UNIVERSITI PUTRA MALAYSIA

STUDY ON THE RELEASE OF ANTHOCYANIN INCORPORATION IN SAGO STARCH SMART FILM

NURSYABII AFINI MOHD SHAIDI

**Ip
FSPM 2019 19**

STUDY ON THE RELEASE OF ANTHOCYANIN INCORPORATION IN SAGO STARCH SMART FILM

By

NURSYABII AFINI BINTI MOHD SHAIDI

A Project Report Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor of Bioindustrial Science in the Faculty of Agriculture and Food Sciences University Putra Malaysia Bintulu Sarawak Campus

2019

© COPYRIGHT UPM

ABSTRACT

Smart packaging is a promising form of food packaging that offers a great economical potential due to consumer demand for a packaging that accommodate a hectic way of life. A packaging with pH colour indicator can be made by incorporating colour indicator into food package matrices whilst applying a bio switch concept to respond automatically to changes (external stimuli) in the environment. A new pH indicator film was developed by using sago starch and the natural colorants extracted from red cabbage known as anthocyanin with antioxidant properties for food active packaging. The present work aimed to develop the formulation of sago starch film in which the pH colour indicator (range of percentage) were incorporated into the polymeric material. The sago starch film with anthocyanin showed colour variations to different pH values. Scanning Electron Microscopy (SEM) analysis showed the compatibility of the starch with anthocyanin extract and the morphology of the film. Fourier Transform Infrared Spectroscopy Analysis (FTIR) shows no chemical changes compared to control film. Colour variations of pH indicator films were measured by a colour meter after immersion in different buffer solution. Film showed a decreasing trend in lightness when there is incorporation of anthocyanin. Therefore, the developed pH indicator films could be made used as a diagnostic tool for the detection of food spoilage.

© COPYRIGHT UPM

ABSTRAK

Pembungkusan pintar adalah satu bentuk pembungkusan makanan yang menjanjikan dan menawarkan potensi ekonomi yang besar kerana permintaan pengguna terhadap pembungkusan untuk menampung jalan hidup yang sibuk. Pembungkus dengan penunjuk warna pH boleh dibuat dengan memasukkan petunjuk warna ke dalam matriks pakej makanan untuk bertindak secara automatik kepada perubahan (rangsangan luar) dalam persekitaran. Kajian mengenai pembungkusan dengan penunjuk pH telah dikembangkan dengan menggunakan kanji sagu dan pewarna semula jadi yang diekstrak dari kubis merah yang dikenali sebagai antosianin dengan sifat antioksidan untuk pembungkusan aktif makanan. Kerja-kerja ini bertujuan untuk meluaskan lagi kajian tentang pembungkusan yang diperbuat daripada kanji sagu di mana penunjuk warna pH (julat peratusan) dimasukkan ke dalam bahan polimer. Pembungkus kanji sagu dengan antosianin menunjukkan variasi warna kepada nilai pH yang berbeza. Analisis Spektroskopi Imbasan Elektron SEM) menunjukkan keserasian kanji dengan ekstrak antosianin dan morfologi pembungkus. Analisis Spektrosokopi Inframerah Transformasi Fourier (FTIR) tidak menunjukkan perubahan kimia apabila dibandingkan dengan pembungkus kawalan. Variasi warna pembungkus penunjuk pH diukur dengan meter warna selepas rendaman dalam larutan penimbal yang berbeza. Pembungkus menunjukkan satu corak penurunan dalam bentuk kecerahan apabila terdapat penambahan antosianin. Oleh itu, pembungkus penunjuk pH yang maju boleh digunakan sebagai alat diagnostik untuk mengesan kerosakan makanan.

© COPYRIGHT UPM

ACKNOWLEDGEMENTS

I would like to show my indebtedness to Allah S.W.T because with His blessing I would be able to complete my Final Year Project at the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

A special appreciation and million thanks to my supervisor, Dr. Nozieana Binti Khairuddin for all her supports, guidance, brilliant ideas, cooperation, patience and time spent for this project and report writing.

My deepest gratefulness also goes to the laboratory assistant, Puan Siti Fatimah Razali for her guidance, assistance and suggestion throughout my project to be done. To all my lecturers, my friends especially Nasuuha Binti Hashim who always give encouragement, support, advice and help at all aspects during I am conducting this project.

Last but not least, I would like to thank to my family members especially my parents and my siblings for their supports, prayers and countless love that helped me to complete this project.

Thank you.

Wassalam.

© COPYRIGHT UPM

APPROVAL SHEET

I certify that this project report entitled of "The Study on the Release of Anthocyanin Incorporation in Sago Starch Smart Film" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

____

Dr. Nozieana Binti Khairuddin
Supervisor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus

____

Assoc. Prof. Dr. Shahrul Razid Sarbini
Dean
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus

Date:

© COPYRIGHT UPM

TABLE OF CONTENT

© COPYRIGHT UPM

© COPYRIGHT UPM

LIST OF TABLES

© COPYRIGHT UPM

LIST OF FIGURES

© COPYRIGHT UPM

LIST OF ABBREVIATION

FIB	Focused Ion Beam
FTIR	Fourier Transform Infrared Spectroscopy
MC	Moisture Content
SEM	Scanning Electron Microscopy

© COPYRIGHT UPM

CHAPTER 1

INTRODUCTION

1.1 Background

Natural colorants of anthocyanin are widely used in the food industry in order to replace synthetic dyes. There are so many safety issues arise regarding on the usage of synthetic colorants as food additives. Depending on their molecular structure and pH value, the anthocyanin are characterize by a wide spectrum of colour tone ranging from orange, through red, to purple and blue. Degenhardt *et al.* (2000), Giusti and Wrostland (2003) found that the interest in anthocyanin not only limit from their colouring effect but also from their beneficial properties, including anti-oxidizing activity, improvement in the tightness of capillary blood vessels and prevention of thrombocyte aggregation, all of which reduce the risk of circulatory disease.

Chemically speaking, anthocyanin are glycosides of one of several forms of anthocyanidin (aglycone), which differ from one another in the position of substitution of hydroxyl and methoxy-groups in the *b*-ring of the flavylium cation. In plant products, anthocyanin occur in the form of mono-, di- and triglycosides. Anthocyanin glycoside residues are frequently acylated with phenolic acids. Both glycosidation and acylation of glycoside residues increase anthocyanin stability. The principal aglycone of red cabbage is cyanidin, which occurs as cyanidin 3-sphoroside-5-glucoside, acylated with sinapic, ferulic, malonic and *p*-coumaric acids.

© COPYRIGHT UPM

Besides that, the concern on the usage of biodegradable plastic has increased due to elevation fossil fuel prices and at the same time awareness of the negative impact from plastic waste also increase. This caused increased in interest to develop a study in order to produce edible and biodegradable film or the materials that have potential to extend the shelf life and the quality of the food (Jongjareonrak *et al.* 2006). According to Sothornvit and Pitak (2007) this alternative way on edible and biodegradable film usage can replace synthetic polymers which can benefits both manufacturers and consumers. This type of film can reduced the amount of non-renewable conventional synthetic polymer packaging materials and can used ingredients of agricultural derived products. Cao *et al.* (2007) claimed that the combinations of the components such as polysaccharides, lipids and protein can be used to make edible films. There are several studies on the usage of sorbitol and glycerol as plastcizers in forming starch or gelatin based.

© COPYRIGHT UPM

1.2 Problem Statement

Anthocyanin have natural dyes which have raised a growing interest due to their wide range of colour. The presence of anthocyanin normally related to attractive and colourful fruits such as grapes, strawberries, raspberries, pomegranates mangoes, figs, red cabbage and sweet potato. For this study, anthocyanin from red cabbage (*Brassica oleracea*) is extracted as an indicator in film packaging. Anthocyanin extracts from red cabbage have higher stability compared to the other source of fruits and vegetables.

Besides, starch-based film production has been reported in several studies, but somehow there is no studies reported on the sago starch film with the incorporation of anthocyanin from red cabbage extraction as natural dyes to assure food safety and quality. Apart from that, the sago starch film as a smart film with pH indicator is not only cheap and environmental friendly but also can reduce tons of waste.

1.3 Objectives

The objectives for this study are :

1. To determine the optimum formulation of smart film of sago starch film/anthocyanin.
2. To study the colour changes of the film incorporated with anthocyanin from red cabbage towards pH changes.
3. Release of the anthocyanin.

© COPYRIGHT UPM

CHAPTER 2

LITERATURE REVIEWS

2.1 Anthocyanin

Anthocyanin in Greek (*anthos* = flower and *kianos* = blue) are abundant in most of the vascular plants. As a natural water soluble pigments, anthocyanin are harmless and easy to incorporate in aqueous media. Anthocyanin are bio active compound with water-soluble vacuolar pigments that are present in many fruits, vegetables and their products. They may appear red, purple or blue depending on their pH. They occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. By adding sugars, anthocyanin are derived from anthocyanidin. In flowers with anthocyanin pigment accumulation, the coloration that is provided by anthocyanin may attract a wide variety of animal pollinators, while in fruits, the same coloration will be a major factor in seed dispersal especially by attracting herbivorous animal to the potentially-edible fruits.

Besides of their colour attribute, Liu *et al.* (2004) reported that anthocyanin are very beneficial to health as potent antioxidant and to improve visual acuity. According to Hou (2003) anthocyanin have range from inhibition of DNA damage in cancer cells in vitro because they have range of biological activities that may produce in term of health benefits. Next, Konczak and Zhang (2004) claims that a significant property of anthocyanin is their antioxidant activity, which plays an important role in the prevention of neuronal and cardiovascular illness, cancer, diabetes among others. Lule and Xia (2005) writes that there are several studies focusing on the effect of anthocyanin in cancer treatments, on human nutrition (Stinzing and Carle 2004) and its biological activity (Kong *et al.* 2003). Furthermore, the interest in anthocyanin

© COPYRIGHT UPM

2 0 JUL 2023
PERPUSTAKAAN
UPM KAMPUS BINTULU

derives not only from their beneficial properties including improving in the tightness of capillary blood vessels, and prevention of thrombocyte aggregation, all of which reduce the risk of circulatory disease (Degenhardt *et al.* 2000, Giusti and Wrolstad 2003, Tsai *et al.* 2002).

Basically, anthocyanin are widely used in food industry as an alternative way to replace synthetic colorants. Anthocyanin have high potential in the food industry as it is safe and effective food colorants. According to Giusti and Wrolstad (2003) world are now concern on the safety of synthetic pigments, leading to a reduction in the number of permitted food colorants. This situation lead to further research in natural colorants increasing, mainly because of the apparent lack of toxicity and eco-friendliness. pH sensitive dyes like bromophenol blue and chlorophenol red (Dong *et al.* 2008) can be utilized to develop pH indicators, although for food applications, the usage of synthetic chemicals compounds is avoided due to their potential that have harmful effects on human beings (Zhang *et al.* 2014). In this study anthocyanin are very important as a visual pH indicators in one type of intelligent food packaging system due to several factors which are small in size, have great sensitivity and low costs (Gupta and Sharma 1998). Zhang *et al.* (2014) stated that visual pH indicators consists of a pH sensitive dye and a solid. Anthocyanin may be used as pH indicators because their color changes with pH. They can be red or pink in acidic solutions ($pH < 7$), purple in neutral solutions ($pH \sim 7$), and ranges from blue to green to yellow in alkaline solutions ($pH > 7$), and colorless in very alkaline solutions, where the pigment is completely reduced.

© COPYRIGHT UPM

2.2 Red Cabbage *(Brassica oleracea)*

Brassica oleracea are categorized in Brassicaceae family which is a kind of cabbage also known as red or purple cabbage. Their leaves are in dark red or dark purple coloured. Somehow, the pH value of the soil will affect the colour of the plants due to a pigment known as anthocyanin. The leaves will grow more reddish in acidic soils while in neutral soils they will grow more purple and produce rather greenish-yellow coloured cabbages in alkaline soil. Furthermore, red cabbage juice can be extracted to be homemade pH indicator as their turning red in acid, green or yellow when in basic solutions. According to Chigurupati *et al.* (2002) there is an investigations to study if it is possible to use anthocyanin as an indicator of changes in the pH value in pharmaceutical preparations. This shows that anthocyanin usage not only limit in food industry but can benefits medical sector as well. Dyrby *et al.* (2001) writes that unlike the majority of the anthocyanin extracted from berry fruits, the colorant obtained from red cabbage can be used to colour food articles over a wide pH range, not only in acidic but also neutral ones. According to Dorota and Janusz (2007) stated that anthocyanin are currently used in beverages, candies, dry mixed concentrates, chewing gum, yogurt and sauces as they have potential to provide natural alternative to synthetic colorants. Therefore, anthocyanin is an alternative way to replace synthetic blue dyes. Anthocyanin from red cabbage (*Brassica oleracea*) are one of the sources that responsible for coloration of food since it is unique, display colour over a very broad pH range. In addition to that, the major problems of anthocyanin extracted from many natural sources suggested is that their instability during processing and storage (Monica and Ronald 1996).

© COPYRIGHT UPM

2.3 Sago Starch

The usage of plastic approximately growth 5 % annually and it shows an increasing dramatically in the world. Due to low cost, have good mechanical properties in terms of tensile strength and elongation, people more prefer on some synthetic packaging material such as polyethylene terphthalate (PET) and polvinylchloride (PVC) and others. Somehow, their usage should be restricted due to non-biodegradability even they have good properties plastic material. In a way, the usage of synthetic plastic can pose to environmental problems. There is intensive studies on the biodegradable polymers such as starch. In recent years, many researchers keep focus to develop biodegradable polymers as an alternative to replace synthetic polymer-based packaging materials. In a way, this is so called green packaging materials or bioplastic. This packaging film have potential with good physical, mechanical and barrier properties. Besides, certain food like fresh foods is more suitable to use biodegradable polymers. This film packaging made up of starch can be related to it being renewable, abundant and low cost. Most food contamination exist on the food surface, therefore packaging has an important role to control food contamination, including protection against food borne pathogenic diseases. The film can be as primary packaging as the first layer of plastic that easily can detect contamination when there is food spoilage. Biologically active compounds such as antioxidants, vitamins, antimicrobial agents, and flavoring agents are incorporated into the biodegradable packaging to give an active packaging system. Among the starch materials, sago starch is relatively unknown. It is obtained from an uncommon source *(Metroxylon sagu* palmtree) in Southeast Asia at a very low cost compared with common starches.

© COPYRIGHT UPM

2.4 Smart Packaging

Food packaging traditionally include 4 categories: protection, containment, communication and convenience but somehow this characteristic do not achieved society's quality standards. Apart from here a new concepts of smart packaging have been developed as world now are very concern on safety and quality of food products. Pereira *et al.* (2015) defined that smart packaging is a food packaging system that can monitor and inform food conditions to consumers in real time. Smart packaging refers to the incorporation of additives into the package with the aim of maintaining or extending the product quality and shelf life. In order to develop this smart packaging concepts, pH changes plays an important role to inform the spoilage of the food (Silva-Pereira *et al.* 2015). There are so many studies toward the development of visual pH indicator especially by using natural sensing dyes from fruits or vegetables. In this study, anthocyanin is used as natural colorants to be as pH indicator in sago starch smart film. This natural pigments is used because it is more safer and more eco-friendly to consumer. The anthocyanin can be as incorporated in the film as primary packaging (the first layer of the plastic) which is contained in a secondary or tertiary packaging. Taking into account as a smart film, the pH indicator of the anthocyanin will correspond to any changes if the food under spoilage process. This way can contribute to reduce the number of waste and bring less toxicity to the environment. According to European Bioplastic (2013) global production capacities of bioplastic were 1492 million tons in 2012, 1622 million tons in 2013 and is predicted to be 6731 million tons in 2018. This bioplastic sago starch smart film is edible, biodegradable, easy to degrade in soil, less toxicity and eco-friendly to the environment.

© COPYRIGHT UPM

CHAPTER 3

MATERIALS AND METHOD

3.1 Sample Collection

The performed experiments required the extraction of red cabbage *(Brassica oleracea)* as pH indicator. The red cabbage was purchased from local market.

3.2 Buffer Solution Preparation

In this study, it can be divided into three parts which were extraction of anthocyanin from crude red cabbage, preparation of buffer solutions for various pH and colour measurement for juice anthocyanin for acidic, neutral and basic conditions. Buffer solutions for 13 different pH values were purchased. The accurate pH for each buffer solutions was measured with a portable pH meter. The spectral behaviour of anthocyanin is dependent on pH and substances present in the solutions may influences the colour.

3.3 Anthocyanin Extraction

Red cabbage leaves were shredded and soaked in 20% ethanol for 24 hours. Then it was boiled for 5 - 10 minutes until purple colour appeared.

3.4 Film Formation

Sago starch-based film were prepared by casting onto the petri dish. The film were incorporated with red cabbage extraction. The film were prepared with six different concentration. All components to make the film include sago starch, distilled water and glycerol as plasticizer were mixed and heated for 20 minutes at ambient temperature with controlled stirring. The mixed was heated to ensure starch

© COPYRIGHT UPM

1900132740

gelatinization is formed. The mixed solution were casted onto the petri dish which were dried at room temperature. The thickness of the resultant film was ~ 1 mm.

3.5 Scanning Electron Microscopy (SEM)

Surface and cross sectional morphology of the film were examined using scanning electron microscopy with a Focused Ion Beam (FIB) Zeiss Crossbeam 340 in order to investigate the morphology of the casted film.

3.6 Moisture Content

Moisture content (MC) with different films concentration was determined using the standard method of the International Association of Official Analytical Chemistry (AOAC 1995). The films were dried in an oven at 100℃ for 24 hours. Water content was calculated as :

$$MC = \frac{m_f - m_i}{m_i} x100\%$$

(Equation 1)

3.7 Colour Measurement using Colour Reader Hunter L, a and b

In this study, the colour changes from the film indicators were measured through the lid with a colour meter (Colour reader CR-10, Japan) and expressed as Hunter L, a and b values. The colour parameters (l, a and b) were determined using white patterns. The total differences of colour (ΔE) was calculated to the equation

© COPYRIGHT UPM

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

Where, $\Delta L = L_{standard} - L_{sample}$; $\Delta a = a_{standard} - a_{sample}$; $\Delta b = b_{standard} - b_{sample}$

(Equation 2)

3.8 Colour Measurement of Film Incorporated with Indicators

Colour changes of the films incorporated with anthocyanin were measured with a Colour Reader CR10 (Konica Minolta CR10, Japan), and expressed as Hunter system (L, *a* and *b*) values. The prepared films were cut into circular disc in diameter of 2 cm and were place on a clean petri dish. The release of the colour were recorded in L, *a* and *b* values.

3.9 Chemical Characterization using Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to study the chemical composition and chemical bonding presence in the prepared films. The analysis was carried out using a Recorder Spectra model FTIR Nicolet MAGNA-IR 860 spectrometer; Thermo Fisher Scientific, Inc; USA) and recorded at a 4 cm^{-1} resolution with a total of 64 scans. To measured pH indicator film. Chemical composition changes of sago starch smart film were determined

© COPYRIGHT UPM

CHAPTER 4

RESULTS

4.1 Red Cabbage Extraction and Buffer Solution

The natural colorant of red cabbage were extracted and poured with buffer solution range from pH 1 to pH 13. The colour changes were observed and recorded.

Figure 4.1 50 ml of red cabbage extraction were prepared

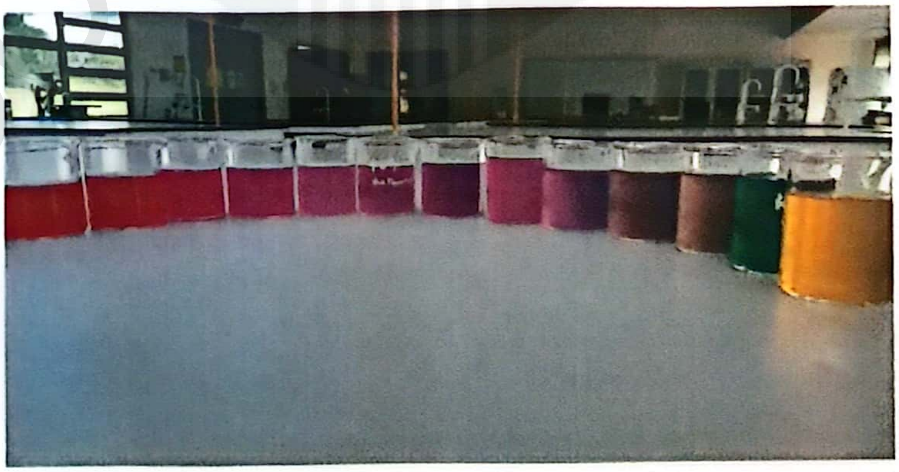
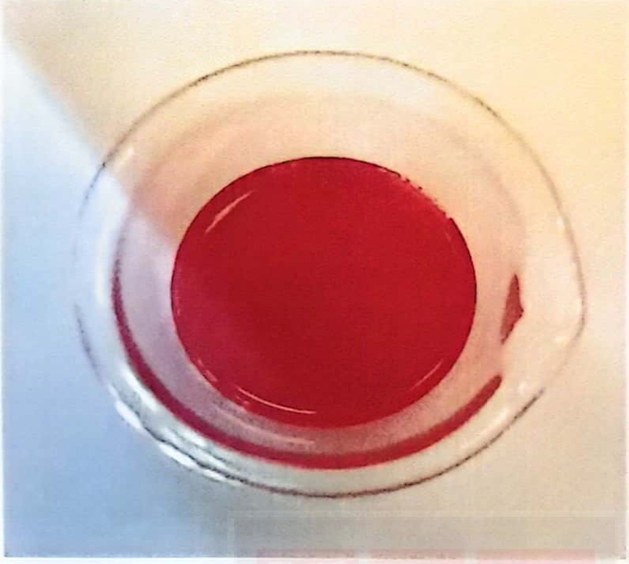
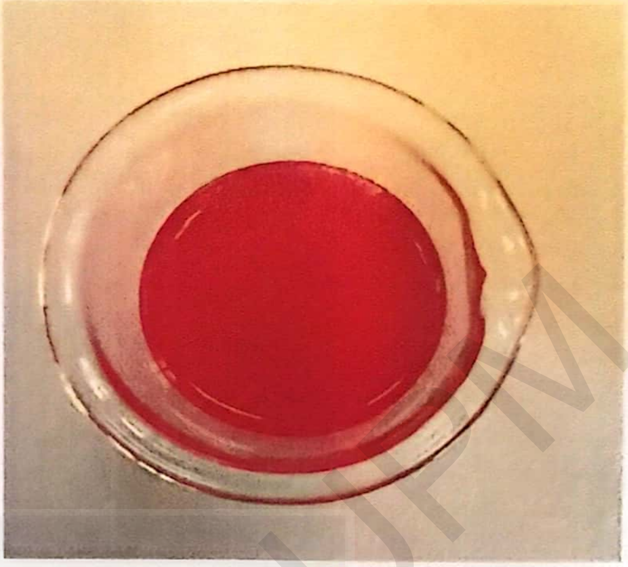


Figure 4.2 From left the solution in red is pH 1 and solution turns yellow at pH 13

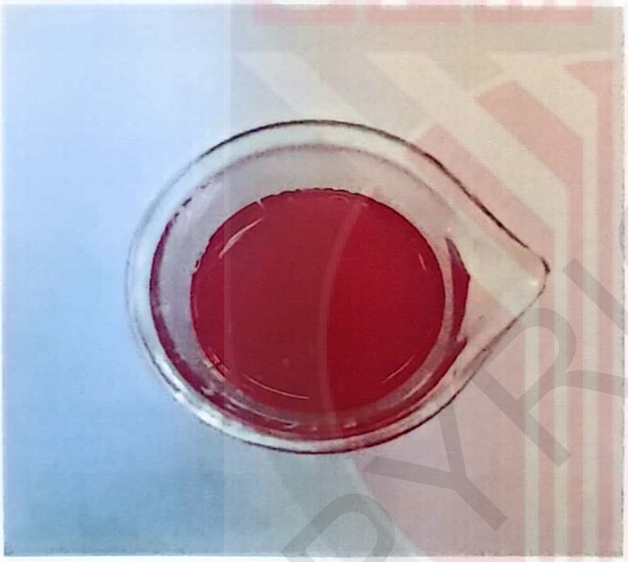
© COPYRIGHT UPM



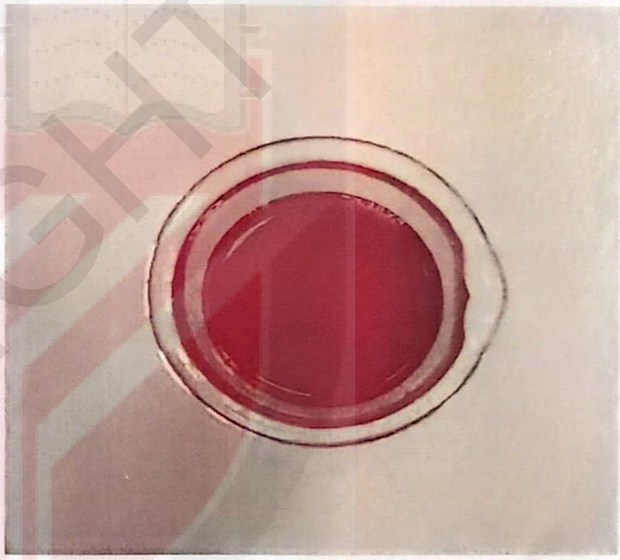
pH 1



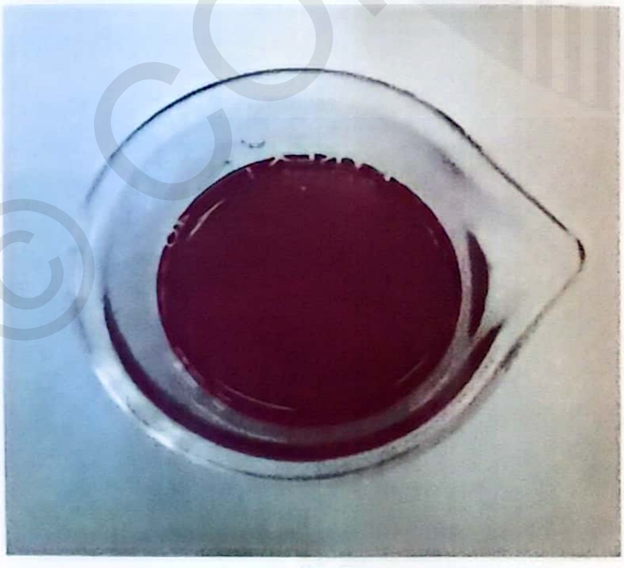
pH 2



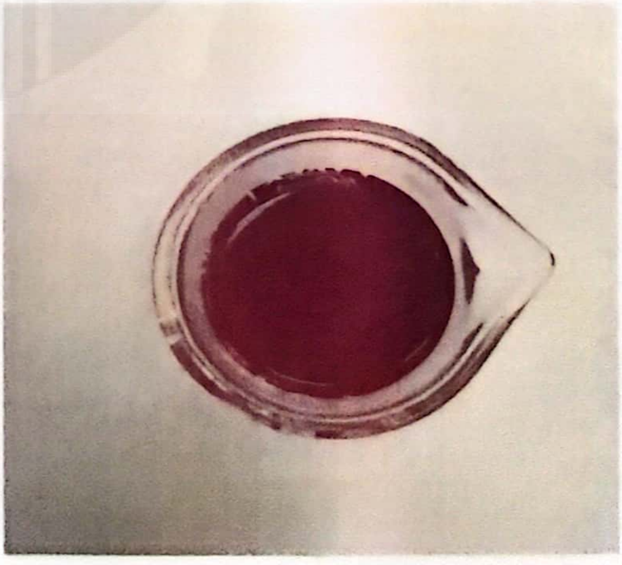
pH 3



pH 4

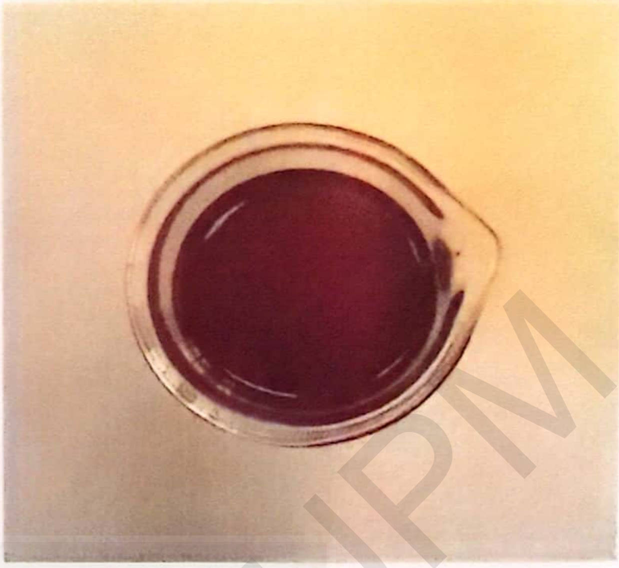


pH 5

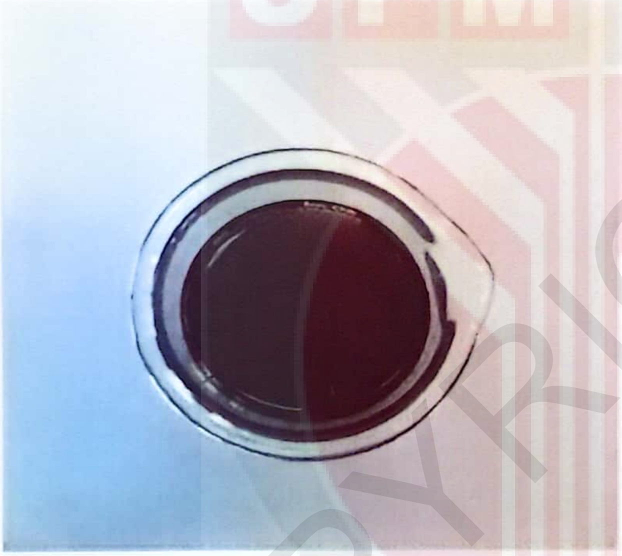


pH 6

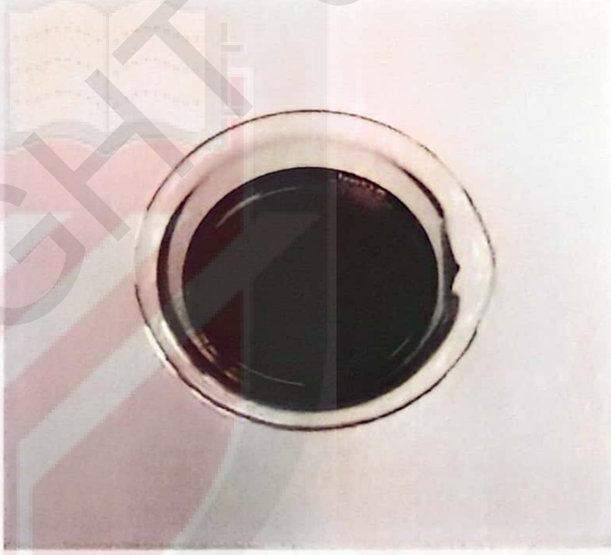
pH 7



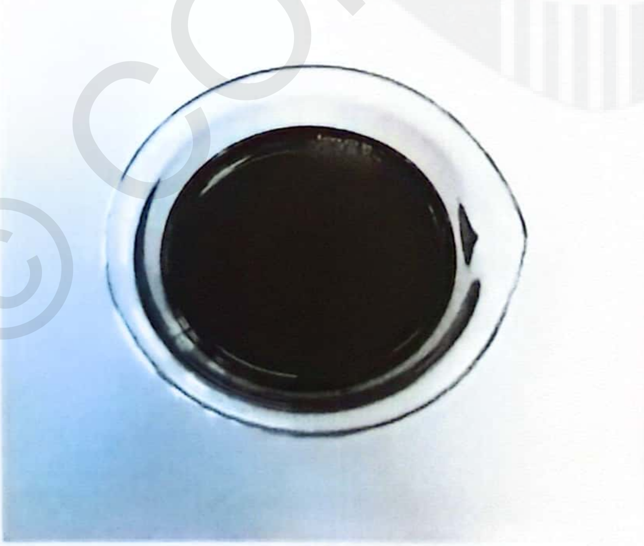
pH 8



pH 9



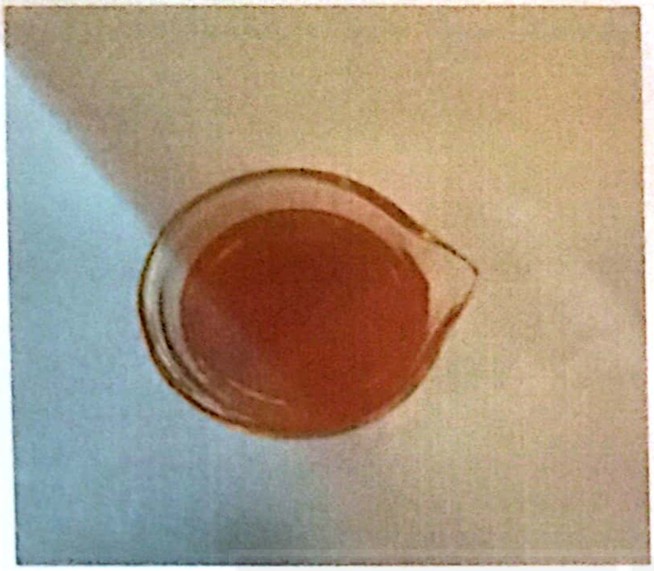
pH 10



pH 11

pH 12

© COPYRIGHT UPM



pH 13

Figure 4.3 The anthocyanin extraction change it colour in different pH solution

© COPYRIGHT UPM

4.2 Film with pH Indicator

The sago starch were prepared with the incorporation of red cabbage extraction as pH indicator. The concentration were different range from 0 g, 10 g, 20 g, 30 g, 40 g and 50 g. Thus the film were labeled as S0, S1, S2, S3, S4 and S5 respectively.

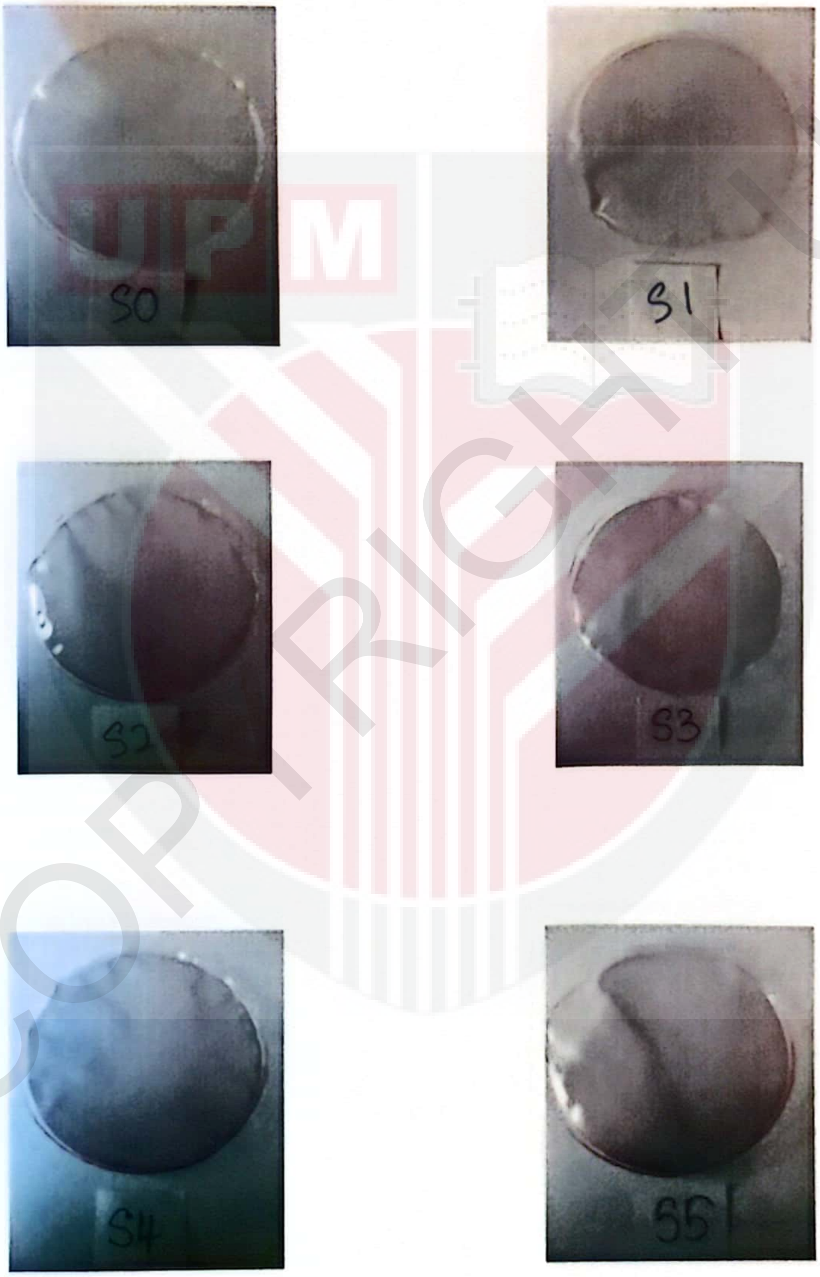


Figure 4.4 Film made of sago starch with different concentration

© COPYRIGHT UPM

4.3 Scanning Electron Microscopy (SEM)

SEM showed the compatibility of the starch with anthocyanin extract and the morphology of the film.

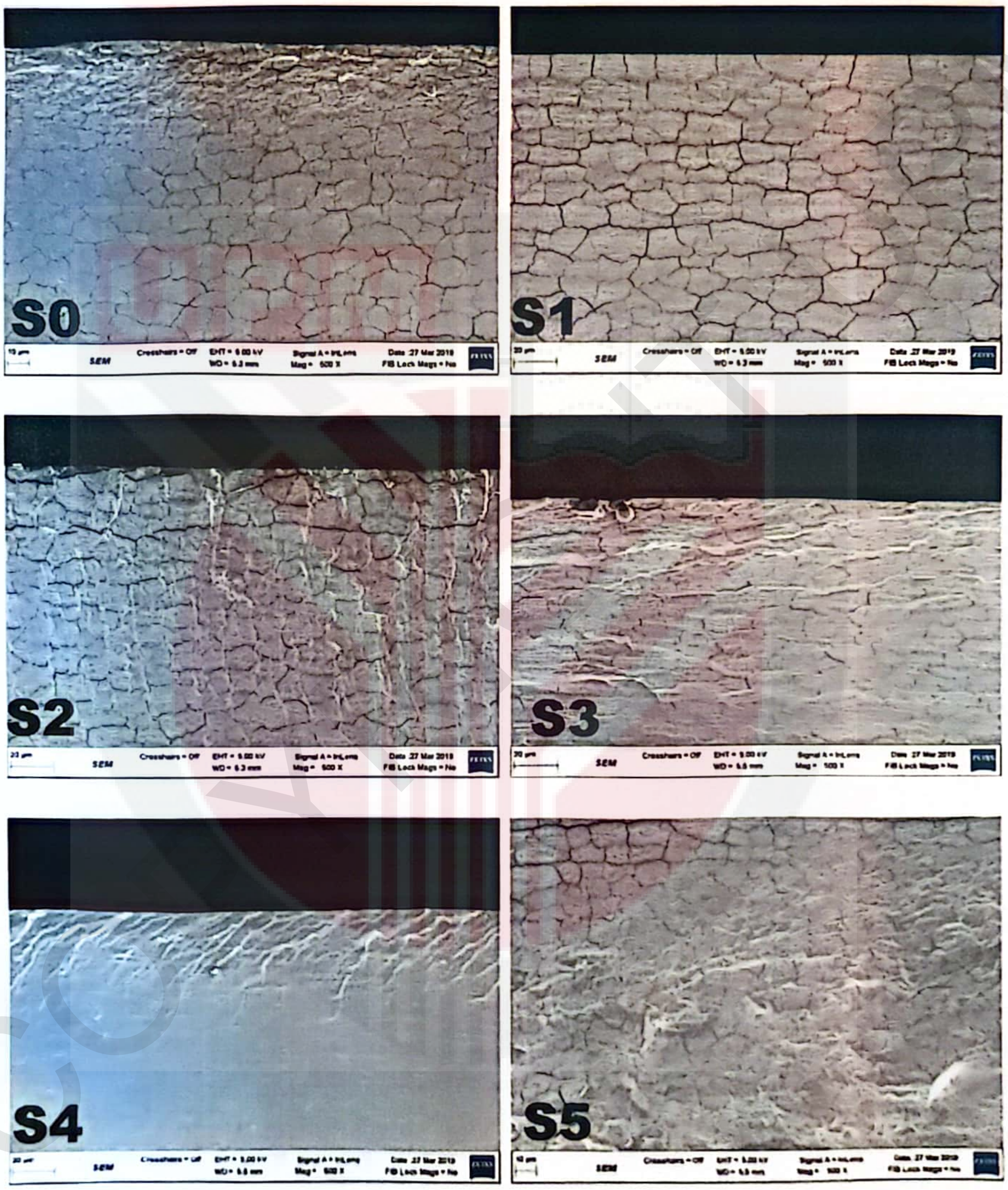


Figure 4.5 Cross sectional morphology of the film by SEM with 500x magnification

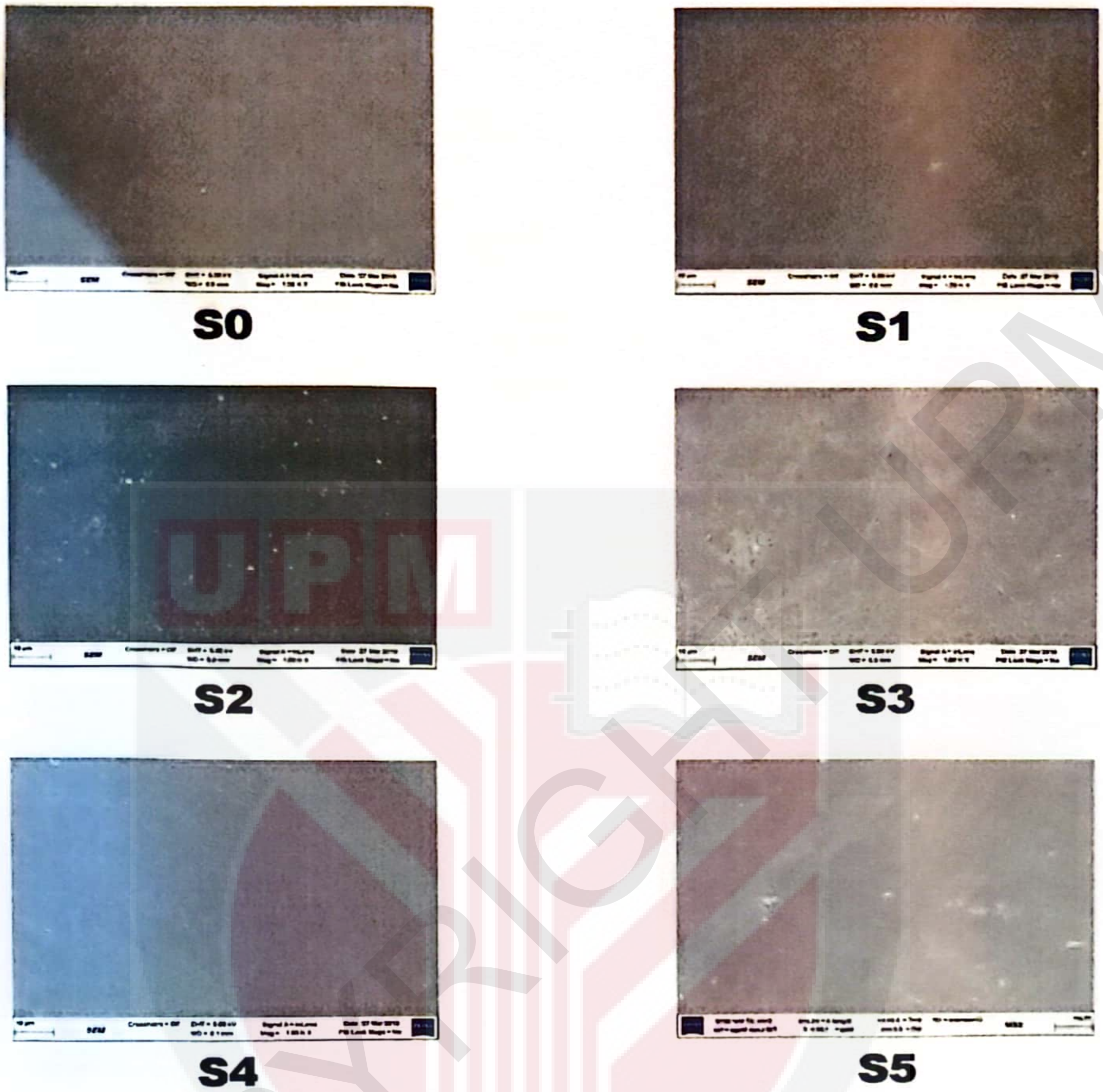


Figure 4.6 Surface morphology and composition of the sample from S0 to S5

© COPYRIGHT UPM

4.4 Moisture Content

Moisture content of the film is observed after 24 hour for oven dried. The initial and the final weight is recorded to calculate the moisture content based on the formula.

Figure 4.7 Film sample after 24 hour oven dried

© COPYRIGHT UPM

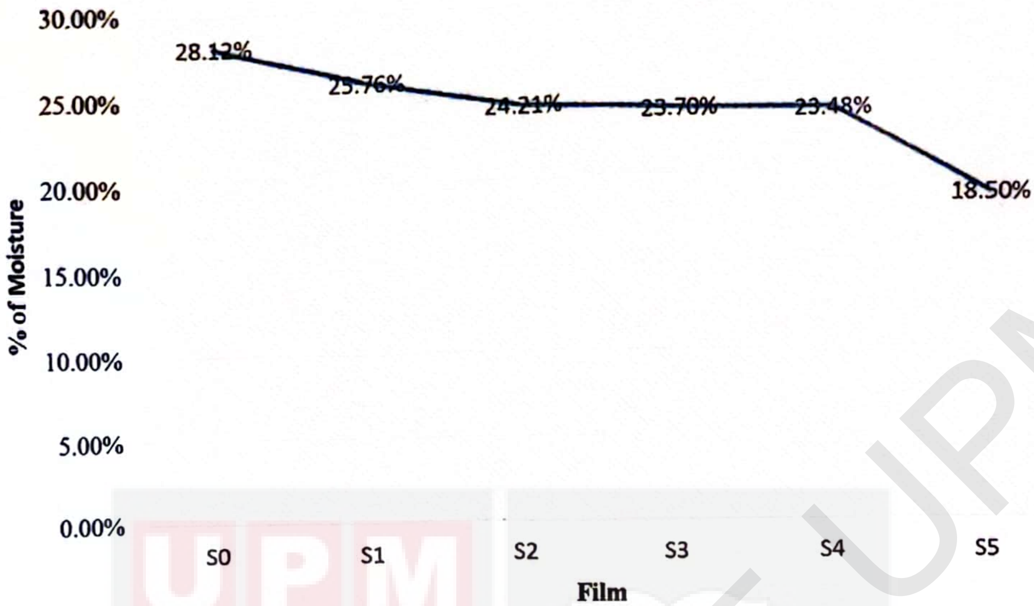
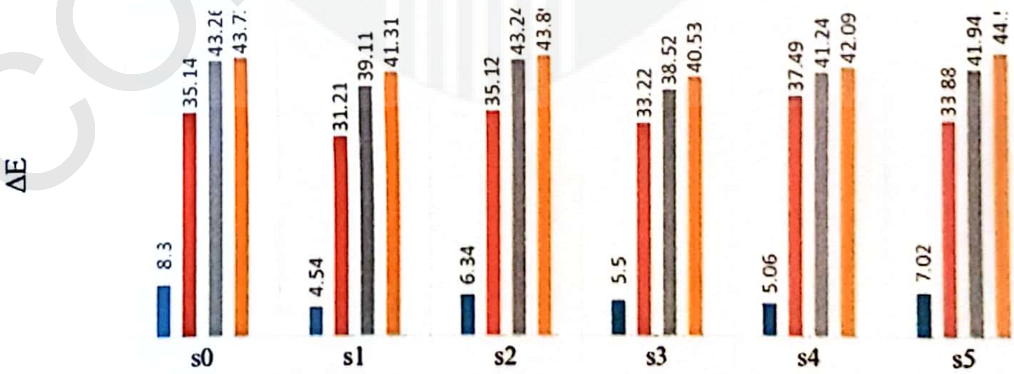


Figure 4.8 Moisture Content (MC) of the films

4.5 Colour Analysis

The pH indicator film with anthocyanin is observed on the release of the colour. The film is soaked in 15 ml of distilled water and observed for every 8 hours within 32 hours. The results were expressed in L* (lightness), a* (red to green), b* (yellow to blue). The total difference were calculated based on the equation.



	s0	s1	s2	s3	s4	s5
8h	8.3	4.54	6.34	5.5	5.06	7.02
16h	35.14	31.21	35.12	33.22	37.49	33.88
24h	43.26	39.11	43.24	38.52	41.24	41.94
32h	43.73	41.31	43.89	40.53	42.09	44.5

FILM

Figure 4.9 Release of colour in film after 32 hours

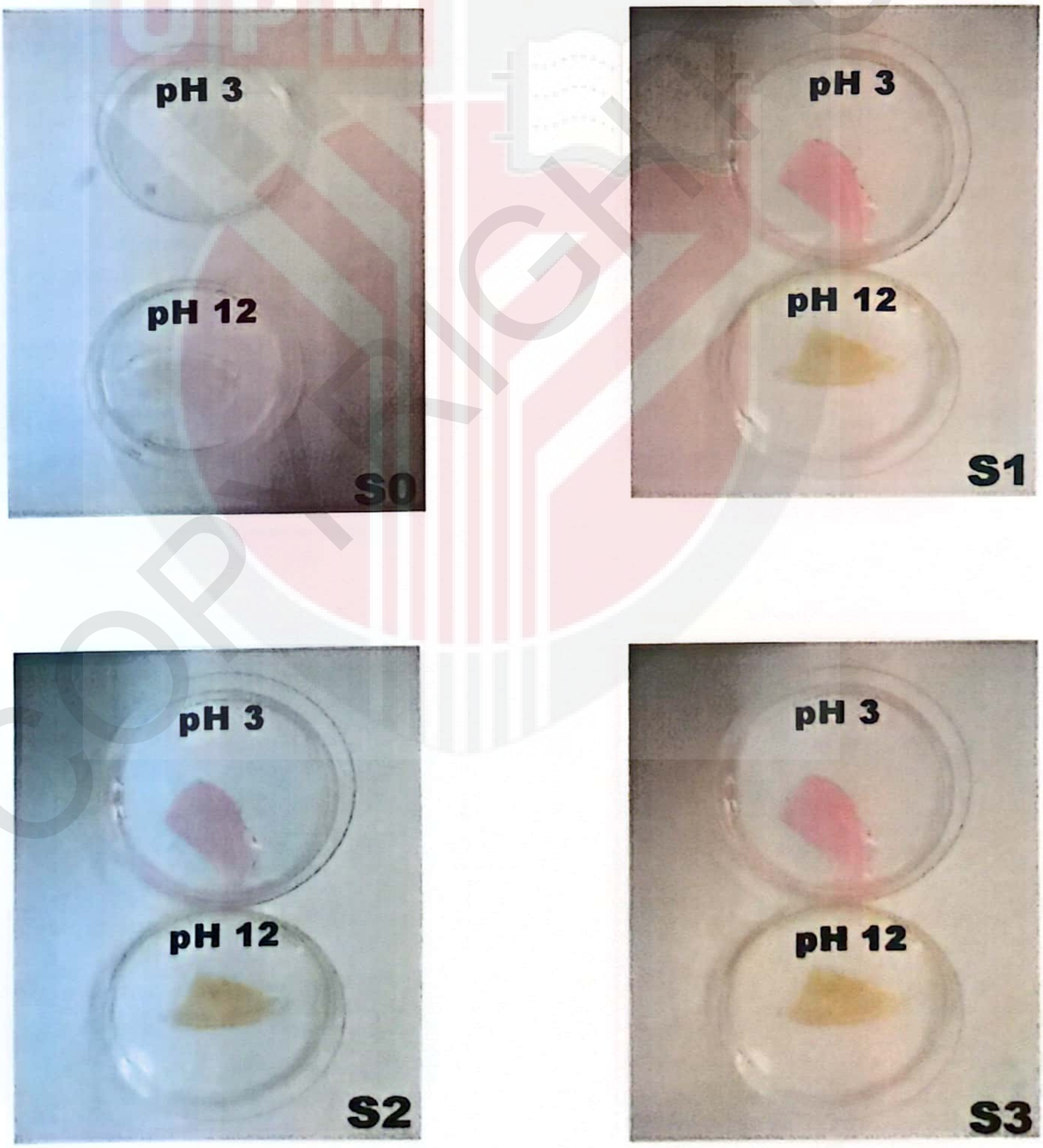
© COPYRIGHT UPM

$$\Delta \boldsymbol{E} = \sqrt{(\Delta \boldsymbol{L})^2 + (\Delta \boldsymbol{a})^2 + (\Delta \boldsymbol{b})^2}$$

Where, $\Delta L = L_{standard} - L_{sample}$; $\Delta a = a_{standard} - a_{sample}$; $\Delta b = b_{standard} - b_{sample}$

4.6 Colour Change of Film in Acidic and Alkaline Solution

The film were tested in acidic and basic medium as it could be use as intelligent packaging and correspond to the pH changes.



© COPYRIGHT UPM

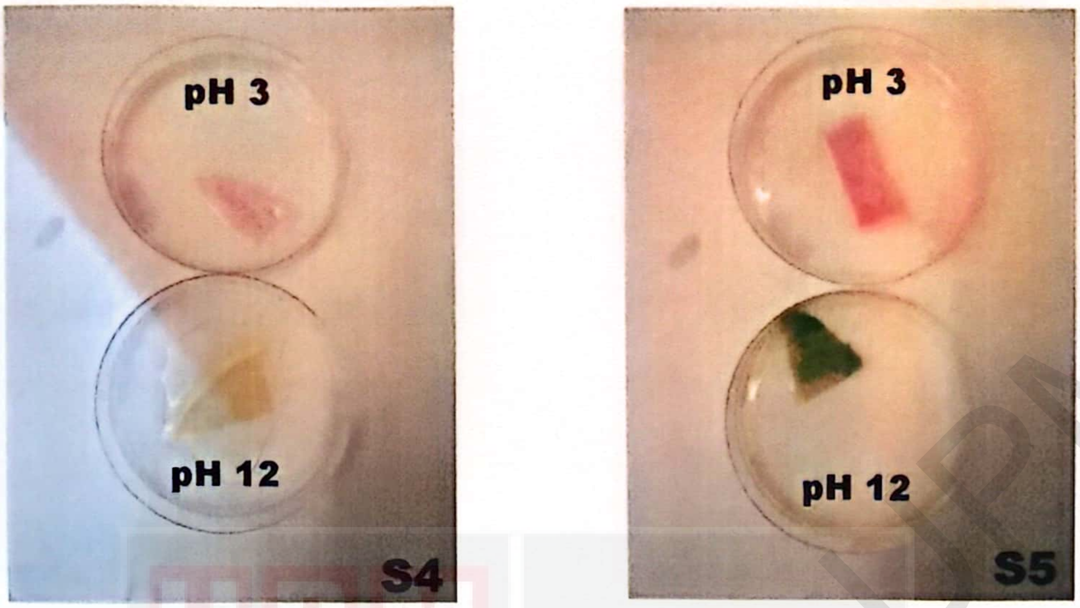


Figure 4.10 The film labeled from S0 to S5 were soaked in acidic and basic medium.

The results were expressed in colour parameters of L* (lightness), a* (red to green) and b* (yellow to blue). It shows that parameters a* shows an increasing trend as the film change into red when immersed into pH 3. Parameters b* shows as increasing trend as well as the film turns into yellow when soaked in the pH 12 buffer solutions.

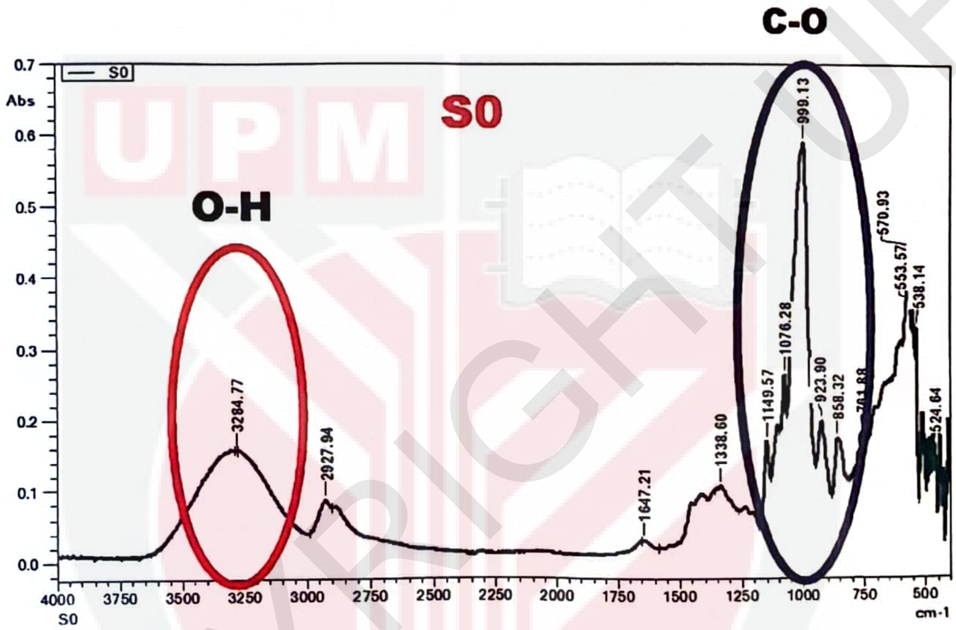
Table 1 Summary of total difference colour of the film in acidic and basic medium.

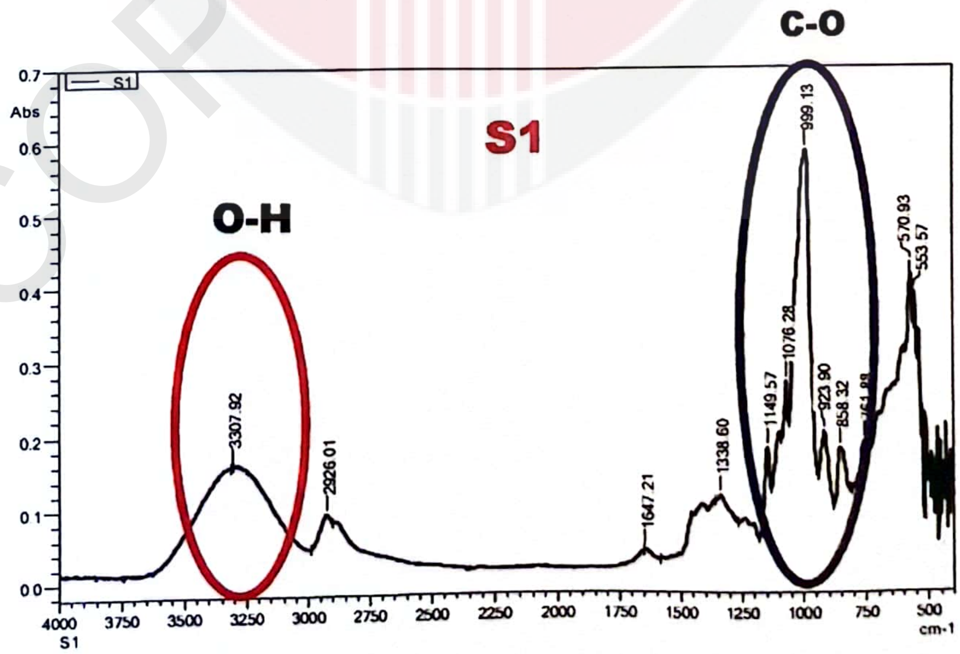
Film		**pH 3**			**pH 12**	
Parameter	**L***	**a***	**b***	**L***	**a***	**b***
Standard	92.8	-0.7	-4.6	92.8	-0.7	-4.6
S0	80.7	1.9	2.8	80.5	0.1	-3.0
S1	79.5	6.5	-3.9	75.5	-2.2	5.0
S2	79.6	14.1	-7.7	85.4	-0.2	8.1
S3	77.5	13.6	-5.9	80.2	-2.1	13.8
S4	74.6	17.5	-7.6	82.0	-1.6	17.6
S5	67.4	22.1	-7.6	65.7	-8.7	28.7

© COPYRIGHT UPM

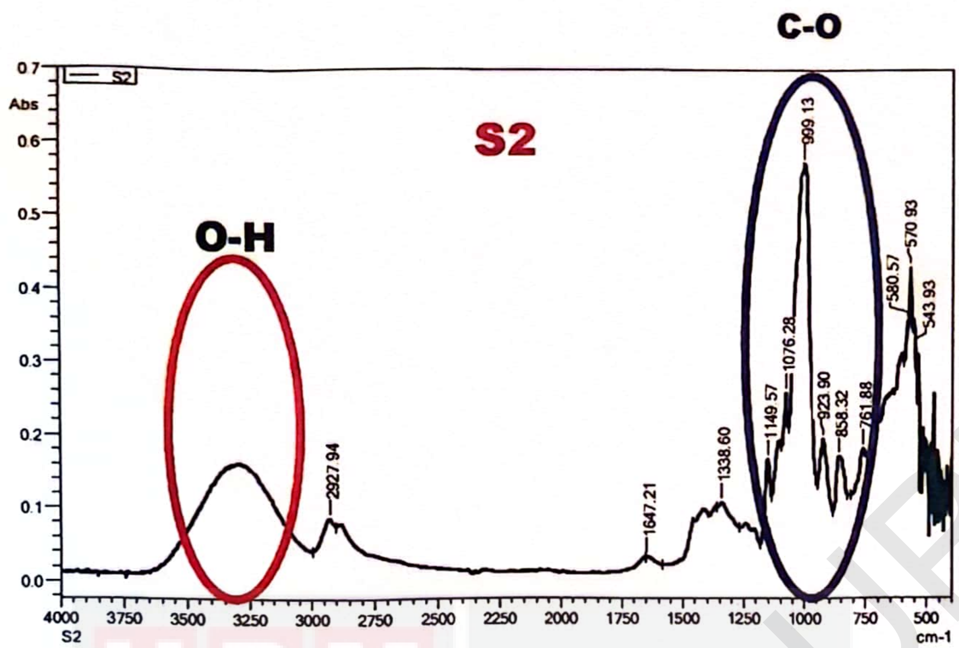
4.7 Chemical Characterization using Fourier Transform Infrared Spectroscopy (FTIR)

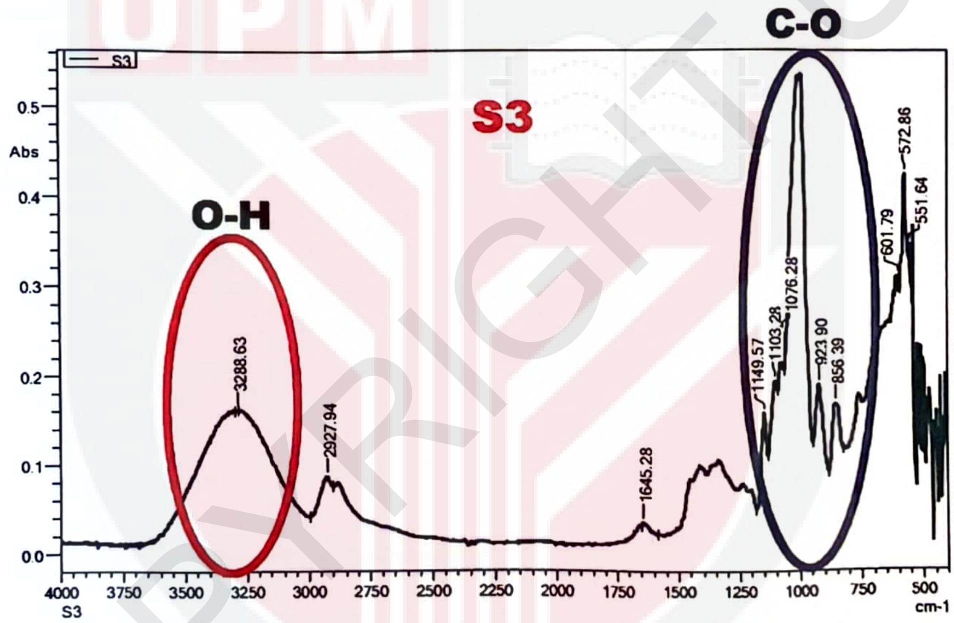
FTIR analysis was carried out to analyse the functional group in the film. Film contain O-H at 3250 cm^{-1} to 3330 cm^{-1} and C-O at 990 cm^{-1} to 1000 cm^{-1} respectively. There is no chemical changes of the film with the incorporation of anthocyanin from S1 until S5 compared to control film S0.

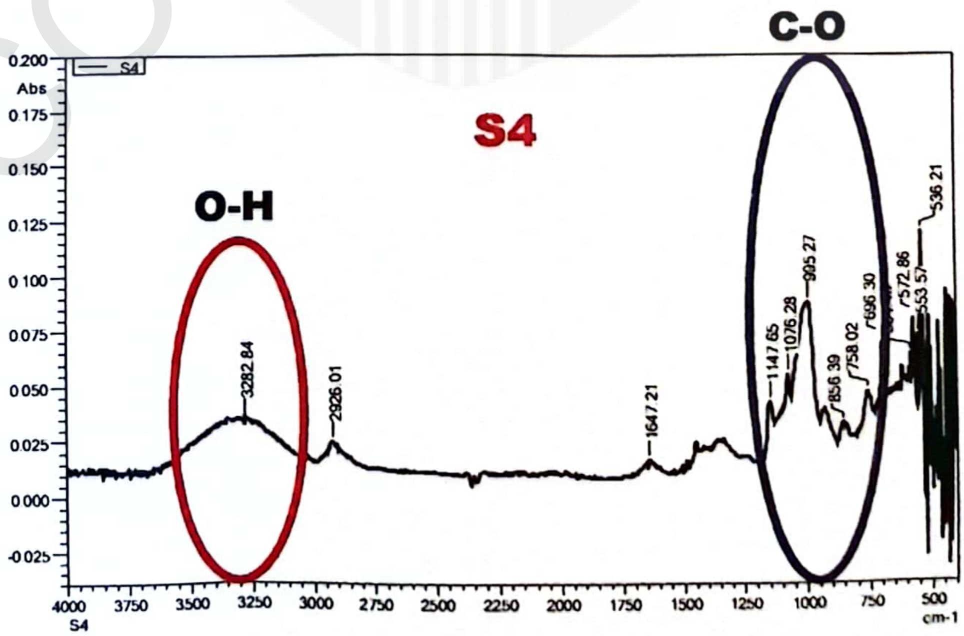




© COPYRIGHT UPM







© COPYRIGHT UPM

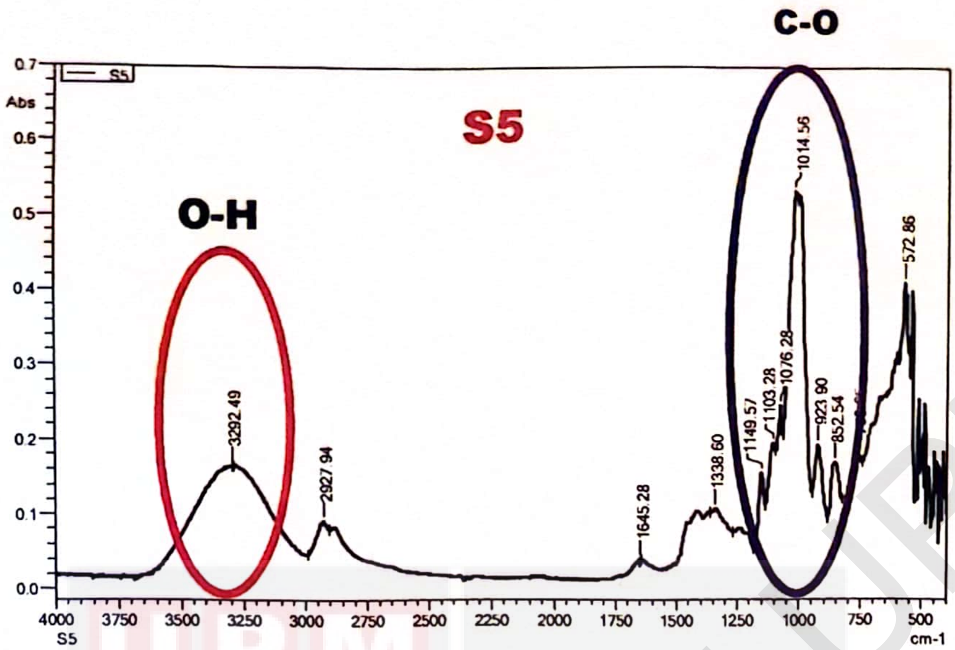


Figure 4.11 FTIR profiles of Sago Starch Film

4.8 Film Contaminated Fungus

The film was contaminated with fungus during drying session. The changes on the film into red and blue shows that the film correspond to the pH changes as the fungus can grow in slight acidic medium and basic medium. The fungus can developed multiple mechanism to adopt pH variations.

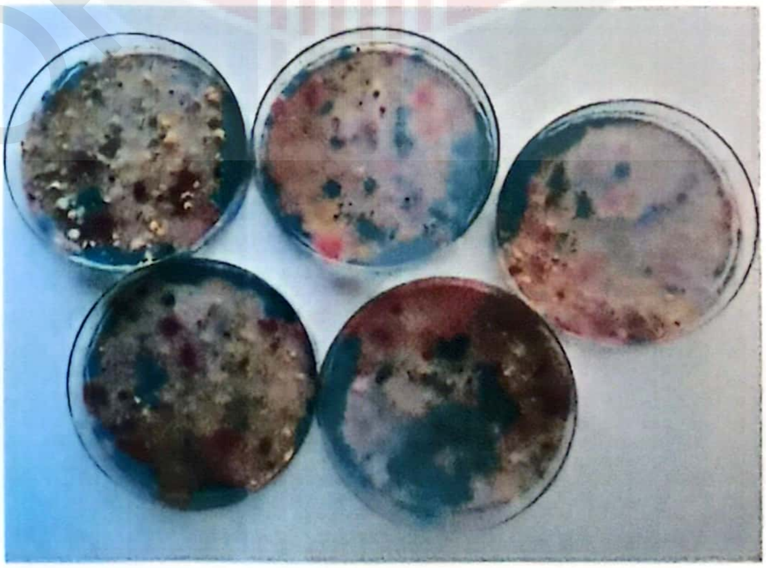


Figure 4.12 Film sample S0 to S5 were contaminated with fungus

© COPYRIGHT UPM

CHAPTER 5

DISCUSSION

5.1 Red Cabbage Extraction and Buffer Solution

The stability of the anthocyanin from the extraction of red cabbage can be affected by several factors such as pH. Based on Clydesdale (1988) and Markakis (1982) anthocyanin have pigment vary from red, purple and blue which is unstable if there is any changes in pH. From the analysis, the buffer solution starting from pH 2 to pH 13 have been poured into the 50 ml of anthocyanin extraction. Figure 4.2 shows that the extraction of anthocyanin of the red cabbage changed from red to yellow. It shows that the anthocyanin turned red at low pH (acidic condition) which lower than 4. Then, it turned into purple and pink at pH of 5, 6, 7 and 8 respectively. Next, the anthocyanin had tendency to change from purple to green and yellow at higher pH (alkaline). As the pH is higher, the pH value of anthocyanin will provide colour fading of colourless, yellow, purple and blue.

The stability and the equilibrium of the anthocyanin was influenced by the pH. From the results, pH plays an important roles for the colour of the anthocyanin. The anthocyanin can be red in acidic, violet in neutral and turn into blue in alkaline solution. This happen due to the structure of anthocyanin which known as flavylium. The cyanidin molecule is protonated and will forms positive anion or cation in low pH. When the pH increases the molecules become deprotonated where it will forms negative ion or anion. This is the reason why anthocyanin can be the pH indicator in the smart packaging film. The principal aglycone of red cabbage is cyanidin, which

© COPYRIGHT UPM

occurs as cyanidin 3-sophoroside-5-glucoside and cyanidin3,5diglucoside, acylated with sinapic, frulic, malonic and p-coumaric acids (Hrazdina *et al.* 1977).

5.2 Film with pH Indicator

Figure 4.4 shows that six different films with different concentration of anthocyanin extraction. The film was made up of sago starch. Starch is usually being used to make bioplastic. They are widely used in research due to their bio compatibility, low toxicity and fast degrade. The mixture of the film is the sago starch, distilled water and glycerol that help to give plasticizer characteristic and the incorporation of anthocyanin as the pH indicator. S0, S1, S2, S3, S4 and S5 represent 0 g, 10 g, 20 g, 30 g, 40 g, and 50 g respectively of red cabbage to extract the anthocyanin where ethanol was used as the solvent. S0 with zero concentration of anthocyanin gives a translucent white film. S1 to S5 sample film have incorporation of anthocyanin as pH indicator. The purple colour slightly apparent as it is increases from S1 to S5.

5.3 Scanning Electron Microscopy (SEM)

The film were examined on the surface and the cross-sections using SEM under the accelerating voltage of 5kV and being observed under 500 magnifications based on figure 4.5. The surface of the film also can be seen form the figure 4.6 as the sample from sample S1 to S5 do not have much different from S0. The incorporation of different concentration anthocyanin do not influenced the morphology of the film. SEM showed the compatibility of the starch with anthocyanin extract and the morphology of the film.

© COPYRIGHT UPM

5.4 Moisture Content

Moisture of the film were examined by calculate the water content using standard method of the International Association of Official Analytical Chemistry. From the Table 1, there is a decreasing trend in the film with the incorporation of anthocyanin when moisture content was evaluated. S0 with zero concentration of anthocyanin have the highest moisture content with 28.12%. On the other hand, S5 with the highest concentration of anthocyanin have the lowest moisture content which is 18.50%. This result is due to slightly higher hydrophobicity in this film. The film may be taken as hydrophobic material as as there is a trend towards greater hydrophobicity in the film containing of red cabbage extraction. According to Medina Jaramillo *et al.* (2016) the increasing of hydrophobocity in the materials that having natural extracts was expected.

5.5 Colour Analysis

The colour changes of the film indicator were examined for every 8 hours until it become constant after 32 hours. The film at 2 cm x 2 cm were soaked in 15 ml of distilled water within the 32 hours in the petri dish. Based on the results, it shows that there is an increasing trend in the colour intensity. This shows that the film with incorporation of anthocyanin which is in purple release their colour. The differences of the colour parameter in the film were measured by using a colour meter. The parameter were expressed as L* (lightness), a* (red to green) and b*(yellow to blue).

5.6 Changes of the Film in Acidic and Basic Medium

Colour changes of the film in different aqueous media were observed. The film were casted 2 cm x 2 cm diameter were placed in petri dish containing 5 ml of acidic solution (pH = 3) and alkaline solution (pH = 12). The colour of pH indicator have tendency to be red when the parameter a* values is above zero. Taking into account the film as a smart packaging, the colour changes in acidic and alkaline buffer solution were measured using colour meter. The film immediately turn into red in acidic buffer and changed into green in alkaline buffer solution. These results are relevant when considering the anthocyanin as pH indicator in the film. This is an important components where the film have the ability to generate colour of a coating that is in direct contact with food especially when they begin to deteriorate. Besides, it is also important for the film to change their colour in basic medium. Most of the pathogenic bacteria grow in foods of neutral to alkaline.

From the result obtained, the incorporation of anthocyanin in the film will influence the colour of the film. The addition of anthocyanin shows a decreasing trend in lightness parameter. In pH 3 the lightness reduced from 80.7 in S0 to 67.4 in S5. The same thing happen to pH 12. Basically, the film itself have reduced in lightness when there is addition of anthocyanin. Film sample S0 do not have any alteration in colour changes. This film act as control without any incorporation of anthocyanin. Film sample S1 to S5 immediately changed colour when immersed into the buffer solution. This results proven that the pH indicator film will change in colour in acidic or basic medium and relevant to current research to be known as smart packaging.

© COPYRIGHT UPM

5.7 Chemical Characterization using Fourier Transform Infrared Spectroscopy (FTIR)

From the FTIR profiles the addition of the anthocyanin do not influence the chemical changes in the film when compared to control film. From the profiles, there is the appearance of O-H functional group within range 3250 cm^{-1} to 3330 cm^{-1} . The C-O functional group also can be seen from 990cm^{-1} to 1000 cm^{-1} . There is not much changes in the physical and chemical characteristic of the film with the incorporation of anthocyanin.

5.8 Film Contaminated with Fungus

The film need to be dried withing two to three days before can be peeled off from the petri dish at room temperature. During drying sessions, all the film were contaminated with fungus. The reason is the film is being exposed to contaminated environment. The fungus can growth at any temperature where the best growth is in moist environment and at 25 to 30℃ which is room temperature. Somehow, in the present of oxygen also be one of the factor that encourage for the growth of the fungus. Another reason is the petri dish is not being sterile by 70 % of ethanol before the homogenized of film solution were poured. Figure 4.11 shows that the growth of fungus result in slightly changed in the colour of the film. The fungus can developed multiple mechanism to adopt pH variations. They can grow at slight acidic pH values but however at pH below 2 or above 8 the fungus cannot grow. Based on the results, the film changed into slightly pink which fall in range of pH 4 to pH 6.

© COPYRIGHT UPM

CHAPTER 6

CONCLUSION

As the conclusion, the optimum formulation of sago starch smart film with pH indicator is obtained. Film with incorporation of anthocyanin from the red cabbage have colour changes toward pH thus it can inform when the food under spoilage process. The film with the incorporation of the anthocyanin which is in purple colour also release their colour In this study, using biodegradable materials such as sago starch and red cabbage extraction as a calorimetric pH indicator film was newly developed. The smart film with the incorporation of anthocyanin were utilized as natural colorants in the packaging. The film also biodegradable that make them fast to degrade in soil and less toxicity compared to synthetic plastic that pose environmental pollution everywhere. This shows that the smart film is environmentally friendly materials. This film is non-toxic and they can generate colour at variations of pH which make the film is relevant for intelligent food packaging materials. Smart film with the colour changes which act as indicator shows the film present a simple and visual method in order to detect quality changes of food product, since the pH values of food changed under spoilage process.

This development can used the film as primary packaging (where it can be the layer that come into direct contact with the food product), which is contained in a secondary or tertiary packaging. In this case, pH effects of other materials contacted with the coated food during shipping and handling is avoided. In other words, when there is deterioration in food products, the film can immediately respond to any changes under spoilage process. Therefore, the use of films containing the

© COPYRIGHT UPM

anthocyanin from the red cabbage extraction with products that lower their pH when spoilage occur, could contribute to the consumer for a better food quality control. Thus, the proposed indicators has a great potential as a diagnostic tool to assure food safety and quality.

As recommendation to develop this study, the film can be incorporated with mango peel extract. The addition of mango peel extract can produce continuous and compact film micro-structure as it can influence the changes of mechanical behaviour. Uniformly dispersed of the mango peel extract in the film matric can strengthen the film network. Thus, it can leads to a stronger film compared to sago starch film alone. Besides, the sago starch film also can be incorporated with extraction of cellulose nanofiber film from banana stem. This way contributed to improvement in the film strength rather than when depends on sago starch alone. Therefore, further research should be explore the potency of these film as an effective active packaging for food applications.

© COPYRIGHT UPM

REFERENCES

AOAC.1995. Official methods of analysis. Washington, D; Official Methods of Analysis. Association of Official Analytical Chemists 934,5-17.

Cao, N., Fu, Y., and He, J. 2007. Preparation and physical properties of soy protein isolate and gelatin composite films. *Food Hydrocolloids*, 21(7) : 1153-1162.

Chigurupati, N., Saiki, L., Gayser, Ch., Jr., and Dash, A. K. 2002. Evaluation of red cabbage dye as a potential natural color for pharmaceutical use. *International Journal of Pharmaceutics*, 241 : 293–299

Clydesdale F M. 1998. Color: origin, stability, measurement and quality in Storage Stability. Taub, I.A. and Singh, R. P (Ed) (New York: CRC Press LCC)

Degenhardt, A., Knapp, H., and Winterhalter. 2000. Separation and purification of anthocyanins by high-speed countercurrent chromatography and screening for antioxidant activity. *Journal of Agriculture and Food Chemistry*, 48(2) : 338-343

Dong, S., Luo, M., Peng, G., and Cheng, W. 2008. Broad range pH sensor based on sol–gel entrapped indicators on fibre optic. Sensors and Actuators B-Chemical, 129(1) : 94–98.

Dorota, W.T., Janusz, C. 2007. Colour changes of a preparation from red cabbage during storage in a model system. *Food Chemistry*, 104 : 709–714.

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

European Bioplastic. Bioplastic facts and figure 2013. http://.corbion.com/media/203221/cubp.factsfigures.bioplastics.2013.pdf (Accessesd 26 January 2019)

Giusti, M M., and Wrostland, R. E. 2003. Acylated anthocyanin from edible sources and their application in food systems. *Biochemical Engineering Journal*, 14 : 217-225

Gupta, B. D., and Sharma, S. 1998. A long-range fiber optic pH sensor prepared by dye doped sol–gel immobilization technique. *Optics Communications*, 154(5–6):282–284.

Hou, D.X. 2003. Potential mechanism of cancer chemoprevention by anthocyanin. Current Molecular Medicine 3:149-159

Hrazdina, G., Itrdale, H., and Marrick, L. R..1977. Anthocyanin composition of Brassica olerecea cv. Red Danish. *Phytochemistry*, 16, 297.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., and Tanaka, M. 2006. Effects of plasticizers on the properties of edible films from skin gelatin of big eye snapper and brown stripe red snapper. *European Food Research Technology*, 222 : 229-235.

© COPYRIGHT UPM

Konczak, I., and Zhang, W. 2004. Anthocyanin-more than nature's colours. *Journal of Biomedicine and Biotechnology*, 2004 (5) : 239-240.

Kong, J. M., Chia L. S., Goh, N. K., Chia, T. F., and Brouillard, R. 2003. Analysis and biological activities of anthocyanin. *Phtochemistry*, 64(5) : 923-933.

Liu, X., Xioa, G, Chen, W., Xu Y., Wu, J. 2004. Quantifications and purification of mulberry anthocyanin with macroprous resins. *Journal Biomedicine Biotechnology* 326-331.

Lule, S. U., and Xia, W. 2005. Food and phenolics, pros and cons : a review. *Food Reviews International*, 21(4) : 367-388.

Markakis. 1982 Anthocyanins as food additives: Anthocyanins as food colours (New York: Academic Press)

Medina Jaramillo, C., Gutiérrez, T. J., Goyanes, S., Bernal, C., and Famá, L. 2016. Biodegradability and plasticizing effect of yerba mate extract on cassava starch ediblefilms. *Carbohydrate Polymers*, 151 : 150–159.

Monica, G.M., Ronald, E.W. 1996. Characterization of red radish anthocyanins. *Journal Food Science* 2 (61) : 322–326.

Pereira, V. A., de Arruda, I. N. Q., and Stefani, R. 2015. Active chitosan/PVA films with anthocyanins from *Brassica oleraceae* (Red Cabbage) as time-temperature indicators for application in intelligent food packaging. *Food Hydrocolloids*, 43:180–188.

Silva-Pereira, M. C., Teixeira, J. A., Pereira-Junior, V. A., and Stefani, R. 2015. Chitosan/corn starch blend films with extract from *Brassica oleracea*e (red cabbage) as a visual indicator of fish deterioration. *Food Science and Technology*, 61(1):258–262.

Sothornvit, R., and Pitak, N. 2007. Oxygen permeability and mechanical properties of banana films. *Food Research Internationa*l, 40(3) : 365-370.

Stinzing, F. C., and Carle, R. 2004. Functional properties of anthocyanin and betalains inplants, food and in human nutrition. *Trends in Food Science and Technology*, 15(1), 19-38

Tsai, P. J., McIntosh, J., Pearce, P., Camden, B., and Jordan, B. R. 2002. Anthocyanin and antioxidant capacity in Roselle (Hibiscus sabdariffa L.) extract. *Food Research International*, (35) : 351-356.

Zhang, X. H., Lu, S. S., and Chen, X. 2014. A visual pH sensing film using natural dyes from *Bauhinia blakeana* Dunn. *Sensors and Actuators BioChemica*l, 198:268–273.

© COPYRIGHT UPM

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Study on the Release of Anthocyanin Incorporation in Sago Starch Smart Film" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus Sarawak and published in the form approved by the Faculty.

© COPYRIGHT UPM

____

NURSYABII AFINI BINTI MOHD SHAIDI

DATE :