Development of anthocyanin-rich jelly by Thai mulberry (*Morus alba*) fruit powder

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Abstract

Mulberry fruit is well known as a good source of anthocyanins with many biological activities. This study was investigated the physico-chemical properties of Thai mulberry (*Morus alba*) fruits powder (MFP) and use as an ingredient to developing an anthocyanin-rich jelly. The physical and chemical properties and sensory evaluation of anthocyanin-rich jelly by varying four levels of MFP at 0, 13, 20 and 26% (w/w) were determined. The results showed that MFP contains carbohydrate (73.46%), protein (10.24%), fat (1.74%), and dietary fiber (11.29%). The acidity, total soluble solid and pH were 6.43%, 13.72% and 4.28, respectively. MPF exhibited the antioxidant activities by 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assay of 1.20 µmol trolox equivalent (TE)/100g dry weight (DW), 166.16 µmol TE/g DW and 595.11 µmol TE/g DW, respectively. The result of total phenolic contents (TPCs) using Folin-Ciocalteu method was 26.88 mg GAE/g DW and total anthocyanin was 6.13 mg cyanidin-3-glucoside equivalents/g DW. As the results of anthocyanin-rich jelly, the increase of MFP resulted in the increasing of total soluble solid, acidity and hardness. For color measurement, lightness (L*) was decreased but redness (a*) and yellowness (b*) were increased. The sensory results showed anthocyanin-rich jelly from 13% MFP obtained the highest overall liking score of 7.5 (like moderately to like very much). The antioxidant activities, TPCs and total anthocyanin content of the developed jelly with MFP were significantly higher ($p<0.05$) than the jelly without MFP. Therefore, anthocyanin-rich jelly could be developed as functional food with consumer acceptability.

Keywords: Jelly, mulberry fruit, anthocyanin, antioxidant activity, total phenolic

Introduction

Mulberry belongs to the genus Morus of the family Moraceae and it is grown wild or cultivated in all regions such as Asia, Europe, North and South America and Africa. In general, there are three types of mulberry, including white (*Morus alba*), black (*Morus nigra*) and red (*Morus rubra*). However, the color of mulberry fruits cannot be used to identify the mulberry species (Ozguven & Ozcelik, 2013; Hamid & Joshi, 2016). Mulberry tree has been cultivated traditionally for their leaves (food for silkworms (*Bombyx mori L.*) and animal fodder) and as ornamental trees (Vijayan, 2010); however, due to its nutritive value, the mulberry fruit is nowadays consumed in both fresh and processed forms, such as juice, jams, syrups, beverages, natural dyes, or dried fruits (Ercisli & Orhan, 2007; Gundogdu et al. 2011; Sánchez, 2000). In previous studies, mulberry fruits have been reported to exhibit a variety of biological activities, such as anti-thrombotic (Yamamoto et al. 2006), antioxidant (Kim et al. 1999; Naderi, Asgary et al. 2004), antimicrobial (Takasugi et al. 1979), anti-inflammation (Kim & Park, 2006) and neuroprotective effects (Kanget al. 2006). These activities are generated by anthocyanins, which are a group of naturally occurring phenolic compounds that are responsible for the color of mulberries. Cyanidin-3-glucoside and cyanidin-3-rutinoside are the major anthocyanins (Liu et al. 2004).

Processing can alter and often damage anthocyanin and its antioxidant properties in fruit and vegetable. Maceration, heating, and various separation steps can result in oxidation, thermal degradation, leaching, and other events that can lead to lowering the levels of antioxidants in processed foods compared with the fresh ones (Kalt, 2005). Nevertheless, in contrast, there are also recent studies which have shown that the antioxidant levels of processed food products, derived from e.g. tomato (Capanoglu et al. 2008), and sour cherry (Toydemir et al. 2013) are maintained by the high recovery of the compounds during processing or may even enhanced by the appearance of new compounds which have a higher antioxidant capacity (Nicoli, Anese & Parpinel, 1999). Therefore, this study aimed to investigate nutritive values, physical properties and total phenolic contents, total anthocyanin content and antioxidant activities by various assays including ORAC, DPPH and FRAP from Thai mulberry (*Morus alba*). In addition, anthocyanin-rich jelly was developed using sensory evaluation and determined its physico-chemical properties.

Materials and methods

Raw materials

Thai mulberry fruit (*Morus alba*) powder (MFP) from fully mature fruits by Freeze-drying were obtained from Kamnulchul Farming Co., Ltd., Thailand and kept at -20°C until studies. Other ingredients used for jelly included carrageenan, locust bean gum, and citric acid (Chemipan Corporation Co., Ltd., Thailand) and sugar (Mitr Phol Sugar Corporation., Ltd., Thailand).

Jelly processing

The jelly formulation consisted of 19 g of sugar, 0.2 g of citric acid, 0.85 g of carrageenan, 0.25 g of locust bean gum, and 3-7 g of mulberry fruit powder. Distill water was added to mulberry fruit powder. Sugar previously dissolved in boiling water with jelly agents was warmed up to 80°C for 20 min or until clear solution was obtained. Then, MFP dispersion, citric acid and flavor were added into the clear solution. The dispersion was then heated again for approximately 3 min at 80°C. In order to obtain a desirable gel consistency, the end point was judged as the point at which the total soluble solids of the viscous dispersion reached 72ºBrix, as determined by a hand refractometer (ATAGO Co. Ltd., Tokyo, Japan). The dispersion was then poured into a round mold and cooled at 4°C for 24 h to produce a jelly. The produced jelly was then freeze-dried, subsequently ground into a fine powder using a grinder to a size of 60 messes, and stored at −20°C in an aluminum foil container for analysis.

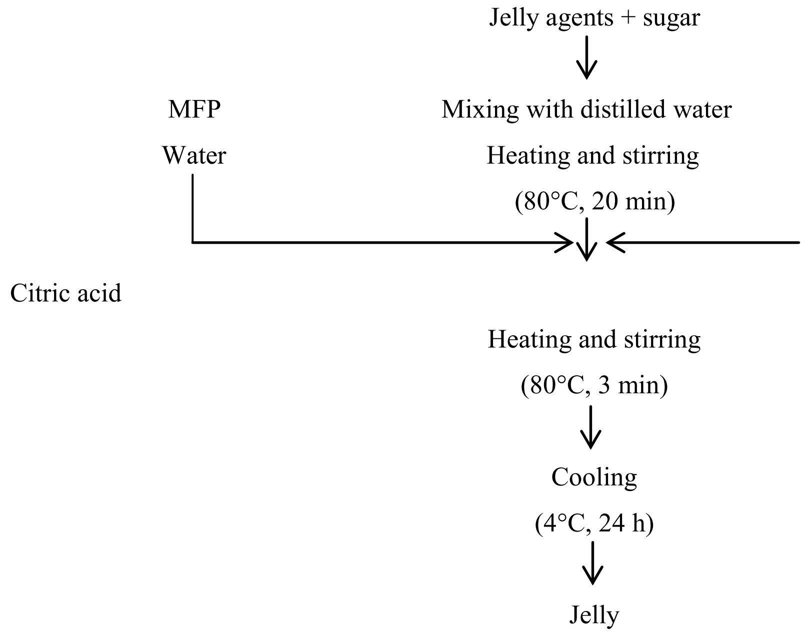

Figure 1 Flow diagrams for production of mulberry fruit powder jelly.

Physico-chemical analysis

The physico-chemical properties of mulberry fruit powder (MFP) and jelly samples were analyzed at the Laboratory of Food Science and Food Chemical unit, Institute of Nutrition, Mahidol University.

Proximate analysis: the moisture content, ash, protein, fat, carbohydrate, dietary fiber and sugar were determined using standard method of AOAC (2016). The calorie content was calculated based on the contents of protein, fat and carbohydrate.

Soluble solids, pH, total acidity and color: the soluble solids were evaluated through a digital pocket refractometer (ATAGO Co. Ltd., Tokyo, Japan) and expressed in °Brix. The pH was measured using a digital pH meter (METTLER TOLEDO, USA). Five g of MFP was dispersed with 25 mL deionized water at 25°C after pH meter calibration with standard buffers pH 4 and 7 (Kha et al., 2010). In case of jelly, pH meter was introduced electrode directly into the jelly. Total acidity was measured by titration 10% solution of product with 0.1 N NaOH up to pH 8.1 and recorded as mg citric acid/100 mL (Mutlu et al., 2018). The color of samples were analyzed by a HunterLab ColorFlex EZ spectrophotometer using C.I.E. LAB (L^*, a^*, b^*) system, whereas L^* is lightness, a^* is redness/greenness, and b^* is yellowness/blueness.

Texture: texture properties of jelly were evaluated using Texture Analyzer (TA.XT plus, Stable Micro System Ltd., YL, UK). The method was modified from Lee et al. (2010). Hardness, springiness, cohesiveness, gumminess and chewiness of mulberry jelly were determined using radiuses cylinder probe P/0.5. Ten mulberry jellies with 15×15×10 mm^3 were individually compressed using the penetration mode under conditions of pre-test speed of 1.5 mm/s, a test speed 1.5 mm/s, post-test speed 10 mm/s, 50% strain.

Sensory evaluation: Nine point hedonic test (1=dislike extremely, 9=like extremely) was followed for conducting the sensory evaluation of mulberry jelly. The panels of thirty judges evaluated the product for sensory parameters including appearance, color, odor, flavor, texture and overall acceptability. Ten gram of mulberry jelly was served in a capped transparent plastic cup which was coded with 3-digit numbers. The panelists were asked to rinse their mouth with water in between the sample (Amerine et al. 1965).

Total phenolic contents and antioxidant activities: Approximately 50 mg of mulberry fruit powder was mixed with 1 mL distilled water. However, 40% ethanol was used to extract jelly samples. After thoroughly mixing (1min) with Vortex mixer, the mixture was heat in the water bath for 2 h at 50°C. The residue was extracted after it was re-mixed and centrifuge at 4600 rpm for 15 min at 4°C. The clear supernatant was separated for the analysis of total phenolic compounds using the Folin-Ciocalteu colorimetric method (Ainsworth & Gillespie, 2007) and the results were expressed in gallic acid equivalents per gram dry weight (mg GAE/g DW) of sample. Antioxidant activity were determined using the DPPH-radical scavenging assay (Fukumoto & Mazza, 2000) , ORAC (oxygen radical absorbance capacity) assay (Ou et al. 2001) and FRAP assays (Benzie & Strain, 1996) and the results were expressed as Trolox equivalents per gram dry weight (TE/g DW).

Total anthocyanin: Total anthocyanins were determined by pH differential method (Giusti & Wrolstad, 2005) using a spectrophotometer. The pigments were extracted by combined 1 g of each sample with 12 mL HCl (1%). After incubation for 48 h at 3 to 5°C, the tubes were centrifuged at 4000 rpm. Samples were filtered and stored at –20° C until analysis. Absorbance was measured at 530 nm and 657 nm in buffers at pH 1.01 and 4.5 using $A = [(A530 – A657)\ pH\ 1.0 - (A530 – A657)\ pH\ 4.5]$ with a molar extinction coefficient of 29.600. Results were expressed as mg of cyanidin-3-glucoside equivalents (CGE) in g dry weight (mg cy-3-glue/ 100 g-1 DW).

Statistical analysis

The data were expressed as mean ± standard deviation. Data were analyzed using an analysis of variance and t-test. Duncan multiple range test was used to determine significant difference among the various samples in triplicate. Data was analyzed using the software, Statistical Package for Social Science (SPSS) version 19 SPSS Inc., Chicago, II, USA at the 0.05 level.

Results and discussion

Physico-chemical properties of mulberry fruits powder (MFP)

The proximate composition of mulberry fruits powder is shown in **Table 1**. Similar results regarding the chemical compositions of dried mulberry were previously reported by Sadia et al. (2013). The principal component of Mulberry fruits (*Morus alba*) powder var. Chiang Mai was carbohydrates (73.46%), particularly total sugars (31.98%). Ozguven & Ozcelik (2013) found that the ripen mulberry contains the highest amount of total sugar which is related to the sensory acceptability. Moreover, MFP contained dietary fiber (11.29%) and protein (10.24%), similar to some staple foods; this implies that MFP may also be a source of important nutrients (Marconi et al. 1997). The several studied reported that dried mulberry (*Morus alba*) contains higher amount of protein than strawberry (Ozcan & Hacıseferogulları, 2007) and sun berry (Patel et al. 2011). The dietary fiber consisted of soluble dietary fiber (1.27%) and insoluble dietary fiber (10.03%). The relevant health organizations recommend ingesting 18-32 g of dietary fiber per day (Spiller, 1986), and food products formulated to contain abundant dietary fiber are currently being developed (Grigelmo et al. 1999). Therefore, the MFP may be considered to be an alternative source of dietary fiber or a specialty food ingredient for use in the production of functional food products. The moisture content of MFP was 9.99% which was in line with the quality standard of Thai community product standard (Thai industrial standards institute, 2016).

The MFP pH value was 3.29 which indicate that the products are from a food technology point of view, within a safe range in terms of microbial development. According to Azeredo (2012), food with pH lower than 4.0 are classified as very acid foods. Total acidity was related to pH and reported at 6.34%. Moreover, the soluble solid of MFP was 13.72°Brix. The color of MFP was 21.78 of brightness, 10.36 of redness and 3.30 of yellowness. This could be explained that the color of the powders influenced by the maturity of fruit ripening (Lee & Hwang, 2017).

Table 1 Physico-chemical properties of mulberry fruits powder (MFP)[1].

Physico-chemical properties	MFP
Energy (kcal/100g)	350.43±11.92
Moisture (%)	9.99±3.07
Protein (g/100g)	10.24±0.40
Total fat (g/100g)	1.74±0.05
Total carbohydrate (g/100g)	73.46±2.68
Ash (g/100g)	4.58±0.04
Dietary fiber (g/100g)	11.29±1.55
Soluble dietary fiber (g/100g)	1.27±0.18
Insoluble dietary fiber (g/100g)	10.03±1.37
Total sugars (g/100g)	31.98±8.82
pH	3.29±1.82
Total acidity (mg/100ml)	6.34±0.32
Soluble solids (°Brix)	13.72±0.90

Physico-chemical properties	MFP
Color	
*L**	21.78±4.92
*a**	10.36±4.45
*b**	3.30±1.61

[1] the data are displayed as having a mean±SD.

Total phenolic contents, antioxidant activities and total anthocyanins of mulberry fruits powder (MFP)

Table 2 shows total phenolic contents, antioxidant activities and total anthocyanin of mulberry fruits powder (MFP). MPF exhibited the antioxidant activities by 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assay of 1.20 µmol trolox equivalent (TE)/100g dry weight (DW), 166.16 µmol TE/g DW and 595.11 µmol TE/g DW, respectively. The result of total phenolic contents (TPCs) using Folin-Ciocalteu method was 26.88 mg GAE/g DW and total anthocyanin was 6.13 mg cy-3-glue/ 100 g-1 DW. Equally to our results, Lee & Hwang (2017) found similar level of total anthocyanins in Korean *Morus alba* L. However, Aljane & Sdiri (2016) presented the lower TPCs and total anthocyanins in mulberry fruit (*Morus alba*).

Table 2 Total phenolic contents, antioxidant activities and total anthocyanins of mulberry fruits powder (MFP)**[1]**.

Sample	Total phenolic contents (mg GAE/g DW)	Antioxidant activities			Total anthocyanins (mg cy-3-glue/ 100 g-1 DW)
		DPPH radical scavenging assay (umol TE/100g DW)	FRAP assay (umol TE/g DW)	ORAC assay (umol TE/g DW)	
MFP	26.88± 1.76	1.20 ± 0.06	166.16±16.39	595.11±63.59	6.13±0.59

[1]the data are displayed as having a mean±SD.

Effect of MFP on physico-chemical properties of jelly

Data pertaining to physico-chemical charateristics of different mulberry fruit jelly formulas was presented in **Table 3**. The control jelly showed the lowest pH (2.91) and total acidity (0.17g/100g) which due to no MFP was added in the formula as compared to other formulas. The hardness of the jellies increased significantly with increase in the quantities of MFP added to the jellies. Considering the fact that the other conditions known to affect the formation of jelly, such as cooking time, temperature, and concentration of sugar were fixed in this study, the amount of MFP added profoundly influenced the hardness of the gels. Chandra & Shamasundar (2014) were also reported that the hardness of jelly could be increased as the pH decreased. Moreover, the jellies produced in this study exhibited hardness values lower than the commercial fruit jelly (848 g), probably as the result of gel additives, like pectin. For the color measurement, lightness (*L**) was decreased but redness (*a**) and yellowness (*b**) were increased in the mulberry jelly when compared with the control formula.

Table 3 Physico-chemical properties of mulberry fruits jelly (MFJ)[1,2].

Physico-chemical properties	Jelly (control)	Jelly with mulberry fruits powder (%)		
		13	20	26
pH	2.91±0.02[c]	3.74±0.03[b]	4.02±0.03[a]	4.06±0.02[a]
Total acidity (g/100g)	0.17±0.02[d]	0.32±0.02[c]	0.40±0.03[b]	0.62±0.02[a]
Hardness (g)	-1.69±1.00[d]	524.98±6.84[c]	592.39±13.83[b]	604.05±18.44[a]
Adhesiveness (g.mm)	-2.94±0.88[a]	-15.95±2.78[c]	-27.19±1.19[d]	-10.74±1.03[b]
Springiness (mm)	0.57±0.06[d]	0.85±0.02[b]	0.90±0.003[a]	0.82±0.02[c]
Chewiness (g.mm)	-0.23±0.10[c]	224.96±9.11[a]	224.22±2.39[a]	164.64±7.84[b]
Color				
*L**	16.99±0.04[a]	4.28±0.03[b]	4.08±0.04[c]	4.11±0.01[c]
*a**	-0.66±0.03[d]	11.96±0.05[a]	9.55±0.05[b]	10.64±0.04[c]
*b**	-0.09±0.02[d]	2.88±0.02[a]	1.96±0.05[c]	2.40±0.04[b]

[1]the data are displayed as having a mean±SD. [2]Different letters in same row indicate significant difference at $p<0.05$ using one way ANOVA followed by Duncan multiple range test.

Total phenolic contents, antioxidant activities and total anthocyanins of mulberry fruit jelly

Phenolic compounds are plant constituents that possess antioxidant activity and prevent the decomposition of hydroperoxides into free radicals (Pokorny, Yanishlieva & Gordon, 2001). The total phenolic contents, antioxidant activities and total anthocyanins of mulberry fruit jelly are shown in **Table 4**. The mulberry jellies contained higher amount of total phenolic contents than the control formula. This was due to the MFP addition. Moreover, it was found that the amount of phenolic is higher than the calculation which might because of the heat processing. The heat treatment might induce the changes in extractabilities of the phenolics and flavonoids due to the disruption of the plant cell wall, and consequently, the enhanced release of the bound polyphenolic and flavonoid compounds (Choi et al. 2006). A similar result was noted in the study of Khatun et al. (2006), reporting that the phenolic contents of spices increased by a factor of approximately 2-4 after cooking. Moreover, the antioxidant activities of mulberry jellies were determined by DPPH radical scavenging, FRAP and ORAC assay. The results showed that antioxidant activities increased with increases in the MFP concentration in the jellies, a trend which was similar to those of TPCs of the jellies. Therefore, the antioxidant activities of mulberry jellies were attributed to the contributions of TPCs. The total anthocyanins of the jelly with 13%MFP estimated to be 4.51 mg cy-3-glue/ 100 g-1 DW was found to increase to 5.25 and 5.54 mg cy-3-glue/ 100 g-1 DW in jelly with 20 and 26% MFP, respectively. In addition, it was found the antioxidant activities and total anthocyanins remained after jelly production.

Table 4 Total phenolic contents, antioxidant activities and total anthocyanins of mulberry fruit jelly[1,2].

Sample	Total phenolic contents (mg GAE/g DW)	Antioxidant activities			Total anthocyanins (mg cy-3-glue/ 100 g-1 DW)
		DPPH radical scavenging assay (umol TE/100g DW)	FRAP assay (umol TE/g DW)	ORAC assay (umol TE/g DW)	
Control	2.93±0.12^c	0.17±0.02^c	4.11±0.39^d	ND	ND
13% MFP	21.66±1.13^b	3.43±0.11^b	117.13±5.23^c	500.03±23.78^b	4.51±0.26^c
20% MFP	22.50±1.75^b	3.55±0.25^b	124.09±4.92^b	532.21±52.95^b	5.25±0.20^b
26% MFP	25.64±0.95^a	4.12±0.30^a	140.84±5.05^a	600.60±24.34^a	5.54±0.24^a

[1] the data are displayed as having a mean±SD. [2]Different letters in same column indicate significant difference at $p<0.05$ using one way ANOVA followed by Duncan multiple range test. ND=not detected

Sensory evaluation of mulberry fruit jelly

Sensory evaluation of jellies with different levels of MFP were performed on 6 different attributes such as appearance, color, odor, taste, texture and overall liking using 9-point hedonic scale (**Table 5**). There was no significant difference in all sensory attributes between jellies with different levels of MFP. However, the results showed the tendency of adding more MFP could result in decreased in all the attributes. Therefore, 13% MFP was recommended to use in the production of anthocyanin-rich jelly because it obtained the highest overall liking score of 7.5 (like moderately to like very much).

Table 5 Sensory evaluation of mulberry fruits jelly[1,2,3].

	Sensory attributes	Jelly (control)	Jelly with mulberry fruits powder (%)		
			13	20	26
Before test	Appearance	7.33±1.30^a	7.47 ±0.94^a	7.27±1.11^a	7.07±1.20^a
	Color	7.30±1.26^{ab}	7.70±0.84^a	7.40±1.04^{ab}	7.00±1.49^b
After test	Odor	7.00±1.20^a	7.23±1.01^a	7.20±0.85^a	6.83±1.21^a
	Taste	6.77±1.57^a	7.53±1.20^a	7.37±0.89^a	7.17±0.95^a
	Texture	7.27±1.08^a	7.33±0.71^a	7.00±1.11^a	6.77±1.55^a
	Overall liking	6.93±1.23^a	7.47±0.78^a	7.30±0.88^a	7.30±1.09^a

[1]All data were represented as mean±SD. [2]Different letters in same row indicate significant difference at $p<0.05$ using one way ANOVA followed by Duncan multiple range test. [3]9-points hedonic scale (1=Dislike extremely, 5=Neither Like nor Dislike, 9=Like Extremely.

Conclusions

Mulberry fruit powder could be use as ingredient to the production of delightful, nutritious and delicious jellies. Anthocyanin-rich jelly with 13% MFP was found to be accepted by the consumer. Moreover, the developed jelly was contained higher total phenolic contents, antioxidant activities and total anthocyanins than the control formula. This development will not only improve the nutrients of the product but it helps to increase the values of the ingredient and the product. Therefore, it might be concluded that mulberry fruit powder is one of functional ingredient which can use in food products in order to increase applications of such value-added food ingredients.

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