when added too high histidine. At higher histidine concentration, the metabolism may rapidly reach the stage of monacolin K production. The post metabolic pathway may cause the transformation of monacolin K to other related molecules. This was shown by [7], the presence of monacolin K related molecule with the molecular weight of 358 after the production of monacolin K. This result could be explained the reducing of monacolin K content in air limitation condition due to the post metabolic pathway.

TABLE III
MONACOLIN K CONTENT IN RED YEAST RICE

	Histidine (mg)	Total monacolin K (mg)
AN	-	3.47
AN – AL	-	2.73
AN^1	156	1.48
AN^1 - AL	156	3.23
AN^2	312	1.21
AN^2 - AL	312	5.72
AN^3	625	0.98
AN^3 - AL	625	1.42
AN^4	1250	2.63
AN^4 - AL	1250	1.77

C. Determination of Citrinin Content

In order to make red yeast rice safe to be taken, the product should be prepared with decreasing of citrinin content. The ethanol extracts of all sample after HPLC analysis showed undetectable amount under the methods used. Citrinin content was found to be under 24462 ng/g or less than the limit of quantization (LOQ) in all dried red yeast rice. In Japan recommended concentration of citrinin is 200 ng/g [8]. To meet the limit of quantization at 200 ng/g, about more than 100 times of preprared red yeast rice or more than 100 times of extractions might be needed. Therefore, Increase of the starting rice grains and accumulation of the extracts were recommended. Hajjaj et al. [9] studied the effect of amino acids on red pigments and citrinin production in *Monascus* ruber. Amino acids were used as sole nitrogen sources to examine their effects on the production of water-soluble red pigments and citrinin by *Monascus ruber* ATCC 9 6218 cultivated on chemically defined media. In general, when glycine, tyrosine, arginine, serine, or histidine was used as sole nitrogen sources, they favored the production of red pigments, and restricted the synthesis of the mycotoxin. In contrast, the production of citrinin was enhanced in media supplemented with glutamate, alanine, or proline. Histidine was found to be the most valuable amino acid as it resulted in the highest production of red pigments and almost completely eliminated the formation of mycotoxin.

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