



UNIVERSITI PUTRA MALAYSIA

***COLOUR RESPONSE AND RELEASE KINETICS OF ANTHOCYANIN
INCORPORATED IN SWEET POTATO-BASED FILMS***

AIN SYAFIQAH AHMAD KAMAL

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**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
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ABSTRACT

Anthocyanin is a natural additive used in the pH indicator biopolymer films for smart packaging applications to monitor the quality and freshness of food products and at the same time to prevent food waste. However, due to the solubility nature of anthocyanin, migration of anthocyanin from packaging to food may occur. Hence, the migration of anthocyanin needs to be studied. In this study, purple sweet potato starch (SPS) and sweet potato peel (SPP) based indicator films were solvent cast at different loadings of commercial anthocyanin (ANT) (0%, 1%, 1.5% & 2% w/v) to study the colour response of the SPS and SPS/SPP (6:4 ratio) films containing anthocyanin and to investigate the migration and release kinetics of anthocyanin. The SPS/ANT and SPS/SPP/ANT films were immersed in different pH buffer solutions ranging from pH 1 to pH 12 to evaluate the colour response of the films. Colour changes of the SPS/ANT and SPS/SPP/ANT films were studied on direct food contact using chicken meat and cherry tomato samples for 2 days at room temperature. To examine the migration of anthocyanin from the SPS/ANT and SPS/SPP/ANT films, water, 3% acetic acid, 50% ethanol, and 95% ethanol(v/v) were used as food simulants. The kinetic of colour changes of anthocyanin in 3% (v/v) acetic acid solution was studied for the SPS and SPS/SPP films with 1.5% ANT at 8°C, 25°C, 40°C, and 50°C. The concentration of anthocyanin migrated was determined based on the absorbance value measured at wavelength 520 nm by using UV – VIS spectrophotometer. In this study, both of the SPS/ANT and SPS/SPP/ANT films demonstrated colour changes ranging from bright red at pH 1.0 to pH 4.0, purple at pH 5.0 to pH 8.0, blue at pH 9.0 to pH 10.0, and green at pH 11.0 to pH 12.0 when immersed at different pH. As well as when both films were tested in direct contact with the chicken meat and cherry tomato, the films have changed their colour to blueish-green colour on the second day indicating

pH towards alkaline. The anthocyanin was discovered to be migrated faster in the 3% acetic acid than in water, 50% ethanol, and 95% ethanol solutions. The results revealed that the SPS/SPP/ANT film released anthocyanin faster than the SPS/ANT film as the SPS/SPP/ANT film showed greater diffusion coefficient in each case. From this study, the diffusion of anthocyanin from SPS/ANT films was discovered to have required higher activation energy which is 134.76 kJ/mol compared to SPS/SPP/ANT films as the activation energy was recorded at 130.87 kJ/mol. In conclusion, the release kinetics of anthocyanin from the SPS/ANT and SPS/SPP/ANT films followed the first-order kinetic model and diffusion model and both films show good potential to be developed as pH indicator packaging.

ABSTRAK

Antosianin adalah bahan tambahan semula jadi yang digunakan dalam filem biopolimer penunjuk pH untuk aplikasi pembungkusan pintar untuk memantau kualiti dan kesegaran produk makanan dan pada masa yang sama untuk mengelakkan pembaziran makanan. Walau bagaimanapun, kerana sifat larut antosianin, migrasi antosianin dari pembungkusan ke makanan mungkin berlaku. Oleh itu, penghijrahan antosianin perlu dikaji. Dalam kajian ini, filem penunjuk berasaskan kanji ubi keledak ungu (SPS) dan kulit ubi keledak (SPP) dilarutkan dalam pelarut dengan kandungan antosianin komersial (ANT) yang berbeza (0%, 1%, 1.5% & 2% w/v) untuk mengkaji tindak balas warna filem SPS dan SPS / SPP (nisbah 6:4) yang mengandungi antosianin dan untuk menyiasat migrasi dan kinetik lepasan antosianin. Filem SPS/ANT dan SPS/SPP/ANT direndam dalam larutan penimbal pH yang berbeza mulai dari pH 1 hingga pH 12 untuk menilai tindak balas warna filem. Perubahan warna filem SPS/ANT dan SPS/SPP/ANT dikaji pada hubungan makanan langsung menggunakan sampel isi ayam dan tomato ceri selama 2 hari pada suhu bilik. Untuk memeriksa penghijrahan antosianin dari filem SPS/ANT dan SPS/SPP/ANT, air, 3% asid asetik, 50% etanol, dan 95% etanol (v/v) digunakan sebagai simulator makanan. Kinetik perubahan warna antosianin dalam larutan asid asetik 3% (v/v) dikaji untuk filem SPS dan SPS/SPP dengan 1.5% ANT pada 8°C, 25°C, 40°C, dan 50°C. Kepekatan antosianin yang termigrasi ditentukan berdasarkan nilai serapan yang diukur pada panjang gelombang 520 nm dengan menggunakan spektrofotometer UV - VIS. Dalam kajian ini, kedua-dua filem SPS/ANT dan SPS/SPP/ANT menunjukkan perubahan warna mulai dari merah terang pada pH 1.0 hingga pH 4.0, ungu pada pH 5.0 hingga pH 8.0, biru pada pH 9.0 hingga pH 10.0, dan hijau pada pH 11.0 hingga

pH 12.0 ketika direndam pada pH yang berbeza. Seperti halnya ketika kedua film tersebut diuji secara langsung dengan daging ayam dan tomat ceri, filem-filem tersebut telah berubah warna menjadi warna biru kehijauan pada hari kedua yang menunjukkan pH menuju alkali. Antosianin didapati berpindah lebih cepat dalam asid asetik 3% daripada di dalam air, 50% etanol, dan larutan etanol 95%. Hasil kajian menunjukkan bahawa filem SPS/SPP/ANT mengeluarkan antosianin lebih cepat daripada filem SPS/ANT kerana filem SPS/SPP/ANT menunjukkan pekali penyebaran yang lebih besar dalam setiap kes. Dari kajian ini, penyebaran antosianin dari filem SPS/ANT didapati memerlukan tenaga pengaktifan yang lebih tinggi iaitu 134.76 kJ/mol berbanding filem SPS/SPP/ANT yang mempunyai tenaga pengaktifan pada 130.87 kJ/mol. Kesimpulannya, kinetik pelepasan antosianin dari filem SPS/ANT dan SPS/SPP/ANT mengikuti model kinetik dan model resapan pertama dan kedua-dua filem menunjukkan potensi yang baik untuk dikembangkan sebagai penunjuk pH pembungkusan.

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LIST OF ABBREVIATIONS

ANT	Anthocyanin
CA	Content Anthocyanin
EO	Essential Oil
HPMC	Hydroxypropyl Methylcellulose
LRM	<i>Lycium ruthenicum</i> Murr
MC	Methylcellulose
NFC	Nanofibrillated cellulose
PLA	Poly(lactic) Acid
PHB	Polyhydroxybutyrate
SPS	Sweet Potato Starch
SPP	Sweet Potato Peel

CHAPTER 1

INTRODUCTION

1.1 Background

Various food products are currently available on the market, such as fresh, frozen, processed, and ready-to-eat food products. Food technology also has evolved year by year to provide convenience to the consumer which has been leading to the creation of new food products. To ensure the safety and quality of those food products during storage and distribution, one of the important factors that need to be considered is food packaging. Food packaging does not only contain information about the food but also play important role in protecting and preserving the food, extend its shelf life, reduce the environmental effect of a product by reducing food waste (Brennan et al., 2020). There are varieties of food packaging that have been used in the market such as glass, paper, metal, and commonly used is plastic.

Plastics that have been used as food packaging can be categorized into two which are non-biodegradable and biodegradable. Currently, most of the types of plastics food packaging that have been used are petroleum-based plastics such as polyethylene (PE), polypropylene (PP), polytetrafluoroethylene (PTFE), polyester (PS), nylon, and epoxy. These plastics are categorized as non-biodegradable. Meanwhile for biodegradable plastics, one of the examples is biopolymers. Biopolymers can either be developed through direct extraction from biomass such as thermoplastic starch or

directly produced from naturally or genetically modified organisms such as poly(lactic acid) (PLA). Native starch lacks thermoplastic characteristics; nevertheless, when plasticizers, heat processes, and shear stress are added, native starch gelatinizes and shows its thermoplastic nature which is called thermoplastic starch (Zhang et al., 2013). Usually, a plasticizer such as glycerol is added to thermoplastic starch to improve the tensile properties (Marichelvam et al., 2019). Thermoplastic starch is also degradable and is easy to be developed (T. Jiang et al., 2020). The development of thermoplastic starch as biopolymer films is convenient and cheap as starch is a renewable source and can be found in common plants such as rice, corn, sweet potato, potato, and sago.

The development of biopolymer films today has become more advanced as more added value compounds or additives have been incorporated into the films such as anthocyanin which act as a pH indicator. This type of film is categorized as a smart packaging as it not only functioned as basic packaging but also able to reflect the freshness of the food packaged. Smart packaging is defined as a form of food packaging that makes use of chemicals, electrical, electronic, or mechanical technology, or any combination of these technologies. Smart packaging, in particular, is the use of technology to add features to packaging, so that it becomes an essential component of the entire product (Nicoletti & Serrone, 2017).

Anthocyanins are a group of polyphenolic pigments that are widely present in plants such as purple sweet potato, red cabbage, and blueberries. Anthocyanins change their colour depending on the pH of a solution (Khoo et al., 2017). The pH of food changes with the spoilage of the food product. Therefore, anthocyanin can reflect the

real-time pH of the food by displaying its color change, hence, protecting the consumer from foodborne disease.

The purpose of this study is to develop sweet potato starch (SPS) and peel (SPP) based biodegradable packaging incorporated with commercial purple sweet potato anthocyanin (ANT). This study aims to characterize the SPS and SPS/SPP films containing ANT particularly on the physical properties and colour response to various pH ranges. In addition, to investigate the migration and release kinetics of ANT from the films into different food simulants and temperatures.

1.2 Problem Statement

Although food packaging is important in preserving and protecting food products, however, food packaging has caused negative effects on the environment as most of the food packages are made from non-biodegradable petroleum-based plastics that require a long time to fully decompose and this results in environmental pollution. It has been reported by the World Wide Fund for Nature (2020), about 4.8 to 12.7 million tonnes of plastics end up in the ocean per year, most of which flow into the Indian and Pacific Oceans, where many agricultural lands and countries are located and they expected that the amount of plastic waste will rise four times between 2010 and 2050. Moreover, the usage of petroleum-based plastic can cause global warming as it has high CO₂ emissions compared to bioplastic (Gironi & Piemonte, 2011).

One of the alternatives in reducing the usage of petroleum-based plastic as food packaging is shifting to biodegradable biopolymer (e.g. starch) which mostly is made from renewable sources. However, the major downside of biopolymer is its properties and strength are weaker than the petroleum-based plastic. Because of its hydrophilic nature, starch has limited water stability and high moisture sensitivity, limiting the

development of starch-based products (Ribba et al., 2017). Thermoplastic starch is also reported to have poor mechanical properties and processability.

Food is a daily human necessity; it is a requirement of human survival. As a result, food safety is critical to ensuring the health of people. If food safety is overlooked, millions of people are at risk of suffering foodborne illness, in worse case can lead to fatality. According to World Health Organization (2020) Every year, an estimated 600 million individuals—nearly one in every ten people in the world—fall ill after consuming contaminated food, and 420 000 dies. The risk of consuming unsafe food can be avoided by informing consumers of the information of food through the packaging. Although every food packaging such as milk has an expiration date, sometimes the current quality and safety of the food cannot be ascertained. Therefore, it is important to develop smart packaging that has an indicator or sensor to monitor the quality of food.

For instance, the addition of anthocyanin as a pH indicator into films to develop smart packaging. However, the addition of this additive has the potential to migrate into the food in contact. For example, due to the solubility of anthocyanins, the inclusion of this pigment into the films can diffuse to the surface of food, particularly food with high moisture content. Therefore, the migration and release kinetics of anthocyanin should be conducted to predict and analyse the release behaviour of anthocyanin. Nevertheless, there is limited study on the migration and release kinetics of anthocyanin as a pH indicator incorporated with starch-based film.

1.3 Objectives

The objectives of this research are;

1. To study the colour response of films at different pH solutions and direct food contact with chicken and cherry tomato sample.
2. To study the migration of anthocyanin from SPS and SPS/SPP films using different food simulants.
3. To investigate the release kinetics of anthocyanin for the SPS and SPS/SPP films.

1.4 Scope of the Study

The scope of the study is to examine the physical properties of the ANT incorporated SPS and SPS/SPP films in terms of thickness and visual appearance. Also, to evaluate the colour response of films to different pH (1 to 12) ranges and after in direct food contact with chicken and cherry tomato sample. Then, the migration of anthocyanin in different food simulants will be analysed for the SPS and SPS/SPP films with fixed loading of ANT. After that, the kinetics analysis will be studied by analysing the migration of anthocyanin from SPS and SPS/SPP films with fixed loading of ANT in 3% acetic acid at different temperature for 3 hours. SPS will be extracted from purple sweet potato. In this study, different loading of ANT will be added to the films to act as a pH indicator for reflecting the freshness of the food.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable Packaging

Biodegradable packaging is described as packaging that will degrade under the action of microorganisms such as moulds, fungi, and bacteria over a given period and environment (Niaounakis, 2015). The degradation of biodegradable occurs when the polymer decomposed into carbon dioxide, methane, water, inorganic compounds, or biomass, where the primary process is the enzymatic activity of microorganisms.

Biopolymer means either the plastic is sourced from bio-based material or biodegradability of the plastic. The first category is renewable (non-fossil fuels) and this type of material can be classified into two which are biodegradable and non-biodegradable. Although derived from bio-based, some of the biopolymers are non-biodegradable such as aminoundecanoic acid-derived polyamide (PA 11) and laurolactam-derived polyamide (PA 12) (Niaounakis, 2015). The second category, which is plastic can degrade despite the source of the material either fossil fuels or bio-based. Examples of plastic under the second category were poly (butylene succinate) (PBS) and poly (vinyl alcohol) (PVOH).

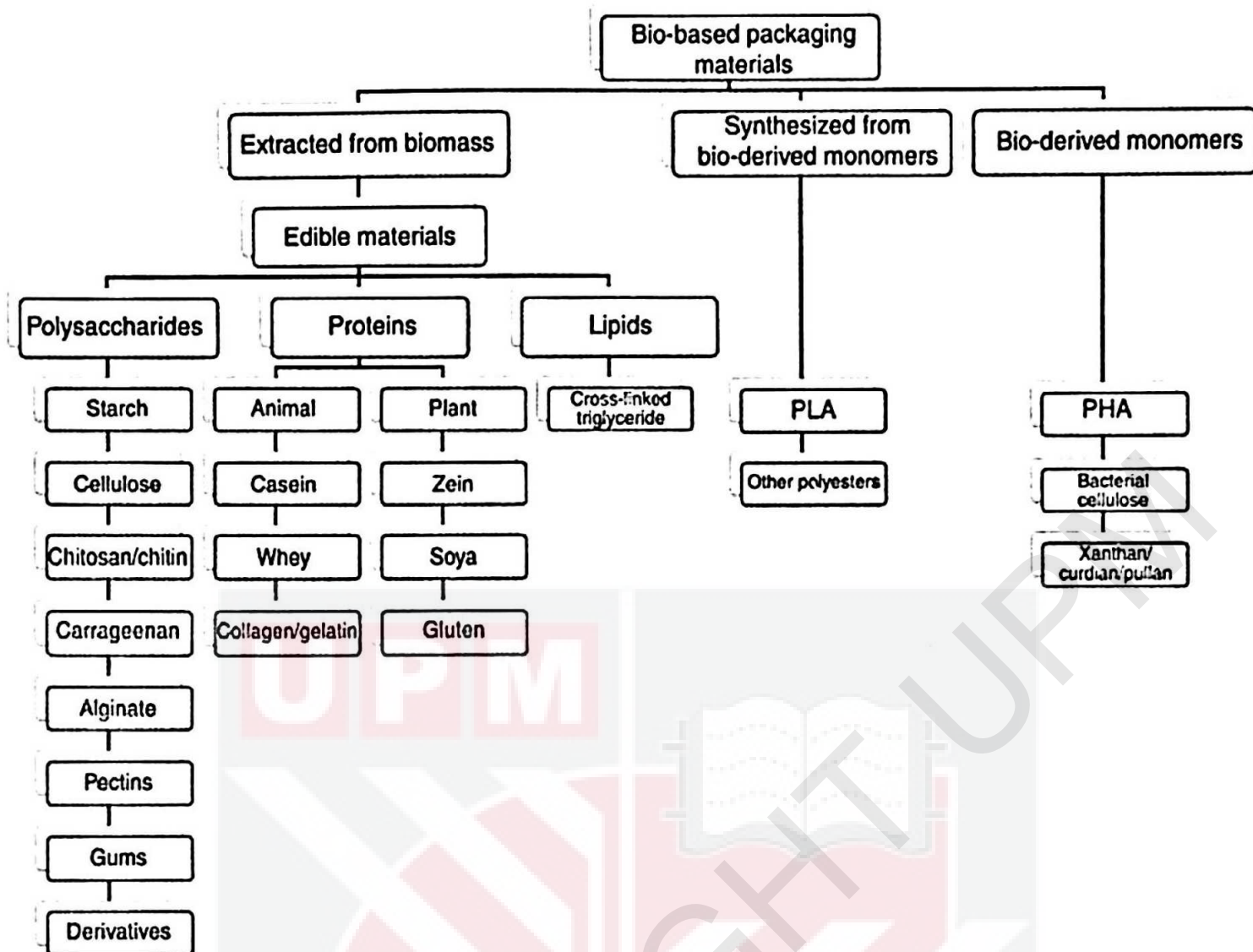


Figure 2.1 Biobased polymers. Reprinted from Ramos et al. (2018)

Figure 2.1 shows the source of bio-based packaging materials which can be classified into three categories. The first category is through extraction from biomass where edible packaging can be developed through some of this material. Biomass extraction will be divided into three groups of material which is through polysaccharides materials such as starch and cellulose, secondly through proteins from animals or plants, and lastly through lipids. Under the first category, the development is more direct as it can be extracted from biomass. The second category is synthesized from bio-derived monomers such as PLA, while the last category is through bio-derived monomers such as PHA, and xanthan.

2.2 Starch-based Films

Starch-based films are relatively low cost as these can be developed from plants sources that are rich in starch such as rice, sago, and sweet potato starch. Aside from being biodegradable, starch-based films are also easy to develop due to the starch sources are cheaper, easy to get, and renewable. Because native starch is brittle and hydrophilic in nature, it is unsuitable for a variety of applications such as plastic bags, food packaging, and so forth. Starch, on the other hand, may be turned into thermoplastic by the application of a plasticizer in conjunction with the combined effects of temperature and shear (Khan et al., 2017). To develop starch-based films, the granular structure of starch molecules needs to be destructed by using water as a swelling agent (Niaounakis, 2015). The chemical structure of starch as shown in Figure 2.2.

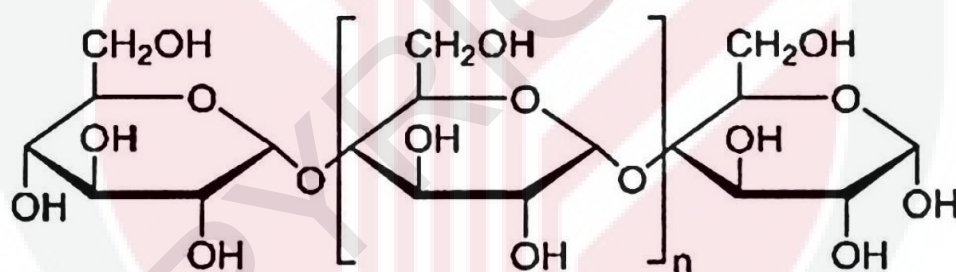


Figure 2.2: Chemical Structure of Starch

However, according to Muller et al. (2017), the starch-based films are sensitive to water, and their film characteristics are highly reliant on moisture content, resulting in very poor mechanical properties. Thus, numerous initiatives have been made to resolve these drawbacks, such as the application of co-biopolymers or other secondary compounds to enhance the mechanical and tensile properties of the film (Thakur et al., 2019).

Table 2.1: Current research of starch-based films

Film	Major Findings	Author
Rice Starch/ carboxymethyl chitosan	- Rice starch-based blend films containing different carboxymethyl chitosan content had stronger mechanical properties, transparency, and thermal stability than rice starch-based alone - carboxymethyl chitosan did not affect the permeability of oxygen and water vapour.	Suriyatem et al. (2018)
Corn and cassava starch-based film	- The microstructure, particle size, and roughness of the starch source affected the viscosity of the film-forming suspensions, enabling the alteration of the film-forming matrix during the drying process. - This alteration would influence the physicochemical and mechanical properties of the film samples.	Luchese et al. (2017)
Sago starch and glycerol	- The tensile strength increased when the starch content was increased. This was accompanied by a significant reduction in tensile strain and an increase in tearing strength. - Increasing the plasticizer concentration resulted in a decrease in tensile and tearing strength as well as an increase in tensile strain.	Edhirej et al., (2017)

2.3 Sweet Potato

Sweet potatoes (*Ipomoea batatas*) are dicotyledonous plants belonging to the morning-glory family (*Convolvulaceae*). It is one of the important crops in the developing world and is considered staples food in some countries. Sweet potato tubers have long, tapered, and smooth skin that can be yellow, orange, red, brown, purple, and/or beige, with flesh that can range from beige to white, red, pink, violet, yellow, orange, or purple depending on the varieties (Mu & Singh, 2019). Besides the tuber, the leaves and stems of sweet potatoes are edible and nutritious. Depending on

the variety, sweet potato tubers and leaves are high in carbohydrates, protein, dietary fibre, lipids, polyphenols, carotenoids, vitamins, and mineral components such as potassium and calcium. For example, purple sweet potato is rich in anthocyanin, a colour pigment that also can be found in berries, onions, kidney beans, pomegranates, and grapes. Meanwhile, orange and yellow-fleshed sweet potatoes are rich in carotenoids which can be used to treat vitamin A deficiency (Mu & Singh, 2019). Sweet potatoes may also be used as a raw material to extract various functional components that have a greater nutritional value.

In Malaysia, there are various sweet potatoes cultivars such as *Gendut*, *Telong*, *Jalomas*, *VitAto* (Figure 2.3), and many more (S. L. Tan, 2015). These varieties have yellow and orange flesh colour respectively. Meanwhile, the newly introduced cultivars from the Malaysian Agriculture Research and Development Institute (MARDI), *Anggun 1* as shown in (Figure 2.3), *Anggun 2*, and *Anggun 3* have purple flesh which is rich in anthocyanin. According to studies by Zulkifli et al. (2021) on the proximate composition of Malaysian local sweet potatoes, they found that *VitAto* has a higher content of protein and starch compare to *Anggun* and White varieties. It is also found that crude fibre and protein content in the skin of mentioned sweet potato varieties is higher than their cortex or cambium ring and central parenchyma. This shows that the skin of sweet potatoes can be utilized for other applications such as the extraction of starch or fibre.

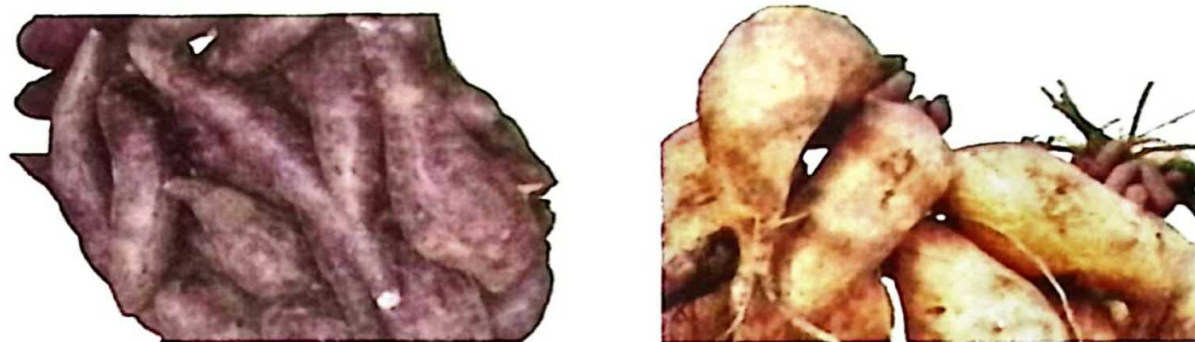


Figure 2.3: *Anggun 1* Sweet Potato (Left) and *VitAto* Sweet Potato (Right).
Reprinted from (BERNAMA, 2019)

Regarding the properties of starch from sweet potatoes with different flesh colour, Soison et al. (2015) found that pasting and thermal characteristics of starches were significantly affected by the flesh colour of sweet potato varieties. The authors reported that purple flesh sweet potato starch (Torperk) showed the lowest gelatinization temperature (70.3 °C) and enthalpy (12.0 J/g), and good swelling power (27.5 g/g), whereas yellow-fleshed sweet potato starch (Kaset) was more stable to high temperatures and shear stress, and it had a lower tendency to retrograde than other varieties. As the starch granules are heated in excess water above the initial gelatinization temperature, they swelled as hydrogen bonds in amorphous areas are broken, resulting in dramatic alterations (BeMiller, 2007). Due to the differences in granule size and starch, protein, and amylose concentration, the functional parameters such as pasting behaviour, gelatinization temperature, swelling power, and solubility are significantly affected as well. Sweet potato starch also showed increased swelling power, and water solubility when the heating temperature of the starch was increased (Zhu & Wang, 2014).

There are various applications of sweet potato starch, for example, sweet potato starch is used to produce isomaltose-oligosaccharides (IMOs) which is a sugar substitute from the enzymatic reaction of starch. Sweet potato starch also has been used as a tablet binder for pharmacological and medical uses (Zhu & Wang, 2014).

Researches on the development of biodegradable films from the sweet potato starch are listed below:

Table 2.2: Current research of sweet potato starch-based films

Major Ingredients	Major Findings	Author
Sweet potato starch, glycerol, sorbitol	- The researchers discovered that increasing the plasticizer content increased the percentage of elongation, water solubility, and water vapor permeability of sweet potato starch films. - The highest percentage elongation and water vapor permeability values were obtained in glycerol-plasticized films.	Ballesteros-Mártinez et al. (2020)
Sweet potato starch, sweet potato peel, Glycerol, Anthocynin	- Sweet potato starch incorporated with sweet potato peel films (SPS/SPP) was found to have greater thickness, higher water solubility and water vapor permeability rate compared to sweet potato starch-based films (SPS). - SPS films showed that it had better tensile strength and thermal stability compared to SPS/SPP films.	Ananthan (2020)
Sweet Potato Starch, 2-Octen-1-ylsuccinic an- hydride, Oregano Oil	- The findings showed that usage of octenylsuccinated of sweet potato starch (OSS) significantly delayed the coagulation of oil droplets during the film casting process and improved their consistency in the dried film. - Octenylsuccination or incorporation of oregano essential oil independently reduced film strength, hardness, moisture content, solubility in water, and water vapor permeability while enhancing film elongation less than their combination.	Li et al. (2018)

A prior study by Borah et al. (2017) has demonstrated that the sweet potato peel may be used to make a biodegradable film. The benefit of utilizing the peel is that it reduces manufacturing processes, waste, and, most significantly production costs.

2.4 Smart Packaging

Smart packaging is a new technology in packaging which provides systems with integrated sensor technologies used in food, pharmaceuticals, and many other types of products. It can be defined as packaging that can detect environmental changes and, in turn, notify users of the changes and packaging that are interactive or responsive (Han et al., 2005). Smart packaging is used to prolong shelf life, monitor freshness, show quality details, and increase product and consumer protection (Schaefer & Cheung, 2018). Han et al. (2005) stated that there are four application systems based on their category under smart packaging. Firstly, packaging that can provide convenience to the user through quality distribution and preparation methods. Secondly, the one that improves the quality and value of the product (quality indicators, temperature, and time-temperature indicators, and gas concentration indicators). Next, those that can alter the properties of gas permeability and smart packaging that provide security against fraud, forgery, and tampering.

Active packaging meanwhile is described as a packaging system that reacts with food by "deliberately incorporating components that would release or absorb substances into or from the packaged food or the food environment" (Yildirim & Röcker, 2018). Active packaging usually being used by incorporated antimicrobial ingredients (e.g. essential oil) or active compounds to prolong the shelf life of the food by inhibits bacteria growth in the food. In contrast, indicators are grouped under intelligent packaging, for example, incorporation of anthocyanin in packaging can act as pH indicator for food (see Figure2.4).



Figure 2.4: Example of smart packaging with pH sensor

2.5 Anthocyanin

Anthocyanins are glycosylated polyphenolic compounds of several colours ranging from orange, red, and purple to blue in flowers, seeds, fruits, and vegetative tissues (Y. Liu et al., 2018). Anthocyanins are pigments that are usually derived from plants and are soluble in the polar solvent. Among the fruits that are rich in anthocyanin are purple sweet potato, red cabbage, blueberry, and grape skin. Apart from being able to impart colour to the plants or plant products with which they exist, anthocyanin also plays a function in flowering plants other than being as attractive as it can serve as antioxidants, phytoalexins, or antibacterial agents (Kong et al., 2003). Kong et al. (2003) reported that anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (as shown in Figure 2.5) and the number of hydroxyl groups, the nature and number of sugars connected to the molecule, the position of this attachment, and the structure and number of aliphatic or aromatic acids attached; all contribute to the variations among different anthocyanins.

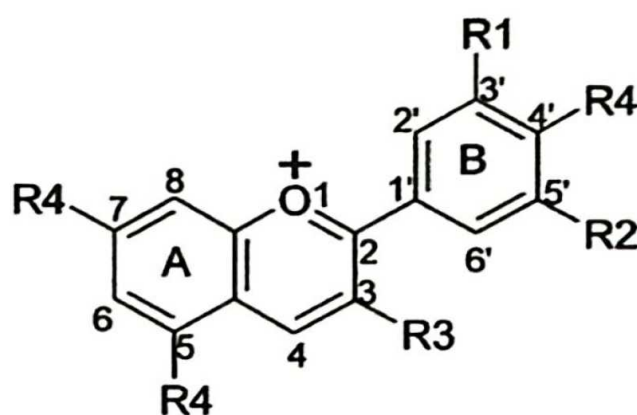


Figure 2.5: Chemical Structure of Anthocyanin. R1 and R2 are H, OH, or OCH₃; R3 is a glycosyl or H; and R4 is OH or a glycosyl. Reprinted from Kong et al. (2003).

Due to the molecular composition of anthocyanin containing ionic nature, the colour of anthocyanins is affected by the pH of a solution (Turturică et al., 2015). In an acidic condition, some of the anthocyanins turn red while at neutral pH anthocyanins have a purple hue colour and the hue shifts to blue when the pH condition increases (Khoo et al., 2017). Figure 2.6 shows the anthocyanin aqueous solution as the pH changes.

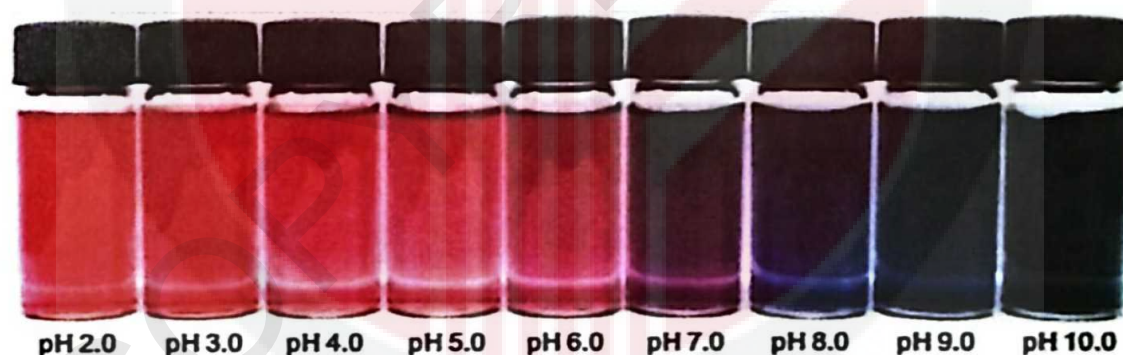


Figure 2.6: Optical representation of anthocyanin aqueous solutions with various pH values (2–10). Reprinted from Choi et al. (2017).

Turturică et al. (2015) stated the mechanism of anthocyanin colour changes and reported in a previous study that four anthocyanin structures exist in equilibrium in aqueous media: flavylium cation, carbinol pseudo-base, quinoidal base, and chalcone. The red-coloured flavylium cation is the dominating species at pH 1.0 while it has been observed that the uncharged blue quinoidal unstable species predominates between pH values of 2.0 and 4.0, and with increasing pH it results in the formation

of an anionic quinoidal species. They also mentioned that the carbinol pseudo-base and chalcone structures were discovered at higher pH levels (5.0 and 6.0) and the acylated anthocyanins have remarkable stability at pH levels over 5.0.

Anthocyanin has often been used as a food additive. The additive of E163, for example, is a commercial supplement produced from fruit anthocyanins such as grape skin. It is a purple food additive used to make purple jams, confectionaries, and drinks. According to Khoo et al. (2017), anthocyanin also has been used as a nutraceutical and traditional medicine bioactive component as it has long been employed as a phytopharmaceutical, appetite stimulant, choleric agent, and in the treatment of a variety of other illnesses. Aside from being used as a consumable product, due to its ability to change the colour with the pH, studies on anthocyanin as a pH indicator into the films have been conducted (Table 2.3).

Table 2.3: Current study on anthocyanin incorporated starch-based films

Film	Source of Anthocyanin	Major Findings	Author
Sago starch	Torch ginger	- After the addition of anthocyanin in sago starch-based films, the thickness and moisture content of the films increased while the permeability of water vapour decreased. - The film demonstrated its ability to change colour (pink to green) over a broad pH range (pH 4–pH 9)	(Xue Mei et al., 2020)
Cassava Starch	Grape Skin	- Anthocyanin greatly decreased resistance, flexibility, and thermoplastic starch rigidity. - Different concentrations of anthocyanin did not cause any significant difference in the mechanical	(Vedove et al., 2020)

		properties of films
		• The addition of anthocyanin has increased the moisture content, which may lead to the increase in thickness
Agar/Potato Starch	Sweet Potato	• The pH indicator films (Choi et al., 2017) displayed changes in pH and the spoilage point of animal samples, from red to green.

2.6 Migration of Anthocyanin

Plasticizers, thermal stabilizers, active ingredients, and anthocyanin are commonly added to packaging materials to increase strength and functionality. However, the presence of these substances may result in chemical migration into food that comes into contact with the packaging. Migration is also referred to as the diffusion of chemicals from a zone of greater concentration (the food-contact layer) to one of lower concentration (typically the food surface). Diffusion of chemical compounds from polymers is a complex process that is affected by several factors, including the concentration of chemicals in packaging film and food, the type of the foods, temperature, and the time over which contact occurs (Bhunia et al., 2013). The migration process is split into four key steps: diffusion of chemical compounds through polymers, the release of dispersed molecules from polymer surfaces, sorption of compounds at the plastic–food contact, and release of compounds in food (Ferrara et al., 2001).

According to Úbeda et al. (2017), it is important to control the migration of substances because if a significant number of migrants are transferred to foodstuffs, they may reach the levels that are hazardous to health and can also change the

composition and qualities of food such as colour, odour, flavour, and texture, reducing the product's shelf life. Moreover, to assure food safety, food packaging often must meet with legislation that has previously been established such as food contact materials (FCMs) which already set the overall migration limits (OML), specific migration limits (SML), and/or maximum permissible quantity (QM) for particular substances. If the substances are not on the list, their migration must not be determined to be more than 60 mg/kg food simulant. Therefore, to predict the migration of a chemical into food over time, the release kinetics of a substance may be evaluated.

The migration of chemical from the films can be explained via mechanism diffusion. Interstitial diffusion and substitutional diffusion are the two methods through which diffusion of molecules can occur. Considering the atom is the impurities in the matrix. The atom can sit on the matrix itself, as a replacement for one of the bulk material's atoms as in Figure 2.7, or, if tiny enough, in an interstice (interstitial) as in Figure 2.8.

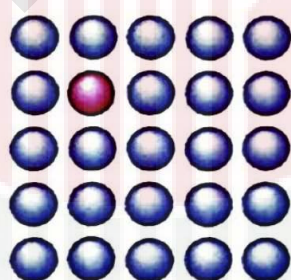


Figure 2.7: Substitutional atom position in matrix. (*Diffusion Mechanisms*, n.d.)

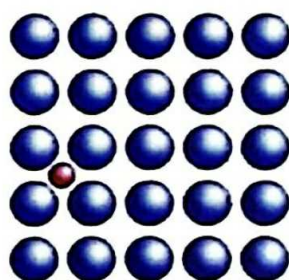


Figure 2.8: Interstitial atom position in matrix. (*Diffusion Mechanisms*, n.d.)

According to Substitutional diffusion happens only when there is a vacancy. A vacancy in the lattice is a "missing atom." If there is a vacancy, one of the nearby atoms can migrate into it, producing a vacancy on the spot that the atom has just left. In the same way that an atom has an equal chance of migrating into any neighboring atomic site, any of the surrounding atoms has an equal chance of going into the vacancy. It is frequently helpful to understand about this mechanism in terms of vacancy diffusion rather than atom diffusion. Meanwhile, for the interstitial diffusion, the diffusing atom is on an interstice rather than a lattice site. Unless otherwise already occupied, the diffusing atom is free to migrate to any neighboring interstice. The ease with which a diffusing atom may travel into an interstice therefore determines the rate of diffusion.

To evaluate the amount of chemical migration from packaging into food, food simulants, rather than actual foodstuffs, are used. Because chemical analysis is simplified and food simulants are employed as food substitutes, therefore, chemical detection and quantification required the development of specific analytical techniques for each chemical of interest, which must be tailored to each food or food simulant type (Muncke, 2013)

2.7 Food Simulants

Food simulants are food replacements that represent the properties of food products. Usually, food simulants are used to test on the packaging to analyse the migration of any material from the packaging to the packaged foods. The selection of food simulants depends on the type of foods that will be tested. Food simulants differ in their chemical characteristics, reflecting various food categories such as hydrophilic (water-based), lipophilic (fatty foods), and amphiphilic (foods with both watery and fatty properties) (Muncke, 2013). For example, distilled water is used as food simulants to represent aqueous foods, while 3% acetic acid is used to represent only acidic food, and ethanol 10% is used to represent alcoholic food. Meanwhile, to represent fatty foods, vegetable oil or 95% ethanol are used as food simulants as shows in Figure 2.9: Food simulant guide. Reprinted from Simoneau (2009).

Food type	Food simulant	Abbreviation
Aqueous food (pH > 4.5)	Distilled water	Simulant A
Acidic foods (pH ≤ 4.5)	Acetic acid 3% in water (w/v)	Simulant B
Alcoholic foods	Ethanol 10% in water (v/v) Ethanol X% adjust % for foods with >10%	Simulant C
Fatty foods	Rectified olive oil or other fatty food simulants	Simulant D
Dry foods	none	None
Milk products	Ethanol 50% in water (v/v)	

Figure 2.9: Food simulant guide. Reprinted from Simoneau (2009)

2.8 Release Kinetics

Artificial food colorant restrictions have resulted in the significant study on natural pigments in recent years. Anthocyanin pigments are responsible for the red, purple, and blue colours of many plants, and sweet potato has just subsequently been discovered as a possible source of anthocyanin. It is critical to investigate the migration of anthocyanin into food for a commercial production process to determine the release behaviour of the anthocyanin. As a result, the diffusion coefficient value should be determined to predict anthocyanin release behaviour under certain circumstances such as at different storage temperatures and pH of the media.

According to Nagy (2012), the diffusion coefficient is frequently defined as the ratio of flux density to the negative of the gradient of concentration in the direction of diffusion, followed by Fick's law:

$$J_i = -D_i \frac{d\phi}{dy} \quad (1)$$

(1) where J denotes the transfer rate ($\text{kg/m}^2\text{s}$), ϕ is the concentration in the membrane (kg/m^3), and y is the spatial coordinate in the diffusion direction (m). D_i is the diffusion coefficient, d/dy is the concentration gradient, and it may be considered as the driving force for diffusion. In a conclusion, the diffusion coefficient means that the substance's mass diffuses across a unit surface in a unit of time with a concentration gradient of unity.

The diffusion coefficient of a substance in a packaging can be determined using various models such as diffusion model Equation (2) and (3) as suggested by

Crank (1975). Where Equation (2) for short-term diffusion $\frac{m_t}{m_\infty} < 0.6$, and Equation (3) for long-term diffusion $\frac{m_t}{m_\infty} > 0.6$.

$$\frac{m_t}{m_\infty} = 4 \left(\frac{Dt}{\pi l^2} \right)^{1/2} \quad (2)$$

$$\frac{m_t}{m_\infty} = 1 - \left(\frac{8}{\pi^2} \right) \exp \left(\frac{\pi^2 Dt}{l^2} \right) \quad (3)$$

Whereby, $\frac{m_t}{m_\infty}$ is the mass fraction of mass of substance at the time divided by mass of substance at equilibrium ($t=\infty$), D is diffusion coefficient, l is the thickness of the plane (film), and t is the time. Previously there are several studies on release kinetics of additive in films as shown in Table 2.4 which using different model to analyse the release behaviour of additive from films.

Table 2.4: Previous Study on Release Kinetics of Addictive in Films

Films	Substance Studied	Major Findings	Author
κ-carrageenan	Extract from <i>Lycium ruthenicum</i> Murr (LRM) (Natural pigment)	- The release of LRM was described using zero-order kinetics, first-order kinetics, second-order kinetics, Higuchi model, and Hitson-Crowell model. - The release of LRM from films followed Fickian's behaviour, which was driven by the diffusion mechanism rather than the swelling process.	J. Liu et al. (2019)
Chitosan	Anthocyanin	- When fitted to the Higuchi model, all films were controlled by Fick's law. - The films demonstrated different release mechanisms, including a Fickian and a non-Fickian behaviour.	Carlos-Salazar, Max J. (2017)

PLA and PLA/Kenaf	Thymol	• The release of thymol was described using first-order kinetics, diffusion modelling, and Fick's law modelling. • The rate of thymol release into 950 mL/L ethanol/water at various temperatures exhibits Fickian behaviour. • In each case, the PLA/Kenaf composite film has a greater diffusion coefficient.	Tawakkal et al. (2016)
Heat pressed and MC-HPMC coated starch-based films.	Carvacrol, Thymol and Linalool	• A first-order and diffusion model successfully characterized the kinetics of the overall release of carvacrol, thymol, and linalool from heat pressed and MC-HPMC coated starch-based films.	Kuorwel et al. (2013)

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter comprises detailed information on raw materials and methods that will be used for the study. The procedure for the preparation of SPS/ANT (sweet potato starch/anthocyanin) and SPS/SPP/ANT (sweet potato starch/ sweet potato peel/anthocyanin) films have also been discussed in this chapter. The methods used to examine the physical properties (thickness and colour) of both types of films and the colour responses of the films as pH indicator has also been explained. Moreover, the methodology used for investigating the migration and release kinetics of anthocyanin from both films to the food simulants were described. The overall process flow of this study can be referred in Figure 3.1.

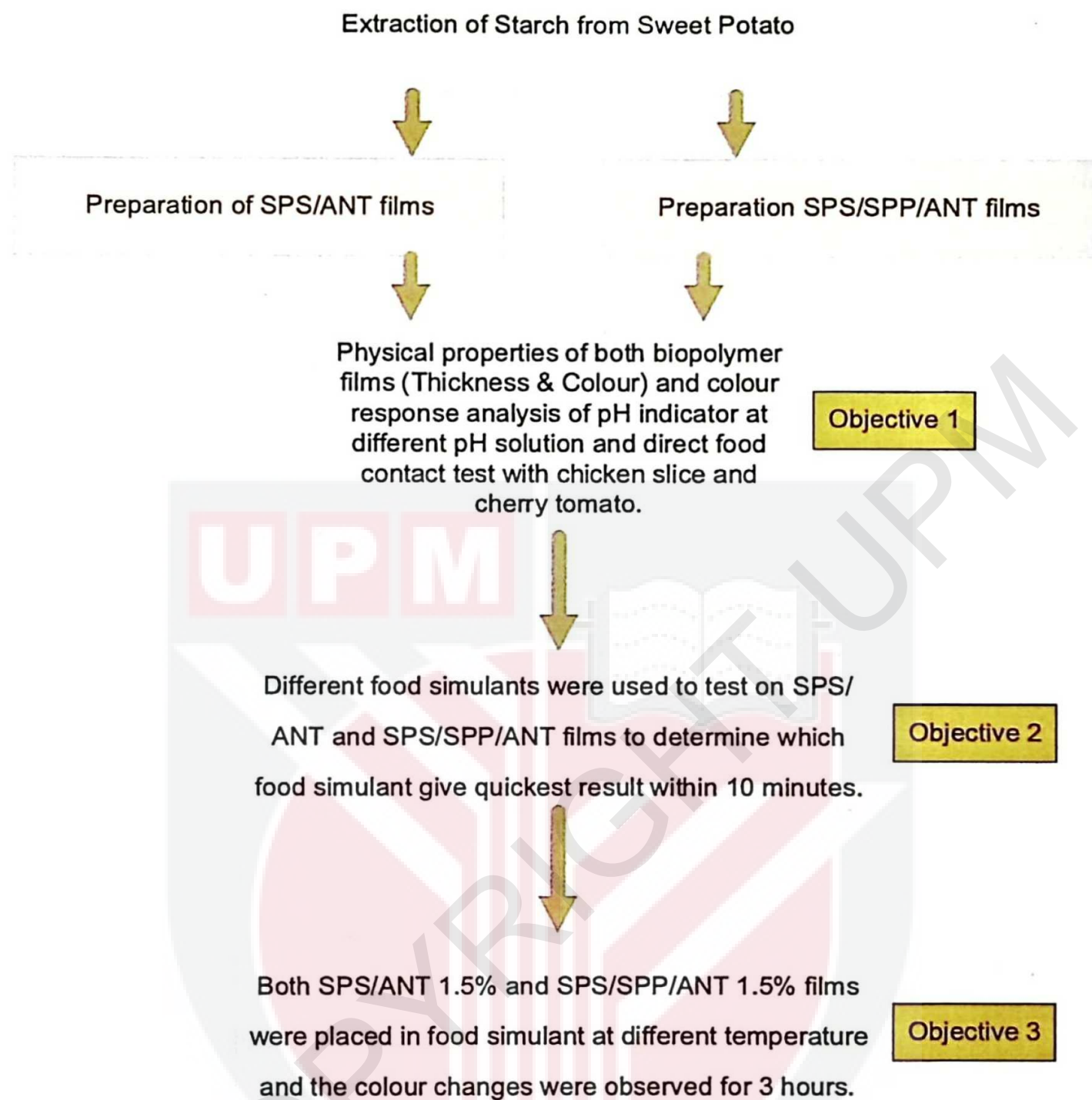


Figure 3.1: Methodology Overall Process Flow

3.2 Raw Material

The sweet potato was obtained from a farm located at Serendah, Selangor Darul Ehsan. The starch was extracted from the sweet potato and the sweet potato peel (Figure 3.2) was prepared in the laboratory. Commercial anthocyanin (Figure 3.3) was purchased from Green Bio-Tech, Malaysia. The glycerol, 3% acetic acid, ethanol 95%, and buffer solutions from pH 1 to pH 12 were obtained from R&M Chemicals, Malaysia. Meanwhile litmus paper was purchase from Merck KGaA, Germany.



Figure 3.2: Peeled sweet potato and sweet potato peel



Figure 3.3: Commercial anthocyanin powder

3.2.1 Preparation of sweet potato starch (SPS) and sweet potato peel (SPP)

The starch extraction method was adapted from Soison et al. (2015) with slight modification. One kg of sweet potato was washed, peeled, and cut into a smaller size (3 mm thickness). Then the sweet potato cuts were blended in a blender by adding 1:15 (w/w) of distilled water ratio. The slurry was filtered using a muslin cloth to remove the fibre of the sweet potato. The filtrate was collected and centrifuged (Hettich Zentrifugen Universal 320, Germany) at 9000 rpm for about 3 minutes. The supernatant was discarded and distilled water was added again to proceed with the centrifugation to remove the heavy particle and the colour particle from the sedimented starch as shown in Figure 3.4. The process continued several times in the same manner until the sedimented starch solution turned white. The sediment starch was dried in a convection oven (Jepo Tech OF-22FW, Korea) at 50°C for 24 hours. After drying, the starch was ground using a grinder and was sieved using 100-micrometer mesh sieve size (Retsch AS2001, Germany).



Figure 3.4: Sweet potato starch suspension

For preparing the peel powder, the sweet potato peels were dried in the oven at 50°C for 24 hours. The dehydrated peels were then ground and sieved using 100-micrometer mesh sieve size (Retsch AS2001, Germany). Both the SPS and SPP were stored in polyethylene bags.

3.3 Preparation of SPS and SPS/SPP Films incorporated with anthocyanin

The SPS films were prepared by adding 4g of sweet potato starch in 100ml of distilled water. Then, the mixture was heated and stirred using a hotplate (04009951780017, DAIHAN Scientific Co, Korea). When it reached 40°C, 25% glycerol (volume of glycerol/weight of SPS) was added. The stirring and heating continued around 30 minutes at temperature 75°C-85°C until the gelatinisation occurred as shown in Figure 3.5.



Figure 3.5: Gelatinisation of SPS

The solution was left to be cooled at room temperature until the solution temperature reach 40°C and different loadings (0%, 1%, 1.5%, and 2%) of ANT were added to the mixture and mixed using magnetic stirrer for another 15 minutes. After that, the solution was sonicated (S-450D Branson Digital Sonifier, India) for 10 minutes to eliminate air bubbles (see Figure 3.6).



Figure 3.6: Sonification of SPS

The solution was then filtered with a muslin cloth and poured 35 ± 1 mL into the petri dish. The petri dish was kept at room temperature (25°C) for 3 days to dry the SPS films (see Figure 3.7). Each solution preparation can produce two replications of films. The steps were repeated for preparing the SPS/SPP films with different loadings (0%, 1%, 1.5%, and 2%) of ANT and named as SPS/SPP/ANT. The ratio of the SPS and SPP was 60:40 for the SPS/SPP/ANT film formulations (see Table 3.1).



Figure 3.7: Drying process of SPS Films

Table 3.1 : SPS and SPS/SPP Films Formulation

Type of Films	Content		
	SPS (g)	SPP (g)	Anthocyanin (%)
SPS	4	0	0
SPS/ANT 1%	4	0	1
SPS/ANT 1.5%	4	0	1.5
SPS/ANT 2%	4	0	2
SPS/SPP	2.4	1.6	0
SPS/SPP/ANT 1%	2.4	1.6	1
SPS/SPP/ANT 1.5%	2.4	1.6	1.5
SPS/SPP/ANT 2%	2.4	1.6	2

3.4 Physical Properties

3.4.1 Film Thickness

The film thickness was measured at five points using a hand-held micro-meter (Mitituyo, Japan) with an accuracy of 0.001mm. The average thickness value was measured in mm.

3.4.2 Colour

The colour of the SPS/ANT and SPS/SPP/ANT films with the size 15cm in diameter was measured using a Hunter Lab Colorimeter (USP 1431 Ultrascan Pro, USA). The films were placed in the sample port and reading were taken. The colour parameters determined were based on CIE L^* , a^* , and b^* scales, where L^* value indicates the lightness from black (0) to white (100); a^* indicates the chromatic

coordinates were $-a^*$ towards green and $+a^*$ towards redness. Meanwhile, b^* indicates chromatic coordinates towards blueness $-b^*$ with towards yellowness $+b^*$ (Gutiérrez et al., 2015). The standard white plate was used for calibration. The colour difference (ΔE) was calculated using equation (4)

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (4)$$

where, $L^* = 99.67$, $a^* = -0.06$, and $b^* = -0.12$ are the colour values of the standard white plate. The measurements for each film have been measured in five different positions.

3.5 Colour Response Analysis of pH Indicator

The SPS and SPS/SPP with and without anthocyanin films were immersed in 15 mL of buffer solutions (pH 1 to 12) for 10 minutes to observe the colour changes after direct contact with different pH solutions. The films were then removed and the colour was measured in a wet condition. The colour parameters L^* , a^* , and b^* of the films were recorded using the portable colorimeter (Precise Colour Reader WR-18, China). The colour difference (ΔE) was calculated using equation (4) where, L^* , a^* , and b^* of colour of films at pH7 was used as reference to compare the colour of films at pH 1 to pH12 (Halász & Csóka, 2018). Three replicates for each sample were conducted.

3.6 Direct Food Contact Test

To test the performance of the films as pH indicators in direct contact with food, the films were cut into 3 cm × 6 cm shapes. Then, one gram of chicken slice samples was wrapped with the films and stored in petri dishes for 2 days at room temperature (see Figure 3.8). The pH of the chicken slice without any films was measured using litmus paper at 0 days, 1 day, and 2 days (see Figure 3.9). The films'

colour shifting was physically observed (Alizadeh-Sani et al., 2021). The steps then were repeated by using cherry tomato as the samples.



Figure 3.8: Chicken slice samples wrapped with films

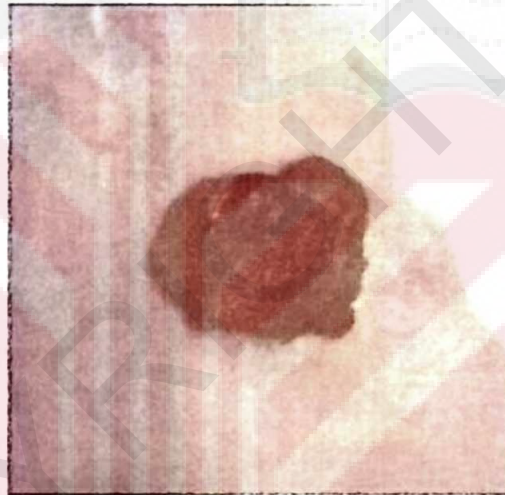


Figure 3.9: Chicken slice without films

3.7 Migration of Anthocyanin

3.7.1 Standard Curve

To develop standard curve for anthocyanin absorbance in different food simulant at different concentration, four different anthocyanin concentration solutions was prepared (0.01%, 0.02%, 0.05%, and 0.1% w/v). To prepare the 0.01% w/v of anthocyanin solution, 0.01 gram of anthocyanin was diluted with 100 mL of distilled water in volumetric flask. The absorbance value of the solution was measured at wavelength 520nm using UV – VIS spectrophotometer (SQ2810, USA). The step then

was repeated for 0.02%, 0.05%, and 0.1% w/v of anthocyanin solutions. Then, the step was repeated by replacing distilled water with 3% acetic acid, 50% ethanol solution, and 95% ethanol solution. A standard curve was generated by plotting the absorbance of anthocyanin versus concentration of anthocyanin.

3.7.2 Migration Analysis

For the analysis of the migration of anthocyanin from both SPS/ANT and SPS/SPP/ANT films, three food simulants were prepared to be tested on the films. The food simulants were 3% acetic acid as a resemblance for acidic food (pH 2.9), 50% ethanol solution (pH 5.3) as a resemblance for low-fat food, and 95% ethanol solution (pH 6.4) as a resemblance for fatty food, and lastly distilled water (pH 7) to represent aqueous food. To perform the analysis, the films were cut into 4 cm × 1.5 cm size. Then the films samples were immersed into a centrifuge tube containing 10 ml of each food simulant. The colour changes of the food simulants were observed for 10 minutes to determine the food simulant that caused the colour of anthocyanin to leach out faster from the films. The absorbance value of the food simulant was measured at wavelength 520nm using UV – VIS spectrophotometer (SQ2810, USA).

3.8 Release Kinetics of Anthocyanin

The method used to study the release kinetics of anthocyanin from the SPS/ANT and SPS/SPP/ANT films was adapted from Tawakkal et al. (2016) with a slight modification. To perform the study, the food simulant that caused the colour of anthocyanin to leach out faster was used to test the kinetics. The films were prepared by cutting into 4 cm×1.5 cm size and were immersed into a centrifuge tube containing 50 ml of food simulant. Samples (film and simulant) were then placed in a chiller, set at 8°C. The absorbance value of the food simulant was recorded at wavelength 520 nm

using a UV – VIS spectrophotometer (SQ2810, USA) for 3 hours for every 15 minutes interval. The amount of released anthocyanin was calculated using a standard curve and the results were presented in mg/ml of food simulant. The method was repeated for samples at room temperature (25°C) and afterward at a higher temperature where the samples were introduced into a water bath at 40°C and 50°C. The data recorded were tabulated in a spreadsheet and analysis was performed on the data by using three data analysis treatments: (i) kinetic modelling, (ii) diffusion model in accordance with Tawakkal et al. (2016), and (iii) diffusion activation energy.

3.8.1 Kinetic Modelling

The colour changes of anthocyanin were described in the zero-order and first order kinetic models. The most precise model-fitting kinetic study was determined based on the coefficient of determination (R^2). Zero-order kinetic models have been represented as in equation (5)

$$\frac{m_t}{m_\infty} = kt \quad (5)$$

Whereby m_t denotes the mass of anthocyanin released from the film at time t , m_∞ denotes the quantity of anthocyanin released from the film at equilibrium ($t = \infty$), and k is the zero-order rate constant. A straight line with a slope of k should be obtained when plotting m_t/m_∞ vs time. Meanwhile, equations (6) were used for first-order kinetic model analysis.

$$\ln \left(1 - \frac{m_t}{m_\infty} \right) = -k_1 t \quad (6)$$

$$v_0 = m_\infty k_1 \quad (7)$$

Where m_t denotes the mass of anthocyanin released from the film at time t , m_∞ denotes the quantity of anthocyanin released from the film at equilibrium ($t = \infty$), and k_1 is the first-order rate constant. While, v_0 (7) is initial release rates.

According to Equation (6), a straight line with a slope of $-k_1$ should be obtained when plotting $\ln (1 - m_t/m_\infty)$ vs time. Equation (6) was used to determine the apparent first-order rate constants, and Equation (7) from Kuorwel et al. (2013) was used to calculate the anthocyanin initial release rates, v_0 .

3.8.2 Diffusion Model

For short term migration: $\frac{m_t}{m_\infty} < 0.6$, equation (8) was employed.

$$\frac{m_t}{m_\infty} = 4 \left(\frac{Dt}{\pi l^2} \right)^{1/2} \quad (8)$$

A plot of m_t/m_∞ vs $t^{1/2}$ should result in a straight line where the diffusion coefficient, D can be determined. While for long term migration: $\frac{m_t}{m_\infty} > 0.6$, Equation (9) was employed.

$$\frac{m_t}{m_\infty} = 1 - \left(\frac{8}{\pi^2} \right) \exp \left(\frac{\pi^2 Dt}{l^2} \right) \quad (9)$$

A plot of $\ln (1 - m_t/m_\infty)$ vs t should result in a straight line is $-k_2$ which is equivalent to $(\pi^2 D)/l^2$, while $\ln (8/\pi^2)$ is the y-intercept.

Whereby,

m_t : mass of anthocyanin released from the film at time t

m_∞ : quantity of anthocyanin released from the film at equilibrium ($t = \infty$)

l : Film thickness

t : Time taken

3.8.3 Diffusion Activation Energy

The effect of temperature on anthocyanin release rate was modelled in conjunction with the Arrhenius equation (Suppakul, 2004):

$$D = D_0 \exp\left(\frac{E_a}{RT}\right) \quad (10)$$

Whereby,

D : Diffusion coefficient

D_0 : Pre-exponential Component

E_a : Activation energy of diffusion process

R : Ideal gas constant

T : Absolute Temperature

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter focuses on the physical characterization of the SPS and SPS/SPP films containing anthocyanin including thickness and appearance of the film at 100% starch for the SPS film, and 60% starch and 40% peel for the SPS/SPP film with the concentration of anthocyanin at 1%, 1.5%, and 2%. Next, the colour response of the SPS and SPS/SPP films with anthocyanin at different pH ranging from 1 to 12 and direct food contact using chicken and cherry tomato samples for 2 days at room temperature. This chapter also examined the migration of anthocyanin using different food simulants particularly distilled water, acetic acid, ethanol 50%, and ethanol 95% for 10 minutes at room temperature. Lastly, the release kinetics of anthocyanin at different temperatures 8°C, 25°C, 40°C, and 50°C in the acidic food simulant were performed.

4.2 Physical Characterization of SPS and SPS/SPP Anthocyanin Film

4.2.1 Thickness

Figure 4.1 shows the average thickness of the SPS and SPS/SPP films with different anthocyanin loadings from 1 to 2% w/v. The thickness for the SPS films at 1%, 1.5%, and 2% anthocyanin content was 0.1117 mm, 0.1259 mm, and 0.1273 mm respectively. Meanwhile, the thickness of the SPS/SPP films was 0.1161 mm, 0.1424 mm, and 0.1465 mm respectively. As expected, the thickness of the SPS and SPS/SPP films without anthocyanin (control) was lower than the SPS and SPS/SPP films with anthocyanin as the thickness of the SPS films without anthocyanin was 0.1014 mm while SPS/SPP films without anthocyanin was 0.1059 mm.

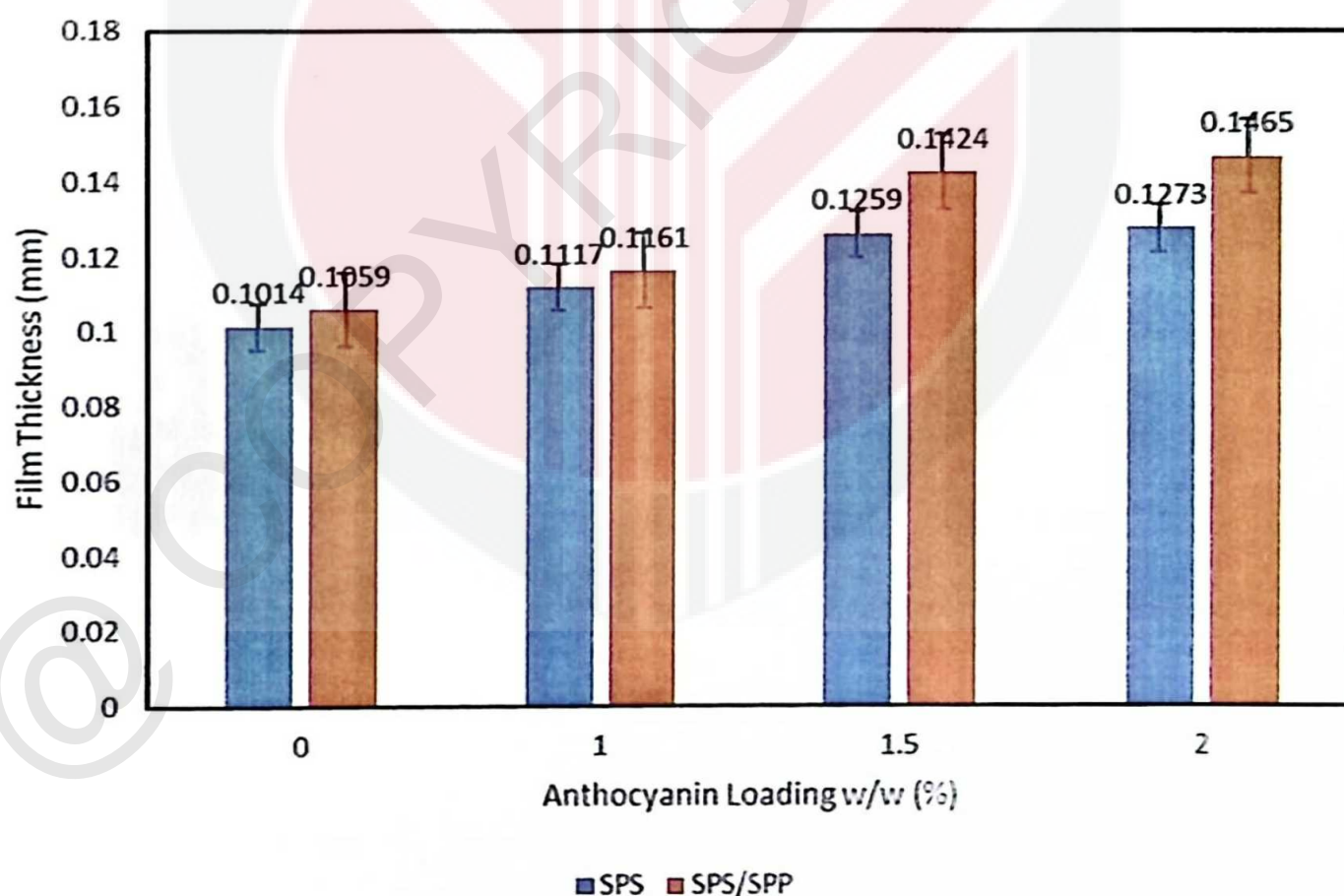


Figure 4.1: Thickness of SPS and SPS/SPP films with different anthocyanin loadings

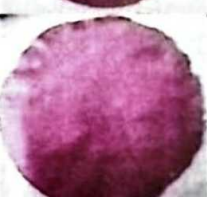
The SPS/SPP/ANT films at different loadings displayed greater thickness compared to the SPS/ANT films. This is because the presence of solid content such as fibre in the peel for the SPS/SPP films possibly make the films thicker. According to Shaari et al. (2021), the peel of sweet potato does not only contain starch, but also it has dietary fibre, crude fat, cellulose and protein. Furthermore, based on the study by Mei et al. (2010), the average amount of cellulose, lignin, pectin, and hemicellulose in the sweet potato fibre was 31.19%, 16.85%, 15.65%, and 11.38% (dry basis), respectively. The presence of fibre in the sweet potato peel might attributed to an increase in solid content, as the same amount of film-casting solution was used (Ananthan, 2020). Along with the increment of anthocyanin loading, both the SPS and SPS/SPP films shows the higher thickness value than lower anthocyanin loading. This indicated that the quantity of anthocyanin incorporated in the SPS and SPS/SPP films had an effect on their thicknesses. The anthocyanin was widely dispersed in the film matrix, a small quantity of anthocyanin (1 wt% on a starch basis) added onto the SPS and SPS/SPP films may become compact in the films. However, higher amounts of anthocyanin (1.5 % and 2 % on a starch basis) might resulted in more complex matrices, resulting in significant thicknesses of the films (Yong et al., 2019). A study by G. Jiang et al. (2020) who prepared films from carboxymethyl-cellulose/starch and purple sweet potato anthocyanin found a similar result whereby the films thickness increases as the anthocyanin loading increased.

4.2.2 Colour of Film

From the Table 4.1, it was found that the colour of the SPS film without anthocyanin was clear and transparent since the sweet potato starch used was white in colour. Meanwhile, the colour of the SPS/SPP film without anthocyanin was yellowish

which resulting to higher value of b^* . This might be due to the presence of SPP resulting it to turn yellowish-brown. In Table 4.1, it shows that the ΔE value of the SPS/ANT films was higher as the anthocyanin loading increased than SPS/ANT with lower anthocyanin loading. The addition of anthocyanin into the film had caused the films to turns slightly darker as the L^* parameter shows lower value. A similar trend was observed for the SPS/SPP films when added with anthocyanin whereby the ΔE value increases as the anthocyanin loading increased which mainly affected by the value of L^* and b^* . Moreover, the b^* value deceases with increasing of anthocyanin loading indicating that the yellowish of films was reduced. Overall, the colour of the SPS/ANT films was more purplish than the SPS/SPP/ANT films which has purplish-brown colour.

Table 4.1: Colour of the SPS and SPS/SPP films at different anthocyanin loadings.

Type of Film	ANT (%)	Colour Parameters			ΔE	Sample
		L^*	a^*	b^*		
SPS	0	91.74 $\pm$ 0.1	-0.94 $\pm$ 0.02	5.63 $\pm$ 0.1	9.76 $\pm$ 0.1	
	1	68.81 $\pm$ 0.3	16.15 $\pm$ 0.2	1.04 $\pm$ 0.3	34.79 $\pm$ 0.4	
	1.5	63.54 $\pm$ 0.5	21.52 $\pm$ 1.7	-1.43 $\pm$ 0.5	42.10 $\pm$ 1.3	
	2	60.78 $\pm$ 0.4	21.98 $\pm$ 0.3	-3.23 $\pm$ 0.1	44.74 $\pm$ 0.5	
SPS/SPP	0	66.99 $\pm$ 1.0	10.78 $\pm$ 0.6	36.23 $\pm$ 0.5	50.02 $\pm$ 1.1	

1	51.76 ±0.7	22.34 ±1.0	18.73 ±0.3	56.09 ±1.0	
1.5	51.88 ±0.8	29.13 ±1.9	12.26 ±0.2	57.35 ±1.4	
2	43.35 ±0.6	23.54 ±0.5	12.23 ±1.0	62.26 ±0.5	

4.3 Colour Response of SPS/ANT and SPS/SPP/ANT Films

Table 4.2, Table 4.3, Table 4.4, and Table 4.5 show the colour changes and colour parameters of the SPS/ANT films at various pH buffer solutions ranging from pH 1.0 (acidic) to pH 12.0 (alkaline). From Table 4.3, 4.4 and 4.5, after exposure the SPS/ANT films to different pH solutions, a noticeable colour shift was observed in the films, ranging from bright red at pH 1.0 to pH 4.0 (acidic), purple at pH 5.0 to pH 8.0, blue at pH 9.0 to pH 10.0, and green at pH 11.0 to pH 12.0 (alkaline). The ΔE values were calculated using the colour parameters of the SPS film in pH 7 as reference. As expected, no colour response was observed for the SPS films without anthocyanin at different pH solutions (see Table 4.2).

Table 4.2: The colour changes and colour parameters of SPS films at different pH buffer solutions.

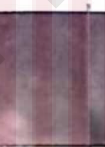
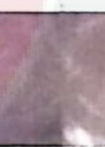
Films	pH	Colour Parameters			ΔE	Sample
		L^*	a^*	b^*		
SPS	1	85.14	2.09	0.22	1.94	
	2	84.28	1.93	0.20	2.77	
	3	86.16	1.98	0.56	0.89	
	4	86.68	2.11	1.40	0.47	
	5	85.02	2.08	1.33	1.93	
	6	88.52	1.97	0.06	1.86	
	7	86.92	2.10	1.00	0.00	
	8	85.87	1.97	1.15	1.08	
	9	82.83	1.62	0.25	4.19	
	10	86.97	1.74	1.25	0.44	
	11	79.31	1.47	2.48	7.78	
	12	85.43	1.55	2.63	2.28	

Table 4.3: The colour changes and colour parameters of SPS/ANT 1% films at different pH buffer solutions.

Films	pH	Colour Parameters			ΔE	Sample
		L^*	a^*	b^*		
SPS/ANT 1%	1	44.10	32.18	1.63	26.12	
	2	46.35	23.42	0.42	17.89	
	3	48.16	22.12	2.18	16.79	
	4	47.52	12.86	1.72	8.30	
	5	46.10	10.71	3.44	5.42	
	6	45.75	8.95	3.29	3.84	
	7	43.20	6.19	4.10	0.00	
	8	52.76	5.73	3.40	9.60	
	9	45.34	0.60	2.46	6.21	
	10	48.32	-2.15	6.14	10.00	
	11	37.52	-0.35	16.84	15.40	
	12	46.96	1.45	25.23	21.98	

Table 4.4: The colour changes and colour parameters of SPS/ANT 1.5% films at different pH buffer solutions.

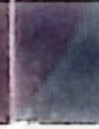
Films	pH	Colour Parameters			ΔE	Sample
		L^*	a^*	b^*		
SPS/ANT 1.5%	1	48.90	43.83	1.11	34.19	
	2	50.75	39.62	1.98	29.78	
	3	41.48	44.85	-0.19	36.86	
	4	47.68	26.84	-1.22	18.31	
	5	50.70	19.68	-0.25	10.73	
	6	59.90	13.08	3.04	7.97	
	7	52.61	9.95	3.86	0.00	
	8	52.39	8.57	1.47	2.77	
	9	48.77	-1.22	-3.77	14.07	
	10	59.32	-5.11	6.88	16.76	
	11	48.15	-3.42	32.88	32.26	
	12	54.26	-0.35	40.50	38.10	

Table 4.5: The colour changes and colour parameters of SPS/ANT 2% films at different pH buffer solutions.

Films	pH	Colour Parameters			ΔE	Sample
		L^*	a^*	b^*		
SPS/ANT 2%	1	34.47	52.94	3.92	39.23	
	2	35.04	54.88	5.37	41.37	
	3	38.98	36.45	-0.09	23.39	
	4	36.02	39.16	-2.62	25.45	
	5	39.70	31.45	-1.11	18.91	
	6	50.36	16.24	3.16	18.64	
	7	32.27	14.06	-0.79	0.00	
	8	39.78	10.00	1.49	8.83	
	9	31.99	-2.26	-3.51	16.55	
	10	39.96	-6.11	8.91	23.66	
	11	34.64	0.55	27.57	31.50	
	12	42.07	10.71	52.86	54.63	

From Table 4.3, Table 4.4, and Table 4.5, it demonstrates that as the anthocyanin loading increases, the range of the ΔE value was significantly broader showing that at higher anthocyanin loading will causing the colour difference is wider. The SPS/ANT 1% film has a range of ΔE value from 3.84 to 26.12. Meanwhile, the SPS/ANT 1.5% film has range of ΔE value from 2.77 to 38.10, and for the SPS/ANT 2%, the range of ΔE value was from 8.83 to 54.63. This indicates that the colour changes of the SPS/ANT films were getting broader as the anthocyanin loading increased. It can be concluded that the SPS/ANT 2% film displayed visibly good colour changes than the SPS/ANT 1% and 1.5% film as the ΔE value is broader indicating wider colour changes.

Table 4.6, Table 4.7, Table 4.8, and Table 4.9 show the colour changes and colour parameters of the SPS/SPP films with and without anthocyanin at various pH buffer solutions ranging from pH 1.0 (acidic) to pH 12.0 (alkaline). From the table, a similar and noticeable colour shift was found in the SPS/SPP films, ranging from bright red at pH 1.0 to pH 4.0 (acidic), purple at pH 5.0 to pH 8.0, blue at pH 9.0 to pH 10.0, and green at pH 11.0 to pH 12.0 (alkaline). The ΔE values were calculated using the colour parameters of the SPS/SPP film in pH 7 buffer solution as reference.

Table 4.6: The colour changes and colour parameters of SPS/SPP film at different pH buffer solutions.

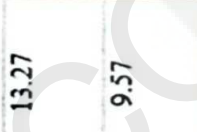
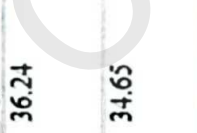
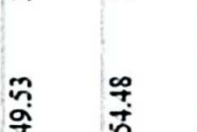
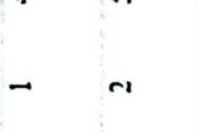
Films	pH	Colour Parameters			ΔE	Sample
		L^*	a^*	b^*		
SPS/SPP	1	49.53	21.97	36.24	13.27	
	2	54.48	18.14	34.65	9.57	
	3	59.44	14.32	30.96	9.40	
	4	57.25	13.16	31.55	6.92	
	5	51.56	11.71	32.48	2.27	
	6	51.95	12.69	36.45	5.88	
	7	51.25	9.73	31.42	0.00	
	8	50.08	10.67	33.93	2.92	
	9	53.04	8.73	34.46	3.67	
	10	49.61	7.95	38.16	7.16	
	11	40.09	28.16	65.02	39.92	
	12	37.73	38.96	69.54	49.91	

Table 4.7: The colour changes and colour parameters of SPS/SPP/ANT 1% film at different pH buffer solutions.

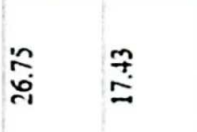
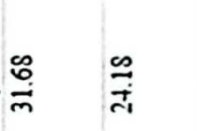
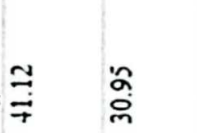
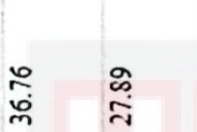
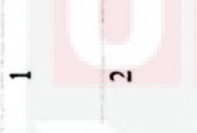
Films	pH	Colour Parameters			ΔE	Sample
		L^*	a^*	b^*		
SPS/SPP/ANT 1%	1	36.76	41.12	31.68	26.75	
	2	27.89	30.95	24.18	17.43	
	3	37.50	33.09	23.45	18.45	
	4	28.74	28.82	25.27	15.05	
	5	39.02	19.10	23.92	6.27	
	6	42.71	15.41	24.04	8.22	
	7	34.75	15.04	26.07	0.00	
	8	37.53	13.71	28.96	4.23	
	9	34.45	7.55	27.65	7.66	
	10	44.37	0.76	30.19	17.70	
	11	25.61	41.93	52.48	38.78	
	12	40.61	10.55	44.92	20.24	

Table 4.8: The colour changes and colour parameters of SPS/SPP/ANT 1.5% film at different pH buffer solutions.

Films	pH	Colour Parameters			ΔE	Sample
		L*	a*	b*		
SPS/SPP/ANT 1.5%	1	29.90	40.91	44.13	36.55	
	2	39.09	34.16	28.80	22.69	
	3	38.48	31.49	26.55	19.69	
	4	27.10	25.45	27.14	18.61	
	5	44.65	18.43	25.30	8.31	
	6	39.87	17.42	29.73	7.93	
	7	39.56	12.00	23.96	0.00	
	8	31.87	15.00	29.91	10.17	
	9	38.20	6.96	27.26	6.18	
	10	31.16	11.49	36.02	14.71	
	11	26.68	47.31	60.23	52.23	
	12	13.45	2.36	54.73	41.49	

Table 4.9: The colour changes and colour parameters of SPS/SPP/ANT 2% film at different pH buffer solutions.

Films	pH	Colour Parameters			ΔE	Sample
		L*	a*	b*		
SPS/SPP/ANT 2%	1	24.61	33.57	28.10	19.21	
	2	19.10	29.30	35.90	21.83	
	3	21.84	28.55	26.02	15.15	
	4	23.49	30.26	24.86	15.66	
	5	21.78	26.77	25.44	13.58	
	6	25.89	23.22	27.70	9.87	
	7	29.24	16.00	21.86	0.00	
	8	30.27	15.60	22.91	1.53	
	9	27.34	7.37	19.38	9.17	
	10	19.48	-1.06	42.48	28.48	
	11	15.70	4.31	50.31	33.60	
	12	16.80	2.45	67.66	49.35	

From the Table 4.6, Table 4.7, Table 4.8, and Table 4.9, it was found that the SPS/SPP films without anthocyanin (see Table 4.6) shows colour changes as the films were immersed at different pH buffer solutions. Interestingly, the colour changes of the SPS/SPP film were due to the present of natural anthocyanin in the sweet potato peel powder. Shaari et al. (2020) reported that the peel of sweet potato contains approximately 5.21 mg of anthocyanin per 100 dry basis. As the natural anthocyanin present in a small amount from the sweet potato peel powder; hence, colour changes of the SPS/SPP film without addition of anthocyanin was observed. Nevertheless, the colour of the film was less visible than the colour changes of the SPS/SPP film with anthocyanin.

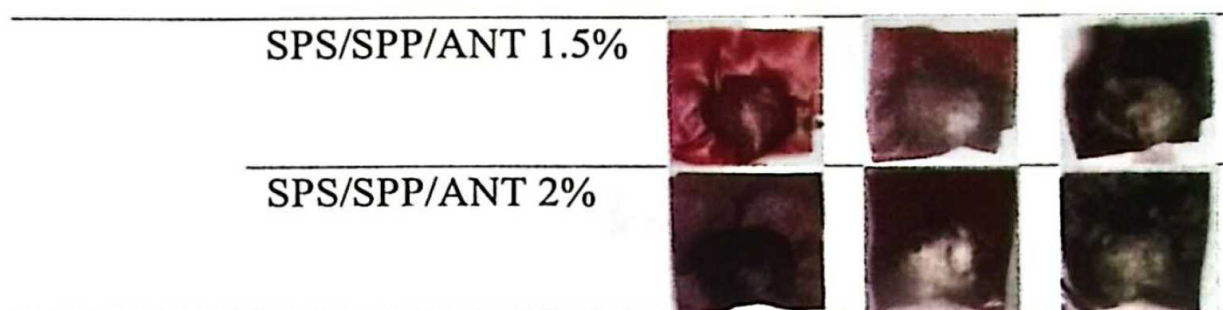
The observation from Table 4.6, Table 4.7, Table 4.8, and Table 4.9 SPS/SPP/ANT demonstrate a similar trend as for the SPS/ANT films in Table 4.3 Table 4.4, and Table 4.5 whereby as the anthocyanin loading increases, the range of ΔE value was significantly increased. The SPS/SPP/ANT 1% film has range of ΔE value from 4.23 to 38.78, meanwhile SPS/SPP/ANT 1.5% has range of ΔE value from 6.18 to 52.23, and at SPS/SPP/ANT CA 2%, the range of ΔE value was from 1.53 to 49.35. This indicates that the colour changes of the SPS/SPP/ANT films were getting broader as the anthocyanin loading increased. Overall, it can be concluded that the SPS/ANT 2% film exhibited noticeable good colour changes than the SPS/ANT 1%, 1.5% films and SPS/SPP/ANT as the range of ΔE value is much broader indicating the colour changes is more significant.

4.4 Colour Changes of Film on Direct Food Contact

Direct food contact testing was performed to observe the pH colour changes of film on chicken meat and cherry tomato (*Lycopersicon esculentum*) samples for a detailed assessment on the functionality of the SPS/ANT and SPS/SPP/ANT films as pH indicator films. The observation of colour changes of films was performed until the day the films showing the first colour changes. In this test, it was found that the films started to change their colour changes after 2 days with direct contact with food. Table 4.10 shows the physical appearance of the film after 2 days in contact with chicken meat at room temperature.

Table 4.10: Direct food contact on chicken meat sample for 2 days at room temperature

Day		0	1	2
pH		6	8	9
Film Type	Blank			
	SPS			
	SPS/ANT 1%			
	SPS/ANT 1.5%			
	SPS/ANT 2%			
	SPS/SPP			
	SPS/SPP/ANT 1%			



After 2 days, the pH of the chicken meat was increasing towards alkaline pH indicating that the chicken was spoiled. According study by Baston et al. (2008), the pH of chicken can be increased due to proteolysis during the aging process. It was found that after 24 hours (1 day) in contact with the chicken meat, both the SPS/ANT and SPS/SPP/ANT films started to swell and absorb water that been leach out from the chicken. After second day in contact with chicken slice, the SPS/ANT 1% and SPS/SPP/ANT 1%, 1.5%, and 2% films has changed its colour from purple to blueish-green which reflecting the pH of the chicken meat which was alkaline.

Table 4.11 shows the physical appearance of the SPS/ANT and SPS/SPP/ANT films after 2 days in contact with cherry tomato at room temperature. After 2 days, the pH of cherry tomato sample continues to increase towards alkaline due to the hydrolysis of organic acid in cherry tomato, suggesting that the cherry tomato was spoiled (Tigist et al., 2013). Nazri (2020) investigated on the in-vivo test of PLA/EO and PLA/NFC/EO film using cherry tomato also shown that the pH of films has shifted towards alkaline as the contact time increased. After 24 hours (1 day) of contact with cherry tomato, both the SPS/ANT and SPS/SPP/ANT films start to swell as they absorbed water from the cherry tomato. After second day in contact with the sample, a blue-green spot can be observed in the SPS/ANT 1.5%, SPS/ANT 2%, SPS/SPP/ANT 1%, and SPS/SPP/ANT 2% films which reflecting the pH of cherry tomato was shifting towards alkaline.

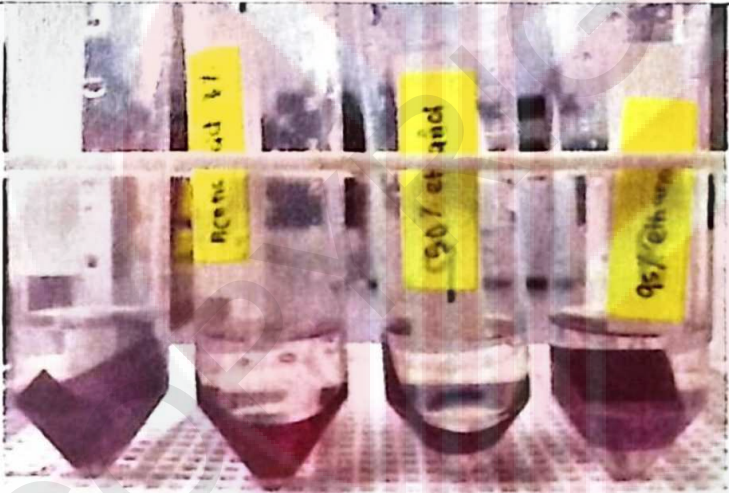
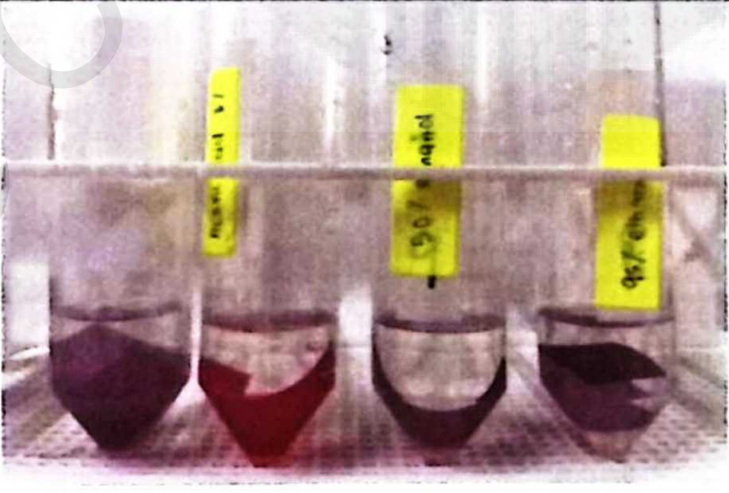
Table 4.11: Direct food contact on cherry tomato sample for 2 days at room temperature

Day		0	1	2
pH		4	5	9
Film Type	Blank			
	SPS			
	SPS/ANT 1%			
	SPS/ANT 1.5%			
	SPS/ANT 2%			
	SPS/SPP			
	SPS/SPP/ANT 1%			
	SPS/SPP/ANT 1.5%			
	SPS/SPP/ANT 2%			

4.5 Migration of Anthocyanin

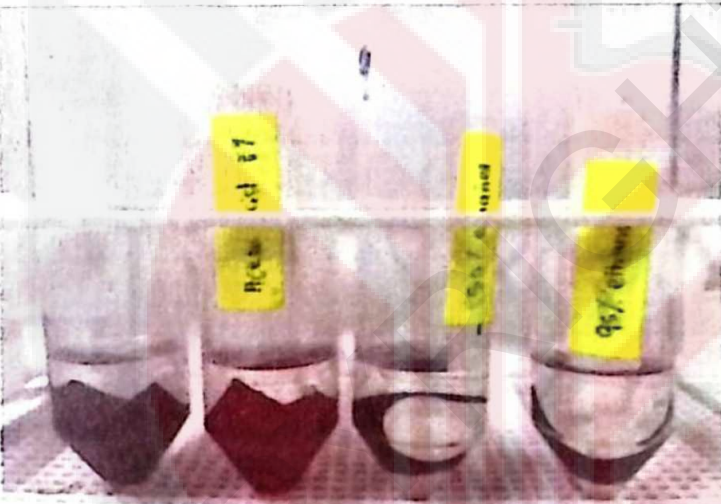
SPS/ANT and SPS/SPP/ANT films with a different loading (0%, 1%, 1.5%, and 2%) of ANT was immersed into different food simulants and the migration of anthocyanin was observed in different food simulants including distilled water, 3% v/v acetic acid solution, 50% v/v ethanol solution, and 95% v/v ethanol solution for 10 minutes at room temperature. No replication was performed due to time limitations. Table 4.12 and Table 4.13 show the migration of anthocyanin from the SPS/ANT 2% and SPS/SPP/ANT 2% films into different food simulants.

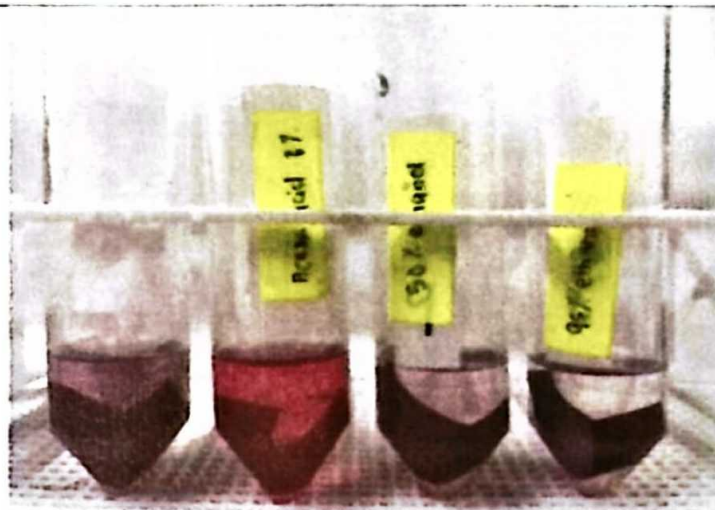
Table 4.12: The migration of anthocyanin from the SPS/ANT 2% films at different food simulants for 10 minutes and at 25°C

Time (min)	Image	Findings
0		- The 3% acetic acid solution has a slight pink hue. - No changes of colour between distilled water, 50% ethanol solution, and 95% ethanol solution.
5		- In 3 % acetic acid solution, the intensity of the pink hue rises. - Distilled water shows a slight purple colour. - No change of colour in 50 % ethanol solution and 95 % ethanol solution.

10		• In 3 % acetic acid solution, the pink hue was getting more intense. • In, distilled water the intensity of the purple colour increasing. • No visible change of colour in 50% and 95 % ethanol solution.
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Table 4.13: The migration of anthocyanin from the SPS/SPP/ANT 2% films at different food simulants for 10 minutes and at 25°C.

Time (min)	Image	Findings
0		• Anthocyanin starts to diffused into the 3% acetic acid solution and slight purple colour are observed in distilled water. • No changes of colour between 50% ethanol solution, and 95% ethanol solution.
5		• In 3 % acetic acid solution, the intensity of the pink hue rises. • Distilled water shows slight purple colour. • No changes of colour in 50 % ethanol solution and 95 % ethanol solution.



- In 3 % acetic acid solution, the pink hue was getting more intense.
- In distilled water, the intensity of the purple colour increasing.
- No visible change of colour in 50% and 95 % ethanol solution.

According to Turturică et al. (2015), the ionic nature of anthocyanins, variations in pH levels may cause changes in their molecular structure. As the pH of 3% acetic acid food simulant was 2.9 (acidic); thus, pink colour solution can be observed indicating that the anthocyanin had leached out from both the SPS/ANT and SPS/SPP/ANT films. Meanwhile, anthocyanin migration into distilled water remains purple in colour since the pH of distilled water was neutral.

Figure 4.2 shows the amount of anthocyanin migrated per food simulant volume at different anthocyanin loadings from the SPS/ANT film at the 10 minutes.

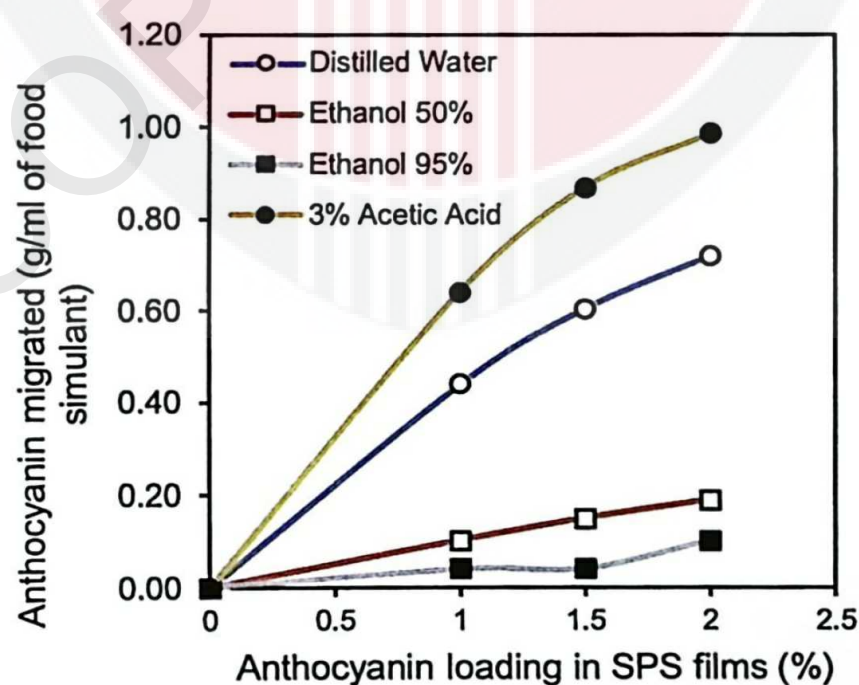


Figure 4.2: Migration analysis of SPS/ANT films at 25°C and for 10 minutes measured at wavelength of 520 nm.

Among the four simulants, the 3% acetic acid food simulant showed the highest value of anthocyanin that has been migrated from the SPS films into the simulant followed by distilled water, ethanol 50% and ethanol 95%. Higher amount of anthocyanin content was observed in the 3% acetic acid and distilled water simulants. This is due to the nature of anthocyanin that soluble in water and organic solvent (Khoo et al., 2017). The 3% acetic acid simulant was recorded as the highest amount of anthocyanin migrated than the other food simulants because the anthocyanin solubility in water increased at lower pH levels due to strong protonation (Khoo et al., 2017). Meanwhile, simulants such as ethanol 50% and ethanol 95% demonstrated the lowest anthocyanin migration after 10 minutes due to the different type and polarity of simulants, the swelling ratio, and the solubility of the films which influence the rate of anthocyanin release from the films (Ali zadeh-Sani et al., 2021; Lee et al., 2018).

Figure 4.3 shows the amounts of anthocyanin migrated per food simulant volume at different anthocyanin loadings from the SPS/SPP/ANT film after 10 minutes.

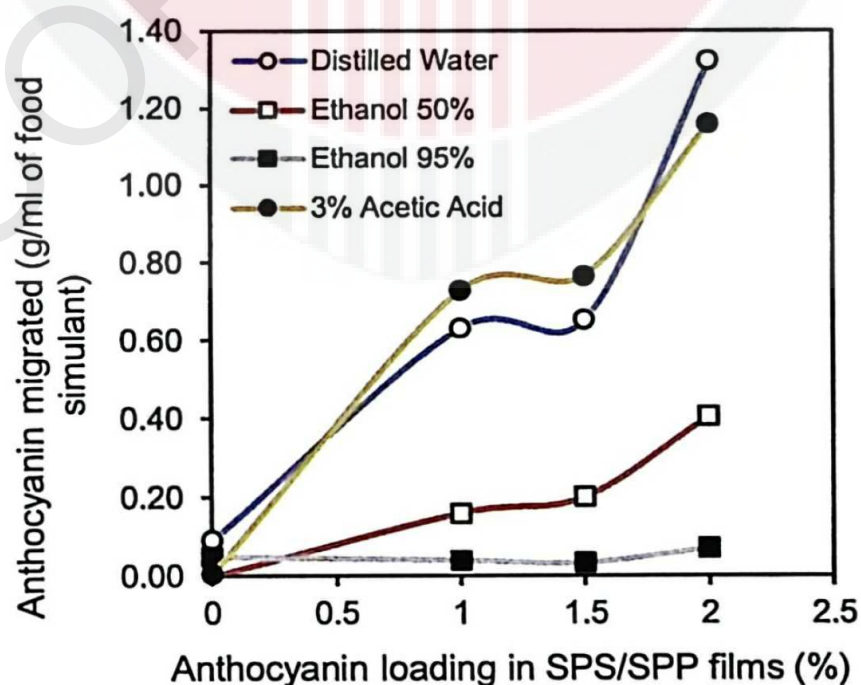


Figure 4.3: Migration analysis of SPS/SPP/ANT films at 25°C and for 10 minutes measured at wavelength of 520 nm.

A similar finding can be observed for the migration of anthocyanin from the SPS/SPP/ANT films. It was found that the 3% acetic acid and distilled water simulants recorded the higher amount of anthocyanin migrated than the 50% ethanol and 95% ethanol simulants. Another observation from the migration of anthocyanin from the SPS/SPP/ANT films was that distilled water simulant demonstrated high anthocyanin migration same as 3% acetic acid simulant. This might be due to the presence of peel (fibre) the SPS/SPP films, the fibre was hydrophilic and easily to absorb water, causing swelling and creating voids that allow the anthocyanin to migrate (Mohanty et al., 2005).

In overall, the 3% acetic acid simulant was found to leached out the anthocyanin from the SPS and SPS/SPP films faster than the other simulants. A study from Andretta et al. (2019), reported that the absorbance values in the migration experiments for the ethanol solution applied as a fatty food simulant were lower, indicating anthocyanin hydrophilicity. This finding has been discovered in other studies on migration of anthocyanin (Agustinelli et al., 2021; Singh et al., 2020).

4.6 Release Kinetics of Anthocyanin

Release kinetics of anthocyanin was performed using SPS/ANT 1.5% and SPS/SPP/ANT 1.5% immersed in 3% acetic acid for 3 hours. 3% acetic acid was used as a food simulant to study the anthocyanin released as it found that anthocyanin was stable at lower pH (acidic) (Tang et al., 2019). In migration analysis was conducted before also found that anthocyanin was migrated faster in 3% acetic acid compared to the other food simulants. Hence, 3% acetic acid was used as the food simulant to study the release of anthocyanin from SPS/ANT 1.5% and SPS/SPP/ANT 1.5% to food simulant. No replication was performed due to time limitations. The release kinetics of anthocyanin was studied using zero-order kinetic model, first-order kinetic model, and diffusion model.

4.6.1 Overall Kinetic Analysis

Figure 4.4 and Figure 4.5 show the mass fraction of anthocyanin release from the films to 3% acetic acid simulant using zero-order kinetic model. Both the SPS/ANT and SPS/SPP/ANT films, demonstrated initial rapid release for 15 minutes as the slope was steeper and followed by continuous release before achieving standstill value. The rapid release of anthocyanin in the first 15 minutes was due to the present of anthocyanin on or near to the surface of the films. A similar phenomenon had been reported on migration of pigment from films by J. Liu et al. (2019) where each release curve of *Lycium ruthenicum* Murr indicated a 10-minute burst release followed by a slow release before reaching a plateau. It also has been observed as the temperature increase, the faster the anthocyanin release reached the equilibrium state. Because as at the same time (min), higher mass fraction was recorded at high temperature compare to at lower temperature. For example, at 60th minutes after SPS/ANT and

SPS/SPP/ANT immersed in 3% acetic acid, it was found that the highest mass fraction of anthocyanin released was recorded at temperature 50°C followed by at temperature 40°C and 25°C (see Figure 4.4 and Figure 4.5). While at 8°C recorded the lowest mass fraction of anthocyanin at the same time. This result is in agreement by Tawakkal et al. (2016) on the release of thymol from poly(lactic acid) (PLA) and PLA/kenaf fibre film whereby the thymol released at higher temperature with 95% ethanol simulant reached equilibrium faster.

The rate of anthocyanin released at different temperatures can be observed using first-order model as shown in Figure 4.6 and Figure 4.7 whereby at higher temperature, the slope of $\ln(1-m_t/m_\infty)$ versus time was getting steeper indicating the rate of anthocyanin released increased. The value of rate constant of anthocyanin from first-order model, k_1 was obtained from the slope was presented in Table 4.14 together with the rate constant value of zero-order model, k .

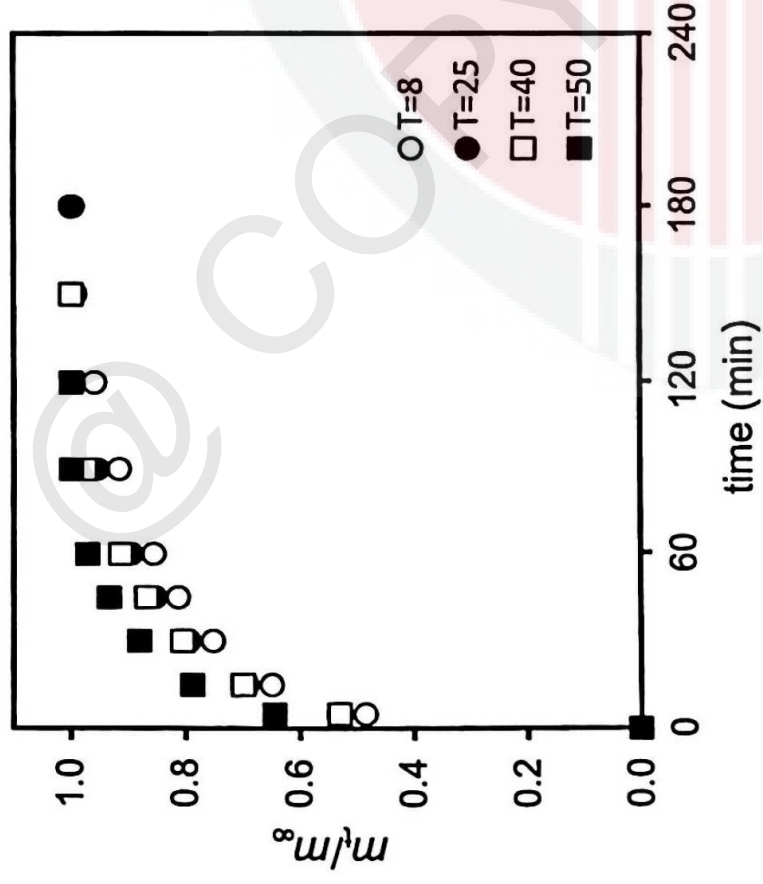


Figure 4.4: Mass fraction m_t/m_∞ versus t for the anthocyanin release from SPS/ANT film to 3% acetic acid simulant

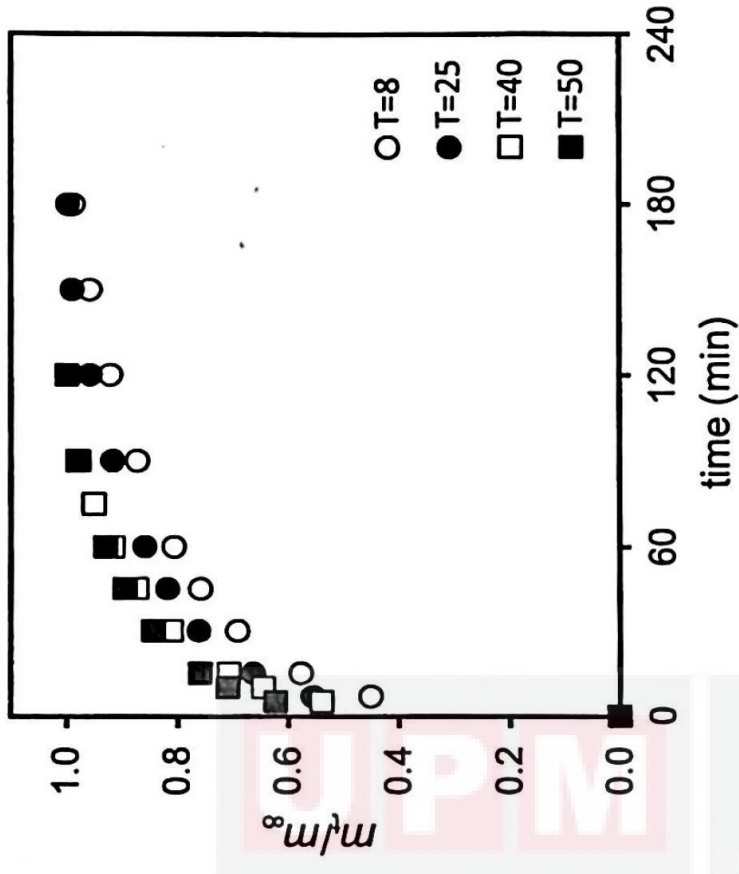


Figure 4.5: Mass fraction m_t/m_∞ versus t for the anthocyanin release from SPS/SPP/ANT film to 3% acetic acid simulant.

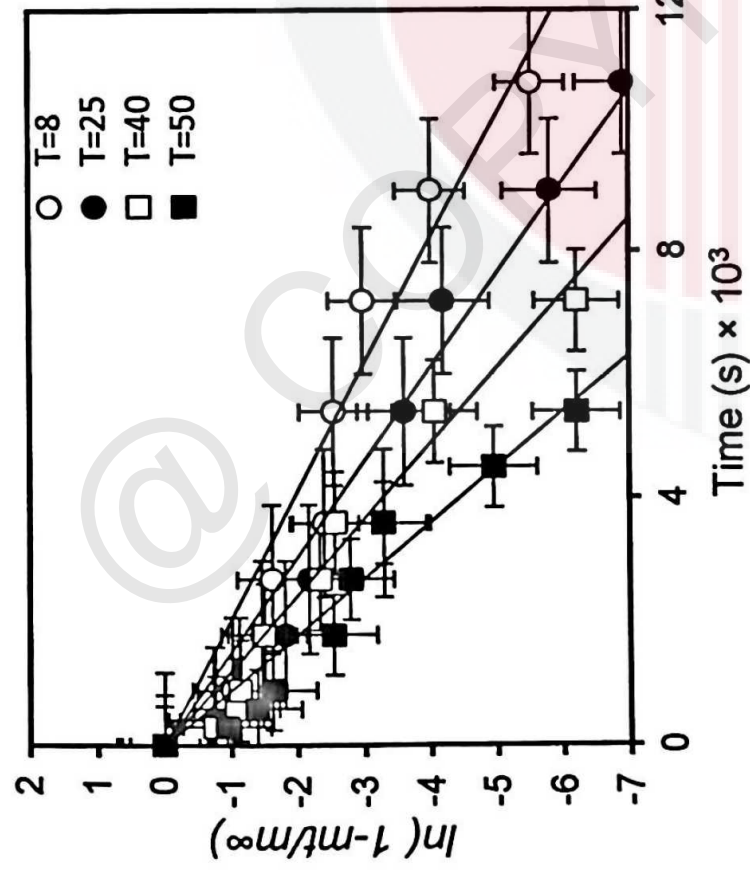


Figure 4.6: $\ln(1-m_t/m_\infty)$ versus t for the anthocyanin release from SPS/ANT film into 3% acetic acid simulant.

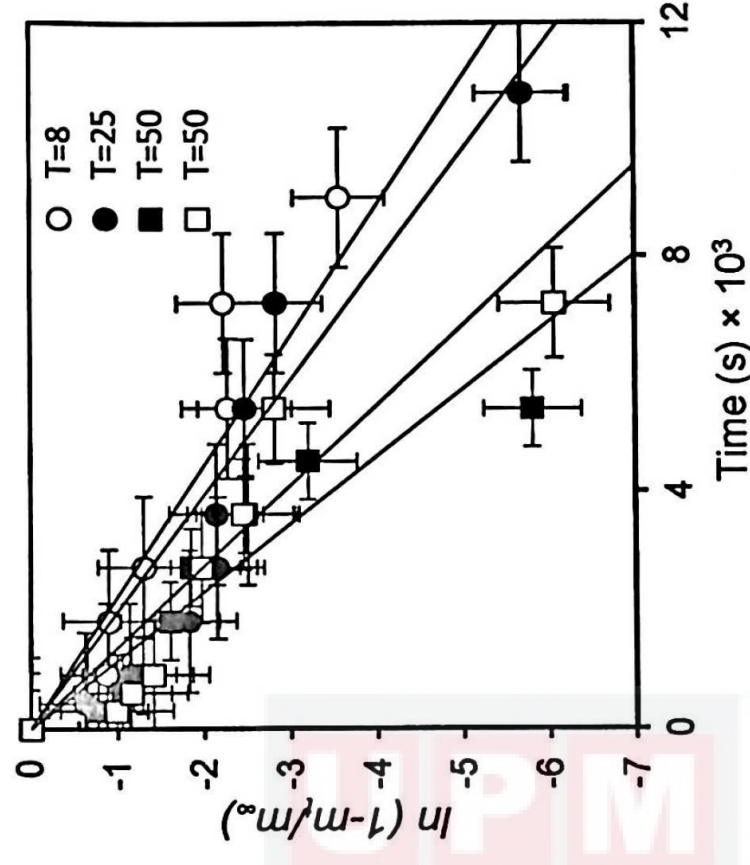


Figure 4.7: $\ln(1-m_t/m_\infty)$ versus t for the anthocyanin release from SPS/SPP/ANT film into 3% acetic acid simulant.

Table 4.14: Overall Kinetic Analysis

Film Type	Temperature (°C)	Zero Order Kinetic Analysis	R ²	First Order Kinetic Analysis		R ²
		$k_0 \times 10^{-3}$ (s ⁻¹)		$v_0 \times 10^5$ (mg s ⁻¹)	$k_1 \times 10^5$ (s ⁻¹)	
SPS	8	0.1290	0.7652	13.4	48.8	0.9739
	25	0.1316	0.7758	18.9	65.6	0.9829
	40	0.2052	0.7094	37.3	82.1	0.9884
	50	0.2504	0.7428	44.8	111.2	0.9772
SPS/SPP	8	0.1248	0.8125	13.2	45.1	0.9514
	25	0.1278	0.7587	15.1	51.0	0.9512
	40	0.2576	0.7255	25.2	73.8	0.9529
	50	0.1978	0.7071	35.6	82.7	0.9819

In Table 4.14, the overall kinetic analysis data for both zero-order and first-order model was measured and calculated using Equation (5),(6), and(7). From Table 4.14, it was observed that the release of anthocyanin from both the SPS and SPS/SPP films to the food simulant was fit into the first-order model than the zero-order model as the regression value was higher R² (0.9884 → 0.9512). Similar outcomes had been reported by J. Liu et al. (2019) which release behaviour of Lycium ruthenicum Murr from κ-carrageenan film was compliment with first-order model.

From Table 4.14, it was found that the release of anthocyanin from the SPS/ANT films recorded higher k₁ value than the SPS/SPP films. Apart from that, the SPS/ANT films also recorded slightly higher value of initial release of anthocyanin, v₀ than the SPS/SPP films. Hence, the first-order model predicted that anthocyanin in the SPS/ANT films was released at a higher rate than in the SPS/SPP/ANT films as the value of k₁ and v₀ of SPS/ANT is higher than SPS/SPP/ANT.

4.6.2 Diffusion Analysis

Table 4.15 shows the diffusion analysis data obtained using diffusion model as in Equation (6)for short term migration and Equation (7) for long term migration. The diffusion coefficient of diffusion analysis was obtained via the slope of Equation (6) and (7). From Table 4.15, most of the data exhibit good fit with diffusion analysis model as it has R^2 value which indicating the reliability to the model. From the result, as the temperature increases, the diffusion coefficient was increased. This finding is in agreement with Kuorwel et al. (2013) where it was found that as the temperature of the isooctane (fatty food simulant) increased, the diffusion coefficient of essential oils from the starch-based films was increased.

According to Agustinelli et al. (2021), the increment of diffusion coefficient value was due to the increase in the mobility of migrants with temperature, migration was also improved at higher temperatures. Moreover, for the short-term diffusion, it was found that the SPS/SPP/ANT films demonstrated higher value of diffusion coefficient value than the SPS/ANT films. As expected, the diffusion rates of film such as SPS/SPP/ANT were greater than diffusion rates of the SPS/ANT films. This might be due to the presence of peel (fibre) in the polymer matrix, which produces voids and allows anthocyanin to be released from the films. This observation is consistent with the findings by Tawakkal et al. (2016) who reported that the diffusion coefficient for thymol released from PLA-containing kenaf fibre films to 95 % and 15 % ethanol was greater than the PLA films.

Table 4.15: Diffusion Analysis of SPS and SPS/SPP films.

Film Type	Temperature (°C)	Diffusion analysis			
		Short Term	R^2	Long Term	R^2
		$D \times 10^{13}/\text{m}^2 \text{ s}^{-1}$		$k_2 \times 10^5/\text{s}^{-1}$	
SPS	8	21.3	0.9297	4.00	0.9453
	25	21.6	0.9285	5.44	0.9766
	40	29.0	0.9879	7.58	0.9675
	50	51.3	0.9660	9.41	0.9560
SPS/SPP	8	11.9	0.9376	3.10	0.8326
	25	21.7	0.9686	3.91	0.8857
	40	40.1	0.9824	5.06	0.7585
	50	53.3	0.9377	6.86	0.9685

4.6.3 Diffusion Activation Energy

Figure 4.8 shows the plot of $\ln D$ versus $1/T$ which obtained from diffusion analysis for short-term diffusion in Table 4.15. It was found that the diffusion coefficient changes as the temperature change. By using Arrhenius equation as in Equation (10), the y-intercept of the plot was the value of $\ln D_0$ which used to measure the activation of energy of the anthocyanin release as shown in Table 4.16.

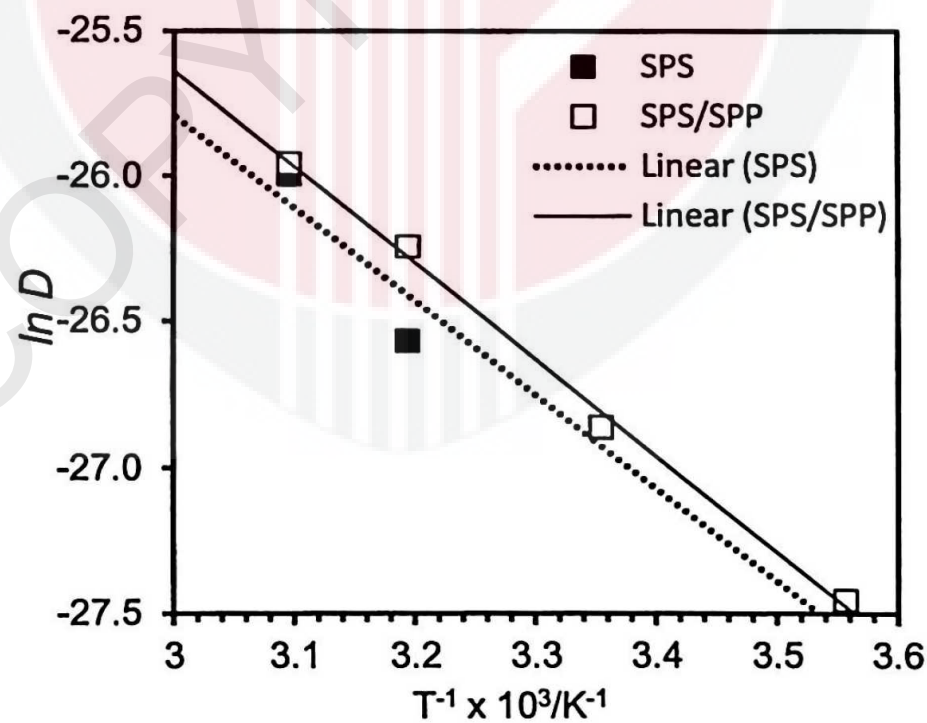


Figure 4.8: Diffusion activation energy of the SPS/ANT and SPS/SPP/ANT films.

Table 4.16: Activation energy of SPS/ANT and SPS/SPP/ANT films

Film Type	Activation Energy, E_a (kJ/mol)	R²
SPS/ANT	134.76	0.9026
SPS/SPP/ANT	130.87	0.9968

Based on the Table 4.16 the activation energy for the SPS/ANT film was slightly greater than the SPS/SPP/ANT film, owing to the strong interaction between the SPS and anthocyanin, which is probably weaker in the presence of the SPP. This trend result was observed by Tawakkal et al. (2016), whereby they discovered that the thymol released to 95% ethanol solution from the PLA containing kenaf fibre had a lower E_a than the PLA alone which is 76.2 kJ/mol and 90.6 kJ/mol respectively. As the nature of anthocyanin which is not volatile as essential oil, it was presumably required more energy to release the anthocyanin from the films than the energy required for essential oil to release from the matrix films. To the best of the authors' knowledge, research on the release kinetics of anthocyanin from the starch-based films is relatively scarce, while several kinetics of colour change of biopolymer films containing anthocyanin have been reported (Ananthan (2020)). Apart from that, there a several studies on the release of anthocyanin from films but using different types of biopolymer including chitosan film cross-linked with sodium tripolyphosphate by Carlos-Salazar, Max J. (2017) and novel konjac glucomannan films with oxidized chitin nanocrystals by Wu et al. (2020). In both of the studies, they are focusing on the release behaviour of anthocyanin with no report mentioned in term of the activation energy of the release of anthocyanin from the films.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

The SPS and SPS/SPP films showed distinct physical properties in terms of thickness and colour. The thickness of the SPS/ANT and SPS/SPP/ANT films increases as the anthocyanin loading increased. The colour parameter of the SPS/SPP films displayed lower value in terms of (L^*), higher value in (a^*) and (b^*) than the SPS film in which indicate that the addition of sweet potato peel resulting darker films with yellowish hue. Meanwhile, as the anthocyanin content increases, the films was observed to exhibit more intense purple colour. Meanwhile, when immersed in different pH buffer solutions the SPS/ANT and SPS/SPP/ANT films demonstrated noticeable colour changes. When tested in direct food contact after 2 days at room temperature for chicken meat and cherry tomato samples, both of SPS/ANT and SPS/SPP/ANT films shows noticeable colour changes where the bluish-green spot was found on the surface of the films.

Higher concentration of anthocyanin was found in the 3% acetic acid simulat followed by distilled water, 50% ethanol, and 95% ethanol simulants, as in the migration analysis. The release kinetics of anthocyanin from the SPS/ANT and SPS/SPP/ANT films fit into the first-order model and diffusion model as the regression coefficient was high. By using first-order model, it was reflected that the anthocyanin

was released higher in the SPS/ANT films than the SPS/SPP/ANT films. However, through the diffusion model, the SPS/SPP/ANT film demonstrated higher diffusion coefficient for short-term diffusion and low long-term diffusion than the anthocyanin released from the SPS/ANT films. The diffusion activation energy demonstrated that the SPS/ANT films required more energy than the SPS/SPP/ANT films.

5.2 Recommendations

Studies on the migration of anthocyanin from the SPS and SPS/SPP films into food simulant can be conducted over a longer period of time, about 8 to 10 hours, to guarantee that the final migration data is the equilibrium data that can be studied using Fick's second law model. In this studies, absorbance of anthocyanin in food simulant was only measured at wavelength 520 nm as this the peak absorbance of anthocyanin in acidic media (acidic food simulant); however, to measure absorbance of anthocyanin in another food simulant such as distilled water and ethanol may require measurement at different value of wavelength.

It was discovered that the SPS/ANT and SPS/SPP/ANT films have the potential to change colour when placed in direct contact with food; however, the mould was easily formed on the films if it was exposed to excess moisture. Thus, the addition of active ingredient such as essential oil can be performed to improve the shelf life of films as it may capable to prevent mould formation. Characterization of the SPS/ANT and SPS/SPP/ANT films at different anthocyanin concentration with and without addition of essential oil can be explored in order to evaluate the mechanical, thermal, and morphological properties.

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APPENDICES

A-1: Film Thickness of SPS and SPS/SPP Films

Type of Film	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Mean	SD	SEM
SPS	0.1052	0.1092	0.0999	0.0935	0.0993	0.1014	0.0060	0.003
SPS CA 1%	0.1019	0.1180	0.1178	0.1023	0.1185	0.1117	0.0088	0.004
SPS CA 1.5%	0.1282	0.1198	0.1304	0.1191	0.1320	0.1259	0.0060	0.003
SPS CA 2%	0.1182	0.1422	0.1205	0.1280	0.1277	0.1273	0.0094	0.004
SPS/SPP	0.1029	0.0995	0.1018	0.1093	0.1161	0.1059	0.0067	0.003
SPS/SPP CA 1%	0.1178	0.1129	0.1130	0.1175	0.1194	0.1161	0.0030	0.001
SPS/SPP CA 1.5%	0.1332	0.1306	0.1465	0.1447	0.1569	0.1424	0.1070	0.005
SPS/SPP CA 2%	0.1543	0.1572	0.1330	0.1388	0.1494	0.1465	0.0103	0.005

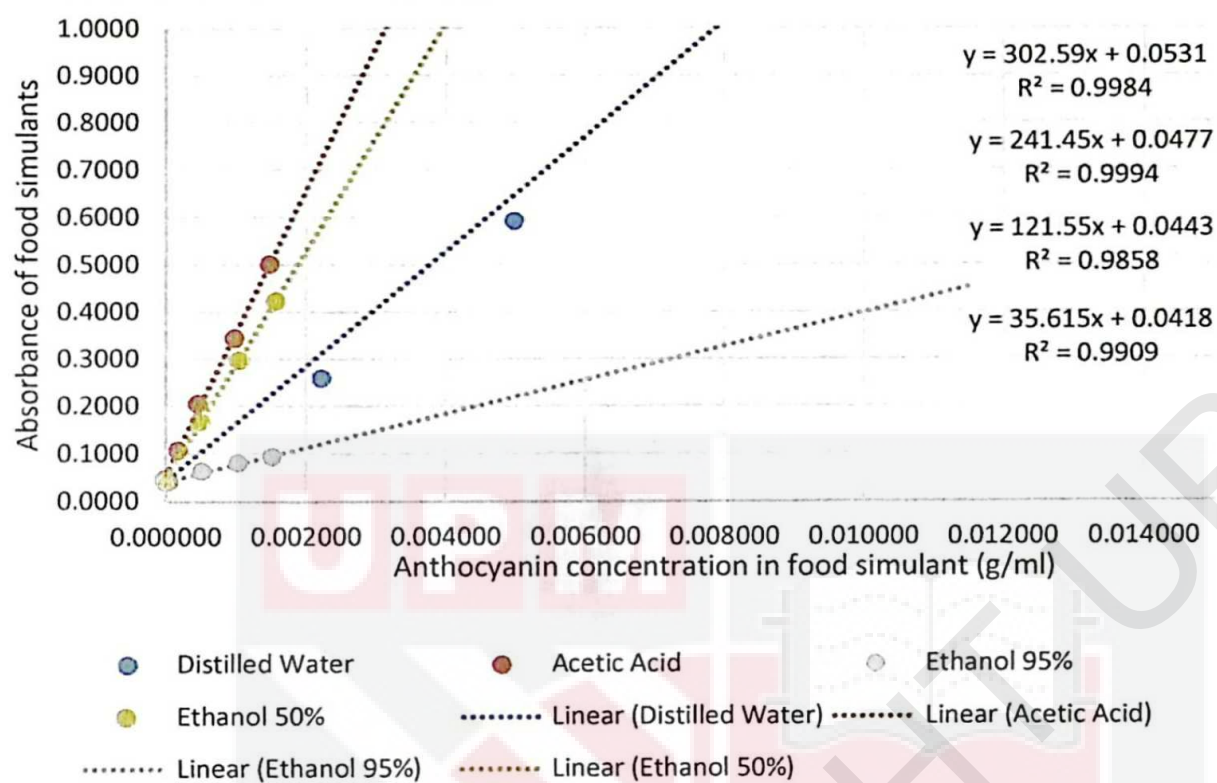
A-2: Colour of SPS and SPS/SPP Anthocyanin Films

Film Type		L	a	b	E
SPS	R1	91.86	-0.90	5.33	9.48
	R2	92.05	-0.97	5.49	9.43
	R3	91.62	-0.94	5.96	10.05
	R4	91.43	-0.99	5.82	10.12
	R5	91.74	-0.92	5.55	9.71
	AVG	91.74	-0.94	5.63	9.76
SPS 1%	R1	69.61	15.65	0.74	33.84
	R2	68.11	16.46	1.66	35.58
	R3	69.47	15.81	1.48	34.07
	R4	68.44	16.41	1.06	35.25
	R5	68.44	16.41	0.28	35.22
	AVG	68.81	16.15	1.04	34.79
SPS 1.5%	R1	62.08	28.10	-2.58	46.96
	R2	63.47	20.61	-2.04	41.64

	R3	64.91	18.73	-1.43	39.45
	R4	62.69	21.23	-1.57	42.61
	R5	64.54	18.92	0.49	39.85
	AVG	63.54	21.52	-1.43	42.10
SPS 2%	R1	61.21	22.02	-3.42	44.39
	R2	61.69	21.09	-3.11	43.49
	R3	59.73	22.32	-3.52	45.83
	R4				
	R5	60.47	22.49	-2.86	45.23
	AVG	60.78	21.98	-3.23	44.74

Film Type		L	a	b	E
SPS/SPP	R1	68.84	9.04	35.32	47.77
	R2	68.32	10.44	35.42	48.48
	R3	68.53	10.61	35.67	48.56
	R4	65.60	11.09	36.96	51.52
	R5	63.64	12.71	37.80	53.77
	AVG	66.99	10.78	36.23	50.02
SPS/SPP 1%	R1	54.24	19.54	18.19	52.67
	R2	51.51	20.63	18.96	55.70
	R3	52.12	23.97	19.10	56.56
	R4	50.66	23.26	19.32	57.58
	R5	50.25	24.28	18.06	57.93
	AVG	51.76	22.34	18.73	56.09
SPS /SPP 1.5%	R1	48.85	31.54	13.10	61.21
	R2	52.48	32.47	12.15	58.54
	R3	52.73	28.19	11.96	56.02
	R4	52.19	31.29	11.97	58.09
	R5	53.17	22.17	12.12	52.89
	AVG	51.88	29.13	12.26	57.35
SPS/SPP 2%	R1	42.22	24.50	10.54	63.30
	R2	42.97	24.40	12.19	62.88
	R3	45.51	22.31	16.03	60.70
	R4	42.98	24.25	11.91	62.76
	R5	43.06	22.23	10.50	61.67
	AVG	43.35	23.54	12.23	62.26

A-3: Standard Curve of Food Simulants



A-4: Migration of Anthocyanin in different Food Simulant

Distilled Water		Absorbance				Ant Migrated /vol of food simulant (g/ml)	Total Ant Migrated/ Mass of food simulant (mg/kg)
Film Type	Ant %	R1	R2	R3	AVG		
B	0	0.0440	0.0441	0.0440	0.0440	0.00	0
SPS	1	0.0979	0.0981	0.0978	0.0979	0.44	441.25
	1.5	0.1200	0.1165	0.1169	0.1178	0.60	604.69
	2	0.1323	0.1319	0.1315	0.1319	0.72	720.69
SPS/ SPP	0	0.0555	0.0554	0.0553	0.0554	0.09	91.32
	1	0.1212	0.1211	0.1209	0.1211	0.63	631.56
	1.5	0.1241	0.1242	0.1230	0.1238	0.65	653.78
	2	0.2054	0.2051	0.2051	0.2052	1.32	1323.74

Ethanol 50%		Absorbance				Ant Migrated /vol of food simulant (g/ml)	Total Ant Migrated/ Mass of food simulant (mg/kg)
Film Type	Ant %	R1	R2	R3	AVG		
B	0	0.0445	0.0442	0.0442	0.0443	0.00	0.00
SPS	1	0.0724	0.0719	0.0727	0.0723	0.10	111.62
	1.5	0.0843	0.0838	0.0838	0.0840	0.15	164.34
	2	0.0939	0.0939	0.0934	0.0937	0.19	208.59
SPS/ SPP	0	0.0485	0.0469	0.0467	0.0474	0.00	0.00
	1	0.0862	0.0861	0.0861	0.0861	0.16	174.15
	1.5	0.0973	0.0953	0.0960	0.0962	0.20	219.77
	2	0.1469	0.1449	0.1455	0.1458	0.41	444.37

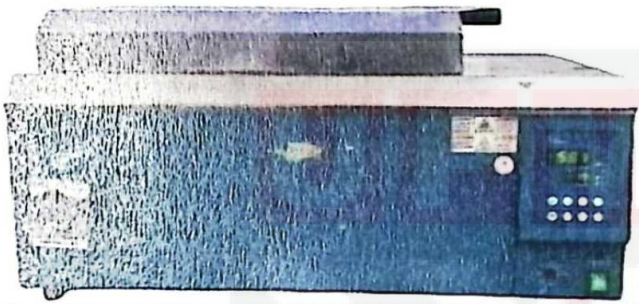
Ethanol 95%		Absorbance				Ant Migrated /vol of food simulant (g/ml)	Total Ant Migrated/ Mass of food simulant (mg/kg)
Film Type	Ant %	R1	R2	R3	AVG		
B	0	0.0433	0.0434	0.0434	0.0434	0.00	0.00
SPS	1	0.0433	0.0432	0.0433	0.0433	0.04	51.16
	1.5	0.0433	0.0432	0.0434	0.0433	0.04	52.32
	2	0.0454	0.0455	0.0455	0.0455	0.10	127.89
SPS/ SPP	0	0.0436	0.0435	0.0435	0.0435	0.05	60.46
	1	0.0430	0.0432	0.0433	0.0432	0.04	47.67
	1.5	0.0432	0.0429	0.0429	0.0430	0.03	41.86
	2	0.0443	0.0443	0.0442	0.0443	0.07	86.04

3% Acetic Acid		Absorbance				Ant Migrated /vol of food simulant (g/ml)	Total Ant Migrated/ Mass of food simulant (mg/kg)
Film Type	Ant %	R1	R2	R3	AVG		
B	0	0.0476	0.0477	0.0477	0.0477	0.00	0.00
SPS	1	0.2472	0.2468	0.2467	0.2469	0.64	610.55
	1.5	0.3157	0.3164	0.3159	0.3160	0.87	828.25
	2	0.3518	0.3518	0.3518	0.3518	0.99	941.03
SPS/ SPP	0	0.0550	0.0545	0.0544	0.0546	0.01	4.83
	1	0.2741	0.2734	0.2735	0.2737	0.73	694.88
	1.5	0.2853	0.2847	0.2850	0.2850	0.77	730.58
	2	0.4032	0.4047	0.4038	0.4039	1.16	1105.17

A-5: Equipment used for films preparation and testing.

Equipment	Details
	Digimatic Micrometer, Mitutoyo, Japan
	Portable Colorimeter, FRU Precise Colour Reader WR-18, China
	AS200, Retsch, 153 Dusseldorf, Germany
	Magnetic stir (04009951780017, DAIHAN Scientific Co, Korea)

	Haunter Lab colorimeter (USP 1431 UltraScan Pro, USA)
	Cutter Press (GT-7016-A, GOTECH, Taiwan)
	S-450D Branson Digital Sonifier, India
	UV – VIS spectrophotometer (SQ2810, USA).

	Centrifuge (007930-10, Andreas Hettich GmbH & Co, Germany)
	Shaking Water Bath