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Quality and acceptability of *Monascus* biopigment beverage

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Abstract

The study aimed to develop a *Monascus* biopigment beverage, which was made from the extract of *Monascus*-fermented rice, and evaluate its quality and acceptability as a functional drink. In addition, the stability of secondary metabolites, monacolin K and citrinin, on different acid concentrations (0.2, 0.4 and 0.6% w/v), various temperature treatments (30, 60 and 90°C), and daylight exposure for 30 days was determined. A method of enhancing monacolin K to citrinin ratio was included in the extraction process by partial heating of *Monascus*-fermented rice with ethanol at 65°C for 60 minutes. Preference ranking test and quality scoring were performed for the preliminary and final screening, respectively, of beverage formulations. On the other hand, quantification of monacolin K and citrinin in stability studies was done using high-performance liquid chromatography (HPLC) with UV-Vis spectrophotometer. Results showed that two formulations, both with the lowest amount of *Monascus* extract added (7.2 mL per 250 mL), were significantly acceptable and comparable but the sample with less sugar and acidulant contents (9.7% sugar, 0.3% acidulant) was preferred and analyzed for physico-chemical tests. The developed beverage had a pH of 2.8, titratable acidity (TA) of 0.31% expressed as citric acid while 0.32% as malic acid, total soluble solids (TSS) of 11°Brix, 2,2 diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity of 16.66%, and total phenolic content of 4.13 mg catechin (per 250 mL beverage). No visible peak was observed in both monacolin K (lovastatin equivalent) and citrinin determination. The metabolites were shown to decrease when the extract was subjected to different acid concentrations and temperature treatments as well as when exposed to daylight for 30 days. Conversely, increase in the ratio of monacolin K to citrinin was observed in partial heating of *Monascus*-fermented rice, thus the need to include in the extraction method. The study suggested that aside from imparting color, *Monascus* biopigment could be incorporated in the beverages to improve its functional properties.

Introduction

Color is an important characteristic of food. It creates impressions on the taste and flavor of food and its safety as well. With this information, color is added to food for the following purposes: (a) to replace color lost during processing, (b) to enhance the present color, (c) to minimize batch-to-batch variations, and (d) to color the uncolored food (Mortensen, 2006). Based on the National Academy of Sciences (1961), food colors, whether natural or synthetic origin, are commonly used in processed foods to increase the acceptability and attractiveness of these products. This was proven by the studies of Dizon (1983) and Mamucod (2011) on Philippine native sliced pork (tocino) and sausage (longganisa), respectively. Both results showed that the addition of pigments from *Monascus purpureus* was found to be more acceptable than the non-colored version of the products and significantly comparable to the nitrate-treated ones.

Monascus purpureus is one of the four species of the genus *Monascus* which belongs to the group of Ascomycetes, particularly to the family of Monascaceae (Pattanagul *et al*., 2007). It is divided into four species namely, *M. pilosus, M. purpureus, M. ruber,* and *M. froridanus* (Lee *et al*., 2008). The common names of the fungal product are *Monascus*-fermented products, red rice, angkak, red leaven, benikoji in Japan, hung-chu, hong qu and zhitai in China, rotschimmelreis in Europe, and red mold in USA (Pattanagul *et al*., 2007). For many centuries in China, red rice has been a traditional Chinese food additive used as a coloring and flavoring agent (Ng and Shyu, 2004). Its special effects and application on food have been recorded in ancient Chinese records (Lee *et al*., 2006).

Many studies suggested that *Monascus*-fermented rice, aside from its color, has beneficial health and clinical impact. A research revealed that *Monascus*-

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fermented products had a strong antioxidant action which can help protect our cells against the effects of dangerous free radicals (Kim *et al.*, 2008). Likewise, Mamucod (2011) reported that crude *Monascus* extract was a rich source of phenolics which was comparable to other plant sources with high amounts of the compound. Moreover, based on accumulating scientific reports on the medical or functional food effects, *Monascus*-fermented red rice extract, which contains monacolin, has positive effects in inflammation, bone fractures, glucose tolerance, Alzheimer disease, and anti-cancer progression (Ho and Pan, 2009). The most recognized health benefit of monacolin is its antihypercholesterolemic effect which can alleviate the problem of increasing number of cardiovascular death. These researches have shown that *Monascus*-fermented product contains multifunctional compounds making it as an important topic in the field of functional food (Lee *et al.*, 2006).

Recent research and development on *Monascus*-fermented products include the incorporation of the fermented red rice pigment in beverages or drinks (Srianta *et al*., 2014). One of the studies was from Kim *et al.* (2008) which involved the application of fermented rice in the preparation of rice beverages. The product showed greater reducing power, scavenging and chelating abilities, and higher total phenolic content than the uninoculated rice beverage counterpart.

The combined beneficial effect of *Monascus* biopigment on lipid levels and against oxidative damage due its strong antioxidant properties could transform an ordinary beverage into a functional one upon incorporation of the compound. Therefore, the study developed a *Monascus* biopigment beverage, which was made from the extract of Monascus-fermented rice, and evaluated its quality and acceptability as a functional drink. Moreover, the research determined the effects of different acid concentrations, various temperature treatments, and daylight exposure for 30 days on the stability of monacolin K and citrinin in *Monascus* extract.

Materials and Methods

Preparation of Monascus-fermented rice

The optimized method developed by Dizon (1983) with some modifications was used in the preparation of *Monascus*-fermented rice. A C4 variety of rice *(Oryza sativa)* was purchased from a local market in Los Baños, Laguna, Philippines. The rice was soaked in water for 10 hours, drained, spread onto cheesecloth, and steamed for 30 min. Fifty grams (50 g) of steamed rice was transferred into a flask and the moisture content was adjusted to approximately 35%. The flasks were then sterilized at 121°C for 30 min and were allowed to cool before adding the spore suspension from *Monascus purpureus* Went with 5 mL of sterilized distilled water. The samples were incubated for 10 days at 26 to 30°C. The resulting fermented rice was oven-dried at 55°C for 24 to 48 hours. The dried *Monascus*-fermented rice were transferred in clean and dry plastic bags and stored at refrigerated temperature.

Extraction of Monascus-fermented rice

The optimized method developed by Mamucod (2011) with some modifications was used in the extraction of *Monascus* pigment. The fermented rice was ground (Shimono blender) and sieved (No. 40 USA Standard Testing Sieve). For every 1 g of the sample, 10 mL of 70% ethanol was added. The mixture was shaken for 1 hr then heated in a boiling water bath for another 1 hr at 65°C with continuous shaking. After cooling, the sample was centrifuged at 4000 rpm (International Centrifuge) for 15 min. The supernatant was collected, and the ethanol was removed by concentration in rotary vacuum evaporator at temperature between 50°C to less than 60°C. The resulting crude extract was used for the different tests and for the preparation of *Monascus* biopigment beverage.

Development of Monascus biopigment beverage

A preliminary screening without *Monascus* extract was done to get the proper combination of sugar and acidulant. The formulations were based on total soluble solids (10 to 13°Brix). The acidulants used were combinations of citric and malic acids in a ratio of 50:50. The amounts of acidulants added were 0.2, 0.3, and 0.4%. Each was mixed with different amounts of sugar (8.6 to 11.8%), 0.05% natural identical apple flavor (International Flavors and Fragrances), and purified water producing 250-mL beverages. Twelve (12) combinations were formulated and served into two batches. The first batch consisted of combinations with 10 and 11°Brix while the second composed of combinations with 12 and 13°Brix. Each batch of the samples was evaluated by preference ranking test with 25 experienced panelists using a 6-point scale, 1 as the most preferred and 6 as the least preferred. The most preferred combination in each set was used for the development of *Monascus* biopigment beverage.

The recommended supplementation of 2400 mg a day of *Monascus*-fermented rice (Heber *et al.*, 1999) was the basis of the dosage of *Monascus* extract that

was made into a beverage. Other variables were dosages of 3600 and 4800 mg. For every 1 g of dried *Monascus*-fermented rice, 10 mL of 70% ethanol was mixed. The extract was first concentrated in rotary evaporator before adding in the different formulations. For 2400, 3600, and 4800 mg of powdered *Monascus*-fermented rice, the computed amounts extracted were 7.2, 10.8, and 14.4 mL, respectively. Pasteurization, using the method of Alvarez *et al.* (2009), was performed after mixing all ingredients.

Sensory evaluation of Monascus biopigment beverage

Sensory evaluation of *Monascus* biopigment beverage was conducted at the Food Science Cluster, University of the Philippines Los Baños, College, Laguna, Philippines. The samples were prepared by pouring approximately 20 mL of cold beverages in sensory glasses, coded properly using three-digit random numbers, and presented in random order. The samples were evaluated by 15-member experienced panelists. Using quality scoring, the panelists discriminate the formulated samples based on color, flavor, aroma, aftertaste, sweetness, sourness, and overall acceptability. The most acceptable combination scored by the panelists was subjected to physico-chemical analyses.

Physico-chemical analyses of Monascus biopigment beverage

The physico-chemical properties of *Monascus* biopigment beverage were determined in terms of pH, total soluble solids (TSS), titratable acidity (TA), total phenolic content, antioxidant activity, and monacolin K and citrinin contents. A pen type pH meter (Milwaukee pH 600) calibrated with standard buffer, pH 7.0 and a hand-held refractometer (Atago Master-M) were used to measure pH and total soluble solids (TSS) expressed in ^{o}Brix, respectively. Titratable acidity (TA) was analyzed by titrating the sample with standardized 0.1 N sodium hydroxide (NaOH) solution to a pH of 8.1 and the values obtained was computed as percent (%) titratable acidity. The total phenolic content was determined using Folin-Ciocalteu Phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) method (Tanqueco *et al*., 2007). The antioxidant scavenging activity was measured using 2,2 diphenyl-1-picryl hydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) radical according to the method of Duan *et al.* (2007) with some modifications. Quantifying monacolin K and citrinin was done using high-performance liquid chromatography (HPLC) with UV-Vis spectrophotometer. Chromatographic separation was performed using Shimadzu LC-20AD isocratic system consisting of a SPD-2AV Prominence UV/VIS detector, a 20 µL sample loop injector and a 150 x 4.6 mm Shi-pack CLC-ODS C18 guard column. The optimum mobile phase of acetonitrile-water-trifluoroacetate (55 + 45 + 0.05, v/v) was used (Lee *et al.*, 2006). The eluent was pumped at a flow rate of 0.7 mL min^{-1} for monacolin K while 1.0 mL min^{-1} for citrinin. UV detection was set at 238 nm and 330 nm for monacolin K and citrinin, respectively. The contents of monacolin K (mg L^{-1}, as lovastatin equivalent) and citrinin (mg L^{-1}) were obtained from calibrated standard curve. Different concentrations of lovastatin and citrinin dissolved in acetonitrile were prepared and were also analyzed using HPLC-UV.

Stability of monacolin K and citrinin

The effects of different concentrations of acid, varying temperature conditions, and daylight exposure on monacolin K and citrinin of *Monascus* extract were determined. For the effect of acid, 3 tubes with 10 mL of extract were added separately with 0.2, 0.4, and 0.6% citric acids and thoroughly mixed using a test tube mixer. On the other hand, for the effect of temperature on monacolin K and citrinin of *Monascus* extract at constant time of 30 minutes, aliquots of 10 mL *Monascus* extract were put into screw-cap test tubes and heated in a water bath at 30, 60 and 90^{o}C. The heated vials were removed immediately after treatment and then cooled rapidly with cold water. Lastly, for the effect of daylight exposure, 2 tubes containing 10 mL of the extract were stored at room temperature for 30 days. One was covered with aluminum foil and the other was left uncovered. All samples were stored in refrigerator (4±1^{o}C) until the day of analysis using HPLC-UV.

Enhancement of monacolin K to citrinin ratio

One (1) gram of *Monascus*-fermented rice was mixed with ethanol and heated at 65^{o}C for 60 minutes to enhance the monacolin K to citrinin ratio, as recommended by Lee *et al*. (2007). Another 1 g was extracted using the same process, but without subjecting to partial heating in a water bath. The changes in monacolin K and citrinin contents were analyzed using HPLC-UV.

Statistical analysis

All laboratory analyses were performed in triplicates and the results were expressed as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the significant differences of all data gathered

Table 1. Mean sensory scores of the formulations with TSS of 10 to 13°Brix

*Formulation	Mean Score	*Formulation	Mean Score
10°Brix		12°Brix	
8.6:0.4	3.76[a]	10.6:0.4	2.64[b]
8.7:0.3	3.88[a]	10.7:0.3	3.20[b]
8.8:0.2	4.36[a]	10.8:0.2	4.56[a]
11°Brix		13°Brix	
9.6:0.4	2.76[b]	11.6:0.4	2.84[b]
9.7:0.3	2.52[b]	11.7:0.3	3.24[b]
9.8:0.2	3.72[a]	11.8:0.2	4.52[a]

Mean values with different letters in the same group are significantly different from each other at $p<0.05$.
*Sugar (%): Acidulant (%)
Range of scores: 1, most preferred to 6, least preferred.

Table 2. Mean sensory scores of the different formulations of *Monascus* biopigment beverage

*SAMPLE	Sensory attributes						
	Color	Aroma	Flavor	After-taste	Sweet-ness	Sour-ness	Over-all Acceptability
11°Brix							
9.7:0.3,7.2	6.07[d]	7.89[a]	10.43[a]	5.15[de]	8.69[ab]	7.03[a]	10.68[a]
9.7:0.3,10.8	11.08[b]	8.73[a]	7.70[b]	9.89[ab]	6.85[bc]	7.19[a]	5.93[c]
9.7:0.3,14.4	13.31[a]	9.07[a]	7.17[b]	11.70[a]	5.74[c]	7.17[a]	3.96[d]
12°Brix							
10.6:0.4,7.2	4.23[e]	7.77[a]	10.83[a]	4.85[e]	10.02[a]	7.97[a]	11.22[a]
10.6:0.4,10.8	7.75[c]	8.33[a]	9.17[ab]	7.21[cd]	9.01[ab]	7.67[a]	8.20[b]
10.6:0.4,14.4	10.29[b]	8.92[a]	7.59[b]	8.35[bc]	8.15[ab]	8.28[a]	6.38[c]

Mean values with different letters in the same group are significantly different from each other at $p<0.05$.
*Sugar:Acidulant (%), *Monascus* extract added (mL).
Range of scores: color: 0-pale pink, 15-dark red; aroma: 0-no apple scent, 15-strong apple scent; flavor: 0-no apple flavor, 15-full apple flavor; aftertaste: 0-imperceptible, 15-very perceptible; sweetness: 0-bland, 15-very sweet; sourness: 0-bland, 15-very sour; over-all acceptability: 0-unacceptable, 15-highly acceptable.

statistically. The least significant difference (LSD) test was used to compare treatment means at 5% level of significance using Statistical Package for the Social Sciences (SPSS) version 10.0.

Results and Discussion

Preliminary screening of beverage formulation

The mean sensory scores of 25 panelists for each batch of preference ranking test are shown in Table 1. Among the six beverage formulations in the first batch, all samples with 10°Brix showed significantly comparable results to one another and were the least preferred by the panelists. Moreover, most of the panelists favored samples with higher amount of acidulant than the formulations with only 0.2% acid added. This preference in more acidic samples was also observed in the second batch of formulations.

The result of the second batch of formulations with TSS of 12 and 13°Brix was comparable to the first where samples containing 0.2% acid were the least preferred by the panelists. Since most of the favored formulations in both batches had insignificant results with another sample/s in the same group ($p<0.05$), the two formulations with the lowest mean sensory scores were considered and used for the succeeding preparations and development of *Monascus* biopigment beverage. The two most preferred samples had TSS of 11 and 12°Brix with sugar: acidulant percentage values of 9.7: 0.3 and 10.6: 0.4, respectively.

Sensory evaluation of Monascus biopigment beverage

Quality scoring was used as the method for discrimination of the different formulations of *Monascus* biopigment beverage using 15-cm scale. The attributes tested were color, aroma, flavor, aftertaste, sweetness, sourness, and over-all acceptability. The mean scores of the two most preferred sugar and acidulant combinations added with different concentrations of *Monascus* extract are shown in Table 2.

Among the six formulated beverages, four samples were distinctly differentiated by the panelists in terms of color. Generally, the higher the amount of *Monascus* extract added, the more reddish the product was. This was shown by the increasing mean sensory scores of the samples as the volume of *Monascus* extract increased. However, the formulation with 10.6% sugar, 0.4% acidulant, and

14.4 mL *Monascus* extract (12°Brix) was found to be insignificantly different to the formulation with 9.7% sugar, 0.3% acidulant, and 10.8 mL extract (11°Brix). This unrecognizable color difference between the two samples may be accounted to the amount of acidulant added. Lin *et al.* (2008) reported that the stability of *Monascus* pigments was seriously affected by pH, and the red pigment was found to be less stable in acidic condition. The effect may be due to the acid acceleration of water interaction with pigments resulting to breaking of ester linkages in rubropunctamine or monascorubramine, the red pigments produced by *Monascus* sp. (de Carvalho *et al*., 2005). Thus, the higher the amount of acidulant added in the sample, the lesser the perception of red color.

In terms of aroma, all samples had mean sensory scores of more than half of the 15-cm line scale but the difference between samples was generally undistinguishable. Results also showed that the concentration of *Monascus* extract added did not significantly influence the aroma perception of the samples. However, there was an increasing trend of mean sensory scores as the amount of *Monascus* extract added increased. This may be attributed to the different color intensities of the products which, according to Mahony (2011), have a direct correlation with odor intensity; thus, the dark-colored samples perceived to have higher aroma intensities than the light-colored and pale products.

The flavor of the samples was influenced significantly by the amount of *Monascus* extract added. There was a decreasing trend of flavor perception as the amount of *Monascus* extract added increased. This was shown by the low mean sensory scores obtained from the samples incorporated with higher amount of extract. Most panelists commented on the bitter and slight astringent taste of the more reddish samples. This may be accounted to the high total phenolic content which according to Jackson (2000) has a direct influence on the perception of bitterness and astringency. In general, samples with 7.2 mL *Monascus* extract were found to be more acceptable in terms of flavor perception than 10.8 and 14.4 mL concentrations of *Monascus* extract.

As what was reported in flavor perception, bitter and slight astringent tastes were perceived by most of the panelists, especially to the samples with darker colors. The result of this study was comparable to Mamucod (2011) wherein samples incorporated with high concentration of *Monascus* extract (2 and 3% w/v) were markedly found to have perceptible aftertaste. The aftertaste was due to the bitter and astringent attributes of the added *Monascus* extract, and majority of the panelists did not find these characteristics as acceptable. This was evident in the lower over-all acceptability mean sensory scores of the more reddish samples with higher aftertaste mean scores. In addition, based on the trend of the result, the aftertaste decreased as more acidulant and sugar were added to the product. This was manifested in the samples with TSS of 12°Brix which showed lower aftertaste mean score values than the samples with TSS of 11°Brix when compared in accordance with the amount of *Monascus* extract added.

The samples with TSS of 12°Brix were perceptibly sweeter than the samples with TSS of 11°Brix. However, as the amount of *Monascus* extract added was increased, the perception of sweetness by the panelists slightly shifted to the bland side of the scale. Likewise, the sample with 7.2 mL *Monascus* extract having 11°Brix showed comparable mean score, in terms of sweetness attribute, to the samples with 10.8 and 14.4 mL *Monascus* extract which both had a TSS of 12°Brix. This implied that the bitter taste of *Monascus* extract had a tendency to mask the sweetness of the formulated products.

No significant difference among the formulations was found in terms of perceiving sourness. However, samples with 0.4% acidulant still had higher mean score values than samples with 0.3% acid. The range of mean scores was from 7.02 to 8.28 which was equivalent to subjective rating as slightly to moderately sour.

Two formulations, both containing 7.2 mL *Monascus* extract, were found to be significantly acceptable and comparable. Between the two, the formulation with 9.7% sugar was preferred and used for the succeeding analyses due to its lower sugar content than the other. Aside from economical reason, the product formulated was intended for adults who are recommended to take less consumption of concentrated sugars to prevent increasing the risk of acquiring lifestyle diseases, specifically cardiovascular diseases. Generally, as the concentration of *Monascus* extract increased, the bitter aftertaste affected the acceptability scores of the samples as perceived by the panelists.

Physico-chemical properties of Monascus biopigment beverage

Physico-chemical properties of *Monascus* biopigment beverage are shown in Table 3. The developed beverage had TSS, pH and titrable acidity of 11°Brix, 2.8 and 0.31% (expressed as citric acid) to 0.32% (expressed as malic acid), respectively. On the other hand, the DPPH radical scavenging activity

Table 3. Physico-chemical properties of *Monascus* biopigment beverage

Physico-chemical properties						
pH	TA (%)	TSS (°Brix)	Total Phenolics (mg catechin/ 250mL)	Anti-oxidant Activity (%)	Monacolin K (mg/mL)	Citrinin (µg/mL)
2.8±0.1	0.31±0.01* 0.32±0.01**	11	4.13±1.17	16.66± 0.01	ND	ND

Data are means±S.D.
*expressed as citric acid
**expressed as malic acid
ND, not detected

and total phenolic content of *Monascus* beverage were 16.66% and 4.13 mg catechin per 250 mL, respectively. The standard, BHA, for antioxidant had percentage scavenging activities of 48.05, 73.87, and 86.29% for 15, 30, and 75 µM, respectively. Previous studies such as that of Mamucod (2011) reported that *Monascus* extract was a good metal chelator because it had site specific scavenging activity (77.57%) which was significantly greater than the well-recognized antioxidants such as BHT, BHA, and vitamin E. However, in the study, the scavenging activity and phenolic content of the developed beverage were relatively low which may be due to the minute amount of *Monascus* extract (7.2 mL) added and diluted in water to produce a 250-mL acceptable beverage. Same reason may be accounted for the no visible peak observed in both monacolin K and citrinin determination. The absence of peak means that the amount of monacolin K and citrinin in the product was too small to quantify. According to Heber *et al*. (1999), aside from monacolin K, there were other monacolin-related active compounds in *Monascus*-fermented rice which may contribute to the lipid-lowering effect of the product; however, in this study, only monacolin K was analyzed. The unquantified amount of citrinin determined, on the other hand, was a good indicator of its possible safe intake.

Stability of monacolin K and citrinin

The strain of *Monascus purpureus* used in developing *Monascus* biopigment beverage yields 1.45 mg monacolin K (lovastatin equivalent) and 1.20 mg citrinin per liter of *Monascus* extract, as shown in Figure 1a. Since the amount of monacolin K measured in the study was relatively low, it would be necessary to determine the stability of this compound in different processing treatments. On the other hand, data regarding citrinin stability gives information on how the compound will be degraded since there is little information on the fate of citrinin during processing (EFSA, 2012).

The effect of different concentrations of acid, ranging from 0 (control) to 0.6%, on the levels of monacolin K and citrinin in *Monascus* extract is shown in Figure 1a. It was observed that the amounts of monacolin K and citrinin decreased as the concentration of acid incorporated in *Monascus* extract increased. The control, with no acid added, had a pH of 4.2. When 0.2% acid was added to the extract, the resulting pH was 3.6; monacolin and citrinin were significantly decreased by 68% and 64%, respectively. On the other hand, in the extract with 0.4% acid (pH 3.2), the decline in monacolin K was not significantly different with that of the 0.2% acid concentration, but citrinin content significantly decreased by 49%. Much lower concentrations of the compounds were obtained when 0.6% acid was added to the extract, with resulting pH of 2.9. There were 79% and 85% significant reduction in the amounts of monacolin K and citrinin, respectively, in reference to the control. Wang and Xu (2009) reported that the solubility of monacolin K in water decreased under acidic condition. This resulted to the formation of precipitate, which was also observed in the developed product. According to Wang (2008), at pH 4 to 9, only minute amount of precipitate was formed. This means that at pH less than 4 and greater than 9, a significant decrease in monacolin K level can be observed which was evident in the experiment. On the other hand, citrinin had the same fate as that of monacolin K when subjected to different acid concentrations. Xu *et al*. (2006) reported that citrinin can be degraded in acidic or alkaline solutions.

The effect of temperature on monacolin K and citrinin at constant heating time of 30 minutes is shown in Figure 1b. A significant reduction on the amounts of monacolin K and citrinin in *Monascus*

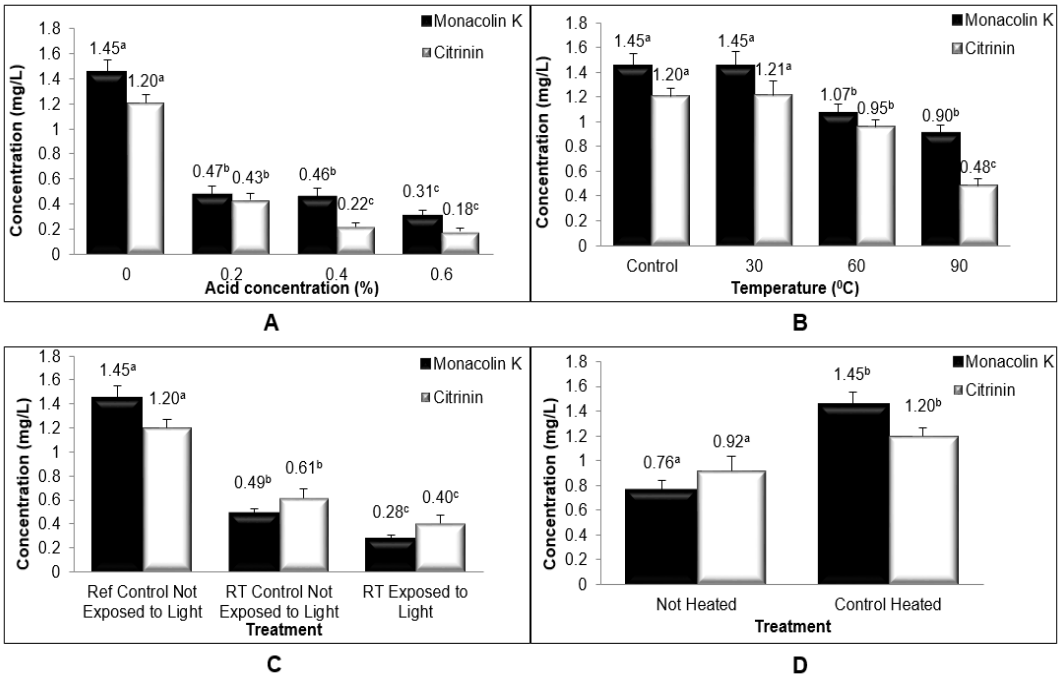

Figure 1. Effects of (a) different acid concentrations, (b) temperature, (c) daylight exposure, and (d) partial heating during biopigment extraction process on the amounts of monacolin K (lovastatin equivalent) and citrinin in *Monascus* extract

Ref, refrigerated; RT, room temperature

Mean values with different letters in the same group are significantly different from each other at $p<0.05$.

extract was observed at more than 30°C of heating. At 60°C, monacolin K and citrinin were decreased significantly by 26% and 21%, respectively, when compared to the control. However, when the compounds were subjected to 90°C for 30 minutes, there was 60% significant decreased in the amount of citrinin, from 1.2 mg L^{-1} to 0.48 mg L^{-1}. The sudden decrease in the amount of citrinin indicated that the compound was more sensitive to heat than monacolin K which only had 38% reduction after processing at 90°C. Ou *et al*. (2009) reported that as heat temperature increased from 85 to 121°C, the content of monacolin K decreased significantly. Furthermore, monacolin K was stable under low temperature treatments, such as with pasteurization processing. The decreased amount of monacolin K when exposed at increasing temperature values may be due to the destruction of the compound by heat treatment (Wang and Xu, 2009). This can also be the explanation of the instability of citrinin in aqueous solution as affected by heat. But based on Lee *et al*., (2007), citrinin is more unstable at high temperature than monacolin K. Xu *et al.* (2006) reported that after boiling in water, concentration of citrinin in *Monascus* extract was dramatically decreased by 50% in 20 minutes of heating. Comparable result was observed in the study where more than half of citrinin was lost when the extract was heated at 90°C for 30 minutes.

The effect of daylight exposure on monacolin K and citrinin in *Monascus* extract is shown in Figure 1c. There were 43% and 34% decrease in the amounts of monacolin K and citrinin, respectively, when the extract exposed to daylight and stored at room temperature was compared from the extract not exposed to light but stored at the same condition. Existing literature reports highlighted similar results that direct exposure to light reduced the amounts of monacolin K and citrinin in aqueous solution. Xu *et al.* (2006) also noted the slight destruction of monacolin K when exposed to light for a long time. Same as monacolin K, Weindenborner (2001) reported that citrinin was unstable during prolonged exposure to light. The reduction in the contents of monacolin K and citrinin may be attributed to the photoactivated catalysis in which light was absorbed, and its energy was used to excite molecules (Suppan, 1994) leading to a faster decomposition of the compounds. Results also showed that longer storage time at room temperature decreased the amounts of monacolin K and citrinin. However, faster reduction in the concentrations of the compounds was observed in light conditions than in dark environment. Almost half of the amounts of the compounds were lost when the extract was stored at room temperature for 30 days compared to the refrigerated control. The decline in the amounts of monacolin K and citrinin after long time storage can be accounted to the natural

decomposition of the compounds (Wang, 2008).

Enhancement of monacolin K to citrinin ratio

Aside from avoidance of light because of its effect on the amounts of monacolin K and citrinin, heating with ethanol at 65°C for 60 minutes was included in the extraction process. This was done to revalidate the method recommended by Lee *et al.* (2007) and to enhance the ratio of monacolin K to citrinin. The effect of partial heating on the concentrations of monacolin K and citrinin in *Monascus* extract is shown in Figure 1d.

There was a tremendous increase of 91% in monacolin K of the sample that was heated in a water bath for 1 hr. The initial amount of monacolin K without subjecting to heat treatment was 0.76 mg L^{-1}. The amount significantly increased to 1.45 mg L^{-1} when heating in water bath procedure was added. Likewise, citrinin content also increased from 0.92 mg L^{-1} to 1.2 mg L^{-1}. From 0.83 monacolin/citrinin ratio in not heated samples, the values increased to 1.21 (46% increase) after subjecting the sample to partial heating during the extraction process. Heat is usually used to accelerate the breakage of cells and obtain higher levels of extraction especially for intracellular samples (Wu *et al*., 2011). A study by Ajdari *et al.* (2011) confirmed that heating enhanced the amount of monacolin K extraction with continuous agitation using methanol and ethanol as extracting solvents.

Conclusion

The study was conducted to develop a *Monascus* biopigment beverage and evaluate its quality and acceptability as a functional drink. The developed beverage contained 7.2 mL *Monascus* extract, 9.7% sugar, 0.3% acidulant, and 0.05% natural identical apple flavor. On the other hand, stability studies showed decrease amounts of secondary metabolites, monacolin K and citrinin, when subjected to different acid concentrations, temperature treatments, as well as when exposed to daylight for 30 days. Nevertheless, an increase in the ratio of monacolin K to citrinin was observed when partial heating of *Monascus*-fermented rice was included in the process. The results of physico-chemical analyses and stability studies suggested that aside from imparting color, *Monascus* biopigment could be incorporated in the beverages to improve its functional properties.

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