ANALYSIS OF ANTHOCYANIN COLOUR STABILITY FROM FRUITS OF *IXORA SIAMENSIS*

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ABSTRACT

This studies are to identify the potential of fruits from *Ixora siamensis* (“pokok jejarum”) as a new source of antocyanin natural colourant and to evaluate the suitability of the anthocyanin natural colourant with ferulic acid (FA) stabilizing agent when blended with poly(vinyl) alcohol (PVA) to form a coating system. Furthermore, the aim is to analyse colour stability of anthocyanin colourant in a coating system in terms of pH and co-pigmentation under UV-B exposure by CIE colour system. The natural anthocyanin colourant from *Ixora siamensis* was extracted by using acidified methanol for crude extraction. Further purification of crude extraction was performed by using liquid-liquid partition extraction and ion exchange column chromatography. Different proportions of ferulic acid as stabilizing agent were added in order to improve and enhance the resistance towards the UV-B irradiation during exposure period. FA added colourant was mixed with PVA to develop coating system. To test the colour stability of crude and purified anthocyanin colourant and anthocyanin-PVA blend towards UV-B irradiation, CIE colour analysis was conducted. CIE results obtained were analysed in terms of L* C* H^{o} a* b* colour coordinate values. Total colour difference (ΔE) and saturation (s) of colour were determined in order to evaluate the visual colour variation. Based on the results obtained, the colour of the untreated anthocyanin colourant and anthocyanin-PVA blend was susceptible to UV degradation during 93 days of exposure. The addition of 2% FA at pH 3 performed better colour stability. CIE results also showed that the colour variation of anthocyanin and anthocyanin-PVA blend definitely influenced by pH variation.

ABSTRAK

Kajian ini bertujuan untuk mengenal pasti potensi buah-buahan daripada *Ixora siamensis* (“pokok jejarum”) sebagai sumber baru antosianin pewarna semulajadi diguna untuk menilai kesesuaian sebagai antosianin pewarna semulajadi dengan asid ferulik (FA) untuk menstabilkan ejen apabila dicampur dengan alkohol poli (vinil) (PVA) bagi membentuk sistem salutan. Seterusnya analisis kestabilan warna daripada antosianin pewarna dalam sistem lapisan terhadap kesan pH dan bersama-pigmentasi di bawah UV-B menggunakan sistem warna CIE. Antosianin pewarna semulajadi daripada *Ixora siamensis* yang diekstrak menggunakan metanol berasid digelar pengekstrakan mentah. Penulenan selanjutnya daripada pengekstrakan mentah dilakukan dengan menggunakan kaedah pengekstrakan cecair-cecair partition dan kromatografi pertukaran ion. Perkadaran yang berbeza asid ferulik sebagai ejen menstabilkan telah ditambah untuk memperbaiki dan meningkatkan ketahanan ke arah penyinaran UV-B dalam tempoh penyimpanan. FA yang ditambahkan kepada pewarna dicampurkan dengan PVA untuk membentuk sistem salutan. Untuk menguji kestabilan warna mentah dan yang ditulenkan dengan antosianin pewarna dan campuran antosianin-PVA diuji kesan penyinaran UV-B, dilakukan analisis warna CIE. Keputusan CIE yang diperolehi dianalisis dari segi nilai L* C * H º a* b* warna koordinat. Perbezaan warna (ΔE) dan ketepuan warna (s) ditentukan untuk menilai perubahan warna visual. Berdasarkan keputusan yang diperolehi, pewarna antosianin dan antosianin-PVA campuran yang tidak dirawat adalah mudah terdegradasi terhadap kesan UV semasa 93 hari pendedahan. Penambahan 2% FA pada pH 3 dapat menambahkan kestabilan warna yang lebih baik. Keputusan CIE juga menunjukkan bahawa perubahan warna antosianin dan antosianin-PVA campuran sangat dipengaruhi oleh kesan perubahan pH.

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LIST OF SYMBOLS AND ABBREVIATIONS

OCCAP Oxford Climate Change Action Plan

FA Ferulic acid

TFA Trifluoroacetic acid

PVA Poly(vinyl) alcohol

UV-B Ultraviolet-B

CIE Commission Internationale del'Eclairage

CHAPTER 1: INTRODUCTION

1.1. Background

The coating industries have been driven toward the production of new products with lower cost, better performance and environmental friendly. The word “coating” describes a dry continuous film that resulted after applying a liquid material onto a substrate surface by a process of choice. A coating is composed of four types of material; resins, pigments, solvents and additives. Coatings may be described as clear, pigmented, metallic, glossy, and by their functions which include corrosion, abrasion and skid resistant, and decoration (Weiss, 1997). The primary purpose of a coating is to protect a substrate from being corroded by the environment. Different substrates may require different types of coatings materials. Materials that adhere to metal may not bond to plastic. Therefore, coatings for metals will have different component of materials from glass, plastics or wood coatings.

The paint and coatings industry has encountered many technology changes. The automotive industry has experienced more than 95% changes in the paint and coating formulations applied to their products. Plastic parts in automobiles are coated to withstand chemical and solvent attacks, over-exposure to ultraviolet light and prevent contact with abrasive materials. Coatings for metal casings and transformers are required for chemical resistance, corrosion, hardness and humidity resistance. Wood coatings require blocking and detergent resistance, sandability, and resistance to grain raising. Calculators, typewriters and analytical instruments require coatings that are strong to chemical and solvent resistance and can adhere to plastics (Gutoff and Cohen, 2006).

1.2. Problem Statement

The environment stress has driven the coating industry to develop coatings that utilize less expensive raw materials (Gutoff and Cohen, 2006). The problem of increasing material costs and consumer demands to keep prices low is evident in the paint and coating industry. Synthetic coatings can be toxic and may cause death or permanent injury (OCCAP). The increasing demand for more environment-friendly and safer coatings has become a major concern. There is a lack of information about materials suitable for making cheap, safe and good performing coatings that will provide desirable appearance, durability and resistance to degradation. Colour is one of the important criteria that represent the coating quality. Natural colourant is preferred for alternative in replacement of synthetic colourant since it is more environmental friendly. Furthermore, according to Castaneda-Ovando et al. (2009), there were progressively studying the natural colourant advantages due to the toxicity effects in human caused by synthetic colourant.

1.3. Objectives of Study

The objectives of the study are:

a) To identify the potential of fruits from *Ixora siamensis* as a new source of anthocyanin natural colourant.

b) To evaluate the suitability of the anthocyanin natural colourant with and without ferulic acid (FA) stabilizing agent when blended with poly(vinyl) alcohol (PVA) to form a coating system.

c) To analyse colour stability of anthocyanin colourant in a coating system as well as the anthocyanin extraction in terms of pH and co-pigmentation effect under UV-B exposure by CIE colour system.

1.4. Scope of Study

The shift in consumer expectations for higher quality and performance with lower cost has led to exploration and development of paints using natural raw materials that can act as alternatives to the synthetic ones. In order to accomplish this target, research is required to obtain safe coating materials that will give good coating characteristics and performance. Colourant is one of the basic ingredients for coating production. This work therefore tries to develop new coating components derived from plant pigments in order to solve the demand in substituting the synthetic colourant. Plant pigments that have been identified for this research is the anthocyanin colourant obtained from fruits of *Ixora siamensis,* locally known as "pokok jejarum". Further study on the suitability of this natural colourant as a raw material in coating system is needed as it is more economical and environmental friendly colourant. Nevertheless, it is well known that natural colourant are less stability compared to the synthetic ones, therefore it is important to enhance the colour stability of natural colourant from fruits of *Ixora siamensis* in a coating system. Poly(vinyl) alcohol (PVA), a synthetic resin will be used as it is a water-soluble polymer and capable of water absorption.

Chapter Two of this dissertation represents the literature review regarding the coating components and materials used in this project. Chapter Three presents the sample

preparations and the techniques used to study the colour characteristics of the samples. Chapter Four displays the results obtained from colour analysis of the crude and purified anthocyanin extraction under UV-B irradiation by using CIE system. Chapter Five represents results for the coloured PVA coatings using crude and purified colourants. Chapter Six discuss results obtain and Chapter Seven concludes the dissertation with some suggestions for further works that may enhance and improve the performance of coating system produced.

CHAPTER 2: LITERATURE REVIEW

2.1. Natural Pigment

Pigments are chemical compounds that absorb light in the visible region. The colour is due to a chromophore that captures light energy and excites an electron from a lower to a higher orbital. The non-absorbed energy is reflected and/or refracted to be captured by the eye. Neural impulses are then generated and transmitted to the brain where they are interpreted as a colour (Hari et al., 1994).

Bauernfeind (1981) classified pigments by their origin. Pigments are classified as natural and synthetic. Natural pigments are produced by living organisms such as plants, animals, fungi, and microorganisms. Synthetic pigments are produced by man. Natural and synthetic pigments are organic compounds. The trend to replace synthetic colourants with natural pigments has been initiated many years ago. The strong demand by consumers on the use of natural products (Jackman and Smith, 1996) is the driving force behind this trend.

In terms of stability, natural pigments are less stable compared to synthetic colourants, but their development and utilization has attracted much attention. Natural pigments from plants have substituted synthetic dyes in the food and pharmaceutical industry as they do not have negative effect on health. Positive consumer response makes it worthwhile to develop alternative sources of colourants. There are varieties of compounds adequate for colourant, such as the water-soluble anthocyanins, betalains, as well as the oil soluble carotenoids and chlorophylls. This dissertation will limit itself to anthocyanins.

2.2. Anthocyanins

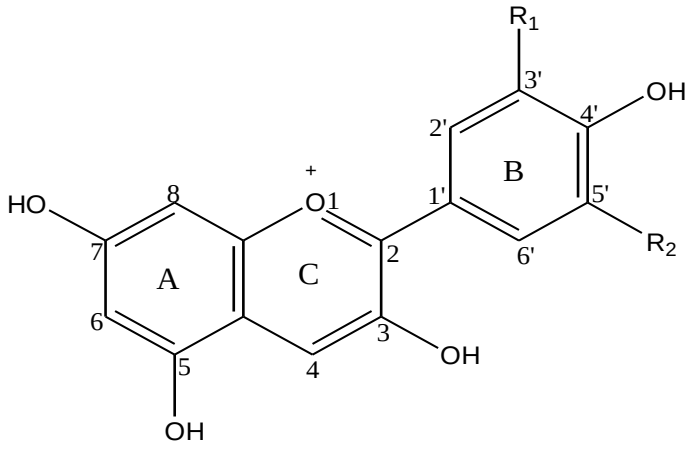
Anthocyanin is one of flavonoid chemical group. It is one of the major groups of natural pigments, after chlorophyll, which is visible to the human eye. Anthocyanins are vacuolar pigments. In flowers, anthocyanins can be found mainly in epidermal cells, and only occasionally in the mesophylls. Anthocyanins absorb light towards the red and are responsible for blue, purple, violet, magenta, red, and orange plant colouration (Jackman and Smith, 1996). The range of colours depends on the degree of anthocyanidin oxygenation and the nature as well as the number of substituents for example sugar moieties added to these chromophores (Schwinn and Davies, 2004). Anthocyanidin is the central chromophore of anthocyanin.

Generally, anthocyanins have a C15 skeleton with a chromane ring bearing a second aromatic ring B in position 2 (C3-C6-C3) and with one or more sugar molecules bonded at different hydroxylated positions of the basic structure. Therefore, anthocyanins are substituted glycosides of salts of phenyl-2-benzopyrilium which known as anthocyanidins (Swain and Bate-Smith, 1962). The type of anthocyanins in plant varies as some ornamental plants (*Dianthus and Petunia*) present only one main type of anthocyanin whereas others (*Rosa, Tulipa, Verbena)* have mixtures. Some fruits contain only one anthocyanin e.g cyanidin as in apple. Delphinidin is another single anthocyanin found in eggplant and pomegranate. Some fruits have two main anthocyanins i.e. cyanidin and peonidin. Examples are cherry sweet and cranberry. Another example of a fruit with several anthocyanins is grape (Delgado-Vargas et al., 2000).

2.2.1. Structure of Anthocyanins

The anthocyanidins are the basic structure of anthocyanins. The anthocyanidins (or aglycons) consist of an aromatic ring [A] bonded to an heterocyclic ring [C] that contains an oxygen atom, which is also bonded by a carbon–carbon bond to a third aromatic ring [B] (Konczak and Zhang, 2004). The structure is illustrated in Figure 2.1. When the anthocyanidins are found in their glycoside form (bonded to a sugar moiety) they are known as anthocyanins. There are more than 540 anthocyanin pigments in nature. The structural variation can be attributed to the glycosidic substitution at the 3' and 5' positions of the aglycons. Possible acylation of sugar residues with organic acids also contribute to the variations. Anthocyanidins are almost glycosylated in the 3-position, though glycosylation in other positions and in more than one position at a time has been encountered. The sugar moiety may be acylated with aliphatic or aromatic acids.

There are 19 types of anthocyanidins, aglycons or chromophores of anthocyanins. Only six of these 19 are major ones. These are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin as seen in Figure 2.2 (Anderson and Francis, 2006). The differences among these aglycons are in the number and position of hydroxyl groups and/or methyl ether groups. Due to this, anthocyanin classification is done in accordance to the number of sugar molecules constituting the molecules. Many anthocyanins have ester bonds between sugars and organic acids. Acylated anthocyanin is an example. In nature the most common acyl groups are coumaric, caffeic, ferulic, synaptic and oxalic (Francis, 1989).



Aglycon	Substitution pattern		λ_{max}(nm)
	R_1	R_2	Visible spectra
Pelargonidin	H	H	494 (orange)
Cyanidin	OH	H	506 (orange-red)
Delphinidin	OH	OH	508 (blue-red)
Peonidin	OCH_3	H	506 (orange-red)
Petunidin	OCH_3	OH	508 (blue-red)
Malvidin	OCH_3	OCH_3	510 (blue-red)

Figure 2.1: Structural and spectral characteristics of the major naturally occurring aglycons (Wrolstad et al., 2005)

2.2.2. The Physical and Chemical Properties of Anthocyanins

The isolated anthocyanins are very unstable and susceptible to degradation (Giusti and Wrolstad, 2003). Anthocyanin stability is affected by factors such as pH, exposure temperature, chemical structure, concentration, light, oxygen, solvents, enzymes, flavonoids, proteins and metallic ions. Stabilisation of anthocyanin has been the main focus of recent studies. This is due to their abundance, beneficial effects and potential as alternative to artificial colourants (Rein, 2005).

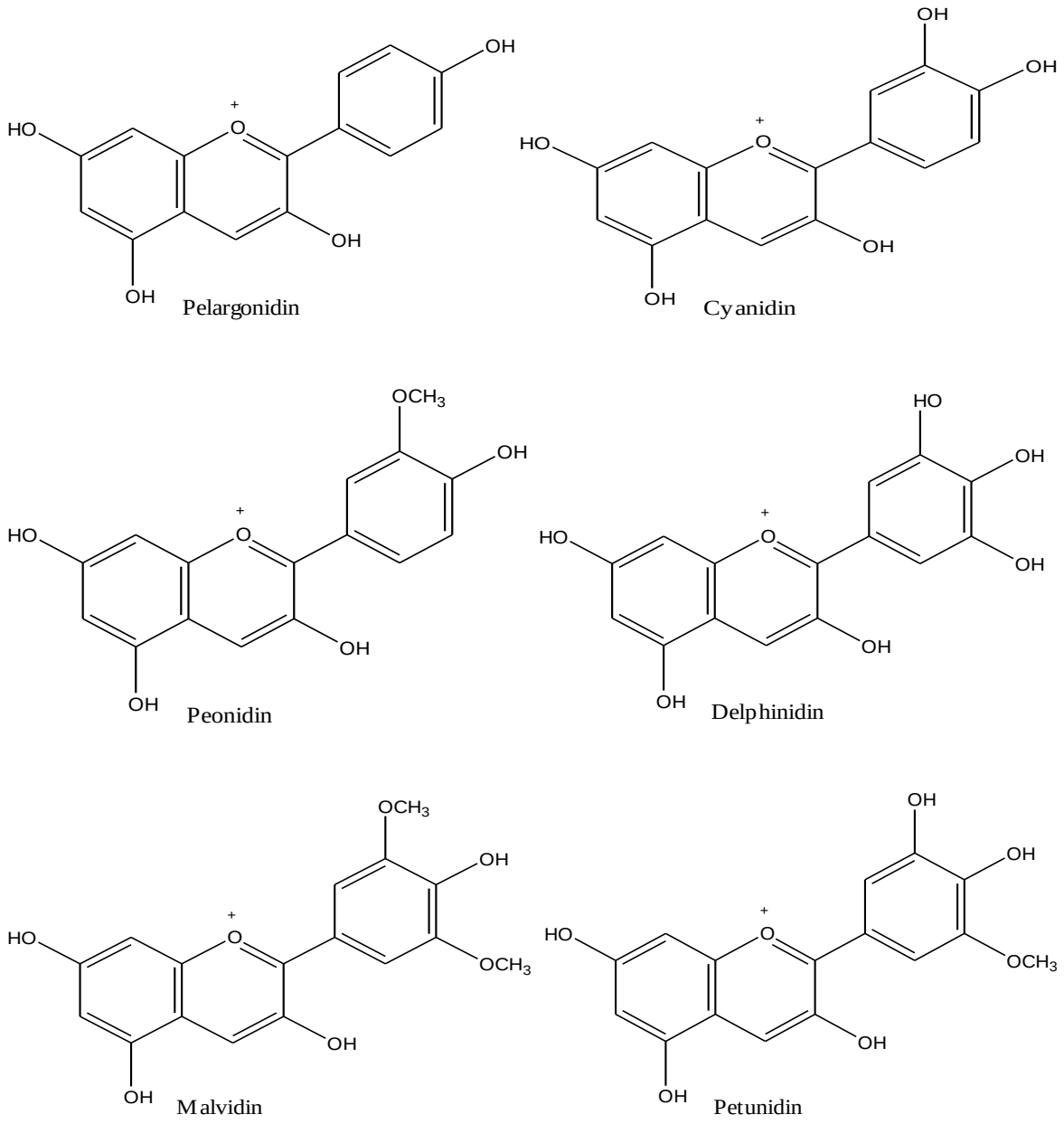


Figure 2.2: The most important natural anthocyanidins (Rein, 2005)

Anthocyanins exist in different chemical forms depending on pH of the solution (Figure 2.3) (Kennedy and Waterhouse, 2000). At pH 1, the flavylium cation (red colour) is predominant and contributes to purple and red colours (Figure 2.3A). At pH between 2 and

4, the quinoidal blue species are predominant (Figure 2.3B–D). At pH between 5 and 6 only two colourless species a carbinol pseudobase (Figure 2.3E) and a chalcone (Figure 2.3F) can be observed. At pH higher than 7, the anthocyanins are degraded depending on their substituent groups (Figure 2.4). At pH values between 4 and 6, four structural forms of anthocyanin coexist: flavylium cation, anhydrous quinoidal base, colourless carbinol base and the pale yellow chalcone. The equilibrium between the quinoidal bases and carbinol occurs via the flavylium cation as shown in Figure 2.3 (D, A and E structures). When the pH is increased, the amount of anhydrous base also increases and under more acidic conditions, the predominant species is the red flavylium ion (Cooper-Driver, 2001). The anthocyanidins stability is influenced by the ring B substituents. The presence of additional hydroxyl or methoxyl groups decreases the aglycon stability in neutral media. Pelargonidin is the most stable anthocyanidin (Fleschhut et al., 2006). In contrast with aglycons, monoglycosides, and mostly, diglycosides derivatives are more stable in neutral pH conditions. This is because the sugar molecules avoid the degradation of instable intermediaries into phenolic acid and aldehyde compounds (Fleschhut et al., 2006), Figure 2.4. Acidity also affects the stability of anthocyanins, which are rather unstable at weakly acidic to alkaline pH (Mortensen, 2006).

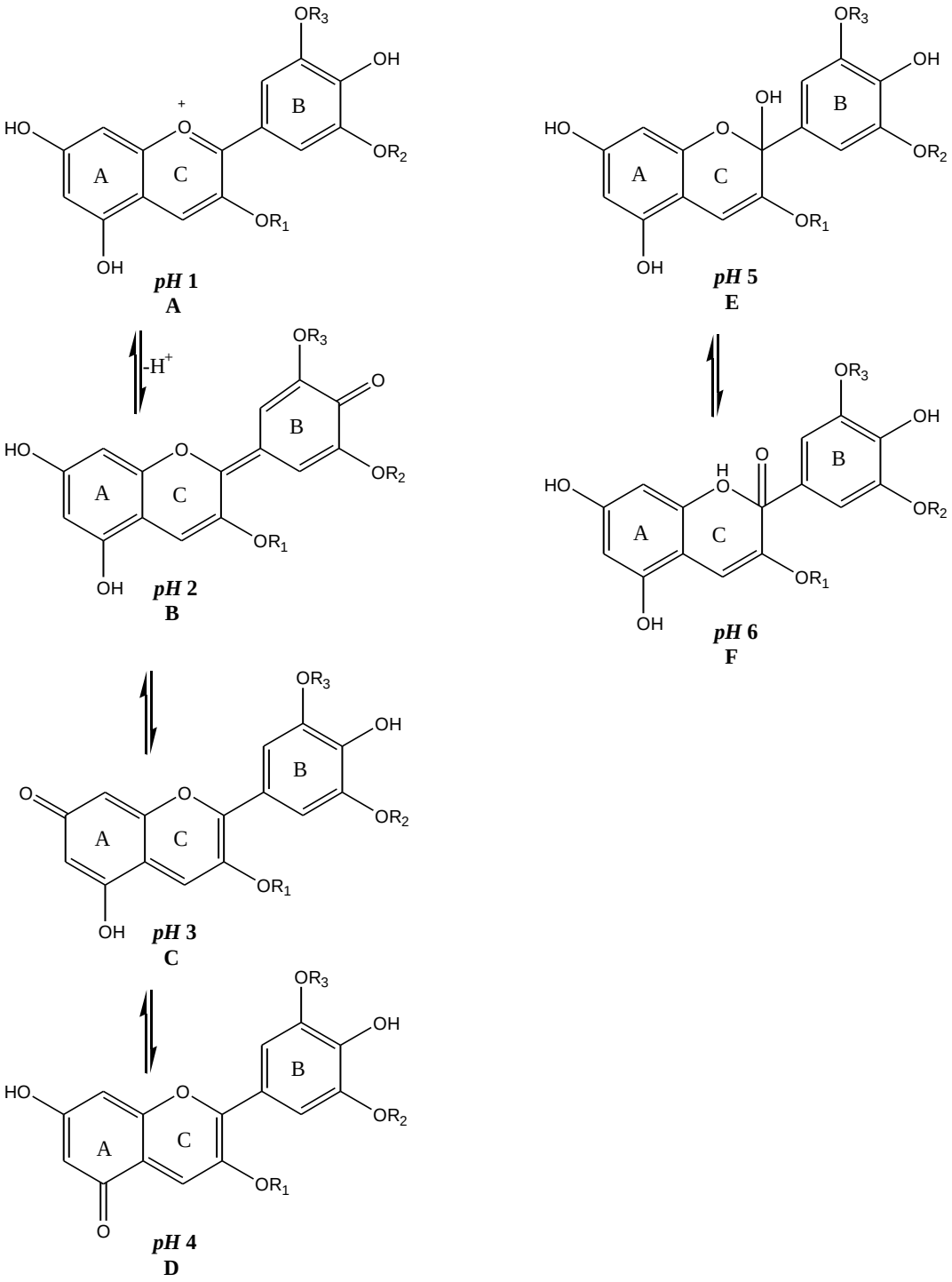


Figure 2.3: Anthocyanins chemical forms depending on pH. Where R1=H or saccharide, R2 AND R3=H or Methyl (Castaneda-Ovando et al., 2009)

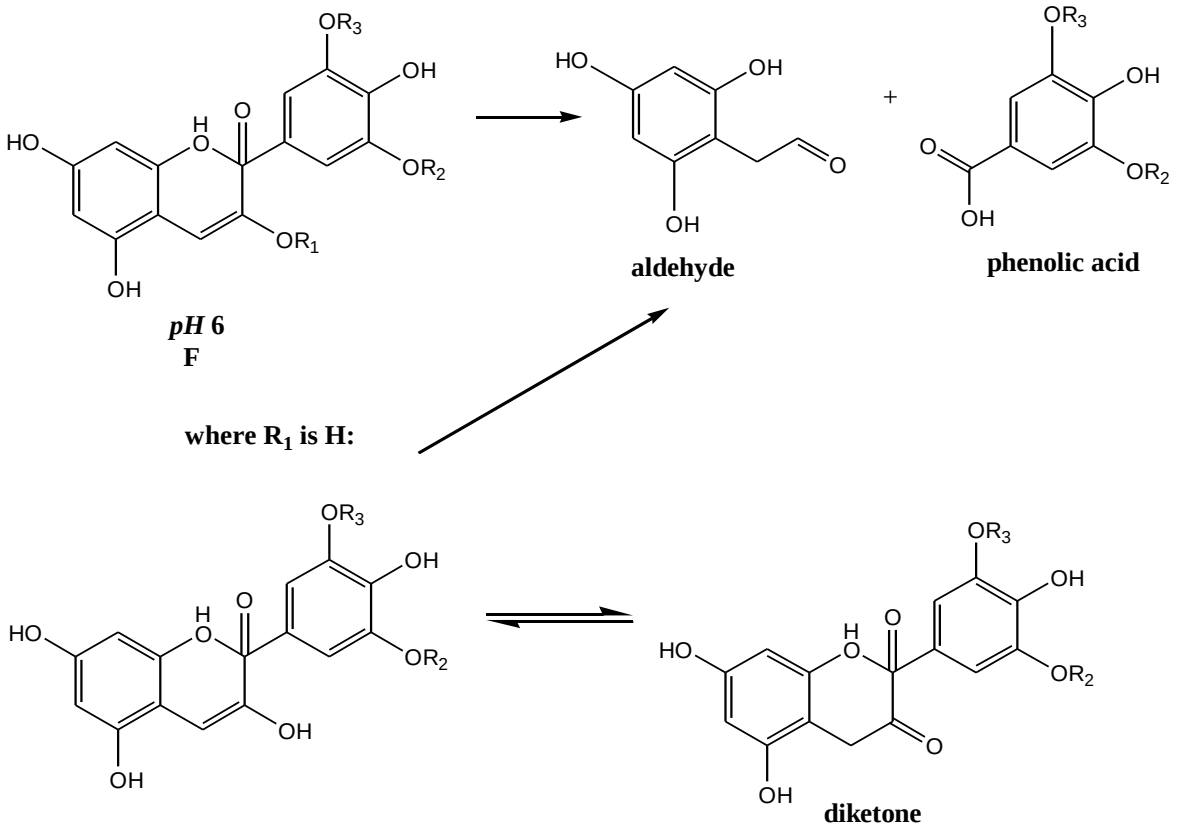


Figure 2.4: Degradation reaction of anthocyanins. Where R1=H or saccharide, R2 AND R3=H or Methyl (Castaneda-Ovando et al., 2009)

The colour of anthocyanins depends on the substitution pattern in the anthocyanin molecule. Increasing the number of hydroxyl groups will deepen the colour to a more bluish shade while increasing the number of methoxyl groups' increases redness (Mortensen, 2006). According to Tanaka et al. (2008), the larger the number of hydroxyl groups on the B-ring, the bluer the colour. O-methylation of anthocyanins has a slight reddening effect. The glycosyl moieties of anthocyanins are commonly modified by aliphatic (malonic, acetic, or succinic) and/or aromatic (hydroxycinnamic or hydroxybenzoic) acyl moieties. Aromatic acylation causes a blue shift and stabilizes anthocyanins. Anthocyanins modified with multiple aromatic acyl moieties (poly-acylated

anthocyanins) (Honda and Saito, 2002) often show a stable blue colour via intermolecular stacking. Aliphatic acylation does not change the colour but increases the stability and solubility (Mortensen, 2006).

Anthocyanins are susceptible to degradative reactions. Stability of this pigment depends on their structure and the composition of the matrix in which they exist (Delgado-Vargas and Paredes-Lopez, 2002). Acylation of sugar residues with cinnamic acids increases pigment stability. Enzymes such as polyphenoloxidase, peroxidase, and glycosidase can greatly affect anthocyanins. Ascorbic acid will accelerate anthocyanin degradation (Skrede et al., 2000). Anthocyanins will condense with other phenolic compounds to form coloured polymeric pigments.

Temperature is one of the factors that lead to the anthocyanin degradation which increases with increasing of temperature especially during storage. The increasing of solid content during heating accelerates the degradation kinetics of anthocyanin as the reacting molecules become closer when a product is concentrated. Thermal degradation is dependent on time and temperature of storage conditions, which increases with increasing storage temperature (Patras, 2010). Increasing temperature at pH from 2 to 4 will induces the loss of glycosyl moieties of anthocyanin due to the hydrolysis of the glycosidic bond. The browning of the anthocyanin is attributed to the chalcone formation which is the first step in thermal degradation of anthocyanins. Continuous consequences are transformation into brown product, especially in the presence of oxygen (Markakis et al., 1982). Light exposure will accelerates and promote pigment destruction. According to Bakhshayeshi et

al. (2006), UV-irradiation leads to anthocyanin destruction in four *Malus* varieties which is also similar to research reported by Palamadis and Markakis (1978) who's discovered the destruction of 50% grapes anthocyanin pigments under light exposure. The UV irradiation degradation during storage can be prevented by presence of co-pigmentation in anthocyanin solution (Abyari et al., 2006; Setareh et al., 2007).

Co-pigmentation is another factor that contributes to colour shade as it may cause a red-shift in the anthocyanin absorption, giving more bluish colour, an increase in absorption (Mortensen, 2006) and stability of anthocyanins. Co-pigmentation of anthocyanins with other compounds is the main mechanism of colour stabilisation in plants (Davies and Mazza, 1993; Mazza and Brouillard, 1990). Co-pigments rich in p-electrons are able to associate with electron poor flavylium ions. This association gives protection for the water nucleophilic attack in position 2 of the flavylium and for peroxides and sulphur dioxide in the position 4. Co-pigments are generally colourless. Co-pigments interact with anthocyanin to produce a hyperchromic effect and a bathochromic shift in the UV-visible region of the absorption spectrum. Co-pigments can be flavonoids, alkaloids, amino acids, organic acids, nucleotides, polysaccharides, metals or another anthocyanin.

There are several ways that anthocyanin-co-pigment interaction can be carried out depending on the interacting species. If the co-pigment is other anthocyanin, a self-association or an intramolecular co-pigmentation is formed. When the interaction is with a metal, a complexation is said to occur. In the case of co-pigments with free electron pairs, an intermolecular co-pigmentation takes place. In the most complex case, the co-pigment

can be an aglycon, sugar, co-pigment and protons all at the same time. For phenolic compound co-pigment, the interaction is transitory because of the lack of chemical bonds. Intermolecular stacking by self-association or with flavones or flavonols stabilizes anthocyanins and causes a bathochromic shift (blueing and intensifying of colour) (Tanaka et al., 2008). Studies about colour stability in plants suggest that the blue colours are due to a complexation between anthocyanins and some metals such as Al, Fe, Cu and Sn or Mg and Mo.

The total resulting co-pigmentation is based in two effects (Dangles and Brouillard, 1992), which are the formation of the π-π complex which causes changes in the spectral properties of the molecules in the flavylium ion, lead to increasing the absorption intensity (hyperchromic effect) and its wavelength (bathochromic shift) and the stabilisation of the flavylium form by the π complex displaces the equilibrium in such way that the red colour increases. Therefore, the magnitude of the co-pigmentation effect is pH dependent, because at low pH values, all the anthocyanin molecules are in flavylium form, and at high pH values, the anthocyanin is in its carbinol pseudobase form, which is colourless. The co-pigmentation effect is evident under weakly acid conditions (pH 4–6) where anthocyanins exist in its colourless forms.

Colour is generally evaluated by spectrophotometry. Flavonoids show high absorbance between 250 and 270 nm (UV region). Anthocyanins have intense absorption between 520 and 560 nm (Delgado-Vargas et al., 2000). Different aglycons have different $\boldsymbol{\lambda}_{\text{vis-max}}$ ranging from 520 nm for pelargonidin to 546 nm for delphinidin. From the shape of the

spectrum information on the number and position of glycosidic and cinnamic acid can be obtained. The absorbance ratio at 440 nm to $\lambda_{vis\text{-}max}$ is almost twice for anthocyanins with glycosidic substitutions in position 3 compared to those with substitutions in positions 3 and 5 or position 5 only. The presence of cinnamic acid acylation can be confirmed from the absorption band between 310 and 360 nm ranges. The absorbance ratio in this range to $\lambda_{vis\text{-}max}$ will give an estimation of the number of acylating groups. UV-visible spectroscopy can also detect glycosylation on B-ring, because the spectrum of the glycosylated B-ring will be hypochromic shifted compared to the unglycosylated B-ring (Harborne and William, 1976).

In most fruits and vegetables, pigments are located in cells near the surface (Jackman et al., 1987). Like flavonoids, anthocyanins have aromatic rings that consist of polar substituent groups (hydroxyl, carboxyl, and methoxyl) and glycosyl residues that altogether produce a polar molecule (Delgado-Vargas et al., 2000). Pigment extraction involves acidic solvents to denature the cell tissue membranes and simultaneously dissolve the pigments. Although the acid tends to stabilize the pigments (anthocyanins), it may also change their native form in the tissue by breaking associations with metals, co-pigments, or other factors. Concentration procedures may also cause acid hydrolysis of labile acyl and sugar residues. Extraction using acidified methanol produces a significantly higher amount of anthocyanins compared to the use of aqueous acetone. The extraction with acidified methanol is twice more efficient than extraction with aqueous acetone. To minimize pigment decomposition, weaker organic acids (e.g., formic, acetic, citric, or tartaric acids) or smaller amounts (0.5% to 3%) of more volatile acids (e.g., trifluoroacetic acid) can be used. These acids can then

be removed during pigment concentration (Jackman and Smith, 1996). If the extracted material is too dilute the methanol must be evaporated in vacuum at 30° to 40°C. Anthocyanins are heat sensitive. The anthocyanin-containing extracts are then purified (Jackman and Smith, 1996).

2.2.3. Application of Anthocyanins

There are now increasing interest to use anthocyanin in pharmacology. Anthocyanins play a role in reduction of coronary heart disease (Bridle and Timberlake, 1997). The consumption of wine flavonoids has been correlated with low incidences of coronary heart diseases (French paradox). Anthocyanins can also increase visual acuity. Apart from that, anthocyanins also have antioxidant (Wang et al., 1997) and anticancer properties (Kamei et al., 1995). The easily oxidised compounds are the best antioxidants. This is important because these compounds can donate electrons. Anthocyanins have potential application in the food industry as safe and effective food colourants (Strack and Wray, 1993).

2.3. Compositions of Coatings

The industries encompassing Paints, Inks, Coatings and Adhesives (PICA) are closely interrelated. They are based on the same raw material components. Coatings are used for the protection of objects from destructive external attacks and for decoration. The basics coating components are vehicle (consists of binder and solvent system), and pigments. In addition modern coatings may contain additives such as dispersing agents, wetting agents, viscosity controlling agents, anti-settling agents, anti-skinning agents, antioxidants,

antifoaming agents, adhesion promoters, desiccants, driers, biocides, light stabilizers (Tracton, 2006).

2.3.1. Binders

The binder is the key component of the coating. It includes natural and synthetic resins, vegetable oils and natural fats. These are the film forming materials in coatings. The main function of binders is to hold the different components together and to adhere the film to the applied surface. The basic properties of coatings such as drying, gloss, hardness, durability, flexibility, abrasion resistance, impact resistance, chemical resistances, adhesion, are governed by the binder system used in coating formation (Tracton, 2006). Resins are generally solid, sticky materials. Different types of resins used will result in different coating characteristics.

2.3.2. Natural Resins

Natural resins from plants are lipid-soluble mixtures of volatile and nonvolatile terpenoid and/or phenolic secondary compounds. Natural terpenoid resins have been used as adhesives, hydro-repellents and coating and sealing agents (Modugno et al., 2006). Due to their antitoxic and antioxidant properties, they were also added to wine. Natural resins have high viscosity, semisolids or solid and are insoluble in water (Colombini and Modugno, 2009).

Terpenoids are made up of isoprene units, a 5-carbon compound. Triterpenoids are 30-carbon substances with ring systems and functional groups. Triterpenoid resins mainly

consist of triterpenoids and a proportion of polymeric material (van Der Doelen et al., 1998). Triterpenoid resins have excellent adhesive properties, good solubility in oil of turpentine, and varnishes from triterpenoid resins are yellow to a lesser extent compared to varnishes made with diterpenoid resins (Colombini et al., 2000).

Dammar is an example of triterpenoid resin. It is a yellowish, easily brittle resin with clean edges. “Damar” is the Malay word for resin. The resin has good optical properties and low acidity. It is derived from various species of the genus *Hopea* and *Shorea* from the *Dipterocarpaceae* family. The dammar mainly comprises tetracyclic triterpenoids with minor amounts of pentacyclic triterpenoids. It also contains a polymeric fraction, polycadinene or β-resene as in Figure 2.5. Dammar resin triterpenoids undergo oxidation with ageing (van Der Doelen et al., 1998).

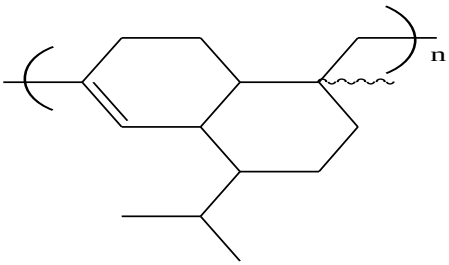


Figure 2.5: Molecular structure of polycadinene (van Der Doelen et al., 1998)

Ageing and exposure to light profoundly change the composition of a resin. As a result of light exposure oxidized species, high molecular weight material (due to condensation) and light-induced radical reactions are produced. These transformations depend on exposure time to light, light wavelength and thickness of the resin layer (van Der Doelen et al.,

1998). However, their main problem is that they deteriorate rather quickly. The pronounced yellowing of thick varnish layers can significantly change the appearance of a coating. Brittle cracking degrades optical properties and protective function of the varnishes. The oxidized terpenoid films are soluble only in polar solvents, which may also affect the coating layer (Colombini et al., 2000). Due to this, synthetic resins were chosen as raw material for coating production.

2.3.3. Synthetic Resins

Synthetic resins are viscous materials capable of hardening with similar properties to natural resins. They are manufactured by esterification or soaping of organic compounds. Epoxy resin is manufactured through polyaddition or polycondensation reactions. Epoxy resin is twice stronger than concrete. Epoxy resin show good properties in resistant to acid, alkali and organic solvents but they have a tendency to yellow with ageing. Polyurethane on the other hand, shows tendency to yellow, depolymerise as well as toxicity of isocyanates (Colombini and Modugno, 2009).

Synthetic coatings and varnishes have for the most part replaced natural paint varnishes. The modern polymers used for this purpose include ketonic, acrylic and metacrylic resins. The polymers have good refractive index, resistance to yellowing and high glass transition temperature. Low glass transition temperatures can lead to loss of transparency and gloss. One of the most widely used polymers is acrylic and ethyl-methyl methacrylate copolymer (Colombini and Modugno, 2009). In this dissertation, poly(vinyl) alcohol (PVA), synthetic resin was used as a binder in a coating system.

2.3.4. Poly(vinyl) Alcohol (PVA)

Poly(vinyl) alcohol (PVA) is a widely used synthetic biomaterial that is non-toxic, water-soluble, biocompatible, and biodegradable with excellent mechanical properties (Paradossi et al., 2003). It is widely used in adhesives, coatings, sealants, coatings, textiles, plastics etc. PVA is commercially produced from poly(vinyl) acetate. The physical characteristics and its specific functional uses depend on the degree of polymerization and the degree of hydrolysis (Saxena, 2004).

2.3.5. Structures of PVA

The primary raw material used in the manufacture of poly(vinyl) alcohol is the vinyl acetate monomer. PVA is manufactured through polymerization addition, and the polymer is built on a carbon-chain backbone, with a hydroxyl group (–OH) on every other carbon (Figure 2.6(a)). This is followed by partial hydrolysis (Figure 2.6(b)). The process of hydrolysis is based on the partial replacement of ester group in vinyl acetate with the hydroxyl group (-OH), and is completed in the presence of aqueous sodium hydroxide, following gradual addition of the aqueous saponification agent. PVA is precipitated, washed and dried. The degree of hydrolysis is determined by the time point at which the saponification reaction is stopped (Saxena, 2004).

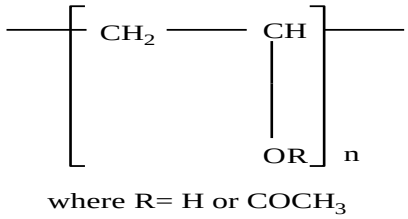


Figure 2.6: Structure of PVA (partially hydrolyzed) (Saxena, 2004)

2.3.6. The Physical and Chemical Properties of PVA

The physicochemical properties of polymers are dependent on the type of physical bonds across the polymer chains as well as the type of chemical bonds along the polymer chains. In the case of polymers with strong polar intermolecular interactions such as PVA the molecular aggregation has a significant effect on the physical properties, which is greatly affected by conformation of polymer molecules (Shafee and Naguib, 2003).

PVA are widely used in industry because of its high capability to absorb water (Shafee and Naguib, 2003; Li et al., 2005). The abundance of hydroxyl group (–OH) along each polymer strand allows it to form hydrogen bonds, making poly(vinyl) alcohol very soluble in water. Due to this, PVA is characterized by strong hydrophilic and hydrogen bonding character. Another important feature of PVA is biodegradability. PVA possess unique characteristics such as excellent film-forming ability, emulsifying, and adhesive properties. PVA is also resistant to oil and grease as well as odorless and nontoxic. It has high tensile strength and flexibility, and performed high oxygen and aroma barrier properties. However these properties are dependent on humidity, in other words, with higher humidity more water is absorbed. The water acts as a plasticiser, results in reducing of its tensile strength, but increase its elongation and tear strength. PVA is fully degradable (Zhu and Qian, 2007).

2.3.7. Application of PVA

PVA has found applications in the food industry as a binding and coating agent. It is a film coating agent especially in applications where moisture barrier are required and is a component in food supplement tablet coating formulation. The viscosity of PVA allows

application as coatings for tablets, capsules and objects with high solid contents. The food products in which PVA is intended to be used should have neutral pH and stored at temperatures that would not result in PVA breakdown (Saxena, 2004). PVA can form polymeric blends with starch and used as a new material in packaging. PVA blended with collagen is used in biomedical applications. Meanwhile, PVA blends with poly (acrylic acid) can be used as polymer electrolytes (Yang et al., 2008).

2.4. Pigment

Pigment is the substance that gives colour. Pigments can be classified into different classes: inorganic, organic, organometallic and metallic pigments. Colour is produced when pigment and binder is dissolved in a solvent. The quality of coatings depends on the particle size of the pigment (Hradil et al., 2003). Particle size affects natural colour strength, transparency or opacity, exterior durability, solvent resistance and other properties. Natural pigment from anthocyanin pigment which is water soluble, have been chosen as a source of pigment in production of coating. The anthocyanin pigments were obtained from plant species of *Ixora siamensis.*

In this dissertation, fruits from *Ixora siamensis* were chosen as the source of the anthocyanin pigment. *Ixora siamensis* is locally known as “pokok jejarum” and it is a genus of the *Rubiaceae* family, which comprises about 300-400 species, (Fosberg and Sachet, 1989) with the greatest diversity in Asia, particularly Malaysia. The genus is well known for its cultivated ornamentals (Figure 2.7), with their beautiful clusters of flowers in different shades of red, pink or yellow (De Block, 1998). *Ixora siamensis* are short bushy

plants and are sometimes trimmed into hedges. *Ixora siamensis* are short bushy plants and are sometimes trimmed into hedges. The fruits (Figure 2.8) are eaten and the flowers are used as a flavouring agent. However, due to the commercialisation of this species as ornamental plants, the utilisation of its fruit has been overlooked. The potential of the fruit as a source of natural colourants has yet to be explored.

(a)

(b)

Figure 2.7: (a) Flower of *Ixora siamensis* and (b) Fruits of *Ixora siamensis*

2.5. Solvents

The function of solvent is to reduce viscosity of the binder to ease the processing and wetting of pigment. Thus, the less solvent in the coating, the higher the quality and better the coverage. In the early 1970s, more than 90 % of the paint and coatings sold worldwide are low solid (5-20 % by weight) solvent borne coatings. The solvents are volatile organic compounds (VOC) that play a major role in global warming and toxic photochemical ozone harmful to plants, animals as well as man (Weiss, 1997). Thus, there is a need to develop

coatings which contain less VOC. Solvents that are generally used in coatings technology are aromatic and aliphatic hydrocarbons, esters of acetic acid, glycol ethers, alcohols, and ketones. Most present day coatings, as well as water-borne coatings, still contain at least some volatile organic solvents. In replacement to toxic volatile solvents, distilled water is used as solvent in water-borne coatings. The main advantages of water-borne coatings are that they do not generate cleanup solvents, do not show distinct stroke marks and does not permeate the room with the strong smell that other coating does.

2.6. Additives

Additives are materials that constitute a small percentage of the coatings that are included to modify coating properties such as durability, appearance, lifespan of countless products, coating rheology and pigment wetting. Otherwise, it also can improve properties of the cured film against corrosion resistance and UV durability (Florio and Miller, 2004). In this work, ferulic acid (FA) has been used to modify properties of the coating system.

Pure FA is a yellowish powder (Figure 2.9). FA belongs to the family of hydroxycinnamic acid ($C_{10}H_{10}O_4$). It is a substance found in the seeds and leaves of most plants. FA has antioxidant properties that make it an important anti-aging supplement. Due to its phenolic nucleus and an extended side chain conjugation, it forms a resonance stabilized phenoxy radical, potentiates it ability to neutralize free radicals (superoxide, nitric oxide and hydroxyl radicals) which can cause oxidative damage of cell membranes and DNA. FA helps to prevent damage caused by ultraviolet light (UV), as itself UV exposure led to an increase in antioxidant potency of FA. Other uses include applications in controlling

diabetes, cardiovascular disease, cancer, neuroprotection, bone degeneration, menopause, and immunity as well as in cosmecticeuticals applications (Sahelian, 2003).

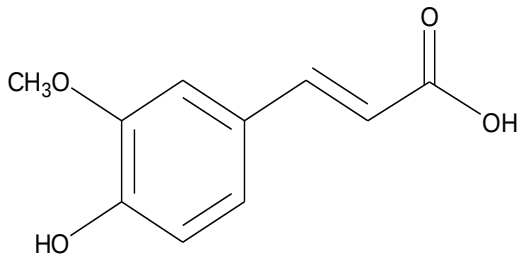


Figure 2.8: Structure of FA

CHAPTER 3: METHODOLOGY

3.1. Materials

This chapter focuses on preparation method of the extraction and purification of natural anthocyanin colourant as well as the formulation of coating system made from the blend of anthocyanin extract with synthetic resin, poly(vinyl) alcohol (PVA). The fruits of *Ixora siamensis* were chosen as the source of natural colourant which were collected in Banting, Selangor. The fruits were sealed in polyethylene bags, covered with aluminium foil and kept in a freezer (-18 °C) before analysis to maintain the quality of extraction. The anthocyanin colourant was used in both crude (unpurified) and purified forms to compare the performance of the colourant. Solvents used for anthocyanin extraction were methanol and trifluoroacetic acid (TFA) procured from Sigma Aldrich. Distilled water was used for preparation of water-borne coating made from PVA resin and anthocyanin extract. PVA supplied by Sigma Aldrich has relative molecular weight (MW) 46000. Ferulic acid (Sigma) was used to improve stability of the samples.

3.2. Crude Anthocyanin Colourant

The fruits of *Ixora siamensis* (300 g) were ground using mortar and pestle. Anthocyanins were extracted using methanol containing 0.5% trifluoracetic acid (TFA) (v/v). TFA was added to improve the extraction yield because direct methanolic extraction provided very poor yield. The extraction was performed in a cubicle containing ice to avoid hydrolysis of acyl groups in the anthocyanin structure and degradation. The crude extract or unpurified anthocyanin colourant was centrifuged at 10,000 rpm for 15 minutes. The supernatant liquid was then filtered using Whatman No 1 filter paper to remove any traces of residues

and the methanol content was fully removed by evaporation under reduced pressure at low temperatures (<30ºC). Pictorially, the beginning and end of the procedure can be presented as in Figure 3.1.

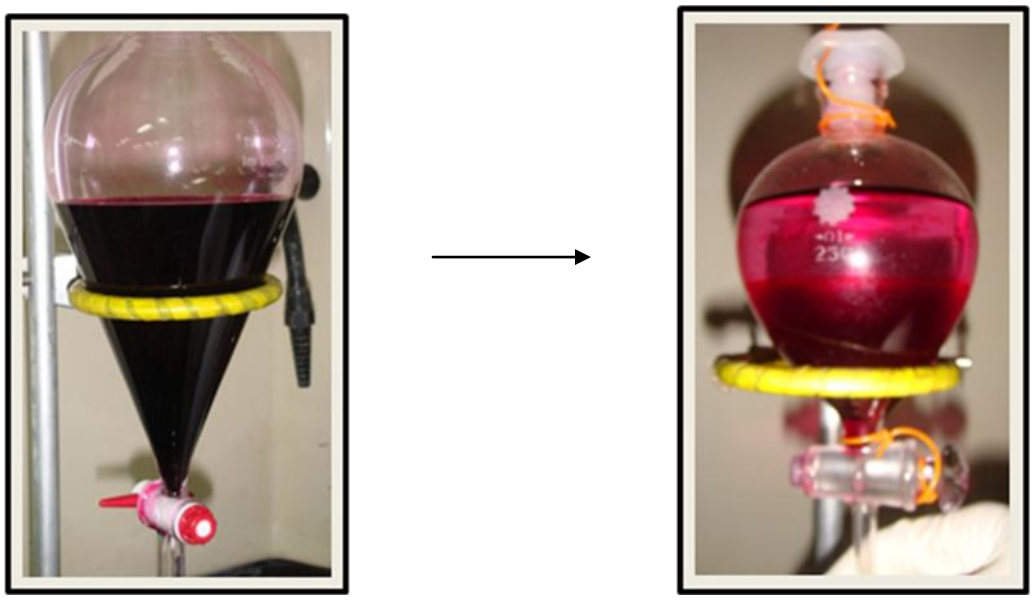
The anthocyanin colourant from fruits of *Ixora siamensis* were extracted with acidified methanol containing 0.5% trifluoroacetic acid (TFA) (v/v)

After extraction, the extract was centrifuged at 10,000 rpm for 15 minutes and the methanol was removed by evaporation

Figure 3.1: Crude anthocyanin extraction

3.3. Purification of Anthocyanin Colourant

After extracting anthocyanin using methanol containing 0.5% trifluoroacetic acid (TFA) (v/v), the solution were evaporated in a vacuum chamber until only 50% of the initial methanol volume remained. The concentrated solution was then washed several times with ethyl acetate to remove chlorophylls, stilbenoids, flavonoids and other non-polar compounds from the mixture. The aqueous solution were again evaporated in a vacuum chamber until 50% of the initial methanol volume remained. Pictorially, part of the purification procedure is shown in Figure 3.2.



After evaporation, the samples were washed with ethyl acetate using separating funnel for separation of polar and non-polar compounds

Through the separation process, the portion at lower part which contain polar molecules were collected before continuing further process

Figure 3.2: Anthocyanin colourant purification

After this treatment, the polar extract will contain anthocyanin and other water soluble impurities like free sugars and aliphatic acids. These impurities were removed using Amberlite XAD-7HP column chromatography. The polar extracts were added into the column. The column was then washed with distilled water (pH 7) several times to remove the free sugar and aliphatic acid. This solution was thrown away. After this step, the column was washed with acidified methanol and the filtrate collected. To further remove adsorbed anthocyanin from the amberlite column, the column was also washed with absolute methanol. After the filtrate from the column has become clear, indicating all the purified anthocyanin has been collected, the column was washed again with 50% acidified methanol containing 0.5% (v/v) TFA followed with distilled water (pH 7) before use in

purifying another batch of unpurified anthocyanin. The collected solutions were evaporated for two days under reduced pressure in a vacuum chamber at low temperatures (<30ºC) to concentrate and fully remove the methanol content. The flowchart in Figure 3.3 summarizes the natural colourant purification.

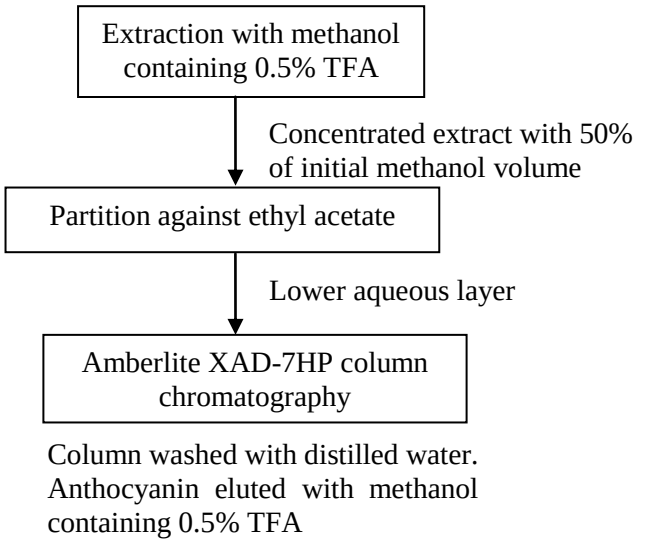


Figure 3.3: Summary of anthocyanin colourant purification

3.4. Sample preparation for colour analysis

3.4.1. Anthocyanin colourant from fruits of *Ixora siamensis*

Colour analysis stability of natural colourants can be improved by adjusting their pH and adding stabilizing agents such as citric, tartaric, gallic and ferulic acid. In this study, ferulic acid (FA) was used as stabilizing agent. To the original extraction of crude and purified anthocyanin solutions, different amounts of FA (1, 2, 3, 4 and 5 vol %) were then added. The colour analysis of the crude and purified anthocyanins with and without FA was determined using Commission Internationale de l'Eclairage (CIE) system. From this analysis, the most stable colour in terms of FA content was obtained. Other than stabilizing

agents, pH also play important role in colour stability. To another set of the original crude and purified anthocyanin extracts, the pH of the solutions was varied by adding different amounts of 1M HCl and 1M NaOH to adjust the original pH to 1, 3, 5, 7, 9 and 11. This is done to determine the type of colour that can be obtained. With this, all samples at different pH as well as the original pH were subjected to colour analysis study. The most stable solution in terms of pH was obtained. From these results, the composition exhibiting the most stable colour in terms of FA content and pH was determined. Finally, to the original crude and purified anthocyanin solutions the best FA amount was added to another set of original extract and the pH was again adjusted to 1, 3, 5, 7, 9 and 11 using the same method as before. The colour stability of these samples as well as the original solution was again determined using CIE colour analysis study. All samples were prepared in triplicate.

3.4.2. Anthocyanin-PVA blends from fruits of *Ixora siamensis*

Anthocyanin colourant both for crude and purified were blend with 30% poly(vinyl) alcohol (PVA) respectively, in order to form a coating system. Similar samples preparations as in anthocyanin colourant were repeated for crude and purified anthocyanin-PVA blends which were being applied onto glass slides. The crude and purified anthocyanin-PVA blends samples were kept overnight in the dark for curing process.

3.5. CIE colour analysis study

3.5.1. Colour analysis measurement

The anthocyanin colourants crude and purified were put into transparent glass bottle before being placed under UV-B lamp. The crude and purified anthocyanins blended with PVA

that have been coated on glass also being placed under UV-B lamp. 10 mm optical path quartz cuvettes were used for colour extraction analysis of liquid samples. All samples prepared were exposed to UV-B irradiation of intensity 17.55 lux. The UV-B lamp emits radiation of wavelength 312 nm. The distance between the samples and the light source was fixed at 5 cm. Spectral curves were recorded with a Shimadzu 3101 spectrophotometer (regular transmission, from 380 to 780 nm with a 2 nm bandwith) and analysed using colour analysis software (CIE system).

3.5.2. Colourimetric calculation

Colour analysis was performed on colourimetric calculation using CIE system. According to Birse (2007), the use of absorbance profiles and $\boldsymbol{\lambda}_{max}$ value can be difficult for an inexperienced person to understand. This is because the $\boldsymbol{\lambda}_{max}$ value requires an understanding of absorbance values, wavelengths and colours before making an adequate judgement. The varying degrees of absorbance at different wavelengths may imply that the colour observed is not simply blue or green. For example, a spectrum may show high absorbance in the red, but different proportions of yellow, green and violet regions are also absorbed. The colour observed may not be simply red, but red-brown. CIELab colour software is a more appropriate measurement to determine the colour of natural colourant, and is precisely described using CIELab colour coordinates.

From the transmittance spectrum curves, the X, Y and Z tristimulus values were computerized for a couple of CIE illuminant/observer conditions: D65 (diffuse daylight type) and A (tungsten light), both for the ‘suplementary” or 2°, CIE observer, according to

the weighted ordinate method. L*, a* and b* were calculated from the tristimulus value (X, Y, Z) which serve as the backbone of all colour mathematical models. The location of colour, in the CIELAB colour space, is defined by a three dimensional cartesian coordinate system. Along the vertical axis, L* is a measure of lightness from completely opaque (0) to completely white (100). Simply, the L* value can be used to describe the lightness of the colour. The hue circle, used to describe the colour in the horizontal plane where a* is a measure of redness (or –a* of greenness and b* is a measure of yellowness (or –b* of blueness) (Fig. 3.4) (Birse, 2007). On the chromaticity circle in Figure 3.4, hue angle values are stepped counterclockwise from h_{ab} O°-360° (magenta-red) across a continuously fading hue circle, the other remarkable values of which are 90° (yellow), 180° (bluish-green) and 270° (blue) (Gonnet, 1998).

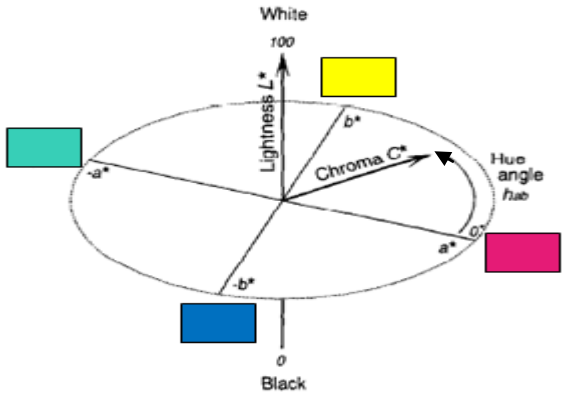


Figure 3.4: CIELab colour space describing colour in three dimensions, luminance, L*, the red-green axis, a*, and the blue-yellow axis, b* (Gonnet, 1998)

The chromaticity (C*) corresponds to the brightness of the colour and is generally observed by how intense the colour is. The chromaticity (Equation 3.1) is derived from a* and b* coordinates, and is calculated using Pythagoras' theorem. The hue angle (H°) is used to

describe the colour in the horizontal plane where a* is a measure of redness (or –a* of greenness and b* is a measure of yellowness (or –b* of blueness). Hue angle (Equation 3.2), is calculated from a* and b* values using trigonometric ratios. Both chromaticity and hue angle are calculated based in Figure 3.5 (Birse, 2007). Other additional values derived from CIE colour coordinates are total colour difference, (ΔE) (Equation 3.3) and saturation (s) (Equation 3.4). Total colour difference (ΔE) is a combination of the changes of three components (chromaticity, hue and lightness) while saturation (s) is the colourfulness of an area visualized by an observer which is determined as the proportion of chromaticity to lightness (Birse, 2007).

$$\mathbf{C^*} = \sqrt{a^{*2} + b^{*2}} \qquad \textbf{(3.1)}$$

$$\mathbf{H^\circ} = \tan^{-1}(a^* | b^*) \qquad \textbf{(3.2)}$$

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad \textbf{(3.3)}$$

$$S = C^*/L^* \qquad \textbf{(3.4)}$$

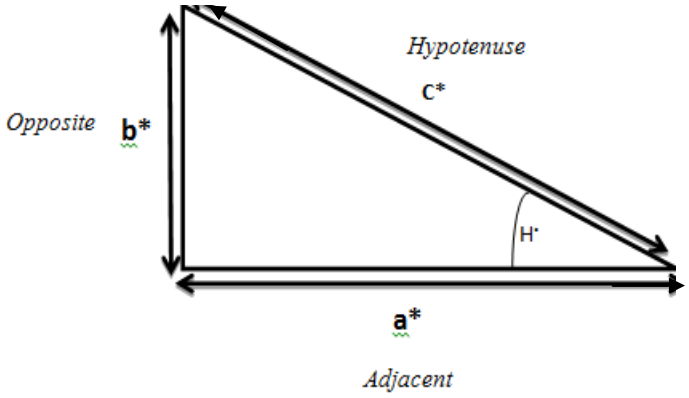


Figure 3.5: Trigonometric relationship involving the known sides a* and b* used to derive the chromaticity, C* and hue angle, H◦ respectively (Birse, 2007)

3.6. Experimental design and statistical analysis

A completely randomized design with three replications was used. Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences). Differences between means were tested using analysis of variance (ANOVA) with significant level of $P<0.05$ in order to find whether there is a relationship between amount of FA added and pH variation according to Duncan test.

CHAPTER 4: EXPERIMENTAL RESULTS ON COLOUR ANALYSIS OF LIQUID ANTHOCYANIN COLOURANT

4.1. Introduction

Colour is one of the most important attributes of product appearance that defines the quality of the products and has a decisive influence on acceptance or rejection by consumers. Thus, it is important to maintain colour appearance during UV exposure. This chapter focuses on the colour measurement analysis studies of the crude and purified anthocyanin colourant that were exposed to the fixed 17.55 lux intensity of UV-B irradiation. The effect of pH and FA co-pigmentation on the colour for all samples before and after exposure to UV-B irradiation during the three month of exposure period were observed and investigated.

4.2. Colour analysis on crude anthocyanin colourant from *Ixora siamensis*

4.2.1. Effect of ferulic acid (FA) addition on visual colour variation

Figure 4.1 presents the colour parameters CIE L* variables of crude anthocyanin colourant from *Ixora* added with different percentages (from 1% to 5%) of ferulic acid (FA). Initially, lightness percentage (L*) of crude anthocyanin colourant observed decrease from sample without presence of FA (65.099 ± 0.020) until sample with presence of 2% FA (46.815 ± 0.007). However, the L* values increased when the percentage of FA increased being (47.104 ± 0.011) for sample with 3% FA and (63.891 ± 0.010) when FA added was 5%. Furthermore, during exposure the L* parameter values for crude anthocyanin without addition of FA increase continually from zero time of exposure (65.099 ± 0.020) until the third month of exposure (77.662 ± 0.018). In contrast, the lightness percentages for crude anthocyanin with addition of FA decreased (to darker colour) from zero time of exposure

until second month of exposure before increase at the third month of exposure. A slight decrease in L* over two months of exposure was exhibited by the crude anthocyanin colourant with addition of 5% FA, the initial L* which was (63.891 ± 0.010) and decreased to (63.132 ± 0.012), followed by the colourant added with 4% FA the L* of which was (61.706 ± 0.012) that decreased to (60.167 ± 0.009). The highest decrease in L* (darker colour) was experienced by crude anthocyanin colourant with addition of 2% FA which decreased from (46.815 ± 0.007) to (35.021 ± 0.007). After three month of exposure, the crude anthocyanin colourant without addition of FA exhibited the highest L* value of (77.662 ± 0.018), while the lowest L* was exhibited by sample with addition of 2% FA (48.923 ± 0.008).

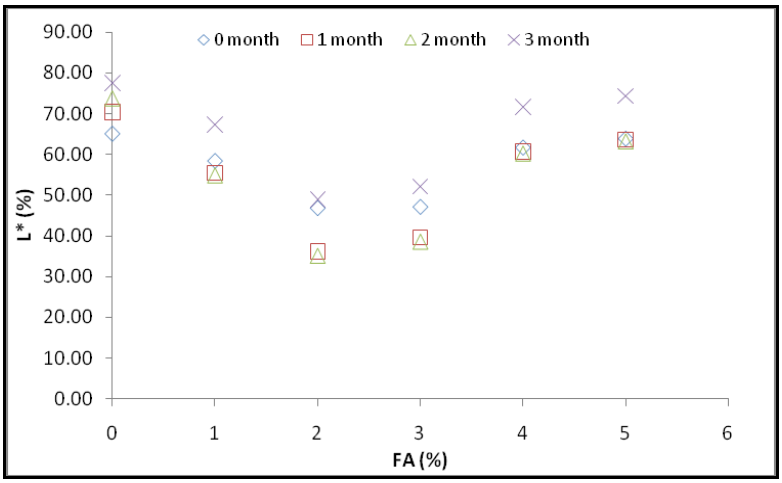


Figure 4.1: Relationship between percentage of FA and L* values (%) for crude colourant *Ixora siamensis* during three month of exposure

The chromaticity (C*) values in the beginning and at the end of exposure was shown in Figure 4.2. The C* values of the crude anthocyanin colourant at zero time of exposure were observed to increase from the sample without addition of FA (28.639 ± 0.016) to the

sample with addition 2% FA the C* value of which was (33.056 ± 0.008). C* then decreased to (32.294 ± 0.012) for sample with 3% FA and further decreased to (28.162 ± 0.012) for sample added with 5% FA. In addition, the C* value for crude anthocyanin without addition of FA decreased continuously during storage from zero time of exposure with C*=(28.639 ± 0.016) until the end of exposure (up to three month) with C*=(25.265 ± 0.012). On the other hand, C* values for crude anthocyanin with addition of FA increased from zero time exposure until second month of exposure before decreasing on the third month of exposure. The crude anthocyanin sample added with 2% FA exhibited the highest C* (brightest colour) over two month of exposure from (33.056 ± 0.008) to (40.027 ± 0.008). A small increase in C* was recorded for sample with presence of 5% FA, where the initial C* value increased from (28.162 ± 0.012) to (29.663 ± 0.012). After three month of exposure, the crude anthocyanin colourant without addition of FA experienced the lowest C* of (25.265 ± 0.012) while the highest C* was exhibited by the sample with addition of 2% FA, C*=(31.258 ± 0.011).

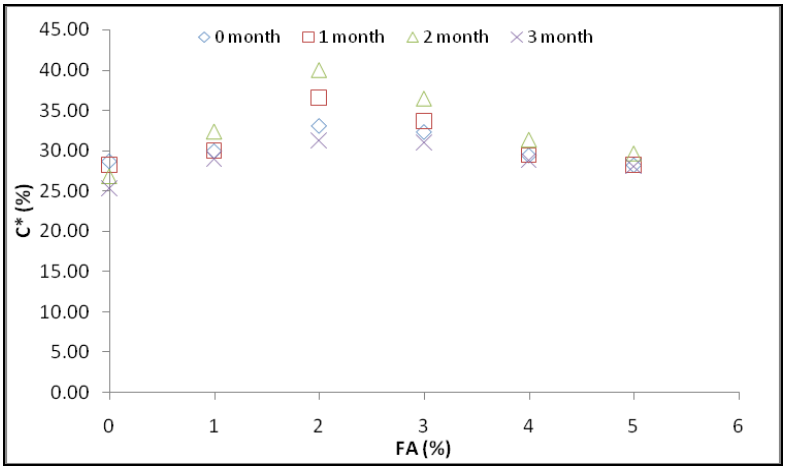
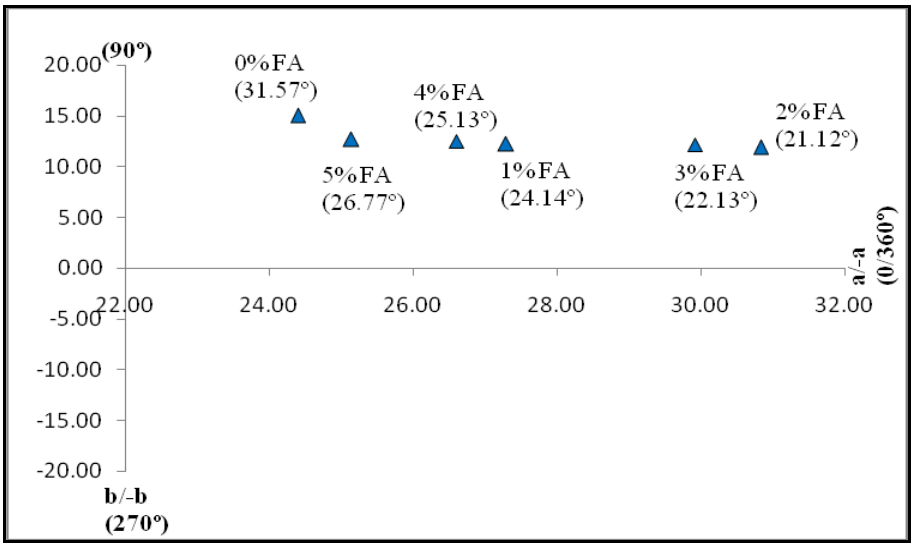


Figure 4.2: Relationship between percentage of FA and C* values (%) for crude colourant *Ixora siamensis* during three month of exposure

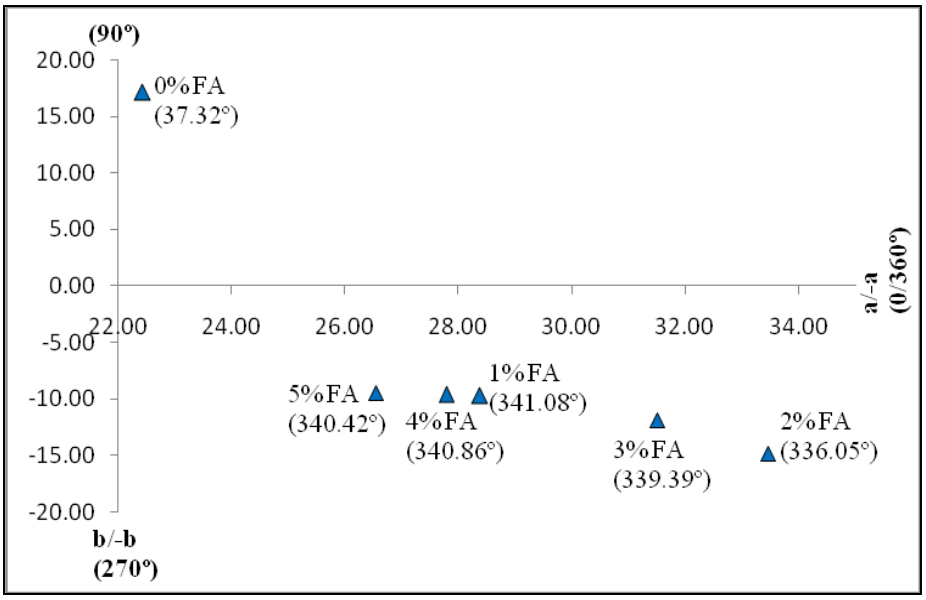
Figure 4.3 shows the initial hue of h°. h° shows a decrease for the crude anthocyanin colourant without addition of FA at (31.568 ± 0.014)° until the sample with 2% FA with h°=(21.120 ± 0.007)°. On further addition of FA, h° increased to (22.126 ± 0.012)° for sample with 3% FA and continued to increase up to addition of 5% FA when h°=(26.766 ± 0.012)°. The initial hue angle (h_{ab})° of crude anthocyanin colourant without presence of FA is (31.568 ± 0.014)° with coordinate a* as (24.401 ± 0.014) and coordinate b* as (14.993 ± 0.016). At the end of UV exposure, h_{ab}°=(81.326 ± 0.014)° but with a lower a* coordinate, a*=(3.810 ± 0.014) and higher b* coordinate of (24.977 ± 0.018). The CIE a* value is a measure of redness when positive and greenness when negative, while b* is a measure of yellowness when positive and that of blueness when negative. Hence, the crude anthocyanin solution without presence of FA has become less redness and more yellowness. In contrast, immediately after addition of FA to the crude anthocyanin colourant and after first month of exposure, a significant increment of the hue angle ranging from (24.144 ± 0.013)° to (341.080 ± 0.015)°, (21.120 ± 0.007)° to (336.050 ± 0.012)° , (22.126 ± 0.012)° to (339.390 ± 0.013)°, (25.130 ± 0.014)° to (340.860 ± 0.013)° and (26.766 ± 0.012)° to (340.420 ± 0.015)°., respectively for 1, 2, 3, 4 and 5% FA before decreasing again after the third month of exposure, as shown in Figure 4.3. For solutions with presence of FA, the hue angle moved clockwise (negative) into blue region during the second month of exposure and is most significant for solution with addition of 2% FA since the initial hue angle (21.120 ± 0.007)° moved to (336.050 ± 0.012)° with a*=(30.836 ± 0.011) that moved to (33.455 ± 0.011) and b*=(11.911 ± 0.006) that moved to (-14.854 ± 0.007). On the second month of exposure, coordinate a* increased to (34.954 ± 0.005) and b* to (-19.504 ± 0.004) with hue angle to (330.830 ± 0.010)°. However, towards the end of

storage, during the third month of exposure, the hue angle moved counterclockwise into red region with hue angle (29.068 ± 0.008)°, while a* moved backward to lower positive (27.321 ± 0.007) and more positive of b*=(15.187 ± 0.006). In addition, the gradual degradation of red colour, visually observed in all systems, is more significant for crude anthocyanin solution without FA addition. As the h° increase tonality changes from red to yellow tints can be observed. The h° angle of crude solutions with FA was higher than that of the FA free crude solution over two months of exposure. The FA added solution showed vivid purple colours, especially for solution with 2% FA, before turning back to show red colour tonalities again.



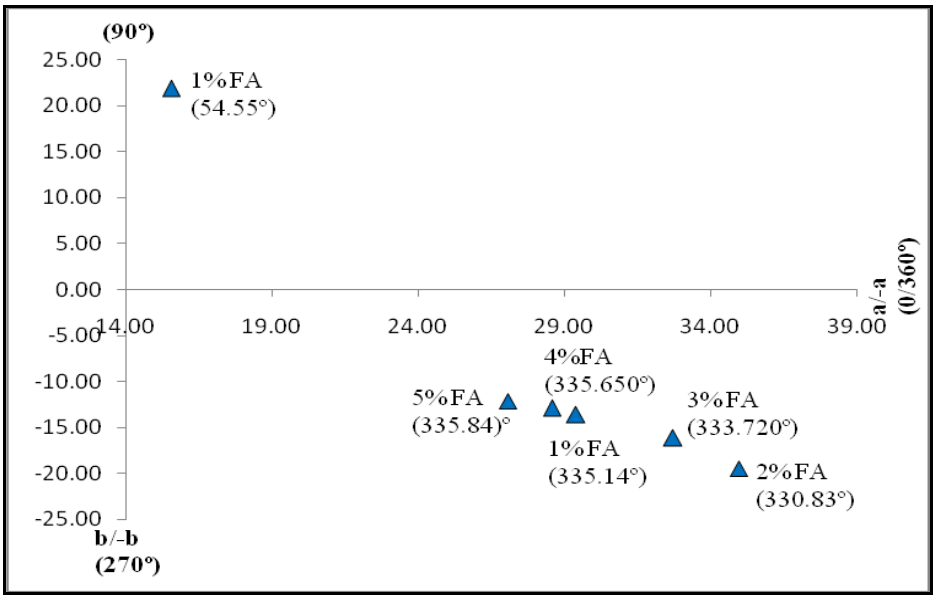
(a)

Figure 4.3: Relationship between percentage of FA and H° with a*b* coordinate for crude colourant *Ixora siamensis* during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



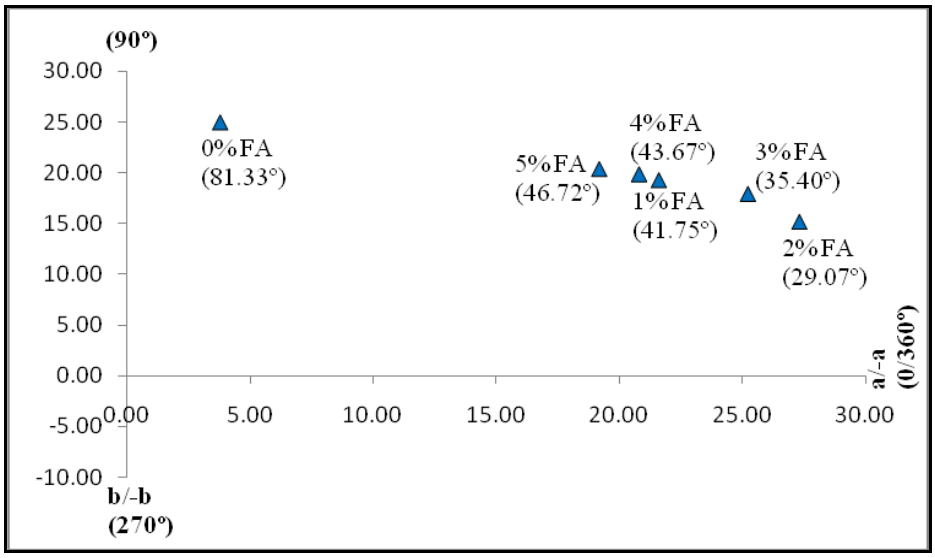
(b)

'Figure 4.3, continued'



(c)

'Figure 4.3, continued'



(d)

'Figure 4.3, continued'

Table 4.1 shows the total colour difference (ΔE), which is a combination of the changes of the three components (chrome, hue, and lightness). ΔE was the greatest for the crude anthocyanin colourant with addition of 2% FA with ΔE_1=28.978, at first month of exposure. The sample exhibited a lower colour change (ΔE_3=5.247) at the end of exposure. The other solutions with added FA demonstrated a similar trend which is highest before exposure (zero time) and lowest at the end of exposure from ΔE_1=22.182 to ΔE_3=12.767, ΔE_1=25.236 to ΔE_3=8.983, ΔE_1=22.173 to ΔE_3=13.690, and ΔE_1=22.168 to ΔE_3=14.357, respectively for 1, 3, 4 and 5% of FA. In contrast, the ΔE for anthocyanin colourant without FA was the lowest before exposure (zero time) (ΔE_1=6.121) but increased to ΔE_3=14.357 at the end of exposure. For all FA added samples ΔE was higher than that of the crude anthocyanin colourant, with the highest ΔE for 2% FA added samples. This sample also exhibited highest saturation parameter. At zero time s_0=0.7061 for the 2% FA added

sample. The saturation increased with increasing exposure time until the second month of exposure (s_2=1.1429) before dropping to s_3=0.5936 at the third month of exposure. The Other FA added samples also showed similar trend, but with smaller value. The saturation or s parameter is the colourfulness of an area visualized by an observer which is determined as the proportion of chromaticity to lightness. The crude anthocyanin colourant exhibits the lowest saturation parameter before exposure (zero time), (s_0=0.4399) and continues to decrease at the end of exposure with s_3=0.3253 as presented in Table 4.1. The subscript showed the beginning of each monthly exposure period.

Table 4.1: Total colour differences (ΔE) and saturation crude colourant *Ixora siamensis* as affected by the addition of FA

FA (%)	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
0	s_0=0.4399	s_1=0.4000	s_2=0.3639	s_3=0.3253	ΔE_1=6.121	ΔE_3=26.105
1	s_0=0.5121	s_1=0.5407	s_2=0.5908	s_3=0.4297	ΔE_1=22.182	ΔE_3=12.767
2	s_0=0.7061	s_1=1.0162	s_2=1.1429	s_3=0.6389	ΔE_1=28.978	ΔE_3=5.247
3	s_0=0.6856	s_1=0.8524	s_2=0.9470	s_3=0.5936	ΔE_1=25.236	ΔE_3=8.983
4	s_0=0.4761	s_1=0.4846	s_2=0.5213	s_3=0.4014	ΔE_1=22.173	ΔE_3=13.690
5	s_0=0.4408	s_1=0.4427	s_2=0.4699	s_3=0.3761	ΔE_1=22.168	ΔE_3=14.357

4.2.2. Effect of pH on visual colour variation

Figure 4.4 presents values of the colour parameters CIE L* of crude anthocyanin colourant from *Ixora* at different pH 1, 3, 5, 7, 9 and 11. The initial pH of the crude is 3.5. After zero

exposure time, lightness percentage (L*) of crude anthocyanin colourant increased from sample at pH 1 (58.826 ± 0.015) until sample at pH 5 (66.191 ± 0.014). However, the L* values started to decrease when pH of the sample increased from 7 (L*=61.083 ± 0.015) until pH 11 (L*=57.989 ± 0.019). In addition, during storage the L* parameter for crude anthocyanin for all pH increases continuously from zero exposure time until the end of exposure. According to the figure, sample at pH 11 exhibited to the lowest L* values before exposure (zero time) (57.989 ± 0.019) while towards the end of exposure L* increase rapidly to the highest value of others (91.438 ± 0.010). In contrast, the lightness percentage at the beginning for crude anthocyanin at pH 1 was (58.826 ± 0.015) while it gradually increases with increasing exposure time and at the third month of exposure the L* values was the lowest compared to others (69.928 ± 0.014). This indicated that after three months of exposure the colour of sample at pH 11 was the lightest (higher L*) while the crude anthocyanin at pH 1 resulted in brighter or darker colours (lower L*), followed by sample at pH 3, and 3.5 which were (72.980 ± 0.014) and (77.662 ± 0.018) respectively.

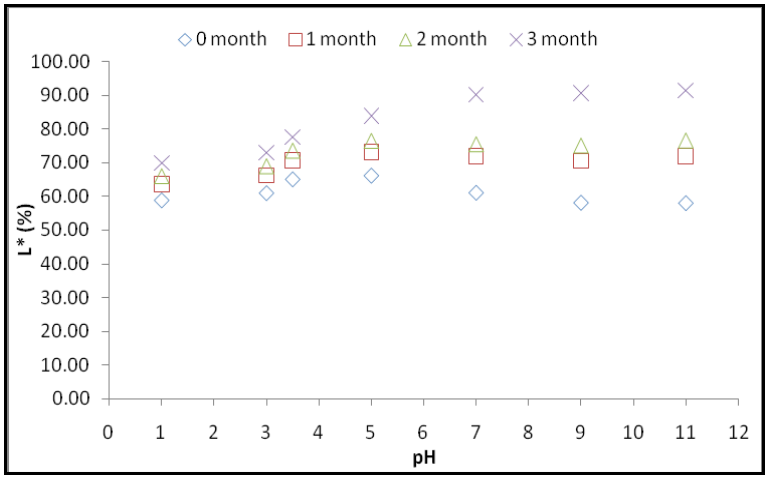


Figure 4.4: Relationship between pH variation and L* values (%) for crude colourant *Ixora siamensis* during three month of exposure

The chromaticity (C*) values in the beginning and at the end of exposure are shown in Figure 4.5. The initial (zero time of exposure) chromaticity C* for crude anthocyanin colourant decreased with increasing pH from pH 1 (39.381 ± 0.012) until pH 7 (12.167 ± 0.015) before increasing at pH 9 (43.201 ± 0.020) and decreasing again at pH 11 (15.089 ± 0.016). In addition, the C* value for crude anthocyanin for all pH were observed to decrease continuously from zero time of exposure until the end of exposure (up to three months). On the other hand, the highest C* value for the acidic crude anthocyanin was exhibited by sample at pH 1 (C*=39.381 ± 0.012) at the beginning of as well as at the end of exposure (third month of exposure) with the values of C*=34.157 ± 0.010. Eventhough sample at pH 9 showed higher C* value at the beginning and end of exposure, the sample exhibited the phenomena of browning towards the end of exposure, as can be seen in Figure 4.5. The lowest C* values at zero time was exhibited by sample at pH 11 (15.089 ± 0.016) while after three month of exposure sample at pH 11 also exhibited the lowest C* values (11.839 ± 0.016). The colour was also brownish.

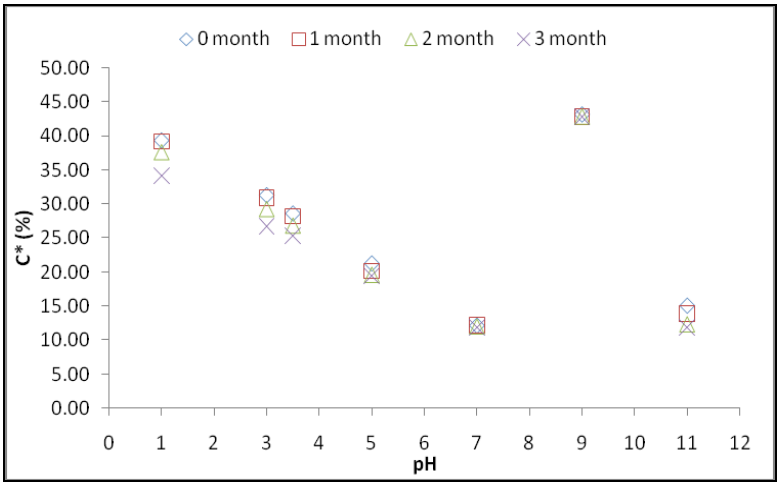
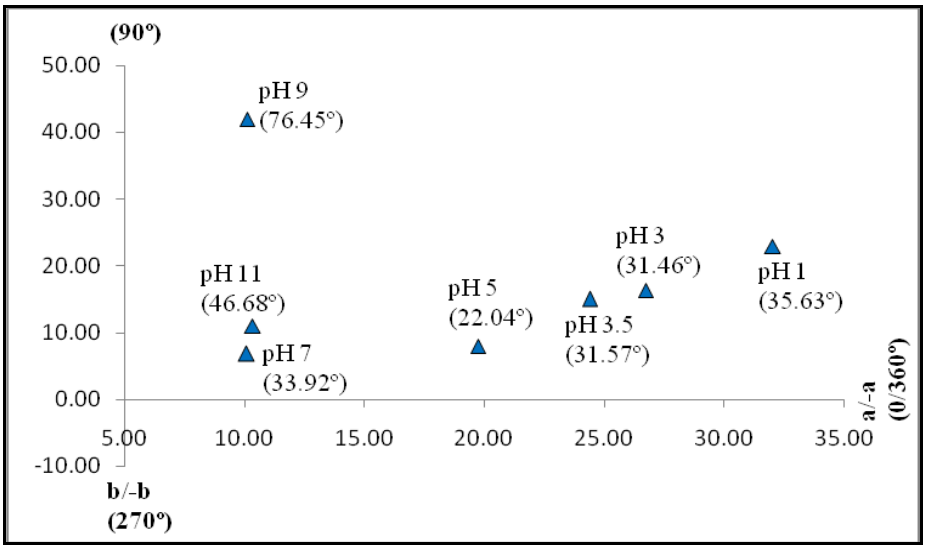


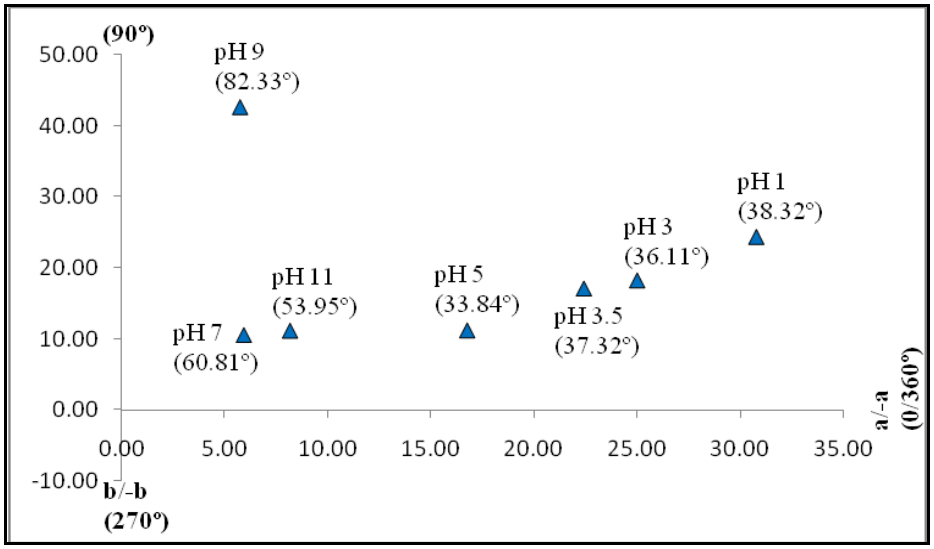
Figure 4.5: Relationship between pH variation and C* values (%) for crude colourant *Ixora siamensis* during three month of exposure

Hue is another parameter that affects colour quality. From Figure 4.6, the initial hue angle, h^o for the crude anthocyanin colourant for all pH decreased from sample at pH 1 hue angle h°=(35.625 ± 0.012)° until sample at pH 5 h°=(22.042 ± 0.011)° and begins to increase at pH 7 h°=(33.916 ± 0.012)° until pH 9 h°=(76.445 ± 0.014)° before decreasing again at pH 11 h°=(46.678 ± 0.015)°. The hue angle of crude anthocyanin colourant for all pH increased significantly from the beginning of exposure until the third month of exposure, ranging from (35.625 ± 0.012)° to (68.024 ± 0.015)°, (31.459 ± 0.013)° to (75.431 ± 0.013)°, (31.568 ± 0.014)° to (81.326 ± 0.014)°, (22.042 ± 0.011)° to (87.016 ± 0.009)°, (33.916 ± 0.012)° to (85.507 ± 0.015)°, (76.445 ± 0.014)° to (89.032 ± 0.010)° and (46.678 ± 0.015)° to (86.537 ± 0.014)° for pH 1, 3, 3.5, 5, 7, 9 and 11 respectively as presented in Figure 4.6. During the three months of exposure, the crude anthocyanin colourant at pH 9 exhibited the highest hue angle values of (76.445 ± 0.014)° with a*=10.125 ± 0.014 and b*=41.998 ± 0.019. This is followed by sample at pH 11 with hue angle, h°=(46.678 ± 0.015)°, a*=(10.353 ± 0.012) while the coordinate of b* increased to (10.978 ± 0.014). After three months of exposure, sample at pH 9 again contributed to the highest hue angle of (89.032 ± 0.010)° but a* coordinate moved back to (0.723 ± 0.013) and b* slightly increased to (42.791 ± 0.014). The hue angle for sample at pH 11 h°=(86.537 ± 0.014)°, the a* moved to (0.715 ± 0.013) and b* to (11.818 ± 0.018). In addition, the hue angle of sample at pH 1 is (35.625 ± 0.012)° with highest a* of (32.011 ± 0.013) and b* value of (22.939 ± 0.015) at zero time, while at the end of exposure the hue angle increased to (68.024 ± 0.015)°, with a* at (12.782 ± 0.014) and b* at (31.676 ± 0.012). The gradual degradation of red colour, visually observed in all pH, experienced by crude anthocyanin is

accompanied by the tonality changes from red to brown-yellow tints as the hº increased during experiment time and is significant for samples at higher pH (pH 9 and 11).

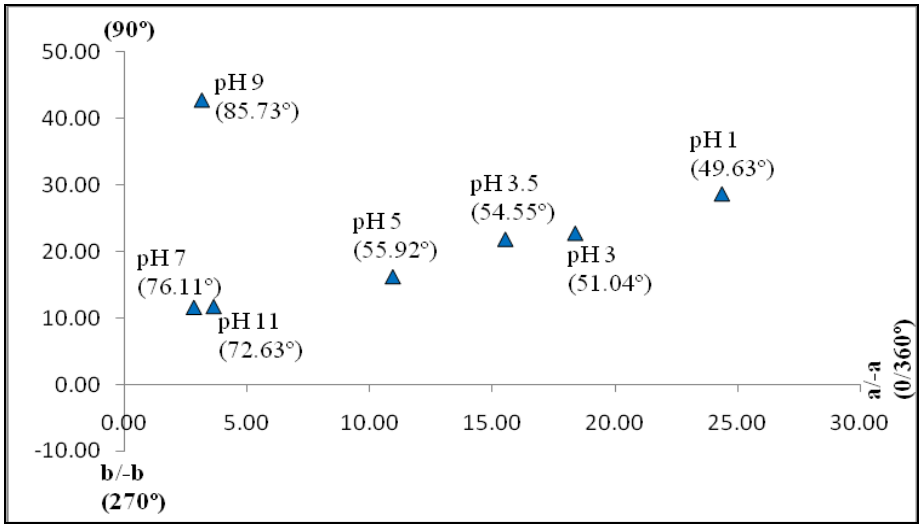


(a)



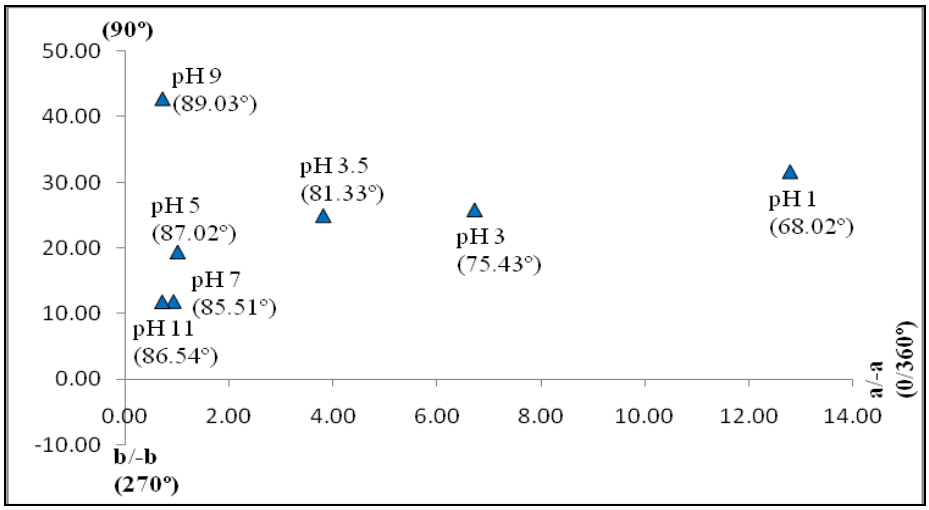
(b)

Figure 4.6: Relationship between pH variation and H◦ with a*b* coordinate for crude colourant *Ixora siamensis* during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 4.6, continued'



(d)

'Figure 4.6, continued'

Table 4.2 lists the total colour difference (ΔE), which was lowest for crude anthocyanin colourant at pH 1 which ΔE_1=4.959, during first month of exposure and is still the lowest at the end of exposure (ΔE_3=23.861). In contrast, the total colour difference of crude anthocyanin colourant at pH 11 was the highest at zero time (ΔE_1=13.990) and at the end of

exposure ΔE_3=34.820. Other crude anthocyanin colourant of different pH demonstrated a similar trend in colour change being low before exposure at zero time and higher at the end of exposure from ΔE_1=5.715 to ΔE_3=25.192, ΔE_1=6.121 to ΔE_3=26.105, ΔE_1=8.184 to ΔE_3=28.174, ΔE_1=12.034 to ΔE_3=31.027 and ΔE_1=13.071 to ΔE_3=33.924 for pH 3, 3.5, 5, 7 and 9 respectively. In addition, the crude anthocyanin colourant at pH 1 exhibited the highest saturation parameter. At time zero s_0=0.6694 that decreased with increasing exposure time until the end of three months with s_3=0.4885. Though saturation of sample at pH 9 was higher in the beginning, the colour tends to turn into brown. Other crude anthocyanin colourants with different pH also showed similar trend. Crude anthocyanin colourant at pH 11 exhibited the lowest saturation. At time zero, s_0=0.2602 and continuously decreased towards the end of exposure with s_3=0.1295 as seen in Table 4.2.

Table 4.2: Total colour differences (ΔE) and saturation crude colourant *Ixora siamensis* as affected by pH

pH	TIME (Month) 0	1	2	3	ΔE_1	ΔE_3
pH 1	s_0=0.6694	s_1=0.6190	s_2=0.5691	s_3=0.4885	ΔE_1=4.959	ΔE_3=23.861
pH 3	s_0=0.5134	s_1=0.4686	s_2=0.4236	s_3=0.3660	ΔE_1=5.715	ΔE_3=25.192
pH 3.5	s_0=0.4399	s_1=0.4000	s_2=0.3639	s_3=0.3253	ΔE_1=6.121	ΔE_3=26.105
pH 5	s_0=0.3217	s_1=0.2761	s_2=0.2558	s_3=0.2319	ΔE_1=8.184	ΔE_3=28.174
pH 7	s_0=0.1992	s_1=0.1690	s_2=0.1581	s_3=0.1319	ΔE_1=12.034	ΔE_3=31.027
pH 9	s_0=0.7435	s_1=0.6098	s_2=0.5703	s_3=0.4719	ΔE_1=13.071	ΔE_3=33.924
pH 11	s_0=0.2602	s_1=0.1929	s_2=0.1603	s_3=0.1295	ΔE_1=13.990	ΔE_3=34.820

4.2.3. Effect of addition 2% ferulic acid (FA) and pH on visual colour variation

Figure 4.7 displays the results of colour parameters CIE L* for crude anthocyanin colourant from *Ixora* with addition of 2% FA and at different pH values. From previous results, the 2% FA act as a good colour enhancer and stabilizer. The initial (zero time) lightness percentage (L*) of crude anthocyanin colourant containing 2% FA with altered pH (initial pH (3.3), pH 1, 3, 5, 7, 9 and 11) were observed to decrease from sample at pH 1 (L*=48.170 ± 0.006) until sample at pH 5 (L*=44.531 ± 0.006). L* increased from pH 7 (L*=46.081 ± 0.009) until pH 9 (L*=51.813 ± 0.007) before decreasing again at pH 11 (L*=43.240 ± 0.008). In addition, during exposure the L* parameter values for crude anthocyanin at pH 3, 3.3 and 5 were observed to decrease (darker colour) from zero time L* value until the second months of exposure before increasing again at the third month of exposure. The significant decrease in L* value over two months of exposure was exhibited by the crude anthocyanin colourant at pH 3, with the initial L*=47.315 ± 0.005 that decreased to 34.886 ± 0.004. This is followed by sample at pH 3.3, with L* decreasing from (46.815 ± 0.007) to (35.021 ± 0.007) and pH 5 from (44.531 ± 0.006) to (L*=36.273 ± 0.010). In contrast, at other pH values (pH 1, 7, 9 and 11), L* continues to increase from zero time of exposure until the end of exposure where L* ranges from (48.170 ± 0.006) to (52.982 ± 0.005), (46.081 ± 0.009) to (62.995 ± 0.009), (51.813 ± 0.007) to (73.429 ± 0.010), and (43.240 ± 0.008) to (72.999 ± 0.011) respectively. After three months of exposure, the crude anthocyanin colourant containing 2% FA at pH 9 exhibited the lightest colour with highest L* of (73.429 ± 0.010), followed with sample at pH 11 (L*=72.999 ± 0.011), while the lowest L* (darkest colour) was exhibited by samples at pH 3 with (L*=48.725 ± 0.005).

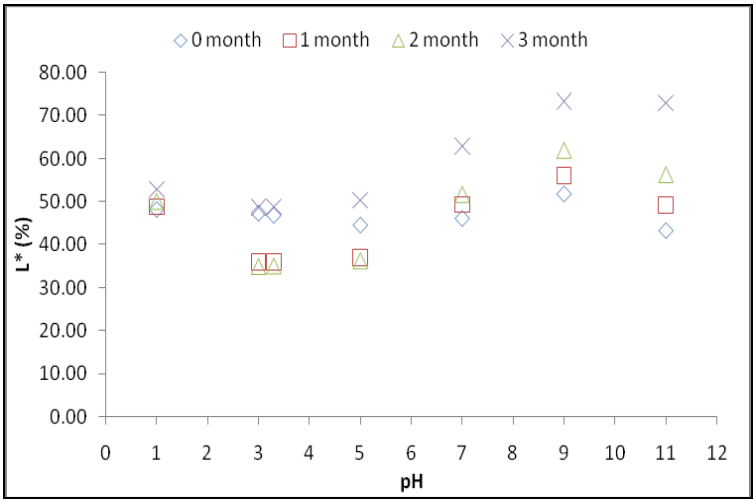


Figure 4.7: Relationship between pH variation and L* values (%) for crude colourant *Ixora siamensis* containing 2% FA during three month of exposure

The chromaticity (C*) values of crude anthocyanin colourant with altered pH (initial pH (3.3), pH 1, 3, 5, 7, 9 and 11) in the beginning and at the end of exposure are shown in Figure 4.8. The C* value of the crude anthocyanin colourant containing 2% FA at altered pH (pH 3, 3.3 and 5) at zero time of exposure were observed to increased continuously until the second month of exposure, in which C* increased significantly (brightest colour) for sample at pH 3. The C* value increased from (34.977 ± 0.007) to (41.864 ± 0.006) before decreasing at the third month of exposure (C*=32.865 ± 0.005). This trend is followed by sample at pH 3.3. The initial C* value (33.056 ± 0.008) increased on the second month of exposure (C*=40.027 ± 0.008) before decreasing at end of exposure time at C*=31.258 ± 0.011. For sample at pH 5, C* increased from (26.809 ± 0.006) to (34.646 ± 0.008) and decreased on the third month of exposure with C*=26.595 ± 0.008. For samples with pH variations (pH 1, 7, 9 and 11), C* decreased continuously from zero time of exposure until the third month of exposure ranging from (43.364 ± 0.005) to (26.456 ±

0.008), (12.982 ± 0.008) to (12.752 ± 0.008), (45.734 ± 0.011) to (43.977 ± 0.008), (17.403 ± 0.007) to (14.554 ± 0.009), respectively. Nevertheless, after three months of exposure, the crude anthocyanin colourant at pH 3 experienced the highest C* of (32.865 ± 0.005). Eventhough the crude anthocyanin colourant at pH 9 also experienced higher C* values, browning of the samples indicate degradation. The lowest C* value was exhibited by samples at pH 7 C*=12.752 ± 0.008 and pH 11 C*=14.554 ± 0.009.

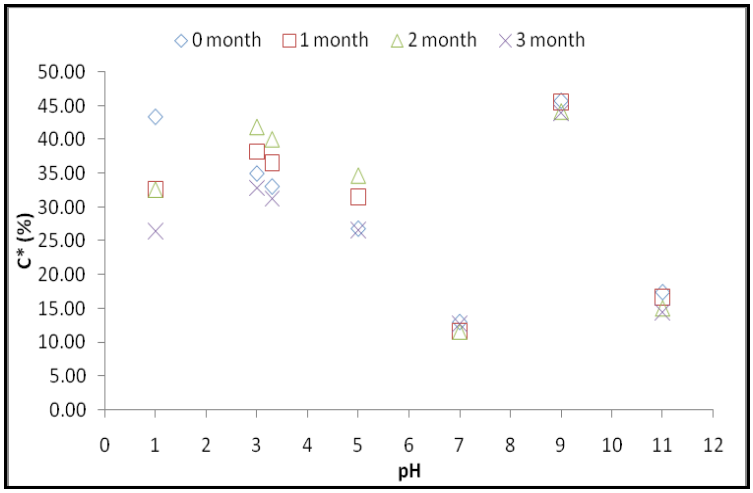
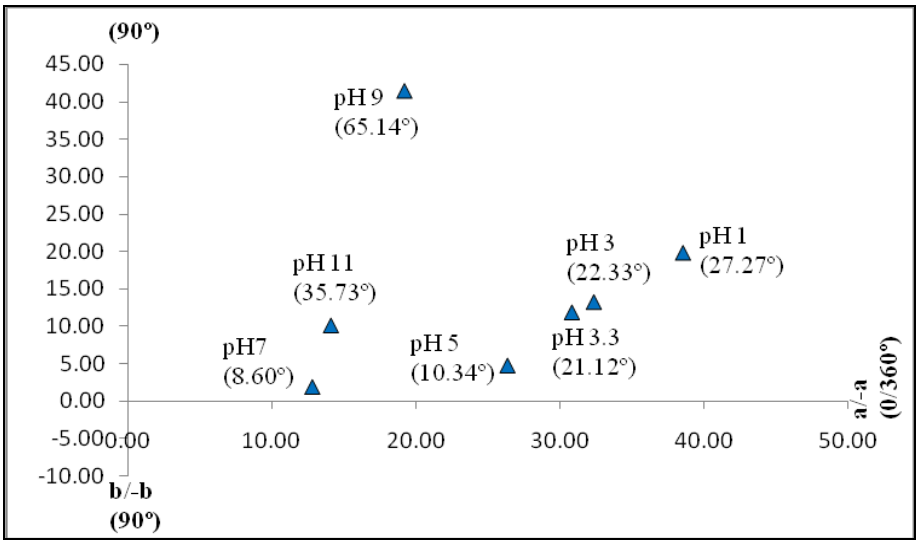


Figure 4.8: Relationship between pH variation and C* values (%) for crude colourant *Ixora siamensis* containing 2% FA during three month of exposure

The hue angle, hº values for the crude anthocyanin colourant containing 2% FA at different pH are shown in Figure 4.9. The initial hue angle decreased from the value for sample at pH 1 h°=(27.267 ± 0.005)° until sample at pH 7 h°=(8.598 ± 0.009)°. The hue angle starts to increase from pH 9 with h°=(65.138 ± 0.007)° and decreases again at sample pH 11 with h°=(35.734 ± 0.010)°. From Figure 4.9, it can be noted that the hue angle for crude anthocyanin colourant with pH 3, 3.3 and 5 moves clockwise into blue region from the zero

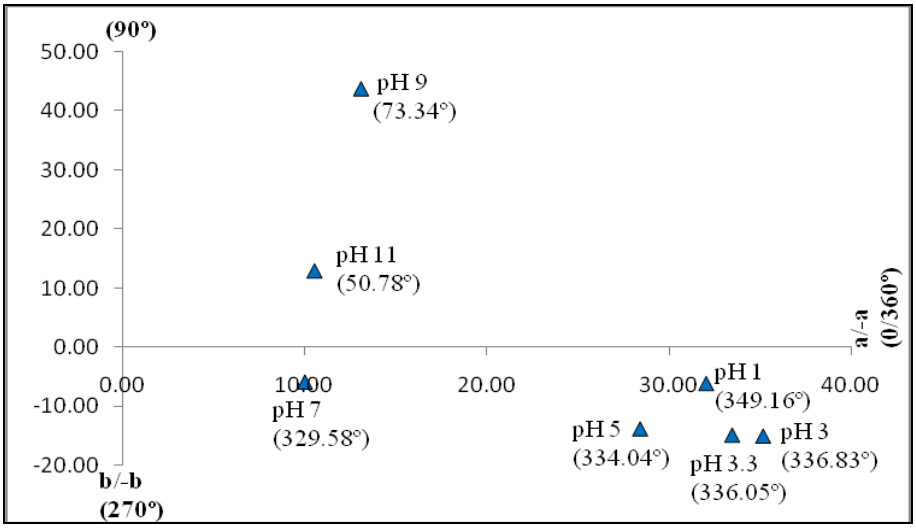
time of exposure until the second month of exposure, ranging from hue angle (22.325 ± 0.004)° with a*=(32.356 ± 0.006) and b*=(13.287 ± 0.008) to hue angle (331.410 ± 0.007)° with more positive a* (36.762 ± 0.004) and negative b* value (-20.030 ± 0.008) for sample at pH 3 and hue angle of (21.120 ± 0.007)° with positive a* (30.836 ± 0.011) and b* value (11.911 ± 0.006) moved to (330.830 ± 0.010)° with more positive a* (34.954 ± 0.005) and negative b* value (-19.504 ± 0.004) while for sample at pH 3.3 and hue angle of (10.335 ± 0.007)° with positive a* (26.374 ± 0.006) and b* value (4.810 ± 0.007) moved to (329.030 ± 0.009)° with more positive a* (29.709 ± 0.008) and negative b* value (-17.826 ± 0.009) for sample at pH 5. At the third month of exposure, the parameters of samples with pH 3, 3.3 and 5 moved counterclockwise into red tonalities with hue angle of (26.212 ± 0.008)° with less positive a* of (29.486 ± 0.008) and b*=(14.517 ± 0.006) for sample at pH 3. For sample with pH 3.3, the hue angle was (29.068 ± 0.008)° with less positive a* of (27.321 ± 0.007) and b*=(15.187 ± 0.006). For sample at pH 5, the hue angle was (34.882 ± 0.007)° with less positive a* of (21.817 ± 0.010) and b*=(15.210 ± 0.005). For sample at pH 1 and 7, the hue angle also moved clockwise into the blue region but only during the first month of exposure since at the second month of exposure the samples have already moved counterclockwise into red tonalities and continues until the third month of exposure. In contrast, during the third months of exposure the crude anthocyanin colourant at pH 9 and 11 directly moved counterclockwise from the first month of exposure until the third month of exposure and the hue angle approaches the yellow region, to higher h^o. At time zero, the hue angle for sample at pH 9 was the highest (65.138 ± 0.007)° with a*=(19.228 ± 0.010) and b*=(41.496 ± 0.013) while after three month of exposure, sample at pH 9 again contributed to the higher hue angle of (88.694 ± 0.011)° but a* become less positive with

a*=(1.002 ± 0.006). The value of b* increased slightly to (43.966 ± 0.011). In addition, sample at pH 3 experienced lower hue angle of (22.325 ± 0.004)° with a*=(32.356 ± 0.006) and b*=(13.287 ± 0.008) at zero time. At the end of exposure, the hue angle was lowest at (26.212 ± 0.008)°, with highest a* value of (29.486 ± 0.008) and b* value of (14.517 ± 0.006). The gradual degradation of red colour, visually observed for crude anthocyanin colourant was accompanied by the tonality changes from red to brown-yellow tints and black colour as the hº increased with time. This is significant for samples at higher pH (pH 7, 9 and 11). Furthermore, the hº values of lower pH (pH 1, 3, 3.3 and 5) showed vivid purple colours, especially for sample at pH 3, before turning back again into red colour tonalities at the end of exposure.



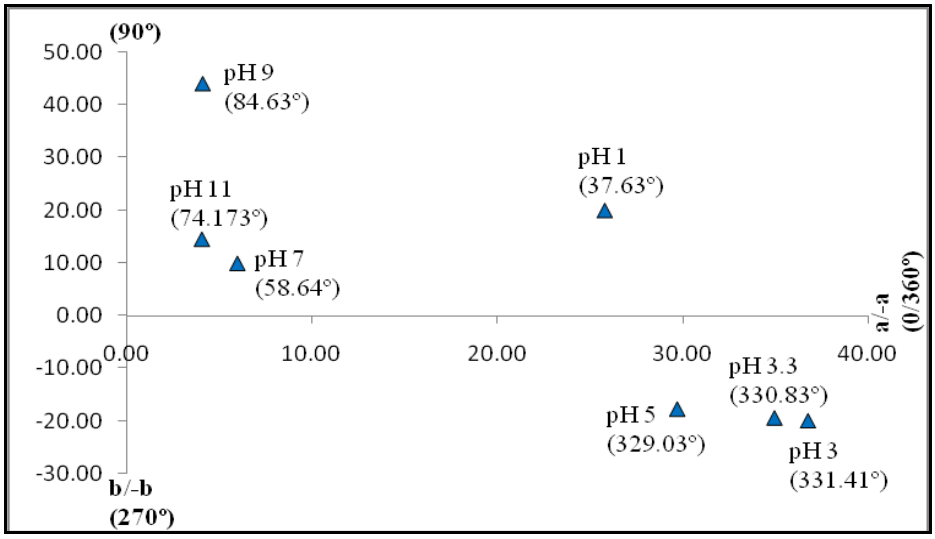
(a)

Figure 4.9: Relationship between pH variation and H◦ with a*b* coordinate for crude colourant *Ixora siamensis* containing 2% FA during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



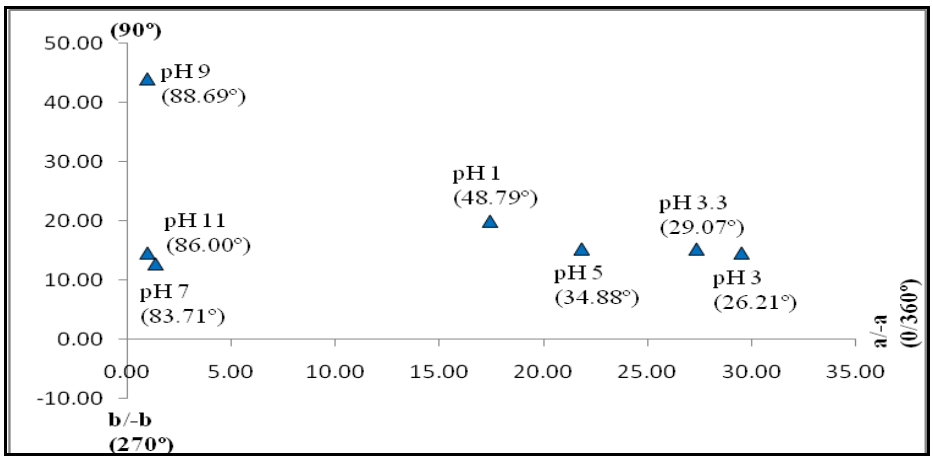
(b)

'Figure 4.9, continued'



(c)

'Figure 4.9, continued'



(d)

'Figure 4.9, continued'

Table 4.3 showed the total colour difference (ΔE), which was the greatest for the crude anthocyanin colourant containing 2% FA at pH 3 where ΔE_1=30.625, at first month of exposure while lower colour change at the end of exposure (ΔE_3=3.426). Other crude anthocyanin colourant demonstrated a similar trend in change of the colour difference, the highest being at zero time and lower towards the end of exposure from ΔE_1=26.813 to ΔE_3=21.657, ΔE_1=28.978 to ΔE_3=5.247 and ΔE_1=20.170 to ΔE_3=12.653 for pH 1, 3.3 and 5 respectively. In contrast, the ΔE of crude anthocyanin colourant at pH 7, 9 and 11 were lower at zero time but increased at the end of exposure period showing degradation. The crude anthocyanin colourant containing 2% FA at pH 3 exhibited the highest saturation index at zero time storage, s_0=0.7061, which increased with increasing exposure time until the second month of exposure (s_2=1.1429). Finally at the third month of exposure, (s_3=0.6745) the saturation index dropped. Eventhough saturation index for sample at pH 1 is also high; it decreased with increasing of exposure time. The sample with pH 9 tends to turn into brown as can be seen in Table 4.3. Sample at pH 11 exhibits the lowest saturation

index, which at zero time, (s_0=0.4025) and continuous to decrease towards the end of exposure (s_3=0.1994).

Table 4.3: Total colour differences (ΔE) and saturation of crude colourant *Ixora siamensis* with addition of 2% FA as affected by pH

pH	TIME (Month) 0	1	2	3	ΔE_1	ΔE_3
pH 1	s_0=0.9002	s_1=0.6669	s_2=0.6509	s_3=0.4993	ΔE_1=26.813	ΔE_3=21.657
pH 3	s_0=0.7392	s_1=1.0618	s_2=1.2000	s_3=0.6745	ΔE_1=30.625	ΔE_3=3.426
pH 3.3	s_0=0.7061	s_1=1.0162	s_2=1.1429	s_3=0.6389	ΔE_1=28.978	ΔE_3=5.247
pH 5	s_0=0.6020	s_1=0.8518	s_2=0.9551	s_3=0.5278	ΔE_1=20.170	ΔE_3=12.653
pH 7	s_0=0.2817	s_1=0.2351	s_2=0.2231	s_3=0.2024	ΔE_1=8.934	ΔE_3=23.069
pH 9	s_0=0.8827	s_1=0.8131	s_2=0.7127	s_3=0.5989	ΔE_1=7.840	ΔE_3=28.382
pH 11	s_0=0.4025	s_1=0.3381	s_2=0.2660	s_3=0.1994	ΔE_1=7.538	ΔE_3=32.810

4.3. Colour analysis on purified anthocyanin colourant from *Ixora siamensis*

4.3.1. Effect of ferulic acid (FA) addition on visual colour variation

Figure 4.10 presents the results of the colour parameters CIE L* of purified anthocyanin colourant from *Ixora* added with different percentages (from 1% to 5%) of ferulic acid (FA). Initially at zero time, the lightness percentage (L*) of purified anthocyanin colourant decreased from sample without presence of FA (71.185 ± 0.014) until sample with 2% FA (55.125 ± 0.007). The L* values start to increase when percentage of FA increased being (57.284 ± 0.010) for sample with 3% FA added and (69.074 ± 0.014) when FA added was

5%. During exposure, the L* parameter values for purified anthocyanin without addition of FA increase continually from zero time of exposure (71.185 ± 0.014) until the third month of exposure (89.364 ± 0.017). On the other hand, the lightness percentages for purified anthocyanin with addition of FA decreased (to darker colour) from zero time of exposure until second month of exposure before increase at the third month of exposure. Slightly decrease in L* over two months of exposure was exhibited by the purified anthocyanin colourant with addition of 5% FA, the initial L* of which was (69.074 ± 0.014) and decreased to (65.481 ± 0.012), followed by the colourant added with 4% FA the L* of which was (66.888 ± 0.016) that decreased to (61.672 ± 0.016). The highest decrease in L* (darker colour) was experienced by the purified anthocyanin colourant with addition of 2% FA. L* decreased from (55.125 ± 0.007) to (44.152 ± 0.011). After three month of exposure, the purified anthocyanin colourant without addition of FA exhibited the highest L* value (lightest colour) of (89.364 ± 0.017), while the lowest L* (darker colour) was exhibited by the sample with addition of 2% FA (58.309 ± 0.010).

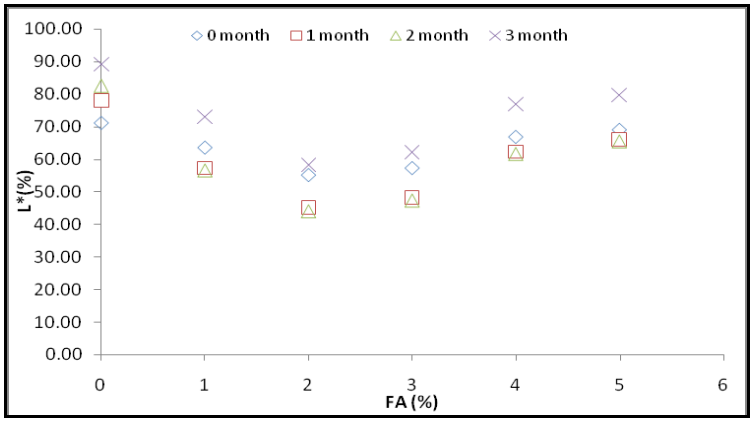


Figure 4.10: Relationship between percentage of FA and L* values (%) for purified colourant *Ixora siamensis* during three month of exposure

In addition, the chromaticity (C*) values in the beginning and at the end of exposure was shown in Figure 4.11. The initial (zero time of exposure) C* values of the purified anthocyanin colourant were observed to increase from the sample without addition of FA (23.350 ± 0.016) to the sample with addition 2% FA the C* value of which was (31.678 ± 0.012). C* then decreased to (28.707 ± 0.011) for sample with 3% FA and further decreased to (23.424 ± 0.020) for sample added with 5% FA. The C* for purified anthocyanin colourant without addition of FA decreased continuously during exposure, from zero time of exposure with C*=23.350 ± 0.016 until the end of exposure (up to three month) with C*=21.391 ± 0.013. In contrast, the C* values for purified anthocyanin colourant with addition of FA increased from zero time exposure until second month of exposure before decreasing on the third month of exposure. The purified anthocyanin sample added with 2% FA exhibited the highest C* (brightest colour) over two month of exposure from (31.678 ± 0.012) to (38.673 ± 0.006). There was small increase in C* values recorded for sample with presence of 5% FA, where the zero time C* value increase from (23.424 ± 0.020) to (25.963 ± 0.014). After three month of exposure, the purified anthocyanin colourant without addition of FA experienced the lowest C* of (21.391 ± 0.013) while the highest C* was exhibited by the sample with addition of 2% FA, C*=29.442 ± 0.007.

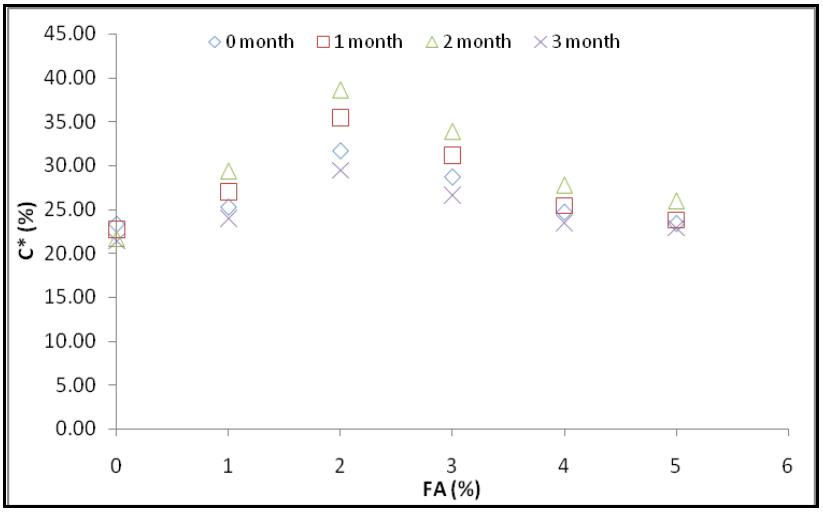
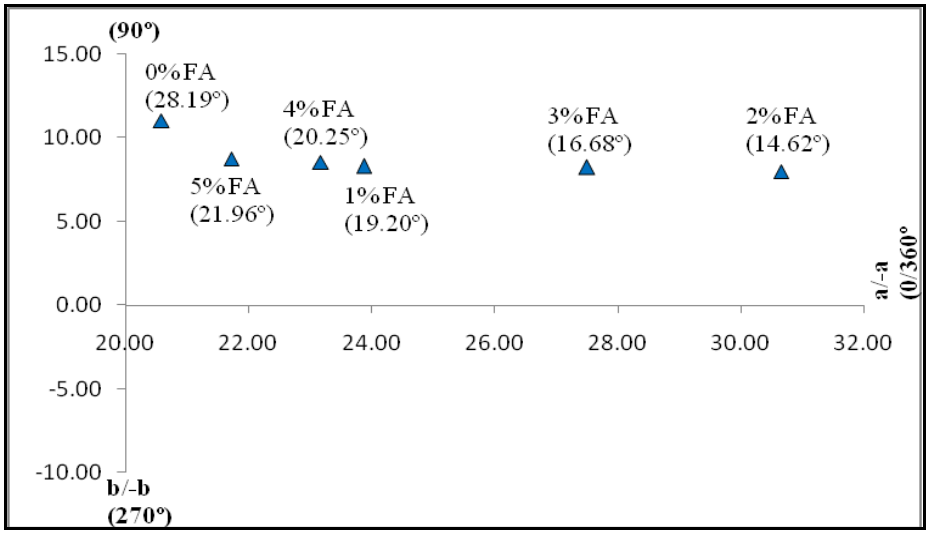


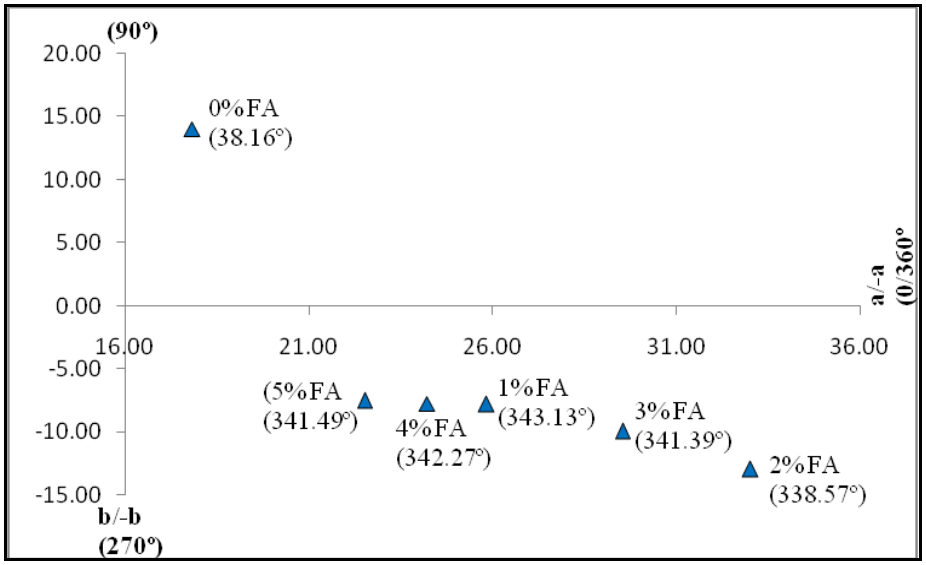
Figure 4.11: Relationship between percentage of FA and C* values (%) for purified colourant *Ixora siamensis* during three month of exposure

Figure 4.12 shows the initial hue of h°. h° shows a decrease for the purified anthocyanin colourant without addition of FA at (28.188 ± 0.014)° until the sample with 2% FA with h°=(14.616 ± 0.009)°. On further addition of FA, h° increased increase to (16.684 ± 0.014)° for sample with 3% FA and continual to increase up to 5% FA when h°=(21.958 ± 0.012)°. The initial hue angle (h_{ab})° of purified anthocyanin colourant without presence of FA is (28.188 ± 0.014)° with coordinate a* as (20.581 ± 0.013) and coordinate b* as (11.030 ± 0.018). At the end of exposure, hue angle value (h_{ab})°=(85.804 ± 0.011)°, but with a lower a* coordinate, a*=(1.565 ± 0.019) and higher b* coordinate of (21.334 ± 0.018). In contrast, immediately after addition of FA to the purified anthocyanin colourant and after first month of exposure, a significant increment of the hue angle ranging from (19.196 ± 0.014)° to (343.130 ± 0.016)°, (14.616 ± 0.009)° to (338.570 ± 0.008)°, (16.684 ± 0.014)° to (341.390 ± 0.013)°, (20.254 ± 0.011)° to (342.270 ± 0.013)° and (21.958 ± 0.012)° to (341.490 ± 0.016)° respectively for 1, 2, 3, 4 and 5% FA before decreasing again after the

third month of exposure, as shown in Figure 4.12. For solutions with presence of FA, the hue angle moved clockwise (negative) into blue region during the second month of exposure and is most significant for solution with addition of 2% FA since the initial hue angle, (14.616 ± 0.009)° moved to (338.570 ± 0.008)° with a*=(30.653 ± 0.009) that moved to (33.018 ± 0.011) and b*=(7.994 ± 0.011) that moved to (-12.955 ± 0.011). On the second month of exposure, coordinate a* increased to (34.459 ± 0.012) and b* to (-17.557 ± 0.012) with hue angle to (333.000 ± 0.009)°. However, towards the end of exposure, during the third month of storage, the hue angle moved counterclockwise into red region with hue angle (25.540 ± 0.011)°, while a* moved backward to lower positive (26.565 ± 0.010) and more positive of b* value (12.694 ± 0.013). In addition, the gradual degradation of red colour, visually observed in all systems is more significant for purified anthocyanin colourant solutions without FA addition. As the h° increases, tonality changes from red to yellow tints can be observed. The hue angles of purified solutions with FA were higher than that of the FA free purified solution over two months of exposure. The FA added solutions showed vivid purple colours, especially for solution with 2% FA, before turning back to show red colour tonalities again.

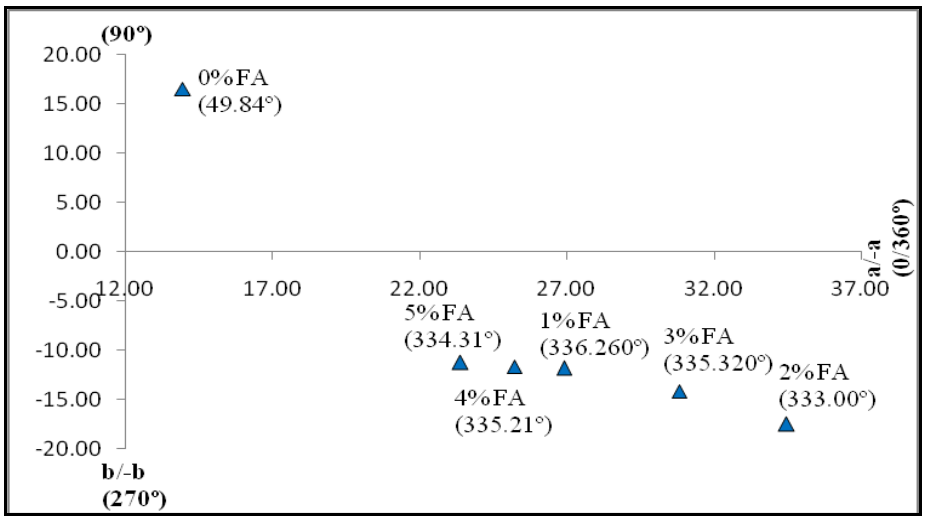


(a)



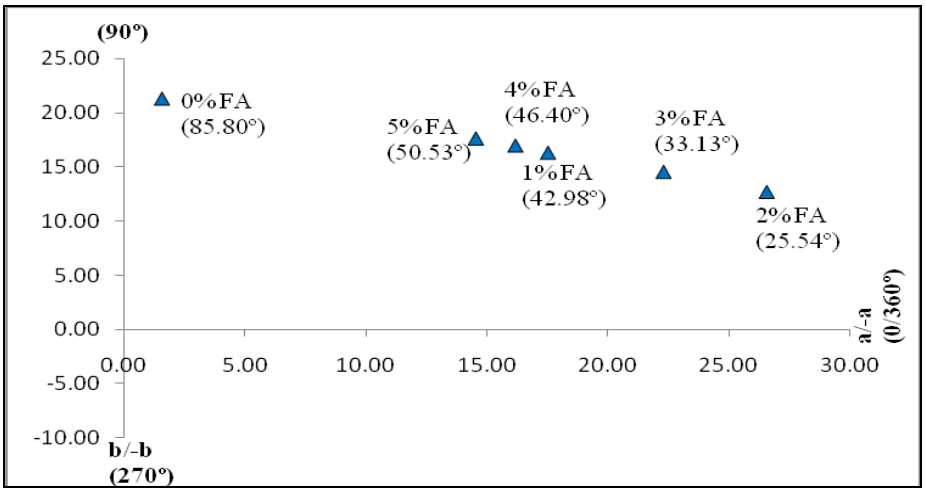
(b)

Figure 4.12: Relationship between percentage of FA and H◦ with a*b* coordinate for purified colourant *Ixora siamensis* during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 4.12, continued'



(d)

'Figure 4.12, continued'

From Table 4.4, ΔE was the highest for the purified anthocyanin colourant with addition of 2% FA with ΔE_1=23.329, at first month of exposure. The sample exhibited a lower colour change (ΔE_3=6.996) at the end of exposure. The other solution with added FA demonstrated a similar trend, which is highest before exposure (zero time) and lowest at the

end of exposure from ΔE_1=17.402 to ΔE_3=13.958, ΔE_1=20.395 to ΔE_3=9.600, ΔE_1=16.942 to ΔE_3=14.982, and ΔE_1=16.577 to ΔE_3=15.654, respectively for 1, 3, 4 and 5% of FA. In contrast, ΔE for purified anthocyanin colourant without FA was the lowest before exposure (zero time) (ΔE_1=8.089) but increased to ΔE_3=28.253 at the end of exposure. For all FA added samples, ΔE was higher than that of the purified anthocyanin colourant, with the highest value ΔE for 2% FA added samples. This sample also exhibited the highest saturation parameter at zero time, s_0=0.5747 for the 2% FA added sample. The saturation increased with increasing of exposure time until the second month of exposure (s_2=0.8759) before decreased to (s_3=0.5049) at the end of storage. Other FA added samples also showed similar trend, but with smaller value. The purified anthocyanin colourant without addition of FA exhibits the lowest saturation parameter before exposure (zero time) (s_0=0.3280) and continues to decrease at the end of exposure with s_3=0.2394 as presented in Table 4.4.

Table 4.4: Total colour differences (ΔE) and saturation of purified colourant *Ixora siamensis* as affected by the addition of FA

FA (%)	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
0	s_0=0.3280	s_1=0.2900	s_2=0.2623	s_3=0.2394	ΔE_1=8.089	ΔE_3=28.253
1	s_0=0.3976	s_1=0.4703	s_2=0.5191	s_3=0.3277	ΔE_1=17.402	ΔE_3=13.958
2	s_0=0.5747	s_1=0.7858	s_2=0.8759	s_3=0.5049	ΔE_1=23.329	ΔE_3=6.996
3	s_0=0.5011	s_1=0.6456	s_2=0.7151	s_3=0.4274	ΔE_1=20.395	ΔE_3=9.600
4	s_0=0.3693	s_1=0.4078	s_2=0.4504	s_3=0.3043	ΔE_1=16.942	ΔE_3=14.982
5	s_0=0.3391	s_1=0.3590	s_2=0.3965	s_3=0.2867	ΔE_1=16.577	ΔE_3=15.654

4.3.2. Effect of pH on visual colour variation

Figure 4.13 displays the results of the colour parameters CIE L* of purified anthocyanin colourant from *Ixora* at different pH 1, 3, 5, 7, 9 and 11. The initial pH of purified is 3.6. After zero exposure time, the lightness percentage (L*) of purified anthocyanin colourant increased from sample at pH 1 (67.951 ± 0.013) until sample at pH 5 (71.264 ± 0.011). However, the L* values started to decrease when pH of the sample increased from pH 7 (L*=65.173 ± 0.014) until pH 11 (L*=61.275 ± 0.014). In addition, during exposure the L* parameter for purified anthocyanin colourant for all pH increases continuously from zero storage time until the end of storage. From the figure, sample at pH 11 exhibited to the lowest L* values before exposure (zero time) (61.275 ± 0.014) while towards the end of exposure L* increased rapidly to the highest value of (98.109 ± 0.012). In contrast, the lightness percentage at the beginning for purified anthocyanin at pH 1 was (67.951 ± 0.013) while it gradually increases with increasing exposure time and at the third month of exposure the L* values was the lowest compared to others (83.344 ± 0.013). This inferred that after three months of exposure the colour of sample at pH 11 was the lightest (higher L*) while the purified anthocyanin at pH 1 resulted in brighter or darker colours (lower L*), followed by sample at pH 3, and 3.6 which were (85.019 ± 0.011) and (89.364 ± 0.017) respectively.

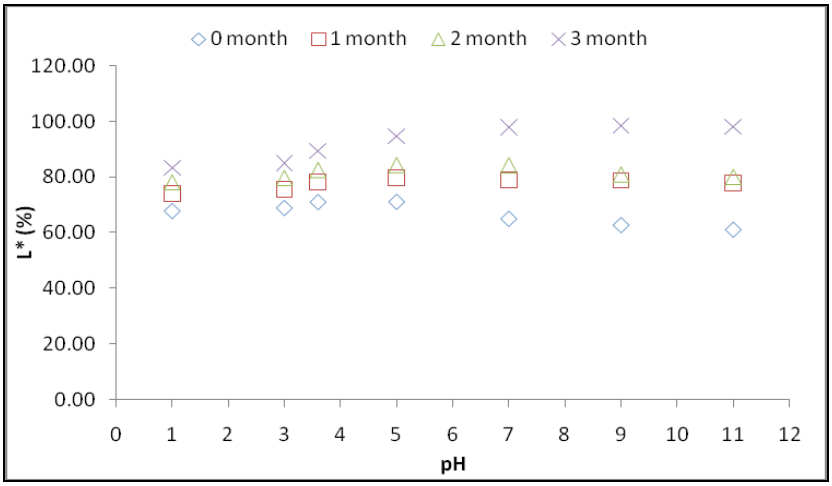


Figure 4.13: Relationship between pH variation and L* values (%) for purified colourant *Ixora siamensis* during three month of exposure

Furthermore, the chromaticity (C*) values in the beginning and at the end of storage are shown in Figure 4.14. The initial (zero time of exposure) chromaticity C* for the purified anthocyanin colourant decreased with increasing pH from pH 1 (33.297 ± 0.014) until pH 7 (12.063 ± 0.012) before increasing at pH 9 (30.660 ± 0.012) and decreasing again at pH 11 (12.515 ± 0.010). In addition, the C* value for purified anthocyanin colourant for all pH were observed to decrease continuously from zero time of exposure until the end of exposure (up to three months). In addition, the highest C* value for acidic purified anthocyanin colourant was exhibited by sample at pH 1 (C*=33.297 ± 0.014) at the beginning as well as the end of exposure (third month of exposure) with the values of C*=28.164 ± 0.015. Eventhough sample at pH 9 showed higher C* value at beginning and end of exposure, the sample already experienced the phenomena of browning from earlier towards the end of storage. The lowest C* values at zero time was exhibited by sample at pH 11 (12.515 ± 0.010) while after three month of exposure sample at pH 11 also exhibited the lowest C* values (8.807 ± 0.014), due to the colour loss.

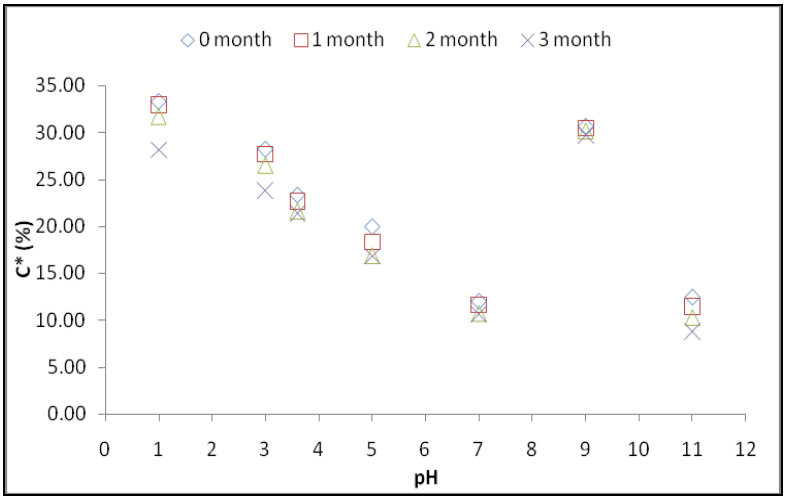
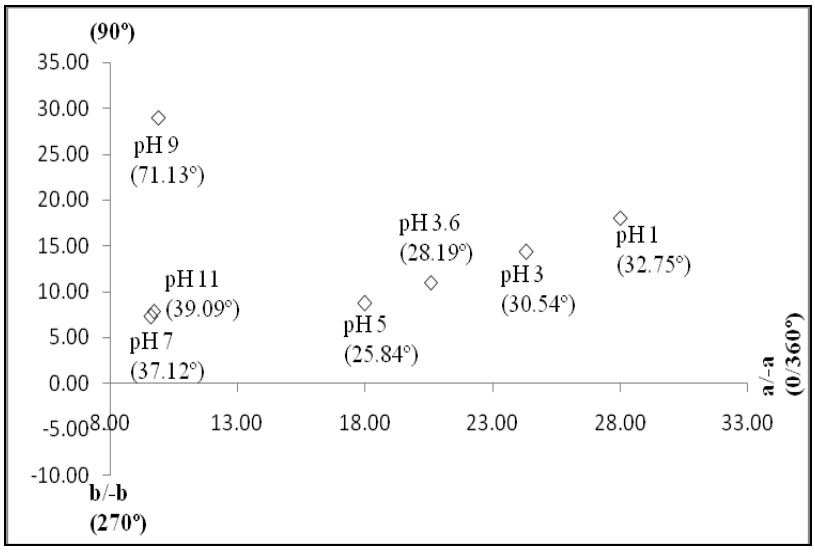


Figure 4.14: Relationship between pH variation and C* values (%) for purified colourant *Ixora siamensis* during three month of exposure

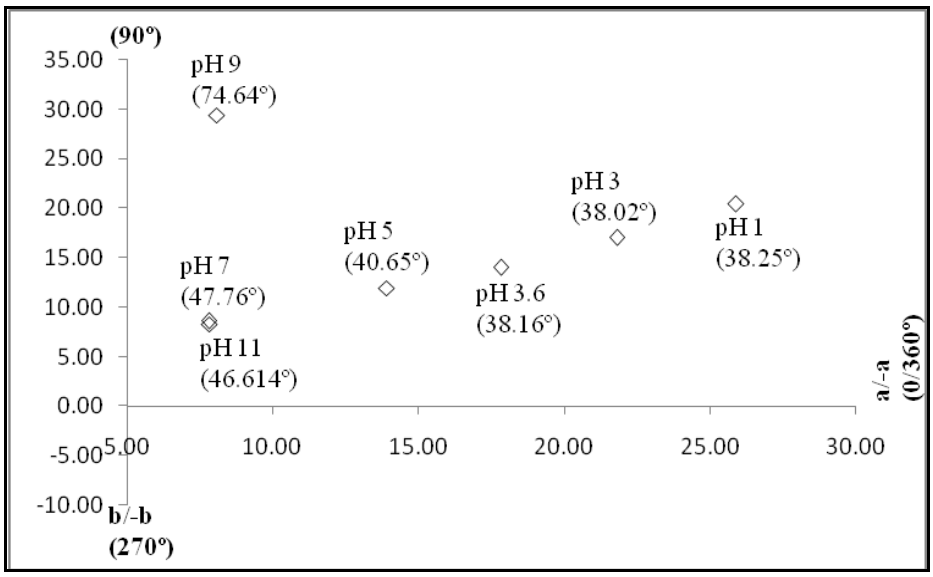
From Figure 4.15, the initial of hue angle, hº for purified anthocyanin colourant for all pH decreased from sample at pH 1 h°=(32.749 ± 0.016)° until sample at pH 5 h°=(25.838 ± 0.012)° and begins to increase at pH 7 h°=(37.123 ± 0.013)° until pH 9 h°=(71.132 ± 0.016)° before decreasing again at pH 11 h°=(39.085 ± 0.016)°. The hue angle of purified anthocyanin colourant for all pH increased significantly from the beginning of exposure until the third month of exposure, ranging from (32.749 ± 0.016)° to (68.786 ± 0.015)°, (30.543 ± 0.012)° to (77.128 ± 0.011)°, (28.188 ± 0.014)° to (85.804 ± 0.011)°, (25.838 ± 0.012)° to (86.557 ± 0.012)°, (37.123 ± 0.013)° to (85.675 ± 0.016)°, (71.132 ± 0.016)° to (88.263 ± 0.013)° and (39.085 ± 0.016)° to (85.186 ± 0.016)° for pH 1, 3, 3.6, 5, 7, 9 and 11 respectively as presented in Figure 4.15. During the three months of exposure, the purified anthocyanin sample at pH 9 exhibited the highest hue angle of (71.132 ± 0.016)° with a*=(9.915 ± 0.016) and b*=(29.013 ± 0.014). This is followed by sample at pH 11 with hue angle h°=(39.085 ± 0.016)°, a*=(9.715 ± 0.016) while the coordinate of b* to

(7.891 ± 0.017). After three months of exposure, sample at pH 9 again contributed to the highest hue angle of (88.263 ± 0.013)°, but the a* coordinate moved back to lower positive a*=(0.901 ± 0.014) and b* slightly increased to (29.726 ± 0.015) The hue angle for sample at pH 11=(85.186 ± 0.016)°, the a* moved to lower positive (0.739 ± 0.010) and b* to (8.776 ± 0.018). In addition, the hue angle of sample at pH 1 is (32.749 ± 0.016)° with highest a* of (28.005 ± 0.010) and b* value of (18.013 ± 0.014) at zero time, while at the end of exposure the hue angle increased to (68.786 ± 0.015)°, with a* value at (10.191 ± 0.014) and b* at (26.256 ± 0.015). The gradual degradation of red colour, visually observed in all pH, experienced by purified anthocyanin colourant is accompanied by the tonality changes from red to brown-yellow tints as hº increased during experiment time and is significant for samples at higher pH (pH 9 and 11).



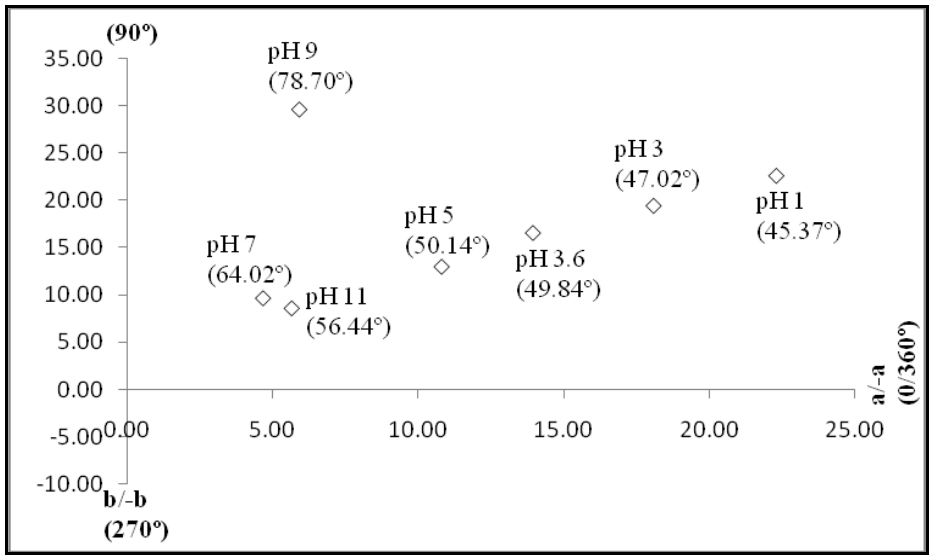
(a)

Figure 4.15: Relationship between pH variation and H◦ with a*b* coordinate for purified colourant *Ixora siamensis* during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



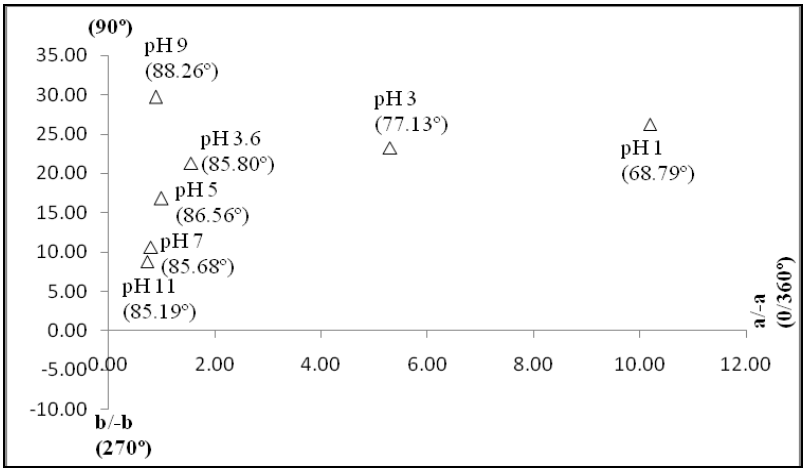
(b)

'Figure 4.15, continued'



(c)

'Figure 4.15, continued'



(d)

'Figure 4.15, continued'

Table 4.5 showed the total colour difference (ΔE), which was lowest for purified anthocyanin colourant at pH 1 which ΔE_1=6.978, during first month of exposure and is still the lowest at the end of exposure (ΔE_3=24.944). In contrast, the ΔE of purified anthocyanin colourant at pH 11 was the highest at zero time (ΔE_1=16.822) and at the end of exposure ΔE_3=37.922. Other purified anthocyanin colourant of different pH demonstrated a similar trend in colour change being low before exposure (zero time) and higher at the end of exposure from ΔE_1=7.483 to ΔE_3=26.361, ΔE_1=8.089 to ΔE_3=28.253, ΔE_1=10.041 to ΔE_3=30.049, ΔE_1=14.001 to ΔE_3=34.070 and ΔE_1=16.014 to ΔE_3=36.689 for pH 3, 3.6, 5, 7 and 9 respectively. In addition, the purified anthocyanin colourant at pH 1 exhibited the highest saturation parameter at time zero (s_0=0.4900) that decreased with increasing exposure time until the end of three months with (s_3=0.3379). Though saturation of sample at pH 9 was higher in the beginning, the colour tends to turn into brown. Other purified anthocyanin colourant with different pH showed similar trend. Purified anthocyanin

colourant at pH 11 exhibited the lowest saturation at time zero, (s_0=0.2042) and drastically decreased towards the end of exposure with (s_3=0.0898) as seen in Table 4.5.

Table 4.5: Total colour differences (ΔE) and saturation of purified colourant *Ixora siamensis* as affected by pH

pH	TIME (Month) 0	1	2	3	ΔE_1	ΔE_3
pH 1	s_0=0.4900	s_1=0.4447	s_2=0.4068	s_3=0.3379	ΔE_1=6.978	ΔE_3=24.944
pH 3	s_0=0.4088	s_1=0.3663	s_2=0.3334	s_3=0.2803	ΔE_1=7.483	ΔE_3=26.361
pH 3.6	s_0=0.3280	s_1=0.2900	s_2=0.2623	s_3=0.2394	ΔE_1=8.089	ΔE_3=28.253
pH 5	s_0=0.2806	s_1=0.2294	s_2=0.2002	s_3=0.1780	ΔE_1=10.041	ΔE_3=30.049
pH 7	s_0=0.1851	s_1=0.1469	s_2=0.1269	s_3=0.1090	ΔE_1=14.001	ΔE_3=34.070
pH 9	s_0=0.4874	s_1=0.3864	s_2=0.3737	s_3=0.3020	ΔE_1=16.014	ΔE_3=36.689
pH 11	s_0=0.2042	s_1=0.1461	s_2=0.1280	s_3=0.0898	ΔE_1=16.822	ΔE_3=37.922

4.3.3. Effect of addition 2% ferulic acid (FA) and pH on visual colour variation

Figure 4.16 displays the results of the colour parameters CIE L* for purified anthocyanin colourant from *Ixora* with addition of 2% FA and at different pH values. From previous results, the 2% FA acts as good colour enhancer and stabilizer. The initial (zero time of exposure) lightness percentage (L*) of purified anthocyanin colourant containing 2% FA with altered pH (initial pH (3.4), pH 1, 3, 5, 7, 9 and 11) were observed to increase from sample at pH 1 (L*=50.321 ± 0.005) until sample at pH 3 (L*=55.626 ± 0.008) and decreasing until pH 7 (L*=50.402 ± 0.008). L* increased at pH 9 (L*=59.124 ± 0.008)

before decreasing again at pH 11 (L*=50.554 ± 0.011). In addition, during exposure the L* parameter values for purified anthocyanin at pH 3, 3.4 and 5 were observed to decrease (darker colour) from initial L* value until the second month of exposure before increasing during the third month of exposure. The significant decrease in L* over two months of exposure was exhibited by the purified anthocyanin colourant at pH 3, with the initial L*=55.626 ± 0.008 that decrease to 44.237 ± 0.011. This is followed by the sample with pH 3.4, with L* decreasing from (55.125 ± 0.007) to (44.152 ± 0.011) and pH 5 from (L*=51.842 ± 0.012) to (L*=44.124 ± 0.014). In contrast, at other pH values (pH 1, 7, 9 and 11), L* continues to increase from zero time of exposure until the end of exposure where L* ranges from (50.321 ± 0.005) to (62.442 ± 0.010), (50.402 ± 0.008) to (69.574 ± 0.012), (59.124 ± 0.008) to (82.199 ± 0.012), and (50.554 ± 0.011) to (81.555 ± 0.011) respectively. After three months of exposure, the purified anthocyanin colourant containing 2% FA at pH 9 exhibited the lightest colour with highest L* of (82.199 ± 0.012), while the lowest L* (darkest colour) was exhibited by samples at pH 3 with (L*=57.667 ± 0.009).

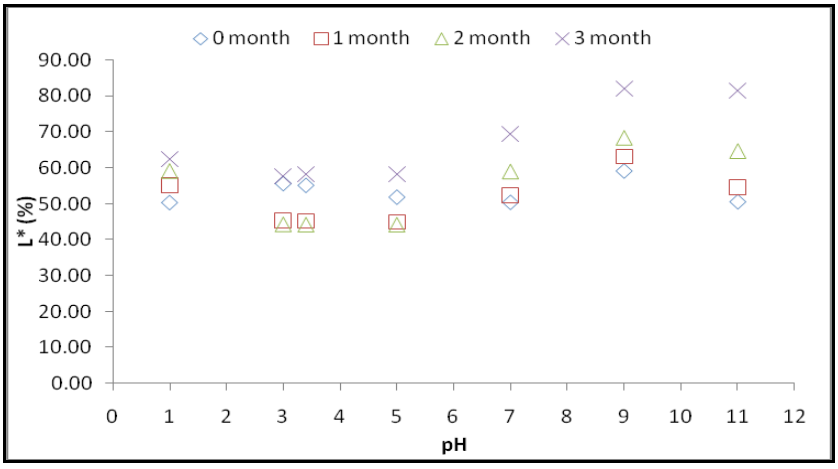


Figure 4.16: Relationship between pH variation and L* values (%) for purified colourant *Ixora siamensis* containing 2% FA during three month of exposure

The chromaticity (C*) values of purified anthocyanin colourant with altered pH (initial pH (3.4), pH 1, 3, 5, 7, 9 and 11) in the beginning and at the end of exposure are shown in Figure 4.17. The initial (zero time of exposure) C* value of the purified anthocyanin colourant containing 2% FA at altered pH (pH 3, 3.4 and 5) were observed to increase continuously until the second month of exposure, in which C* increased significantly (brightest colour) for sample at pH 3. The C* value increased from (33.263 ± 0.012) to (40.728 ± 0.004) before decreasing at the third month of exposure (C*=31.408 ± 0.010). This trend is followed by sample at pH 3.4. The initial C* value of (31.678 ± 0.012) increased on the second month to (C*=38.673 ± 0.006) before decreasing at the end of exposure time at C*=29.442 ± 0.007. For sample at pH 5, C* increased from (26.456 ± 0.014) to (33.439 ± 0.008) and decreased on the third month of exposure with C*=25.986 ± 0.008. For samples with other pH variations (pH 1, 7, 9 and 11), C* decreased continuously from zero time of exposure until the third month of exposure ranging from (40.716 ± 0.008) to (26.993 ± 0.011), (12.366 ± 0.013) to (11.337 ± 0.008), (38.114 ± 0.006) to (35.499 ± 0.004) and (15.522 ± 0.011) to (11.789 ± 0.014). Nevertheless, after three months of exposure, the purified anthocyanin colourant at pH 3 experienced the highest C* of (31.408 ± 0.010). Eventhough the purified anthocyanin colourant at pH 9 also experienced higher C* values browning of the sample indicate degradation. The lowest C* value was exhibited by samples at pH 7 (C*=11.337 ± 0.008) and pH 11 (C*=11.789 ± 0.014).

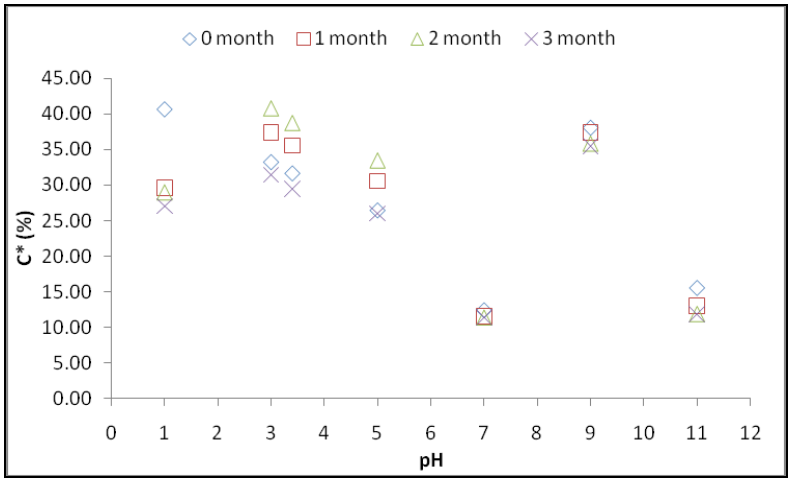
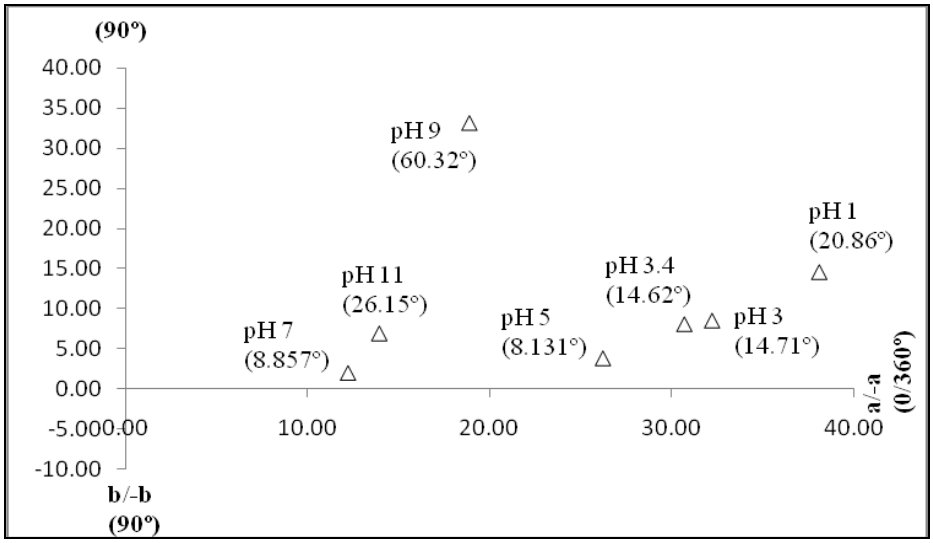


Figure 4.17: Relationship between pH variation and C* values (%) for purified colourant *Ixora siamensis* containing 2% FA during three month of exposure

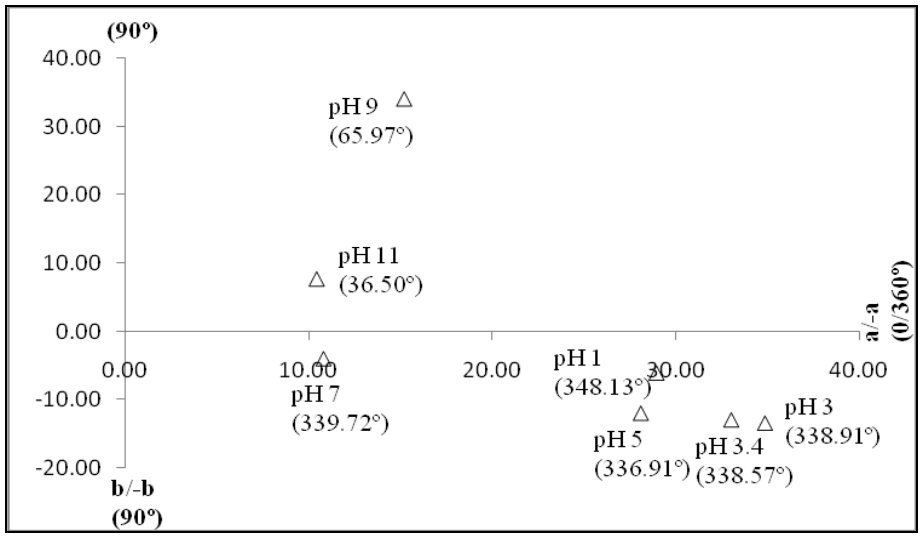
Additionally, the initial exposure of hue angle, hº values of the purified anthocyanin colourant containing 2% FA with different pH variation were observed decreased from sample at pH 1 (20.855 ± 0.012)° until sample at pH 7 (8.857 ± 0.012)°, whereas started to increase at pH 9 (60.316 ± 0.009)° and decrease again at pH 11 (26.145 ± 0.008)°. According to Figure 4.18, it can be noted that the hue angle of purified anthocyanin colourant with pH variation (pH 3, 3.4 and 5) continually moved clockwise into blue region from the zero time of exposure until the second month of exposure, ranging from hue angle (14.714 ± 0.008)° with positive a* (32.173 ± 0.011) and b* value (8.449 ± 0.010) moved to hue angle (333.390 ± 0.009)° with more positive a* (36.417 ± 0.009) and negative b* value (-18.238 ± 0.010) for sample at pH 3, hue angle of (14.616 ± 0.009)° with positive a* (30.653 ± 0.009) and b* value (7.994 ± 0.011) moved to (333.000 ± 0.009)° with more positive a* (34.459 ± 0.012) and negative b* value (-17.557 ± 0.012) for sample at pH 3.4 and hue angle of (8.131 ± 0.007)° with positive a* (26.191 ± 0.010) and b* value (3.742 ± 0.011) moved to (331.470 ± 0.010)° with more positive a* (29.381 ± 0.010) and negative

b* value (-15.968 ± 0.012) for sample at pH 5. At the third month of exposure, the corresponding pH (pH 3, 3.4 and 5) moved counterclockwise into red tonalities which hue angle were (21.931 ± 0.009)° with lower positive a* (29.136 ± 0.008) and b* value (11.731 ± 0.006) for sample at pH 3, hue angle of (25.540 ± 0.011)° with lower positive a* (26.565 ± 0.010) and b* value (12.694 ± 0.013) for sample at pH 3.4, whereas for sample at pH 5 the hue angle was (34.217 ± 0.010)° with lower positive a* (21.488 ± 0.007) and b* value (14.613 ± 0.008). For sample at pH 1 and 7, the hue angle also moved clockwise into the blue region but only during the first month of exposure since at the second month of exposure the samples have already moved counterclockwise into red tonalities and continues until the third month of exposure. In contrast, during the three months of exposure the purified anthocyanin colourant at pH 9 and 11 directly moved counterclockwise from the first month of exposure until the third month of exposure and the hue angle approaches the yellow region, to higher h°. At time zero, the hue angle for sample at pH 9 was the highest which h°=(60.316 ± 0.009)° with a*=(18.875 ± 0.012) and b*=(33.113 ± 0.007) while after three month of exposure, sample at pH 9 again contributed to the higher hue angle overall (88.371 ± 0.010)° but a* become less positive with a* value=(1.009 ± 0.013). The value of b* increased slightly to (35.485 ± 0.012). In addition, sample at pH 3 experienced lower hue angle of (14.714 ± 0.008)° with a*=(32.173 ± 0.011) and b*=(8.449 ± 0.010) at zero time. At the end of exposure the hue angle was lowest at (21.931 ± 0.009)°, with highest a* value of (29.136 ± 0.008) and b* value (11.731 ± 0.006). The gradual degradation of red colour, visually observed for purified anthocyanin colourant was accompanied by the tonality changes from red to brown-yellow tints and black colour as the hº increased with time. This is significant for samples at higher pH (pH 7, 9 and 11)

as can be seen in Figure 4.18. Furthermore, the h° values of lower pH (pH 1, 3, 3.4 and 5) showed vivid purple colours, especially for sample at pH 3, before turning back again into red colour tonalities at the end of exposure.

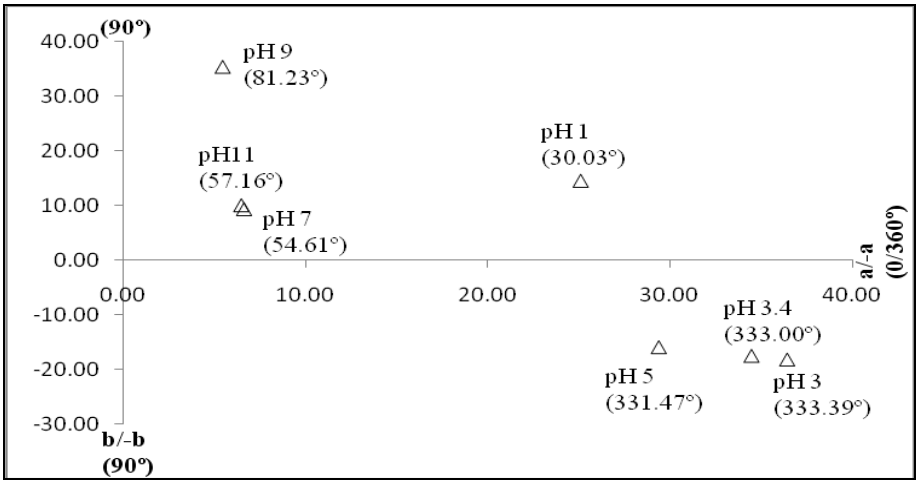


(a)



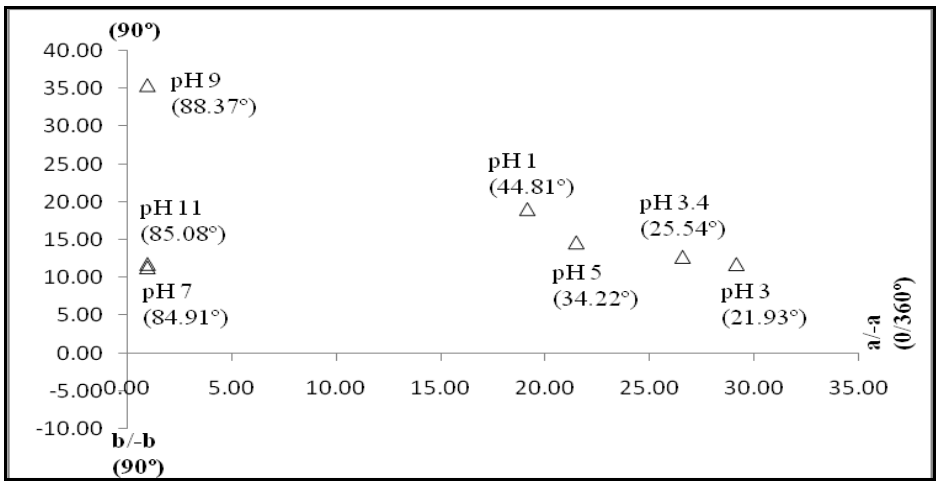
(b)

Figure 4.18: Relationship between pH variation and H◦ with a*b* coordinate for purified colourant *Ixora siamensis* containing 2% FA during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 4.18, continued'



(d)

'Figure 4.18, continued'

Table 4.6 showed the total colour difference (ΔE), which was the greatest for the purified anthocyanin colourant containing 2% FA at pH 3 where ΔE_1=24.373, at first month of exposure while lower colour change at the end of exposure (ΔE_3=4.915). Other purified anthocyanin colourant demonstrated a similar trend in ΔE, the highest being at zero time

and lower towards the end of exposure from ΔE_1=23.028 to ΔE_3=22.903, ΔE_1=23.329 to ΔE_3=6.996 and ΔE_1=17.275 to ΔE_3=13.470 for pH 1, 3.4 and 5 respectively. In contrast, the ΔE of purified anthocyanin colourant at pH 7, 9 and 11 were lower at zero time but increased at the end of storage period showing degradation. The purified anthocyanin colourant containing 2% FA at pH 3 exhibited the highest saturation index at the zero time, (s_0=0.5980), which increased with increasing exposure time until the second month of exposure (s_2=0.9207). Finally, at the third month of exposure, (s_3=0.5446) the saturation index dropped. Eventhough saturation index for sample at pH 1 is also high; it decreased with increasing of exposure time. The sample with pH 9 tends to degrade into brown as can be seen in Table 4.6. Sample at pH 11 exhibit the lowest saturation index, which at zero time (s_0=0.3070) and continues decrease towards the end of exposure (s_3=0.1445).

Table 4.6: Total colour differences (ΔE) and saturation of purified colourant *Ixora siamensis* with addition of 2% FA as affected by pH

pH	TIME (Month) 0	1	2	3	ΔE_1	ΔE_3
pH 1	s_0=0.8091	s_1=0.5356	s_2=0.4966	s_3=0.4323	ΔE_1=23.028	ΔE_3=22.903
pH 3	s_0=0.5980	s_1=0.8257	s_2=0.9207	s_3=0.5446	ΔE_1=24.373	ΔE_3=4.915
pH 3.4	s_0=0.5747	s_1=0.7858	s_2=0.8759	s_3=0.5049	ΔE_1=23.329	ΔE_3=6.996
pH 5	s_0=0.5103	s_1=0.6797	s_2=0.7578	s_3=0.4461	ΔE_1=17.275	ΔE_3=13.470
pH 7	s_0=0.2453	s_1=0.2198	s_2=0.1943	s_3=0.1629	ΔE_1=6.377	ΔE_3=24.114
pH 9	s_0=0.6446	s_1=0.5910	s_2=0.5245	s_3=0.4319	ΔE_1=5.537	ΔE_3=29.279
pH 11	s_0=0.3070	s_1=0.2379	s_2=0.1849	s_3=0.1445	ΔE_1=5.380	ΔE_3=33.943

CHAPTER 5: EXPERIMENTAL RESULTS ON COLOUR ANALYSIS OF ANTHOCYANIN-PVA BLENDS

5.1. Introduction

Colour is one of the most important attributes of product appearance that defined the quality of the products and it has a decisive influence on acceptance or rejection by consumers. Thus, it is important to maintain the colour appearance during exposure regardless to environmental and other factor influences. This chapter focused on the colour measurement analysis studies of the coating system made from crude and purified anthocyanin colourant blend with PVA that were exposed to fixed 17.55 lux intensity of UV-B irradiation. The influences of pH and co-pigmentation reaction of ferulic acid (FA), as colour enhancer on transformation of colour for all samples before and after exposed to UV-B irradiation during three month of exposure were observed and investigated.

5.2. Colour analysis on crude anthocyanin from *Ixora siamensis* blended with PVA

5.2.1. Effect of addition ferulic acid (FA) on visual colour variation

Table 5.1 presents the results of the colour parameters CIE L* of crude anthocyanin-PVA blends from *Ixora* added with different percentages (from 1% to 5%) of ferulic acid (FA). Initially at zero time, the lightness percentage (L*) of crude anthocyanin-PVA blends decreased from sample without presence of FA (64.288 ± 0.013) until sample with 2% FA (45.971 ± 0.012). The L* values start to increase when percentage of FA increased being (46.267 ± 0.016) for sample with 3% FA added and (63.059 ± 0.014) when FA added was 5%. During exposure, the L* parameter values for crude anthocyanin-PVA blends without addition of FA increase continually from zero time of exposure (64.288 ± 0.013) until the

third month of exposure (76.206 ± 0.012). On the other hand, the lightness percentages for crude anthocyanin-PVA blends with addition of FA decreased (to darker colour) from zero time of exposure until second month of exposure before increase at the third month of exposure. Slightly decrease in L* over two months of exposure was exhibited by the crude anthocyanin-PVA blends with addition of 5% FA, the initial L* of which was (63.059 ± 0.014) and decreased to (55.063 ± 0.020), followed by the samples added with 4% FA the L* of which was (60.873 ± 0.015) that decreased to (69.862 ± 0.015). The highest decrease in L* (darker colour) was experienced by the crude anthocyanin-PVA blends with addition of 2% FA. L* decreased from (45.971 ± 0.012) to (32.762 ± 0.013). After three month of exposure, the crude anthocyanin-PVA blends without addition of FA exhibited the highest L* value (lightest colour) of (76.206 ± 0.012), while the lowest L* (darker colour) was exhibited by the sample with addition of 2% FA (46.998 ± 0.014). The trend can be further observed in Figure 5.1.

Table 5.1: Statistical summary of CIE L* colour data for crude anthocyanin-PVA blends with addition of FA

CIE value	Time (month)	FA (%)	Mean $_a$ ± s.e.	Minimum	Maximum
L*	0	0	64.288_{7} ± 0.013	64.265	64.311
		1	57.575_{10}± 0.015	57.549	57.602
		2	45.971_{20}± 0.012	45.949	45.992
		3	46.267_{19}± 0.016	46.239	46.294
		4	60.873_{9} ± 0.015	60.846	60.899
		5	63.059_{8}± 0.014	63.035	63.083
	1	0	69.289_{5} ± 0.015	69.262	69.315
		1	47.862_{16} ±0.018	47.831	47.893
		2	34.773_{22} ±0.012	34.753	34.793
		3	35.662_{21} ±0.010	35.644	35.680
		4	52.456_{13} ±0.014	52.431	52.481
		5	55.764_{11} ±0.019	55.731	55.797
	2	0	72.291_{3} ± 0.016	72.263	72.319
		1	46.877_{18} ±0.013	46.854	46.900
		2	32.762_{24} ±0.013	32.740	32.784
		3	33.819_{23} ±0.017	33.789	33.849
		4	51.653_{14} ±0.016	51.626	51.681
		5	55.063_{12} ±0.020	55.029	55.098
	3	0	76.206_{1} ± 0.012	76.186	76.226
		1	65.573_{6} ± 0.019	65.539	65.606
		2	46.998_{17} ±0.014	46.974	47.021
		3	50.153_{15} ±0.009	50.137	50.169
		4	69.862_{4} ± 0.015	69.836	69.889
		5	72.873_{2} ± 0.012	72.853	72.893

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with addition of FA

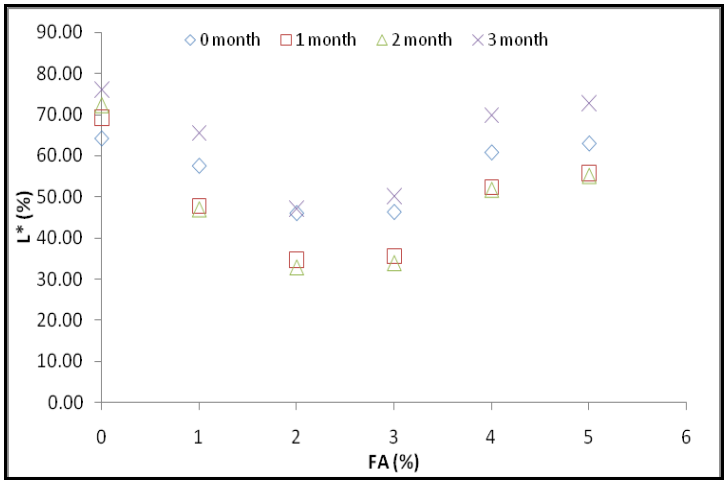


Figure 5.1: Relationship between percentage of FA and L* values (%) for crude anthocyanin-PVA blends during three month of exposure

In addition, the chromaticity (C*) values in the beginning and at the end of exposure was shown in Table 5.2. The initial (zero time of exposure) C* values of the crude anthocyanin-PVA blends were observed to increase from the sample without addition of FA (38.703 ± 0.010) to the sample with addition 2% FA the C* value of which was (43.739 ± 0.010). C* then decreased to (42.950 ± 0.011) for sample with 3% FA and further decreased to (38.570 ± 0.015) for sample added with 5% FA. The C* for crude anthocyanin-PVA blends without addition of FA decreased continuously during exposure, from zero time of exposure with C*=38.703 ± 0.010 until the end of exposure (up to three month) with C*=29.169 ± 0.017. The trend can be further observed in Figure 5.2. In contrast, the C* values for crude anthocyanin-PVA blends with addition of FA increased from zero time exposure until second month of exposure before decreasing on the third month of exposure. The crude anthocyanin-PVA sample added with 2% FA exhibited the highest C* (brightest colour) over two month of exposure from (43.739 ± 0.010) to (52.030 ± 0.012). There was small increase in C* values recorded for sample with presence of 5% FA, where the zero time C*

value increase from (38.570 ± 0.015) to (40.360 ± 0.017). After three month of exposure, the crude anthocyanin-PVA blends without addition of FA experienced the lowest C* of (29.169 ± 0.017) while the highest C* was exhibited by the sample with addition of 2% FA, C*=(41.392 ± 0.009).

Table 5.2: Statistical summary of CIE C* colour data for crude anthocyanin-PVA blends with addition of FA

CIE value	Time (month)	FA (%)	Mean $_a$ ± s.e.	Minimum	Maximum
C*	0	0	38.703_{15} ±0.010	38.685	38.720
		1	40.466_{12} ±0.020	40.432	40.500
		2	43.739_{6} ± 0.010	43.721	43.757
		3	42.950_{7} ± 0.011	42.930	42.969
		4	39.877_{14} ±0.018	39.846	39.908
		5	38.570_{16} ±0.015	38.544	38.596
	1	0	37.926_{18} ±0.012	37.904	37.947
		1	41.865_{9} ± 0.014	41.842	41.889
		2	47.716_{3} ± 0.006	47.705	47.727
		3	45.334_{4} ± 0.013	45.312	45.356
		4	40.587_{11} ±0.012	40.567	40.607
		5	38.685_{15} ±0.017	38.655	38.714
	2	0	34.770_{21} ±0.014	34.746	34.794
		1	44.256_{5} ± 0.009	44.240	44.272
		2	52.030_{1} ± 0.012	52.010	52.050
		3	49.181_{2} ± 0.011	49.162	49.200
		4	42.568_{8} ± 0.013	42.545	42.591
		5	40.360_{13} ±0.017	40.331	40.389
	3	0	29.169_{22} ±0.017	29.139	29.199
		1	38.201_{17} ±0.011	38.182	38.221
		2	41.392_{10} ±0.009	41.376	41.407
		3	40.482_{12} ±0.012	40.461	40.503
		4	37.699_{19} ±0.012	37.679	37.719
		5	36.951_{20} ±0.013	36.929	36.973

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with addition of FA

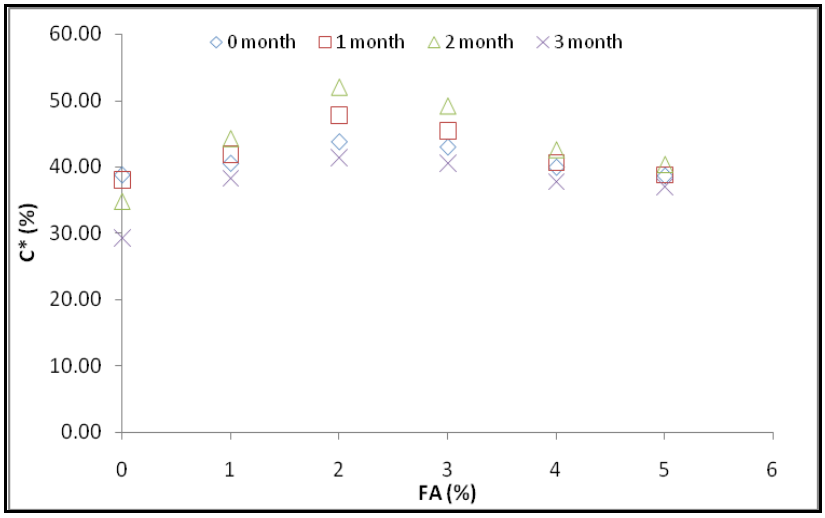


Figure 5.2: Relationship between percentage of FA and C* values (%) for crude anthocyanin-PVA blends during three month of exposure

Table 5.3 shows the initial hue of h°. h° shows a decrease for the crude anthocyanin-PVA blends without addition of FA at (23.072 ± 0.015)° until the sample with 2% FA with h°=(15.890 ± 0.008)°. On further addition of FA, h° increased to (16.680 ± 0.016)° for sample with 3% FA and continual to increase up to 5% FA when h°=(19.461 ± 0.016)°. The initial hue angle (h_{ab})° of crude anthocyanin-PVA blends without presence of FA is (23.072 ± 0.015)° with coordinate a* as (35.608 ± 0.013) and coordinate b* as (15.168 ± 0.012). At the end of UV exposure, h_{ab}°=(57.766 ± 0.012)°, but with a lower a* coordinate, a*=(15.558 ± 0.015) and higher b*coordinate of (24.674 ± 0.022). In contrast, immediately after addition of FA to the crude anthocyanin-PVA blends and after first month of exposure, a significant increment of the hue angle ranging from (17.845 ± 0.015)° to (345.410 ± 0.014)°, (15.890 ± 0.008)° to (340.770 ± 0.007)°, (16.680 ± 0.016)° to (343.860 ± 0.010)°, (18.484 ± 0.018)° to (345.420 ± 0.011)° and (19.461 ± 0.016)° to (345.030 ± 0.016)° respectively for 1, 2, 3, 4 and 5% FA before decreasing again after the third month

of exposure, as shown in Figure 5.3. For samples with presence of FA, the hue angle moved clockwise (negative) into blue region during the second month of exposure and is most significant for samples with addition of 2% FA since the initial hue angle, (15.890 ± 0.008)° moved to (340.770 ± 0.007)° with a*=(42.068 ± 0.014) that moved to (45.055 ± 0.011) and b*=(11.976 ± 0.013) that moved to (-15.713 ± 0.007). On the second month of exposure, coordinate a* increased to (47.457 ± 0.012) and b* to (-21.330 ± 0.006) with hue angle to (335.790 ± 0.009)°. However, towards the end of exposure, during the third month of storage, the hue angle moved counterclockwise into red region with hue angle (20.966 ± 0.007)°, while a* moved backward to lower positive (38.652 ± 0.012) and more positive of b* value (14.811 ± 0.006). In addition, the gradual degradation of red colour, visually observed in all systems is more significant for crude anthocyanin-PVA blends without FA addition. As the h° increases, tonality changes from red to yellow tints can be observed. The hue angles of crude anthocyanin-PVA with FA were higher than that of the FA free crude anthocyanin-PVA over two months of exposure. The FA added samples showed vivid purple colours, especially for samples with 2% FA, before turning back to show red colour tonalities again.

Table 5.3: Statistical summary of CIE H°a*b* colour data for crude anthocyanin-PVA blends with addition of FA

CIE value	Time (month)	FA (%)	Mean $_a$ ± s.e.	Minimum	Maximum
H°	0	0	23.072_{17}± 0.015	23.046	23.097
		1	17.845_{21}± 0.015	17.819	17.872
		2	15.890_{23}± 0.008	15.876	15.904
		3	16.680_{22}± 0.016	16.652	16.707
		4	18.484_{20}± 0.018	18.452	18.515
		5	19.461_{19}± 0.016	19.434	19.488
	1	0	26.664_{15}± 0.014	26.640	26.689
		1	345.410_{1}±0.014	345.386	345.435
		2	340.770_{6}±0.007	340.758	340.783
		3	343.860_{3}±0.010	343.842	343.877
		4	345.420_{1}±0.011	345.401	345.439
		5	345.030_{2}±0.016	345.002	345.059
	2	0	38.318_{11}± 0.014	38.295	38.342
		1	340.510_{7}±0.012	340.489	340.532
		2	335.790_{9}±0.009	335.775	335.805
		3	338.500_{8}±0.015	338.474	338.527
		4	341.070_{5}±0.015	341.043	341.096
		5	341.140_{4}±0.019	341.106	341.173
	3	0	57.766_{10}±0.012	57.745	57.787
		1	30.165_{14}± 0.015	30.139	30.192
		2	20.966_{18}± 0.007	20.954	20.978
		3	25.427_{16}± 0.012	25.406	25.449
		4	31.397_{13}± 0.017	31.368	31.426
		5	33.228_{12}± 0.016	33.201	33.255

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with addition of FA

'Table 5.3, continued'

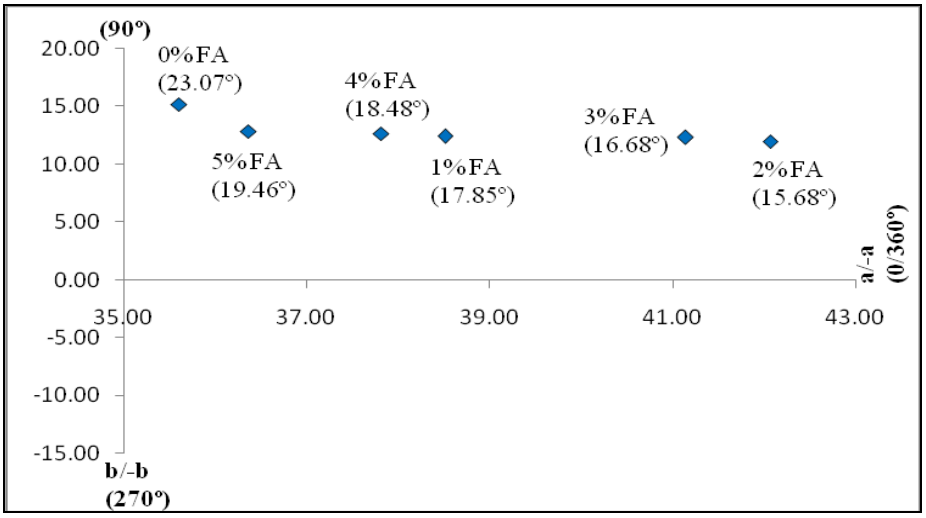
CIE value	Time (month)	FA (%)	Mean $_a$ ± s.e.	Minimum	Maximum
a*	0	0	35.608_{18}± 0.013	35.586	35.630
		1	38.519_{12}± 0.019	38.486	38.551
		2	42.068_{5} ± 0.014	42.045	42.092
		3	41.143_{7} ± 0.011	41.124	41.163
		4	37.820_{14}± 0.011	37.801	37.838
		5	36.367_{17}± 0.015	36.342	36.393
	1	0	33.893_{19}± 0.018	33.861	33.925
		1	40.517_{8} ± 0.016	40.489	40.544
		2	45.055_{3} ± 0.011	45.035	45.074
		3	43.550_{4} ± 0.015	43.523	43.576
		4	39.281_{10}± 0.009	39.265	39.297
		5	37.374_{15}± 0.011	37.354	37.393
	2	0	27.280_{23}± 0.017	27.251	27.309
		1	41.722_{6} ± 0.015	41.696	41.749
		2	47.457_{1} ± 0.012	47.436	47.478
		3	45.762_{2} ± 0.016	45.734	45.789
		4	40.268_{9} ± 0.014	40.243	40.292
		5	38.195_{13}± 0.015	38.168	38.221
	3	0	15.558_{24}± 0.015	15.532	15.583
		1	33.028_{20}± 0.016	33.000	33.057
		2	38.652_{11}± 0.012	38.632	38.673
		3	36.561_{16}± 0.015	36.536	36.587
		4	32.179_{21}± 0.012	32.159	32.199
		5	30.910_{22}± 0.011	30.891	30.929

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with addition of FA

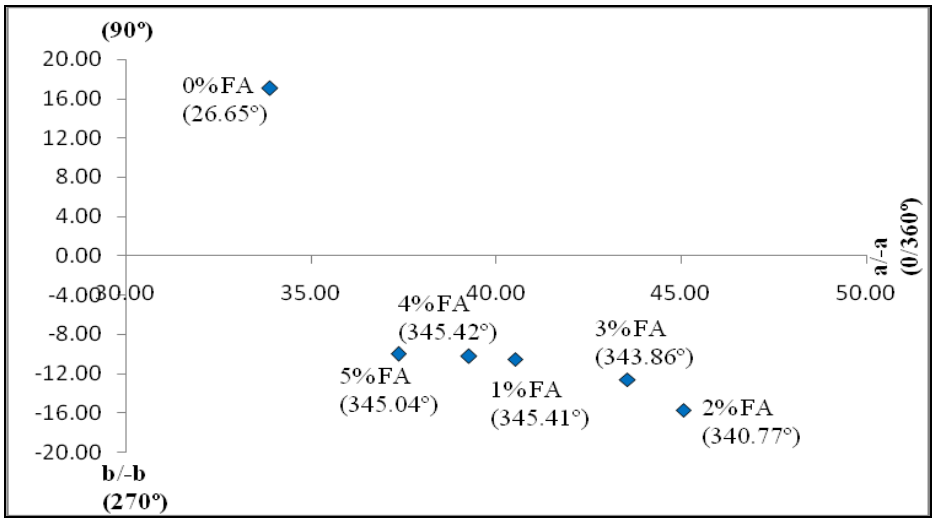
'Table 5.3, continued'

CIE value	Time (month)	FA (%)	Mean $_a$ ± s.e.	Minimum	Maximum
b*	0	0	15.168_{11}±0.012	15.146	15.189
		1	12.401_{19}± 0.011	12.381	12.420
		2	11.976_{21}± 0.013	11.954	11.998
		3	12.328_{20}± 0.015	12.301	12.354
		4	12.643_{17}± 0.012	12.622	12.664
		5	12.851_{16}± 0.016	12.824	12.878
	1	0	17.020_{9} ± 0.018	16.989	17.052
		1	-10.542_{22}±0.016	10.514	10.569
		2	-15.713_{10}±0.007	15.701	15.725
		3	-12.596_{18}±0.009	12.580	12.611
		4	-10.215_{23}±0.011	10.196	10.234
		5	-9.989_{25}± 0.014	9.965	10.013
	2	0	21.559_{2} ± 0.017	21.529	21.589
		1	-14.761_{13}±0.011	14.742	14.781
		2	-21.330_{3}± 0.006	21.319	21.340
		3	-18.017_{7}± 0.010	18.000	18.034
		4	-13.805_{14}±0.008	13.791	13.820
		5	-13.044_{15}±0.013	13.021	13.067
	3	0	24.674_{1} ± 0.022	24.636	24.713
		1	19.196_{6} ± 0.016	19.169	19.224
		2	14.811_{12}± 0.006	14.801	14.822
		3	17.382_{8} ± 0.012	17.362	17.402
		4	19.640_{5} ± 0.012	19.619	19.662
		5	20.249_{4} ± 0.016	20.221	20.277

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with addition of FA

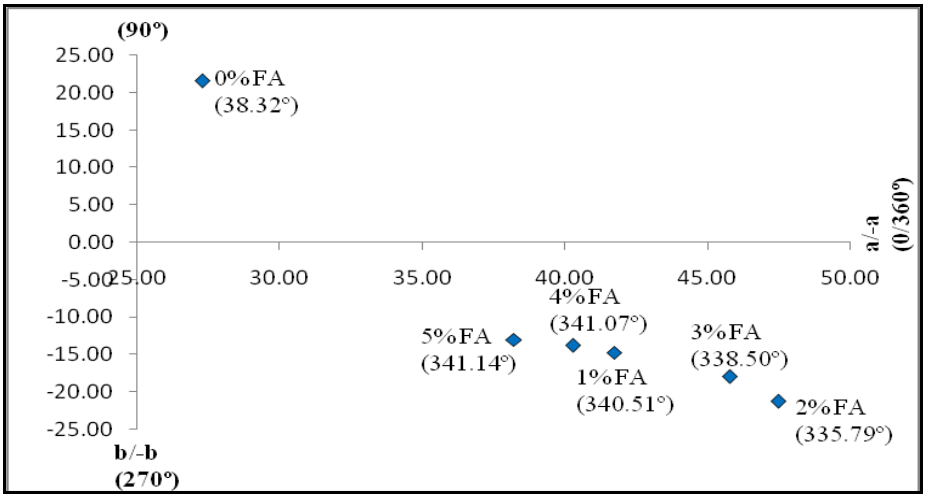


(a)



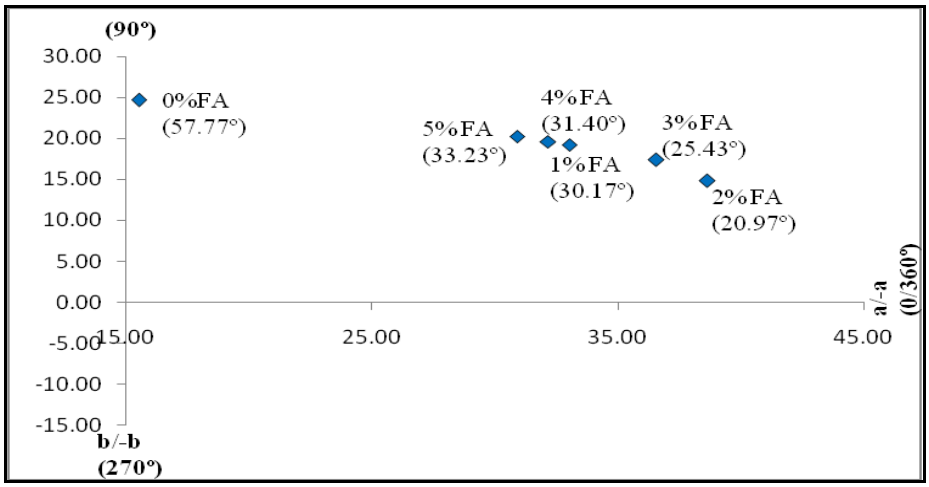
(b)

Figure 5.3: Relationship between percentage of FA and H◦ with a*b* coordinate for crude anthocyanin-PVA blends during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 5.3, continued'



(d)

'Figure 5.3, continued'

From Table 5.4, ΔE was the greatest for the crude anthocyanin-PVA blends with addition of 2% FA with ΔE_1=30.017, at first month of exposure. The sample exhibited a lower colour change (ΔE_3=4.556) at the end of exposure. The other solution with added FA demonstrated a similar trend, which is highest before exposure (zero time) and lowest at the

end of exposure from ΔE_1=24.994to ΔE_3=11.844, ΔE_1=27.193 to ΔE_3=7.851, ΔE_1=24.402 to ΔE_3=12.711, and ΔE_1=23.998 to ΔE_3=13.447, respectively for 1, 3, 4 and 5% of FA. In contrast, ΔE for crude anthocyanin-PVA blends without FA was the lowest before exposure (zero time) (ΔE_1=5.602) but increased to ΔE_3=25.187 at the end of exposure. For all FA added samples, ΔE was higher than that of the crude antocyanin-PVA blends with the highest value ΔE for 2% FA added samples. This sample also exhibited the highest saturation parameter at zero time, s_0=0.9514 for the 2% FA added sample. The saturation increased with increasing of exposure time until the second month of exposure (s_2=1.5881) before decreased to (s_3=0.8807) at the end of storage. Other FA added samples also showed similar trend, but with smaller value. The crude anthocyanin-PVA blends without addition of FA exhibits the lowest saturation parameter before exposure (zero time) (s_0=0.6020) and continues to decrease at the end of exposure with s_3=0.3828 as presented in Table 5.4.

Table 5.4: Total colour differences (ΔE) and saturation of crude anthocyanin-PVA blends as affected by the addition of FA

FA (%)	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
0	s_0=0.6020	s_1=0.5474	s_2=0.4810	s_3=0.3828	ΔE_1=5.602	ΔE_3=25.187
1	s_0=0.7028	s_1=0.8747	S_2=0.9441	s_3=0.5826	ΔE_1=24.994	ΔE_3=11.844
2	s_0=0.9514	s_1=1.3722	s_2=1.5881	s_3=0.8807	ΔE_1=30.017	ΔE_3=4.556
3	s_0=0.9283	s_1=1.2712	s_2=1.4542	s_3=0.8072	ΔE_1=27.193	ΔE_3=7.851
4	s_0=0.6551	s_1=0.7737	s_2=0.8241	s_3=0.5396	ΔE_1=24.402	ΔE_3=12.711
5	s_0=0.6116	s_1=0.6937	s_2=0.7330	s_3=0.5071	ΔE_1=23.998	ΔE_3=13.447

5.2.2. Effect of pH on visual colour variation

Table 5.5 displays the results of the colour parameters CIE L* of crude anthocyanin-PVA blends from *Ixora* at different pH 1, 3, 5, 7, 9 and 11. The initial pH of crude anthocyanin-PVA is 3.8. After zero exposure time, the lightness percentage (L*) of crude anthocyanin-PVA blends increased from sample at pH 1 (58.019 ± 0.011) until sample at pH 5 (65.377 ± 0.016). However, the L* values started to decrease when pH of the sample increased from pH 7 (L*=60.266 ± 0.014) until pH 11 (L*=57.181 ± 0.014). In addition, during exposure the L* parameter for crude anthocyanin-PVA for all pH increases continuously from zero storage time until the end of storage. From the Figure 5.4, sample at pH 11 exhibited to the lowest L* values before exposure (zero time) (57.181 ± 0.014) while towards the end of exposure L* increased rapidly to the highest value of (89.884 ± 0.019). In contrast, the lightness percentage at the beginning for purified anthocyanin-PVA at pH 1 was (58.019 ± 0.011) while it gradually increases with increasing exposure time and at the third month of exposure the L* values was the lowest compared to others (68.092 ± 0.016). This inferred that after three months of exposure the colour of sample at pH 11 was the lightest (higher L*) while the crude anthocyanin-PVA at pH 1 resulted in brighter or darker colours (lower L*), followed by sample at pH 3, and 3.8 which were (71.292 ± 0.016) and (76.206 ± 0.012) respectively.

Table 5.5: Statistical summary of CIE L* colour data for crude anthocyanin-PVA blends with different pH

CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
L*	0	1	58.019_{26}± 0.011	57.999	58.038
		3	60.162_{25}± 0.012	60.141	60.184
		3.8	64.288_{22}± 0.013	64.265	64.311
		5	65.377_{19}± 0.016	65.349	65.406
		7	60.266_{24}± 0.014	60.242	60.289
		9	57.285_{27}± 0.012	57.265	57.306
		11	57.181_{28}± 0.014	57.157	57.204
	1	1	61.601_{23}± 0.011	61.582	61.619
		3	64.577_{20}± 0.013	64.554	64.599
		3.8	69.289_{15}± 0.015	69.262	69.315
		5	71.662_{11}± 0.011	71.642	71.681
		7	70.149_{13}± 0.015	70.123	70.175
		9	68.829_{16}± 0.016	68.801	68.857
		11	69.898_{14}± 0.016	69.871	69.925
	2	1	64.384_{21}± 0.010	64.366	64.402
		3	67.394_{18}± 0.016	67.365	67.422
		3.8	72.291_{10}± 0.016	72.263	72.319
		5	74.945_{6} ± 0.013	74.923	74.967
		7	73.933_{8} ± 0.018	73.902	73.963
		9	73.618_{9} ± 0.013	73.595	73.641
		11	74.551_{7} ± 0.018	74.520	74.583
	3	1	68.092_{17}± 0.016	68.065	68.120
		3	71.292_{12}± 0.016	71.265	71.320
		3.8	76.206_{5} ± 0.012	76.186	76.226
		5	82.375_{4} ± 0.015	82.349	82.402
		7	88.608_{3} ± 0.010	88.591	88.625
		9	88.719_{2} ± 0.014	88.695	88.742
		11	89.884_{1} ± 0.019	89.851	89.916

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with different pH

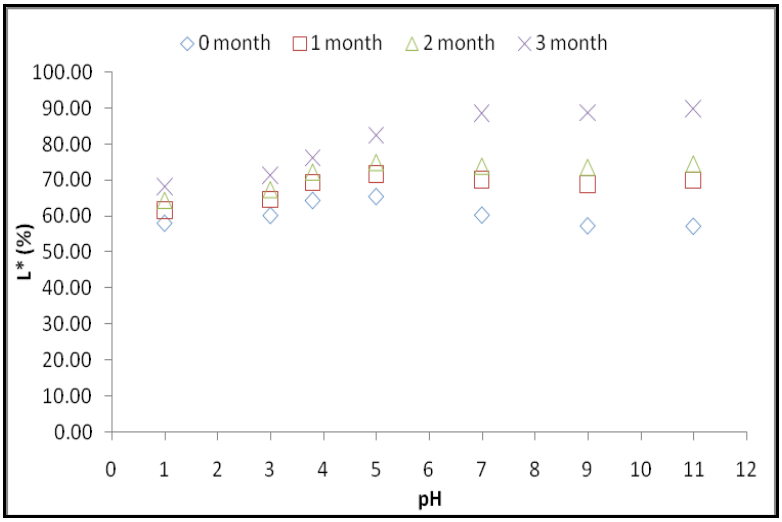


Figure 5.4: Relationship between pH variation and L* values (%) for crude anthocyanin-PVA blends during three month of exposure

In addition, the chromaticity (C*) values in the beginning and at the end of storage are shown in Table 5.6. The initial (zero time of exposure) chromaticity C* for the crude anthocyanin-PVA blends decreased with increasing pH from pH 1 (49.180 ± 0.009) until pH 7 (18.468 ± 0.015) before increasing at pH 9 (45.212 ± 0.013) and decreasing again at pH 11 (18.202 ± 0.012). In addition, the C* value for crude anthocyanin-PVA blends for all pH were observed to decrease continuously from zero time of exposure until the end of exposure (up to three months), as presented in Figure 5.5. In addition, the highest C* value for acidic crude anthocyanin-PVA was exhibited by sample at pH 1 (C*=49.180 ± 0.009) at the beginning as well as the end of exposure (third month of exposure) with the values of C*=39.780 ± 0.013. Eventhough sample at pH 9 showed higher C* value at beginning and end of exposure, the sample exhibited the phenomena of browning towards the end of storage. The lowest C* values at zero time was exhibited by sample at pH 11 (18.202 ±

0.012) while after three month of exposure sample at pH 11 also exhibited the lowest C* values (13.094 ± 0.015), due to the colour loss.

Table 5.6: Statistical summary of CIE C* colour data for crude anthocyanin-PVA blends with different pH

CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
C*	0	1	49.180_{1} ± 0.009	49.164	49.196
		3	41.497_{8} ± 0.015	41.471	41.522
		3.8	38.703_{11}± 0.010	38.685	38.720
		5	31.907_{15}± 0.015	31.881	31.932
		7	18.468_{21}± 0.015	18.442	18.494
		9	45.212_{4} ± 0.013	45.189	45.235
		11	18.202_{22}± 0.012	18.181	18.223
	1	1	49.075_{2} ± 0.012	49.053	49.096
		3	40.923_{9} ± 0.013	40.901	40.946
		3.8	37.926_{12}± 0.012	37.904	37.947
		5	30.108_{17}± 0.012	30.087	30.129
		7	16.863_{23}± 0.013	16.841	16.886
		9	44.397_{5} ± 0.014	44.373	44.421
		11	16.745_{24}± 0.011	16.725	16.764
	2	1	46.100_{3} ± 0.012	46.079	46.121
		3	37.586_{13}± 0.016	37.558	37.615
		3.8	34.770_{14}± 0.014	34.746	34.794
		5	26.445_{19}± 0.014	26.421	26.469
		7	15.412_{26}± 0.014	15.387	15.436
		9	43.994_{6} ± 0.017	43.965	44.024
		11	15.466_{25}± 0.019	15.432	15.499
	3	1	39.780_{10}± 0.013	39.757	39.802
		3	31.390_{16}± 0.012	31.369	31.411
		3.8	29.169_{18}± 0.017	29.139	29.199
		5	23.114_{20}± 0.016	23.086	23.142
		7	14.393_{27}± 0.019	14.359	14.426
		9	43.546_{7} ± 0.020	43.512	43.581
		11	13.094_{28}± 0.015	13.068	13.119

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with different pH

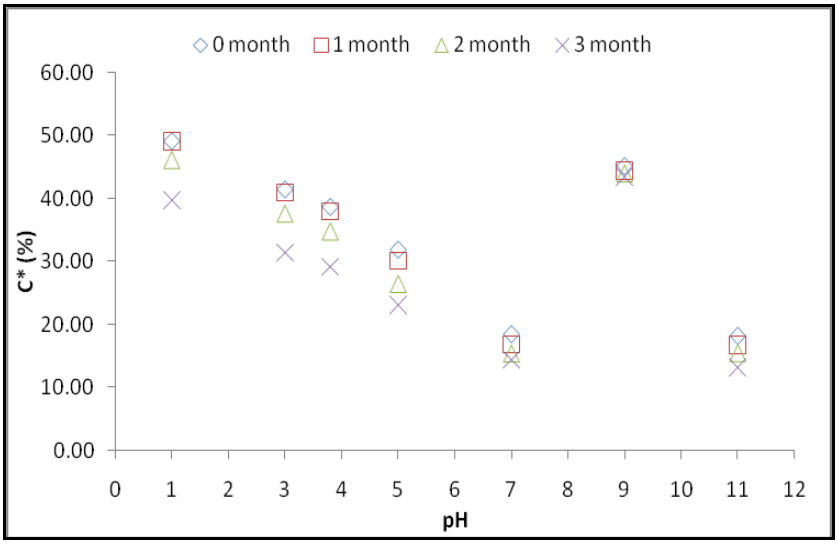


Figure 5.5: Relationship between pH variation and C* values (%) for crude anthocyanin-PVA blends during three month of exposure

From Table 5.7, the initial of hue angle, hº for crude anthocyanin-PVA blends for all pH decreased from sample at pH 1 h°=(28.001 ± 0.011)° until sample at pH 5 h°=(14.845 ± 0.018)° and begins to increase at pH 7 h°=(22.215 ± 0.014)° until pH 9 h°=(68.941 ± 0.017)° before decreasing again at pH 11 h°=(37.937 ± 0.016)°. The hue angle of crude anthocyanin-PVA blends for all pH increased significantly from the beginning of exposure until the third month of exposure, ranging from (28.001 ± 0.011)° to (51.149 ± 0.014)°, (23.445 ± 0.014)° to (53.508 ± 0.013)°, (23.072 ± 0.015)° to (57.766 ± 0.012)°, (14.845 ± 0.018)° to (56.197 ± 0.016)°, (22.215 ± 0.014)° to (55.261 ± 0.013)°, (68.941 ± 0.017)° to (80.664 ± 0.016)° and (37.937 ± 0.016)° to (65.832 ± 0.017)° for pH 1, 3, 3.8, 5, 7, 9 and 11 respectively as presented in Figure 5.6. During the three months of exposure, the crude anthocyanin-PVA sample at pH 9 exhibited the highest hue angle of (68.941 ± 0.017)° with a*=(16.246 ± 0.014) and b*=(42.193 ± 0.014). This is followed by sample at pH 11 with hue angle h°=(37.937 ± 0.016)°, a*=(14.356 ± 0.014) while the coordinate of b* to (11.191

± 0.017). After three months of exposure, sample at pH 9 again contributed to the highest hue angle of (80.664 ± 0.016)°, but the a* coordinate moved back to lower positive a*=(7.064 ± 0.012) and b* slightly increased to (42.970 ± 0.014) The hue angle for sample at pH 11=(65.832 ± 0.017)°, the a* moved to lower positive (24.954 ± 0.014) and b* to (11.947 ± 0.016). In addition, the hue angle of sample at pH 1 is (28.001 ± 0.011)° with highest a* of (43.423 ± 0.014) and b* value of (23.090 ± 0.013) at zero time, while at the end of exposure the hue angle increased to (51.149 ± 0.014)°, with a* value at (24.954 ± 0.014) and b* at (30.981 ± 0.016). The gradual degradation of red colour, visually observed in all pH, experienced by crude anthocyanin-PVA blends is accompanied by the tonality changes from red to brown-yellow tints as h^o increased during experiment time and is significant for samples at higher pH (pH 9 and 11).

Table 5.7: Statistical summary of CIE H°a*b* colour data for crude anthocyanin-PVA blends with different pH

CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
H°	0	1	28.001_{21}± 0.011	27.982	28.021
		3	23.445_{24}± 0.014	23.420	23.470
		3.8	23.072_{25}± 0.015	23.046	23.097
		5	14.845_{28}± 0.018	14.814	14.875
		7	22.215_{26}± 0.014	22.191	22.240
		9	68.941_{4} ± 0.017	68.911	68.971
		11	37.937_{16}± 0.016	37.909	37.965
	1	1	29.000_{20}± 0.013	28.978	29.022
		3	25.838_{23}± 0.012	25.816	25.859
		3.8	26.664_{22}± 0.014	26.640	26.689
		5	21.780_{27}± 0.017	21.751	21.810
		7	38.980_{14}± 0.017	38.951	39.010
		9	74.265_{3} ± 0.011	74.246	74.283
		11	42.947_{13}± 0.015	42.921	42.974
	2	1	37.521_{17}± 0.011	37.502	37.540
		3	36.087_{19}± 0.010	36.069	36.105
		3.8	38.318_{15}± 0.014	38.295	38.342
		5	37.132_{18}± 0.018	37.101	37.162
		7	48.972_{12}± 0.012	48.951	48.993
		9	77.506_{2} ± 0.011	77.487	77.526
		11	50.480_{11}± 0.017	50.451	50.509
	3	1	51.149_{10}± 0.014	51.125	51.172
		3	53.508_{9} ± 0.013	53.485	53.531
		3.8	57.766_{6} ± 0.012	57.745	57.787
		5	56.197_{7} ± 0.016	56.169	56.224
		7	55.261_{8} ± 0.013	55.239	55.284
		9	80.664_{1} ± 0.016	80.635	80.692
		11	65.832_{5} ± 0.017	65.803	65.861

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with different pH

'Table 5.7, continued'

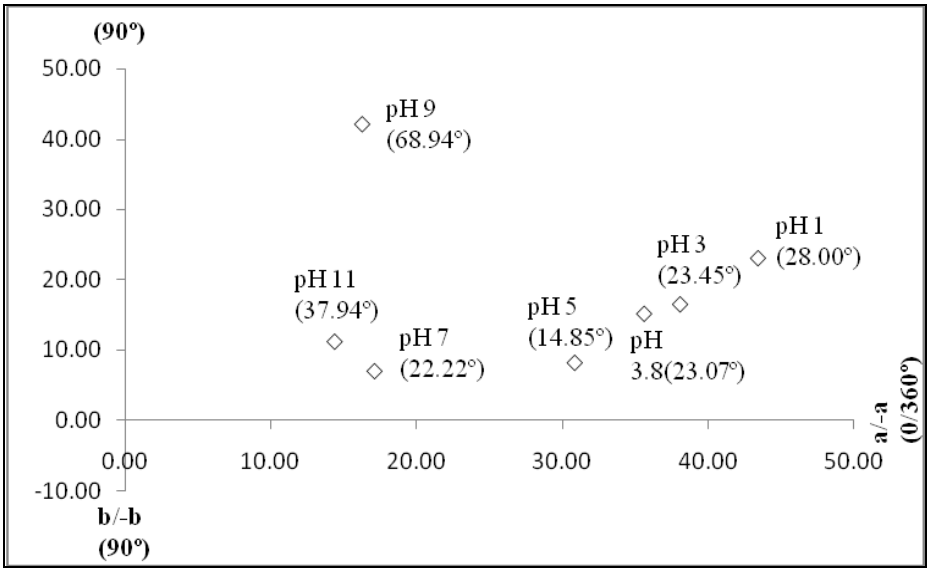
CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
a*	0	1	43.423_{1} ± 0.014	43.398	43.447
		3	38.071_{3} ± 0.008	38.057	38.086
		3.8	35.608_{6} ± 0.013	35.586	35.630
		5	30.842_{8} ± 0.017	30.813	30.872
		7	17.098_{15}± 0.014	17.073	17.123
		9	16.246_{16}± 0.014	16.221	16.271
		11	14.356_{18}± 0.014	14.332	14.379
	1	1	42.922_{2} ± 0.015	42.895	42.948
		3	36.832_{4} ± 0.015	36.805	36.858
		3.8	33.893_{7} ± 0.018	33.861	33.925
		5	27.959_{10}± 0.015	27.934	27.985
		7	13.109_{19}± 0.009	13.094	13.124
		9	12.040_{22}± 0.018	12.009	12.071
		11	12.257_{21}± 0.014	12.232	12.281
	2	1	36.563_{5} ± 0.012	36.542	36.585
		3	30.374_{9} ± 0.011	30.354	30.393
		3.8	27.280_{11}± 0.017	27.251	27.309
		5	21.083_{13}± 0.016	21.056	21.111
		7	10.117_{23}± 0.014	10.093	10.141
		9	9.517_{25} ± 0.013	9.494	9.539
		11	9.842_{24} ± 0.018	9.812	9.873
	3	1	24.954_{12}± 0.014	24.929	24.978
		3	18.668_{14}± 0.012	18.647	18.689
		3.8	15.558_{17}± 0.015	15.532	15.583
		5	12.859_{20}± 0.009	12.843	12.874
		7	8.202_{26} ± 0.016	8.175	8.229
		9	7.064_{27} ± 0.012	7.043	7.086
		11	5.361_{28} ± 0.016	5.332	5.389

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with different pH

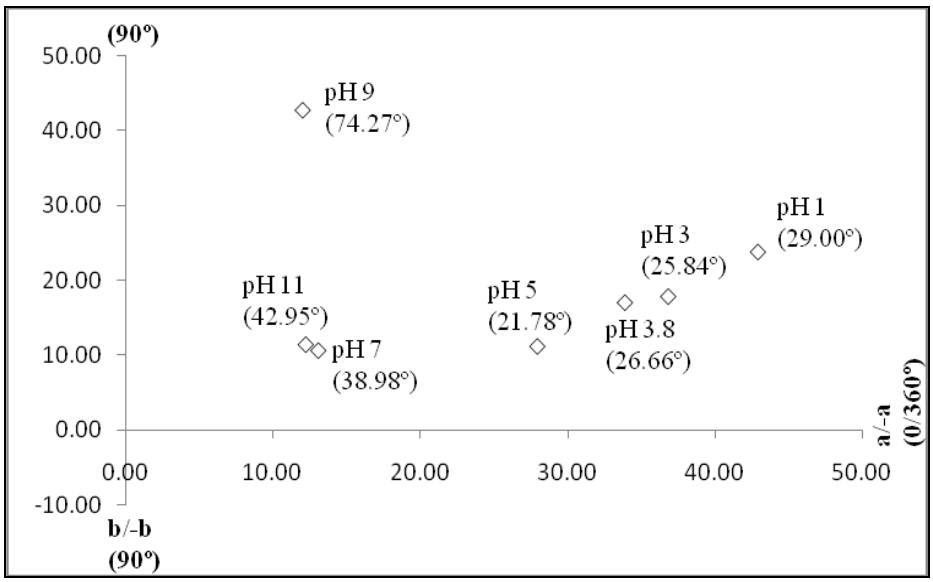
'Table 5.7, continued'

CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
b*	0	1	23.090_{9} ± 0.013	23.068	23.113
		3	16.511_{15}± 0.008	16.496	16.525
		3.8	15.168_{17}± 0.012	15.146	15.189
		5	8.175_{24} ± 0.015	8.148	8.201
		7	6.983_{25} ± 0.016	6.954	7.011
		9	42.193_{3} ± 0.014	42.170	42.217
		11	11.191_{22}± 0.017	11.161	11.221
	1	1	23.793_{8} ± 0.019	23.759	23.826
		3	17.836_{13}± 0.013	17.813	17.859
		3.8	17.020_{14}± 0.018	16.989	17.052
		5	11.172_{22}± 0.012	11.151	11.193
		7	10.608_{23}± 0.014	10.584	10.632
		9	42.734_{2} ± 0.012	42.712	42.755
		11	11.409_{21}± 0.013	11.387	11.431
	2	1	28.078_{5} ± 0.013	28.055	28.101
		3	22.139_{10}± 0.010	22.121	22.156
		3.8	21.559_{11}± 0.017	21.529	21.589
		5	15.964_{16}± 0.015	15.938	15.989
		7	11.627_{20}± 0.015	11.601	11.653
		9	42.953_{1} ± 0.015	42.926	42.979
		11	11.931_{18}± 0.012	11.911	11.951
	3	1	30.981_{4} ± 0.016	30.953	31.010
		3	25.236_{6} ± 0.012	25.215	25.257
		3.8	24.674_{7} ± 0.022	24.636	24.713
		5	19.207_{12}± 0.010	19.191	19.224
		7	11.828_{19}± 0.015	11.801	11.854
		9	42.970_{1} ± 0.014	42.945	42.994
		11	11.947_{18}± 0.016	11.918	11.975

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends with different pH

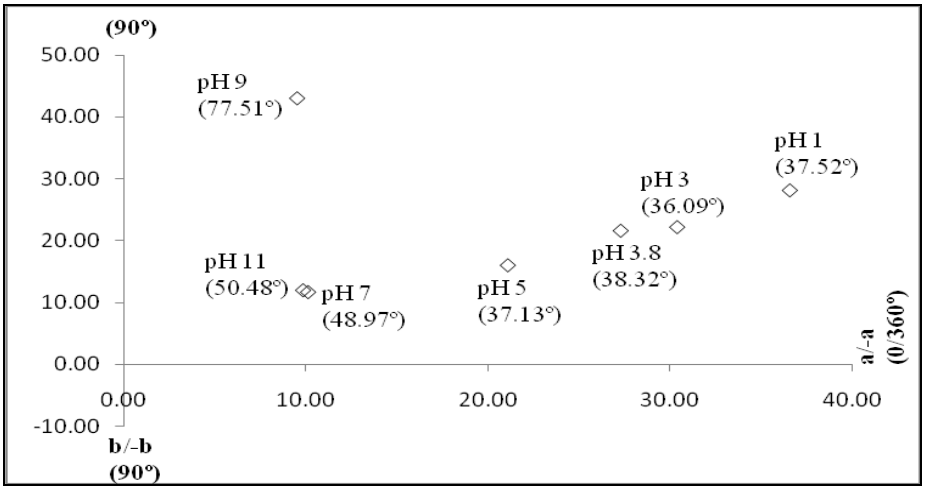


(a)



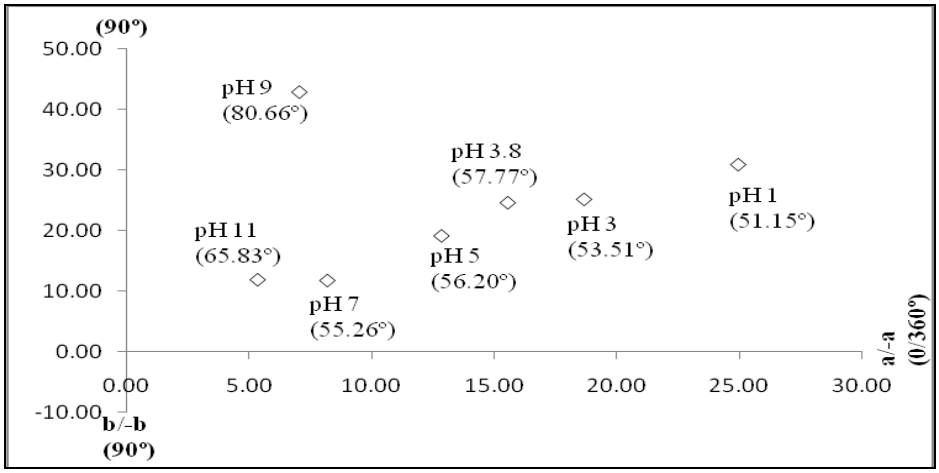
(b)

Figure 5.6: Relationship between pH variation and H◦ with a*b* coordinate for crude anthocyanin-PVA blends during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 5.6, continued'



(d)

'Figure 5.6, continued'

Table 5.8 presents the total colour difference (ΔE), which was lowest for crude anthocyanin-PVA blends at pH 1 which ΔE_1=3.684, during first month of exposure and is still the lowest at the end of exposure (ΔE_3=22.469). In contrast, the ΔE of crude anthocyanin-PVA blends at pH 11 was the highest at zero time (ΔE_1=12.891) and at the

end of exposure ΔE_3=33.926. Other crude anthocyanin-PVA of different pH demonstrated a similar trend in colour change being low before exposure (zero time) and higher at the end of exposure from ΔE_1=4.773to ΔE_3=24.010, ΔE_1=5.602 to ΔE_3=25.187, ΔE_1=7.536 to ΔE_3=27.093, ΔE_1=11.257 to ΔE_3=30.098 and ΔE_1=12.298 to ΔE_3=32.757 for pH 3, 3.8, 5, 7 and 9 respectively. In addition, the crude anthocyanin-PVA blends at pH 1 exhibited the highest saturation parameter at time zero (s_0=0.8476) that decreased with increasing exposure time until the end of three months with (s_3=0.5842). Though saturation of sample at pH 9 was higher in the beginning, the colour tends to turn into brown. Other crude anthocyanin-PVA blends with different pH showed similar trend. Crude anthocyanin-PVA blends at pH 11 exhibited the lowest saturation at time zero, (s_0=0.3183) and drastically decreased towards the end of exposure with (s_3=0.1457) as seen in Table 5.8.

Table 5.8: Total colour differences (ΔE) and saturation of crude anthocyanin-PVA blends as affected by pH

pH	TIME (Month) 0	1	2	3	ΔE_1	ΔE_3
pH 1	S_0=0.8476	S_1=0.7967	S_2=0.7160	S_3=0.5842	ΔE_1=3.684	ΔE_3=22.469
pH 3	S_0=0.6897	S_1=0.6337	S_2=0.5577	S_3=0.4403	ΔE_1=4.773	ΔE_3=24.010
pH 3.8	S_0=0.6020	S_1=0.5474	S_2=0.4810	S_3=0.3828	ΔE_1=5.602	ΔE_3=25.187
pH 5	S_0=0.4880	S_1=0.4201	S_2=0.3529	S_3=0.2806	ΔE_1=7.536	ΔE_3=27.093
pH 7	S_0=0.3064	S_1=0.2404	S_2=0.2085	S_3=0.1624	ΔE_1=11.257	ΔE_3=30.098
pH 9	S_0=0.7892	S_1=0.6450	S_2=0.5976	S_3=0.4908	ΔE_1=12.298	ΔE_3=32.757
pH 11	S_0=0.3183	S_1=0.2396	S_2=0.2074	S_3=0.1457	ΔE_1=12.891	ΔE_3=33.926

5.2.3. Effect of addition 2% ferulic acid (PVA) and pH on visual colour variation

Table 5.9 displays the results of colour parameters CIE L* for crude anthocyanin-PVA blends from *Ixora* with addition of 2% FA and at different pH values. From previous results, the 2% FA act as a good colour enhancer and stabilizer. The initial (zero time) lightness percentage (L*) of crude anthocyanin-PVA blends containing 2% FA with altered pH (initial pH (3.7), pH 1, 3, 5, 7, 9 and 11) were observed to decrease from sample at pH 1 (L*=47.326 ± 0.011) until sample at pH 5 (L*=43.682 ± 0.010). L* increased from pH 7 (L*=45.237 ± 0.009) until pH 9 (L*=50.967 ± 0.012) before decreasing again at pH 11 (L*=42.396 ± 0.012). In addition, during exposure the L* parameter values for crude anthocyanin-PVA blends at pH 3, 3.7 and 5 were observed to decrease (darker colour) from zero time L* value until the second months of exposure before increasing again at the third month of exposure. The significant decrease in L* value over two months of exposure was exhibited by the crude anthocyanin-PVA blends at pH 3, with the initial L*=46.471 ± 0.009 that decreased to 31.388 ± 0.013. This is followed by sample at pH 3.7, with L* decreasing from (45.971 ± 0.012) to (32.762 ± 0.013) and pH 5 from (43.682 ± 0.010) to (L*=48.971 ± 0.011). In contrast, at other pH values (pH 1, 7, 9 and 11), L* continues to increase from zero time of exposure until the end of exposure where L* ranges from (47.326 ± 0.011) to (52.027 ± 0.012), (45.237 ± 0.009) to (54.765 ± 0.014), (50.967 ± 0.012) to (70.858 ± 0.009), and (42.396 ± 0.012) to (71.013 ± 0.008) respectively. After three months of exposure, the crude anthocyanin-PVA blends containing 2% FA at pH 11 exhibited the lightest colour with highest L* of (71.013 ± 0.008), while the lowest L* (darkest colour) was exhibited by samples at pH 3 with (L*=47.492 ± 0.010). The trend can be further observed in Figure 5.7.

Table 5.9: Statistical summary of CIE L* colour data for crude anthocyanin-PVA blends containing 2% FA with different pH

CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
L*	0	1	47.326_{22}± 0.011	47.307	47.345
		3	46.471_{16}± 0.009	46.456	46.486
		3.7	45.971_{17}± 0.012	45.949	45.992
		5	43.682_{19}± 0.010	43.665	43.699
		7	45.237_{21}± 0.009	45.222	45.253
		9	50.967_{8} ± 0.012	50.947	50.988
		11	42.396_{20}± 0.012	42.375	42.416
	1	1	48.890_{14}± 0.011	48.871	48.910
		3	33.680_{25}± 0.009	33.665	33.696
		3.7	34.773_{23}± 0.012	34.753	34.793
		5	34.401_{24}± 0.009	34.386	34.416
		7	47.252_{18}± 0.009	47.237	47.267
		9	59.884_{6} ± 0.011	59.865	59.902
		11	50.797_{15}± 0.013	50.774	50.820
	2	1	49.147_{10}± 0.009	49.132	49.162
		3	31.388_{28}± 0.013	31.366	31.410
		3.7	32.762_{26}± 0.013	32.740	32.784
		5	32.488_{27}± 0.014	32.465	32.512
		7	54.765_{9} ± 0.014	54.741	54.790
		9	64.782_{3} ± 0.010	64.765	64.799
		11	60.794_{5} ± 0.012	60.773	60.815
	3	1	52.027_{7} ± 0.012	52.007	52.048
		3	47.492_{13}± 0.010	47.475	47.510
		3.7	46.998_{12}± 0.014	46.974	47.021
		5	48.971_{11}± 0.011	48.952	48.990
		7	61.956_{4} ± 0.013	61.934	61.979
		9	70.858_{1} ± 0.009	70.843	70.874
		11	71.013_{2} ± 0.008	71.000	71.027

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA containing 2% FA with different pH

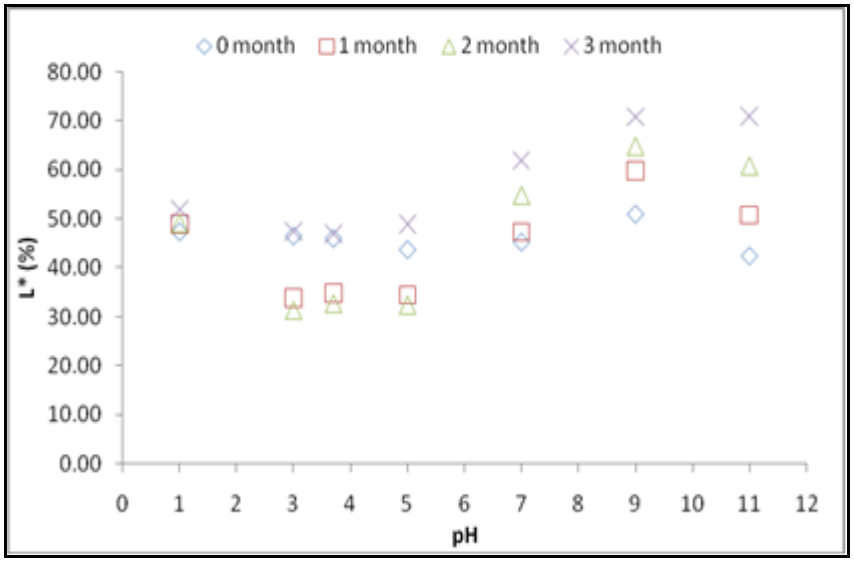


Figure 5.7: Relationship between pH variation and L* values (%) for crude anthocyanin-PVA blends containing 2% FA during three month of exposure

The chromaticity (C*) values of crude anthocyanin-PVA blends containing 2% FA with altered pH (initial pH (3.7), pH 1, 3, 5, 7, 9 and 11) in the beginning and at the end of exposure are shown in Figure 5.8. The C* value of the crude anthocyanin-PVA containing 2% FA at altered pH (pH 3, 3.7 and 5) at zero time of exposure were observed to increased continuously until the second month of exposure, in which C* increased significantly (brightest colour) for sample at pH 3. The C* value increased from (45.587 ± 0.013) to (55.047 ± 0.012) before decreasing at the third month of exposure (C*=43.554 ± 0.012). This trend is followed by sample at pH 3.7. The initial C* value (43.739 ± 0.010) increased on the second month (C*=52.030 ± 0.012) before decreasing at end of exposure time at C*=41.392 ± 0.009. For sample at pH 5, C* increased from (37.344 ± 0.012) to (45.874 ± 0.011) and decreased on the third month of exposure with C*=35.785 ± 0.010. For samples with pH variations (pH 1, 7, 9 and 11), C* decreased continuously from zero time of exposure until the third month of exposure ranging from (52.785 ± 0.010) to (34.945 ±

0.011), (20.286 ± 0.013) to (14.434 ± 0.010), (50.184 ± 0.011) to (46.675 ± 0.012), (22.702 ± 0.010) to (15.495 ± 0.011), respectively. Nevertheless, after three months of exposure, the crude anthocyanin-PVA blends at pH 3 experienced the highest C* of (43.554 ± 0.012). Eventhough the crude anthocyanin-PVA at pH 9 also experienced higher C* values, browning of the samples indicate degradation. The lowest C* value was exhibited by samples at pH 7 C*=14.434 ± 0.010 and pH 11 C*=15.495 ± 0.011. Detailed results on chromaticity can be further observed in Table 5.10.

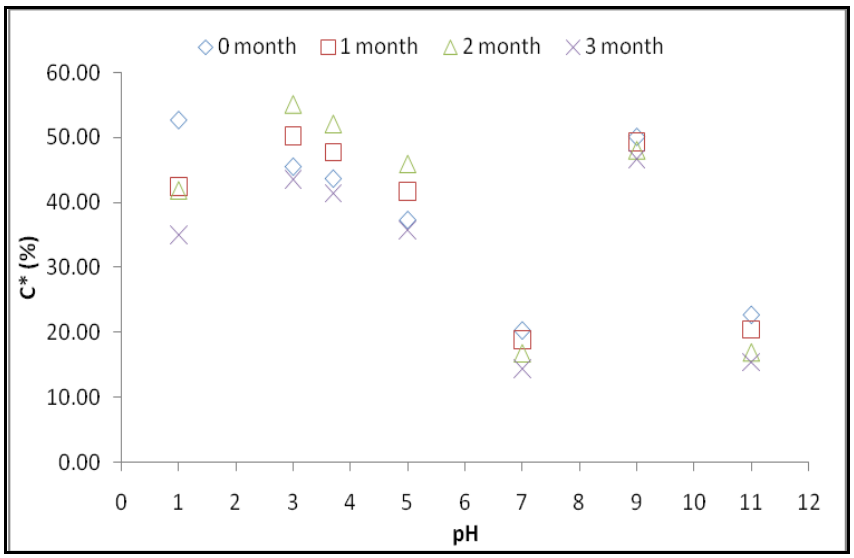


Figure 5.8: Relationship between pH variation and C* values (%) for crude anthocyanin-PVA blends containing 2% FA during three month of exposure

Table 5.10: Statistical summary of CIE C* colour data for crude anthocyanin-PVA blends containing 2% FA with different pH

CIE value	Time (month)	pH	$Mean_a \pm$ s.e.	Minimum	Maximum
C*	0	1	$52.785_3 \pm 0.010$	52.768	52.802
		3	$45.587_8 \pm 0.013$	45.565	45.609
		3.7	$43.739_{10} \pm 0.010$	43.721	43.757
		5	$37.344_{18} \pm 0.012$	37.324	37.365
		7	$20.286_{21} \pm 0.013$	20.264	20.308
		9	$50.184_6 \pm 0.011$	50.165	50.203
		11	$22.702_{22} \pm 0.010$	22.684	22.719
	1	1	$42.507_{16} \pm 0.012$	42.487	42.528
		3	$50.289_4 \pm 0.008$	50.275	50.302
		3.7	$47.716_5 \pm 0.006$	47.705	47.727
		5	$41.758_{11} \pm 0.008$	41.745	41.772
		7	$18.848_{23} \pm 0.012$	18.828	18.868
		9	$49.316_9 \pm 0.009$	49.301	49.332
		11	$20.455_{24} \pm 0.012$	20.434	20.476
	2	1	$41.807_{17} \pm 0.010$	41.790	41.825
		3	$55.047_1 \pm 0.012$	55.025	55.068
		3.7	$52.030_2 \pm 0.012$	52.010	52.050
		5	$45.874_7 \pm 0.011$	45.856	45.893
		7	$16.704_{25} \pm 0.010$	16.687	16.721
		9	$47.969_{13} \pm 0.015$	47.943	47.994
		11	$16.849_{26} \pm 0.010$	16.832	16.865
	3	1	$34.945_{20} \pm 0.011$	34.926	34.963
		3	$43.554_{12} \pm 0.012$	43.534	43.574
		3.7	$41.392_{15} \pm 0.009$	41.376	41.407
		5	$35.785_{19} \pm 0.010$	35.769	35.802
		7	$14.434_{27} \pm 0.010$	14.417	14.451
		9	$46.675_{14} \pm 0.012$	46.654	46.696
		11	$15.495_{28} \pm 0.011$	15.476	15.514

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a \pm$ standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends containing 2% FA with different pH

Additionally, the initial exposure of hue angle, h^o values of the crude anthocyanin-PVA containing 2% FA with different pH variation were observed decreased from sample at pH 1 (22.185 ± 0.012)° until sample at pH 5 (8.793 ± 0.011)°, whereas started to increase at pH 7 (12.089 ± 0.014)° until pH 9 and (61.133 ± 0.010)°, before decrease again at pH 11 (32.593 ± 0.011)°. According to Table 5.11, it can be noted that the hue angle of crude anthocyanin-PVA with pH variation (pH 3, 3.7 and 5) continually moved clockwise into blue region from the zero time of exposure until the second month of exposure, ranging from hue angle (17.031 ± 0.006)° with positive a* (43.588 ± 0.014) and b* value (13.352 ± 0.012) moved to hue angle (336.450 ± 0.015)° with more positive a* (50.464 ± 0.012) and negative b* value (-21.991 ± 0.012) for sample at pH 3, hue angle of (15.890 ± 0.008)° with positive a* (42.068 ± 0.014) and b* value (11.976 ± 0.013) moved to (335.790 ± 0.009)° with more positive a* (47.457 ± 0.012) and negative b* value (-21.330 ± 0.006) for sample at pH 3.7 and hue angle of (8.793 ± 0.011)° with positive a* (36.906 ± 0.013) and b* value (5.709 ± 0.013) moved to hue angle of (334.240 ± 0.012)° with more positive a* (41.317 ± 0.009) and negative b* value (-14.615 ± 0.008) for sample at pH 5. At the third month of exposure, the parameters of samples with pH 3, 3.7 and 5 moved counterclockwise into red tonalities with hue angle of (19.242 ± 0.007)° with less positive a* of (41.12 ± 0.005) and b*=(14.354 ± 0.012) for sample at pH 3. For sample with pH 3.7, the hue angle was h°=(20.966 ± 0.007)° with less positive a* of (38.652 ± 0.012) and with b*=(14.811 ± 0.006). For sample at pH 5, the hue angle was (24.517 ± 0.010)° with less positive a* of (32.559 ± 0.014) and b*=(14.850 ± 0.010). For sample at pH 1 and 7, the hue angle also moved clockwise into the blue region but only during the first month of exposure since at the second month of exposure the samples have already moved

counterclockwise into red tonalities and continues until the third month of exposure. In contrast, during the third months of exposure the crude anthocyanin-PVA blends at pH 9 and 11 directly moved counterclockwise from the first month of exposure until the third month of exposure and the hue angle approaches the yellow region, to higher h^o. At time zero, the hue angle for sample at pH 9 was the highest (61.133 ± 0.010)° with a*=(24.228 ± 0.009) and b*=(43.949 ± 0.014) while after three month of exposure, sample at pH 9 again contributed to the higher hue angle of (83.399 ± 0.016)° but a* become less positive with a*=(5.365 ± 0.007). The value of b* increased slightly to (46.366 ± 0.005). In addition, sample at pH 3 experienced lower hue angle of h°=(17.031 ± 0.006)° with value a*=(43.588 ± 0.014) and b*=(13.352 ± 0.012) at zero time. At the end of exposure the hue angle was lowest at (19.242 ± 0.007)°, with highest a* value of (41.121 ± 0.005) and b* value of (14.354 ± 0.012). The detailed in trend can be clear observed in Figure 5.9. The gradual degradation of red colour, visually observed for crude anthocyanin-PVA blends was accompanied by the tonality changes from red to brown-yellow tints and black colour as the h^o increased with time. This is significant for samples at higher pH (pH 7, 9 and 11). Furthermore, the h^o values of lower pH (pH 1, 3, 3.7 and 5) showed vivid purple colours, especially for sample at pH 3, before turning back again into red colour tonalities at the end of exposure.

Table 5.11: Statistical summary of CIE H°a*b* colour data for crude anthocyanin-PVA blends containing 2% FA with different pH

CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
H°	0	1	22.185_{23}± 0.012	22.165	22.205
		3	17.031_{25}± 0.006	17.021	17.042
		3.7	15.890_{26}± 0.008	15.876	15.904
		5	8.793_{28} ± 0.011	8.774	8.812
		7	12.089_{27}± 0.014	12.065	12.113
		9	61.133_{14}± 0.010	61.115	61.151
		11	32.593_{19}± 0.011	32.575	32.612
	1	1	350.090_{1}±0.009	350.075	350.105
		3	341.440_{3}±0.010	341.422	341.457
		3.7	340.770_{4}±0.007	340.758	340.783
		5	339.510_{5}±0.010	339.494	339.527
		7	341.130_{2}±0.005	341.121	341.139
		9	66.111_{12}± 0.006	66.101	66.122
		11	40.408_{16}± 0.012	40.386	40.429
	2	1	28.575_{21}± 0.010	28.557	28.593
		3	336.450_{6}±0.015	336.425	336.476
		3.7	335.790_{7}±0.009	335.775	335.805
		5	334.240_{8}±0.012	334.220	334.260
		7	36.930_{18}± 0.004	36.923	36.938
		9	73.793_{10}± 0.011	73.773	73.812
		11	57.799_{15}± 0.012	57.778	57.819
	3	1	35.080_{17}± 0.009	35.065	35.096
		3	19.242_{24}± 0.007	19.230	19.254
		3.7	20.966_{22}± 0.007	20.954	20.978
		5	24.517_{20}± 0.010	24.501	24.534
		7	60.773_{13}± 0.015	60.748	60.799
		9	83.399_{9} ± 0.016	83.371	83.427
		11	69.277_{11}± 0.013	69.255	69.299

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends containing 2% FA with different pH

'Table 5.11, continued'

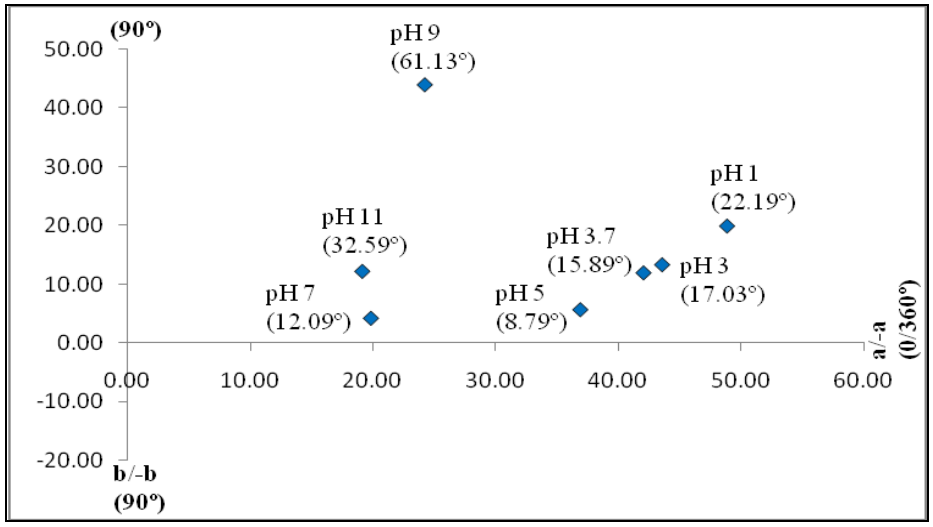
CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
a*	0	1	48.878_{2} ± 0.008	48.865	48.892
		3	43.588_{4} ± 0.014	43.564	43.612
		3.7	42.068_{5} ± 0.014	42.045	42.092
		5	36.906_{11}± 0.013	36.883	36.928
		7	19.837_{16}± 0.010	19.821	19.854
		9	24.228_{15}± 0.009	24.212	24.243
		11	19.127_{19}± 0.013	19.104	19.149
	1	1	41.873_{10}± 0.010	41.856	41.891
		3	47.676_{2} ± 0.009	47.661	47.692
		3.7	45.055_{3} ± 0.011	45.035	45.074
		5	39.117_{8} ± 0.009	39.101	39.133
		7	17.836_{18}± 0.009	17.821	17.851
		9	19.971_{17}± 0.010	19.954	19.988
		11	15.576_{21}± 0.014	15.552	15.599
	2	1	36.715_{12}± 0.010	36.699	36.732
		3	50.464_{1} ± 0.012	50.443	50.485
		3.7	47.457_{2} ± 0.012	47.436	47.478
		5	41.317_{6} ± 0.009	41.301	41.333
		7	13.353_{20}± 0.010	13.336	13.371
		9	13.388_{22}± 0.014	13.364	13.412
		11	8.979_{23} ± 0.009	8.963	8.994
	3	1	28.597_{14}± 0.007	28.584	28.609
		3	41.121_{7} ± 0.005	41.112	41.130
		3.7	38.652_{9} ± 0.012	38.632	38.673
		5	32.559_{13}± 0.014	32.535	32.583
		7	7.048_{24} ± 0.008	7.035	7.061
		9	5.365_{25} ± 0.007	5.353	5.376
		11	5.483_{26} ± 0.010	5.465	5.501

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends containing 2% FA with different pH

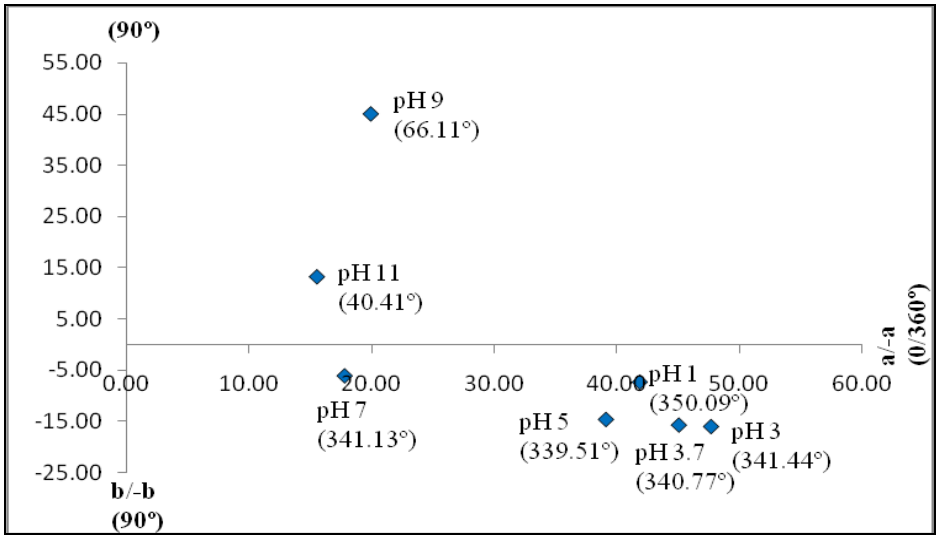
'Table 5.11, continued'

CIE value	Time (month)	pH	Mean $_a$ ± s.e.	Minimum	Maximum
b*	0	1	19.932_{9} ± 0.005	19.923	19.941
		3	13.352_{21}± 0.012	13.331	13.373
		3.7	11.976_{22}± 0.013	11.954	11.998
		5	5.709_{26} ± 0.013	5.687	5.732
		7	4.249_{27} ± 0.009	4.234	4.265
		9	43.949_{4} ± 0.014	43.925	43.974
		11	12.229_{20}± 0.010	12.211	12.247
	1	1	-7.315_{23} ± 0.006	7.304	7.326
		3	-16.001_{11}±0.010	15.985	16.018
		3.7	-15.713_{12}±0.007	15.701	15.725
		5	-14.615_{14}±0.008	14.601	14.630
		7	-6.093_{25} ± 0.016	6.064	6.121
		9	45.092_{3} ± 0.007	45.079	45.104
		11	13.260_{19}± 0.004	13.254	13.267
	2	1	19.997_{9} ± 0.012	19.976	20.018
		3	-21.991_{5}± 0.012	21.970	22.013
		3.7	-21.330_{7}± 0.006	21.319	21.340
		5	-19.934_{8}± 0.007	19.922	19.945
		7	10.037_{24}± 0.015	10.011	10.062
		9	46.063_{2} ± 0.009	46.047	46.079
		11	14.258_{16}± 0.007	14.246	14.269
	3	1	20.084_{6} ± 0.012	20.064	20.105
		3	14.354_{18}± 0.012	14.333	14.375
		3.7	14.811_{17}± 0.006	14.801	14.822
		5	14.850_{10}± 0.010	14.832	14.868
		7	12.597_{15}± 0.018	12.566	12.628
		9	46.366_{1} ± 0.005	46.357	46.375
		11	14.493_{13}± 0.010	14.476	14.511

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on crude anthocyanin-PVA blends containing 2% FA with different pH

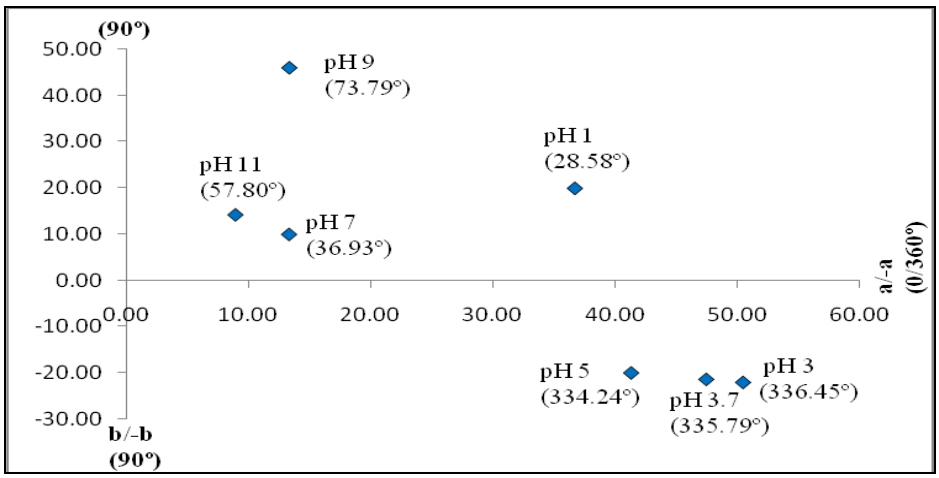


(a)



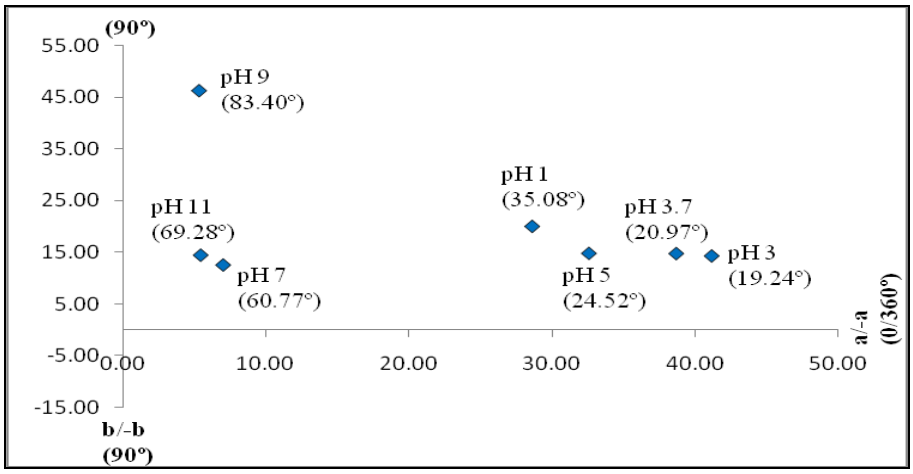
(b)

Figure 5.9: Relationship between pH variation and H◦ with a*b* coordinate for crude anthocyanin-PVA blends containing 2% FA during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 5.9, continued'



(d)

'Figure 5.9, continued'

Table 5.12 showed the total colour difference (ΔE), which was the greatest for the crude anthocyanin-PVA containing 2% FA at pH 3 where ΔE_1=32.279, at first month of exposure while lower colour change at the end of exposure (ΔE_3=2.994). Other crude anthocyanin-PVA blends demonstrated a similar trend in ΔE, the highest being at zero time and lower

towards the end of exposure from ΔE_1=28.176 to ΔE_3=20.819, ΔE_1=30.017 to ΔE_3=4.556 and ΔE_1=22.452 to ΔE_3=11.420 for pH 1, 3.7 and 5 respectively. In contrast, the ΔE of crude anthocyanin-PVA blends at pH 7, 9 and 11 were lower at zero time but increased at the end of storage period showing degradation. The crude anthocyanin-PVA containing 2% FA at pH 3 exhibited the highest saturation index at the zero time, (s_0=0.9810), which increased with increasing exposure time until the second month of exposure (s_2=1.7538). Finally, at the third month of exposure, (s_3=0.9104) the saturation index dropped. Eventhough saturation index for sample at pH 1 is also high; it decreased with increasing of exposure time. The sample with pH 9 tends to degrade into brown as can be seen in Table 5.12. Sample at pH 11 exhibit the lowest saturation index, which at zero time (s_0=0.5355) and continues decrease towards the end of exposure (s_3=0.2182).

Table 5.12: Total colour differences (ΔE) and saturation of crude anthocyanin-PVA blends as affected by pH with addition of 2% FA

pH	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
pH 1	s_0=1.1153	s_1=0.8694	s_2=0.8506	s_3=0.6717	ΔE_1=28.176	ΔE_3=20.819
pH 3	s_0=0.9810	s_1=1.4931	s_2=1.7538	s_3=0.9104	ΔE_1=32.279	ΔE_3=2.994
pH 3.7	s_0=0.9514	s_1=1.3722	s_2=1.5881	s_3=0.8807	ΔE_1=30.017	ΔE_3=4.556
pH 5	s_0=0.8549	s_1=1.2139	s_2=1.4120	s_3=0.7307	ΔE_1=22.452	ΔE_3=11.420
pH 7	s_0=0.4484	s_1=0.3989	s_2=0.3050	s_3=0.2330	ΔE_1=10.725	ΔE_3=22.644
pH 9	s_0=0.9846	s_1=0.8235	s_2=0.7405	s_3=0.6587	ΔE_1=9.947	ΔE_3=27.519
pH 11	s_0=0.5355	s_1=0.4027	s_2=0.2771	s_3=0.2182	ΔE_1=9.179	ΔE_3=31.784

5.3. Colour analysis on purified anthocyanin from *Ixora siamensis* blended with PVA

5.3.1. Effect of addition ferulic acid (FA) on visual colour variation

Table 5.13 presents the results of the colour parameters CIE L* of purified anthocyanin-PVA blends from *Ixora* added with different percentages (from 1% to 5%) of ferulic acid (FA). Initially at zero time, the lightness percentage (L*) of purified anthocyanin-PVA blends decreased from sample without presence of FA (70.356 ± 0.015) until sample with 2% FA (54.283 ± 0.005). The L* values start to increase when percentage of FA increased being (56.449 ± 0.015) for sample with 3% FA added and (68.244 ± 0.018) when FA added was 5%. During exposure, the L* parameter values for purified anthocyanin-PVA blends without addition of FA increase continually from zero time of exposure (70.356 ± 0.015) until the third month of exposure (87.797 ± 0.016). On the other hand, the lightness percentages for purified anthocyanin-PVA blends with addition of FA decreased (to darker colour) from zero time of exposure until second month of exposure before increase at the third month of exposure. Slightly decrease in L* over two months of exposure was exhibited by the purified anthocyanin-PVA blends with addition of 5% FA, the initial L* of which was (68.244 ± 0.018) and decreased to (61.640 ± 0.014), followed by the samples added with 4% FA the L* of which was (66.057 ± 0.017) that decreased to (59.241 ± 0.011). The highest decrease in L* (darker colour) was experienced by the purified anthocyanin-PVA blends with addition of 2% FA. L* decreased from (54.283 ± 0.005) to (41.794 ± 0.010). After three month of exposure, the purified anthocyanin-PVA blends without addition of FA exhibited the highest L* value (lightest colour) of (87.797 ± 0.016),

while the lowest L* (darker colour) was exhibited by the sample with addition of 2% FA (56.880 ± 0.010). The trend can be further observed in Figure 5.10.

Table 5.13: Statistical summary of CIE L* colour data for purified anthocyanin-PVA blends with addition of FA

CIE value	Time (month)	FA (%)	$Mean_a$ ± s.e.	Minimum	Maximum
L*	0	0	70.356_{7} ± 0.015	70.331	70.382
		1	62.758_{10}± 0.014	62.734	62.782
		2	54.283_{18}± 0.005	54.274	54.293
		3	56.449_{17}± 0.015	56.423	56.475
		4	66.057_{9} ± 0.017	66.028	66.086
		5	68.244_{8} ± 0.018	68.213	68.274
	1	0	76.693_{4} ± 0.011	76.674	76.713
		1	54.101_{19}± 0.012	54.079	54.122
		2	42.691_{23}± 0.011	42.672	42.711
		3	45.446_{21}± 0.014	45.421	45.471
		4	59.854_{14}± 0.014	59.831	59.878
		5	62.191_{11}± 0.011	62.172	62.209
	2	0	80.696_{2} ± 0.014	80.672	80.719
		1	53.412_{20}± 0.011	53.393	53.432
		2	41.794_{24}± 0.010	41.776	41.812
		3	44.648_{22}± 0.016	44.621	44.675
		4	59.241_{15}± 0.011	59.223	59.260
		5	61.640_{12}± 0.014	61.617	61.664
	3	0	87.797_{1} ± 0.016	87.769	87.824
		1	71.889_{6} ± 0.014	71.864	71.913
		2	56.880_{16}± 0.010	56.864	56.897
		3	61.511_{13}± 0.012	61.491	61.531
		4	76.175_{5} ± 0.016	76.148	76.203
		5	79.125_{3} ± 0.014	79.101	79.148

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with addition of FA

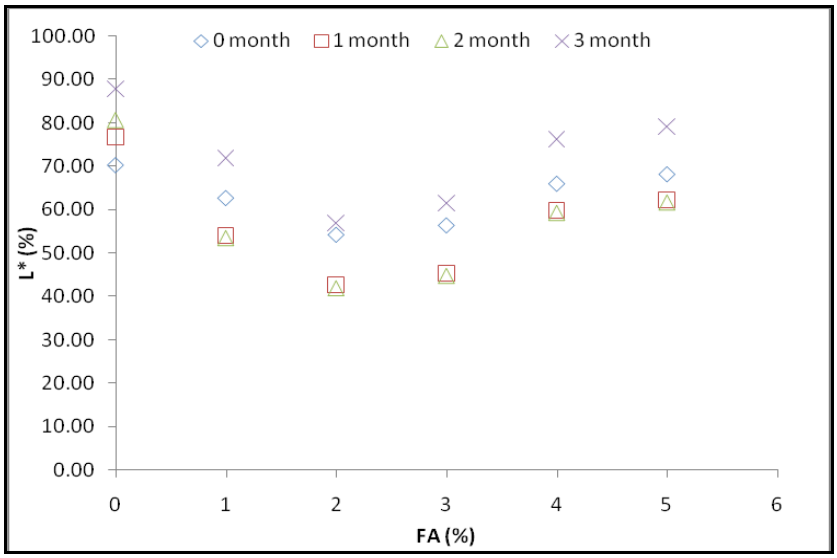


Figure 5.10: Relationship between percentage of FA and L* values (%) for purified anthocyanin-PVA blends during three month of exposure

In addition, the chromaticity (C*) values in the beginning and at the end of exposure was shown in Figure 5.11. The initial (zero time of exposure) C* values of the purified anthocyanin-PVA blends were observed to increase from the sample without addition of FA (31.803 ± 0.012) to the sample with addition 2% FA the C* value of which was (40.688 ± 0.015). C* then decreased to (37.659 ± 0.016) for sample with 3% FA and further decreased to (32.190 ± 0.018) for sample added with 5% FA. The C* for purified anthocyanin-PVA blends without addition of FA decreased continuously during exposure, from zero time of exposure with C*=31.803 ± 0.012 until the end of exposure (up to three month) with C*=24.103 ± 0.020. The detailed can be further observed in Table 5.14. In contrast, the C* values for purified anthocyanin-PVA blends with addition of FA increased from zero time exposure until second month of exposure before decreasing on the third month of exposure. The purified anthocyanin-PVA sample added with 2% FA exhibited the highest C* (brightest colour) over two month of exposure from (40.688 ± 0.015) to (48.551

± 0.011). There was small increase in C* values recorded for sample with presence of 5% FA, where the zero time C* value increase from (32.190 ± 0.018) to (34.989 ± 0.014). After three month of exposure, the purified anthocyanin-PVA blends without addition of FA experienced the lowest C* of (24.103 ± 0.020) while the highest C* was exhibited by the sample with addition of 2% FA, C*=37.757 ± 0.015, which lead to more vivid colour.

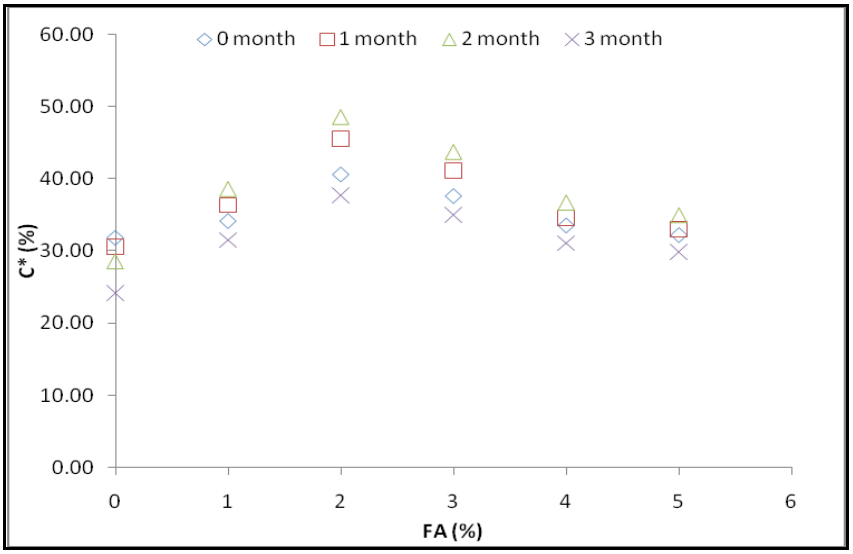


Figure 5.11: Relationship between percentage of FA and C* values (%) for purified anthocyanin-PVA blends during three month of exposure

Table 5.14: Statistical summary of CIE C* colour data for purified anthocyanin-PVA blends with addition of FA

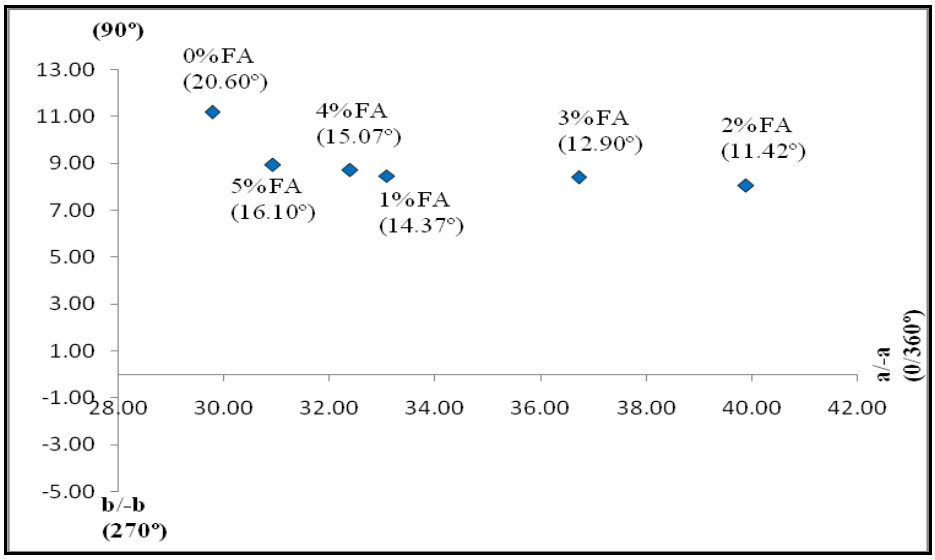
CIE value	Time (month)	FA (%)	$Mean_a \pm$ s.e.	Minimum	Maximum
C*	0	0	$31.803_{17} \pm 0.012$	31.781	31.824
		1	$34.153_{13} \pm 0.016$	34.126	34.180
		2	$40.688_{5} \pm 0.015$	40.663	40.714
		3	$37.659_{8} \pm 0.016$	37.632	37.687
		4	$33.535_{14} \pm 0.011$	33.517	33.554
		5	$32.190_{16} \pm 0.018$	32.160	32.221
	1	0	$30.519_{20} \pm 0.017$	30.489	30.548
		1	$36.383_{10} \pm 0.018$	36.352	36.415
		2	$45.645_{2} \pm 0.013$	45.623	45.667
		3	$41.186_{4} \pm 0.012$	41.165	41.206
		4	$34.546_{12} \pm 0.014$	34.521	34.571
		5	$32.963_{15} \pm 0.012$	32.941	32.984
	2	0	$28.620_{22} \pm 0.011$	28.601	28.639
		1	$38.631_{6} \pm 0.018$	38.600	38.662
		2	$48.551_{1} \pm 0.011$	48.532	48.571
		3	$43.740_{3} \pm 0.017$	43.711	43.769
		4	$36.739_{9} \pm 0.013$	36.717	36.761
		5	$34.989_{11} \pm 0.014$	34.965	35.012
	3	0	$24.103_{23} \pm 0.020$	24.069	24.137
		1	$31.508_{18} \pm 0.010$	31.490	31.525
		2	$37.757_{7} \pm 0.015$	37.732	37.783
		3	$34.991_{11} \pm 0.014$	34.966	35.015
		4	$31.082_{19} \pm 0.018$	31.051	31.112
		5	$29.845_{21} \pm 0.013$	29.822	29.867

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a \pm$ standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with addition of FA

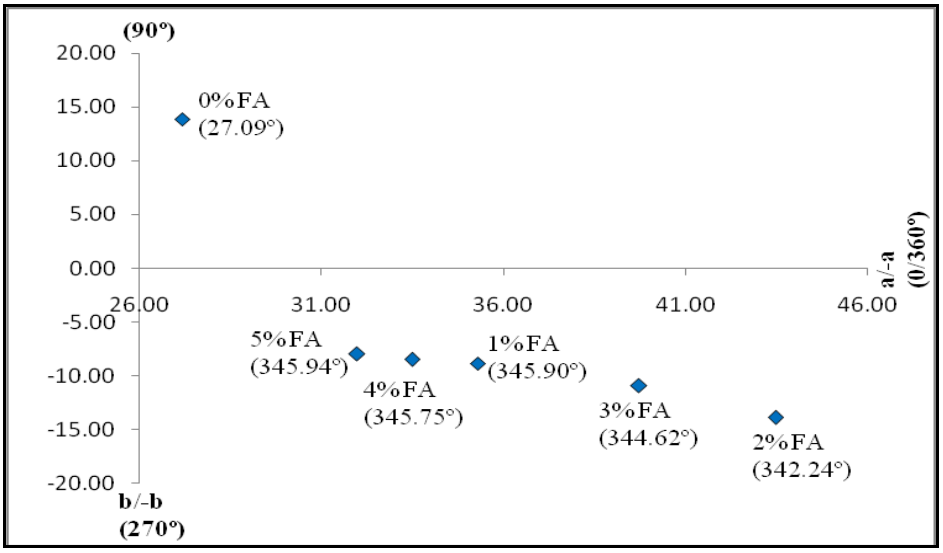
Figure 5.12 shows the initial hue of h°. h° shows a decrease for the purified anthocyanin-PVA blends without addition of FA at (20.598 ± 0.018)° until the sample with 2% FA with h°=(11.420 ± 0.005)°. On further addition of FA, h° increased to (12.895 ± 0.015)° for sample with 3% FA and continual to increase up to 5% FA when h°=(16.096 ± 0.017)°.

The initial hue angle (h_{ab})° of purified anthocyanin-PVA blends without presence of FA is (20.598 ± 0.018)° with coordinate a* as (29.770 ± 0.013) and coordinate b* as (11.198 ± 0.016). At the end of exposure, h_{ab}°=(61.846 ± 0.015)°, but with a lower a* coordinate, as a*= 11.373 ± 0.016 and higher b*coordinate of (11.189 ± 0.016). In contrast, immediately after addition of FA to the purified anthocyanin-PVA blends and after first month of exposure, a significant increment of the hue angle ranging from (14.371 ± 0.016)° to (345.900 ± 0.014)°, (11.420 ± 0.005)° to (342.240 ± 0.012)°, (12.895 ± 0.015)° to (344.620 ± 0.017)°, (15.067 ± 0.015)° to (345.750 ± 0.016)° and (16.096 ± 0.017)° to (345.940 ± 0.014)° respectively for 1, 2, 3, 4 and 5% FA before decreasing again after the third month of exposure, as shown in Table 5.15. For samples with presence of FA, the hue angle moved clockwise (negative) into blue region during the second month of exposure and is most significant for samples with addition of 2% FA since the initial hue angle, (11.420 ± 0.005)° moved to (342.240 ± 0.012)° with a*=(39.883 ± 0.016) that moved to (43.472 ± 0.010) and b*=(8.057 ± 0.014) that moved to (-13.918 ± 0.009). On the second month of exposure, coordinate a* increased to (44.861 ± 0.009) and b* to (-18.566 ± 0.012) with hue angle to (337.510 ± 0.007)°. However, towards the end of exposure, during the third month of storage, the hue angle moved counterclockwise into red region with hue angle (18.054 ± 0.013)°, while a* moved backward to lower positive (35.898 ± 0.015) and more positive of b* value (11.702 ± 0.009). In addition, the gradual degradation of red colour, visually observed in all systems is more significant for purified anthocyanin-PVA blends without FA addition. As the h° increases tonality changes from red to yellow tints can be observed. The hue angles of purified anthocyanin-PVA with FA were higher than that of the FA free purified-PVA over two months of exposure. The FA added samples showed vivid purple

colours, especially for samples with 2% FA, before turning back to show red colour tonalities again.

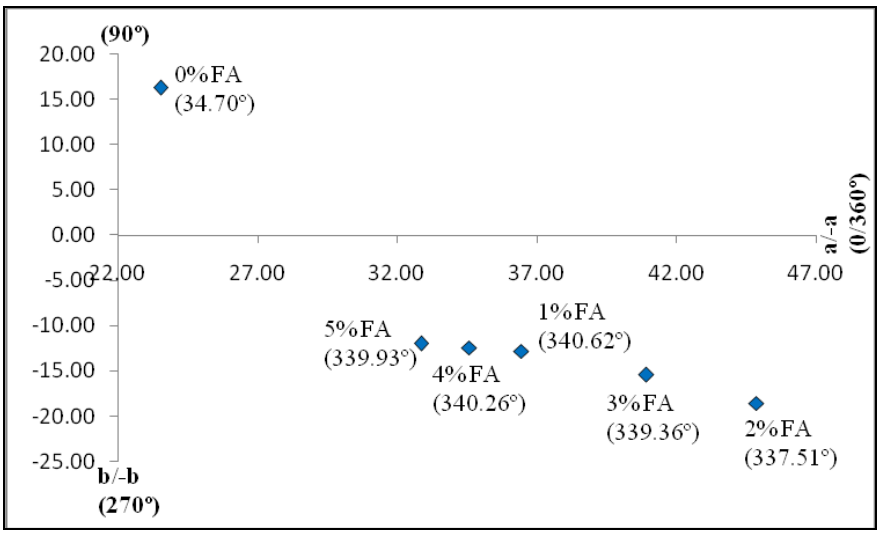


(a)



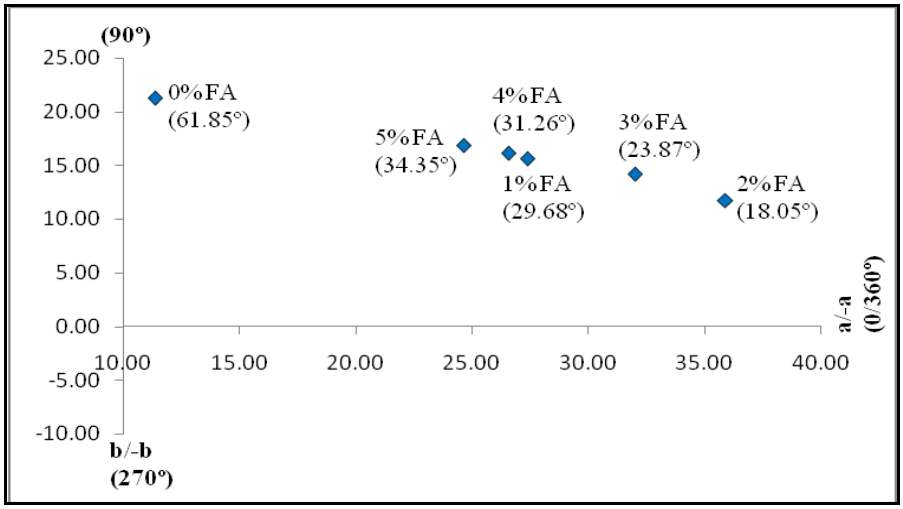
(b)

Figure 5.12: Relationship between percentage of FA and H◦ with a*b* coordinate for purified anthocyanin-PVA blends during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 5.12, continued'



(d)

'Figure 5.12, continued'

Table 5.15: Statistical summary of CIE H°a*b* colour data for purified anthocyanin-PVA blends with addition of FA

CIE value	Time (month)	FA (%)	$Mean_a$ ± s.e.	Minimum	Maximum
H°	0	0	20.598_{17}± 0.018	20.567	20.628
		1	14.371_{21}± 0.016	14.344	14.398
		2	11.420_{23}± 0.005	11.411	11.429
		3	12.895_{22}± 0.015	12.868	12.921
		4	15.067_{20}± 0.015	15.041	15.092
		5	16.096_{19}± 0.017	16.067	16.125
	1	0	27.085_{15}± 0.015	27.059	27.111
		1	345.900_{1}±0.014	345.875	345.925
		2	342.240_{4}±0.012	342.220	342.260
		3	344.620_{3}±0.017	344.591	344.649
		4	345.750_{2}±0.016	345.722	345.779
		5	345.940_{1}±0.014	345.916	345.963
	2	0	34.704_{11}± 0.012	34.682	34.725
		1	340.620_{5}±0.020	340.586	340.654
		2	337.510_{9}±0.007	337.498	337.521
		3	339.360_{8}±0.015	339.334	339.385
		4	340.260_{6}±0.012	340.239	340.281
		5	339.930_{7}±0.012	339.909	339.952
	3	0	61.846_{10}± 0.015	61.820	61.872
		1	29.682_{14}± 0.016	29.655	29.709
		2	18.054_{18}± 0.013	18.032	18.076
		3	23.870_{16}± 0.014	23.847	23.894
		4	31.260_{13}± 0.010	31.243	31.278
		5	34.346_{12}± 0.012	34.324	34.367

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with addition of FA

'Table 5.15, continued'

CIE value	Time (month)	FA (%)	$Mean_a \pm$ s.e.	Minimum	Maximum
a*	0	0	$29.770_{17} \pm 0.013$	29.747	29.792
		1	$33.085_{12} \pm 0.017$	33.056	33.114
		2	$39.883_{4} \pm 0.016$	39.856	39.910
		3	$36.710_{6} \pm 0.011$	36.691	36.728
		4	$32.383_{14} \pm 0.013$	32.361	32.406
		5	$30.929_{16} \pm 0.016$	30.901	30.956
	1	0	$27.172_{19} \pm 0.016$	27.144	27.199
		1	$35.287_{9} \pm 0.014$	35.263	35.312
		2	$43.472_{2} \pm 0.010$	43.454	43.489
		3	$39.712_{5} \pm 0.013$	39.690	39.735
		4	$33.484_{11} \pm 0.012$	33.462	33.505
		5	$31.976_{15} \pm 0.013$	31.954	31.999
	2	0	$23.529_{22} \pm 0.020$	23.495	23.563
		1	$36.444_{7} \pm 0.014$	36.420	36.467
		2	$44.861_{1} \pm 0.009$	44.846	44.876
		3	$40.935_{3} \pm 0.014$	40.911	40.960
		4	$34.582_{10} \pm 0.017$	34.552	34.612
		5	$32.867_{13} \pm 0.014$	32.843	32.891
	3	0	$11.373_{23} \pm 0.016$	11.344	11.401
		1	$27.374_{18} \pm 0.018$	27.343	27.406
		2	$35.898_{8} \pm 0.015$	35.872	35.923
		3	$31.998_{15} \pm 0.014$	31.974	32.021
		4	$26.570_{20} \pm 0.010$	26.552	26.587
		5	$24.642_{21} \pm 0.019$	24.610	24.675

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a \pm$ standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with addition of FA

'Table 5.15, continued'

CIE value	Time (month)	FA (%)	$Mean_a \pm$ s.e.	Minimum	Maximum
b*	0	0	$11.189_{14} \pm 0.016$	11.161	11.216
		1	$8.477_{19} \pm 0.013$	8.454	8.499
		2	$8.057_{21} \pm 0.014$	8.032	8.082
		3	$8.405_{20} \pm 0.015$	8.380	8.431
		4	$8.718_{18} \pm 0.014$	8.695	8.742
		5	$8.925_{16} \pm 0.018$	8.893	8.957
	1	0	$13.896_{9} \pm 0.013$	13.873	13.919
		1	$-8.863_{17} \pm 0.015$	8.836	8.889
		2	$-13.918_{9} \pm 0.009$	13.903	13.933
		3	$-10.922_{15} \pm 0.012$	10.901	10.942
		4	$-8.503_{19} \pm 0.016$	8.475	8.531
		5	$-8.008_{22} \pm 0.014$	7.983	8.032
	2	0	$16.295_{4} \pm 0.014$	16.271	16.319
		1	$-12.815_{10} \pm 0.012$	12.793	12.836
		2	$-18.566_{2} \pm 0.012$	18.545	18.586
		3	$-15.414_{7} \pm 0.013$	15.391	15.437
		4	$-12.406_{11} \pm 0.015$	12.381	12.432
		5	$-12.002_{12} \pm 0.012$	11.981	12.022
	3	0	$21.252_{1} \pm 0.015$	21.225	21.278
		1	$15.603_{6} \pm 0.018$	15.572	15.634
		2	$11.702_{13} \pm 0.009$	11.686	11.718
		3	$14.160_{8} \pm 0.014$	14.136	14.184
		4	$16.130_{5} \pm 0.012$	16.109	16.151
		5	$16.839_{3} \pm 0.014$	16.815	16.862

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
$Mean_a \pm$ standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with addition of FA

From Table 5.16, ΔE was the greatest for the purified anthocyanin-PVA blends with addition of 2% FA with $\Delta E_1=25.103$, at first month of exposure. The sample exhibited a lower colour change ($\Delta E_3=5.992$) at the end of exposure. The other solution with added FA demonstrated a similar trend, which is highest before exposure (zero time) and lowest at the end of exposure from $\Delta E_1=19.506$ to $\Delta E_3=12.914$, $\Delta E_1=22.441$ to $\Delta E_3=8.997$, $\Delta E_1=18.337$

to ΔE_3=13.824, and ΔE_1=18.013 to ΔE_3=14.851, respectively for 1, 3, 4 and 5% of FA. In contrast, ΔE for purified anthocyanin-PVA blends without FA was the lowest before exposure (zero time) (ΔE_1=7.364) but increased to ΔE_3=27.275 at the end of exposure. For all FA added samples, ΔE was higher than that of the purified antocyanin-PVA blends with the highest value ΔE for 2% FA added samples. This sample also exhibited the highest saturation parameter at zero time, s_0=0.7495 for the 2% FA added sample. The saturation increased with increasing of exposure time until the second month of exposure (s_2=1.1617) before decreased to (s_3=0.5689) at the end of storage. Other FA added samples also showed similar trend, but with smaller value. The purified anthocyanin-PVA blends without addition of FA exhibits the lowest saturation parameter before exposure (zero time) (s_0=0.4520) and continues to decrease at the end of exposure with s_3=0.2745 as presented in Table 5.16.

Table 5.16: Total colour differences (ΔE) and saturation of purified anthocyanin-PVA blends as affected by the addition of FA

FA (%)	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
0	s_0=0.4520	s_1=0.3979	s_2=0.3547	s_3=0.2745	ΔE_1=7.364	ΔE_3=27.275
1	s_0=0.5442	s_1=0.6725	s_2=0.7233	s_3=0.4383	ΔE_1=19.506	ΔE_3=12.914
2	s_0=0.7495	s_1=1.0692	s_2=1.1617	s_3=0.6638	ΔE_1=25.103	ΔE_3=5.992
3	s_0=0.6671	s_1=0.9063	s_2=0.9797	s_3=0.5689	ΔE_1=22.441	ΔE_3=8.997
4	s_0=0.5077	s_1=0.5772	s_2=0.6202	s_3=0.4080	ΔE_1=18.337	ΔE_3=13.824
5	s_0=0.4717	s_1=0.5300	s_2=0.5676	s_3=0.3772	ΔE_1=18.013	ΔE_3=14.851

5.3.2. Effect of pH on visual colour variation

Table 5.17 displays the results of the colour parameters CIE L* of purified anthocyanin-PVA blends from *Ixora* at different pH 1, 3, 5, 7, 9 and 11. The initial pH of purified anthocyanin-PVA is 3.9. After zero exposure time, the lightness percentage (L*) of purified anthocyanin-PVA blends increased from sample at pH 1 (67.122 ± 0.014) until sample at pH 5 (70.435 ± 0.012). However, the L* values started to decrease when pH of the sample increased from pH 7 (L*=64.344 ± 0.017) until pH 11 (L*=60.449 ± 0.016). In addition, during exposure the L* parameter for purified anthocyanin-PVA for all pH increases continuously from zero storage time until the end of storage. From the Figure 5.13, sample at pH 11 exhibited to the lowest L* values before exposure (zero time) (60.449 ± 0.016) while towards the end of exposure L* increased rapidly to the highest value of (96.222 ± 0.014). In contrast, the lightness percentage at the beginning for purified anthocyanin-PVA at pH 1 was (67.122 ± 0.014) while it gradually increases with increasing exposure time and at the third month of exposure the L* values was the lowest compared to others (81.759 ± 0.014). This can be noted that after three months of exposure the colour of sample at pH 9 (96.727 ± 0.015) was the lightest (higher L*) followed with sample at pH 11 (96.2223 ± 0.014) while the purified anthocyanin-PVA at pH 1 resulted in brighter or darker colours (lower L*), followed by sample at pH 3, and 3.9 which were (83.9256 ± 0.014) and (87.7975 ± 0.016) respectively.

Table 5.17: Statistical summary of CIE L* colour data for purified anthocyanin-PVA blends with different pH

CIE value	Time (month)	pH	$Mean_a$ ± s.e.	Minimum	Maximum
L*	0	1	67.122_{25}± 0.014	67.097	67.147
		3	68.228_{24}± 0.015	68.202	68.255
		3.9	70.356_{23}± 0.015	70.331	70.382
		5	70.435_{22}± 0.012	70.414	70.456
		7	64.344_{26}± 0.017	64.315	64.373
		9	62.080_{27}± 0.017	62.051	62.109
		11	60.449_{28}± 0.016	60.421	60.477
	1	1	72.229_{21}± 0.015	72.203	72.255
		3	74.201_{20}± 0.013	74.178	74.224
		3.9	76.693_{17}± 0.011	76.674	76.713
		5	78.332_{12}± 0.011	78.314	78.351
		7	77.240_{15}± 0.013	77.218	77.263
		9	77.057_{16}± 0.019	77.023	77.090
		11	76.320_{18}± 0.015	76.295	76.346
	2	1	75.938_{19}± 0.015	75.912	75.965
		3	78.022_{14}± 0.012	78.001	78.044
		3.9	80.696_{10}± 0.014	80.672	80.719
		5	82.715_{7} ± 0.014	82.691	82.738
		7	81.838_{8} ± 0.013	81.816	81.860
		9	78.954_{11}± 0.013	78.931	78.976
		11	78.221_{13}± 0.013	78.198	78.244
	3	1	81.759_{9} ± 0.014	81.735	81.783
		3	83.925_{6} ± 0.014	83.901	83.949
		3.9	87.797_{5} ± 0.016	87.769	87.824
		5	93.477_{4} ± 0.013	93.455	93.499
		7	96.661_{2} ± 0.016	96.634	96.689
		9	96.727_{1} ± 0.015	96.701	96.753
		11	96.222_{3} ± 0.014	96.197	96.247

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA with different pH

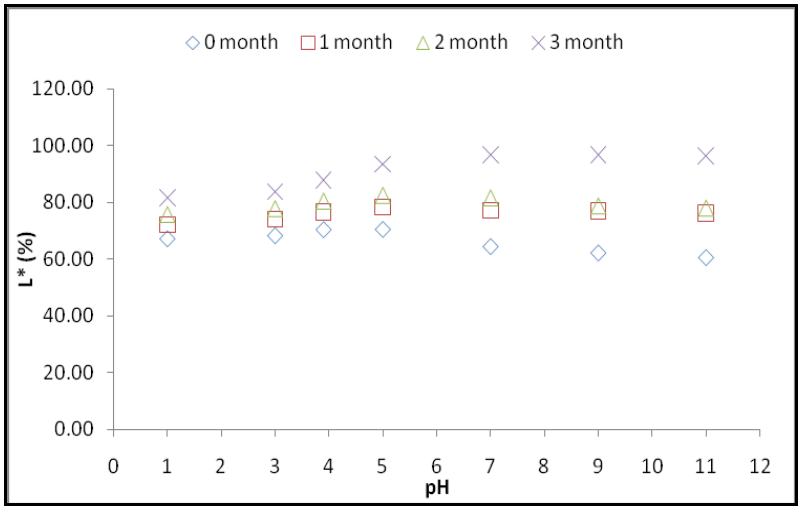


Figure 5.13: Relationship between pH variation and L* values (%) for purified anthocyanin-PVA blends during three month of exposure

In addition, the chromaticity (C*) values in the beginning and at the end of storage are shown in Table 5.18. The initial (zero time of exposure) chromaticity C* for the purified anthocyanin-PVA blends decreased with increasing pH from pH 1 (41.395 ± 0.017) until pH 7 (17.479 ± 0.012) before increasing at pH 9 (32.860 ± 0.011) and decreasing again at pH 11 (16.073 ± 0.018). In addition, the C* value for purified anthocyanin-PVA blends for all pH were observed to decrease continuously from zero time of exposure until the end of exposure (up to three months), as presented in Figure 5.14. In addition, the highest C* value for acidic purified anthocyanin-PVA was exhibited by sample at pH 1 (C*=41.395 ± 0.017) at the beginning as well as the end of exposure (third month of exposure) with the values of C*=32.943 ± 0.016. Eventhough sample at pH 9 showed higher C* value at beginning and end of exposure, the sample exhibited the phenomena of browning towards the end of storage. The lowest C* values at zero time was exhibited by sample at pH 11

(16.073 ± 0.018) while after three month of exposure sample at pH 11 also exhibited the lowest C* values (10.670 ± 0.020), due to the colour loss.

Table 5.18: Statistical summary of CIE C* colour data for purified anthocyanin-PVA blends with different pH

CIE value	Time (month)	pH	$Mean_a$ ± s.e.	Minimum	Maximum
C*	0	1	41.395_{1} ± 0.017	41.365	41.425
		3	36.506_{4} ± 0.012	36.485	36.526
		3.9	31.803_{10}± 0.012	31.781	31.824
		5	26.705_{16}± 0.011	26.685	26.724
		7	17.479_{21}± 0.012	17.459	17.499
		9	32.860_{8} ± 0.011	32.841	32.880
		11	16.073_{23}± 0.018	16.042	16.104
	1	1	40.854_{2} ± 0.012	40.834	40.875
		3	35.577_{5} ± 0.015	35.551	35.603
		3.9	30.519_{12}± 0.017	30.489	30.548
		5	24.405_{17}± 0.010	24.387	24.422
		7	16.642_{22}± 0.012	16.621	16.664
		9	32.466_{9} ± 0.018	32.435	32.497
		11	14.900_{24}± 0.013	14.878	14.922
	2	1	39.118_{3} ± 0.013	39.095	39.141
		3	33.800_{6} ± 0.017	33.771	33.829
		3.9	28.620_{14}± 0.011	28.601	28.639
		5	22.479_{19}± 0.012	22.458	22.499
		7	14.788_{25}± 0.014	14.763	14.812
		9	31.737_{11}± 0.014	31.713	31.762
		11	13.350_{26}± 0.016	13.321	13.378
	3	1	32.943_{7} ± 0.016	32.915	32.972
		3	27.517_{15}± 0.012	27.496	27.537
		3.9	24.103_{18}± 0.020	24.069	24.137
		5	18.889_{20}± 0.013	18.866	18.912
		7	12.777_{27}± 0.018	12.745	12.809
		9	30.407_{13}± 0.020	30.372	30.443
		11	10.670_{28}± 0.020	10.636	10.705

(Note: Means with the different subscript numbers are significantly different at P<0.05)
$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with different pH

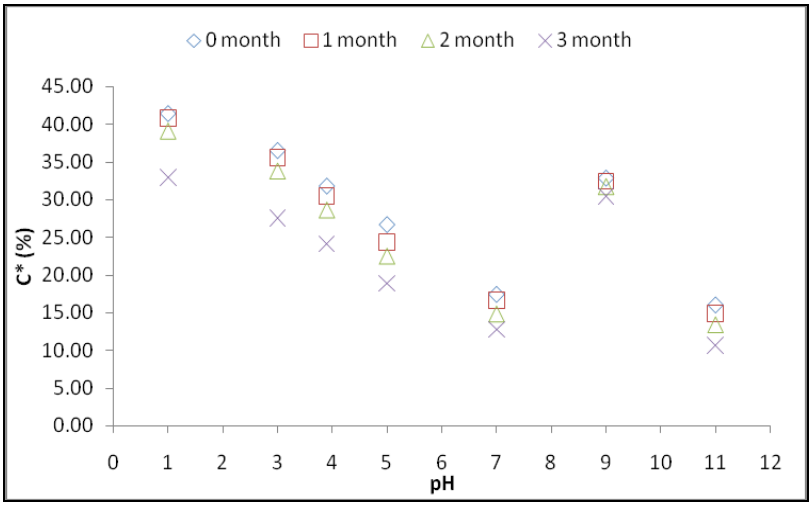


Figure 5.14: Relationship between pH variation and C* values (%) for purified anthocyanin-PVA blends during three month of exposure

From Table 5.19, the initial of hue angle, hº for purified anthocyanin-PVA blends for all pH decreased from sample at pH 1 h°=(26.038 ± 0.014)° until sample at pH 5 h°=(19.430 ± 0.011)° and begins to increase at pH 7 h°=(25.263 ± 0.013)° until pH 9 h°=(62.636 ± 0.015)° before decreasing again at pH 11 h°=(30.112 ± 0.015)°. The hue angle of purified anthocyanin-PVA blends for all pH increased significantly from the beginning of exposure until the third month of exposure, ranging from (26.038 ± 0.014)° to (52.605 ± 0.011)°, (23.411 ± 0.010)° to (57.340 ± 0.011)°, (20.598 ± 0.018)° to (61.846 ± 0.015)°, (19.430 ± 0.011)° to (62.675 ± 0.014)°, (25.263 ± 0.013)° to (55.119 ± 0.016)°, (62.636 ± 0.015)° to (77.581 ± 0.018)° and (30.112 ± 0.015)° to (55.113 ± 0.010)° for pH 1, 3, 3.9, 5, 7, 9 and 11 respectively as presented in Figure 5.15. During the three months of exposure, the purified anthocyanin-PVA sample at pH 9 exhibited the highest hue angle of (62.636 ± 0.015)° with a*=(15.104 ± 0.016) and b*=(29.184 ± 0.017). This is followed by sample at pH 11 with hue angle=(30.112 ± 0.015)°, a*=(13.904 ± 0.018) while the coordinate of b*

to (8.064 ± 0.018). After three months of exposure, sample at pH 9 again contributed to the highest hue angle of (77.581 ± 0.018)°, but the a* coordinate moved back to lower positive a*=(6.539 ± 0.017) and b* slightly increased to (29.696 ± 0.022). The hue angle for sample at pH 11 h°=(55.113 ± 0.010)°, the a* moved to lower positive (6.103 ± 0.015) and b* to (8.753 ± 0.017). In addition, the hue angle of sample at pH 1 is (26.038 ± 0.014)° with highest a* of (37.194 ± 0.016) and b* value of (18.172 ± 0.016) at zero time, while at the end of exposure the hue angle increased to (52.605 ± 0.011)°, with a* value at (20.007 ± 0.012) and b* at (26.173 ± 0.012). The gradual degradation of red colour, visually observed in all pH, experienced by purified anthocyanin-PVA blends is accompanied by the tonality changes from red to brown-yellow tints as h^o increased during experiment time and is significant for samples at higher pH (pH 9 and 11).

Table 5.19: Statistical summary of CIE H˚a*b* colour data for purified anthocyanin-PVA blends with different pH

CIE value	Time (month)	pH	$Mean_a$ ± s.e.	Minimum	Maximum
H˚	0	1	26.038_{22}± 0.014	26.014	26.063
		3	23.411_{24}± 0.010	23.393	23.429
		3.9	20.598_{25}± 0.018	20.567	20.628
		5	19.430_{26}± 0.011	19.411	19.450
		7	25.263_{23}± 0.013	25.241	25.285
		9	62.636_{4} ± 0.015	62.611	62.662
		11	30.112_{17}± 0.015	30.087	30.138
	1	1	29.085_{19}± 0.015	29.059	29.112
		3	28.190_{20}± 0.017	28.161	28.219
		3.9	27.085_{21}± 0.015	27.059	27.111
		5	29.175_{18}± 0.013	29.152	29.197
		7	30.557_{16}± 0.017	30.528	30.586
		9	65.336_{3} ± 0.014	65.311	65.361
		11	34.384_{13}± 0.020	34.348	34.419
	2	1	34.308_{14}± 0.012	34.288	34.328
		3	34.212_{15}± 0.017	34.182	34.242
		3.9	34.704_{12}± 0.012	34.682	34.725
		5	35.026_{11}± 0.016	34.999	35.053
		7	39.820_{10}± 0.016	39.793	39.847
		9	68.858_{2} ± 0.014	68.834	68.883
		11	40.418_{9} ± 0.020	40.384	40.452
	3	1	52.605_{8} ± 0.011	52.586	52.625
		3	57.340_{6} ± 0.011	57.321	57.358
		3.9	61.846_{5} ± 0.015	61.820	61.872
		5	62.675_{4} ± 0.014	62.651	62.699
		7	55.119_{7} ± 0.016	55.091	55.147
		9	77.581_{1} ± 0.018	77.551	77.612
		11	55.113_{7} ± 0.010	55.096	55.129

(Note: Means with the different subscript numbers are significantly different at P<0.05)
$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with different pH

'Table 5.19, continued'

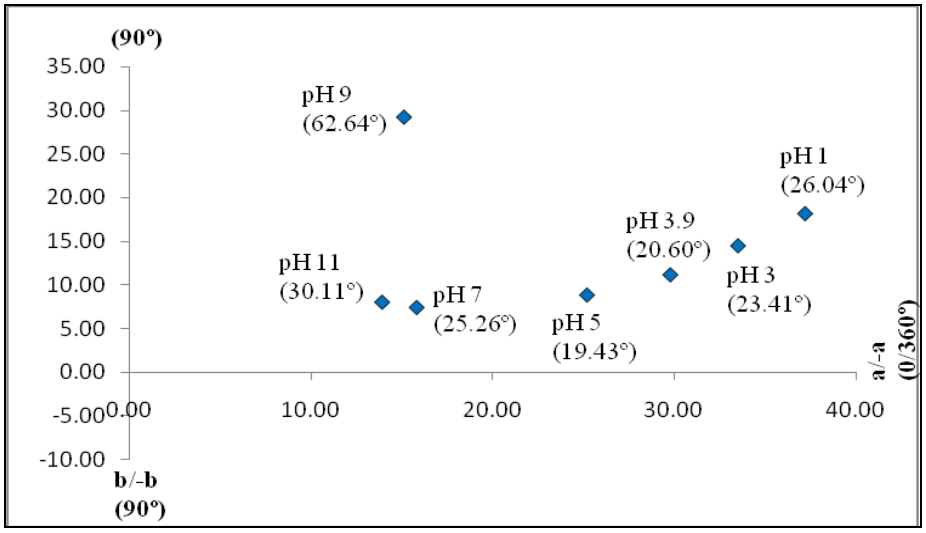
CIE value	Time (month)	pH	Mean$_a$ ± s.e.	Minimum	Maximum
a*	0	1	37.194_{1} ± 0.016	37.167	37.221
		3	33.501_{3} ± 0.014	33.476	33.525
		3.9	29.770_{6} ± 0.013	29.747	29.792
		5	25.185_{9} ± 0.016	25.158	25.213
		7	15.808_{14}± 0.010	15.791	15.825
		9	15.104_{15}± 0.016	15.077	15.132
		11	13.904_{18}± 0.018	13.873	13.936
	1	1	35.703_{2} ± 0.011	35.684	35.722
		3	31.357_{5} ± 0.015	31.331	31.383
		3.9	27.172_{8} ± 0.016	27.144	27.199
		5	21.309_{11}± 0.012	21.288	21.329
		7	14.331_{17}± 0.010	14.314	14.349
		9	13.548_{19}± 0.014	13.523	13.573
		11	12.297_{20}± 0.020	12.262	12.331
	2	1	32.312_{4} ± 0.013	32.289	32.334
		3	27.952_{7} ± 0.012	27.931	27.973
		3.9	23.529_{10}± 0.020	23.495	23.563
		5	18.408_{13}± 0.009	18.392	18.424
		7	11.358_{22}± 0.014	11.333	11.383
		9	11.447_{21}± 0.014	11.423	11.472
		11	10.164_{23}± 0.012	10.142	10.185
	3	1	20.007_{12}± 0.012	19.987	20.027
		3	14.850_{16}± 0.011	14.832	14.869
		3.9	11.373_{22}± 0.016	11.344	11.401
		5	8.671_{24} ± 0.014	8.646	8.695
		7	7.307_{25} ± 0.011	7.289	7.326
		9	6.539_{26} ± 0.017	6.509	6.569
		11	6.103_{27} ± 0.015	6.077	6.129

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with different pH

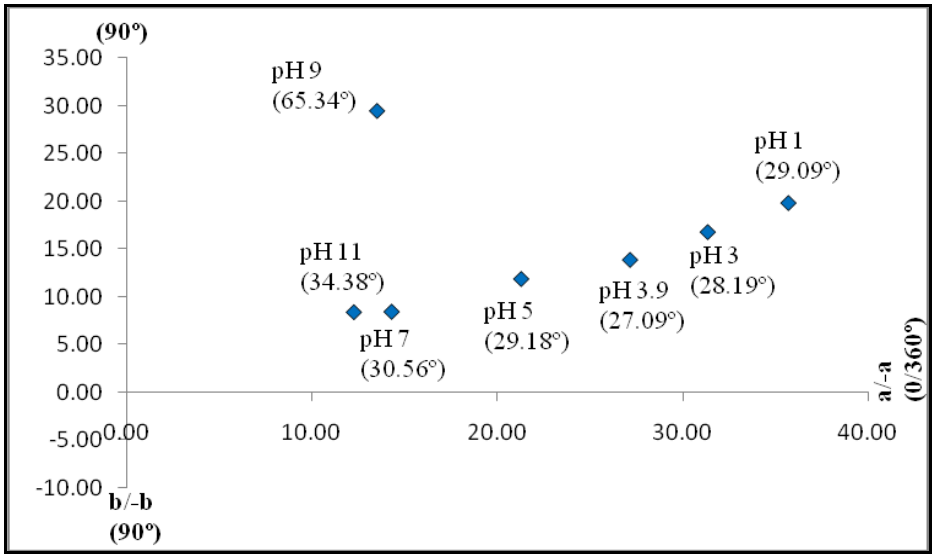
'Table 5.19, continued'

CIE value	Time (month)	pH	Mean$_a$ ± s.e.	Minimum	Maximum
b*	0	1	18.172_{11}± 0.016	18.144	18.199
		3	14.505_{14}± 0.010	14.487	14.522
		3.9	11.189_{18}± 0.016	11.161	11.216
		5	8.884_{21} ± 0.012	8.862	8.905
		7	7.460_{27} ± 0.014	7.435	7.485
		9	29.184_{4} ± 0.017	29.154	29.213
		11	8.064_{26} ± 0.018	8.033	8.094
	1	1	19.860_{9} ± 0.014	19.836	19.884
		3	16.807_{12}± 0.013	16.785	16.829
		3.9	13.896_{15}± 0.013	13.873	13.919
		5	11.897_{17}± 0.014	11.872	11.921
		7	8.461_{24} ± 0.018	8.429	8.493
		9	29.505_{3} ± 0.022	29.467	29.542
		11	8.415_{25} ± 0.012	8.395	8.435
	2	1	22.049_{7} ± 0.015	22.023	22.075
		3	19.005_{10}± 0.009	18.989	19.020
		3.9	16.295_{13}± 0.014	16.271	16.319
		5	12.902_{16}± 0.012	12.881	12.922
		7	9.470_{20} ± 0.020	9.436	9.504
		9	29.601_{2} ± 0.016	29.573	29.628
		11	8.656_{23} ± 0.015	8.631	8.682
	3	1	26.173_{5} ± 0.012	26.153	26.193
		3	23.167_{6} ± 0.014	23.143	23.191
		3.9	21.252_{8} ± 0.015	21.225	21.278
		5	16.782_{12}± 0.014	16.757	16.806
		7	10.482_{19}± 0.017	10.453	10.511
		9	29.696_{1} ± 0.022	29.659	29.734
		11	8.753_{22} ± 0.017	8.724	8.782

(Note: Means with the different subscript numbers are significantly different at P<0.05)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends with different pH

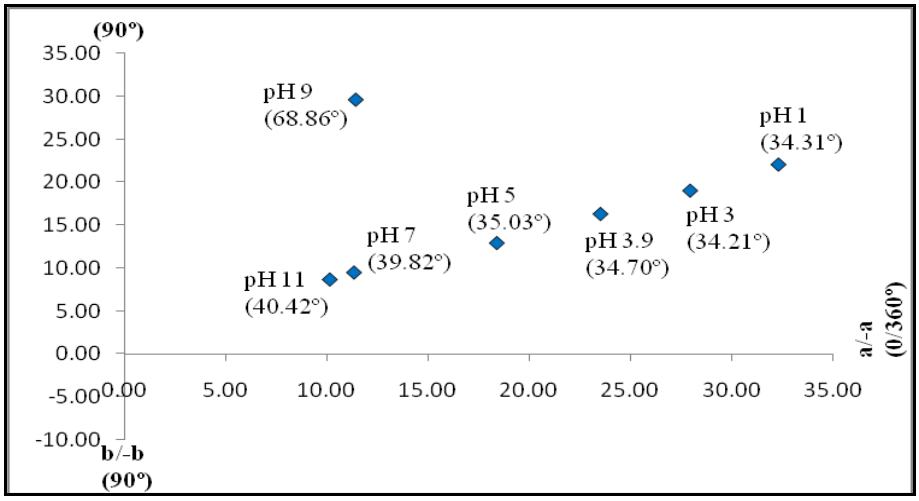


(a)



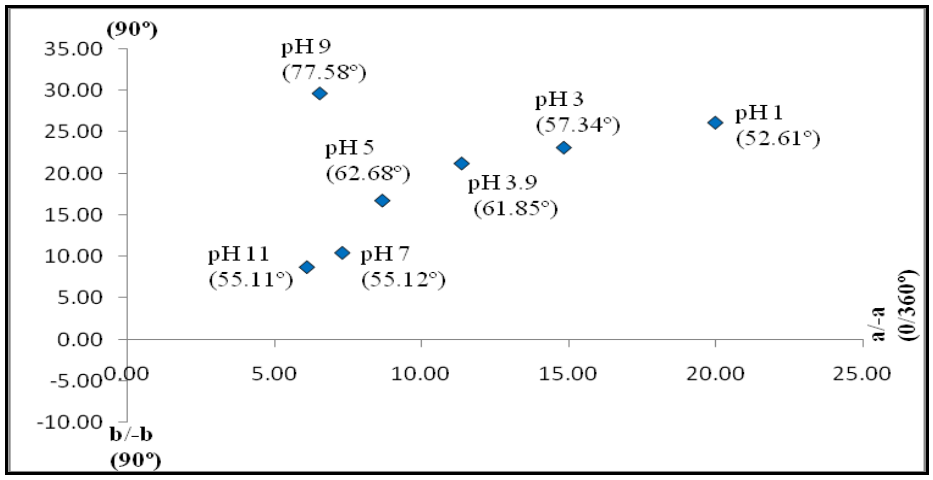
(b)

Figure 5.15: Relationship between pH variation and H◦ with a*b* coordinate for purified anthocyanin-PVA blends during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 5.15, continued'



(d)

'Figure 5.15, continued'

Table 5.20 presents the total colour difference (ΔE), which was lowest for purified anthocyanin-PVA blends at pH 1 which ΔE_1=5.582, during first month of exposure and is still the lowest at the end of exposure (ΔE_3=23.951). In contrast, the ΔE of purified anthocyanin-PVA blends at pH 11 was the highest at zero time (ΔE_1=15.956) and at the

end of exposure ΔE_3=36.620. Other purified anthocyanin-PVA of different pH demonstrated a similar trend in colour change being low before exposure (zero time) and higher at the end of exposure from ΔE_1=6.751 to ΔE_3=25.871, ΔE_1=7.364 to ΔE_3=25.187, ΔE_1=7.536 to ΔE_3=27.093, ΔE_1=11.257 to ΔE_3=30.098 and ΔE_1=12.298 to ΔE_3=27.275 for pH 3, 3.9, 5, 7 and 9 respectively. In addition, the purified anthocyanin-PVA blends at pH 1 exhibited the highest saturation parameter at time zero (s_0=0.6167) that decreased with increasing exposure time until the end of three months with (s_3=0.4029). Though saturation of sample at pH 9 was higher in the beginning, the colour tends to turn into brown. Other purified anthocyanin-PVA blends with different pH showed similar trend. Purified anthocyanin-PVA blends at pH 11 exhibited the lowest saturation at time zero, (s_0=0.2659) and drastically decreased towards the end of exposure with (s_3=0.1109) as in Table 5.20.

Table 5.20: Total colour differences (ΔE) and saturation of purified anthocyanin-PVA blends as affected by pH

pH	TIME (Month)				ΔE_1	ΔE_3
	0	1	2	3		
pH 1	S_0=0.6167	S_1=0.5656	S_2=0.5151	S_3=0.4029	ΔE_1=5.582	ΔE_3=23.951
pH 3	S_0=0.5351	S_1=0.4795	S_2=0.4332	S_3=0.3279	ΔE_1=6.751	ΔE_3=25.871
pH 3.9	S_0=0.4520	S_1=0.3979	S_2=0.3547	S_3=0.2745	ΔE_1=7.364	ΔE_3=27.275
pH 5	S_0=0.3791	S_1=0.3116	S_2=0.2718	S_3=0.2021	ΔE_1=9.299	ΔE_3=29.428
pH 7	S_0=0.2716	S_1=0.2155	S_2=0.1807	S_3=0.1322	ΔE_1=13.019	ΔE_3=33.553
pH 9	S_0=0.5293	S_1=0.4213	S_2=0.4020	S_3=0.3144	ΔE_1=15.061	ΔE_3=35.694
pH 11	S_0=0.2659	S_1=0.1952	S_2=0.1707	S_3=0.1109	ΔE_1=15.956	ΔE_3=36.620

5.3.3. Effect of addition 2% ferulic (FA) and pH on visual colour variation

Table 5.21 displays the results of the colour parameters (CIE L*) of purified anthocyanin-PVA from *Ixora* as affected by the addition of 2% FA and with different pH values. From previous results, 2% FA act as good colour enhancer and stabilizer. The initial (zero time of exposure) lightness percentage (L*) of purified anthocyanin-PVA containing 2% FA with altered pH (initial pH (3.8), pH 1, 3, 5, 7, 9 and 11) were observed increasing from sample at pH 1 (L*=49.479 ± 0.009) until sample at pH 3 (L*=54.784 ± 0.011) while decreasing until pH 7 (L*=49.560 ± 0.010), increasing at pH 9 (L*=58.282 ± 0.010) before decreasing again at pH 11 (L*=49.712 ± 0.007). In addition, during exposure the L* parameter for purified anthocyanin-PVA at pH 3, 3.8 and 5 decreased (darker colour) from initial L* value until second month of exposure before increased at the third month of exposure. The significant decreased in L* value over two month of exposure was obtained by purified anthocyanin-PVA at pH 3, which the initial L*=(54.784 ± 0.011) decrease to (41.156 ± 0.012), followed by sample at pH 3.8, L* decreased from (54.283 ± 0.005) to (41.794 ± 0.010) and pH 5 L* decreased from (51.001 ± 0.009) to (41.344 ± 0.011). In contrast, other pH values (pH 1, 7, 9 and 11) continually increased from zero time of exposure until the third month of exposure ranging from (49.479 ± 0.009) to (60.996 ± 0.012), (49.560 ± 0.010) to (67.981 ± 0.008), (58.282 ± 0.010) to (80.198 ± 0.010), and (49.712 ± 0.007) to (79.549 ± 0.010) respectively. After three month of exposure, the purified anthocyanin-PVA containing 2% FA at pH 9 exhibited the lightest colour with highest L* values which (80.198 ± 0.010), while the lowest values (darker colour) gained by samples at pH 3 with (55.991 ± 0.015). The trend can be seen in Figure 5.16.

Table 5.21: Statistical summary of CIE L* colour data for purified anthocyanin-PVA blends containing 2% FA with different pH

CIE value	Time (month)	pH	Mean$_a$ ± s.e.	Minimum	Maximum
L*	0	1	49.479_{22}± 0.009	49.464	49.494
		3	54.784_{16}± 0.011	54.765	54.803
		3.8	54.283_{17}± 0.005	54.274	54.293
		5	51.001_{19}± 0.009	50.986	51.017
		7	49.560_{21}± 0.010	49.543	49.576
		9	58.282_{8} ± 0.010	58.265	58.299
		11	49.712_{20}± 0.007	49.701	49.724
	1	1	55.045_{14}± 0.013	55.023	55.068
		3	42.287_{25}± 0.007	42.276	42.299
		3.8	42.691_{23}± 0.011	42.672	42.711
		5	42.342_{24}± 0.011	42.322	42.361
		7	51.721_{18}± 0.011	51.703	51.740
		9	63.804_{6} ± 0.010	63.786	63.821
		11	54.924_{15}± 0.010	54.908	54.941
	2	1	58.046_{10}± 0.013	58.023	58.069
		3	41.156_{28}± 0.012	41.135	41.176
		3.8	41.794_{26}± 0.010	41.776	41.812
		5	41.344_{27}± 0.011	41.325	41.364
		7	58.218_{9} ± 0.008	58.205	58.232
		9	68.093_{3} ± 0.010	68.076	68.109
		11	64.105_{5} ± 0.012	64.085	64.125
	3	1	60.996_{7} ± 0.012	60.975	61.018
		3	55.991_{13}± 0.015	55.965	56.017
		3.8	56.880_{12}± 0.010	56.864	56.897
		5	56.998_{11}± 0.015	56.972	57.025
		7	67.981_{4} ± 0.008	67.968	67.995
		9	80.198_{1} ± 0.010	80.182	80.215
		11	79.549_{2} ± 0.010	79.532	79.567

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends containing 2% FA with different pH

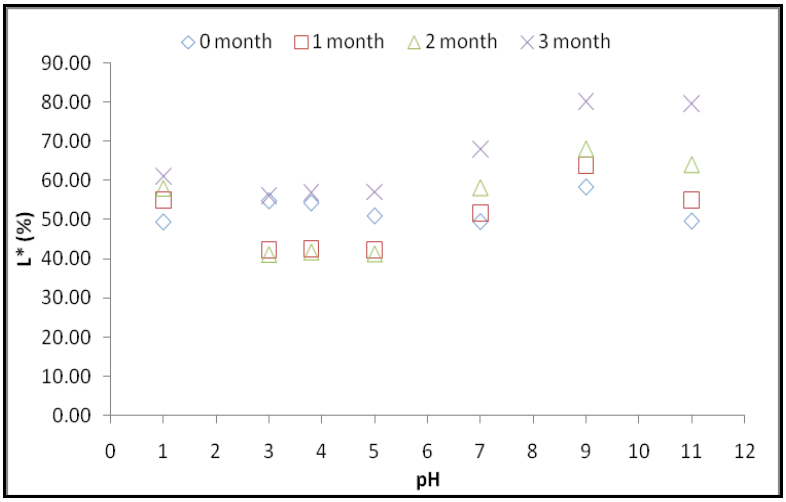


Figure 5.16: Relationship between pH variation and L* values (%) for purified anthocyanin-PVA blends containing 2% FA during three month of exposure

Meanwhile, the chromaticity (C*) values of purified anthocyanin-PVA with altered pH (initial pH (3.8), pH 1, 3, 5, 7, 9 and 11) in the beginning and at the end of exposure are shown in Table 5.22. The initial (zero time of exposure) C* values of the purified anthocyanin-PVA containing 2% FA at altered pH (pH 3, 3.8 and 5) were observed increased continuously until second month of exposure, in which significant increased (brightest colour) for sample at pH 3 as the initial C* values increased from (41.289 ± 0.014) to (50.201 ± 0.007) before decreasing at the third month of exposure (39.086 ± 0.015). This trend is followed by the sample at pH 3.8 which increasing from initial C* value (40.688 ± 0.015) to second month C* value (48.551 ± 0.011) before decreasing at end of exposure (37.757 ± 0.015) as well as sample at pH 5 which increasing from (34.630 ± 0.006) to (42.322 ± 0.007) and decreasing at the third month of exposure (33.316 ± 0.008). In contrast, other pH variation (pH 1, 7, 9 and 11) decreased continuously from zero time of exposure until the third month of exposure ranging from (47.174 ± 0.012) to

(32.560 ± 0.011), (19.470 ± 0.010) to (15.170 ± 0.008), (42.965 ± 0.011) to (38.664 ± 0.016) and (19.331 ± 0.011) to (15.256 ± 0.014). Nevertheless, after three month of exposure, the purified anthocyanin-PVA at pH 3 experienced the highest C* values (39.086 ± 0.015) which exhibit more vivid colour (brighter colour). Meanwhile the lowest in C* value obtained by samples at pH 11 (13.899 ± 0.014) and pH 7 (15.170 ± 0.008), exhibit dull colours.

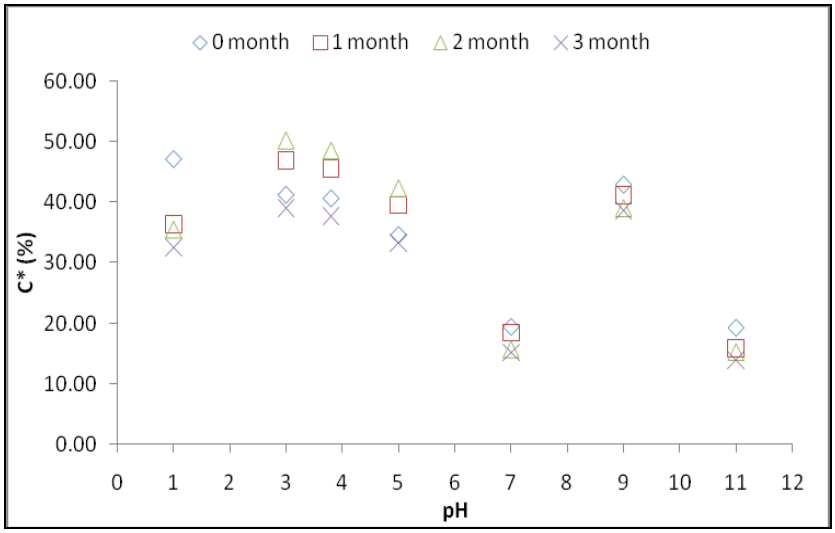


Figure 5.17: Relationship between pH variation and C* values (%) for purified anthocyanin-PVA blends containing 2% FA during three month of exposure

Table 5.22: Statistical summary of CIE C* colour data for purified anthocyanin-PVA blends containing 2% FA with different pH

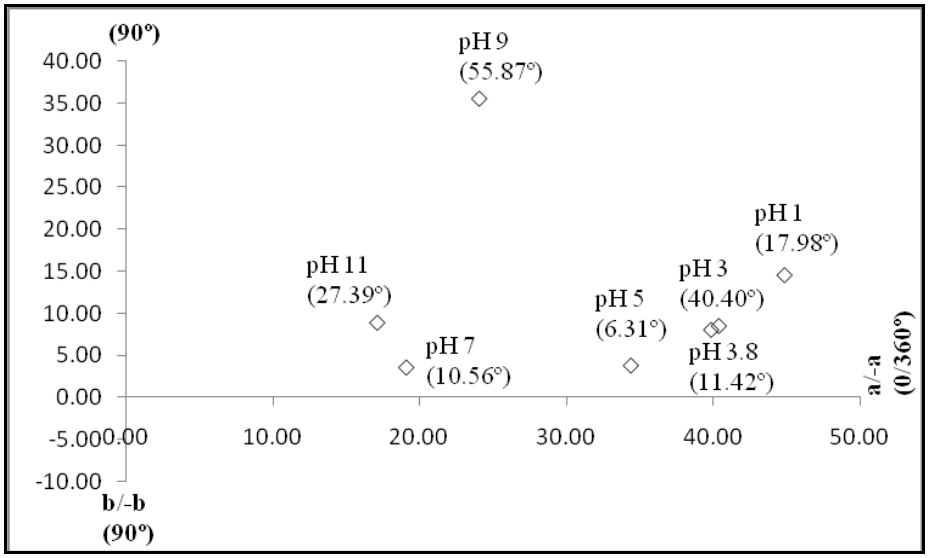
CIE value	Time (month)	pH	$Mean_a \pm$ s.e.	Minimum	Maximum
C*	0	1	$47.174_3 \pm 0.012$	47.153	47.195
		3	$41.289_8 \pm 0.014$	41.265	41.312
		3.8	$40.688_{10} \pm 0.015$	40.663	40.714
		5	$34.630_{18} \pm 0.006$	34.619	34.641
		7	$19.470_{21} \pm 0.010$	19.453	19.486
		9	$42.965_6 \pm 0.011$	42.946	42.985
		11	$19.331_{22} \pm 0.011$	19.312	19.351
	1	1	$36.363_{16} \pm 0.012$	36.343	36.383
		3	$47.005_4 \pm 0.012$	46.984	47.026
		3.8	$45.645_5 \pm 0.013$	45.623	45.667
		5	$39.558_{11} \pm 0.015$	39.532	39.584
		7	$18.469_{23} \pm 0.014$	18.445	18.492
		9	$41.147_9 \pm 0.015$	41.121	41.174
		11	$15.796_{24} \pm 0.007$	15.784	15.809
	2	1	$35.507_{17} \pm 0.007$	35.495	35.519
		3	$50.201_1 \pm 0.007$	50.189	50.213
		3.8	$48.551_2 \pm 0.011$	48.532	48.571
		5	$42.322_7 \pm 0.007$	42.310	42.333
		7	$15.706_{25} \pm 0.010$	15.689	15.722
		9	$39.039_{13} \pm 0.011$	39.019	39.058
		11	$15.256_{26} \pm 0.014$	15.232	15.279
	3	1	$32.560_{20} \pm 0.008$	32.546	32.575
		3	$39.086_{12} \pm 0.015$	39.061	39.112
		3.8	$37.757_{15} \pm 0.015$	37.732	37.783
		5	$33.316_{19} \pm 0.008$	33.303	33.329
		7	$15.170_{27} \pm 0.008$	15.156	15.184
		9	$38.664_{14} \pm 0.016$	38.635	38.692
		11	$13.899_{28} \pm 0.014$	13.875	13.923

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

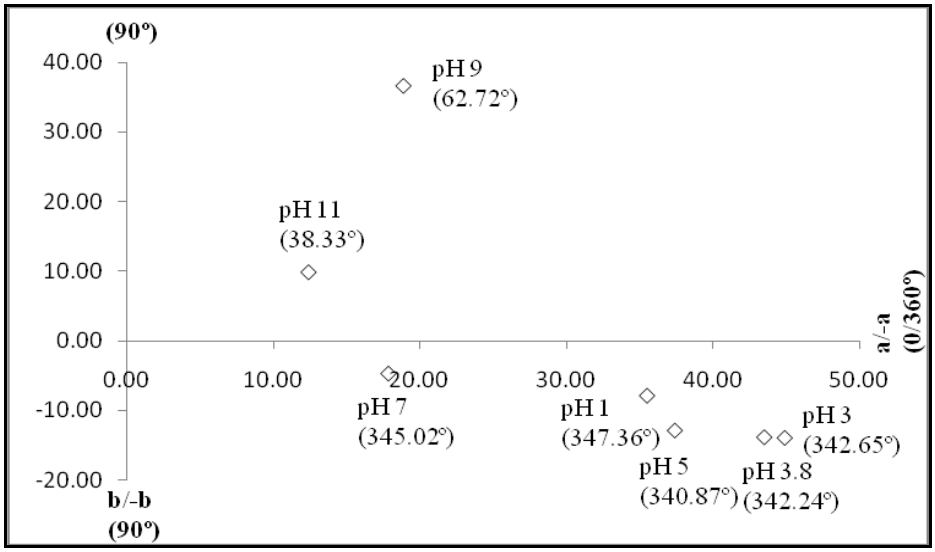
$Mean_a \pm$ standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends containing 2% FA with different pH

Additionally, the initial exposure of hue angle, h^o values of the purified anthocyanin-PVA containing 2% FA with different pH values were observed decreased from sample at pH 1 (17.978 ± 0.012)^o until sample at pH 5 (6.308 ± 0.012)^o, whereas started to increase at pH 7 (10.559 ± 0.014)^o until pH 9 and (55.872 ± 0.015)^o, before decrease again at pH 11 (27.389 ± 0.012)^o. According to Figure 5.18, it can be noted that the hue angle of purified anthocyanin-PVA with pH values (pH 3, 3.8 and 5) continually moved clockwise into blue region from the zero time of exposure until the second month of exposure, ranging from hue angle (11.896 ± 0.011)^o with positive a* (40.403 ± 0.008) and b* value (8.512 ± 0.007) moved to hue angle of (337.750 ± 0.012)^o with more positive a* (46.467 ± 0.013) and negative b* value (-19.001 ± 0.010) for sample at pH 3, hue angle of (11.420 ± 0.005)^o with positive a* (39.883 ± 0.016) and b* value (8.057 ± 0.014) moved to (337.510 ± 0.007)^o with more positive a* (44.861 ± 0.009) and negative b* value (-18.566 ± 0.012) for sample at pH 3.8 and hue angle of (6.308 ± 0.012)^o with positive a* (34.421 ± 0.011) and b* value (3.805 ± 0.007) moved to (336.370 ± 0.011)^o with more positive a* (38.775 ± 0.010) and negative b* value (-16.961 ± 0.009) for sample at pH 5. At the third month of exposure, the corresponding pH (pH 3, 3.8 and 5) moved counterclockwise into red tonalities which hue angle were (16.316 ± 0.007)^o with lower positive a* (37.512 ± 0.007) and b* value (10.981 ± 0.009) for sample at pH 3, hue angle of (18.054 ± 0.013)^o with lower positive a* (35.898 ± 0.015) and b* value (11.702 ± 0.009) for sample at pH 3.8, whereas for sample at pH 5 the hue angle was (25.703 ± 0.007)^o with lower positive a* (30.020 ± 0.011) and b* value (14.450 ± 0.012). Meanwhile for sample at pH 1 and 7 the hue angle also moved clockwise into blue region but only at the first month of exposure since at the second month of exposure the sample have already moved counterclockwise

into red tonalities until third month of exposure. In contrast, during three month of exposure the purified anthocyanin-PVA at pH 9 and 11 directly moved counterclockwise start from first month of exposure until third month of exposure approaching yellow region, to the higher h^o values. Further detailed values can be seen in Table 5.23. Before exposure (zero time), the hue angle for sample at pH 9 was the highest $(55.872 \pm 0.015)^o$ with positive a* value (24.105 ± 0.008) and positive b* values (35.566 ± 0.013) while after three month of exposure, sample at pH 9 again contributed to the higher hue angle overall $(81.744 \pm 0.011)^o$ while moved drastically backward to lower positive a* value (5.552 ± 0.011) and slightly increased of b* values (38.264 ± 0.011). In addition, sample at pH 3 experienced lower hue angle of $(11.896 \pm 0.011)^o$ with positive a* value (40.403 ± 0.008) and positive b* value (8.512 ± 0.007) before exposure (zero time), while at the end of exposure the hue angle was the lowest $(16.316 \pm 0.007)^o$, with highest a* value (37.512 ± 0.007) and lowest b* value (10.981 ± 0.009). The gradual degradation of red colour, visually observed for purified anthocyanin-PVA blends was accompanied by the tonality changes from red to brown-yellow tints and black colour as the h^o increased with time. This is significant for samples at higher pH (pH 7, 9 and 11). Furthermore, the h^o values of lower pH (pH 1, 3, 3.8 and 5) showed vivid purple colours, especially for sample at pH 3, before turning back again into red colour tonalities at the end of exposure.

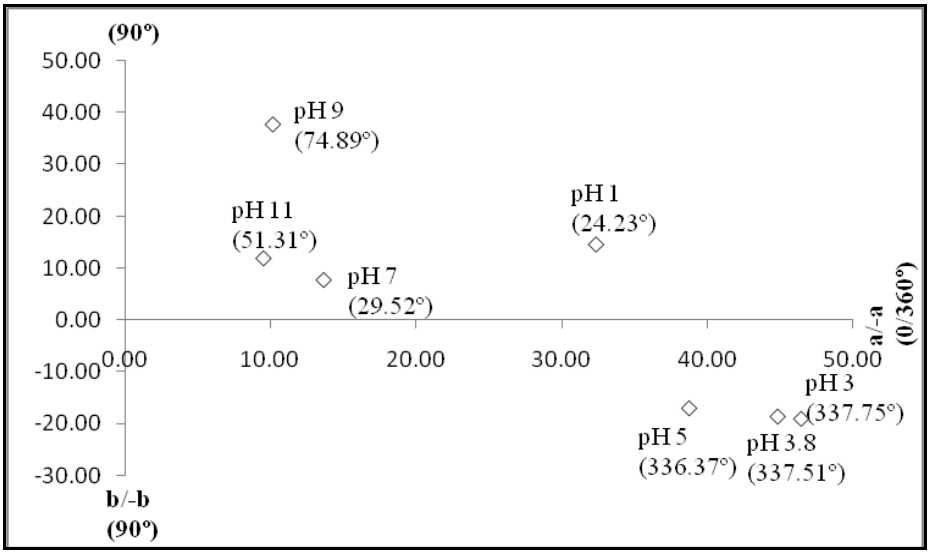


(a)



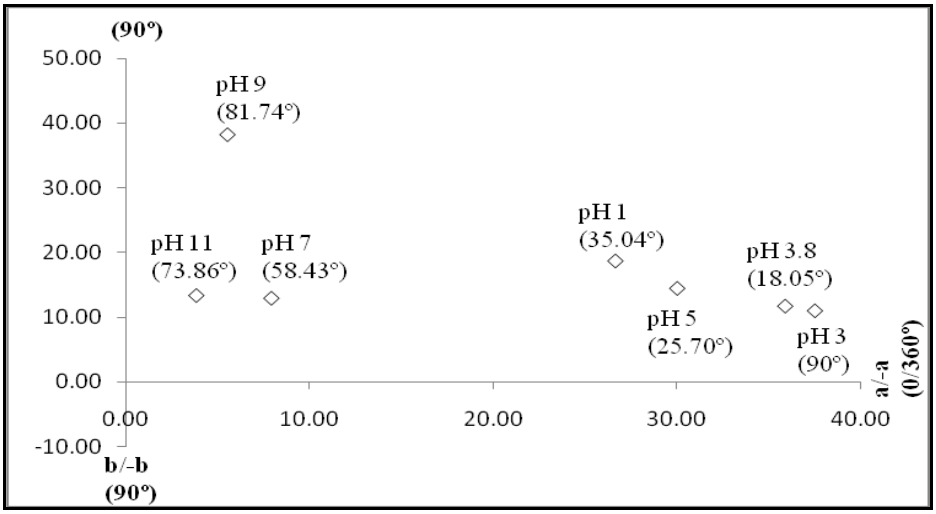
(b)

Figure 5.18: Relationship between pH variation and H◦ with a*b* coordinate for purified anthocyanin-PVA blends containing 2% FA during (a) zero time of exposure, (b) first month of exposure, (c) second month of exposure, (d) third month of exposure



(c)

'Figure 5.18, continued'



(d)

'Figure 5.18, continued'

Table 5.23: Statistical summary of CIE H˚a*b* colour data for purified anthocyanin-PVA blends containing 2% FA with different pH

CIE value	Time (month)	pH	$Mean_a$ ± s.e.	Minimum	Maximum
H˚	0	1	17.978_{23}± 0.012	17.958	17.998
		3	11.896_{25}± 0.011	11.876	11.915
		3.8	11.420_{26}± 0.005	11.411	11.429
		5	6.308_{28} ± 0.012	6.286	6.329
		7	10.559_{27}± 0.014	10.534	10.584
		9	55.872_{14}± 0.015	55.846	55.899
		11	27.389_{19}± 0.012	27.368	27.411
	1	1	347.360_{1}±0.012	347.340	347.381
		3	342.650_{3}±0.014	342.626	342.675
		3.8	342.240_{4}±0.012	342.220	342.260
		5	340.870_{5}±0.009	340.854	340.886
		7	345.020_{2}±0.015	344.995	345.046
		9	62.717_{12}± 0.009	62.701	62.733
		11	38.326_{16}± 0.012	38.305	38.348
	2	1	24.225_{21}± 0.014	24.200	24.250
		3	337.750_{6}±0.012	337.729	337.771
		3.8	337.510_{7}±0.007	337.498	337.521
		5	336.370_{8}±0.011	336.351	336.389
		7	29.524_{18}± 0.013	29.502	29.547
		9	74.886_{10}± 0.018	74.854	74.918
		11	51.314_{15}± 0.006	51.303	51.325
	3	1	35.043_{17}± 0.006	35.032	35.054
		3	16.316_{24}± 0.007	16.304	16.329
		3.8	18.054_{22}± 0.013	18.032	18.076
		5	25.703_{20}± 0.007	25.691	25.715
		7	58.430_{13}± 0.009	58.415	58.445
		9	81.744_{9} ± 0.011	81.725	81.762
		11	73.863_{11}± 0.016	73.835	73.891

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

$Mean_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends containing 2% FA with different pH

'Table 5.23, continued'

CIE value	Time (month)	pH	$Mean_a \pm$ s.e.	Minimum	Maximum
a*	0	1	$44.871_2 \pm 0.010$	44.854	44.888
		3	$40.403_4 \pm 0.008$	40.389	40.416
		3.8	$39.883_5 \pm 0.016$	39.856	39.910
		5	$34.421_{11} \pm 0.011$	34.402	34.441
		7	$19.141_{16} \pm 0.012$	19.121	19.161
		9	$24.105_{15} \pm 0.008$	24.091	24.118
		11	$17.164_{19} \pm 0.016$	17.137	17.192
	1	1	$35.482_{10} \pm 0.012$	35.461	35.504
		3	$44.868_2 \pm 0.013$	44.845	44.891
		3.8	$43.472_3 \pm 0.010$	43.454	43.489
		5	$37.376_8 \pm 0.014$	37.352	37.399
		7	$17.842_{18} \pm 0.012$	17.821	17.862
		9	$18.861_{17} \pm 0.010$	18.843	18.878
		11	$12.392_{21} \pm 0.008$	12.378	12.407
	2	1	$32.381_{12} \pm 0.015$	32.354	32.407
		3	$46.467_1 \pm 0.013$	46.445	46.489
		3.8	$44.861_2 \pm 0.009$	44.846	44.876
		5	$38.775_6 \pm 0.010$	38.758	38.793
		7	$13.667_{20} \pm 0.008$	13.653	13.682
		9	$10.179_{22} \pm 0.013$	10.156	10.201
		11	$9.536_{23} \pm 0.008$	9.522	9.551
	3	1	$26.658_{14} \pm 0.009$	26.643	26.674
		3	$37.512_7 \pm 0.007$	37.501	37.524
		3.8	$35.898_9 \pm 0.015$	35.872	35.923
		5	$30.020_{13} \pm 0.011$	30.001	30.038
		7	$7.942_{24} \pm 0.010$	7.924	7.959
		9	$5.552_{25} \pm 0.011$	5.533	5.571
		11	$3.863_{26} \pm 0.012$	3.842	3.884

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)

$Mean_a \pm$ standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends containing 2% FA with different pH

'Table 5.23, continued'

CIE value	Time (month)	pH	Mean$_a$ ± s.e.	Minimum	Maximum
b*	0	1	14.561_{9} ± 0.010	14.543	14.578
		3	8.512_{21} ± 0.007	8.501	8.524
		3.8	8.057_{22} ± 0.014	8.032	8.082
		5	3.805_{26} ± 0.007	3.792	3.817
		7	3.568_{27} ± 0.010	3.551	3.584
		9	35.566_{4} ± 0.013	35.543	35.588
		11	8.893_{20} ± 0.009	8.878	8.908
	1	1	-7.956_{23} ± 0.008	7.943	7.970
		3	-14.012_{11}±0.006	14.001	14.022
		3.8	-13.918_{12}±0.009	13.903	13.933
		5	-12.958_{14}±0.013	12.935	12.981
		7	-4.773_{25} ± 0.005	4.764	4.783
		9	36.570_{3} ± 0.014	36.546	36.593
		11	9.796_{19} ± 0.016	9.769	9.824
	2	1	14.570_{9} ± 0.015	14.543	14.596
		3	-19.001_{5}± 0.010	18.984	19.018
		3.8	-18.566_{7}± 0.012	18.545	18.586
		5	-16.961_{8}± 0.009	16.945	16.977
		7	7.740_{24} ± 0.009	7.724	7.756
		9	37.689_{2} ± 0.010	37.671	37.706
		11	11.909_{16}± 0.012	11.889	11.929
	3	1	18.696_{6} ± 0.010	18.680	18.713
		3	10.981_{18}± 0.009	10.965	10.996
		3.8	11.702_{17}± 0.009	11.686	11.718
		5	14.450_{10}± 0.012	14.430	14.470
		7	12.925_{15}± 0.012	12.904	12.945
		9	38.264_{1} ± 0.011	38.245	38.282
		11	13.352_{13}± 0.013	13.329	13.375

(Note: Means with the different subscript numbers are significantly different at $P<0.05$)
Mean$_a$ ± standard error calculated from colour measurements repeated in triplicate on purified anthocyanin-PVA blends containing 2% FA with different pH

Table 5.24 showed the total colour difference (ΔE), which was the greatest for the purified anthocyanin-PVA containing 2% FA at pH 3 where ΔE_1=26.143, at first month of exposure while lower colour change at the end of exposure (ΔE_3=3.989). Other purified anthocyanin-

PVA blends demonstrated a similar trend in ΔE, the highest being at zero time and lower towards the end of exposure from ΔE_1=25.023 to ΔE_3=21.942, ΔE_1=25.103 to ΔE_3=5.992 and ΔE_1=19.097 to ΔE_3=12.986, for pH 1, 3.8 and 5 respectively. In contrast, the ΔE of purified anthocyanin-PVA blends at pH 7, 9 and 11 were lower at zero time but increased at the end of storage period showing degradation. The purified anthocyanin-PVA containing 2% FA at pH 3 exhibited the highest saturation index at the zero time, (s_0=0.7537), which increased with increasing exposure time until the second month of exposure (s_2=1.2198). Finally, at the third month of exposure, (s_3=0.6981) the saturation index dropped. Eventhough saturation index for sample at pH 1 is also high; it decreased with increasing of exposure time. The sample with pH 9 tends to degrade into brown as can be seen in Table 5.24. Sample at pH 11 exhibit the lowest saturation index, which at zero time (s_0=0.3889) and continues decrease towards the end of exposure (s_3=0.1747).

Table 5.24: Total colour differences (ΔE) and saturation of purified anthcyanin-PVA blends as affected by pH with addition of 2% FA

pH	TIME (Month) 0	1	2	3	ΔE_1	ΔE_3
pH 1	s_0=0.9534	s_1=0.6606	s_2=0.6117	s_3=0.5338	ΔE_1=25.023	ΔE_3=21.942
pH 3	s_0=0.7537	s_1=1.1116	s_2=1.2198	s_3=0.6981	ΔE_1=26.143	ΔE_3=3.989
pH 3.8	s_0=0.7495	s_1=1.0692	s_2=1.1617	s_3=0.6638	ΔE_1=25.103	ΔE_3=5.992
pH 5	s_0=0.6790	s_1=0.9342	s_2=1.0236	s_3=0.5845	ΔE_1=19.097	ΔE_3=12.986
pH 7	s_0=0.3929	s_1=0.3571	s_2=0.2698	s_3=0.2231	ΔE_1=8.714	ΔE_3=23.501
pH 9	s_0=0.7372	s_1=0.6449	s_2=0.5733	s_3=0.4821	ΔE_1=7.681	ΔE_3=28.841
pH 11	s_0=0.3889	s_1=0.2876	s_2=0.2380	s_3=0.1747	ΔE_1=7.124	ΔE_3=32.970

CHAPTER 6: DISCUSSIONS

Anthocyanins have useful potential as natural colourants due to their unique and attractive colours as well as beneficial health effects. However, the usage is limited since the colour and stability of the anthocyanin pigments is dependent on various factors including structure and concentration of the pigments, pH, temperature, light intensity, quality and the presence of other compounds called co-pigments (Rein, 2005). Transformation of these pigments to other forms by enzymes, oxidation, light, temperature, heating, UV irradiation, for example during exposure could cause colour change from red to brown which has a negative impact on product appearance. This study examines the colour stability of anthocyanin solutions and coating system containing anthocyanin in terms of pH effect and co-pigmentation under exposure of UV-B irradiation.

UV irradiation is one of factors that induce anthocyanin degradation. In this study, UV-B lamp was used to accelerate the degradation kinetics of anthocyanin during exposition to UV-B light. After intervals of one month, the colour stability was analysed using spectroscopy with CIE colour analysis system. The CIE colour analysis system is very effective for measuring colour differences and tracking colour changes during exposure. CIELab colour values are more appropriate indicators for the state of the colour since it can be used to describe all colours that man can see. In CIELab system, colours can be precisely described using CIE colour coordinates. L* is a measure of lightness from completely black (0) to completely white (100). Simply, the L* value can be used to describe how faded is the colour. Chromaticity (C*), describes the vividness or dullness of

a colour while hue angle derived from the a* and b* coordinate. Hue is expressed on a 360° grid where 0° indicates bluish-red, 90° indicates yellow, 180° indicates green and 270° indicates blue. (ΔE) is a total colour difference, in which is a combination of the changes of the three components (chrome, hue, and lightness). Meanwhile, saturation, s is the colourfulness of an area visualized by an observer which is determined as the proportion of chromaticity to lightness. Colour indices (Cie L* C* H^o a* b*) as well as ΔE and saturation (s) derived from CIE measurements nowdays, are increasingly being reported in natural colourant research articles. Colour analysis by using L* C* H^o a* b* precisely describe colour better than absorption spectroscopy. Therefore it is advantages to use for colour analysis stability in both liquid and solid forms.

This study includes liquid and solid samples which exposed under UV-B irradiation. The liquid state can be classified into two groups, which the group of crude or unpurified anthocyanin solution, while the other is purified anthocyanin solution which obtained through purification process. These two groups of liquid samples are further divided into FA added anthocyanin colourant and without FA anthocyanin colourant (free FA). The aim of this study is to obtain the most stabilizing composition consisting of anthocyanin colourant and best amount FA with best pH condition. According to the results obtained from colour analysis stability for the untreated crude and purified anthocyanin colourant from fruits from *Ixora siamensis*, anthocyanins colourants are unstable and easily susceptible to degradation process which lead to the colour loss as indicated by high L* with low C* value. The loss and fading of colour can be attributed by the change in saturation (s) and total colour differences during exposure of 93 days. This was the result of

a strong degradation effect of UV-B light on anthocyanin colourant from *Ixora*. The saturation for both crude and purified colourants decreased indicating colour faded at the end of exposure while changes in (ΔE) occur as the initial colour change to more yellow tonalities. Thus, these results obtained correlated with study by Laleh et al. (2006).

Moreover, Janna et al. (2007) stated that daylight or short wavelength, and incandescent lamp or long wavelength can possible to affect anthocyanin colourant in different solutions. These results are in agreement with that study by Bakhshyashi et al. (2006) which found that UV irradiation leads to anthocyanin destruction. The UV degradation of anthocyanin leads to a bleaching of the colour. When comparing between crude and purified anthocyanins, the crude extract obtained higher saturation (s) value compared to the purified ones. This can be predicted that natural impurities can help to stable the crude anthocyanin colourant. This can correlate with the smaller (ΔE) (change into yellow tonalities) for crude extract. The application of PVA into the crude and purified anthocyanin colourant forms coating system when applied in glass slides. The colour analysis results obtained showed that over UV-B exposure for 93 days, the L* value increased with low C*. When comparing between the coatings of crude anthocyanin-PVA and purified anthocyanin-PVA, the crude anthocyanin-PVA results showed that lower L* and (ΔE) (change into yellow tonalities) and high s and C* values. While between the PVA added colourant and colourant in liquids sample, the PVA added colourant showed lower L* and (ΔE) with higher s and C* value. This results show that PVA itself helps to improve colour stability of the crude and purified extracs. Furthermore, the results indicate that the PVA is a good potential protective coating material as it protects the colour of anthocyanin

and possibly is able to delay colour faded and loss of the anthocyanin colourant against UV-B irradiation. In terms of CIE coordinate, crude samples have the higher position in Cartesan coordinate system. The colour of the crude is redder than purified colourants as indicated by the more positive b value compared to the purified. This suggests that the crude extract is more redder than the purified extract at the end of the UV-B irradiation exposure. These also similar for the PVA containing crude extract. Thus, it again shows the capability of PVA as a good protective coating as it able to delay the colour degradation during exposure.

Due to lower colour stability of anthocyanin colourant and anthocyanin-PVA for both crude and purified, thus enhancements were needed in order to increase the colour stability of all samples. It is known that molecular co-pigmentation of anthocyanins with other compounds, known as co-pigments is the main colour-stabilising mechanism. Although co-pigment alone usually colourless, but when added to an anthocyanin solution it greatly enhances the colour of solution (Bakowska et al., 2003). In this work, different percentages (1, 2, 3, 4 and 5%) of the cinnamic acid-type (ferulic acid) (FA), as a co-pigment were added to the solution of the anthocyanin colourant and anthocyanin-PVA blend for both crude and purified to improve and enhancing the colour stability towards UV-B irradiation influences. Ferulic acid (FA) is known as a light absorber and is used in cosmetic application to block light. Thus, FA was added in order to protect the anthocyanin colourant against UV light degradation.

According to the result obtained towards the colour stability of anthocyanin and anthocyanin-PVA for both crude and purified with the presence of FA, the addition of FA to the anthocyanin and anthocyanin-PVA blend observed gave positive impact in improving the colour of the mixture by enhancing colour brightness (C*) as well as the saturation (s) with the decreasing of lightness (L*) values throughout 93 days of exposure. Moreover, the colour difference (ΔE) and hue (h_{ab}) are more larger for the first and second month of exposure as the colour of anthocyanin extraction and anthocyanin-PVA blend for both crude and purified approaching blue region due to the blueing effect, which also claimed by Rein (2005), Birse (2007) and turning into more purplish colour. However, the colour turn back into its original colours which mean redness in colour with decreasing of saturation (s) and colour chromaticity (C*) while increasing the lightness (L*) values at the third month of exposure. The colour change possibly due to the formation of a longer chromophore via an intermolecular interaction between the co-pigment and anthocyanin compound, which in this study is the interaction between samples and ferulic acid (FA) (Rein, 2005).

The results in this study correlated with other finding by Kucharska et al. (1998) and Bakowska et al. (2003) which previously investigated the influence of UV irradiation on the stability of the anthocyanin-co-pigment complex and found that with the presence of co-pigment in anthocyanin solutions will inhibited the degradation influence of UV on anthocyanin colour stability. Besides act as a co-pigment, FA can also work as UV absorber in preventing the degradation of anthocyanin colourant and anthocyanin blended with PVA as UV absorbers itself act in such a way that dissipate the absorbed energy as to

cause no degradation or colour change in the medium its protect. There are several mechanisms on the actions of UV absorber which are; converting electronic excitation energy into thermal energy, via fast, reversible intermolecular proton transfer reaction; or functioning as radical scavenger as well as functioning as singlet oxygen quenchers (Eva and Imre, 1996). Furthermore, the strong capabilities of ferulic acid as UV absorber is due to its phenolic nucleus and an extended side chain conjugation, which readily forms a resonance, stabilized phenoxy radical. Thus, the UV absorb by FA stable the phenoxy radical formation thereby potentiates its ability to terminate free radical chain reactions. Moreover, according to Sahelian (2003), FA itself can helps to prevent damage caused by ultraviolet light since exposure to ultraviolet light actually increases the antioxidant potency of ferulic acid.

Furthermore, Bakowska et al. (2003), Abyari et al. (2006) and Setareh et al. (2007) who also studied influence of UV irradiation time on anthocyanin-co-pigment complex found that presence of co-pigment in anthocyanin solution significantly inhibited UV-irradiation degradation over a period of time, especially with tannic acid as co-pigment followed with ferulic acids (FA), which were in agreement with this work. In addition, from the results of colour stability of *Ixora* with FA added, it immediately realised that the crude and purified anthocyanin colourant and anthocyanin-PVA blend with addition of 2% FA exhibited greater co-pigmentation effect than other samples. It means that 2% FA used in this work sufficient in preventing the UV degradation and stabilized the colour of the samples better than other, thus resulted in the biggest enhancement on colour brightness (C*) and saturation (s) which lead to decreasing in Lightness (L*) value from the first until the

second month of exposure. The change in colour difference (ΔE) and hue (h_{ab}) were larger compared to others for the two month duration as the colour are rapidly approaching blue region and turn into deeply purplish colour due to the blueing effect from the co-pigmentation reactions, before turn back into the original colour at the end of exposure. The result of addition of co-pigments (FA) at five concentration levels showed that the outcome of co-pigmentation is dependent on molar ratio, which is in good agreement with previous research by Asen et al. (1972), Davis and Mazza, (1993). Because of the anthocyanin concentration was constant in each solution, it seems obvious that the CIE parameter effects depended on the concentration of co-pigment, which also found that ferulic acid were the best co-pigment (Abyari et al., 2006) and (Setareh et al., 2007).

However, when increase the percentages of FA from 3% up to 5%, the co-pigmentation reaction start to reduce, in which there were lower increase in colour brightness (C*) and saturation (s) with higher in Lightness (L*) values compared to 2% FA added. There were also less colour change (ΔE) into purplish colour during two month of exposure and higher decrease in C* and s value with higher increase in L* value at the end of exposure compared to 2% FA. This trend can be support by Hoshino et al. (1980) which found that, when the co-pigment concentration exceeds a certain level, no further changes colour properties can be observed; therefore the molar ratio cannot be raised to an unlimited extent. This explained the reason of addition 3% until 5% FA enhance lower co-pigmentation reaction than the 2% FA added. Thus, the results show the effectiveness of 2% FA in improving the colour properties of the samples and proceed for the colour analysis with pH varied.

The UV-B stability studies were further evaluated for all samples with different pH (initial pH, pH 1, 3, 5, 7, 9 and 11) under UV-B irradiation for 93 days. For this colour study, it was found that crude and purified for ancyanin colourant and anthocyanin-PVA blend experienced rapidly decrease in colour brightness (C*) and saturation (s) with increasing in lightness (L*) rapidly from first month until the end of exposure time (up to 93 days). This can be defined that the colour of the crude and purified of anthocyanin and coating systems for all pH dramatically (larger H^o) tend to degrade into brown and yellower colour and for sample at higher pH obviously experienced faded in colour.

However, results showed that samples at pH 1 experienced slowly decrease in colour brightness (C*) and saturation (s) throughout 93 days of exposure. It can be shown that the most acidic solution, pH 1 were the most coloured in terms of lower in L* (lightness), higher in C* (chromaticity), saturation (s), more redness (a*) and less yellow (b*) which lead to the lower value of hue compared to other pH. From the observation, it can be predict that as the pH was increase, the colour was spreading caused by an important loss of saturation and increased hue shifts to yellower. The results showed in agreement with Gonnet (1999) as the most acidic solutions were the most coloured at each concentration. As the pH was increased, the general trend was a colour fading caused by an important loss of saturation and an increased lightness, coupled with hue shifts (to yellower tonalities). The author also found results that high L* with low C* were observable for all the solutions. Saturation was the most influential parameter in these variations, the loss of chromaticity caused by increasing the pH. Furthermore, anthocyanin which used as natural colourant in this research are known to display a huge variety of colour variation in the pH

ranges from 1-14. This is due to the ionic nature of anthocyanins which enables the changes of the molecule structure according to the prevailing pH, thus resulting in different colours and hues at different pH values (Brouillard, 1982; von Elbe and Schwartz, 1996).

The pH value was important when determining the colour of samples. This is because anthocyanins can be found in different chemical forms which depend on the pH of the solution (Kennedy and Waterhouse, 2000). According to Bakhshayeshi et al. (2006) and Abyari et al. (2006), another factor which affects the stability of anthocyanins is the pHs as increasing pH cause greater destruction of anthocyanin in samples. At pH below 2, anthocyanins exist primarily in the form of flavylium cations which were stable only in highly acidic conditions. While at pH values between 2 and 4, the quinoidal blue species are predominant whereas when the pH increases from 5 to 6, this flavylium cation are labile and will lose the proton upon nucleophilic attack by water will transfer to the colourless carbinol pseudobase and chalcone pseudobase. Moreover, higher pH can cause fading the colour and decrease in colour stability of the products. The results are also in agreement with Córtes et al. (2006) who have stated that at alkaline pH the flavyl cation begins to hydrate, convert into colourless carbinol or pseudobase in equilibrium, with the open form of chalcone which is also colourless. Thus, it can be concluded that samples with higher pH experienced lower colour stability.

The colour study were continued by adding the best amount of UV absorber with varied pH (initial pH, pH 1, 3, 5, 7, 9 and 11) exposed under 17.55 lux intensity of UV-B irradiation for 93 days of exposure. From previous results, it can be clarified that samples with

addition of 2% FA contributed to the highest colour enhancement in terms of chromaticity (C*), saturation (s), lightness (L*), hue (h_{ab}) and colour difference (ΔE), as well as experience longer colour remained, therefore was used in order to improve the lower colour stability performance of anthocyanin colourant for both crude and purified at different pH (initial pH, pH 1, 3, 5, 7, 9 and 11) through co-pigmentation reaction with FA as explained before. As a result, the crude and purified anthocyanin colourant containing 2% FA with altered pH experienced increase in colour brightness (C*) and saturation (s) while lowering lightness (L*) value for the two month of exposure. There were also change in colour difference (ΔE) and hue (h_{ab}) were higher into blue region contribute to deep in purple colour. The colour turn back into the original colour (less redness) in colour for lower pH while the colour degrades and turn into browning upon the higher pH. Approaching the end of exposure, the change in colour difference (ΔE) and hue (h_{ab}) (into yellow region) were lower for acidic pH while higher for alkaline pH. pH 3 exhibited the highest enhancement with addition of 2% FA in terms of all colour parameter (L* C* H^o a* b*) throughout the three month of exposure.

The results can be correlated to research by Gonnet (1999) which stated that whatever were the pH and the pigment concentration, the co-pigmentation resulted in darker colour (lower L*) and enhanced saturation level with higher C* in most solutions. Birse (2007), which also study this phenomena obtained the similar result with darker in colour (low L* values) and increase colour brightness (high C* values). The results from this dissertation followed similar pattern except for the samples at higher pH. Thus, it can be summarised that co-pigmentation significantly influences by pH values. The results obtained from the crude

and purified anthocyanin-PVA blend also performed the same pattern during three month of exposure. The colour chromaticity (C*) and saturation (s) increase for the two month of exposure, decrease in lightness (L*) and obtained higher colour difference (ΔE) and hue (h_{ab}) into blue region contribute to the purple colour due to the co-pigmentation reaction between with FA. At the third month of exposure, the crude and purified experience the increase in L* value while decrease in saturation (s) and colour chromaticity (C*) and change back into the original colour with lower colour difference (ΔE) (shift to yellower region). The crude and purified anthocyanin-PVA blend exhibited highest improvement at pH 3 compared to other. The result from this study can be supported with previous research reported by Abyari et al. (2006) and Setareh et al. (2007) who's stated that optimum pH range for the co-pigmentation of anthocyanin is between approximately 3 and 5.

CHAPTER 7: CONCLUSION AND SUGGESTION FOR FURTHER WORKS

It is evident that natural colourant from anthocyanin easily experienced degradation and susceptible to environmental factor such as pH, UV irradiation and temperature which will limit its application and marketability. Therefore there need to conduct research in order to evaluate the colour properties of the natural colourant especially during exposure time and with the results obtained will directly find the techniques to improve and enhancing the properties. In this study, CIE colour analysis was performed in order to evaluate the colour stability of both anthocyanin extraction and coating system. Colour of products is important for quality attribute. Measuring colour in terms of CIE parameter can be used to monitor colourant degradation. This is because it can be used to describe all colours that man can see. The colours can be accurately describe by using CIE parameter (L* C* H° a* b*) and analysed in terms of ΔE and s. Thus, this dissertation involves UV-B degradation study for 93 days by using CIE system.

In this study, crude anthocyanin colourant from *Ixora siamensis* showed better stability towards UV-B degradation compared to purified anthocyanin. The presence of 2% ferulic acid (FA) as co-pigment increases the colour stability in terms of enhancing the colour brightness (C*) and saturation (s) during third month of exposure compared to other amount of FA, whereas the untreated exhibited the lowest stability overall. For samples with altered pH, the colour stability resulting in pH dependent and pH 1 exhibit the higher colour stability and remained longer colour compared to others, which already experienced significant colour loss during exposure. While for the samples with 2% FA added, varied in

pH, results showed that the samples at pH 3 contribute to the higher enhancement of CIE parameter in which the colour brightness (C*) and saturation (s) increased tremendously compared before FA added. However, the crude of anthocyanin shows higher colour stability compared to purify.

When blended the anthocyanin-containing colourant with PVA, the CIE results obtained revealed that the colour stable and was not really influenced by addition of polymer, thus make it suitable for the production of coloured polymer. Similarly, for coating system blend with PVA, the crude anthocyanin system performed better colour stability than the purified anthocyanin coating system. The crude coating system experienced highest colour brightness (C*) and saturation (s) since the beginning of exposure compared to the purified ones. Without presence of ferulic acid as co-pigment, the colour stability of crude anthocyanin-PVA blend was better at pH 1. The presence of 2% FA increase the colour stability in all CIE colour parameter (L* C* H^o a* b*) while others experienced colour fading and degradation. In the presence of 2% FA as best colour enhancer, crude anthocyanin-PVA blended at pH 3 was higher in colour stability towards UV-B irradiation during 93 days of exposure. This is advantages as the use of crude extract were not depend on high cost compared to purified anthocyanin which need higher cost and time consuming. As a conclusion, the CIE measurements are effective tool in describing colour properties since it indistinctly covered simultaneous attributes of colour, lightness and chromaticity which lead to influential in the appreciation of products' colours.

An overall CIE colour analysis showed that the best samples exhibited by crude phase containing 2% ferulic acid (FA) at pH 3. However, the colour stability of the samples also affected by the negative effect of UV-B irradiation during 93 days of exposure eventhough FA UV absorber have included during the preparation of both colourant and coating system. For further works it is recommended to look into other natural UV absorber which can improve and gives highest stability to anthocyanin colourant and coating system against UV irradiation especially for outdoor purposes with direct natural weathering. Other suggestion is to look for other natural sources of resin in order to form environmentally coating system with natural colourant. For the extraction of natural colourant, it is suggested to look into isolation process by high performance liquid chromatography (HPLC) in order to obtain the individual anthocyanin pigment or used the lower cost of producing individual anthocyanin. Suitable stabilizing agents need to be added to improve the lower colour stability of the purification anthocyanin. There also need to find more sources of anthocyanin colourant as it have potential to be used in replacement of synthetic colours due to less toxic and not harmful.

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APPENDIX A

Statistical output (SPSS) of CIE analysis colourant for L* (Duncan Test)

			Subset for alpha = 0.05																						
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	14	3	35																							
	8	3		36																						
	15	3			39																					
	9	3				39																				
	2	3					47																			
	3	3						47																		
	20	3							49																	
	21	3								52																
	13	3									55															
	7	3										55														
	1	3											58													
	16	3												60												
	10	3													61											
	4	3														62										
	17	3															63									
	11	3																64								
	5	3																	64							
	0	3																		65						
	19	3																			67					
	6	3																				70				
	22	3																					72			
	12	3																						74		
	23	3																							74	
	18	3																								78
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX B

Statistical output (SPSS) of CIE analysis colourant for C* (Duncan Test)

			Subset for alpha = 0.05																			
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Duncan[a]	18	3	25																				
	12	3		27																			
	23	3			28																		
	5	3				28																	
	11	3				28																	
	6	3				28																	
	0	3					29																
	22	3						29															
	19	3							29														
	4	3								29													
	10	3								29													
	17	3									30												
	1	3										30											
	7	3											30										
	21	3												31									
	20	3													31								
	16	3														31							
	3	3															32						
	13	3																32					
	2	3																	33				
	9	3																		34			
	15	3																			36		
	8	3																				37	
	14	3																					40
	Sig.		1	1	1	.05	1	1	1	.10	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX C

Statistical output (SPSS) of CIE analysis colourant for h° (Duncan Test)

			Subset for alpha = 0.05																					
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	2	3	21																							
	3	3		22																						
	1	3			24																					
	4	3				25																				
	5	3					27																			
	20	3						29																		
	0	3							32																	
	21	3								35																
	6	3									37															
	19	3										42														
	22	3											44													
	23	3												47												
	12	3													55											
	18	3														81										
	14	3															331									
	15	3																334								
	13	3																	335							
	16	3																		336						
	17	3																			336					
	8	3																				336				
	9	3																					339			
	11	3																						340		
	10	3																							341	
	7	3																								341
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX D

Statistical output (SPSS) of CIE analysis colourant for a* (Duncan Test)

			Subset for alpha = 0.05																					
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Duncan[a]	18	3	3.8																						
	12	3		16																					
	23	3			19																				
	22	3				21																			
	19	3					22																		
	6	3						22																	
	0	3							24																
	5	3								25															
	21	3									25														
	11	3										27													
	4	3											27												
	17	3												27											
	1	3													27										
	20	3													27										
	10	3														28									
	7	3															28								
	16	3																29							
	13	3																	29						
	3	3																		30					
	2	3																			31				
	9	3																				32			
	15	3																					33		
	8	3																						33	
	14	3																							35
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	.14	1	1	1	1	1	1	1	1	1	1

APPENDIX E

Statistical output (SPSS) of CIE analysis colourant for b* (Duncan Test)

			Subset for alpha = 0.05																				
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Duncan[a]	11	3	9.4																						
	10	3		10																					
	7	3			10																				
	9	3				12																			
	2	3					12																		
	17	3						12																	
	3	3						12																	
	1	3							12																
	4	3								12															
	5	3									13														
	16	3										13													
	13	3											14												
	8	3												15											
	0	3													15										
	20	3														15									
	15	3															16								
	6	3																17							
	21	3																	18						
	19	3																		19					
	14	3																			20				
	22	3																				20			
	23	3																					20		
	12	3																						22	
	18	3																							25
	Sig.		1	1	1	1	1	.23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX F

Statistical output (SPSS) of CIE analysis colourant-PVA blends for L* (Duncan Test)

			Subset for alpha = 0.05																					
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	14	3	33																							
	15	3		34																						
	8	3			35																					
	9	3				36																				
	2	3					46																			
	3	3						46																		
	13	3							47																	
	20	3								47																
	7	3									48															
	21	3										50														
	16	3											52													
	10	3												52												
	17	3													55											
	11	3														56										
	1	3															58									
	4	3																61								
	5	3																	63							
	0	3																		64						
	19	3																			66					
	6	3																				69				
	22	3																					70			
	12	3																						72		
	23	3																							73	
	18	3																								76
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX G

Statistical output (SPSS) of CIE analysis colourant-PVA blends for C* (Duncan Test)

			Subset for alpha = 0.05																				
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Duncan[a]	18	3	29																					
	12	3		35																				
	23	3			37																			
	22	3				38																		
	6	3					38																	
	19	3						38																
	5	3							39															
	11	3								39														
	0	3								39														
	4	3									40													
	17	3										40												
	1	3											40											
	21	3											40											
	10	3												41										
	20	3													41									
	7	3														42								
	16	3															43							
	3	3																43						
	2	3																	44					
	13	3																		44				
	9	3																			45			
	8	3																				48		
	15	3																					49	
	14	3																						52
	Sig.		1	1	1	1	1	1	1	.34	1	1	.39	1	1	1	1	1	1	1	1	1	1	1

APPENDIX H

Statistical output (SPSS) of CIE analysis colourant-PVA blends for H° (Duncan Test)

			Subset for alpha = 0.05																				
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Duncan[a]	2	3	16																						
	3	3		17																					
	1	3			18																				
	4	3				18																			
	5	3					19																		
	20	3						21																	
	0	3							23																
	21	3								25															
	6	3									27														
	19	3										30													
	22	3											31												
	23	3												33											
	12	3													38										
	18	3														58									
	14	3															336								
	15	3																339							
	13	3																	341						
	8	3																		341					
	16	3																			341				
	17	3																				341			
	9	3																					344		
	11	3																						345	
	7	3																							345
	10	3																							345
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.63

APPENDIX I

Statistical output (SPSS) of CIE analysis colourant-PVA blends for a* (Duncan Test)

			Subset for alpha = 0.05																					
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	18	3	16																							
	12	3		27																						
	23	3			31																					
	22	3				32																				
	19	3					33																			
	6	3						34																		
	0	3							36																	
	5	3								36																
	21	3									37															
	11	3										37														
	4	3											38													
	17	3												38												
	1	3													39											
	20	3														39										
	10	3															39									
	16	3																40								
	7	3																	41							
	3	3																		41						
	13	3																			42					
	2	3																				42				
	9	3																					44			
	8	3																						45		
	15	3																							46	
	14	3																								47
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX J

Statistical output (SPSS) of CIE analysis colourant-PVA blends for b* (Duncan Test)

			Subset for alpha = 0.05																					
	FA	N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Duncan[a]	11	3	10																							
	10	3		10																						
	7	3			11																					
	2	3				12																				
	3	3					12																			
	1	3						12																		
	9	3							13																	
	4	3								13																
	5	3									13															
	17	3										13														
	16	3											14													
	13	3												15												
	20	3													15											
	0	3														15										
	8	3															16									
	6	3																17								
	21	3																	17							
	15	3																		18						
	19	3																			19					
	22	3																				20				
	23	3																					20			
	14	3																						21		
	12	3																							22	
	18	3																								25
	Sig.		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

APPENDIX K

Publications of works:

A. F. Mohd-Adnan, N. A. Mat Nor, N. Aziz, R. M. Taha, (2011), "Colour analysis of potential natural colourant from *Ixora siamensis* and *Melastoma malabathricum*", Material Research Innovations, Vol. 15 pp. 176-183 (ISI-Publish)

N. Aziz, N. A. Mat Nor, A. F. Mohd-Adnan, R. M. Taha, A. K. Arof, (2012), "Study of anthocyanin stability derived from the fruit pulp of *Melastoma malabathricum* in a coating system", Pigment & Resin Technology, Vol. 41 Iss: 4 pp. 223-229 (ISI-Publish)

N. A. Mat Nor, N. Aziz, A. F. Mohd-Adnan, R. M. Taha, A. K. Arof, (2012), "Effects of UV-B irradiation on poly(vinyl) and *Ixora siamensis* anthocyanin-coated glass", Pigment & Resin Technology, Vol. 42 Iss: 3 (ISI-Waiting for publication)


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Colour analysis of potential natural colourant from *Ixora siamensis* and *Melastoma malabathricum*

A. F. Mohd-Adnan*[1], N. A. Mat Nor[2], N. Aziz[2] and R. M. Taha[1]

Anthocyanins are an important group of natural pigments that are responsible for many colours in plants. The variation in colour, depending on the pH, makes them a unique source of natural colourant. In this study, pigments from the fruits of *Ixora siamensis* and the fruit pulps of *Melastoma malabathricum* were extracted using trifluoroacetic acid–methanol solution. Spectral measurements (380–780 nm) were performed using visible spectroscopy with colour analysis software at different pHs (initial extracts were 1, 5, 7, 9 and 11). The colours of the solutions were expressed as colourimetric coordinates in the Commission Internationale de l'Eclairage (CIE) laboratory scale using L^* (lightness), C^* (chroma), H° (hue angle notation h_{ab}), $a^*/-a^*$ (redness and greenness) and $b^*/-b^*$ (blueness and yellowness) for the D65/2°CIE Illuminant/Observer condition. In this work, the colour parameters were observed for natural colourant with and without blending with polyvinyl alcohol for both species (*Ixora siamensis* and *Melastoma malabathricum*). The relationships between the colour parameters (colourimetric indexes and CIELab variables) with pH variation and species dependence were discussed in this paper.

Keywords: Natural colourant, Anthocyanins, Colourimetric indexes, pH, Colour measurement, CIELab

Introduction

There has been much interest in the development of new natural colourants, which is apparently due to the strong consumer demand for more natural products, at least in some countries. The current consumer preference for naturally derived colourants is associated with their image of being healthy and of good quality. Natural colourants have become increasingly popular with consumers because synthetic colourants tend to be perceived as undesirable and harmful; some are considered to be responsible for allergenic and intolerance reactions.[1] According to Zhang *et al.*,[2] the development of new and alternative sources of natural colourants is worthwhile as the demand for natural colourants increases. Scientific research on the chemistry of colours, in the theoretical and applied level, is essential in order to improve the colourants from plants. The need to avoid the use of synthetic colourants and move towards the use of natural colours has also increased research during the past decades.

Anthocyanins are natural pigments that are widely distributed in nature. Anthocyanin colour molecules are subclasses of flavonoid. They are responsible for the red, purple and blue pigments in many flowers, fruits and vegetables. Anthocyanins are highly unstable and easily susceptible to the degradation process. The stability of anthocyanins is affected by pH, storage temperature, presence of enzymes, light, oxygen, structure and concentration of the anthocyanins and the presence of other compounds, such as other flavonoids, proteins and minerals.[3] Anthocyanins belong to the flavonoid group of polyphenols. They have a $C_6C_3C_6$ skeleton typical of flavonoids. Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium cation, for example the flavylium cation in very acidic solutions.[4]

A solution of anthocyanin may exhibit different colours, depending on the pH of the solution.[5] As the pH increases, the anthocyanic nucleus is affected by important structural changes,[6] causing a dramatic loss of absorptivity in the visible region.[7,8] Below the pH 2 level, anthocyanins appear red due to the presence of flavylium cations, whereas at pH 6, the flavylium cation is converted into purple quinonoidal bases. Consequently, an adequate description of the colour variations in anthocyanins caused by co-pigmentation or pH requires the following:

(i) that the spectral variations considered should be those affecting the entire spectral curve, not only its visible λ_{max}
(ii) that the three cited colour attributes should be employed
(iii) that these should refer to one (or more) light source(s) and observer(s) condition(s).[9]

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Study of anthocyanin stability derived from the fruit pulp of *Melastoma malabathricum* in a coating system

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Abstract

Purpose – The purpose of this paper is to evaluate the stability of anthocyanin colorant with and without ferulic acid (FA) stabilising agent in a polyvinyl alcohol (PVA) binder coating system.

Design/methodology/approach – The anthocyanin colorant was extracted using methanol acidified with 0.5% trifluoroacetic acid (TFA). FA was added to improve thermal stability of the colorant. The FA added colorant was mixed with PVA to develop a coating system. To test the ability of the coating mixture to withstand heat in the liquid state, spectroscopic studies were carried out in the visible region of the electromagnetic spectrum when the liquid samples had cooled down to room temperature after being heated at 80 and 90°C for 30 minutes. This procedure was repeated six times until a total heating time of 180 minutes has been accomplished. The liquid samples were also coated on glass slides, cured and then stored in different incubators at 30, 40 and 50°C. The visible spectrum was taken everyday for 30 days to study the effect of storage temperature. Spectroscopic results were analysed in terms of intensity rate percentage (IRP).

Findings – In the liquid state, the anthocyanin-PVA mixture without FA showed lower absorbance compared to the mixture containing FA after heating at 80 and 90°C. This shows that FA can enhance the intensity of absorbance of the liquid coating mixture. The mixtures containing FA show increase in absorbance with increase in heating time. The same results are obtained for the coating on glass substrate where FA containing coatings show increase in IRP with time for all storage temperatures. Coating with 1% FA content showed better enhancement and stability.

Research limitations/implications – The colour of the untreated samples quickly faded during heating and storage at different temperatures. In this study, the addition of 0.5% and 1% FA stabilised and enhanced the colour intensity at 30, 40 and 50°C. Further improvements may find the mixture suitable as paint or coating materials and as nail varnish.

Practical implications – The results indicate the possibility of applying the FA stabilised anthocyanin-PVA, colorant-binder composition in a coating system.

Originality/value – The use of anthocyanin from *M. Malabathricum* as a colourant in a coating system or nail varnish is original. Anthocyanin pigments are normally used as colorant in foods.

Keywords Coatings technology, Colour fastness, Plants, Anthocyanin, Melastoma malabathricum, Ferulic acid, Polyvinyl alcohol, Intensity rate percentage

Paper type Research paper

Introduction

Melastoma malabathricum is a shrub that belongs to the Melastomatacea family and it is locally known as "pokok senduduk". It has oblong leaves, purple flowers and deep purplish-blue fruits. Fruits of *M. malabathricum* are technically classified as berries. The seeds are orange in colour (Wong, 2008).

Fruit pulp of *M. malabathricum* contains anthocyanin (Janna *et al.*, 2006). Anthocyanins are natural, water-soluble and non-toxic compounds suitable for a wide range of applications. Anthocyanins have become well-known alternatives to synthetic dyes (Andersen and Jordheim, 2006; Espin *et al.*, 2000). However, anthocyanins are susceptible to colour deterioration during storage. This delays their potential for commercialisation (Cabrita *et al.*, 2000; Cai *et al.*, 1998; Mazza and Brouillard, 1990; Tsai *et al.*, 2002). According to Mazza and Brouillard (1990), the colour stability of anthocyanins depends on a combination of factors, such as the structure and concentration of the anthocyanin, pH, temperature, light and the presence of complexing agents such as phenols and metals. In the food industry, for example, the thermal impact during processing enhances the formation

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Pigment & Resin Technology

Effects of UV-B irradiation on poly (vinyl alcohol) and Ixora siamensis anthocyanins-coated glass

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Manuscript Type:	Original Article
Keywords:	Anthocyanins, Ixora siamensis, co-pigment, UV-B, glossiness, UV-Visible spectroscopy

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Dear Professor Arof,

Manuscript ID PRT-12-2010-0114.R1 entitled "Effects of UV-B irradiation on poly (vinyl alcohol) and Ixora siamensis anthocyanins-coated glass" which you submitted to Pigment & Resin Technology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

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Article Title	Effects of UV-B irradiation on poly (vinyl alcohol) and Ixora siamensis anthocyanins-coated glass

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