CHEMICAL ENGINEERING TRANSACTIONS
VOL. 56, 2017
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Jiří Jaromír Klemeš, Peng Yen Liew, Wai Shin Ho, Jeng Shiun Lim

ISBN 978-88-95608-47-1; **ISSN** 2283-9216
DOI: 10.3303/CET1756176

Physicochemical Properties and Storage Stability of Margarine Containing Anthocyanins from Roselle Calyces

Dayang Norulfairuz Abang Zaidel*[a], Nur Shakira Sahat[a], Yanti Maslina Mohd Jusoh[a], Ida Idayu Muhamad[b]

[a]Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia,
[b]IJN-UTM Cardiovascular Engineering Centre, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia.
dayang@cheme.utm.my

Addition of encapsulated anthocyanins in margarine formulation improves 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 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 (88:12, 86:14 and 84:16). The blend formulation was compared with commercial margarine and the stability of the margarine was determined by using chemical, physical and texture stability analysis and. Margarine containing non-encapsulated anthocyanin showed improved stability compared with encapsulated anthocyanin.

1. Introduction

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). Anthocyanin is one of the example colorant widely used as natural colorant in food industry. Anthocyanin from different sources gives different processing and storage stability such as storage temperature, light, pH, concentration, ascorbic acid, sugar and oxygen (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 (Lule and Xia, 2005), lower blood pressure and improve the digestive system in humans (Muhammad and Shakib, 1995).

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).

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).

Please cite this article as: Zaidel D.N.A., Sahat N.S., Jusoh Y.M.M., Muhamad I.I., 2017, Physicochemical properties and storage stability of margarine containing anthocyanins from roselle calyces, Chemical Engineering Transactions, 56, 1051-1056 DOI:10.3303/CET1756176

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

2. Materials and methods

2.1 Preparation of Roselle samples

The sample preparations for Roselle were performed according to Tsai et al. (2001) with modification. The fresh Roselle calyces (*Hibiscus sabdariffa L.*) which was obtained from Johor (FAMA Pontian) were dried at 50 °C for 36 h and stored at 25 °C. The dried Roselle calyces were shredded into small calyces by using food shredder and put in dry wide-mouthed bottle containers, covered and labeled.

2.2 Extraction of anthocyanins

The extraction of anthocyanins was performed according to Looi (2008) with modification. The shredded Roselle calyces were added in distilled water in different beaker. The mixtures were stirred and heated at 35 °C on hotplate for an hour until a red colour appeared. The extracted solution was poured through the coffee filter to remove the pieces of Roselle. The procedures were replicated three times with the same roselle. For third time replication, the samples were soaked and stored in refrigerator for 24 h until all anthocyanins were almost fully extracted or the fibre become colourless through observation. 25 mL of the extracted solutions were collected in universal bottle for concentration determination, respectively while the least was collected in a beaker.

2.3 Encapsulation of anthocyanins using microwave-assisted technique

The encapsulation of anthocyanins was performed according to Abbasi and Rahimi (2006) with modification. Anthocyanins (roselle extract) as core material were mixed well with Maltodextrin at ratio 1:10 (v/v) inside a round glass plate. Mixtures were place in a domestic microwave oven (1,200 W) until the wall materials started to melt or burnt.

2.4 Blend Preparation

The oil blend was performed according to Ming et al. (1999) with modification. Liquefied palm stearin (PS), palm oil (PO) and palm kernel oil (PKO) were mixed in proportions of 70:10:20 respectively, identified by the mass ratio of the blend.

2.5 Margarine Formulation

The formulation of margarine was performed as described by Kim et al. (2006) with modification. The ingredients (w/w %) were lipid phase, 84 % (oil blend, 79.5 % and soy lecithin fluid, 0.5 %) and aqueous phase, 16 % (distilled water, 9 %; anthocyanins, 9 % and table salt, 2 %). The lipids and water were heated to 60 °C separately and combined into a table top homogenizer. The mixture was emulsified by vigorously mixing for 60 min. Commercial synthetic butter flavour was added to cover any distinction in the aroma of the samples. The mixture was crystallized using a water bath for 15 min. The solid emulsion then was refrigerated overnight at 20 ºC. The overall steps are repeated for other two lipid-to-aqueous ratios: 86:14 and 88:12, different type of encapsulated material according to the formulation table in Table 1. The margarines were placed into plastic tubs and stored at two different temperatures 25 °C and room temperatures.

Table 1: Formulation Table of Margarine

Sample	Factor 1 Oil-to-aqueous Ratio	Factor 2 Type of Encapsulated Material
1	84/16	Non-encapsulated
2	84/16	Maltodextrin
3	86/14	Non-encapsulated
4	86/14	Maltodextrin
5	88/12	Non-encapsulated
6	88/12	Maltodextrin

2.6 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.

2.7 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 where time is recorded.

2.8 Determination of Fatty Acid (FA) Profile by Gas Chromatograph (GC)

FAs composition of PO/PS binary fat blends were determined by following the same procedure reported by Saadi et al. (2011). 1 µL was taken carefully from the top layer and injected manually at 20 °C in the splitless mode. Then, the sample prepared was chromatographed to determine the FAs composition. By using the retention time of their TAG standards used (St. Louis, MO, USA), the FAs composition were identified and calculated.

2.9 Analysis of Solid Fat Content (SFC) of margarine

Nine tubes were used for each sample. Each sample was tempered at 70 °C for 30 min, followed by chilling at 0 °C for 90 min and then kept at the desired temperatures for 30 min 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).

2.10 Determination of melting point

The capillary tubes were placed in cold water then heated (0.5°C/min) until the level of the fat matter rises in the capillary tube. The melting temperature was noted (ISO International Standard, 2002b).

3. Results and discussion

3.1 Extraction of anthocyanins

After three replications of extraction by using 100 % distilled water method, it was expected that all anthocyanins were fully extracted from roselle. This was shown from the red solution obtained and after three replication of extraction the roselle obtained was becoming colourless fibre. Total concentration of anthocyanin obtained 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. The physical properties of extracted anthocyanins from Roselle was shown in Table 2.

Table 2: Physical properties for extracted anthocyanins from Roselle.

Total soluble solid (°Brix)	10.8±1.0
Total anthocyanin content (mg/L)	135.33±2.0
Colour parameters	
L*	41.4±1.0
*a**	+0.3±2.0
*b**	+8.6±2.0
C*	8.6±1.0
H°	88.1±1.0

3.2 Encapsulation of anthocyanins

Table 3 shows the physical properties for encapsulated anthocyanins from roselle using maltodextrin as the wall material. The encapsulated anthocyanins from roselle had low moisture content and were soluble fast in water.

Table 3: Physical properties for encapsulated anthocyanins from roselle using maltodextrin as wall material.

Moisture Content (%)	7.2409±0.0001
Dissolution test (s)	5±1
Color parameters	
L*	51.0±1.0
*a**	+4.4±2.0
*b**	+6.9±2.0
C*	8.2±1.0
H°	56.6±1

The moisture content reported was low, low moisture content is good for the stability of encapsulated anthocyanins. The encapsulated anthocyanins were soluble in 5s which is suitable for application in margarine formulation process. Hooe (2009) in their work, has reported that maltodextrin showed the greatest protecting effect for anthocyanins stability because the percentage of anthocyanins degradation is lower compared to anthocyanins encapsulated with Gum Arabic. In solution state, degradation of anthocyanins encapsulated by maltodextrin is lower rather than anthocyanins encapsulated by Gum Arabic and non-encapsulated anthocyanins.

3.3 Fatty acid content analysis

From Figure 2, the percentage of fatty acid of C12%, R12% and RE12% are almost similar. These shows that addition of anthocyanins did not affect the composition of fatty acid of margarine formulated. However, the lauric acid(C12) and myristic acid (C14) composition lower than in Planta. However, palmitic acid (C16) and oleic acid (C18:1) composition are slightly higher than in Planta. The linoleic acid (C18:2) composition are almost similar with Planta. The results were almost similar for solid/liquid ratio of 14% and 16%. These emphasizes that the fatty acid composition did not effected by the addition of anthocyanins.

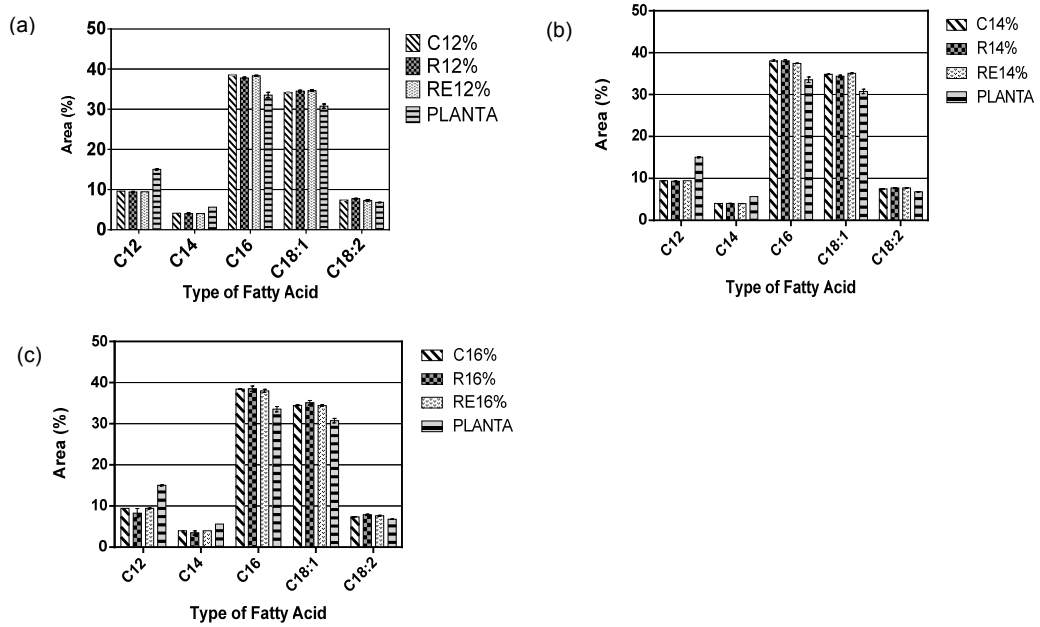

Figure 2: Fatty Acid Content (%) margarine formulated for (a) solid/liquid ratio 12 % (b) solid/liquid ratio 14 % (c) solid/liquid ratio 16 %

3.4 Solid fat content of margarine containing anthocyanins

The SFC rates of the samples varied with the temperature. Indeed, it decreases with decreasing temperature. The values obtained for the margarine are close whatever the temperature. This proves that the margarines had the same composition and therefore their preparation was made according to the good manufacturing practices. At 20 °C, the SFC were more than 10 %, at 30 °C, they were above 5 %. At 40 °C, the values were very low (below 5 %). These results were in agreement with those obtained by Karleskind (1992). At 37 °C SFC must be less than 6 %. In the present study, margarine had already a SFC lower than 6% at 30°C which indicates that these margarines melts easily in the mouth.

At 20 °C, all margarines have more than 10 % SFC and shows more than 20 % SFC at 25 °C. At 30 °C, the SFC percentages is recorded at very low percentage which is lower than 5 % as shown in Figure 3. These results is coherent with the agreement on producing a good spreadibility and texture of margarine formulation.

3.5 Slip melting point analysis

The melting points give an indication of the temperature at which margarine should be smooth in the mouth. Figure 4 shows changes in SMP of different margarines formulated throughout 12-week storage period. All the margarines are due to the polymorphic transformation from the β' to the β form. Food systems with β- form crystals are usually firmer with higher SMP due to the stronger crystal network required to link the large-

needle crystal (Zhang et al., 2009). The melting point international standard range of margarine are between 28 °C and 34 °C which implies that margarine can melt rapidly in the mouth and be firm at room temperature to resist to mechanical work during its spreadability. In Figure 4, the melting points of the margarine formulated were not significantly different and fluctuates throughout the storage time. The melting points of the margarine formulated were above than 28 °C. R12% and R14% show a stable SMP value within 12 weeks storage time. However, RE14% and RE16% shows a significance fluctuations during the storage time.

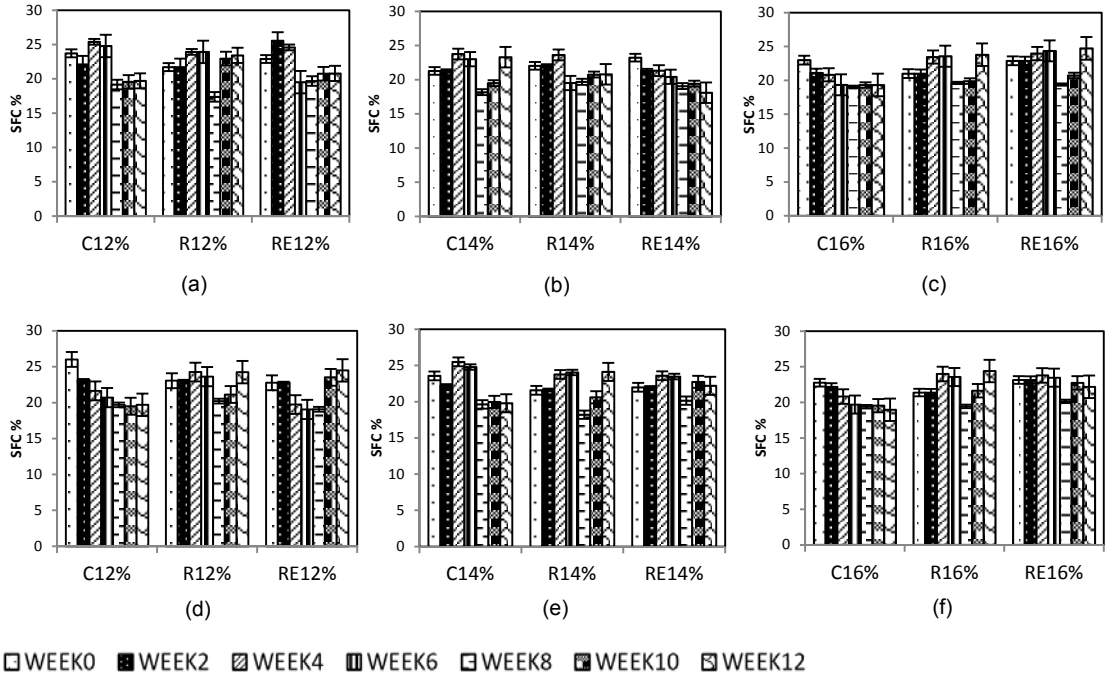

Figure 3: Solid fat content (%) margarine with 25°C storage temperature at 20°C (a) solid/liquid ratio 12% (b) solid/liquid ratio 14% (c) solid/liquid ratio 16; Solid fat content (%) of margarine with 30°C storage temperature at 20°C (d)solid/liquid ratio 12% (e) solid/liquid ratio 14% (f) solid/liquid ratio 16%

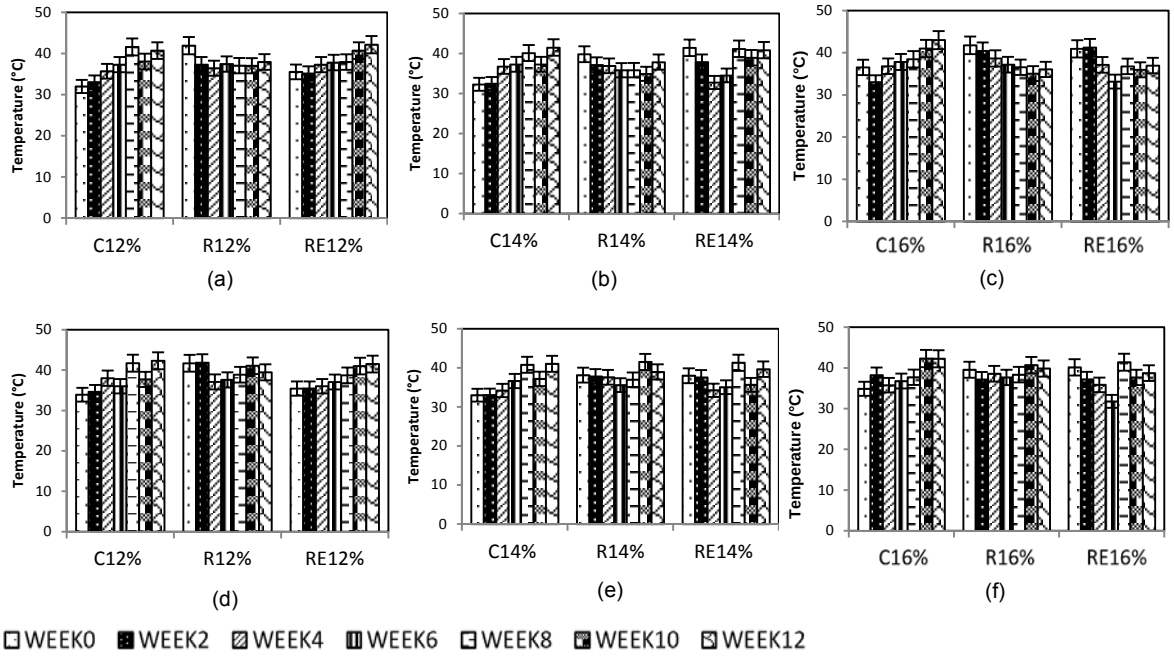

Figure 4: Slip Melting Points of margarine formulated with storage temperature at 25 °C (a) solid/liquid ratio 12 % (b) solid/liquid ratio 14 % (c) solid/liquid ratio 16 %; Slip Melting Points of margarine formulated with storage temperature at 30 °C (d) solid/liquid ratio 12 % (e) solid/liquid ratio 14% (f) solid/liquid ratio 16 %

4. Conclusions

Total concentration of anthocyanin obtained 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 100mL 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. The moisture content reported was low which is good for stability of encapsulated anthocyanins. At 20 °C, all margarine have more than 10% solid fat content and shows more than 20 % SFC at 25 °C. At 30 °C the SFC percentages recorded at very low percent which is lower than 5 °C which is follow the agreement on producing a good spreadibility and texture of margarine formulation. The melting points of the margarine formulated were above than 28 °C. R12% and R14% show a stable SMP value within 12 weeks' storage time. So the best formulated margarine is R12% (solid/liquid ratio 88:12) which has a suitable SFC an SMP value compared to commercial margarine.

Acknowledgments

The authors would like to acknowledge Universiti Teknologi Malaysia and Ministry of Higher Education for the financial support under Research University Grant Scheme (Q.J130000.2544.06H39) and Fundamental Research Grant Scheme (R.J130000.7844.4F447).

References

Abbasi S., Rahimi S., 2008, Microwave-assisted Encapsulation of Citric Acid using Hydrocolloids, International Journal of Food Science and Technology 43, 1226-1232.

Duke J.A., Ducellier M.J.B.J., 1993, Handbook of Medicinal Spices, CRC, Florida, USA, 53-55.

Haghi A.K. Amanifard N., 2008, Analysis of Heat and Mass Transfer during Microwave Drying of Food Products, Brazilian Journal of Chemical Engineering 25, 491-501.

Hui K.H., 2009, Microwave-Assisted Encapsulation of Anthocyanins from Roselle (Hibicus Sabdarifa L.) and its Thermal Stability Analysis, MSc Thesis, Universiti Teknologi Malaysia, Johor, Malaysia.

Looi M.L., 2008, Nano Encapsulation of Anthocyanins from Brassica Oleracea, MSc Thesis, Universiti Teknologi Malaysia, Johor, Malaysia.

Lule S.U., Xia W., 2005, Food Phenolics, Pros and Cons: A Review, Food Review International 21, 367-388.

Rein M., 2005. Copigmentation Reactions and Color Stability of Berry Anthocyanins, PhD Thesis, University of Helsinki, Helsinki, Finland.

Saadi S., Ariffin A.A., Ghazali H.M., Abdulkarim M.S., Huey C.B., Miskandar, M.S., 2012, Crystallisation Regime of w/o Emulsion [e.g. Multipurpose Margarine] Models During Storage, Food Chemistry 133, 1485-1493.

Stintzing F.C., Carle R., 2004, Functional Properties of Anthocyanins and Betalains in Plants, Food, and in Human Nutrition, Trends in Food Science and Technology 15, 19-38.

Tsai P., McIntosh J., Pearce P., Camden B., Jordan B.R., 2002, Anthocyanins and Antioxidant Capacity in Roselle (Hibiscus Sabdariffa L.) Extract, Food International Research 35, 351-356.