COLOR STABILITY OF NATURAL COLORANT ON BLUE PEA FLOWERS

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"I hereby declare that I have read this thesis and in my opinion this thesis has fulfilled the qualities and requirements for the award of Bachelor of Chemical Engineering"

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A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

MAY 2010

"I declare that this thesis entitled "Color Stability of Natural Colorant on Blue Pea Flowers" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree"

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To my beloved parents, my family members, & friends

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ABSTRACT

The use of natural colorants is highly increasing in the food industry due to strong consumer demand for more natural products. On the other hand, synthetic colorant give harms to consumers that may affect health because of the unsuitable chemicals contained. Color stability of certain product is important to maintain its appearance that differs in many aspects. This study aimed at determining the color stability of blue pea flower based on the storage days, the use of different solvents and its concentrations. The blue pea flower was kept soaked at room temperature with each methanol and ethanol as solvents and an addition of maltodextrin as stabilizer every day for 7 days. Solutions of colors and solvents, methanol and ethanol were then heated at boiling point of each solvent, 64.7°C and 78.4°C, respectively. The samples obtained was analyzed and tested by using a programmed spectrophotometer, giving results of its qualities and changes of the color. Based on the results, methanol with concentration of 20 percent and storage days at the 7^{th} day is at its best in maintaining the color stability. The value of L and a* are quite consistent through the days while b* value undergo little changes that shows its stability compared to other parameters. Thus, the color stability of the blue pea flower based on data collected is suitable to be a natural colorant with a high stability to replace synthetic colorant.

ABSTRAK

Kestabilan warna bagi sesetengah produk adalah penting untuk mengekalkan pandangan yang berbeza dalam pelbagai aspek. Penelitian ini bertujuan untuk menentukan kestabilan warna biru bunga telang berdasarkan hari simpanan, penggunaan pelarut dengan kepekatan yang berbeza. Bunga telang direndam pada suhu bilik dengan metanol dan etanol sebagai pelarut dan penambahan maltodekstrin sebagai penstabil setiap hari selama 7 hari. Larutan kedua-dua warna dan pelarut kemudian dipanaskan pada takat lebur masing-masing. Sampel dianalisis dan diuji, dan keputusan bagi kualiti dan perubahan warna diperolehi. Berdasarkan keputusan, metanol dengan kepekatan 20 peratus dan hari simpanan pada hari ke-7 adalah parameter yang terbaik dalam menjamin kestabilan warna. Nilai L* dan a* cukup konsisten sepanjang hari simpanan manakala nilai b* mengalami sedikit perubahan yang mempengaruhi kestabilannya. Dengan demikian, kestabilan warna biru bunga telang berdasarkan data yang dikumpul adalah sesuai menjadi pewarna semulajadi dengan kestabilan yang tinggi untuk menggantikan pewarna sintetik.

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LIST OF ABBRIEVIATION/TERMINOLOGY/SYMBOLS

mm – millimeter

nm – nanometer

L* - lightness

a* - green to red

b* - blue to yellow

cm – centimeter

v/v – volume per volume

°C – Degree Celcius

ml – milliliter

% - percentage

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Colorants are widely used for variety of applications. Some are used in processes of manufacturing colored products. Colorants are also being used as additives in foods, drugs, and cosmetics. As for food colorant, it can be categorized into two kinds of colorant which are natural colorant and synthetic colorant. Natural colorant is the color that occurs naturally. It can be discovered mostly from the fruits and vegetables. Natural colorant can be classified into several types such as chlorophylls, carotenoids and anthocyanins. Chlorophylls are green pigment that appears mostly in vegetables and green fruits. Carotenoids are the pigments which distribute the color of red to orange and anthocyanins are the pigment of colors that distribute in blue to violet pigments. Meanwhile, synthetic colorant is manmade colorants that are made in a laboratory. Synthetic colorants made in a controlled atmosphere without any impurities which makes it dominate the manufacturing industries. In this study, these two types of colorant can be extracted from its material by undergo certain process such as extraction process (Epp and Sarguis, 2000).

A major problem with colorants is that they tend to fade when being exposed. Stabilizer is functioned to maintain the quality of the colorants that are placed in any types of environment. There are many types of stabilizers used in the

industries in order to maintain the color's stability. One of the most effective stabilizers called maltodextrin. Maltodextrin can be classified as a sweet polysaccharide or can be described as sugar contained by hydrolysis of natural corn or starches. This stabilizers came in as powder that contains little amount of fiber, fat and protein (Santostrading.com.au).

Spectrophotometer is an absorbance spectroscopy that is able to measure the amount of light at a specific wavelength that passes through a medium. This instrument can be classified into two types. A single-beam spectrophotometer which is the simplest spectrophotometer and it was minimized by using double-beam spectrophotometer. Spectrophotometer can be used in many methods as CIE lab is one of the best method. CIE lab method is functioned to give precise definition of the color of a test sample. This method based on light, object and detector. CIE lab is able to describe the pigment concentrations by using its calorimetric scale and light or observer conditions (Gonnet, 2001). The CIE lab method will determine the properties of the certain amount of colors and measure its color stability of a certain sample.

Color stability is defined as the ability of a certain light source to maintain its color properties especially in its appearances over time. The stability of color for each source is majorly different. The colors development of a certain products will differ in a certain degrees according to its properties. In this study, blue pea flowers will be used as the sample. This flowers also known as *Clitoria Ternatia* which a fast tropical climber plant in tropical collimates, especially in Asian countries. The flowers have the vivid deep blue color that shows its most striking features. It is believed to have been brought to India in the 17^{th} century (Vargas and Lopez, 2003). The colors are widely used in colorant industries such as for food and dyes. There are many uses of this flower's color in around the world. Each tradition uses it in their specific ways.

1.2 Problem Statements

Colors are vey important aspect that can highly give influences to consumers which they can be tempted to buy a good quality of colored products. Colors can be one of the factors of marketing technique to the manufacturers as it is widely used in the industries such as on foods, cosmetics, coloring and dying process. Some colored products may change in color due to outdoor exposure. This is mostly because of the color properties in the product itself that interact with the surrounding. Some qualities of colors are poor and this gives a lot of effects to consumers and also to manufacturers, in health and economics. The colors need to be maintained in a good condition so that its quality can be assured as it will gives satisfaction to consumers. Other than that, some of colored products might give harm to consumers. As for the products that have been colored synthetically, it may contain unwanted chemicals that can affect the consumers, specifically in health. Thus, in order to have a better controlled in its quality, measurement and appearance, color stability need to be observed and improved on natural colorant.

1.3 Objectives

The purpose of this study is to determine the color stability of Blue Pea flowers by using CIE lab method based on storage days and the use of different solvents and concentrations.

1.4 Scope of Research

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

i. Blue Pea flowers.
ii. Analysis of colors using CIE Lab method.
iii. Pigments of colors that contributes to the color of Blue Pea flowers.
iv. Effect of the concentration and solvents difference based on storage days.
v. Difference in color properties based on the storage days.

1.5 Rationale and Significance

There are some rationales and significances in determining the color stability of natural colorants. In order to ensure the quality of the desired colors a natural colorant should be used in the industries. Since the demand of natural colorants is much higher than synthetic colorants, the usage of these colorants is used widely. Therefore, certain aspects need to be taken in order to maintain the safety of its usage and gives the satisfaction to the consumers.

CHAPTER 2

LITERATURE REVIEW

2.1 Colorant

Colorant comes from the name of color which does not exist by itself but it depending on the source of light. Color can be categorized into two stages. First, it consists of pure physical phenomenon which required three elements – source of light, an object and detector. Second, incompletely known process occurs where the eye receptors transmit the information that the brain will interpret as color. All colors perceived by the human eye are associated with the light radiation in specified range of values as shown in Table 2.1. As for light, it has different wavelengths radiation. Visible light where its wavelength is between 380-750 mm, are very important to color appreciation (Vargas and Lopez, 2003).

Table 2.1: Light radiation of colors in specified range of values perceived by the human eye (Vargas and Lopez, 2003).

Colors	**Wavelengths radiation, λ (mm)**
Violet-blue	$380 < \lambda < 480$
Green	$480 < \lambda < 560$
Yellow	$560 < \lambda < 590$
Orange	$590 < \lambda < 630$
Red	$630 < \lambda < 750$

Color is often the first notable characteristic of a food and it influences the expectations of consumers buying the product and also influences food handlers or manufacturers who make quality-related decisions. More specifically, color predetermines our expectations and perceptions of flavor and taste. Color is often used as an indicator of food quality due to short evaluation times and cost savings (Vargas and Lopez, 2003).

2.1.1 Food Colorant

Food colorant means any substance which restored the color in a foodstuff or any product that requires coloring, which contains natural component. Food coloring is used both in commercial food production and in domestic cooking. They are tested for safety by various bodies around the world and sometimes different bodies have different views on food color safety. Food colorant can be classified into two which are natural colorant and synthetic colorant (Socaciu, 2008).

2.1.2 Natural Colorant

Natural colorant can be defined as colors that appear in fruits, vegetables or flowers due to the chemicals that occurs naturally. There are several types of natural colorant pigments.

First are chlorophylls. They are the green pigments of leafy vegetables. They also give green color to the skin of apples and other fruits. In the second are carotenoids which contribute to the red, yellow, and orange colors in many fruits and vegetables. This group of compounds includes carotenes, which are strictly hydrocarbons. Third are the anthocyanins. They are responsible for the blue, pink,

red, and violet colors in fruits and vegetables. This group of natural colorants is sensitive to pH, but is fairly heat stable and resists fading in daylight. This group is playing an increasingly larger colorant role for various foods (Epp and Sarguis, 2000).

2.1.3 Synthetic Colorant

Synthetic colorant is color that is scientifically made by human being. Most consumers find a colorless food unappealing, which is why colorants are added. Some foods are colored synthetically because they have no color or their natural color has been altered or faded during processing or storage. Currently, seven synthetic colorants are approved by the government for use in food. Table 2.2 below shows approved colors to be used in colorant industries with its functional group.

Table 2.2: Colors and its functional group (Epp and Sarguis, 2000)

Color	**Functional group**
2 Reds	#3 and #4
2 Blues	#1 and #2
2 Yellows	#5 and #6
1 Green	#3

These seven colorants are grouped by the color-giving chemical functional group they contain. Just as with any substance, the chemical structure of each these colorants determine its' characteristics, for example if it is water soluble or not. Water-soluble colorants are useful in water-based foods, but not in fatty foods such as salad dressings and ice cream. Therefore a special form of colorant is prepared by attaching the water soluble colorant to an insoluble material (Epp and Sarguis, 2000)

2.2 Spectrophotometer

Spectrophotometer is an instrument that functioned to measure the amount of light of a specified wavelength which passes through a medium. According to Beer's Law, the amount of light absorbed by a medium is proportional to the concentration of the absorbing material or solute present. Thus, the concentration of a colored solute in a solution may be determined in the lab by measuring the absorbency of light at a given wavelength. Wavelength or lambda is measured in nm. The spectrophotometer allows selection of a wavelength pass through the solution. Usually, the wavelength that has been chosen corresponds to the absorption maximum of the solute. Absorbency is indicated with a capital A (Christian, 1986).

Christian (1986) works also tells that spectrophotometer can be classified into two types which are single-beam spectrophotometer and double-beam spectrophotometer. In a single-beam spectrophotometer, there is a single beam from the light source. The general configuration of a single beam spectrophotometer is shown in Figure 2.1. The reference standard is measured to standardize the instrument, and then removed. For the single beam configuration to perform well, the light source, detector and electronics must be reasonably stable over time.

As in double beam spectrophotometer, the beam from the light source is split into two. Figure 2.2 shows the general configuration of a double beam spectrophotometer. One beam illuminates the reference standard and the other illuminates the sample. The beam may be combined before they reached the monochromator. The splitting of the beam is normally accomplished in one of two manners which are statically with a partially-transmitting mirror or similar device; or by attenuating the beams using moving optical and mechanical devices. Double beam instruments become popular in the early days of spectrophotometry due to its instability of light sources, detectors and the associated electronics (Christian, 1986).

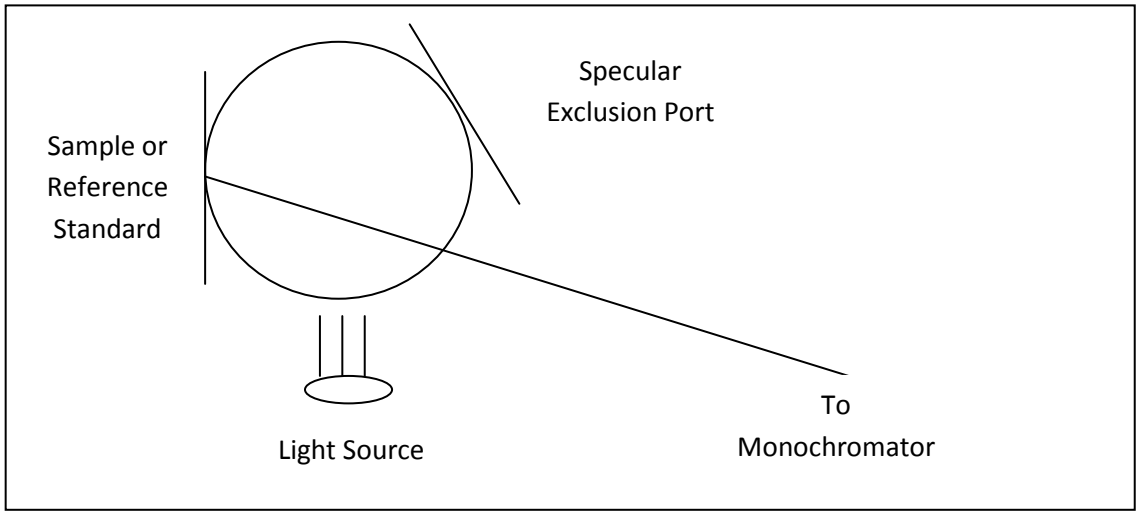


Figure 2.1: General configuration of a single beam spectrophotometer (Christian, 1986).

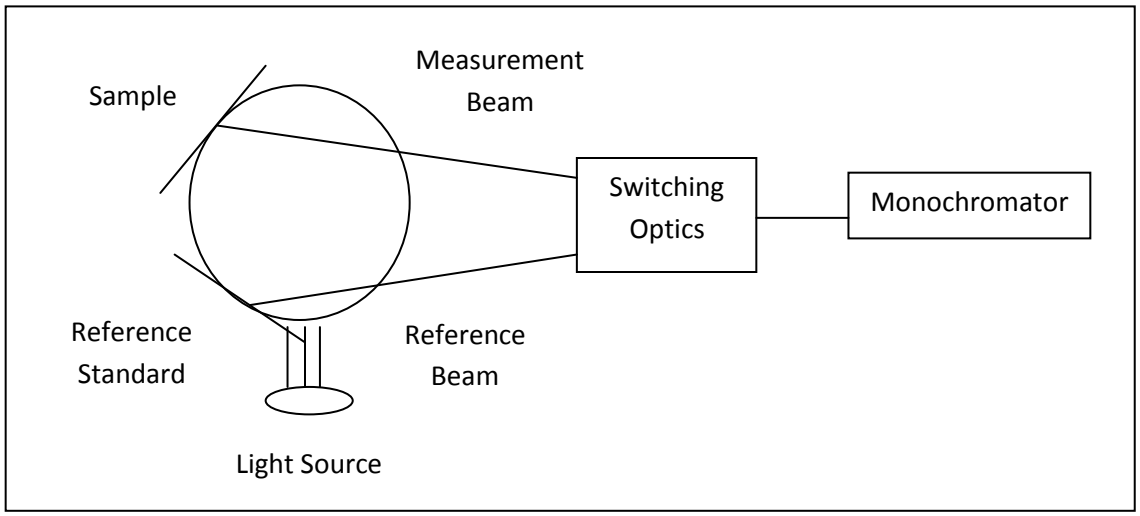


Figure 2.2: General configuration of a double beam spectrophotometer (Christian, 1986).

2.3 Color Stability

Color stability can be defined as the ability of any light source to maintain its color and appearance properties over its life. The main purpose of color stability is actually to obtain an appealing product color. Colors will interact with other components to achieve its stability and by that, it is very important to know the interactions between them. Temperature can be one of the parameters that can give influences to the color stability. Also, its shade and the process recommended to attain its final quality, packaging and shelf life requirements are important too (Vargas and Lopez, 2003).

2.3.1 CIE Lab

In 1986, the classical method was established by the "Commission Internationale de L'Eclairage'' (CIE) where it was based on the determination of tristimulus values which means the magnitudes of three standard stimuli needed to match a given sample of light, on which is based three-dimensional space called the CIE-xy space. But, this commission has adopted a new color space called the CIELAB space, as a better measurement of color (Magarino and Sanjose, 2003). This three-dimensional color space is a non-linear transformation of the CIE XYZ tristimulus values and each colour is defined by its coordinates as shown in Table 2.3.

Table 2.3: Coordinates used in CIE lab method with its appearances of colors (Magarino and Sanjose, 2003).

Coordinates	**Colors /Appearances**
L*	Lightness
a*	Green - Red
b*	Blue - Yellow
C*	Metric Chroma
h	Hue Angle
E*	Color Change

These parameters are calculated by using the CIELAB calculations formula shown on the Equations 2.1-2.6 below (Senthilkumar, 2007).

$$L* = 116(Y/Y_n)^{\frac{1}{3}} - 16 \quad (2.1)$$

$$a* = 500\left[(X/X_n)^{\frac{1}{3}} - (Y/Y_n)^{\frac{1}{3}}\right] \quad (2.2)$$

$$b* = 200\left[(Y/Y_n)^{\frac{1}{3}} - (Z/Z_n)^{\frac{1}{3}}\right] \quad (2.3)$$

$$C*_{ab} = (a^2 + b^2)^{\frac{1}{2}} \quad (2.4)$$

$$h° = \tan^{-1}(b^*/a^*) \quad (2.5)$$

$$\Delta E^*_{ab} = \left(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}\right)^{\frac{1}{2}} \quad (2.6)$$

This system is based on the three elements that are involved in color evaluation which are source of light, object and detector. The main objective of the CIE lab system is to obtain the calorimetric results that valid for normal people with the normal vision (Vargas and Lopez, 2003). Furthermore, this method is the most precise to measure the color and the most useful in the characterization and

differentiation of color. Therefore, CIE method provides a more precise definition of color than the other methods, since CIE method uses measurements over the complete visible spectrum, similar to the one perceived by the human eye. Figure 2.3 shows the framework of CIE Lab Color Model. The coordinates will be able to show which color for a certain samples are in depending on the calculations made by using the equation mentioned above.

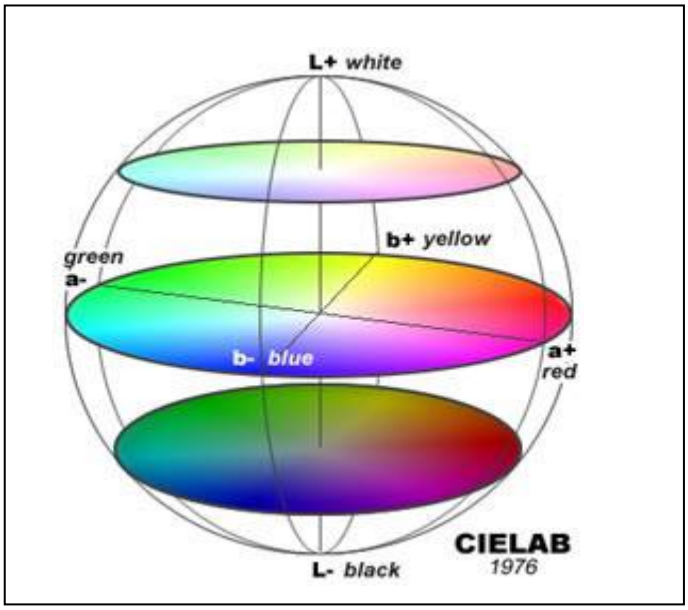


Figure 2.3: Framework of CIELAB Color Model (Personales.upv.es).

2.3.2 Stabilizer

Color is easily faded when exposed to sunlight or any other electromagnetic radiation. It is believed that most of the fading colorants when exposed to the light are due to oxidation or reduction of the colorants upon the environmental conditions in which the colorants is placed (Nohr *et al*., 1999).

Stabilizer is functioned to maintain the quality of the colorants that are placed in any types of environment. There are various factors that affect the fading

of the colorants, which are temperature, humidity, gaseous reactants (including O_2, O_3, SO_2 and NO_2), water soluble and nonvolatile photodegradation products. Nohr *et al.* (1999) have studied that generally, the most unstable colorants will be more faded efficiently by visible light while those of higher lightfastness were degraded mainly by ultraviolet light. The influence of stabilizer can be extremely important.

2.3.2.1 Maltodextrin

Maltodextrin can be described as sugar obtained by hydrolysis of natural corn or rice starches that are easily digestible. As shown in Figure 2.4, maltodextrin is classified as a sweet polysaccharide. It is one of the colorant stabilizers that are able to extend the life of the colors appearances and also can be used as flavor enhancer in candies and chocolate. Maltodextrin is a convenient source of energy that contained approximately only 4 calories per gram. It supplies carbohydrate for nutritional beverages. While containing sweet qualities, maltodextrin is considered to contain fewer calories than sugar (Santostrading.com.au).

In producing maltodextrin, natural enzymes and acids help to break down the starch even further. The end result is a simple white powder that contains extremely small amounts of fiber, fat and protein. Most of maltodextrin can be considered as inorganic since it is not produced by organic farming methods but they are produced on conventional farms (Santostrading.com.au).

Figure 2.4: Maltodextrin Stabilizer

2.4 Blue Pea Flowers

The Blue Pea flowers also called as Butterfly Pea or Pigeon Wings flowers is a fast tropical climber plant in tropical collimates which bring it as a native to Asian tropical. Although it can be found growing in our region, the Blue Pea has been introducing to Africa, Australia and some other countries. This flower grows as vine or creeper that blooms in only 6 weeks from seed and it bloom all year long. It needs intermediate temperatures, but it can be suited in our hot Asia temperatures (Jain *et al.*,2003).

Figure 2.5: Blue Pea Flower (Wikipedia, 2010)

2.4.1 Description

The Blue Pea is also known as *Clitoria Ternatea Linn.* as its scientific name is a perennial herbaceous plant. Its leaves with 4cm long by 3cm wide are elliptic and obtuse that can easily gives its pretty blue color as shown in Figure 2.5. The leaves pinnated into 5 to 9 leaflets and it often grow into thick foliage. Its flat pods contain 6 to 10 seeds in each pod which will pop black seeds when mature. The pods are 5-10 cm long, straight and sharply beaked (Mukherjee *et al*., 2008). The seeds are yellowish-brown or blackish in color and oval in shape. The flowers last for only 24 hours as it is very soft and tender. It requires little care when cultivated. The most striking feature of this flower is its vivid deep blue colors with light yellow markings, solitarily.

Figure 2.6: The Pods of Blue Pea flower (Wikipedia, 2010)

This flower is believed to have been brought to India in the 17th century, then to Europe and much later to the tropics. The root is fixed with nitrogen that can be used to improve the soil quality. The appealing flowers are home-grown organic air-dried. It is also non-toxic flowers that contains chemical free which leads to a natural dyes for food, mostly (Pwee and Leng, 1999).

2.4.2 Uses and Advantages

Blue Pea flower is commonly used in Southeast Asia as food colorants and natural dyes. As the colorant for food particularly, it is used in the making of cakes, "*kuih*" and dumplings where a tinge of the blue color will be added. For an example, in Malay cooking, the flower will undergo an aqueous extraction to obtain its blue color in making "kuih tekan". As in Thailand, a syrupy blue drink called "*nam dok anchan*" is made by using the flowers. Meanwhile, in Burma the flowers are used as food where they are dipped in batter and fried (Wikipedia, 2010). As a dye, it is popular amongst the health conscious who do not appreciate artificial dyes. The dyes can also be used as litmus while the plant itself as matting.

In Asian, the Indians use this flower as medicine. Its roots and seeds are made into a drug forms called “Sankhapushpi” and they used it as tonic of the nerves. The roots are usually used as a tonic to children for improving mental faculties and muscular strength. The roots and stems are also very useful for the treatment of snakebite and scorpion sting in India. Eventhough there is limited information available on local and traditional uses of the Blue Pea flowers in Americas, but in Cuba, the mixture of roots in a bottle of water is believe to be able to promote menstruation and induce uterine contractions. In addition, by combining the flowers and roots in a bottle of wine will be able to treat clorosis and against liver and intestinal problems (Mukherjee *et al*., 2008).

The Blue Pea flower is also being used in hair treatment. In ancient Thai herbal medicine the flowers are believed to be able to treated hair loss and prematurely grey hair. It works by promoting the blood circulation in the scalp, nourishing and strengthening the hair follicles. Its roots, seeds n leaves are commonly used in Ayurvedic system of medicine. The roots and seeds have powerful laxative effects, the flowers are used to make collyrium and the leaves are used in Madagascar to relieve joint pains. This flower contains antifungal proteins and has been shown to be homologous to plant defensins. This flower also discovered to be able to enhance memory in rats by undergo some tests and experiments. As reported, when the aerial and root parts of the Blue Pea flower were extracted with alcohol, it produced significant memory retention and the root parts were found to be more effective (Jain *et al*., 2003). Table 2.4 shows the traditional medicinal properties of Blue Pea flowers from its roots, leaves and seeds.

Table 2.4: Traditional medicinal properties of Blue Pea Flower (Mukherjee et al., 2008).

Root	**Leave**	**Seed**
Dementia	Otalgia	Cathartic
Hemicranias	Hepatopathy	
Burning sensation		
Leprosy		
Inflammation		
Leucoderma		
Bronchitis		
Asthma		
Pulmonary tuberculosis		
Ascites		
Fever		

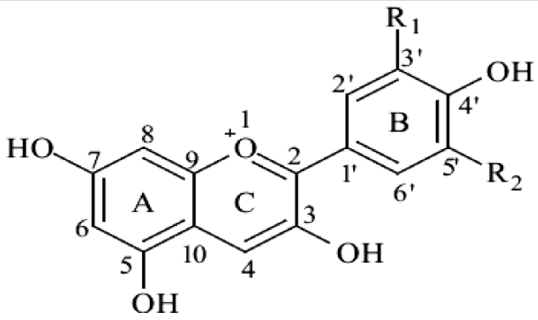
2.4.3 Anthocyanin Pigments

Anthocyanins are the natural colorant in food industry. It represents the plant pigments that responsible for various colors found in flowers, fruits and autumn foliage. The purpose of determination of anthocyanins is to improve the quality control of these natural products found in fruits. Also, it can help the manufacturers in their quality control processes. Anthocyanins are widely distributed in higher plants especially in fruits and flowers (Ovando *et al*., 2009).

Their use has been limited because of the relative instability and low extraction percentage. Anthocyanins are water-soluble and less stable than carotenoids which are the other types of colorant. Meanwhile, they are the most

important pigments in the vascular plants because of their harmless and easy incorporation in aqueous media which gives more interests for its use as natural water soluble colorants. The basic structure of anthocyanins is anthocyanidins where it can be found in their glycoside forms which are bonded to a sugar moiety (Ovando *et al*., 2009).

Anthocyanins that have been isolated are highly stable and very susceptible to degradation. One of the stability factors for anthocyanins are pH where it can be found in different forms that depends on the pH of the solution. Other factors that affect their stability are the storage temperature, chemical structure, concentration, light, oxygen, solvents, presence of enzymes, flavanoids, proteins and metallic ions (Reins, 2005). Another significant property of anthocyanins is their antioxidant activity, which plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes, among others (Ovando *et al*., 2009). Figure 2.7 shows the six most common anthocyanidin skeletons.



Name/ Skeletons	R1	R2
Delphinidin	OH	OH
Petunidin	OCH_3	H
Cyanidin	OH	H
Pelargonidin	H	H
Peonidin	OCH_3	H
Malvidin	OCH_3	OCH

Figure 2.7: Chemical structure of anthocyanidins (Wang and Stoner, 2008).

There are eight anthrocyananins are found in Butterfly Pea flowers which helps in blood circulations especially in scalp area and help promote hair growth.

Table 2.5: Anthocyanins found in Blue Pea flowers (Mukherjee *et al*., 2008).

Anthocyanins	
Ternatins	C1, C2, C3, C4, C5 and D3
Pre-ternatins	A3 and C4

Six ternatins from the flowers were partly characterized as highly acylated dephinidin derivatives. Deacylternatin was determined as delphinidin 3,3’,5’-tri-O-b-D-glucopyranoside (Mukherjee *et al*., 2008).

2.5 The Summary of Previous Studies

Table 2.6: Summary of previous studies

Journals / **Findings**	**(Ayadi *et al*., 2003)**	**(Harivaindaran *et al*., 2008)**	**(Patil *et al*., 2009)**
Raw Material	Red Cabbage	Dragon Fruits	Red Radish
Solvent	Methanol	Distilled water	Pure water, water with hydrochloric acid(1-2%), water with ethanol(0-80%), water with hydrochloric acid mixed water with ethanol
Parameters/ Variables	pH (1.1-10.5)	Temperature, time, light exposure and pH	Solvents, concentrations of solvents
Conclusion	• The pigments had reddish nuances at the lowest pH values. • Starting at pH 7.3, the colors of sample start to change gradually towards bluish-tone. • In higher pH, the colors of samples tend to change and disappear rapidly.	• The samples being extracted at 100°C at pH 5 and heated for 5 minutes to obtain the highest yield. • In the thermal treatment, the pigment experiencing degradation and fluctuating stability. • Prolonged heating gives effect to betacyanin's retention and stability.	• Maximum anthocyanin content obtained by using 50% of ethanol that mixed with acidified water. • The result samples were then undergo a dealcoholization by using membrane pertraction to remove alcohol contained.

Journals / Findings	(Nisha *et al*., 2004)	(Aubert *et al*., 2001)	(Hurtado *et al.*, 2009)
Raw Material	Green Spinach	Grape-marcs, elderberry, purple carrot, red cabbage, red radish and blackcurrant	Tamarillo Fruits
Solvent	Water with salt	Hydrochloric acid (HCl), ethyl acetate, methanol,	Methanol with acetic acid
Parameters/ Variables	Temperatures, treatment time, salt weight percentage	Raw materials, pH, solvents, storage time	pH 2.0-6.2
Conclusions	• Stabilization achieved at 2% NaCl at 60 minutes of heating at temperature 80°C. • As the treatment time increases, the values of L* and –a* decreased consistently while b* is not consistent.	• Red radish was relatively stable at all pH values. It is much more resistant to heating. • Red radish extracts with HCl was slowly increases and remains in red-color area under 15°C after p6h of storage • At certain pH, color stability mainly depends on the anthocyanin's structure and phenolic compounds.	• The jelly and peeling extracts show reddish hues at the lowest pH. • As the pH values increases to 6.2, the color of both extracts change to orange and red-purple hues, respectively • Peeling extract has higher stability due to higher polymeric anthocyanins content. • This shows that anthocyanins is a component that more stable to pH changes

CHAPTER 3

METHODOLGY

3.1 Introduction

The method used is a simple extraction process. This method is used since it is the easiest and the simplest method to prepare. Several precautions steps are taken such as doing research about the ignition source of the chemicals, do not start the flame and used the condenser to prevent the ethanol from being vaporized freely to the surrounding. The experimental studies are divided into two sections which are sample preparation and sample analysis. Samples preparation includes from weighing the samples and undergoes an extraction process while sample analysis includes the stability test of the color sample.

3.2 Materials and Solvents

The material used in this study is 70 grams of dried Blue Pea flowers or as in its scientific name called *Clitoria Ternatea Linn.*. The solvent used in this study are ethanol and methanol for the extraction process. Both solvents were being used in different concentrations which are 10% v/v, 20% v/v, 30% v/v and 40% v/v.

3.3 Apparatus

To analyze the samples, a spectrophotometer modeled HACH DR/4000 was used as the equipment. This equipment analyzed the properties of colors contained in the sample qualitatively and quantitatively. For analysis and stability purpose, CIE lab method was used to characterized the colors and measure the components contained.

3.4 Sample Preparation

In the preparation to analyze the sample, the dried Blue Pea flowers were being weighed for 1gram and put into the 20 ml test tube and 0.5 grams of stabilizer was also being put into the test tube. Both were then being soaked with methanol and ethanol, respectively, each day for 7 days. The flowers were soaked with concentrated solvents with 10, 20, 30 and 40 percent for about 15 ml. Each sample was then being wrapped with aluminum foils to be kept in a room with temperature 25°C.

When the samples were successfully being kept for 7 days, they were then being heated in order to remove the methanol and ethanol contained in the samples. The flower that was being soaked together with the solvents was also taken out before the heating process. In heating process, the sample solution was heated based on the boiling point of ethanol and methanol which are 78.4°C and 64.7°C, respectively. This heating process is to obtain the true color of the samples without any other chemicals involved while analyzing them with the spectrophotometer. The samples were then being wrapped back in order to prevent it from being affected by the environment such as lights.

Before the sample solutions being analyze using spectrophotometer, it was first being diluted in order to be able to be read by the equipment. The samples were diluted 10 times from the actual concentration of the samples solution by using distilled water.

3.5 Stability Test

In this study, a HACH spectrophotometer modeled DR/4000 was used to obtain the color stability. The programmed based on COLOR, Trismulus Values and Chromaticity Coordinates was chosen and the procedure of this program has been attached in Appendix. This program managed to give the value of CIE Lab parameters in order to obtain the color stability of the samples.

On the day 8 of the experiment, each sample that was wrapped was taken out to undergo the stability test. The samples were placed into the spectrophotometer apparatus to obtain the value of CIE Lab. The blank solution was prepared as a controller. The equipment was being operated for about 5 to 10 minutes for every four samples. Thus, in reading all of the samples, it takes about 3 hours to be completed.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This research project involved analysis of data to get the idea of the color stability under certain parameters which are type of solvents, concentration of solvents and storage days of the material.

4.2 Various Concentration of Solvent Analysis

Concentration of solvents analysis plays an important role in determining the changes of CIE Lab parameters that give effect in maintaining the color of the Blue Pea flowers . Each solvent with concentrations 10, 20, 30 and 40 percent volume per volume has been used in this research project.

4.2.1 Methanol with various concentrations

Figures in Section 4.2.1, show the changes of stability for each CIE Lab's parameters when the samples were soaked using methanol as a solvent. CIE Lab parameters consist of L*, a* and b* which represent lightness, red to green and yellow to blue, respectively.

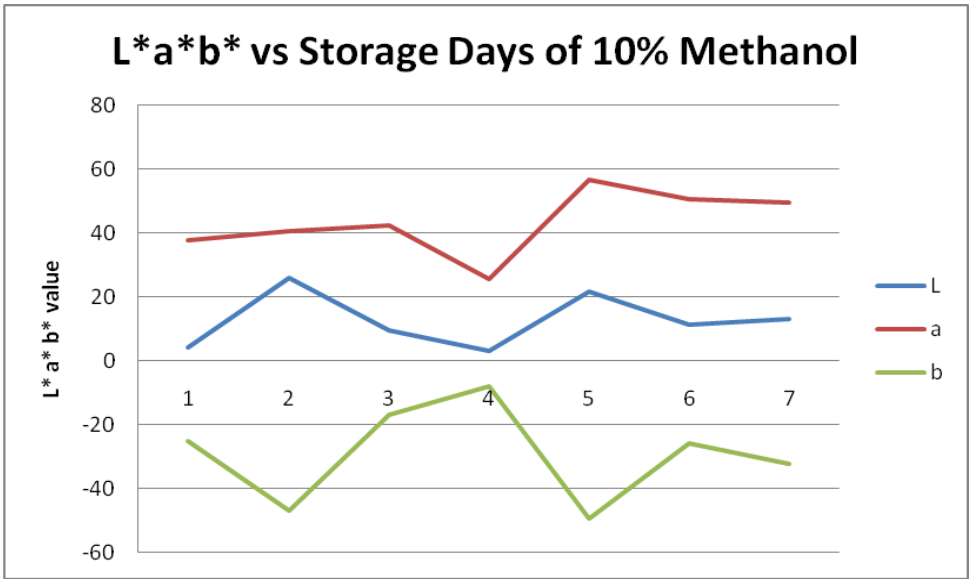


Figure 4.1: Graph of L* a* b* values versus Storage Days for 10% of Methanol

Figure 4.1 illustrate the changes of CIE Lab parameters at different storage days. It can be seen that the value of L* and a* are quite consistent. For L* values, the values keep increase and decrease in each day but when it comes to day seven, the value is quite similar with value in day one. For a* values, the colors becomes more reddish at first but quite unstable within day three to day 6 before achieved the stability at day seven. For b* value, it is clearly shows the inconsistency in color. Starting from day one to two, the blue color contained in the samples are increase.The negative b* values are highly decreased only at day two and day five which have decreases the pigment of blue colors in the samples given the assumptions on the anthocyanin pigment contained are also decreased. This shows the unstability of the samples when reacting with this concentration. The changes in

each color's stability may affect by the concentration and that are not suitable in maintaining the color stability of the Blue Pea flowers.

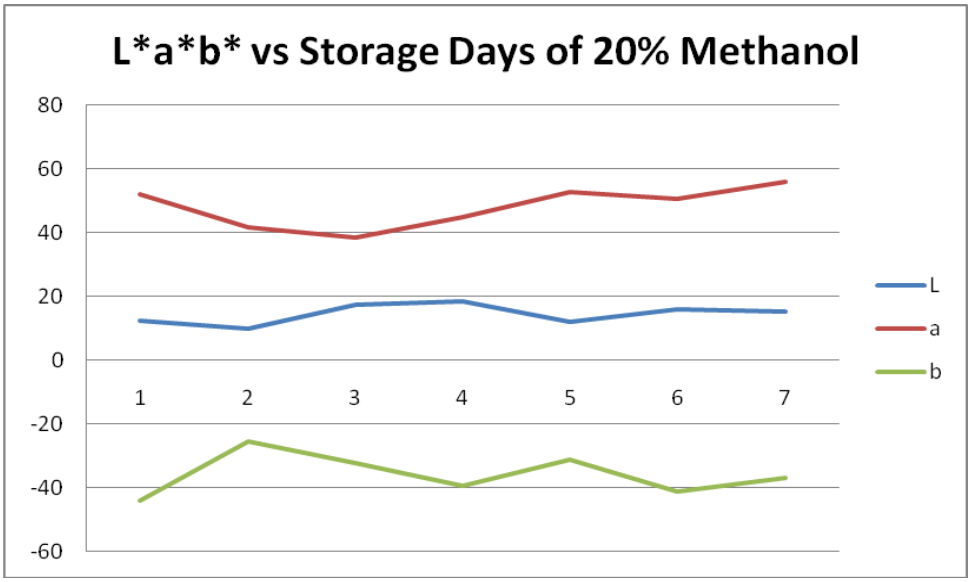


Figure 4.2: Graph of L* a* b* values versus Storage Days for 20% of Methanol

Figure 4.2 shows that L* a* b* values versus storage days for 20% of methanol. From the graph, the values of L* and a* seems to be more stable as the day of storage increases. This shows that the lightness and the red color contained in the samples are able to be maintained when mixed with the concentrated methanol. As for b* values, beginning from day one until two, the negative values of b* decreases. It shows that the anthocyanin pigment contained that contribute to the blue color of the flower is decrease. The rate of extration of the pigment by the concentrated solvents may have increased. From day two until day four, the –b* values increases which shown that the samples gives a higher anthocyanin contained as day increases. Even though the b* values are less stable compare to L* and a* values, this proves that the stability of colors in the flowers, by taking the b* values as our priority (blue color contained) are shown.

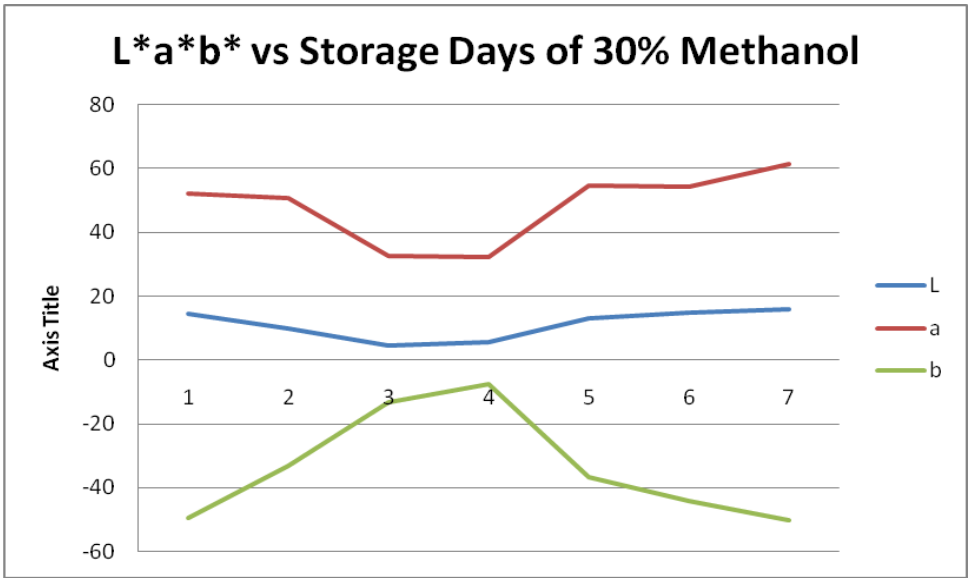


Figure 4.3: Graph of L* a* b* values versus Storage Days for 30% of Methanol

The stability in lightness was clearly shown in Figure 4.3 where the L* values are maintain from day one until day seven. The lightness of the samples might have slightly decreased from day two to day five but in the mean time, the samples managed to maintain it's lightness at day three to four which valued 4.43 and 5.61, respectively. As for a* values, the red color was stable from day one until day two, but it was clearly decreased until day four. After that, from day five to day seven, the red color contained tend to increase. For b* values, the blue color in the samples are obviusly being unstable. It shows that from day one until day four, the blue colors contained decreases. On the other hand, from day five to day seven, the blue color tend to increases again. This have shown the colors inconsistency in 30% of methanol. Based on the b* values, methanol as a solvent at 30% concentration may not be the best solvent in extraction process in this study.

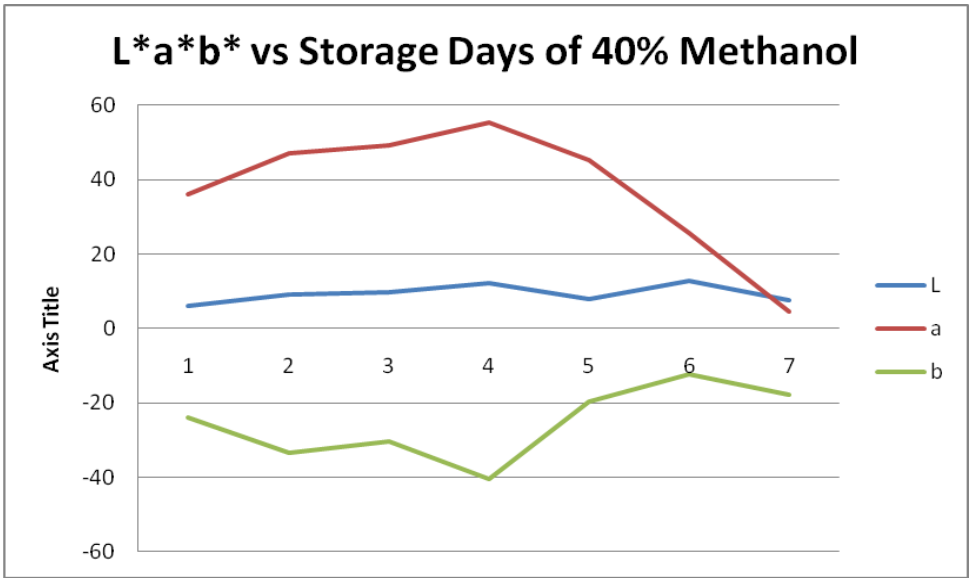


Figure 4.4: Graph of L* a* b* values versus Storage Days for 40% of Methanol

Figure 4.4 shows the changes in Cie Lab parameters as the days of storage increases. As usual, the L* values were maintained from day one until day seven which shows the lightness of the sample are stable. This proves that the samples were being kept away form any source of light successfully. On the other hand, the a* values show a huge changes in the sample. It can be seen that the a* values tend to increase from day one until day four which shows the red color contained in the samples are increase. But on day five onwards, the value are obviously decreased which gives the opposite result for the color contained. Same goes as the values of b* where the blue color in the samples were very inconsistent as shown in the graph. The samples becomes more bluish from day one until day four (inconsistently). From day five to seven, the color contained decreased which makes the sample were less bluish. As shown clearly, the use of methanol with 40% concentration of solvents are not suitable in maintaining the color stability because of the huge difference obtained during the analysis process.

4.2.2 Ethanol with various concentrations

Figures in Section 4.2.2 show the changes of stability for each CIE Lab's parameters when the samples were soaked using ethanol as a solvent. CIE Lab parameters consist of L*, a* and b* which represent lightness, red to green and yellow to blue, respectively.

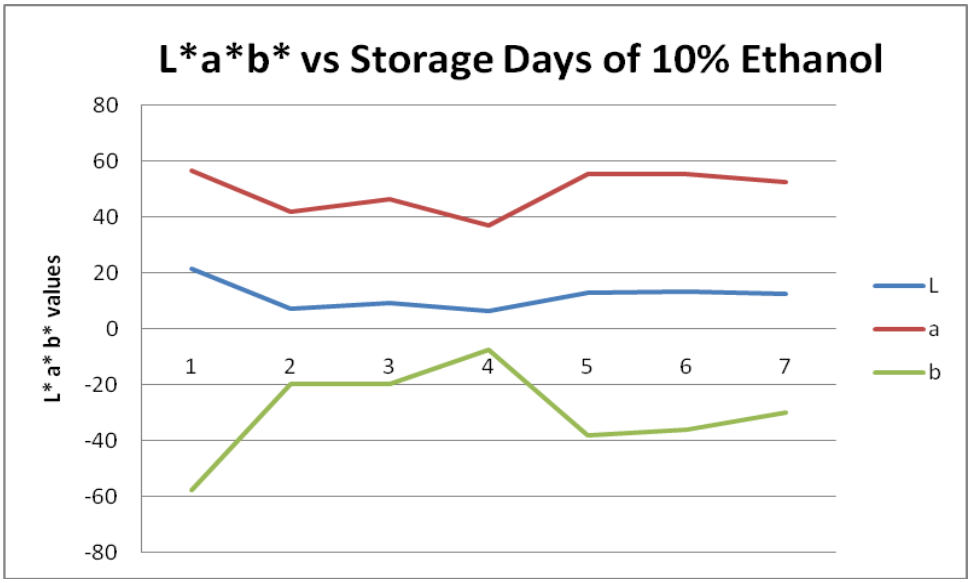


Figure 4.5: Graph of L* a* b* values versus Storage Days for 10% of Ethanol

Figure 4.5 clearly shows the graph of CIE Lab's parameters; L*, a* and b* versus storage days by using ethanol as a solvent at 10% concentration. It can be seen that the L* values are the most stable compared to others. The lightness went slightly decreased from day one to day two but able to maintain stable from day three onwards. As for a* and b* values, both line are quite similar. For a* values, it seems to be more stable than b* values. In this case, a* values are more likely in red color of ranges. For -b* values, on day one of stability test, the values are highly increased when it comes to day two. This are the same as the a* values on day four where it is highly increased when it comes to day five. The few days onwards, b* values seems to be more stable which leads the samples are all in blue range of colors. Thus, from the graph, it barely shows the stability in the samples as the b*

values are clearly less stable. Thus, this concentrated solvents are not suitable in maintaining the color stability in the samples.

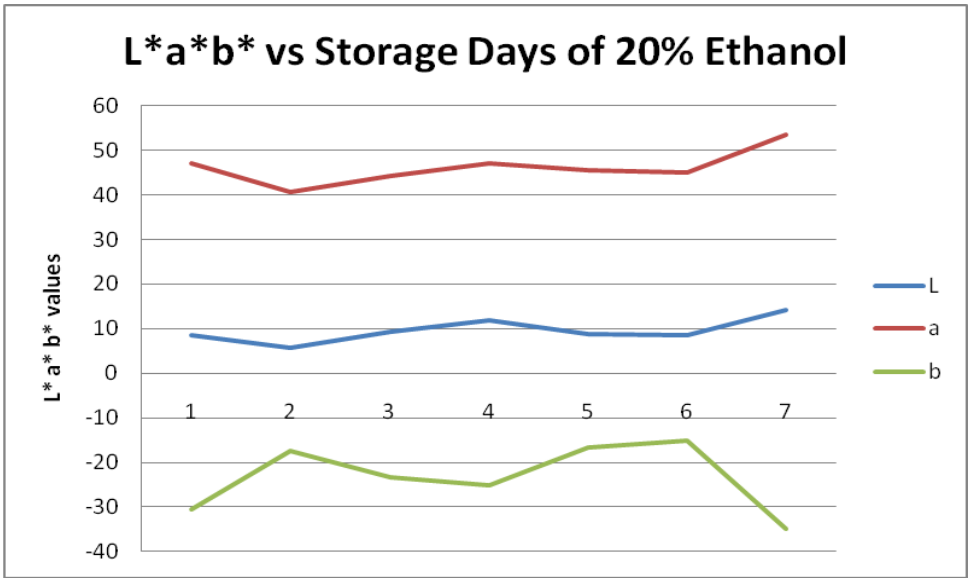


Figure 4.6: Graph of L* a* b* values versus Storage Days for 20% of Ethanol

From Figure 4.6, it shows that L* and a* values are maintained. The L* values shown above conclude that the lightness of the flowers are still maintain even with a 10% difference in concentrations compared to Figure 4.5. For a* values, the values are slightly increased compared with ethanol in 10%. This shows that the color of the flowers by referring to the a* values is being more reddish. As for b* values, by comparing these values with the values in Figure 4.5, b* values with 20% ethanol is much stable than 10% of ethanol. The differences of values within the 7 days is not very large. Still, the b* values shown the blue color of range. By comparing the suitablility of the concentrations for ethanol, concentrations of 20% is more likely suitable to be able in maintaining the color stability compared to 10% of ethanol.

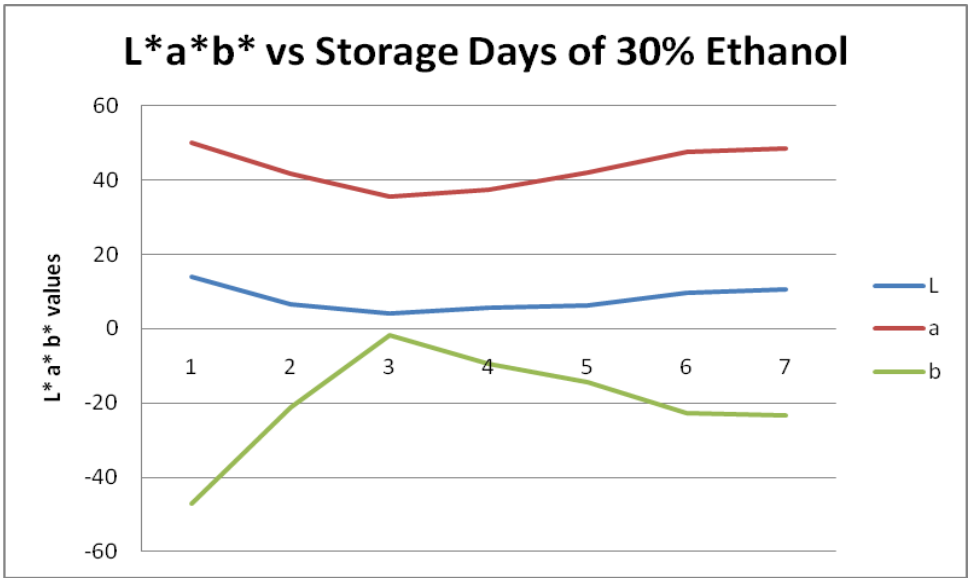


Figure 4.7: Graph of L* a* b* values versus Storage Days for 30% of Ethanol

Figure 4.7 shows that L* a* b* values versus storage days for 30% of ethanol. From the graph, the values of L* and a* shows the similar stability in their range of colors, respectively. Both values tend to decreased from day one to day three which give the samples less lightness and less of red colors. As the day increases, the color involved between this two values are back at its value as in day one. This shows that both L* and a* values are stable within the range of seven days. For values of b*, the color contained undergo more changes compared to the previous graph. This shows that from day one to day three, the values of b* are highly increased from -21.3 to -1.76. It is clearly shows that at day three, the samples may have been reacted with the solvents that affect its color to be more to white color. As from day four to day six, the values decreased consistently and it became stable at day six and seven. This shows that the blue color contained in the samples increased. By comparing the suitablility of the concentrations for ethanol, concentrations of 30% is less suitable to be able to maintain the color stability.

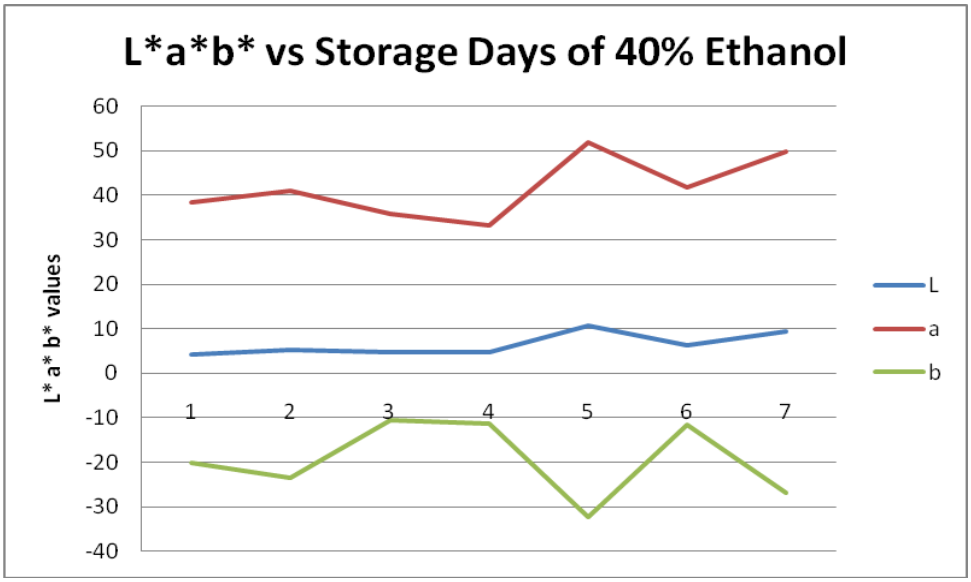


Figure 4.8: Graph of L* a* b* values versus Storage Days for 40% of Ethanol

Figure 4.8 shows the Cie Lab parameters values changes as the storage days increases. For L* values, the lightness of the samples manage to stay stable. Only at day 5, the color may have been interact with the concentration of the solvents that caused it to increase its lightness. On the other hand, the a* values which is from day one to day four shows a little stability. As from day five to day seven, the colors of the sample seems to be inconsistent. This may caused by the concentrations of the solvents that may affect the properties of the colors in the samples. For b* values, it is obviously shown that the blue color contained in the samples are highly inconsistent. A temporary stability was achieved only from day three to day four. Thus, the concentration of ethanol at 40% can not stabilize the color of the samples.

4.3 Different Types of Solvents Analysis (20% concentration)

Analysis for different types of solvents is very important in order to identify the best solvents used in maintaining the color stability of the flower. In this research project, two types of solvents are used which are methanol and ethanol. Both

solvents have their own properties and characteristics that may contribute to the changes of color of the flower.

4.3.1 L* value Analysis

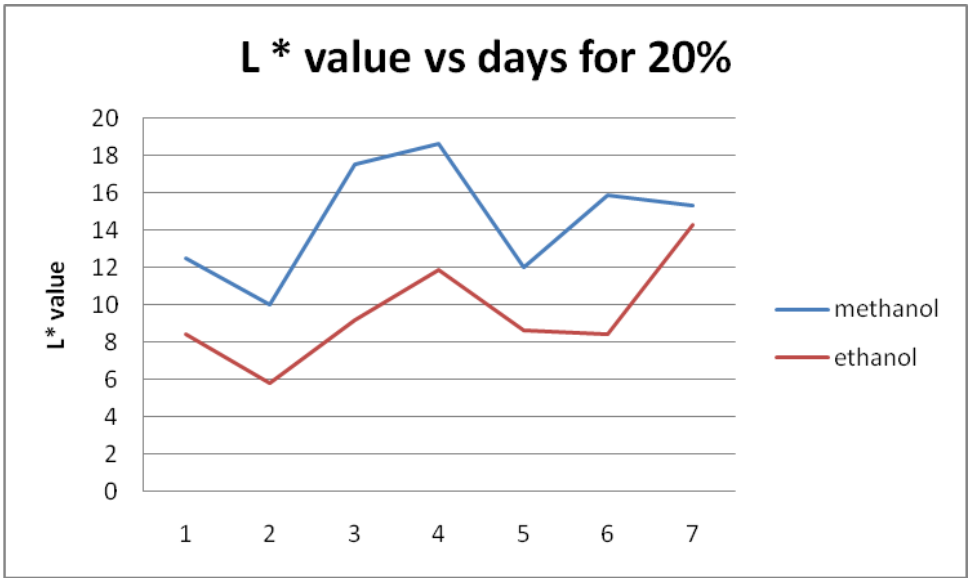


Figure 4.9: Graph of L* value versus Storage Days

Figure 4.9 shows the graph of the lightness (L*) versus storage days by using methanol and ethanol as the solvents at 20% of concentration. It can be seen that the L* values changed when storage days increased. For methanol, the value of L* decreased just when reached day two. This shows that the flowers undergo some changes in color which reduce its lightness from the actual condition. From day two until day four, the L* value for methanol increased. This changes shows the color of the flower becomes lighter by referring to the L* value (white to black). Both changes occurred again from day four onwards.

For ethanol, the L* values decreased from day one until day two. This shows that the flower becomes darker when it was kept soaked by using ethanol as the

solvent. From day two until day four, the L* values are consistently increased. which shows that there have been reactions between the colors of the flower and solvents. At this point, the degradation of the lightness is clearly shown where the color tends to be lighter or whiter. The similar changes of color happened from day five onwards.

4.3.2 a* value Analysis

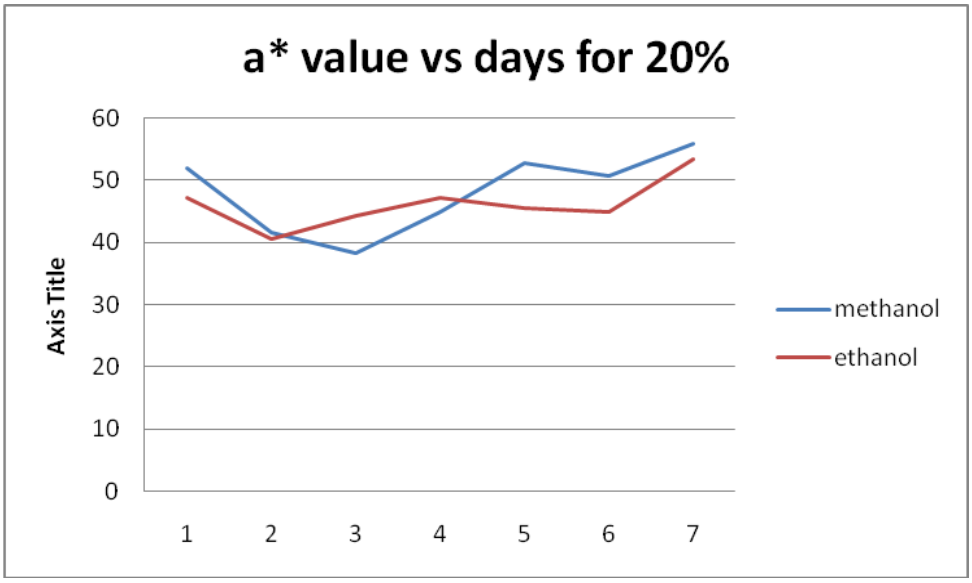


Figure 4.10: Graph of a* value versus storage days

Graph of a* value versus storage days is illustrated in Figure 4.10. The a* values consist of positive a* which represent the red pigments while negative a* represent the green pigments. For methanol, the values of a* decreases from day one until day three. This shows that the samples are being less redish. As from day four onwards, the valus seems to be increased. This shows that the reddish color contained in the samples increased. For ethanol, the samples being less reddish from day one to day two. But, from day three until day seven, the a* values seems to be inconsistently increased. This shows the inconsistency of the solvent in maintaining

the reddish color for the samples. Thus, for a* values, the samples are not really stable in its reddish color with both solvents.

4.3.3 b* value Analysis

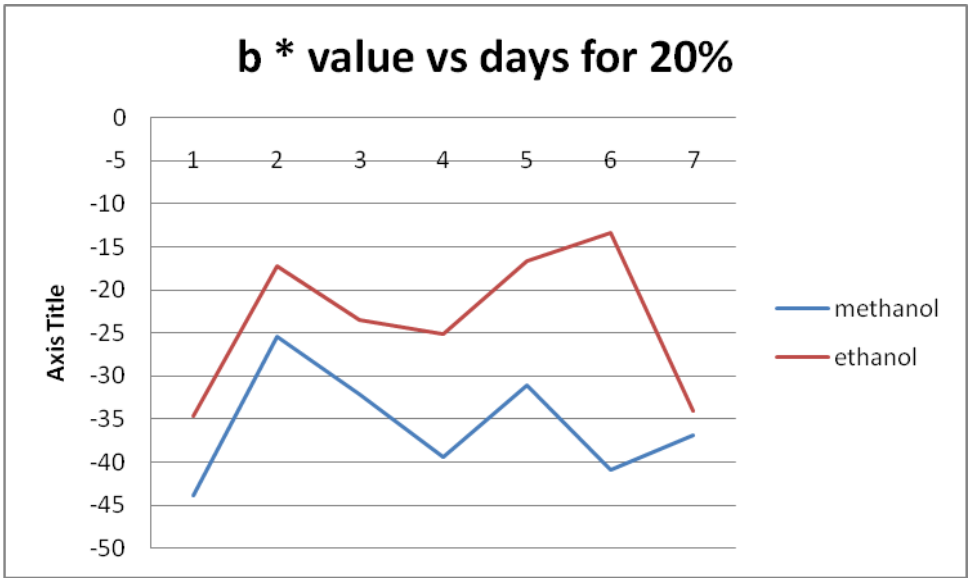


Figure 4.11: Graph of b* value versus Storage Days

Figure 4.11 shows that changes in b* values with differ in solvents. The b* values consist of positive b* which represent the yellow while negative b* represent the blue pigments. As shown in the graph, different values of b* plotted for both solvents. For methanol, the color of the flower contained less blue pigments since the value of -b* in the graph decreased in day two. As from day two to day three, the -b* value tend to increase until day four which caused the blue color of the flower increased. The value of b* decreased inconsistently from day four until day seven. For ethanol, the b* values increased where the lines plotted quite similarly with methanol. From day three to four, the values of –b are quite stable with gradient at 1.69. fom day five onwards, the blue color contained are obviously not stable. So, based on the fluctuates of b* values for both solvents was clearly shown that methanol is more consistent compared to ethanol.

4.4 Storage Days Analysis

Analysis for storage days have been made in order to identify which day that gives the most stable in color. In this study, the samples were being kept for seven days and observed. Each day of storage may give a different stability on the colorant. From this research, storage day at seven have been noticed to be able to maintain the color of the blue pea flowers.

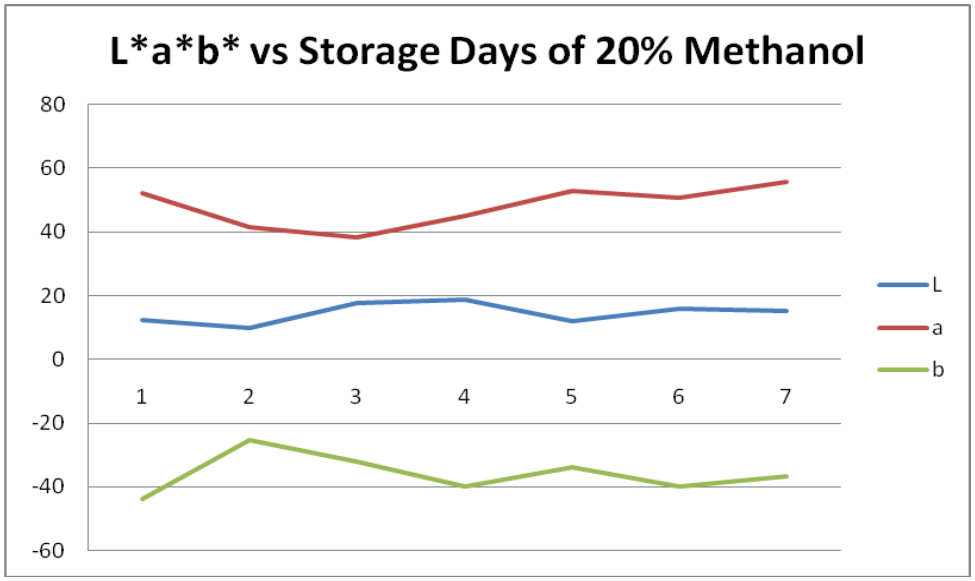


Figure 4.12: Graph of L* a* b* values versus Storage Days for 20% of Methanol

As in Figure 4.12 shown above, starting form day one to two, all of the CIE Lab's parameters have shown an unstable changes. This occurred maybe because of the range of storage days is too short to have it being stabilize. The values of L* and a* are quite the same in its flow. The only difference is the values which are opposite. The L* value seems to achieved its stability on day 6 with 1.99 difference in values while a* achieved its stability on day seven with difference in 0.53. As for b*, starting from day one, the stability are very poor but as it came to day six, it seems to be more stabilized. This shows that the b* values stability increases as the storage days increases. Thus, the stability for b* value have been found at day seven with difference value at 3.05. In this analysis, the b* values is set to be the priority in

discover the best storage days as the b* values contribute to the bluish color of the flower. So, the best storage day is at seventh day.

CHAPTER 5

CONCLUSION & RECOMMENDATION

5.1 Conclusion

In this study, the usage of methanol in the simple extraction process has been discovered as the best solvents in extracting the colors of the Blue Pea flowers. Thus, the stability of the colors for Blue Pea flowers have clearly shown as the most stables in L*, a* and b* values. The studies of four variables of concentrations which are 10%, 20%, 30% and 40% resulting in 20% is the most suitable concentration for maintaining the color stability. Both result for solvents and concentration selection was brought forward to determine the suitable storage days.

The result for storage days that gives the most stable in color for the Blue Pea flower is at day 7 with gradient 3.05. The addition of stabilizers is to maintain and stabilize the color changes over the parameters set. There might be an error while the solutions were being diluted before being analyzed that caused the stability rates.

5.2 Recommendations for Further Studies

It is recommended that the solvents used should be mixed or added with a little amount of acidic solvents. This is in order to give a higher rate of extraction of the colors from the blue pea petals. This may give more of color contained in the flowers. Thus, the stability rate might also improve. The types of extractions are also recommended by using centrifuge, membrane or filtration. These processes may be able to produce higher percentage of extraction.

In addition, there should be a dealcoholized process where this process will remove the alcohol contained in the samples. This are clearly will show the stability of color by confirming that there are no alcohol contained that might affect the stability. Other than that, further studies should be made with strictly controlled environment and conditions. This is in order to have more precise results at the end of the research.

Lastly, the safety aspect should be taken more seriously since this study includes hazardous chemicals with specific concentrations. This is to ensure the safety while doing this research.

REFERENCES

Aubert, C.M., Dangles, O. and Amiot, M.J. (2001). *Color Stability of Commercial Anthocyanin-Based Extracts in Realation to The Phenolic Composition. Protective Effects by Intra- and Intermolecular Copigmentation.* Food Chemistry 49, 170-176

Ayadi, N., Lejeune, F., Charrier, F., Charrier, B. and Merlin, A. (2003). *Color Stability of Heat-Treated Wood During Artificial Weathering*. European Journal of Wood and Wood Products 61, 221-226.

Christian, G.D. (1986). *Analytical Chemistry*. (4th ed.). University of Michigan. Wiley.

Epp, D.N. and Sarguis, M. (2000). *Palette of color – The chemistry of food dyes*. Miami University Middletown: Terrific Science Press.

Gonnet, J.F. (2000). *Colour Effects of Co-Pigmentation of Anthocyanin Revisited-3. A Further Description Using CIELAB Differences and Assessment of Matched Colours Using The CMC Model.* Food Chemistry 75, 473-485.

Harivaindran, K. V., Rebecca, O. P. S. and Chandran, S. (2008). *Study of Optimal Temperature, pH and Stability of Dragon Fruit Peel for Use as Potential Natural Colorant*. Pakistan Journal of Biological Sciences 11(18), 2259-2263.

Hurtado, N.H., Morales, A.L., Miret, M.L.G., Gilete M.E. and Heredia F.J. (2009). *Color, pH Stability and Antioxidant Activity of Anthocyanin Rutinosides Isolated from Tamarillo Fruit* (*Solanum betaceum Cav*.). Food Chemistry 117, 88-93.

Jain, N.N., Ohal, C.C., Shroff, S.K., Bhutada, R.H , Somani, R.S., Kasture, V.S. and Kasture, SB. (2003). *Clitoria Ternatea and the CNS.* Pharmacology, Biochemistry and Behaviour 75, 529-536.

Magarino, S.P. and Sanjose, M.L.G. (2003). *Application of Absorbance Values Used in Wineries for Estimating CIE Lab Parameters in Red Wines.* Food Chemistry 81, 301-306.

Mukherjee, P.K., Kumar, V., Kumar, N.S., Heinrich, M. (2008). *The Ayurvedic Medicine Clitoria Ternatea- From Traditional Use to Scientific Assessment*. Journal of Ethnopharmacology 120, 291-301.

Nisha, P., Singhal, R.S. and Pandit, A.B. (2004). *A Study on The Degradation Kinetics of Visual Green Colour in Spinach* (*Spinacea Oleracea L*.) *and The Effect of Salt Therein*. Journal of Food Engineering 64, 135-142.

Nohr, R.S. and MacDonald, J.G. (1999). *US Patent No. 6,168,655 B1*. Washington DC: US Patent and Trademark Office.

Ovando, A.C., Hernandez, L.P., Hernandez, E.P., Rodriguez, J.A and Vidal, C.AG. (2009). *Chemical Studies of Anthocyanins: A Review.* Food Chemistry 113, 859-871.

Patil, G., Madhusudhan, M. C., Babu, B. R. and Raghavarao, K.S.M.S. (2009). *Extraction, Dealcoholization and Concentration of Anthocyanin from Red Radish*. Chemical Engineering and Processing 48, 364-369.

Pwee, T. and Leng, A.S. (1999). *Singapore, Our Maritime City*. National Library Board, Singapore. National Library Board.

Rein, M. (2005). *Copigmentation Reactions and Color Stability of Berry Anthocyanins.* Academic Dissertation. University of Helsinki.

Santos Natural Food Store. (2010). What is a Maltodextrin?

Senthilkumar, M. (2007). *Modelling of CIELAB Values in Vinyl Sulphone Dye Application Using Feed-Forward Neural Networks*. Dyes and Pigments 75, 356-361.

Socaciu, C. (2008). *Food Colorants: Chemical and Functional Properties*. Boca Raton, FL. CRC Press.

Vargas, F.D. and Lopez O.P. (2003). *Natural Colorants for Food and Nutraceuticals Uses*. CRC Press.

Wang, L.S. and Stoner, G.D. (2008). *Anthocyanins and Their Role in Cancer Prevention*. Cancer Letters 269, 281-290.

WORLD WIDE WEB

http://en.wikipedia.org/wiki/Clitoria_ternatea

http://personales.upv.es

http://santostrading.com.au

APPENDIX A

Experimental Diagram

Figure A.1: Dried Blue Pea Flowers

Figure A.2: HACH Spectrophotometer model DR/4000

Figure A.3: Samples with ethanol before heat

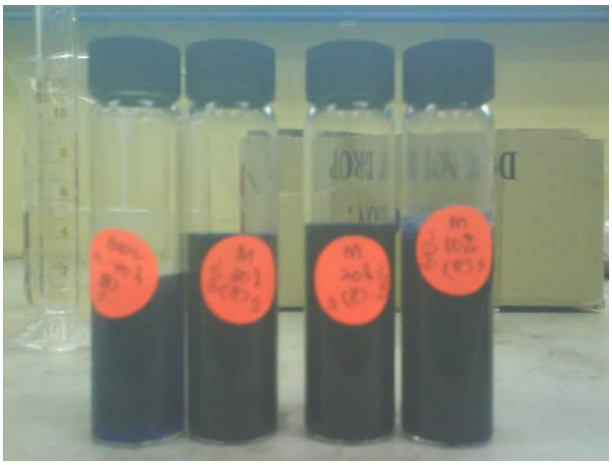


Figure A.4: Samples with methanol before heat

Figure A.5: Samples with ethanol after diluted

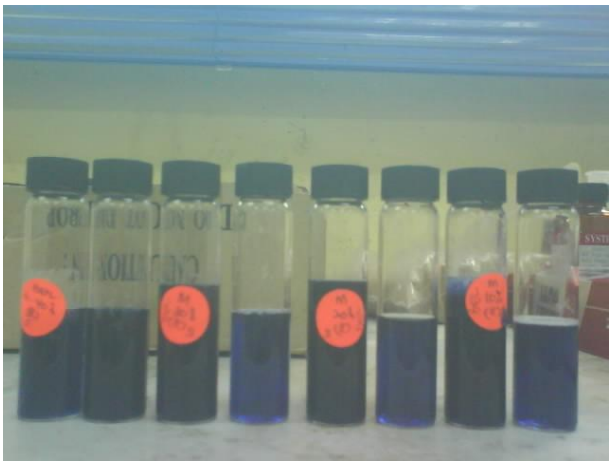


Figure A.6: Samples with methanol after diluted

Figure A.7: The placement in the spectrophotometer

APPENDIX B

Values of CIE Lab parameters (L*, a* and b*) for the analyzed samples by using HACH spectrophotometer DR/4000

Table B.1: L*, a* and b* Values for Concentrated Methanol

% / Days	CIE Lab Parameters	10	20	30	40
1	L*	4.12	12.47	14.58	5.98
	a*	37.83	52.05	52.32	36.12
	b*	-25.01	-43.92	-49.47	-24
2	L*	26.02	9.98	9.75	9.02
	a*	40.61	41.61	50.58	47.16
	b*	-46.79	-25.45	-33.26	-33.28
3	L*	9.49	17.53	4.43	9.6
	a*	42.5	38.39	32.72	49.13
	b*	-16.98	-32.06	-13.15	-30.41
4	L*	3.04	18.61	5.61	12.32
	a*	25.76	45.01	32.37	55.21
	b*	-7.92	-39.96	-7.48	-40.28
5	L*	21.63	12.03	12.98	7.79
	a*	56.74	52.72	54.62	45.12
	b*	-49.21	-33.91	-36.54	-19.75
6	L*	11.4	15.88	14.9	12.86
	a*	50.79	50.73	54.16	25.79
	b*	-25.84	-39.92	-44.33	-12.22
7	L*	13	15.35	15.94	7.72
	a*	49.56	55.82	61.52	4.53
	b*	-32.22	-36.87	-50.21	-17.71

Table B.2: L*, a* and b* Values for Concentrated Ethanol

% / Days	CIE Lab Parameters	10	20	30	40
1	**L***	21.49	8.45	14.1	4.16
	a*	56.69	47.19	50.01	38.42
	b*	-57.38	-30.62	-47	-20.21
2	**L***	7.4	5.79	6.52	5.2
	a*	41.96	40.66	41.66	40.88
	b*	-19.49	-17.3	-21.25	-23.55
3	**L***	9.44	9.21	4.05	4.73
	a*	46.51	44.33	35.58	35.8
	b*	-19.74	-23.41	-1.76	-10.45
4	**L***	6.31	11.05	5.62	4.83
	a*	36.91	47.11	37.32	33.14
	b*	-7.47	-25.19	-9.37	-11.32
5	**L***	13.11	8.06	6.14	10.71
	a*	55.37	44.63	42.1	51.91
	b*	-38	-16.58	-14.35	-32.28
6	**L***	13.5	8.22	9.71	6.38
	a*	55.21	43.96	47.6	41.85
	b*	-35.97	-15.62	-22.81	-11.6
7	**L***	12.37	12.3	10.44	9.48
	a*	52.74	50.39	48.34	49.81
	b*	-29.99	-35.02	-23.33	-26.95