

**QUALITATIVE AND QUANTITATIVE ANALYSIS OF NATURAL
COLORANTS FROM BANANA BRACTS**

FATIAH HAYATI BINTI ABDUL SANI

UNIVERSITI MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

**JUDUL : QUALITATIVE AND QUANTITATIVE ANALYSIS OF NATURAL
COLORANTS FROM BANANA BRACTS**

SESI PENGAJIAN : 2010/2011

Saya **FATIAH HAYATI BINTI ABDUL SANI**

(HURUF BESAR)

mengaku membenarkan tesis PSM ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut :

1. Tesis adalah hakmilik Universiti Malaysia Pahang.
2. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (✓)

☐ **SULIT** (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

☐ **TERHAD** (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

☒ **TIDAK TERHAD**

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PENYELIA)

Alamat Tetap : No. 48, Jalan Setia Jasa
Taman Setia Jasa,
28000 Temerloh,
Pahang Darul Makmur

Fathie bt Ahmad Zakil

Tarikh : **04 DISEMBER 2010**

Tarikh: **04 DISEMBER 2010**

CATATAN : *

**

Potong yang tidak berkenaan.

Jika tesis ini **SULIT** atau **TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai **SULIT** atau **TERHAD**.



Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Lapuran Projek Sarjana Muda (PSM).

“I declare that I have read this thesis and in my opinion this thesis is adequate in terms of scope and quality for the purpose awarding a Bachelor’s Degree of Chemical Engineering.”

Signature :
Supervisor’s Name : FATHIE BT. AHMAD ZAKIL
Date : 04 DECEMBER 2010

**QUALITATIVE AND QUANTITATIVE ANALYSIS OF NATURAL
COLORANTS FROM BANANA BRACTS**

FATIAH HAYATI BINTI ABDUL SANI

A report submitted in partial fulfillment of the
requirement for the award of degree the of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

DECEMBER 2010

DECLARATION

I declare that this thesis 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.

Signature :

Author's Name : FATIHAH HAYATI BINTI ABDUL SANI

Date : 04 DECEMBER 2010

DEDICATION

*Dedicated, in appreciation for support,
encouragement and understanding to my beloved family,
my love one, friends and my supervisor.*

ACKNOWLEDGEMENT

First of all, I would like to express my heartily gratitude to my research supervisor, Ms Fathie binti Ahmad Zakil for her guidance, advices, efforts, supervision and enthusiasm given throughout the progress of this research.

I wish to thank Madam Sazwani for her advice and guidance during handling the rotay evaporator equipment. I am also would like to thank Mr Zulhabri for his assistance and co-operation in helping me using UV-Vis spectrophotometer equipment in the lab.

I would like to express my sincere appreciation to my parents for their support through all these years. Without them, I would not be able complete this research. Besides, I would like to thank my beloved Mahadi Azizi for his encouragement and supporting me in completing my research. Thanks for all the love and care.

ABSTRACT

Natural colorants are preferable nowadays due to their beneficial health values. Natural colorants have been used as one of the colorants in food. The banana bracts, scientifically known as *Musa X paradisiaca* will be used in this study as the raw material. This study aimed on determining the best concentration of methanol in methanol-water, the best temperature to maintain the colorants in the samples extracted and the best storage condition. The banana bract layers will be washed and cut into smaller pieces. The colorant contains in banana bracts, known as anthocyanins will be extracted by blending 10g of banana bracts into the solvent until the bracts fully blended in a blender. Then, the sample extracted will be filtered to remove the residue of banana bract layers. Rotary evaporator were used to remove all methanol from the solutions extracted. Every sample was stored for 3, 6, 9, 12 and 15 days in a dark place. The characteristics of colorants were analyzed by using UV-Vis spectrophotometer. Qualitative analysis was based on the ability of the colorants to stands for 15 days. From the analysis, the best concentration of methanol in methanol- water if the sample was stored in the light was 30% at the temperature of 70°C. When the sample was stored in the dark, the best concentration of methanol in methanol-water was 40% at the temperature of 50°C. Qualitatively, all the samples could last up to 15 days.

ABSTRAK

Pewarna semulajadi menjadi pilihan pada hari ini kerana faedah nilai kesihatannya. Pewarna semulajadi telah digunakan sebagai salah satu pewarna di dalam makanan. Jantung pisang, yang secara saintifiknya dikenali sebagai *Musa X Paradisiaca*, akan digunakan sebagai bahan mentah di dalam kajian ini. Kajian ini bertujuan untuk menentukan kepekatan metanol terbaik di dalam metanol-air, suhu terbaik untuk mengekalkan pewarna di dalam sampel yang diekstrak dan keadaan simpanan terbaik. Lapisan jantung pisang akan dikeringkan di bawah cahaya matahari setelah dibersihkan dan dipotong kecil. Pewarna yang terkandung di dalam jantung pisang, dikenali sebagai *anthocyanins*, akan diekstrak dengan cara menghancurkan 10 gram jantung pisang ke dalam pelarut sehingga semua jantung pisang hancur sepenuhnya. Kemudian, sampel yang diekstrak akan ditapis untuk membuang sisa-sisa lapisan jantung pisang. Penyejat berputar telah digunakan untuk membuang semua metanol daripada larutan yang diekstrak. Setiap sampel akan disimpan selama 3, 6, 9, 12 dan 15 hari di dalam tempat yang gelap. Ciri-ciri pewarna dianalisa dengan menggunakan UV-Vis spectrophotometer. Analisis kualitatif adalah berdasarkan kemampuan pewarna untuk tahan selama 15 hari. Berdasarkan analisis, kepekatan metanol yang terbaik di dalam metanol-air jika sampel disimpan di dalam terang adalah 30% pada suhu 70°C. Apabila sampel disimpan di dalam gelap, kepekatan metanol terbaik adalah 40% pada suhu 50°C. Secara kualitatif, semua sampel boleh tahan sehingga 15 hari.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLE	ix
	LIST OF FIGURE	x
	LIST OF SYMBOLS/ABBREVIATIONS	xi
	LIST OF APPENDICES	xii
 1	 INTRODUCTION	
	1.1 Background of Study	1
	1.2 Problem Statement	2
	1.3 Objective	3
	1.4 Scope	3
	1.5 Rationale and Significance	4
 2	 LITERATURE REVIEW	
	2.1 Banana bracts	5
	2.2 Anthocyanins	6
	2.3 Natural colorants	8
	2.4 Rotary Evaporator	8

	2.5	Color Measurement	10
	2.5.1	UV-Visible Spectrophotometer	11
3		METHODOLOGY	
	3.1	Material and Solvents	13
	3.1	Apparatus	13
	3.3	Sample Preparation	14
	3.4	Extraction	14
	3.5	Storage and Analysis	15
4		RESULTS AND DISCUSSION	
	4.1	Introduction	18
	4.2	Observations	18
	4.2.1	Results by Percentage of Methanol	20
	4.3	Concentration of Anthocyanin	24
	4.3.1	Concentration for Optimum Value	24
5	4.4	Conclusion	26
		CONCLUSION	
	5.1	Conclusion	27
	5.2	Recommendations	28
		REFERENCES	29
		APPENDICES	32

LIST OF TABLES

TABLE NO.	TITLE	PAGE
4.1	Table of observations on colorants extracted	19

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Banana bracts	5
2.2	General anthocyanin structure	7
2.3	Optical System Diagram for UV-Visible spectrophotometer	11
3.1	Sample preparation	14
3.2	Extracted samples	15
3.3	Rotary evaporator	15
3.4	Samples to be stored	16
3.5	UV- Visible Spectrophotometer	16
3.6	Samples for UV-Vis spectrophotometer	17
4.1	Graph absorbance versus temperature for 10% methanol with light	20
4.2	Graph absorbance versus temperature for 10% methanol without light	20
4.3	Graph absorbance versus temperature for 20% methanol with light	21
4.4	Graph absorbance versus temperature for 20% methanol without light	21
4.5	Graph absorbance versus temperature for 30% methanol with light	22
4.6	Graph absorbance versus temperature for 30% methanol without light	22
4.7	Graph absorbance versus temperature for 40% methanol with light	23
4.8	Graph absorbance versus temperature for 40% methanol without light	23

LIST OF SYMBOLS/ABBREVIATIONS

g	-	gram
ml	-	milliliter
%	-	percentage
°C	-	degree Celsius

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A.1	Banana bracts	32
A.2	Anthocyanins chemical structure	32
A.3	Optical System Diagram for UV-Visible spectrophotometer	33
A.4	Sample preparation	33
A.5	Sample extracted	34
A.6	Rotary Evaporator	34
A.7	Samples to be stored	35
A.8	UV-Vis spectrophotometer	35
A.9	Samples for UV-Vis spectrophotometer	36
B.1	Table of observations on colorants extracted	37

CHAPTER 1

INTRODUCTION

1.1 Background of study

Natural colorants were used as attraction to food products. The demands for natural colorants become higher due to safety issues. During ancient time, those people used saffron for its flavor and colorants. In 19th century, many problems occur as a result from the usage of food colorants that made from minerals. Lead chromate and copper sulphate which were used as food colorants during that time had caused deaths because they were contaminated with arsenic. The use of synthetic colorants is becoming less important as they are unsafe and can cause health problems (Vargas and Lopez, 2003).

Nowadays, color additives are widely used as to (1) restore the original food appearance, (2) ensure color uniformity, (3) intensify colors normally found in food, (4) protect other components (such as antioxidants), (5) obtain the best food appearance, (6) preserve characteristics associated with food, and (7) help as a visual characteristic of food quality (Vargas and Lopez, 2003).

Food colorants may be divided into three types, depending on their source (Vargas and Lopez, 2003):

- (a) Natural colors, organic colorants derived from natural edible sources such as anthocyanins;
- (b) Nature-identical colors, manufactured by chemical synthesis so as to be identical to colorants found in nature (e.g. β -carotene and riboflavin);
- (c) Synthetic colors that do not occur in nature, produced by chemical synthesis (e.g. tartrazine and carmoisine).

In this study, banana bracts (*Musa X paradisiaca*) are used as raw material to extract its natural colorants which is anthocyanins. Most bananas have red, purple or violet bracts although a few are acyanic- green or yellow. The variation in bract color is correlated with the composition of the anthocyanins present, which is distinctive of species and sub species (Duran *et al.*, 2000). Banana bracts were usually thrown away as waste during harvesting season back then. Recently, anthocyanin pigments in banana bracts were found as potential food colorants. Anthocyanins have become a good choice for food colorants as it is easily dissolve in water. New sources of anthocyanins with high stability and low cost are desired as natural food colorants (Duran *et al.*, 2000).

1.2 Problem Statement

Synthetic dyes were more preferable than natural colorants. There were lots of studies on natural colorants before but they were not used widely. This is because natural colorants are unstable as they are easily influenced by pH value, temperature and presence of oxygen, enzymes and condensation reactions. In this study, the qualitative and quantitative properties of natural colorants will be determined.

The objective of doing this study is to determine and to select which sample can extract the most colorants from the banana bracts and last the longest. Temperature and concentration of methanol in methanol-water will be the parameters

to determine the best sample of colorants so that it can be used for food industry in Malaysia.

1.3 Objectives

The purpose of this study is to determine the best storing temperature of sample and the best concentration of methanol in methanol-water during extraction process in order to see which sample can be stored longer and the color can stands longer. In this study, anthocyanin is the colorant contains in banana bracts.

1.4 Scope of Study

Raw material that is to be used in this experiment is banana bracts. Banana bracts are very easy to get as it comes from banana tree. The colored layers of banana bracts are easily obtained because banana is one of local fruits. The layers of banana bracts are taken and washed. Banana bracts need to be dried so that the moisture is eliminated before processing them.

In the extraction process, anthocyanins were extracted with varied concentration of methanol in methanol-water. Methanol concentrations to be used in this study are 10%, 20%, 30% and 40%. The layers of banana bracts are blended with the solvent in a blender.

After the blending process is done, all samples will be stored in the water bath for 30 minutes at room temperature (30°C), 50°C, 60°C, 70°C and 80°C. Four samples will be stored at room temperature to see the ability of the color to stands for 15 days.

After extraction is completed, every sample will be put in a rotary evaporator to eliminate all the methanol contains in every sample. Then, every sample will be put in the water bath accordingly. UV-Visible spectrophotometer is used to determine the color absorbance of the sample.

1.5 Rationale and Significance of this Study

The rationale of doing this study is banana bracts are easily found. The layers of banana bracts are usually thrown away when people want to cook them. Rather than wasting them, they can be useful as a natural source of natural colorants. This source of anthocyanins is low cost making it desired as natural food colorants.

The significance of doing this study is to investigate the best condition to extract colorants from banana bracts in order to make it last long. If the colorants can last long, it can be used for a longer period. Besides, this study is done to determine the intensity of colorants exist in banana bracts. The optimum anthocyanins in a sample will be chosen to be the best sample and can be used as colorants in food production. The outcomes from this study can also open up opportunities to increase the demand of natural colorants for food industry in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.6 Banana bracts

Banana bracts or well-known as banana hearts are a part of banana tree with purple layers of skin. In Malaysia, the people especially Malays used banana bracts for culinary purposes. Banana bracts are also available at supermarkets as Malaysia is one of the banana's producers. Since the bracts of banana are widely available and have been traditionally used as food without apparent toxic effect, they could be a potential source of anthocyanins (Duran *et al.*, 2000) to be natural colorants.



Figure 2.1: Banana bracts (laocook.com)

Most bananas have red, purple or violet bracts although a few are acyanic-green or yellow. The variation in bract color is correlated with the composition of the anthocyanins present, which is distinctive of species and subspecies (Durán *et al.*, 2000).

2.2 Anthocyanins

Anthocyanins are natural colorants which have raised a growing interest due to their extensive range of colours, innocuous and beneficial health effects. Despite the great potential of application that anthocyanins represent for food, pharmaceutical and cosmetic industries, their use has been limited because of their relative instability and low extraction percentages. Currently, most investigations on anthocyanins are focused on solving these problems, as well as their purification and identification (Ovando *et al.*, 2008). The isolated anthocyanins are highly instable and very susceptible to degradation (Giusti & Wroslad, 2003). Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, flavonoids, proteins and metallic ions (Rein, 2005).

Anthocyanins are flavonoid phenolic compounds, widely distributed among fruits, berries and flowers, providing attractive colours, such as orange, red and blue. These pigments are water-soluble and this property facilitates their incorporation into numerous aqueous food systems. These qualities make anthocyanins attractive natural colorants. Moreover, it has been demonstrated that, in addition to their colourful characteristics, anthocyanins possess some positive therapeutic effects, mainly associated with their antioxidant properties (Longo and Vasapollo, 2004). Anthocyanins have recently received increasing attention as natural colorants in food systems, as a consequence of the social trend toward the consumption of natural products instead of synthetic ones. Thus, new sources of pigments, such as anthocyanins, with high colorant power, stability and low cost are nowadays desired (Longo and Vasapollo, 2004).

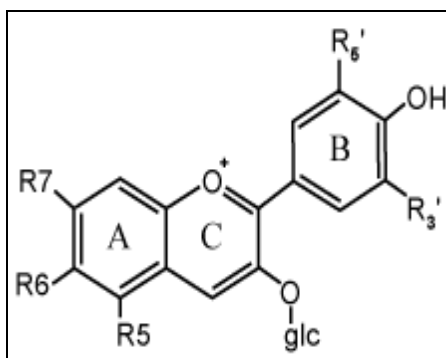


Figure 2.2: General anthocyanin structure (www.micro-ox.com)

The anthocyanin structures produce a great range of colors from scarlet to blue that are clearly represented in flowers and fruits, although they are also present in leaves and storage organs. Anthocyanins are common in higher plants but are absent in some lower plants such as liverworts and algae (Vargas and López, 2003).

According to the numbering system used by the Codex Alimentarius Commission, anthocyanins (any anthocyanin-derived colorant) are listed as a natural colorant by the European Union (EU) legislation as product E163. With respect to the US, the FDA (Food and Drug Administration) has a different list of “natural” colors that do not require certification (without any FD & C numbers), and anthocyanins can be obtained either from “grape color extract”, “grape skin extract”, “fruit juices or vegetable juices”. Nevertheless, the use of anthocyanins in food products is restricted to some products varying among countries. Usually, the USA is the most restrictive country regarding the use of anthocyanin colorants (Gould *et al.*, 2009).

Anthocyanins are interesting pigments regarding their chromatic features. The oldest anthocyanin extract used in the food industry is enocyanin obtained from red grape pomace and marketed in Italy since 1879. Nowadays, grape extracts are often used as colorants for sugar confectionary, dairy products, ice creams, etc (Gould *et al.*, 2009).

2.3 Natural colorants

Food coloring (colouring) is any substance that is added to food or drink to change its color. Food coloring is used both in commercial food production and in domestic cooking (www.wikipedia.org). Natural colorants are colors that can be obtained from natural sources such as fruits and vegetables. Colors that occur in these natural sources are caused by the content in them.

Natural colorants were not an option for food colorants back then because of its stability. They are water-soluble, which facilitates their incorporation into aqueous food systems. They are widespread in nature and are innocuous. These qualities make them attractive natural colorants. However, some factors, such as the low stability when compared with synthetic dyes have limited their use (Durán *et al.*, 2001).

Colour is an important factor in the acceptability of a food product. The safety of synthetic colorants has previously been questioned, leading to a reduction in the number of permitted colorants. Due to this limitation and to the worldwide tendency towards the consumption of natural products, the interest in natural colorants has increased significantly (Durán *et al.*, 2001).

2.4 Rotary Evaporator

The rotary evaporator is a device for gently and efficiently evaporating solvents from a mixture. It consists of a heated rotating vessel (usually a large flask) which is maintained under a vacuum through a tube connecting it to a condenser. The rotating flask is heated by partial immersion in a hot water bath. The flask's rotation provides improved heat transfer to the contained liquid; the rotation also strongly reduces the occurrence of 'bumps' caused by superheating of the liquid. The solvent vapors leave the flask by the connecting tube and are condensed in the condenser section. The condenser section is arranged so that the condensed vapors drain into

another flask where they are collected. It is a very efficient way of rapidly removing large quantities of solvent. The major use in chromatography is the recovery of non-volatile solutes in preparative chromatography and the recovery of solvents for recycling. The device is also used for preparing coated supports for gas chromatography. For this application a weighed amount of support is placed in the flask, the required amount of stationary phase dissolved in excess of solvent is added to the solid and mixture tumble dried. This procedure has two advantages; as well as drying the support it ensures a very even distribution of the stationary phase throughout the support (chromatography-online.org).

A vacuum is a key component of this device, and is used to aid in the evaporation of the solvent. A vacuum will lower the air pressure in the space above a liquid, thereby lowering the boiling point of the liquid being heated. Often the mixture is very sensitive and highly reactive so excessive heating would not be ideal. The vacuum allows the user to temporarily alter the physical properties of the sample so that solvents can be extracted from a variety of mixtures that would otherwise be impossible. The simplest type of vacuum used on a rotary evaporator is a water aspirator. Picture a tube that is wider at the top and narrows as it goes down, with an opening at the side. When water is channeled downwards, it increases speed, and the pressure is lowered - creating a vacuum effect through the opening at the side. A more complex method would involve a regulated mechanical vacuum pump (article-niche.com).

Rotation is not absolutely necessary for the evaporation process to take place. However, the centrifugal forces allow the solvent to rise and be spread thinly over a larger surface, which makes it easier to extract. Also, the rotation helps reduce “bumping.” Bumping is what happens when two substances come together, like water and ethanol, and the total amount is reduced. This is due the difference in the size of the molecules (article-niche.com).

The rotary evaporator has evolved over the years to become more and more advanced, and while features and capabilities of different models vary widely, modern rotary evaporators still consist of seven primary components (article-niche.com):

- The motor that rotates the sample (contained in either a flask or vial)
- Hot water bath to heat the sample
- The vacuum used to lower the pressure
- A vapor duct to capture the solvent
- Condenser that cools the solvent
- Flask to collect the solvent after it condenses
- A mechanism to quickly remove the sample from the hot water bath

2.5 Color Measurement

The principal attributes of object colors are hue, lightness, and saturation (Vargas and López, 2003). Hue is the quality that we normally identify with a color name such as red, green, and blue. Lightness is a term related to the concept of light and dark by considering color as a source of reflected light. Lightness is the light reflected by a surface in comparison to a white surface, under similar conditions of illumination. A related term is brightness, but this is used for the total light from the illuminant or reflected from a surface. Lightness and brightness are grouped in the term value, although lightness and value are commonly used interchangeably. Saturation is the clarity or purity of a color. Also, it can be understood as the intensity of hue in comparison to its own brightness. A saturated color looks clear and bright, but an unsaturated color appears pale, muddy, or dull (Vargas and López, 2003).

2.5.1 UV-Visible Spectrophotometer

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length, b , and the concentration, c , of the absorbing species. Beer's Law states that

$A = \epsilon bc$, where ϵ is a constant of proportionality, called the absorptivity (teaching.shu.ac.uk).

Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. For example, the absorption that is observed in the UV region for the carbonyl group in acetone is of the same wavelength as the absorption from the carbonyl group in diethyl ketone (teaching.shu.ac.uk).

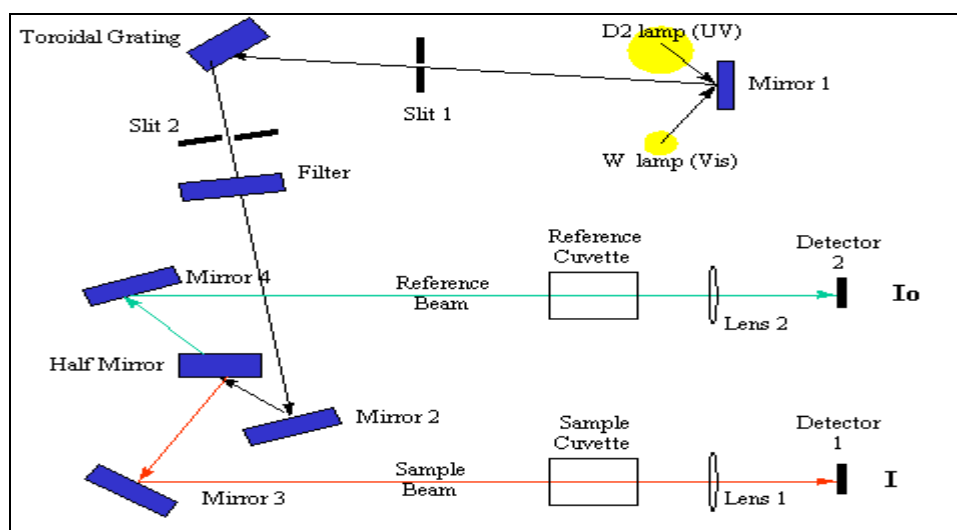


Figure 2.3: Optical System Diagram for UV-Visible spectrophotometer

The UV-Visible spectrophotometer uses two light sources, a deuterium (D_2) lamp for ultraviolet light and a tungsten (W) lamp for visible light. After bouncing off a mirror (mirror 1), the light beam passes through a slit and hits a diffraction grating. The grating can be rotated allowing for a specific wavelength to be selected. At any specific orientation of the grating, only monochromatic (single wavelength) successfully passes through a slit. A filter is used to remove unwanted higher orders

of diffraction. (Recall the experiment you did last semester on Atomic Spectra) The light beam hits a second mirror before it gets split by a half mirror (half of the light is reflected, the other half passes through). One of the beams is allowed to pass through a reference cuvette (which contains the solvent only), the other passes through the sample cuvette. The intensities of the light beams are then measured at the end.

CHAPTER 3

METHODOLOGY

3.1 Materials and Solvents

The banana bract species in this study is named *Musa X paradisiaca* from Malaysia that bought from the night market in Gambang. The solvent used to extract the colorants is methanol and water. Methanol and water will be mixed and used to extract the colorants from banana bracts.

3.2 Apparatus

For drying process, banana bracts layers will be washed and dried to remove the moisture content in the banana bracts. Besides, it is easier to store a dry banana bract to prevent it from being damaged. In the extraction process, methanol-water is used to pull out the colorants from the banana bracts. A blender was used to blend the layer of banana bracts. Absorbance of the colorants will be determined by using UV- Visible Spectrophotometer. Another equipment to be used is rotary evaporator. This equipment is needed to remove the content of methanol in all samples of extracted banana bract colorants.

3.3 Sample Preparation

The colored layers of banana bracts are to be taken. They need to be washed with cold water to remove any dirt on the layers. The layers will be cut into smaller pieces before the blending process. Then, wipe the banana bracts to remove all the water. The banana bracts can now be used for extraction procedure.



Figure 3.1: Sample preparation

3.4 Extraction

After the solvents are prepared, extraction process can be done. 10 g of layers of banana bracts will be put in the solvents and will be blended in a blender. The concentration for every sample will be different; 10%, 20%, 30% and 40% methanol in methanol-water.



Figure 3.2: Extracted samples

3.5 Storage and analysis

When the extraction process was finished, the solvent will be removed by using Büchi rotary evaporator. By using rotary evaporator, methanol was eliminated. The temperature used for the elimination of methanol is 70°C , which is higher than the boiling point of methanol. After all methanols were eliminated, the samples were stored.



Figure 3.3: Rotary evaporator

After the elimination of methanol, the samples will be put in a water bath at different temperature. The temperature used was varied ; 50°C, 60°C, 70°C and 80°C. All samples were stored in the light and in the dark.

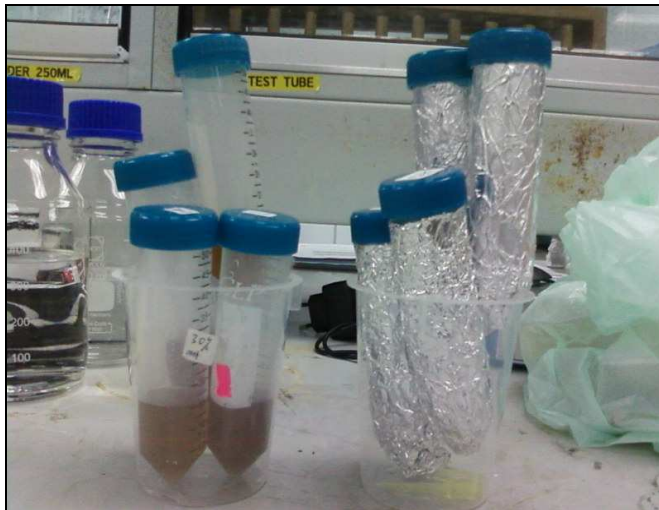


Figure 3.4: Samples to be stored

After every 30 minutes, samples that were stored in the light and in the dark were analyzed by using UV-Vis spectrophotometer. UV-Vis spectrophotometer analyzed the samples and showed the value of absorbance for each of the samples. The wavelength used was 700 nm. From the value of absorbance, concentration of colorants in the samples was determined.



Figure 3.5: UV- Visible Spectrophotometer

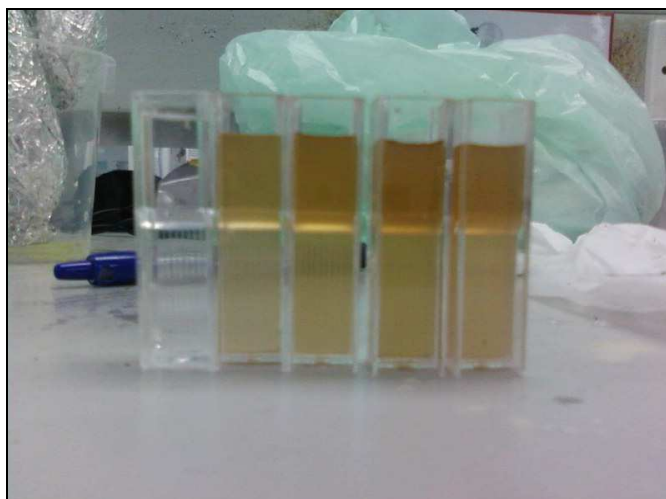


Figure 3.6: Samples for UV-Vis spectrophotometer

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

For this chapter, there are four aspects being considered. The aspects are the concentration of methanol in methanol- water solution, storage temperature, storage condition and the ability of the colorants to stand for 15 days.

4.2 Observations

After the extraction process, elimination of methanol was made by using rotary evaporator. Then, all samples were stored in a water bath at different temperature and storage condition. Table 4.1 shows the observation on the absorbance of the colorants after analysis by using UV-Vis spectrophotometer.

Table 4.1: Table of observations on colorants extracted.

Percentage of methanol (%)	Temperature (°C)	Storage condition	Time (min)	Absorbance (Au)
10	50	With light	30	0.571
		Without light	30	0.698
	60	With light	30	0.618
		Without light	30	0.633
	70	With light	30	0.617
		Without light	30	0.636
	80	With light	30	0.609
		Without light	30	0.595
20	50	With light	30	0.992
		Without light	30	0.950
	60	With light	30	0.999
		Without light	30	0.937
	70	With light	30	1.021
		Without light	30	1.026
	80	With light	30	1.012
		Without light	30	1.015
30	50	With light	30	1.151
		Without light	30	1.194
	60	With light	30	1.156
		Without light	30	1.305
	70	With light	30	1.186
		Without light	30	1.257
	80	With light	30	1.185
		Without light	30	1.282
40	50	With light	30	1.086
		Without light	30	1.302
	60	With light	30	1.060
		Without light	30	1.038
	70	With light	30	1.060
		Without light	30	1.048
	80	With light	30	1.076
		Without light	30	1.080

From the observations, the highest absorbance was obtained from the sample that extracted by 30% methanol in methanol-water solution at the temperature of 70°C when the sample stored in the light with a value of 1.186 Au. For the samples that were stored in the dark, the highest absorbance was obtained from the sample that extracted by 30% methanol in methanol- water solution at the temperature of 60°C with an absorbance value of 1.305 Au.

4.2.1 Results by Percentage of Methanol

(a) 10% (with light)

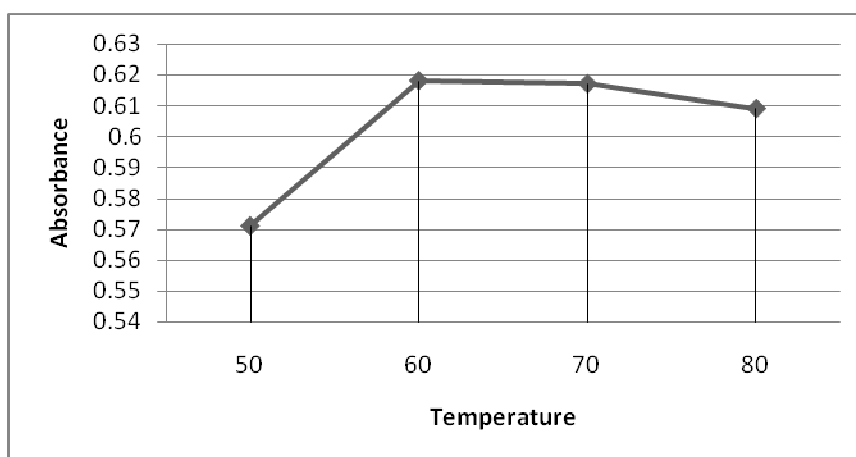


Figure 4.1: Graph absorbance versus temperature for 10% methanol with light

(b) 10% (without light)

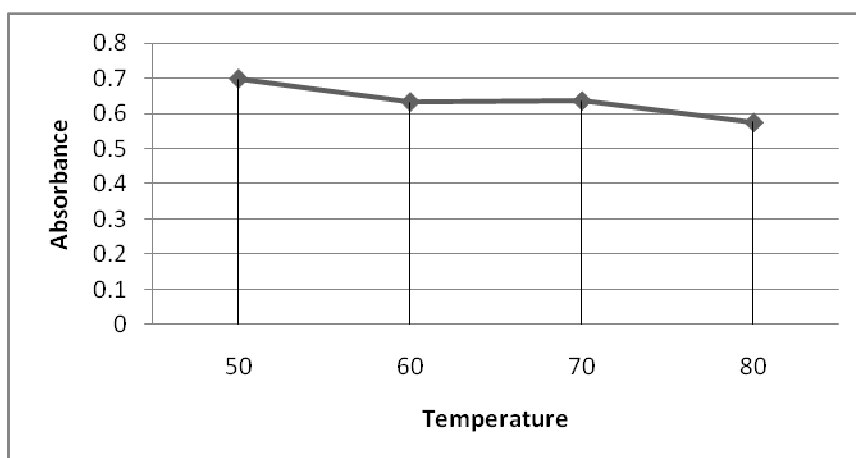


Figure 4.2: Graph absorbance versus temperature for 10% methanol without light

c) 20% (with light)

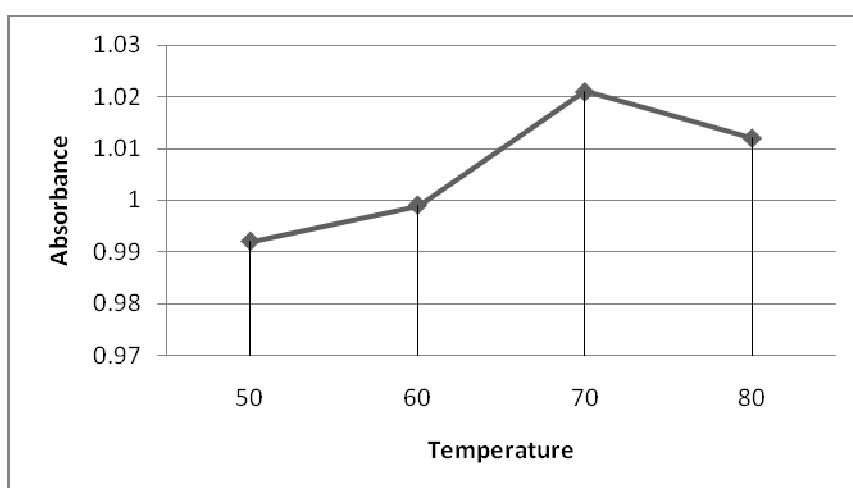


Figure 4.3: Graph absorbance versus temperature for 20% methanol with light

(d) 20% (without light)

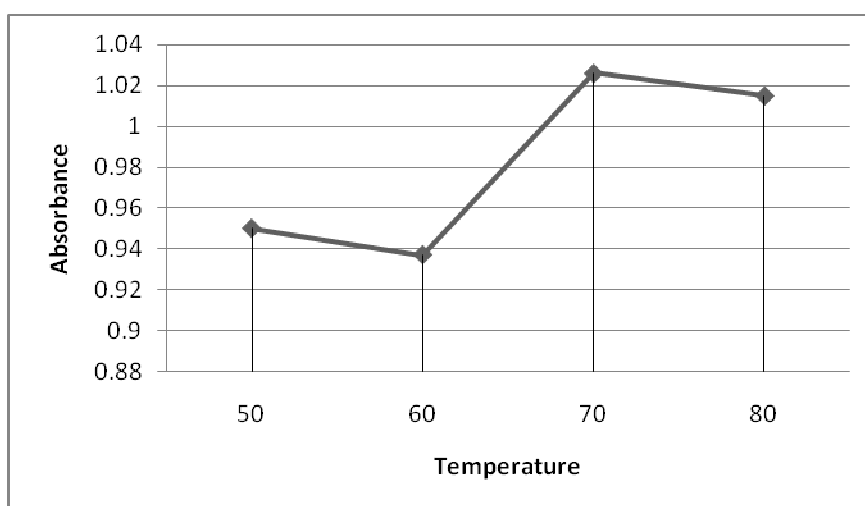


Figure 4.4: Graph absorbance versus temperature for 20% methanol without light

(e) 30% (with light)

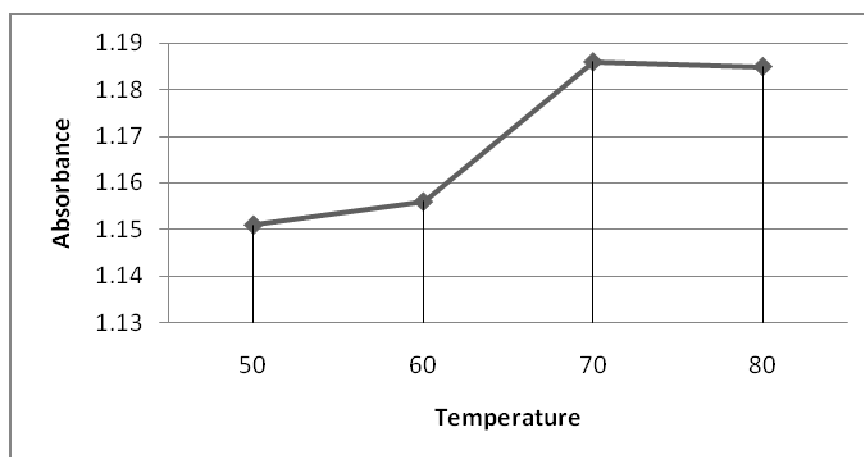


Figure 4.5: Graph absorbance versus temperature for 30% methanol with light

(f) 30% (without light)

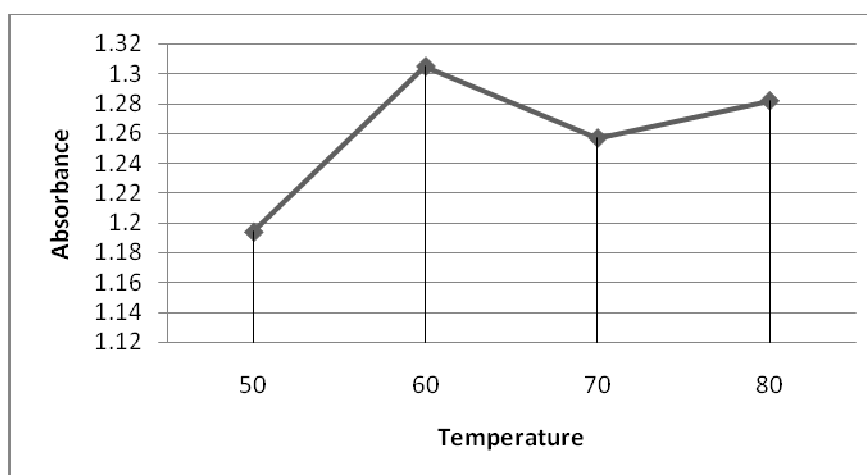


Figure 4.6: Graph absorbance versus temperature for 30% methanol without light

(g) 40% (with light)

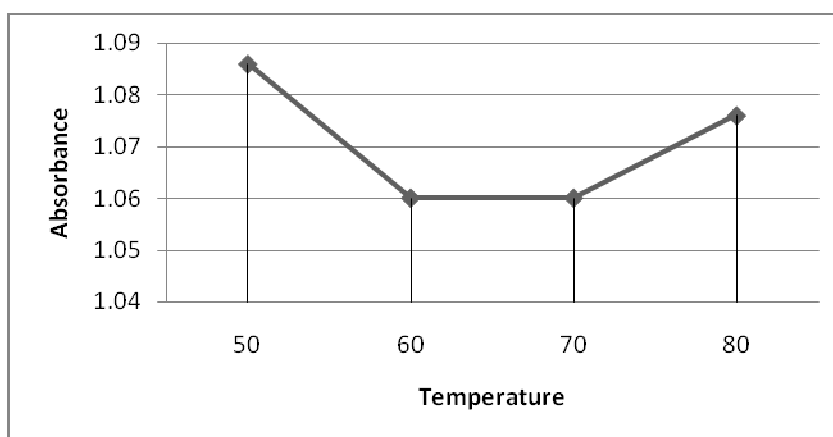


Figure 4.7: Graph absorbance versus temperature for 40% methanol with light

(h) 40% (without light)

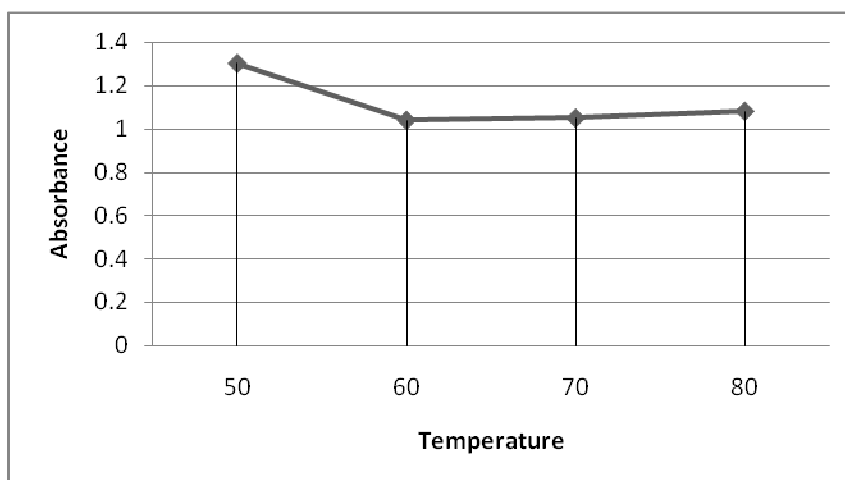


Figure 4.8: Graph absorbance versus temperature for 40% methanol without light

Generally, all of the graph above showing the value of absorbance at different temperature and storage condition. The values were fluctuated as the content of colorants varied in every sample.

For the results obtain in the table above, every sample will have different readings of absorbance. This is because every sample will contain different content of colorants. Some sample will have high content of colorants and some sample will contain low content of colorants.

Different storage temperature are used to analyze which sample can stands for 30 minutes and still have high content of colorants. When the value of absorbance of sample is higher, then the content of colorants is higher.

There were two different storage condition used in this study. Some samples were stored in the light and some samples were stored in the dark. Based on the results obtained, highest absorbance was obtained when the sample was stored in the dark. This is because when the sample is stored in the dark, the colorants will not degrade.

4.3 Concentration of Anthocyanins

From the value of absorbance, concentration of colorants can be calculated. The formula used to determine the concentration of colorants in banana bracts is:

$$\text{Monomeric anthocyanin pigment (mg/liter)} = (A \times MW \times DF \times 1000) / (\epsilon \times 1)$$

Where;

A = Absorbance

ϵ = Cyd-3-glu molar absorbance (26900)

MW = anthocyanin molecular weight (449.2)

DF = dilution factor

4.3.1 Concentration for Optimum Value

The best concentration of methanol when the sample stored in the light is 30% at the temperature of 70°C. The absorbance value is 1.186. From the formula above, concentration of colorants can be calculated.

$$\begin{aligned}\text{Monomeric anthocyanin pigment (mg/liter)} &= \frac{1.186 \times 449.2 \times 1 \times 1000}{26900 \times 1} \\ &= 19.8049 \text{ mg/liter}\end{aligned}$$

When the sample stored in the dark, the best concentration of methanol to extract the colorant is 30% at the temperature of 60°C. The concentration of colorants is calculated as below:

$$\begin{aligned}\text{Monomeric anthocyanin pigment (mg/liter)} &= \frac{1.305 \times 449.2 \times 1 \times 1000}{26900 \times 1} \\ &= 21.7920 \text{ mg/liter}\end{aligned}$$

Based on the concentration calculated for both condition, the best condition to store the colorants from banana bracts will be in the dark.

4.4 Conclusion

From the results obtained, it can be concluded that the best condition to extract the colorants from banana bracts is at the concentration of methanol 30% by storing the colorant at the temperature of 60°C and in the dark place. The colorants can also stands until 15 days without losing the colors.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Banana bracts have been recognized as one of a good source for natural colorants. Natural colorants can be used to replace synthetic dye as the synthetic dye is dangerous to human health. Since the bracts of banana are widely available and have been traditionally used as food without apparent toxic effect, they could be a potential source of anthocyanins (Durán *et al.*, 2000).

Overall, this study gives results in terms of best solvent concentration, best storage temperature and best storage condition. The best methanol percentage in methanol-water solution was 30% in 100ml of total solution. The best storage temperature was 60°C and should be stored in the dark place.

5.2 Recommendations

To achieve a better result for the further study, there are several recommendations that need to be considered. To obtain a more accurate readings of the percentage of colorants exist in the extracted sample, CIE $L^*a^*b^*$ colorimeter can be used. The three parameters in the model represent the lightness of the color (L^* , $L^*=0$ yields black and $L^*=100$ indicates white), its position between magenta and green (a^* , negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b^* , negative values indicate blue and positive values indicate yellow).

In addition, to know better about the content of sample extracted, FTIR can be used as it can detect the content of the sample. Anthocyanin is a general name for the colorant exists in banana bracts. To know the specific type of anthocyanin existed in the banana bracts, FTIR is a good equipment to be used.

Therefore, all the information that has been obtained from this study can be used as a reference in further research.

REFERENCES

- Araceli Castañeda-Ovando, Ma. de Lourdes Pacheco-Hernández, Ma. Elena Páez Hernández, José A. Rodríguez, Carlos Andrés Galán-Vidal. Chemical studies of anthocyanins: A review. *Journal of Food Chemistry*. 113 (2009) 859–871.
- E. Alexandra Pazmino Duran, M. Monica Giusti, Ronald E. Wrolstad (2000). Anthocyanins from banana bracts (*Musa X Paradisiaca*) as potential food colorants. *Food Chemistry*. 73, 327-332.
- E. Alexandra Pazmino Duran, M. Monica Giusti, Ronald E. Wrolstad, M. Beatriz A. Gloria (2001). *Food Chemistry*. 75, 211-216.
- Emille R.B.A Prata, Leandro S. Oliveira (2006). *Fresh Coffee Husks as a Potential Sources of Anthocyanins*. 40, 1555-1560.
- Francisco Delgado-Vargas, Octavio Paredes-López (2003). *Natural Colorants for Food and Nutraceutical Uses*. CRC Press.
- Fuleki, T. Francis, F.J., *Quantitative Methods for Anthocyanins*. 2. Determination of Total Anthocyanin and Degradation Index for Cranberry Juice, *Journal Food Science*, Vol. 33, (1968) pp 78-83.
- G.H Laleh, H. Frrydoonfar, R. Heidary, R. Jameei, S. Zare (2006). *The Effect of Light, Temperature, pH and Species on Stability of Anthocyanin Pigments in Four Berberis Species*. Asian Network for Scientific Information.

- Ganapathi Patil, M.C Madhusudhan, B. Ravindra Babu, K.S.M.S Raghavarao (2006). *Extraction, Dealcoholization and Concentration of Anthocyanin From Red Raddish*. 43, 364-369.
- Giusti, M.M., & Wrolstad, R.E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, 14(3), 217-225.
- Giusti, M.M., Rodriguez-Saona, Wrolstad, R.E. *Molar Absorptivity and Color Characteristics of Acylated and Non-Acylated Pelargonidin-Based Anthocyanins*. Vol. 47, No. 11, pp 4631-4637.
- Kasipong Kitdamrongsont, Pongsagon Pothavorn, Sasivimon Swangpol, Siripope Wongniam, Kanokporn Atawongsa, Jisnuson Svasti, Jamorn Somana (2008). *Anthocyanin Composition of Wild Bananas in Thailand*. 56, 10853- 10857.
- Kevin Gould, Kevin Davies, Chris Winefield (2009). *Anthocyanins: Biosynthesis, Functions, and Applications*. Springer Science+Business Media.
- Kiattisak Duangmal, Busararat Saicheua, Suchitra Sueeprasan (2004). *Roselle Anthocyanins as a Natural Food Colorant and Improvement of its Color Stability*. *AIC 2004*
- Luiga Longo, Giuseppe Vasapollo (2004). *Extraction and Identification of Anthocyanins from Smilax aspera L. Berries*. *Food Chemistry*. 94,226-231.
- M. Monica Giusti, Ronald E. Wrolstad (2001). *Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy*. F1.2.1- F1.2.13.
- Nezhet Turker, Salih Aksay, H. Ibrahim Ekiz (2004). *Effect of Storage Temperature on the Stability of Anthocyanins of a Fermented Black Carrot (Daucus carota var. L.) Beverage: Shalgam*. 52, 3807-3813.

Pritam Chattopadhyay, Sandipan Chatterjee, Sukanta K. Sen (2008).

Biotechnological Potential of Natural Food Grade Biocolorants. 17, pp 2972- 2985.

R.E. Wrolstad, R.W. Durst, J. Lee, Tracking colour pigment changes in anthocyanin products. *Trends Food Sci. Technol.* 16 (2005) 423-428.

R.E Wrolstad. *Color and Pigment Analyses in Fruit Products, Agricultural Experiment Station*. Station Bulletin 624.

Rein, M. (2005). *Copigmentation reactions and color stability of berry anthocyanins*. Helsinki: University of Helsinki. pp. 10-14.

Tunde Vatai, Mojca Škerget, Zeljko Knez, Sabine Kareth, Manuel Wehowski, Eckhard Weidner. Extraction and formulation of anthocyanin-concentrates from grape residues. *Journal of Supercritical Fluids*. 45 (2008) 32–36

<http://www.wikipedia.org> Food colorants

<http://www.wikipedia.org> Lab Color Space

<http://laocook.com> Banana bracts

<http://www.micro-ox.com> Anthocyanins

<http://linocolor.com> CIE L*a*b* Color Space

<http://www.chromatography-online> Rotary Evaporator

<http://article-niche.com> Rotary Evaporator

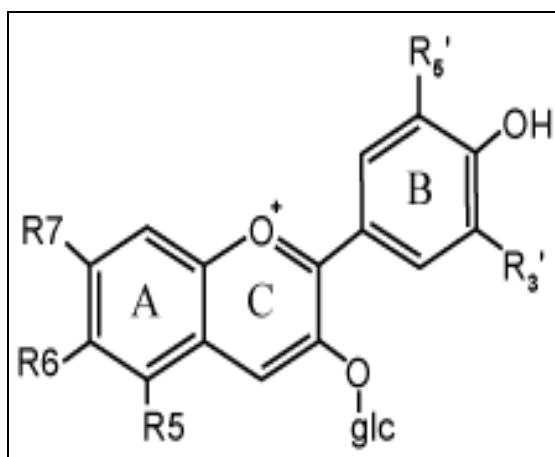
<http://teaching.shu.ac.uk> UV-Vis Spectrophotometer

<http://141.161.23.43> UV-Vis Spectrophotometer

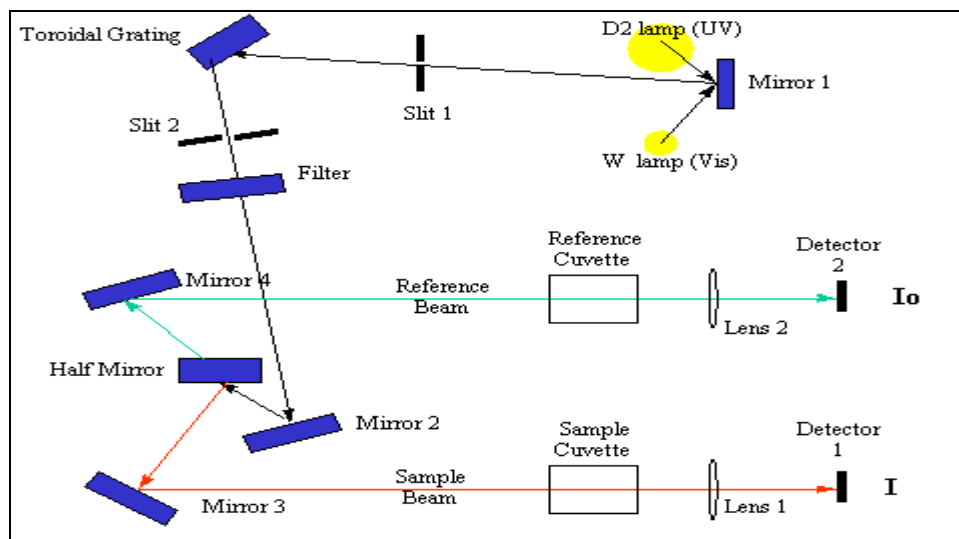
APPENDIX A



Appendix A.1: Banana bracts



Appendix A.2: Anthocyanins chemical structure



Appendix A.3: Optical System Diagram for UV-Visible spectrophotometer



Appendix A.4: Sample preparation



Appendix A.5: Sample extracted



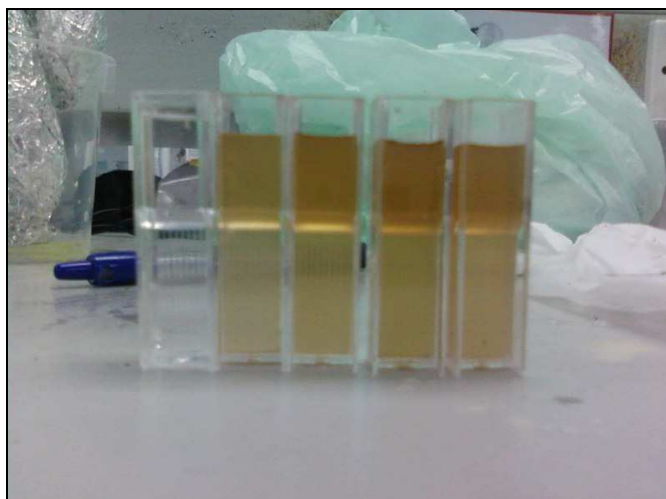
Appendix A.6: Rotary Evaporator



Appendix A.7: Samples to be stored



Appendix A.8: UV-Vis spectrophotometer



Appendix A.9: Samples for UV-Vis spectrophotometer

APPENDIX B

Appendix B.1: Table of observations on colorants extracted

Percentage of methanol (%)	Temperature (°C)	Storage condition	Time (min)	Absorbance (Au)
10	50	With light	30	0.571
		Without light	30	0.698
	60	With light	30	0.618
		Without light	30	0.633
	70	With light	30	0.617
		Without light	30	0.636
	80	With light	30	0.609
		Without light	30	0.595
20	50	With light	30	0.992
		Without light	30	0.950
	60	With light	30	0.999
		Without light	30	0.937
	70	With light	30	1.021
		Without light	30	1.026
	80	With light	30	1.012
		Without light	30	1.015
30	50	With light	30	1.151
		Without light	30	1.194
	60	With light	30	1.156
		Without light	30	1.305
	70	With light	30	1.186
		Without light	30	1.257
	80	With light	30	1.185
		Without light	30	1.282
40	50	With light	30	1.086
		Without light	30	1.302
	60	With light	30	1.060
		Without light	30	1.038
	70	With light	30	1.060
		Without light	30	1.048
	80	With light	30	1.076
		Without light	30	1.080