BEHAVIOUR AND PERFORMANCE OF ANTHOCYANNINS *Brassica oleracea* AND THEIR CO-PIGMENTS IN FOOD HYDROCOLLOIDS

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ABSTRACT

Anthocyannins are pigments with attractive colours, ranging from red at low pH, to blue and green at high pH. Anthocyannins can be found in a number of natural sources, including strawberries, sweet potatoes, yams, grapes, and red cabbages. In this work, the effects of ultraviolet (UV) radiation on the degradation of anthocyannins from red cabbages, incorporated in food hydrocolloids will be investigated, in terms of anthocyannin colour stability and change in concentration. To study short term and long term effects of UV exposure to anthocyannins in food application, three types of UV radiation, namely UV-A, UV-B, and UV-C were used. The UV effects were demonstrated by increasing the frequency of the radiation. In the first part of the work, the anthocyannins were extracted from red cabbages, and purified using double chromatographies, namely ion exchange chromatography and size exclusion chromatography. The later extracts the pigment better than the former, as distinct fraction colours were observed during the process. The anthocyannin extract was freeze-dried and stored at - 20°C until used in food hydrocolloids, namely agar, carrageenan and gelatine for the UV study. Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) was also performed on the purified anthocyannins, and the results confirmed the presence of cyanidins in the sample. pH differential method was done to calculate the total monomeric content of anthocyannins in the red cabbage sample, prior to UV exposure. The pure anthocyannin was applied in food hydrocolloids, namely agar, carrageenan and gelatine for the UV study. In the second part of the work, colorimetric study i.e. CIELAB and chemometric study i.e. FT-IR spectroscopy were performed to evaluate anthocyannin colour stability, and change in anthocyannin concentration, respectively. The anthocyannin samples were mixed with copigments, namely cinnamic acid, ferulic acid, and gallic acid, to study the performance of anthocyannins with the copigments. In the colorimetric study, all hydrocolloid samples containing anthocyannin

became fader throughout 35 days of storage under exposure to UV radiations as the radiations were destructive, and cross-linking of hydrocolloids was enhanced under UV exposure, which caused changes in hue and lightness. The effects became more pronounced from UV-A to UV-B and UV-C, as the frequency of radiation was increased. However, samples containing anthocyannin-copigment complexes were duller than samples without copigment, as the water contained in the hydrocolloids suppressed copigmentation. In the FT-IR spectroscopy, concentration of anthocyannins was shown to generally decrease during the 35 days of storage under exposure to UV radiations and the effects became more pronounced from UV-A to UV-B and UV-C, due to destructive effects of UV radiations. In conclusion, UV-C has the greatest effect on the degradation of anthocyannin, followed by UV-B and UV-A, in a given period of time. This means that when anthocyannins are incorporated in food, the degradation becomes increasingly faster from UV-A to UV-B and UV-C. In the future, it may be possible to manipulate the chemical bonds and functional groups of anthocyannin to generate new colours, which can then be applied for industrial use, such as in textile dyeing.

ABSTRAK

Antosianin merupakan pigmen dengan warna-warna yang menarik, daripada merah pada pH yang rendah, hingga biru dan hijau pada pH yang tinggi. Antosianin boleh didapati dari pelbagai sumber semulajadi, termasuklah strawberi, keledek, keladi, anggur, dan kubis merah. Dalam kajian ini, kesan sinaran ultraviolet (UV) terhadap degradasi antosianin, yang diletakkan di dalam hidrokoloid makanan disiasat, dari segi kestabilan warna antosianin dan perubahan kepekatan. Untuk mengkaji kesan jangka pendek dan jangka panjang sinaran UV terhadap antosianin dalam pengaplikasian makanan, tiga jenis sinaran UV iaitu UV-A, UV-B dan UV-C telah digunakan. Kesan UV ditunjukkan dengan meningkatkan frekuensi sinaran. Dalam kajian pertama, antosianin diekstrak daripada kubis merah, dan ditulenkan melalui dua kromatografi iaitu kromatografi pertukaran ion dan kromatografi penyisihan saiz. Kromatografi kedua mengekstrak pigmen tersebut dengan lebih baik berbanding yang pertama kerana warna jalur yang berbeza telah diperhatikan semasa proses tersebut. Ekstrak antosianin dikering-bekukan dan disimpan pada -20°C sehingga digunakan di dalam hidrokoloid makanan iaitu agar-agar, karagenan dan gelatin untuk kajian UV. Kromatografi cecair-spektrometri jisim/spektrometri jisim (LC-MS/MS) juga dijalankan terhadap antosianin yang telah ditulenkan, dan keputusannya mengesahkan kehadiran sianidin. Kaedah pembezaan pH dijalankan untuk menghitung jumlah kandungan monomer antosianin di dalam sampel kubis merah, sebelum didedahkan kepada UV. Antosianin yang telah ditulenkan diaplikasikan dalam hidrokoloid makanan iaitu agar-agar, karagenan dan gelatin untuk kajian UV. Dalam kajian kedua, kajian kolorimetri iaitu CIELAB, dan kajian kemometri iaitu spektroskopi FT-IR telah dijalankan untuk menilai masing-masing kestabilan warna antosianin, dan perubahan kepekatan antosianin. Sampel antosianin dicampurkan dengan kopigmen-kopigmen iaitu asid sinamik, asid ferulik, dan asid galik, untuk mengkaji prestasi antosianin bersama kopigmen. Dalam kajian kolorimetri, semua sampel hidrokoloid yang

mengandungi antosianin, semakin pudar sepanjang 35 hari penyimpanan di bawah pendedahan sinaran UV, kerana sinaran tersebut merosakkan, dan penyilangan hidrokoloid bertambah kuat di bawah sinaran UV, yang menyebabkan perubahan rona dan kecerahan. Kesan tersebut semakin menyerlah daripada UV-A kepada UV-B dan UV-C, memandangkan frekuensi sinaran meningkat. Walau bagaimanapun, sampel yang mengandungi kompleks antosianin-kopigmen lebih pudar berbanding sampel tanpa kopigmen, kerana kandungan air di dalam hidrokoloid merencatkan kopigmentasi. Dalam spektroskopi FT-IR, kepekatan antosianin berkurangan secara amnya sepanjang 35 hari penyimpanan di bawah pendedahan sinaran UV dan kesan tersebut semakin menyerlah daripada UV-A kepada UV-B dan UV-C, atas kesan kerosakan oleh sinaran UV. Kesimpulannya, UV-C memberikan kesan terbesar terhadap degradasi antosianin, diikuti oleh UV-B dan UV-A, dalam tempoh tertentu. Hal ini bermakna apabila antosianin digabungkan ke dalam makanan, degradasi menjadi semakin pantas daripada UV-A kepada UV-B dan UV-C. Pada masa hadapan, terdapat kemungkinan untuk memanipulasi ikatan kimia dan kumpulan berfungsi antosianin untuk menghasilkan warna-warna baru, yang kemudiannya dapat digunakan dalam industri, contohnya pencelupan tekstil.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	:	Analysis of variance
ATR	:	Attenuated total reflectance
CHD	:	Coronary heart disease
CIELAB	:	Commission Internationale d'Eclairge L*a*b*
CVD	:	Cardiovascular disease
DNA	:	Deoxyribonucleic acid
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
DSSC	:	Dye-sensitised solar cell
FDA	:	Food and Drug Administration
FT-IR	:	Fourier Transform Infrared
GMP	:	Good manufacturing practices
HPLC-DAD-MS/MS	:	High performance liquid chromatography-diode array detector-mass spectrometry/mass spectrometry
HPTLC	:	High performance thin layer chromatography
JECFA	:	Joint FAO/WHO Expert Committee on Food Additives
LC-MS/MS	:	Liquid chromatography-mass spectrometry/mass spectrometry
LDL	:	Low-density lipoprotein
MIR	:	Middle infrared
NIR	:	Near infrared
ORAC	:	Oxygen radical absorbance capacity
ROS	:	Reactive oxygen species
SPSS	:	Statistical Package for the Social Sciences

TTI	:	Time temperature indicator
UHPLC	:	Ultra high performane liquid chromatography
UV	:	Ultraviolet
ΔA	:	Hyperchromic effect
$\Delta\lambda$	:	Bathochromic effect
η	:	Light-to-electricity conversion efficiency
λ_{max}	:	Wavelength with maximum absorbance

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CHAPTER 1: INTRODUCTION

1.1 Background

Brassica oleracea var. *capitata* f. *rubra*, also known as red cabbage is originally found to grow in the Mediterranean region and south western Europe (Arapitsas & Turner, 2008). To date, it grows in regions all over the world. It belongs to the family of *Brassicaceae* (Arapitsas & Turner, 2008). Previous epidemiological studies reported that the *Brassica* species prevent cardiovascular diseases and several types of cancer (Arapitsas & Turner, 2008). Red cabbage is also valued for its medicinal purposes to treat headaches, gout, diarrhoea, and peptic ulcers (Arapitsas & Turner, 2008). Apart from that, it also prevents chronic and degradative diseases such as heart disease and cancer, neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, as well as preventing aging process (Arapitsas & Turner, 2008; Castaneda Ovando et al., 2014).

It was found that the presence of anthocyannins account for the health attributes (Chandrasekhar et al., 2012). Anthocyannins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium (flavylium) salts (Chandrasekhar et al., 2012). They have high antioxidant activity, which significantly prevents the aforementioned diseases (Castaneda Ovando et al., 2014). Anthocyannins are also the natural pigments which are responsible for attractive colours of fruits such as grapes, strawberries, raspberries, pomegranates, mangoes, figs, red cabbage, and sweet potato (Chandrasekhar et al., 2012). Anthocyannins from red cabbage exhibit colour over a very broad pH range, from red at low pH to blue and green at high pH (Chandrasekhar et al., 2012). Their apparent lack of toxicity and eco-friendliness means that they can be used as a natural substitute to synthetic colourants (Chandrasekhar et al., 2012). Due to these reasons, red cabbage was selected as the source of anthocyannins in this study.

In the study conducted by Chandrasekhar et al. (2012), anthocyannin was extracted through solid-liquid partition in the ratio of 1:2, and different types of solvents were used namely water, acidified water, mixture of ethanol with water and acidified water, methanol, acidified methanol, acetone, and 70% aqueous acetone. This step was followed by pH differential method to estimate the anthocyannin concentration in all extracts (Chandrasekhar et al., 2012). An additional step was included in our study, which was the liquid-liquid partition. This was done to further remove lipids, chlorophylls, stilbenoids, less polar flavonoids and other non-polar compounds (Anuar et al., 2013).

Like any other natural pigments, the colour stability of anthocyannins is of major concern when they are used as natural colourants. Factors known to affect pigment stability include heat, oxygen, pH, light and presence of complexing agents such as metals and phenols (Bakowska et al., 2003; Vonelbe et al., 1981). Studies made by Markakis et al. (1957) exhibit first order reaction kinetics following the heat degradation of pelargonidin 3-monoglucoside, the major anthocyannin in strawberries. The rate was pH and oxygen dependent (Vonelbe et al., 1981). Later studies show that heat degradation of anthocyannins depend on their aglycone and its sugar moiety (Vonelbe et al., 1981). Impurities such as sugars, sugar alcohols, organic acids, amino acids and proteins accelerate anthocyannin degradation during processing and storage (Chandrasekhar et al., 2012).

On the other hand, molecular copigmentation of anthocyannins with other compounds such as flavonoids, alkaloids and metals, greatly enhance the colour of anthocyannin solution (Bakowska et al., 2003). These copigments have electron-rich pi systems. Hence, they are able to associate with the relatively electron-poor flavylium ion (Bakowska et al., 2003). This association protects the flavylium ion from nucleophilic addition of water,

which can convert the ion into the colourless pseudobase, resulting in the loss of colour (Bakowska et al., 2003). Copigmentation of anthocyannins causes a hyperchromic effect (ΔA) which increases colour intensity, and a bathochromic shift ($\Delta\lambda$) which consists of a shift of the maximum absorbance wavelength (Bakowska et al., 2003).

In this study, we investigated the effects of UV exposure to anthocyannin and its copigments. Samples of anthocyannin in food hydrocolloids were stored under UV radiations for 35 days to observe the physical changes in the colour of the samples. To determine the colour stability in anthocyannin, a colorimetric study was conducted. The hue, saturation and lightness variations of differently copigmented anthocyannin solutions in food hydrocolloids were studied using the CIELAB colour scale (Gonnet, 2001). This ground of colorimetry was established by the Commission Internationale d'Eclairage (CIE) (Grad at al., 2013). CIE L*a*b* or CIELAB is the most uniform colour model to describe colours visible to human eyes (Grad et al., 2013). To determine the degradation of anthocyannin, a chemometric study was conducted. Fourier Transform Infrared (FT-IR) spectroscopy was used to determine total anthocyannin prior to and upon UV exposure (Rasines Perea et al., 2015). The concentration of anthocyannin contained in the hydrocolloids was further quantified by an additional reference analysis (Romera Fernandez et al., 2012).

Research questions in this study are:

1) How do we extract and purify anthocyannins derived from red cabbage *B. oleracea*?
2) What are the effects on anthocyannins and copigments when subjected to UV treatment and how do we measure them?
3) What is the degradation kinetics of anthocyannins and their copigments upon UV treatment?

4) How is the performance of anthocyannins when applied in food and what are the effects of copigmentation on the pigments?

1.2 Research Objectives

The objectives of this study are:

1) to extract and purify anthocyannins from red cabbage *B. oleracea*.
2) to evaluate the behaviour, performance and degradation kinetics of anthocyannins and their copigments in food application when subjected to environmental treatments, and the effects of copigmentation.

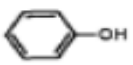
CHAPTER 2: LITERATURE REVIEW

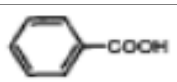
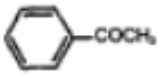
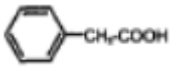
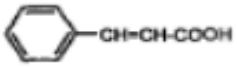
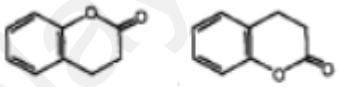
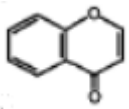
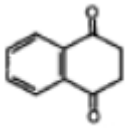
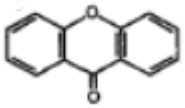
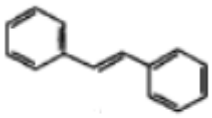
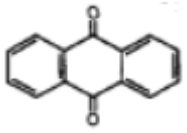
2.1 Polyphenols

Phenolic compounds are one of plants secondary metabolite. They arise from two main synthetic pathways, namely the shikimate pathway and the acetate pathway (Bravo, 1998). Shikimic acid and acetic acid are the precursors of many phenolic compounds such as anthocyannins (Delgado Vargas et al., 2000). Natural polyphenols are primarily conjugated, with their hydroxyl groups linked to one or more sugar residues. The sugar unit may also directly link to an aromatic carbon atom. The associated sugars may be monosaccharides such as glucose, disaccharides such as galactose and rhamnose, or even oligosaccharides such as glucuronic and galacturonic acids. It is also common to find linkages of other compounds such as carboxylic and organic acids to the phenol compounds (Bravo, 1998).

Depending on their basic chemical structure, polyphenols can be classified into several different classes (Bravo, 1998). Table 2.1 shows the classification of the main polyphenolic compounds based on their basic chemical structure. One of these classes is flavonoids, which account for the building of anthocyannin skeleton.

Table 2.1: Main classes of polyphenolic compounds (Bravo, 1998)

Class	Basic skeleton	Basic structure
Simple phenols	C_6	
Benzoquinones	C_6	

Phenolic acids	C_6—C_1	
Acetophenones	C_6—C_2	
Phenylacetic acids	C_6—C_2	
Hydroxycinnamic acids	C_6—C_3	
Phenylpropenes	C_6—C_3	
Coumarins, isocoumarins	C_6—C_3	
Chromones	C_6—C_3	
Naftoquinones	C_6—C_4	
Xanthones	C_6—C_1—C_6	
Stilbenes	C_6—C_2—C_6	
Anthraquinones	C_6—C_2—C_6	
Flavonoids	C_6—C_3—C_6	
Lignans, neolignans	$(C_6$—$C_3)_2$	
Lignins	$(C_6$—$C_3)_n$	

2.2 Flavonoids

Flavonoids are benzopyran derivatives with two aromatic rings bonded by a C3 unit, the central pyran ring (Delgado Vargas et al., 2000). To date, flavonoids are found in a variety of plant taxa, which include the lignin containing angiosperms, gymnosperms and ferns (Markham & Porter, 1969). As shown in Table 2.1, flavonoids have the C_6-C_3-C_6 skeleton. Flavonoids can be divided into 13 classes based on colour and the oxidation state of the pyran ring. They are anthocyannins, aurons, chalcones, yellow flavonols, flavones, colourless flavonols, flavanones, dihydroflavonols, dihydrochalcones, leucoanthocyanidins, catechins, flavans, as well as isoflavonoids. They are water soluble and are widely distributed in vascular plants. They can also undergo modifications such as hydroxylation, methylation, acylation and glycosylation. Anthocyannins are known to be the most important flavonoids, after chlorophyll, as they give colours ranging from scarlet to blue in fruits, petals, leaves and roots of plants (Delgado Vargas et al., 2000).

2.3 Anthocyannins in *B. oleracea*

Red cabbage, or also scientifically known as *B. oleracea*, is now widely grown in North and Central Europe, North America, China and Japan (Piccaglia et al., 2002; Wiczkowski et al., 2014). It is rich in anthocyannins and thus is red coloured. It has a complex pattern due to glucosylation of anthocyanidin with two different sugar moieties and acylation with several aromatic acids. With cyanidin as the only aglycone, the dominant anthocyannin structures in red cabbage are cyanidin-3,5-diglucoside and cyanidin-3-sophoroside-5-glycoside. These structures are further acylated with sinapic acid, ferulic acid, *p*-coumaric acid, caffeic acid or malonic acid (Dyrby et al., 2001). Figure 2.1 shows the chemical structure of an acylated anthocyannin. In Italy, anthocyannins from red cabbage are an alternative to grape pomace in the food industry because the latter contains

residues of sulphur dioxide and thereby can trigger allergenicity in sensitive people. The acylation in red cabbage anthocyannins also provides greater stability to heat and light, than that of grape anthocyannins (Piccaglia et al., 2002). Moreover, anthocyannins from red cabbage are unique such that they are coloured over a very broad pH range. Red cabbage anthocyannins vary from red at low pH to blue and green at high pH. A study conducted by Dyrby et al. (2001) showed that red cabbage anthocyannins have low sensitivity to photodegradation between pH 3 and 7. Previous study reported an increase in anthocyannin content in plant tissues when the plants are nutrient deficient, thus a reduced supply of fertiliser may improve cabbage pigment production. While oversupply of nitrogen decreases pigment concentration in grapes, potassium and phosphorus starvation increases anthocyannin production without markedly affecting yield of cabbages (Piccaglia et al., 2002).

In a study conducted by Wiczkowski et al. (2014), they observed that anthocyannin profile from red cabbages was affected by genotype. Five red cabbage varieties characterised by the bulbs of a spherical shape and a conical shape, were analysed using HPLC-DAD-MS/MS method. The extract of red cabbages showed radical scavenging activity and antiradical potential which differ significantly across varieties. The red cabbage varieties also possess their own anthocyannin fingerprint and specific antioxidant capacity (Wiczkowski et al., 2014).

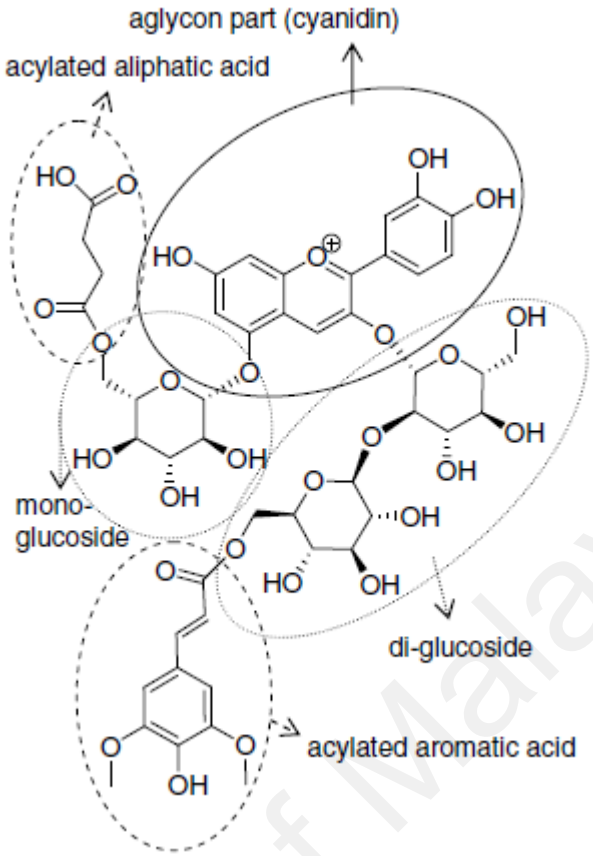
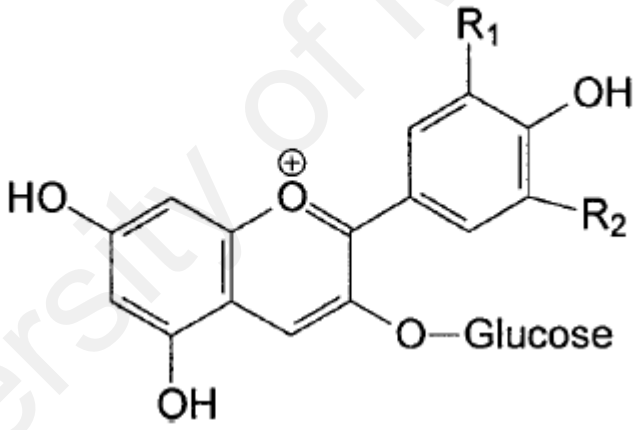


Figure 2.1: Chemical structure of an acylated anthocyannin (Arapitsas & Turner, 2008)

2.4 Structure of anthocyannins

The term anthocyannin is coined from the Greek *anthos* which means a flower, and *kyanos* which means dark blue (Delgado Vargas et al., 2000). Anthocyannins are glycosides with an aglycone or anthocyanidin C_6—C_3—C_6 skeleton (Clifford, 2000). They are derived from 2-phenylbenzopyrylium(flavylium) salts by substituting glycosides (Brouillard et al., 1991; Delgado Vargas et al., 2000). Anthocyannins are intensely coloured especially under acidic conditions. This characteristic is attributed to the long chromophore of eight conjugated double bonds with a positive charge (He & Giusti, 2010). Colours of anthocyannin are determined by the number of hydroxyl groups, the degree of methylation of these hydroxyl groups, the nature and number of sugar moieties attached to the pigment and the position of attachment, as well as the nature and

number of aliphatic or aromatic acids attached to the sugar moieties (Brouillard et al., 1991). Figure 2.2 shows the structure of anthocyanidin or aglycone. The hydroxyl and methoxyl groups on R_1 and R_2 determine the type of anthocyannin. 25 different aglycones exist, which differ in the number and position of hydroxyl and methyl ether groups (He & Giusti, 2010). With at least one sugar moiety, they give rise to anthocyannin compounds. As shown in Figure 2.2, pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin are the most common anthocyannins found in plants (He & Giusti, 2010). Cyanidin can be found in apples, cherries, figs and peaches, whereas delphinidin can be found in eggplants and pomegranates, and peonidin can be found in sweet cherries and cranberries (Delgado Vargas et al., 2000).



R_1	R_2	Anthocyanin
H	H	Pelargonidin-3-glucoside
OH	H	Cyanidin-3-glucoside
OH	OH	Delphinidin-3-glucoside
OCH_3	H	Peonidin-3-glucoside
OCH_3	OH	Petunidin-3-glucoside
OCH_3	OCH_3	Malvidin-3-glucoside

Figure 2.2: The structure of anthocyanidin (Clifford, 2000)

Hundreds of anthocyannins may vary from one another due to differences in their chemical structures. These include the number and position of hydroxyl and methoxyl substituents, the identity, number and position at which sugars are attached to the skeleton, as well as the extent of sugar acylation and the acylating agents identity (Clifford, 2000). An increase in the number of hydroxyl groups attached to anthocyannin skeleton enhances blueness whereas an increase in the number of methoxyl groups enhances redness (Delgado Vargas et al., 2000). Sugars most commonly encountered attached to anthocyannins are glucose, galactose, rhamnose, xylose, arabinose and fructose. They are found in the form of 3-glycosides or 3,5-diglycosides. Among other occurrences of glycosides attached to anthocyannin skeleton are rutinosides (6-O-α-$_{L}$-rhamnosyl-$_{D}$-glucosides), sophorosides (2-O-β-$_{D}$-glucosyl-$_{D}$-glucosides), sambubiosides (2-O-β-$_{D}$-xylosyl-$_{D}$-glucosides), 3,7-diglycosides and 3-triosides (Clifford, 2000). Anthocyannins can thus be classified based on the number of sugar moieties attached to the skeleton such as monosides, biosides and triosides. Taking into account the vast diversity of sugar and the possible structural points of glycosylation, the number of probable anthocyannin compounds is increased (Delgado Vargas et al., 2000; Dufour & Sauvaitre, 2000).

In addition, many anthocyannins also show ester linkages between the sugar and organic acids in their structure. They are known as acylated anthocyannins. The most common acylating agents include cinnamic acids such as caffeic, *p*-coumaric and ferulic from the shikimate pathway, as well as aliphatic acids such as acetic, malic, malonic, oxalic and succinic (Delgado Vargas et al., 2000). Acylated anthocyannins can be found in red cabbage, red lettuce, blood orange and elderberries among others (Clifford, 2000).

2.5 Properties of anthocyannins

2.5.1 Antioxidant activity

Reports have shown that flavonoids such as anthocyannins possess antioxidant activities (Iversen, 1999). These activities are crucial in developing their roles and functions in scavenging free radicals, as well as treating and preventing diseases (Fenglin et al., 2004; Iversen, 1999). It was found that the hydroxyl groups at the 3'- and 4'- positions of the B-ring have the highest antioxidant activity. They protect ascorbic acid against oxidation by chelating metal ions (Delgado Vargas et al., 2000). Aglycone flavonoids are also potent antioxidants. Anthocyannins show a strong antioxidant activity in which they prevent the oxidation of ascorbic acid and protect against free radicals. They also show inhibitory activity against oxidative enzymes. Besides, anthocyannins demonstrate scavenging activity against $^{\bullet}OH$ and O_2^{-}. $^{\bullet}OH$ scavenging is better with aglycone with high number of OH groups in the B-ring (Delgado Vargas et al., 2000). On the other hand, O_2^{-} scavenging is does not depend on glycosylation state but improves with the number of hydroxyl groups (Delgado Vargas et al., 2000).

The antioxidative activities of several pure compounds and plant extracts can be determined by measuring the oxygen consumption or the production of hydroperoxides or other degradation products (Brand Williams et al., 1995). Brand-Williams et al. (1995) evaluated the antioxidative activity of some phenolic compounds by reacting them with a stable radical namely 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) in a methanol solution. The DPPH free radical scavenging activity is widely used in screening bioactive compound (Fenglin et al., 2004; Lu et al., 2010; Sharma & Bhat, 2009). The reduction of $DPPH^{\bullet}$ was followed by monitoring the decrease in its absorbance at 515 nm. The radical form of $DPPH^{\bullet}$ absorbs at this wavelength but the absorption decreases and disappears when it is reduced by an antioxidant (Brand Williams et al., 1995). This disappearance is the result of a colour change from purple to yellow.

The antioxidant scavenges the radical by donating hydrogen to form the reduced DPPH-H (Espin et al., 2000). The reaction can be summarised in the following equation:

$$DPPH^{\bullet} + (AH)_n \longrightarrow DPPH\text{---}H + (A^{\bullet})_n$$

where *n* is the possible number of radical scavenger species. The newly-formed ($A^{\bullet}$) can then render stable molecules by radical disproportionation (Espin et al., 2000).

In vitro, anthocyannins are potent antioxidants. Reactive oxygen species (ROS) such as free radicals, singlet oxygen and peroxides can cause oxidative damage if overly produced in the body. Cellular defence systems fight against ROS to provide protection to the body, but 1% of ROS can escape daily elimination and cause cellular oxidative damage, which in turn increases oxidative stress (Lau et al., 2006). Anthocyannins efficiently quench these by terminating their chain reactions (He & Giusti, 2010). Wang et al. (2008) conducted the oxygen radical absorbance capacity (ORAC) assay to study the antioxidant activity of 14 anthocyannins and their glycosylated derivatives in aqueous phase at neutral pH. Cyanidin 3-glucosides showed the highest ORAC values, which are 3.5 times as potent as Trolox, which is a water-soluble vitamin E analog. Cyanidin 3-glucoside and its aglycone cyanidin had similar antioxidant potency as vitamin E, when tested in rabbit erythrocyte membrane and rat liver microsomal systems. Pelargonidin, which exhibited the lowest ORAC values, was still as potent as Trolox (He & Giusti, 2010). In human erythrocytes treated with hydrogen peroxide, anthocyannins from red wine fractions greatly lowered ROS in the red blood cells (He & Giusti, 2010).

Anthocyannins also show antioxidant potency *in vivo*. Cyanidin 3-glucosides efficiently attenuated the biomarker changes in rat liver injury, induced by hepatic ischemia-reperfusion (He & Giusti, 2010). In a different study, rats were fed vitamin E-deficient diets. After 12 weeks, they were supplemented with purified anthocyannin-rich extracts.

Anthocyannins greatly improved plasma antioxidant capacity, and decreased the level of hydroperoxides and 8-oxo-deoxyguanosine, which were responsible for lipid peroxidation and DNA damage respectively (He & Giusti, 2010).

2.5.2 Antibacterial activity

Plant flavonoids have shown antibacterial activity as a defence mechanism against pathogens. Anthocyannins such as cyanidin and peonidin glycosides exhibited growth inhibition of *Xanthomonas oryzae* pv, which is one of the major rice pathogens (Delgado Vargas et al., 2000). Four anthocyannins extracted from Finnish berry extracts which were pelargonidin chloride, cyanidin chloride, delphinidin chloride and cyanidin-3-glucoside, were found to effectively inhibit the Gram-negative *Escherichia coli* strain CM 871, a DNA repair-deficient strain (He & Giusti, 2010). In another study, anthocyannin fraction from berries was potent in reducing the viability of *Salmonella enterica serovar Typhimurium*. This effect was due to the ability of anthocyannins in inducing the release of lipopolysaccharides from the outer membrane of Gram-negative bacteria (He & Giusti, 2010).

2.5.3 Health attributes

Large consumptions of anthocyannins are believed to be safe to humans. Based on previous toxicological studies, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that extracts containing anthocyannins have very low toxicity (He & Giusti, 2010). In 1982, the acceptable daily intake for humans was 2.5 mg kg^{-1} body weight per day (He & Giusti, 2010). Anthocyannins have been used in traditional herbal medicines used by North American Indians, the Europeans and the Chinese (Konczak & Zhang, 2004). Beneficial effects from anthocyannins consumption include visual acuity enhancement, coronary heart disease reduction, protection against age-related declines in neurological dysfunction, maintenance of normal vascular

permeability, anticarcinogenic, antimutagenic, anti-inflammatory, and antioxidative (Ju & Howard, 2003). The consumption of wine flavonoids among Italian subjects was correlated with low incidence of coronary heart diseases. Anthocyannins are also important agents in minimising the risk of cancer and heart disease (Delgado Vargas et al., 2000). Dragsted et al. (1993) reported the presence of cancer-protective factors in fruits and vegetables, which contain polyphenol compounds (Dragsted et al., 1993; Fenglin et al., 2004). Previous epidemiologic studies showed a decrease in the incidence of cardiovascular disease (CVD), coronary heart disease (CHD) and stroke, with an increase in the consumption of fruits and vegetables. The Nurses' Health Study and the Health Professionals' Follow-up Study showed an equivalent of 4% in reduction of CHD for every 1-serving per day increase in the intake of fruits and vegetables (Etherton et al., 2002).

Anthocyannins, being dietary antioxidants, have the ability to increase serum antioxidant capacity, thereby providing protection against low-density lipoprotein (LDL) and cardiovascular diseases. In a study conducted by Matsumoto et al. (2002), oral administration of black currant anthocyannins caused a rapid increase in plasma antioxidant capacity (He & Giusti, 2010). Moreover, anthocyannins also possess anti-inflammatory activity. In an *in vivo* study by Rossi et al. (2003), rats with carrageenan-induced lung inflammation were tested for the therapeutic efficacy of blackberry anthocyannins. It was found that all parameters of inflammation were effectively minimised by anthocyannins (He & Giusti, 2010). In addition, anthocyannins also possess anticarcinogenic activity based on in vitro evidence. Anthocyannins extracted from flower petals were found to be potent in the inhibition of cell growth in a human malignant intestinal carcinoma-derived cell line (He & Giusti, 2010).

In another study reported by Tsuda et al. (2008), anthocyannins were found to ameliorate the function of adipocytes, which in turn prevent metabolic syndrome and obesity. Anthocyannins from black soybean were found to reverse the weight gain of rats fed high-fat diet (He & Giusti, 2010). Obesity can lead to relative inadequacy of insulin in late stages of type 2 diabetes. Since anthocyannins can prevent obesity, they are also able to control type 2 diabetes. In vivo oxidation leads to diabetes. Anthocyannins hindered the development of in vivo oxidation as increased plasma and liver biomarker oxidation were observed in diabetic rats when fed boysenberry anthocyannins (He & Giusti, 2010). In a study conducted by Jayaprakasam et al. (2005), anthocyannins and anthocyanidins showed the ability to trigger the secretion of insulin. Cyanidin-3-glucoside and delphinidin-3-glucoside were the most effective insulin secretagogues among the anthocyannins and anthocyanidins tested (Jayaprakasam et al., 2005). Kramer reported that anthocyannins improve eye vision. In a study involving healthy human subjects, feeding black currant anthocyannin concentrate resulted in the reduction of the dark adaptation threshold (He & Giusti, 2010).

2.5.4 Colour stability of anthocyannins

Loss of anthocyannin pigments can cause colour deterioration in plants. Anthocyannins are chemically unstable and can easily change from their natural red or blue colour to undesirable brown colour (Daraving et al., 1968; Kirca et al., 2003). They can also be destructed easily during food processing (Delgado Vargas et al., 2000). Maccarone et al. (1985) observed that microwave pasteurisation, and addition of tartaric acid and glutathione improved the stability of anthocyannins in blood orange juice (Kirca et al., 2003; Maccarone et al., 1985). The major factors which affect the colour stability of anthocyannins include temperature, pH, light, phenolic compounds, sulphur dioxide, sugar and sugar degradation products, as well as molecular oxygen and ascorbic acid

(Cemeroglu et al., 1994; Daraving et al., 1968; Delgado Vargas et al., 2000; Kirca et al., 2003).

2.5.5 Temperatures

As reported by Cemeroglu et al. (1994) and Kirca et al. (2003), the degradation of anthocyannins from sour cherry juice and blood orange juice follows first order reaction with respect to temperatures. The degradation was faster in concentrates than in juices as the rate of chemical reactions accelerates when molecules become closer (Kirca et al., 2003). They observed an excellent correlation between the percent anthocyannin retention and storage time or heating time at all tested temperatures (Cemeroglu et al., 1994). Arrhenius model was used to calculate the effect of temperature on the rate constant of anthocyannin degradation:

$$k = K_0 e^{-Ea/RT}$$

where k is the rate constant, K_0 is the frequency factor, E_a is the activation energy, R is the gas constant, and T is temperature in kelvin (Kirca et al., 2003). It was found that the values of rate constant were temperature dependent. They also found that the activation energies E_a for anthocyannin degradation in sour cherry concentrates were higher at higher concentrations. The higher activation energy implies that anthocyannin is rapidly degraded at a small temperature change. Thus, it can be concluded that anthocyannins in concentrate (E_a = 19.14 kcal/mole) are more susceptible to thermal degradation than those of single-strength juice (E_a = 16.37 kcal/mole) (Cemeroglu et al., 1994). The commercial processing of blood orange into juice is also not recommended unless accompanied by copigmentation or another means to provide stability to anthocyannins (Kirca et al., 2003).

In addition, Tinsley et al. (1960) reported first order kinetics of thermal degradation of anthocyannins from strawberries, as influenced by a variety of sugars and sugar degradation products under nitrogen or air (Daraving et al., 1968). Lamort et al. (1968) also reported first order kinetics of thermal degradation of anthocyannins from red raspberry (Daraving et al., 1968).

2.5.6 pH

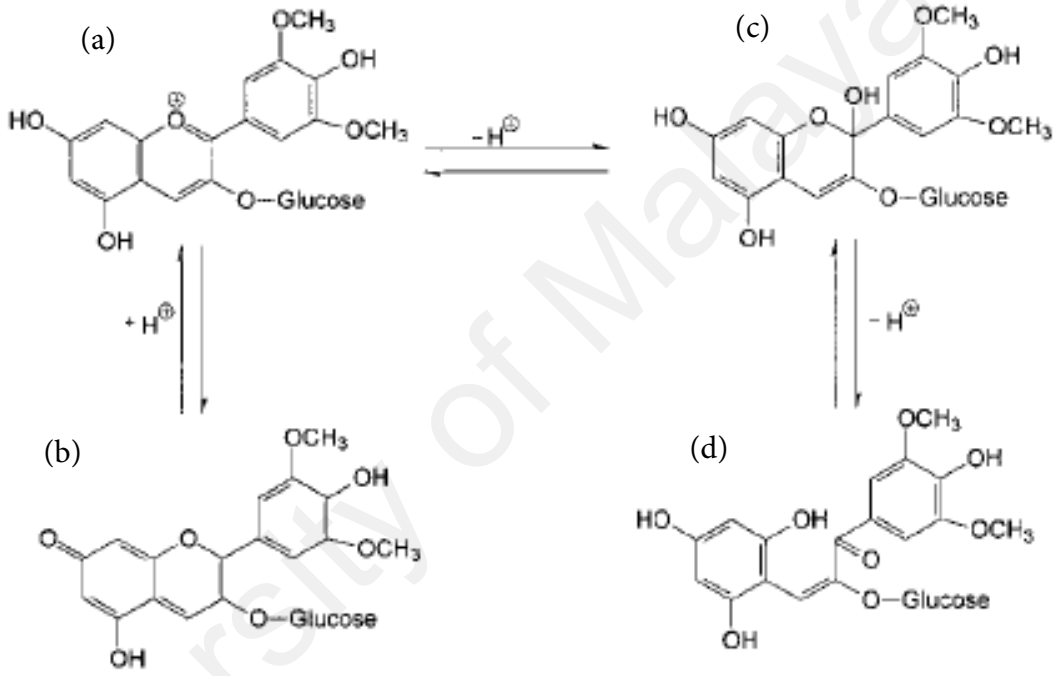


Figure 2.3: Anthocyannin as an equilibrium of (a) flavylium cation, (b) quinonoidal base, (c) carbinol pseudobase and (d) chalcone (Clifford, 2000)

Depending on the extent of acidity or alkalinity, anthocyannins can adopt distinct chemical structures. Except in strongly acidic aqueous medium, the stability of flavylium cation is affected by pH-dependent--transformations (Dufour & Sauvaitre, 2000). Studies have found that there is an equilibrium between the flavylium (2-phenylbenzopyrylium) cation and carbinol in acidic media (Brouillard & Dubois, 1977). Figure 2.3 shows the occurrence of anthocyannins in plant vacuoles as an equilibrium of four different

molecular species (Dasneves et al, 1993). These are the red-coloured basic flavylium cations with three secondary structures, the blue quinonoidal base, the colourless carbinol and chalcone pseudobase. Each of these species has a number of rapidly interconverting tautomeric forms. The chalcone may also be present in both cis and trans forms (Clifford, 2000).

Brouillard et al. (1977) reported structural transformations of anthocyannins in acidic aqueous media (pH 1 to 6) at 4 °C. At pH 3 and below, anthocyannins are orange or red and exist as a flavylium cation. As pH increases, kinetic and thermodynamic competition takes place between proton transfer reaction and hydration reaction of the flavylium cation, related to the aglycone acidic hydroxyl groups. (Fossen et al., 1998). This competition predominantly favours deprotonation (He & Giusti, 2010). In the first reaction, there is an extremely fast proton transfer between the quinonoidal base and the flavylium cation (Brouillard & Delaporte, 1977). The deprotonation of the flavylium cation resulted in the formation of anhydro bases (Brouillard & Dubois, 1977; Dufour & Sauvaitre, 2000). This formation of highly coloured ionised anhydro bases was observed at pH 8 to 10 (Delgado Vargas et al., 2000). By nucleophilic addition of water, these bases quickly hydrate, yielding the carbinol pseudobase. This is the second reaction which occurs at pH 5 to 7 (Brouillard & Delaporte, 1977; Brouillard & Dubois, 1977; Dufour & Sauvaitre, 2000). As the pH increases further to 12, the pyrylium ring opens and carbinol hydrolyses rapidly to fully ionised chalcone (Brouillard & Dubois, 1977; Delgado Vargas et al., 2000; Dufour & Sauvaitre, 2000).

In another study, the kinetic chalcone was reported to show a characteristic of an intermediary species, yielding the final degradation products. These are 3,4,5-trihydroxybenzoic acid from carbinol pseudobase and 2,4,6-trihydroxybenzaldehyde from quinonoidal base (Dasneves et al., 1993). It was also reported that instant

acidification causes reverse transition from carbinol pseudobase to flavylium cation (He & Giusti, 2010).

2.5.7 Light

Generally, light has a negative effect on anthocyannin, hence light exposure to natural coloured drink must be avoided. A light-exposed peel of an apple has lower anthocyannin than a shaded peel, thus light intensity has a profound effect on the colour of an apple (Delgado Vargas et al., 2000). Samples containing monoglycosides are usually the least stable, with diglycosides intermediate in stability. The most light stable of anthocyannins are acylated diglucosides. In contrast, the light stability of samples containing anthocyannins from radish was exceptionally high (Giusti & Wrolstad, 1996).

In a study conducted by Giusti et al. (1996), samples containing anthocyannins from radish which were exposed to light had a lower anthocyannin content than those stored in the dark, during a year of storage. The monomeric anthocyannin content from syrup samples which have higher anthocyannin concentration, decreased at a higher rate upon light exposure than those stored in the dark (Giusti & Wrolstad, 1996). Other study by Markakis et al. (1996) reported that the half-life of anthocyannins from grape pomace which was incorporated into a carbonated drink at 22 °C, was 3.3 times shorter when exposed to light, than those stored in the dark at 20 °C. It was also reported that light may promote the formation of polymers with higher molecular weight, favouring material precipitation (Giusti & Wrolstad, 1996).

2.5.8 Copigmentation

Copigments are structurally unrelated compounds such as flavonoids or non-flavonoid phenols, amino acids and organic acids (Darias Martin et al., 2001). Presence of co-pigments can alter the colour of anthocyannins. The same anthocyannins may have different colours when co-pigments are added to one of them (Brouillard et al., 1991). A

co-pigment is not normally coloured but can greatly enhance the colour of anthocyannins in aqueous solutions (Brouillard et al., 1991). Copigments can also form a clustered colour with colourless forms of anthocyannins. Reactions between anthocyannins and organic compounds found in higher plants cause colour changes in fruits, vegetables and flowers. A copigment displaces the colourless free anthocyannins in preference of the coloured forms, and can cause colour intensification at high concentration (Darias Martin et al., 2001). The effect of the co-pigment on anthocyannins can be estimated from the increase in the bathochromic shift and hyperchromic shift of the visible λ_{max} (Brouillard et al., 1989; Giusti & Wrolstad, 1996). The effect depends on the type and concentration of co-pigment, pH of the medium, temperature and metals (Brouillard et al., 1991). The equilibrium of copigmentation may be written as:

Free anthocyannins + Copigmentation cofactors ⟷ Copigmented anthocyannins

Anthocyannins can react with various compounds such as amino acids, benzoic acids, alkaloids, coumarin, cinnamic acids and other flavylium compounds. They form a weak association which is known as intermolecular copigmentation. On the other hand, intramolecular copigmentation arises due to the acylation in the molecule. The acyl groups in the copigment avoid the formation of the hydrated species, hence protecting the coloured flavylium cation from nucleophilic attack of water molecule. This protection is due to the stacking of the aromatic residues of acyl groups with the pyrylium group of anthocyannins. It was also reported that at least two constituents of acyl groups are needed for good colour stability in neutral or acidic media (Giusti & Wrolstad, 1996). As anthocyannins form covalent bonds with the copigments, intramolecular is therefore more effective than intermolecular copigmentation. Anthocyannins can also self-associate or form complexes with metals (Delgado Vargas et al., 2000; Dufour & Sauvaitre, 2000), as

shown in Figure 2.4. In addition, previous reports have demonstrated an increase in thermal stability by copigmentation, presence of glucoside substituents, as well as their acylation (Dasneves et al., 1993).

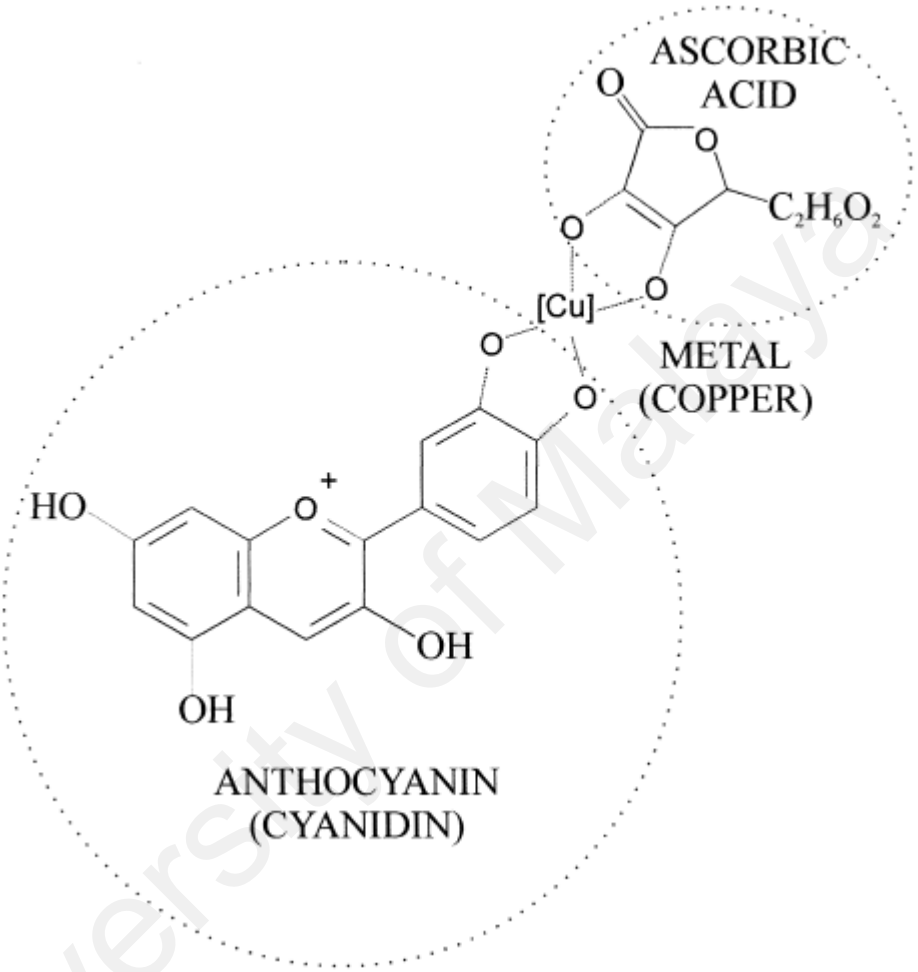


Figure 2.4: Anthocyannin-copper metal complexes (Delgado Vargas et al., 2000)

2.6 Applications

2.6.1 Food processing

In food industry, anthocyannins are used as a marker for good manufacturing practices (GMP) (Delgado Vargas et al., 2000). They are used to evaluate the adulteration of some pigmented products. In prune juice production, the reaction between anthocyannins and phenolic compounds produces brown colour. Some manufacturers may adulterate the prune juice with other fruit juices to improve its colour. A high level of anthocyannins indicates an adulteration as pure prune juice may only contain traces of anthocyannins (Delgado Vargas et al., 2000). Apart from that, anthocyannins are also important in determining the authenticity of fruit jams. Using anthocyannin profiles, it was found that labelled black cherry jams were apparently prepared using the less expensive common red cherries. Moreover, analysis of the relation between pelargonidin and cyanidin 3-glucoside proved that blackberry jams were adulterated with strawberries. Since anthocyannins are quite stable during jam manufacture, their use is very efficient in detecting food product adulteration (Delgado Vargas et al., 2000). On top of that, instead of monomeric anthocyannin pigments, high levels of polymeric colour in red raspberry juices imply that the samples were adulterated (Delgado Vargas et al., 2000).

Anthocyannins are also a potential candidate in replacing synthetic red dyes such as Ponceau 4R and FD&C Red No. 40 in food industry (Espin et al., 2000; Fossen et al., 1998; Giusti & Wrolstad, 1996). Both legislative action and consumer demand have caused an increase for natural colourants over synthetics in their food as the former is healthful (Giusti & Wrolstad, 1996). The main use of anthocyannins is in the manufacture of beverages and soft drink because only anthocyannins from enocyanin and lees are approved by the FDA in the U.S.A (Delgado Vargas et al., 2000).

2.6.2 Dye-sensitised solar cell

Dye-sensitised solar cell (DSSC) is a photovoltaic device used to convert light energy to electricity. DSSC is composed of a photosensitiser, a transparent conductive oxide glass, TiO_2 film and an electrolyte. Recently, ruthenium polypyridyl complex has been used as the photosensitiser and more than 10% improvement in the light-to-electricity conversion efficiency (η) was observed. Regardless of this fact, ruthenium is a trace element, and can become too expensive and less accessible if widely used. Ruthenium also threatens the environment as it is a heavy metal (Chien & Hsu, 2013). Being environmentally friendly, as well as having easy accessibility and high absorption in the visible region, anthocyannins are potential candidates to substitute ruthenium. In comparison with ruthenium-based dyes, anthocyannins are nontoxic, metal free, widely available and are inexpensive. They also have sufficient hydroxyl groups to bind TiO_2 nanocrystallites. Moreover, they are able to inject electrons into TiO_2 conduction band at an extremely fast rate when excited with visible light (Chien & Hsu, 2013).

In a study conducted by Chien et al. (2013), DSSC performance in relation with the pH value of anthocyannin extract was studied. Under their experimental condition, η reached maximum at pH 8, and decreased towards both acidic and basic ends (Chien & Hsu, 2013). DSSC performance in relation to the concentration of anthocyannin extract at a constant pH of 8 was also investigated. Generally, η showed an increase with increasing anthocyannin concentration. The best performance was observed at 3 mM, and further concentration increment did not yield better results. Increasing concentration of anthocyannin increases the number of bound dye molecules, which in turn increases the photocurrent (Chien & Hsu, 2013). In previous studies, DSSC performance in relation with immersion time in the anthocyannin extract at a constant pH of 8 was also investigated. Electrodes were immersed in the anthocyannin extract for at least 16 hours in order to completely cover TiO_2 particles with anthocyannin molecules. It was observed

that η steadily increased with immersion time and reached the maximum at 15 minutes. Further immersion resulted in a drop in η which then became stabilised between 1 and 16 hours (Chien & Hsu, 2013).

2.6.3 Textile dyeing

There has been a growing interest in the use of natural dyes in technical textile dye-houses since the past decade (Bechtold et al., 2007). Textile scientists have grown their interest in using natural phenolic compounds as a functional finishing agent and colourant for textile materials (Hong, 2015). Natural dyes are potential candidates in substituting the role of synthetic dyes in textile industry as the latter are known to be toxic and hazardous to both human health and the environment. To date, many natural dyes are being produced in Asian countries and are used in most countries in the world (Guemruekcue et al., 2008). Pawlak et al. (2006) highlighted the use of anthocyannin from elderberries as colouring matters in textile dyeing and mural painting (Pawlak et al., 2006).

In a study conducted by Bechtold et al. (2007), they investigated the amount of anthocyannin which can be extracted from grape pomace from different grape varieties. They found that the concentrations of anthocyannin vary with grape varieties. An optimised dyeing procedure which includes pre-mordanting with anthocyannin gave an intensive red colour on cotton fabric. They also found that the drying step was shown to yield high anthocyannin concentration. Thus, anthocyannin content from dried grape pomace was sufficient for it to serve as the raw material in textile dyeing (Bechtold et al., 2007).

In another study conducted by Hong (2015), waste water containing anthocyannins generated from the cooking of black rice was used in fabric dyeing. Multifibre fabrics were dyed with the black rice extract. It was found that fibres with amide group such as

silk, as well as nylon 66 absorbed the dye more than other fibres. This finding showed that protein fibres absorb dyes more efficiently than cellulose fibres. Cationisation with N-trimethylammonium chloride improves the dyeability of cellulose fibre (Hong, 2015). In addition, the antioxidant activities of anthocyannin from black rice can protect human skin from ultraviolet (UV) irradiation (Hong, 2015).

CHAPTER 3: MATERIALS AND METHODS

3.1 Source of materials

Fresh mature red cabbages, *B. oleracea* were purchased from the local market (source for local supermarket, Australian origin). To keep them fresh and obtain good quality of the extracts, they were stored at -20 °C until used. Chemical reagents namely the undenatured ethanol (99.8%), methanol (99.8%) and acetic acid which were used as the extraction solvents were purchased from Systerm. Ethyl acetate which was used in liquid-liquid partition was also purchased from Systerm. Potassium chloride and sodium acetate used in pH differential method were purchased from Sigma Aldrich. Carrageenan (USA) and gallic acid (97.5 – 102.5%) used in UV treatment were purchased from Sigma, ferulic acid (99%) from Aldrich, cinnamic acid (>99%) and gelatine from R&M Chemicals (USA), and certified molecular biology agarose from Bio-Rad. The chromatography resin Amberlite XAD7HP used in ion exchange was purchased from Sigma while Sephadex LH-20 used in size exclusion was purchased from GE Healthcare. Deionised water used in size exclusion chromatography was purified at 18.2 MΩ cm^{-1} (Barnstead RO & Deionised Systems).

3.2 Extraction and identification of major anthocyannins in *B. oleracea* (study 1)

3.2.1 Extraction of anthocyannins

The leaves of fresh red cabbage were chopped and cut into small pieces. They were pre-soaked in the acidified extraction solvent and stored overnight at 10 °C to ease the extraction of anthocyannins. The extraction solvent used in this study was undenatured ethanol containing 0.5% (v/v) acetic acid. The purpose of extraction was to extract maximum pigment with minimal adjuncts and minimal degradation or alteration of anthocyannins (Patil et al., 2009). A combination of organic solvent and acid was used in extraction to give a high yield of anthocyannins while preserving its quality to be used in

food processing. The selection of the best extraction solvent is crucial to ensure health safety and maximum yield of anthocyannin. Acidified ethanol was chosen as the extraction solvent because the anthocyannin extract was meant to be used in food processing (Chandrasekhar et al., 2012). Solvents such as methanol were not chosen due to the toxicity of methanolic solutions, and acetone for being hazardous to health (Patil et al., 2009; Spagna et al., 2003). Extraction with ethanol alone was also not preferable because a little water was needed to extract the hydrophilic anthocyannin (Patil et al., 2009). The presence of acetic acid stabilised the anthocyannin pigment and lowered the pH to a level where the absorbance of anthocyannins was at their maximum (Patil et al., 2009). A study conducted by Patil et al. demonstrated an increase in anthocyannin content with an increase in acid content, but a high concentration of acid was not favourable in food application (Chandrasekhar et al., 2012; Patil et al., 2009).

The pre-soaked red cabbage leaves were then homogenised by a blender (7012G, Waring Products, Inc.). Red cabbage juice was obtained by filtration using cheese cloth, followed by filtration with filter papers (Whatman, grade No. 4) to remove cell debris and other undissolved substances. The filtrate was heated in water bath at 50 °C for 15 minutes to remove anthocyannin-degrading enzymes. The organic solvent was removed from the filtrate using a rotary evaporator (IKA, RV 10 basic) at 50 °C.

Upon completion of solid-liquid partition, the juice of anthocyannin was further subjected to liquid-liquid partition, where ethyl acetate was used in the extraction process. The aqueous anthocyannin solution was washed several times by partition against three times volume of ethyl acetate (1:3) in a glass separation funnel to remove lipids, chlorophylls, stilbenoids, less polar flavonoids and other non-polar compounds (Andersen et al., 2005; Anuar et al., 2013; Tanchev, 1976). This step was done in a fume hood. Successive partition against ethyl acetate was performed to obtain maximum yield of anthocyannin

pigment from the juice, in accordance with the study conducted by Jin et al. (2010). The synergistic effects of both solid-liquid and liquid-liquid partition ensure the highest possible yield of anthocyannin extract from the red cabbage *B. oleracea* in this study. Since the presence of alcohol in the anthocyannin juices may limit the pigment application as food colourant (Patil et al., 2009), both the acidified ethanol and ethyl acetate were removed from the juice using the rotary evaporator at 50 °C, where the anthocyannin extract was obtained. The extract was freeze-dried and stored at -20 °C to minimise the degradation of the anthocyannin pigment during storage. The whole procedure was conducted under dim light. These steps were carried out as described by (Anuar et al., 2013).

Figure 3.1: Liquid-liquid partition in anthocyannin extraction

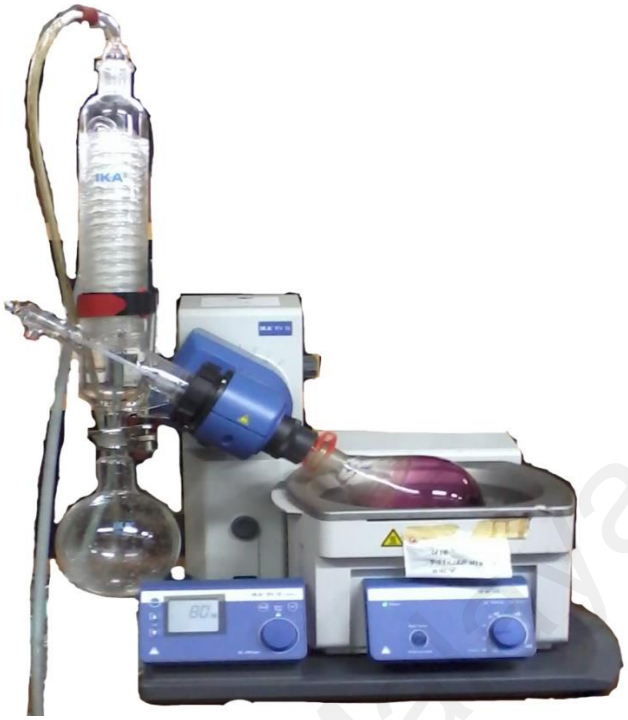


Figure 3.2: Anthocyannin extraction using a rotary evaporator

3.2.2 Purification of anthocyannins

3.2.2.1 Ion exchange chromatography

Double chromatographies were performed on the anthocyannin extract to obtain the purest possible sample to be used in the UV studies and for structural identification. Ion exchange chromatography was selected as the initial purification step. This type of chromatography has been used to separate ionic species through interactions between the ions and a charged stationary phase (Johnson, 2014). It has a large sampling capacity with moderate cost (Cummins et al., 2011). The mobile phase is an aqueous buffer system, into which we introduced the anthocyannin sample. In this study, we used distilled water and acidified ethanol (0.5% v/v acetic acid) as the mobile phase. On the other hand, the stationary phase is usually an inert organic matrix which is chemically derivatised with ionisable functional groups carrying a displaceable oppositely charged counterion (Cummins et al., 2011). In this study, Amberlite XAD7HP resin was used as the stationary

phase. Amberlite XAD7 is a non-ionic acrylic ester resin with moderate polarity. It adsorbs and releases ionic species through hydrophobic and polar interactions, under isocratic conditions. Amberlite XAD7HP was used because it has high surface area and similar polarity to anthocyannins, hence the high adsorption and desorption capacities (Chandrasekhar et al., 2012).

There are two categories of ion exchange functional groups; the anion-exchangers and the cation-exchangers. Strong ion-exchangers are fully ionised over a broad pH range, while weak ion-exchangers are only partially ionised over a narrow pH range. According to Cummins et al. (2011), commercial resins such as Amberlite are generally strong anions, normally have quarternary ammonium as the ion exchange group, and work around the pH between 2 to 12 (Cummins et al., 2011). In this study, isocratic elution was performed by using a single buffer, the acidified ethanol (0.5% v/v acetic acid), throughout the entire separation. Anthocyannins molecules were only loosely adsorbed to Amberlite XAD7HP. The separation was achieved by relative speeds of molecular migration over the column. This technique was found to be time-consuming but no gradient-forming apparatus was required (Cummins et al., 2011). Ion exchange is also a non-denaturing strategy hence will remain useful in molecular characterisation (Fekete et al., 2015).

The crude extract of anthocyannin was filtered through filter papers (polyamide, Sartorius, pore size 0.45 µm) to reduce the viscosity of the extract. Hundred milliliters of the filtrate was introduced to a 24/29 fractionating column (Favorit) packed with Amberlite XAD7HP resin (Andersen et al., 2005). The column was activated first with distilled water three times the bed volume. The column was then rinsed with distilled water between eight to twelve times the bed volume. This step removed free sugars, aliphatic acids and other non-flavonoid compounds, those which were designated as

impurities. Anthocyannin and other flavonoids were then eluted with undenatured ethanol containing 0.5% (v/v) acetic acid in isocratic elution. To remove the acidified ethanol prior to further purification, the eluate was concentrated using a rotary evaporator (IKA, RV 10 basic) at 50 °C. The concentrated eluate was freeze-dried and stored at -20 °C until use. Figure 3.3 shows the separation of anthocyannin molecules in ion exchange chromatography.

Figure 3.3: Ion exchange chromatography

3.2.2.2 Size exclusion chromatography

The second purification step involves size exclusion chromatography. Size exclusion or gel filtration chromatography was performed to separate molecules with different molecular sizes. Other names which also refer to this technique are gel permeation, gel exclusion and molecular sieve chromatography. This purification technique is advantageous in which the mild mobile phase conditions allow for molecule characterisation with minimal change in the conformational structure (Fekete et al., 2014). In this technique, molecules are partitioned between a mobile phase and stationary

phase, which comprises of a porous matrix of relative sizes (Duong Ly et al., 2014; O'Fagain et al., 2011). Practically, when samples are loaded in the column, molecules with larger size than the pores will be excluded from the beads, hence migrate relatively quickly through the column. On the other hand, molecules with smaller size than the pores will migrate much more slowly. This, in turn, causes molecules to elute in order of decreasing molecular size (Fekete et al., 2014; O'Fagain et al., 2011). Generally, we assumed that all the molecules in the sample are of the same symmetrical shape so the order of elution will strictly be of decreasing order in size.

Unlike other types of chromatography, the stationary phase in size exclusion does not bind with the elements of the mobile phase. This can prevent unnecessary damage to fragile anthocyannin molecules, ensuring high recovery of the activity (O'Fagain et al., 2011). Moreover, the use of long column results in high resolution (Duong Ly et al., 2014). In this study, we used the C26/40 column, which has an internal diameter of 26 mm and column length of 40 cm. Size exclusion was also used as a final purification step after at least one other purification step, in this study, the ion exchange chromatography. It was not used as the initial purification step due to the existence of different molecules with similar size (Duong Ly et al., 2014). In addition, the matrix used must be inert to the separating molecules to avoid partial adsorption of the molecules to the matrix, which can result in the retardation of the molecular migration, as well as tailed peaks (O'Fagain et al., 2011). In this study, we used Sephadex LH-20 resin as the matrix. This resin is a polysaccharide network, crosslinked from the hydroxypropylated bead-form dextran Sephadex G-25, with fractionation range between 1 to 5 kDa (O'Fagain et al., 2011). Gradient elution was performed, which involved variations in the concentration of the acidified ethanol (0.5% v/v acetic acid) to give a generally high resolution (Cummins et al., 2011)

Some drawbacks of this purification technique were noted. Sephadex from GE Healthcare was itself a carbohydrate, thus milligram quantities of anthocyannin molecules might be shedded into the mobile phase. Secondly, since sugars like anthocyannin are amphipathic with hydrophobic ring structure and hydrophilic functional groups, there might be non-specific interactions with the matrix materials (O'Fagain et al., 2011). Nonetheless, this technique is still essentially useful in purifying anthocyannin molecules.

Freeze-dried anthocyannin extract weighing 0.05 g was dissolved in a mixture of distilled water and undenatured ethanol containing 0.5% (v/v) acetic acid in the ratio of 4:1. 10 ml of the anthocyannin solution was introduced to a C26/40 column (GE Healthcare) packed with Sephadex LH-20 resin (Andersen et al., 2005). The column was activated first with a mixture of distilled water and undenatured ethanol containing 0.5% (v/v) acetic acid in the ratio of 4:1, one-time bed volume. Gradient elution of anthocyannin fractions was performed by increasing the concentration of the acidified ethanol, varying from 20% to 100% (v/v) (Chandrasekhar et al., 2012). The elution was performed at a flow rate of 3 ml/min and the fractions were collected for every 15 ml/tube.

Figure 3.4 shows the fractionation of the anthocyannin samples in size exclusion chromatography. Three distinct fractions were observed upon the elution of the sample through the column. The middle fraction, which was the darkest fraction, was collected and concentrated using a rotary evaporator (IKA, RV 10 basic) at 50 °C. The extract was then freeze-dried and stored at -20 °C until used for the structural identification and the subsequent UV studies, which will be discussed later in this chapter.

Figure 3.4: Size exclusion chromatography

3.2.3 Total monomeric anthocyannin content measurement

To determine the total monomeric anthocyannin content in the anthocyannin sample prior to the UV treatment, pH differential spectroscopic method was employed. This method is advantageous in which it has an outstanding possibility to perform quantitative analysis (Tonutare et al., 2014). Quantitative analysis methods need calibration with high purity compounds of the analysed samples. However, high purity anthocyannins are expensive (Wrolstad et al., 2005) and commercially available standards of anthocyannins are lacking (Garcia-Falcon et al., 2007). In this study, we used the anthocyannin samples which have been purified through ion exchange and size exclusion chromatographies to determine the total monomeric anthocyannin content.

Based on the molar absorptivity or molar extinction coefficient (ε) of anthocyannins, we can calculate the total anthocyannin content in a solvent and pH of choice. Molar absorptivity measures how strongly the anthocyannin pigment absorbs light at a certain wavelength in a specific solvent at a specific pH value (Ahmadiani et al., 2016).

Previously, many literatures have reported a number of different molar absorptivities from decades ago. These data, however, have many discrepancies and inaccuracies due to the limitation in the preparation of crystalline anthocyannins in a pure form (Ahmadiani et al., 2016). The number of anthocyannins in which molar absorptivity has been determined, is limited (Wrolstad et al., 2005). A study conducted by Ahmadiani et al. has identified a number of molar absorptivities ranging from 30,247 to 1057 for red cabbage cyanidin-based anthocyannins in acidified methanol (0.1% HCl) at buffer pH ranging from 1 to 9 (Ahmadiani et al., 2016). It should also be noted that, in employing pH differential method, molar absorptivities determined in aqueous solutions should be used instead of those determined in acidic ethanol or methanol due to solvent effects (Wrolstad et al., 2005). If the total anthocyannin content is expressed as cyanidin-3-glucoside, the frequently used molar absorptivity values are 26,900 and 29,600 (Tonutare et al., 2014). For the purpose of this study, we used the value of 26,900 as the molar absorptivity, in accordance to the study conducted by (Anuar et al., 2013).

The electronic structure of anthocyannin molecule gives the wavelength at which anthocyannins have maximum absorbance (λ_{max}) at a given pH, and the total UV-Vis spectral characteristics. It also determines the pigment colour characteristics (Ahmadiani et al., 2016). In this study, samples of freeze-dried anthocyannin from size exclusion chromatography were separately diluted in 0.025 M of aqueous potassium chloride buffer at pH 1.0, and in 0.4 M of sodium acetate buffer at pH 4.5, where the measurement of absorbance was taken at λ_{max} of pH 1.0 solution. The two buffer solutions have different absorbance due to the monomeric anthocyannins (Wrolstad et al., 2005). Monomeric anthocyannins can undergo reversible structural transformations, in which they exist in the form of coloured oxonium at pH 1.0, and in the form of colourless hemiketal at pH 4.5 (Lee et al., 2005). Polymerised anthocyannin pigments and non-enzymic browning

pigments were omitted from the calculation as they do not undergo reversible transformation with pH (Wrolstad et al., 2005)

The total monomeric anthocyannin content prior to UV treatment was determined using pH differential method as described by Lee et al. and Vankar et al. (Lee et al., 2005; Vankar & Shukla, 2011). The anthocyannin content was determined using Beer-Lambert Law (Yang et al., 2009). The absorbance was measured at various wavelengths using Jasco V-730 UV-Vis spectrophotometer (Jasco Inc., USA). The absorbance was calculated as

$$A = (A_{max} - A_{700})_{\text{pH 1.0}} - (A_{max} - A_{700})_{\text{pH 4.5}}$$ (Chien & Hsu, 2013). Equation 3.1

The following equation was used to calculate the total anthocyannin content as cyanidin-3-glucoside equivalents:

$$\text{Anthocyannin content (g/100 g)} = \frac{[A \times MW \times DF \times V \times 100]}{[\varepsilon \times l \times m]}$$ Equation 3.2

where A is the absorbance calculated using Equation 3.1, MW is the molecular weight (449.2 g/mol for cyanidin-3-glucoside), DF is the dilution factor, V is the solvent volume brought to sample stock solution (in L), ε is the molar extinction coefficient (26900 L mol^{-1} cm^{-1} for cyanidin-3-glucoside), l is the path length (1 cm), and m is the freeze-dried sample weight (in g) (Klimaviciute et al., 2015).

3.2.4 Structural identification of anthocyannins using LC-MS/MS

Due to the lack of pure and structurally defined available standards of acylated cyanidins, the analysis of red cabbage anthocyannin was a difficult task (Arapitsas & Turner, 2008). In this study, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was performed to identify and confirm the type of anthocyannin contained in the red cabbage *B. oleracea*. High performance thin layer chromatography (HPTLC),

high-performance liquid chromatography (HPLC), and LCMSMS are common analytical techniques used to determine qualitatively and quantitatively single phenols. These techniques are favourable for being sufficiently sensitive and precise (Kumar et al., 2013). Nonetheless, HPLC analysis often requires additional treatments prior to the HPLC technique because HPLC method uses UV detection and many phenols show UV spectra with λ_{max} in a narrow range, in which can cause interferences. In contrast, LC-MS/MS can provide useful structural information and allow compound identification. This is true especially when standard reference compounds are not available, and when the generated peaks have similar retention time (Kumar et al., 2013).

The identification and characterisation of anthocyannin pigment have normally employed the tandem mass spectrometry (MS/MS) techniques such as the product-ion analysis, precursor ion analysis and neutral loss analysis (Anuar et al., 2013; Arapitsas & Turner, 2008). A number of researchers used the peak spectral characteristic λ_{vis}, λ_{acyl}, and their corresponding absorptivities to identify the anthocyannins as either monosides or biosides, and as either monoacylated or diacylated. E_{440}/E_{vis} absorptivity ratio of 29% to 35% indicates a monoside while 15% to 24% indicates a bioside. E_{acyl}/E_{vis} absorptivity ratio of 53% to 69% indicates monoacylation while 98% to 128% indicates diacylation (Arapitsas & Turner, 2008). In addition, λ_{acyl} in the 320 nm to 333 nm range indicates either sinapic or ferulic acid while in the 310 nm to 315 nm range indicates *p*-coumaric acid (Dyrby et al., 2001). Table 3.1 shows the molecular weight of common aglycones, sugars, and acylated groups in anthocyannins.

Table 3.1: Molecular weight (Da) of common anthocyanidins, sugars, and acylated groups in anthocyannins (Wu & Prior, 2005)

Compound	MW	MW—H_2O
Anthocyanidin		
Pelargonidin	271	
Cyanidin	287	
Peonidin	301	
Petunidin	303	
Delphinidin	317	
Malvidin	331	
Monosaccharide		
Pentose		
Arabinose	150	132
Xylose	150	132
Rhamnose	164	146
Hexose		
Glucose	180	162
Galactose	180	162
Disaccharide		
Sambubiose (2—*O*—*β*—D—xylosyl—D—glucose)	312	294
Lathyrose (2—*O*—*β*—D—xylosyl—D—galactose)	312	294
Rutinose (6—*O*—α—L—rhamnosyl—D—glucose)	326	308
Sophorose (2—*O*—*β*—D—glucosyl—D—glucose)	342	324
Laminaribiose (3—*O*—*β*—D—glucosyl—D—glucose)	342	324
Gentiobiose (6—*O*—*β*—D—glucosyl—D—glucose)	342	324

Acylated group		
Aliphatic acid	60	42
Acetic acid	74	56
Propionic acid	90	72
Oxalic acid	104	86
Malonic acid	118	100
Succinic acid	134	116
Malic acid		
Aromatic acid	138	120
p-hydroxybenzoic acid	164	146
p-coumaric acid	180	162
caffeic acid	194	176
ferulic acid	224	206
sinapic acid		

A sample of freeze-dried anthocyannin from size exclusion chromatography was sent to Advanced Chemistry Solutions (Malaysia) for anthocyannin structural identification. Ultra High Performance Liquid Chromatography (UHPLC) was performed on the sample using Agilent 1290 series. Three sample extracts were not reconstituted in any solvent but injected directly with injection volume of 10 µL. The sample was then analysed using AB Sciex 5500QTrap, equipped with Phenomenex Synergi Fusion column (100 mm x 2.1 mm x 3 µm). The solvents used were (A) water with 0.1% (v/v) formic acid and (B) acetonitrile with 0.1% (v/v) formic acid, establishing the following gradient: 5% B to 95% B from 0.01 minute to 10 minutes, hold for two minutes, and back to 10% B in 0.1 minute and re-equilibrated for 3 minutes. The tandem mass spectrometry (MS/MS) was

operated in a positive ion mode under the following conditions: voltage IS of 5500 V, source temperature at 500 °C, desolvation gas at 40 psi, source gas at 40 psi, scan range between 100 to 1000 m/z for full scan and 50 to 1000 m/z for MS/MS scan. Mass fragmentations were based on journal references and ACD/Labs advanced chemometrics mass fragmentations predictive software (Jampani & Naik, 2014; McDougall et al., 2007; Pang et al., 2004).

3.3 Effects of UV treatment on the colour stability and concentration of anthocyannins in *B. oleracea* (study 2)

3.3.1 Sample preparation

Food hydrocolloids; agar, carrageenan and gelatine were initially dissolved in distilled water to give 0.2% (w/v) agar solution, 1% (w/v) carrageenan solution and 6% (w/v) gelatine solution. Cinnamic acid, ferulic acid and gallic acid as copigments were also dissolved in distilled water to give 0.1% (w/v) solution each. Freeze-dried anthocyannin from size exclusion chromatography weighing 0.05 g was dissolved in the food hydrocolloids and the copigments respectively to give a final concentration of 0.53 g/L without copigments, and 0.424 g/L with copigments. Hydrocolloids without anthocyannin serve as the first control. Hydrocolloids with anthocyannin kept in a dark room serve as the second control. All samples were made in triplicates.

In this study, the effects of UV radiation and presence of copigments were investigated. Samples of each hydrocolloid with anthocyannin but no copigment, and samples with anthocyannin and different copigments were exposed to 100% UV-A radiation at 10 °C for 24 hours/day for 35 days. The distance between the samples and the UV light source was fixed at 7.3 cm. Another triplicates of each hydrocolloid without anthocyannin and copigment were also exposed to 100% UV-A at 10 °C to serve as the first control. The experiment was repeated with UV-B and UV-C. Another triplicates of each hydrocolloid

with anthocyannin but no copigment were kept in a dark room at 10 °C to serve as the second control.

Table 3.2: Samples and controls under investigation

Triplicates	Sample contents
Sample 1	Hydrocolloid + anthocyannin (UV)
Sample 2	Hydrocolloid + anthocyannin + cinnamic acid (UV)
Sample 3	Hydrocolloid + anthocyannin + ferulic acid (UV)
Sample 4	Hydrocolloid + anthocyannin + gallic acid (UV)
Control 1	Hydrocolloid (UV)
Control 2	Hydrocolloid + anthocyannin (dark room)

3.3.2 Colorimetric study

CIE *L**, *a**, *b** is a rectangular coordinate system which has been used to communicate numerical colours (Berns, 2014). Owing its name to the Commission Internationale d'Eclairage (Bakker et al., 1986), the CIELAB coordinates were previously and successfully used to describe the colour of berries and flowers, including green-yellow, pink, red, red-grey, red-dark violet, blue-black, and red-black (Liang et al., 2011). The *L**, *a**, *b** values describe the colour space in three dimensions. Based on Figure 4.13, *L** is a vertical axis which measures lightness, assigning values from 0 to 100 to describe samples from completely opaque to completely transparent (Bakker et al., 1986). *a** and *b** are axes perpendicular to each other which describe samples from red to green, and from blue to yellow, respectively (Liang et al., 2011). The hue angle, h_{ab} and the chroma, C^*_{ab} are calculated and derived from the values of *a** and *b** (Bakker et al., 1986; Liang et al., 2011). The hue is how our eyes perceive a colour, while the chroma describes the vividness or the dullness of a colour. Chroma is also known as saturation.

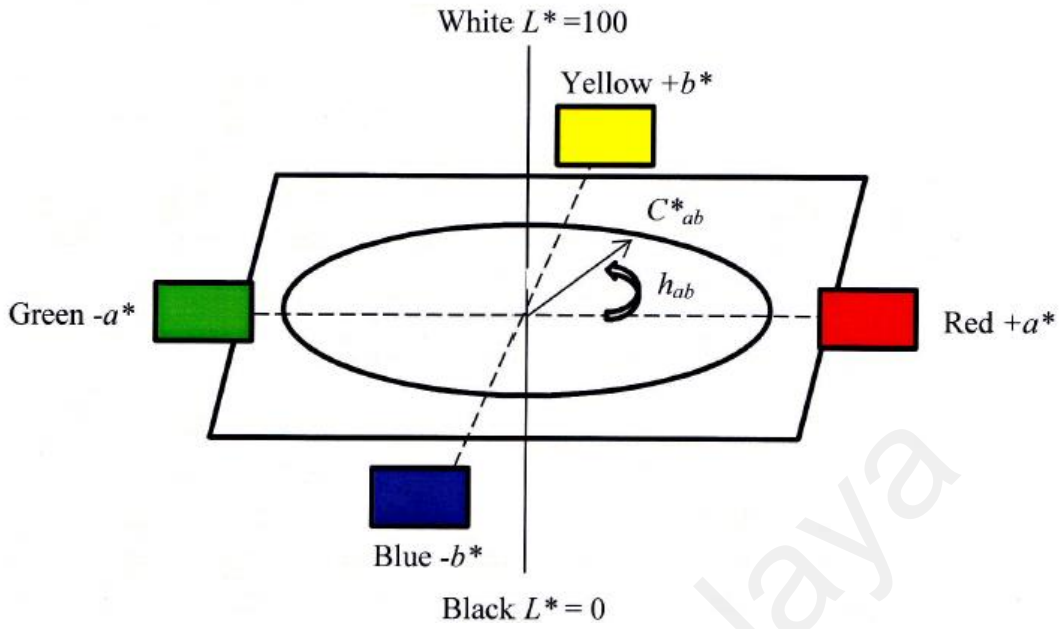


Figure 3.5: CIELAB colour space chart (Anuar et al., 2013)

The CIELAB spectrophotometer gathers and filters the wavelengths of light reflected from an object. The colours are assigned to numeric values, and recorded as points across the visible spectrum. This gives the spectral curve. The reflectance curve can then be obtained. The data on the reflectance curve is then multiplied by the CIE standard illuminant, such as D65 (daylight). The product is further multiplied by the CIE standard observer, which gives the tristimulus values of XYZ. These values can identify a colour numerically (Anuar et al., 2013). Figure 3.6 summarises the working principle of the spectrophotometer.

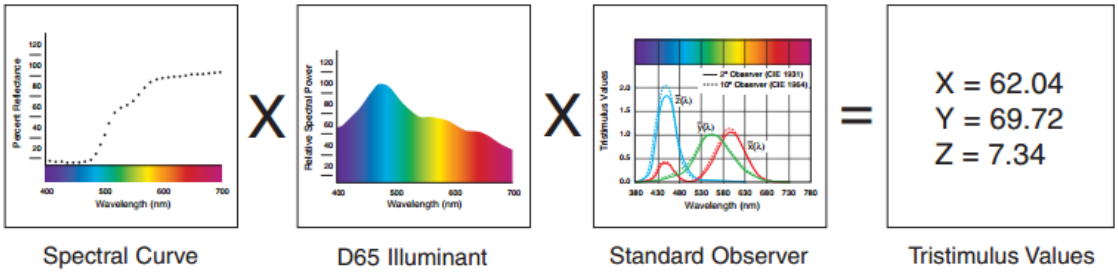


Figure 3.6: The derivation of tristimulus values (Anuar et al., 2013)

The changes in colour in anthocyannin upon UV exposure were determined using the CIELAB colorimeter, equipped with Ava Spec-2048 spectrometer and Ava Soft 8.2 Software. The calculation takes into account the D65 standard illuminant which corresponds to the natural daylight, and 10° standard observer. The parameters L^* (lightness), a^* (redness) and b^* (yellowness) were used to calculate the values of C^*_{ab} (chroma) and h_{ab} (hue angle).

3.3.3 Chemometric analysis

To account for the changes in the concentration of anthocyannin in the hydrocolloid samples upon exposure to UV radiation, we performed the Fourier Transform Infrared (FT-IR) spectroscopy. When molecules absorb infrared radiation, they are excited to a higher energy state. They absorb at selected frequencies, which correspond to the stretching and vibrational frequencies of most covalent bonds. These molecules absorb at the frequencies of infrared radiation, which match the natural vibrational frequencies of the molecules. The absorbed energy then increases the amplitude of the vibrational motions of the bonds (Pavia et al., 2001). However, only bonds with a dipole moment which change with time can absorb infrared radiation. Thus, only asymmetric bonds can give an infrared spectrum.

The optical pathway of modern infrared spectrometers produces a pattern known as an interferogram. This is a complex signal, containing all the frequencies which make up the infrared spectrum (Pavia et al., 2001). Infrared spectra normally consist of overlapping infrared bands. These overlapping bands often cause a problem because the widths of individual absorption bands are normally larger than the separation between neighbouring bands (Markovich & Pidgeon, 1991). Mathematical techniques such as curve-fitting, derivative spectroscopy, and Fourier deconvolution were used to overcome this problem. Fourier deconvolution is a powerful technique, which provides the most information on

band structure. To obtain a spectrum of a compound, researcher first collects the background noise, which consists of infrared-active atmospheric gases, carbon dioxide and water vapour. When the compound of interest is placed into the beam, the resulting spectrum contains both the absorption bands for the compound and the background. The computer software will then automatically subtract the background noise from the sample spectrum, producing only the spectrum of the analysed compound (Pavia et al., 2001). Nonetheless, researchers need to make the correct choice of the spectral and the deconvolution parameters. Artefacts such as water vapour band intensities, and background noise are enhanced by this technique, hence may be mistaken for real spectra (Markovich & Pidgeon, 1991).

FT-IR technology in the middle infrared (MIR) region has demonstrated better determination of constituents and properties of molecules with a higher accuracy, when compared with near infrared (NIR) region (Rasines Perea et al., 2015). Previously, researchers have proposed several techniques in FT-IR spectroscopy to study the characterisation and classification of wines based on geographical origin, grape variety, and manufacturing technique. FT-MIR was first applied in the quantification of anthocyannins by Versari et al. (2015) for total anthocyannins, and Soriano et al. (2015) for individual anthocyannins (Rasines Perea et al., 2015). To date, FT-MIR, together with advances in multivariate data analysis, have been an important tool in wine analysis. FT-MIR has been used to study important chemical parameters for wine quality analysis, including alcoholic degree and total acidity (Romera Fernandez et al., 2012).

Pereira et al. studied the characterisation of a Time-Temperature Indicator (TTI) based on a PVA/Chitosan polymers containing anthocyannin to indicate changes in food quality at various storage temperatures. They reported a strong absorption band in the FT-IR spectrum of anthocyannin extract, with a maximum at 1015 cm^{-1}, which corresponds to

aromatic ring C—H deformation. The second absorption bands were recorded at 1650 cm^{-1} and 1455 cm^{-1}, which correspond to the stretching vibration of C==C aromatic ring. Another absorption band with a maximum at 1233 cm^{-1} was also recorded, which corresponds to the stretching of pyran rings. In addition, bands between 1300 cm^{-1} and 1380 cm^{-1} correspond to C—O angular deformations of phenols (Pereira & Stefani, 2015).

The changes in anthocyannin concentration upon UV exposure were determined using the Perkin Elmer Spectrum 400 FT-IR spectrophotometer with a single reflectance horizontal attenuated total reflectance (ATR) cell equipped with a diamond crystal. The data were recorded in the spectral range from 650 to 4000 cm^{-1} by accumulating 4 scans with a resolution of 4 cm^{-1} (Pereira & Stefani, 2015).

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Extraction and identification of major anthocyannins in *B. oleracea* (study 1)

4.1.1 Extraction and purification of anthocyannins

In this study, 9 kg of red cabbage *B. oleracea* produced 10.63 g of freeze-dried anthocyannin extract after performing ion exchange chromatography. This gave a yield of 0.12% (w/w). Anthocyannin extract from ion exchange chromatography weighing 8 g was subjected to size exclusion chromatography. This step further produced 1.68 g of freeze-dried anthocyannin extract, which gave a yield of 21.00% (w/w).

4.1.2 Total monomeric anthocyannin content measurement

Figure 4.1 shows the spectral characteristics of crude anthocyannin extract of *B. oleracea* in pH 1.0 and pH 4.5 buffers. This figure clearly shows that the λ_{max} of anthocyannins from red cabbage *B. oleracea* was 515 nm with absorbance of 0.62 in potassium chloride buffer at pH 1.0. It was observed that the curvature of pH 1.0 was remarkable in the visible region between 450 nm and 600 nm. On the other hand, there was a small absorption band between 450 nm and 610 nm for pH 4.5. In accordance to the study conducted by (Lee et al., 2005), the absorbance measurements were performed within 3 minutes upon the dilution of the samples in buffers. This is crucial to avoid misleading measurement since absorbance increases with time upon dilution.

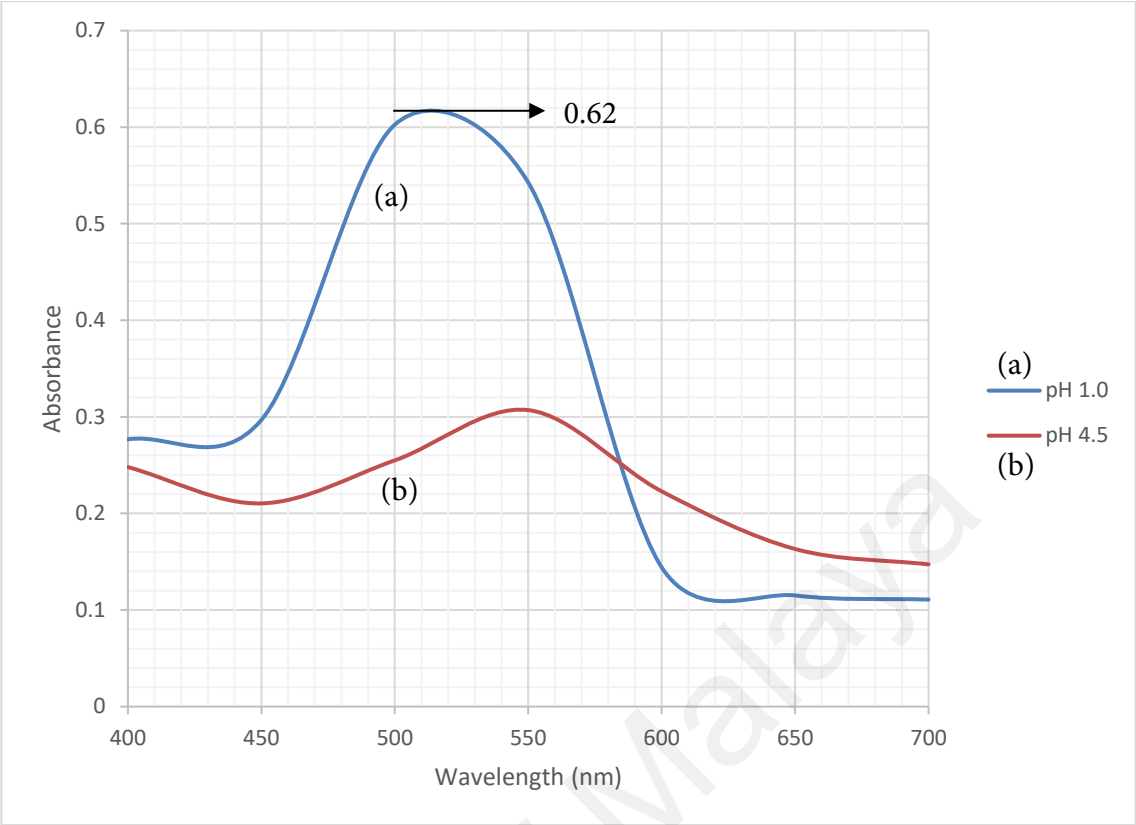


Figure 4.1: Spectral characteristics of crude anthocyannin extract of red cabbage *B. oleracea* in pH 1.0 and pH 4.5 buffers

Based on Figure 4.1, 515 nm was chosen as the λ_{max} to calculate the total monomeric anthocyannin prior to UV treatment (See Section 3.2.3). Since it is customary to use the molecular weight and molar absorptivity of the major anthocyannin in the sample matrix (Wrolstad et al., 2005), the total monomeric anthocyannin content was calculated as cyanidin-3-glucoside equivalent. Using Equation 3.1, the absorbance value of 0.3494 was used to calculate the total monomeric anthocyannin content. Using Equation 3.2, the total monomeric anthocyannin content was determined to be 117 mg per 100 g fresh weight of red cabbage *B. oleracea*, where DF was 100, V was 0.01 L, and m was 0.05 g.

4.1.3 Structural identification of anthocyannins using LC-MS/MS

Structural identification of anthocyanins using LC-MS/MS was performed by Advanced Chemistry Solutions. The identification was made based on MS/MS spectrum produced

and was matched to the library. All fragment ions which were found similar to the library is a positive identification, thus the compounds were named accordingly. At times, the compound was unable to be identified exactly but the predictive software categorised the compounds into classes based on the fragment ions. For instance, in dimer or trimer anthocyanin compounds, fragment ion of m/z 289 indicates that the compound contains cathechin fragment, which is in anthocyanin class. There is no individual reference available for each identified compound. The library used is an internal database compiled with Sciex instruments, and was built based on compound standards. Since the detection and identification were based on mass spectrometry, UV chromatogram was not produced in this analysis.

Table 4.1 summarises the chromatographic profile of anthocyannins from red cabbage *B. oleracea* shown in Appendix A1. All the identified anthocyannins have cyanidin as the aglycone part, a finding similar to a number of published data (Arapitsas & Turner, 2008; Charron et al., 2007; Dyrby et al., 2001; Wu & Prior, 2005). As for the sugar part, disaccharides (sophorose) of hexose (glucose) were identified. For the acylated groups, aromatic acids (ferulic and sinapic acids) were identified. By combining MS and MS/MS data, peak 5 was identified as cyanidin 3-sophoroside-5-glucoside. The data indicated a fragment pattern of $[M+H]^+$ *m/z* 773, with MS/MS *m/z* 611, 449, and 287 $[cyanidin]^+$. In fact, all 10 peaks indicated the same fragment ion *m/z* 287 which corresponds to $[cyanidin]^+$. Peak 2 was identified as cyanidin-3(6-feruloyl)-sophoroside-5-glucoside. The data indicated a fragment pattern of $[M+H]^+$ *m/z* 920, with MS/MS *m/z* 758, 449, 287 $[cyanidin]^+$ and 207 $[sinapic\ acid - H_2O]^+$. Peak 4 was identified as cyanidin-3(6-sinapyl)-sophoroside-5-glucoside. The data indicated a fragment pattern of $[M+H]^+$ *m/z* 920 with MS/MS *m/z* 758, 450, 287 $[cyanidin]^+$ and 207 $[sinapic\ acid - H_2O]^+$. Peak 9 was identified as cyanidin 3-(6-feruloylsophoroside)-5-glucoside. The data indicated a fragment pattern of $[M+H]^+$ *m/z* 950 with MS/MS *m/z* 788 and 287 $[cyanidin]^+$. Peak 10

was identified as cyanidin-3-(6-sinapyl)-sophoroside-5-glucoside. The data indicated a fragment pattern of $[M+H]^+$ *m/z* 979 with MS/MS *m/z* 817, 449, 316 and 287 $[cyanidin]^+$. Finally, peaks 1, 3, 6, 7 and 8 were identified as cyanidin-3(6-sinapyl)-sophoroside-5(6-sinapyl)-glucoside. Peak 1 and 3 have the same fragment pattern of $[M+H]^+$ *m/z* 980 with MS/MS *m/z* 818, 450 and 287 $[cyanidin]^+$, but with different retention times. Peak 6 and 7 also have the same fragment pattern of $[M+H]^+$ *m/z* 979 with MS/MS *m/z* 818, 450 and 287 $[cyanidin]^+$, again with different retention times. Peak 8 has a fragment pattern of $[M+H]^+$ *m/z* 979 with MS/MS *m/z* 817, 449, 287 $[cyanidin]^+$ and 207 [sinapic acid – $H_2O]^+$.

Table 4.1: Identification of major anthocyannins in *B. oleracea*

Peak	RT (min)	$[M+H]^+$ *m/z*	MS/MS *m/z*	Identification
1	1.33	980	818, 450, 287	Cyanidin-3(6-sinapyl)-sophoroside-5(6-sinapyl)-glucoside
2	1.81	920	758, 449, 287, 207	Cyanidin-3(6-feruloyl)-sophoroside-5-glucoside
3	2.11	980	818, 450, 287	Cyanidin-3(6-sinapyl)-sophoroside-5(6-sinapyl)-glucoside

4	2.51	920	758, 450, 287, 207	Cyanidin-3(6-sinapyl)-sophoroside-5-glucoside
5	2.71	773	611, 449, 287	Cyanidin 3-sophoroside-5-glucoside
6	3.32	979	818, 450, 287	Cyanidin-3(6-sinapyl)-sophoroside-5(6-sinapyl)-glucoside
7	4.11	979	819, 450, 287	Cyanidin-3(6-sinapyl)-sophoroside-5(6-sinapyl)-glucoside
8	6.21	979	817, 449, 287, 207	Cyanidin-3(6-sinapyl)-sophoroside-5(6-sinapyl)-glucoside
9	6.71	950	788, 287	Cyanidin 3-(6-feruloylsophoroside)-5-glucoside
10	7.81	979	817, 449, 316, 287	Cyanidin-3-(6-sinapyl)-sophoroside-5-glucoside

4.2 Effects of UV treatment on the colour stability and concentration of anthocyannins in *B. oleracea* (study 2)

4.2.1 Exposure to UV-A

The wavelength of UV-A ranges from 400 nm to 320 nm, the longest between the three types of UV radiation. Table 4.2 shows the mean values of the colour parameters for the non-enhanced sample (without copigment), enhanced samples (with copigments), and non-pigmented sample of agar hydrocolloid under exposure of UV-A during 35 days of storage. At day 0, all samples had not been exposed to UV radiation yet. Agar containing anthocyannin-cinnamic acid complex was observed to be the lightest sample with the highest L^* value (73.520 ± 2.260). On the other hand, agar containing anthocyannin-gallic acid complex were the darkest sample with the lowest L^* value (60.180 ± 2.082). Upon storage, the samples of agar containing anthocyannin, agar containing anthocyannin-gallic acid complex, and agar containing anthocyannin kept in a dark room (control) were observed to become darker, with a general trend of decreasing L^* values. At the end of the storage, agar (control) sample was the lightest sample with the highest L^* value (87.163 ± 3.063), while agar containing anthocyannin sample was the darkest with the lowest L^* value (39.880 ± 6.930).

Table 4.3 shows the mean value of the colour parameters for the samples of carrageenan hydrocolloid under the exposure of UV-A during 35 days of storage. At day 0, the carrageenan (control) sample was the lightest sample with the highest L^* value (68.317 ± 2.367). This was followed by carrageenan sample containing anthocyannin-gallic acid complex with L^* value (53.630 ± 9.610). Upon storage, samples containing anthocyannin, anthocyannin-ferulic complex and anthocyannin-gallic acid complex became darker, with a general decrease of L^* values. Both control samples, and sample containing anthocyannin-cinnamic complex became lighter with increasing L^* values. At the end of

the storage, the carrageenan (control) sample was the lightest sample the highest *L** value (68.467 ± 2.860) while carrageenan containing anthocyannin sample was the darkest with lowest *L** value (35.197 ± 5.043).

Table 4.4 shows the mean value of the colour parameters for the samples of gelatine hydrocolloid under the exposure of UV-A during 35 days of storage. At day 0, the gelatine sample containing anthocyannin-gallic acid complex was the lightest sample with the highest *L** value (91.750 ± 0.821). Upon storage, samples of gelatine containing anthocyannin, and anthocyannin-cinnamic acid complex became lighter with a general increase of *L** values. On the other hand, all other samples, including the two controls, became darker with a general decrease in *L** values. At the end of the storage, the gelatine sample containing anthocyannin kept in a dark room (control) was the darkest sample with the lowest *L** value (44.300 ± 26.579), followed by gelatine sample containing anthocyannin-gallic acid complex (72.823 ± 8.047). On the other hand, gelatine (control) sample was the lightest sample with the highest *L** value (86.733 ± 1.056).

Hue angle was also affected by UV exposure and addition of copigments. Table 4.2 shows the relationship between the hue, *a** and *b** values for agar samples under exposure of UV-A. For agar sample containing anthocyannin, the hue angles were above 300° from day 0 to day 35, except on day 21, which was (229.330 ± 198.320). From day 0 to day 35, it was observed that all *a** values were positive, which indicated redness, and all *b** values were negative, which indicated blueness. For agar sample containing anthocyannin-cinnamic acid complex, the hue angle was (9.840 ± 9.231) at day 0, and started increasing up to (330.237 ± 3.881) at day 14, and decreased again to (146.963 ± 167.749) at the end of the storage. For agar sample containing anthocyannin-ferulic acid complex, the hue angle started with (234.947 ± 202.443) at day 0, increasing throughout the storage period, and dropped again until (44.123 ± 7.776) at day 28. For agar sample

containing anthocyannin-gallic acid complex, the hue angle was the lowest at day 7 (224.213 ± 194.010), and highest at day 28 (349.220 ± 4.417). For agar (control) sample, the highest hue angle was observed at day 7 (155.437 ± 151.351), and lowest at day 14 (66.133 ± 3.489). For agar sample containing anthocyannin kept in a dark room (control), the hue angles were similar to that of agar sample containing anthocyannin exposed to UV-A. It was also observed that all a^* values for all samples were positive throughout the storage period.

Table 4.3 shows the change in hue angle for carrageenan samples exposed to UV-A. For carrageenan sample containing anthocyannin, h_{ab} was the lowest at day 35 (55.583 ± 19.662), and the highest at day 7 (177.490 ± 25.096). All b^* values were positive, indicating yellowness of the sample. For carrageenan sample containing anthocyannin-cinnamic acid complex, the lowest h_{ab} value was observed at day 35 (57.680 ± 34.823), and highest at day 0 (289.563 ± 5.332). All a^* values were positive, indicating redness of the sample. For carrageenan sample containing anthocyannin-ferulic acid complex, h_{ab} value was the lowest at day 35 (67.780 ± 16.469), and highest at day 0 (296.477 ± 2.757). For carrageenan sample containing anthocyannin-gallic acid complex, h_{ab} was the lowest at day 35 (37.850 ± 26.025), and highest at day 14 (210.123 ± 163.277). All a^* values were positive, indicating redness of the sample. For carrageenan (control) sample, h_{ab} was lowest at day 35 (38.543 ± 15.927), and highest at day 7 (217.223 ± 123.369). For carrageenan sample containing anthocyannin kept in a dark room (control), h_{ab} was the lowest at day 35 (78.820 ± 8.218), and highest at day 7 (106.850 ± 6.548). All b^* values were positive, indicating yellowness of the sample.

Table 4.4 shows the change in hue angle for gelatine samples under exposure of UV-A. For gelatine sample containing anthocyannin, h_{ab} was the lowest at day 14 (90.013 ± 61.677), and highest at day 0 (289.157 ± 19.500). For gelatine sample containing

anthocyannin-cinnamic acid complex, h_{ab} was the lowest at day 28 (160.093 ± 15.231), and highest at day 35 (309.380 ± 31.287). For gelatine sample containing anthocyannin-ferulic acid complex, h_{ab} was the lowest at day 28 (90.493 ± 27.475), and highest at day 0 (321.660 ± 26.032). For gelatine sample containing anthocyannin-gallic acid complex, h_{ab} was the lowest at day 7 (81.190 ± 10.114), and highest at day 0 (319.140 ± 29.171). All a^* values were positive, indicating redness of the sample. For gelatine (control) sample, h_{ab} was the lowest at day 14 (52.880 ± 43.109), and highest at day 28 (135.667 ± 125.434). For gelatine sample containing anthocyannin kept in a dark room (control), h_{ab} was the lowest at day 21 (84.300 ± 7.670), and highest at day 0 (289.157 ± 19.500).

To study the colour stability of anthocyannin samples, C^*_{ab} or chromaticity was also taken into account. From Table 4.2, at day 0 of exposure to UV-A, the agar (control) sample gave the dullest colour with the lowest C^*_{ab} value (4.477 ± 0.047), whereas the agar sample containing anthocyannin-gallic acid complex was the brightest with the highest C^*_{ab} value (16.140 ± 2.462). Upon storage, it was observed that all agar samples except agar (control) sample became brighter with a general increase in their C^*_{ab} values. At the end of the storage, agar sample containing anthocyannin had the brightest colour with the highest C^*_{ab} value (23.080 ± 5.346). On the other hand, the agar (control) sample was the dullest sample with the lowest C^*_{ab} value (3.947 ± 1.443), followed by agar sample containing anthocyannin-cinnamic acid complex (6.877 ± 4.508).

From Table 4.3, we can observe the change in chromaticity for carrageenan samples under UV-A exposure. At day 0, samples of carrageenan containing anthocyannin, and anthocyannin-cinnamic acid complex were the brightest with the highest C^*_{ab} value (21.687 ± 1.336), whereas the dullest sample was carrageenan (control) with the lowest C^*_{ab} (5.010 ± 1.261). Upon storage, all samples except carrageenan containing anthocyannin-gallic acid complex became duller with a general decrease in C^*_{ab} values.

At the end of the storage, samples of carrageenan containing anthocyannin, and anthocyannin-ferulic acid complex, were the brightest with the highest C^*_{ab} values of (11.963 ± 4.218) and (11.457 ± 1.930) respectively. On the other hand, carrageenan (control) sample was the dullest with the lowest C^*_{ab} value (1.990 ± 0.295), followed by carrageenan containing anthocyannin kept in a dark room (control) (5.060 ± 1.794) and carrageenan containing anthocyannin-cinnamic acid complex (5.710 ± 0.745).

Based on Table 4.4, we can see the change in chromaticity in gelatine sample under exposure to UV-A. At day 0, gelatine sample containing anthocyannin-gallic acid complex was the dullest with the lowest C^*_{ab} value (0.697 ± 0.251), whereas gelatine samples containing anthocyannin (including the control) were the brightest with the highest C^*_{ab} value (2.910 ± 2.832). Upon storage, samples of gelatine containing anthocyannin, anthocyannin-ferulic acid complex, and anthocyannin-gallic acid complex became brighter with a general increase in C^*_{ab} values. At the end of the storage, gelatine sample of anthocyannin-gallic acid complex was the brightest with the highest C^*_{ab} value (17.617 ± 8.247), while gelatine sample with anthocyannin-cinnamic acid complex was the dullest with the lowest C^*_{ab} value (1.777 ± 1.111).

Table 4.2: The relationship between the colour parameters of agar hydrocolloid samples containing anthocyannin and UV-A exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Agar + anthocyannin	0	64.010 ± 3.795	9.287 ± 1.429	-3.480 ± 2.192	340.663 ± 8.452	9.993 ± 2.136	
	7	53.177 ± 7.043	39.040 ± 19.529	-11.737 ± 4.009	333.870 ± 3.865	27.097 ± 10.432	
	14	47.230 ± 2.200	18.547 ± 1.782	-9.370 ± 2.276	333.197 ± 6.464	20.867 ± 1.702	
	21	38.907 ± 18.105	38.603 ± 3.019	-7.237 ± 6.526	229.330 ± 198.320	39.643 ± 2.859	
	28	34.470 ± 7.946	22.767 ± 3.334	-5.047 ± 1.991	347.697 ± 3.627	23.353 ± 3.576	
	35	39.880 ± 6.930	23.033 ± 5.368	-1.403 ± 0.430	356.330 ± 1.442	23.080 ± 5.346	
Agar + anthocyannin + cinnamic acid	0	73.520 ± 2.260	8.157 ± 2.289	1.190 ± 1.016	9.840 ± 9.231	8.313 ± 2.123	
	7	60.003 ± 24.345	16.763 ± 8.598	-4.543 ± 7.001	226.723 ± 191.835	17.877 ± 9.794	
	14	58.783 ± 1.313	11.033 ± 0.333	-6.340 ± 1.077	330.237 ± 3.881	12.750 ± 0.711	
	21	56.873 ± 19.661	10.917 ± 7.313	-8.093 ± 7.151	326.773 ± 10.955	13.697 ± 10.008	

‘Table 4.2, continued’

	28	49.893 ± 21.093	12.870 ± 4.920	2.827 ± 2.686	135.397 ± 193.977	13.483 ± 4.380	
	35	70.393 ± 8.215	4.573 ± 2.677	4.643 ± 4.511	146.963 ± 167.749	6.877 ± 4.508	
Agar + anthocyanin + ferulic acid	0	66.570 ± 3.789	8.837 ± 1.167	-0.863 ± 1.083	234.947 ± 202.443	8.917 ± 1.263	
	7	56.760 ± 42.284	25.543 ± 5.434	-9.473 ± 6.315	340.390 ± 12.584	27.630 ± 6.110	
	14	64.180 ± 3.135	13.643 ± 2.534	-5.157 ± 1.932	339.730 ± 3.557	14.603 ± 3.045	
	21	61.417 ± 21.509	10.550 ± 3.777	-2.090 ± 3.374	231.197 ± 200.223	10.997 ± 4.202	
	28	72.963 ± 0.988	28.957 ± 37.129	7.293 ± 2.277	44.123 ± 7.776	10.360 ± 1.862	
	35	72.760 ± 7.533	10.850 ± 2.691	7.247 ± 12.066	139.253 ± 158.011	15.827 ± 5.700	
Agar + anthocyanin + gallic acid	0	60.180 ± 2.082	14.860 ± 1.653	-6.070 ± 2.745	338.550 ± 7.698	16.140 ± 2.462	
	7	61.463 ± 16.485	27.537 ± 12.926	-10.333 ± 10.224	224.213 ± 194.010	29.817 ± 15.361	

	14	37.457 ± 7.117	30.817 ± 4.237	-14.293 ± 5.811	335.830 ± 6.901	34.120 ± 6.038
	21	50.327 ± 11.538	29.403 ± 9.906	-9.553 ± 1.033	340.940 ± 5.138	30.980 ± 9.662
	28	47.523 ± 4.654	28.820 ± 7.080	-5.147 ± 1.196	349.220 ± 4.417	29.340 ± 6.798
	35	56.600 ± 2.962	19.237 ± 9.612	0.137 ± 1.726	241.467 ± 202.164	19.293 ± 9.594
Agar (control)	0	73.160 ± 4.062	0.430 ± 0.200	4.450 ± 0.044	84.513 ± 2.549	4.477 ± 0.047
	7	85.457 ± 3.461	2.010 ± 0.687	2.903 ± 4.775	155.437 ± 151.351	4.697 ± 2.972
	14	74.400 ± 1.163	1.177 ± 0.150	2.670 ± 0.167	66.133 ± 3.489	2.920 ± 0.128
	21	78.837 ± 4.933	0.563 ± 0.404	1.430 ± 3.922	152.337 ± 117.363	3.377 ± 1.395
	28	77.960 ± 0.805	0.643 ± 0.827	2.543 ± 1.152	72.660 ± 19.839	2.743 ± 1.027
	35	87.163 ± 3.063	2.037 ± 0.387	3.237 ± 1.838	53.703 ± 19.920	3.947 ± 1.443
Agar + anthocyanin in a	0	64.010 ± 3.795	9.287 ± 1.429	-3.480 ± 2.192	340.663 ± 8.452	9.993 ± 2.136
	7	60.687 ± 2.986	9.810 ± 2.594	-7.653 ± 2.298	322.240 ± 2.515	12.450 ± 12.430

dark room (control)	14	66.800 ± 4.294	10.163 ± 2.487	-2.970 ± 2.145	238.310 ± 180.227	10.640 ± 3.011	
	21	51.660 ± 6.790	32.347 ± 6.488	-9.670 ± 3.666	343.647 ± 3.541	33.800 ± 7.159	
	28	59.620 ± 6.712	16.697 ± 6.711	-8.853 ± 2.876	331.657 ± 1.770	18.907 ± 7.274	
	35	54.023 ± 4.023	16.937 ± 4.611	-5.040 ± 5.459	225.093 ± 190.611	18.157 ± 4.997	

Table 4.3: The relationship between the colour parameters of carrageenan hydrocolloid samples containing anthocyannin and UV-A exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Carrageenan + anthocyannin	0	45.753 ± 10.090	0.893 ± 2.525	13.597 ± 1.998	87.287 ± 10.524	13.773 ± 2.080	
	7	28.900 ± 5.970	-5.267 ± 4.291	1.277 ± 1.492	177.490 ± 25.096	5.510 ± 4.370	
	14	36.713 ± 7.811	-3.660 ± 2.221	5.503 ± 3.192	125.240 ± 11.564	6.713 ± 3.606	
	21	38.943 ± 3.747	-2.053 ± 0.822	12.007 ± 1.408	99.903 ± 4.593	12.207 ± 1.303	
	28	34.500 ± 10.634	2.283 ± 1.179	8.387 ± 3.515	74.037 ± 6.945	8.727 ± 3.585	
	35	35.197 ± 5.043	5.797 ± 0.717	10.073 ± 5.420	55.583 ± 19.662	11.963 ± 4.218	
Carrageenan + anthocyannin + cinnamic acid	0	38.080 ± 5.261	8.417 ± 0.997	-19.960 ± 1.503	292.927 ± 3.222	21.687 ± 1.336	
	7	30.240 ± 4.359	9.217 ± 3.047	-25.573 ± 1.591	289.563 ± 5.332	27.260 ± 2.357	
	14	41.273 ± 2.736	2.417 ± 3.680	-7.150 ± 4.426	281.463 ± 26.118	7.900 ± 5.002	
	21	48.030 ± 15.684	4.253 ± 2.750	0.957 ± 3.601	144.913 ± 165.391	5.437 ± 2.180	
	28	44.477 ± 7.167	2.610 ± 0.436	7.220 ± 4.592	62.663 ± 23.263	7.937 ± 3.894	

	35	59.470 ± 18.374	2.663 ± 2.905	4.283 ± 1.674	57.680 ± 34.823	5.710 ± 0.745	
Carrageenan + anthocyannin + ferulic acid	0	47.670 ± 5.847	6.063 ± 1.821	-12.483 ± 4.619	296.477 ± 2.757	13.883 ± 4.932	
	7	40.500 ± 8.234	-2.633 ± 3.810	-6.787 ± 4.105	260.260 ± 36.739	7.857 ± 4.286	
	14	37.790 ± 11.512	-6.277 ± 2.705	0.847 ± 2.322	173.893 ± 18.967	6.567 ± 2.872	
	21	38.663 ± 8.107	-2.423 ± 2.301	5.157 ± 3.539	122.443 ± 37.446	6.503 ± 1.738	
	28	38.107 ± 8.820	0.477 ± 2.425	17.203 ± 2.285	89.087 ± 7.567	17.313 ± 2.392	
	35	38.743 ± 5.603	3.950 ± 2.182	10.447 ± 2.953	67.780 ± 16.469	11.457 ± 1.930	
Carrageenan + anthocyannin + gallic acid	0	53.630 ± 9.610	3.380 ± 1.058	3.973 ± 2.294	46.190 ± 23.109	5.517 ± 1.255	
	7	21.810 ± 11.689	1.430 ± 1.222	-0.337 ± 3.697	133.177 ± 133.167	3.260 ± 1.569	
	14	17.880 ± 2.887	2.147 ± 1.571	-1.007 ± 2.320	210.123 ± 163.277	3.270 ± 0.450	
	21	35.027 ± 5.450	4.520 ± 1.076	10.300 ± 4.467	65.320 ± 3.906	11.270 ± 4.525	

	28	26.133 ± 10.182	3.810 ± 2.136	11.113 ± 9.705	66.467 ± 12.488	11.850 ± 9.759	
	35	36.050 ± 7.576	5.933 ± 1.655	5.373 ± 4.431	37.850 ± 26.025	8.663 ± 2.434	
Carrageenan (control)	0	68.317 ± 2.367	0.787 ± 0.599	4.937 ± 1.196	81.823 ± 5.530	5.010 ± 1.261	
	7	73.757 ± 7.032	0.820 ± 0.464	0.127 ± 3.695	217.223 ± 123.369	2.943 ± 1.383	
	14	71.747 ± 10.577	0.393 ± 0.441	0.490 ± 3.811	209.800 ± 111.707	2.777 ± 1.936	
	21	60.717 ± 18.146	1.077 ± 0.230	1.857 ± 0.475	58.880 ± 10.884	2.173 ± 0.318	
	28	56.443 ± 8.746	0.853 ± 0.324	2.963 ± 0.702	74.330 ± 2.923	3.083 ± 0.759	
	35	68.467 ± 2.860	1.483 ± 0.182	1.250 ± 0.600	38.543 ± 15.927	1.990 ± 0.295	
Carrageenan + anthocyannin in a dark room (control)	0	45.753 ± 10.090	0.893 ± 2.525	13.597 ± 1.998	87.287 ± 10.524	13.773 ± 2.080	
	7	54.027 ± 1.134	-2.390 ± 0.839	8.037 ± 1.211	106.850 ± 6.548	8.423 ± 1.096	
	14	44.567 ± 2.894	-1.477 ± 0.754	8.287 ± 2.613	100.887 ± 7.453	8.460 ± 2.517	
	21	63.090 ± 5.135	-0.520 ± 1.576	13.690 ± 1.901	92.413 ± 6.423	13.760 ± 1.911	

	28	58.387 ± 5.420	1.817 ± 1.289	5.947 ± 3.814	72.710 ± 6.520	6.237 ± 3.983
	35	51.790 ± 10.590	1.037 ± 0.943	4.920 ± 1.677	78.820 ± 8.218	5.060 ± 1.794

Table 4.4: The relationship between the colour parameters of gelatine hydrocolloid samples containing anthocyannin and UV-A exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Gelatine + anthocyannin	0	85.650 ± 37.294	1.480 ± 2.221	-2.390 ± 1.977	289.157 ± 19.500	2.910 ± 2.832	
	7	79.750 ± 6.007	0.700 ± 0.835	0.383 ± 1.299	142.253 ± 163.433	1.470 ± 0.287	
	14	74.380 ± 4.070	-1.537 ± 3.331	3.443 ± 2.579	90.013 ± 61.677	4.723 ± 2.376	
	21	75.283 ± 16.358	-4.203 ± 1.231	11.850 ± 7.057	111.427 ± 5.808	12.620 ± 7.037	
	28	72.340 ± 3.609	-2.257 ± 0.934	11.163 ± 8.087	107.027 ± 12.659	11.567 ± 7.751	
	35	83.830 ± 4.216	1.243 ± 0.271	1.703 ± 4.965	241.380 ± 137.514	3.717 ± 3.269	
Gelatine + anthocyannin + cinnamic acid	0	46.063 ± 35.775	0.140 ± 0.121	-2.510 ± 2.551	286.973 ± 27.669	2.550 ± 2.498	
	7	79.387 ± 2.645	-0.133 ± 0.335	-0.257 ± 0.474	234.277 ± 75.810	0.547 ± 0.080	
	14	72.440 ± 4.065	-2.147 ± 1.565	-0.733 ± 0.583	210.473 ± 33.513	2.460 ± 1.198	
	21	72.627 ± 7.665	-2.063 ± 0.704	-1.373 ± 0.601	214.503 ± 17.397	2.563 ± 0.471	
	28	69.300 ± 5.406	-3.910 ± 2.176	1.050 ± 0.286	160.093 ± 15.231	4.117 ± 1.999	

	35	81.707 ± 1.529	0.950 ± 1.036	-1.337 ± 0.934	309.380 ± 31.287	1.777 ± 1.111	
Gelatine + anthocyannin + ferulic acid	0	83.863 ± 6.446	0.980 ± 0.324	-1.337 ± 1.695	321.660 ± 26.032	1.767 ± 1.547	
	7	77.780 ± 3.212	1.537 ± 1.035	0.803 ± 1.402	140.443 ± 166.651	2.157 ± 0.758	
	14	86.790 ± 3.517	-1.250 ± 1.289	5.450 ± 1.799	104.577 ± 15.499	5.717 ± 1.669	
	21	84.530 ± 8.923	-0.903 ± 1.869	6.903 ± 2.321	94.723 ± 13.683	7.093 ± 2.491	
	28	84.590 ± 2.366	-0.260 ± 1.673	5.177 ± 3.593	90.493 ± 27.475	5.447 ± 3.392	
	35	78.583 ± 7.959	0.637 ± 0.624	1.080 ± 3.411	252.317 ± 144.043	2.497 ± 2.243	
Gelatine + anthocyannin + gallic acid	0	91.750 ± 0.821	0.480 ± 0.286	-0.440 ± 0.280	319.140 ± 29.171	0.697 ± 0.251	
	7	74.937 ± 4.205	0.647 ± 0.682	3.540 ± 0.738	81.190 ± 10.114	3.627 ± 0.815	
	14	46.637 ± 7.786	2.760 ± 2.110	23.557 ± 6.253	83.953 ± 3.865	23.750 ± 6.425	
	21	68.343 ± 4.550	0.680 ± 1.443	21.137 ± 8.816	88.997 ± 3.763	21.170 ± 8.838	

	28	45.367 ± 17.139	2.267 ± 3.573	23.513 ± 8.785	86.027 ± 6.939	23.730 ± 9.065	
	35	72.823 ± 8.047	1.557 ± 1.995	17.520 ± 8.097	86.073 ± 3.685	17.617 ± 8.247	
Gelatine (control)	0	90.287 ± 1.450	0.993 ± 0.279	1.010 ± 0.419	44.240 ± 7.340	1.423 ± 0.472	
	7	83.687 ± 2.955	1.007 ± 0.081	0.323 ± 1.940	135.180 ± 140.171	1.887 ± 0.316	
	14	80.063 ± 1.462	0.823 ± 0.524	1.173 ± 1.459	52.880 ± 43.109	2.177 ± 0.727	
	21	82.940 ± 5.673	-0.230 ± 0.168	0.810 ± 0.936	148.693 ± 75.186	0.953 ± 0.770	
	28	80.207 ± 6.208	-0.063 ± 0.524	0.860 ± 1.434	135.667 ± 125.434	1.077 ± 1.311	
	35	86.733 ± 1.056	1.787 ± 0.236	-0.900 ± 0.416	334.140 ± 7.690	2.010 ± 0.390	
Gelatine + anthocyannin in a dark room (control)	0	64.183 ± 37.294	1.480 ± 2.221	-2.390 ± 1.977	289.157 ± 19.500	2.910 ± 2.832	
	7	74.173 ± 3.352	-2.903 ± 1.396	-0.557 ± 0.652	196.480 ± 23.046	3.077 ± 1.127	
	14	84.950 ± 1.915	-0.017 ± 1.656	0.117 ± 1.272	187.470 ± 130.169	1.647 ± 0.551	
	21	86.917 ± 3.339	0.153 ± 0.156	2.297 ± 1.022	84.300 ± 7.670	2.313 ± 1.006	

	28	88.050 ± 1.600	2.603 ± 1.060	0.830 ± 1.073	143.647 ± 186.892	2.910 ± 0.829	
	35	44.300 ± 26.579	0.280 ± 0.854	2.283 ± 3.886	161.283 ± 120.784	3.753 ± 1.619	

4.2.2 Exposure to UV-B

Unlike UV-A, the wavelength of UV-B ranges from 320 nm to 290 nm. Table 4.5 shows the mean values of the colour parameters for the samples of agar hydrocolloid under the exposure of UV-B during 35 days of storage. At day 0, agar sample containing anthocyannin-cinnamic acid complex, and the agar (control) sample were the lightest sample with the highest L^* value of (73.520 ± 2.260) and (73.160 ± 4.062) respectively. Upon storage, agar samples containing anthocyannin, and anthocyannin-cinnamic acid complex became darker with a general decrease in L^* values. On the other hand, the agar sample (control) became lighter with a general increase in L^* value. At the end of the storage, agar sample containing anthocyannin kept in a dark room (control) was the darkest with the lowest L^* value (54.023 ± 4.023) while the agar (control) sample remained the lightest with highest L^* value (83.047 ± 5.942).

Table 4.6 shows the mean value of the colour parameters for the samples of carrageenan hydrocolloid under the exposure of UV-B during 35 days of storage. At day 0, the carrageenan (control) sample was the lightest with the highest L^* value (68.317 ± 2.367), followed carrageenan sample containing anthocyannin-gallic acid complex (53.630 ± 9.610). Upon storage, samples of carrageenan containing anthocyannin, anthocyannin-cinnamic acid complex, anthocyannin-ferulic acid complex, and anthocyannin-gallic acid complex became lighter with a general increase in L^* values. At the end of the storage, carrageenan sample containing anthocyannin kept in a dark room (control) was the darkest sample with the lowest L^* value (51.790 ± 10.590), while carrageenan sample containing anthocyannin-cinnamic acid complex was the lightest with the highest L^* value (71.133 ± 1.880).

Table 4.7 shows the mean value of the colour parameters for the samples of gelatine hydrocolloid under the exposure of UV-B during 35 days of storage. At day 0, gelatine

sample with anthocyannin-gallic acid complex was the lightest with the highest L^* value (91.750 ± 0.821), followed by gelatine (control) sample (90.287 ± 1.450). Upon storage, samples of gelatine containing anthocyannin, anthocyannin-cinnamic acid complex, and anthocyannin-ferulic acid complex became lighter with a general increase in L^* values, while gelatine sample containing anthocyannin-gallic acid complex became darker with a decrease in L^* value. At the end of the storage, gelatine sample containing anthocyannin kept in a dark room (control) was the darkest sample with the lowest L^* value (44.300 ± 26.579), while gelatine (control) sample remained the lightest sample with the highest L^* value (94.897 ± 2.689).

Table 4.5 shows the change in hue angle for agar samples under UV-B exposure. For agar sample containing anthocyannin, h_{ab} started with very high value (340.663 ± 8.452) at day 0, and dropped until (150.330 ± 168.694) at day 35. All a^* values were positive, indicating redness of the sample, while all b^* values except at day 35 was negative, indicating blueness of the sample. For agar sample containing anthocyannin-cinnamic acid complex, h_{ab} started with very low value (9.840 ± 9.231) at day 0, then increased up to (346.887 ± 7.966) at day 21, then dropped again to (39.647 ± 21.624). For agar sample containing anthocyannin-ferulic acid complex, h_{ab} was the lowest at day 14 (15.753 ± 8.402), and highest at day 21 (344.407 ± 7.204). For agar sample containing anthocyannin-gallic acid complex, h_{ab} was the highest at day 21 (351.943 ± 7.519), and lowest at day 28 (43.710 ± 20.970). For agar (control) sample, h_{ab} did not vary much throughout the storage period, with the lowest h_{ab} at day 21 (62.207 ± 3.790), and highest at day 0 (84.513 ± 2.549). In addition, all a^* values for all samples were positive throughout the storage period, indicating redness of the samples.

Table 4.6 shows the change in hue angle for carrageenan samples under UV-B exposure. For carrageenan sample containing anthocyannin, h_{ab} was the lowest at day 28 (66.890 ±

16.960), and highest at day 7 (153.717 ± 91.204). All *b** values were positive, and all *a** values except at day 7, were positive, indicating a blend of redness and yellowness of the sample. For carrageenan sample containing anthocyannin-cinnamic acid complex, h_{ab} started with the highest value (292.927 ± 3.222) at day 0, then dropped until (39.327 ± 8.878) at day 28. For carrageenan sample containing anthocyannin-ferulic acid complex, h_{ab} started with the highest value (296.477 ± 2.757) at day 0, then dropped until (70.517 ± 4.366) at day 35. All *a** values except at day 21, were positive, indicating redness of the sample. For carrageenan sample containing anthocyannin-gallic acid complex, h_{ab} started with the lowest value (46.190 ± 23.109) at day 0, then started increasing until (229.593 ± 134.770) at day 35. All *a** values were positive, indicating redness of the sample. For carrageenan (control) sample, h_{ab} was the highest at day 7 (123.783 ± 28.171), and lowest at day 35 (58.520 ± 29.516).

Table 4.7 shows the change in hue angle for gelatine samples under exposure of UV-B. For gelatine sample containing anthocyannin, h_{ab} started with the highest value at day 0 (289.157 ± 19.500), then dropped until (118.130 ± 10.449) at day 35. For gelatine sample containing anthocyannin-cinnamic acid complex, h_{ab} started with the highest value at day 0 (286.973 ± 27.669), then dropped until (72.513 ± 20.540) at day 28. All *b** values except at day 0 were positive, indicating yellowness of the sample. For gelatine sample containing anthocyannin-ferulic acid complex, h_{ab} started with the highest value at day 0 (321.660 ± 26.032), then dropped until (82.217 ± 28.942) at day 35. For gelatine sample containing anthocyannin-gallic acid complex, h_{ab} started with the highest value at day 0 (319.140 ± 29.171). h_{ab} was the lowest at day 7 (72.870 ± 6.365). All *b** values except at day 0 were positive, indicating yellowness of the sample. For gelatine (control) sample, h_{ab} was the lowest at day 7 (33.270 ± 23.528), and highest at day 21 (119.820 ± 11.150).

As shown in Table 4.5, the change in chromaticity can be observed for agar samples under the exposure of UV-B. At day 0, agar sample containing anthocyannin-gallic acid complex was the brightest with the highest C^*_{ab} value (16.140 ± 2.462). On the other hand, agar (control) sample was the dullest with the lowest C^*_{ab} value (4.477 ± 0.047), followed by agar containing anthocyannin-cinnamic acid complex (8.313 ± 2.123). Upon storage, samples of agar containing anthocyannin, anthocyannin-cinnamic acid complex, and anthocyannin-ferulic acid complex became brighter with a general increase in C^*_{ab} values. At the end of the storage, agar sample containing anthocyannin was the brightest with the highest C^*_{ab} value (17.407 ± 4.731), while agar (control) sample was the dullest with the lowest C^*_{ab} value (3.723 ± 0.623), followed by agar sample containing anthocyannin-cinnamic acid complex (9.280 ± 1.209).

Based on Table 4.6, we can observe the change in chromaticity of carrageenan samples under exposure to UV-B. At day 0, carrageenan sample containing anthocyannin-cinnamic acid complex was the brightest with the highest C^*_{ab} value (21.687 ± 1.336), whereas carrageenan (control) sample was the dullest with the lowest C^*_{ab} value (5.010 ± 1.261), followed by carrageenan sample containing anthocyannin-gallic acid complex (5.517 ± 1.255). Upon storage, samples of carrageenan containing anthocyannin, anthocyannin-cinnamic acid complex, anthocyannin-ferulic acid complex, and carrageenan (control) became duller with a general decrease in C^*_{ab} values. At the end of the storage, carrageenan sample containing anthocyannin was the brightest with the highest C^*_{ab} value (13.233 ± 4.364), while carrageenan (control) sample was the dullest with the lowest C^*_{ab} value (3.327 ± 2.249), followed by carrageenan sample containing anthocyannin-gallic acid complex (5.363 ± 3.769).

From Table 4.7, the change in chromaticity in gelatine samples under the exposure to UV-B can be observed. At day 0, gelatine sample containing anthocyannin-gallic acid

complex was the dullest with the lowest C^*_{ab} value (0.697 ± 0.251), whereas samples of gelatine containing anthocyannin, and anthocyannin-cinnamic acid complex were the brightest with the highest C^*_{ab} values of (2.910 ± 2.832) and (2.550 ± 2.498) respectively. Upon storage, all samples generally became brighter with an increase in their C^*_{ab} values. At the end of the storage, gelatine sample containing anthocyannin was the brightest with the highest C^*_{ab} value (10.007 ± 2.635), whereas gelatine sample containing anthocyannin-ferulic acid complex was the dullest with the lowest C^*_{ab} value (3.090 ± 1.529).

Table 4.5: The relationship between the colour parameters of agar hydrocolloid samples containing anthocyannin and UV-B exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Agar + anthocyannin	0	64.010 ± 3.795	9.287 ± 1.429	-3.480 ± 2.192	340.663 ± 8.452	9.993 ± 2.136	
	7	61.900 ± 9.268	21.460 ± 8.151	-9.377 ± 6.426	338.237 ± 6.453	23.517 ± 10.042	
	14	62.750 ± 2.819	13.087 ± 3.948	-6.637 ± 2.866	333.863 ± 4.118	14.700 ± 4.770	
	21	59.870 ± 4.888	12.230 ± 2.299	-1.580 ± 1.444	352.797 ± 6.182	12.380 ± 2.361	
	28	58.503 ± 11.890	20.627 ± 9.105	-0.513 ± 6.327	124.287 ± 191.137	21.370 ± 8.761	
	35	54.670 ± 6.457	12.957 ± 6.337	6.697 ± 10.836	150.330 ± 168.694	17.407 ± 4.731	
Agar + anthocyannin + cinnamic acid	0	73.520 ± 2.260	8.157 ± 2.289	1.190 ± 1.016	9.840 ± 9.231	8.313 ± 2.123	
	7	69.723 ± 0.908	7.753 ± 0.460	-1.903 ± 0.887	346.130 ± 6.668	8.020 ± 0.349	
	14	63.300 ± 2.044	12.020 ± 2.381	-5.693 ± 1.289	334.707 ± 1.376	13.303 ± 2.687	
	21	69.857 ± 6.811	11.650 ± 4.961	-2.850 ± 2.592	346.887 ± 7.966	12.077 ± 5.325	
	28	74.777 ± 3.272	6.983 ± 0.572	8.073 ± 0.399	49.167 ± 3.254	10.690 ± 0.342	

	35	67.410 ± 4.682	6.633 ± 1.333	5.850 ± 3.392	39.647 ± 21.624	9.280 ± 1.209	
Agar + anthocyanin + ferulic acid	0	66.570 ± 3.789	8.837 ± 1.167	-0.863 ± 1.083	234.947 ± 202.443	8.917 ± 1.263	
	7	57.617 ± 18.684	17.767 ± 8.736	-7.743 ± 6.556	338.533 ± 16.302	19.887 ± 9.450	
	14	73.730 ± 4.889	6.660 ± 1.273	1.790 ± 0.724	15.753 ± 8.402	6.943 ± 1.092	
	21	56.830 ± 11.128	21.993 ± 5.339	-5.777 ± 1.678	344.407 ± 7.204	22.847 ± 4.910	
	28	88.323 ± 9.940	7.340 ± 3.374	13.130 ± 6.310	57.277 ± 25.781	15.963 ± 2.877	
	35	69.883 ± 10.895	9.457 ± 6.570	7.167 ± 3.925	40.733 ± 26.840	12.920 ± 4.395	
Agar + anthocyanin + gallic acid	0	60.180 ± 2.082	14.860 ± 1.653	-6.070 ± 2.745	338.550 ± 7.698	16.140 ± 2.462	
	7	51.940 ± 12.755	27.703 ± 10.945	-6.733 ± 3.660	346.920 ± 2.383	28.523 ± 11.490	
	14	68.647 ± 1.652	9.987 ± 2.421	0.237 ± 2.122	243.390 ± 193.507	10.170 ± 2.196	

	21	65.623 ± 1.655	14.963 ± 3.071	-1.923 ± 1.519	351.943 ± 7.519	15.167 ± 2.866	
	28	75.377 ± 5.758	8.583 ± 0.650	9.327 ± 5.567	43.710 ± 20.970	13.193 ± 3.355	
	35	63.117 ± 4.770	4.750 ± 2.712	14.100 ± 2.646	72.017 ± 8.141	14.987 ± 3.102	
Agar (control)	0	73.160 ± 4.062	0.430 ± 0.200	4.450 ± 0.044	84.513 ± 2.549	4.477 ± 0.047	
	7	78.590 ± 1.361	1.443 ± 0.295	5.190 ± 1.021	73.737 ± 6.598	5.407 ± 0.891	
	14	81.887 ± 7.293	0.603 ± 0.306	4.700 ± 0.822	82.897 ± 2.501	4.740 ± 0.852	
	21	78.557 ± 6.215	2.793 ± 0.341	5.310 ± 0.554	62.207 ± 3.790	6.007 ± 0.524	
	28	81.673 ± 4.071	2.677 ± 0.405	9.330 ± 1.448	73.643 ± 4.165	9.723 ± 1.322	
	35	83.047 ± 5.942	0.423 ± 0.287	3.690 ± 0.672	82.743 ± 6.172	3.723 ± 0.623	

Table 4.6: The relationship between the colour parameters of carrageenan hydrocolloid samples containing anthocyannin and UV-B exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Carrageenan + anthocyannin	0	45.753 ± 10.090	0.893 ± 2.525	13.597 ± 1.998	87.287 ± 10.524	13.773 ± 2.080	
	7	49.507 ± 0.631	-0.493 ± 0.127	1.227 ± 3.511	153.717 ± 91.204	2.870 ± 1.609	
	14	61.110 ± 2.095	0.110 ± 0.537	10.893 ± 4.238	88.710 ± 2.727	10.900 ± 4.232	
	21	58.140 ± 15.095	2.017 ± 0.654	9.577 ± 4.893	77.153 ± 2.831	9.793 ± 4.914	
	28	58.227 ± 16.164	4.330 ± 1.477	11.987 ± 4.791	66.890 ± 16.960	13.047 ± 3.690	
	35	58.860 ± 6.240	3.567 ± 1.039	12.680 ± 4.535	72.860 ± 7.423	13.233 ± 4.364	
Carrageenan + anthocyannin + cinnamic acid	0	38.080 ± 5.261	8.417 ± 0.997	-19.960 ± 1.503	292.927 ± 3.222	21.687 ± 1.336	
	7	46.510 ± 8.086	4.633 ± 1.959	-18.583 ± 4.696	283.723 ± 2.591	19.170 ± 5.000	
	14	59.660 ± 3.991	-0.007 ± 0.451	-6.277 ± 3.324	269.850 ± 5.665	6.290 ± 3.314	
	21	51.693 ± 9.936	-0.760 ± 2.130	-5.103 ± 2.344	255.273 ± 32.974	5.647 ± 1.464	
	28	69.787 ± 6.350	4.503 ± 1.729	3.517 ± 0.900	39.327 ± 8.878	5.753 ± 1.772	

	35	71.133 ± 1.880	1.983 ± 0.215	6.813 ± 2.567	72.110 ± 7.299	7.130 ± 2.442	
Carrageenan + anthocyannin + ferulic acid	0	47.670 ± 5.847	6.063 ± 1.821	-12.483 ± 4.619	296.477 ± 2.757	13.883 ± 4.932	
	7	44.010 ± 3.139	4.587 ± 1.390	-17.803 ± 3.084	284.230 ± 2.061	18.390 ± 3.329	
	14	60.557 ± 3.554	3.817 ± 0.430	-8.850 ± 0.217	293.320 ± 2.410	9.647 ± 0.261	
	21	53.440 ± 8.951	-1.433 ± 0.736	-0.957 ± 8.486	206.497 ± 88.148	6.567 ± 3.507	
	28	64.390 ± 10.677	4.390 ± 1.202	6.123 ± 7.490	160.283 ± 165.841	8.930 ± 4.808	
	35	65.587 ± 2.622	2.630 ± 0.563	7.463 ± 1.053	70.517 ± 4.366	7.927 ± 1.044	
Carrageenan + anthocyannin + gallic acid	0	53.630 ± 9.610	3.380 ± 1.058	3.973 ± 2.294	46.190 ± 23.109	5.517 ± 1.255	
	7	43.623 ± 6.470	5.593 ± 1.290	-10.380 ± 1.293	298.210 ± 4.559	11.817 ± 1.563	
	14	59.503 ± 2.065	3.893 ± 0.186	1.807 ± 1.882	142.983 ± 185.264	4.537 ± 0.592	
	21	56.060 ± 7.884	0.700 ± 1.337	6.483 ± 1.821	82.680 ± 14.417	6.640 ± 1.663	

	28	62.013 ± 4.084	3.460 ± 0.947	9.613 ± 1.383	70.130 ± 5.545	10.250 ± 1.340	
	35	65.837 ± 6.743	0.300 ± 1.247	-0.317 ± 7.455	229.593 ± 134.770	5.363 ± 3.769	
Carrageenan (control)	0	68.317 ± 2.367	0.787 ± 0.599	4.937 ± 1.196	81.823 ± 5.530	5.010 ± 1.261	
	7	69.897 ± 6.045	-0.443 ± 0.270	0.943 ± 0.801	123.783 ± 28.171	1.130 ± 0.646	
	14	70.980 ± 1.361	1.400 ± 0.140	3.717 ± 0.797	68.977 ± 2.850	3.977 ± 0.782	
	21	79.050 ± 13.096	-1.960 ± 0.798	6.393 ± 2.557	110.400 ± 16.587	6.833 ± 2.048	
	28	66.743 ± 3.564	1.477 ± 0.853	3.927 ± 1.770	69.070 ± 6.585	4.210 ± 1.911	
	35	68.963 ± 5.641	0.970 ± 0.513	2.993 ± 2.554	58.520 ± 29.516	3.327 ± 2.249	

Table 4.7: The relationship between the colour parameters of gelatine hydrocolloid samples containing anthocyannin and UV-B exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Gelatine + anthocyannin	0	85.780 ± 37.294	1.480 ± 2.221	-2.390 ± 1.977	289.157 ± 19.500	2.910 ± 2.832	
	7	80.627 ± 5.734	-0.403 ± 1.377	-0.973 ± 0.454	264.677 ± 62.193	1.523 ± 0.543	
	14	80.377 ± 2.815	-2.287 ± 0.667	3.063 ± 0.650	126.427 ± 2.183	3.823 ± 0.920	
	21	98.450 ± 0.485	-3.070 ± 0.336	6.707 ± 0.522	114.713 ± 3.943	7.390 ± 0.364	
	28	85.850 ± 28.269	3.013 ± 0.081	3.003 ± 5.821	145.610 ± 145.540	6.070 ± 2.393	
	35	69.937 ± 11.790	-4.430 ± 1.013	8.837 ± 3.092	118.130 ± 10.449	10.007 ± 2.635	
Gelatine + anthocyannin + cinnamic acid	0	46.063 ± 35.775	0.140 ± 0.121	-2.510 ± 2.551	286.973 ± 27.669	2.550 ± 2.498	
	7	92.837 ± 3.893	-1.293 ± 0.974	0.050 ± 0.584	167.203 ± 34.134	1.417 ± 0.888	
	14	85.283 ± 1.960	-1.450 ± 0.427	1.377 ± 0.245	135.927 ± 11.319	2.027 ± 0.275	
	21	68.223 ± 22.893	-3.283 ± 1.888	6.707 ± 1.276	116.437 ± 15.603	7.663 ± 0.906	

	28	69.477 ± 23.061	1.327 ± 1.960	4.537 ± 1.768	72.513 ± 20.540	4.960 ± 1.890
	35	87.610 ± 9.312	-3.283 ± 3.000	6.270 ± 4.476	119.927 ± 25.948	7.673 ± 3.984
Gelatine + anthocyannin + ferulic acid	0	83.863 ± 6.446	0.980 ± 0.324	-1.337 ± 1.695	321.660 ± 26.032	1.767 ± 1.547
	7	88.087 ± 5.954	-0.543 ± 0.811	-0.423 ± 0.983	173.480 ± 86.015	1.000 ± 0.918
	14	92.550 ± 2.145	0.787 ± 0.482	1.993 ± 0.252	68.810 ± 11.778	2.173 ± 0.291
	21	85.287 ± 4.625	-3.207 ± 0.389	6.377 ± 0.906	116.980 ± 5.064	7.153 ± 0.757
	28	73.600 ± 23.431	1.493 ± 3.743	5.620 ± 1.511	73.677 ± 35.829	6.677 ± 0.457
	35	91.690 ± 5.746	-0.113 ± 0.990	2.933 ± 1.659	82.217 ± 28.942	3.090 ± 1.529
Gelatine + anthocyannin + gallic acid	0	91.750 ± 0.821	0.480 ± 0.286	-0.440 ± 0.280	319.140 ± 29.171	0.697 ± 0.251
	7	85.333 ± 4.239	1.217 ± 0.260	4.160 ± 0.998	72.870 ± 6.365	4.357 ± 0.904
	14	83.977 ± 4.573	0.077 ± 0.086	7.510 ± 2.660	89.247 ± 0.883	7.513 ± 2.656
	21	72.387 ± 7.607	-1.613 ± 1.861	7.247 ± 3.339	98.683 ± 12.201	7.500 ± 3.598

	28	59.333 ± 23.718	4.783 ± 2.577	15.523 ± 14.645	172.910 ± 157.577	18.420 ± 10.385	
	35	87.357 ± 10.703	-1.367 ± 0.538	8.147 ± 4.833	103.390 ± 11.563	8.343 ± 4.643	
Gelatine (control)	0	90.287 ± 1.450	0.993 ± 0.279	1.010 ± 0.419	44.240 ± 7.340	1.423 ± 0.472	
	7	88.320 ± 2.430	0.957 ± 0.190	0.780 ± 0.635	33.270 ± 23.528	1.293 ± 0.492	
	14	92.270 ± 2.989	0.453 ± 0.086	3.003 ± 0.613	81.363 ± 1.071	3.037 ± 0.610	
	21	94.177 ± 2.821	-2.040 ± 0.547	3.873 ± 2.007	119.820 ± 11.150	4.430 ± 1.916	
	28	87.570 ± 3.674	1.253 ± 2.489	5.873 ± 1.972	80.443 ± 25.529	6.350 ± 1.941	
	35	94.897 ± 2.689	-0.663 ± 0.150	3.657 ± 1.351	101.567 ± 5.629	3.727 ± 1.313	

4.2.3 Exposure to UV-C

Unlike UV-A and UV-B, the wavelength of UV-C ranges from 290 nm to 100 nm, the shortest amongst all. Table 4.8 shows the mean values of the colour parameters for the samples of agar hydrocolloid under the exposure of UV-C during 35 days of storage. At day 0, samples of agar containing anthocyannin-cinnamic acid complex, and agar (control) were the lightest with the highest L^* values of (73.520 ± 2.260) and (73.160 ± 4.062) respectively. Upon storage, all samples except agar (control) became darker with a general decrease in L^* values. At the end of the storage, the agar (control) sample was the lightest with the highest L^* value (75.550 ± 9.372), followed by agar sample containing anthocyannin-cinnamic acid complex (63.703 ± 3.982). On the other hand, the agar sample containing anthocyannin kept in a dark room (control) was the darkest with the lowest L^* value (54.023 ± 4.023).

Table 4.9 shows the mean values of the colour parameters for the samples of carrageenan hydrocolloid under the exposure of UV-C during 35 days of storage. At day 0, carrageenan (control) sample was the lightest with the highest L^* value (68.317 ± 2.367), followed by carrageenan sample containing anthocyannin-gallic acid complex (53.630 ± 9.610). Upon storage, samples of carrageenan containing anthocyannin, anthocyannin-cinnamic acid complex, and anthocyannin-ferulic acid complex became lighter with a general increase in L^* values while carrageenan sample containing anthocyannin-gallic acid complex became darker with a general decrease in its L^* value. At the end of the storage, carrageenan (control) sample remained the lightest sample with the highest L^* value (70.640 ± 4.845), followed by carrageenan sample containing anthocyannin-ferulic acid complex (60.733 ± 3.143). On the other hand, carrageenan sample containing anthocyannin-gallic acid complex was the darkest with the lowest L^* value (46.170 ± 0.225).

Table 4.10 shows the mean values of the colour parameters for the samples of gelatine hydrocolloid under the exposure of UV-C during 35 days of storage. At day 0, gelatine sample containing anthocyannin-gallic acid complex was the lightest with the highest L^* value (91.750 ± 0.821). Upon storage, samples of gelatine containing anthocyannin, and anthocyannin-cinnamic acid complex became lighter with a general increase in L^* values. On the other hand, gelatine sample containing anthocyannin-gallic acid complex became darker with a general decrease in L^* value. At the end of the storage, gelatine (control) sample was the lightest with the highest L^* value (86.490 ± 4.177), followed by gelatine sample containing anthocyannin-gallic acid complex (71.830 ± 19.163). In contrast, gelatine sample containing anthocyannin kept in a dark room (control) was the darkest with the lowest L^* value (44.300 ± 26.579).

Table 4.8 shows the change in hue angle for agar samples under UV-C exposure. For agar sample containing anthocyannin, h_{ab} remained in the 300° quadrant. All a^* values were positive, while all b^* values were negative, indicating a blend of redness and blueness of the sample. For agar sample containing anthocyannin-cinnamic acid complex, h_{ab} started with the lowest value at day 0 (9.840 ± 9.231), and increased until (347.397 ± 1.235) at day 28. All a^* values were positive, while all b^* values except at day 14 were negative, indicating a blend of redness and blueness of the sample. For agar sample containing anthocyannin-ferulic acid complex, h_{ab} started with (234.947 ± 202.443) at day 0, then increased until (336.007 ± 4.134) at day 35. All a^* values were positive, while all b^* values were negative, indicating a blend of redness and blueness of the sample. For agar sample containing anthocyannin-gallic acid complex, h_{ab} started with (338.50 ± 7.698), remained stable until day 28, then dropped to (223.803 ± 193.372) at day 35. All a^* values were positive, while all b^* values were negative, indicating a blend of redness and blueness of the sample. For agar (control) sample, h_{ab} was the highest at day 7 (159.653 ± 122.421), and the lowest at day 28 (50.230 ± 12.459).

Table 4.9 shows the change in hue angle for carrageenan samples under exposure of UV-C. For carrageenan sample containing anthocyannin, h_{ab} was the highest at day 14 (110.710 ± 34.303), and the lowest at day 28 (57.907 ± 13.890). All b^* values were positive, indicating yellowness of the sample. For carrageenan sample containing anthocyannin-cinnamic acid complex, h_{ab} started with the highest value at day 0 (292.927 ± 3.222), then dropped until (152.407 ± 112.762) at day 35. For carrageenan sample containing anthocyannin-ferulic acid complex, h_{ab} started with the highest value at day 0 (296.477 ± 2.757), then dropped until (45.887 ± 21.469) at day 28. For carrageenan sample containing anthocyannin-gallic acid complex, h_{ab} started with the lowest value at day 0 (46.190 ± 23.109), then increased until (322.917 ± 27.490) at day 14, then dropped again until (171.537 ± 159.503) at day 35. All a^* values were positive, indicating redness of the sample. For carrageenan (control) sample, h_{ab} was the highest at day 7 (91.947 ± 6.241), and the lowest at day 28 (34.870 ± 11.349).

Table 4.10 shows the change in hue angle for gelatine samples under UV-C exposure. For gelatine sample containing anthocyannin, h_{ab} started with the highest value at day 0 (289.157 ± 19.500), then dropped until (76.003 ± 2.238) at day 28. All a^* values except at day 7 were positive, indicating redness of the sample. For gelatine sample containing anthocyannin-cinnamic acid complex, h_{ab} started with the highest value at day 0 (286.973 ± 27.669), then dropped until (117.617 ± 164.625) at day 28. For gelatine sample containing anthocyannin-ferulic acid complex, h_{ab} was the highest at day 0 (321.660 ± 26.032), and the lowest at day 21 (166.650 ± 156.974). All a^* values except at day 7 were positive, indicating redness of the sample. For gelatine sample containing anthocyannin-gallic acid complex, h_{ab} was the highest at day 0 (319.140 ± 29.171), and the lowest at day 14 (83.170 ± 1.764). For gelatine (control) sample, h_{ab} started with the lowest value at day 0 (44.240 ± 7.340), and increased until (231.140 ± 129.882) at day 35.

As shown in Table 4.8, change in chromaticity for agar samples under the exposure of UV-C was observed. At day 0, agar (control) sample was the dullest with the lowest C^*_{ab} value (4.477 ± 0.047), whereas agar sample containing anthocyannin-gallic acid sample was the brightest with the highest C^*_{ab} value (16.140 ± 2.462). Upon storage, all samples except agar (control) became brighter with a general increase in their C^*_{ab} values. At the end of the storage, agar sample containing anthocyannin was the brightest with the highest C^*_{ab} value (20.297 ± 7.412), while the dullest sample was agar (control) with the lowest C^*_{ab} value (0.947 ± 0.665).

From Table 4.9, we observed the change in chromaticity for carrageenan samples under the exposure of UV-C. At day 0, carrageenan (control) sample and carrageenan containing anthocyannin-gallic acid complex were the dullest with the lowest C^*_{ab} values of (5.010 ± 1.261) and (5.517 ± 1.255) respectively. On the other hand, carrageenan sample containing anthocyannin-cinnamic acid was the brightest with the highest C^*_{ab} value (21.687 ± 1.336). Upon storage, all samples except carrageenan containing anthocyannin-gallic acid complex became duller with a general decrease in their C^*_{ab} values. At the end of the storage, carrageenan (control) sample was the dullest with the lowest C^*_{ab} (2.273 ± 0.254), followed by carrageenan sample containing anthocyannin-cinnamic acid complex (4.357 ± 2.634). On the other hand, carrageenan sample containing anthocyannin was the brightest with the highest C^*_{ab} value (8.850 ± 2.834).

Table 4.10 shows the change in chromaticity for gelatine samples under UV-C exposure. At day 0, gelatine sample containing anthocyannin-gallic acid complex was the dullest with the lowest C^*_{ab} value (0.697 ± 0.251), whereas samples of gelatine containing anthocyannin, and anthocyannin-cinnamic acid complex were relatively brighter with higher C^*_{ab} values of (2.910 ± 2.832) and (2.550 ± 2.498) respectively. Upon storage, samples of gelatine containing anthocyannin, anthocyannin-ferulic acid complex, and

anthocyannin-gallic acid complex became brighter with a slight increase in their C^*_{ab} values. At the end of the storage, gelatine (control) sample was the dullest with the lowest C^*_{ab} value (0.693 ± 0.112), followed by gelatine sample containing anthocyannin-cinnamic acid complex (1.960 ± 1.732). On the other hand, gelatine sample containing anthocyannin was the brightest with the highest C^*_{ab} value (11.943 ± 9.727).

Table 4.8: The relationship between the colour parameters of agar hydrocolloid samples containing anthocyannin and UV-C exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Agar + anthocyannin	0	64.010 ± 3.795	9.287 ± 1.429	-3.480 ± 2.192	340.663 ± 8.452	9.993 ± 2.136	
	7	50.620 ± 4.786	12.010 ± 5.544	-12.117 ± 5.560	314.493 ± 2.654	17.070 ± 7.813	
	14	59.640 ± 15.599	15.280 ± 7.509	-7.760 ± 6.247	338.353 ± 14.617	17.323 ± 9.261	
	21	53.300 ± 1.265	27.933 ± 4.199	-13.490 ± 2.324	334.240 ± 1.784	31.030 ± 4.705	
	28	51.710 ± 9.135	24.287 ± 5.630	-11.213 ± 1.050	334.740 ± 3.467	26.777 ± 5.530	
	35	55.160 ± 1.853	17.987 ± 5.977	-8.690 ± 6.220	337.003 ± 14.510	20.297 ± 7.412	
Agar + anthocyannin + cinnamic acid	0	73.520 ± 2.260	8.157 ± 2.289	1.190 ± 1.016	9.840 ± 9.231	8.313 ± 2.123	
	7	52.383 ± 5.919	10.030 ± 6.110	-10.423 ± 6.409	314.190 ± 2.837	14.473 ± 8.833	
	14	91.760 ± 1.776	7.477 ± 1.947	8.387 ± 2.041	48.367 ± 2.549	11.243 ± 2.766	
	21	62.047 ± 1.359	18.397 ± 2.250	-9.347 ± 0.747	332.863 ± 3.903	20.667 ± 1.914	
	28	69.893 ± 1.521	9.987 ± 1.251	-2.230 ± 0.285	347.397 ± 1.235	10.233 ± 1.265	

	35	63.703 ± 3.982	11.377 ± 2.904	-3.913 ± 1.004	341.003 ± 1.001	12.030 ± 3.063	
Agar + anthocyanin + ferulic acid	0	66.570 ± 3.789	8.837 ± 1.167	-0.863 ± 1.083	234.947 ± 202.443	8.917 ± 1.263	
	7	61.070 ± 0.643	7.080 ± 0.997	-4.207 ± 1.548	329.997 ± 5.288	8.263 ± 1.660	
	14	62.917 ± 11.331	18.013 ± 8.570	-11.163 ± 7.045	329.443 ± 3.604	21.223 ± 11.011	
	21	65.267 ± 1.690	23.007 ± 4.721	-9.150 ± 3.324	338.643 ± 3.908	24.800 ± 5.494	
	28	72.947 ± 11.738	11.573 ± 5.210	-7.590 ± 0.709	323.877 ± 14.193	14.060 ± 4.303	
	35	54.533 ± 4.308	15.883 ± 4.283	-7.113 ± 2.487	336.007 ± 4.134	17.437 ± 4.765	
Agar + anthocyanin + gallic acid	0	60.180 ± 2.082	14.860 ± 1.653	-6.070 ± 2.745	338.550 ± 7.698	16.140 ± 2.462	
	7	46.533 ± 3.311	20.023 ± 0.964	-13.030 ± 1.144	326.983 ± 1.830	23.900 ± 1.295	
	14	48.137 ± 1.120	36.823 ± 3.018	-20.233 ± 2.830	331.297 ± 1.993	42.030 ± 3.887	
	21	62.393 ± 3.595	21.927 ± 4.588	-8.620 ± 1.767	338.480 ± 1.387	23.567 ± 4.880	

	28	58.823 ± 4.869	22.820 ± 9.327	-10.437 ± 5.415	336.213 ± 2.787	25.110 ± 10.722	
	35	57.797 ± 5.940	13.217 ± 1.635	-4.143 ± 3.709	223.803 ± 193.372	14.133 ± 2.111	
Agar (control)	0	73.160 ± 4.062	0.430 ± 0.200	4.450 ± 0.044	84.513 ± 2.549	4.477 ± 0.047	
	7	52.670 ± 35.054	0.327 ± 0.497	1.227 ± 2.387	159.653 ± 122.421	2.310 ± 0.598	
	14	82.933 ± 3.496	1.933 ± 0.625	1.130 ± 4.866	134.737 ± 142.029	4.427 ± 1.496	
	21	85.883 ± 4.874	0.597 ± 0.147	3.617 ± 0.153	80.710 ± 2.043	3.667 ± 0.176	
	28	79.570 ± 1.568	2.633 ± 0.476	3.257 ± 0.932	50.230 ± 12.459	4.247 ± 0.570	
	35	75.550 ± 9.372	0.523 ± 0.467	0.743 ± 0.570	51.693 ± 25.566	0.947 ± 0.665	

Table 4.9: The relationship between the colour parameters of carrageenan hydrocolloid samples containing anthocyannin and UV-C exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Carrageenan + anthocyannin	0	45.753 ± 10.090	0.893 ± 2.525	13.597 ± 1.998	87.287 ± 10.524	13.773 ± 2.080	
	7	56.470 ± 3.501	-1.450 ± 0.639	5.900 ± 2.533	105.710 ± 10.919	6.140 ± 2.374	
	14	58.570 ± 13.918	-0.783 ± 1.265	6.667 ± 4.764	110.710 ± 34.303	7.077 ± 4.089	
	21	59.560 ± 2.248	-1.303 ± 1.190	13.217 ± 2.833	96.347 ± 6.224	13.333 ± 2.714	
	28	56.177 ± 11.659	2.320 ± 0.681	4.513 ± 2.736	57.907 ± 13.890	5.127 ± 2.680	
	35	59.970 ± 9.370	1.657 ± 0.653	8.693 ± 2.767	79.307 ± 1.793	8.850 ± 2.834	
Carrageenan + anthocyannin + cinnamic acid	0	38.080 ± 5.261	8.417 ± 0.997	-19.960 ± 1.503	292.927 ± 3.222	21.687 ± 1.336	
	7	37.390 ± 8.224	-3.973 ± 0.900	-12.113 ± 5.134	250.897 ± 4.385	12.767 ± 5.128	
	14	38.800 ± 4.227	1.737 ± 2.348	-17.067 ± 6.004	274.550 ± 5.116	17.203 ± 6.233	
	21	58.460 ± 12.243	-3.740 ± 1.195	-0.400 ± 4.690	180.433 ± 53.577	5.300 ± 1.596	
	28	47.513 ± 8.750	3.607 ± 2.168	-10.127 ± 8.283	291.987 ± 6.516	10.780 ± 8.508	

	35	49.180 ± 10.943	0.683 ± 0.681	0.863 ± 5.758	152.407 ± 112.762	4.357 ± 2.634	
Carrageenan + anthocyannin + ferulic acid	0	47.670 ± 5.847	6.063 ± 1.821	-12.483 ± 4.619	296.477 ± 2.757	13.883 ± 4.932	
	7	41.477 ± 5.864	-3.933 ± 0.300	-8.787 ± 1.795	245.473 ± 3.355	9.637 ± 1.740	
	14	44.073 ± 5.947	-2.073 ± 2.572	-8.030 ± 11.892	138.243 ± 118.073	11.797 ± 6.529	
	21	60.763 ± 3.204	0.497 ± 2.117	13.630 ± 8.455	90.950 ± 7.440	13.713 ± 8.534	
	28	50.737 ± 4.580	4.323 ± 0.519	5.297 ± 3.219	45.887 ± 21.469	7.083 ± 2.343	
	35	60.733 ± 3.143	0.860 ± 1.013	6.597 ± 1.897	83.770 ± 5.971	6.680 ± 2.024	
Carrageenan + anthocyannin + gallic acid	0	53.630 ± 9.610	3.380 ± 1.058	3.973 ± 2.294	46.190 ± 23.109	5.517 ± 1.255	
	7	46.397 ± 3.523	5.910 ± 2.200	-6.873 ± 8.804	210.717 ± 171.547	10.220 ± 7.002	
	14	30.387 ± 11.249	11.010 ± 4.315	-10.020 ± 8.841	322.917 ± 27.490	15.763 ± 7.522	
	21	44.897 ± 9.508	6.500 ± 5.186	0.570 ± 14.527	145.910 ± 140.999	12.663 ± 7.814	

	28	48.607 ± 1.880	5.383 ± 1.100	-1.860 ± 7.579	227.093 ± 150.366	8.443 ± 0.590	
	35	46.170 ± 0.225	1.637 ± 1.150	3.950 ± 3.882	171.537 ± 159.503	5.070 ± 2.293	
Carrageenan (control)	0	68.317 ± 2.367	0.787 ± 0.599	4.937 ± 1.196	81.823 ± 5.530	5.010 ± 1.261	
	7	73.540 ± 3.092	-0.090 ± 0.257	2.790 ± 0.894	91.947 ± 6.241	2.800 ± 0.885	
	14	71.710 ± 5.231	1.183 ± 0.603	1.883 ± 2.105	43.427 ± 37.022	2.577 ± 1.488	
	21	77.787 ± 2.405	0.543 ± 0.237	5.880 ± 0.563	84.743 ± 2.194	5.907 ± 0.567	
	28	69.787 ± 6.075	1.563 ± 0.460	1.117 ± 0.556	34.870 ± 11.349	1.953 ± 0.593	
	35	70.640 ± 4.845	0.297 ± 0.217	2.247 ± 0.277	82.093 ± 6.264	2.273 ± 0.254	

Table 4.10: The relationship between the colour parameters of gelatine hydrocolloid samples containing anthocyannin and UV-C exposure

Sample	Day	L^*	a^*	b^*	h_{ab}	C^*_{ab}	Colour
Gelatine + anthocyannin	0	85.780 ± 37.294	1.480 ± 2.221	-2.390 ± 1.977	289.157 ± 19.500	2.910 ± 2.832	
	7	73.467 ± 3.860	-2.427 ± 0.578	-0.173 ± 0.579	185.117 ± 13.322	2.477 ± 0.578	
	14	73.300 ± 2.259	0.747 ± 0.611	0.157 ± 0.828	147.270 ± 155.081	0.993 ± 0.667	
	21	69.440 ± 18.912	1.177 ± 4.312	15.650 ± 16.049	93.927 ± 12.441	15.923 ± 16.294	
	28	70.090 ± 15.117	3.833 ± 1.335	15.557 ± 6.081	76.003 ± 2.238	16.030 ± 6.202	
	35	68.730 ± 3.735	0.260 ± 0.811	11.870 ± 9.813	99.297 ± 19.883	11.943 ± 9.727	
Gelatine + anthocyannin + cinnamic acid	0	46.063 ± 35.775	0.140 ± 0.121	-2.510 ± 2.551	286.973 ± 27.669	2.550 ± 2.498	
	7	76.117 ± 1.821	-1.163 ± 1.981	-0.673 ± 0.240	247.640 ± 53.302	1.610 ± 1.672	
	14	75.907 ± 6.866	0.393 ± 0.905	0.530 ± 2.362	219.937 ± 118.607	1.883 ± 1.317	
	21	87.077 ± 1.799	-0.797 ± 0.967	1.643 ± 1.155	119.993 ± 28.878	2.037 ± 1.021	
	28	84.017 ± 10.029	2.403 ± 1.584	0.627 ± 2.789	117.617 ± 164.625	3.170 ± 2.117	

	35	66.613 ± 29.793	0.007 ± 0.886	0.700 ± 2.686	240.610 ± 118.097	1.960 ± 1.732
Gelatine + anthocyanin + ferulic acid	0	83.863 ± 6.446	0.980 ± 0.324	-1.337 ± 1.695	321.660 ± 26.032	1.767 ± 1.547
	7	76.407 ± 2.901	-2.320 ± 0.575	0.197 ± 0.042	174.897 ± 1.961	2.327 ± 0.569
	14	87.083 ± 3.193	0.787 ± 0.440	-0.207 ± 0.870	236.550 ± 150.073	1.117 ± 0.257
	21	87.900 ± 1.889	0.323 ± 0.144	0.660 ± 0.687	166.650 ± 156.974	0.863 ± 0.441
	28	86.707 ± 8.226	1.633 ± 0.587	-4.910 ± 4.393	191.553 ± 159.726	5.547 ± 3.704
	35	67.093 ± 30.222	0.220 ± 0.674	-0.090 ± 5.320	217.003 ± 114.493	4.017 ± 2.156
Gelatine + anthocyanin + gallic acid	0	91.750 ± 0.821	0.480 ± 0.286	-0.440 ± 0.280	319.140 ± 29.171	0.697 ± 0.251
	7	75.147 ± 1.265	-0.963 ± 0.798	1.760 ± 0.358	117.553 ± 22.931	2.123 ± 0.200
	14	78.727 ± 19.812	0.853 ± 0.561	6.863 ± 3.029	83.170 ± 1.764	6.917 ± 3.073
	21	89.953 ± 4.162	-0.257 ± 0.595	8.063 ± 2.390	91.047 ± 4.537	8.080 ± 2.391
	28	86.810 ± 0.856	-0.200 ± 0.602	-5.987 ± 0.821	267.627 ± 6.306	6.013 ± 0.793
	35	71.830 ± 19.163	0.513 ± 1.423	8.103 ± 7.407	145.010 ± 100.543	8.343 ± 7.168

Gelatine (control)	0	90.287 ± 1.450	0.993 ± 0.279	1.010 ± 0.419	44.240 ± 7.340	1.423 ± 0.472
	7	77.700 ± 3.382	-0.067 ± 0.225	0.853 ± 0.315	94.777 ± 13.906	0.870 ± 0.324
	14	94.480 ± 1.323	1.307 ± 0.506	2.193 ± 1.197	54.640 ± 21.204	2.660 ± 0.913
	21	86.170 ± 4.679	0.737 ± 0.186	1.740 ± 0.269	66.580 ± 7.640	1.907 ± 0.201
	28	92.777 ± 3.106	2.497 ± 0.357	0.277 ± 0.638	124.757 ± 192.894	2.563 ± 0.371
	35	86.490 ± 4.177	0.303 ± 0.186	-0.173 ± 0.728	231.410 ± 129.882	0.693 ± 0.112

Owing to its shortest wavelength, UV-C has the greatest destructive effects on the hydrocolloids sample, followed by UV-B and UV-A. In this study, hydrocolloids which are agar, carrageenan and gelatine, were cross-linked to anthocyanidin. This cross-linking can take place at random places, as shown in Figure 4.2 and Figure 4.3. In Figure 4.2, random positions labelled in red ink indicate the possibility of cross-linking between hydrocolloids and anthocyanidin. Figure 4.3, on the other hand, shows an example of chemical structure when this cross-linking takes place. This cross-linking can affect the lightness and hue of the samples over time. When the samples were exposed to UV-radiations, this further changes lightness and hue over time, as the radiations can break the cross-linking. This phenomenon explains the fluctuations in the values of lightness and hue, and hence the chromaticity.

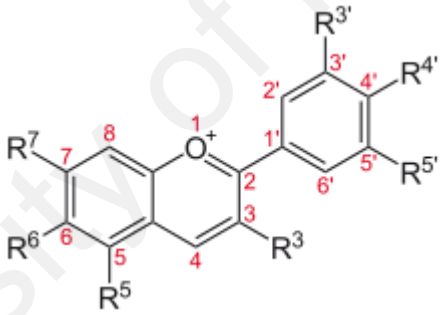


Figure 4.2: Chemical structure of anthocyanidin

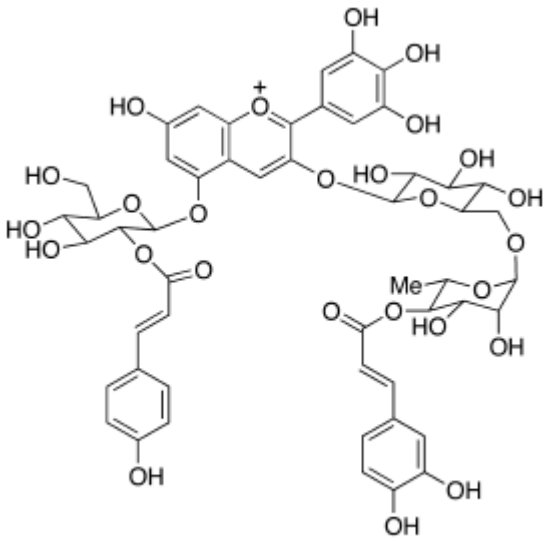


Figure 4.3: Chemical structure of 3,5-diglycoside anthocyannin

In addition, it was also observed that addition of copigments caused dullness to the samples, when they were supposed to enhance the samples. The copigments used which are cinnamic acid, ferulic acid and gallic acid, are phenolic acids. This dullness may be the result of the reaction between the acids and the hydrocolloids. Both the acids and the hydrocolloids contain water, which can disturb the action of the acids as copigments. As a result, the copigments did not enhance the sample, and caused dullness to the samples. The cross-linking of the hydrocolloids, anthocyanidin, and the acids under UV radiations may also have a role in contributing to the sample dullness. Figure 2.1 (see section 2.3) shows an example of chemical structure illustrating the cross-linking between the three.

On top of that, it was also observed that the incorporation of anthocyannin into the hydrocolloids gave different colours, as shown in their h_{ab} values. Carrageenan also showed the darkest colour when incorporated with anthocyannin, followed by agar and gelatine. These observations might owe to their respective chemical structures, and hence the melting points. When preparing for the hydrocolloid gel, we observed that

carrageenan set at around 40 °C to 50 °C, agar set at around 30 ° C to 45 °C, while gelatine set at 0 °C to 5 °C. Overall, carrageenan being the firmest hydrocolloid among the three, showed the strongest colour while gelatine showed the weakest colour, prior to UV treatment. Due to this factor, the anthocyannin degradation was most pronounced in carrageenan, followed by agar and gelatine, in all UV types.

Liang et al. (2011) in their studies on berry skin anthocyannins, found high correlations between *L**, *b** and hue. *L**and *b** values declined but *a** value increased as the berry skin colour became darker, in pink and red grape cultivars. However, there was no significant difference in *L**and *b** values in red-dark violet, red-black and blue-black cultivars. Overall, the CIELAB study that they conducted produced good results in distinguishing grape berry skin colour (Liang et al., 2011).

4.3 Effects of UV treatment on the colour stability and concentration of *B. oleracea* (study 2)

4.3.1 Chemometric analysis

The concept of calibration has been widely used in analytical chemistry. In this study, we also applied this concept in the FT-IR spectroscopy. This was done to predict the concentration of anthocyannin, contained in the hydrocolloid samples. An additional reference analysis of hydrocolloid samples was included. Reference analysis gave a more accurate result, despite being time consuming (Romera Fernandez et al., 2012). Figure 4.4 gives the calibration curve, which shows the relationship between the concentration of anthocyannin, and the absorbance recorded in FT-IR. This curve was drawn based on the average absorbance from three agar samples containing anthocyannin, at frequency 1036 cm^{-1}, which corresponds to the C—O bond which gave the highest absorbance peak in the spectrum. The vibration of a C—O bond can be traced between 1300 cm^{-1} and 1000 cm^{-1} (Pavia et al., 2001). The curve shows a linear relationship between the two, with R^2

value of 0.9784. From this figure, it was demonstrated that the concentration of anthocyannin is directly proportional to the absorbance. Further analysis of the samples exposed under UV radiation would be based on this calibration curve.

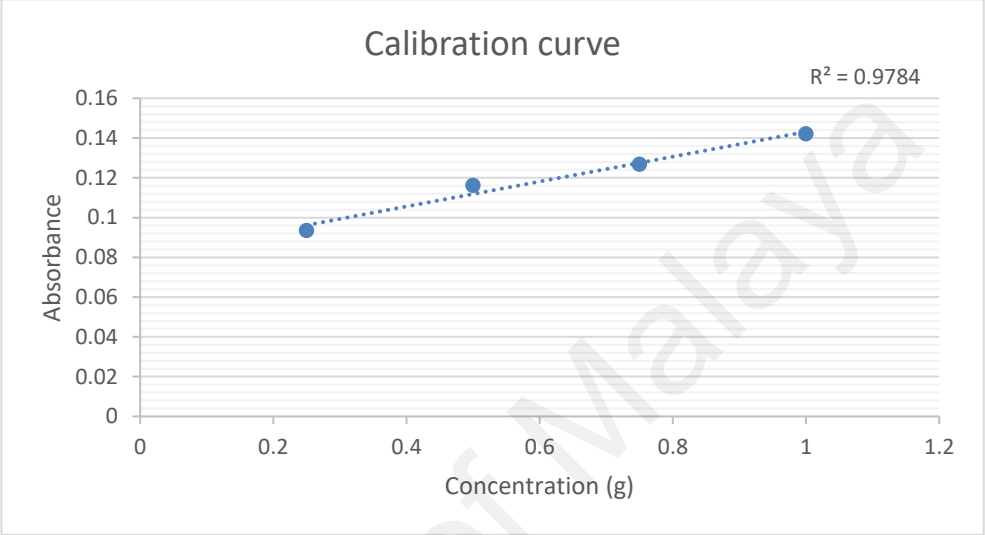


Figure 4.4: The calibration curve showing the relationship between the concentration of anthocyannin and the absorbance recorded in FT-IR

Figures 4.5, 4.6 and 4.7 show the change in concentration of anthocyannin in agar samples under UV-A, UV-B and UV-C exposure, respectively. These figures were derived from the abovementioned calibration curve. Based on Table 4.11, this relationship can be analysed quantitatively. For agar sample containing anthocyannin, the concentration started at 2.619 g at day 0 under UV-A exposure. It was then observed that the concentration generally decreased until 0.430 g at day 28, with an exception at day 14. This might indicate the formation of new bonds due to prolonged exposure to UV light. Under UV-B exposure, the concentration generally decreased from day 7 to day 28, which was from 3.629 g to 0.814 g. The concentration however started increasing again at day 35. On the other hand, under UV-C exposure, the concentration also generally decreased

from day 0 to day 35, with a very small increase at day 14 and day 35, which were considered as negligible.

In this study, we coordinated anthocyanidin with agar for the purpose of food application. As shown in Figure 4.2, there are multiple random positions on anthocyanidin, that can be coordinated with agar, as labelled with red ink. Figure 4.3 shows an example of structure when glycosides coordinate with anthocyanidin at positions 3 and 5. When this coordination takes place, the vibrational frequency of the C—O bond may change. This would result in weaker vibration of the bond, and thus shift the wavenumber to a lower value, as wavenumber is directly proportional to energy (Pavia et al., 2001). However, the calibration curve that this analysis is based on, merely accounts for the vibration at wavenumber 1036 cm^{-1}. As cross-linking with agar decreases the vibration of C—O bond, and hence the wavenumber, we were not able to capture this vibration and thus was not illustrated in the calibration curve. This may explain data anomalies that we observed at day 14 of UV-A, and at day 35 of UV-B.

For agar samples containing anthocyannin-cinnamic acid complex, the concentration started at 2.124 g at day 0 under UV-A exposure. The concentration then decreased to 1.379 g at day 21, with an exception at day 7. It then increased again to 2.091 g at day 28 before decreasing to 1.601 g at day 35. Under UV-B exposure, the initial concentration of 2.124 g at day 0 decreased to 1.344 g at day 21, with an exception at day 14. It then increased again to 2.425 g at day 35. Under UV-C exposure, the initial concentration of 1.325 g at day 0 showed a large drop to 0.026 g at day 7 before increasing to 1.180 g at day 14. It further dropped to 0.797 g at day 28 before increasing to 1.764 g at day 35.

For agar samples containing anthocyannin-ferulic acid complex, under UV-A exposure, the concentration fluctuated in the 35-day storage period, with the lowest at day 0 with 1.188 g, and the highest at day 21 with 2.513 g. Under UV-B exposure, the concentration

generally increased from day 0 to day 35, with an exception at day 14 and day 28. Under UV-C exposure, the concentration started with the highest value at day 0 with 3.423 g. It generally decreased until day 35, with an exception at day 21.

For agar samples containing anthocyannin-gallic acid complex, under UV-A exposure, the concentration generally increased from day 0 with 1.099 g to day 21 with 3.686 g. It then decreased to 1.029 g at day 35. Under UV-B exposure, the concentration generally decreased from day 0 with 1.099 g to day 21 with 0.736 g, with an exception at day 7. It then increased to 2.146 g at day 35. Under UV-C exposure, the concentration generally decreased during the 35-day storage period, with an exception at day 14 and day 35.

Cinnamic acids, ferulic acids and gallic acids are phenolic acids. In addition to agar, these phenolic acids are also able to coordinate or cross-link with anthocyanidin, and causes a change in the vibration of the C—O bond. An example of such chemical structure is shown in Figure 2.1 (see section 2.3). When these acids cross-link with anthocyanidin, they shift the vibration to a lower wavenumber. Similar to the case of the previous sample, only the wavenumber 1036 cm^{-1} was used to derive the calibration curve, that this analysis is based on. Hence, when the wavenumber fell to a value lower than this, we were not able to capture the vibration. In addition, when the agar samples containing anthocyannin-phenolic acid complex were exposed too long under UV radiations, the cross-linking might eventually break. This, on the other hand, increased the vibration of the C—O bond, and shifted the wavenumber to a higher value. Again, we could not capture this vibration. These two phenomena explain the sudden drop in anthocyannin concentration in the samples, as we could not illustrate the true concentration in the graphs below.

For the control, we used agar sample containing anthocyannin, kept in a dark room. This control sample was not exposed to any type of UV radiation for 35 days. The control perfectly met our expectation on the anthocyannin concentration, to decrease over time.

Overall, it was observed that exposure to UV-C radiation has caused the greatest degradation to anthocyannin, with most concentration recorded were below 1 g for all agar samples. Owing to its shortest wavelength in the range of 290 nm to 100 nm, it was proposed that UV-C has the greatest destructive effect among all UV types. On the other hand, it was also observed that there was only a slight difference in terms of anthocyannin concentration, between exposure to UV-A and UV-B. With shorter wavelength in the range of 320 nm to 290 nm, UV-B was proposed to cause greater effect of destruction of anthocyannin, when compared to UV-A, which has the longest wavelength of 400 nm to 320 nm. In this study, it was important to incorporate the pigment in situ with the hydrocolloid due to the need to monitor the pigment concentration during its application and processing. The crosslinking in the hydrocolloid has an influence on its opacity, hence will interfere with the degradation of the pigment mixed with the hydrocolloid (Pereira & Stefani, 2015).

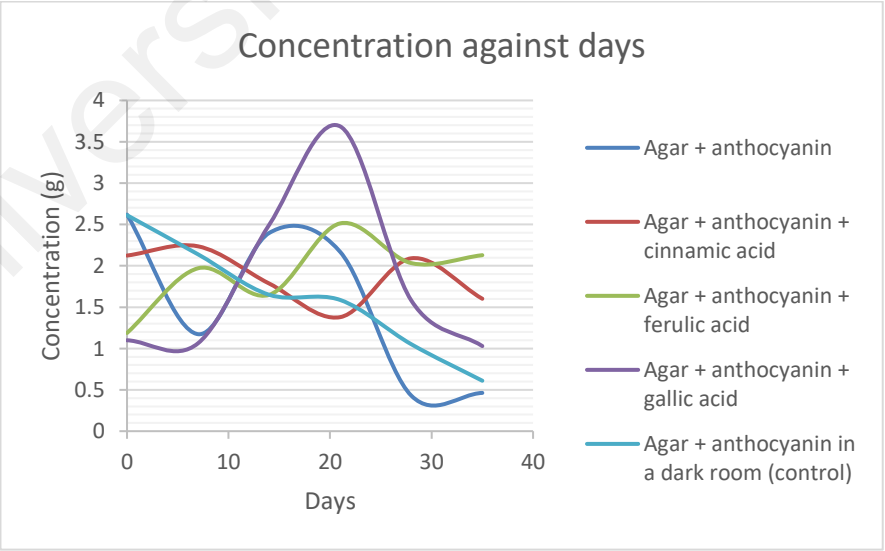


Figure 4.5: The graph of concentration of anthocyannin in agar samples against days under UV-A exposure

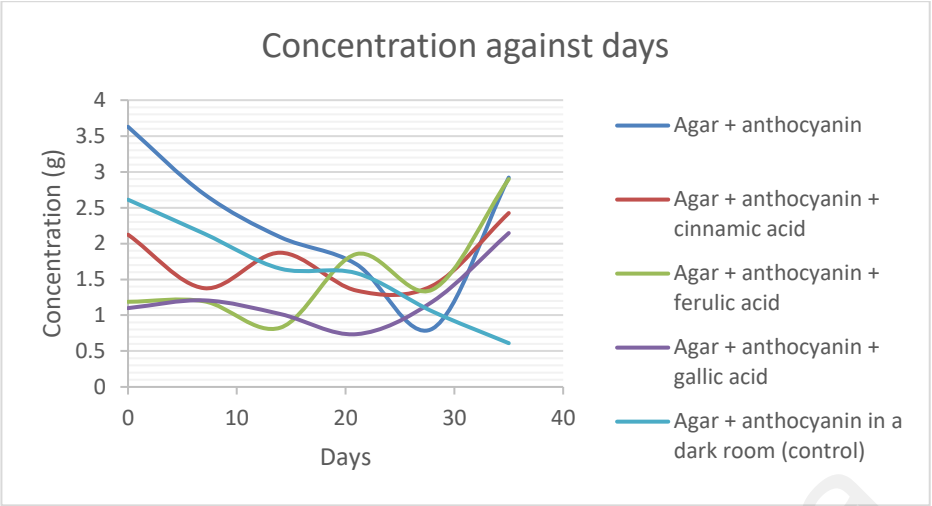


Figure 4.6: The graph of concentration of anthocyannin in agar samples against days under UV-B exposure

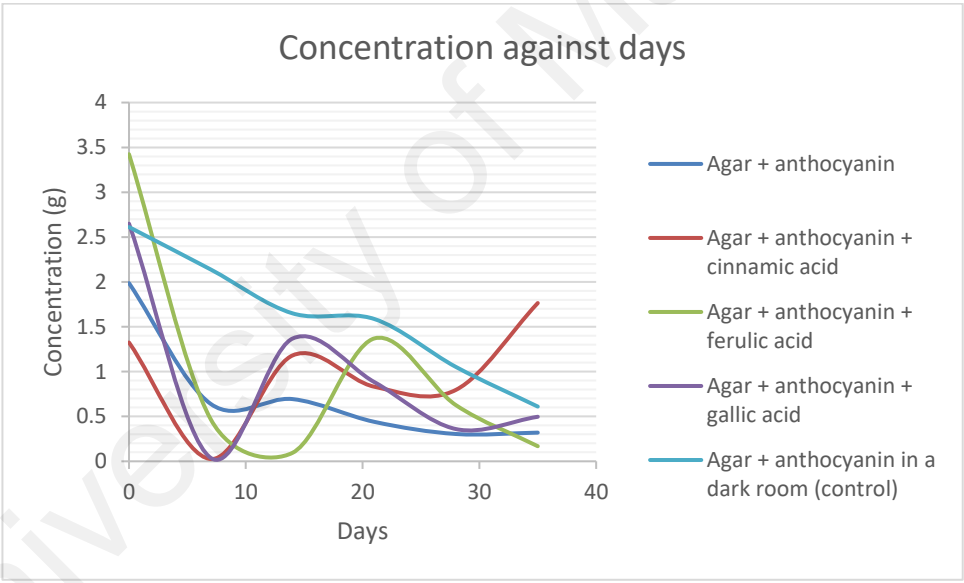


Figure 4.7: The graph of concentration of anthocyannin in agar samples under UV-C exposure

Table 4.11: The relationship between concentration of C—O bonds in agar samples and exposure to different types of UV radiations

Sample	Day	Concentration (g)		
		UV-A	UV-B	UV-C
Agar + anthocyannin	0	2.619	3.629	1.987
	7	1.173	2.689	0.640
	14	2.397	2.089	0.694
	21	2.168	1.711	0.440
	28	0.430	0.814	0.305
	35	0.463	2.921	0.320
Agar + anthocyannin + cinnamic acid	0	2.124	2.124	1.325
	7	2.236	1.377	0.026
	14	1.786	1.872	1.180
	21	1.379	1.344	0.832
	28	2.091	1.413	0.797
	35	1.601	2.425	1.764
Agar + anthocyannin + ferulic acid	0	1.188	1.188	3.423
	7	1.970	1.194	0.468
	14	1.648	0.826	0.095
	21	2.513	1.853	1.370
	28	2.032	1.358	0.626
	35	2.129	2.899	0.168

Agar + anthocyannin + gallic acid	0	1.099	1.099	2.649
	7	1.064	1.207	0.038
	14	2.495	1.017	1.368
	21	3.686	0.736	0.884
	28	1.581	1.191	0.359
	35	1.029	2.146	0.497
Agar + anthocyannin kept in a dark room (control)	0		2.612	
	7		2.138	
	14		1.650	
	21		1.587	
	28		1.052	
	35		0.610	

CHAPTER 5: CONCLUSIONS

In this study, red cabbages *B. oleracea* was used in the extraction process due to their high content of anthocyannin pigments. The identification anthocyannin contained in this plant has been carried out by using LC-MS/MS. While lacking pure anthocyannin standards, the identification of anthocyannin was achieved. Anthocyannin identification done by this method revealed that *B. oleracea* is rich in cyanidin, and that cyanidin is the only type of anthocyannin present in this plant.

Total monomeric anthocyannin content was also determined prior to the UV study. It was worth noted that from the pH differential method conducted, 117 mg of anthocyannin can be extracted from 100 g of fresh red cabbages *B. oleracea*. There might be shortcomings which caused the initial degradation of anthocyannin during its storage, that resulted in this low amount. However, if these are overcome, more anthocyannin can be extracted from this plant, and hence the plant can be deemed suitable to be the source of anthocyannin for industrial use.

The CIELAB study was a colorimetric study conducted to determine the colour stability of anthocyannin when applied in food hydrocolloids and exposed to different types of UV radiations. It was observed that the colour was not stable both when applied to these food hydrocolloids and exposed to UV radiations. The cross-linking between anthocyannin and hydrocolloids can take place at random places, which in turn changes the hue and lightness over time. Exposure to UV radiation can also randomly break the cross-linking, and this further changes both the hue and lightness. However, it can still be concluded that the colour of anthocyannin became duller under exposure of all three types of UV radiation, with the fastest fading in colour being under exposure of UV-C.

It was also noted that in this study, copigmentation did not enhance the colour of anthocyannin when applied in food hydrocolloids. Copigmentation further cross-linked anthocyannin and hydrocolloids with the phenolic acids. The presence of water in the acids might prevent the copigments from enhancing the colour of anthocyannin, hence causing the dullness of the sample. From these findings, it can be concluded that exposure to UV radiations have a destructive effect on anthocyannin in terms of colour stability, both in short term, as demonstrated by UV-A, and in long term, as demonstrated by UV-C.

FT-IR was then performed to determine the change in concentration of anthocyannin in the hydrocolloid samples under UV exposure over time. For the purpose of this study, we did an additional reference analysis by drawing a calibration curve to determine the concentration of anthocyannin, by using the absorbance data recorded in the FT-IR spectra. The wavenumber 1036 cm^{-1} was chosen to reflect the analysis of the findings. This wavenumber corresponds to the vibration in the C—O bond of anthocyanidin. As the agar hydrocolloid, the phenolic acids and anthocyanidin cross-link, and at several random positions, the vibration in the bond might be weaker, and thus shifted the wavenumber to a lower value. If the new wavenumber was lower than 1036 cm^{-1}, the true concentration of anthocyannin was not reflected, and hence a sudden drop of concentration in anthocyannin was observed during the 35-day storage period.

Apart from that, as the UV radiations became more intense, and the samples were exposed long enough to the radiation, these cross-linking might break at several random positions. This occurrence caused the vibration in the bond to become stronger, and hence shifted the wavenumber to a higher value. Similarly, if the new wavenumber was higher than 1036 cm^{-1}, the true concentration of anthocyannin was not reflected, and again causing a sudden drop in the concentration of anthocyannin during the 35-day storage period. From

these findings, it can be concluded that exposure to UV radiations have a destructive effect on anthocyannin in terms of concentration, both in short term, as demonstrated by UV-A, and in long term, as demonstrated by UV-C.

Based on our finding, it can be proposed that anthocyannins are useful in dealing with issues such as food adulteration and substitute for synthetic dyes in food. Due to its high colour intensity, the anthocyannin profile can be used to check if food manufacturers have made adulterations. This is useful even in long terms as anthocyannins take quite a while to deteriorate. As proved by other studies, anthocyannin being a natural colourant, is also a safe substitute for synthetic dyes in food. For this reason, anthocyannin becomes a great option to be used in food, in addition to the fact that it only slowly degrades over time.

From these findings, it was proposed that for the future work, we can combine anthocyannin with other pigments to see the formation of a new colour and study its stability. In addition, we can also manipulate the chemical bonds and functional groups of anthocyannin to generate new colours, which can then be applied for industrial use, such as in textile dyeing.

REFERENCES

Ahmadiani, N., Robbins, R., Collins, T., & Giusti, M. M. (2016). Molar absorptivity (ε) and spectral characteristics of cyanidin-based anthocyanins from red cabbage. *Food Chemistry, 197*(Pt A), 900-906.

Andersen, O. M., Torskangerpoll, K., & Andersen, Ø. (2005). Colour stability of anthocyanins in aqueous solutions at various pH values. *Food Chemistry, 89*(3), 427-440.

Anuar, N., Saat, N., Aziz, N., Taha, R., Mohd Adnan, A., & Mat Taha, R. (2013). Optimization of Extraction Parameters by Using Response Surface Methodology, Purification, and Identification of Anthocyanin Pigments in Melastoma malabathricum Fruit. *The Scientific World Journal, 2013*, 1-10.

Arapitsas, P., & Turner, C. (2008). Characterisation of anthocyanins in red cabbage using high resolution liquid chromatography coupled with photodiode array detection and electrospray ionization-linear ion trap mass spectrometry. *Food Chemistry, 109*(1), 219-226.

Bakker, J., Bridle, P., & Timberlake, C. F. (1986). Tristimulus Measurements (CIELAB 76) of Port Wine Color. *Vitis, 25*(2), 67-78.

Bakowska, A., Kucharska, A. Z., & Oszmianski, J. (2003). The effects of heating, UV irradiation, and storage on stability of the anthocyanin–polyphenol copigment complex. *Food Chemistry, 81*(3), 349-355.

Bechtold, T., Mahmud Ali, A., & Mussak, R. (2007). Anthocyanin dyes extracted from grape pomace for the purpose of textile dyeing. *Journal of The Science of Food and Agriculture, 87*(14), 2589-2595.

Berns, R. (2014). Extending CIELAB: Vividness, Vab *, depth, Dab *, and clarity, Tab *. *Color Research And Application, 39*(4), 322-330.

Brand Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science & Technology, 28*(1), 25-30.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*(11), 317-333.

Brouillard, R., & Delaporte, B. (1977). Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. *Journal of The American Chemical Society, 99*(26), 8461-8468.

Brouillard, R., & Dubois, J. E. (1977). Mechanism of the structural transformations of anthocyanins in acidic media. *Journal of The American Chemical Society, 99*(5), 1359-1364.

Brouillard, R., Mazza, G., Saad, Z., Albrechtgary, A. M., Cheminat, A., & Albrecht-Gary, A. M. (1989). The co-pigmentation reaction of anthocyanins: a microprobe for the structural study of aqueous solutions. *Journal of The American Chemical Society, 111*(7), 2604-2610.

Brouillard, R., Wigand, M. C., Dangles, O., & Cheminat, A. (1991). pH and solvent effects on the copigmentation reaction of malvin with polyphenols, purine and pyrimidine derivatives. *Journal of The Chemical Society, Perkin Transactions 2*(8), 1235-1241.

Castaneda Ovando, A., Castañeda Ovando, A., Contreras Lopez, E., Contreras-López, E., Castañeda Ovando, A., Galán Vidal, C., Páez Hernández, M. (2014). Purification of Anthocyanins with o-Dihydroxy Arrangement by Sorption in Cationic Resins Charged with Fe(III). *Journal of Chemistry, 2014*, 1-9.

Cemeroglu, B., Velioglu, S., & Isik, S. (1994). Degradation Kinetics of Anthocyanins in Sour Cherry Juice and Concentrate. *Journal of Food Science, 59*(6), 1216-1218.

Chandrasekhar, J., Madhusudhan, M. C., & Madhusudhan, M. C. (2012). Extraction of anthocyanins from red cabbage and purification using adsorption. *Food And Bioproducts Processing, 90*(C4), 615-623.

Charron, C., Clevidence, B., Britz, S., & Novotny, J. (2007). Effect of Dose Size on Bioavailability of Acylated and Nonacylated Anthocyanins from Red Cabbage (*Brassica oleracea* L. Var. *capitata*). *Journal of Agricultural And Food Chemistry, 55*(13), 5354-5362.

Chien, C. Y., & Hsu, B. D. (2013). Optimization of the dye-sensitized solar cell with anthocyanin as photosensitizer. *Solar Energy, 98*, 203-211.

Clifford, M. (2000). Anthocyanins - nature, occurrence and dietary burden. *Journal of The Science of Food And Agriculture, 80*(7), 1063-1072.

Cummins, P., Dowling, O., O'Connor, B., Oconnor, B., Walls, D., & Loughran, S. T. (2011). *Ion-Exchange Chromatography: Basic Principles and Application to the Partial Purification of Soluble Mammalian Prolyl Oligopeptidase. Protein Chromatography: Methods and Protocols* (Vol. 681).

Daraving. G, G., Cain, R. F., Cain, & Daravingas, R. F. (1968). Thermal Degradation of Black Raspberry Anthocyanin Pigments in Model Systems. *Journal of Food Science, 33*(2), 138-142.

Darias Martin, J., Diaz, E., Darias Martín, J., Carrillo, M., Díaz, E., & Boulton, R. (2001). Enhancement of red wine colour by pre-fermentation addition of copigments. *Food Chemistry, 73*(2), 217-220.

Dasneves, H. C., Furtado, P., Figueiredo, P., & Pina, F. (1993). Photochemical and thermal degradation of anthocyanidins. *Journal of Photochemistry And Photobiology. A, Chemistry, 75*(2), 113-118.

Delgado Vargas, F., Jimenez, A. R., Paredes Lopez, O., Jiménez, A. R., & Paredes López, O. (2000). Natural Pigments: Carotenoids, Anthocyanins, and Betalains — Characteristics, Biosynthesis, Processing, and Stability. *Critical Reviews In Food Science And Nutrition, 40*(3), 173-289.

Dragsted, L. O., Strube, M., & Larsen, J. C. (1993). Cancer-protective factors in fruits and vegetables: biochemical and biological background. *Pharmacology & Toxicology, 72 Suppl 1*, 116-135.

Dufour, C., & Sauvaitre, I. (2000). Interactions between Anthocyanins and Aroma Substances in a Model System. Effect on the Flavor of Grape-Derived Beverages. *Journal of Agricultural And Food Chemistry, 48*(5), 1784-1788.

Duong Ly, K., Gabelli, S., & Lorsch, J. (2014). *Gel Filtration Chromatography (Size Exclusion Chromatography) of Proteins. Laboratory Methods in Enzymology: Protein Part C* (Vol. 541).

Dyrby, M., Westergaard, N., & Stapelfeldt, H. (2001). Light and heat sensitivity of red cabbage extract in soft drink model systems. *Food Chemistry, 72*(4), 431-437.

Espin, J. C., Garcia Viguera, C., Espín, J., Soler Rivas, C., Wichers, H., & García Viguera, C. (2000). Anthocyanin-Based Natural Colorants: A New Source of Antiradical Activity for Foodstuff. *Journal of Agricultural And Food Chemistry, 48*(5), 1588-1592.

Fekete, S., Beck, A., Veuthey, J. L., & Guillarme, D. (2014). Theory and practice of size exclusion chromatography for the analysis of protein aggregates. *Journal of Pharmaceutical And Biomedical Analysis, 101*, 161-173.

Fekete, S., Beck, A., Veuthey, J. L., & Guillarme, D. (2015). Ion-exchange chromatography for the characterization of biopharmaceuticals. *Journal of Pharmaceutical And Biomedical Analysis, 113*, 43-55.

Fenglin, H., Ruili, L., Bao, H., & Liang, M. (2004). Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. *Fitoterapia*, *75*(1), 14-23.

Fossen, T., Cabrita, L., & Andersen, O. (1998). Colour and stability of pure anthocyanins influenced by pH including the alkaline region. *Food Chemistry, 63*(4), 435-440.

Garcia-Falcon, M. S., Perez Lamela, C., Martinez Carballo, E., Simal Gandara, J., Garciafalcon, M., Perezlamela, C., Simalgandara, J. (2007). Determination of phenolic compounds in wines: Influence of bottle storage of young red wines on their evolution. *Food Chemistry*, *105*(1), 248-259.

Giusti, M. M., & Wrolstad, R. (1996). Radish Anthocyanin Extract as a Natural Red Colorant for Maraschino Cherries. *Journal of Food Science, 61*(4), 688-694.

Gonnet, J. F. (2001). 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*(4), 473-485.

Grad, M., Simu, G., Muntean, S., & Ilia, G. (2013). Synthesis, characterization and colour determination using CIELAB colour space of stilbene dyes. *Journal of The Iranian Chemical Society*, *10*(4), 807-816.

Guemruekcue, G., Oezguer, M., & Gueltekin, C. (2008). Extraction of anthocyanin pigments from red onion (Alliumcepa L.) and dyeing woolen fabrics. *Asian Journal of Chemistry*, *20*(4), 2891-2902.

He, J., & Giusti, M. M. (2010). *Anthocyanins: Natural Colorants with Health-Promoting Properties* (Vol. 1).

Hong, K. (2015). Preparation and properties of cotton and wool fabrics dyed by black rice extract. *Textile Research Journal*, *85*(18), 1875-1883.

Iversen, C. K. (1999). Black Currant Nectar: Effect of Processing and Storage on Anthocyanin and Ascorbic Acid Content. *Journal of Food Science, 64*(1), 37-41.

Jampani, C., & Naik, A. (2014). Purification of anthocyanins from jamun (Syzygium cumini L.) employing adsorption. *Separation And Purification Technology, 125*, 170-178.

Jayaprakasam, B., Vareed, S., Olson, L. K., & Nair, M. (2005). Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits. *Journal of Agricultural And Food Chemistry, 53*(1), 28-31.

Jin, Z. M., Bi, H. Q., Liang, N. N., & Duan, C. Q. (2010). An Extraction Method for Obtaining the Maximum Non-Anthocyanin Phenolics from Grape Berry Skins. *Analytical Letters, 43*(5), 776-785.

Johnson, B. (2014). Using Ion Exchange Chromatography To Separate and Quantify Complex Ions. *Journal of Chemical Education, 91*(8), 1212-1215.

Ju, Z., & Howard, L. (2003). Effects of Solvent and Temperature on Pressurized Liquid Extraction of Anthocyanins and Total Phenolics from Dried Red Grape Skin. *Journal of Agricultural And Food Chemistry, 51*(18), 5207-5213.

Kirca, A., Cemeroglu, B., Kırca, A., & Cemeroğlu, B. (2003). Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chemistry, 81*(4), 583-587.

Klimaviciute, R., Navikaite, V., Jakstas, V., & Ivanauskas, L. (2015). Complexes of dextran sulfate and anthocyanins from Vaccinium myrtillus: Formation and stability. *Carbohydrate Polymers, 129*, 70-78.

Konczak, I., & Zhang, W. (2004). Anthocyanins—More Than Nature's Colours. *Journal of Biomedicine and Biotechnology, 2004*(5), 239-240.

Kris Etherton, P., Hecker, K., Bonanome, A., Coval, S., Binkoski, A., Hilpert, K., Etherton, T. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine, 113*(9), 71-88.

Kumar, D. S., Banji, D., Harani, A., Rao, A. T., & Rao, S. N. (2013). Screening of Polyphenolic Compounds in Echinochloa crusgalli Roxb Extracts by Various Analytical Techniques. *Asian Journal of Chemistry, 25*(17), 9848-9852.

Lau, F., Shukitt Hale, B., & Joseph, J. (2006). Beneficial effects of berry fruit polyphenols on neuronal and behavioral aging. *Journal of The Science of Food And Agriculture, 86*(14), 2251-2255.

Lee, J., Durst, R., & Wrolstad, R. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *Journal of AOAC International, 88*(5), 1269-1278.

Liang, Z., Sang, M., Fan, P., Wu, B., Wang, L., Yang, S., & Li, S. (2011). CIELAB Coordinates in Response to Berry Skin Anthocyanins and Their Composition in Vitis. *Journal of Food Science, 76*(3), C490-C497.

Lu, L. Z., Zhou, Y. Z., Zhang, Y. Q., Ma, Y. L., Zhou, L. X., Li, L., He, T. Z. (2010). Anthocyanin extracts from purple sweet potato by means of microwave baking and acidified electrolysed water and their antioxidation in vitro. *International Journal of Food Science & Technology, 45*(7), 1378-1385.

Maccarone, E., Maccarrone, A., & Rapisarda, P. (1985). Stabilization of Anthocyanins of Blood Orange Fruit Juice. *Journal of Food Science, 50*(4), 901-904.

Markham, K. R., & Porter, L. J. (1969). Flavonoids in the green algae (chlorophyta). *Phytochemistry, 8*(9), 1777-1781.

Markovich, R., & Pidgeon, C. (1991). Introduction to Fourier transform infrared spectroscopy and applications in the pharmaceutical sciences. *Pharmaceutical Research, 8*(6), 663-675.

McDougall, G., Fyffe, S., Dobson, P., & Stewart, D. (2007). Anthocyanins from red cabbage – stability to simulated gastrointestinal digestion. *Phytochemistry, 68*(9), 1285-1294.

O'Fagain, C., Cummins, P., O'Connor, B., O'Fágáin, C., ÓFágáin, C., Oconnor, B., Loughran, S. T. (2011). *Gel-Filtration Chromatography. Protein Chromatography: Methods And Protocols* (Vol. 681).

Pang, X. Q., Zhang, Z., Xuequn, P., Yang, C., Ji, Z., & Jiang, Y. (2004). Purification and structural analysis of anthocyanins from litchi pericarp. *Food Chemistry, 84*(4), 601-604.

Patil, G., Madhusudhan, M. C., Babu, B. R., Ravindra Babu, B., & Ravindra Babu, B. (2009). Extraction, dealcoholization and concentration of anthocyanin from red radish. *Chemical Engineering And Processing, 48*(1), 364-369.

Pavia, D. L., Lampman, G. M., & Kriz, G. S. (2001). *Introduction to spectroscopy : a guide for students of organic chemistry* (3rd ed.). Fort Worth: Saunders College Pub.

Pawlak, K., Puchalska, M., Miszczak, A., Rosloniec, E., Jarosz, M., & Rosłoniec, E. (2006). Blue natural organic dyestuffs—from textile dyeing to mural painting. Separation and characterization of coloring matters present in elderberry, logwood and indigo. *Journal of Mass Spectrometry, 41*(5), 613-622.

Pereira, V., & Stefani, R. (2015). Active chitosan/PVA films with anthocyanins from Brassica oleraceae (Red Cabbage) as Time–Temperature Indicators for application in intelligent food packaging. *Food Hydrocolloids, 43*, 180-188.

Piccaglia, R., Marotti, M., & Baldoni, G. (2002). Factors influencing anthocyanin content in red cabbage (Brassica oleracea varcapitata L f rubra (L) Thell). *Journal of The Science of Food And Agriculture, 82*(13), 1504-1509.

Rasines Perea, Z., Prieto Perea, N., Romera Fernandez, M., Berrueta, L. A., Gallo, B., & Romera Fernández, M. (2015). Fast determination of anthocyanins in red grape musts by Fourier transform mid-infrared spectroscopy and partial least squares regression. *European Food Research And Technology, 240*(5), 897-908.

Romera Fernandez, M., Berrueta, L. A., Garmon Lobato, S., Gallo, B., Vicente, F., Romera Fernández, M., Moreda, J. M. (2012). Feasibility study of FT-MIR spectroscopy and PLS-R for the fast determination of anthocyanins in wine. *Talanta, 88*, 303-310.

Sharma, O., & Bhat, T. (2009). DPPH antioxidant assay revisited. *Food Chemistry, 113*(4), 1202-1205.

Spagna, G., Barbagallo, R. N., Todaro, A., Durante, M. J., & Pifferi, P. G. (2003). A method for anthocyanin extraction from fresh grape skin. *Italian Journal of Food Science, 15*(3), 337-346.

Tanchev, S. (1976). Products of Thermal Degradation of The Anthocyanins Cyanidin 3 Glucoside Cyanidin 3 Rutinoside And Cyanidin 3 Sophoroside. *Die Nahrung, 20*(10), 889-893.

Tonutare, T., Moor, U., & Szajdak, L. (2014). Strawberry Anthocyanin Determination by pH Differential Spectroscopic Method - How To Get True Results? *Acta Scientiarum Polonorum. Hortorum Cultus, 13*(3), 35-47.

Vankar, P., & Shukla, D. (2011). Natural dyeing with anthocyanins from *Hibiscus rosa sinensis* flowers. *Journal of Applied Polymer Science, 122*(5), 3361-3368.

Vonelbe, J. H., Attoe, E. L., & Elbe, J. H. (1981). Photochemial Degradation of Betanine and Selected Anthocyanins. *Journal of Food Science, 46*(6), 1934-1937.

Wiczkowski, W., Topolska, J., & Honke, J. (2014). Anthocyanins profile and antioxidant capacity of red cabbages are influenced by genotype and vegetation period. *Journal of Functional Foods, 7*, 201-211.

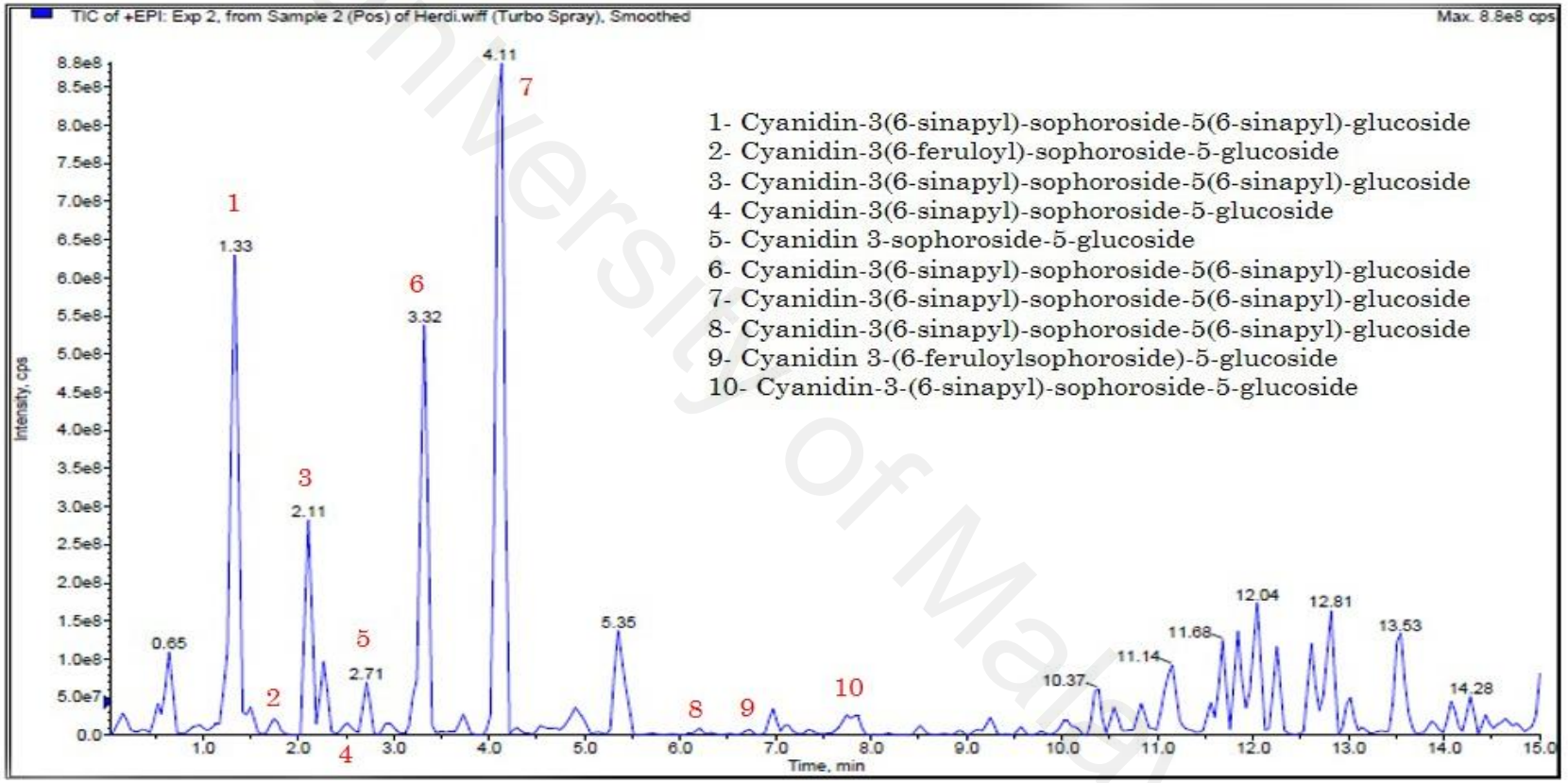
Wrolstad, R., Durst, R., & Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. *Trends in Food Science & Technology, 16*(9), 423-428.

Wu, X., & Prior, R. (2005). Identification and Characterization of Anthocyanins by High-Performance Liquid Chromatography−Electrospray Ionization−Tandem Mass Spectrometry in Common Foods in the United States: Vegetables, Nuts, and Grains. *Journal of Agricultural And Food Chemistry, 53*(8), 3101-3113.

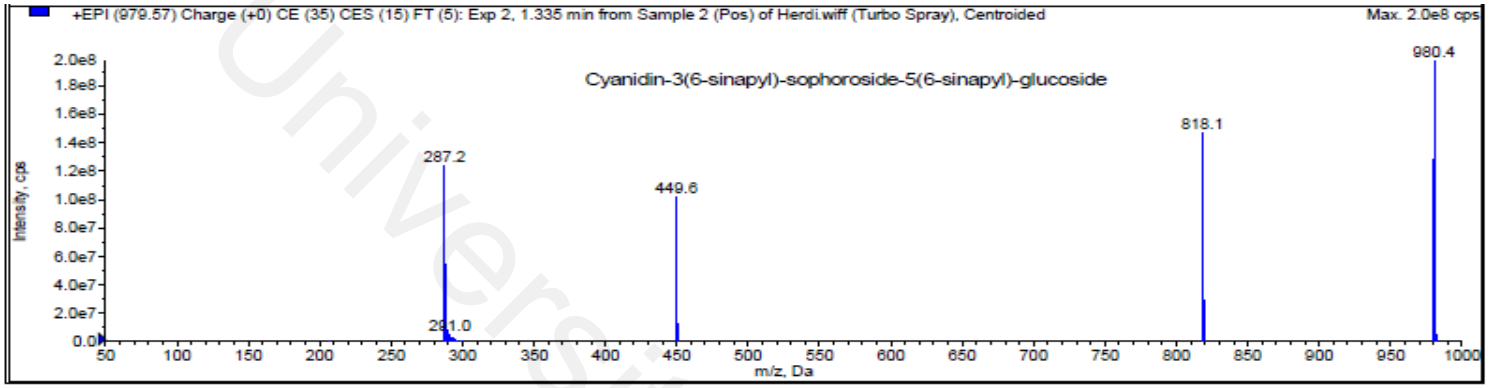
Yang, Z., Chen, Z., Yuan, S., Zhai, W., & Piao, X. (2009). Extraction and identification of anthocyanin from purple corn (*Zea mays* L.). *International Journal of Food Science & Technology, 44*(12), 2485-2492.

APPENDIX

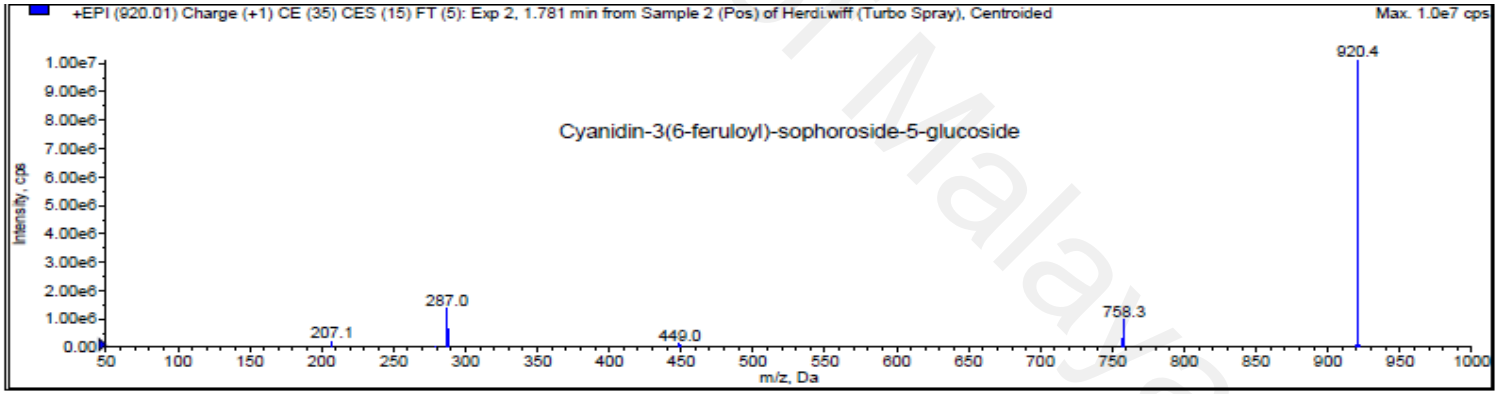
Appendix A 1 - Full chromatogram of *B. oleracea*



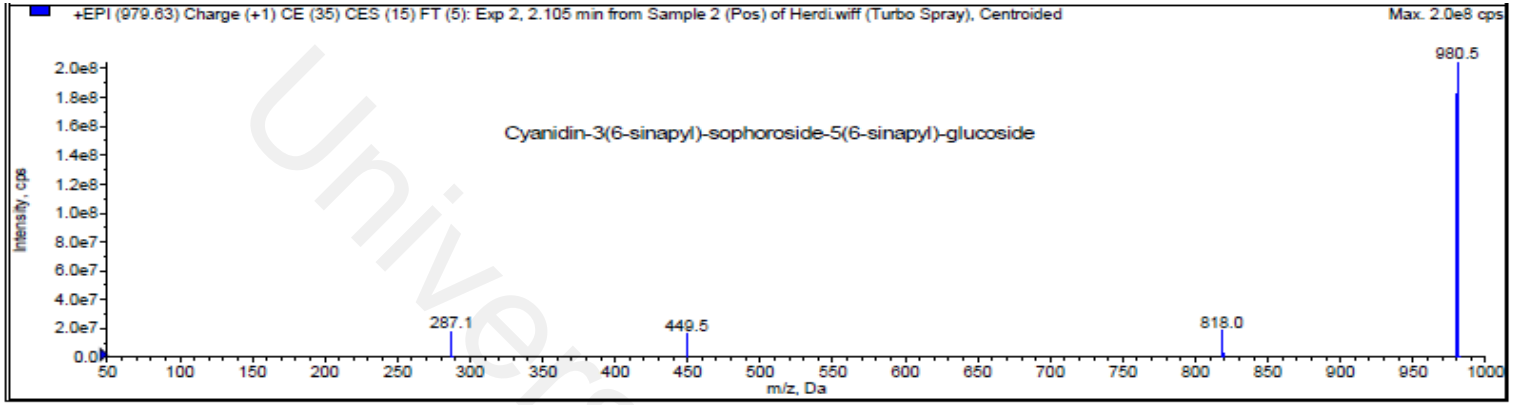
APPENDIX A2 – MS/MS OF CYANIDIN-3(6-SINAPYL)-SOPHOROSIDE-5(6-SINAPYL)-GLUCOSIDE (PEAK 1)



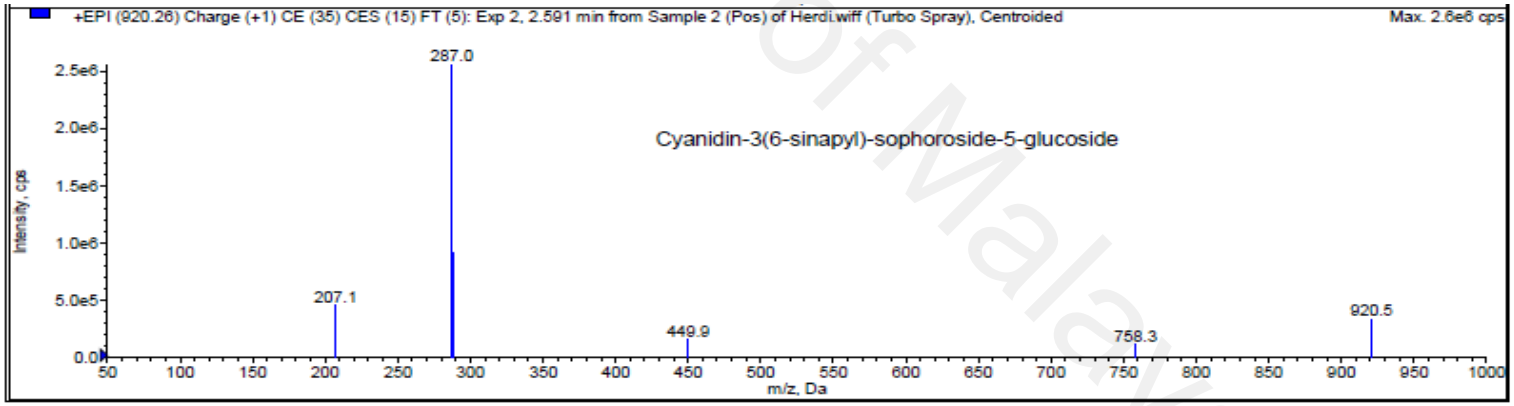
APPENDIX A3 – MS/MS OF CYANIDIN-3(6-FERULOYL)-SOPHOROSIDE-5-GLUCOSIDE (PEAK 2)



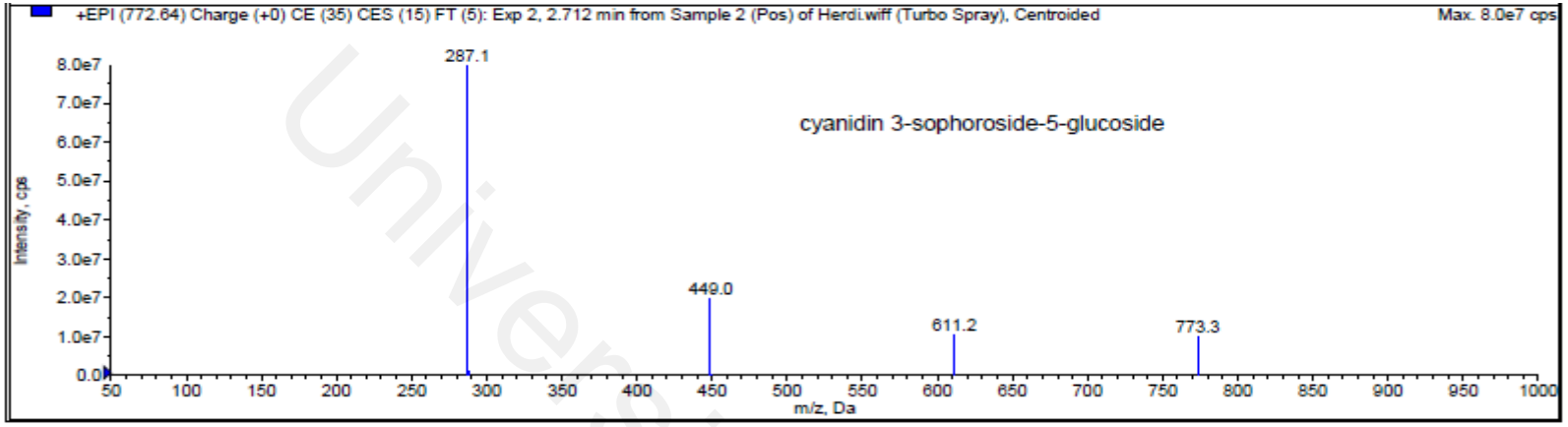
APPENDIX A4 – MS/MS OF CYANIDIN-3(6-SINAPYL)-GLUCOSIDE (PEAK 3)



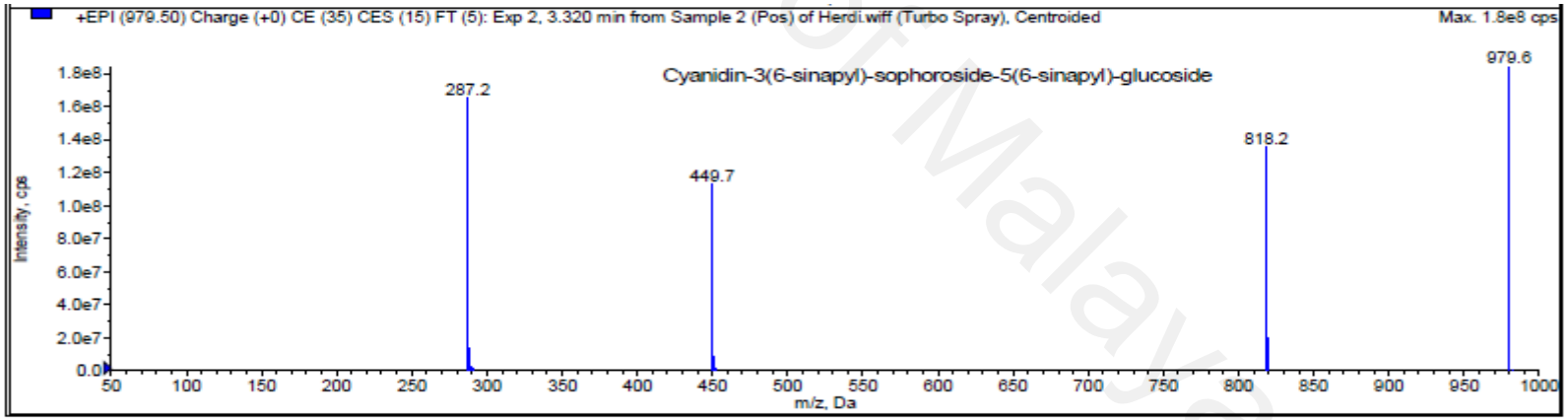
APPENDIX A5 – MS/MS OF CYANIDIN-3(6-SINAPYL)-SOPHOROSIDE-5-GLUCOSIDE (PEAK 4)



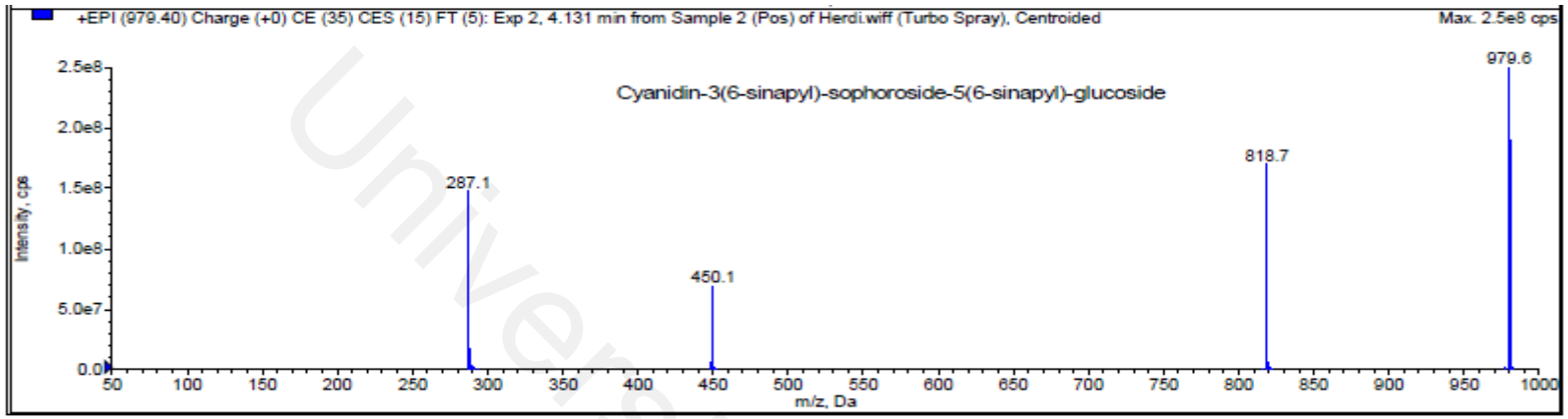
APPENDIX A6 – MS/MS OF CYANIDIN 3-SOPHOROSIDE-5-GLUCOSIDE (PEAK 5)



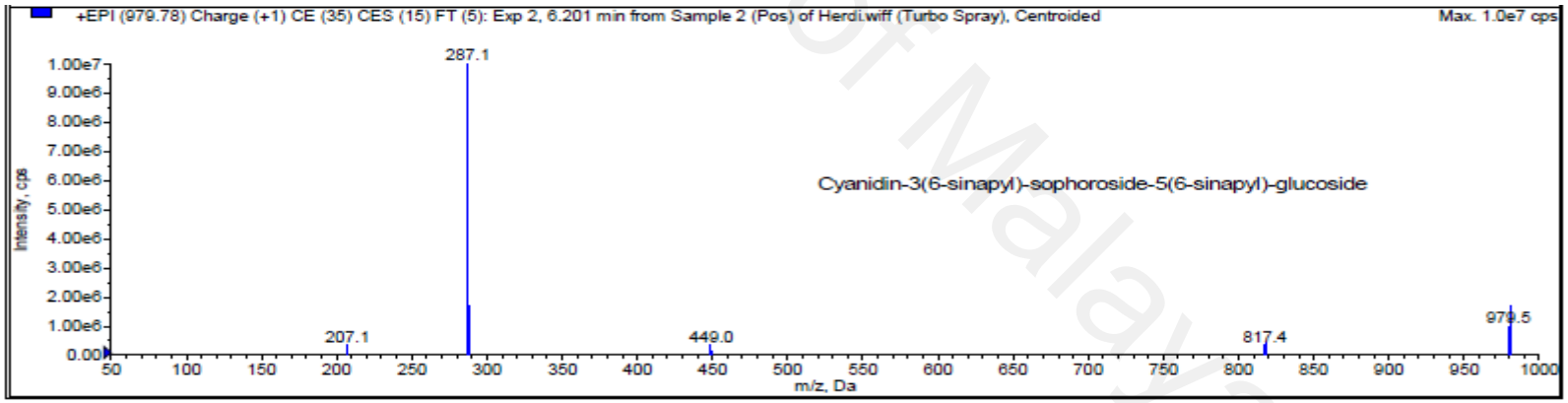
APPENDIX A7 – MS/MS OF CYANIDIN-3(6-SINAPYL)-SOPHOROSIDE-5(6-SINAPYL)-GLUCOSIDE (PEAK 6)



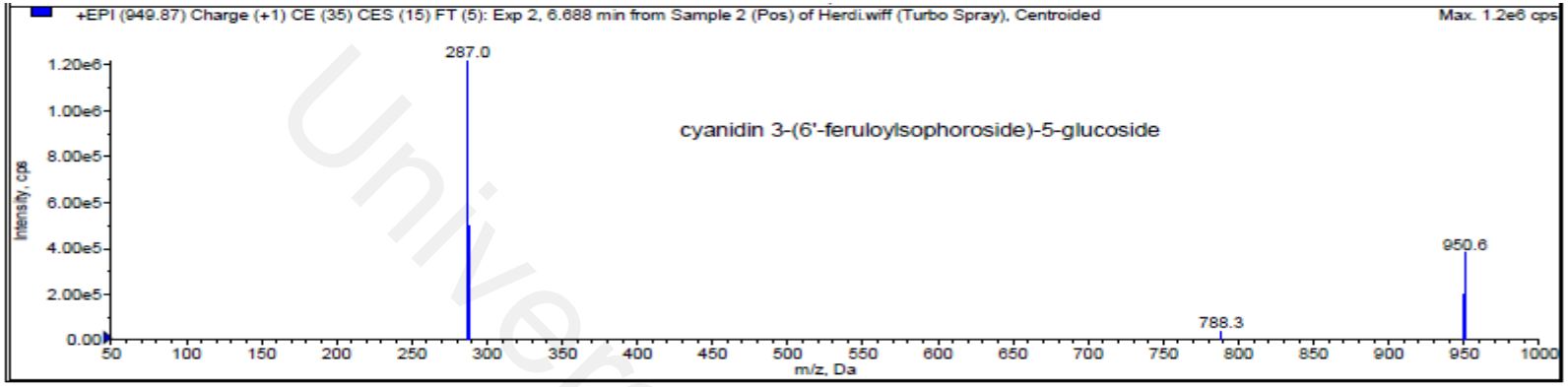
APPENDIX A8 – MS/MS OF CYANIDIN-3(6-SINAPYL)-SOPHOROSIDE-5(6-SINAPYL)-GLUCOSIDE (PEAK 7)



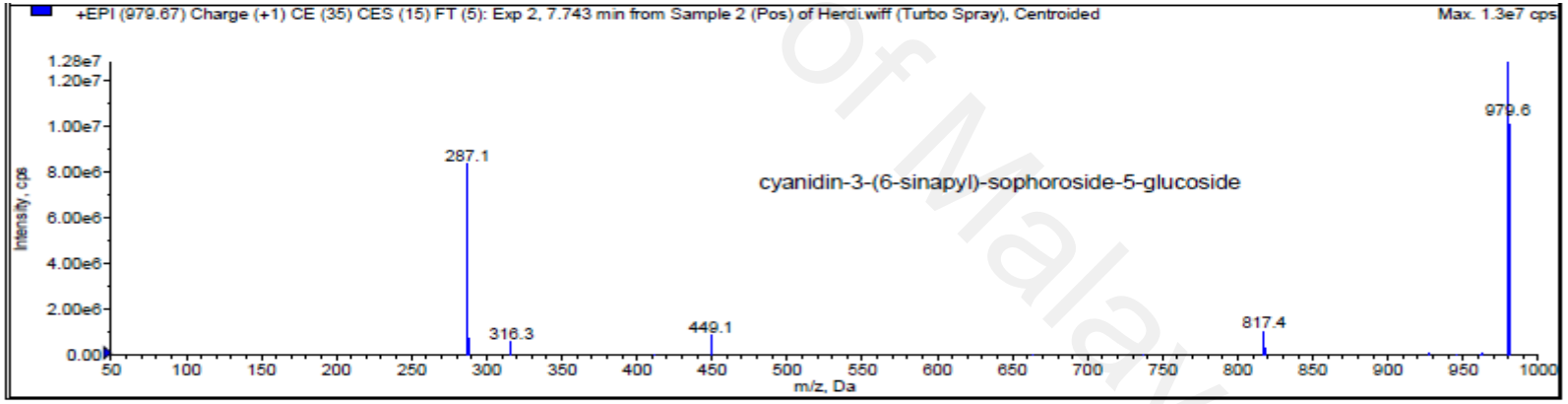
APPENDIX A9 – MS/MS OF CYANIDIN-3(6-SINAPYL)-SOPHOROSIDE-5(6-SINAPYL)-GLUCOSIDE (PEAK 8)



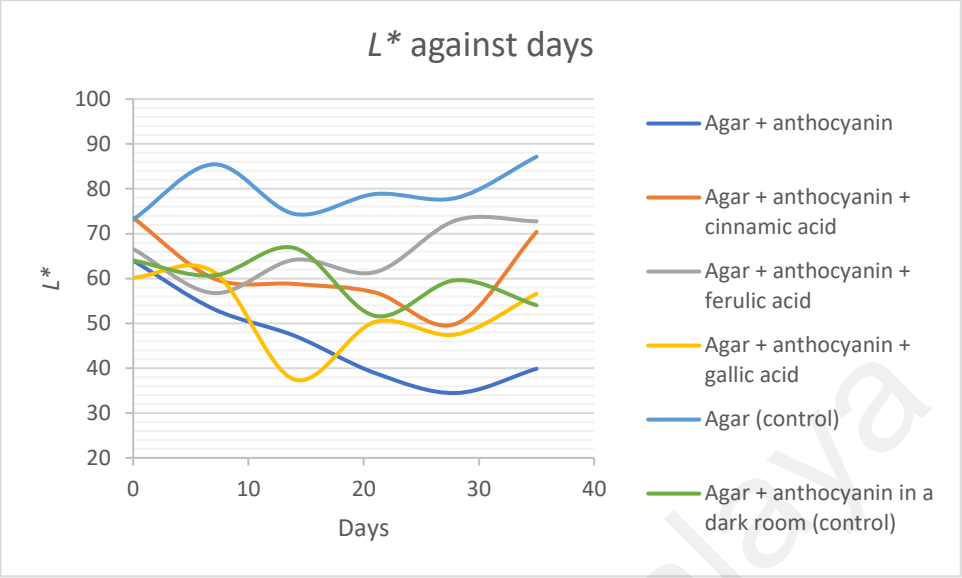
APPENDIX A10 – MS/MS OF CYANIDIN 3-(6-FERULOYLSOPHOROSIDE)-5-GLUCOSIDE (PEAK 9)



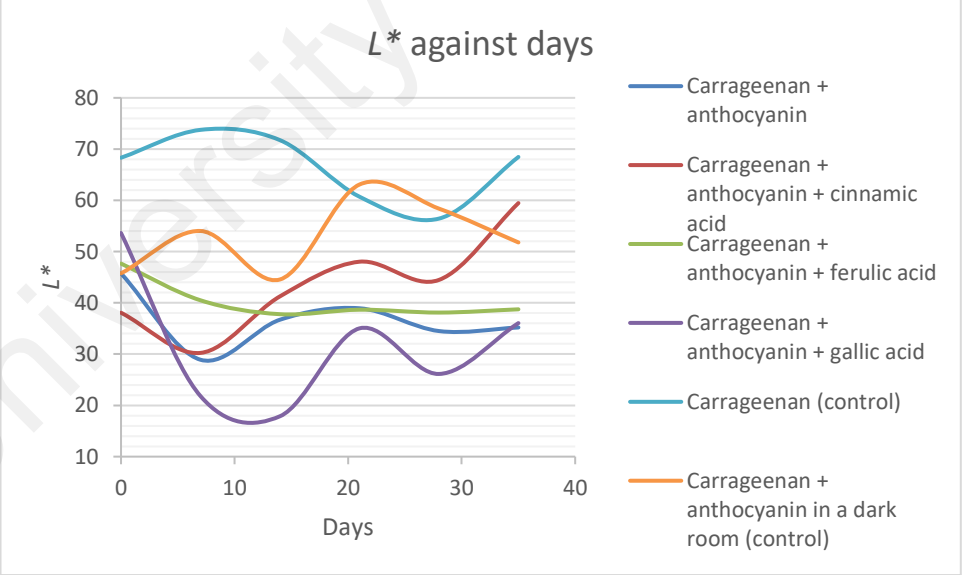
APPENDIX A11 – MS/MS OF CYANIDIN-3-(6-SINAPYL)-SOPHOROSIDE-5-GLUCOSIDE (PEAK 10)



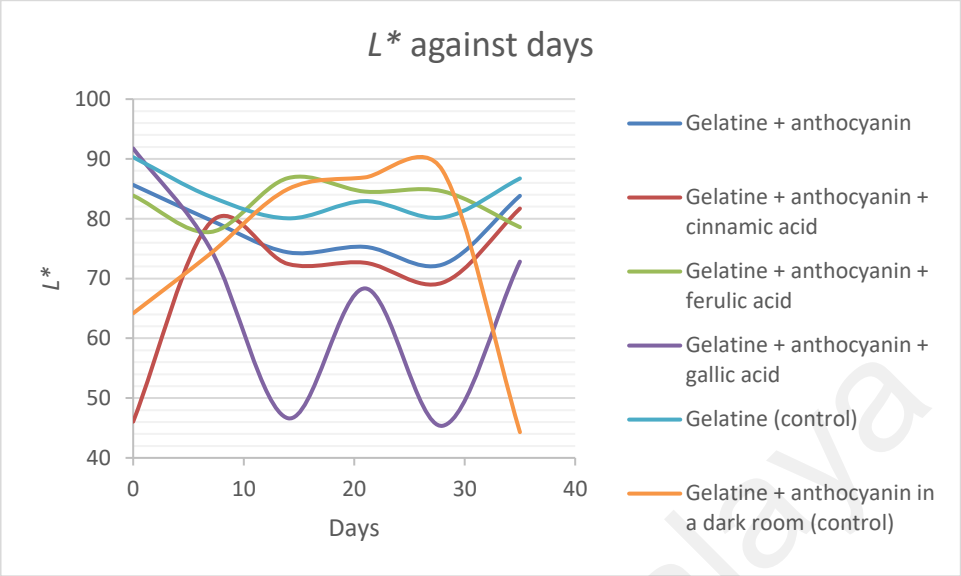
APPENDIX B1 – THE GRAPH OF *L** AGAINST DAYS FOR AGAR SAMPLE UNDER UV-A EXPOSURE



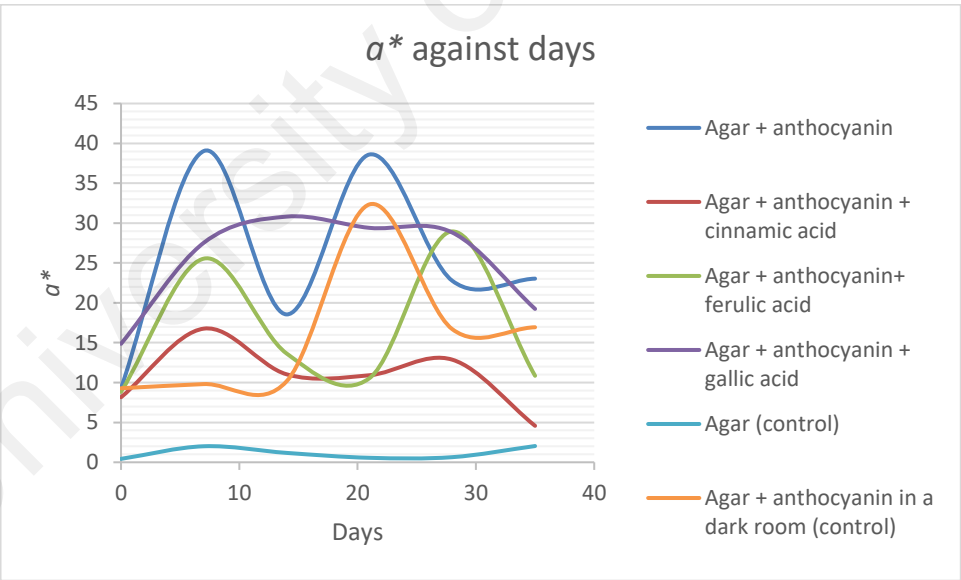
APPENDIX B2 – THE GRAPH OF *L** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-A EXPOSURE



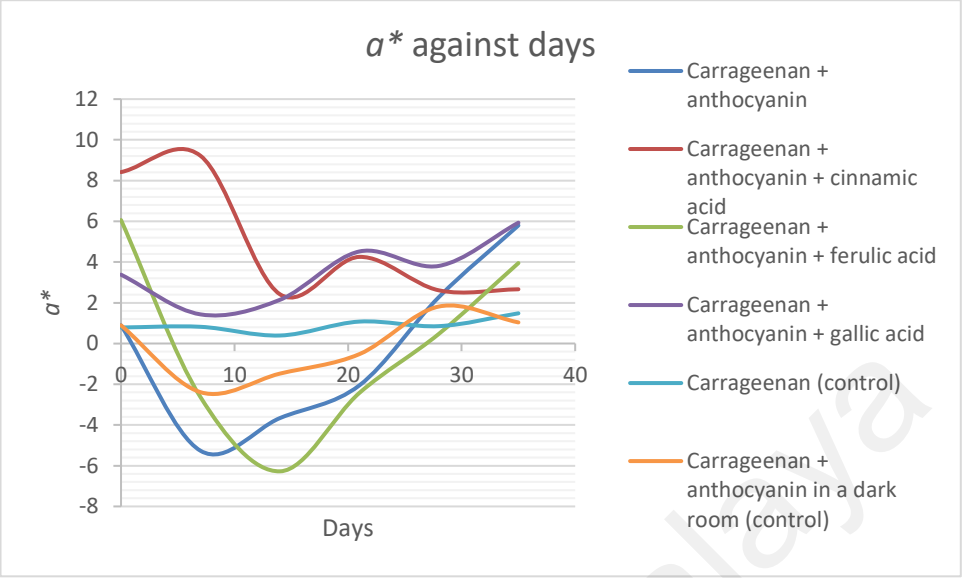
APPENDIX B3 - THE GRAPH OF *L** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-A EXPOSURE



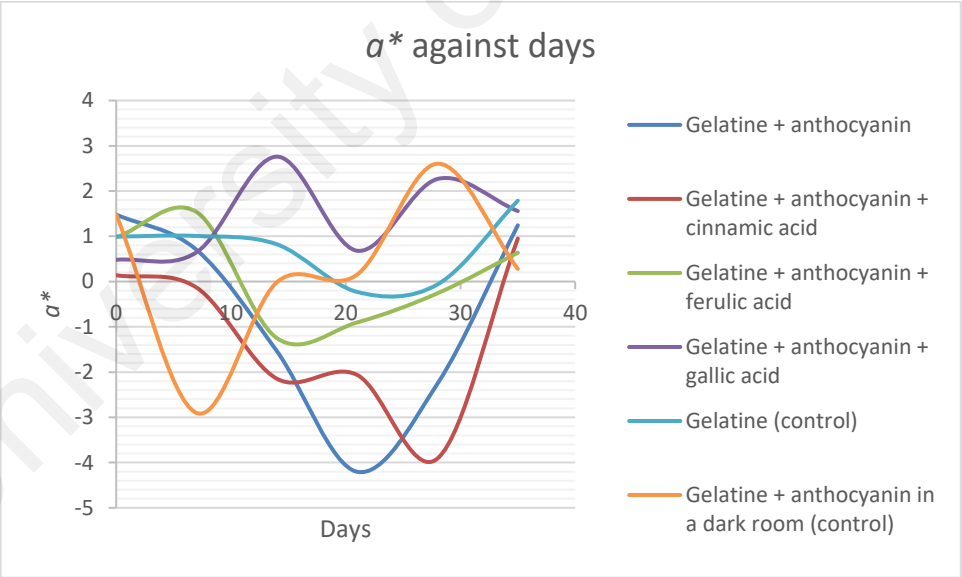
APPENDIX B4 – THE GRAPH OF *a** AGAINST DAYS FOR AGAR SAMPLE UNDER UV-A EXPOSURE



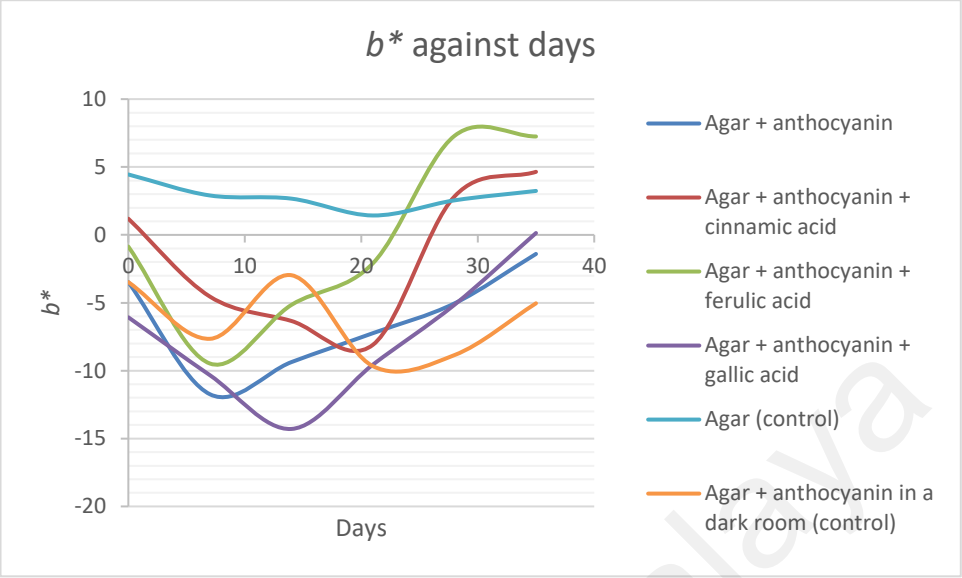
APPENDIX B5 - THE GRAPH OF *a** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-A EXPOSURE



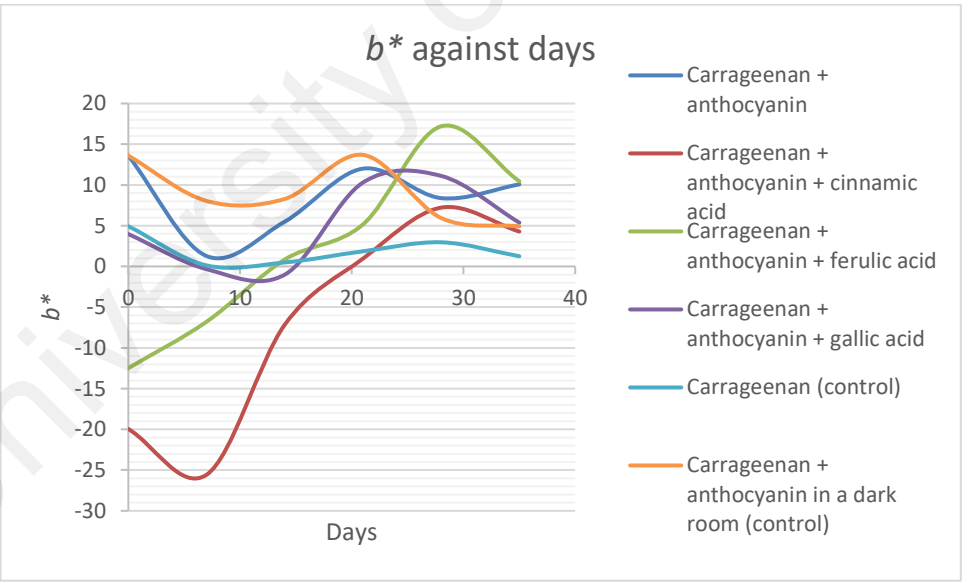
APPENDIX B6 - THE GRAPH OF *a** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-A EXPOSURE



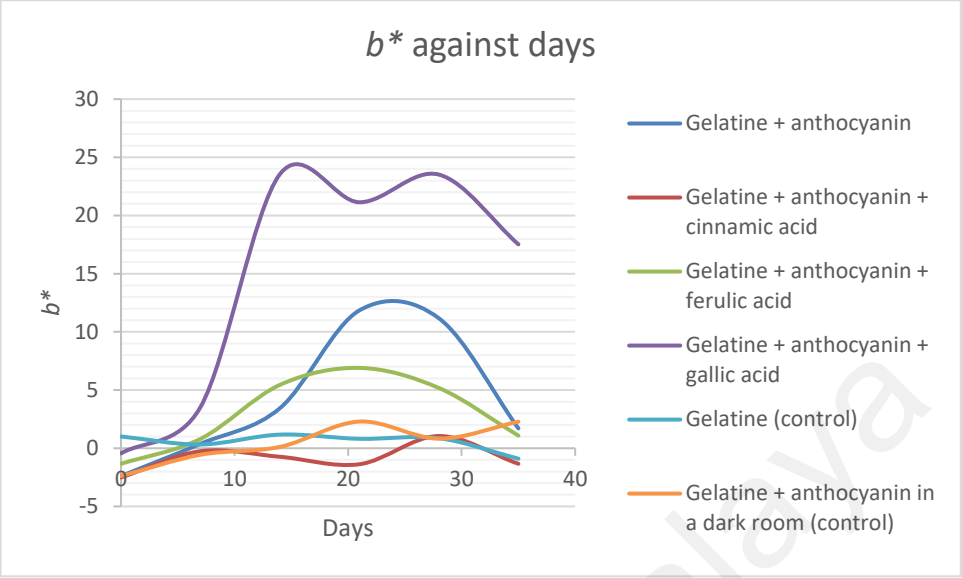
APPENDIX B7 - THE GRAPH OF *b** AGAINST DAYS FOR AGAR SAMPLE UNDER UV-A EXPOSURE



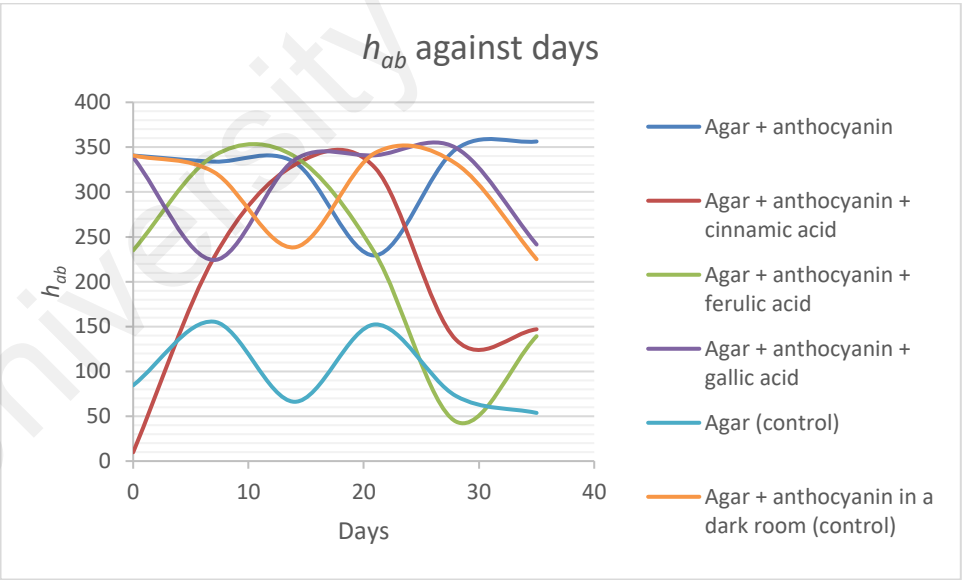
APPENDIX B8 - THE GRAPH OF *b** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-A EXPOSURE



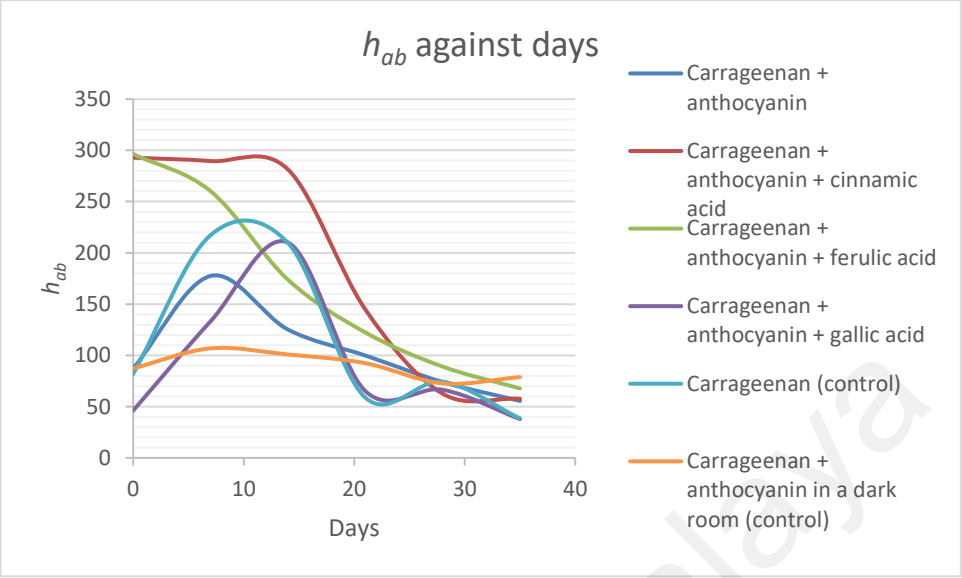
APPENDIX B9 - THE GRAPH OF *b** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-A EXPOSURE



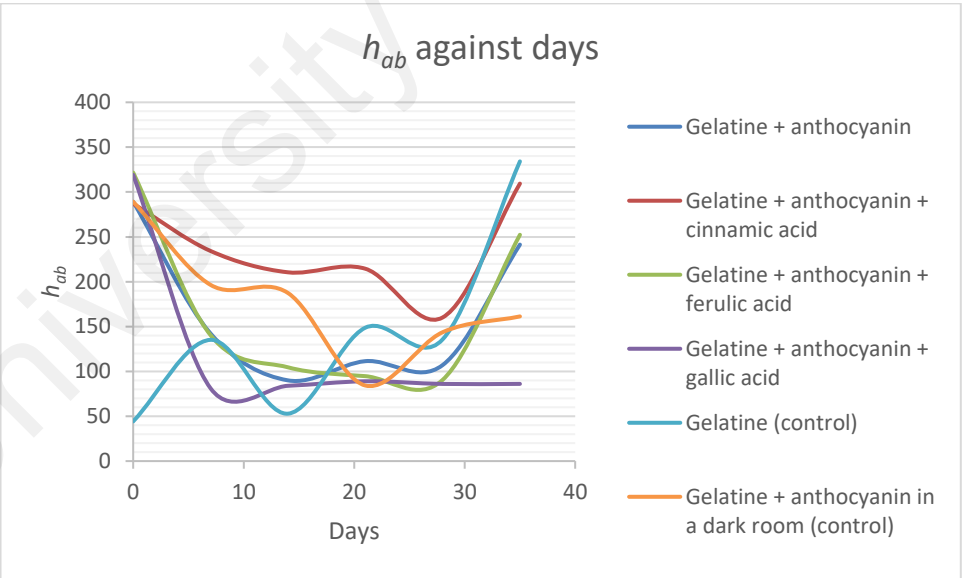
APPENDIX B10 - THE GRAPH OF h_{ab} AGAINST DAYS FOR AGAR SAMPLE UNDER UV-A EXPOSURE



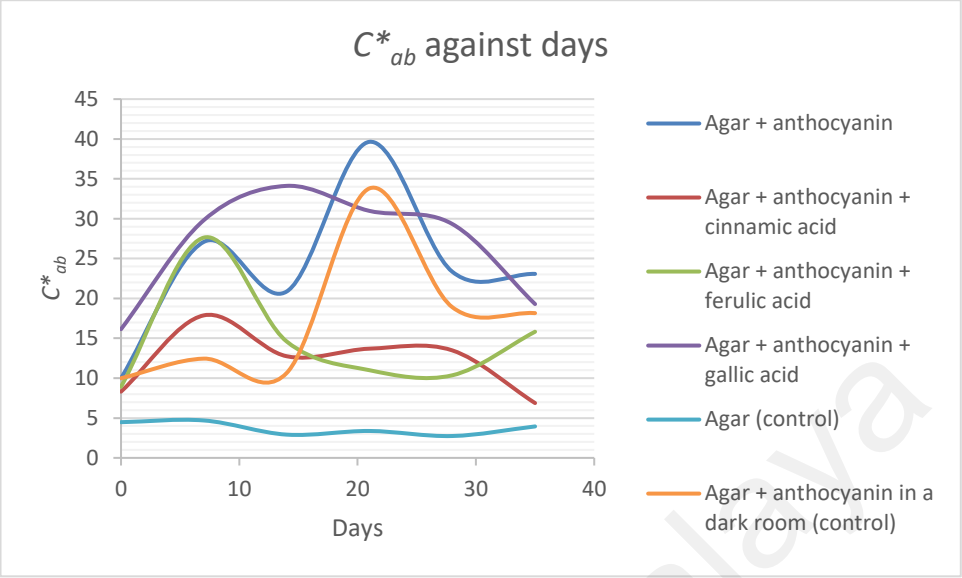
APPENDIX B11 - THE GRAPH OF h_{ab} AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-A EXPOSURE



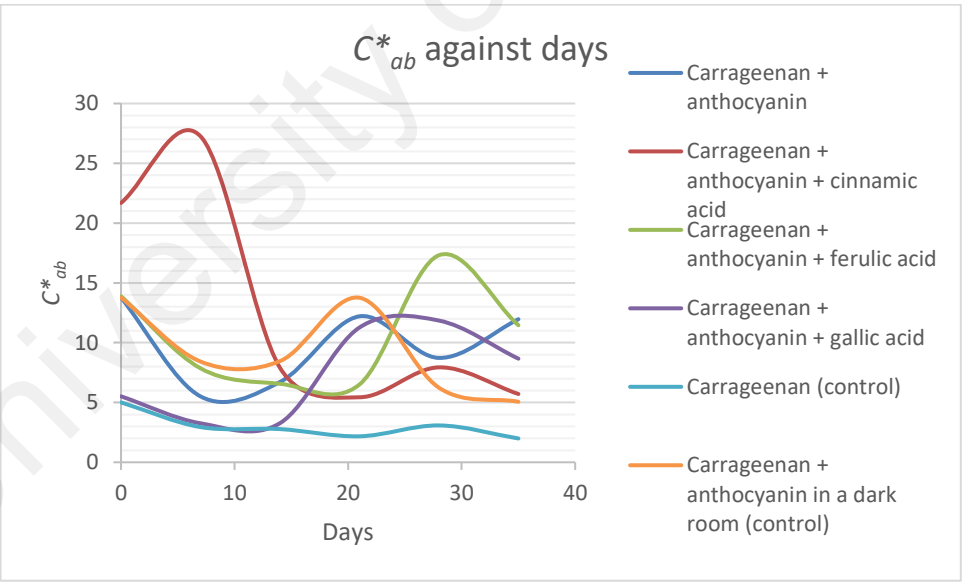
APPENDIX B12 - THE GRAPH OF h_{ab} AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-A EXPOSURE



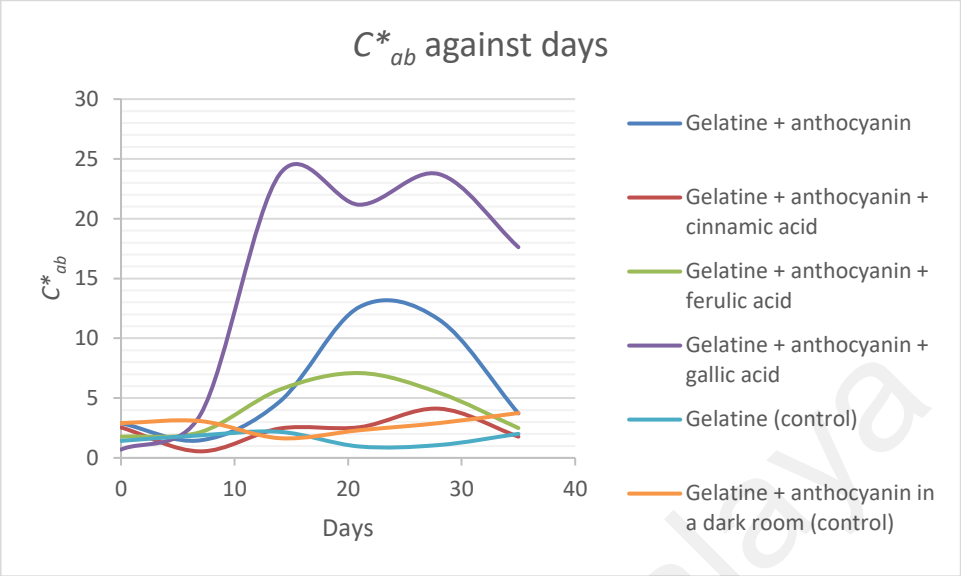
APPENDIX B13 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR AGAR SAMPLE UNDER UV-A EXPOSURE



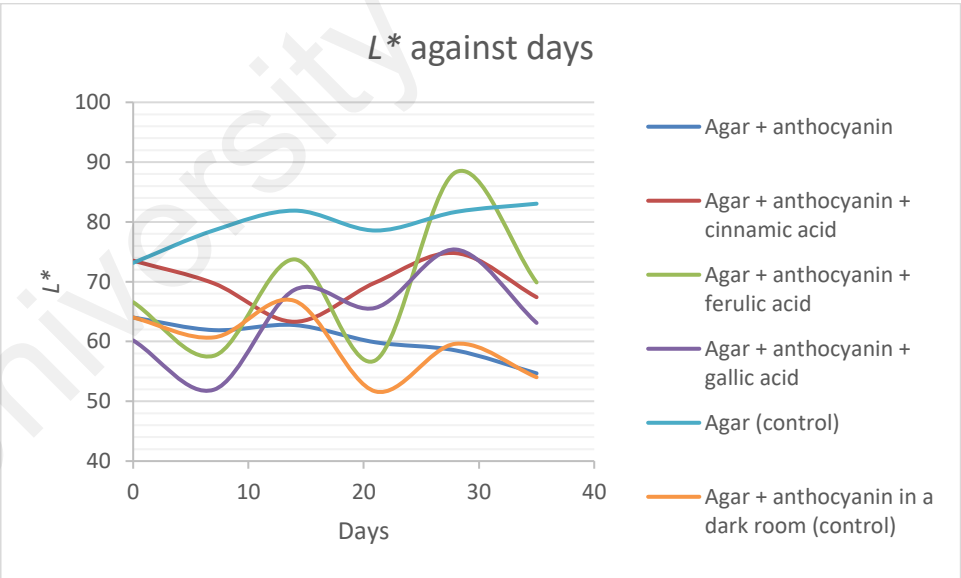
APPENDIX B14 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-A EXPOSURE



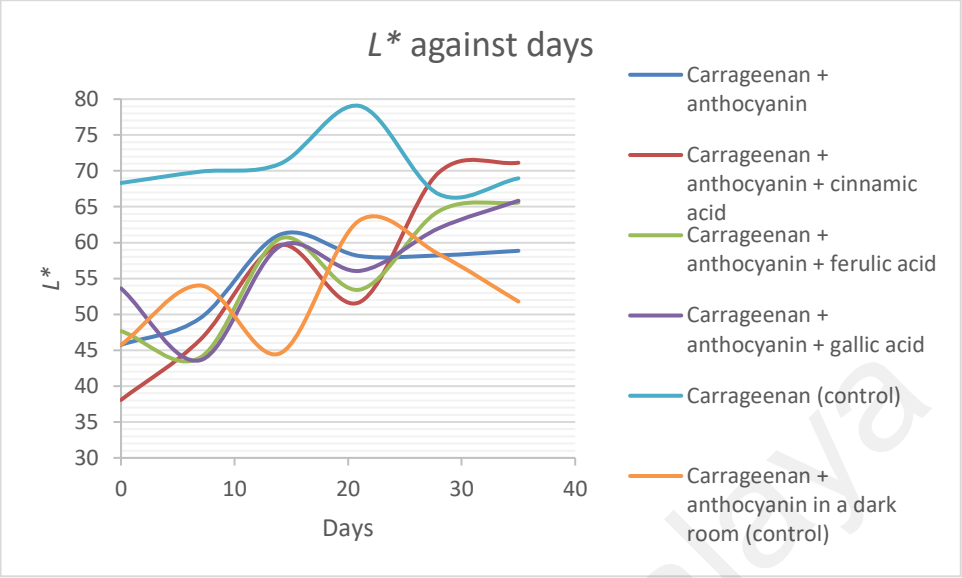
APPENDIX B15 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-A EXPOSURE



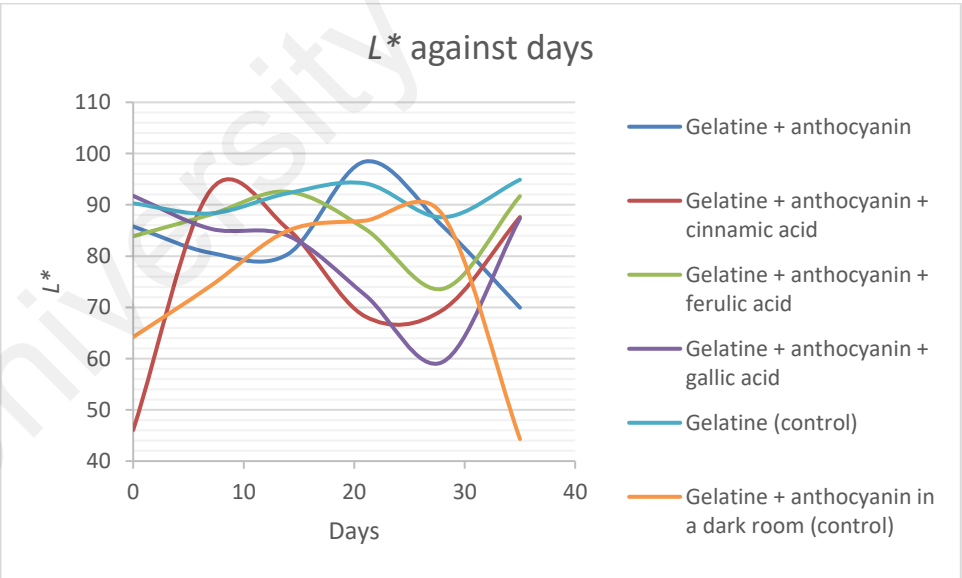
APPENDIX B16 - THE GRAPH OF L^* AGAINST DAYS FOR AGAR SAMPLE UNDER UV-B EXPOSURE



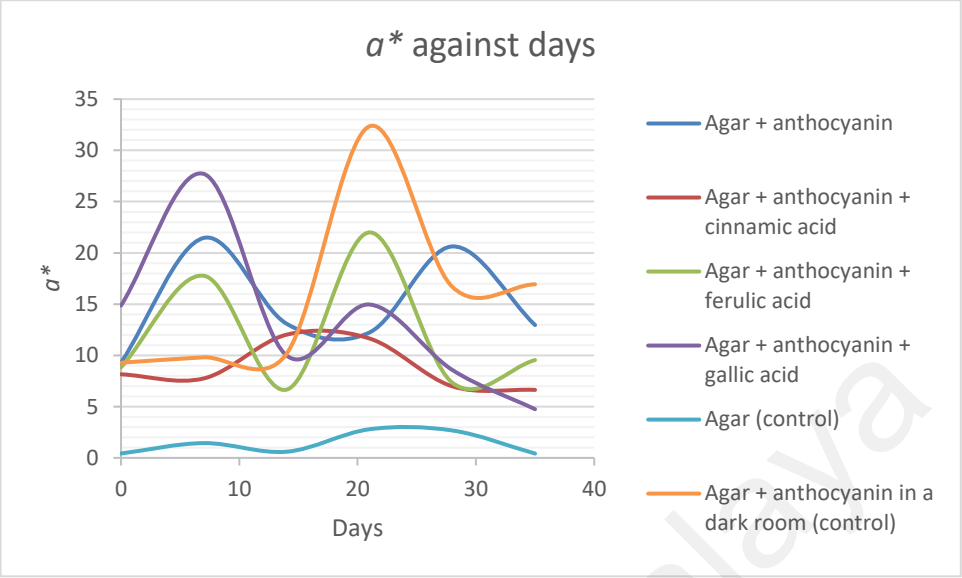
APPENDIX B17 - THE GRAPH OF *L** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-B EXPOSURE



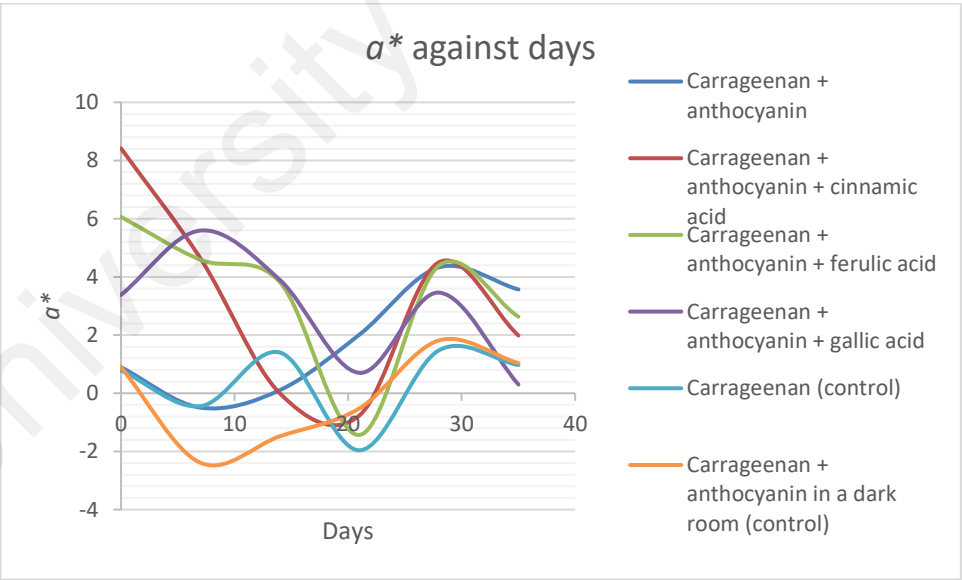
APPENDIX B18 - THE GRAPH OF *L** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-B EXPOSURE



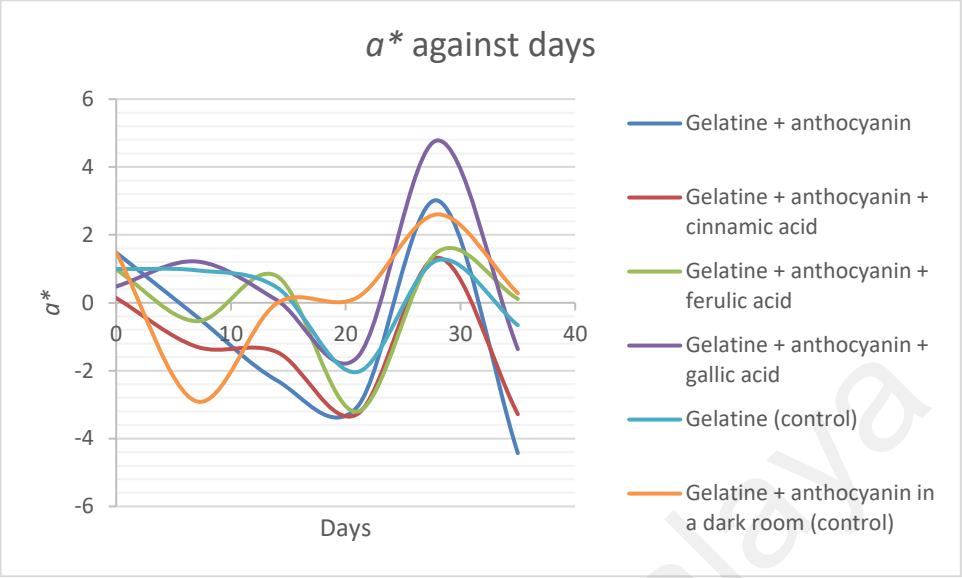
APPENDIX B19 - THE GRAPH OF *a** AGAINST DAYS FOR AGAR SAMPLE UNDER UV-B EXPOSURE



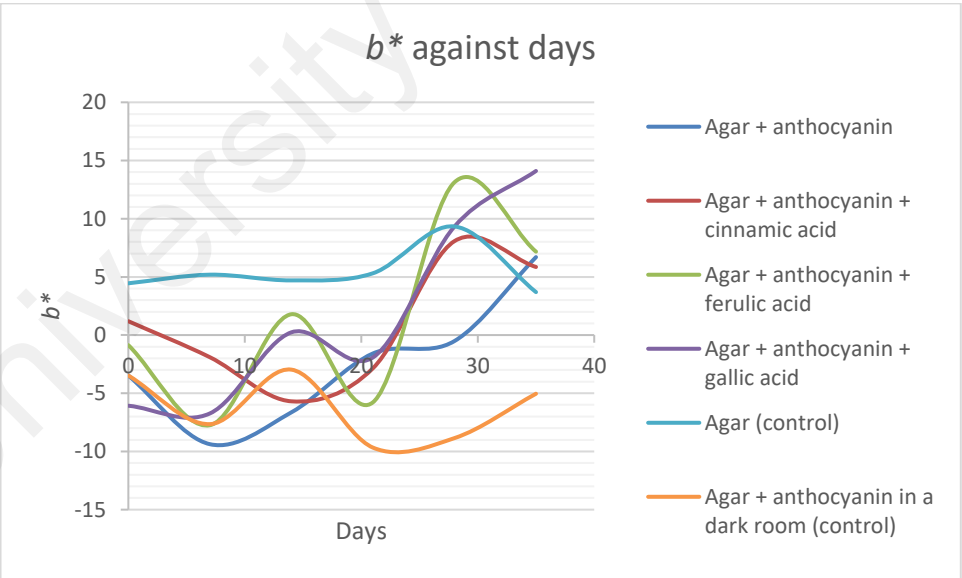
APPENDIX B20 - THE GRAPH OF *a** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-B EXPOSURE



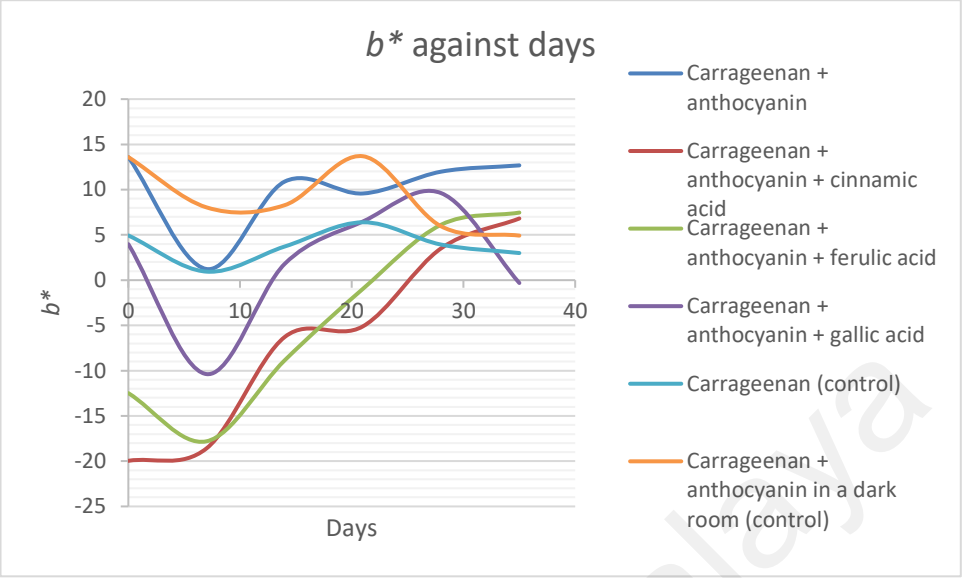
APPENDIX B21 - THE GRAPH OF *a** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-B EXPOSURE



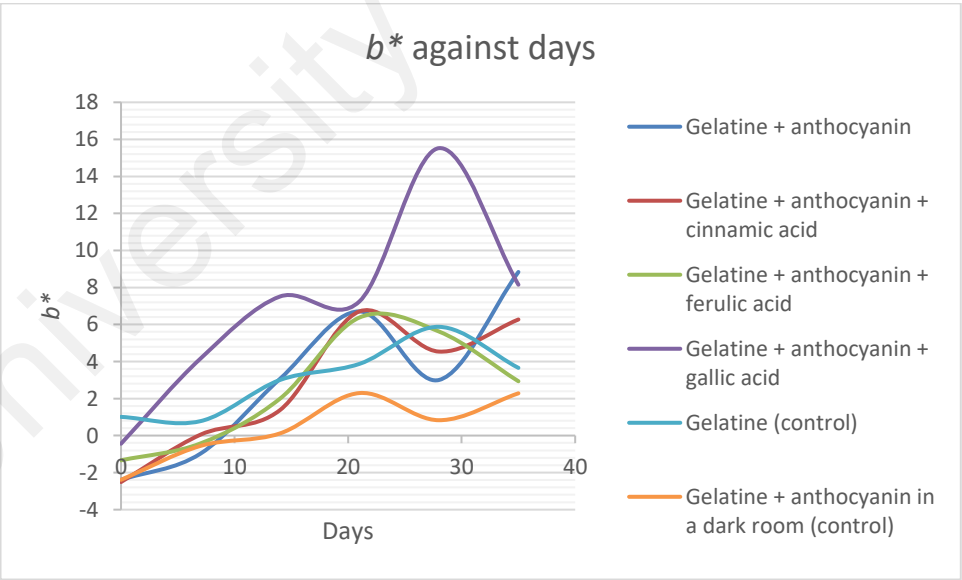
APPENDIX B22 - THE GRAPH OF *b** AGAINST DAYS FOR AGAR SAMPLE UNDER UV-B EXPOSURE



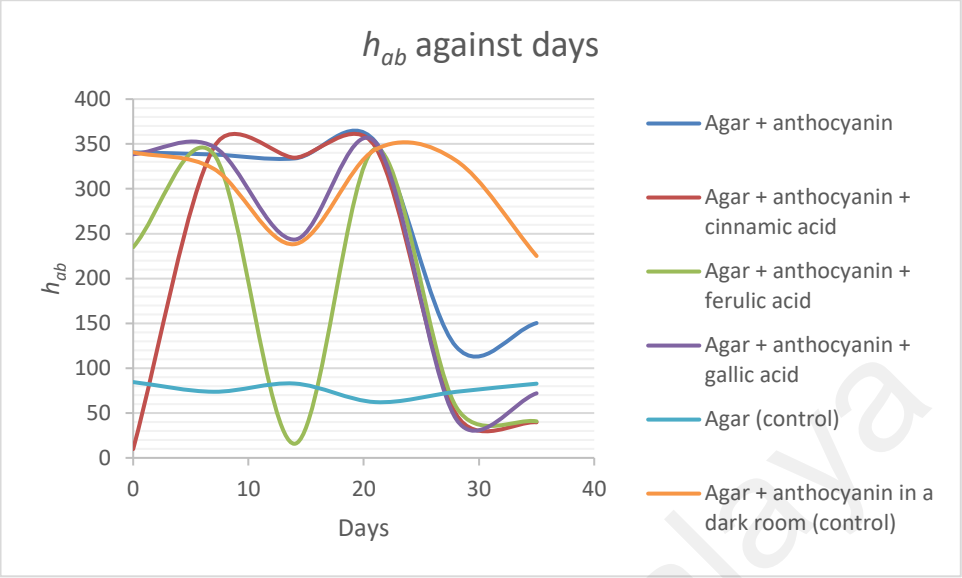
APPENDIX B23 - THE GRAPH OF *b** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-B EXPOSURE



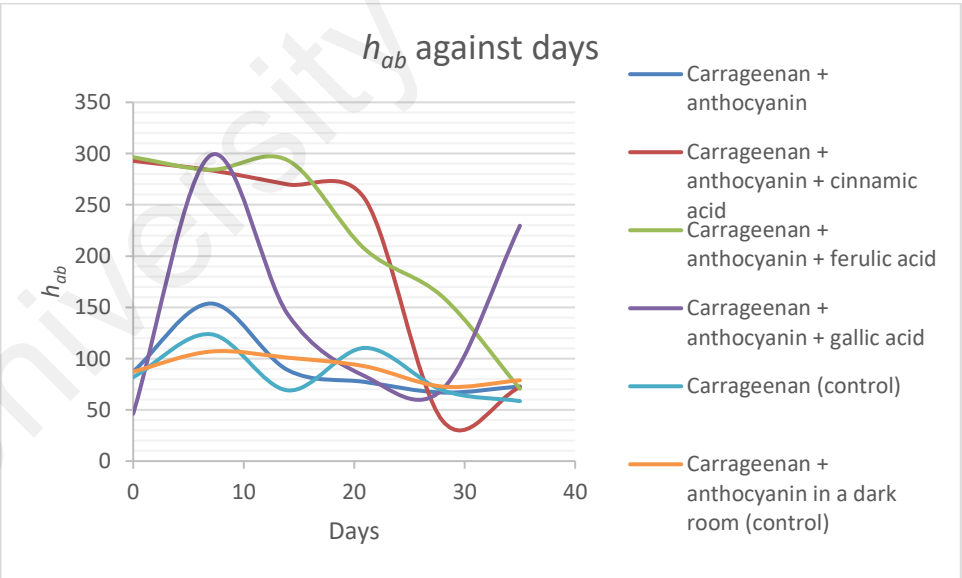
APPENDIX B24 - THE GRAPH OF *b** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-B EXPOSURE



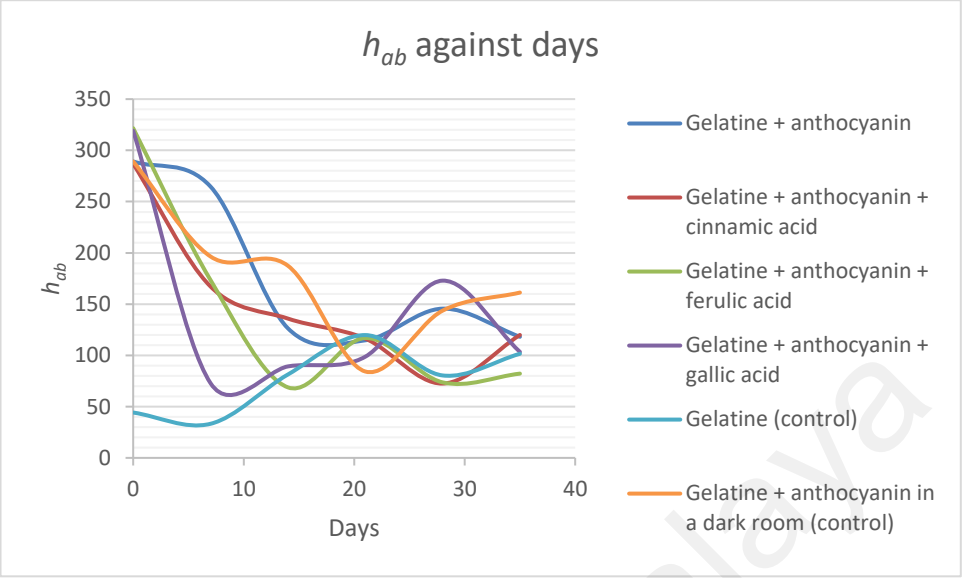
APPENDIX B25 - THE GRAPH OF h_{ab} AGAINST DAYS FOR AGAR SAMPLE UNDER UV-B EXPOSURE



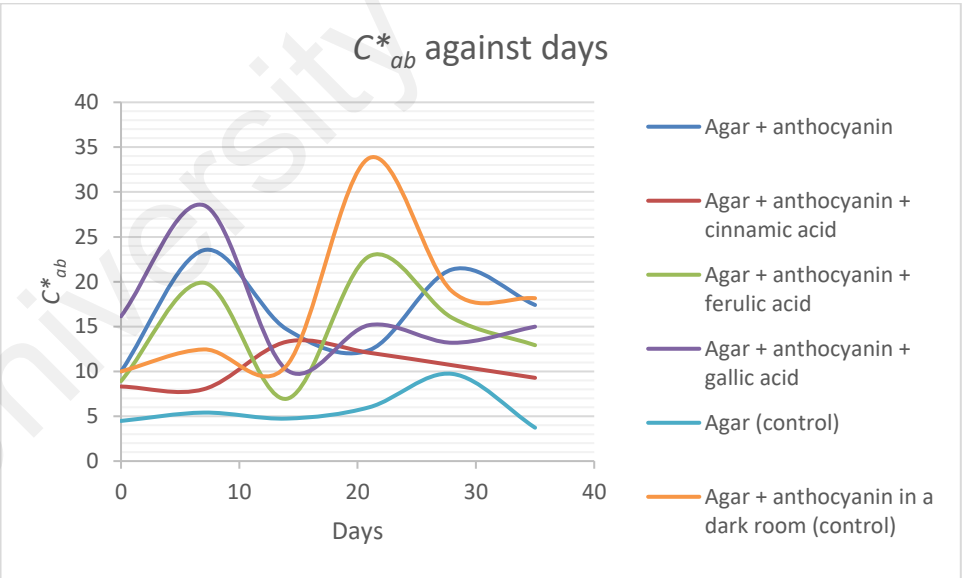
APPENDIX B26 - THE GRAPH OF h_{ab} AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-B EXPOSURE



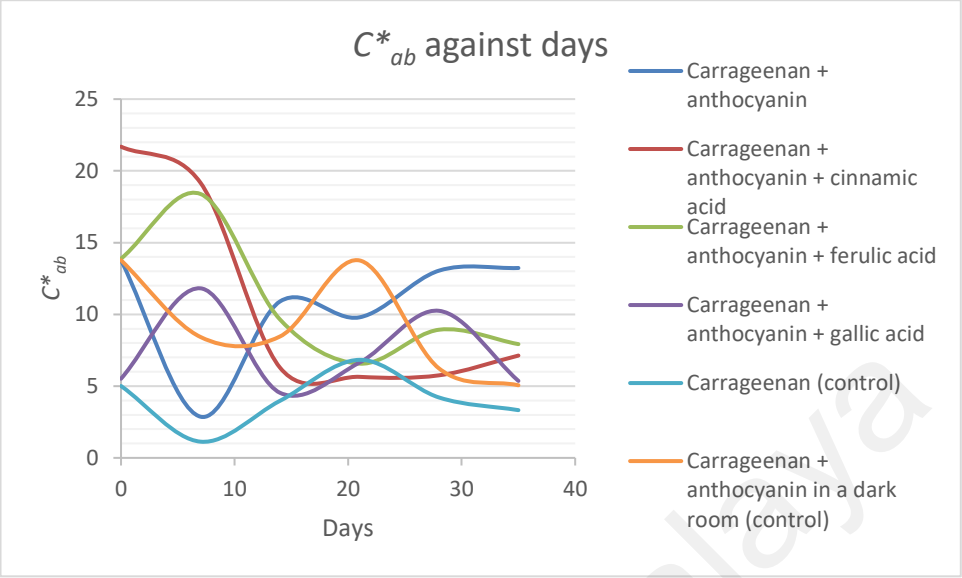
APPENDIX B27 - THE GRAPH OF h_{ab} AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-B EXPOSURE



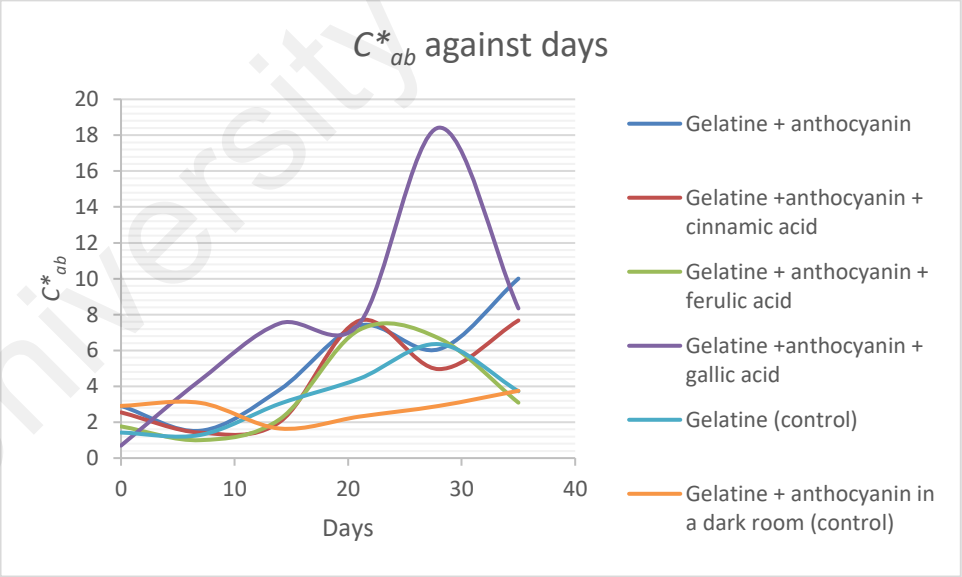
APPENDIX B28 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR AGAR SAMPLE UNDER UV-B EXPOSURE



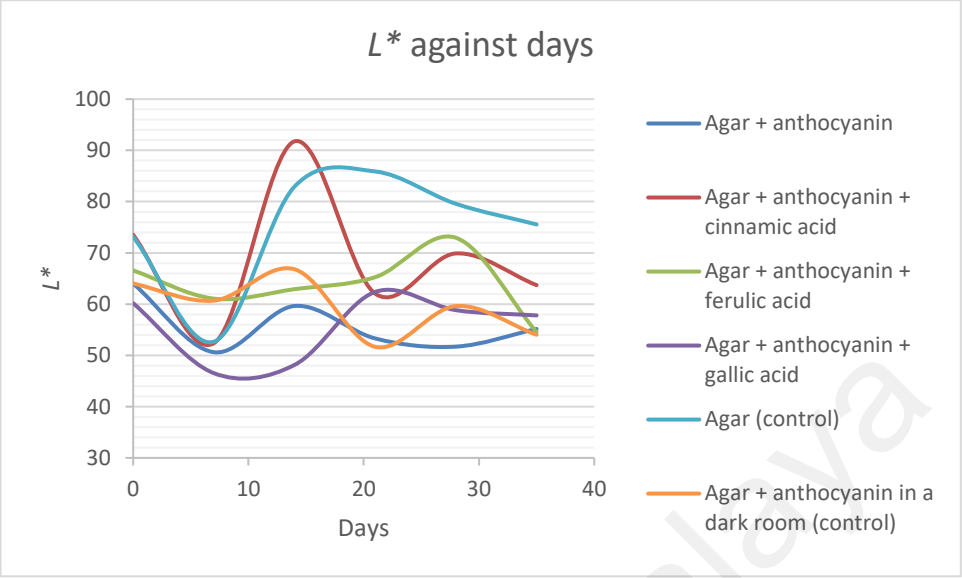
APPENDIX B29 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-B EXPOSURE



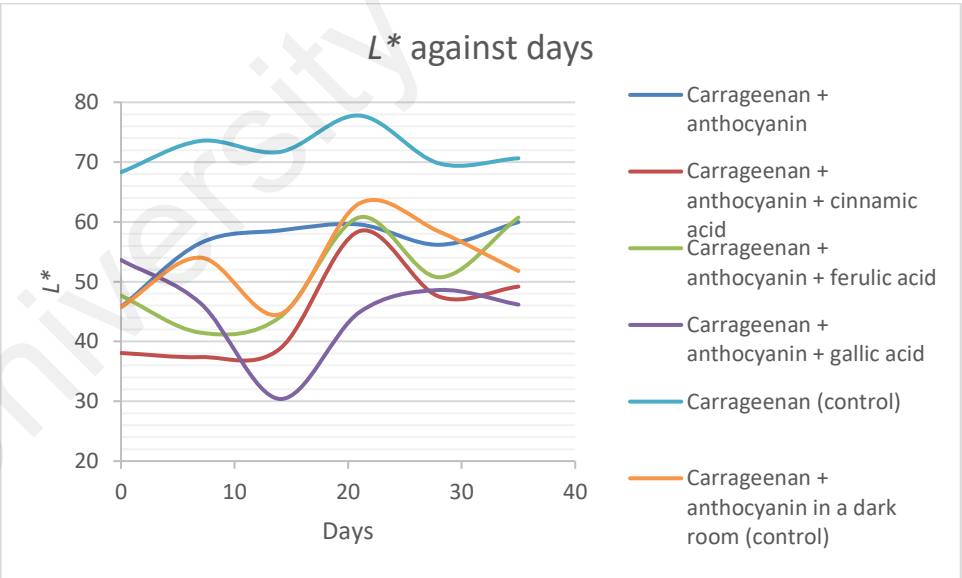
APPENDIX B30 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-B EXPOSURE



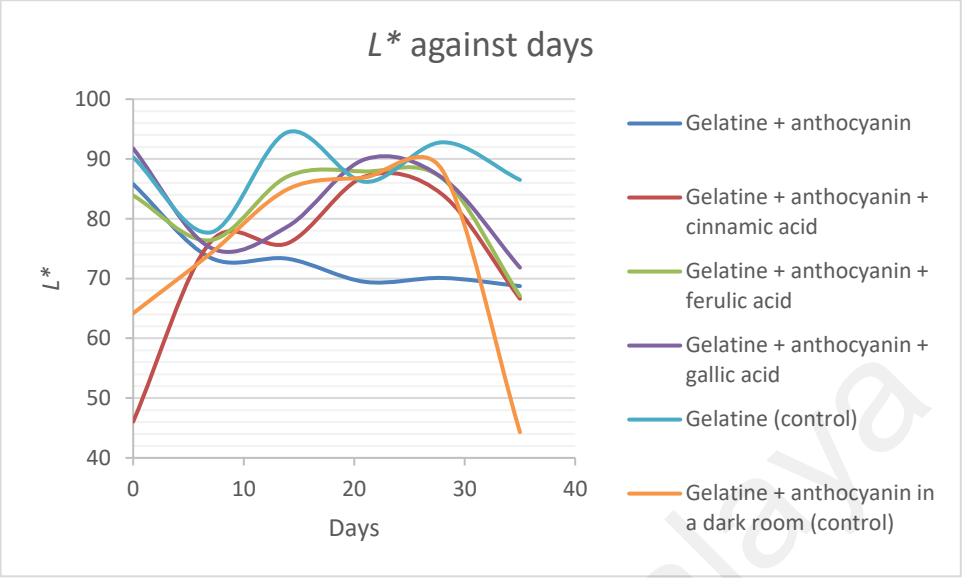
APPENDIX B31 - THE GRAPH OF *L** AGAINST DAYS FOR AGAR SAMPLE UNDER UV-C EXPOSURE



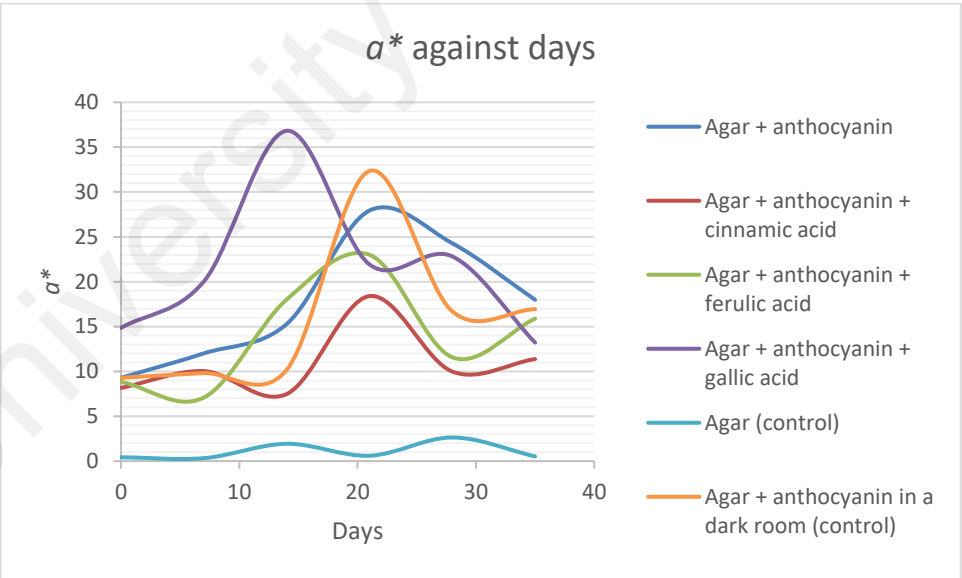
APPENDIX B32 - THE GRAPH OF *L** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-C EXPOSURE



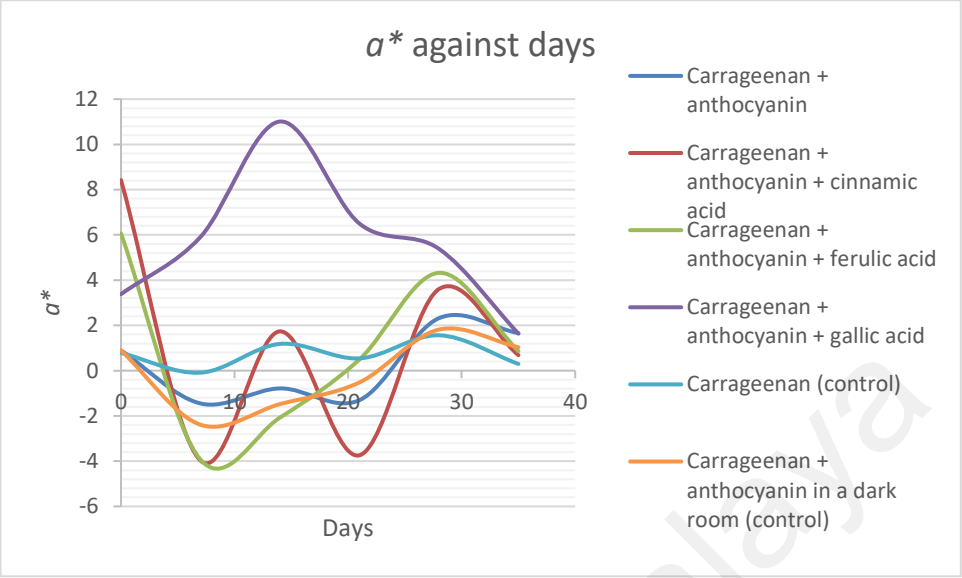
APPENDIX B33 - THE GRAPH OF *L** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-C EXPOSURE



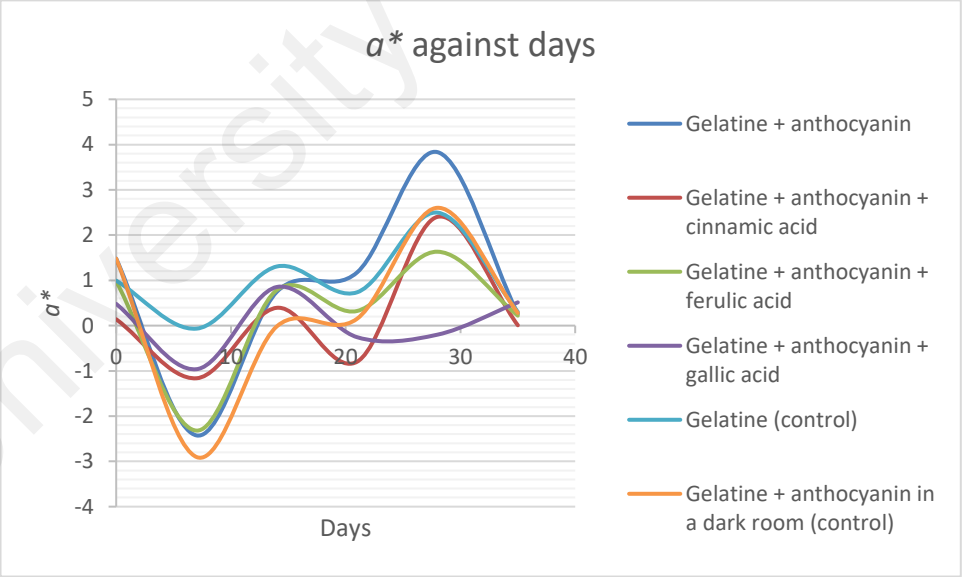
APPENDIX B34 - THE GRAPH OF *a** AGAINST DAYS FOR AGAR SAMPLE UNDER UV-C EXPOSURE



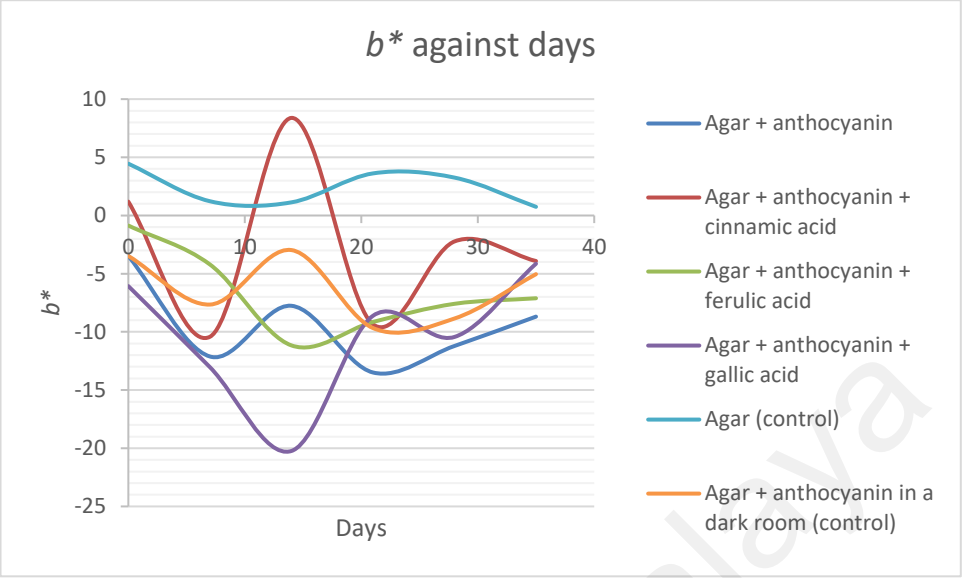
APPENDIX B35 - THE GRAPH OF *a** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-C EXPOSURE



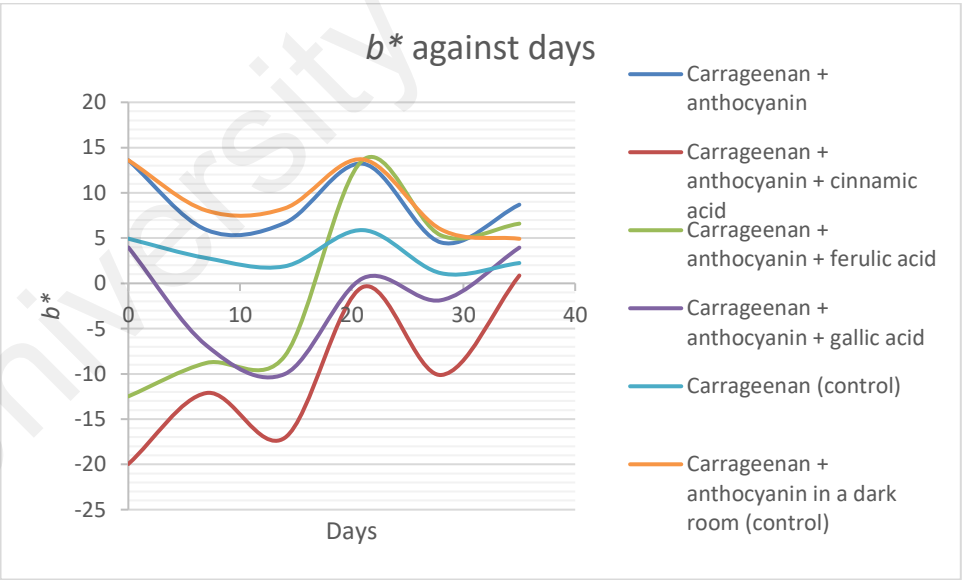
APPENDIX B36 - THE GRAPH OF *a** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-C EXPOSURE



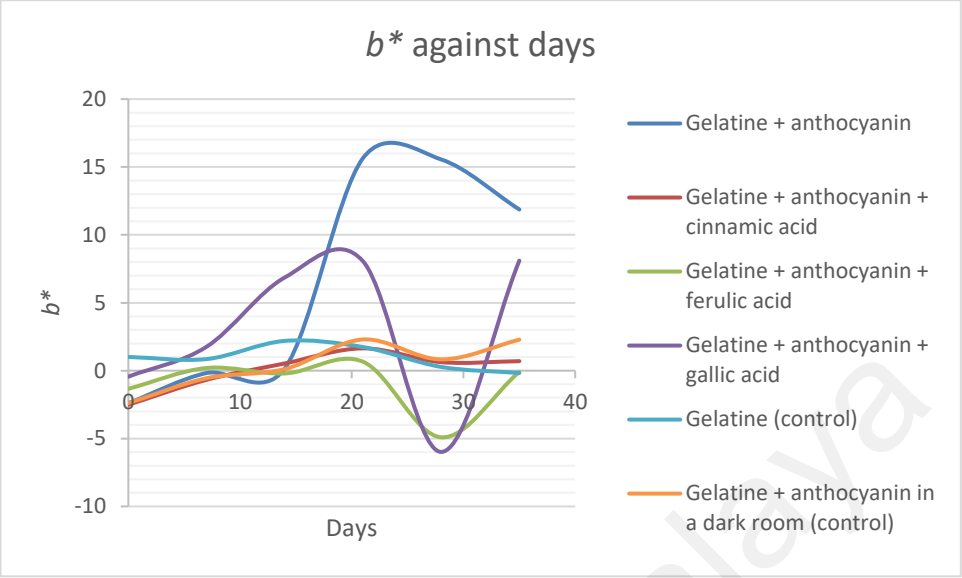
APPENDIX B37 - THE GRAPH OF *b** AGAINST DAYS FOR AGAR SAMPLE UNDER UV-C EXPOSURE



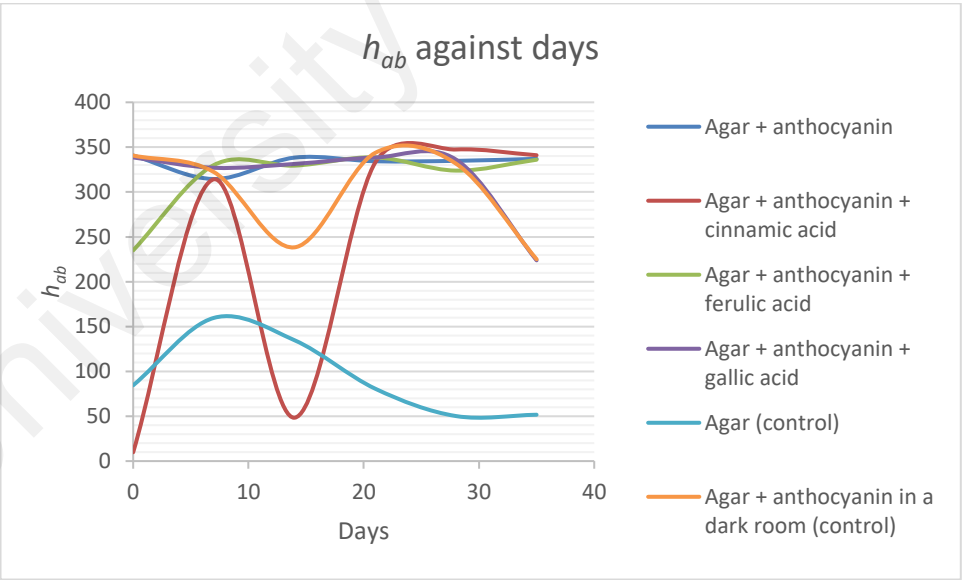
APPENDIX B38 - THE GRAPH OF *b** AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-C EXPOSURE



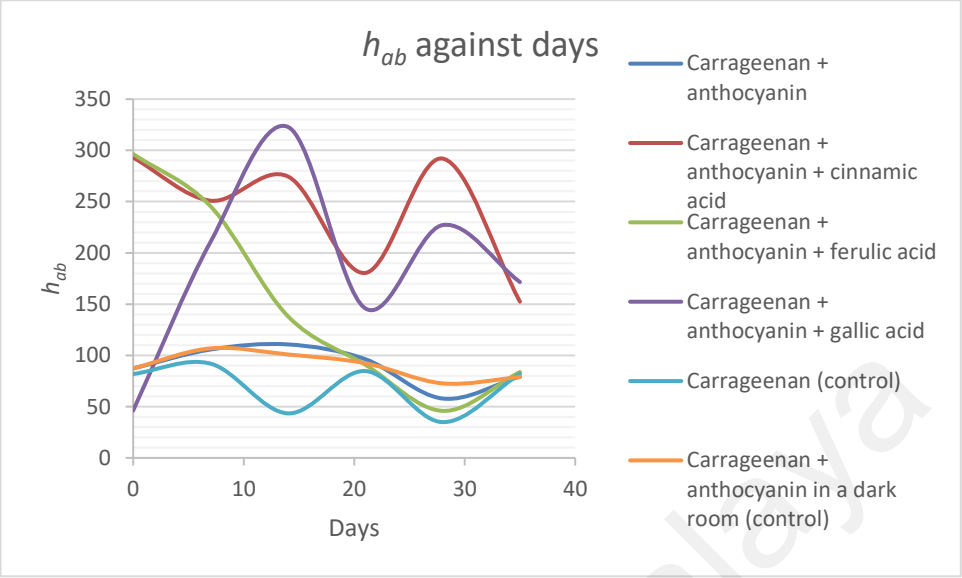
APPENDIX B39 - THE GRAPH OF *b** AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-C EXPOSURE



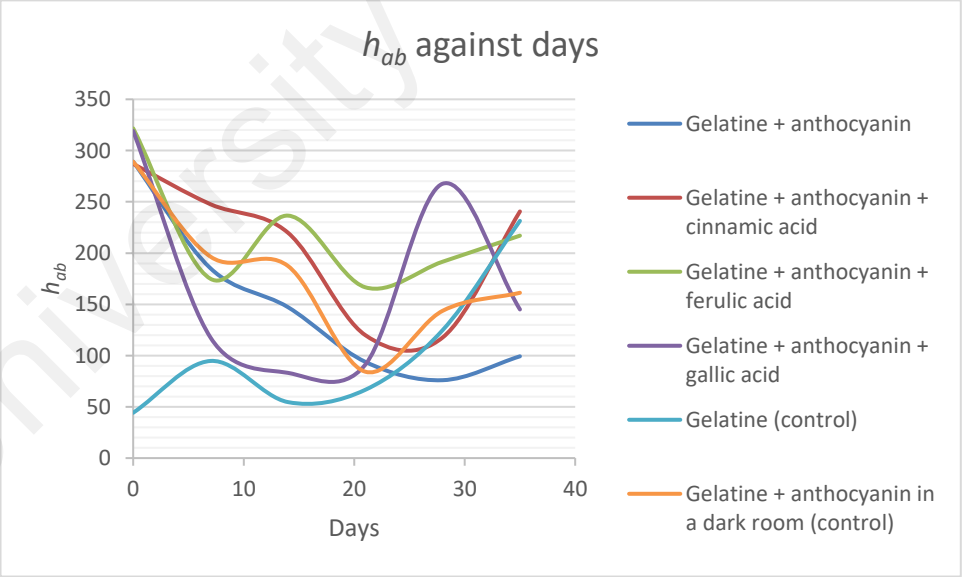
APPENDIX B40 - THE GRAPH OF h_{ab} AGAINST DAYS FOR AGAR SAMPLE UNDER UV-C EXPOSURE



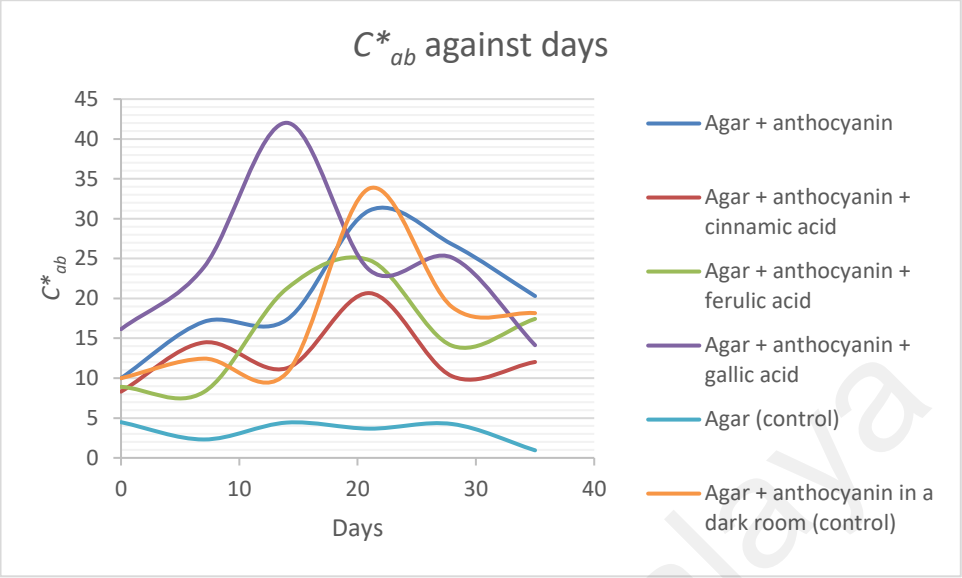
APPENDIX B41 - THE GRAPH OF h_{ab} AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-C EXPOSURE



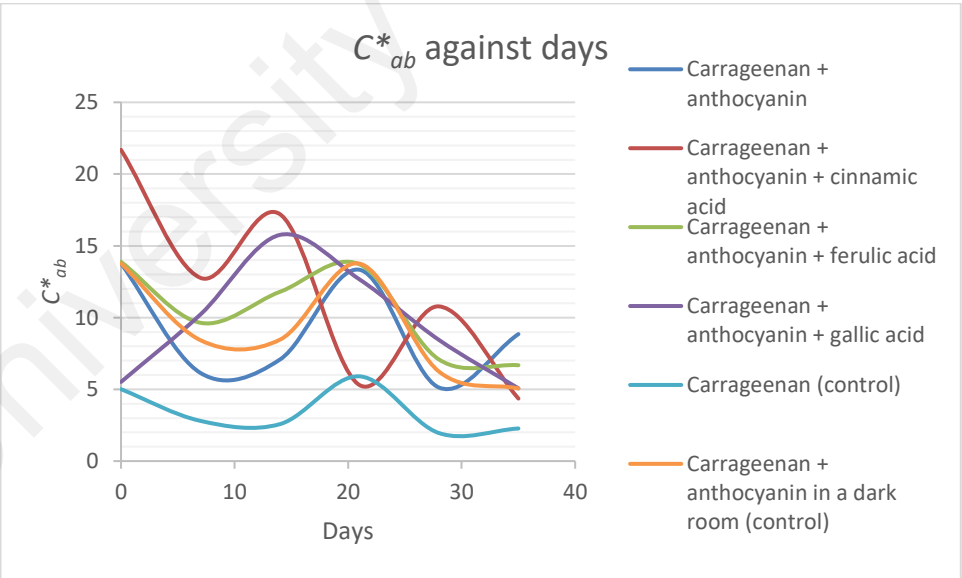
APPENDIX B42 - THE GRAPH OF h_{ab} AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-C EXPOSURE



APPENDIX B43 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR AGAR SAMPLE UNDER UV-C EXPOSURE



APPENDIX B44 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR CARRAGEENAN SAMPLE UNDER UV-C EXPOSURE



APPENDIX B45 - THE GRAPH OF C^*_{ab} AGAINST DAYS FOR GELATINE SAMPLE UNDER UV-C EXPOSURE