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Encapsulation of Anthocyanin from Roselle and Red Cabbage for Stabilization of Water-in-Oil Emulsion

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Abstract

Addition of encapsulated anthocyanins in margarine formulation improved the stability of margarine. In this study, investigation on the storage and stability characteristics of water-in-oil emulsion, i.e. margarine, containing encapsulated or non-encapsulated anthocyanins from roselle and red cabbage was carried out. Encapsulation of anthocyanins was performed using microwave-assisted technique to study the dispersion and stability of anthocyanins in the margarine. Margarines were formulated with three different oil-to-aqueous ratios (75:25, 80:20 and 85:15). The stability of the margarine was determined by using Solid Fat Content, thermal and phase stability analysis. Margarine containing encapsulated anthocyanin showed improved stability compared to non-encapsulated anthocyanin.

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Keywords: Anthocyanin; emulsion; margarine; stability

1. Introduction

Margarine has become one of the choices for table spread, bakery, and pastry for many people for reasons of either health or economics (Vaisey-Genser, 2003; Saadi et al., 2012). Margarine is a water-in-oil emulsion, in which the oil phase consists of both liquid oil; crystalline fat at room temperature which consists of one liquid being dispersed into another liquid (Ramisetty and Shyamsunder, 2011). The stability of margarine depends on many

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factors such as the liquid to oil phase ratio, temperature of crystallization, size of water droplets, type of emulsifier, storage temperature, presence of salt or preservative and method of manufacturing.

There have been increased attractions in the development of food colorants from natural or natural-derived alternatives as the synthetic pigments are increasingly rejected by the consumer (Stintzing and Carle, 2004). The concern is increasing as the synthetic colorants or antioxidants have been reported to provide an implication on cardiovascular disease (Stintzing and Carle, 2004). Anthocyanin is one of the example colorant widely used as natural colorant in food industry. Aanthocyanins can be widely found as it comprise the largest group of water soluble pigments in the plant kingdom and are especially characteristic of the angiosperms or flowering plants such as cherry, blueberry, apple, acai berry, avocado, guava, kiwifruit, roselle, mango and dragon fruit (Duangmal et al., 2004; Ersus and Yurdagel, 2006; Looi, 2008). Anthocyanin from different sources gives different processing and storage stability such as storage temperature, light, pH, concentration, ascorbic acid, sugar and oxygen (Markakis, 1982; Tsai and Huang, 2004; Rein, 2005).

Recently, the biological activities of anthocyanin, such as antioxidant activity, protection from atherosclerosis and anticarcinigenic activity, aphrodisiac properties (Duke and Ducellier, 1993) have been investigated, and reported that anthocyanins can provide some beneficial effects in the treatment of disease such as neuronaland cardiovascular illnesses, cancer and diabetes (Tsai et al., 2002; Lule and Xia, 2005; Nichenamentla, et al., 2006), lower blood pressure and improve the digestive system in humans (Muhammad and Shakib, 1995). As the consequences, anthocyanin as food colorant is becoming increasingly important not only do they contribute to the aesthetic value but also they tend to yield potential positive health effects.

Several studies have reported that the isolated anthocyanins are highly instable and very susceptible to degradation. Stability of anthocyanins is affected by several factors such as pH, SO*,storage temperature, chemical structure, concentration, light, oxygen, solvents, copigmentation and 'thin film' effects, the presence of enzymes, flavonoids, proteins and metallic ions (Rein, 2005; Markakis, 1982). When heating, the anthocyanins become paler because the equilibrium between the four anthocyanin species shifts towards the colourless carbinol base and chalcone forms.

Encapsulation is a useful means of protecting colorant from degradation and preventing premature colour development. Microencapsulating a pigment in matrix suitable for given application improves the stability of the color both in bulk and in food formulations while ensuring end-product functionality. Encapsulated natural colours have better heat, light and pH stability. Hence, encapsulation of colour has longer shelf life, improved stability over a wider pH range and the prevention colour development during storage while delivering the intended colour upon preparation. Currently, there are high interests of using microwave technique for encapsulation among food researchers due to its high potential capabilities such as shorter drying time, low cost, improved product quality and also have flexibility in producing a variety of dried product (Haghi and Amanifard, 2008).

This study aims to investigate the storage and stability characteristics of water-in-oil emulsion, i.e. margarine, containing encapsulated anthocyanins from roselle and red cabbage. Comparison between margarines formulated containing non-encapsulated and encapsulated anthocyanins were investigated.

Nomenclature

T_{mc}	melting completion temperature
T_{oc}	crystallization onset temperature
SFC	Solid Fat Content
DSC	Differential Scanning Calorimeter

2. Materials and methods

2.1. Preparation of roselle and red cabbage samples

Fresh roselle calyces (*Hibiscus Sabdariffa L.*) and red cabbage (*Brassica Oleracea*), which was obtained from Gunung Jerai, Kedah (Organic Authentic Farm), were dried at 50 °C for 36 hours and stored at 25 °C. The dried red cabbage were shredded and put in dry wide-mouthed bottle containers, cover and label (Ersus and Yurdagel, 2006).

2.2. Extraction of anthocyanins

The extraction of anthocyanins will be performed according to Looi (2008) with modification. The shredded roselle calyces and red cabbages were added in distilled water in different beaker. The mixtures were stirred and heat at 35 °C on hotplate for an hour until purple color appeared. The extracted solution was poured through the coffee filter to remove the pieces of cabbage. The procedure was replicated three times. For third time replication, the sample was soaked and stored in refrigerator for 24 hour until all anthocyanins were almost fully extracted or the fiber become colorless through observation. The extracted solutions (25 mL) were collected in universal bottle for concentration determination, respectively while the least was collected in a beaker. The extracted solvents were evaporated at 50 °C under vacuum (Looi, 2008).

2.3. Encapsulation of anthocyanins using microwave-assisted technique

Anthocyanins (roselle and red cabbage extracts) as core materials were mixed well with Maltodextrin at ratio 1:10 (v/v) inside a round glass plate. Mixtures were placed in a domestic microwave oven (1100 W) up to seconds. The process proceeds until the wall materials started to melt or burn (Abbasi and Rahimi, 2008).

2.4. Determination of color parameters

Colour parameters (*L, a*, b*, C* and H) were measured using colorimeter (Konica Minolta). The sample was placed in glass plate. The start button was pressed when the colorimeter was ready to evaluate. The *L, a*, b*, C* and H value appeared at the screen of colorimeter and was recorded.

2.5. Dissolution test

Encapsulated sample (50 mg powder) was mixed with one mL distilled water at room temperature in a test tube. The time taken for powder to dissolve in water was recorded. The analysis was repeated three times.

2.6. Formulation of margarine

Aqueous phase was prepared by blending water (47.5%), extracted non-encapsulated anthocyanins (47.5%), and salt (5%). Oil phase was prepared by blending palm oil and palm stearin (60:40) (99.5%) and emulsifier (0.5%). The oil phase and liquid phase were heated to 60 °C. Both oil and aqueous phase were blended vigorously in homogenizer with varied oil phase-to-aqueous phase ratio (75:25; 80:20; 85:15) for 15 minutes. The liquid emulsion was crystallized for 15 minutes in ice cream maker and then refrigerated overnight. The margarines were tempered at room temperature for 4hour and vigorously mixed with hand mixer. The margarine samples were placed into plastic tubes and stored at room temperature (Young and Morr, 1996). The steps were repeated for margarines containing encapsulated anthocyanins from roselle and red cabbage.

2.7. Analysis of Solid Fat Content (SFC) of margarine

Nine tubes were used for each sample. Each sample was tempered at 70 °C for 30 minutes, followed by chilling at 0 °C for 90 minutes and then kept at the desired temperatures for 30 minutes prior to measurements. The melting, chilling and holding of the samples were carried out in pre-equilibrated thermo-stated baths. The SFC was measured within the temperature ranges 5 to 40 °C (Saadi et al., 2012).

2.8. Analysis of thermal properties of margarine

Samples weighing from 3 – 15 mg, scaled in an aluminum pen were heated to 70 °C for 15 minutes in a Differential Scanning Calorimeter (DSC) (Perkins-Elmer Diamond DSC, Shelton, CT, USA) to ensure that no residual nuclei remained. The samples were then cooled from melt (70 °C) at 5 °C/min to -30°C and held for 15 minutes before heating the samples to 70 °C again at 5 °C/min for the melting thermo-grams (Saadi et al., 2012).

2.9. Determination of phase stability of margarine

The margarine (10 ml sample) was stored in a graduated 17 x 100 mm tube at room temperature for 7 days and 14 days. The height of the total system and height of the lower opaque phase (dispersed water droplets) were measured to determine the volume fraction of the sediment. The appearance of the emulsion was documented by photographs (OLYMPUS Digital Camera C-4040ZOOM, Japan) (Knoth et al., 2005).

3. Results and discussion

3.1. Extraction of anthocyanins

From the extraction process, red solution was obtained from roselle while purple solution was obtained from red cabbage and at the end the red cabbage became colourless fibre. Total concentration of pure water extraction anthocyanin for roselle was 135.33 ± 2.0 mg/L. The first concentration of extracted anthocyanin with 200 mL pure water was 186.55 ± 2.0 mg/L; second concentration of extraction with 100 mL pure water was 117.95 ± 2.0 mg/L, and third extraction of anthocyanin from the same sample was 59.91 ± 2.0 mg/L.

Total concentration of pure water extraction anthocyanin for red cabbage was 152.33 ± 2.0 mg/L. The first concentration of extracted anthocyanin with 200 mL pure water was 192.85 ± 2.0 mg/L; second concentration of extraction with 100 mL pure water was 126.15 ± 2.0 mg/L, and third extraction of anthocyanin from the same sample was 58.91 ± 2.0 mg/L. Table 1 shows the physical properties of anthocyanins extracted from roselle and red cabbage. Both plants contained almost the same amount of anthocyanin extracted by using distilled water extraction. The results showed that anthocyanin concentration in roselle was lesser than in red cabbage.

Table 1. Physical properties for extracted anthocyanins from roselle and red cabbage.

	Roselle	Red cabbage
Total soluble solid (°Brix)	10.8 ± 1.0	9.9 ± 1.0
Total anthocyanin content (mg/L)	135.33 ± 2.0	152.33 ± 2.0
Color parameters		
L*	41.4 ± 1.0	42.7 ± 1.0
*a**	+0.3 ± 2.0	+8.3 ± 2.0
*b**	+8.6 ± 2.0	+10.8 ± 2.0
C*	8.6 ± 1.0	13.6 ± 1.0
H°	88.1 ± 1.0	52.5 ± 1.0

3.2. Encapsulation of anthocyanins

The anthocyanins were encapsulated by using microwave-assisted approach utilising maltodextrin as wall material. From previous research maltodextrin showed the greatest protecting effect for anthocyanins stability (Hui, 2009). In solution state, maltodextrin reduced the degradation of anthocyanins rather than naked anthocyanins. Table 2 shows the physical properties for encapsulated anthocyanins from roselle and red cabbage. Encapsulated anthocyanins from roselle had lower moisture content and soluble faster in water compared to encapsulated anthocyanins from red cabbage.

Table 2. Physical properties for encapsulated anthocyanins from roselle and red cabbage.

	Roselle	Red cabbage
Moisture Content (%)	0.72409 ± 0.00001	3.11529 ± 0.00001
Dissolution test (s)	5 ± 1	7 ± 1
Color parameters		
L*	51.0 ± 1.0	48.3 ± 1.0
*a**	+4.4 ± 2.0	+4.7 ± 2.0
*b**	+6.9 ± 2.0	+7.9 ± 2.0
C*	8.2 ± 1.0	9.2 ± 1.0
H°	56.6 ± 1.0	59.4 ± 1.0

3.3. Solid Fat Content of margarine containing anthocyanins

Solid Fat Content (SFC) was performed to determine the specific volume for solid and liquid at specific temperature. For standard margarine the solid contents were approximately 15 - 35% at 25 °C, more than 10% at 20 °C and below 10% at 33.3 °C to avoid oil separation (Rao et al., 2001). The results showed that all margarines followed the criterion stated by Rao et al. (2001) at 20 °C which have more than 10% and at 25 °C which have 15 - 35% SFC (Fig. 1). However, at 33 °C all the margarines have more than 10% SFC.

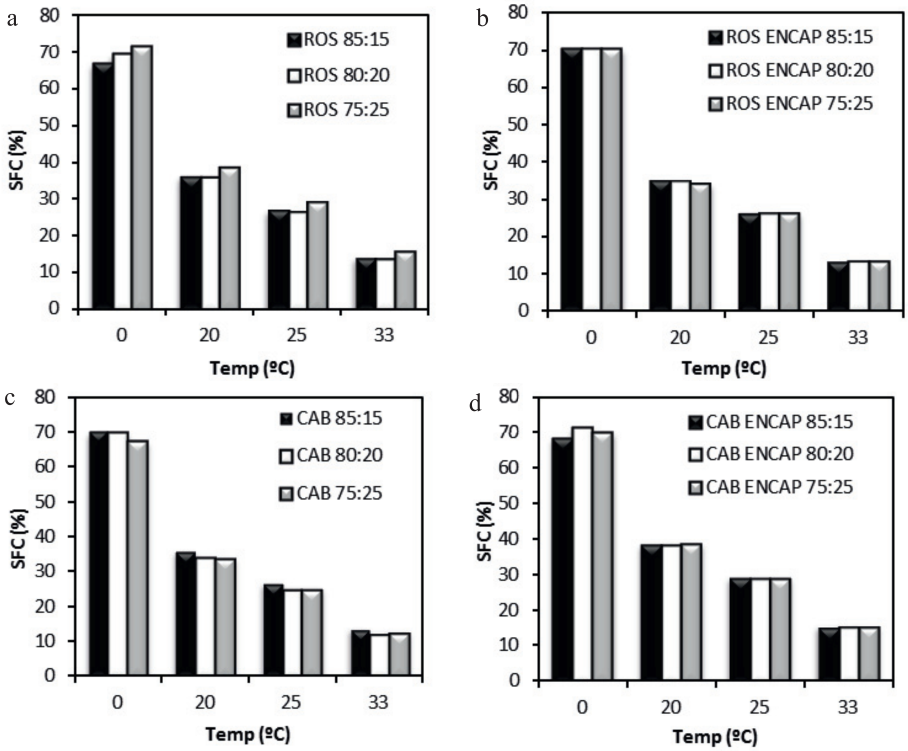

Fig. 1. SFC analysis for margarine containing (a) non-encapsulated anthocyanins from roselle; (b) encapsulated anthocyanins from roselle; (c) non-encapsulated anthocyanins from red cabbage; (d) encapsulated anthocyanins from red cabbage.

The best margarine was the margarine with lowest SFC percentage at 33 °C. The results indicated that the margarine at oil-to-aqueous ratio of 85:15 has the lowest SFC percentage at 33 °C and have been chosen as the best margarine formulation based on SFC (Fig. 1). Fig. 2 shows the comparison of different types of margarine at oil-to-aqueous ratio 85:15 to choose the best margarine formulation. From the graph plotted, the margarine containing encapsulated red cabbage was the best formulation as it has the lowest SFC percentage at 33 °C.

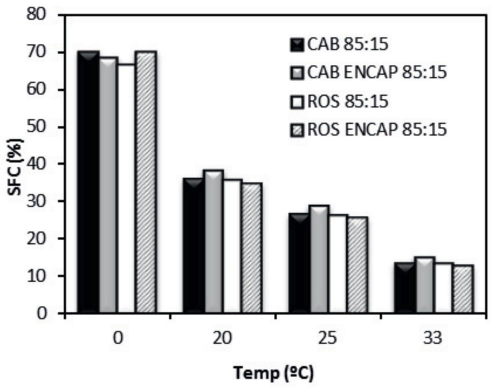

Fig. 2. SFC for different types of margarine at oil-to aqueous ratio 85:15 containing non-encapsulated and encapsulated anthocyanins.

3.4. Thermal properties of margarine containing anthocyanins

DSC is the thermo-analytical technique that is used to assess the thermal behaviour and phase transition protocols occurred during blending of palm oil with palm stearin. It gives an indication on the heat flows and temperatures variation related to the materials transition (Dollimore, 1996). Thermal properties of the margarine at different oil-to-aqueous ratio containing anthocyanins from roselle and red cabbage showed the melting completion temperature (T_{mc}) and onset crystallisation temperature (T_{oc}) of the margarine (Table 3).

Table 3. Thermal behaviour for margarine containing anthocyanins.

Anthocyanin source	Encapsulation	Oil-to-aqueous ratio	Melting Completion Temperature (T_{mc})	Onset Crystallization Temperature (T_{oc})
Roselle	Non-Encapsulated	85:15	46.55	23.07
		80:20	47.40	23.24
		75:15	47.23	22.26
	Encapsulated	85:15	46.60	22.87
		80:20	46.85	22.84
		75:15	46.66	22.00
Cabbage	Non-Encapsulated	85:15	47.01	22.35
		80:20	46.59	22.68
		75:15	46.34	21.92
	Encapsulated	85:15	47.26	22.92
		80:20	47.35	22.91
		75:15	47.43	22.17

Fat and oils do not have a distinct melting point but rather a melting range because of the different fatty acids (FAs) present. The T_{mc} depends on the amount and type of FAs. The best margarine is margarine that has the highest T_{mc} but the lowest T_{oc}. This property indicates that the margarine will melt very rapidly at body temperature to ensure a 'quick getaway' in the mouth with minimum gumminess (Vaisey-Genser, 2003). The T_{mc} and T_{oc} were approximately similar between margarines (Table 3). Based on the result obtained, the margarines containing encapsulated red cabbage showed the best properties since they have high T_{mc} and low T_{oc}.

3.5. Phase stability analysis of margarine containing anthocyanins

Lower values of sedimentation stability indicate the lower extent of sedimentation (phase separation) which means higher stability of the emulsions. Fig. 5 shows the sedimentation stability of margarine after 7 days and 14 days at room temperature. At room temperature, the oil separations increased after day 4 until day 14 for all margarines (Fig. 3). The lowest sedimentation stability was at oil-to-aqueous ratio 85:15 and the highest sedimentation stability was at oil-to-aqueous ratio 75:25. Margarines containing encapsulated anthocyanins were more stable than margarine containing non-encapsulated anthocyanins as the sedimentation stability was lower. This result indicates that the anthocyanins affected the stability of the margarine as the encapsulated anthocyanins were more stable in the margarine compared to non-encapsulated anthocyanins.

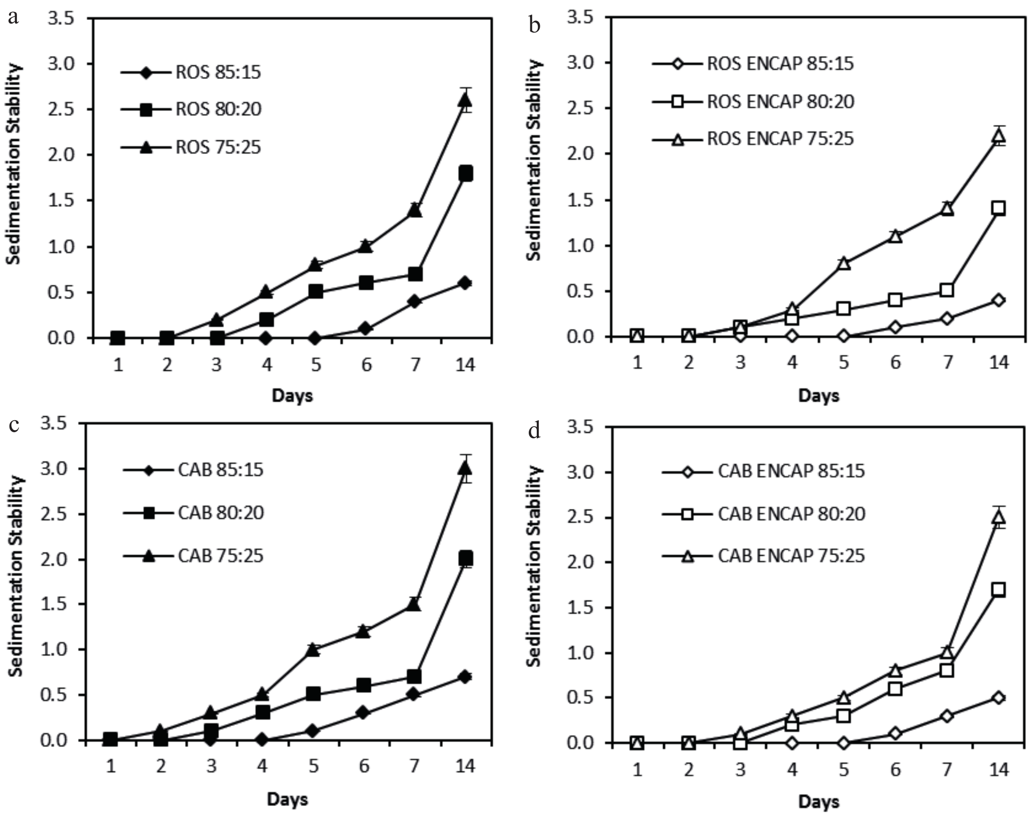

Fig. 3. Stability test at room temperature for margarine containing (a) non-encapsulated anthocyanins from roselle (b) encapsulated anthocyanins from roselle (c) non-encapsulated anthocyanins from red cabbage (d) encapsulated anthocyanins from red cabbage.

Previous research done by Tan et al. (2014) focused on the effects of the particle concentration oil-to-aqueous ratio and salt concentration on emulsion type on the stability of the emulsions. It has been highlighted that the coalescence or deformation of droplets which leads to phase separation increases as the fraction of aqueous phase increases (Tan et al., 2014). The inadequate addition of aqueous phase on water-in-oil emulsion may affect the stabilization of the emulsion.

4. Conclusions

For extraction of anthocyanins by using 100% distilled water extraction method, roselle and red cabbage had high total anthocyanins content. Encapsulated anthocyanins from roselle had low moisture content and soluble fast in water. Solid Fat Content (SFC) analysis shows that the best margarine was margarine containing encapsulated red cabbage as it follows the criterion at 20 °C and 25 °C and has the lowest SFC percentage at 33 °C. From DSC analysis, the margarine contained encapsulated anthocyanins red cabbage at oil-to-aqueous ratio 85:15 and oil-to-aqueous ratio 80:20 showed the best properties since they have high melting completion temperature and low onset crystallisation temperature. From stability test we can conclude that the anthocyanins affected the stability of the margarine as the addition of encapsulated anthocyanins in the margarine formulation improved the stability of the margarine. The encapsulated anthocyanins that have higher moisture content and solubility will produce margarine with higher sedimentation stability which means low stability.

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