

## Storage stability of monascal adlay

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### Abstract

*Monascus purpureus* was inoculated into cooked adlay and new products, monascal dehulled adlay (MDA) and monascal polished adlay (MPA), were produced after fungal fermentation. MDA contained higher protein and lower carbohydrate contents than MPA. However, no difference was found in crude ash, fat and fibre contents. During storage at 25 °C, the acid and peroxide values of these two products significantly increased at month six. At months 0–3, the contents of coixenolide, monacolin K and  $\gamma$ -aminobutyric acid in MDA and MPA were in the range of 532–540  $\mu\text{g g}^{-1}$ , 1.05–1.13  $\text{mg g}^{-1}$  and 489–497  $\mu\text{g g}^{-1}$ , respectively, but at month six, decreased to 487–497  $\mu\text{g g}^{-1}$ , 0.71–0.84  $\text{mg g}^{-1}$  and 397–419  $\mu\text{g g}^{-1}$ , respectively. Three major fatty acids found were in descending order of oleic, linoleic and palmitic acids. At month six, the percentages of oleic and linoleic acids significantly decreased. Based on the results obtained, MDA and MPA were slightly different in proximate composition and comparable in their content of functional components and their fatty acid profiles. Also, these two products were shelf stable at 25 °C for three months.

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**Keywords:** Adlay; *Monascus*; Coixenolide; Monacolin K;  $\gamma$ -Aminobutyric acid; Storage

### 1. Introduction

The fungal genus *Monascus* has been used to prepare red fermented rice (anka, red koji) as a food colorant and traditional medicine in oriental countries for centuries (Bau & Mo, 1975). Metabolic products from fermentation of *Monascus* species are commonly utilized as pigments or as antimicrobial agents. The components isolated from the fungus exert several biological actions and produce hypocholesterolemic (Endo, 1979, 1980), liver-protective and antitumor effects (Aniya, Yokomakura, Yonamine, Nagamine, & Nakanishi, 1998; Yasukawa, Takahashi, Yamanouchi, & Takido, 1996). Monacolin K, also called lovastatin, mevicolin and mevacor, one of the secondary metabolites from *Monascus* species, has been demonstrated as a specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in cholesterol biosynthesis (Al-

berts et al., 1980; Endo, 1980). Accordingly, *Monascus*-fermented products have been used as a functional dietary supplement to reduce cholesterol levels in the human body (Tobert et al., 1982).

$\gamma$ -Aminobutyric acid (GABA) in the aqueous extract of *Monascus*-fermented rice has been shown decrease blood pressure in vivo (Kohama et al., 1987; Kushiro et al., 1996) without affecting electrolyte metabolism or the activity of angiotensin-I-converting enzyme (Tsuji et al., 1992). In addition, dimeric acid and polyketide pigments isolated from red koji have shown several physiological functions (Aniya et al., 2000; Izawa et al., 1997).

Adlay (Chinese pearl barley, soft-shelled Job's tears, *Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a grass crop that has long been used in traditional Chinese medicine and as a nourishing food, due to its high nutritional value and special biological and functional effects on the human body. Adlay is widely planted in Taiwan, China, and Japan, where it is considered a healthy food supplement. According to the ancient Chinese medical book *Pen-Tsao-Kang-Mu* (Li, 1596), the seed of adlay was used in China for the treatment of warts, chapped skin,

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rheumatism and neuralgia, and as an anti-inflammatory or antihelminthic agent. Coixenolide [1-methyl-2-(*cis*-9-hexadecenoyloxy) propyl *trans*-11-octadecenoate, C<sub>38</sub>H<sub>70</sub>O<sub>4</sub>], which was isolated from the adlay seed, exhibited antitumor activity towards Ehrlich ascites sarcoma in mice (Tanimura, 1961; Ukita & Tanimura, 1961). Numerous recent reports have also indicated that the consumption of adlay seed is beneficial to the human body (Chiang, Cheng, Chiang, & Chung, 2000; Kondo, Nakajima, Nozoe, & Suzuki, 1998; Kuo, Shih, Kuo, & Chiang, 2000; Otsuka, Hirai, Nagao, & Yamaski, 1988; Tsai, Yang, & Hsu, 1999; Yang & Tsai, 1998).

Both the fungus *Monascus* and adlay possess functional components effective in maintaining human health. The fungus was inoculated into cooked adlay and a new monascal product was then produced after the colonization of fungal mycelia. The functional components of adlay are still present in the fermented adlay products along with those produced by the fungus (Chang, 2001). However, lipid oxidation and changes in the functional components are indicators of storage quality of this monascal adlay. Therefore, our objective was to evaluate the composition changes in monascal adlay products during storage at 25 °C for six months, including monascal polished adlay (MPA) and monascal dehulled adlay (MDA). The composition changes of acid and peroxide values and fatty acid composition of the adlay oil and the functional components such as monacolin K, GABA and coixenolide were determined at 0, 1, 2, 3 and 6 months. The proximate composition of MPA and MDA was also analysed before storage.

## 2. Materials and methods

### 2.1. Monascal adlay products

Polished adlay [Taichung Selected No.4 (TCS 4)] (PA) was purchased at a local market in Taichung City, Taiwan. Dehulled adlay (DA) was obtained from a farm at Erhlin, Changhua County, Taiwan. *Monascus purpureus* Went (CCRC 31498) was obtained from the Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu City, Taiwan. The fungus was inoculated onto malt extract agar (Difco) and incubated at 25 °C for 72 h. After pure culture was obtained, the mycelium was re-inoculated into potato dextrose broth (Difco) and incubated at 25 °C for 7 days. The culture was then homogenized in a Waring blender and inoculated into two kinds of autoclaved adlay, PA and DA, respectively, at an inoculation rate of 5%.

New corresponding products, MPA and MDA, respectively, were then produced after the colonization of fungal mycelia for 7 days at 25 °C. Two *Monascus*-colonized adlay products were air-dried in an oven at 40

°C. For each product, three dried samples (~50 g each) were randomly selected and ground using a mill (Retsch ultra centrifugal mill and sieving machine, Haan, Germany) to obtain a coarse powder (20 mesh).

### 2.2. Proximate analysis and storage study

The proximate compositions of two kinds of monascal adlay products, including moisture, carbohydrate, crude ash, crude fat, crude fibre and crude protein, were determined according to AOAC (1990) methods. The nitrogen factor used for crude protein calculation was 5.83 (AOAC, 1990). The carbohydrate content was calculated by subtracting the contents of crude ash, fat, fiber and protein from 100% of dry matter and expressed as the percentage of dry weight. Two monascal adlay products were stored at room temperature (25 °C) and three samples from each product were taken at month 0, 1, 2, 3 and 6 for quality study.

### 2.3. Acid and peroxide value determination

Crude oil was extracted from monascal adlay products in the Soxhlet apparatus using diethyl ether according to the method of AOAC (1990). The acid value (AV) and peroxide value (POV) of products were determined by the method of AOAC (1990) and expressed as mg of potassium hydroxide per gram of sample and as milliequivalents of peroxide per kg of sample, respectively.

### 2.4. Coixenolide assay

Coixenolide was determined by measuring 2,3-butanediol liberated from coixenolide by acid-catalyzed transesterification using the method modified from Chen, Chang, and Chiang (1995). The crude oil extracted from monascal adlay products (1 g) was dissolved in 20 ml of 7 % (w/w) methanolic HCl solution, and the mixture was then refluxed in a water bath at 100 °C for 4 h. 1,5-Pentanediol (Sigma Chemical Co., St. Louis, MO) was added to the reaction mixture as an internal standard after acid-catalyzed transesterification. The mixture was cooled and neutralized with 30% methanolic sodium methoxide (Sigma). The salt thus precipitated was filtered and the filtrate was rotary evaporated to a final volume of 2 ml.

The concentrated methanolic solution was analysed using a Hewlett–Packard 5890A Series II gas chromatograph (GC) equipped with a flame ionisation detector (FID). A fused silica capillary column (30 m × 0.25 mm ID) coated with Stabilwax (0.25 µm film thickness, Restek Corp., Bellefonte, PA) was used. The carrier gas was nitrogen at a flow rate of 30 cm/min. The column temperature was programmed from 100 to 250 °C at 10 °C min<sup>-1</sup>. Injector and detector temperatures

were both 250 °C. Coixenolide content was converted from the 2,3-butanediol content, which was quantified by comparing its peak area to that of the internal standard (Sigma).

### 2.5. Fatty acid composition determination

The crude oil extracted from monaschal products was methylated according to the method of Metcalfe, Schmitz, and Pelka (1966). The fatty acid composition was analysed using a Hitachi G-3000 GC equipped with an FID. A stainless steel column (2 m × 1.8 mm ID) packed with GP10% SP2330 adsorbed on 100/120 Chromosorb W/AW (Supelco Co., Bellefonte, PA) was used. The carrier gas was nitrogen at a flow rate of 25 ml min<sup>-1</sup>. The column temperature was programmed from 105 to 200 °C at 2 °C min<sup>-1</sup>. Injector and detector temperatures were both 250 °C. Fatty acid methyl ester mix (C8–C24, Supelco) was used as the external standards for peak identification. Each fatty acid was expressed as the percentage of total fatty acid peak area.

### 2.6. Monacolin K assay

Monaschal adlay powder (0.5 g) was dissolved in 10 ml of 75% aqueous ethanol, sonicated for 60 min, centrifuged at for 10 min, and filtered through a 0.45 µm CA non-sterile filter (3 mm, Lida, Corp., Kenosha, WI). The filtrate was injected onto a high-performance liquid chromatograph (HPLC). The HPLC system consisted of a Hitachi L-6000 pump, a Rheodyne 7161 injector, a 20 µl sample loop, a Hitachi D-2500 chromato-integrator, a Hitachi L-4000 UV detector, and a Prodigy 5 ODS-2 column (4.6 × 250 mm, 5 µm, Phenomenex Inc., Torrance, CA). The mobile phase was acetonitrile (LC grade, Tedia Co., Fairfield, OH)/deionised water, 70:30 (v/v) at a flow rate of 1 ml min<sup>-1</sup> and UV detection at 254 nm was used. Monacolin k was quantified by the calibration curve of the authentic standard (Lovacor, Hsien-Tai Pharmaceuticals Inc., Tainan, Taiwan).

### 2.7. $\gamma$ -Aminobutyric acid (GABA) assay

Monaschal adlay powder (500 mg) was shaken with 50 ml of 0.1 N HCl solution for 45 min at ambient temperature and filtered through Whatman No. 4 filter paper. The filtrate was then passed through a filter unit (13 mm, Lida), and filtered using 0.45 µm CA non-sterile filter (Lida). This filtrate was mixed with *o*-phthalaldehyde reagent (Sigma) in an Eppendorf tube, shaken to facilitate derivatisation and then immediately injected onto the HPLC.

The HPLC system was the same as for monacolin K analysis but included a Hitachi F-1050 fluorescence detector with fluorescence excitation at 340 nm and emission at 450 nm. The mobile phases were A, 50 mM

sodium acetate (pH 5.7) containing 0.5% tetrahydrofuran; B, deionised water; and C, methanol (Mau, Chyau, Li, & Tseng, 1997). The gradient was A:B:C 83:0:17 to 33:0:67 for 0–37 min, 0:33:67 for 37–40 min, and 0:100:0 for 40–43 min at the flow rate of 1.2 ml min<sup>-1</sup> (Mau et al., 1997). GABA was quantified by the calibration curve of the authentic standard (Sigma).

### 2.8. Statistical analysis

For each monaschal adlay product, three samples were used for the determination of every quality attribute. The experimental data were subjected to an analysis of variance for a completely random design as described by Steel, Torrie, and Dickey (1997), to determine the least significant difference among means at the level of 0.05.

## 3. Results and discussion

Moisture content was higher in MDA than in MPA (Table 1). MDA contained higher protein and lower carbohydrate contents than MPA. However, no difference was found in crude ash, fat and fibre contents. Generally, one of the beneficial effects of dehulled grain over polished grain was the higher content of crude fibre (7.85–8.72% vs. 6.30–6.45%) (Tsai & Chiang, 1993). Surprisingly, both crude fibre contents in MDA and MPA were similar and much higher than those in raw materials before fungal fermentation.

As compared to dehulled and polished adlay in Chang (2001), carbohydrate contents were much lower in the monaschal adlay (72.3–72.9% vs. 37.0–42.8%). In addition, Tsai and Chiang (1993) reported that dehulled and polished adlay contained 74.5% and 77.9% carbohydrate, respectively. The reduction in carbohydrate content was apparently due to the growth of fungal mycelia, which consumed carbohydrate to generate energy and synthesize biomaterials. Accordingly, contents

Table 1  
Proximate composition of two monaschal adlay products before storage

Component <sup>a</sup>	Content <sup>b</sup> (%)	
	MDA <sup>c</sup>	MPA <sup>c</sup>
Moisture	14.2 ± 0.23a	12.6 ± 0.15b
Dry matter	85.8 ± 0.23b	87.4 ± 0.15a
Carbohydrate	37.0 ± 1.96b	42.8 ± 1.18a
Crude ash	6.87 ± 0.67a	6.97 ± 0.54a
Crude fat	15.4 ± 1.41a	13.6 ± 1.36a
Crude fibre	15.9 ± 1.67a	14.4 ± 0.98a
Crude protein	24.8 ± 1.58a	22.3 ± 0.68b

<sup>a</sup> Moisture and dry matter were presented based on fresh weight, others were presented based on dry weight.

<sup>b</sup> Each value is expressed as mean ± SD (*n* = 3). Means with different letters within a row are significantly different (*p* < 0.05).

<sup>c</sup> MDA, monaschal dehulled adlay; MPA, monaschal polished adlay.

of crude ash, fat, fibre and protein in the monascal products were correspondingly increased. Also, high contents of crude fat, fibre and protein in the monascal adlay partially resulted from the mycelial growth. However, using rice as a substrate, *Monascus*-fermented rice contained 6.0% of moisture, 73.4% of carbohydrate, 0.8% of fibre, 2.8% of fatty acids and 14.7% of protein (Ma et al., 2000). Unexpectedly, in addition to their functional components, it seemed that the monascal adlay products became a better nutraceutical grain and were a good source of fat, fibre and protein as compared to conventional monascal rice, which only contained the functional components, monacolin K and GABA, formed from the growth of *Monascus* sp.

During storage at 25 °C, the acid values of monascal adlay products were low and similar at months 0 to 2 (6.35–7.08 mg KOH g<sup>-1</sup>), but increased at month three and doubled at month six (Table 2). The peroxide values were similar at months 0–3 (3.70–4.01 meq kg<sup>-1</sup>) but almost doubled at month six. Also, no difference in acid and peroxide values between the two products was found. However, at month six, these acid and peroxide values were still low. Since the acid and peroxide values are indices of hydrolytic rancidity and early stages of oxidation, the results showed that these two monascal adlay products were shelf stable for at least three months at 25 °C.

Huang and Chiang (1997) found that at months 0, 3 and 6, the acid values of dehulled adlay stored at 25 °C were 4.6, 23.1 and 25.8 mg KOH g<sup>-1</sup>, respectively, whereas the peroxide values were 2.3, 3.9 and 5.7 meq kg<sup>-1</sup>, respectively. Obviously, at months three and six, the acid values of MDA and MPA were much lower than those of dehulled adlay found in Huang and Chiang (1997). It seemed that monascal adlay products were resistant to hydrolytic rancidity. One reason for this discrepancy is that liberated fatty acids originally present in the raw adlay might have been consumed by the growing fungus. However, the peroxide values of MDA and MPA were similar to those of dehulled adlay

in Huang and Chiang (1997). It seemed that monascal adlay did not produce any antioxidant effective in inhibiting the formation of peroxides.

The contents of coixenolide in MDA and MPA were in the range of 532–540 µg g<sup>-1</sup> at months 0–3, but decreased to 487–497 µg g<sup>-1</sup> at month six. Since the acid and peroxide values increased significantly at month six, the reduction in the coixenolide content might be as a result of hydrolytic rancidity and oxidation of adlay oil. However, at months 0, 3 and 6, the coixenolide content in dehulled adlay stored at 25 °C were 564, 285 and 226 µg g<sup>-1</sup>, respectively, (Huang & Chiang, 1997). At month zero, the coixenolide contents in MDA and MPA were similar to that in dehulled adlay (Huang & Chiang, 1997). However, after three and six months of storage at 25 °C, the coixenolide content in dehulled adlay significantly dropped (Huang & Chiang, 1997). It was revealed that adlay after monascal fermentation might produce some components effective in preventing the degradation of coixenolide.

Three major fatty acids found in MDA and MPA were in descending order of oleic, linoleic and palmitic acids, which represent predominantly monounsaturated, polyunsaturated and saturated fatty acids, respectively, (Tables 3 and 4). The percentage of palmitic acid was higher in MPA (21.4%) than in MDA (18.2%) before storage, whereas the percentages of oleic and linoleic acids were comparable for two monascal adlay products. For both MDA and MPA, the fatty acid profiles were similar after storage for three months. However, at month six, the percentages of mono- and polyunsaturated fatty acids, mainly oleic and linoleic acids significantly decreased due to lipid oxidation as evidenced by the increased acid and peroxide values. Therefore, the percentage of saturated fatty acids, mainly palmitic acid, was correspondingly increased. Based on this result, the products were not recommended for storage at 25 °C for 6 months.

Huang, Chen, and Chiang (1994) reported that oleic (48.8–49.7%), linoleic (27.9–29.3%) and palmitic acids

Table 2  
Acid and peroxide values and coixenolide content of monascal adlay products during storage at 25 °C for six months

Product <sup>a</sup>	Month 0	1	2	3	6
Acid value <sup>b</sup> (mg KOH g <sup>-1</sup> )					
MDA	a6.35 ± 0.71C	a6.51 ± 0.92C	a6.45 ± 0.62C	a8.69 ± 1.02B	a13.7 ± 2.18A
MPA	a6.51 ± 0.44C	a6.67 ± 0.71C	a7.08 ± 0.93B	a7.51 ± 0.64B	a16.0 ± 1.67A
Peroxide value <sup>b</sup> (meq kg <sup>-1</sup> )					
MDA	a3.70 ± 0.41B	a3.73 ± 0.32B	a3.85 ± 0.62B	a3.98 ± 1.02B	a6.57 ± 1.59A
MPA	a3.80 ± 0.47B	a3.91 ± 0.54B	a3.97 ± 0.73B	a4.01 ± 0.34B	a8.54 ± 1.97A
Coixenolide content <sup>b</sup> (µg g <sup>-1</sup> )					
MDA	a537 ± 6A	a534 ± 4A	a540 ± 5A	a538 ± 3A	a497 ± 3B
MPA	a532 ± 4A	a534 ± 3A	a532 ± 3A	a533 ± 4A	a487 ± 6B

<sup>a</sup> MDA, monascal dehulled adlay; MPA, monascal polished adlay.

<sup>b</sup> Each value is expressed as mean ± SD (*n* = 3). Means with different capital letters within a row are significantly different (*p* < 0.05). Means with different small letters within a column are significantly different (*p* < 0.05).

Table 3  
Fatty acid composition in monascal dehulled adlay during storage at 25 °C for six months

Fatty acid (Carbon no.: double bond)	% Total fatty acid <sup>a</sup>				
	Month 0	1	2	3	6
Tetradecanoic (14:0)	0.02 ± 0.01c	0.02 ± 0.01c	0.04 ± 0.01ab	0.03 ± 0.01bc	0.05 ± 0.01a
Palmitic (16:0)	18.2 ± 3.18b	18.1 ± 1.37b	18.8 ± 2.53b	19.0 ± 1.69b	24.9 ± 1.69a
Palmitoleic (16:1)	0.23 ± 0.02ab	0.18 ± 0.04b	0.21 ± 0.07b	0.20 ± 0.05b	0.26 ± 0.06a
Margaric (17:0)	0.77 ± 0.03a	0.79 ± 0.12a	0.78 ± 0.09a	0.70 ± 0.15a	0.88 ± 0.25a
Stearic (18:0)	2.38 ± 0.36b	2.34 ± 0.53b	2.44 ± 0.27b	2.38 ± 0.39b	3.67 ± 0.69a
Oleic (18:1)	45.1 ± 3.90a	44.2 ± 2.93a	43.9 ± 3.45a	44.3 ± 3.96a	39.3 ± 4.57b
Linoleic (18:2)	30.5 ± 2.41a	31.3 ± 1.27a	30.9 ± 1.56a	30.8 ± 2.33a	28.4 ± 1.67b
Linolenic (18:3)	1.55 ± 0.27ab	1.69 ± 0.25a	1.65 ± 0.36a	1.49 ± 0.09b	1.27 ± 0.18c
Arachidic (20:0)	0.49 ± 0.06b	0.53 ± 0.08a	0.42 ± 0.09c	0.47 ± 0.07b	0.59 ± 0.05a
Henicosanoic (21:0)	0.37 ± 0.05ab	0.41 ± 0.03a	0.33 ± 0.09bc	0.27 ± 0.04c	0.37 ± 0.05ab
Behenic (22:0)	0.26 ± 0.05b	0.26 ± 0.05b	0.30 ± 0.03a	0.24 ± 0.02bc	0.21 ± 0.03c
Tetracosanoic (24:0)	0.15 ± 0.08a	0.18 ± 0.02a	0.20 ± 0.04a	0.17 ± 0.03a	0.15 ± 0.02a
SFA <sup>b</sup>	22.6 ± 3.82b	22.6 ± 2.21b	23.3 ± 3.15b	23.2 ± 2.40b	30.8 ± 2.79a
MUFA <sup>b</sup>	45.4 ± 3.92a	44.4 ± 2.97a	44.1 ± 3.52a	44.5 ± 4.01a	39.5 ± 4.63b
PUFA <sup>b</sup>	32.0 ± 2.68a	33.0 ± 1.52a	32.6 ± 1.92a	32.3 ± 2.42a	29.7 ± 1.85b

<sup>a</sup> Each value is expressed as mean ± SD ( $n = 3$ ). Means with different letters within a row are significantly different ( $p < 0.05$ ).

<sup>b</sup> SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 4  
Fatty acid composition in monascal polished adlay during storage at 25 °C for six months

Fatty acid (Carbon no.: double bond)	% Total fatty acid <sup>a</sup>				
	month 0	1	2	3	6
Tetradecanoic (14:0)	0.09 ± 0.01b	0.10 ± 0.02b	0.08 ± 0.01b	0.09 ± 0.02b	1.57 ± 0.38a
Palmitic (16:0)	21.4 ± 2.36c	22.8 ± 2.37b	22.9 ± 3.69b	23.8 ± 3.26b	25.4 ± 5.69a
Palmitoleic (16:1)	0.21 ± 0.01ab	0.23 ± 0.03a	0.19 ± 0.02bc	0.21 ± 0.05ab	0.17 ± 0.07c
Margaric (17:0)	0.91 ± 0.12b	0.95 ± 0.07b	0.95 ± 0.06b	0.91 ± 0.09b	1.82 ± 0.14a
Stearic (18:0)	1.70 ± 0.57a	1.60 ± 0.37a	1.67 ± 0.26a	1.76 ± 0.09a	1.85 ± 0.34a
Oleic (18:1)	43.6 ± 3.54a	42.8 ± 3.29a	42.7 ± 3.67a	42.5 ± 2.73a	40.1 ± 3.42b
Linoleic (18:2)	29.3 ± 3.19a	28.9 ± 2.76a	28.7 ± 1.96a	28.0 ± 1.26a	26.0 ± 3.12b
Linolenic (18:3)	1.37 ± 0.05a	1.22 ± 0.07c	1.31 ± 0.05ab	1.28 ± 0.09bc	1.12 ± 0.13d
Arachidic (20:0)	0.52 ± 0.02b	0.51 ± 0.13b	0.47 ± 0.07b	0.50 ± 0.06b	0.69 ± 0.09a
Henicosanoic (21:0)	0.52 ± 0.03b	0.49 ± 0.05b	0.53 ± 0.03b	0.56 ± 0.03b	0.63 ± 0.04a
Behenic (22:0)	0.29 ± 0.06b	0.26 ± 0.02b	0.25 ± 0.03b	0.24 ± 0.05b	0.38 ± 0.06a
Tetracosanoic (24:0)	0.21 ± 0.01b	0.19 ± 0.09b	0.18 ± 0.07b	0.19 ± 0.05b	0.25 ± 0.03a
SFA <sup>b</sup>	25.6 ± 3.18c	26.9 ± 3.12b	27.0 ± 4.22b	28.1 ± 3.65b	32.6 ± 6.85a
MUFA <sup>b</sup>	43.8 ± 3.55a	43.0 ± 3.32a	42.9 ± 3.69a	42.7 ± 2.78a	40.3 ± 3.49b
PUFA <sup>b</sup>	30.7 ± 3.24a	30.1 ± 2.83a	30.1 ± 2.01a	29.3 ± 1.35a	27.1 ± 3.25b

<sup>a</sup> Each value is expressed as mean ± SD ( $n = 3$ ). Means with different letters within a row are significantly different ( $p < 0.05$ ).

<sup>b</sup> SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

(13.2–13.9%) were three major fatty acids in polished adlay. Apparently, after fungal fermentation, MDA and MPA showed slightly different fatty acid profiles. In addition, the fatty acid composition in *Monascus*-fermented rice was calculated to be linoleic (21.8%), palmitic (19.7%), oleic (17.6%) and linolenic acids (12.7%) (Ma et al., 2000). Obviously, the fatty acid profile of *Monascus*-fermented rice was notably different from those of MDA and MPA. It seemed that the substrate used was the major factor contributing to the discrepancy in the fatty acid profiles.

During storage of MDA and MPA at 25 °C for three months, the contents of monacolin K and GABA were

in the range of 1.05–1.13 mg g<sup>-1</sup> and 489–497 µg g<sup>-1</sup>, respectively (Table 5). No difference was found in the contents of monacolin K and GABA between MDA and MPA. However, at month 6, the contents of monacolin K and GABA decreased to 0.71–0.84 mg g<sup>-1</sup> and 397–419 µg g<sup>-1</sup>, respectively. Like coixenolide and fatty acid composition, the reduction in the monacolin K and GABA contents might be related to lipid degradation.

Since both *Monascus*-fermented rice and adlay are used as foods, the newly developed monascal adlay products are undoubtedly consumed as a traditional food. The only concern about their safety for human

Table 5  
Contents of monacolin K and  $\gamma$ -aminobutyric acid (GABA) of monascad adlay products during storage at 25 °C for six months

Product <sup>a</sup>	Month 0	1	2	3	6
Monacolin K content <sup>b</sup> (mg g <sup>-1</sup> )					
MDA	a1.12 ± 0.11A	a1.07 ± 0.05A	a1.05 ± 0.04A	a1.13 ± 0.02A	a0.84 ± 0.08B
MPA	a1.09 ± 0.14A	a1.10 ± 0.07A	a1.06 ± 0.10A	a1.09 ± 0.10A	a0.71 ± 0.06B
GABA content <sup>b</sup> (μg g <sup>-1</sup> )					
MDA	a497 ± 5A	a494 ± 4A	a496 ± 2A	a489 ± 4A	a419 ± 6B
MPA	a489 ± 4A	a492 ± 3A	a489 ± 6A	a493 ± 3A	b397 ± 5B

<sup>a</sup> MDA, monascad dehulled adlay; MPA, monascad polished adlay.

<sup>b</sup> Each value is expressed as mean ± SD ( $n = 3$ ). Means with different capital letters within a row are significantly different ( $p < 0.05$ ). Means with different small letters within a column are significantly different ( $p < 0.05$ ).

consumption is the possible existence of citrinin (Blanc et al., 1995). However, citrinin was not present in these products at the detection limit of 1 μg g<sup>-1</sup> (Chang, 2001).

Based on the results obtained, these two inoculated adlay products were slightly different in proximate composition and comparable in their composition of functional components and fatty acids. Also, these two products were shelf stable at 25 °C for three months. In order to maintain their quality for a prolonged time, storage temperatures lower than 25 °C, such as 4 °C, was strongly recommended to lower the inevitable lipid oxidation. In addition, the study on the physiological effects of these two new products with the combination of the functional components from *Monascus* species and adlay on humans would be an area worthy of investigation.

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## References

- Alberts, A. W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E., Patchett, A., Monaghan, R., Currie, S., Stapley, E., Albers-Schnoberg, G., Hensens, O., Hirschfield, J., Hoogsteen, K., Liesch, J., & Springer, J. P. (1980). Mevinolin, a higher potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase. *Proceedings of the National Academy of Sciences of the USA*, 77, 3957–3961.
- Aniya, Y., Ohtani, I. I., Higa, T., Miyagi, C., Gibo, H., Shimabukuro, M., Nakanishi, H., & Taira, J. (2000). Dimeric acid as an antioxidant of the mold, *Monascus anka*. *Free Radical Biology and Medicine*, 28, 999–1004.
- Aniya, Y., Yokomakura, T., Yonamine, M., Nagamine, T., & Nakanishi, H. (1998). Protective effect of the mold *Monascus anka* against acetaminophen-induced liver toxicity in rats. *Japanese Journal of Pharmacology*, 78, 79–82.
- AOAC., 1990. Official methods of analysis (15th ed.) Washington, DC: Association of Official Analytical Chemists.
- Bau, Y. S., & Mo, C. F. (1975). The uses and culturing methods of *Monascus purpureus* Went. *New Asia College Academy Annual*, 17, 335–357.
- Blanc, P. J., Laussac, J. P., Le Bars, J., Le Bars, P., Loret, M. O., Pareilleux, A., Prome, D., Prome, J. C., Santerre, A. L., & Goma, G. (1995). Characterization of monascidin A from *Monascus* as citrinin. *International Journal of Food Microbiology*, 27, 201–213.
- Chang, H.L., (2001). Evaluation of taste quality, antioxidant properties and storage stability of monascad adlay. Master's thesis, National Chung-Hsing University, Taichung, Taiwan.
- Chen, Y.-F., Chang, T.-W., & Chiang, W. (1995). Quantification of coixenolide in adlay by measuring 2,3-butanediol. *Food Science*, 22, 327–363.
- Chiang, W., Cheng, C. Y., Chiang, M. T., & Chung, K. T. (2000). Effects of dehulled adlay on the culture of some microbiota and their metabolism in the gastrointestinal tract of rats. *Journal of Agricultural and Food Chemistry*, 48, 829–832.
- Endo, A. (1979). Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. *Journal of Antibiotics*, 32, 852–854.
- Endo, A. (1980). Monacolin K, a new hypocholesterolemic agent that specifically inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Journal of Antibiotics*, 33, 334–336.
- Huang, S.-L., Chen, Y.-F., & Chiang, W. (1994). Amino acids, fatty acids and proximate composition of the seed of adlay. *Food Science*, 21, 67–74.
- Huang, S.-L., & Chiang, W. (1997). Changes of coixenolide content, acid value, peroxide value and fatty acid composition of the oil in adlay seed during storage. *Food Science*, 24, 365–375.
- Izawa, S., Harada, N., Watanabe, T., Kotokawa, N., Yamamoto, A., Hayatsu, H., & Arimoto-Kobayashi, S. (1997). Inhibitory effects of food-coloring agents derived from *Monascus* on the mutagenicity of heterocyclic amines. *Journal of Agricultural and Food Chemistry*, 45, 3980–3984.
- Kohama, Y., Matsumoto, S., Mimura, T., Tanabe, N., Inada, A., & Nakanishi, T. (1987). Isolation and identification of hypotensive principles in red-mold rice. *Chemical & Pharmaceutical Bulletin*, 35, 2484–2489.
- Kondo, Y., Nakajima, K., Nozoe, S., & Suzuki, S. (1998). Isolation of ovulatory-active substances from crops of Job's tears (*Coix lachryma-jobi* L. var. *mayuen* (Roman.) Stapf.). *Chemical & Pharmaceutical Bulletin*, 36, 3147–3152.
- Kuo, C. C., Shih, M. C., Kuo, Y. H., & Chiang, W. (2000). Antagonism of free-radical-induced damage of adlay seed and its antiproliferative effect in human histolytic lymphoma U937. *Journal of Agricultural and Food Chemistry*, 49, 1564–1570.
- Kushiro, T., Hashida, J., Kawamura, H., Mitsubayashi, H., Saito, T., Suzuki, Y., Takahashi, N., Ishii, T., Kimura, T., Tsuji, K., Tanabe, N., Asano, K., Abe, S., & Tarui, S. (1996). Clinical effects of benkoji in mild essential hypertension—a multi-center double-blind comparison with placebo. *Nippon Jinzo Gakkai Shi*, 38, 625–633.
- Li, S.-C., (1518–1593). Pen Tsao Kang Mu (Systematic Pharmacopoeia); China, 1596.

- Ma, J., Li, Y., Ye, Q., Li, J., Hua, Y., Ju, D., Zhang, D., Cooper, R., & Chang, M. (2000). Constituents of red yeast rice, a traditional Chinese food and medicine. *Journal of Agricultural and Food Chemistry*, *48*, 5220–5225.
- Mau, J.-L., Chyau, C.-C., Li, J.-Y., & Tseng, Y.-H. (1997). Flavor compounds in straw mushrooms *Volvariella volvacea* harvested at different stages of maturity. *Journal of Agricultural and Food Chemistry*, *45*, 4726–4729.
- Metcalfe, L. D., Schmitz, A. A., & Pelka, J. R. (1966). Rapid preparation of fatty acid ester from lipid for gas chromatographic analysis. *Analytical Chemistry*, *38*, 514–515.
- Otsuka, H., Hirai, Y., Nagao, T., & Yamaski, K. (1988). Antiinflammatory activity of benzoxaninoids from roots of *Coix lachryma-jobi* L. var. *mayuen*. *Journal of Natural Products*, *51*, 74–79.
- Steel, R. G., Torrie, J. H., & Dickey, D. A. (1997). *Principles and procedures of statistics: A biometrical approach*. Singapore: McGraw-Hill.
- Tanimura, A. (1961). Studies on the anti-tumor component in the seeds of *Coix lachryma-jobi* L. var. *mayuen* (Roman.) Stapf. II. The structure of coixenolide. *Chemical & Pharmaceutical Bulletin*, *9*, 47–53.
- Tobert, J. A., Bell, G. D., Birtwell, J., James, I., Kukovetz, W. R., Pryor, J. S., Buntinx, A., Holmes, I. B., Chao, Y. S., & Bolognese, J. A. (1982). Cholesterol-lowering effect of mevinolin, an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, in healthy volunteers. *Journal of Clinical Investigation*, *68*, 913–919.
- Tsai, C. E., Yang, L. J., & Hsu, H. C. (1999). Ingestion of adlay may reduce liver fat accumulation in hamsters of fed high fat diets. *Food Science*, *26*, 265–276.
- Tsai, C.-M., & Chiang, W. (1993). Extrusion-cooking processing of Job's tears flour. *Journal of Chinese Agricultural Chemical Society*, *31*, 490–496.
- Tsuji, K., Ichikawa, T., Tanabe, N., Abe, S., Tarui, S., & Nakagawa, Y. (1992). Effects of two kinds of koji on blood pressure in spontaneously hypertensive rats. *Nippon Nogeikagaku Kaishi*, *66*, 1241–1246.
- Ukita, T., & Tanimura, A. (1961). Browning reaction products produced by the reaction between sugars and amino acids. VII. Decomposition of lipid hydroperoxide by the browning products. *Nippon Shokuhin Kogyo Gakkaishi*, *15*, 187–191.
- Yang, L. J., & Tsai, C. E. (1998). Effect of adlay on plasma lipids in hamsters. *Food Science*, *25*, 265–276.
- Yasukawa, K., Takahashi, M., Yamanouchi, S., & Takido, M. (1996). Inhibitory effect of oral administration of *Monascus* pigment on tumor promotion in two-stage carcinogenesis in mouse skin. *Oncology*, *53*, 247–249.