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Stability of mao (*Antidesma bunius* (L.) Spreng) powder in different food process models

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Abstract

Products with natural ingredients are now more preferred, due to people being more health conscious. Mao *(Antidesma bunius)* is an excellent source of phytochemicals which provide a bright attractive color and possible health benefits. This research studied the stability of mao powders under food process models (pH: 3, 5, 7, 9; heating conditions: no heating, pasteurized and sterilized; and sucrose addition: 0% and 10%). The results showed that color, browning index, total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant activities (ABTS and FRAP assay) varied depending on the conditions. The stability of anthocyanins in mao powders depended on the pH and temperature. The no heating treatment at pH 3-7 and pasteurization at pH 3 had the highest TAC (2.38-2.43mg/g fw) ($p \leq 0.05$), while the highest phenolic value and antioxidant activities were found in solutions at pH 9 in sterilized condition.

Introduction

Color is an important attribute of any food or beverage as it can indicate quality as well as improve the appearance; therefore synthetic dyes are commonly use in the food industry even though their safety is still uncertain, since color has a great impact on consumers buying decisions. Nowadays, consumers with health concerns prefer foods with natural colorants; as a result, natural colorants have become increasingly needed (Giusti and Wrolstad, 2003). Nevertheless, the use of natural colorants is limited because their stability depends on various factors such as pH, heat, oxygen, light intensity, co-pigments, metal ions, ascorbic acid, sugars, enzymes and sulfur dioxide (Bakowska-Barczak, 2005; He and Giusti, 2010).

Among several pigments found in nature, anthocyanins are widely used as a replacement for synthetic colorant (Lapornik *et al*., 2005; Amelia, 2013). They exist in many parts of plants such as the leaves, flowers, fruits, stems and roots (Mazza and Miniati, 1993). Anthocyanins are a class of phenolic, which exhibit different colors ranging through pink, red, purple and blue depending on their pH (Cevallos-Casals and Cisneros-Zevallos, 2004; Reyes and Cisneros-Zevallos, 2007; Iosub *et al.*, 2012). They are one of the most interesting natural colorants due to their bright attractive colors, water solubility and many health benefits. They have been used for protective and therapeutic application against many diseases such as hypertension, cardiovascular disease, diabetes, nervous system or immune system disorders, inflammation, cancer and vision disorders (Lazze *et al.*, 2004; Motohashi and Sakagami, 2008; Ruenroengklin *et al.*, 2008).

Mao or mamao fruit (*Antidesma bunius* (L.) Spreng) is a tropical fruit in the Phyllanthaceae family, generally found in Africa, Australia, groups of islands in the Pacific Ocean and tropical Asia. They are grown in many area of Thailand especially in Northeastern Thailand (Butkhup and Samappito, 2008; Puangpronpitag *et al.*, 2011). The ripe mao fruit has a deep purple color and a sweet and sour taste with slightly astringency. Anthocyanins in mao fruit, identified as cyanidin, pelargonidin, delphinidin and malvidin (Jorjong *et al*., 2015), have been reported to have antioxidant activity (Nuengchamnong and Ingkaninan, 2010). As a result, anthocyanin extract from mao fruit could be another source of natural colorants with health benefits.

Anthocyanins are phytochemicals which are widely used in functional foods due to their attractive color and several health benefits. The objectives of

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this study were to investigated the stability of mao powder in a food process model (various pH, heating condition and sucrose addition) on color, anthocyanin content, phenolic compound and antioxidant capacity in order to provide guidance for its use in food products, with the results being recommendations for each food system of suitable conditions that maintained the health benefits from mao powder.

Materials and Methods

Materials

Maodong or mamaodong (family Phyllanthaceae, genus bunius) was purchased from an orchard in Loei province, Northeastern Thailand. Mao fruits with dark red and purple color were selected and washed with water to remove any remaining sand or dirt and to reduce the initial microbial load, soaked with chlorine (50 ppm) for 30 mins and washed with water again. Later, the washed mao fruits were sealed in plastic bags and kept in the dark in a freezer (temperature about -18°C) until use, in order to slow down anthocyanin degradation.

Chemicals

Maltodextrin with DE 10-12 (Thai Food and Chemical, Thailand) was used as the carrier agent. Folin-Ciocalteu reagent, gallic acid, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), iron(II) sulfate, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium chloride, iron(III) chloride, boric acid, citric acid, sodium carbonate and disodium hydrogenphosphate were purchased from Ajax Finechem (Auckland, New Zealand), hydrochloric acid from Merck (Darmstadt, Germany) and sodium acetate hydrate from Carlo Erba (Milan, Italy). All water used was deionized using a WaterPro Ro (Labconco Corporation, Kansas City, MO, USA).

Preparation of mao powders

Frozen mao fruits were transferred to a refrigerator (4°C) overnight before use, and later the fruits were squeezed using a Green Star GS 1000 twin gear juice extractor (Tribest, USA). The juice was vacuum filtrated through a 230-mesh silk screen, mixed with maltodextrin in order to reduced stickiness and agglomerates after freeze drying (mao juice: maltodextrin = 65 : 35 w/w), frozen at -80°C, and later freeze-dried using a Thermo Scientific Supermodulyo-230 freeze dryer (Thermo Fisher Scientific, Waltham, MA, USA) at -65°C under a pressure of 6.5 x 10^{-1} mbar for 29 hr. After freeze-drying, the dried sample was ground into powder, packed in aluminum foil and kept in a desiccator for further analysis.

Stability test of mao powders

The experimental design for the mao powder stability test was planned using a full factorial with three factors; sucrose addition at 2 levels (10% sucrose added, no sucrose added), pH at 4 levels (3.0, 5.0, 7.0 or 9.0) and heating condition at 3 levels (no heating, pasteurized at 65°C for 30 mins, at pH 3 sterilized at 100°C for 15 mins, and at pH 5-9 sterilized at 121°C for 15 mins). Mao powders were dissolved in different pH buffers at a concentration of 25mg/mL, with or without 10% sucrose added. Then, each solution was subjected to the different heating conditions. After that, each treatment was analyzed for its total anthocyanin content (TAC), total phenolic content (TPC), color value using the CIE L^* C^* $h°$ system and antioxidant activities (ABTS and FRAP assays).

Color

The color parameters *(L^*, a^*, b^*, C^*, $h°$)* were measured in transmission mode in a cuvette with a 10 mm path length using a Minolta CM-3500d spectrophotometer (Konica Minolta Inc., Tokyo, Japan), under a standard illuminant D65, standard observer 10°.

The color values (L^*, a^* and b^*) were used to calculate the browning index (Palou *et al.*, 1999). The formulae are shown in Equation (1) and (2)

$$\text{Browing index (BI)} = \frac{[100\times(X-0.31)]}{0.172} \quad (1)$$

where:

$$X = \frac{(a^*+1.75L^*)}{(5.645L^*+a^*-3.012b^*)} \quad (2)$$

Total anthocyanin content (TAC)

The TAC was determined using the pH-differential method (Worsltad *et al.*, 2005). Absorbance was measured using a spectrophotometer-UV/VIS Uvmini-1240 (Shimadzu, Japan) at 510 and 700 nm. Distilled water was used as the blank. The TAC was calculated using Equation (3) and expressed as cyanidin-3-glucoside in mg/g sample.

$$\text{Total anthocyanin content } \left(\frac{\text{mg}}{\text{g}}\right) = \frac{A\times MW\times DF\times 1000\times v}{\varepsilon\times l\times g} \quad (3)$$

where A is absorbance = $(A_{510}\text{-}A_{700})_{pH1.0}$ - $(A_{510}\text{-}A_{700})_{pH\ 4.5}$, ε is cyanidin-3-glucoside molar absorbance (26,900 L/(mol·cm)), l is the cell path length (1 cm), MW is the molecular weight of anthocyanin (449.2

g/mol), v is the solution volume (L), g is weight of sample (g) and DF is the dilution factor.

Total phenolic content (TPC)

The TPC was determined using a modified colorimetric Folin-Ciocalteu method (Wolfe *et al.*, 2008). Deionized water 0.5 mL and mao solution 0.125 mL were added to a test tube. Folin-Ciocalteu reagent 0.125 mL was added to the mixture, which was shaken and incubated at room temperature (about 25°C) for 6 mins. Later, an aliquot of 7% sodium carbonate solution 1.25 mL was added into the test tube, and the mixture was diluted to 3mL with deionized water. The mixture was allowed to develop color for 90 mins, and the absorbance was read at 760 nm using the spectrophotometer. The measurement was compared to a standard curve of gallic acid concentrations, which were expressed in mg equivalents gallic acid/g sample.

ABTS free radical scavenging activity

The method for investigating ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging activity was modified from Re *et al.* (1999). The ABTS radical cation was generated by mixing 7 mM ABTS and 2.45 mM potassium persulfate solution at a proportion of 2:1; the mixture was kept in the dark and refrigerated for 12 hr. The ABTS solution was diluted with ethanol until its absorbance at 734 nm was in the range 0.700 ± 0.02. Later, 2 mL of ABTS solution and 20 µL of mao solution were reacted in the dark for 6 mins. The absorbance was measured at 734 nm using the spectrophotometer. The results were calculated as the percentage of free radical scavenging using Equation. (4) and compared to a standard curve of trolox concentrations, which were expressed in mg trolox equivalents/g sample.

$$\%\ \text{ABTS inhibition} = \frac{[(Ac\text{-}Abc)-(As\text{-}Abs)]}{(Ac\text{-}Abc)} \times 100 \qquad (4)$$

where Ac, Abc, As and Abs are the absorbance of the control, the blank control, the sample and the blank sample, respectively.

Ferric reducing ability of plasma (FRAP)

The FRAP assay was modified from Benzie and Strain (1996), Kubola and Siriamornpun (2011). The FRAP reagent was generated by mixing 300 mM acetate buffer: iron reagent solution: $FeCl_3 \cdot 6H_2O$: deionized water at the ratio of 100:10:10:12 (v/v). The FRAP reagent 1.8 mL was heated to 37°C, then deionized water 180 µL and mao solution 60 µL were added. The mixture was shaken and incubated at 37°C for 4 mins. The absorbance was measured at 593 nm using spectrophotometer. The measurement was compared to a standard curve of $FeSO_4$ concentrations and expressed in µM $FeSO_4$/g sample.

Statistical analysis

Analysis of variance (ANOVA) and t test was used for determination of significant variables using the SPSS statistical program version 12 (SPSS Inc., Chicago, IL, USA) at a confidence level superior to 95% ($p \leq 0.05$). Differences between means were evaluated using Duncan's new multiple range test. All measurements were done in triplicate.

Results and Discussion

In order to determine potential applications of mao powder to a food product as an alternative to artificial colorants and their beneficial effects, it is important to study the stability of mao powder against thermal food processing conditions, which involve heating to temperatures from 50 to 150°C, depending on the pH of the product (Patras *et al.,* 2010). Among several factors, the pH and heating conditions affected the anthocyanin stability the most (Kalt *et al*., 2000; Cevallos-Casals and Cisneros-Zevallos, 2003; Moldovan *et al.*, 2012). Moreover, sucrose addition was reported to be a good anthocyanin protector (Wrolstad *et al.*, 1990; Tsai *et al*., 2004). The influence of the pH, heating conditions and sucrose addition on the color, anthocyanins and phenolic contents and the antioxidant activities is presented below.

Color

For the no heating condition, as the pH changed, the solution color changed because anthocyanins are sensitive to pH changes; their structures could be resonant and the color change is derived from this. At low pH (pH 1-3), the anthocyanins were in the flavylium cation form, with a red color. At pH 4-5, hydroxylation of the flavylium cation occurred rapidly to form the colorless carbinol pseudobase. As the pH increased (pH 6-7), quinoidal base form existed as a mixture due to proton loss and the color changed to blue or violet. Furthermore at pH 7-8, the hydration of the flavylium cation toward chalcone produced a yellow color and at pH 8-10, anthocyanins formed highly colored ionized anhydro bases (Lapidot *et al.,* 1999; Friedman and Jurgens, 2000).

Figure 1 illustrates the colors of the mao solution in the food process models and clearly shows that the color of the mao solution varied with pH. At pH 3, the solution color was red under all conditions (h°=9.96-13.31), as the anthocyanin color was the

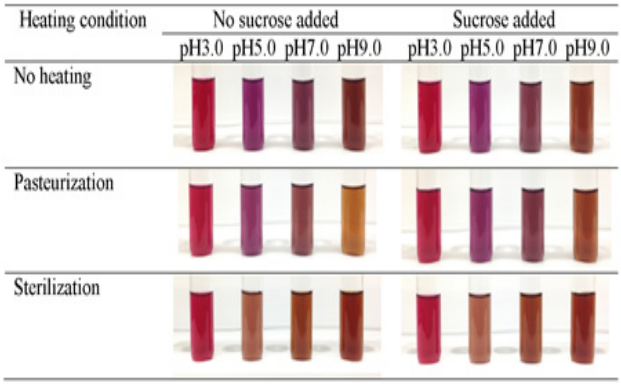

Figure 1 Diagram illustrates color of mao solution in food process models with different pH (3.0, 5.0, 7.0 and 9.0), heating conditions (no heating, pasteurization and sterilization) and sucrose addition (0% and 10%)

most stable at low pH values (Bakowska-Barczak, 2005; Hubbermann *et al*., 2006). At pH 5, the solution color was purple (h°=337.15-339.35), when the solution was treated under pasteurized conditions (65°C for 30 mins), it still had purple color, but once the heating condition increased up to 121°C for 15 mins (sterilized conditions), the solution turned brown (h°=48.91-49.65). At pH 7-9, the color of the solution changed from purple to light brown and dark brown (h°=16.04-64.94) after subjected to heating. Amelia *et al.* (2013) found that the color intensity of anthocyanins extracts from Buni fruits was stable at acidic pH (≤5) and unstable at pH 7. The results were in agreement with those from Cevallos-Casals and Cisneros-Zevallos (2004), who reported that bathochromic shifts were observed for all colorants at pH>5. Moreover, they explained that the color degradation included an increase in absorbance due to browning, a decrease in absorbance due to the formation of colorless carbinol bases, and the effect of bathochromic shifts was due to the anthocyanin structure evolving into less stable forms.

The heating condition is one of the most important factors that influence anthocyanin color. The results clearly showed that it had an effect on the color of solution and as the heating condition became more intense, the color changed noticeably. However, the buffer solution was stable at pH 3; as the anthocyanins were in the flavylium cation form which is relatively stable (Pina *et al.*, 2015).

The browning index (BI) of mao solution with different pH, heating conditions and sucrose addition are shown in Table 1. The t-test analysis of the BI of mao solution with and without sucrose addition indicated that sucrose addition up to 10% had no effect on the browning of mao solution with different pH and heating conditions. Nevertheless, at pH 5-9 under sterilization conditions, the BI values were

Table 1 Browning index of mao solution in food process models with different pH (3.0, 5.0, 7.0 and 9.0), heating conditions (no heating, pasteurization and sterilization) and sucrose addition (0% and 10%)

Heating condition	Sucrose addition ^{ns}							
	No sucrose added				Sucrose added			
	pH 3.0	pH 5.0	pH 7.0	pH 9.0	pH 3.0	pH 5.0	pH 7.0	pH 9.0
No heating	152.49	23.16	38.98	177.14	155.84	21.90	37.34	154.94
Pasteurization	162.93	37.43	73.30	193.09	160.74	36.77	64.39	183.42
Sterilization	159.73	97.59	413.09	750.63	170.13	101.22	486.53	746.31

$^{*\,ns}$ t-test indicated no significant difference between mean values of treatments with and without sucrose ($p \leq 0.05$)

significantly higher than those under other heating conditions. According to Ma *et al.* (2012), the total color difference *(ΔE*)* of *S. chinensis* fruit in solutions gradually accrued with increased heating temperature. The solutions with and without sucrose presented slightly different color and samples that contained added sucrose showed a more slightly brown color. Malien-Aubert *et al.* (2001) studied the stability of commercial anthocyanin-based extracts (purple carrot, red radish, red cabbage, grape-marc, elderberry, black currant and chokeberry) in sugar drink (100 g/L sucrose, 500 g/L citric acid) and non-sugar drink models at different pH values (3, 4 and 5) and the drinks were placed in a hot air steam cabinet at 50°C for 72 hr. They found similar results in the sugar and non-sugar drinks for the same pH but the BI increased as the pH increased. Sadilova *et al*. (2009) reported that in most cases, the differences between the L^* values of black carrot, elderberry and strawberry juices at pH 3.5 with or without saccharide (glucose, fructose and sucrose) concentrations of 50 g/L supplementation were not significant, as were the BI of supplemented and unsupplemented juices.

Total anthocyanin content (TAC)

Statistical analysis revealed that sucrose addition had no influence on the TAC of mao powders. This result was in agreement with Hubbermann *et al.* (2006), who reported no difference in anthocyanin heat stability between sugar (invert sugar syrup 10°Brix) and the non-sugar drink models using commercial anthocyanin colorants. Rubinskiene *et al.* (2005) studied the influence of 10-40% sucrose addition to solutions of spray-dried black currant solutions heated at 70°C for 2hr and found that the addition of 10% and 20% sucrose reduced the thermostability of pigments but at 40% sucrose addition, there was a positive effect on the stability of pigments. Tsai *et al.* (2008) also investigated anthocyanin stability in a model system (pH 3.2) which contained anthocyanins

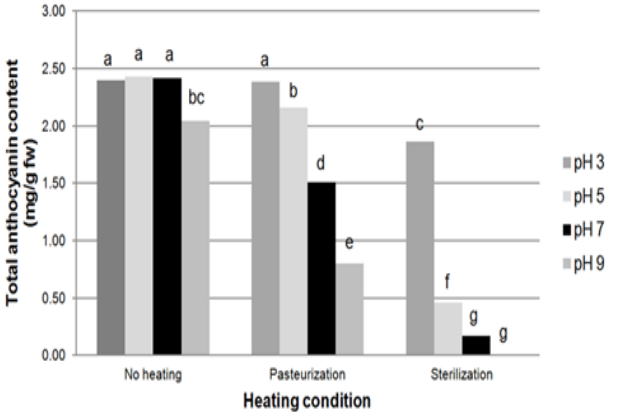

Figure 2 Total anthocyanin content (mg/g fw) of mao solution under pH (3.0, 5.0, 7.0 and 9.0) and heating conditions (no heating, pasteurization and sterilization)
* a-f significantly different mean values of treatments ($p \leq 0.05$)

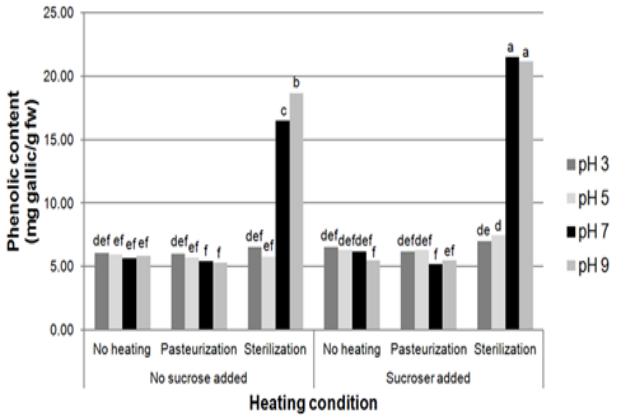

Figure 3 Total phenolic content (mg gallic/g fw) of mao solution under pH (3.0, 5.0, 7.0 and 9.0) and heating conditions (no heating, pasteurization and sterilization)
* a-f significantly different mean values of treatments ($p \leq 0.05$)

from roselles with different sucrose concentrations (20%, 40%, 60%). When the solutions were heated at different temperatures (30-60°C) for 96 hr, they found that increasing temperature had an effect on the degradation of anthocyanins, while sucrose was a good anthocyanin protector, especially at a high sucrose concentration.

The TAC values of mao solution under different pH and heating conditions were in the range 0.01-2.43 mg/g fw (Figure 2). The no heating treatment at pH 3-7 and pasteurized at pH 3 had the highest TAC which was significantly different ($p \leq 0.05$) from other treatments. Both pasteurized and sterilized conditions decreased the TAC as the pH increased. Therefore, increasing the heating conditions influenced TAC degradation. In addition, the TAC was rarely measurable in the solutions which were sterilized at high pH (pH 5-9). Similar trends were observed from several studies of anthocyanin stability in black carrots (Kirca *et al.*, 2007), purple corn, red sweet potato (Cevallos-Casals and Cisneroe-Zevallos, 2004), spinach vine fruit (Basella rubra) (Ozela *et al.*, 2007) and European cranberry bush fruit (Moldovan *et al*., 2012) at various pH levels during heating and they all found that increasing the temperature and pH led to degradation of the anthocyanins.

Total phenolic content (TPC)

The TPC values of mao solution under different pH and heating conditions are shown in Figure 3, with values in the range 5.18-21.50 mg gallic/g fw. The highest phenolic values (21.16-21.50 mg gallic/g fw) were reported in the solutions with 10% sucrose at pH 7 and 9 under sterilized conditions. Even though the anthocyanins were susceptible to high pH and temperature, the phenolic compounds indicated different results likely because in mao fruit, there are various phenolic compounds—for example flavoniods ([+]-catechin, [-]-epicatechin, rutin, myricetin, resveratol, lutelin, quercetin, naringenin and kaempferol), phenolic acids (gallic, caffeic, vanillic, ellagic and ferulic acids) and tannins (procyanidin B1 and B2) (Samappito and Butkhup, 2008; Jorjong *et al*., 2015). Moreover, it has been reported that some phenolic compounds—for example (-)-catechin, ferulic acid, rutin—were resistant to pH-induced degradation because their structures are more complex (Friedman and Jurgens, 2000). In addition, results obtained by HPLC from quinoa extracts found that the content of vanillic and ferulic acids, quercetin and kaempferol increased with temperature increasing from 100-145°C (Carciochi *et al.*, 2016).

Antioxidant activity determined using ABTS free radical scavenging activity and ferric reducing ability of plasma (FRAP) assay

The effects of pH and heating conditions on antioxidant activities were similar to those of the TPC. Since anthocyanins are within a class of the phenolics, even though they degraded, the mao powders contained a large number of structurally different antioxidants.

From Table 2, the antioxidant activities of mao powders were determined using ABTS and FRAP assay showed that the antioxidant efficiency of no heating and pasteurized solutions decreased when the pH value increased. At the same pH value, as temperature increased up to pasteurized condition, their antioxidant activities were not significantly different ($p > 0.05$). Xiong *et al*. (2006) reported the effect of pH (2.4, 3.6, 4.5, 5.5 and 6.8) and temperature (60°C, 80°C and 100°C) on black currant stability and found that increasing the pH and temperature could lead to a decrease in the ferric reducing ability, as 55% of the initial antioxidant activity was lost at

Table 2 Antioxidant activities of mao solution under pH (3.0, 5.0, 7.0 and 9.0) and heating conditions (no heating, pasteurization and sterilization)

Antioxidant assay	Heating condition	pH			
		3.0	5.0	7.0	9.0
ABTS	No heating	15.01±2.33^{efg}	16.83±0.96^{de}	13.26±1.22^{g}	10.38±1.91^{h}
(mg trolox	Pasteurize	15.34±1.55^{def}	17.12±1.21^{d}	14.37±1.39^{fg}	11.46±2.90^{h}
equivalents/g)	Sterilize	15.83±1.87^{def}	21.05±1.46^{c}	30.47±3.22^{b}	44.74±3.58^{a}
FRAP	No heating	108.45±4.25^{de}	105.20±3.89^{e}	82.14±2.10^{f}	51.78±4.47^{g}
(μM $FeSO_4$/g)	Pasteurize	112.43±4.35^{cd}	107.16±6.92^{de}	82.39±3.53^{f}	56.29±7.29^{g}
	Sterilize	116.33±1.83^{c}	115.21±3.41^{c}	159.48±7.56^{b}	258.31±4.18^{a}

$^{*\ a-h}$ significantly different mean values of treatments ($p \leq 0.05$)
ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric reducing ability of plasma

pH 6.8 and 100°C within 2hr. Additionally, Kalt *et al.* (2000) studied the antioxidant capacity of lowbush bluberry and found that it had a high correlation with anthocyanin (R =0.92) and the phenolic content (R =0.95), and the antioxidant capacity was higher at pH 1 than at pH 4 and 7 and furthermore, their products required less processing and had a higher antioxidant capacity.

In contrast with the sterilized conditions, increasing the pH value resulted in higher antioxidant efficiency. For both the ABTS and FRAP assays, mao solution sterilized at pH 9 showed the highest antioxidant activity of 44.74 mg trolox equivalents/g fw. and 258.31 μM $FeSO_4$/g fw., respectively. Arabshahi-D *et al.* (2007) explained that thermal processing at 100°C for 15 mins can induce the formation of compounds with antioxidant properties or improve the antioxidant activities of mint leaves and a significant increase in antioxidant activities was observed when the pH increased from 4 to 9.

Conclusion

The results from this study showed that the stability of anthocyanins from mao powders depended on the pH and temperature. Increasing the pH and temperature promoted anthocyanin degradation, while sucrose addition hardly had any effect on them, and high pH and temperature enhanced the phenolic compound and antioxidant activities. This research recommends the use of mao powders as a food colorant with no heating at pH 3-7 and pasteurized conditions at pH 3 since under these conditions the samples still had an attractive color, high anthocyanin content and high phenolic compound and high antioxidant activities.

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