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Original Article

Pigment stability in Chaba maple (*Hibiscus acetosella* Welw. Ex Hiern.) petals extract as natural food and cosmetic colorants

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Abstract

The goal of this study was to investigate the stability of Chaba maple (*Hibiscus acetosella* Welw. Ex Hiern.) petal extracts with different extraction methods. Natural colorants from the petals of Chaba maple were extracted by maceration (MDE) and freeze dry (FDE) method. The stability of compounds in the extract was studied under different environmental conditions by varying the pH, temperature and light exposure. Percent degradation of the color pigments was calculated. The results showed that lower pH and high temperatures of 50 °C and 80 °C increases the stability of pigments in both MDE and FDE. Notably, MDE was significantly more stable than FDE, when exposed to light. Overall, pH, temperature and light exposure affect the stability of the extracts, though MDE was found to be more stable than FDE. However, since solvent contamination may cause toxicity in MDE, FDE may be a more suitable natural colorant for food and cosmetic products.

Keywords: Chaba maple (*Hibiscus acetosella* Welw. Ex Hiern.), anthocyanin, stability, food colorant, cosmetic colorant

1. Introduction

Anthocyanins, a group of flavonoids, have strong potential for use as natural food and cosmetic colorants. The characteristic colorants are orange, red, purple and blue. These pigments are widely applied in food, drink and cosmetics industries, due to their ability to turn color. Anthocyanins are interesting due to their nutraceutical and pharmaceutical effects, i.e. antioxidant, antityrosinase, antiimflammatory, antidiabetes, anti-obesity, antimicrobial and anticancer properties (Khoo, Azlan, Tang, & Lim, 2017; Lin, Gong, Song, & Cui, 2017; Mahmad *et al.*, 2018; Vendrame & Klimis-Zacas, 2015). However, the stability of anthocyanins are affected by structure, pH, temperature, light, oxygen, sugar and copigments (Devi, Saravanakumar, & Mohandas, 2012; Moonjung & Eunok, 2013; Rakkimuthu, Palmurugan, & Shanmugapriya, 2016).

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Chaba maple is a Thai name of *Hibiscus acetosella* Welw. Ex Hiern. (Malvaceae), which have other local names such as African rosemallow, false roselle, maroon mallow, cranberry hibiscus and red leaved hibiscus that are recognized in Africa, Latin America, Asia and Europe (Mahr, 2008). Chaba maple is an ornamental plant and is also consumed as food. The leaves and flower are purple-red in color. The flower and leaf extracts were shown in previous studies to contain significant amounts of flavonoid and phenolic content, and also has antioxidant activity (Thungmungmee, Dumrong phuttidecha, Noysang, & Techaoei, 2016; Thungmungmee, Wisidsri, & Khobjai, 2018). Other flavonoids apart from aglycones and anthocyanins were also found in the *Hibiscus* flower including delphinidin, cyanidin, petunidin, myricetin, pelargonidin, malvidin, quercetin and kaempferol (Puckhaber, Stipanovic, & Bost, 2002).

In cosmetic and food industries, anthocyanins from Chaba maple, coming from a natural origin is considered and attractive substitute for synthetic dyes. There are very few studies on stability of anthocyanins in Chaba maple. This research, therefore, aims to investigate how environmental

factors (e.g. pH, temperature and light exposure) affect the stability of Chaba maple petal anthocyanins that were extracted by different methods.

2. Materials and Methods

2.1 Samples

Fresh *Hibiscus acetosella* Welw. Ex Hiern. or Chaba maple grown in Pathum Thani, Thailand were collected on September, 2018. The petals from flowers were selected to evaluate the stability. The plant specimen was identified as BK No. 070332 by Plant Varieties Protection Office, Bangkok, Thailand.

2.2 Sample extraction

The anthocyanin from Chaba maple petal was extracted by two methods, maceration and freeze-drying which modified from Sipahli, Mohanlall, & Mellem (2017) and Mar *et al.* (2020). For maceration, the dried petals were macerated with 85% formic acid:methanol (3:97) and shacked at 250 rpm for 2 hrs at room temperature, followed by vacuum filtration. Methanol was removed by a rotary evaporator under 45 °C and the extract (MDE) was stored in -20 °C. For freeze-drying, the fresh petals were boiled with distilled water at 80°C for 15 min. The filtration was filtrated and then frozen down to -80 °C for 8 hrs. The frozen-extract (FDE) was dried using a freeze dryer (Labconco, Missouri, USA) for five days. All extracts were calculated with percentage of yield followed Equation 1:

$$\%yield = [\text{weight of extract powder (g)} / \text{weight of matter (g)}] \times 100 \quad (1)$$

2.3 Stability studies

2.3.1 pH stability

In the pH study, buffers at nine pH values (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) were prepared using 0.1 M HCl, 0.2 M HCl, 0.1 M acetic acid, 0.2 M KCl, 0.1 M sodium acetate, 0.1 M Na_2HPO_4. They were used for analysis as follows: The 100 µl of extracts (10 mg/ml dissolved in water) was aliqoted in to 900 µl of buffer solutions in various pH values and mixed together. The absorbance of each extracts was detected immediately and after 1, 6, 24, 48 and 72 h using a visible spectrophotometer in 520 nm. Percentage degrada tion of pigment was calculated followed Equation 2:

$$\%degradation\ of\ pigment = (Abs_{control}-Abs_{sample}/Abs_{control}) \times 100 \quad (2)$$

2.3.2 Temperature stability

The effect of temperature on extracts was determined according to Sipahli, Mohanlall, & Mellem (2017) with slightly modification. An aliquot of 0.1 ml of extract was made up to 25 ml with distilled water. The stability of pigment was done with samples inside capped glass bottle covered with aluminum foil and kept in 50 °C and 80 °C for 6 hrs. The absorbance of extracts was read at 520 nm using microplate reader (Glomax-Multi Detection System, USA). Percentage degradation of pigment was calculated.

2.3.3 Light stability

The method by Sipahli, Mohanlall, & Mellem (2017) was used with some modifications. The 100 µl of extract (10 mg/ml dissolved in water) was added into 900 µl of distilled water in centrifuge tube. The samples were separated into two sets. The first set was incubated in a closed box at 28±2 °C under 20 W fluorescence light, while the second set was wrap with aluminum foil (dark condition) and incubated in the same closed box. Absorbance of extracts was read at 520 nm using microplate reader at 0, 24, 48 and 72 hrs. Percentage degradation of pigment was calculated.

2.4 Statistical analysis

Data are reported as the mean±standard deviation of three replicate experiments. The stabilities were analyzed by T-test and one way ANOVA followed by Tukey's (Post Hoc) using SPSS 22.0 Software. Significance was accepted at $p<0.05$.

3. Results and Discussion

3.1 Sample extraction

The %yield of the extract from macerated dried petals (MDE) with acidified methanol and the extract from freeze-dry (FDE) was 9.08 and 3.34 respectively. MDE showed purple-red viscous liquid and FDE showed purple-red powder. Anthocyanins are polar molecules, and then the most extractants used in the extractions are aqueous mixtures of methanol or ethanol or acetone and acidified methanol or ethanol (Chandrasekhar, Madhusudhan, & Raghavarao, 2012). Previous studies demonstrated anthocyanins extracted from *H. acetosella* using acidified ethanol and water (Marco, Poppi, Scarminio, & Tauler (2011); Mar *et al.* (2020). Some research supported that methanol is the most efficient method in anthocyanin extractions (Kapasakalidis, Rastall, & Gordon, 2006). The methanol extraction of wine grape pomace showed the highest anthocyanin pigments compared with ethanol and water extraction, respectively (Metivierf, Francisf, & Clydesdale (2006). Castaneda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal (2009) observed that acylated anthocyanins and 3,5-diglc extraction need to use weakly acid media to avoid their hydrolysis. In our studied, the extractions revealed that weakly acid solvent as acidified methanol produced higher %yield of MDE.

3.2 Stability studies

3.2.1 pH stability

pH is the one of factor that affect to anthocyanin stability. The color of both extracts are red at acidic condition (pH 1-3) and purple when higher pH value (pH 4-7) to blue at pH 8-9 followed in Figure 1. The stability of anthocyanin in extracts was determined by %degradation of pigment. The

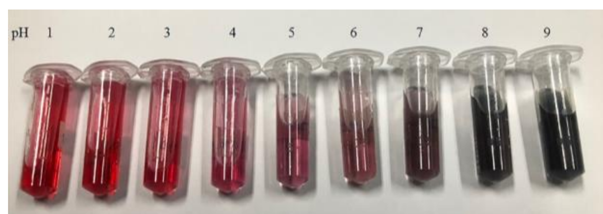

Figure 1. Color of MDE in variation pH

results showed that anthocyanin was more stable in lower pH than upper pH in both MDE and FDE. Percentage of degradation of pigment was low at pH 1-5 as shown in Figure 2 and 3. After 1 hr incubation, both of extracts decreased significantly at pH above 5.0. At pH 7-9, the pigment in MDE and FDE was degraded immediately when incubate for 1 h to 6 hrs and stabilized after 24 h to 72 hrs. The color stability depended on pH of solution related with anthocyanin structure. The red-colored pigments at acidic condition are predominantly in the form of flavylium cations (Khoo, Azlan, Tang, & Lim, 2017). At low pH, flavylium cations formed anthocyanin to be highly soluble in water, thus there are more stable at lower solution (pH 1-3). Moreover these ions may interact with co-pigments which can stabilize the pigment (Sipahli, Mohanlall, & Mellem, 2017). At increasing pH, anion quinonoidal species were formed while flavylium cation decreased. This result caused the color fading and increase %degradation of pigment at pH 4-5. Remarkably, MDE and FDE decreased significantly at pH above 5.0 as same as anthocyanins in black carrot (Kirca, Ozkan, & Cemeroglu, 2007). At pH 6-7, the quinonoid anions are formed from deprotonation of quinonoidal anion. Then purple color were presented. The color as well as %degradation of pigment of the extracts was evidently changed in alkaline solution (pH 8-9) due to their instability. For our results were similar to many research. For example, anthocyanins in red onion extract stable at low pH (pH 1) and unstable at high pH (pH 9) (Oancea & Draghici, 2013). The crude pigments from *Hibiscus sabdariffa* degraded slower at acidic solution (Sipahli, Mohanlall, & Mellem, 2017). Rose flower anthocyanins showed red color and stabilized at low pH. Meanwhile, the blue color was occurred at high pH (Wahyuningsih, Wulandari, Wartono, Munawaroh, & Ramelan, 2017). The most common anthocyanins in plants are cyaniding, delphinidin, pelargonidin, ponidin, malvidin and petunidin. For anthocyanin stability, cyanidin and delphinidin are stable in acidic condition. Likewise, peonidin (3-*O*-methyllated anthocyanin) has red at low pH and change to deep blue at pH 8 (Torskangerpoll & Andersen, 2005). In previous studied, flowers of *Hibiscus* species showed anthocyanins such as cyananidin, delphinidin, malvidin, petunidin, pelargonidin (Puckhaber, Stipanovic, & Bost, 2002). Therefore, Chaba maple extract might be have these compound.

3.2.2 Temperature stability

Higher temperatures, 50 °C and 80 °C, had similar effects to anthocyanin stability of both extracts. The color intensity of the extracts was decreased and then the color change to uncolored form. The %degradation of pigment of

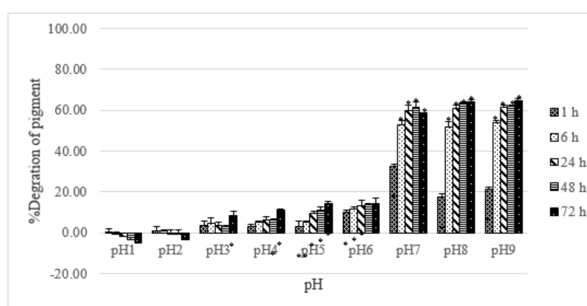

Figure 2. Effect of pH on stability of MDE anthocyanins extracted after incubated for 1, 6, 24, 48, 72 hrs compared with start time (*$p < 0.05$).

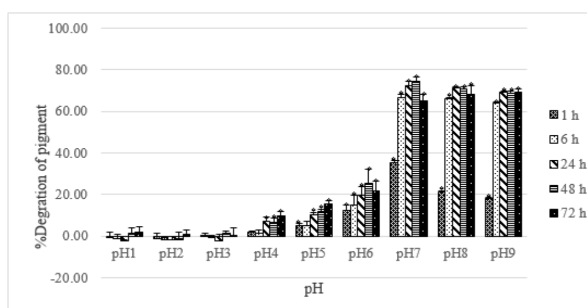

Figure 3. Effect of pH on stability of FDE anthocyanins extracted after incubated for 1, 6, 24, 48, 72 hrs compared with start time (*$p < 0.05$).

the extracts at 50 °C and 80 °C was increased significantly when compared with room temperature condition (Figure 4). Moreover, at 80 °C, both of MDE and FDE were the highest degradation. These experimental results were in agreement with many studied. For example, Liu, Tao, Liu, Pan, & Lv (2018) found that the half-life of blueberry total anthocyanin decreased rapidly from 60 °C and the degradation rate was fast when temperature increased. Devi, Saravanakumar, & Mohandas (2012) reported that high temperature affected to red sorghum bran stability. The temperature at 60-80°C degraded six anthocyanins and total anthocyanin in black rice bran colorant powder followed first-order reaction kinetics (Loypimai, Moongngarm, & Chottanom, 2016). However there was slightly different with Sipahli, Mohanlall, & Mellem (2017). Most of anthocyanins from *Hibiscus sabdariffa* were degraded at 50 °C and no further degraded when heated up to 80 °C. It could be concluded that temperature was also an important factor which influenced anthocyanin stability.

3.2.3 Light stability

Both of MDE and FDE were degraded under light as shown by %degradation of pigment of the extracts (Figure 5). Under light condition, MDE gradually degraded from 24 h to 72 h while it was not degraded in dark condition. Noticeably, the pigment in FDE had degraded more than 25% in dark and light condition. The pigment in FDE declined and unchanged until 72 hrs. However, MDE was significantly stable than FDE in duration of experiment. There were many studied the effect of light to plant anthocyanin stability. For

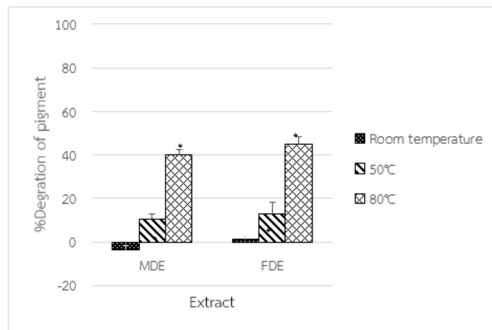

Figure 4. Effect of temperature on stability of MDE and FDE anthocyanins extracted compared with start time (*p < 0.05).

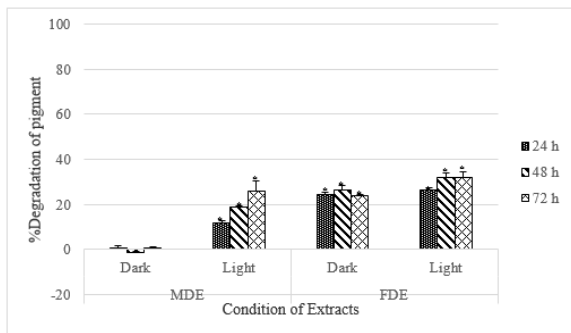

Figure 5. Effect of light on stability of MDE and FDE anthocyanins extracted after incubated for 24, 48 and 72 hrs compared with start time (*p < 0.05).

anthocyanins stability in *H. acetosella*, UV radiation with varies pH accelerated anthocyanin kinetic degradation (Marco, Poppi, Scarminio, & Tauler, 2011). Anthocyanin pigment degradation in *Cocculus hirsutus* fruit extracts slowly degraded in dark and highly degraded under light (Rakkimuthu, Palmurugan, & Shanmugapriya, 2016). Blackberry anthocyanins degraded under light exposure and storage at darkness by the second order reaction kinetics (Contreras-Lopez, Castañeda-Ovando, González-Olivares, Añorve-Morga, & Jaimez-Ordaz, 2014). Likewise, the percentage of destruction of anthocyanin in four *Berberis* species for 84 days had decrease in both of the presence or absence of light. In the presence of light, anthocyanin had stable more than in the absence of light (Laleh, Frydoonfar, Heidary, Jameei, & Zare, 2006). The research of Sipahli, Mohanlall, & Mellem (2017) showed that anthocyanin *H. sabdariffa* extract with light retained significantly more than dark condition over 6 hrs. These are related with our result. Then, the extracts should be kept in dark condition for stabilizing anthocyanins. The flowers of *Hibiscus* species revealed that delphinidin were found (Puckhaber, Stipanovic, & Bost 2002). This compound is more soluble in methanol than water. Delphinidin is the most soluble in methanol and followed by water. This might be a reason why the stability of MDE is higher than of FDE (Kumoro, Retnowati, & Budiyati, 2010).

4. Conclusions

Chaba maple (*Hibiscus acetosella* Welw. Ex Hiern.) is a potentially valuable source of natural food and cosmetic colorant. The pH, temperature and light had a great influence on the stability of anthocyanin pigment in FDE, whereas MDE was significantly more stable across different test conditions. However, due to the known toxicity of methanol contaminants in food and cosmetic products, FDE would be a more favorable ingredient for these purposes. Results from this study would provide essential information regarding appropriate conditions for extraction, storage and further formulation constraints of FDE and MDE into food and cosmetic products.

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References

Castaneda-Ovando, A., Pacheco-Hernandez, L., Paez-Hernandez, E., Rodriguez, J. A., & Galan-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry, 113*, 859-871. doi:10.1016/j.foodchem.2008.09.001

Chandrasekhar, J., Madhusudhan, M. C., & Raghavarao, K. S. M. S. (2012). Extraction of anthocyanins from red cabbage and purification using adsorption. *Food and Bioproducts Processing, 90*, 615–623. doi:10.1016/j.fbp.2012.07.004

Contreras-Lopez, E., Castañeda-Ovando, A., González-Olivares, L. G., Añorve-Morga, J., & Jaimez-Ordaz, J. (2014). Effect of light on stability of anthocyanins in ethanolic extracts of *Rubus fruticosus*. *Food and Nutrition Sciences, 5*, 488-494. doi:10.4236/fns.2014.56058

Devi, P. S., Saravanakumar, M., & Mohandas, S. (2012). The effects of temperature and pH on stability of anthocyanins from red sorghum (*Sorghum bicolor*) bran. *African Journal of Food Science, 6*(24), 567-573. doi:10.5897/AJFS12.052

Kapasakalidis, P. G., Rastall, R. A., & Gordon, M. H. (2006). Extraction of polyphenols from processed black currant (Ribes nigrum L.) residues. *Journal of Agricultural and Food Chemistry, 54*(11), 4016–4021. doi:10.1021/jf052999l

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food and Nutrition Research, 61*(1), 1-21. doi:10.1080/16546628.2017.1361779

Kirca, A., Ozkan, M., & Cemeroglu, B. (2007). Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. *Food Chemistry, 101*, 212-218. doi:10.1016/j.foodchem.2006.01.019

Kumoro, A. C., Retnowati, D. S., & Budiyati, C. S. (2010). Solubility of delphinidin in water and various organic solvents between (298.15 and 343.15) K. *Journal of Chemical and Engineering Data,* 55(7), 2603–2606. doi:10.1021/je900851k

Laleh, G. H., Frydoonfar, H., Heidary, R., Jameei, R., & Zare, S. (2006). The effect of light, temperature, pH and species on stability of anthocyanin pigments in four berberis species. *Pakistan Journal of Nutrition, 5*(1), 90-92. doi:10.3923/pjn.2006.90.92

Lin, B. W., Gong, C. C., Song, H. F., & Cui, Y. Y. (2017). Effects of anthocyanins on the prevention and treatment of cancer. *British Journal of Pharmacology, 174*(11), 1226-1243. doi:10.1111/bph.13627

Liu, Y., Liu, Y., Tao, C., Liu, M., Pan, Y., & Lv, Z. (2018). Effect of temperature and pH on stability of anthocyanin obtained from blueberry. *Journal of Food Measurement and Characterization, 12*(3), 1744-1753. doi:10.1007/s11694-018-9789-1

Loypimai, P., Moongngarm, A., & Chottanom, P. (2016). Thermal and pH degradation kinetics of anthocyanins in natural food colorant prepared from black rice bran. *Journal of Food Science and Technology, 53*(1), 461-470. doi: 10.1007/s13197-015-2002-1

Mahmad, N., Taha, R. M., Othman, R., Abdullah, S., Anuar, N., & Elias, H. (2018). Anthocyanin as potential source for antimicrobial activity in *Clitoria ternatea* L. and *Dioscorea alata* L. *Pigment and Resin Technology, 47*, 490-495. doi:10.1108/PRT-11-2016-0109

Mahr, S. (2008, April 21). *Hibiscus acetosella* Retrieved from https://wimastergardener.org/article/hibiscus-acetosella/

Mar, J. J., Silva, L. S., Lira, A. C., Kinupp, V. F., Yoshida, M. I., & Moreira, W. P., (2020). Bioactive compounds-rich powders: Influence of different carriers and drying techniques on the chemical stability of the *Hibiscus acetosella* extract. *Powder Technology, 360*, 383-391. doi:10.1016/j.powtec.2019.10.062

Marco, P. H., Poppi, R. J., Scarminio, I. S., & Tauler, R. (2011). Investigation of the pH effect and UV radiation on kinetic degradation of anthocyanin mixtures extracted from *Hibiscus acetosella*. *Food Chemistry, 125*, 1020–1027. doi:10.1016/j.foodchem.2010.10.005

Metivierf, R. P., Francisf, J., & Clydesdale, M. R. (2006). Solvent extraction of anthocyanins from wine pomace. *Journal of Food Science, 45*(4), 1099-1100. doi:10.1111/j.1365-2621.1980.tb07534.x

Moonjung, K., & Eunok, C. (2013). Stability of anthocyanins and sugars during heating for low sugar Meoru (*Vitis coignetiea*) jam-making under singlet oxygen. *Food Science and Biotechnology, 22*(3), 645-650. doi:10.1007/s10068-013-0126-5

Oancea, S., & Draghici, O. (2013). pH and thermal stability of anthocyanin-based optimised extracts of romanian red onion cultivars. *Czech Journal of Food Sciences, 31*(3), 283–291. doi:10.17221/302/2012-CJFS

Puckhaber, L. S., Stipanovic, R. D., & Bost, G. A. (2002). Analyses for flavonoid aglycones in fresh and preserved *Hibiscus* flowers. In J. Janick & A. Whipkey (Eds.). *Trends in new crops and new uses* (pp. 556-563). Alexandria, VI: ASHS Press.

Rakkimuthu, R., Palmurugan, S., & Shanmugapriya, A. (2016). Effect of temperature, light, pH on the stability of anthocyanin pigments in *Cocculus hirsutus* fruits. *International Journal of Multi disciplinary Research and Modern Education, 2*(2), 91-96. doi:10.5281/zenodo.61818

Sipahli, S., Mohanlall, V., & Mellem, J. J. (2017). Stability and degradation kinetics of crude anthocyanin extracts from *H. sabdariffa*. *Food Science and Technology, 37*(2), 209-215. doi:10.1590/1678-457x.14216

Thungmungmee, S., Dumrongphuttidecha, T., Noysang, C., & Techaoei, S. (2016). The quantity of total phenolic and flavonoid in *Hibiscus acetosella*. *Proceeding of the Academic Science and Technology Conference,* 319-323.

Thungmungmee, S., Wisidsri, N., & Khobjai, W. (2018). Antioxidant activities of Chaba maple (*Hibiscus acetosella*) flower extract. *Applied Mechanics and Materials, 886,* 34-39. doi:10.4028/www.scientific.net/AMM.886.34

Torskangerpoll, K., & Andersen, M. (2005). Colour stability of anthocyanins in aqueous solutions at various pH values. *Food Chemistry, 89*(3), 427–440. doi:10.1016/j.foodchem.2004.03.002

Vendrame, S., & Klimis-Zacas, D. (2015). Anti-inflammatory effect of anthocyanins via modulation of nuclear factor-jB and mitogen-activated protein kinase signaling cascades. *Nutrition Reviews, 73*(6), 348–358. doi:10.1093/nutrit/nuu066

Wahyuningsih, S., Wulandari, L., Wartono, M. W., Munawaroh, H., & Ramelan, A. H. (2017). The effect of pH and color stability of anthocyanin on food colorant. *IOP Conference Series: Materials Science and Engineering, 193*(1), 1-9. doi:10.1088/1757-899X/193/1/012047