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Conventional and ultrasound-assisted extraction of anthocyanin from red and purple roselle (*Hibiscus sabdariffa* L.) calyces and characterisation of its anthocyanin powder

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Abstract

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Introduction

Roselle (*Hibiscus sabdariffa* L.) is a perennial plant in Indonesia, and its calyces are widely used in health-promoting drink due to its high antioxidant content. Roselle contains 1.5 g total anthocyanin per 100 g dry weight of flower calyces (Duangmal *et al.*, 2008). Anthocyanin is commonly utilised in the food industry to replace synthetic red food colorants. Anthocyanin and its derivatives have been listed in the Codex Alimentarius Commission in the European Union as a natural colouring agent with the code of E163. Anthocyanin from roselle has been previously extracted using various solvents such as water (Mohd-Esa *et al.*, 2010; Cissé *et al.*, 2011; Cissé *et al.*, 2012; Aishah *et al.*, 2013; Serrano-Cruz *et al.* 2013; and Zaidel *et al.*, 2014), methanol (Mohd-Esa *et al.*, 2010), ethanol acidified by HCl (Duangmal *et al.*, 2008) ethanol acidified by mixture of HCl and citric acid (Selim *et al.*, 2008) and also

in the combination of instant pressure drop system solvent (Amor and Allaf, 2009). Maximum yield of anthocyanin extraction from roselle calyces by maceration extraction (ME) was found to be 88% (Cissé *et al.*, 2012). However, ME is time-consuming. Hence, a more efficient method such as ultrasonic-assisted extraction (UAE) for anthocyanin extraction is an advantage. UAE requires low energy and less time (Chemat *et al.*, 2011), and has been used for the extraction of anthocyanin from sugar beet molasses (Chen *et al.*, 2015), *Aronia melanocarpa* (D'Alessandro *et al.*, 2014) and *Delonix regia* (Adjé *et al.*, 2010).

Anthocyanin extracts from roselle are usually obtained in the liquid form which has low colour stability, is degradable and has shorter shelf life (Tiwari *et al.*, 2008; Korca and Ustun 2009; Cissé *et al.*, 2011; Zaidel *et al.*, 2014). Solid product of anthocyanin extracts is expected to be more stable and have longer shelf life. Therefore the present work was aimed to

produce anthocyanin powder as a food colorant in a more stable form (powdered microcapsules with the matrix-microcapsule system) using UAE followed by freeze drying. In Indonesia, the dominant varieties are the red- and purple-coloured roselle. Since there is no information available yet on the investigation of the effect of different extraction techniques on the anthocyanin composition in the red and purple roselle as well as the comparison of ME and UAE, the present work was focused on the investigation of UAE and ME performance in anthocyanin extraction from the red and purple roselle. Furthermore, the anthocyanin-based food colorant powdered form was then characterised and compared to commercial synthetic red food colorant.

Materials and methods

Anthocyanin extraction

Red and purple roselle were purchased from Roselle Garden in Blitar, East Java, Indonesia. The specimen is deposited in Kebun Raya Bogor Herbarium. The roselle calyces were tray-dried at 50°C for 36 h. Distilled water (aquadest) and technical grade ethanol (Multi Kimia Raya, Semarang) were used as the extraction solvents. Two methods of extraction comprising of UAE (Melecchi *et al.*, 2006) and ME were applied to extract the anthocyanin from the roselle calyces. The ratios of material to solvent were 1:10 and 1:15 (w/v). UAE and ME were carried out at room temperature ($\pm 25^\circ\text{C}$). UAE was conducted at a frequency of 40 kHz for 30 min, while ME was carried out by soaking the roselle calyces in the solvent at room temperature ($\pm 25^\circ\text{C}$) for 24 h. The liquid extracts obtained from both UAE and ME were then filtered. Anthocyanin content in the extracts was analysed based on total anthocyanin content. The total anthocyanin content was defined as mg of cyanidin-3-glucoside. The determination of total anthocyanin content was performed at pH 1.0 and 4.5 using UV-Vis spectrophotometer (UV Mini 1240 Shimadzu) at wavelengths of 515 and 700 nm. The total anthocyanin content was determined based on the following equation (Giusti and Wrostand, 2001):

$$\text{Total Anthocyanin (mg/L)} = \frac{[(A_{515} - A_{700})_{\text{pH}1.0} - (A_{515} - A_{700})_{\text{pH}4.5}] \cdot \text{M.W.} \cdot 1,000}{\epsilon \cdot l}$$

where A = absorbance, MW = molecular weight of cyanidin-3-glucoside (449.2 g/mole), ϵ = the molar extinction coefficient for cyanidin-3-glucoside (26,900 L/mole/cm) and l = path-length (cm)

Microencapsulation of anthocyanin

The liquid extracts from UAE and ME were centrifuged and evaporated (Rotary Vacuum Evaporator IKA RV 10). Maltodextrin was added as a stabiliser (10% v/v) to prevent anthocyanin degradation. The mixture was then homogenised using IKA homogeniser (T-10 Basic Ultra Turrax) at 11,000 rpm for 1 min. Next, the mixture was frozen overnight and subjected to freeze drying (Heto Powerdry LL 1500) at -100°C for approximately 24 h (Pérez-Gregorio *et al.*, 2011).

Characterisation of anthocyanin powder

The obtained freeze dried product (anthocyanin powder) was ground into fine powder and refrigerated at 4°C in an air-tight container prior to further analysis. The powder was characterised based on its size and size distribution of powder, and were also analysed for anthocyanin content, moisture content, solubility and colour value. The powder size was evaluated by using images obtained from Scanning Electron Microscope (FEI Type Inspect-S50). The powder size was then measured based on the microscopic images using an ImageJ software (National Institutes of Health, USA). Manual measurements were required in order to provide a precise powder size. The analysis of moisture content was determined by AOAC method (oven-drying method). The solubility of powders was evaluated based on the method developed by Daramola and Osanyinlusi (2006). The moisture content and solubility evaluation of commercial synthetic red food colorant (Ponceau 4R CI 16255) were also conducted. The colour value of powder was calculated using the following equation:

$$E = \frac{10 \cdot A \cdot F}{W}$$

where E = colour value, F = dilution factor to adjust absorbance (in the range of 0.3 to 0.7) and w = weight of powder sample (g). To evaluate the colour stability, the powder was put in closed container, kept in the dark, and the colour values were determined for 4 days.

In addition, the colour intensity was also evaluated by a chromameter (Colour Reader CR-400/410) based on Hunter's Lab Colorimetric System. Hunter notation system is characterised by L^* (lightness), a^* (redness), and b^* (yellowness). The lightness characteristics are white = 100, black = 0. The value of a^* ($+a^*$ = red, $-a^*$ = green). The value of b^* ($+b^*$ = yellow, $-b^*$ = blue). $C = ((a^*)^2 + (b^*)^2)^{1/2}$ and $h_o = (\tan^{-1}(b^*/a^*))$. The chromameter was calibrated by white calibration plate. Twenty-five

grams sample powder was placed into a transparent container prior to analysis. The L^* , a^* and b^* values were read in triplicate for each sample (Caliskan and Dirim, 2016).

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Statistical analysis

All experiments were carried out in triplicate, and the results were represented by means \pm standard deviations (SD). The statistical analysis was conducted by one-way analysis of variance (ANOVA) using MSeExcel Office 2013. The statistical significance was determined using the Least Significant Difference (LSD) t-test, with acceptable statistical significance at $p < 0.05$.

Results and discussion

The anthocyanin contents obtained through different extraction methods at various solute to solvent ratios is shown in Figure 1. It is apparent that UAE yielded significantly ($p < 0.05$) higher results as compared to ME. Similar trend was also found in the anthocyanin extraction from grape and strawberry (Chen *et al.*, 2007; Vilku *et al.*, 2008). UAE is a modern extraction method which provides higher efficiency in shorter time. This method requires fewer solvents (only 1/6 of the organic solvent compared to conventional extraction methods) without the possibility of solvent loss. UAE has also been reported to be a reliable process owing to its capability to preserve the chemical structure, particles and compound materials used (Alupului *et al.*, 2009). The ultrasonic method at a frequency of 42 kHz can break up live cells, accelerating the mass transfer of bioactive compounds from the cells into the solvent (Chemat *et al.*, 2011). The study also showed that ultrasound cavitation generated fracture

and mechanically break the cell wall to increase the material transfer (Liu *et al.*, 2010).

Extraction by ultrasound waves also offers several advantages (Alupului *et al.*, 2009). The ultrasound waves assist extraction by generating micro-cavitation in the liquid surrounding the plant material and heating it, thereby releasing the extract. An increase in the extract diffusion occurs by desolating the cell wall, thereby releasing the compounds in it. Based on Figure 1, it was found that purple roselle produced more anthocyanin content than red roselle. Properties and colour of anthocyanin in the plant tissue are affected by several factors such as the amount of pigment, location, the number of hydroxyl and methoxyl groups, as well as co-pigmentation (Markakis, 1982). Anthocyanin pigments from plant tissues can be classified into pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin (Stintzing and Carle, 2004). Roselle anthocyanin usually comes in the forms of delphinidin-3-sambubioside, cyanidin-3-sambubioside, cyanidin-3-glucoside and delphinidin-3-glucoside (Amor and Allaf, 2009). Anthocyanin obtained from purple roselle calyces (blue-purple colour) was predicted to have more stable structure than the red calyces. The blue-purple colour is more stable due to intermolecular co-pigmentation with the presence of colourless matrix component called flavones. Flavones may assist in retaining the anthocyanin colour by stabilising the quinoidal base (Stintzing and Carle, 2004). The co-pigment protects flavylum ions in the purple anthocyanin structure (Castañeda-Ovando *et al.*, 2009) resulting in a more anthocyanin content from purple roselle.

Figure 1 also confirms that the use of water resulted in higher content of anthocyanin in liquid extract as compared to ethanol ($p < 0.05$). This might

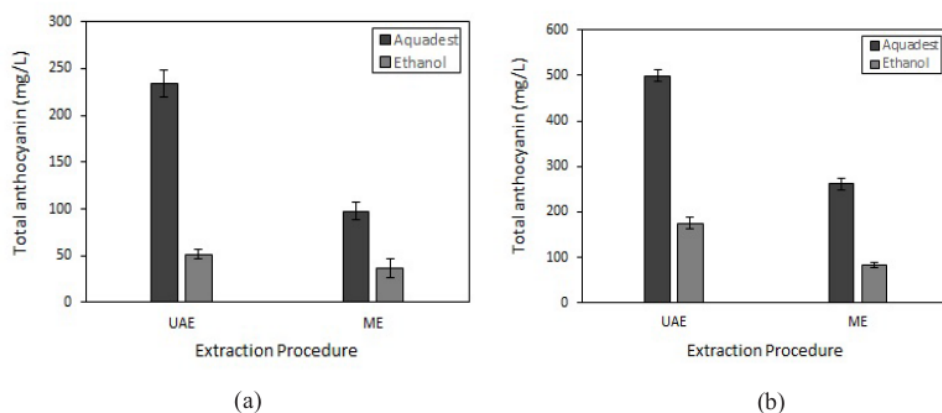


Figure 1. Total anthocyanin content (mg/L) of roselle by UAE and ME at solute to solvent ratio of 1:15 with different solvents and types of roselle: (a) red roselle (b) purple roselle

be due to the characteristic of non-polar solvents which have low dielectric constant. In contrast, polar solvents have high dielectric constant. High polarity solvents have better performance in extracting polar chemical compounds than low polarity solvents (Falleh *et al.*, 2012). Water is a polar solvent having polarity number (dielectric constant) of 80.370, while ethanol is a semi-polar solvent with the polarity number of 24.30 (Weast and Astle, 1982). This could be the reason why water is better to extract anthocyanin than ethanol. Water is generally used for the extraction of high polarity components, such as carbohydrates, glycosides and amino acids (Shirsath *et al.*, 2012). Anthocyanin has a glycoside structure, hence more suitable to be extracted using water as the solvent. Moreover, in UAE, the cavitation will increase for solvents that have low vapour pressure, high viscosity and high surface tension (Vardanega *et al.*, 2014). Data showed that the vapour pressure and surface tension of water are higher than that of ethanol.

Figure 2 shows the anthocyanin contents at solute to solvent ratio of 1:10. The Figure confirms that higher solute to solvent ratio significantly ($p < 0.05$) yielded higher anthocyanin content from the roselle extract. This is a general behaviour during extraction. By increasing the volume of solvent, the dissolved extracted materials would be higher thus resulting in higher extraction yield. In addition, the mass transfer parameter is also affected by the volume of solvent. The higher the solvent volume results in larger mass transfer and more accelerated diffusion into the medium (solvent) (Xu *et al.*, 2016). Similar results were also found in extraction of anthocyanin by UAE from mulberry (Zou *et al.*, 2011), antioxidant from

Jatropha integerrima (spicy jatropha) flowers (Xu *et al.*, 2016), phenolics from wine lees (Tao *et al.*, 2014) and ursolic acid from *Ocimum sanctum* (holy basil) leaves (Vetal *et al.*, 2012).

Characterisation of anthocyanin colorant powder from roselle

In the present work, it was found that the shape and size of the anthocyanin powder were not uniform, and anthocyanin was distributed as matrix microcapsules. The powder obtained from freeze-drying exhibited porous and hygroscopic characteristics. On the contrary, encapsulation by spray-drying was spherical due to nozzle spray (Ersus and Yurdagel, 2007). The anthocyanin powder had average particle size of 4.24 μm and 3.94 μm from red and purple roselle, respectively. The powder size would affect the physical properties of the product, such as solubility, moisture content and colour profile.

The moisture content and solubility of the anthocyanin powder are displayed in Table 1. It is apparent that the moisture content of the anthocyanin powder was higher than the commercial synthetic red colorant. In general, freeze-drying generates a product with higher moisture content than spray-dried products (Fang and Bhandari, 2012). Solubility is another important parameter to determine the quality of food colorant powder. The high solubility of powder colorant has a correlation with good colouring ability. The solubility of the anthocyanin powder of roselle in the present work was found to be close to the solubility of the synthetic food colorant powder. The addition of maltodextrin improved the water solubility of the product. The maltodextrin facilitates the increase of water content in the

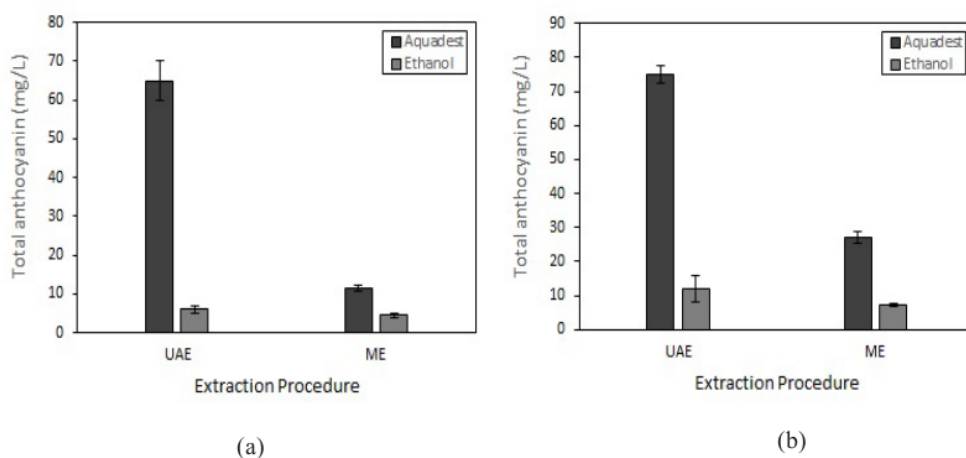


Figure 2. Total anthocyanin content (mg/L) of roselle by UAE and ME at solute to solvent ratio of 1:10 with different solvents and types of roselle: (a) red roselle (b) purple roselle

monolayer which has an effect on the improvement of their solubility (Canuto *et al.*, 2014). However, adding maltodextrin decreased the hygroscopicity of the solid product thereby producing more stable and dry product (Mosquera *et al.*, 2010).

Table 1. Moisture content and solubility of anthocyanin food colorant powder from roselle and commercial synthetic food colorant

Colorant	Moisture content (%)	Solubility (%)
Red roselle	10.29	96.92
Purple roselle	9.67	97.44
Synthetic food colorant	7.33	97.00

The colour intensity of anthocyanin from roselle calyces as well as commercial synthetic red food colorant is displayed in Table 2. Based on the Table, anthocyanins from roselle significantly ($p < 0.05$) yielded higher L^* values as compared to the commercial synthetic red food colorant. On the other hand, the a^* and b^* values of the anthocyanin powder were significantly ($p < 0.05$) lower as compared to the commercial synthetic food colorant.

Table 2. Colour Intensity of anthocyanin food colorant powder from roselle and commercial synthetic food colorant

Colorant	Colour Intensity		
	$*L$	$*a$	$*b$
Red roselle	42.61 ± 1.8	24.79 ± 0.8	7.35 ± 0.3
Purple roselle	43.91 ± 0.25	24.40 ± 0.41	5.56 ± 0.36
Synthetic food colorant	36.87 ± 1.01	27.49 ± 0.47	9.27 ± 0.58

The colour stability of the anthocyanin powder is displayed in Table 3 which reveals that the colour value of the anthocyanin powder decreased day by day. Comparing to the commercial synthetic red food colorant, the natural anthocyanin colour had less performance in the term of colour stability. The commercial synthetic red food colorant showed a stable value for five days with a colour value around 7102.8. Based on Table 3, it is predicted that the colour value would start to diminish after five and six days for the anthocyanin product from red and

purple roselle, respectively. With the reduction of colour value, the antioxidant activity also decreases. The stability of anthocyanin colour is influenced by enzymatic and non-enzymatic aspects. The non-enzymatic aspect includes pH, light, oxygen, sugar and temperature (Jackman and Smith, 1996), while enzymatic decolourisation has attributed to the release of anthocyanidins from their sugar moieties to glycosidases and destabilise the aglycon degradation into a colourless product. Peroxidase-catalysed and phenolates (phenoloxidasases, polyphenol oxidasases) are also involved in anthocyanin degradation. In general, the phenolases enzyme indirectly degrades anthocyanin and oxidises phenolase compounds into o-benzoquinone which then oxidises anthocyanin into a colourless product (Markakis, 1982).

Light is another primary factor having an important role in anthocyanin degradation. Light has certain energy to stimulate the occurrence of photochemical reaction in anthocyanin (Jackman and Smith, 1996). The second carbon ring will open due to photochemical reactions of anthocyanin molecule. Finally, these reactions would form colourless compounds indicating the complete degradation of anthocyanin (Markakis, 1982). In addition, oxygen can also stimulate anthocyanin degradation, either directly or indirectly. Directly, oxygen triggers anthocyanin oxidation and generates a colourless compound which degrades the stability of anthocyanin (Gradinaru *et al.*, 2003).

Conclusion

Anthocyanin extraction using UAE was confirmed to be of superior performance than ME. The purple roselle yielded higher anthocyanin content as compared to the red roselle. In addition, it was also demonstrated that the use of water as solvent improved the extraction ability. The evaluation of colour verified that the colour intensity of anthocyanin food colorant powder diminished day by day. Anthocyanin powder from red and purple roselle calyces with natural food colorant properties was successfully produced. However, further research should be carried out in order to evaluate the performance of anthocyanin powder in food product application.

Table 3. Colour values of anthocyanin food colorant powder from roselle and commercial synthetic food colorant

Colorant	Colour Value				
	Day 0	Day 1	Day 2	Day 3	Day 4
Red roselle	83.53	46.85	33.90	33.05	29.41
Purple roselle	195.39	107.75	91.57	70.79	57.30
Synthetic food colorant	7,102.76	7,102.76	7,102.76	7,100.55	7,098.34

Acknowledgement

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