

## Effect of monosodium glutamate and peptone on antioxidant activity of monascal waxy corn

<sup>1</sup>Kraboun, K., <sup>1</sup>Tochampa, W., <sup>2</sup>Chatdamrong, W. and <sup>1,\*</sup>Kongbangkerd, T.

<sup>1</sup>Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand

<sup>2</sup>Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

### Article history

Received: 28 July 2012

Received in revised form:

15 October 2012

Accepted: 26 October 2012

### Abstract

The effects of monosodium glutamate (MSG) and peptone on antioxidant activity, monacolin K and citrinin contents, pigment intensity and glucosamine content of monascal waxy corn produced by *Monascus purpureus* TISTR 3090 at 25°C were investigated. The contents of peptone and MSG used as nitrogen sources were equated to 0.25, 0.50, 0.75 and 1.00% nitrogen content. The addition of MSG or peptone to waxy corn affected the *Monascus* fermentation, leading to higher antioxidant activity, pigment intensity and glucosamine content, monacolin K and citrinin contents compared to the control (no nitrogen source added). The production of pigment, monacolin K and citrinin contents and antioxidant activity were maximum on day 12 of fermentation while glucosamine content was maximum on day 8. The highest antioxidant activity was obtained using 12.08% MSG content, which equated to 1.00% nitrogen content. Pearson's correlation coefficients of monascal waxy corn supplemented with MSG and peptone treatments gave good negative correlations with pigment intensity and DPPH IC<sub>50</sub> value and pigment intensity and chelating ability on ferrous ions IC<sub>50</sub>. Meanwhile, a good positive correlation was found between pigment intensity and monacolin K content. Therefore, pigment intensity could be a good indicator for antioxidant activity of monascal waxy corn.

### Keywords

MSG

peptone

antioxidant activity

monascal waxy corn

© All Rights Reserved

### Introduction

Fermentation of *Monascus* sp. normally produces pigments and some significant antioxidants e.g., monacolin K,  $\gamma$ -Aminobutyric acid (GABA), dimeric acid and flavonoids (Chairote *et al.*, 2008), while citrinin, a secondary metabolite produced during fermentation, is harmful to humans and animals (Dufosse *et al.*, 2005). The main compound from *Monascus* fermentation is monacolin K which is commercially called lovastatin, mevinolin and mevacor. It has been demonstrated as a specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in cholesterol biosynthesis (Endo, 1980). The reductase enzyme produces mevalonyl-CoA which is an important step in synthesizing cholesterol (Hajjaj *et al.*, 2001; Su *et al.*, 2003). Therefore, it helps to decrease blood pressure (Wang *et al.*, 1997; Li *et al.*, 2004). In the United States, statin drugs e.g., lovastatin, mevinolin

and mevacor, with antioxidant properties similar to monacolin K, are used as cholesterol-lowering drugs (Erdogrul and Azirak, 2004; Journoud and Jones, 2004). There are some reports of monascal products with high antioxidant activity e.g., monascal adlay (Tseng *et al.*, 2006) and monascal rice (Yang *et al.*, 2006).

Normally, white rice without germ has been used as a solid substrate for monascal rice production which is rich in carbohydrate but lacked of some essential amino acids, fatty acids and other minerals (Dufosse *et al.*, 2005; Jiranuntakul *et al.*, 2011) reported that fatty acids and nitrogen sources promoted *Monascus* pigment production but suppressed citrinin production. For this standpoint, white rice seems inappropriate for monascal rice production.

Nitrogen is a nutritional source and is required for microbial growth (Broder and Koehler, 1980; Lin and Demain, 1991). Babitha *et al.* (2007) reported addition of 1% MSG or peptone to jackfruit seed

\*Corresponding author.

Email: [teerapornk@nu.ac.th](mailto:teerapornk@nu.ac.th)

powder promoted *Monascus* growth and pigment production. Moreover, MSG and peptone have been used as a supplement for *Monascus* fermentation using grape waste broth as a substrate. Silveira *et al.* (2008) investigated the effects of MSG and peptone on pigment production and *M. purpureus* growth, and found that nitrogen from MSG and peptone was important for increasing pigment and *Monascus* growth.

Waxy corn, *Zea mays* var. *ceratina*, has high amylopectin but low amylose contents, while the lipid and ash contents are higher than those of polished rice (Jiranuntakul *et al.*, 2011). Therefore, it should be suitable as an alternative substrate for *Monascus* pigment production because of various nutrients and the price is relatively low. Nevertheless, the use of waxy corn as a substrate for *Monascus* fermentation has never been reported.

The objectives of this research were to study nitrogen concentrations from different sources as additives to waxy corn for *Monascus* fermentation, and their effects on antioxidant activity. The relationships between metabolites and antioxidant activities of monascal waxy corn were also investigated.

## Materials and Methods

### Microorganism

Lyophilized *Monascus purpureus* TISTR 3090 was purchased from the Thailand Institute of Scientific and Technological Research (TISTR). The strain was cultivated on potato dextrose agar (PDA) (Merck, Darmstadt, Germany) at 25°C for 7 days. After a pure culture was obtained, the mycelium was re-inoculated into PDA slant at 25°C for 7 days before being used for production of monascal waxy corn.

### Raw materials

Waxy corn (*Zea mays* var. *ceratina*) was harvested between 67 and 70 days after planting in Sukhothai province, Thailand. It was peeled and cleaned and the seeds were removed and stored at -18°C prior to being used for *Monascus* fermentation.

### Chemicals

Monacolin K, citrinin, monosodium glutamate (MSG), peptone, 2,4,6-tripyridyls-triazine (TPTZ), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (St. Louis, MO). All chemicals and solvents were analytical reagent grade.

### Monascal waxy corn preparation

The *Monascus* fermentation followed the modified method of Yang *et al.* (2006). One hundred grams of cleaned waxy corn seeds with different contents of MSG or peptone were put into a flask. Peptone and MSG were used as nitrogen sources with different concentrations, equivalent to 0.25, 0.50, 0.75 and 1.00% nitrogen. The calculated amounts of peptone and MSG were 2.08, 4.16, 6.25 and 8.33% (w/w) and 3.01, 6.04, 9.06 and 12.08% (w/w), respectively. The mixtures were sterilized in an autoclave at 121°C for 15 min and then left at ambient temperature. Spore suspension of *M. purpureus* was prepared from actively growing slants in sterile water and diluted to a concentration of 10<sup>6</sup> spores/ml. A 5 ml aliquot of the spore suspension was inoculated into sterilized waxy corn, and incubated at 25°C for 20 days. The fermented products were dried in an oven at 40°C for 24 h. A fine powder (20 mesh) was obtained using a mill (Retsch ultracentrifugal mill and sieving machine (Haan, Germany)).

### Sample extraction for antioxidant activity assay

The extraction method described by Yang *et al.* (2006) was used with some modifications. A 10 g sample was extracted in a shaker with 100 ml of methanol at 170 rpm for 24 h and the solution was filtered through Whatman no.4 filter paper. The residue was then extracted with two additional 100 ml portions of methanol as described above. The combined methanolic extracts were then evaporated at 40°C to dryness. The dried product was used for analysis of antioxidant activities.

### Pigment intensity

One gram of monascal waxy corn was extracted with 5 ml methanol using a rotary shaker at 170 rpm for 1 h. The extract was then filtered through Whatman no.4 filter paper to remove suspended solids and the supernatant was analysed by a spectrophotometer (Thermo spectrophotometer model Genesys 20) against a methanol blank. The pigment concentration was measured at 500 nm (Yongsmith *et al.*, 2000). Pigment intensity was calculated from the following equation.

$$\text{Pigment intensity} = \frac{A_{500} \times \text{dilution factor} \times \text{Volume of methanol}}{\text{Unit /g dry weight} \times \text{Weight of sample (g)}}$$

### Glucosamine content

The fungal growth was estimated by determining the amount of N-acetyl glucosamine released by acid hydrolysis of chitin, present in the mycelia cell wall. One gram of dried sample was washed with 50 ml of 5 M H<sub>2</sub>SO<sub>4</sub> under agitation for 15 min. The mixture

was then centrifuged at 5,000 rpm for 10 min and was rinsed twice with distilled water. For chitin hydrolysis to N-acetyl glucosamine, the washed sample was incubated with 10 ml of 10 M HCl at 20°C for 16 h. After dilution with 40 ml distilled water, the hydrolysis proceeded during autoclaving for 2 h at 130°C. The hydrolysate was neutralized to pH 7.0 with 10 M NaOH and subsequently with 0.5 M NaOH. The neutralized sample of 1 ml was mixed with 1 ml acetyl acetone reagent and incubated in a boiling water bath for 20 min. After cooling, 6 ml of ethanol was added and followed by the addition of 1 ml of Ehrlich reagent and incubated at 65°C for 10 min. The optical density was read at 530 nm against the reagent blank. N-Acetyl glucosamine (Sigma) was used as a standard (Babitha *et al.*, 2007).

#### *Trolox equivalent antioxidant capacity (TEAC)*

For ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay, antioxidant activity of monascal waxy corn extracts against ABTS.<sup>+</sup> radical, was evaluated spectrophotometrically by a slightly modified method of Re *et al.* (1999). The TEAC assay is based on the scavenging of ABTS.<sup>+</sup> radical converting into a colourless product. The degree of decolorisation induced by a compound is related to that induced by trolox, giving the "TEAC value". The ABTS.<sup>+</sup> radical was produced by the reaction between 2 ml of 7 mM ABTS solution and 40 µl of 2.45 mM potassium persulphate solution, stored in the dark at room temperature for 16 h. Before usage, the ABTS.<sup>+</sup> solution was diluted to get an absorbance of 0.700 ± 0.025 at 734 nm with ethanol. For the assay, the resulting solution was mixed with 300 µl of sample of each monascal waxy corn extract (1-20 mg/ml). The absorbance was read at 30°C after exactly 6 min. The obtained absorbance of samples was compared with a standard curve from the corresponding readings of Trolox (0.4-0.04 mM). The total antioxidant capacities (TAC) were estimated as trolox equivalents (TEAC) by interpolation to 50% inhibition (TEAC<sub>50</sub>).

#### *Ferric reducing/antioxidant power (FRAP)*

The procedure was adapted from Benzie and Strain (1996). This method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM FeCl<sub>3</sub> and 25 ml of 0.3 M acetate buffer, pH 3.6. The reagents were freshly prepared and warmed at 37°C. Each aliquot (1-20 mg/ml) of 40 µl sample supernatant was mixed with 2.2 ml of distilled

water and 1.8 ml of FRAP reagent. The absorbance of the reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37°C for 10 min. IC<sub>50</sub> value (mg extract/ml) is the effective concentration at which the reducing power was 50% obtained by interpolation from linear regression analysis.

#### *DPPH radical scavenging activity*

The scavenging activity (H/e-transferring ability) against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was measured spectrophotometrically by following Velazquez *et al.* (2003). Each 1-20 mg/ml of aliquot of 40 µl appropriately diluted extracts mixed with 200 µl of 0.02 mM DPPH solution and methanol 4 ml. Samples were kept for 15 min at room temperature and the absorbance was measured at 517 nm. The absorbance of a blank sample containing the same amount of solvent was also measured. The extent of decolourisation is calculated as percentage reduction of absorbance, and this is determined as a function of concentration and calculated relative to the 0.1-0.01 mM of equivalent trolox concentration. The radical scavenging activity is expressed in mmol of equivalent Trolox per gram of sample (mmol Trolox/ml) with interpolation to 50% inhibition (IC<sub>50</sub>).

#### *Chelating ability on ferrous ions*

Chelating ability on ferrous ions was evaluated spectrophotometrically by a slightly modified method of Kuo *et al.* (2009). Three hundred µl of 2 mM FeSO<sub>4</sub>.H<sub>2</sub>O were mixed with 1-20 mg/ml of each aliquot of 500 µl test samples before addition of 600 µl of 5 mM ferrozine. After the incubation at room temperature for 10 min, 5 ml of ethanol was added and the absorbance was measured at 562 nm. IC<sub>50</sub> value (mg extract/ml) is the effective concentration at which ferrous ions were chelated by 50 % by interpolation from linear regression analysis.

#### *Monacolin K analysis*

An 0.5 g sample was extracted with 25 ml of 70% ethanol at 50°C for 2 h, followed by filtration through a 0.2 µm membrane (Chayawat *et al.*, 2009) and the extract was analysed by HPLC. The HPLC system consisted of Shimadzu LC-10AT VP Liquid Chromatograph, a FCV-10AL VP pump, an LDC Analytical SpectroMonitor 3100 detector set at 238 nm and an LDC Analytical CI-4100 integrator. A chromatography column Ascentis C18, 5µm, 250×4.6 mm was connected to a 20 µl loop injector. An isocratic mobile phase of acetonitrile:water in the ratio of 65:35 (by vol.) was used. The flow rate and temperature were 1.0 ml/min and 28°C, respectively

(Friedrich *et al.*, 1995). Monacolin K dissolved in 70% ethanol was used as a standard.

### Citrinin analysis

Citrinin analysis was described by Lim *et al.* (2010). A 1 g sample was extracted with a solution (acetone : ethyl acetate = 1:1, v/v) at 65°C for 90 min under vigorous shaking. The supernatant was obtained by centrifugation at 1,600g for 10 min followed by filtration through a 0.45 µm PTEE filter unit (National Scientific, Rockwood, TN). The citrinin was determined by HPLC using a chromatography column Ascentis C18 column (4.6 x 250 mm). The mobile phase consisted of methanol/acetonitrile/0.1% phosphoric acid (3:3:4, v:v:v) and the analysis was performed with a fluorescence detector set at excitation and emission wavelengths of 330 and 500 nm, respectively. The flow rate was 0.6 ml/min and the sample was spiked to confirm the presence of citrinin.

### Statistical analysis

All determinations were performed in triplicate and results were expressed as the mean + standard deviation calculated using spreadsheet software Microsoft Excel. The data were analysed by an analysis of variance ( $p < 0.05$ ) and means separated by Duncan's multiple range test. The relationship among the antioxidant capacity, antioxidant content, pigment intensity and glucosamine content of different monascal waxy corn samples, as well as different antioxidant capacity assays, was analysed by Pearson correlation coefficients. The results were processed by SPSS 16.0 for Windows.

## Results and Discussion

### Pigment intensity and glucosamine content

Pigment intensity and glucosamine content of monascal waxy corn supplemented with different contents of MSG and peptone are shown in Figure 1 and 2, respectively. Both pigment intensity and glucosamine content increased with increasing peptone and MSG contents. This indicated that nitrogen sources promote the growth and pigment production of fungi (Vidyalakshmi *et al.*, 2009). On day 12, pigment intensity of every treatment reached a maximum and was stable afterwards. Meanwhile, glucosamine content also reached a maximum on day 8 and then decreased until the end of fermentation. The result affirmed that the pigment is a secondary metabolite since it was increasingly produced following the growth of fungus. These results were similar to the finding of Babitha *et al.* (2007).

An increased content of MSG and peptone

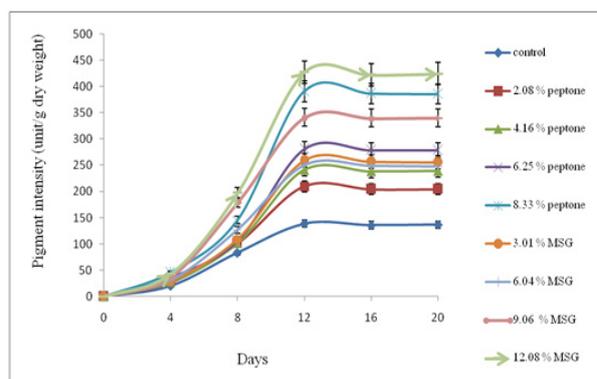


Figure 1. Pigment intensity of monascal waxy corn during *Monascus* fermentation with different contents of MSG and peptone. Each value is expressed as mean  $\pm$  standard deviation (n = 3)

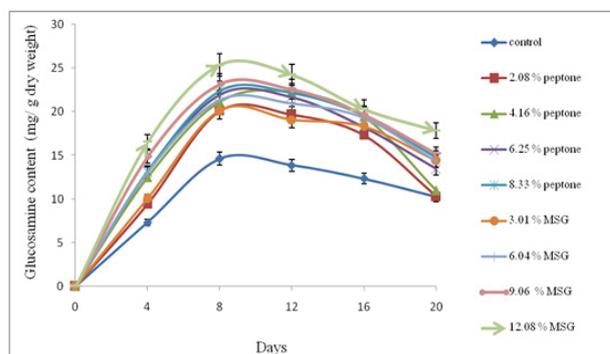


Figure 2. Glucosamine contents of monascal waxy corn during *Monascus* fermentation with different contents of MSG and peptone. Each value is expressed as mean + standard deviation (n = 3)

resulted in increasing pigment production and glucosamine content compared to the control (no nitrogen source added). The addition of 12.08% (w/w) MSG (1.00% of nitrogen) provided maximum pigment intensity and glucosamine of 427.3 unit/g dry weight and 25.34 mg/g dry weight, respectively. Compared with the same nitrogen content from peptone (8.33% (w/w)), pigment intensity and glucosamine contents were 36.60 unit/g dry weight and 2.12 mg/g dry weight, respectively, less than those of MSG. Vidyalakshmi *et al.* (2009) reported that monascal rice supplemented with 0.50 % (w/w) MSG fermented with *Monascus ruber* contained higher pigment yield than those supplemented with peptone, yeast extract and ammonium nitrate. Furthermore, Mukherjee and Singh (2011) suggested that the pigment production and growth of *M. purpureus* in submerged fermentation supplemented with 5.00% (w/v) MSG were higher than those with other nitrogen sources, such as peptone,  $KNO_3$ ,  $NH_4NO_3$ ,  $NH_4Cl$ ,  $NaNO_3$ , MSG, and  $(NH_4)_2SO_4$ .

The obtained results confirmed that MSG was the most appropriate additive for pigment production and growth of *M. purpureus* because MSG is stable under heating conditions and is not easily destroyed at high temperatures (Yamaguchi and Ninomiya, 1998),

Table 1. Monacolin K contents of monacal waxy corn during *Monascus* fermentation with different contents of MSG and peptone

Treatment	Monacolin K contents* mg/kg dry weight at different incubation time (days)				
	4	8	12	16	20
control	0.32±0.01 <sup>a</sup>	0.39±0.02 <sup>a</sup>	1.21±0.04 <sup>a</sup>	1.21±0.00 <sup>a</sup>	1.18±0.00 <sup>a</sup>
2.08 % peptone	0.41±0.02 <sup>b</sup>	0.43±0.01 <sup>b</sup>	1.30±0.02 <sup>a</sup>	1.31±0.00 <sup>a</sup>	1.30±0.01 <sup>a</sup>
4.16 % peptone	0.42±0.03 <sup>b</sup>	0.46±0.03 <sup>c</sup>	2.32±0.03 <sup>b</sup>	2.30±0.02 <sup>b</sup>	2.30±0.02 <sup>b</sup>
6.25 % peptone	0.47±0.06 <sup>c</sup>	0.48±0.01 <sup>c</sup>	3.21±0.06 <sup>c</sup>	3.18±0.02 <sup>c</sup>	3.18±0.04 <sup>c</sup>
8.33 % peptone	0.50±0.01 <sup>d</sup>	0.66±0.03 <sup>d</sup>	5.12±0.05 <sup>d</sup>	5.11±0.01 <sup>d</sup>	5.09±0.01 <sup>d</sup>
3.01 % MSG					
	0.51±0.01 <sup>d</sup>	1.23±0.01 <sup>c</sup>	10.12±0.21 <sup>e</sup>	10.13±0.04 <sup>e</sup>	10.11±0.05 <sup>e</sup>
6.04 % MSG	0.53±0.03 <sup>e</sup>	1.58±0.01 <sup>f</sup>	12.79±0.04 <sup>f</sup>	12.78±0.00 <sup>f</sup>	12.7±0.21 <sup>f</sup>
9.06 % MSG					
	0.63±0.01 <sup>f</sup>	2.00±0.01 <sup>e</sup>	13.12±0.03 <sup>e</sup>	13.13±0.02 <sup>e</sup>	13.12±0.01 <sup>e</sup>
12.08 % MSG	1.23±0.01 <sup>e</sup>	3.43±0.01 <sup>b</sup>	16.83±0.12 <sup>b</sup>	16.8±0.02 <sup>b</sup>	16.8±0.01 <sup>b</sup>

\*Different letters in front of means within a row are significantly different (p < 0.05). Different letters behind means within a column are significantly different (p < 0.05)

Table 2. Citrinin contents of monacal waxy corn during *Monascus* fermentation with different contents of MSG and peptone

Treatment	Citrinin contents* µg/kg dry weight at different incubation time (days)				
	4	8	12	16	20
control	0.01±0.00 <sup>a</sup>	0.02±0.00 <sup>a</sup>	0.20±0.01 <sup>a</sup>	0.21±0.02 <sup>a</sup>	0.20±0.00 <sup>a</sup>
2.08 % peptone	0.01±0.00 <sup>a</sup>	0.03±0.00 <sup>b</sup>	0.34±0.02 <sup>b</sup>	0.33±0.02 <sup>b</sup>	0.33±0.02 <sup>b</sup>
4.16 % peptone	0.03±0.00 <sup>b</sup>	0.04±0.00 <sup>b</sup>	0.39±0.01 <sup>c</sup>	0.39±0.02 <sup>c</sup>	0.39±0.01 <sup>c</sup>
6.25 % peptone	0.03±0.00 <sup>b</sup>	0.05±0.00 <sup>c</sup>	0.55±0.04 <sup>d</sup>	0.55±0.04 <sup>d</sup>	0.50±0.02 <sup>d</sup>
8.33 % peptone	0.04±0.00 <sup>b</sup>	0.07±0.00 <sup>d</sup>	0.67±0.01 <sup>e</sup>	0.67±0.04 <sup>e</sup>	0.67±0.03 <sup>e</sup>
3.01 % MSG	0.04±0.00 <sup>b</sup>	0.08±0.00 <sup>d</sup>	1.54±0.04 <sup>f</sup>	1.54±0.02 <sup>f</sup>	1.54±0.01 <sup>f</sup>
6.04 % MSG	0.05±0.00 <sup>b</sup>	0.12±0.02 <sup>e</sup>	1.72±0.05 <sup>e</sup>	1.72±0.01 <sup>e</sup>	1.72±0.01 <sup>e</sup>
9.06 % MSG	0.07±0.00 <sup>c</sup>	0.13±0.01 <sup>e</sup>	2.45±0.02 <sup>b</sup>	2.45±0.03 <sup>b</sup>	2.45±0.02 <sup>b</sup>
12.08 % MSG	0.13±0.01 <sup>d</sup>	0.29±0.03 <sup>f</sup>	3.55±0.02 <sup>i</sup>	3.56±0.01 <sup>i</sup>	3.56±0.02 <sup>i</sup>

\*Different letters in front of means within a row are significantly different (p < 0.05). Different letters behind means within a column are significantly different (p < 0.05)

Table 3. TEAC<sub>50</sub> of ABTS assay of monacal waxy corn during *Monascus* fermentation with different contents of MSG and peptone

Treatment	TEAC <sub>50</sub> values* mmol trolox /ml at different incubation time (days)				
	4	8	12	16	20
control	0.18±0.01 <sup>c</sup>	0.16±0.01 <sup>i</sup>	0.08±0.01 <sup>c</sup>	0.09±0.01 <sup>c</sup>	0.10±0.01 <sup>c</sup>
2.08 % peptone	0.15±0.03 <sup>de</sup>	0.10±0.02 <sup>f</sup>	0.03±0.00 <sup>b</sup>	0.04±0.00 <sup>ab</sup>	0.04±0.00 <sup>b</sup>
4.16 % peptone	0.13±0.02 <sup>cd</sup>	0.09±0.01 <sup>e</sup>	0.03±0.00 <sup>b</sup>	0.04±0.00 <sup>b</sup>	0.04±0.00 <sup>b</sup>
6.25 % peptone	0.10±0.04 <sup>bc</sup>	0.12±0.02 <sup>b</sup>	0.03±0.00 <sup>b</sup>	0.03±0.00 <sup>ab</sup>	0.04±0.00 <sup>b</sup>
8.33 % peptone	0.08±0.00 <sup>ab</sup>	0.07±0.00 <sup>c</sup>	0.03±0.00 <sup>b</sup>	0.03±0.00 <sup>ab</sup>	0.03±0.00 <sup>b</sup>
3.01 % MSG	0.61±0.01 <sup>f</sup>	0.12±0.01 <sup>e</sup>	0.03±0.00 <sup>b</sup>	0.03±0.00 <sup>ab</sup>	0.04±0.01 <sup>b</sup>
6.04 % MSG	0.07±0.00 <sup>ab</sup>	0.07±0.00 <sup>d</sup>	0.04±0.00 <sup>b</sup>	0.04±0.00 <sup>ab</sup>	0.04±0.00 <sup>b</sup>
9.06 % MSG	0.06±0.00 <sup>a</sup>	0.04±0.00 <sup>a</sup>	0.03±0.00 <sup>b</sup>	0.03±0.00 <sup>ab</sup>	0.04±0.00 <sup>b</sup>
12.08 % MSG	0.05±0.00 <sup>a</sup>	0.05±0.00 <sup>b</sup>	0.02±0.00 <sup>a</sup>	0.02±0.00 <sup>a</sup>	0.02±0.00 <sup>a</sup>

\*Different letters in front of means within a row are significantly different (p < 0.05). Different letters behind means within a column are significantly different (p < 0.05)

whereas the amino acids of peptone are partially lost during sterilization (Purcell and Walter, 1982). Therefore, the more remaining nitrogen contents of MSG could be better utilized by *M. purpureus* to produce higher levels of pigment and glucosamine. The lower pigmentation in the product supplemented with peptone was caused by a high glucose concentration which leads to lower growth rates, pigment synthesis and considerable ethanol production (Chen and Johns, 1993; Chen and Johns, 1994). The glucose when consumed is first metabolized to acetyl CoA, which could be channelled into pigment production on entry to the TCA cycle (Wang and Hesseltine, 1979). This suggests that the *Monascus* pigment could serve as a carbon sink by incorporating the carbon of acetyl CoA when glucose is present in excess concentration (Lee et al., 2001).

Monacolin K and citrinin contents

The results of monacolin K and citrinin contents in monacal waxy corn supplemented with peptone and MSG at different contents presented in Table 1 and 2, respectively. monacolin K and citrinin are secondary metabolites from *Monascus* sp. and citrinin is known as a toxic substance to humans and animals. Monacolin K content of every treatment was increased during fermentation as well as increasing peptone and MSG contents. The highest monacolin K contents between 1.30 – 5.12 mg/kg dry weight, were produced on day 12 of fermentation and after that the values were not significantly different (p>0.05) until the end of fermentation. Hence, it could be said that the suitable concentration of nitrogen as a supplement for monacolin K production was 12.08% (w/w) MSG which equated to 1.00% of nitrogen. Chairote et al. (2008) reported that monacal product from glutinous rice cv. Kam by *M. purpureus* incubated at 30°C for 3 weeks contained 3.13 mg/g of monacolin K which was slightly more than that of commercial monacal rice (about 2.00 mg/g). Even though the amylopectin content in glutinous rice is as high as waxy corn (Jiranuntakul et al., 2011), monacolin K content in monacal waxy corn was yet less than that of monacal glutinous rice according to less starchy substrate of waxy corn compared to glutinous rice. On the other hand, Pattanagul et al. (2008) elucidated that monacolin K content from monacal adlay produced from *M. purpureus*, incubated at 32-35°C for 28 days, was 14.97 mg/kg.

Citrinin was increased with incubation time and increasing contents of peptone and MSG until day 12 and then it was stable. The citrinin contents in peptone supplemented monacal product were in the range of 0.34 – 0.67 µg/kg dry weight, whereas those supplemented with MSG were 1.54 – 3.55 µg/kg dry weight. Japan has issued an advisory limit of 200 µg/kg of citrinin in agricultural products for sale. The limit set by the Chinese Food and Drug Administration (FDA) is 20 µg/kg, while the European Union has recommended a citrinin limit of 100 µg/kg (Shi and Pan, 2011). Obviously, the citrinin contents of products in Table 2 were not over the maximum allowance for sale according to Chinese FDA. Furthermore, citrinin contents between 0.20 – 3.55 µg/kg were presented in the control and nitrogen supplemented products, which were less than those produced from monacal rice and monacal adlay products which were 100–300 and 0.26 – 14.64 mg/kg, respectively (Pinthong and Bunsangsom, 2003; Pattanagul et al., 2008).

Table 4. IC<sub>50</sub> of DPPH assay of monacal waxy corn during *Monascus* fermentation with different contents of MSG and peptone

Treatment	IC <sub>50</sub> value* (mmol trolox / ml) at different incubation time (days)				
	4	8	12	16	20
control	<sup>b</sup> 0.33±0.01 <sup>h</sup>	<sup>a</sup> 0.25±0.02 <sup>f</sup>	<sup>a</sup> 0.20±0.02 <sup>g</sup>	<sup>a</sup> 0.18±0.02 <sup>g</sup>	<sup>a</sup> 0.17±0.01 <sup>g</sup>
2.08 % peptone	<sup>b</sup> 0.28±0.01 <sup>f</sup>	<sup>b</sup> 0.15±0.03 <sup>e</sup>	<sup>a</sup> 0.13±0.01 <sup>f</sup>	<sup>a</sup> 0.13±0.07 <sup>f</sup>	<sup>a</sup> 0.13±0.00 <sup>f</sup>
4.16 % peptone	<sup>b</sup> 0.24±0.01 <sup>e</sup>	<sup>a</sup> 0.13±0.05 <sup>d</sup>	<sup>a</sup> 0.11±0.00 <sup>e</sup>	<sup>a</sup> 0.10±0.00 <sup>de</sup>	<sup>a</sup> 0.10±0.02 <sup>de</sup>
6.25 % peptone	<sup>b</sup> 0.21±0.07 <sup>d</sup>	<sup>a</sup> 0.09±0.04 <sup>c</sup>	<sup>a</sup> 0.09±0.00 <sup>d</sup>	<sup>a</sup> 0.09±0.01 <sup>cd</sup>	<sup>a</sup> 0.09±0.00 <sup>d</sup>
8.33 % peptone	<sup>b</sup> 0.15±0.01 <sup>c</sup>	<sup>a</sup> 0.05±0.00 <sup>a</sup>	<sup>a</sup> 0.04±0.00 <sup>b</sup>	<sup>a</sup> 0.05±0.00 <sup>b</sup>	<sup>a</sup> 0.04±0.00 <sup>b</sup>
3.01 % MSG	<sup>a</sup> 0.31±0.03 <sup>g</sup>	<sup>a</sup> 0.12±0.00 <sup>d</sup>	<sup>a</sup> 0.10±0.00 <sup>de</sup>	<sup>a</sup> 0.11±0.00 <sup>de</sup>	<sup>a</sup> 0.11±0.02 <sup>e</sup>
6.04 % MSG	<sup>a</sup> 0.26±0.04 <sup>e</sup>	<sup>b</sup> 0.09±0.02 <sup>c</sup>	<sup>a</sup> 0.07±0.01 <sup>c</sup>	<sup>a</sup> 0.07±0.00 <sup>bc</sup>	<sup>a</sup> 0.07±0.01 <sup>c</sup>
9.06 % MSG	<sup>a</sup> 0.13±0.02 <sup>b</sup>	<sup>b</sup> 0.07±0.00 <sup>b</sup>	<sup>a</sup> 0.06±0.00 <sup>c</sup>	<sup>a</sup> 0.06±0.00 <sup>b</sup>	<sup>a</sup> 0.06±0.00 <sup>c</sup>
12.08 % MSG	<sup>a</sup> 0.10±0.01 <sup>a</sup>	<sup>b</sup> 0.04±0.00 <sup>a</sup>	<sup>a</sup> 0.02±0.00 <sup>a</sup>	<sup>a</sup> 0.02±0.00 <sup>a</sup>	<sup>a</sup> 0.02±0.02 <sup>a</sup>

\*Different letters in front of means within a row are significantly different (p < 0.05). Different letters behind means within a column are significantly different (p < 0.05)

Table 5. IC<sub>50</sub> of FRAP assay of monacal waxy corn during *Monascus* fermentation with different contents of MSG and peptone

Treatment	IC <sub>50</sub> values* (mg extract/ml) at different incubation time (days)				
	4	8	12	16	20
control	<sup>c</sup> 11.11±0.11 <sup>i</sup>	<sup>b</sup> 10.20±0.54 <sup>i</sup>	<sup>a</sup> 9.73±0.22 <sup>h</sup>	<sup>a</sup> 9.43±0.17 <sup>h</sup>	<sup>a</sup> 9.33±0.15 <sup>h</sup>
2.08 % peptone	<sup>c</sup> 5.69±0.12 <sup>h</sup>	<sup>b</sup> 5.21±0.41 <sup>h</sup>	<sup>a</sup> 4.90±0.66 <sup>g</sup>	<sup>a</sup> 4.70±0.17 <sup>g</sup>	<sup>a</sup> 4.80±0.35 <sup>g</sup>
4.16 % peptone	<sup>c</sup> 5.47±0.21 <sup>g</sup>	<sup>b</sup> 3.70±0.01 <sup>g</sup>	<sup>a</sup> 2.13±0.12 <sup>c</sup>	<sup>a</sup> 2.43±0.43 <sup>c</sup>	<sup>a</sup> 2.21±0.12 <sup>c</sup>
6.25 % peptone	<sup>c</sup> 4.53±0.02 <sup>f</sup>	<sup>b</sup> 2.22±0.10 <sup>d</sup>	<sup>a</sup> 1.23±0.02 <sup>c</sup>	<sup>a</sup> 1.24±0.55 <sup>c</sup>	<sup>a</sup> 1.19±0.56 <sup>c</sup>
8.33 % peptone	<sup>c</sup> 3.16±0.26 <sup>e</sup>	<sup>b</sup> 1.55±0.27 <sup>c</sup>	<sup>a</sup> 1.21±0.38 <sup>c</sup>	<sup>a</sup> 1.22±0.15 <sup>c</sup>	<sup>a</sup> 1.23±0.57 <sup>c</sup>
3.01 % MSG	<sup>c</sup> 4.24±0.60 <sup>f</sup>	<sup>b</sup> 3.24±0.41 <sup>h</sup>	<sup>a</sup> 3.10±0.12 <sup>f</sup>	<sup>a</sup> 3.11±0.71 <sup>f</sup>	<sup>a</sup> 3.12±0.39 <sup>f</sup>
6.04 % MSG	<sup>c</sup> 3.40±0.51 <sup>d</sup>	<sup>b</sup> 2.43±0.23 <sup>c</sup>	<sup>a</sup> 1.50±0.10 <sup>d</sup>	<sup>a</sup> 1.56±0.34 <sup>d</sup>	<sup>a</sup> 1.46±0.67 <sup>d</sup>
9.06 % MSG	<sup>c</sup> 2.72±0.03 <sup>b</sup>	<sup>b</sup> 1.37±0.18 <sup>b</sup>	<sup>a</sup> 0.97±0.19 <sup>b</sup>	<sup>a</sup> 0.93±0.15 <sup>b</sup>	<sup>a</sup> 0.98±0.14 <sup>b</sup>
12.08 % MSG	<sup>c</sup> 1.89±0.14 <sup>a</sup>	<sup>b</sup> 0.67±0.05 <sup>a</sup>	<sup>a</sup> 0.09±0.03 <sup>a</sup>	<sup>a</sup> 0.09±0.02 <sup>a</sup>	<sup>a</sup> 0.08±0.00 <sup>a</sup>

\*Different letters in front of means within a row are significantly different (p < 0.05). Different letters behind means within a column are significantly different (p < 0.05)

Table 6. IC<sub>50</sub> of Chelating ability on ferrous ions assay of monacal waxy corn during *Monascus* fermentation with different contents of MSG and peptone

Treatment	IC <sub>50</sub> values (mg extract/g ml) at different incubation time (days)				
	4	8	12	16	20
control	<sup>c</sup> 14.57±1.43 <sup>h</sup>	<sup>b</sup> 11.20±0.34 <sup>f</sup>	<sup>a</sup> 9.73±0.61 <sup>f</sup>	<sup>a</sup> 9.68±0.36 <sup>f</sup>	<sup>a</sup> 9.66±0.36 <sup>f</sup>
2.08 % peptone	<sup>c</sup> 11.83±0.03 <sup>g</sup>	<sup>b</sup> 9.83±0.09 <sup>g</sup>	<sup>a</sup> 7.90±0.69 <sup>g</sup>	<sup>a</sup> 7.83±0.99 <sup>g</sup>	<sup>a</sup> 7.88±0.55 <sup>g</sup>
4.16 % peptone	<sup>c</sup> 11.47±1.00 <sup>h</sup>	<sup>b</sup> 8.70±0.12 <sup>f</sup>	<sup>a</sup> 7.70±0.01 <sup>f</sup>	<sup>a</sup> 7.67±0.48 <sup>f</sup>	<sup>a</sup> 7.54±0.27 <sup>f</sup>
6.25 % peptone	<sup>c</sup> 10.53±0.31 <sup>e</sup>	<sup>b</sup> 7.32±0.07 <sup>d</sup>	<sup>a</sup> 6.22±0.11 <sup>d</sup>	<sup>a</sup> 6.23±0.46 <sup>d</sup>	<sup>a</sup> 6.20±0.38 <sup>d</sup>
8.33 % peptone	<sup>c</sup> 10.16±0.66 <sup>d</sup>	<sup>b</sup> 5.55±0.46 <sup>c</sup>	<sup>a</sup> 4.50±0.66 <sup>b</sup>	<sup>a</sup> 4.45±0.03 <sup>b</sup>	<sup>a</sup> 4.43±0.66 <sup>b</sup>
3.01 % MSG	<sup>c</sup> 10.24±0.76 <sup>d</sup>	<sup>b</sup> 8.94±0.28 <sup>g</sup>	<sup>a</sup> 8.00±1.21 <sup>h</sup>	<sup>a</sup> 7.90±0.60 <sup>h</sup>	<sup>a</sup> 7.90±0.76 <sup>h</sup>
6.04 % MSG	<sup>c</sup> 9.40±0.44 <sup>c</sup>	<sup>b</sup> 7.43±0.38 <sup>e</sup>	<sup>a</sup> 6.80±0.34 <sup>e</sup>	<sup>a</sup> 6.75±0.19 <sup>e</sup>	<sup>a</sup> 6.77±0.26 <sup>e</sup>
9.06 % MSG	<sup>c</sup> 8.87±0.01 <sup>b</sup>	<sup>b</sup> 6.37±1.66 <sup>e</sup>	<sup>a</sup> 5.97±0.77 <sup>e</sup>	<sup>a</sup> 5.94±1.26 <sup>e</sup>	<sup>a</sup> 5.93±0.59 <sup>e</sup>
12.08 % MSG	<sup>c</sup> 5.89±0.12 <sup>a</sup>	<sup>b</sup> 4.67±0.09 <sup>a</sup>	<sup>a</sup> 2.89±0.18 <sup>a</sup>	<sup>a</sup> 2.86±0.76 <sup>a</sup>	<sup>a</sup> 2.85±0.68 <sup>a</sup>

\*Different letters in front of means within a row are significantly different (p < 0.05). Different letters behind means within a column are significantly different (p < 0.05)

### Antioxidant activities of monacal waxy corn

In ABTS assay, TEAC<sub>50</sub> values of monacal waxy corn are shown in Table 3. TEAC<sub>50</sub> values of every treatments were minimum on day 12 and slightly decreased until the end of fermentation (P>0.05). The lower TEAC<sub>50</sub> values were obtained from the increasing contents of peptone and MSG. The lowest TEAC<sub>50</sub> value of 0.02 mmol trolox / ml was found in the fermented product supplemented with 12.08% (w/w) MSG (1% of nitrogen) during day 12 - day 20. However, TEAC<sub>50</sub> value of the control was higher than those of other treatments throughout the fermentation. This implied that the fermented extracts from nitrogen supplemented waxy corns might contain more phenolic hydroxyl groups, which show high electron/hydrogen donors antioxidant activities (Jayaprakasha and Patil, 2007).

The IC<sub>50</sub> of DPPH assay of monacal waxy corn are shown in Table 4. IC<sub>50</sub> values of every treatments tended to decrease after day 4 and were minimum

on day 12. The lower IC<sub>50</sub> values were due to the increased contents of peptone and MSG. The lowest IC<sub>50</sub> value of 0.02 mmol trolox/ml was obtained from the product supplemented with 12.08 % (w/w) MSG. The IC<sub>50</sub> value of the control was higher than those of MSG and peptone at each time point. However, the IC<sub>50</sub> value of the control was less than that of Yang *et al.* (2006), which showed that EC<sub>50</sub> value of scavenging ability on DPPH radicals of monacal polished rice was 0.59 mg extract/ml. These data suggested that addition of nitrogen promoted antioxidant production of monacal rice (Su *et al.*, 2003), which possessed more effective hydrogen donation to oxidants.

In FRAP assay, IC<sub>50</sub> values of monacal waxy corn are shown in Table 5. A similar trend was observed between IC<sub>50</sub> values of FRAP and DPPH assays (P>0.05). The IC<sub>50</sub> values were lower with increasing MSG and peptone contents. Comparing the same nitrogen content between MSG and peptone, average IC<sub>50</sub> value of MSG supplemented products was lower than that of peptone throughout the fermentation. The lowest IC<sub>50</sub> value of 0.09 mg extract/ ml was also obtained from monacal waxy corn supplemented with 12.08% (w/w) MSG. IC<sub>50</sub> value of the control on day 12 was about 100 times higher than that of 12.08% (w/w) MSG. EC<sub>50</sub> values of reducing power of monacal polished rice were 0.79 mg extract/ml (Yang *et al.*, 2006) and 0.78 mg extract/ ml from monacal polished adlay (Tseng *et al.*, 2006), hence, our products presented higher potential in reducing Fe (III)/tripirydyltriazine complex.

IC<sub>50</sub> values of monacal waxy corn from the chelating ability on ferrous ions assay are shown in Table 6. IC<sub>50</sub> values tended to decrease until day 12 of fermentation (P<0.05). The lower IC<sub>50</sub> values obtained from the increasing contents of MSG and peptone. The lowest IC<sub>50</sub> value of 2.89 mg extract/ml was presented in the product supplemented with 12.08% (w/w) MSG. The IC<sub>50</sub> value of the control on day 12 was about 3 times higher than that of 12.08% (w/w) MSG. EC<sub>50</sub> values of chelating ability on ferrous ions of monacal rice and monacal adlay were 3.92 mg extract/ ml and 2.91 mg extract/ ml, respectively (Tseng *et al.*, 2006; Yang *et al.*, 2006). Generally, ferrous ions are the most effective pro-oxidants in the food system and also are quenched by effective Fe<sup>2+</sup> chelating antioxidant (Yamaguchi *et al.*, 1988; Prior *et al.*, 2005). Our results showed stronger chelating ability on Fe<sup>2+</sup> for inhibition of lipid oxidation than the previous studies.

Table 7. Pearson’s correlation coefficients of pigment intensity and antioxidant activity values in monascal waxy corn supple supplemented with different peptone contents on day 12

	Pigment intensity	Glucosamine	TEAC <sub>50</sub>	IC <sub>50</sub> (DPPH)	IC <sub>50</sub> (FRAP)	IC <sub>50</sub> (Chelate Fe <sup>2+</sup> )	Monacolin K
Glucosamine	0.977**						
ABTS	-0.705	-0.961**					
IC <sub>50</sub> (DPPH)	-0.985**	-0.846	0.749				
IC <sub>50</sub> (FRAP)	-0.838	-0.981**	0.922*	0.901*			
IC <sub>50</sub> (Chelate Fe <sup>2+</sup> )	-0.988**	-0.784	0.737	0.970**	0.857		
Monacolin K	0.968**	0.643	-0.525	-0.951*	-0.740	-0.948*	
Citrinin	0.979**	0.796	-0.731	-0.974**	-0.878*	-0.995**	0.951*

\*Correlation is significant at the 0.05 level (2-tailed)

\*\*Correlation is significant at the 0.01 level (2-tailed)

Table 8. Pearson’s correlation coefficients of pigment intensity and antioxidant activity values in monascal waxy corn supplemented with different MSG contents on day 12

	Pigment intensity	Glucosamine	TEAC <sub>50</sub>	IC <sub>50</sub> (DPPH)	IC <sub>50</sub> (FRAP)	IC <sub>50</sub> (Chelate Fe <sup>2+</sup> )	Monacolin K
Glucosamine	0.949*						
ABTS	-0.874	-0.937*					
IC <sub>50</sub> (DPPH)	-0.904*	-0.807	0.635				
IC <sub>50</sub> (FRAP)	-0.875	-0.977**	0.969**	0.669			
IC <sub>50</sub> (Chelate Fe <sup>2+</sup> )	-0.967**	-0.923*	0.808	0.967**	0.831		
Monacolin K	0.922*	0.989**	-0.967**	-0.766	0.987**	-0.903*	
Citrinin	0.995**	0.965**	-0.890*	-0.911*	-0.898*	-0.980**	0.947*

\*Correlation is significant at the 0.05 level (2-tailed)

\*\*Correlation is significant at the 0.01 level (2-tailed)

*Pearson’s correlation among antioxidant activities, pigment intensity, glucosamine content, monacolin K and citrinin contents*

The correlation among antioxidant activities, pigment intensity, glucosamine, monacolin K and citrinin contents was analysed on day 12 because that was when the highest antioxidant activities and monacolin K and citrinin contents were obtained (Table 7 and 8). Good positive correlation was found between pigment intensity and monacolin k, between pigment intensity and citrinin contents and between pigment intensity and glucosamine content. On the other hand, good negative correlation was found between pigment intensity and inhibition of DPPH radical, between pigment intensity and chelating ability on ferrous ions and between monacolin K content and chelating ability on ferrous ions of both products supplemented with peptone and MSG. The results showed that Pearson’s correlation coefficients of the product supplemented with MSG were higher than those of peptone. IC<sub>50</sub> values obtained from different antioxidant activity assays decreased when increasing pigment intensity. This indicated that *Monascus* pigment containing high content of monacolin K could effectively scavenge DPPH radical (r<sup>2</sup> between -0.904 and -0.985) and chelate Fe<sup>2+</sup> (r<sup>2</sup> between -0.967 and -0.988) and it was well related to the growth of *M. purpureus* in waxy corn product supplemented with both MSG and peptone

(r<sup>2</sup> between 0.949 and 0.977).

**Conclusion**

The monascal waxy corn obtained from *M. purpureus* (TISTR 3090) supplemented with nitrogen presented high potential antioxidant activity. The increase of antioxidant activities and monacolin K was influenced by nitrogen sources and concentrations. The highest antioxidant activity and monacolin K values were found in monascal product supplemented with 12.08 % (w/w) MSG. Furthermore, the citrinin contents in monascal waxy corn were less than the maximum allowed level in red fermented rice legislated by the Chinese FDA. Pigment intensity could be a reliable indicator for monacolin K production and antioxidant activity.

**Acknowledgements**

This work was financially supported by the Doctoral Research Scholarship, Faculty of Agriculture, Natural Resources and Environment, Naresuan University. The authors would like to thank all staff of the faculty for maintenance and operation of the scientific laboratory and equipment.

**References**

Babitha, S., Soccol, C.R. and Pandey, A. 2007. Solid-state fermentation for the production of *Monascus* pigments from jackfruit seed. *Bioresource Technology* 98: 1554-1560.

Benzie, I.F.F. and Strain, J.J. 1996. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry* 239: 70–76.

Broder, C.U. and Koehler, P.E. 1980. Pigments produced by *Monascus purpureus* with regard to quality. *Journal of Food Science* 45: 579-579.

Chairote, E., Chairote, G., Nilamsup, H. and Lamyong, S. 2008. The presence and the content of monacolins in red yeast rice prepared from Thai glutinous rice. *World Journal of Microbiological and Biotechnology* 24: 3039-3047.

Chayawat, J., Jareonkitmongkol, S., Songsasen, A. and Yongsmith, B. 2009. Pigments and anti-cholesterol agent production by *Monascus kaoliang* KB 9 and its color mutants in rice solid cultures. *Kasetsart Journal* 43: 696-702.

Chen, M.H. and Johns, M.R. 1993. Effect of pH and nitrogen source on pigment production by *Monascus purpureus*. *Applied Microbiology and Biotechnology* 40: 132-138.

Chen, M.H. and Johns, M.R. 1994. Effect of carbon source on ethanol and pigment production by *Monascus purpureus*. *Enzyme and Microbial Technology* 16:

- 584-590.
- Dufosse, L., Galaup, P., Yaron, A., Arad, S.M., Blanc, P., Murthy, K.N. C. and Ravishankar, G.A. 2005. Microorganisms microalgae as sources of pigments for food use: a scientific oddity or an industrial reality?. Trends in Food Science and Technology 16: 389-406.
- 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.
- Erdogru, O. and Azirak, S. 2004. Review of the study on the red yeast rice (*Monascus purpureus*). Turkish Electronic Journal of Biotechnology 2: 37-49.
- Friedrich, J., Zuzek, M., Bencina, M., Cimerman, A., Strancar, A. and Radez, I. 1995. High-performance liquid chromatographic analysis of mevinolin as mevinolic acid in fermentation broths. Journal of Chromatography A 704: 363-367.
- Hajjaj, H., Niederberger, P. and Duboc, P. 2001. Lovastatin biosynthesis by *Aspergillus terreus* in a chemistry defined medium. Applied Environmental Microbiology 67: 2596-2602.
- Jayaprakasha, G.K. and Patil, S.B. 2007. *In vitro* evaluation of the Antioxidant activities in fruit extracts from citron and blood orange. Food Chemistry 101: 410-418.
- Jiranuntakul, W., Puttanlek, C., Rungsardthong, V., Panchanarong, S. and Uttapap, D. 2011. Microstructural and physicochemical properties of heat-moisture treated waxy and normal starches. Journal of Food Engineering 104: 246-258.
- Journoud, M.L. and Jones, P.J. 2004. Red yeast rice: a new hypolipidemic drug. Life Science 74: 2675-2683.
- Kuo, C.F., Hou, M.H., Wang, T.S., Chyau, C.C. and Chen, Y.T. 2009. Enhanced antioxidant activity of *Monascus pilosus* fermented products by addition of ginger to the medium. Food Chemistry 116: 915-922.
- Lee, B.K., Park, N.H., Piao, H.Y. and Chung, W.J. 2001. Production of red pigments by *Monascus purpureus* in submerged culture. Biotechnology and Bioprocess Engineering 6: 341-346.
- Li, Y.G., Zhang, F., Wang, Z.T. and Hu, Z.B. 2004. Identification and chemical profiling of monacolins in red yeast rice using high-performance liquid chromatography with photodiode array detector and mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 35: 1101-1112.
- Lim, J.Y., Kim, J.J., Lee, D.S., Kim, G.H., Shim, J.Y., Lee, I. and Imm, J.Y. 2010. Physicochemical characteristics and production of whole soymilk from *Monascus* fermented soybeans. Food Chemistry 120: 255-260.
- Lin, C.F. and Demain, A.L. 1991. Effect of nutrition of *Monascus* sp. on formation of red pigment. Applied Microbiology and Biotechnology 36: 70-75.
- Mukherjee, G. and Singh, K.S. 2011. Purification and characterization of a new red pigment from *Monascus purpureus* in submerged fermentation. Process Biochemistry 46: 188-192.
- Pattanagul, P., Pinthong, R., Phianmongkhol, A. and Tharatha, S. 2008. Mevinolin, citrinin and pigments of adlay angkak fermented by *Monascus* sp. International Journal of Food Microbiology 126: 20-23.
- Pinthong, R. and Bunsangsom, C. 2003. Effect of *Monascus* sp. and rice varieties on citrinin in red rice (Thai). Journal of Agricultural Science 168: 4-6.
- Prior, R.L. Wu, X. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53: 4290-4302.
- Purcell, E. A. and Walter, M.W. 1982. Stability of amino acid during cooking and processing of sweet potatoes. Journal of Agriculture and Food Chemistry 30: 443-444.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yanh, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26: 1231-1237.
- Shi, Y.C. and Pan, T.M. 2011. Beneficial effects of *Monascus purpureus* NTU 568-fermented products: a review. Applied Microbiology and Biotechnology 90: 1207-1217.
- Silveira, S.T., Daroit D.J. and Brandelli, A. 2008. Pigment production by *Monascus purpureus* in grape waste using factorial design. LWT-Food Science and Technology 41: 170-174.
- Su, Y.C., Wang, J.J., Lin, T.T. and Pan, T.M. 2003. Production of the secondary metabolites  $\alpha$ -aminobutyric acid and monacolin K by *Monascus*. Journal of Industrial Microbiology and Biotechnology 30: 41-46.
- Tseng, Y.H., Yang, J.H., Chang, H.L., Lee, Y.L. and Mau, J.L. 2006. Antioxidant properties of methanolic extracts from monascal adlay. Food Chemistry 97: 375-381.
- Velazquez, E., Tournier, H.A., Mordujovich de Buschiazco, P., Saavedra, G. and Schinella, G.R. 2003. Antioxidant activity of Paraguayan plant extracts. Fitoterapia 74: 91-97.
- Vidyalakshmi, R., Paranthaman, R., Muruges, S. and Singaravadivel, K. 2009. Stimulation of *Monascus* pigments by intervention of different nitrogen sources. Global Journal of Biotechnology and Biochemistry 4: 25-28.
- Wang, H.L. and Hesseltine, W.C. 1979. Mold-modified foods. In Pepler, H.J. and Perlman, J. (Eds.). Microbial Technology, p. 95-129. New York : Academic Press.
- Wang, J., Lu, Z. and Chi, J. 1997. Multicenter clinical trial of the serum lipid-lowering effects of a *Monascus purpureus* red yeast rice preparation from traditional Chinese medicine. Current Therapeutic Research 58: 964-978.
- Yamaguchi, R., Tatsumi, M.A., Kato, K. and Yoshimitsu, U. 1988. Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. Agricultural and Biological Chemistry 52: 849-850.
- Yamaguchi, S. and Ninomiya, K. 1998. What is umami ?. Food Reviews International 14: 123-138.
- Yang, J.H., Tseng, Y.H., Lee, Y.L. and Mau, J.L. 2006. Antioxidant properties of methanolic extracts from monascal rice. LWT-Food Science and Technology

39: 740-747.

Yongsmith B., Kitprechavanich, V., Chitradon, L., Chairisook, C. and Budda, N. 2000. Color mutants of *Monascus* sp. KB9 and their comparative glucoamylase on rice solid cultures. *Journal of Molecular Catalysis B: Enzymatic* 10: 263-272.