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RESEARCH NOTE

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Natural Occurring Levels of Citrinin and Monacolin K in Korean *Monascus* Fermentation Products

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Abstract The levels of citrinin and monacolin K in ten commercial Korean *Monascus* fermentation products were determined. The products contained citrinin at levels ranging from 0.64 to 112.27 μg/kg, with only 2 exceeding the limit of 50 μg/kg set by the Korea Food and Drug Administration (KFDA). The levels of monacolin K ranged from 0.87 to 1,030 mg/kg, however 6 products contained monacolin K at levels lower than 500 mg/kg, the level required by KFDA to be claimed as a functional food. Therefore, many commercial Korean *Monascus* fermentation products should be considered safe, however many need to be improved before being considered as functional dietary supplements.

Keywords: *Monascus*, *Monascus* fermentation product, monacolin K, citrinin

Introduction

Monascus fermentation products are gaining interest from the public and industries in Korea as they are proven to contain various bioactive compounds. Monacolin K, γ-aminobutyric acid (GABA), and dimerumic acid are major secondary metabolites produced by various *Monascus* sp. that have beneficial physiological effects (1-7). For example, monacolin K, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), is known to lower plasma cholesterol levels (1). However, some *Monascus* fermentation products contain citrinin, which has nephrotoxic and hepatotoxic properties (11).

The main form of commercialized *Monascus* fermentation products is *Monascus* fermentation rice, often called red yeast rice. Red yeast rice prepared by growing *Monascus* on steamed rice has been traditionally used as a natural pigment in red soybean gels, meats, and vegetables, as preservatives, and as *koji* for red wine making in East Asia (2, 12, 13). In addition, similar products are available in capsule or tablet form, and in combinations containing *Monascus* fermentation rice and other grains (*jabkok*), which may also be in powdered form (*sunsik*). In Korea, *Monascus* fermentation rice, *jabgok* and *sunsik* containing *Monascus* fermentation rice are sold as functional foods and manufactured by several companies. The Korea Food and Drug Administration (KFDA) has set the standard levels of monacolin K and citrinin necessary for a product to be sold as a functional food. The minimum acceptable level of monacolin K in functional food products is 500 mg/kg, and the maximum acceptable level of citrinin is 50 μg/kg. In spite of the increasing number of commercialized *Monascus* fermentation products in Korea, there are only a limited number of reports evaluating the levels of monacolin K and citrinin in these products (14). In this study, the levels of monacolin K and citrinin in various commercialized *Monascus* fermentation products available in Korean markets were evaluated by HPLC.

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Materials and Methods

Materials and reagents *Monascus* fermentation products manufactured in Korea were purchased from their manufacturers or from local markets. Their forms are summarized in Table 1. Citrinin and the lactone form of monacolin K were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The acid form of monacolin K was prepared according to the KFDA method, in which the lactone form is converted to the acid form by treatment with 0.05 N NaOH in ethyl alcohol. All solvents used in chromatography were of HPLC grade.

Extraction of citrinin and monacolin K from *Monascus* fermentation products *Monascus* fermentation products (20-30 g) in forms of rice and mixed grains were ground into powder using a blender. Two and half g portions of ground *Monascus* rice powder was resuspended in 20 mL of extraction solvent: acetone/ethyl acetate (50:50, v/v) for citrinin (15) and 80% ethanol for monacolin K (16).

For citrinin analysis, after extraction by shaking for 90 min in a 65°C water bath, the mixture was centrifuged at 5,300×g for 20 min at room temperature. The supernatant was dried in a rotary evaporator with a water bath set at 65°C. The fraction was dissolved in 2.5 mL of ethanol and filtered trough a 0.45 μm filter (PTFE acrodisc syringe filter, 0.45 μm, 25 mm; PALL Life Sciences, East Hills, NY, USA).

For monacolin K analysis, similar extraction conditions were applied except for an extraction time of 60 min at 30°C. After centrifugation at 11,000×g for 15 min at room temperature, 2 mL of supernatant was filtered through a 0.45 μm filter (PTFE acrodisc syringe filter 0.45 μm, 25 mm). Filtrates were immediately applied to HPLC.

Table 1. Citrinin and monacolin K contents in *Monascus* fermentation products

Product	Product form	Citrinin[1] (μg/kg)	Monacolin K[1] (mg/kg)		
			Acid form	Lactone form	Total
A	Rice	8.19±1.46	478.69±0.70	551.32±0.44	1030.01±1.15
B	Rice	86.45±3.05	53.46±1.96	424.86±12.89	478.32±14.85
C	Rice	112.27±16.9	35.89±0.08	331.00±0.40	366.90±0.48
D	Rice	26.51±17.6	76.97±0.09	29.62±0.11	106.59±0.02
E	Rice	4.89±1.10	24.13±0.03	5.91±0.01	30.03±0.03
F	Rice	0.64±0.25	27.16±0.01	11.43±0.01	38.60±0.01
G	Rice	3.83±0.82	148.83±0.11	26.49±0.01	175.33±0.11
H	Rice mixed with other grains	0.83±0.03	1.84±0.02	17.15±0.33	18.98±0.35
I	Mixed powder of rice and other grains	3.77±1.17	0.24±0.00	0.63±0.01	0.87±0.01
J	Rice powder	1.76±0.87	232.74±0.25	127.88±0.17	360.62±0.42

[1]Data are means±SEM derived from 2 separate experiments.

HPLC analysis HPLC analysis of citrinin was performed using a Waters system [515 HPLC pump, 717 plus autosampler, 2475 multi λ fluorescence detector (Waters Corp., New Castle, DE, USA)] equipped with a Hypersil Gold column (4.6×150 mm, 5 μm; Thermo, Waltham, MA, USA). The mobile phase was methanol/acetonitrile/water, pH 2.5 (30/30/40, v/v/v) and run at a flow rate of 0.6 mL/min (17). Chromatograms were monitored using a fluorescence detector set at an excitation wavelength of 330 nm and an emission wavelength of 500 nm. The absorbance data were analyzed using the Waters system empowers software. The quantity of citrinin was calculated by comparing peak height to that of the citrinin standard.

Monacolin K analysis was carried out using a Jasco HPLC (PU-2089 pump, AS-2055 intelligent sampler, UV-2075 detector; Jasco Co., Makuhari Messe, Japan) equipped with a Hypersil Gold column (4.6×150 mm, 5 μm). Samples were separated with a mobile phase of acetonitrile/0.1% phosphoric acid, pH 2.5/water (30/30/40, v/v/v) at a flow rate of 0.5 mL/min (16). Chromatograms were monitored using an UV detector set at 238 nm. The quantity of monacolin K was determined by comparing the peak area with that of the monacolin K standard.

Results and Discussion

Because *Monascus* fermentation products may serve as multi-functional dietary supplements that can also prevent heart disease, their demand is expected to increase. *Monascus* fermentation products should contain sufficient amounts of bioactive compounds for them to exert beneficial physiological effects. In addition, they should not exceed what are considered to be safe levels of citrinin and thus risk having hepatotoxic and nephrotoxic effects. Therefore, we evaluated the levels of citrinin in various Korean *Monascus* fermentation products available in markets.

The various *Monascus* fermentation products were analyzed for the presence of citrinin by HPLC. The citrinin standard was identified by a retention time of 7.2 min (Fig. 1A). The extracts of most products showed several peaks at the retention time expected for citrinin (Fig. 1B), and the citrinin peak was confirmed by spiking extracts with the citrinin standard (Fig. 1C). The *Monascus* fermentation products tested contained citrinin at levels ranging from 0.64 to 112.27 μg/kg (Table 1). Thus all but

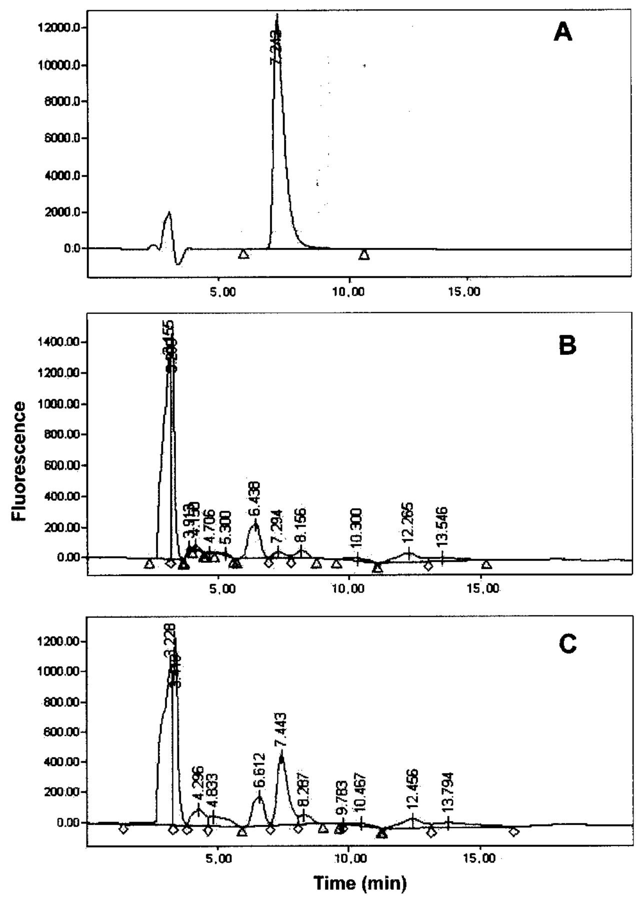

Fig. 1. Citrinin analysis by HPLC. (A) Citrinin standard (2 μg). (B) An extract of product B. (C) The extract of product B spiked with citrinin standard (7 ng).

two of the Korean food products tested contain citrinin at levels lower than 50 μg/kg, the limit set by KFDA. Sabater-Vilar *et al.* (10) reported that *Monascus* fermentation rice obtained from various companies in Europe contained citrinin at levels of 200-17,100 μg/kg. Commercial *Monascus* fermentation products in rice, tablet, and capsule form from Taiwan were shown to contain citrinin levels ranging from 280-6,290 μg/kg (15). In Chinese *Monascus* fermentation rice, the levels of citrinin can be as high as 12,993 μg/kg (14, 18). The levels of citrinin in commercial Korean *Monascus* fermentation products thus seem to be lower than products from other countries. The two Korean products that exceeded KFDA limits could be due to fermentation conditions in the production of particular lots, since fermentation conditions can greatly affect the production of citrinin. In general, Korean *Monascus* fermentation products should be considered as safe, however strict control in their manufacturing should be applied to protect consumers from excessive levels of citrinin.

Because both the acid and lactone forms of monacolin K are known to be beneficial for lowering cholesterol levels *in vivo*, the levels of these two forms of monacolin K in various *Monascus* fermentation products were determined by HPLC. The acid and lactone forms were identified by their retention times of 8.6 and 12.2 min, respectively (Fig. 2). *Monascus* fermentation products contained monacolin K at levels ranging from 0.87 to 1,030 mg/kg (Table 1). Several products contained monacolin K at levels lower than the minimum standard set by the

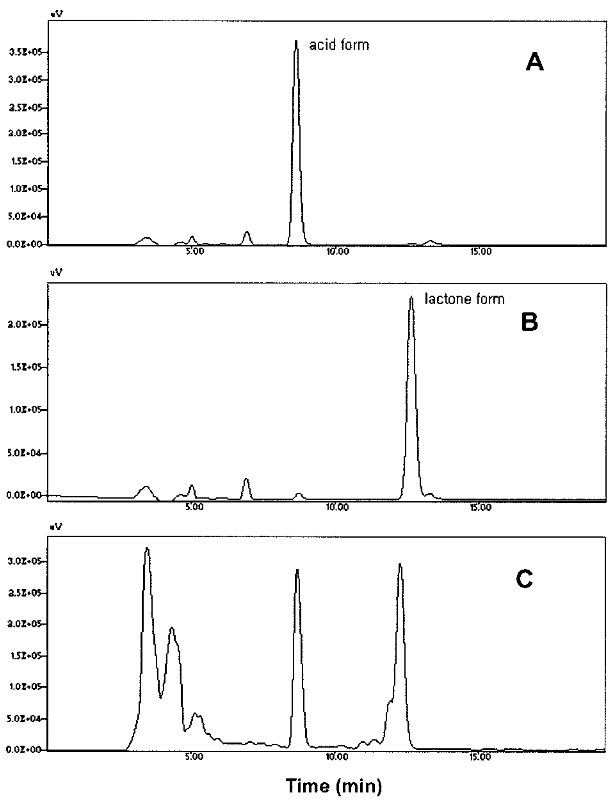

Fig. 2. Monacolin K analysis by HPLC. (A) The acid form of standard monacolin K (0.89 μg); (B) The lactone form of standard monacolin K (1 μg); (C) An extract of product A.

KFDA. *Monascus* processed foods such as the product H and I may contain lower levels of monacolin K than other products since they are produced by mixing a small amount of *Monascus* rice with other food materials. Since *Monascus* fermentation products are generally consumed as dietary supplements for their beneficial cholesterol-lowering effects, monacolin K levels should be increased in several products. Previous analysis of several *Monascus* rice products manufactured in Taiwan, China, and Malaysia showed that they contained the lactone form at levels ranging from 182 to 15,801 mg/kg, and the acid form ranging from 15.7 to 15,577 mg/kg (18). Recently, Huang *et al.* (19) reported that red yeast rice from Fujian province, China contained monacolin K at levels ranging from 310 to 3,100 mg/kg.

In summary, most Korean *Monascus* fermentation products met the standards set by KFDA by not exceeding the limits of citrinin content, and by containing sufficient levels of monacolin K to qualify as functional foods. Several products, however, contained citrinin levels exceeding the KFDA standard, and levels of monacolin K beneath the minimum KFDA standard. Therefore, manufacturers should make the changes necessary to meet these standards and thus produce food products that are safe and contribute to public health.

Acknowledgments

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