INVESTIGATION ON NATURAL BLUE COLORANT OF *CLITORIA TERNATEA l. FABACEAE* (BUNGA TELANG): THE EFFECTS OF STABILIZERS TO EXTRACTS

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Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
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JANUARY 2015

ABSTRACT

Clitoria ternatea Linn Fabaceae which is also known as *bunga telang* in Malaysia, contains many useful functions leading to various activities such as blue food colorant, a cure for cancer and used for treatment from poisonous bite. Demand on the natural blue colorant is increasing due to the health issues from the usage of blue synthetics colorant. The study deals with the identification of phenolic compound in the extract which is anthocyanin by using High-performance Liquid Chromatography (HPLC) at wavelength of 520 nm and cyanidin-3-glucoside as a standard. The color stability of Clitoria ternatea flower is easy to be affected by several factors such as pH and temperature. For that reason, the color stability investigation was performed by combining the extract with two types of stabilizer which are Maltodextrin DE10 **(MD)** and Trehalose **(T)** with ratio 3:10 (w/v). The degradation of anthocyanin content at different temperature was analysed by applying heat treatment on the samples using water bath shaker at 40°C, 60°C, and 80°C for 30 and 45 minutes. From HPLC results, anthocyanin was found at minutes of 2.01 according to retention time order. The total anthocyanin content (TAC) were analysed by using UV-vis spectrophotometer at two wavelengths which 510 nm and 700 nm in two different pH of buffer solution which are pH 1 and pH 4.5. The results showed that the value of TAC were same for extraction at 30 minutes time for all temperatures which 35.54 mg/L but the TAC of 60°C were dramatically decrease to 19.02 mg/L due to the sensitivity anthocyanin towards temperature above 50°C. In stabilizer addition analysis, the study was divided into four samples which are pure **(1)**, **MD (2)**, **T (3)** and, **MD** mixed with **T** (60:40) **(4)**. The samples were tested for storage temperatures of 25°C and 4°C for four weeks. Initially, the TAC (mg/L) for both temperature were same which 80.08 **(1)**, 75.41 **(2)**, 46.88**(3)**, and 106.28 **(4)**. All of the samples for both of temperatures were showed degradation on week of 4. Results showed the TAC was degraded after 30 minutes on the heat treatment analysis. From all these findings, it is concluded that anthocyanin is an unstable compound which can easily be affected by temperature but by adding the stabilizers, there is a potential for blue food colorant application from *Clitoria ternatea* flowers to replace the current usage of blue synthetic colorant.

ABSTRAK

Clitoria Ternatea Linn Fabaceae yang dikenali sebagai bunga telang di Malaysia, mengandungi banyak fungsi yang berguna seperti pewarna makanan biru, penawar kanser dan digunakan sebagai penawar untuk gigitan beracun. Permintaan pewarna biru asli semakin meningkat disebabkan oleh peningkatan isu-isu kesihatan daripada penggunaan pewarna biru sintetik. Kajian ini berkait dalam mengenalpasti antosianin dengan menggunakan HPLC dan cyanidin-3-glucoside sebagai standard. Kestabilan warna bunga telang mudah dipengaruhi oleh faktor seperti pH dan suhu. Oleh itu, kajian terhadap kestabilan warna dilakukan dengan menggabungkan ekstrak dengan dua jenis penstabil iaitu Maltodextrin DE10 (**MD**) dan Trehalosa (**T**) dengan nisbah 03:10 (w / v). Degradasi kandungan antosianin pada suhu yang berbeza dianalisis dengan menggunakan pemanasan haba (40°C, 60°C, dan 80°C) selama 30 dan 45 minit. Dari keputusan HPLC, antosianin dikesan pada 2.01 minit. Jumlah kandungan antosianin (TAC) telah dianalisis dengan menggunakan UV-vis spektrofotometer pada dua jarak gelombang iaitu 510 nm dan 700 nm dan dalam dua pH yang berbeza larutan iaitu pH 1 dan pH 4.5. Hasil kajian menunjukkan bahawa nilai TAC adalah sama untuk pengekstrakan pada masa 30 minit untuk semua suhu yang 35,54 mg / L tetapi TAC pada suhu 60°C telah menurun secara mendadak kepada 19.02 mg / L disebabkan oleh sensitiviti antosianin terhadap suhu melebihi 50 °C . Kajian ini telah dibahagikan kepada empat sampel iaitu ekstrak asli (**1**), ekstrak dicampur dengan **MD** (**2**), ekstrak dicampur dengan **T** (**3**) dan, ekstrak dicampur dengan **MD** dan **T** (60:40) (**4**). Nilai TAC untuk kesemua sampel yang diuji pada suhu penyimpanan 25°C dan 4°C adalah 80.08 (1), 75.41 (2), 46.88 (3), dan 106.28 (4). Semua sampel untuk kedua-dua suhu telah menunjukkan penurunan pada minggu ke-4. Bagi analisis pemanasan haba, keputusan menunjukkan TAC telah menurun selepas 30 minit. Dari semua penemuan ini, didapati bahawa antosianin adalah tidak stabil pada suhu melebihi 50°C dan pewarna biru asli dengan penambahan bahan penstabil mampu untuk mengurangkan kadar penurunan antosianin dan sekaligus mempunyai potensi untuk menggantikan pewarna biru sintetik.

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LIST OF ABBREVIATIONS

DAD	Diode Array Detector
DB	Double Blue
DE	Dextrose Equivalent
FD&C	Foods, Drug and Cosmetics
HPLC	High-performance Liquid Chromatography
LC	Liquid Chromatography
MS	Mass Spectrometry
MT	Metric Tonnes
NMR	Nuclear Magnetic Resonance
TLC	Thin-layer Chromatography
UV	Ultraviolet

1 INTRODUCTION

1.1 Background of study

Color plays an important role in food industries which make the food look more attractive, desirable and healthy in the eyes of consumers. Food coloring is a type of food additives which the most popular used in current industries to produce the fresh food's appearance because the attractive food's appearance can influence the perceptions of consumers towards the flavor of food (Lakshmi G., 2014). There are two types of food colorant which are synthetic food colorant and natural food colorant, which can be in the forms of liquids, powders, pastes and gels (Saleem et *al.*, 2013; Khodjaeva et *al.*, 2013).

Figure 1: Liquid food dyes

Synthetic food colorant were produced chemically and categorized as a complex organic chemical that contain toxic and can give bad effects to the health of the consumers and environmental (Magoulas, 2009). However, the uses of synthetic food colorant or also known as artificial food colorant was still preferable in many companies because it have brighter and greater stability of color compared to natural food colorant and has lower production cost (Kobylewski & Jacobson, 2010). Coca-cola and Pepsi-cola are the example of companies that used synthetic food colorant in their product. They were suspected using caramel coloring in their soft drink products which

contaminated with two cancer-causing chemicals such as 2-methylimidazole and 4-methylimidazole that tend to cause lung, liver and thyroid cancer and also leukaemia to the consumers (F. Jacobson, 2011)

Natural food colorant is coloring that can be obtained from natural sources such as plants, vegetables, animals and minerals. It can be classified into three types which are chlorophyll, carotenoids and anthocyanin. Chlorophyll can be easily obtained by extracted it from plants and vegetables. It also can be produced by photosynthesis in leaves and known as green pigments (Sabir, 2006). In addition, carotenoids also can be produced by the same way of chlorophyll production but the sources of carotenoids are fruits and it was known as yellow-orange-red pigments. Anthocyanin was classified as the red-purple pigments which can be distributed from many fruits and flowers. It can be easily affected by pH, light and also temperature which can fade the color and quality of color (Mortensen, 2006). Most of the companies chose to use synthetic colorant on their product to cut cost of production and also to produce products with attracted appearance.

1.2 Motivation

The motivation to carry out this research is to find a new alternative to the current usage of synthetic blue colorant which is to produce the natural blue food colorant.

In the current commercial market, Food, Drugs and Cosmetic (FD&C) Blue No.1 (Blue 1) and FD&C Blue No.2 (Blue 2) are the synthetic blue colorant used in the foods and cosmetics production in order to maintain the color and produce the attractive appearance of products. Both of them were derived from the petroleum which contains toxic and tend to give diseases to consumers. Many studies on the FD&C Blue No.1 and FD&C Blue No.2 were performed in order to determine the negative effects produced from this synthetic colorant. The most thorough studies of Blue 1 or also known as Brilliant Blue, proved that this color was a carcinogen colorant since the male mice used as the samples in the study shown that the male mice had kidney tumors (Rowland *et. at.,* 2009). Besides that, it also has the potential in neurotoxicity, which tends to give bad effects to the fetus and babies under the age of six months (Lau *et al.*, 2006). FD&C Blue No.2 or also known as Indigo Carmine in widely used in the production of foods was suggested not to use it in the foods because it considered not safe for human consumption (Kobylewski & Jacobson, 2010).

Therefore, this study is carried out to find a suitable alternative to replace the FD&C Blue No.1 and FD&C Blue No. 2 with the natural food colorant for blue color. This study focuses on the extraction of Clitoria Ternatea *l.* Fabaceae (CT) or also known as Butterfly Pea. It is a type of a perennial climb herb which can be classified in four colors which are dark blue, light blue, mauve and white (Hung *et al.*, 2001). Ternatin was the anthocyanin found in the petals of Clitoria Ternatea and it was utilized as food colorant since it has the higher stability (Mukherjee et *al.*, 2008).

1.3 Problem Statement

Nowadays, the food manufacturers give more interest in the production of natural food coloring because of pressure from the market demand. They need to find a new alternative to replace the synthetic food coloring with the natural food coloring. Among the sources of the natural food coloring, there is growing interest from the manufacturers toward the *Clitoria ternatea L.* flowers which also known as the Blue Pea flower to be studied as a natural blue colorant since the synthetic blue colorant used in the current industries have the bad effects towards the consumer's health.

The demand of customers towards the natural food colorant is higher than the synthetic food colorant. It was expected to increase from 8000 MT by the year 2000 to 15000 MT by the year 2015 (Lakshmi G., 2014). The increasing of the report on the health hazards and toxicity of using synthetic colorant was the main cause for the increasing of demand toward the natural food colorant (Santos *et al.*, 2011). The natural food coloring is safe to use in the food production because it gives no allergic reaction on skin plus it was also known as non-carcinogenic colorant in nature (Sinha et *al.*, 2012).

Due to the increasing of demands, the manufacturers need to produce the food dyes in the shortest periods in order to fulfil the customer's requests. There still a problem in producing the natural colorant since the phenolic compounds in the natural colorant were very sensitive towards pH, light and also temperature which when it exposed to light, the color tend to be fade. The study on color stability was carried out in this research work in order to solve the problem faced by the manufacturers.

1.4 Objectives

The objectives of this study are:

- To identify the anthocyanin compound in the extract of *Clitoria ternatea L.* Petals by using High-performance Liquid Chromatography.
- To study the total of anthocyanin content of the extract of *Clitoria ternatea L.* petals at different temperature and time of extraction.
- To encapsulate the anthocyanin compound by using stabilizers and study the color stability of the extract of *Clitoria ternatea l.* petals on the storage days, temperature and during heat treatment.

1.5 Scopes of study

Several scopes have been identified in order to achieve the objective of this study, which are:

i. **Identification of the total anthocyanin content in extract.**
The extract from *Clitoria ternatea L.* petals was analyzed using High-performance Liquid Chromatography (HPLC) at 520 nm of wavelength. Cyanidin-3-glucoside was used as a standard calibration. This analysis was run in order to determine the anthocyanin content in the extract which also known as blue pigments in the colorant.

ii. **Extraction at different temperatures and time.**
Extraction will be performed at 25°C and 60°C for 10, 30 and 60 minutes. The total anthocyanin content was measured by using UV-vis spectrophotometer at two wavelengths which are 510nm and 700nm.

iii. **Encapsulation of extract using stabilizers**
Maltodextrin DE10 and Trehalose will be used as a stabilizer to maintain the quality of the colorants when being placing in any types of conditions. It will reduce the rate of oxidation reaction on extract with oxygen which can reduce the color of extract. Temperature and time will be the parameters that can give influences to the color stability. The color of stability will be studied at the storage time range of 30 days as well as at 4°C and 25°C. The same samples also were studied on the heat treatment at 40°C, 60°C and 80°C. The changes in color with respect to temperatures and time is called color degradation.

1.6 Main contribution of this study

Natural colorants will be widely used in the industries since the demand on this colorant is higher than the synthetic colorants (Lakshmi G., 2014). The studies on stability of natural colorant are still poorly understood. This study was contributed to the understanding of stability natural colorant. The natural blue colorant was contained anthocyanin which known as the unstable compound at higher temperature and long storage time. From this study, the anthocyanin was able to stand at higher temperature and storage time by encapsulated the microparticles of color with the stabilizers. The encapsulating agents used are Maltodextrin DE10 and Trehalose. By combining these two encapsulating agents with the pure extract resulted to the higher percentage of anthocyanin remaining compared to the pure extract combining with maltodextrin or trehalose.

1.7 Organization of this thesis

The structure of the remainder of the thesis is outlined as follow:

Chapter 2 provides a description of the previous studies and books related with this study in order to understand the related aspects such as characteristics and properties of the *Clitoria ternatea* flowers. In addition, *Clitoria ternatea* flowers which produced the natural blue colorant, so, anthocyanin as the blue pigments should be studied in order to know better about the compound. As the color stability has been one of the parameters in this study, stabilizer will be used in order to analyse the color stability. Then, the process of encapsulation and material used to stabilize the samples should be understood in other to proceed with the better ways.

Chapter 3 explains about the methodology applied in this study. The methods used were referred by previous studies. The methods involved are extraction of *Clitoria ternatea* flowers by using water bath shaker and identification of anthocyanin compound by using HPLC. Besides that, the absorbance values of samples were measured by using UV-vis spectrophotometer. The experiment for storage temperature, storage time and heat treatment were using refrigerator and water bath shaker.

Chapter 4 discuss results of this research by plotting the graphs. There are four main results discussed which are identification of anthocyanin by HPLC, the effect of

extraction temperatures and time on the total anthocyanin content, the effect of time and temperature storage on the total anthocyanin content and the effect of heat treatment on the total anthocyanin content. An explanations on the changes occurred in the analysis also was discussed by comparing to the previous studies.

Chapter 5 explains about the conclusion of all the findings involved in this research. Most of the conclusions have similarity with the previous studies related with this research.

2 LITERATURE REVIEW

2.1 Overview

Previous researches and books related with this study have been the references in order to understand the related aspects such as characteristics and properties of the *Clitoria ternatea* flowers. In addition, *Clitoria ternatea* flowers produced the natural blue colorant, so, anthocyanin as the blue pigments should be studied in order to know better about the compound. As the color stability has been one of the parameters in this study, stabilizer will be used to analyse the color stability. Then, the process of encapsulation and material used to stabilize the samples should be understood in other to proceed with the better ways.

2.2 Introduction

This section presents the facts from the previous studies and books about the related aspects for this study.

2.3 Clitoria ternatea Linn Fabaceae

Clitoria ternatea Linn Fabaceae which also commonly known as blue pea or butterfly pea is flowers that can produce blue color from the petals and contain anthocyanin (Mohamad et *al.,* 2011). This flower was belonging to the family *Fabaceae* and subfamily *Papilionaceae* which commonly grows in tropical areas (Gomez & Kalamani, 2003). *Clitoria ternatea* can be classified in four colors which are dark blue, light blue, mauve and white (Hung et *al.*, 2001). But according to Kazuma et *al.*,(2003), they claimed that *Clitoria ternatea* flowers contained five type of petal lines as shown in Figure 2, which are wild-type (a), mauve (b), white (c), Double Blue (d), and bud mutant (e).

Figure 2: Flowers of the Clitoria ternatea lines
(Source : Kazuma et al., 2003)

Clitoria ternatea was categorized as a perennial climber herb plant and can be classified in four colors which are dark blue, light blue, mauve and white (Hung, 2011; Zingare, 2013). It has a detached fine stem about 0.5-3 cm long. The leaves which are elliptic like lanceolate leaflets with 5cm long and 7cm wide. The seeds of *Clitoria ternatea's* flower was normally black or brown in color which about 4.5-7mm long and 3- 4 mm wide. *Clitoria ternatea* which also called as *blue pigeon* is easy to plant and requires only little care. The plant may start flowering 4 months after sowing and will continuous to bloom all year long (Varsha et *al.,* 2012).

In Malaysia, this flower was used widely as a dye to the rice which called as *Nasi Kerabu* and also used to make blue glutinuous rice cake called *Pulut Tai Tai.* Besides that, young pods and flowers of *Clitoria ternatea* were eaten as vegetables in Kerala, India and Philippines. While in Thailand, the blue extract from the flowers was used to make a drink called *nam dok anchan* (Hung et. *al.*, 2011).

In addition, the juice from the flowers was used in order to treat insect's bites and also skin diseases. In India, the stem, root and flower were used in treatment of snakebite and scorpion sting. Besides that, this plant is widely used in Ayurveda medicine; a system of traditional medicine native in India, which was reported as a brain tonic, nervine tonic and laxative which for boosting memory and improving intellect (Zingare et *al.,* 2013).

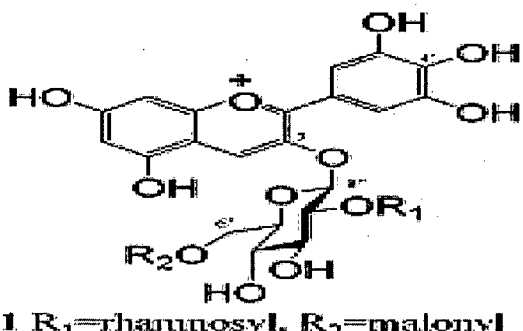
Clitoria ternatea can produce a dark blue color since it contain high amount of anthocyanin and the main of anthocyanin are delphinidin glycoside which attributes to

blue color. Besides that, another anthocyanin was found in the petals of *Clitoria ternatea* is ternatin which has the higher stability in color (Mukherjee et *al.*, 2008). In order to substitute a synthetic colorant was used in current industries, the extract from *Clitoria ternatea* can be a good source of natural colorant to use in food and cosmetics (Tantituvanont et *al.*, 2008).

2.4 Anthocyanin

Anthocyanin can be found in the roots, leaves, fruits and flowers of plants. Anthocyanin also known as a natural pigment which can be a new alternative to replace the synthetic food coloring with the natural food coloring (Khazaei *et. al.*, 2014). It can be analyzed by using classical and modern analysis equipments such as UV-vis spectrophotometer, High-performance Liquid Chromatography (HPLC), Liquid Chromatography (LC), Mass spectrometry (MS), Thin-layer Chromatography (TLC) and Nuclear Magnetic Resonance (NMR) (Oancea and Oprea, 2011).

Anthocyanin was classified as a natural soluble water colorant and non-toxic pigments which unstable in stability and easy to be affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, flavonoids, proteins, and metallic ions (Ovando et *al.*, 2009).



1 R_1=rhamnosyl, R_2=malonyl
2 R_1=H, R_2=malonyl
3 R_1=rhamnosyl, R_2=H
4 R_1=R_2=H

Figure 3: The structures of anthocyanin
(Source : Kazuma et al.,2003)

The color of extract will change according to the changes in pH value. The color changes occur because their chemical structures are changed when pH value changes which the lower the value of pH, the color will tend to turn toward red shaded (Suebkhampet and Sotthibandhu, 2012). The study carried by Vankar and Srivastava

(2010), claimed that the higher value of pH tend to fade the color dye as shown in Figure 4 which at 4.5 of pH value, the blue dye turn to be colorless.

Besides that, the temperature also can give the effect toward the colorant which the higher the temperatures, the faster the rate of degradation of color (Tantituvanont et *al.,* 2008) which degrades it by destroy the flavylium ion. By applying lower temperatures, the rate of degradation of color can be reduced. In additions, the dark condition of storage and oxygen-free packaging also can minimize the rate of color loss.

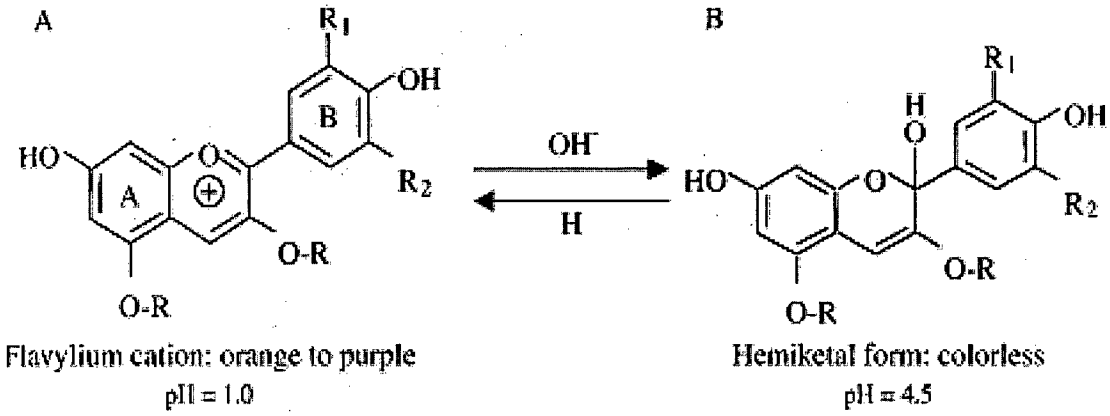


Figure 4: Structures of flavylium cation (A) and hemiketal form (B)
(Source: Vankar and Srivastava, 2010)

In the blue petal, a group of 15 (poly)acylated delphinidin glucoside can be identified and six major anthocyanin ternatin which also known as blue anthocyanin, were found in the petals of *Clitoria ternatea* which are A1, A2, B1, B2, D1 and D2 (Mukherjee et *al.,* 2008).

Anthocyanin is very useful in the health treatment because it contained a powerful antioxidant activity. Antioxidant activity was able to scavenge free radical which means it can reduce the probability of body to get several diseases that related to the reaction of radicals plus the risk of suffered a coronary heart disease and cancer can also be reduced (Zingare et *al.,* 2013).

2.5 Stabilizer

There are many type of stabilizer used in current industries such as *gelatin, Arabic gum, starches, methylcollulose, whey proteins, corn syrups, β-cyclodextrin* and *maltodextrin* which were used as a wall materials in encapsulation process.

Natural colorant has the lower stability and easy to fade when exposed to the light. Besides that, temperature, oxygen and humidity also can affect the color. In order to stabilize and maintain the color, the stabilizer is needed in this study. Sometimes, stabilizer is used to help in reducing the process of degradation of related aspects such as degradation of color. The influence of stabilizers is very vital because the stable colorant have the slowest rate of degradation which means not easily be affected when the light was absorbed (Nohr et *al.*, 1999).

2.6 Encapsulation

Encapsulation is defined as the coating by a polymer on the tiny solid particles, liquid droplets or gaseous compounds in order to control the specific conditions such as the degradation of colour, undesirable odours or tastes.

The process of encapsulation was ease to handle and can offer improve the stability. The products produced from this process will be known as capsules because it was produced in terms of small spheres with diameters about a few micrometers (Gibbs et *al.*, 1999).

The material inside the capsule is referred to as the core, internal phase or fill, whereas the wall is called shell, carrier, capsule, coating agent, wall material and membrane. Encapsulation can be optimized by choosing the optimal wall material, core the wall ratios, techniques and temperatures to give the most stable product (Wilson and Shah, 2007). Various techniques are employed to perform encapsulation, including spray drying.

2.6.1 Maltodextrin DE10

Maltodextrin is a colorant stabilizer which also known as an encapsulating agent. It is able to extend the life of the colorant's appearances. Normally, it was found as creamy-white powder which also known as the most effective carrier in stabilizing the pigments under all the storage conditions. It is a sugar obtained by hydrolysis of starches which a water soluble material and easy to digest or being absorbed as rapidly like glucose. It can help to avoid the powder product from sticking to the walls of spray dryers during the drying process (Langrish et *al.*, 2007).

As shown in Figure 5, maltodextrin consists of D-glucose units which linked with $\alpha(1\rightarrow4)$ glycosidic bonds, was connected in chains of variable length. It contained

dextrose equivalents (DE) of less than 20. The higher the DE value, the shorter the glucose chains, those the higher the sweetness and the solubility.

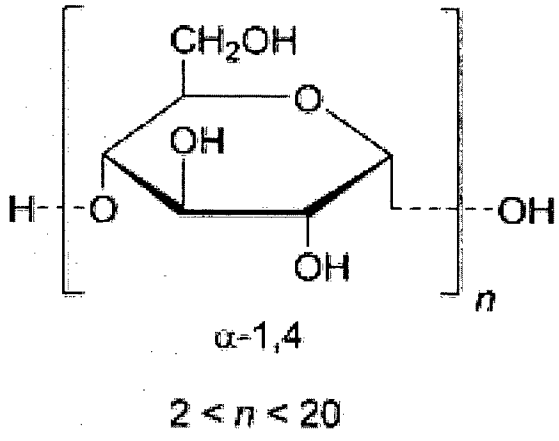


Figure 5: Structure of maltodextrin

2.6.2 Trehalose

Trehalose is categorized as a carbohydrate component of powdered complexes and known as a non-reducing sugar that is not easily hydrolyzed. The molecular formula and weight are $C_{12}H_{22}O_{11}$ and 342.31 respectively.

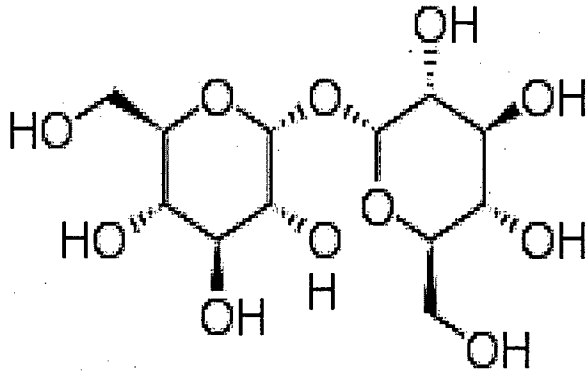


Figure 6: Structure of trehalose

It has high thermo stability and a wide pH stability range and because of nonreducing sugar, it does not show Maillard reaction (reaction toward heat) with amino compounds such as amino acids or proteins. Furthermore, it shows good sweetness like sucrose and in the food industry, it was used as sweetener and stabilizer.

2.7 *The summary of previous studies*

Table 1 Summary of previous studies

Findings	Author	Year	Review
Natural blue colorant	Hung *et. al.*	2011	Blue *Clitoria ternatea L.* flower has relatively high thermal stability and has the potential to be used as natural blue colorant for food, cosmetic and pharmaceutical products and also is a good replacement for synthetic blue colorant.
Demand of customers	Lakshmi	2014	The demand of customers towards the natural food colorant is higher than the synthetic food colorant. It was expected to increase from 8000 MT by the year 2000 to 15000 MT by the year 2015.
Method of study	Vankar and Srivastava	2010	Anthocyanin are normally obtained by extraction from methods currently employed are with methanol, ethanol, acetone, water or mixture as solvents.
Factor affected anthocyanin	Lakshmi	2014	The stability of anthocyanin is easily affected by environmental factors such as storage time, temperature and light.
Stabilizer	Handoyo	2010	*Maltodextrin* as stabilizer was successfully encapsulated and protected anthocyanin extracted from *Clitoria ternatea* flower throughout the heat treatment by spray drying process.

3 MATERIALS AND METHODS

3.1 Overview

This study focused on the extraction of the blue colorant from the *Critoria ternatea* petals. This methods was used the nanopure water as the dilution material since it was contained no hazard chemical. The sample used is the dried flower which has been grinded by using blender. The temperature set up for aqueous extraction was at 60 Celsius of water bath temperature. The analysis equipment used in this study was HPLC and UV-vis spectrophotometer. The extract then was divided into four types which are pure extract, combination of maltodextrin and pure extract, combination of trehalose and pure extract and combination of maltodextrin and trehalose into pure extract. The extract will be analyzed on the degradation of color within the storage day by measuring the value of absorbance of the extract. The color stability of samples also being tested by applying heat treatment on all of the samples.

3.2 Introduction

This section presents the methods used in this research. The blue colorant was extracted by using *Clitoria ternatea* petals. The extract will be analyzed on the degradation of colour within the storage day for 30 days with week as the interval in two temperatures storage which are 25°C and 4°C. The degradation of color stability will be analysed to identify the retention of total anthocyanin content by applied the heat treatment analysis on all of the samples.

3.3 Material

3.3.1 Clitoria ternatea flowers

The flowers were collected around area Kuantan, Pahang. The type of flowers used in this study was Double Blue petals (refer Figure 2 and Figure 7). This type of flower is easy to purchase in Malaysia because it grow widely since it was a wild types of flower. The flowers were dried and blended by using blender and were kept in room temperature which 20°C until processed.

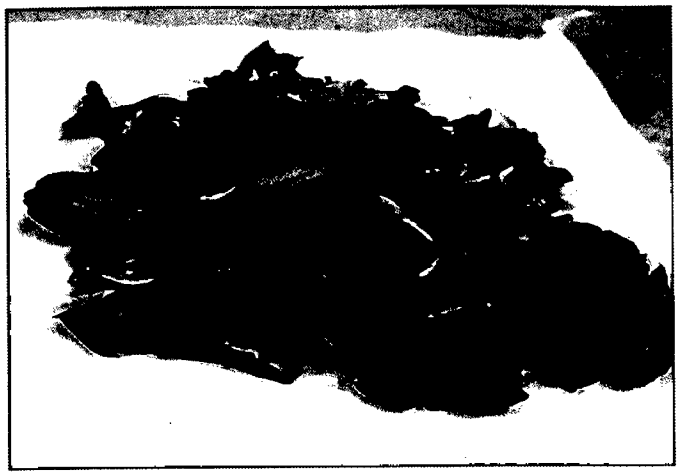


Figure 7: Clitoria ternatea flowers

Figure 8: Seeds of Clitoria ternatea

3.4 Chemicals

i. Nanopure water
ii. Acetonitrile
iii. Cyanidin-3-glucoside

3.4.1 **Buffer solution KCl 0.025M of pH 1.0**

i. Potassium chloride (KCl)
ii. Hydrochloric acid (HCl)

3.4.2 ***Potassium*** **Buffer solution CH_3COONa 0.4 M of pH 4.5**

i. Sodium acetate ($CH_3CO_2Na.3H_2O$)
ii. Hydrochloric acid (HCl)

3.5 Equipments

i. UV-vis Spectrophotometer
ii. High-performance Liquid Chromatography
iii. Vector
iv. Spray drying
v. Water bath
vi. Oven
vii. Refrigerator
viii. Blender

3.6 Apparatus

i. Filter funnel vacuum
ii. Digital mass balance
iii. Thermometer
iv. 5mL of syringes
v. 1000μL of pipette
vi. 0.45μm of nylon filters syringe
vii. Weighing boats
viii. Aluminium foil
ix. Centrifuge tubes
x. 250mL of Amber glass
xi. 100mL of Amber glass
xii. Spatula
xiii. Cuvettes
xiv. Filter papers
xv. Para film

3.7 Glasswares

i. 100mL of beakers
ii. A 1000mL of beaker
iii. 250mL of conical flasks
iv. 1.5mL of vial bottles