Acceptance Notice and Invitation Letter

Dear Alisa Soontornwat,

Thanks for submitting your paper. On behalf of the Organizing Committee of the 6th ICCBES, International Congress on Chemical, Biological and Environmental Sciences (ICCBES 2016), we are pleased to inform you that the following submission has been accepted as one of **Poster Presentation** in this conference:

ID: 1383
Title: In Vitro Gastric And Pancreatic Digestion Of Anthocyanins Extracted From Mulberry Fruit
Author: Alisa Soontornwat
Affiliation: Huachiew Chalermprakiet University

The registration deadline for presenters is **March 20th, 2016.** If we do not receive your registration with payment by this date, your paper will be withdrawn from the conference program and proceedings. If there are any changes in your profile such as e-mail address or affiliations, please log in to the conference system at www.iccbes.org and edit your user information as appropriate. If you have any further questions, please do not hesitate to contact the secretariat of ICCBES by sending your email iccbes@iccbes.org and indicate your **Paper ID which is listed above.**

Please note ICCBES is not authorized to assist with the VISA application beyond providing this invitation letter. Should your application be denied, ICCBES cannot change the decision of the Japan Ministry of Foreign Affairs, nor will ICCBES engage in discussion or correspondence with the MOFA or the Embassy of Japan on behalf of the applicant. No refunds will be issued in any situations.
Please feel free to contact us if any further information is required. We look forward to seeing you at ICCBES in May 10-12, 2016 in Osaka, Japan.

Best regards,

The Organizing Committee of ICCBES

Higher Education Forum
12F.-1, No.129, Sec.1, Fuxing S. Rd., Taipei, Taiwan
Tel: (+886) 2 2740 1498

In vitro gastric and pancreatic digestion of anthocyanins extracted from Mulberry fruit

Alisa Soontornwat[a,*], Chaunpis Jirapong[a], Pornpimon Kanjanavas[a]
Chalermchai Wongs-Aree[b]

[a] Division of Biological Science, Faculty of Science and Technology, Huachiew Chalermprakiet University, Samutprakan 10540, Thailand
E-mail address: alis_peep@hotmail.com

[b] Postharvest Technology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand

Abstract

Mulberry is a good dietary source of anthocyanins and its antioxidant properties with shown the nutraceuticals. Anthocyanins have been recognized to be a potential for preventing chronic degenerative diseases. However, changes of temperature and pH in human digestion may be affected on color and antioxidant activity degradation of consumed anthocyanin extracts. This study was to investigate anthocyanin content, total phenolic compounds and the antioxidant activity during *in vitro* model simulating gastrointestinal digestion of mulberry extract. Results showed that anthocyanin content was in steady level under gastric digestion, whereas total phenolic compounds slightly increased. Anthocyanin content and total phenolic compounds were degraded under a pancreatic condition. Changes in antioxidant activities during the digestion were correlated to the changes in total phenolic compounds as well as the anthocyanin content. This suggests that the anthocyanin and antioxidant activities are evaluated by pH changes and enzyme conditions, appearing in the digestive system.

Keywords: Mulberry fruit, *in vitro* digestion, anthocyanin, antioxidant

1. Background/ Objectives and Goals

Non-communicable diseases (NCDs), also known as chronic diseases are not passed from person to person. The 4 main types of NCDs are cardiovascular diseases, cancers, chronic respiratory diseases and diabetes causing more deaths worldwide than all other causes of death combined (WHO, 2015). Unhealthy diets, tobacco use, exposure to environmental carcinogens and physical inactivity are documented as the major causes of NCDs. The major factor are conduce the production of free radical group such as reactive oxygen and nitrogen species in biochemical reactions in human cells. These presence needs to be neutralized by antioxidants. Therefore it's provide and repair a balanced oxidants-antioxidants in human system.

Polyphenol establish the majority of secondary plant metabolites and also of dietary antioxidants (Bouayed, 2010; Bouayed and Bohn, 2010). Anthocyanins represent an

important group of phenolic compounds called flavonoids. They function as pigments in plants and are nutraceuticals with show the prevent disease through their biological activity that include antioxidant function (Betanzo *et al*. 2014). In Thailand, mulberry fruit widely consumed and is well known as a good source of anthocyanins. In previous studies, mulberry fruits have been reported to biological activities, such as antioxidant (Kim *et al*., 1999; Naderi *et al*., 2004), antimicrobial (Takasugi *et al*., 1979). In addition to studies effect of *in vitro* digestion on dietary polyphenols, e.g. flavonoid and phenolic compound in anthocyanins in raspberry (McDougall *et al*., 2005) However, there is no data about the change of anthocyanin and antioxidant of anthocyanin extract to *in vitro* gastric and pancreatic digestion. The objective of this research was to investigate stability of anthocyanin extract from mulberry and change antioxidant activity during digestion.

2. Methods

Mulberry fruit were harvested from orchards in Phatchabun province, Thailand. Mulberry were selected for uniformity of size and color purple-red; they were freeze dry and stored at -20 ºC. Samples of 50 g (dry weight) were mashed and sonicated for 3 hr. in 500 ml of methanol. The mixture was then filtered through a filter paper (Whatman No. 1). The solvent was then remove using a rotary evaporator (BÜCHI Rotavapor R-205, Japan). Anthocyanin extract from mulberry were stored at -20 ºC for further analysis. This extract was subjected to successive *in vitro* gastric and pancreatic digestion, following method of Ferruzzi *et al*., 2001. The gastric digestion, diluted of anthocyanin extract from mulberry (1:10, anthocyanin extract: NaCl) were adjusted to pH 2.0 with HCl and the mixture was incubated with of pepsin in a shaking bath at 37 ºC for 2 h. The end of the gastric digestion, the pH was brought to 6.5 with $NaHCO_3$ before adding pancreatic and bile salt. The solution was incubated at 37 ºC for 2 h. At different time of gastric and pancreatic digestion (0, 30, 60, 90, 120 min) aliquots of samples were removed for total anthocyanin total phenolic and antioxidant activity analysis. The experiment was performed in 5 replications.

2.1 Total anthocyanin was measured following Giusti and Wrolstad, 2005; Wongs-Aree *et al*, 2006 method. Absorbance was determined at 700 nm using a UV-visible spectrophotometer (Shimadzu, UV-1601, Japan). The total anthocyanin content was calculated using the equation:

Monomeric anthocyanin pigment (mg/liter) = (A x MW x DF x 1000) / (ε x 1)

Where: A= (A λ vis-max - A 700)

DF = dilution factor

MW = molecular weight and molar absorptivity (449.2)

ε = molar extinct coefficient of cyanidin-3-glucoside (26,900 L/mol·cm)

2.2 Total phenolic content of digested fraction were estimated by the Folin-Ciocaiteau method (Singleton and Rossi, 1965). Absorbance was determined at 725 nm using a

UV-visible spectrophotometer. Dfferent concentration of gallic acid were used to generate a standard curve.

2.3 Antioxidants activity was determined by DPPH Radical Scavenging activity (method modifications from Thaipong *et al*., 2006) Ferric reducing antioxidant power assay (Benzie and Strain,1996) and ABTS radical scavenging activities (method modifications from Kriengsak *et al*., 2006)

3. Results

This experiment monitor the release of phytochemical (total phenolic and anthocyanin) from anthocyanin extract of mulberry fruit during digestion. Total anthocyanin content in different stage of digestion shown in Fig 1. The anthocyanin contents was in steady level in the gastric digestion, with 32.41-40.06 mg/ml, whereas total phenolic compounds slightly increase. Our Results, during gastric digestion was stability of anthocyanins under acidic conditions in agreement with those reported for raspberry (McDougall et al., 2005) or pomegranate (Perez-Vicente et al., 2002) anthocyanins. Moreover, Perez-Vicente et al., 2002 reported a small increase in anthocyanins after *in vitro* stomach digestion. The effect of pH on the stability of anthocyanin which affects the stability of anthocyanin is the acidic solution. Flavylium salts are stable only in highly acidic conditions. These salts loose the proton in higher pH and transform into quinoidal base, which is an unstable pigment, and immediately bond to water and form colourless compound called chromenol (Bermúdez-Soto *et al*., 2007). The transition from the acidic gastric to the mild alkaline intestinal was found a dramatic drop in the amount of 9.96 mg/l of anthocyanin content, which was related to total phenolic compounds (Fig 1 and Fig 2). After this initial decline, the incubation with pancreatic solution observed a decrease in the bio-accessibility of anthocyanin whereas total phenolic compounds was slightly increased (Fig2). The high instability of anthocyanins at neutral or slightly basic pH has been observed in other studies (Bermúdez-Soto et al., 2007; McDougall et al., 2005a, 2005b; Pérez-Vicente et al., 2002). Stabilities of polyphenols under gastric conditions is accordance with early absorption from the stomach of intact anthocyanin.

In table 1 the changes in antioxidant activity, measured with both DPPH FRAP and ABTS assay, during the in vitro gastro-intestinal digestion are shown. As the FRAP assay was rise steadily in gastric digestion with different the DPPH and ABTS shown steady level. When the changes in antioxidant activity were measured with the FRAP assay we found a good correlation between the extraction of total polyphenols and the increase in antioxidant activity during gastric digestion. In fact, it is well known from the literature that the radical scavenger activity of polyphenols is strongly pH-dependent with higher pH values which significantly increase this capacity. This increase in the radical scavenger activity has been attributed to the deprotonation of the hydroxyl moieties present on the aromatic rings of the phenolic compounds (Mukai, Oka, Watanabe, Egawa, & Nagaoka, 1997; Tyrakowska et al., 1999).

After this initial decline, the incubation with pancreatic solution observed a sharply decrease in antioxidant activity of DPPH and FRAP assay. In pancreatic digestion, the antioxidant activity was reduce with associate total phenolic compound. The polyphenols with the highest antioxidant activity at acidic pH are not necessarily those with the highest antioxidant activity at the mild alkaline pH value. This suggests that the anthocyanin and antioxidant activities are evaluated by pH changes and enzyme conditions, appearing in the digestive system.

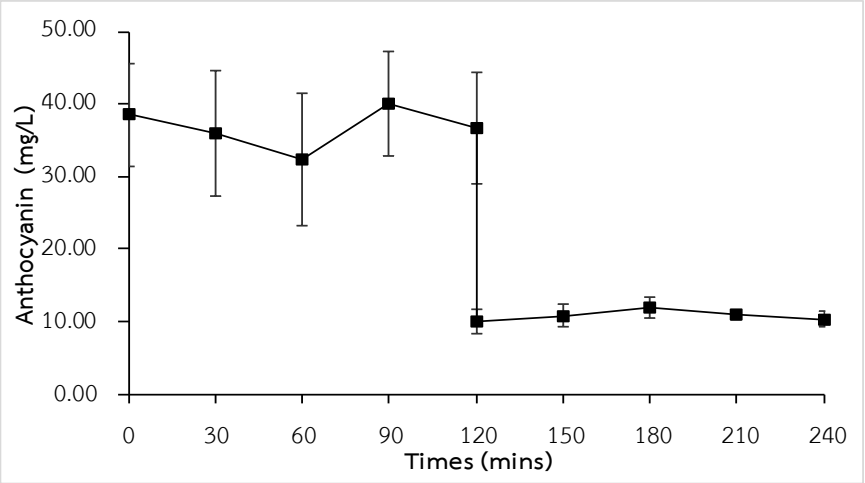

Fig. 1: Change in the amount of anthocyanin extracted during the gastro-intestinal simulated digestion

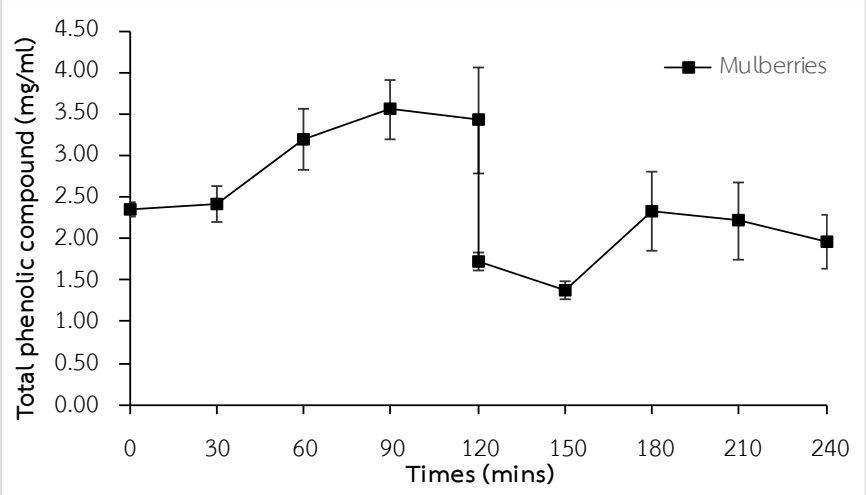

Fig. 2: Change in the amount of total phenolic compound during the gastro-intestinal simulated digestion

Table 1: Antioxidant activity of anthocyanin extracted from mulberry fruit individually subjected to simulated gastric-intestinal digestion.

	Antioxidant activity		
	DPPH assay	FRAP assay	ABTS assay
Gastric digestion			
0 min	0.49±0.045	6.84±1.238	1.12±0.001
30min	0.60±0.027	5.87±0.558	1.12±0.004
60 min	0.55±0.026	8.13±1.506	1.10±0.012
90 min	0.58±0.041	8.11±1.723	1.12±0.001
120 min	0.54±0.064	10.80±1.597	1.09±0.020
Pancreatic digestion			
120 min	0.17±0.012	3.38±0.669	1.02±0.076
150 min	0.18±0.011	7.18±1.491	0.82±0.192
180 min	0.17±0.009	7.53±2.534	0.89±0.143
210 min	0.14±0.026	6.28±1.261	1.07±0.030
240 min	0.17±0.015	3.58±1.458	1.04±0.087

3.3 Acknowledgments

Funding for this research was provided by The Thailand Research fund (TRF), Thailand.

4. References

Benzie, I.F., and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power : the FRAP assay. *Anal. Biochem*, 239: 70-76.

Bermúdez-Soto, M. J., Tomás-Barberán, F. A., & García-Conesa, M. T. (2007). Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to in vitro gastric and pancreatic digestion. *Food Chemistry*, 102, 865–874.

Bouayed, J. (2010). Polyphenols: A potential new strategy for the prevention and treatment of anxiety and depression. *Current Nutrition & Food Science*, 6, 13–18.

Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants – Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine and Cellular Longevity*, 3, 228–237

Ferruzzi, M. G., Failla, M. L., Schwartz, S. J. (2001). Assessment of degradation and intestinal cell uptake of carotenoids and chlorophyll derivatives from spinach puree using an in vitro digestion and caco-2 human cell model. *Journal Agricultural Food Chemistry*, 49, 2082 –2089.

Giusti, M.M. and Ronald, E. W. (2005). Characterization and Measurement of Anthocyaninsby UV-Visible Spectroscopy. *In Handbook of Food Analytical Chemistry:Pigment, Flovors, Texture, and Bioactive Food Components*, 2, 19-31.

Kim, S. Y., Gao, J. J., Lee, W. C., Ryu, K. S., Lee, K. R., & Kim, Y. C. (1999). Antioxidative flavonoids from the leaves of Morus alba. *Archieves of Pharmacal Research*, 22(1), 81–85.

Kriengsak, T., Unaroj, B., Kevin, C., Luis, C.Z. and David,H.B. (2006). Comparison of ABTS, DPPH, Frap, andORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19: 669-675.

McDougall, G. J., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Assessing potential bioavailability of raspberry anthocyanins using an in vitro digestion system. *Journal of Agricultural and Food Chemistry*, 53, 5896–5904.

Mukai, K., Oka, W., Watanabe, K., Egawa, Y., & Nagaoka, S. (1997). Kinetic study of free-radical-scavenging action of flavonoids in homogeneous and aqueous triton X-100 micellar solutions. *Journal of Physical Chemistry A*, 101, 3746–3753.

Naderi, G. A., Asgary, S., Sarraf-Zadegan, N., Oroojy, H., Afshin-Nia, F. (2004). Antioxidant activity of three extracts of Morus nigra. *Phytotherapy Research*, 18(5), 365–369.

Pérez-Vicente, A., Gil-Izquierdo, A., & García-Viguera, C. (2002). In vitro gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. *Journal of Agricultural and Food Chemistry*, 50, 2308–2312.

Singleton,V.L., and Rossi, J. (1965). Colorimetry of Total Phenolic with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Veticulture*, 16,144-158.

Takasugi, M., Nagao, S., Masamune, T., Shirata, A., & Takahashi, K. (1979). Structures of moracins E, F, G, and H, new phytoalexins from diseased mulberry. *Tetrahedron Letter*, 20(48), 4675–4678

Tyrakowska, B., Soffers, A. E. M., Szymcsiak, H., Boeren, S., Boersma, M. G., Leman´ ska, K., et al. (1999). TEAC antioxidant activity of 4-hydroxybenzoates. *Free Radical Biology and Medicine*, 27, 1427–1436.

Wongs-Aree, C., Giusti, M.M. and Schwartz, S.J. (2006). Anthocyanins derived only from Delphinidin in the blue petals of Clitoria ternatea. *Acta Horticulturae*, 712, 437-442.