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Taste Quality of Monascal Adlay

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Monascus purpureus was inoculated into cooked adlay, and a new product was produced after fungal fermentation. Contents of crude ash, fat, fiber, and protein in the inoculated products [monascal polished adlay (MPA) and monascal dehulled adlay (MDA)] were much higher than those in the uninoculated controls [polished adlay (PA) and dehulled adlay (DA)]. Only carbohydrate content was notably higher in DA and PA. The three soluble sugars and polyol found were arabitol, galactose, and glucose. The contents of total soluble sugars and polyol were in the descending order of DA ∼ PA (79.6 and 79.1 mg/g, respectively) > MDA (59.8 mg/g) > MPA (53.5 mg/g). The total free amino acid contents ranged from 8.60 to 14.11 mg/g and occured in the descending order of MDA ∼ MPA > DA > PA. Contents of bitter components (4.07−7.61 mg/g) were high as compared to monosodium glutamate-like and sweet components, in the descending order of MDA ∼ MPA > DA > PA. No flavor 5′-nucleotides were found. On the basis of the results obtained, monascal adlay products might give a bitter perception.

KEYWORDS: ***Monascus*; adlay; monascal adlay; soluble sugars; free amino acids; 5′-nucleotides**

INTRODUCTION

Monascus-fermented rice (anka, red koji) has been used as a part of Chinese food for thousands of years and has also been considered an essential part of wine making and other fermented food products (*1*). Metabolic products from *Monascus* species in the fermentation, including monacolin K, γ-aminobutyric acid, dimerumic acid, and polyketide pigments, were found to exhibit several physiological functions such as hypocholesterolemic, antitoxic, antitumor, neurotransmitting, hypotensive, diuretic, and antimutagenic properties (*2*−*11*). In addition, *Monascus*-fermented products have been used as a functional dietary supplement to reduce cholesterol levels in the human body (*12*).

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 (*13*−*18*). In addition, coixenolide isolated from the adlay seed exhibited antitumor activity toward Ehrlich ascites sarcoma in mice (*19*, *20*). Adlay is widely planted in Taiwan, China, and Japan, where it is considered a healthy food supplement.

Both the fungus *Monascus* and adlay possess functional components effective in improving 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 (*21*). As an ingredient of health or functional food, the chemical composition and nonvolatile taste components of inoculated adlay products may correlate with product acceptability. The taste components are several small, water soluble substances, including soluble carbohydrates, free amino acids, and free 5′-nucleotides (*22*). However, the profile of nonvolatile taste components of inoculated products is not available.

Accordingly, our objective was to examine the nonvolatile taste components of inoculated adlay products [monascal polished adlay (MPA) and monascal dehulled adlay (MDA)] and uninoculated adlay products [polished adlay (PA) and dehulled adlay (DA)], including their proximate compositions, soluble sugars or polyols, free amino acids, and 5′-nucleotides. The differences in the nonvolatile components of inoculated and uninoculated adlay products were also compared.

MATERIALS AND METHODS

Adlay Products. PA [Taichung Selected No. 4 (TCS-4)] was purchased at a local market in Taichung City, Taiwan. 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 reinoculated into potato dextrose broth (Difco) and incubated at 25 °C for 7 days. The culture was then

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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 as well as two uninoculated adlay products that were also autoclaved and used as controls were air-dried in an oven at 40 °C. For each product, three dried samples (~50 g of each) were randomly selected and ground using a mill (Restsch ultra centrifugal mill and sieving machine, Haan, Germany) to obtain coarse powder (20 mesh).

Proximate Analysis. The proximate compositions of four kinds of adlay products, including moisture, crude ash, crude fat, crude fiber, and crude protein, were determined according to the methods of AOAC (*23*). The nitrogen factor used for crude protein calculation was 5.83 (*22*). 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.

Soluble Sugar or Polyol Assay. Soluble sugars or polyols were extracted and analyzed as described by Ajlouni et al. (*24*). Air-dried powder (600 mg) was extracted with 50 mL of 80% aqueous ethanol (95% pure, Taiwan Tobacco & Wine Monopoly Bureau, Taipei), and xylose (50 mg, Sigma Chemical Co., St. Louis, MO) was added as an internal standard. This suspension was shaken for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The residue was washed five times with additional 25 mL portions of 80% ethanol. The combined filtrate was then rotary evaporated at 40 °C and redissolved in deionized water to a final volume of 10 mL. The aqueous extract was passed through a filter unit (13 mm, Lida, Corp., Kenosha, WI), and filtered using 0.45 μm CA nonsterile filter (Lida) prior to injection onto high-performance liquid chromatograph (HPLC).

The HPLC system consisted of a Hitachi L-6000 pump, a Rheodyne 7161 injector, a 20 μm sample loop, a Hitachi D-2500 chromato-integrator, a Shimadzu RID-10A detector, and a Phase Sep-NH_2 column (4.6 mm × 250 mm, 5 μm, Phase Separation Inc., Norwalk, CT). The mobile phase was acetonitrile (LC grade, Tedia Co., Fairfield, OH)/deionized water, 17:3 (v/v) at a flow rate of 2 mL/min. Each sugar or polyol was identified using the authentic compound (Sigma) and quantified by comparing the peak area of the sugar or polyol to that of the internal standard.

Free Amino Acid Assay. Air-dried powder (500 mg) was shaken with 50 mL of 0.1 N HCl (Union Chemical Co., Hsinchu, Taiwan) 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 nonsterile filter (Lida). This filtrate (100 μL) was mixed with *o*-phthalaldehyde reagent (100 μL, Sigma) in an Eppendorf tube, shaken to facilitate derivatization, and then immediately injected onto HPLC.

The HPLC system was the same as for sugar analysis but included a Hitachi F-7485 fluorescence detector with fluorescence excitation at 340 nm and emission at 450 nm and a Prodigy 5 ODS-2 column (4.6 mm × 250 mm, 5 μm, Phenomenex Inc., Torrance, CA). The mobile phases were A, 50 mM sodium acetate (pH 5.7) containing 0.5% tetrahydofuran; B, deionized water; and C, methanol (*25*). 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 (*25*). The flow rate was 1.2 mL/min (*25*). Each amino acid was identified using the authentic amino acid (Sigma) and quantified by the calibration curve of the authentic compound.

5′-Nucleotide Assay. 5′-Nucleotides were extracted and analyzed as described by Taylor et al. (*26*). Air-dried powder (500 mg) was extracted with 25 mL of deionized water. This suspension was heated to boiling for 1 min, cooled, and then centrifuged at 22 200*g* for 15 min. The extraction was repeated once with 20 mL of deionized water. The combined filtrate was then evaporated and filtered prior to HPLC injection in the same manner as in soluble sugar or polyol assay.

The HPLC system was the same as for sugar or polyol assay except for a Hitachi L-4000 UV detector and a Prodigy 5 ODS-2 column (4.6 mm × 250 mm, 5 μm, Phenomenex). The mobile phase was 0.5 M KH_2PO_4/H_3PO_4 (pH 4.0, Wako Pure Chemical Co., Osaka, Japan) at a flow rate of 1 mL/min and UV detection at 254 nm. Each 5′-nucleotide was identified using the authentic 5′-nucleotide (Sigma) and quantified by the calibration curve of the authentic compound.

Table 1. Proximate Composition of Various Adlay Products

component[a]	content[b] (%)			
	DA	MDA	PA	MPA
moisture	12.6 ± 0.2b	14.2 ± 0.2a	11.4 ± 0.2c	12.6 ± 0.2b
dry matter	87.4 ± 0.2b	85.8 ± 0.2c	88.6 ± 0.2a	87.4 ± 0.2b
carbohydrate	72.9 ± 2.4a	37.0 ± 2.0c	72.3 ± 2.2a	42.7 ± 1.2b
crude ash	3.9 ± 0.8b	6.9 ± 0.7a	3.5 ± 0.6b	7.0 ± 0.5a
crude fat	6.4 ± 0.6b	15.4 ± 1.4a	7.7 ± 1.8b	13.6 ± 1.4a
crude fiber	4.5 ± 0.5b	15.9 ± 1.7a	3.4 ± 0.3b	14.4 ± 1.0a
crude protein	12.3 ± 1.5c	24.8 ± 1.6a	13.1 ± 1.2c	22.3 ± 0.7b

[a] Moisture and dry matter were presented based on fresh weight; others were presented based on dry weight. [b] Each value is expressed as mean ± SD (n = 3). Means with different letters within a row are significantly different ($p < 0.05$).

Statistical Analysis. For each 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 et al. (*27*), to determine the least significant difference among means at the level of 0.05.

RESULTS AND DICUSSION

Moisture contents of various adlay products were in the range of 11.4−14.2% (**Table 1**). Contents of crude ash, fat, fiber, and protein in the inoculated products were much higher than those in the uninoculated controls. Only carbohydrate content was notably higher in the uninoculated DA and PA. The reduction in carbohydrate content was apparently due to the growth of fungal mycelia, which consumed carbohydrate to generate energy and synthesize biomaterials. Therefore, contents of other components in the proximate composition of the inoculated products were correspondingly increased. The percentage of crude ash was doubled mainly as a result of the reduction of carbohydrate content. High contents of crude fat, fiber, and protein in the monascal adlay were partially resulted from the mycelial growth. Unexpectedly, in addition to their functional components, the monascal adlay became nutritive grain, an especially good source of protein.

Generally, one of the beneficial effects of dehulled grain over polished grain was the higher content of crude fiber. However, the monascal adlay contained more fiber. The discrepancy in the contents of fiber and other components between DA and PA and MDA and MPA due to refining process was not remarkable. The major component of crude fiber in fungus is chitin, which is an important structural polysaccharide found in the cell wall (*28*−*30*). However, crude fiber was not ingested and ineffective in taste but acted as dietary fiber.

Tsai and Chiang (*31*) noted that dehulled and PA (TCS-4) contained 7.85 and 6.30% of crude fat, 13.83 and 13.26% of crude protein, 2.16 and 1.50% of crude ash, 1.65 and 1.07% of crude fiber, and 74.51 and 77.87% of carbohydrate, respectively. These results were similar to those of DA and PA shown in **Table 1**. However, *Monascus*-fermented rice contained 6.0% of moisture, 73.4% of carbohydrate, 0.8% of fiber, 2.8% of fatty acids, and 14.7% of protein (*32*). It seemed that the monascal adlay products were a good source of fat, fiber, and protein as compared to monascal rice.

Three soluble sugars and polyol found in these four adlay products were arabitol (10.9−21.3 mg/g), galactose (19.7−21.0 mg/g), and glucose (22.2−40.3 mg/g) (**Table 2**). The contents of total soluble sugars and polyol were in descending order of DA ~ PA (79.6 and 79.1 mg/g, respectively) > MDA (59.8 mg/g) > MPA (53.5 mg/g). Obviously, after fungal fermentation, the soluble sugars and polyol were utilized by *Monascus*

Table 2. Content of Soluble Sugars and Polyols of Various Adlay Products

	content[a] (mg/g dry weight)			
sugar or polyol	DA	MDA	PA	MPA
arabitol	18.3 ± 0.2b	12.0 ± 0.1c	21.3 ± 0.7a	10.9 ± 0.1d
galactose	21.0 ± 0.1a	20.6 ± 0.1b	19.7 ± 0.6c	20.4 ± 0.3b
glucose	40.3 ± 0.6a	27.2 ± 0.3c	38.1 ± 0.1b	22.2 ± 0.3d
total	79.6 ± 0.8a	59.8 ± 0.4b	79.1 ± 1.3a	53.5 ± 0.6c

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

Table 3. Content of Free Amino Acids of Various Adlay Products

	content[b] (mg/g dry weight)			
amino acid	DA	MDA	PA	MPA
L-alanine	0.54 ± 0.07b	1.28 ± 0.02a	0.45 ± 0.07b	1.15 ± 0.03a
L-arginine	0.05 ± <0.01ab	0.05 ± 0.01ab	0.07 ± 0.01a	0.03 ± <0.01b
L-aspartic acid	0.41 ± 0.09a	0.06 ± <0.01c	0.20 ± 0.08b	0.22 ± 0.02b
L-glutamic acid	0.19 ± 0.01a	0.21 ± 0.01a	0.21 ± 0.04a	0.26 ± 0.01a
glycine	0.04 ± 0.01a	0.03 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.01a
L-histidine[a]	0.15 ± 0.04b	0.23 ± <0.01a	0.18 ± 0.04ab	0.14 ± 0.01b
L-isoleucine[a]	1.28 ± 0.12ab	1.38 ± 0.37a	0.96 ± 0.05c	1.04 ± 0.02bc
L-leucine[a]	0.82 ± 0.06b	1.32 ± 0.18a	0.77 ± 0.06b	1.40 ± 0.14a
L-lysine[a]	2.16 ± 0.46c	2.96 ± 0.31a	2.45 ± 0.05bc	2.66 ± 0.05b
L-methionine[a]	1.28 ± 0.12c	2.54 ± 0.02a	1.35 ± 0.09c	2.14 ± 0.05b
L-phenylalanine[a]	0.65 ± 0.10c	1.15 ± 0.02b	0.51 ± <0.01d	1.29 ± 0.02a
L-serine	0.09 ± 0.02a	0.04 ± <0.01c	0.08 ± 0.03a	0.06 ± 0.01b
L-threonine[a]	0.51 ± 0.14a	0.23 ± 0.01c	0.35 ± 0.03b	0.13 ± <0.01d
L-tryptophan[a]	0.73 ± 0.24a	0.48 ± 0.05b	nd[c]	0.32 ± 0.04c
L-tyrosine	1.08 ± 0.37b	1.69 ± 0.29a	0.75 ± 0.09c	1.75 ± 0.21a
L-valine[a]	0.20 ± 0.12c	0.46 ± 0.08b	0.23 ± 0.06c	0.68 ± 0.05a
total	10.18 ± 1.98b	14.11 ± 1.41a	8.60 ± 0.72c	13.31 ± 0.73a

[a] Essential amino acid. [b] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$). [c] nd, not detected.

for energy. Evidently, galactose was not a prior energy source for fungal growth. With regard to those in DA and PA, after fungal fermentation, contents of arabitol and glucose in MDA were reduced to 65.6 (12.0/18.3) and 67.5% (27.2/40.3), respectively, whereas those in MPA were reduced more to 51.2 (10.9/21.3) and 58.3% (22.2/38.1), respectively. However, soluble sugars and polyol contained in these four adlay products would contribute a sweet taste (*33*).

The total free amino acid contents in four adlay products ranged from 8.60 to 14.11 mg/g and in the descending order of MDA ∼ MPA > DA > PA (**Table 3**). In DA and PA, isoleucine, lysine, methionine, and tyrosine were four major free amino acids found in high contents (>0.96 mg/g). However, in MDA and MPA, contents of alanine, leucine, and phenylalanine were increased to more than 1 mg/g. **Table 4** tabulated the free amino acids into several classes on the basis of their taste characteristics as described by Komata (*34*). Aspartic and glutamic acids were monosodium glutamate-like (MSG-like) components, which gave the most typical mushroom taste, the umami taste or palatable taste that was the characteristic taste of MSG and 5′-nucleotides (*35*). Unsurprisingly, unlike mushrooms, contents of MSG-like components were relatively low for four adlay products (0.27−0.60 mg/g), in which MDA contained the lowest amount (0.27 mg/g).

Contents of sweet components were also low and ranged from 0.92 to 1.58 mg/g. However, contents of bitter components (4.07−7.61 mg/g) were high as compared to other taste components, in the descending order of MDA ∼ MPA > DA > PA. After fungal fermentation, bitter amino acids, especially leucine and phenylalanine, were released from adlay and resulted

Table 4. Content of Taste Characteristics of Free Amino Acids in Various Adlay Products

	content[b] (mg/g dry weight)			
taste characteristic[a]	DA	MDA	PA	MPA
MSG-like	0.60 ± 0.10a	0.27 ± 0.02c	0.41 ± 0.12b	0.48 ± 0.03b
sweet	1.18 ± 0.24bc	1.58 ± 0.05a	0.92 ± 0.23c	1.38 ± 0.06ab
bitter	5.16 ± 0.81b	7.61 ± 0.74a	4.07 ± 0.32c	7.04 ± 0.38a
tasteless	3.24 ± 0.83c	4.65 ± 0.60a	3.20 ± 0.14c	4.41 ± 0.26b
total	10.18 ± 1.98b	14.11 ± 1.41a	8.60 ± 0.72c	13.31 ± 0.73a

[a] MSG-like, monosodium glutamate-like, Asp + Glu; sweet, Ala + Gly + Ser + Thr; bitter, Arg + His + Ile + Leu + Met + Phe + Trp + Val; tasteless, Lys + Tyr. [b] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).

Table 5. Content of 5′-Nucleotides of Various Adlay Products

	content[b] (mg/g dry weight)			
	DA	MDA	PA	MPA
5′-AMP	nd[c]	0.38 ± 0.01a	nd	0.29 ± <0.01b
5′-UMP	nd	0.26 ± 0.02b	nd	0.34 ± 0.05a
flavor 5′-nucleotides[a]	nd	nd	nd	nd
total	nd	0.64 ± 0.03a	nd	0.63 ± 0.06a

[a] Flavor 5′-nucleotide, 5′-GMP + 5′-IMP + 5′-XMP. [b] Each value is expressed as mean ± SD ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$). [c] nd, not detected.

in high contents of bitter components in monascal adlay products, MDA and MPA. Obviously, the bitter components were predominantly present and may contribute most the taste.

No free 5′-nucleotide was detected in uninoculated adlay products (**Table 5**). Among 5′-nucleotides, only 5′-adenosine monophosphate (5′-AMP) and 5′-uridine monophosphate (5′-UMP) were found in monascal adlay products. Flavor 5′-nucleotides were 5′-guanosine monophosphate (5′-GMP), 5′-inosine monophosphate (5′-IMP), and 5′-xanthosine monophosphate (5′-XMP) (*36*). However, no free flavor 5′-nucleotides were found in these four adlay products.

On the basis of contents of the total soluble sugars and polyol and sweet components of free amino acids including alanine, glycine, serine, and threonine, it was anticipated that the sweetness was consistent with their sugar content and in the descending order of DA ∼ PA > MDA > MPA. The uninoculated adlay products would be sweeter than monascal adlay products. In addition, low MSG-like components and no flavor 5′-nucleotide indicated that the umami taste was not the taste characteristic of these four adlay products. Usually, the bitter taste from the bitter components was masked by the umami taste as well as the sweet taste (*36*). However, it seemed that uninoculated adlay controls had a slightly bitter aftertaste.

Because 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 consumption is the possible existence of citrinin (*37*). However, citrinin was not present in these products at the detection limit of 1 μg/g (*21*).

With lower amounts of the total soluble sugars and polyol and sweet components as well as higher amounts of bitter components, it was anticipated that monascal adlay products might give a bitter perception. This anticipation was consistent with the general recognition for *Monascus*-fermented rice. People in Taiwan have presented anecdotal evidence suggesting that metabolic products from *Monascus* species might be responsible for the bitter taste. Therefore, the bitter taste from bitter components and probably monacolin K and polyketide

pigments might affect consumers' acceptability of these monascal adlay products as functional foods. To determine the relationship of the taste of these monascal adlay products with their soluble components, further sensory evaluation is needed.

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