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Stability Study of Water-in-Oil Emulsion Containing Anthocyanins from Red Cabbage

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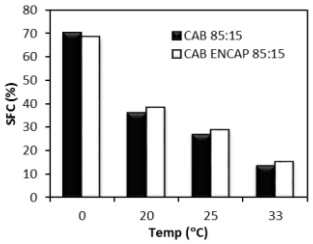
Graphical abstract



Abstract

One of the main factors in the formulation and control of water-in-oil emulsion is the development of desirable solid-to-liquid ratios, achieved by hydrogenation and blending. Formulation of water-in-oil emulsion with bioactive compound could provide additional value and stability to the emulsion for food application for example in margarine. The objectives of this study were to formulate water-in-oil emulsions i.e. margarine containing bioactive compound (anthocyanins) extracted from red cabbage (Brassica oleracea L.) and to study the storage and stability characteristics of the emulsions. The anthocyanins were extracted using hot water extraction method at 60°C with three replications, and encapsulation of anthocyanins was performed using microwave technique to study the dispersion and stability of anthocyanins in the emulsions. In this study, water-in-oil emulsions were formulated with three different oil-to-aqueous ratios (75:25, 80:20 and 85:15) at 60°C for 15 minutes during blending and at 0°C for 30 minutes during crystallization process. The stability of the formulated emulsion was determined by using Solid Fat Content analysis, thermal and phase stability analysis. Total concentration of anthocyanin extracted from red cabbage was 152.33±2.0 mg/L. After 14 days of storage, the emulsion stability was achieved at oil-to-aqueous ratio of 85:15 and with addition of encapsulated anthocyanins. This formulation produced stable emulsion with high melting completion temperature and low onset crystallization temperature.

Keywords: Anthocyanin; emulsion; margarine; red cabbage; stability

1.0 INTRODUCTION

The appreciation of high and good quality food is a part of life's pleasures. Colour gives visual cues to flavor identification and taste thresholds, influencing food preferences, food acceptability and ultimate food choice. Thus, colour plays an important role in enjoyment of food. Margarine has been patented in 1869 as cheap alternative to butter for French workers and armies [1]. By 2000, margarine has become the table spread of choice for many people for reasons of either health or economics. Margarine is a water-in-oil emulsion, in which the oil phase consists of both liquid oil and crystalline fat at room temperature. The stability of margarine depends on many 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 [2]. There have been increased attractions in the development of food colourants from natural sources as alternative to synthetic dyes. Anthocyanin is one of the example colorants widely used as natural colorant in food industry. The anthocyanins 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 [3, 4, 5]. Anthocyanin from different sources gives different stability. The stability of anthocyanins is affected by several factors such as pH, SO*,storage temperature, chemical structure, concentration, light, oxygen, solvents, co-pigmentation and 'thin film' effects, the presence of enzymes, flavonoids, proteins and metallic ions [6]. Recently, the biological activities of anthocyanin, such as antioxidant activity, protection from atherosclerosis and anticarcinigenic activity have been investigated, and shown to have some beneficial evidence that antioxidants have some beneficial effects in the treatment of disease. As the consequences, anthocyanin as food colourant is becoming increasingly important not only do they contribute to the aesthetic value but also they tend to yield potential positive health effects [7]. The objective of this study is to formulate a margarine containing bioactive compound (anthocyanin) from red cabbage (*Brassica oleracea L.*) and to study the storage and stability characteristics of the margarine.

2.0 EXPERIMENTAL

2.1 Preparation of Red Cabbage Extracts

The fresh 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 [8].

2.2 Anthocyanins Extraction

The shredded 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 colour 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 fibre become colourless 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 [9].

2.3 Encapsulation Method

Anthocyanins (red cabbage extract) as core material mix 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 [10].

2.4 Margarine Formulation

Aqueous phase was prepared by blending water (47.5%), extracted anthocyanins (47.5%), and salt (5%). Oil phase is 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 4°C and at room temperature [11].

2.5 Solid Fat Content (SFC) Analysis

Nine tubes are used for each sample. Each sample is 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 are carried out in pre-equilibrated thermostated baths. The SFC is measured within the temperature ranges 5-40°C [12].

2.6 Thermal Properties by DSC Analysis

Samples weighing from 3-15 mg, scaled in an aluminum pen were heated to 70°C for 15 minutes in a Perkin-Elmer Differential Scanning Calorimeter-7 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 [12].

2.7 Determination of Phase Stability

A 10 ml sample of each margarine was stored in a graduated 17 X 100 mm sample tube at 7°C for 7 days and 2 months. 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 is documented by photographs (OLYMPUS Digital Camera C-4040ZOOM, Japan) [13].

3.0 RESULTS AND DISCUSSION

3.1 Extraction of Anthocyanins from Red Cabbage

Table 1 shows the physical properties of anthocyanins extracted from red cabbage. 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 100mL 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.

From previous research, the concentration of extracted anthocyanins from red cabbage was 129.69±4.24 mg/L [9]. The first concentration of extracted anthocyanin with 200 ml pure water was 173.545±1.99 mg/L; second concentration of extraction with 100ml pure water was 116.023±4.05 mg/L, and the third extraction of anthocyanin from the sample was 55.63±8.93 mg/L.

Table 1 Physical properties for extracted anthocyanins from red cabbage

Total soluble solid (°Brix)	9.9±1.0
Total anthocyanin content (mg/L)	152.33±2.0
Colour parameters	
L*	42.7±1.0
*a**	+8.3±2.0
*b**	+10.8±2.0
C*	13.6±1.0
H°	52.5±1.0

From statistical analysis, 350 g of red cabbage contained 51.87 mg of anthocyanin. Compared to the previous study, the concentration of anthocyanins obtained in this study was different due to the different red cabbage varieties and different source. The chemical composition and properties were different for the red cabbage that was retrieved from different places. Red cabbage that was used in this study contained higher concentration of anthocyanins.

3.2 Encapsulation of Anthocyanins by Using Microwave-assisted Approach

The anthocyanins were encapsulated by using microwave-assisted approach with using maltodextrin as wall material. From previous research maltodextrin showed the greatest protecting effect for anthocyanins stability [14]. In solution state, Maltodextrin reduced the degradation of anthocyanins rather than naked anthocyanins. Table 2 shows the physical properties for encapsulated anthocyanins from red cabbage.

Table 2 Physical properties for encapsulated anthocyanins from red cabbage

Moisture Content (%)	3.11529±0.00001
Dissolution test (s)	7±1
Color parameters	
L*	48.3±1.0
*a**	+4.7±2.0
*b**	+7.9±2.0
C*	9.2±1.0
H°	59.4±1.0

3.3 Storage and Stability Characteristics of Margarine containing Anthocyanins

Three analysis have been conducted to determine the storage and stability characteristics of the product that were Solid Fat Content (SFC), thermal properties and phase stability.

3.3.1 Solid Fat Content

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 [15].

The results showed that all margarine follow the criterion stated by Rao *et al*. (2001) [16] at 20°C which have more than 10% and at 25°C which have 15-35% SFC (Figure 1). However, at 33°C all the margarines have more than 10% SFC. 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 (Figure 1(a) and 1(b)). Figure 2 shows the comparison of different type of margarine at oil-to-aqueous ratio 85:15 to choose the best margarine. From the graph plotted, the best margarine contained the encapsulated red cabbage as it has the lowest SFC percentage at 33°C.

3.3.2 Thermal Properties by Differential Scanning Calorimeter (DSC)

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 [16].

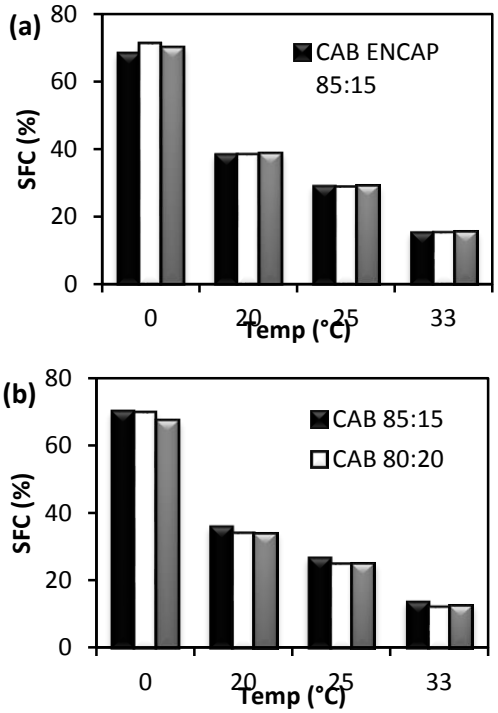


Figure 1 (a) SFC for margarine containing encapsulated red cabbage (b) SFC for margarine containing non-encapsulated red cabbage

Thermal properties of the margarine containing anthocyanins from red cabbage show the melting completion temperature (Tmc) and onset crystallization temperature (Toc) (Table 3). Fat and oils do not have a distinct melting point but rather a melting range because of the different fatty acids (FAs) present. The melting completion temperature depends on the amount and type of FAs. The best margarine is margarine that has the highest melting completion temperature (Tmc) but have the lowest onset crystallization temperature (Toc). It indicates that the margarine will melt very rapidly at body temperature to ensure a 'quick getaway' in the mouth with minimum gumminess [1].

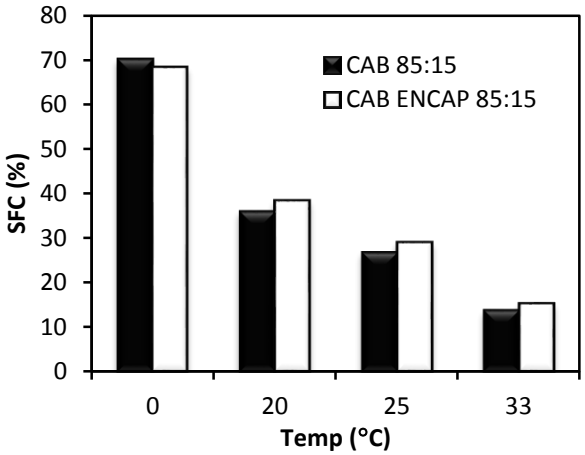


Figure 2 SFC for different type of margarine at oil-to aqueous ratio 85:15

The melting completion temperature (Tmc) and onset crystallization temperature (Toc) is approximately similar between margarines (Figure 3). The best margarine is margarine with encapsulated red cabbage at oil-to-aqueous ratio 85:15 and

oil-to-aqueous ratio 80:20 because they have high melting completion temperature and low onset crystallisation temperature.

Table 3 Thermal behavior for margarine containing anthocyanins

Margarine Formulation	Melting Completion Temperature (Tmc)	Crystallisation Onset Temperature (Tco)
Cabbage Encapsulated 85:15	47.26	22.92
Cabbage Encapsulated 80:20	47.35	22.91
Cabbage Encapsulated 75:25	47.43	22.17
Cabbage Non-Encapsulated 85:15	47.01	22.35
Cabbage Non-Encapsulated 80:20	46.59	22.68
Cabbage Non-Encapsulated 75:25	46.34	21.92

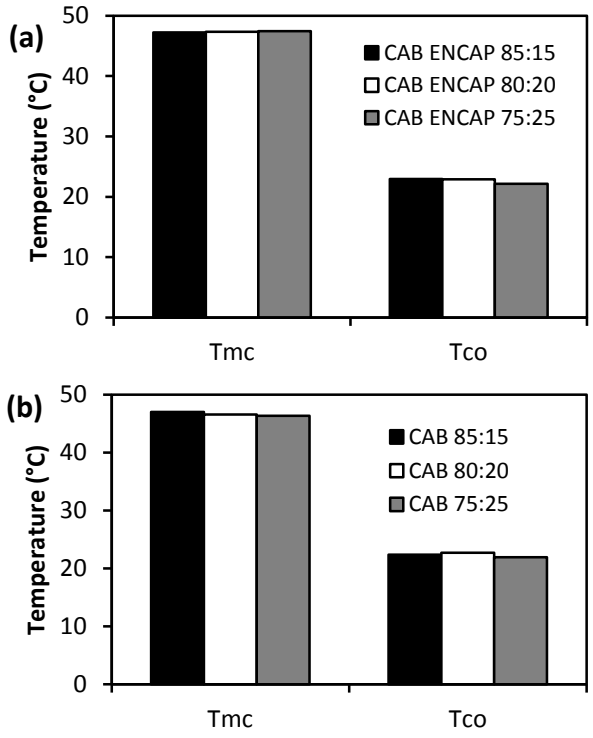


Figure 3 (a) Thermal behaviour for margarine containing encapsulated red cabbage (b) Thermal behaviour for margarine containing non-encapsulated red cabbage

3.3.3 Phase Stability

The lower values of sedimentation stability indicate the lower extent of sedimentation (phase separation) which means the higher stability of the emulsions. Figure 4 shows the sedimentation stability of margarine after 7 days and 14 days at 4ºC. Figure 5 shows the sedimentation stability of margarine after 7 days and 14 days at room temperature. The results showed that the margarine was stable at 4°C as the sedimentation stability was low (Figure 4). At 4°C there were no oil separations for all margarine formulated.

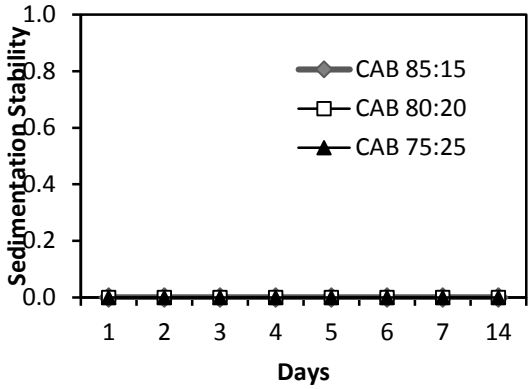


Figure 4 Stability test at 4°C for all margarine

At room temperature the results showed that the oil separations increased after day 4 until day 14 for all margarines (Figure 5). 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. Margarine containing encapsulated red cabbage was more stable than margarine containing non-encapsulated red cabbage 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.

Previous research done by Tan *et al*. (2014) [17] focused on the effects of the particle concentration oil-to-water ratio and salt concentration on emulsion type on the stability of the emulsions.

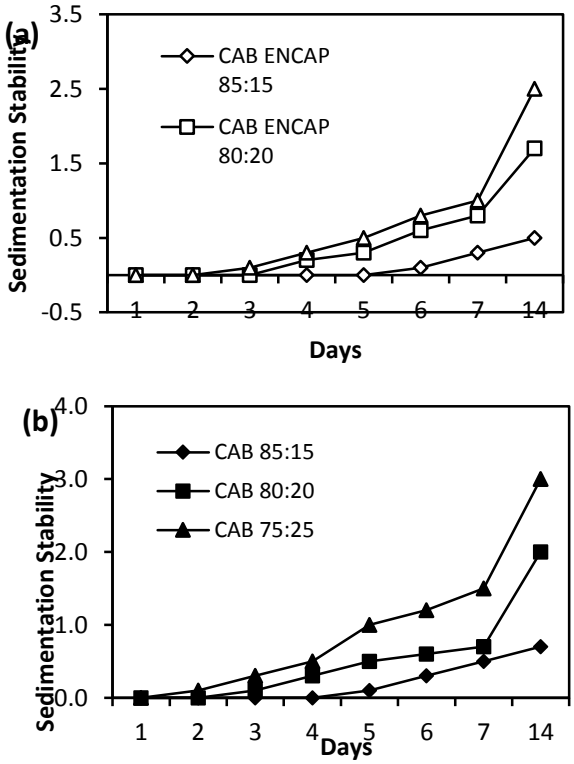


Figure 5 (a) Stability test at room temperature for margarine containing encapsulated red cabbage (b) Stability test at room temperature for margarine containing non-encapsulated red cabbage

It has been highlighted that the coalescence or deformation of droplets which leads to phase separation increases as the fraction of aqueous phase increases [17]. The inadequate addition of aqueous phase on water-in-oil emulsion may affect the stabilization of the emulsion.

4.0 CONCLUSION

For extraction of anthocyanins by using 100% distilled water extraction method, red cabbage had high total anthocyanins content (TAC). Encapsulated anthocyanins from red cabbage 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 best margarine contained encapsulated anthocyanins red cabbage at oil-to-aqueous ratio 85:15 and oil-to-aqueous ratio 80:20 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 encapsulated anthocyanins were more stable in 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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