FORMULATION AND QUALITY EVALUATION OF MARGARINE ENRICHED WITH ANTHOCYANINS EXTRACT FROM ROSELLE

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Specially dedicated to my beloved parents, Sahat bin Daud and S. Rosidah binti Jumat, my siblings and friends for their continuous support, prayers, encouragement and also understanding during my master programme.
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**ABSTRAK**

Penghasilan produk menggunakan mikronutrien yang baik dan pewarna semulajadi berbanding pewarna tiruan telah menjadi tarikan baharu dalam industri makanan dan minuman. Objektif kajian ini dijalankan adalah untuk menghasilkan formula marjerin yang mengandungi bahan bioaktif (antosianin) daripada bunga roselle (*Hibiscus sabdariffa L.*) dan mengkaji ciri-ciri kimia, tekstur dan kestabilan marjerin yang dihasilkan. Marjerin telah diformulasikan dengan tiga nisbah pepejal/cecair yang berbeza (88:12, 86:14 dan 84:16). Antosianin yang dimasukkan ke dalam marjerin adalah dalam bentuk kapsul (serbuk) dan tidak berkapsul (cecair). Antosianin dikapsulkan bersama maltodekstrin dengan kaedah gelombang mikro. Antosianin diekstrak menggunakan kaedah pengekstrakan air suling. Jumlah kepekatan antosianin yang diperoleh adalah 135.33±2.0 mg/L. Antosianin berkapsul daripada roselle mengandungi kelembapan yang rendah dan cepat larut di dalam air. Pada suhu 20 °C, semua marjerin menunjukkan peratus lemak pejal (SFC) melebihi 10% dan melebihi 20% pada suhu 25 °C. Pada suhu 30 °C, peratus SFC didapati lebih rendah daripada 5%. Semua marjerin yang diformulasikan mempunyai takat lebur melebihi 28 °C. R12% (adunan lemak 88%: roselle tidak berkapsul 12%) dan R14% (adunan lemak 86%: roselle tidak berkapsul 14%) menunjukkan titik lebur yang stabil sepanjang 12 minggu masa penyimpanan. R12% dan R16 % (adunan lemak 84%: roselle tidak berkapsul 16%) menunjukkan nilai tekstur yang stabil sepanjang 12 minggu penyimpanan pada kedua-dua suhu penyimpanan. Ini menegaskan bahawa R12% dan R16% mempunyai proses pasca pengerasan yang lambat dengan penambahan antosianin. Daripada semua analisis yang dijalankan, marjerin yang terbaik dipilih adalah marjerin tidak berkapsul dengan nisbah pepejal/cecair 88:12 (R12%) manakala marjerin yang diformulasi bersama antosianin berkapsul (RE12%, RE14% dan RE16%) menunjukkan kestabilan yang lebih rendah.
ABSTRACT

There have been increased attractions in the development of food that contains good micronutrients and natural colourants rather than synthetic colourants. The objectives of this study were to formulate a margarine that contains bioactive compound (anthocyanin) from roselle (*Hibiscus sabdariffa* L.) and to study the chemical, texture and stability characteristics of the margarine. The margarine was formulated with three different solid/liquid ratios (88:12, 86:14 and 84:16). The anthocyanins added in the margarine were in the form of encapsulated (powder) and non-encapsulated (liquid). The anthocyanins were encapsulated with maltodextrin by using microwave. Extraction of anthocyanins was performed using distilled water extraction method. Total concentration of anthocyanin obtained for roselle was 135.33±2.0 mg/L. Encapsulated anthocyanins from roselle had low moisture content and soluble fast in water. At 20°C, all margarine had more than 10% solid fat content (SFC) percentages and showed more than 20% SFC at 25°C. At 30°C the SFC percentages was found to be lower than 5%. The melting points of the margarine formulated were above than 28°C. R12% (fat blend 88%: roselle non-encapsulated 12%) and R14% (fat blend 86%: roselle non-encapsulated 14%) showed a stable slip melting point value within 12 weeks storage time. R12% and R16% (fat blend 84%: roselle non-encapsulated 16%) showed a stable texture values throughout the 12 weeks storage period at both storage temperature. It emphasized that R12% and R16% had a slow post-hardening process with the addition of anthocyanins. From all the analysis done, the best margarine chosen was non-encapsulated margarine with solid/liquid ratio 88:12 (R12%) whereas the margarine formulated with encapsulated anthocyanins (RE12%, RE14% and RE18%) showed a slightly lower stability.
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LIST OF SYMBOLS

\[ \beta \quad - \quad \text{beta} \]
\[ \beta' \quad - \quad \text{Beta prime} \]
\[ \alpha \quad - \quad \text{alpha} \]
CHAPTER 1

INTRODUCTION

1.1 Research Background

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 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.

The appreciation of high and good quality food is a part of life’s pleasures. Flavor identification and taste thresholds, influencing food preferences, food acceptability and ultimate food choice can be modulated by colour (Briddle and Timberblake, 1996). Thus, colour acts an ultimate role in enjoyment and gratification of food.
There have been increased attractions in the development of food colourants 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 colourants or synthetic antioxidants have been reported to provide an implication on cardiovascular disease (Nalsen et al., 2006). Anthocyanin is one of the example colourant 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 (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 anticarcinogenic activity, aphrodisiac properties (Duke and Ducellier, 1993) have been investigated, and shown that anthocyanins can provide some beneficial effects in the treatment of disease such as neuronal and cardiovascular illnesses, cancer and diabetes (Tsai et al., 2002; Lule and Xia, 2005; Nichenametla et al., 2006), lower blood pressure and improve the digestive system in humans (Muhammad and Shakib, 1995). 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. Several authors have confirmed that Hibiscus Sabdariffa L.is a good source of dietary antioxidants, with its calyces containing amounts of anthocyanins as high as 2.5g⁻¹ DW (Aurelio et al., 2008; Juliani et al., 2009). So, addition of roselle in margarine formulated is the research serves as added value in the food properties.
1.2 Problem Statement

Margarine sometimes may not stable at room temperature. There are many factors that may affect the stability of margarine such as the liquid to oil phase ratio, addition of ingredients in formulation or method of manufacturing. Addition of anthocyanin will provide a healthy product and attractive preference in term of additional colour to the margarine. However, anthocyanins as natural food colorants are not stable in food products (Duangmal et al., 2004). The stability of anthocyanins is affected by several factors such as pH, 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). The formulation of margarine containing anthocyanin is challenging as the addition of anthocyanin may alter the stability of the margarine because it may affect the particle size of emulsion. The stability and storage characteristics of margarine formulated can be achieved by a good formulation of margarine. The margarine that will be formulated is a healthy margarine because the oil blend used is natural vegetable oil. In a nutshell, this product has a high potential to prevent cardiovascular, act as anti-aging and can provide goodness for eye, skin, hair and nails. Currently, there are no products in market similar to margarine formulated with anthocyanin specifically with roselle. The product will provide more choices of healthy food with lower cost to the consumer.
1.3 Objective of the Study

The objectives of this study are
1. To formulate a stable margarine containing anthocyanins from Roselle (*Hibiscus sabdariffa* L.).
2. To study the texture, storage and stability characteristics of the margarine produced.

1.4 Scopes of the Study

The scopes of this study encompass:
1. Extraction of anthocyanins from Roselle calyces using hot water extraction method.
2. Encapsulation of anthocyanins from Roselle using microwave-assisted technique.
3. Formulation of margarine containing non-encapsulated anthocyanins and encapsulated anthocyanins from Roselle calyces with three different oil-to-aqueous ratio starting at 16% with increments of 2%.
4. Investigation of texture, storage and stability characteristics of the margarine containing anthocyanins by using its chemical and physical composition.
1.5 Significance of Study

This study investigates the formulation of stable margarine containing anthocyanin from Roselle (*Hibiscus sabdariffa* L.). As the addition of anthocyanin may alter the stability of margarine, the optimum amounts of anthocyanin that can be formulated with the margarine are determined. It is believed that the commercial value of the margarine will keep on increasing due to the content of anthocyanin as natural colourants as well as its antioxidant activity.
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