การเพิ่มมูลค่าของข้าวโดยการแปรรูปเป็นข้าวแดง

Increasing the Value of Rice by Transformation into Red Yeast Rice

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บทคัดย่อ

ข้าวแดงเป็นข้าวที่เกิดจากการหมักโดยเชื้อราโมแนสคัส ผลิตภัณฑ์ข้าวแดงสามารถใช้เป็นวัตถุเจือปนอาหาร สารสี และยาที่มีคุณภาพ จากรายงานวิจัยที่ผ่านมาพบว่าข้าวแดง มีสารเมแทบอไลต์หลายชนิด เช่นสารสีโมแนสคัส (แดง เหลือง และส้ม) และกลุ่มสารออกฤทธิ์ทางชีวภาพ โดยเฉพาะอย่างยิ่ง สารโมนาโคลิน เค สารแกมมาอะมิโนบิวทิริกแอซิด (กาบา) และสารซิตรินินเป็นต้น ปริมาณของสารสีและสารออกฤทธิ์ทางชีวภาพ จะมีปริมาณมากหรือน้อยขึ้นอยู่กับสายพันธุ์ของเชื้อรา สภาวะที่เหมาะสมในการหมัก เป็นต้น ดังนั้นการแปรรูปข้าวและปลายข้าวเป็นข้าวแดงสามารถเพิ่มมูลค่าของข้าวซึ่งเป็นผลผลิตหลักทางการเกษตรของประเทศไทย อย่างไรก็ตามผลิตภัณฑ์ข้าวแดงต้องมีคุณภาพโดยเฉพาะอย่างยิ่งปริมาณซิตรินินเพื่อความปลอดภัยของผู้บริโภค

คำสำคัญ: โมแนสคัส โมนาโคลิน เค แกมมาอะมิโนบิวทิริกแอซิด ซิตรินิน

Abstract

Red yeast rice (RYR) is rice fermented with *Monascus*. RYR products can be used as food additives, coloring materials, and dietary supplements. Many researchers have reported valuable metabolites in RYR, such as *Monascus* pigments (red, yellow, and orange) and other bioactive metabolites, especially monacolin K, g-aminobutyric acid (GABA), and citrinin, etc. The quantities of *Monascus* pigments and bioactive metabolites are dependent on the species of mold and optimum culture condition. Rice is a major agricultural product of Thailand. Transformation of whole rice and broken rice into RYR will provide added value to the rice. However, the quality of RYR products must be controlled, especially citrinin content, for consumer health protection.

Keywords: *Monascus*, monacolin K, γ-aminobutyric acid, citrinin

Introduction

Red Yeast Rice (RYR) is the fermented product of ordinary rice (*Oryza sativa*) with red mold (*Monascus* spp.). Red yeast rice is also called ang-kak, anka, hung-chu, hon-chi, and hong-qu in Chinese and Taiwan, hong-gug in Korean, red koji, benikoji, and akakoji in Japanese, and so on.[1] It has a long history as a flavoring, coloring and preservative in food and a folk medicine in many Asian countries. The RYR product is referred to by different names according to the local languages. Metabolites of RYR are classified on two bases, including *Monascus* azaphilone pigments (red, yellow, and orange) and other bioactive metabolites, especially monakolin K, g-aminobutyric acid (GABA), and citrinin (Figure 1).[1-5] The chemical

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structures of the main bioactive metabolites are shown in Figure 2. Application of RYR has been shown in foodstuffs, beverages, anti-microbial and human health supporting agents, and miscellaneous industries, e.g. textile industries, cosmetic, and pulp, etc.[6]

Monacolin K is a hypocholestromic agent that competitively inhibits the rat-limiting enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the reduction of HMG-CoA to mevalonate during cholesterol biosynthesis.[7,8] GABA is an amino acid transmitter that is present in the inhibitory neurons of the central nervous system. GABA has several antihypertensive and diabetic hyperglycemia prevention activities.[4] Therefore, food products with RYR extract added are claimed to have nutritional and pharmacological benefits. Nitrite and nitrate have been used in the preparation of cured meats for the purposes of anti-bacterial agent and antioxidant. Nitrite is reduced to nitric oxide and reacts with myoglobin to produce nitric oxide myoglobin, which contributes to the characteristic pink cured meat color. Nitrite can also be applied to preserve a desirable meaty flavor. The levels of nitrite and nitrate used in meat curing has arisen because of the possibility of nitrosamine, which is a carcinogen. The residual nitrate and nitrite in fermented meats may form *N*-nitrosamines in the gastrointestinal tract.[9] Therefore, the color enhancement and antioxidative properties make RYR a potent candidate to be applied in meat products. Figure 3 shows the addition of RYR powder in Thai traditional fermented pork (Nham) and northeastern style Thai sausage.[10]

Red yeast rice production

Rice is an important crop. Although Asia is the major rice producer and largest exporter of rice in the world, the price of rice in each year varies. The price of whole rice and broken rice is 30-35 baht (0.9-1.1 $) per kilogram and 10-15 baht (0.3-0.5 $) per kilogram, respectively. Whereas, RYR has a high price of 300-350 baht (9.4-10.9 $) per kilogram, which depends upon its quality. Therefore, whole rice and broken rice could be served as the sustainable raw material for value-added products through fermentation with *Monascus* molds.

Monascus pigments are produced on commercial scales by many *Monascus* species, especially *M. anka*, *M. kaoliang*, *M. pilosus*, *M. purpureus,* and *M. ruber*. The genes responsible for citrinin synthesis (*pksCT*, *ctnA,* and *orf3*) are absent or significantly different in *M. pilosus* and *M. ruber*. The highly conserved *citrinin* gene cluster is in *M. kaoliang* and *M. purpureus*. *Monascus* strains for pigment production could be achieved by genetic engineering and metabolic engineering.[6,11]

RYR products have numerous bioactive metabolites and are completely safe when they are produced under optimal nutritional and environmental conditions to reduce the citrinin contamination (Table 1). Rice for RYR production should have a high content of amylose and low amylopectin. In solid state culture, bioactive compounds are released into rice grains. Figure 4 shows the changing color during solid state fermentation of broken rice at room temperature (30±2ºC) for eight days.[10] The RYR could be achieved by the following fermentation steps: the rice is rinsed and soaked in water, drained, steamed, sterilized, fermented, and dried. The optimal cultivation temperature is in the range of 25-30°C for growth and pigment production for most species, while temperatures above 35°C inhibit monacolins production.[6] As for the initial pH, the ideal range for pigment production is 4.0-7.0. Metal ions, especially Zn^{2+} and Mg^{2+}, greatly affect growth and pigment production of *Monascus* spp.[1] Good aeration provides a good yield of pigment and low citrinin production. *Monascus* spp. generally produce the maximum pigments in darkness and the minimum ones in white light.[1,6] Nitrogen sources, such as amino acids and ammonium salts, are used for good pigment yields, amino acids could be added to the fermentation processes as a precursor for various pigment colors depending on the content ratios of yellow, orange, and red.[2]

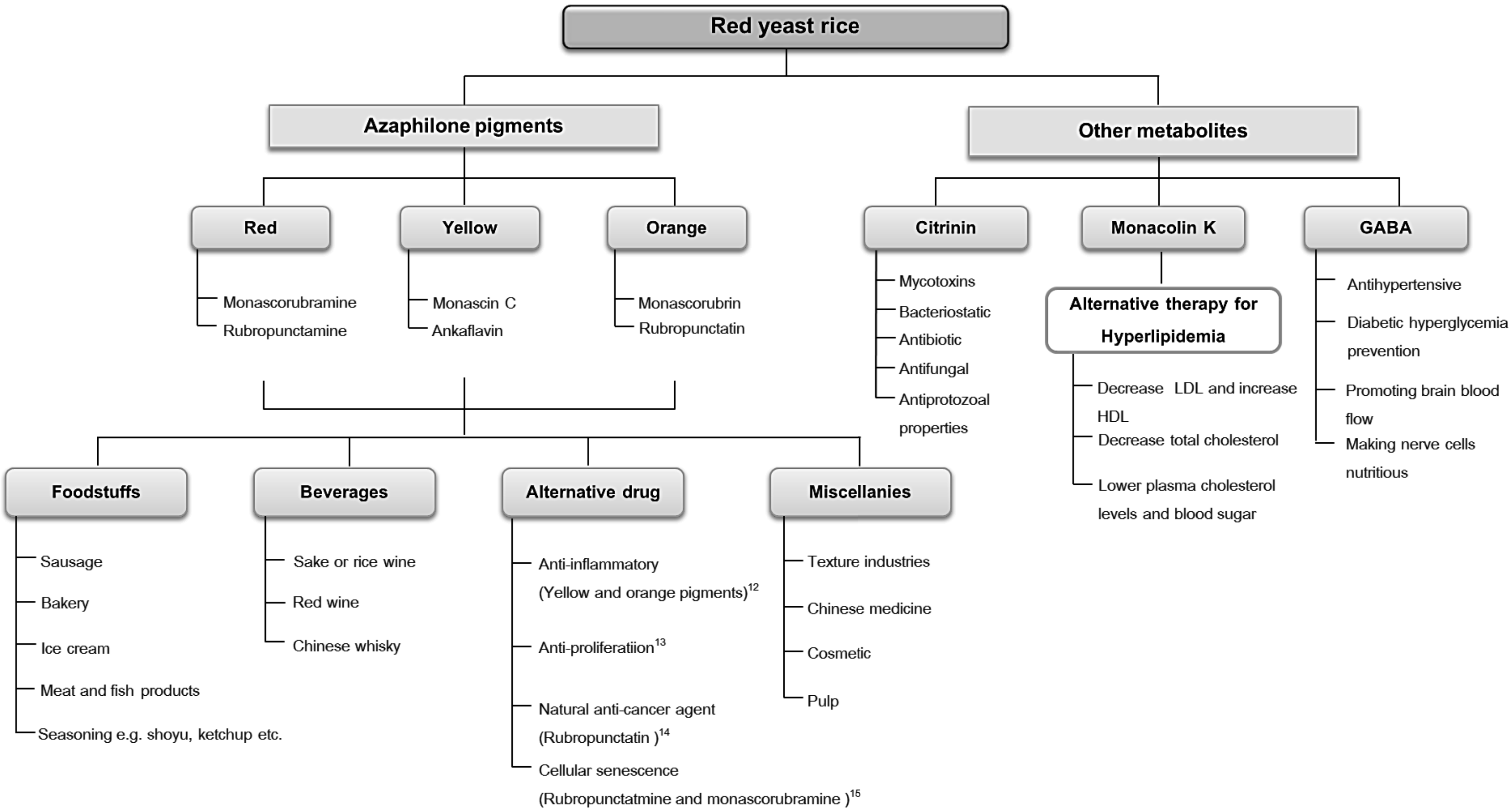

Figure 1 Secondary metabolites of red yeast rice (RYR) are classified on two bases including main pigmented metabolite (red, yellow, and orange) and other bioactive metabolites, especially monakolin K, Azaphilones, g-aminobutyric acid (GABA), and citrinin. Applications of RYR are in foodstuffs, beverages, anti-microbial and human health supporting agents, and miscellaneous industries.[1-5, 12-15]

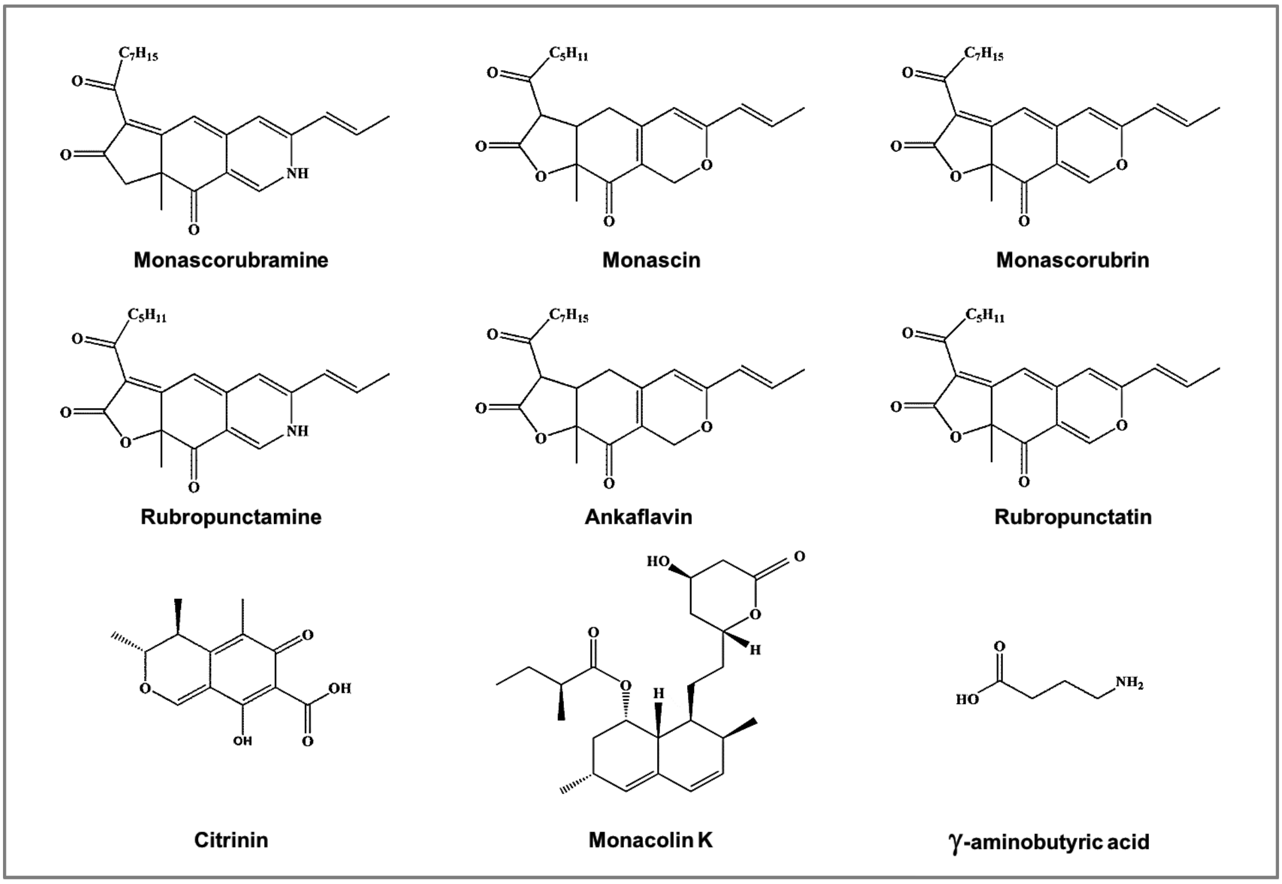

Figure 2 Chemical structure of main bioactive metabolites in red yeast rice, pigmented metabolite (red, yellow, and orange), monakolin K, Azaphilones, g-aminobutyric acid (GABA), and citrinin.[2-5]

Figure 3 *Monascus* sp. on red yeast rice (RYR) (a), powder of RYR for food coloring (b), adding RYR powder in pork (c), comparison of color between meat and meat products with RYR (right) and without RYR (left) of (d) Thai traditional fermented pork (Nham), and (e) northeastern style Thai sausage.[10]

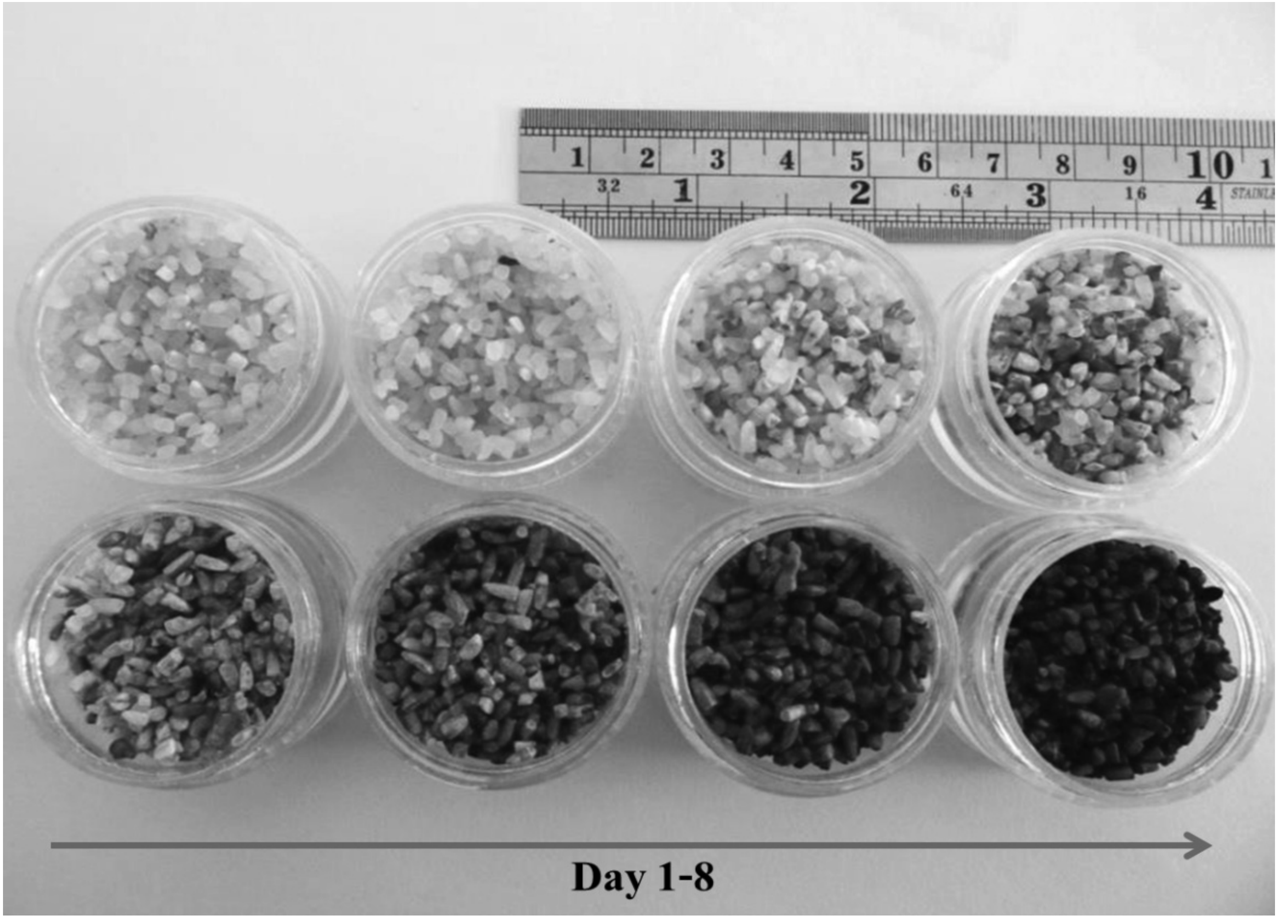

Figure 4 Color Change of red yeast rice during solid state fermentation of broken rice at room temperature (30±2ºC) for eight days.[10]

Table 1. Optimum conditions for fermented cultures in red yeast rice production.

Organism	Conditions	Product Qualities	References
M. purpureus ATCC 16392	*Fermentation condition:* 30°C for 10 days, water content 55%, aeration 20-30 ml/min *Drying process:* 60°C	Monascin 2.93 mg/g Rubropunctatin 7.18 mg/g Ankaflavin 1.18 mg/g Monascorubrin 8.28 mg/g	16
M. purpureus	*Fermentation condition:* 30°C for 14 days, rice bran 5% *Drying process:* 70°C	Lovastratin (mevinolin and monacolin K) 102 mg/kg Red pigment (OD 500 nm) 3.574 AU	17
Monascus sp. LPB 31	*Fermentation condition:* 32°C for 12 days, water content 53%, aeration 1 ml of air/g min (drum rotary fermenter) *Drying process:* 40°C	Red pigment (OD 500 nm) 500 AU/g dry fermentate	11
Coculture: *M. purpureus* MTCC 369 *and* *M. ruber* MTCC 1880	*Fermentation condition:* 29.46°C, pH 6.03 for 13.89 days, water content 55%, aeration 20-30 ml/min *Drying process:* 60°C	Lovastratin (mevinolin and monacolin K) 2.80 mg/g	18
Monascus spp. M12-69 (mutant stain)	*Fermentation condition:* 25°C for 16 days, water content 55-75% *Drying process:* 50°C	Monakolin K 2.52 mg/g Citrinin 0.13 ng/g	19
M. purpureus HD001	*Fermentation condition 30* °C for 14 days *Drying process*: 50 °C	Monakolin K 7,000 mg/g	20
M. pilosus	*Fermentation condition 25±1* °C for 8 weeks (mixing with 40% (w/w) of chopped garlic) *Drying process*: 60 °C	Total phenolic 164.3±5.8 mg GAE/100 g Total flavonoids 507.1±17.0 mg CE/100 g	21

Note: AU = atomic unit, mg = milligram, g = gram, ml = milliliter, mg = microgram, kg = kilogram, mg GAE = milligram gallic acid equivalent, mg CE = milligram catechin equivalent

Table 1. *Continued*

Organism	Conditions	Product Qualities	References
M. purpureus CMU001	*Fermentation condition:* 30°C for 2 and 3 weeks *Drying process:* 65°C for 6 h (Substrate: *Oryza sativa* L. cv. RD6)	Monascin K 33.79 mg/g Compactin 21.98 mg/g	22
Monascus sp. KB9	*Fermentation condition:* 30°C for 15 days, moisture content of rice 38% (w/w) *Drying process:* 50°C	Monacolin K 13,536.61 mg/kg Glucoamylase 189,685.66 Unit/g Glucosamine 4.438 mg/g Red pigment (OD 400 nm) 3,571.97 Unit/g (OD 500 nm) 2,697.71 Unit/g	23
M. purpureus (ATCC 16365, BCC 6131, DMKU, FTCMU) and *M. ruber* (TISTR 3006)	*Fermentation condition:* 32-35°C for 28 days, *Drying process:* 55°C for 3 days	Citrinin 0.26 mg/l Monacolin K 25.03 mg/l Red pigment (OD 500 nm) 3.43 AU	24
The wild type (*M. kaoliang* KB9)	*Fermentation condition:* 28-30°C for 5 weeks, humidity 64% *Drying process:* -	Anti-cholesterol agent 17,892 mg/kg Red pigment (OD 400 nm) 4,834 Unit/g (OD 500 nm) 4,640 Unit/g	25
M. purpureus TISTR 3514	*Fermentation condition:* 30°C for 15 days, moisture content 40-42% *Drying process:* 50°C	Monacolin k 5,900 mg/kg Citrinin 0.26 mg/kg Yellower pigment 1,700 Unit/g	26

See previous page of Table 1

Standard and Regulation on Red Yeast Rice

The Food and Drug Administration (FDA) issued a consumer warning to avoid RYR products because many of these products may contain citrinin.[27] Citrinin is a nephrotoxic in animals with a reported median lethal dose (LD_{50}) of 35 mg/kg.[28] Citrinin in food colorants has been shown to be mutagenic at concentrations between 0.2 and 1.7 ug/g.[29] Therefore, its concentration in supplements should be minimal. In Japan, the maximum allowed level of citrinin in RYR is 200 mg/kg.[27] In Taiwan, the regulatory limits of citrinin in RYR (raw material) and *Monascus* products are 5 mg/kg and 2 mg/kg, respectively.[30] The Chinese national standard (GB 4926-2008) for red kojic rice (powder) was enacted in 2008. This standard requested specific qualities from three aspects (sensory requirements, physicochemical indexes, and health requirements). However, this standard did not mention the limit index for citrinin, although it made a strict requirement on aflatoxin B1 (5 mg/kg).[31] RYR is classified by the Food and Drug Administration (FDA), Thailand as a red food coloring from a natural product, and regulation of citrinin in food products is controlled by legal food control operation. The regulation of the European Community (EC) No. 1881/2006, regards maximum levels as 2000 mg/kg of contaminant citrinin in food supplements based on rice fermented with red yeast *M. purpureus*.[32] Sensory evaluation of Thai traditional fermented pork sausage (Nham)[33], northeastern style Thai sausage[10], smoked sausage, and Chinese sausage[34, 35] that had RYR added as a substitute for nitrite and nitrate indicated that panelists favored the RYR colored meat product. However, applying high RYR levels in products resulted in a darker red. In addition, high RYR may result in citrinin contamination in the food product32.

For citrinin to be analyzed in contaminated samples, it must be extracted and cleaned-up prior to thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), or immunoassay if reliable results are to be obtained.[5] In addition, monacolin K degrades during storage due to the temperature, water activity (a_w) or moisture content, sunlight, and oxygen. The storage of RYR powder at temperatures lower than 30°C under vacuum packaging could enhance retention of monacolin K.[26] Therefore, impurities or contaminant labelling, shelf-life, and packaging of RYR have to be managed and declared to produce trusted high quality products for consumers.

Conclusion

RYR, a fermented product of rice by *Monascus* spp., has been used as a food additive. RYR metabolites provide nutritional and pharmacological benefits, such as monacolin K, GABA, and citrinin, etc. Therefore, whole rice and broken rice could serve as sustainable raw material for value-added products through fermentation with *Monascus* molds. However, RYR production has to be completely safe, such as when produced under optimal nutritional and environmental condition to reduce citrinin contamination. The standardization of metabolites should be provided for the quality control.

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