Synthesis and characterisation of Carboxymethyl Cellulose (CMC) from wheat bran and its application as PE/PET/CMC film

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ABSTRACT

Carboxymethyl cellulose (CMC) is one of cellulose derivatives that can be produced from agro-industrial waste. This biopolymer can be used as ingredient to develop a food packaging. The main aim of this study was to utilise wheat bran produced from milling process into carboxymethyl cellulose. Besides, the purpose of this research is to investigate the effectiveness of CMC as a potential biopolymer that can be used to produce film for packaging materials. CMC from wheat bran was prepared using sodium chloroacetate and NaOH. In this study, six different concentrations of NaOH that ranged from 5% to 30% (w/v) were used with the addition of isopropanol as the supporting medium in etherification process. This process was conducted at 85ºC for 3 hours. CMC obtained in this study was mixed with distilled water and glycerol and applied on PE/PET film to produce PE/PET/CMCS and PE/PET/CMCD films. For CMC, the effect of NaOH used during etherification on yield and physicochemical properties such as degree of substitution (DS) value, CMC content, molecular weight, colour, viscosity and surface morphology of CMC were analysed. CMC extracted with 10% and 15% (w/v) NaOH exhibited the highest value of DS with 0.84 and 0.83 respectively while 61672 and 43012 Da for molecular weight. All CMCs obtained in this study exhibited brighter, reddish and yellowish in colour with moisture content of lower than 8%. On the other hand, physical characteristics of PE/PET/CMC films such as film thicknesses, moisture content, film solubility, moisture absorption, colour, contact angle of films, water vapour permeability and film morphology were investigated. From the results, PE/PET/CMC15D showed the highest values for film thickness, moisture content and film solubility. All films produced were transparent but a bit yellow in colour. PE/PET/CMCS showed higher tensile strength and lower elongation at break compared to PE/PET/CMCD films. However, PE/PET/CMCD exhibited better water vapour permeability lowers than PE/PET film. From the findings obtained in this study, agriculture waste such as wheat bran can be used as a potential source of biopolymer that is capable to be used as a component in multilayered films.

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ABBREVIATIONS

% CM	percentage of Carboxymethyl content
Ag^+	Silver ion
AgCl	Silver chloride
$AgNO_3$	Silver nitrate
AGU	Anhydroglucose units
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
CH_3COOO^-	Peracetic acid
$ClCH_2COONa$	Sodium chloroacetate
CMC	Carboxymethyl cellulose
CMC5	CMC produced using 5% w/v NaOH
CMC10	CMC produced using 10% w/v NaOH
CMC15	CMC produced using 15% w/v NaOH
CMC20	CMC produced using 20% w/v NaOH
CMC25	CMC produced using 25% w/v NaOH
CMC30	CMC produced using 30% w/v NaOH
DAED	Diacetylethylenediamine
DP	Degree of polymerisation
DS	Degree of substitution
EB	Elongation at break
EG	Ethylene glycol
EHEC	Ethyl hydroxymethyl cellulose
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid

HDPE	High density polyethylene
HEC	Hydroxyethyl cellulose
$HOCH_2COONa$	Sodium glycolate
HPMC	Hydroxypropyl methyl cellulose
KOH	Pottasium hydroxide
LDPE	Low density polyethylene
MC	Methyl cellulose
MHPC	Methyl hydroxypropyl cellulose
NaCl	Sodium chloride
$NaClO_2$	Sodium chlorite
NaMCA	Sodium monocloroacetate
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
NSP	Non-starch polysaccharides
PE	Polyethylene
PE/EVOH/PE	Polyethylene/Ethylene vinyl alcohol/Polyethylene
PET	Polyethylene terephthalate
PE/PET	Polyethylene/Polyethylene terephthalate
PE/PET/CMCS	Polyethylene/Polyethylene terephthalate/CMC with single layer coating
PE/PET/CMCD	Polyethylene/Polyethylene terephthalate/CMC with double layer coating
pH	potential of hydrogen
PP/PVDC/PE	Polypropylene/Polyvinylidene chloride/Polyethylene
SEM	Scanning Electron Microscopy
TAED	Tetra Acetyl Ethylene Diamine
Tukey HSD	Tukey’s Honest Significant Difference
UV-VIS	Ultraviolet-visible spectrophotometry
WVP	Water vapour permeability
WVTR	Water vapour transmission rate
YI	Yellowness Index

SYMBOLS

%	percent
% CM	percentage of Carboxymethyl content
$[\eta]$	intrinsic viscosity
A	area
a	greenness – redness
b	yellowness - blueness
cm	centimetre
cm^2	centimetre square
Da	Dalton
dL/g	Decilitre per gram
eV	Electronvolts
g	gram
g/dL	gram per decilitre
g/g	gram per gram
Gg	Gigagram
g/L	gram per litre
g/mL	gram per millilitre
g/h.m.Pa	gram per hour. meter. Pascal
gh/m^2	gram per hour. meter square
h	hour
K	Kelvin
KeV	Kiloelectron-volts
Kg	kilogram
kHz	kiloHertz
L	Lightness
M	millimetre
mg/L	milligram per Litre
mg/ml	milligrams per millilitre
ml	millilitre

mL/g	millilitre per gram
min	minutes
mm	millimetre
mm/min	millimetre per minute
mPa	millipascal
MPa	megapascal
mPas	millipascal seconds
mV	millivolts
n	flow behaviour index
N	Newton
N/mm^2	Newton per square millimetre
nm	nanometre
^{o}	degree
^{o}C	degree Celsius
Rpm	rotation per minutes
s	second
s^{-1}	per second
T	time
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight
x	film thickness
β	Beta
ΔE	Total colour difference
Δp	water vapour pressure difference
Θ	theta
η_{red}	reduced viscosity
η_{rel}	relative viscosity
η_{sp}	specific viscosity
µl	microlitre

CHAPTER 1

INTRODUCTION

1.1 Wheat kernel

Wheat is the primary staple food consumed all over the world. Slavin et al., (1999) stated that wheat is the major consumed grain in the world with 33% of total food followed by rice with 25%. Ortiz and Lafond (2012) reported that wheat is grown widely throughout the world and is consumed in various forms such as bread, biscuits, cereals, pasta, noodles and beer. High demand from consumer towards this staple food has led to an increase of wheat crop production worldwide. Chalamacharla et al., (2018) in their review paper reported that wheat is primarily cultivated in China, India, Russia, USA, France, Canada, Germany, UK, Pakistan, Australia and Ukraine. These countries are the largest world wheat producer and responsible for the production of 758.0 million metric tons from June 2016 to May 2017 with total world consumption of 742132 thousand metric tons (2018) (Foreign Agricultural Service/USDA, 2018). In the United Kingdom itself, the quantity of wheat crop production has increased from 13 316 000 in 1994 to 16 606 000 tonnes in 2014. This value comes only second as the highest consumed commodity to whole fresh cow milk, listed in the top 10 commodities production produced in the United Kingdom.

In 2000, Zohary and Hopf reported that wheat is an annual grass that belongs to Gramineae family under Triticae genus. Common types of wheat cultivated are diploid *T. monococcum* (wild wheat), the tetraploids *T. dicoccum* and *T. durum* (pasta wheat) and the hexaploids *T. aestivum* (bread wheat). Mattern (1990) stated that *T. aestivum* dominates the world production. Wheat kernel is a tiny egg-shaped seed with a turf of hairs known as “beard” formed on top of the seed, while at the other end of the seed, the grain is attached to the stalk is known as germ. Wheat kernel consists of three major components. These are endosperm, bran and germ. Wheat endosperm contains a complex set of proteins that have viscoelastic properties when hydrated and mixed. Dexter and Sarkar (2004) pointed out that white wheat flour originates from starchy endosperm comprises about 85% of the wheat kernel.

Wheat bran is the outer part of the grain and is a good source of dietary fibre and B vitamins. It is formed by pericarp layers consisting of seed coat and nucellar tissue. Epidermis, epicarp, endocarp and testa found in pericarp layers, serve as a mechanism to protect both germ and endosperm. Endosperm acts as energy storage for germ to reproduce. The aleurone layer composed of single layer of cells between endosperm and bran and contains higher amount of arabinoxylans. Overall, wheat grain comprises on average of 58% to 70% starch, 13% water, 9% to 12% protein, 9% fibre and 2% to 5% fat (Hofer, 2015).

1.1.1 Agro-industrial waste from wheat production

The conversion of endosperm as a source of protein into wheat flour in milling process generates agro-industrial wastes such as wheat bran and wheat germ. Wheat bran and wheat germ are large particles size produced after milling process and can be separated by sifting (Deirdre and David, 2012). Recent evidence from Department of Environment Food and Rural Affairs (2017) suggested that, in 2015/2016, 3299.5 thousand tonnes of wheat bran was used to produce animal feeds. This value has increased to 3478.1 thousand tonnes in 2016/2017. As a side note, Taurisano et al, (2014) reported that agricultural waste produced by industry is usually disposed into landfills. These agro-residues are left unattended in open areas to eventually rot and thus are associated with environmental issues.

One of the greatest challenges in wheat production is the abundance of agro-industrial wastes that would result in increased severe soil and air pollution problems resulting from the burning of agro-residues causing health problems such as asthma or other respiratory disorders. Emission of gasses such as nitrous oxide, methane and carbon dioxide from the burning of agro-industrial waste contributes to global warming. As reported by FAO in 2014, the emission of nitrous oxide and methane from burning process of wheat crop residues were recorded at 0.0542 and 2.0909 Gg respectively while carbon dioxide equivalent exhibited the highest value of emission with 60.713 Gg. To overcome the pollution problems caused by waste produced from wheat milling, a critical solution is needed in order to make this waste more usable. This could be achieved by transforming this waste into a new resource that could benefit the community. In recent years, there has been an increasing interest in utilising agro industrial waste into biopolymer, which could be beneficial to the society. However, until now, there has been no detailed investigation on the conversion of wheat bran into cellulose derivatives as well as on the use of this biopolymer as a film. Therefore, this study hopes to provide new insights into such derived biopolymers and process necessary for the conversion. Figure 1.1 shows the image of wheat bran.

Figure 1.1 Wheat bran

1.1.2 Structure and composition of wheat bran

According to Zelch and Ross (1989) and US FDA (2003), wheat bran is defined as the coarse outer layer known as pericarp of the wheat kernel which is separated from the cleaned and scoured kernel during the milling process. The total pericarp has been reported to comprise of roughly 5% of the kernel and consists of approximately 20% cellulose, 6% protein, 2% ash and 0.5% fat with the remainder being non-starch polysaccharides (Arendt and Zannini, 2013). Barron et al., (2007) reported that the outer layer is constitutes of a number of layers surrounded the grain, comprising of about 45 to 50% cell wall material. Another study conducted by Stone and Morell, (2009) stated that, the pericarp is the major tissue of wheat kernel and being more similar in cell wall composition to wheat straw having 30% cellulose, 60% arabinoxylan and 12% lignin. According to Apprich et al., (2013), the outer layers of wheat bran are formed by thick cell walls containing cellulose, cuticle materials and complex xylans with high arabinose to xylose ratios (A/X). Wheat kernel contained the structure of pericarp layer, seed coat and nucellar epidermis. The aleurone layer is located in middle of these three layers and the starchy endosperm.

Table 1.1 provides the percentages of endosperm, germ, bran and total fibre composition in wheat, rice, corn, barley and rye. Hoseney, (2010) reported that the highest component in all grains was endosperm. This ranged between 67% to 90%.The percentages of bran for all major grains is between 7% to 15.6%. The lowest component is germ, which accounts between 3% to 11%. From this table, wheat kernel contains 83% of endosperm, 14% of bran and 3% of germ.

Table 1.1 Composition in major grains (Hoseney, 2010)

Component (%)	Wheat	Rice	Corn	Barley	Rye
Endosperm	83	90	82	81.1	83
Germ	3	2.5	11	3.3	3
Bran	14	7.5	7	15.6	14
Total fibre	12.2	2.8	7.3	17.3	15.1

1.1.3 Wheat bran as a sustainable material

In milling process, wheat grain will be cleaned to remove the impurities. Then, it is tempered or soaked to toughen the outer layers and mellow the starchy endosperm in order to facilitate the separation. The grinding process will take place as to produce finer flour particles while coarser particle are formed as broken wheat and bran. As mentioned by Hassan et al., (2008), this wheat bran represents about 10 to 19% of the wheat kernel, depending on the variety and type of milling process. Due to higher amount of wheat production, the quality of wheat crop residues formed as by-products has also increased. According to FAOSTAT (2017), the total world crop residue from wheat production in 2016 was reported at 9, 640 057 tonnes. The amount of wheat crop residues being burned have increased the environmental burdens and reflects to the emission of nitrous oxide, carbon dioxide and methane produced from burning process with 6.163, 185.5711 and 237.7162 gigagrams respectively. Besides that, wheat bran is also suitable to be used as livestock feed and is sold as raw or in pelleted form with the price of wheat bran being only USD 0.14/kg.

With reference to the high amount of agro-industry waste being burned and used as animal feed, there is a need to utilise innovatively this crop residue, and convert it into other biopolymer sources that could benefit the society. One example of such biopolymers is cellulose derivatives. Furthermore, this agro-industrial waste should be considered as a renewable natural source as it is readily available in abundant volume. It also can be a new source of biopolymer and can be used as main ingredient or even cooperate with other polymers in developing new packaging material. Hence, a potential packaging with better film properties could be an interesting possibility for utilising this waste product.

Previously, a variety of polysaccharides and their derivatives have been used as biodegradable film forming matrixes, due to their availability and low in cost. The development of biopolymer coating based on carboxymethyl cellulose extracted from bran could potentially be a new innovation in food packaging field. The use of bran as a raw material may reduce agro-industry waste in milling industry and hence reduces the environmental burden such as emission of gases from burning process (FAOSTAT, 2017). Previously, wheat bran has been reported to be incorporated into bakery and cereal due to its health promoting properties (Pruckler et al., 2014). Besides, it also had been used as animal feeds since it is palatable to most of the animals (Fuller, 2004). In addition to these applications, wheat bran could also be transformed into biopolymer. Therefore, the significance of this research is that it can help in developing such promising films that can be used as packaging materials. Besides, the current research hopes to provide a reference point and enhance the knowledge of other researchers in food packaging area by improving and creating a new packaging material. The findings obtained in this research are envisaged to be useful for future technology developments in this field.

To date, there is little study made on the synthesis of CMC from wheat bran produced as a waste product. This abundant waste is very often locally available and cheap. In this study, the effect of different concentrations of sodium hydroxide (NaOH) used during CMC production on properties of CMC produced will be discussed. In addition, CMC produced in this study will be used as a coating material applied on top of PE/PET films. This study is very significance in multilayer film development as it helps in creating new potential multilayer film made from PE/PET/CMC.

1.2 Cell wall of plant

Plant cell can be differentiated from animal cell by the presence of a cell wall. The plant cell wall serves a variety of functions such as protecting the intercellular contents. Cell wall provides rigidity to the plant besides providing a porous medium for the circulation and distribution of water, minerals, and other nutrients. In addition, cell wall also serves as a place for molecules to regulate growth and protect the plant from disease (Davidson, 2015). Plant cell wall is usually divided into two categories, which are primary cell wall and secondary cell wall. Primary cell wall is a glycoproteinaceous layer composed of pectin, cellulose, hemicellulose and protein. When the cell grows and differentiates, a new material in a form of mixture with the constituents of the primary cell wall is secreted, leading to the formation of the secondary cell wall. In order to allow cell to extend during its growth, cell wall structure must be able to deform. The mixture of polysaccharides and protein that present in the cell wall helps the cell to do so.

According to Albersheim et al., (2010), cellulose, hemicelluloses and pectin are the major polysaccharides component of plant cell walls commonly known as fibre or non-starch polysaccharides (NSP). The cell wall can be represented as a network of cellulose microfibrils crosslinked by hemicelluloses and embedded in a matrix of pectin polysaccharides. Chanzy (1990) pointed out that xyloglucans are the major component of hemicellulose of higher plant dicotyledons. Apart from its structural properties, hemicelluloses may also have other function such as cell signaling, precursors of signaling molecules and reserve substances. Hemicellulose consists of a large number of different polysaccharides that form a matrix for the cellulose microfibrils which held together by molecular interactions such as hydrogen bonding and Van der Waals forces.

In another study, Perez and Mazeau (2004) stated that pectin constitutes a major component in higher plant dicotyledons contributing about 35% of dry weight to the cell wall. Pectins are a class of galaturonic acid rich polysaccharides including homogalacturonan, xylogalacturonan and rhamnogalacturonan I and II polymers that serve as hydrating agents and cementing material for the cellulosic network. Pectin has a linear 1-4-linked α-D-galacturonate backbone and is responsible for the ion exchange capacity of the cell wall hence control of the ionic environment and pH of the interior of the cell. Some pectin molecules are covalently linked to cellulose and hemicelluloses, contributing to the mechanical properties of the cell wall.

Lignin is the other wall polymer that integrats into the spaces around polysaccharide fibrillar to form lignin polysaccharides. It occurs in the middle of lamella and is the primary cell wall during final stage of cell wall deformation. It is derives from free radical polymerization of alcohols of para-hydroxy cinnamic acid and constitutes about 10% to 30% of the dry weight of wood placing it second after cellulose. According to Perez and Mazeau (2004), lignins also contribute to the mechanical strength of the plant cell wall. Lignin are usually deposited around the cell wall, providing stiffness and protection against pathogens. They are also play a key role in water transport by reducing cell wall permeability (Gupta et al., 2016).

The different compositions of dietary fibre in wheat bran are dependent on the variety of crop, cultivation conditions and the methods employed for separation (De Brier et al., 2015). According to Chalamacharla et al., (2018), cellulose corresponds to 9.3% to 12.1%, while arabinoxylan corresponds to 22.4% to 29.8% of the wheat bran dry weight. Arabinoxylan is a fibre composed of a β-D-xylan backbone with arabinose side chains linked by α-1, 2 and α-1,3 glycosidic linkages. It is estimated that β-glucans correspond to about 2.2% to 2.7% of the dry weight of wheat bran.

1.3 Cellulose

Cellulose is the most abundant renewable biopolymer found in nature and represents about 1.5 x 10^{12} tonnes of the world's total annual biomass production. It is considered as an almost inexhaustible source of raw material (Klemm et al., 2005). Cellulose is the major building block of the cell wall structure of vascular plant such as trees, cotton, flax, sugar beet residues, ramie and cereal straw (Cash and Caputo, 2010; Perez and Mazeau, 2004). It is often occurs in conjunction with lignin and hemicellulose. Commercial cellulose production concentrates on sources such as wood or on the naturally pure source such as cotton linters. According to FAOSTAT (2012), the amount of cotton lint produced in 2010 was 42 million metric tonnes and this makes it as the main source of cellulose produced amongst agricultural products. Besides, cellulose was also found in some of natural products such as 94% in cotton, 77% in hemp, 40-50% in wood and grain straw (Wypych, 2012).

Pappas et al., (2002) mentioned that cellulose could be used for the production of pharmaceuticals, food and chemical feedstock apart from papermaking. Due to the abundant renewable source of polymer in nature, cellulose can also be used as raw material to produce modified cellulose or cellulose derivatives. The use of cellulose and its derivatives in a diverse array of other applications such as films, plastics, coatings, suspension agents and composites, continues to grow worldwide (Kadla and Gilbert, 2000). According to Vásconez et al., (2009), cellulose based materials are being widely used because they offer advantages like edibility, biocompatibility, barrier properties, aesthetic appearance as well as nontoxic, non-polluting and low cost. The use of this bio-based material has been reported in innovating and developing a new food packaging material; providing food safety, extending the shelf life and maintaining the food quality. Ramesh and Tharanathan, (2003) reported that, biopolymer films are generally prepared by using materials such as polysaccharides, proteins and their derivatives which are naturally and abundantly available. In addition, natural biopolymer films have the advantages over synthetic biopolymers that are completely biodegradable by living organisms and are derived from renewable raw materials. Such biopolymers also have desirable overall mechanical and barrier properties against water vapour (Gross and Carla, 2002).

1.3.1 Structure of cellulose

As reported by Rachtanapun et al., (2012), cellulose is a linear polymer made of anhydroglucose units (AGUs) that joined through β-1-4 linkages. This linkage connects carbon number 1 of AGU with carbon number 4 of other AGU to form β-1-4 glycosidic bonds to make a repeating cellobiose unit. An anhydroglucose unit (AGU) within the cellulose chain has three reactive hydroxyl groups at carbon 2, 3 and 6. These reactive groups are able to undergo the typical reactions including esterification, etherification and oxidation (Shubert et al., 2011) to form cellulose derivatives. As reported by Cash and Caputo (2010), the substitution of hydroxyl groups will determine the degree of substitution of cellulose derivatives.

The repeating unit of cellulose is called cellobiose with a length of 1.3 nm (Krassig, 1993). The cellulose molecules is arranged and organized in bundles of microfibrils. These microfibril bundles are held together by hydrogen bonds that are formed between the protruding hydroxyl groups of the glucose molecules (Roberts, 2001). As reported by Carrillo and Kobayashi (2017), these cellulose microfibrils are generally between 0.05 to 0.3μm in diameter with an average diameter of 0.15mm and up to 20 cm in length.

Cellulose chains are set parallel and aligned side-by-side in a specific crystalline arrangement. Festucci-buselli et al., (2007) in a review paper stated that each cellulose microfibril has approximately 36 glucose chains.

There are two hydrogen bonds between C6 hydroxyl and the C2 hydroxyl and between the C5 oxygen and C3 hydroxyl. These bonds are called inter and intra-chain hydrogen bonds that stabilise the glycosidic bond and make the structure stiff. According to Shubert et al., (2011), these cellulose molecules are linked together in a layer and the layers are held together by hydrophobic interactions and weak C-H-O bonds. Hydrophobic forces are important in the cellulose structure since both glucose and cellulose is considered to be very hydrophilic (Gellerstedt & Gunna 2009). According to Nishiyama et al., (2003), the glucose chains are stabilised by intra and inter hydrogen bonding which confers greater stability on microfibrils. Cellulose chains are held in staggered layers on top of another by Van der Waals forces to create a structure called microfibrils. The degree of polymerization of native cellulose of various origins is in the range of 1000 to 30 000 which corresponds to chain lengths from 500 to 15 000 nm.

Jarvis (1984) mentioned that the microfibrils are responsible in constituting the basic framework of the cell wall in providing a great resistance to tensile forces. Kadla and Gilbert, (2000) reported that cellulose molecules have a strong tendency to form inter and intramolecular hydrogen bonds because of the equatorial orientation of the hydroxyl groups and its linear structure. Due to its strong inter and intra molecular hydrogen bonds, cellulose neither melts nor dissolves readily in hot or cold water (Choi et al., 2007) and in most common organic solvents (Hattori et al., 2004; Adinughara et al., 2005). Thus, as to fully utilise cellulose in food industries, it must be converted into its cellulose derivatives.

1.3.2 Method for extracting cellulose from plant waste

Previous studies have proved that there are several methods that can be used to extract cellulose from different plant waste such as wheat bran, wheat straw, rice husk, rice straw, soy hull, sugar beet and durian rind. Most of these methods involve the use of alkaline such as NaOH for alkaline treatment. Cellulose is treated with alkali to swell the polymer. The use of alkaline will disrupt crystalline regions and the subsequent reactions will be uniform. A review authored by Harding et al., (2006) stated that, cellulose ethers are typically produced using NaOH to form an alkali cellulose and subsequently undergone etherification process to produce cellulose derivatives such as carboxymethyl cellulose (CMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), methyl hydroxyethyl cellulose (MHEC), ethyl hydroxymethyl cellulose (EHEC), Methyl cellulose (MC) and methyl hydroxypropyl cellulose (MHPC).

Several studies conducted by Togrul and Arslan, (2003), Abe et al., (2007), Abe and Yano, (2010) and Rosa et al., (2012) demonstrated that fat content in plant waste can be removed using chloroform, toluene, methanol or ethanol through Soxlet method with 6 to 20 hours of extraction. This process is also known as defatting or dewaxing. Furthermore, sodium hydroxide (NaOH) and potassium hydroxide (KOH) with a concentration between 2% to 10% (w/v) are used as an agents to invade the structure of cellulose microfibrils in wheat bran (Wang *et al.,* 2013), wheat straw (Sun *et al.,* 2004), rice husk (Rosa *et al.*, 2012), soy hull (De Rodriquez *et al.,* 2006) and durian rind (Rachtanapun *et al.,* 2012). In order to whiten the colour of cellulose pulp, sodium hypochlorite and hydrogen peroxide (H_2O_2) are used as bleaching agents in the bleaching process in order to dissolve and remove lignin and hemicellulose from cellulose. Tetraacetylethylenediamine (TAED) is used as an accelerator in bleaching process. This process is conducted at pH values of 11.6 at temperatures that ranged between 48^{o}C to 80^{o}C. Samples were purified with acetic acid and concentrated nitric acid as to remove by-products produces during the process. To continue the procedure, most researchers treated and washed their sample with distilled water or ethanol. This is then dried between 50^{o}C to 60^{o}C for 16 to 24 hours to produce the cellulose powder. Table 1.2 shows the comparison of different methods used to extract cellulose from different plant waste conducted by previous researchers.

1.3.3 Alkalisation and etherification of cellulose backbone

Heinze (1998) reported that carboxymethylation is one of the most versatile procedures for cellulose modification as it provides access to bio-based materials with valuable properties such as inspissation, filming, emulsification, suspension, water maintaining and binding. The preparation of CMC involves two reaction stages, which are alkalisation and etherification. Alkalisation also known as mercerisation process happens when pulp is treated with NaOH and ethanol or isopropanol. The function of this treatment is to act as swelling and impregnation stage, as well as to facilitate the penetration of NaOH into the cellulose structure. At this stage, the product is known as alkaline cellulose or (Na-cellulose). Alkaline cellulose is commonly produced by slurrying or mixing the cellulose obtained from chip or pulp (Coffey et al., 2006), with aqueous NaOH solution ranging between 2% to 40% w/v (Heinze, 1998). At this stage, the mixture is held for a predetermined time at a controlled temperature. Treating the cellulose with alkaline solution will disrupt the hydrogen bonding between and within the polymeric strands. This condition will make the majority of the hydroxyl groups available for modification with C2 and C6 hydroxyl sites being the more reactive ones as compared to C3 sites (Samarayanake and Glasser, 1993). Through alkalisation process, hydroxyl groups that present in cellulose chains are stimulated and altered into a more reactive alkaline form. A further effect of the alkaline method is to depolymerise the lignin molecule at cellulose backbone. At the end of the process the produced black liquor is rich in solubilised lignin.

Heinze (1998) also highlighted that large-scale production of CMC is carried out using slurry process; the conversion of alkaline cellulose swollen in aqueous NaOH and organic liquid such as ethanol, isopropanol and acetone together with monochloroacetic acid. Alkaline cellulose is highly reactive towards monochloroacetic acid and the etherification stage normally is carried out at about 50 to 70ºC. Etherification process is the substitution of monochloroacetic acid at cellulose backbone. During this stage, monochloroacetic acid is added to the alkaline cellulose as an etherification agent. Hydroxyl groups in cellulose are usually replaced by carboxymethyl groups in the order of C2>C6>C3. Previous researches by Krassig (1993) and Klemm et al. (2001) established that the reaction between NaOH and monochloroacetic acid will also produce by-products such as sodium glycolate and sodium chloride. In 2006, Pushpamalar et al. reported that the production of CMC is simpler than most other cellulose ethers because all reactions are operated at atmospheric pressure using commercially available reagents. Therefore, CMC has become the largest industrial cellulose ether produced.

Table 1.2: Comparison on different method to extract cellulose from plant waste

Sample	Drying	Extraction	Bleaching	Purification	Washing	Drying	Authors
Wheat bran	60°C, 16 h	3% of NaOH, 80°C, 4 h	Sodium hypochlorite, 2 h	-	Distilled water	60°C	Wang *et al.,* (2013)
Wheat straw	60°C, 16 h	0.5 M aqueous KOH, 35°C, 2.5 h under ultrasonic irradiation – 35 min	2% H_2O_2 + 0.2% TAED solution, pH 11.8, 12h, 48°C	80% acetic acid + concentrated nitric acid	95% ethanol, distilled water	60°C	Sun *et al.,* (2004)
Rice husk	60°C, 16 h	Toluene/ethanol (2:1 v/v), 6 h 121°C – autoclave, 5% aqueous NaOH.	2% H_2O_2 + 0.2% TAED solution, pH 11.8, 12h, 48°C	80% acetic acid + concentrated nitric acid	95% ethanol, distilled water	60°C	Rosa *et al.*, (2012)
Rice straw	60°C, 16 h	Toluene/ethanol (2:1 v/v) – 20 h	1.4% acidified sodium chlorite, pH 3-4, 70°C, 5h	-	Distilled water	60°C	Abe *et al.*, (2007); Abe & Yano (2010); Sun *et al*., (2004).
Soy hull	60°C, 16 h	2% NaOH, 4 h , 100°C	Acetate buffer & 1.7% sodium chlorite - 80°C, 4 h	-	Distilled water	50°C, 12 h	De Rodriquez *et al.,* (2006); Siqueira *et al.*, (2010)
Sugar beet	Sun-dried	Chloroform:methanol (2:1, v/v), 6 h.	-	-	Distilled water and ethanol	50 °C, 16 h	Togrul and Arslan, (2003) and Yasar *et al.,* (2007),
Durian rind	Sun dried	10 g/100 mL NaOH, 100°C, 413 MPa	Hydrogen peroxide	-	Distilled water	55°C, 24 h	Rachtanapun *et al.,* (2012)

1.3.4 Mercerisation and bleaching process of cellulose

Cellulose is a hygroscopic material, insoluble but able to swell in water, dilute in acid and most of the solvent. However, according to Rorrer and Hawley (1993), the solubility of cellulose can be achieved using concentrated acid. The use of alkaline solutions will lead to the swelling and dissolution of hemicellulose that present in the polymer. Mercerisation is defined as the use of caustic soda or NaOH solution to penetrate cellulose microfibrils by causing swelling and subsequently allowing the flow of the solution into the capillary spaces of cellulose structure. This allows the entry to the regions between the crystalline zones. As reported by Dinard et al. (2002), this process is important and is used to activate cellulose for cellulose derivatives production. Hydrogen peroxide (H_2O_2) has been widely used as an environmental-friendly bleaching agent for high yield pulp bleaching processes. H_2O_2 is easily decomposed due to its instability and the catalytic effects of the metal ions that are present in pulp during bleaching process.

Nakasone and Kobayashi (2016) stated that the bleaching process of fibers involves the removal of residual lignin. In 2000, Maes and Delcour revealed that dilute alkaline solutions of H_2O_2 could remove half of the lignin and most of the hemicellulose present in these materials, thus yielding a cellulose-rich, insoluble residue with a high water-absorption capacity and a more open internal structure. On the other hand, bleaching depends on the pH of the reaction mixture with an optimum pH value of 11.5 to 11.6. Due to the fact that association between lignin and polysaccharides is broken, the extraction of hemicellulose from plant cell walls is facilitated. In 1979, Andrews and Sigh stated that conventional peroxide bleaching is typically conducted under conditions of high temperature and pH thus causing a significant fibre strength loss. Therefore, in order to reduce fibre damage, bleaching activator such as Tetraacetylethylenediamine (TAED) is used together with bleaching agent. Using bleaching activator, bleaching process can be conducted at lower temperature and in a shorter time compared to conventional peroxide bleaching (Zhao et al., 2012).

TAED is often used as bleaching activator together with H_2O_2 in bleaching processes to improve bleaching efficiency. TAED is colourless, odourless, non-toxic, stable during storage and easy to handle liquid. In the presence of peroxide at alkaline condition, TAED will generate the anion of peracetic acid. As reported by Zhoa et al., (2012), TAED has the potential to enhance pulp properties such as strength and bulk due to the characteristics of TAED that could improve the oxidation potential of peroxide. This results in faster bleaching and shorter reaction times leads to the reduction in degradation of fibre during bleaching process.

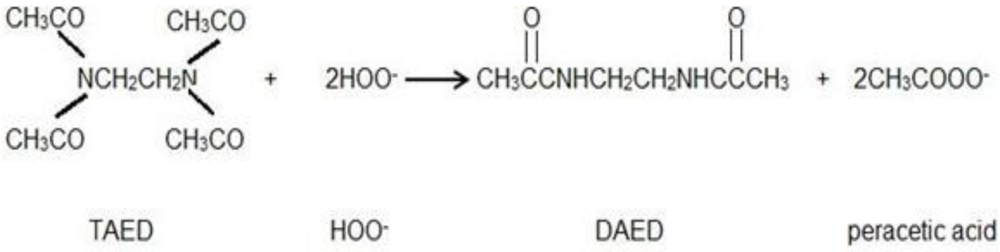

Figure 1.2 Reactions between TAED and HOO-.

During bleaching process, H_2O_2 dissociates to form hydrogen dioxide (HOO-) and hydrogen ion (H+). Figure 1.2 shows the reaction mechanism between HOO- and TAED. It can be seen that, when TAED reacts with HOO- through a process called perhydrolysis, diacetylethylenediamine (DAED) and peracetic acid (CH_3COOO-) are produced. In 2003, it was suggested by Zhoa et al. that CH_3COOO- had better oxidative ability than HOO- due to the delignification reaction that occurs at lower temperature in shorter time. Therefore, as reported by Hashem et al. (2003), H_2O_2 and TAED have been successfully used in cotton fabric bleaching. For that reason, both of these chemicals will be used in this study during bleaching process of wheat bran.

1.4 Carboxymethyl cellulose (CMC) as cellulose derivative

CMC is a cellulose derivative composed of two repeating anhydroglucose units (AGU). As mentioned previously, each AGU contains three hydroxyl groups that can be substituted. Numerous studies have been attempted in order to explain that CMC could be prepared by the activation of cellulose with aqueous NaOH in the slurry of an organic solvent such as isopropanol which reacts with the cellulose and monochloroacetic acid as an etherifying agent. In 1997, Charpentier et al. revealed that the substitution of a carboxymethyl group was slightly predominant at C2 position. Later in 1999, Heinze and Pfeiffer investigated that carboxymethyl groups replaced some of the hydroxyl groups of the glucopyranose units in cellulose and these units are connected via β-1, 4-glucosidic bonds. Another study by Murray (2000) stated that, the average number of carboxymethyl groups substituted at the AGU was known as the degree of substitution (DS). In his study, Murray pinpointed that two main factors influencing the properties of cellulose derivatives are the viscosity and the degree of substitution that have a direct correlation with the degree of polymerisation (DP). This statement later has been highlighted by Heinze and Koschella, (2005), recognising that the hydroxyl groups that were esterified with carboxymethyl indicates the average number of carboxymethyl groups per AGU.

Gennadios, (1997) in his study stated that because of the inherent hydrophilic nature of polysaccharides, cellulose derivatives are poor water vapour barriers and have poor mechanical properties. Thus, the addition of other substances is required to improve the properties of films made from such biopolymers. CMC is a sodium salt with white to cream colour, tasteless, odourless and free flowing powder (Clarke, 2012). It is a linear, long chain, anionic polysaccharide and is the most important water-soluble cellulose derivative (Olaru et al., 1998). Besides that, Ma et al., (2008) also reported that CMC is cellulose derivatives that exhibits thermal gelation and could form excellent films due to its polymeric structure, and high molecular weight. The properties of CMC have been studied extensively in combination with other polymers such as chitosan, gelatin, starch, glucomannan and various other substances such as sunflower oil. As noted by Togrul and Arslan (2003), CMC has been used in a number of edible film formulations due to its non-toxicity, biocompatibility, biodegradability, hydrophilicity and film forming ability.

1.4.1 Preparation of CMC from plant sources

The production of CMC from agricultural waste sources have been studied extensively for cotton linters (Xiquan et al., 1990), sugar beet pulp (Togrul and Arslan, 2003), cashew tree gum (Silva et al., 2004), Cavendish banana pseudo stem (Adinugraha et al., 2005), sago waste (Pushpamalar et al., 2006), papaya peel (Rachtanapun et al., 2007), waste of mulberry paper (Rachtanapun et al., 2007), orange (Yasar et al., 2007) and Mimosa pigra peels (Rachtanapun and Rattanapanone, 2011), durian rind (Rachtanapun et al., 2012) and corn husk (Mondal et al., 2015). Table 1.3 shows the method used by previous researchers to extract CMC from plant waste. From this table, it can be seen that the concentrations of NaOH used during alkalisation process is between 15% to 30% w/v. Most of the processes were carried out at 25°C for 30 to 90 minutes using isopropanol as organic solvent. In addition, the ratios of monochloroacetic acid to cellulose used by previous researchers were between 1.2 to 1.5 g per 1 g of cellulose. Methanol and ethanol were used to wash produced samples and the process was continued with neutralisation process using 90% (v/v) acetic acid producing crude CMC with salt content of sodium glycolate and sodium chloride. As reported by Rinaudo, (2008), these salts can be removed using water-alcohol mixtures such as ethanol or methanol before drying process as to improve the purity content. Adinughara et al., (2005) in their study proved that the optimum condition to synthesis CMC from alkaline cellulose were 15% (w/v) NaOH using 1.2 g (w/w) of monochloroacetic acid at 55°C for 3 hours. These conditions could produce CMC with higher degree of substitution, crystallinity and viscosity. Based on studies conducted by previous researchers such as Adinugraha et al., (2005) and Rachtanapun et al., (2012), different concentrations of NaOH used during alkalisation, and different ratio of monochloroacetic acid added during etherification process can influence the yield and properties of CMC produced. Furthermore, temperature and time also affected the synthesis of CMC derived from plant wastes. Under other conditions, the production of CMC can be conducted only at atmospheric pressure. Figure 1.3 shows the conversion of alkali cellose to CMC.

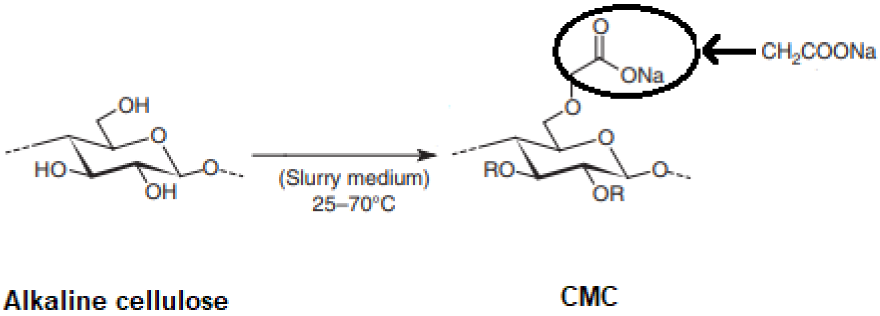

Figure 1.3 The conversion of alkali cellulose to CMC. Substitution of Carboxymethyl group (CH_2COONa) at alkaline cellulose.

Table 1.3: Comparison on method used to synthesis carboxymethyl cellulose from plant waste.

Sample	Concentration of NaOH	Cellulose:NaMCA	Purification	Drying	Authors
Sugar beet pulp	30% NaOH & isopropanol – stirred for 90 min at 25^{o}C	1 : 1.5 g 70^{o}C – 6 hours	70% methanol, neutralised using 90% acetic acid	70^{o}C	Togrul and Arslan, (2003)
Cavendish banana pseudo stem	15% & isopropanol – stirred for 60 min at 25^{o}C	1 : 1.2 g 55^{o}C –3 hours	70% ethanol	60^{o}C	Adinugraha *et al*., (2005)
Sago waste	30% NaOH & isopropanol – stirred for 60 min at 25^{o}C	1 : 1.2 g 45^{o}C –3 hours	70% methanol, neutralized using 90% acetic acid 70% ethanol	60^{o}C	Pushpamalar *et al*., (2006)
Orange peel cellulose	30% NaOH & isopropanol – stirred for 90 min at 25^{o}C	1 : 1.5 g 70^{o}C – 6 hours	70% methanol, neutralised using 90% acetic acid	70^{o}C	Yasar *et al*. (2007)
Cotton fiber	30% & isopropanol – stirred for 5 hours	40%, 600 rpm, 75 °C for 4 h	70% methanol	70^{o}C	Heydarzadeh *et al.,* (2009)
Palm kernel	17.5% NaOH & isopropanol – stirred for 60 min at 30^{o}C	1 : 1.2 g 50^{o}C –2 hours	70% methanol, neutralised using 90% acetic acid, washed with 70% ethanol	60^{o}C	Bono *et al.,* (2009)
Durian rind cellulose	20% NaOH & isopropanol – stirred for 30 min at 25^{o}C	1 : 1.2 g 55^{o}C –3 hours	70% methanol, neutralised using 90% acetic acid, washed with 70% ethanol	55^{o}C	Rachtanapun *et al*., (2012)

1.4.2 Properties of CMC

CMC is soluble in hot or cold water but insoluble in organic solvents. CMC is more hydrophilic if it has a higher degree of substitution. The degree of substitution (DS) is an important parameter in CMC. According to Heinze and Koschella, (2005), the maximum value of DS for CMC is 3.0, but commercially available are from 0.4 to 1.5. Borsa and Racz, (1995) reported that increasing the DS of CMC enhances the solubility in water. Above 0.6, CMC will show good water solubility whereas if it is less than 0.2, CMC will retain the fibrous character of the starting material and is not soluble in water. Solubility in water is dependent on the DS value and the molecular weight. High molecular weight and high DS result in higher solubility. The molecular weight mainly defines the final viscosity of CMC. However, at higher concentration of NaOH, the formation of sodium glycolate increases thus lowering the DS and CMC content (purity) since the NaMCA molecule tend to react with NaOH (Barai et al., 1997).

The viscosity of CMC will increase rapidly with concentration. Long period of heating at high temperature will degrade CMC and permanently reduce viscosity. CMC solution exhibit their maximum viscosity and best stability at pH 7 to 9. Above pH 10.0, the viscosity of solution slightly decreases. Below pH 4.0, the less soluble free acid CMC predominates and viscosity may increase significantly. The addition of solutes such as salts or polar non-solvents will affect the viscosity of CMC solution. If CMC is dissolved in water and the solute is then added, it only has a small effect on viscosities compared when solute is dissolved before the CMC is added as it will inhibit breaking up the crystalline areas and lower the viscosities (Heinze and Koschella, 2005).

As reported by Adinugraha et al. (2005), carboxymethyl groups act as hydrophilic groups therefore with the increase of DS also improves the water holding ability of CMC. To dissolve CMC in water, it is recommended that

CMC is added to water under strong agitation as to avoid any lump formation. Another way to dissolve the CMC is to disperse it in a non-solvent carrier such as oil, glycerine, sugar syrup and then to dilute this suspension in pure water.

1.5 Bio-based polymer

Bio-based polymer is a polymer that has naturally occurred or produced by biological actions. It is derived from plant, animals or microbial biomass and has long chains of molecules that are linked together through a chemical bond (Kanmani et al., 2017). According to Ferreira et al. (2009), polymeric materials derived fully from renewable resources and can be considered sustainable in term of material supply, water and energy use and waste product generation. According to Debeaufort (2014), bio-based polymers can be synthesised from bio-derived monomers such as poly-lactic acid. Besides, these bio-based polymers could also be extracted from a wide range of biomass such as polysaccharides, proteins and lipids. Amongst all of this biomass, polysaccharide group exhibits the widest list of materials and thus contributes to the most abundant source. This includes cellulose, starches, pectin, alginates, carrageenan and chitosan. In addition, bio-based polymers such as Polyhydroalkanoates (PHA) could be produced using microorganisms.

1.5.1 Biopolymers used in packaging

Dick et al., (2015) stated that bio-based polymer packaging materials are normally produced from proteins, polysaccharides, lipid or their blends. In 2014, Debeaufort reported that the interest of the industry for renewable, bio sourced and biodegradable polymers for packaging are increasing significantly. In the last thirteen years ago, only 10 companies were able to invent and retail bio-based packaging films. However at present, more than 250 companies in European countries market their packaging materials made with natural biopolymers. According to Kakoszka et al. (2010), bio-based polymer packaging may also serve as gas, moisture, aroma and lipid barriers that enhance food quality by minimising its deterioration and consequently improving its shelf life.

Previous study has shown that biopolymers could be used as renewable raw materials to be applied as packaging materials. Krochta and Mulder-Johnson (1997) reported that cellulose derivatives such as CMC carries good film-forming characteristics by producing films that are generally odourless, tasteless, flexible, transparent, resistance to oil and fats, water soluble and moderate to moisture and oxygen transmission. Data from several studies suggest that biopolymer film can be prepared using CMC in combination with other biopolymer in order to improve physical, mechanical and barrier properties of films. The used of CMC as a component in biopolymer film has been demonstrated in producing several composite films such as CMC/starch (Ghanbarzadeh et al., 2010), CMC/soy protein (Su et al., 2010), CMC/cassava starch (Tongdeesoontorn et al., 2011), CMC/oleic acid (Ghanbarzadeh and Almasi, 2011), CMC/chitosan (Thomas et al., 2016) and CMC/chicken skin gelatin (Nazmi et al., 2017). Besides these studies, CMC extracted from agriculture waste has been used to develop biopolymer film such as CMC papaya peel/corn starch film (Rachtanapun, 2009), CMC rice stubble/glycerol film (Rodsamran and Sothornvit, 2017) and CMC bagasse pulp film (Kamthai and Magaraphan, 2017).

1.6 Plastics as a film

According to ASTM D 882-02 (2002), the word ‘plastic’ is used to describe the easily deformable state of material. In its everyday use the word ‘plastics’ is more frequently refers to describe the vast range of materials based on macromolecular organic compound. Plastics are used as food packaging materials since they offer a wide range of appearances and performance properties that derive from the inherent features of the individual plastic material (Kirwan and Strawbridge, 2003). Plastic is also used extensively in food industries as they provide choices of transparency, colour, heat resistance, heat sealing and good barrier against moisture and gasses. Besides, these plastics are cost effective, lightweight, and moldable to make different sheets, shapes and structures. Further innovation of plastics are also seen in film that could be used as flexible materials in the form of bags, sachets and pouches as well as cling, stretch and shrink wrapping. In 2003, Kirwan and Strawbridge in their book stated that, plastic film is defined as a film that has the thickness of less than 100µm.

The properties of plastic films and sheets are dependent on the type of plastic as well as the method of manufacturing the film, such as coating or lamination. Coatings are applied to the surfaces of plastic films to improve their heat-sealing and barrier properties, while lamination is a process that combines two or more layers of plastics together with the help of adhesives (Kirwan and Strawbridge, 2003). Over the past few years, the need to produce materials to diminish environmental impact of packaging has been deliberated. Biodegradable polymers have been scrutinised as a new alternative for packaging materials. In 2016, Langhe and Ponting reported that bio-based polymers could not solely meet material properties required of current produced blend, layered or composite products. Wang et al., (2000) demonstrated that it is possible to produce layered films using biodegradable polymers in combination with commercial thermoplastic material. This innovation could be used to achieve collegial property of packaging materials by improving their barriers and mechanical properties.

1.6.1 Multi-layered films in food industry

Food should be packaged as appropriately as possible in order to maintain its visual appearance, texture, flavour, nutritive value as well as its safety. Food packaging can protect the foods from environmental influences such as moisture, light, oxygen, microorganisms, gases, dusts and odours. A good food packaging material must have certain characteristics such as being inert, have perfect barrier properties, be recyclable could overcome deterioration of food and drinks (Pulingundla, 2012).

Multilayer coextruded or laminated flexible films are those that comprises two or more polymers to produce layered composites. It is a notable development in the modern packaging technology. Laminated flexible films can be produced through multistep lamination of polymers, which require separate handling of individual layers, or through extrusion process. Extrusion is a process that allows the simultaneous combination of two or more polymer materials inside a die or feed block to produce layered structures. The development of multi-layered films is important in order to enhance the properties of the final film produced. This overcomes the limitation imposed by the use of a single polymer component that may not meet the entire end use requirement due to the lack of certain properties. According to Tarantili and Kiose (2008), multilayer materials combine a number of desirable properties such as mechanical strength, permeability to gases and water vapour and machinability, provided by different polymer components of the film.

A search in the literature revealed that there are a few studies which developed multilayer films from combination of synthetic and biopolymer layers. In 2012, Kurek found that PET/chitosan/PE multilayer film exhibited similar performance of oxygen permeability to that of PE/EVOH/PE film while Bugnicourt et al. (2013) demonstrated that PET/whey protein/PE exhibited similar results for the water vapour transmission rate.

1.6.2 Polyethylene/Polyethylene Terephthalate (PE/PET) as films

Polyethylene (PE) is a thermoplastic polymer that belongs to the polyolefin family. The properties of PE depend on the degree of chain branching. PE occurs in two main forms, which are low density polyethylene (LDPE) and high density polyethylene (HDPE). In 1984, Schwarzenbach reported that LDPE is extensively used in manufacturing of films. LDPE is produced by free radical initiated polymerisation of ethylene that is carried out under high pressure of 1500 to 3000 atm and at high temperature of 80 to 300^{o}C. Compared to LDPE, HDPE is produced using transition metal catalysts catalysis resulting in a more linear and higher degree of crystallinity compared to LDPE (Ross and MacAdams, 1996).

Polyethylene terephthalate (PET) is a polymer produced from a reaction of ethylene glycol (EG) with tetraphtalic acid (Jabarin, 1996). PET is linear thermoplastic polyester used as synthetic fibre, moulding material, as well as films. The production of PET can be divided in two stages. During the first stage, the process involves the reaction of terephtalic acid with 1, 4-ethanediol at 150^{o}C to produce dimers and trimers with two hydroxyl end groups. This process involves the removal of water. In second stage, the process continues by heating the mixture to 260^{o}C. At this stage, PET is formed through a polycondensation reaction resulting in high degree of polymerisation.

According to McKeen (2011), PET exhibited high glass transition and melting temperature due to the presence of phenylene group in the main chain that imparts the backbone rigidity. PET also carries good barrier properties and has the ability to exist in either an amorphous or a crystalline state. Thin film and fibre of PET are produced by extrusion and quenching at ambient temperature. PET tends to crystallise over time, which affects the properties of the polymer that leads to dimensional changes such as shrinkage or warping (Benson, 2012).

Due to its low cost, good processability, high impact and excellent chemical resistance as well as good electrical insulation properties, PE has been used widely as film in wrapping food, injection and blow moulded parts such as bottles for foodstuffs and household chemicals. As reported by McEwan et al., (2002) in his book entitled ‘Structure and properties of commonly recycled polymers’, PET exhibits a higher value of thermal expansion coefficient, glass transition and melting point compared to LDPE and HDPE. These properties make PET more durable and resistant to most organic solvents and mineral acids (Benson, 2012). Therefore, a combination of PET and PE could improve the properties of films produced. According to Flight Plastic UK, (2017), PE/PET film is an excellent packaging material. It has been used in sealing application as well as to pack dairy product such as cheese. PE/PET film is a transparent film with good barrier properties and a good chemical resistance. It is easily recyclable and environmentally safe. Previous study by Luis et al., (2011) reported that water vapour permeability (WVP) of PET was between 5.80 to 22.92 $gmm^{-2}s^{-1}Pa^{-1}$ while oxygen permeability (OP) was recorded at 0.098 to 0.494 $mlmm^{-2}day^{-1}Pa^{-1}$ at temperature of $23^{o}C$. On the other hand, PE exhibited the values of 1.741 to 3.482 $gmm^{-2}s^{-1}Pa^{-1}$ for WVP and 7.18 $mlmm^{-2}day^{-1}Pa^{-1}$ for OP.

PE/PET can also be used in combination with CMC. Due to the anionic characteristics of CMC, it is water soluble and has excellent film forming properties. It has potential to be used as one of the components in multilayer films. CMC exhibits a hydrophobic polysaccharides backbone with many hydrophilic carboxyl group (Ballesteros et al., 2018). These properties allow CMC film to trap the moisture. On the other hand, the use of CMC on PE/PET film could minimise the water vapor permeability that permeates from surrounding. Therefore, the combination of PE/PET/CMC materials to produce packaging film could significantly enhance the quality of packed food product. This multi-layer film or laminates can be applied to wide range of products such as fruit, vegetable, poultry and meat product.

1.7 Plasticiser as additives used in film making.

In order to produce a film with good mechanical properties, plasticisers need to be blended together with the polymer used. According to Sothornvit and Krochta (2005), plasticisers are defined as low molecular weight, high boiling point, non-separating substances, added to polymers in order to alter and improve their flexibility, extensibility and processability. For example in 1983, Gachter and Muller found that the addition of plasticiser to films or plastics could lower their melting temperature, elastic modulus and transition temperature of polymers without altering their chemical nature of the macromolecules. The degree of flexibility of plasticised material macromolecular depends on the type and the amount of plasticiser added.

In another study conducted by Romero-Bastida et al., (2005), the addition of plasticiser was found to reduce the intermolecular forces between the polymers by interacting with them through hydrogen bonding. As a result, strong intra molecular interactions of polymers are loosened and the mobility of polymer chains is increased. This finding has also been supported by the work of Ghasemlou et al. (2011) who mentioned that when plasticiser was added at the correct level, it improves the mechanical properties of the films. Furthermore, Garcia et al. (2000) in her study highlighted that films produced using plasticisers are more flexible and have significant changes in barrier properties. Additionally, Ghanbarzadeh et al. (2010) reported that plasticisers are usually added to the film forming solutions before casting and drying procedures to overcome brittleness.

For bio-based polymer films, plasticisers can be divided into water-soluble and water insoluble (Siepman et al., 1998) types. The most commonly used plasticisers are glycerol, sorbitol and polyethylene glycol. According to Zhang and Han (2006), polyols have been found to effectively plasticise hydrophilic polymers. In my study, glycerol was used as plasticisers. Addition of glycerol is one of the most broadly used procedures in making of films and has been successfully employed in the production of polysaccharide based films (Ahmadi et al., 2012; Ghasemlou et al., 2011; Piermaria et al., 2011). According to Ghasemlou et al., (2011), glycerol is a suitable plasticiser to be used with water-soluble polymers due to its characteristics of being water soluble, polar with low molecular weight. Compared to glycerol, the use of sorbitol as plasticiser is rather limited due to its tendency to migrate to the surface of the film and its recrystallisation over time. Thus, film produced using sorbitol would lose its homogeneity and become relatively brittle (Li and Huneault, 2011) with time.

1.8 Aim and objectives of the research

1.8.1 Aim

The aim of this research was to utilise wheat bran produced as an agro-industry waste during milling process into cellulose derivatives such as carboxymethyl cellulose (CMC). The use of this agro-industry waste in this manner, not only it could help reducing the environmental issues and supplement its function as an animal feed, but it could also acts as a new source of potential bio-based polymer. Beside the common use of CMC as a food ingredient, this research was conducted in order to also explore the effectiveness of CMC as a potential biopolymer that can be used to develop a new film in food packaging technology. To date, relatively little is known about bio-based polymers that can be used as a component in the multi-layered structure. Therefore, through this study, the production of multi-layered films with biopolymers is envisage as alternative environmentals friendly packaging. Biopolymer coating could also present new applications for the agro-industry waste while safeguarding the performance of multi-layer films as well as enhancing recyclability. The findings obtained from this study are expected to provide and contribute to new knowledge on novel biopolymer materials that could be utilised from agro-industry waste to benefit as well as innovate current food packaging materials with superior physical, mechanical and barrier properties.

1.8.2 Research objectives and research questions

The research objectives (RO) and research questions (RQ) of this study are:

RO1: To synthesis carboxymethyl cellulose (CMC) from alkaline cellulose of wheat bran using different concentration of sodium hydroxide.

RQ1: How does different concentration of sodium hydroxide affect the yield of CMC wheat bran?

RO2: To investigate the effect of different sodium hydroxide concentrations added during etherification process on physicochemical and rheological properties of final CMC produced.

RQ2: How does different concentration of sodium hydroxide used during etherification influence the physical, chemical and rheology properties of CMC?

RO3: To investigate and observe the morphology of alkaline cellulose and CMC wheat bran using Scanning Electron Microscopy and contrast the two.

RQ3: Does the morphology of alkaline cellulose contrast with CMC produced?

RO4: To apply CMC with single or double layer coating on PE/PET film and produce multi-layered films.

RQ4: What are the best conditions (CMC volume, drying temperature and drying time) for CMC that can be used during film development?

RO5: To measure and analyse the physical and mechanical properties of PE/PET/CMC multi-layered films.

RQ5: Does the application of CMC coating on PE/PET film improve the physical and mechanical properties of films?

RO6: To investigate the effect of single or double layer CMC coating on water vapour permeability and film morphology of PE/PET/CMC multi-layered film.

RQ6: How does the addition of CMC coating on PE/PET film influence the water vapour permeability and film morphology?

1.9 Thesis outline

This thesis consists of seven (7) main chapters as follows:

Chapter 1 provides a literature review on wheat bran as agro-industrial waste that has the potential to be used as a sustainable material. It also provides a review highlighting the structure of cellulose, mercerisation and the bleaching process involved in cellulose extraction. This chapter also discusses on preparation and properties of carboxymethyl cellulose produced from other plant sources as well as their importance and application to multi-layer films in food industries. It also presents the aims, research objectives and research questions of this research.

Chapter 2 covers the materials and methods used in this research. The first part of the study demonstrates the synthesis of alkali cellulose and CMC using different concentrations of NaOH. The second part of the study elaborates on the methods used to characterise physical, chemical and rheological properties as well as surface morphology of CMC produced. The third part of this study covers the development of PE/PET/CMC multi-layered films using single or double layer CMC coatings. Furthermore, the methods used to measure physical and mechanical properties, water vapour permeability, morphology and structure of PE/PET/CMC films are discussed in more details. This chapter also explains the methods used to determine normality and homogeneity of the data obtained statistically.

Chapter 3 presents the results of synthesis of alkali cellulose and CMC from wheat bran, using different concentrations of NaOH. It also discusses the effect of NaOH on yield, physicochemical and rheological properties as well as microscopic analysis of CMC using Scanning Electron Microscopy.

Chapter 4 concerns our findings on the development of single or double layer CMC coating on PE/PET films. It also elaborates the effect of NaOH used during etherification of CMC on single or double layer CMC coating applied on PE/PET films. The effects of NaOH were observed and investigated with regards to physical and mechanical properties of films obtained.

Chapter 5 presents the findings on moisture absorption and water vapour properties of films produced. In this chapter, the effect of applied CMC on hydrophobicity or hydrophilicity of film have been investigated and highlighted. Additionally, this chapter also reveals and explores the morphology of films through analysis of surface and cross sectional structure of films produced.

Chapter 6 highlights the general discussion in relation with the findings obtained in Chapter 3, 4 and 5. This chapter connects and correlates the characteristics of CMC produced in Chapter 3 with the properties of films produced that have been discussed in Chapter 4 and 5. This chapter also highlights the effects of NaOH used during etherification of CMC and its potential as a relevant coating solution that can be used to develop films based on its purity, degree of substitution and viscosity.

Chapter 7 provides a summary of the research findings and conclusions based on all studies presented in Chapters 3, 4, 5 and 6. It also considers the limitation of current research work, as well as the implication and contribution of this work to the research development in this fields. Some possible recommendations for future works are also discussed.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Wheat bran brand Jordans was bought from Holland and Barrett, health food shops in Merrion Centre, Leeds. Chemical used in this study were sodium hydroxide pellets (Fisher Chemical), 30% w/v hydrogen peroxide (Fisher Chemical), 99% tetraacetylethylenediamine (Alfa Aesar), 99% acetic acid glacial (VWR Chemicals), 70% nitric acid (Fisher Chemicals), 99.8% ethanol (VWR Chemicals), sodium chloroacetate (Sigma-Aldrich), 99.5% isopropanol (Acros Organics), phenolphthalein (ACS), sodium chloride (Fisher Chemicals), glycolic acid (MP Biomedicals), silver nitrate (Merck, Germany), 2,7-dihydroxynaphthalene 97% (Acros Organics), 37% hydrochloric acid (Fisher Chemicals), acetone (VWR Chemicals), methanol (VWR Chemicals).

2.1.1 Preparation of wheat bran powder

All experiments in this research were conducted in the laboratory of School of Food Science and Nutrition, University of Leeds, unless stated otherwise. For preparation of wheat bran powder, wheat bran was placed on a stainless steel tray and dried in an oven at 60^{o}C for 24 hours in order to eliminate moisture in the sample. After drying, 200 g of dried sample was placed in a plastic jar filled with ceramic ball and crushed using ball mill (Orto Alresa, Madrid) for 2 hours at 20 rpm. It was sieved to pass through an 80 mesh screen using sieve stack to obtain a powder. Wheat bran powder was packed in a plastic bag and stored at room temperature until analysis. Figure 2.1 shows the image of ball mill and plastic jars used in this experiment.

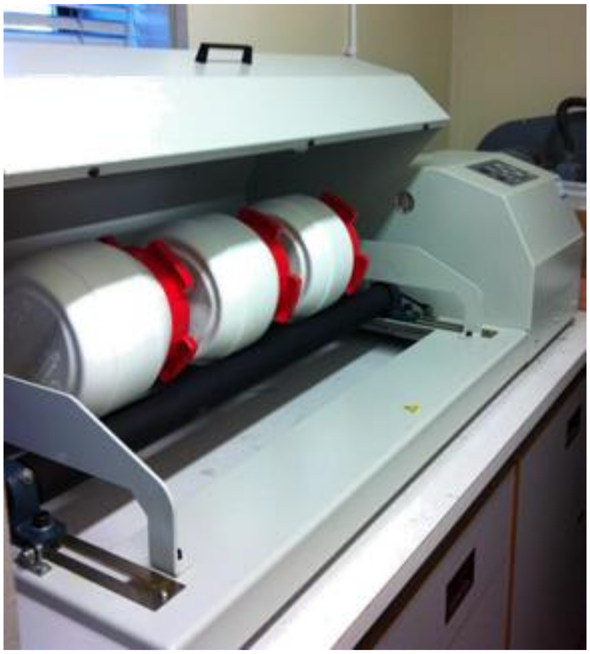

Figure 2.1 Ball mill

2.1.2 Preparation of sodium hydroxide solution

Three gram of NaOH pallet was weighed and placed into the beaker. 100 ml of distilled water was added into the beaker and NaOH pallet was let to dissolve using a stirrer. The concentration of NaOH for prepared solution was 3% (w/v). Similar procedures were used to prepare NaOH solution with the concentrations of 5%, 10%, 15%, 20%, 25% and 30% (w/v).

2.2 Synthesis of alkali cellulose and carboxymethyl cellulose (CMC) from wheat bran

2.2.1 Synthesis of alkali cellulose

Extraction of alkali cellulose from wheat bran was carried out using method by Wang et al., (2013) with a slight modification. At a ratio of 1:10 (w/w), 1 g of wheat bran powder was mixed with 10 g of distilled water and boiled for 10 minutes. Then, using a ratio of 1:20 (w/v),1 g of wheat bran powder was mixed with 20 ml of 3% (w/v) NaOH and left at 85^{o}C for 3 hours. After 3 hours, the mixture of wheat bran and NaOH were filtered and the residue was collected and washed with distilled water to achieve pH 7. Figure 2.2 shows the flow diagram of alkaline cellulose production.

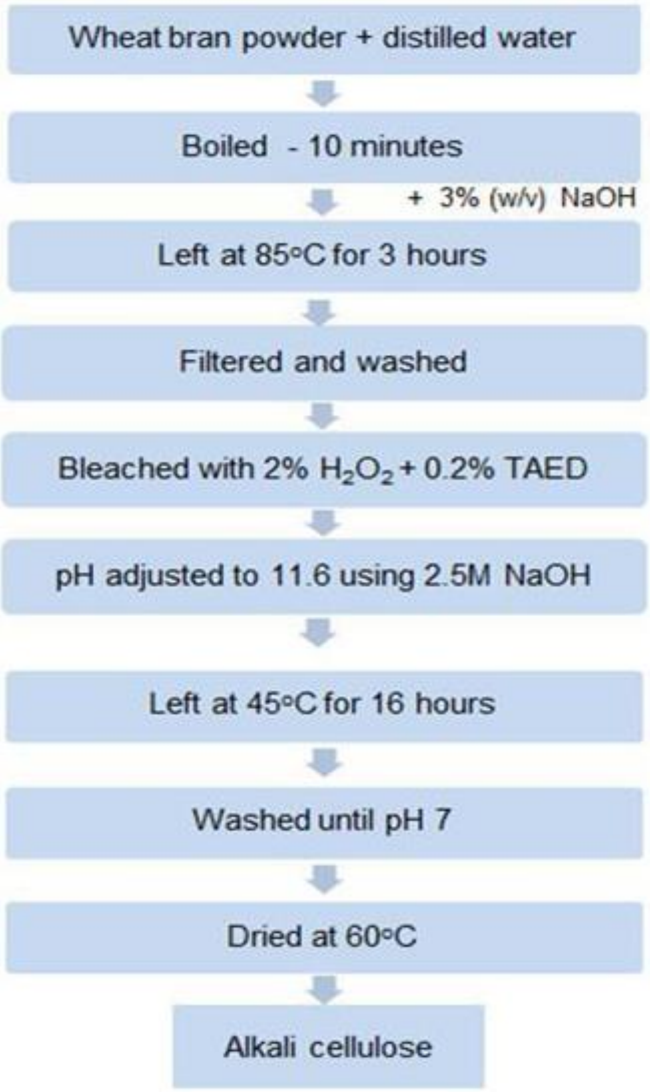

Figure 2.2 Flow diagram of synthesis of alkali cellulose

In order to remove the remaining hemicelluloses and lignin, the mixture of 2% (v/v) hydrogen peroxide (H_2O_2) and 0.2% (w/v) tetraacetylethylenediamine (TAED) solution were added to the pulp. The solutions were mixed together at 1:1 ratio and the pH was adjusted to pH 11.6 with the addition of 2.5M NaOH solution. The mixture obtained was known as liquor. Next, the ratio of liquor to wheat bran pulp was 25:1 (mL/g) and the mixture was left in a water bath at 45°C for 16 hours. The sample was then washed with distilled water until the solution reached a pH of 7. Sample was dried in an oven at 60°C for 16 hours and the yield of alkaline cellulose was calculated as follows:

$$\text{Yield of cellulose} = \frac{\text{Weight of dried cellulose}}{\text{Weight of dried wheat bran}} \times 100 \qquad \text{Eq. 1}$$

Eight batches of alkaline cellulose were extracted in this study. Data obtained were calculated to determine the mean and standard deviation.

2.2.2 Synthesis of CMC through neutralised and etherification process

For the production of CMC, 50 grams of alkaline cellulose powder was mixed with 20 ml of 5% (w/v) NaOH in a beaker. 100 ml of isopropanol was added into the mixture and stirred for 1 hour at 25°C. Next, 6 g of sodium chloracetate was added into the mixture and the temperature was raised up to 55°C for 3 hours. The slurry obtained was then neutralised with 90% of acetic acid glacial and it was filtered using Buchner funnel. The residue which composes of carboxymethyl cellulose was further washed with 60 mL of 70% (v/v) ethanol for five times and was dried at 60°C in an oven until constant weight was achieved. The whole process described for the extraction of CMC was repeated using five other concentrations of NaOH which are 10%, 15%, 20%, 25% and 30% (w/v). The percentage of carboxymethyl cellulose obtained was calculated using equation 2. Figure 2.3 presents the steps involved in the production of CMC from alkaline cellulose.

$$\text{Yield of CMC (\%)} = \frac{\text{Weight of prepared CMC (g)}}{\text{Weight of original cellulose used (g)}} \times 100 \qquad \text{Eq. 2}$$

The averaged mean value of CMC yield for each each treatment was calculated and test for normality and homogeneity of variances as in section 2.9.

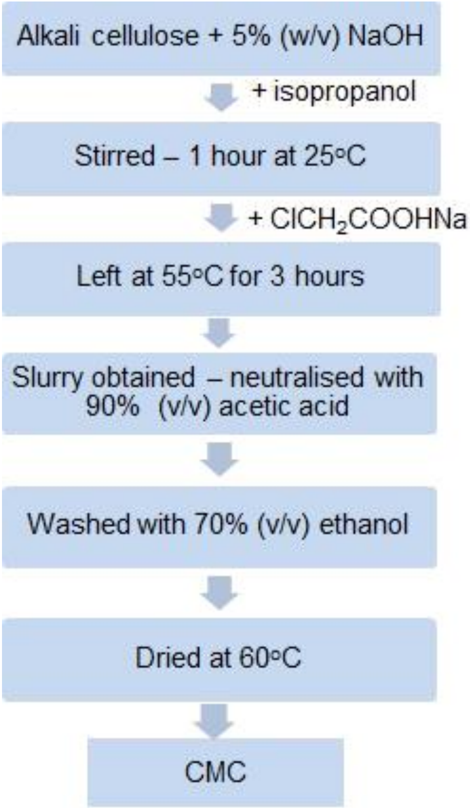

Figure 2.3 Synthesis of CMC

2.3 Characterisation of CMC wheat bran

2.3.1 Analysis on colour and chemical properties of CMC wheat bran

2.3.1.1 Colour measurement using Spectrophotometer

In this analysis, the colour of alkaline cellulose and CMC wheat bran powder was determined by Gretag Macbeth Colour Eye 7000A Spectrophotometer (X-rite) using CIE illuminant of D65 with 10 degree observer. The spectrophotometer was calibrated using white ceramic calibration tile. CMC powder was placed in a cuvette. The value of L, a and b were measured in triplicate. The total colour difference (ΔE) was measured by comparing the colour of alkaline cellulose and CMC wheat bran. Total colour differences and yellowness index (YI) were calculated using equations (3) and (4) adapted from Bolin and Huxsoll, (1991).

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \qquad \text{Eq. 3}$$

$$YI = \frac{142.86}{L} \times b \qquad \text{Eq. 4}$$

where L*, a*, b* are the colour values of alkali cellulose and L, a, b are colour values for CMC wheat bran.

2.3.1.2 Determination on purity of CMC

A method similar to the ASTM method of 2008 which determines the percentages of active ingredient in crude sodium carboxymethyl cellulose as described by Togrul and Arslan (2003) was used. 1.5 g of CMC was added to 100 mL of 80% aqueous methanol solution. The mixture was stirred, kept for 10 minutes and filtered using filtering crucible. The cake formed on filtered paper was washed with 100 mL of fresh 80% aqueous methanol and then dried at 60°C for 24 hours. During washing process, by-product such as sodium chloride and sodium glycolate will be removed (Mondal et al. 2015). The CMC content was calculated as follows:

$$\text{CMC content (\%)} = \frac{W}{W_o} \times 100 \qquad \text{Eq. 5}$$

where W_0 (g) is the weight of sample before washing and W (g) is the weight of dried sample.

2.3.1.3 Determination of sodium glycolate in CMC using UV-VIS Spectrophotometer

The content of sodium glycolate was calculated using the following formula:

$$\text{Sodium glycolate (\%)} = \frac{a \times 1.29}{b} \qquad \text{Eq. 6}$$

where (a*)* is amount of glycolic acid (mg) read from the calibration curve, (b*)* is the dried weight of sample in (g) and 1.29 is a factor for converting glycolic acid into sodium glycolate.

In this experiment, calibration curve for glycolic acid was prepared using the method adapted from Mondal et al., (2015). Different amount of glycolic acid reference solution at concentrations of 0, 1, 2, 3, 4 mg/ml were poured into five graduated 100 ml volumetric flasks. Five millilitre of distilled water and 5 ml of glacial acetic acid were added into each flask and made up with acetone to the filling mark and mixed. In a separate 100 ml graduated flask, a blank with 5 ml of water and 5 ml of glacial acetic acid was prepared using the same procedures. Two millilitre of each solution was pipetted and transferred into 25 ml graduated flasks. Acetone was evaporated in water bath for 20 minutes and cooled until it reached room temperature (23^{o}C). Next, 5 ml of 0.1 g/L 2, 7-dihydroxynaphtalene solution was added, and mixed thoroughly. An additional of 15 ml 2, 7-dihydroxynaphtalene solution was incorporated into the same volumetric flasks and heated in boiling water bath for 20 min. It was then removed from the water bath, cooled and sulphuric acid was added until the filling mark. At this stage, the colour of solution changes from yellow to purple. All the procedures were conducted in a fume cupboard. Then, the absorbance of each sample was measured at 540nm against blank using Jenway UV-VIS spectrophotometer.

To determine the sodium glycolate content, 0.5 g of CMC powder was weighed and transferred into a 100 ml beaker. The sample was added with 5 ml of glacial acetic acid, followed by 5 ml of water and stirred. A further 50 ml of acetone was added while stirring followed by 1 g of sodium chloride. The sample was continuously stirred for several minutes to ensure complete precipitation and then filtered using filter paper. The filtrate was collected in a 100 ml graduated flask and 30 ml of acetone was added to wash the filtered cake. The filtrate was made up to 25 ml with acetone and mixed. Then, 2 ml of sample solution was pipetted and poured into 25 ml graduated flasks. Acetone was evaporated and 2, 7-dihydroxynaphtalene solution was added to all samples. The absorbance was read at 540nm. The quantity of glycolic acid (in mg) was inferred using the calibration curve.

2.3.1.4 Determination of sodium chloride in CMC using silver nitrate titration

Sodium chloride content was determined using a standard ASTM (2008) method. Five grams of CMC extract was placed in 250 ml beaker. Fifty millilitre of water and 5 mL of 30% hydrogen peroxide were added and the beaker was placed in a steam bath, until a clear solution was obtained. Another 5 ml of 30% (w/v) hydrogen peroxide was added as to reduce the viscosity of the solution. Then, 100 ml of water and 10 ml of 15.8 M nitric acid were added into the beaker once the solution was cooled. The solution was then titrated with 0.1N silver nitrate until precipitate was formed. The percentage of sodium chloride was calculated as follows:

$$\text{Sodium chloride (\%)} = \frac{V \times N \times 5.845}{G} \qquad \text{Eq. 7}$$

where *V* is a volume of silver nitrate solution added, *N* is a normality of the silver nitrate solution, *G* is a weight of the sample (g) and *5.845* is a molecular mass of sodium chloride/10.

2.3.1.5 Determination of moisture content in CMC

In this analysis, 3 g of the CMC extract was weighed to the nearest 0.001 g on a petri dish and then placed in an oven at 105^{o}C for 2 hours. After 2 hours, samples were weighed, placed in the oven for 30 minutes and weighed again. The procedure was continued until the weight loss was not more than 5 mg. The percentage of moisture was calculated as follows (ASTM, 2008):

$$\text{Percentage of moisture} = \frac{\text{Weight of sample after dried}}{\text{Weight of sample before dried}} \times 100 \qquad \text{Eq. 8}$$

2.3.1.6 Measurement on degree of substitution of CMC

The degree of substitution of CMC was determined to measure the substitution of carboxymethyl group added to the cellulose backbone which could affect the viscosity and rheology properties, hygroscopicity and salt tolerance of the solution. Five grams of CMC extract was dissolved by stirring 60 ml of 95% ethanol. Then, 10 ml of 2M nitric acid was added and the mixture was stirred using a magnetic stirrer for another 2 minutes. The mixture was heated to boil for 5 minutes, agitated further for another 5 minutes and left to settle for 30 minutes. The supernatant liquid was filtered and the precipitate was washed with 80 ml of 95% ethanol and then with 60 ml of 80% (v/v) ethanol at 60^{o}C. This is followed by washing with 40 ml of methanol. The precipitate was subsequently washed with 40 ml of methanol, before being dried in an oven at 105^{o}C for 3 hours.

0.5 g of CMC extract was placed in a beaker and 100 ml of distilled water was added and stirred. Twenty five millilitre of 0.5N sodium hydroxide was added and heated at 100^{o}C for 15 minutes. The heated solution was titrated with 0.3N hydrochloric acid after 2 drops of phenolphthalein was added to the solution. The titration was stopped when the solution turned to pink colour.

The carboxymethyl content and degree of substitution were calculated using the equation below:

$$\text{Carboxymethyl content (\% CM)} = \frac{(Vo - Vn) \times N \times 0.058}{M} \times 100 \qquad \text{Eq. 9}$$

$$\text{Degree of substitution} = \frac{162 \times \%CM}{5800 - (57 \times \%CM)} \qquad \text{Eq. 10}$$

where V_0 is a volume of HCl used to titrate blank (ml), V_n is a volume of HCl used to titrate sample (ml), N is a normality of HCl used, M is a weight of sample (g), Molecular weight of carboxymethyl group is taken as 58 (Elomaa et al., 2004).

All data obtained in section 2.3.1 were tested for normality and homogeneity of variances before analysed using specific statistical approaches such as Tukey's HSD, Dunnett's T3 and Bonferroni test as in section 2.9.

2.3.2 Rheological properties of CMC wheat bran solution

2.3.2.1 Measurement of reduced viscosity and intrinsic viscosity using Ubbedohle viscometer.

In this method, the solution of CMC was allowed to flow through a capillary and time taken to flow through a given length was measured (t_o). The measured time at a given temperature is proportional to the viscosity of the solution (η_o) at that temperature. The flow time for pure solvent (t_s) was also measured through the same capillary. By using this information, relative viscosity (η_{rel}) can be calculated by applying the following equation:

$$t_o / t_s = \eta_o / \eta_s = \eta_{rel} \qquad \text{Eq. 11}$$

At different concentration of polymer solutions, specific viscosity (η_{sp}) can be calculated as follow:

$$\eta_{sp} = (\eta_o / \eta_s) - 1 \qquad \text{Eq. 12}$$

Reduced viscosity (η_{red}) measures specific capacity of the polymer to elevate viscosity at certain polymer concentration and can be determined using equation below, where c is the polysaccharide concentration that expressed in g/ml:

$$\eta_{red} = \eta_{sp}/c \qquad \text{Eq. 13}$$

Thus, intrinsic viscosity [η] is given by:

$$[\eta] = \lim_{c \to 0} (\eta_{sp}/c) \qquad \text{Eq. 14}$$

According to the Mark-Houwink relationship as stated in equation (15), for every polymer-solvent system, intrinsic viscosity [η] is related to the molecular weight of polymer. However, different polymer has different solvent that need to be used together in order to measure the molecular weight. Each solvent used to dissolve polymer will have different value for constant and polymer shape factor.

In this experiment, 0.5 N NaOH was used as a solvent while CMC obtained in this study is a polymer. 0.5 g of CMC wheat bran was dissolved in 100 ml of 0.5 N NaOH solution to give a concentration of 0.5% (w/v). In this analysis, four different concentrations of CMC wheat bran ranged from 0.5%, 1%, 1.5% and 2% (w/v) were prepared for each CMC wheat bran sample. Blank sample that consists only 100 ml 0.5 N NaOH (0%) was prepared. A sample was poured into the Ubbelodhe viscometer before it was immersed in water bath.

The temperature of water bath was maintained at 25°C±0.1. The times taken by solvent and solution to flow from mark A to B as in Figure 2.4 were recorded. [η] was calculated based on the ratio of efflux time of solution to solvent. The value obtained for intrinsic viscosity can be used to calculate molecular weight of the CMC wheat bran using 'Mark-Houwink-Sakurada' equation.

$$[\eta] = K M^a \qquad \text{Eq. 15}$$

where K is the constant for solvent, a is the polymer shape factor, [η] is the intrinsic viscosity and M is the molecular weight of CMC. The constant value for 0.5 N NaOH (K value) and *'a'* were 5.37×10^{-4}dL/$g^{-1}$and 0.73 respectively. All measurements were carried out in triplicate. The average mean value and standard deviation were calculated.

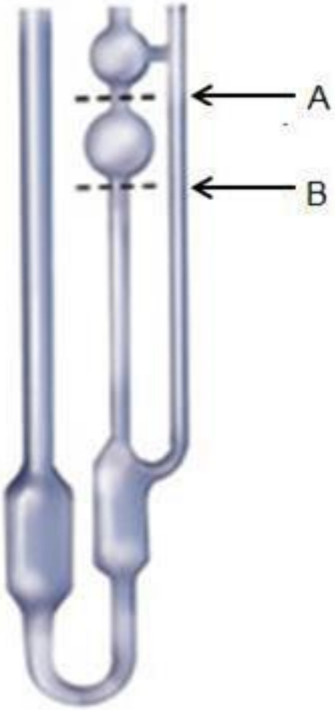

Figure 2.4 Ubbelohde viscometer. Time taken by solution to travel from mark A to B is recorded.

2.3.2.2 Viscosity measurement using rheometer.

In this experiment, dynamic viscosity of CMC solution was measured using Ultra Kinexus Rheometer (Malvern Instrument). CMC solution was prepared by adding 4 g of CMC powder into 100 ml distilled water to give a concentration of 4% (w/v). The solution was mixed for 10 minutes and centrifuged at 1500 rpm for 15 minutes using a centrifuge (Thermo Scientific CL2 Centrifuge), so as to remove the bubble formed during the mixing process. A cone geometry (CP2/60:PL65) with 2^o angle cone, 60 mm diameter and 65 mm plate was used in this experiment while the plate cartridge was used to control the temperature to 25^oC. Sample was placed on plate cartridge and was left for 5 minutes to achieve steady state. The operating gap between cone geometry and plate was set at 0.7 mm and the temperature was set at 25^oC.

Viscosities of CMC were measured using rSpace software (Malvern Instrument) and the starting shear rate was set at 0.1 s^{-1}. Graph of shear strain against shear rate was developed based on the data obtained to calculate shear viscosity. The behaviour of CMC solutions can be investigated from the fitting of Power Law behaviour to the shear stress and shear rate data plotted on log-log plot as shown in Figure 2.5.

2.3.3 Analysis on surface morphology of alkaline cellulose and CMC using Scanning Electron Microscopy (SEM)

Using a secondary electron detector, the effect of NaOH used during etherification process on characteristic of CMC produced can be investigated through surface morphology of sample. In this experiment, samples were placed on mounted stub in Sputter Coater 208 HR (Cressington) as in Figure 2.5 to coat the sample with platinum. The interaction between electron and atom in the sample produces various signals that contain information of sample were determined using Scanning Electron Microscopy (Camscan

Series III). This experiment was conducted at Astbury Biostructure Laboratory, Faculty of Biological Sciences, University of Leeds by authorised staff Martin Fuller.

Figure 2.5 Sputter Coater used to coat sample with platinum.

2.4 Preparation of PE/PET/CMC films and characterisation of films produced

2.4.1 Materials

Materials used in this experiment are CMC extracted using 5%, 10%, 15%, 20%, 25% and 30% (w/v) of NaOH (Fisher Chemical) as in section 2.2.2, glycerol (Fisher Chemical), distilled water and PE/PET bilayer film supplied by ILPRA UK.

2.4.1.1 Preparation of CMC solution

For the preparation of CMC solution, 1 g of CMC was mixed with 100 ml of distilled water. 0.2 ml of glycerol was used as a plasticiser and added into the solution. The mixture was agitated using magnetic stirrer on hot plate at 80^{o}C for 10 min. The solution was left to cool at room temperature and further centrifuged (Thermo Scientific CL2 Centrifuge) at 3000 rpm for 15 minutes in order to remove formed bubble. Similar procedure was used to prepare CMC solution for all CMC wheat bran obtained in section 2.2.2.

2.4.1.2 Preparation of PE/PET/CMC film with single or double layer CMC coating

For a single layer coating, CMC solution was casted onto PE/PET film by pouring 35 ml of CMC solution on PE/PET film. The dimension of PE/PET film was set to be 150 cm^2. The film was placed in the oven and the coating was allowed to dry at 27^{o}C for 12 hours. After 12 hours, the film was taken out from the oven, allowed to cool before covered with aluminium foil and placed in a polyethylene bag.

For double layer CMC coating, after PE/PET/CMC film was dried for 12 hours, another 35 ml of CMC solution was poured and casted onto PE/PET/CMC film. Then, films were dried in an oven at 27^{o}C for another 12 hours. After drying process, all films were allowed to cool before covered with aluminium foil and placed in a polyethylene bag.

2.5 Characterisation of PE/PET/CMC film

2.5.1 Measurement on physical properties of PE/PET/CMC film

2.5.1.1 Film thickness measurement

Thickness of films was measured using a micrometre (Mitutoyo Corp, MDC-1 SB digital micrometre, Japan) with accuracy of 0.001mm. Ten random positions of film were measured and the values obtained were recorded.

2.5.1.2 Determination on moisture content of film

In this analysis, moisture content of PE/PET/CMC films was determined using ASTM (2000) method. The determination of moisture content in PE/PET/CMC film was to measure the quantity of water in film formed. Film was cut into 2 cm x 2 cm dimension and the initial weight of films was recorded. Film was dried in an oven at 90°C for 24 hours until it reached a constant weight. Then, the weight of dried film was recorded and moisture content was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{W_o - W_d}{W_d} \qquad \text{Eq. 16}$$

where W_o is an initial weight of film and W_d is a weight of film after drying.

2.5.1.3 Determination of film solubility

The solubility of PE/PET/CMC films in distilled water was conducted by cutting the film into 1 cm x 3 cm dimension. Method by Shojaee-Aliabadi et al. (2013) was used by drying the film in an oven at 90°C for 24 hours. During this process, water content in film was eliminated through drying process. Then, the weight of film after drying was recorded. This weight is referred to initial weight of film. The film was put into the beaker and 50 ml of distilled water was added. Sample was immersed in water bath and the temperature was set at 25°C for 6 hours. After that, any remaining film was removed by placing the film on filter paper and dried at 90°C for another 24 hours. The weight of dried film was recorded and the percentage of film solubility of film was calculated as follows:

$$\text{Film solubility (\%)} = \frac{W_o - W_d}{W_o} \times 100\% \qquad \text{Eq. 17}$$

where W_o is an initial weight of film and W_d is a weight of dried film.

2.5.1.4 Measurement of moisture absorption

Moisture absorption was measured to determine the uptake of moisture of film from surrounding area thus determining the hydrophilicity or hydrophobicity of film. In this analysis, films were cut into a 2 cm x 2 cm dimension and it was dried in a desiccator that contained silica gel for 24 hours before the analysis was carried out. After 24 hours, the initial weight of films was recorded. All PE/PET/CMC films produced in section 2.4.1.2 were placed in desiccators that contained saturated salt solution. Saturated salt solution was prepared by dissolving NaCl in distilled water until the solution is concentrated. The desiccator was then stored in incubator at 25°C. The weight of the films was measured at 24 hours interval until it reaches constant weight. The percentage of moisture absorption was calculated using following equation:

$$\text{Moisture absorption (\%)} = \frac{W_a - W_o}{W_o} \times 100\% \qquad \text{Eq. 18}$$

where W_a is a weight of film after certain hours and W_o is an initial film weight.

2.5.1.5 Colour and transparency measurement of films

Colour and transparency of film were evaluated using Gretag Macbeth Colour Eye 7000A Spectrophotometer (X-rite) along with CIE illuminant of D65 and a 10 degree of observer. CIELab colour parameters were employed to measure the degree of lightness (L), redness (+a) or greenness (-a) and yellowness (+b) or blueness (-b) of the film. Film specimens were measured on the surface of white standard plate with colour coordinates $L_{standard} = 95.670$, $a_{standard} = 0.001$ and $b_{standard} = 0.464$. The total colour differences (ΔE) and yellowness index were calculated using equation (19) and (20). For transparency value, a spectrum for each film was recorded in wavelength between 250 and 800 nm. The transparency value at 600 nm (T_{600}) was calculated using equation (21) as reported by Han and Floros, (1997). The measurements for colour and transparency of films were conducted in triplicate.

$$\Delta E = [(L_{film} – L_{PET/PE})^2 + (a_{film} – a_{PET/PE})^2 + (b_{film} – b_{PET/PE})^2]^{1/2} \quad \text{Eq. 19}$$

$$YI = \frac{142.86 \times b}{L} \quad \text{Eq. 20}$$

$$\text{Transparency} = \frac{\log (T600)}{X} \quad \text{Eq. 21}$$

where $L_{PET/PE}$, $a_{PET/PE}$, $b_{PET/PE}$ are the colour values of PE/PET film and L_{film}, a_{film}, b_{film} are the colour values for PE/PET/CMC films.

2.5.1.6 Contact angle measurement

The wettability of film was characterised by measuring the contact angle formed by a liquid on the film's surface. Ojagh et al. (2010) reported that contact angle is defined as the angle between the baseline of the drop and the tangent line at the point of contact of the water droplet with the surface. In this study, the wettability of film was determined by sessile drop method. Five microlitre of distilled water was carefully dropped on the surface of film using a microliter syringe (Hamilton). The light source was used as to illuminate the sample and enhance the visibility of the outline of the drop. The image of droplet on PE/PET/CMC film was captured using Nikon Digital Single-lens Reflex camera right after the distilled water has dispensed from the micro syringe. The contact angle measurement was carried out at 23°C. The angle of tangent to the basis of the droplet was measured using ImageJ software (Java). In order to obtain a reliable contact angle for each film sample, this test was repeated six times and the mean value were calculated. All data obtained in section 2.5.1.6 were tested for normality and homogeneity of variances before analysed using specific statistical approaches such as Tukey's HSD, Dunnett's T3 and Bonferroni test as in section 2.9.

2.6 Mechanical properties of PE/PET/CMC wheat bran film using Texture Analyser

2.6.1 Measurement of tensile strength and elongation at break of films

The measurement of tensile strength was performed using a Texture Analyser TA.X.Plus (Stable Micro Systems) testing machine with tensile grips used to hold the films. Film was cut into 1 cm x 9 cm and was clamped between tensile grips with the gap of 5 cm. These tensile grips are responsible to pull both ends of a strip at a specified rate in opposite direction until it breaks. Force and deformation were recorded during extension at 50 mm/min. The weight of the load cell was calibrated at 5 kg. Tensile strength and elongation at break were calculated using equation (22) and (23) as outlined in ASTM D882 (2002). The experiment was carried out at room temperature and five separate film pieces with 1 cm x 9 cm dimensions were used to achieve dependable data. The averaged mean value was calculated while normality and homogeneity of variances for all treatments were analysed as in section 2.9.

$$\text{Tensile strength} = \frac{\text{Maximum force applied (F)}}{\text{Cross sectional area of film (A)}} \qquad \text{Eq. 22}$$

$$\text{Elongation at break (\%)} = \frac{\text{Length of film at deformation}}{\text{Original film length}} \times 100 \qquad \text{Eq. 23}$$

2.7 Measurements of water vapour transmission rate (WVTR) and water vapour permeability (WVP) of PE/PET/CMC film.

The WVTR and WVP of PE/PET/CMC films were measured using the ASTM E96-00 method (2000). This method also known as the ‘cup method’. A permeability cup (Sheen UK) with 3.6 cm diameter was filled with 10 g of silica gel as a desiccant as in Figure 2.6. A film specimen with dimension of 4 cm x 4 cm was placed on the permeability cup and O ring was placed on the film. The cup was sealed tightly and was placed in desiccator that contains saturated salt solution. The desiccator was then placed in an incubator at 25^{o}C with relative humidity of 75%. The cup was weight after 2 hours and subsequent measurements were made at 24, 48, 72, 96 and 102 hours. The changes of moisture gain (Δm) obtained by permeability cup was plotted over time (t). The slope produced by this line was divided by exposed film area (A) as stated in Equation (24):

$$\text{Water vapour transmission rate} = \frac{\Delta m}{\Delta t . A} \qquad \text{Eq. 24}$$

The difference in concentration of water across the film will generate a driving force. This is known as water vapour permeability. The difference of water vapour pressure at 25^{o}C with 75% relative humidity was 2375.80 Pa. This WVP of film can be calculated using Equation (25) as follows:

$$\text{Water vapour permeability} = \frac{\Delta m . x}{\Delta t . A . \Delta p} \qquad \text{Eq. 25}$$

where m is a weight of moisture gain (g), x is a film thickness (mm), A is an area of exposed film surface (m^2), t is time (s) and Δp is water vapour pressure difference between two sides of the films (Pa).

Figure 2.6 Picture of permeability cup (Sheen UK), (b) Permeability cups with saturated salt solution in desiccator.

2.8 Analysis on film morphology and film structure

The microstructure analyses of PE/PET/CMC films with single or double layer CMC were observed and investigated using Scanning Electron Microscopy (Camscan Series III). Surface structure, crystalline formation and the structure of film layers were evaluated in this study. The principles and techniques used in this experiment are similar and already been explained in section 2.3.3. Films were cut into a tiny strip and placed on the stubs. It was then coated with a thin layer of platinum using Sputter Coater 208 HR (Cressington). All samples were observed under high vacuum condition at accelerating voltage of 3kV. The surface and cross-sectional morphology of films were observed at a magnification of 500x and 3000x respectively.

2.9 Statistical analysis

Data obtained in this experiment was analysed using SPSS version 22 (IBM). At first, the normality and homogeinity of data need to be identified. To determine the normality of data, Kolmogorov-Smirnov and Shapiro-Wilk tests can be used as they compare the scores in the sample to a normal distributed set of scores (Field, 2005). If the test is not significant ($p>0.05$) the distribution of sample is normal. However, if the test is significant ($p<0.05$), the distribution of data is not normal. In my analysis, Shapiro-Wilk test was chosen as it is more accurate compared to Kolmogorov-Smimov test (Field, 2005). On the other hand, homogeneity of variance is the assumption means that the variances should be the same throughout the data. In this study, Levene's test was used to determine if the hypothesis of the variances in the groups are equal. Thus, if Levene's test is not significant ($p>0.05$), the variance are said to be equaled. However, if the test is significant ($p<0.05$), the variance is not equal.

Once the distribution and homogeneity of data has been identified, means of sample need be compared through one way analysis of variance (ANOVA): Post hoc multiple comparisons test. One way ANOVA can be used to evaluate situation in which there are several independent variables as this analysis will discover on how independent variables interact with each other. Post hoc multiple comparisons were carried out as to determine the significant differences between means. Depending on the result of the normality and homogeneity tests, the following procedures were used:

i) normal distribution ($p>0.05$) and the homogeneity of variances are equal ($p>0.05$) – classic (or Fisher's) parametric one-way ANOVA and Tukey's Honestly Significant Difference test (Tukey's HSD) test was used. To test the significance differences among sample means.

ii) normal distribution ($p>0.05$) and the homogeneity of variances are not equal ($p<0.05$) – Welch's parametric ANOVA test was chosen and Dunnett's T3 test was used. This pairwise comparison was used when the variances are unequal.

iii) not normal distribution ($p<0.05$) and the homogeneity of variances are equal ($p>0.05$) – the non-parametric Kruskall Wallis one-way ANOVA test was used and continued with Bonferroni correction. A correction is applied as to control the error rate when multiple significance tests are carried out.

In this study, all tests were conducted at 95% confidence level.

CHAPTER 3

SYNTHESIS AND CHARACTERISATION OF CARBOXYMETHYL CELLULOSE FROM WHEAT BRAN

3.1 Introduction

Wheat bran is an abundant industrial by-product generated during milling operation. Wheat is the major grain consumed in the world with 33% of total grain production followed by rice with 25% (Slavin et al., 1999). Bergmans et al., (1966) found that the major constituents in wheat bran are non-starch polysaccharides (NSPs) (46%), starch (10-20%), protein (15-22%) and lignin (48%). The main NSPs present are arabinoxylan followed by cellulose and (1-3), (1-4)-β-glucan with 70%, 24% and 6% respectively (Maes and Delcour, 2001). Because of its high abundance and low in cost, wheat bran is commonly used as an ingredient in human food and livestock feed (Cherubini, 2010).

Cellulose and cellulose derivatives can be produced from wheat bran as a new source of CMC. Cellulose based materials are being widely used as they offer advantages such as being edible, biocompatibility, good barrier properties and have an aesthetic appearance as being nontoxic and non-polluting (Vásconez et al., 2009). Cellulose is a linear, high molecular weight polymer, natural, renewable and biodegradable. It is the main constituent of cell walls of higher plant such as trees, cotton, flax, sugar beet residues, ramie and cereal straw with these plants representing the main sources of cellulose (Perez & Mazeau, 2004; Cash & Caputo, 2010). Cellulose is commonly converted into useful derivatives through etherification process.

CMC can be produced by conversion of alkaline cellulose using sodium hydroxide and organic solvent such as isopropanol together with sodium chloroacetate. CMC is the most important water-soluble cellulose derivative, with many applications in food industry, cosmetics, pharmaceuticals and detergent (Olaru et al., 1998) and as an ingredient used in paper and textile industries. It has been found to have no harmful effects on human health (Schmitt et al., 1998). Other authors reported that CMC can be obtained from agricultural waste such as sugar beet pulp (Togrul & Arslan, 2003), cavendish banana pseudo stem (Adinughara et al., 2005), sago waste (Pushpamalar et al., 2006), orange peel (Yasar et al., 2007), papaya peel (Rachtanapun, 2009), palm kernel cake (Bono et al., 2009), mimosa pigra (Rachtanapun & Rattanapanone, 2011), durian rind (Rachtanapun et al., 2012), cotton gin waste (Haleem et al., 2014), water hyacinth (Saputra et al., 2014) and corn husk (Mondal et al., 2015).

To date, there is no study made on the synthesis of CMC from wheat bran, which is a by-product of milling in the production of refined grains. It is also locally available and cheap. Due to this situation, the aim of this study is to synthesis alkaline cellulose and CMC from wheat bran using various concentrations of NaOH that ranged from 5% to 30% w/v. In this study, the characteristics of CMC produced were discussed based on its physicochemical, rheology and morphology properties. The conversion of wheat bran to cellulose derivatives not only helps in reducing the agriculture waste from milling operation but could also be used as a new source of biopolymer. Furthermore, the aim of this study is to investigate the effect of sodium hydroxide used during etherification process on the characteristics of CMC produced.

3.2 Results and discussion

3.2.1 Alkali cellulose and carboxymethyl cellulose from wheat bran

Synthesis of alkaline cellulose from wheat bran has been described in details under section 2.2.1. The activation reaction between cellulose and NaOH is referred as mercerisation process. For this process, the concentration of NaOH used in this study was 3% (w/v). Previous research by Ibrahim et al., (2011) established that NaOH is responsible for the swelling of alkaline cellulose and it can act as a diluting agent. It also promotes good penetration into the cellulose structure. Hydroxyl ion from NaOH used to extract wheat bran could cause swelling of cellulose and disruption of the intermolecular hydrogen bonds between cellulose and hemicellulose (Bian et al., 2012), thus solubilise hemicelluloses and produces residue enriched with cellulose. This hydroxyl ion (OH^-) from NaOH combines with hydrogen ion (H^+) from hydroxyl group of cellulose molecule to form water (H_2O) molecule. Figure 3.1 represent the reaction that occurred during mercerisation process of cellulose fibre.

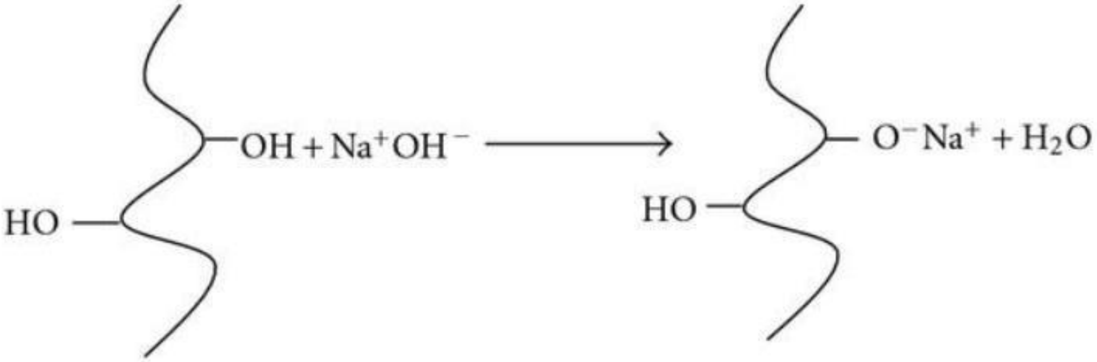

Figure 3.1 Effect of NaOH on cellulose backbone.

In this study, H_2O_2 was used to bleach and brighten the pulp of wheat bran while TAED was used as a bleaching activator. The use of H_2O_2 may improve the delignification process, reduce chlorinated organic compounds and effluent the colour of samples (Sundara, 1998). According to Hsieh et al., (2007), TAED may improve pulp properties such as pulp strength and bulk. Previously, there are a vast number of published studies that describe on the use of H_2O_2 and TAED. Zhao et al. (2012) stated that H_2O_2 is an environmentally bleaching agent that liable for high yield pulp bleaching processes. TAED acts as bleaching activator as bleaching process can be held at lower temperature and thus lessen the fibre damage. Hashem et al. (2003) have demonstrated successfully on the use of TAED as an activator together with H_2O_2 in cotton fabric bleaching. Due to its outstanding benefits, Zhao (2010) and Sain et al. (2009) also studied the application of this process in pulp bleaching. Figure 3.2 provides the images of wheat bran before and after bleaching process obtained during experimental work as elaborated in section 2.2.1.

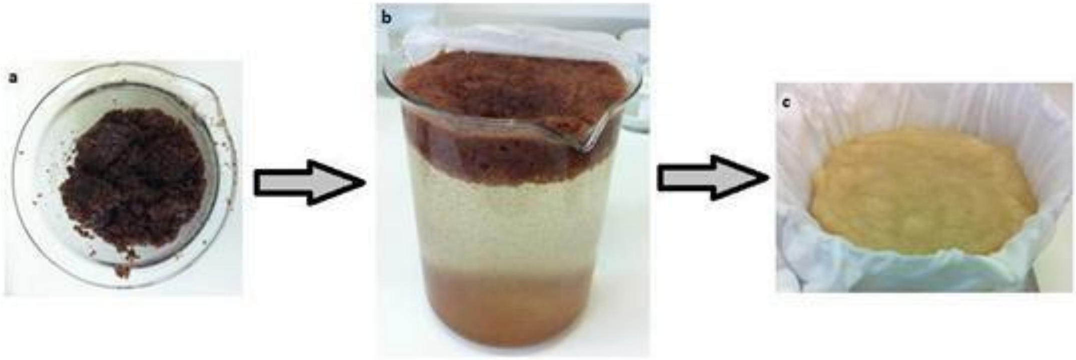

Figure 3.2 (a) Wheat bran after mercerisation process, (b) Wheat bran in H_2O_2 and TAED solutions, (c) Bleached wheat bran.

Hemery et al., (2007) in his reviewed on dry processes to develop wheat fractions reported that cellulose content in wheat bran was 11% of dry matter and it is highly concentrated in pericarp tissue. In 2009, Kamal-Edlin and his friend published a paper on characterisation of industrial rye and wheat bran from the Nordic countries. They found that cellulose in Swedish wheat bran accounts between 9.3 to 12.1% of dry matter. In another study conducted by

Merali et al., (2015), the cellulose content was found to be at 18.5% (w/w). In my study, eight batches of alkaline cellulose have been produced from wheat bran. Overall, the yields of alkaline cellulose obtained from these eight batches of extraction were between 13.02 to 16.83%. The average value of alkali cellulose obtained from this study was 15.03±1.46%. Thus, the value obtained in this study was within the range attained by previous researches. Figure 3.3 (a) shows the pictures of dried wheat bran used while Figure 3.3 (b) shows the image of alkaline cellulose obtained in this study.

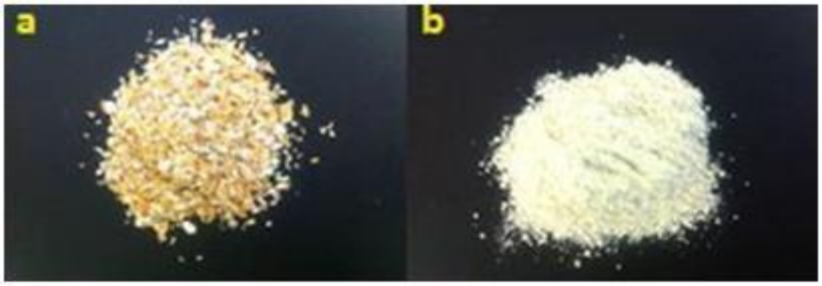

Figure 3.3 (a) Dried wheat bran, (b) Alkaline cellulose

3.2.2 Effect of NaOH concentrations on CMC properties.

3.2.2.1 Yield of CMC

Figure 3.4 illustrates the yield of CMC from wheat bran extracted using different concentrations of NaOH. At 5% (w/v) NaOH concentration, the yield of CMC wheat bran increased from 1.26 g/g up to 1.99 g/g when the concentration of NaOH was at 20% (w/v). However, the yield of CMC wheat bran decreased to 1.79 g/g when the concentration reached 30% (w/v). The percentage yield of CMC wheat bran obtained in this study for all concentrations were more than 1 g/g due to the substitution of carboxymethyl group from sodium chloroacetate at cellulose backbone during etherification process. This result might be explained by the fact that the percentage yield of CMC is computed by dividing the weight of the dry CMC with the weight of cellulose, thus the amount of sodium chloroacetate reacted is not taken into account for the calculation. Therefore, higher percentages of CMC yield were obtained in this study. These results seem to be consistent with other research which found that the yield of CMC was more than 1g/g. Bono et al., (2009) reported that the yield of CMC obtained from palm kernel cake was 1.64 g/g while, Rachtanapun et al., (2012), stated that the yield of CMC extracted from durian rind was 1.65 g/g. Another researches such as Haleem et al., (2014) and Mondal et al., (2015) demonstrated similar findings with 1.44 g/g and 2.4 g/g for waste of cotton ginning and cornhusk respectively.

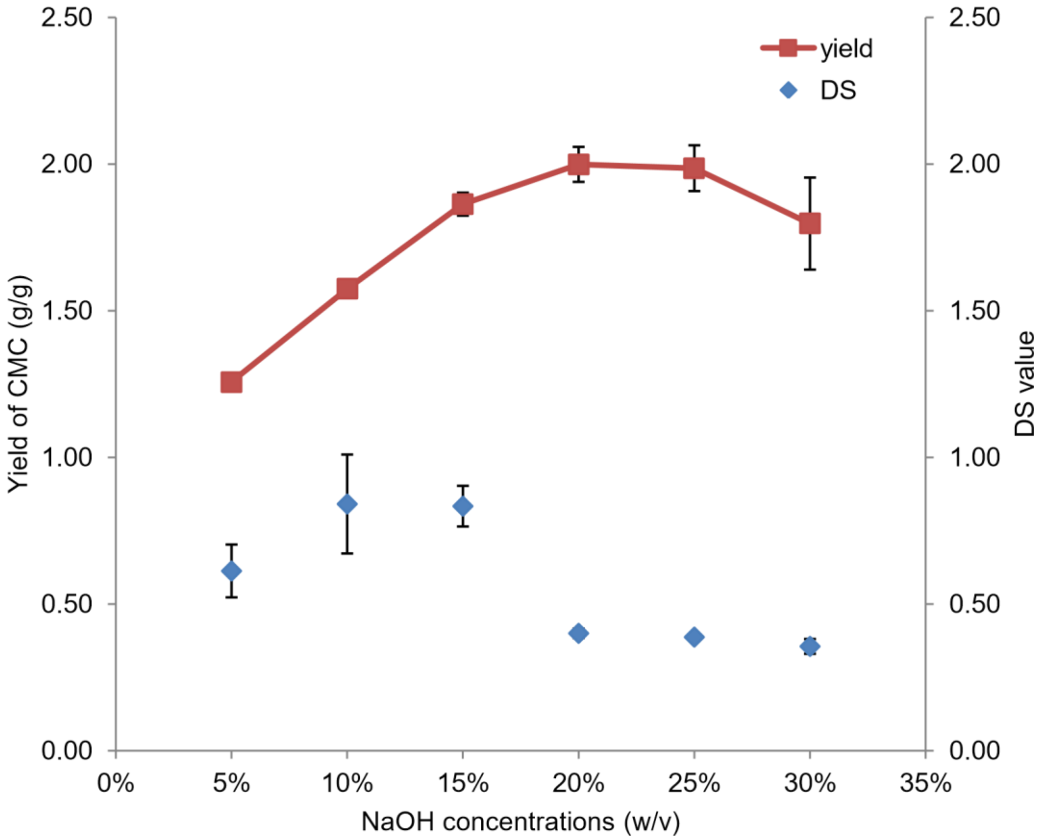

Figure 3.4 Yield and degree of substitution of CMC at different percentage of NaOH concentrations.

(Notes: Data represent the average of triplicate while error bars represent the standard deviation. Yield of CMC was statistically analysed using Dunnett T3 while DS value was using Bonferroni).

3.2.2.2 Degree of substitution (DS)

Carboxymethyl cellulose (CMC) is soluble in hot or cold water but insoluble in organic solvents such as ethanol. CMC is more hydrophilic if it has higher degree of substitution (DS). According to Heinze and Koschella (2005), the maximum value of DS for CMC is 3.0, but commercially available are between 0.4 to 1.5. In 1995, Borsa and Racz, reported that increasing the DS of CMC will enhance the solubility in water. Above 0.6, CMC will show good water solubility while if it less than 0.2, CMC will retain the fibrous character of the starting material and is not soluble in water. According to Keller, (1986) purified CMC is a white to cream colour, tasteless, odourless and free flowing powder. At high concentration of NaOH, the formation of sodium glycolate was increased thus lowering the DS and purity of CMC because partly the sodium chloroacetate molecule tends to react with NaOH (Barai et al., 1997).

Figure 3.4 shows the degree of substitution value for CMC etherified at different concentrations of NaOH. In this study, DS value was measured using potentiometric method as described in section 2.3.1.6. NaOH in aqueous form was added into the CMC solution and excesses NaOH was then titrated back with hydrochloric acid. Pushpamalar et al. (2006) in her published paper on optimisation of reaction conditions for preparing CMC from sago waste suggested that values obtained by potentimetric titration correspond to the absolute values of degree of substitution. As shown in Figure 3.4, the DS values of CMC produced ranged from 0.36 to 0.84. The highest DS value was shown by CMC extracted using 10% (w/v) NaOH while the lowest DS value was shown by CMC extracted with 30% (w/v). At 5% (w/v) NaOH, DS value was recorded at 0.61. This value elevated to 0.84 at 10% (w/v) NaOH and slightly reduces to 0.83 at 15% NaOH concentration. When the concentration was increased to 20% (w/v) NaOH, the DS value was decreased to 0.39 followed by 0.38 and 0.36 for 25% and 30% (w/v) NaOH respectively. Adinughara et al., (2005) and Waring and Parsons, (2001) reported that CMC obtained from the alkalisation of cellulose followed by a carboxymethylation process using sodium chloroacetate usually produce DS values that range from 0.4 to 1.3. Therefore, the DS values demonstrated by CMC in this study were in agreement with results obtained by previous researchers.

From the graph, initially it can be seen that DS values increased when the concentrations of NaOH increased. According to Haleem et al., (2014), the DS value of carboxylic group in CMC is the average number of hydroxyl group in the cellulose structure which was substituted by carboxymethyl groups at C2, C3 and C6. Olaru et al., (1997) reported that crystalline region in cellulose was changed to amorphous and atom at C2, C3 and C6 could be easily accessed by sodium monochloroacetate. However, when concentration of NaOH was at 15% (w/v), the DS values started to decrease at 20% (w/v). On the other hand, the yield of CMC displayed the highest value at 20% (w/v) concentration. This is due to the formation of sodium glycolate as by product in the synthesis of CMC. As reported by Barai et al., (1997), at high concentration of NaOH, polymer degradation was occurred and lowering the DS values. This can be explained by considering the reaction that occurred during carboxymethylation process where two competitive reactions take place simultaneously.

As reported by Pushpamalar et al. (2006), in the first reaction, cellulose hydroxyl and sodium chloroacetate in the presence of NaOH would produce CMC while in second reaction, NaOH reacts with sodium chloroacetate to form sodium glycolate. Based on Figure 3.4, above 15% (w/v) NaOH concentration, the production of sodium glycolate and sodium chloride starts to dominate thus lowering DS value of CMC. Therefore, in this study, it can be stated that increase in NaOH concentration will increase the yield of CMC but will lower the DS value due to the production of by-product. Similar observations have been reported for maize starch (Khalil et al., 1990), Cavendish banana pseudo stem (Adinugraha et al., 2005), sago waste (Pushpamalar et al., 2006), durian rind (Rachtanapun et al., 2012) and sugarcane bagasse (Hong, 2013).

3.2.2.3 Purity of CMC

The purity of CMC content can be measured by washing the crude CMC with 70% ethanol and absolute ethanol in order to remove the salts as described in section 2.3.1.2. The value of CMC purity will indicate the quality, grades and applications of CMC produced. To date, there is a little data published on crude CMC contents and its salts namely sodium chloride and sodium glycolate. A study conducted by Togrul and Arslan, (2003) in the production of carboxymethyl cellulose from sugar beet pulp using isopropyl alcohol as a solvent demonstrated that the purity content of CMC was 57% and 2.55% for sodium chloride content. This result was obtained at the following reaction conditions: 30% (w/v) concentration of NaOH at 30^{o}C for 60 minutes. In a similar paper, isobutyl alcohol was used as a solvent. Using a similar reaction conditions, higher percentage of CMC purity was recorded with 61.4% and 2.38% for sodium chloride. However, no data was reported on sodium glycolate content in their paper.

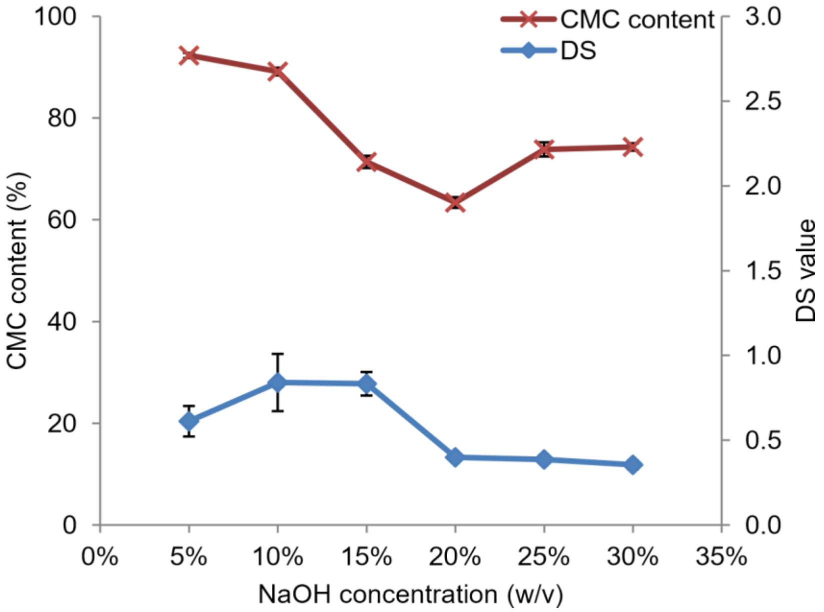

Figure 3.5 CMC content and DS value**.**

(Notes: Data represent means of n=3 measurements while error bars represent the standard deviation. CMC content and DS value were statistically analysed using Bonferroni)

Compared to the findings obtained in my study, the highest purity was observed at CMC extracted with 5% (w/v) NaOH concentration with the value of 92.30% as shown in Figure 3.5. This value decreased when the concentration of NaOH increased to 10% and 20% with the percentages of purity recorded were 89.14% and 63.40% respectively. However, when the concentration of NaOH increased to 25%, the percentage of purity increased to 73.84% and 74.33% at 30% (w/v) concentration. To explain this trend, Tongdeesoontorn et al. (2011) and Yaacob et al. (2011) mentioned that the degradation of cellulose is caused by chemical elimination of water and inter-molecular elimination of hydroxyl groups, which are necessary for carboxymethylation. As the use of NaOH increased during etherification, more sodium glycolate and sodium chloride were produced together with crude CMC. This result is also in accordance with study reported by Barai et al., (1997) who mentioned that higher NaOH influenced the formation of by-products. Besides that in this study, isopropanol was used as a solvent. Hence, the findings obtained in this section was further supports the idea of Pushpamalar et al., (2006) stated that the use of isopropanol as a solvent in efficiency of carboxymethylation reaction is higher as it provides miscibility and accessibility of the etherifying agent to the reaction center of the cellulose chain. Stigsson et al. (2006) also supported this statement and mentioned that cellulose backbone will become more uneven and the amount of substitution units on the C-6 position increased. As reported by Rachtanapun et al., (2012), higher NaOH tend to degrade the cellulose chains by alkaline hydrolysis forming sodium glycolate and sodium chloride as a byproduct, thus causing reduction in DS value. As can be seen in Figure 3.5, CMC extracted with 10% NaOH concentration demonstrated DS value of 0.84 with CMC content of 89.14% which can be considered as the best NaOH concentration for etherification process.

3.2.2.4 Sodium glycolate and sodium chloride content

Sodium glycolate was produced when NaOH react with sodium chloroacetate in etherification process during the production of carboxymethyl cellulose. At this stage, sodium glycolate (Kirk and Othmer, 1967) and sodium chloride will be formed as salts together with CMC. This CMC is also known as crude CMC. During etherification process, alkaline cellulose (Rcell-ONa) will react with sodium chloroacetate ($ClCH_2COONa$) producing sodium carboxymethyl cellulose ($RcellOCH_2COONa$) and sodium chloride (NaCl). However, when cellulose backbone has been fully substituted with carboxymethyl group, the remaining sodium chloroacetate will react with NaOH thus produces sodium glycolate ($OHCH_2COONa$) and sodium chloride (NaCl). This reaction can be seen clearly in Figure 3.6 below. The occurrence of sodium glycolate and sodium chloride content in CMC will influence the purity of CMC. The amount of sodium glycolate produced depends on the amount of sodium chloroacetate as an etherification agent and concentrations of NaOH used in etherification process. Thus, it is very important to investigate and measure the amounts of these salts in the interest of it could affect the behaviour of CMC produced.

(i) $R_{cell}ONa + ClCH_2COONa \longrightarrow R_{cell}OCH_2COONa + NaCl$

Alkali cellulose *Sodium chloroacetate* *NaCMC* *Sodium choride*

(ii) $NaOH + ClCH_2COONa \longrightarrow HOCH_2COONa + NaCl$

Sodium hydroxide *Sodium chloroacetate* *Sodium glycolate* *Sodium chloride*

Figure 3.6 Reactions occurred during etherification process

The results obtained in our study was in an agreement with Barai et al., (1997) who reported that at higher concentration of NaOH, the reaction between sodium chloroacetate and alkaline cellulose tended to shift to the formation of by-product. Sodium glycolate in CMC was calculated using Equation 6 in section 2.3.1.3, where the factor of 1.29 was used to convert glycolic acid to sodium glycolate. Table 3.1 shows the results for glycolic acid content in CMC extracted using different concentration of NaOH. As can be seen from Table 3.1, the percentages of sodium glycolate for all samples ranged from 5.03% to 8.26% with the lowest value was shown by CMC extracted with 5% NaOH with 5.03% of sodium glycolate. The highest sodium glycolate content was displayed by 25% (w/v) NaOH concentration followed by 20%, 15%, and 10% (w/v) concentrations with 8.26%, 8.24%, 8.15% and 7.83% respectively. There was a significance difference between 5% and 10% concentrations. However, no significance differences were observed between 15%, 20% and 25% (w/v) concentrations.

Table 3.1 Sodium glycolate, sodium chloride and moisture content in crude CMC.

NaOH concentrations (%)	Sodium glycolate (%)	Sodium chloride (%)	Moisture (%)
5	5.03 ± 0.03^a	2.67 ± 0.52^a	6.74 ± 0.02^a
10	7.83 ± 0.05^b	3.03 ± 0.81^a	6.78 ± 0.59^a
15	8.15 ± 0.03^c	20.47 ± 0.27^b	5.72 ± 0.76^a
20	8.24 ± 0.03^c	28.36 ± 1.03^c	5.63 ± 0.26^a
25	8.26 ± 0.03^c	17.90 ± 8.26^b	6.14 ± 0.14^a
30	7.83 ± 0.03^b	17.85 ± 1.08^b	5.83 ± 0.55^a

(Notes: Data represent means of n=3 measurements while error bars represent the standard deviation. Different letter (a-c) represent significant different with ($p<0.05$). Sodium glycolate and sodium chloride were statistically analysed using Bonferroni while moisture content was using Tukey HSD)

Excess of amount of NaOH during etherification caused the formation of sodium glycolate due to the inactivation of sodium chloroacetate. At the same time, higher NaOH will decrease the SMCA concentration. As mentioned in Figure 3.6, a side reaction occurs and converts the sodium chloroacetate to sodium glycolate. This condition happened due to the sodium chloroacetate molecules tend to react with NaOH which lead to the destruction of cellulose chain or CMC polymer and decomposition of sodium chloroacetate.

The result obtained in my work on sodium glycolate content has not previously been reported by other researchers who synthesised CMC from plant waste. Adinugraha et al., (2005) have demonstrated the effect of sodium chloroacetate on purity of CMC in cavendish banana pseudo stem only while Togrul and Arslan (2003) have reported on purity and sodium chloride content in sugar beet pulp. Thus, the findings obtained from my study on sodium glycolate content could be used as a future references by other researchers.

Similar with sodium glycolate, sodium chloride is another by-product produces during etherification process of alkaline cellulose. Sodium chloride content can be determined using potentiometric titration with silver nitrate solution by estimating the amount of chloride ions in a solution. During titration process, sodium chloride (NaCl) will react with silver nitrate (AgNO3) solution. Silver ion (Ag+) from silver nitrate will bind with chloride ion (Cl-) to form a precipitate called silver chloride (AgCl) while sodium ion (Na+) will react with nitrate ion (NO3-) to form sodium nitrate (NaNO3) in aqueous. This reaction can be clearly understood by referring to reaction below:

$$NaCl_{(aq)} + AgNO_{3(aq)} \longrightarrow AgCl_{(s)} + NaNO_{3(aq)}$$

NaCl was formed when sodium ion from NaOH used in etherification process react with chloroacetate ion. When the concentration of NaOH increased, more sodium ion is available for chloroacetate ion to bind. Table 3.1 shows the value of NaCl content occurred in CMC after extracting using different concentrations of NaOH. From the graph, CMC extracted with 5% NaOH exhibited the lowest NaCl compared to other sample followed by 10% NaOH with 2.67 ± 0.53 and 3.03 ± 0.81 respectively. This value gradually increased to 20.47 ± 0.27% at 15% NaOH to 28.36 ± 1.03% at 20% NaOH, thus produced the highest CMC that contained NaCl. In contrast, when the concentration of NaOH reached to 25% and 30% w/v, the percentages of NaCl content were reduced to 17.90 ± 1.32% and 17.85 ± 1.09% respectively. This findings also shows that there was a significance difference of NaCl content between all samples with ($p<0.05$) except between CMC extracted with 5% and 10% (w/v) and CMC extracted with 25% and 30% (w/v).

Increased in NaOH concentrations results in lower purity of CMC with increased in the content of by-products. In my study, 20% (w/v) NaOH exhibited the lowest purity of CMC with 63.40%, 28.36% for sodium chloride and 8.24% for sodium glycolate. Previous study reported by Togrul and Arslan (2003) showed that sugar beet pulp extracted using 20% (w/v) NaOH produced CMC with purity of 56.1% and 2.72% of sodium chloride content. The purity of CMC produced from sugar beet was a bit lower compared to the lowest value obtained in my study. This could be due to the different solvent, temperature and amount of sodium chloroacetate used in their study.

The presence of sodium glycolate and sodium chloride will affect the physicochemical properties of CMC produced. As general, these by-products could reduce the lightness of CMC, decrease the DS value and molecular weight and also decrease the viscosity of CMC. Further elaboration on effect of these by products will be explained in section 3.2.2.4 to 3.2.2.7.

3.2.2.4 Moisture content

Moisture content can affect the physical properties of material. In this study, moisture content was determined using thermogravimetric analysis. As described in section 2.3.1.5, thermogravimetric is a method of thermal analysis relates with the changes in physical and chemical properties of material that measured as a function of temperature or time in a controlled atmosphere (ASTM, 2008). According to International Cenological Codex (2009), CMC is granular or fibrous powder, blank, slightly yellowish or greyish in colour, slightly hygroscopic, odourless and tasteless. According to this standard, moisture content of CMC must not contain more than 12%. Table 3.1 shows the moisture content of CMC extracted using different concentrations of NaOH. From the result, moisture content for all samples ranged between 5.63±0.26% to 6.78±0.59%. In my study, all samples exhibited lower moisture content and were below than 12%. From these results, no significance difference was observed between all samples. In 2005, Adinugraha et al., reported that moisture content in CMC produced from Cavendish banana pseudo stem extracted using 15% (w/v) NaOH was 7.37%. They also compared their data with commercial CMC and found that commercial CMC contained 7.54% of moisture. Compared to the study conducted by Mondal et al., (2015), the moisture content for food grade CMC produced from cornhusk was 2.21%. Both of these finding suggests that moisture content of CMC could vary according the source of cellulose, type of solvent and different concentrations of NaOH used during etherification.

3.2.2.5 Colour measurement

Colour is one aspect of appearance that based on visual response to light that consists three dimensions of hue, saturation and lightness. Hue is the first element in the colour-order system illustrated by how can we differentiate red from green or blue from yellow. Saturation is the attribute of colour perception that expresses the amount of departure from a gray of same lightness while lightness is the perception of white objects that distinguished from gray and light coloured objects from dark coloured. Colour measurement involved a physical measurement of light radiated, transmitted or reflected by a specimen under specified condition and mathematically transformed into standardised colorimetric terms (X rite, 2000). When *L* value is equal to 100, it indicates that sample is perfectly white while when *L* value is 0, the colour of sample is black. For *a* value, positive *a* (+*a*) depicts red colour while negative *a* (-*a*) value indicates green colour. Meanwhile for *b* value, positive *b* (+*b*) indicates yellow colour while negative *b* (-*b*) illustrates blue colour of the sample.

In this study, colour measurements were carried out in order to determine the changes of colour due to the carboxymethylation reaction. Table 3.2 presents the values of *L*, *a* and *b* for alkaline cellulose and CMC. From the graph above, alkaline cellulose demonstrated the *L* value of 78.16 ± 0.24, 0.26 ± 0.07 for *a* value and 14.41 ± 0.15 for *b* value. Overall, all CMC samples exhibited *L* values that ranged from 76.11 ± 0.18 to 79.65 ± 0.20. CMC extracted using 25% (w/v) NaOH displayed the highest *L* value and there was a significance difference between 25% (w/v) NaOH with other CMC. When the NaOH concentration increased, the *L* value decreased. However, at 25% (w/v) concentration, the *L* value increased to 79.65 ± 0.20 and decreased to 78.18 ± 0.16 at 30% NaOH concentration. Previous study conducted by Rachtanapun et al., (2012) on CMC extracted from durian rind showed that the *L* value reduced from 83.41 to 77.61 at 50% and 40% (w/v) NaOH concentrations but increased to 79.87 and 82.36 at 50% and 60% (w/v) concentrations. Compared to my study, CMC produced from durian rind were lighter than CMC from wheat bran. In other study by Hutomo et al., (2012) the *L* value for CMC produced from pod husk of cacao (*Theobroma* cacao L.) was reported to be 56.62. This value is a bit lower compared to my study probably due to the bleaching process that performed at a very short time which is only one hour (Hutomo et al., 2012).

On the other hand, the value for *a* ranged from 1.37 ± 0.07 to 0.40 ± 0.03 while for *b*, the results obtained for all CMC samples were between 17.08 ± 0.27 to 22.01 ± 0.54. Alkaline cellulose was significantly difference with other CMC samples for *a* and *b* values. However, there was no significance difference detected between alkaline cellulose with CMC extracted using 30% (w/v) NaOH for *b* value. Compared to alkaline cellulose, all CMC produced in this study demonstrated reddish and yellowish colour. These findings were in agreement with study conducted by Rachtanapun et al., (2012) who proved that CMC from durian rind displayed more yellowish and reddish colour. In their study, at 20% and 30% (w/v) NaOH, the *a* value were 0.32 ± 0.01 and 2.00 ± 0.00 respectively while for *b* value, the results obtained were 19.08 ± 0.03 and 22.90 ± 0.04. Thus, the results obtained in my study have shown some similarity and were in agreement with previous study done by Rachtanapun et al., (2012).

In my work, colour difference (ΔE) was measured between alkaline cellulose and CMC produced. Overall, the differences in colour ranged between 2.34 ± 0.16 to 7.90 ± 0.73. CMC extracted with 15% (w/v) NaOH displayed the highest colour difference amongst all sample due to the higher value recorded in *b* value. This could be due to higher substitution of carboxymethyl group during etherification process (Rachtanapun et al., 2012). In contrast, the lowest value was exhibited by CMC extracted with 30% NaOH. There was a significance difference between 5%, 15% and 30% (w/v) NaOH with other CMC samples however no significance differences were detected between CMC extracted using 20%, 25% and 30% (w/v) NaOH. A possible explanation for this might be due to lower value of degree of substitution exhibited by these samples which relates with carboxymethylation reaction resulting in lower substitution of carboxymethyl group.

Similar trend can also be seen in yellowness index (YI). As shown in Table 3.2, yellowness index for all CMC samples ranged from 29.09 ± 0.34 to 41.10 ± 1.42. According to ASTM (2000), yellowness index indicates the data that describes the changes in colour of a test sample from white to yellow. The highest YI was observed at CMC extracted using 15% (w/v) NaOH. Compared to other samples, CMC extracted with 30% (w/v) NaOH recorded the lowest value for YI. YI is influenced by the *b* value as higher *b* value will result in higher yellowness index or vice versa. Thus, the findings obtained for yellowness index correlates with *b* values.

Table 3.2 Colour value of carboxymethyl cellulose from wheat bran at different NaOH concentrations.

Sample	L	a	b	ΔE	YI
Alkali cellulose	78.16 ± 0.24 ^{b}	0.26 ± 0.07 ^{c}	14.41 ± 0.15 ^{d}	0.00 ± 0.00	26.34 ± 0.35 ^{e}
5% NaOH	77.25 ± 0.64^{bc}	2.15 ± 0.21^{a}	19.61 ± 0.48^{b}	5.63 ± 0.63^{b}	36.28 ± 1.20^{b}
10% NaOH	76.86 ± 0.41^{d}	2.18 ± 0.09^{a}	17.78 ± 0.20^{c}	4.10 ± 0.34^{c}	33.05 ± 0.54^{c}
15% NaOH	76.52 ± 0.78^{d}	1.58 ± 0.29^{b}	22.01 ± 0.54^{a}	7.90 ± 0.73^{a}	41.10 ± 1.42^{a}
20% NaOH	76.11 ± 0.18^{d}	2.40 ± 0.03^{a}	17.08 ± 0.27^{c}	4.00 ± 0.22^{c}	32.06 ± 0.52^{c}
25% NaOH	79.65 ± 0.20^{a}	1.37 ± 0.07^{b}	17.71 ± 0.14^{c}	3.79 ± 0.06^{c}	31.76 ± 0.32^{c}
30% NaOH	78.18 ± 0.16^{b}	2.04 ± 0.04^{a}	15.92 ± 0.20^{d}	2.34 ± 0.16^{d}	29.09 ± 0.34^{d}

(Notes: Data represent means of n=3 measurements while error bars represent the standard deviation. Means in same column with different letters are significantly different ($p<0.05$). Different letter (a-e) represent significant different with ($p<0.05$). All data were statistically analysed using Dunnett T3 except for a value that using Bonferroni)

3.2.2.6 Intrinsic viscosity dan molecular weight of CMC.

Molecular weight of CMC can be measured using dilute solution viscosity method as previously mentioned in section 2.3.2.1. Through dilute solution viscosity method, intrinsic viscosity of the solution can be calculated using Ubbelohde viscometer by measuring the time taken by solution to travel through the viscometer capillary. Ubbelodhe viscometer or also known as capillary viscometer can be used to determine the viscosity of liquid and molecular weight of the polymer. Doublier and Cuvelier, (2006) in their book reported that molecular size of macromolecule can be estimated through determination of intrinsic viscosity via exploration to zero concentration if the concentration is low enough to yield a Newtonian behavior. Meanwhile, according to Durcilene et al., (2007), intrinsic viscosity of a polymer is a measurement of its hydrodynamic volume in solution which depends on its molar mass and structure, the nature of the solvent and the temperature of the medium. It is usually determined from measurement of the viscosity at very low concentration and obtained by extrapolating data to zero concentration using a linear regression. Thus, by knowing the value of intrinsic viscosity, molecular weight of polymer can be estimated using 'Mark-Houwink-Sakurada' with a known constant value (*K*) and polymer shape factor (*a*). Therefore, the value obtained for molecular weight can be used to estimate the size of polymer. As different polymer has different solvent that need to be used together to measure molecular weight, hence each solvent will have different value for constant and polymer shape factor.

In this study, CMC is known as polymer while 0.5N NaOH was used as a solvent. It is important to identify the correct solvent for each polymer as it would affect the results that will be reported. In this experiment, the constant for 0.5N NaOH and polymer shape factor for CMC were 5.37 x 10^{-4}dL/g^{-1} and 0.73 respectively (Whistler et al., 1984). To determine intrinsic viscosity, different concentrations of all CMC samples that ranged from 0.5% to 2% (w/v) were prepared by mixing CMC powder with 0.5N NaOH as a solvent. A details explanation on conducting this experiment has been elaborated in Section 2.3.2.1. The solution of CMC was allowed to flow through viscometer capillary and time taken to flow through a given length was measured (t_o). The measured time at a given temperature is proportional to the viscosity of the solution (η_o) at that temperature. The flow time for pure solvent (t_s) was also been measured through the same capillary. This value is proportional to the viscosity of solvent used (η_s). With the availability of both data, relative viscosity (η_{rel}) can be calculated. Relative viscosity (η_{rel}) is the ratio of viscosity of solution to the viscosity of solvent used and can be described using the following equation:

$$t_o / t_s = \eta_o / \eta_s = \eta_{rel} \qquad \text{Eq. 26}$$

Different concentration of polymer solutions will result in different viscosity. Specific viscosity (η_{sp}) is expresses as the incremental of viscosity due to the presence of the polymer in the solution. It also can be explained as the capacity of a polymer concentration to cause the solution viscosity to increase. Thus, specific viscosity can be calculated by measuring the differences between the ratios of viscosity of solution (η_o) to viscosity of solvent (η_s). This elaboration can be simplified using the following equation:

$$\eta_{sp} = (\eta_o / \eta_s) - (\eta_s / \eta_s) \qquad \text{Eq. 27}$$

Following the equation above, the ratio between specific viscosities of polymer with polymer concentration (c) can be used to estimate the value of reduced viscosity (η_{red}) of polymer. Reduced viscosity measures a specific capacity of the polymer to elevate viscosity at certain polymer concentration and can be determined using equation below. This value was expressed in g/dL.

$$\eta_{red} = \eta_{sp} / c \qquad \text{Eq. 28}$$

Figure 3.7 illustrates the example of value obtained for CMC produced using 10% (w/v) NaOH during etherification process. As we can see in the graph, the value of reduced viscosity was calculated at each CMC concentrations. From the graph, four different concentrations of CMC have been prepared that ranged from 0.5 g/dL to 2.0 g/dL. At 0.5 g/dL, the value for η_{red} was recorded at 2.39 dL/g. This value increased to 3.01 dL/g when the concentration of CMC increased to 1.0 g/dL followed by 3.52 dL/g and 4.49 dL/g at 1.5 and 2.0 g/dL respectively. As the flow time of polymer is proportional to the viscosity of polymer, longer time was required by the polymer with higher CMC concentration and thus increased the value of reduced viscosity. In 2004, Adhikary and Singh have demonstrated a similar pattern of reduced viscosity graph in order to calculate the intrinsic viscosity. However, their study was focused on hydrolysed xanthan gum polyacrylamide. In another study by Vidal and Pawlik (2015), similar trend was also been reported on guar gum samples when the polymer concentration increase from 200 mg/L to 1000 mg/L.

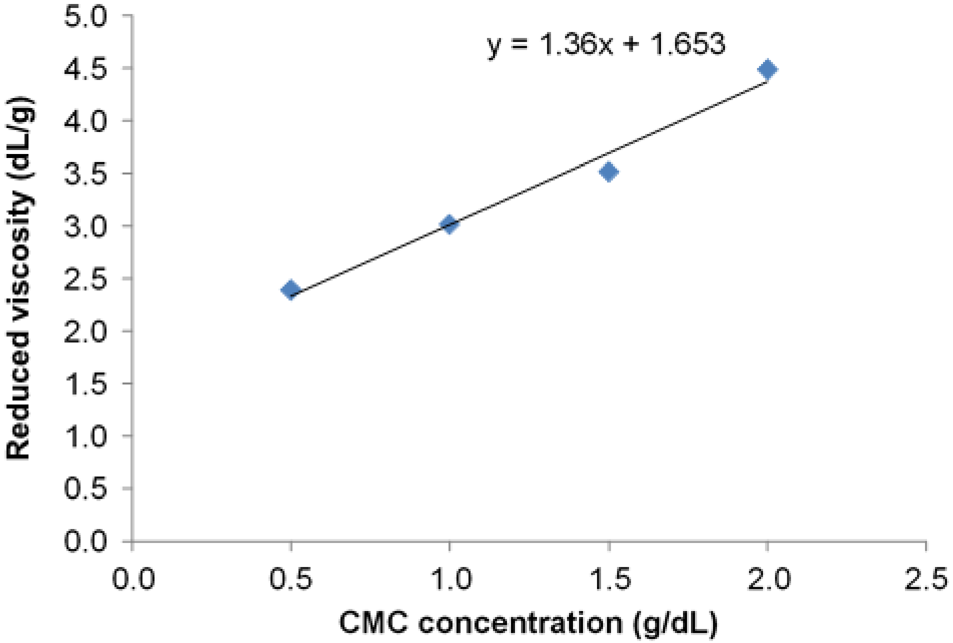

Figure 3.7 Reduced viscosity at of CMC extracted using 10% (w/v) NaOH

The intrinsic viscosity can be determined by extrapolating the plot of reduced viscosity and inherent viscosity towards zero concentration. The intersection between reduced viscosity and inherent viscosity will represent a value for intrinsic viscosity. In order to determine the intrinsic viscosity, inherent viscosity also needs to be calculated. Inherent viscosity is the ratio of logarithm of the relative viscosity to the mass concentration of the polymer. Figure 3.8 shows the example of inherent viscosity plotted against different concentrations of CMC extracted with 10% NaOH (w/v). In this graph, the value for inherent viscosity decreased when the concentration of CMC increased. As in Figure 3.8, the value of inherent viscosity at 0.5 g/dL was recorded at 1.57 dL/g. This value continues to decline to 1.39 dL/g and 1.23 dL/g at CMC concentrations of 1.0 and 1.5 g/dL respectively and further reduced to 1.15 dL/g.

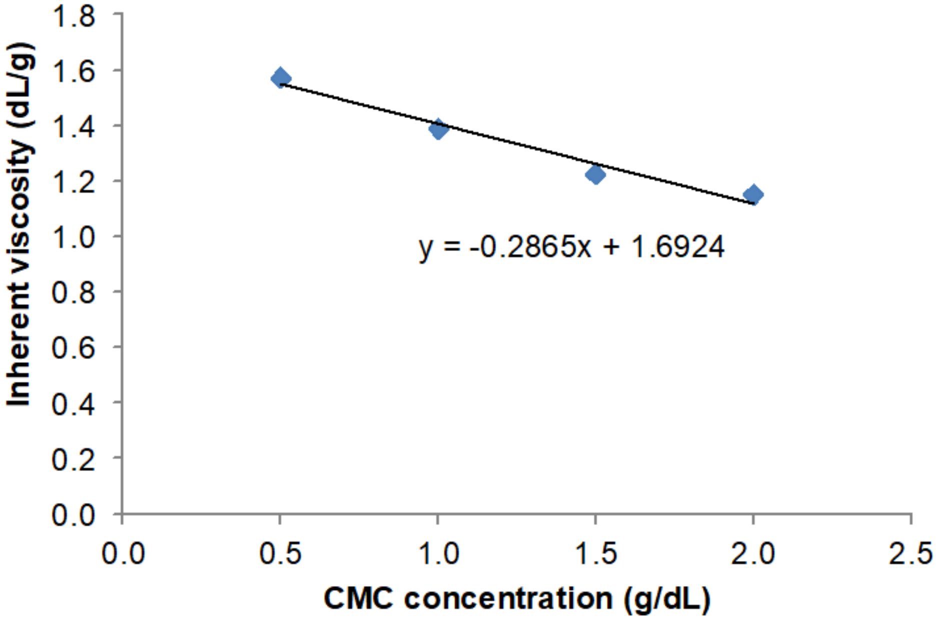

Figure 3.8 Inherent viscosity at of CMC extracted using 10% (w/v) NaOH

As mentioned in previous paragraph, intrinsic viscosity [n] can be determined when graph of reduced viscosity and inherent viscosity were extrapolated towards zero concentration. Therefore, intrinsic viscosity [n] can be represented using equation 29 below:

$$[\eta] = \lim_{c \to 0} (\eta_{sp}/c) \qquad \text{Eq. 29}$$

When both graphs of reduced viscosity and inherent viscosity were plotted towards zero concentration in one graph, Figure 3.9 can be produced. A linear regression of inherent and reduced viscosity produced a common intercept known as intrinsic viscosity. To know the value of intrinsic viscosity which represents by *y* value, the value of *x* is then calculated using equations 30 and 31 as in Figure 3.9.

$$y = 1.36x + 1.653 \qquad \text{Eq. 30}$$

$$y = -0.2865x + 1.6924 \qquad \text{Eq. 31}$$

The calculated value of x is then substituted back into equation 31 to obtain the value of *y* which is equal to intrinsic viscosity [η].

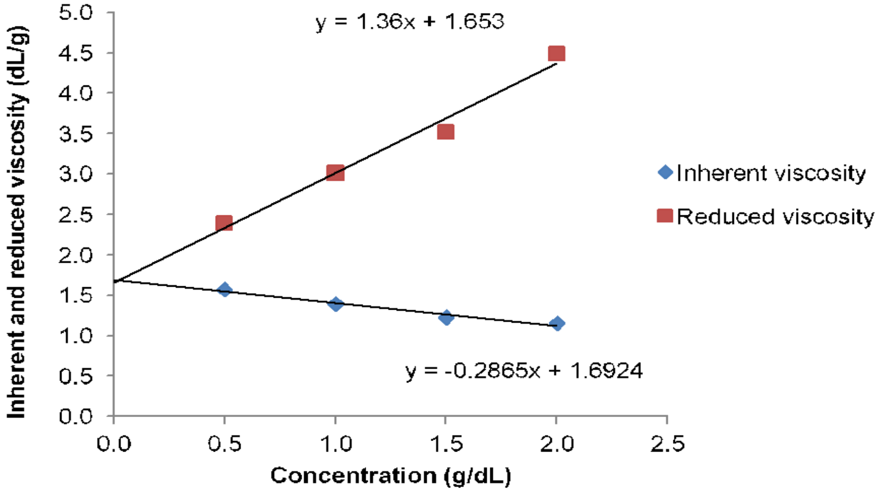

Figure 3.9 Inherent and reduced viscosity of CMC extracted using 10% (w/v) NaOH

From the calculation, intrinsic viscosity for CMC extracted with 10% (w/v) NaOH was 1.6855 dL/g and demonstrated the highest value compared to other CMC. As shown in Figure 3.10, the value of intrinsic viscosity for CMC extracted using 5% (w/v) NaOH increased from 0.4496 to 1.6855 dL/g at 10% (w/v) NaOH. Previously, CMC extracted with 10% and 15% (w/v) NaOH have shown the highest degree of substitution compared to other sample. According to Kaity et al., (2013), this situation might be due to the steric hindrance of carboxymethyl groups and reduction in intermolecular forces. Besides there may be a reduction of the molecular weight due to polymer chain degradation during alkaline kneading of the carboxymethylation process. Qian et al., (2011) in their study reported that intrinsic viscosity measures the hydrodynamic volume occupied by a macromolecule and related to the molecular weight. Therefore, by knowing the value of intrinsic viscosity, molecular weight of CMC produced in this study could be calculated. As reported by previous researchers, factors such as DS value and purity of the CMC have a great influenced on characteristics of CMC produced (Adinugraha et al., 2005 and Mondal et al., 2015). When DS value is higher, the solution become more viscous and the time taken to travel through the viscometer capillary will be longer. Mondal et al., (2015) also reported that, higher DS value results in higher intrinsic viscosity and molecular weight due to the greater number of carboxymethyl groups substituted the hydroxyl groups of the cellulose backbone.

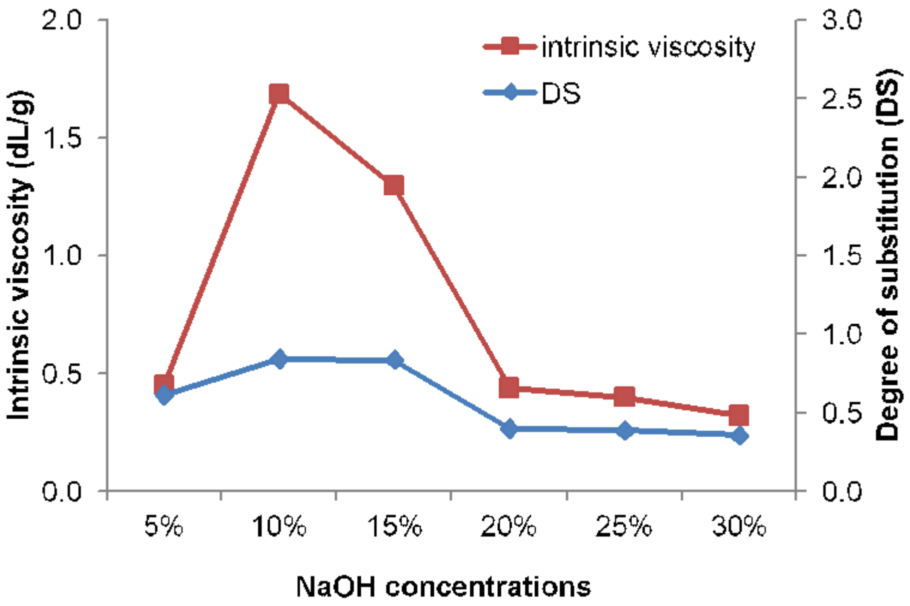

Figure 3.10 Degree of substitution (▲) and intrinsic viscosity (■) of CMC extracted at different concentrations of NaOH

From Figure 3.10, lower DS value also lowered the intrinsic viscosity of CMC. This can be clearly seen at CMC extracted with 20%, 25% and 30% (w/v) that demonstrated 0.4378, 0.3981 and 0.3208 dL/g respectively. Compared to the Figure 3.11, it represents the relationship between DS value and molecular weight of CMC. Increase in DS value will increase molecular weight of CMC. The molecular weight of CMC obtained in this study ranged from 6356 Da to 61672 Da. Due to the higher value obtained in intrinsic viscosity, CMC extracted with 10% (w/v) NaOH shows the highest value of molecular weight followed by CMC extracted with 15% (w/v) NaOH with 61672 Da and 43012 Da respectively. Higher value of molecular weight was due to the increase in hydrodynamic volume by the carboxymethyl group. However, at 20% (w/v) NaOH the molecular weight started to decrease to 9730 Da. As reported by Kaity et al., (2013), this was due to degradation of polymer chain that has been disrupted in highly alkaline medium during the carboxymethylation process. However, compared to CMC extracted with 30% (w/v) NaOH, this sample has shown the lowest molecular weight with only 6356 Da. This is because, the solution formed was more dilute compared to the sample extracted with 10% and 15% (w/v) NaOH and the time needed for the solution to flow through the capillary was shorter. Hence, this indicates that the polymer structure of this CMC is shorter compared to other sample.

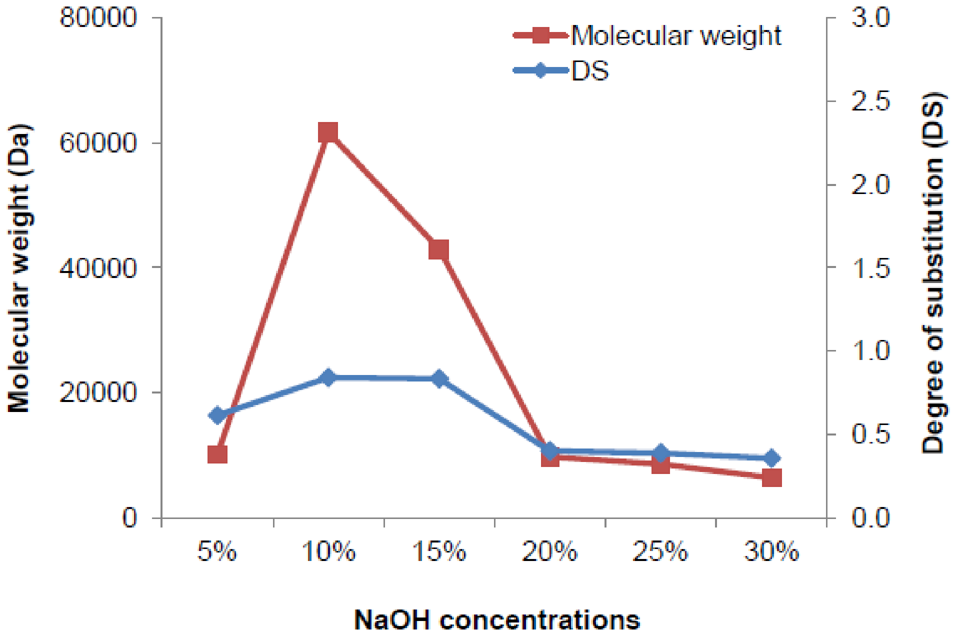

Figure 3.11 Molecular weight (■) and degree of substitution (-▲-) of CMC extracted at different concentrations of NaOH

According to Mulyatno et al., (2017), degree of substitution is the average number of clusters per angydroglucose unit substituted by another group. Cellulose has three hydroxyl groups in each glucose unit meanly at C2, C3 and C6. This hydroxyl group will be substituted with carboxymethyl group of sodium chloroacetate during etherification process. Therefore carboxymethyl groups that substituted at C2, C3 and C6 will modify the cellulose molecule. As previously shown in Figure 3.11, higher molecular weight indicates higher DS value due to the substitution of carboxylmethyl groups at cellulose backbone. Figure 3.12 below represents the molecular structure of CMC substituted with carboxymethyl groups at both C6.

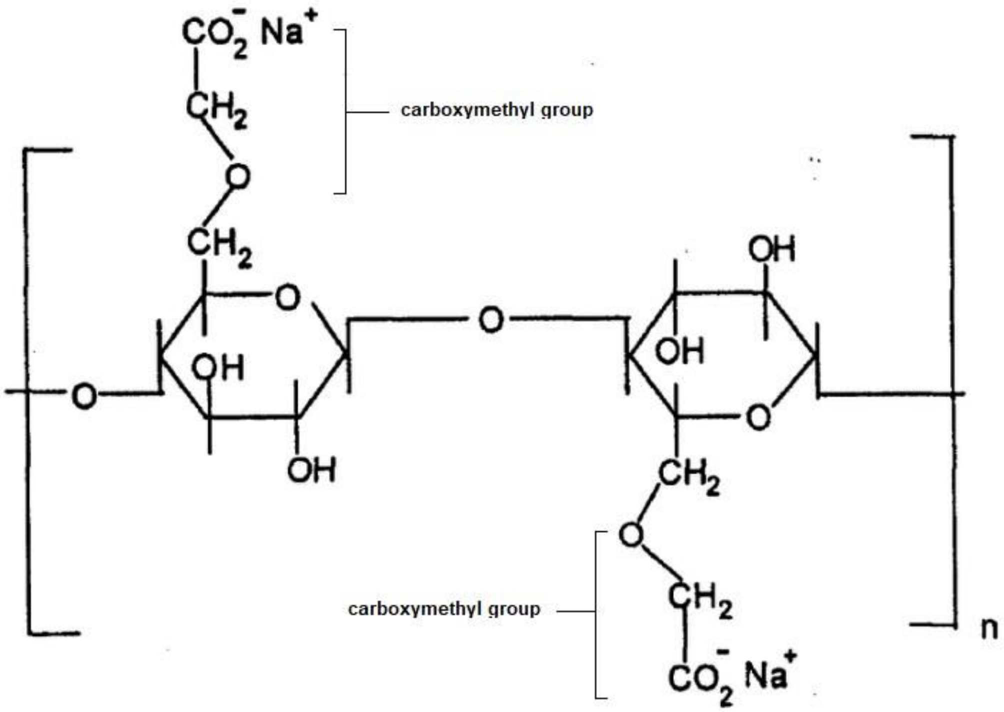

Figure 3.12 Molecular structure of CMC substituted with carboxymethyl groups at C6

To date, no previous study has investigated on the effect of NaOH on molecular weight of CMC. However, Mondal et al., (2015) have demonstrated the effect of different particle size of CMC produced from cornhusk on its molecular weight. In their study, decreased in particle size increased the molecular weight. Nonetheless, the value obtained in his study was also depended on DS value of CMC. On the other hand, molecular weight also indicates the degree of polymerisation (DP). This DP measures the chain length of polymer that influenced by substitution of carboxymethyl group at cellulose backbone. Increase in DP also increases the viscosity of CMC. It also influenced the properties of films produce.

3.2.3 Rheological measurement

In this experiment, rheometer equipped with rSpace software (Malvern Instrument) has been used to measure and analyse the samples. All the conditions involved in this measurement have been described in Section 2.3.2.2. Rheometers are instruments that apply stresses and strain to measure the flow and deformation of material (Rao, 2007). Viscosity measures the ease of flow that related to the amount of force needed to deformed sample at certain deformation rate. Deformation of material can be measured by applying shear. Shearing is the most common deformation technique applied on sample to measure viscosity. Sala and Scholten (2015) reported that shearing is the effect of moving the sample along the surface or by compressing the sample in one direction and becomes compressed in other direction that is known as a compression. In the case of fluids deformed by shear, viscosity is referred as shear viscosity or dynamic viscosity. However, most of food material exhibits a non-Newtonian behaviour that indicated by divergence from a linear relation between shear stress and shear rate.

3.2.3.1 The behaviour of CMC solution

Rheological properties are importance characteristics for fluid or liquid as it study the flow behaviour of materials. In this study, the effect on different concentration of NaOH used in etherification process was investigated as it could affect the rheological properties of CMC produced. This is due to the changes in CMC molecular structures due to the substitution of carboxymethyl groups at cellulose backbone. Besides, the formation of by-products such as sodium chloride and sodium glycolate could also contribute to the rheological properties of CMC solution produced.

Figure 3.13 shows the graph of shear stress towards shear rate of 4% (w/v) CMC solution etherified with different NaOH concentrations. From the graph, all CMC samples showed an increment of shear stress over shear rate. Amongst all samples, CMC etherified with 10% (w/v) NaOH showed the highest shear stress followed by 15% (w/v) NaOH. CMC etherified with 5%, 20%, 25% and 30% (w/v) demonstrated a lower shear stress. This could be due to the formation of sodium glycolate and sodium chloride in CMC content that affect the purity and viscosity of CMC. Increased amount of sodium glycolate and sodium chloride will reduce the shear stress of CMC produced. This is due to the fact that sodium glycolate and sodium chloride are hygroscopic materials. Therefore, these salts will attract and absorb more water molecules and produced a dilute cellulose gum. Another factor that can be associated with higher shear stress was the DS value. In this study, the DS value exhibited by CMC etherified with 10% and 15% (w/v) NaOH were amongst the highest with 0.84 and 0.83 respectively. Compared to other CMC samples, lower DS value were exhibited by CMC etherified using 5%, 20%, 25% and 30% (w/v) of NaOH that ranged from 0.36 to 0.61.

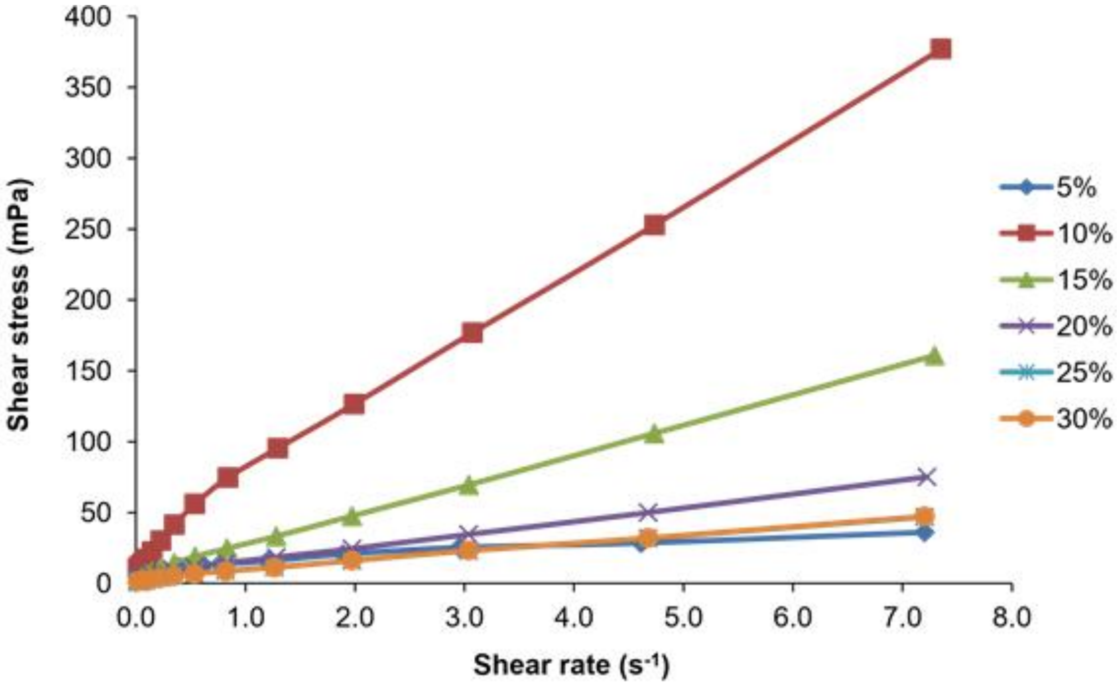

Figure 3.13 Shear stress against shear rate of 4% (w/v) CMC etherified with different concentrations of NaOH

Increased in DS value will contribute to higher shear stress of CMC. This is because increasing DS makes CMC more hydrophilic and readily disaggregated in water (Aqualon, 1999) due to higher carboxymethyl groups that substituted the hydroxyl groups in cellulose backbone at C2, C3 and C6. If CMC exhibited higher DS value, this means that higher carboxymethyl groups from sodium chloroacetate are available in cellulose structure thus resulting in the formation of viscous solution when mixed with water. On the other hand, decreased in DS value resulted in lower shear stress hence exhibited dilute CMC solution. This statement is true for CMC extracted using 5%, 20%, 25% and 30% (w/v) NaOH that displayed lower degree of substitution and shear stress as shown in Figure 3.14.

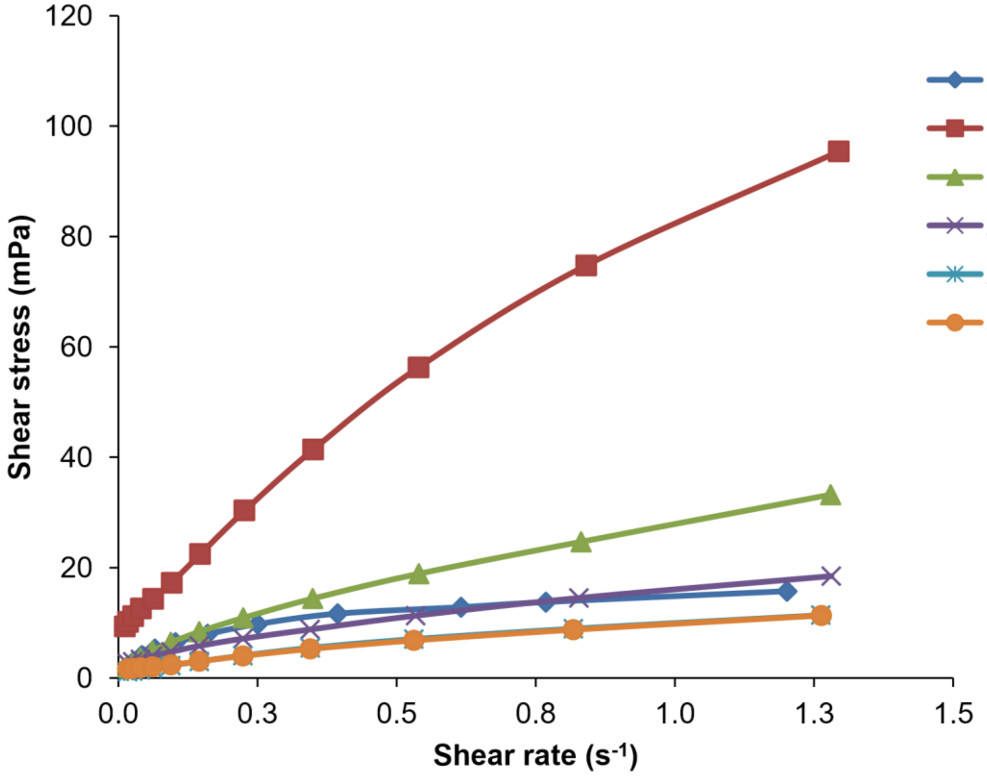

Figure 3.14 Shear stress of CMC solutions at lower shear rate for 4% (w/v) CMC

As shown in Figure 3.14, CMC etherified with 10% and 15% (w/v) NaOH exhibited an obvious curved lines compared to other samples. These curved lines are also known as pseudoplastic behaviour (Togrul and Arslan, 2003). Pseudoplastic behaviour happens due to the long chain CMC molecules that tend to orient themselves in the direction of flow. Thus, pseudoplastic behaviour is categorised as non - Newtonian fluids. Compared to Newtonian fluids, this solution demonstrated as viscosity independent of shear stress thus producing a linear line. In comparison with CMC etherified with 10% and 15% (w/v) NaOH, other CMC solution also exhibited a pseudoplastic behaviour. However, the curved that formed by these solution as in Figure 3.14 were not conspicuous. In order to confirm and investigate the behaviour of CMC solutions produced in this experiment, the Power law model was used to identify the flow behaviour of CMC solutions. Thus, data of shear stress and shear rate were plotted on log-log plot calculation. The value obtained from the slope of log-log plot is known as the flow behaviour index (n) which indicates the behaviour of solutions.

As reported by Togrul and Arslan (2003) in their published paper on rheological properties of CMC produced from sugar beet pulp, when n value is less than 1 (n<1) fluids exhibits the behaviour of shear thinning while shear thickening behaviour will be demonstrated when n value is more than 1 (n>1). Compared to Newtonian behaviour, n value will equal to 1 (n=1), which means that the shear stress of sample is proportional to the shear rate. Shear thinning is a term used to describe the characteristics of fluids with decreasing of viscosity towards shear rate. It is also known as pseudoplastic behaviour. Compared to shear thickening, the behaviour of fluid is indicated by the increment of viscosity towards shear rate. It is also known as dilatant.

Figure 3.15 shows the graph of shear stress against shear rate on log-log plot for 4% (w/v) CMC. From the graph, the values obtained for (n) for all samples were between 0.4355 to 0.6954. All of these values were lower than 1 thus indicates that all sample demonstrated shear thinning behaviour (n < 1). Therefore, the results obtained from this calculation proved that all samples prepared at 4% (w/v) CMC concentration exhibited shear thinning behaviour. In this study, the DS value for CMC etherified with 10% and 15% (w/v) NaOH were higher compared to other samples with 0.84 and 0.83 respectively.

Consequently, they also exhibited the highest viscosities compared to other samples. Hence, increased in DS value demonstrated more shear thinning or pseudoplastic behaviour of CMC solutions. The findings obtained in this experiment was in accordance with the results reported by Togrul and Arslan (2003), Yasar et al., (2007) and Karatas and Arslan (2016) on rheological behaviour of CMC from sugar beet pulp, CMC from orange peel and CMC produced from grapefruit peel respectively. All of these authors reported similar findings that CMC exhibited a shear thinning or pseudoplastic behaviour.

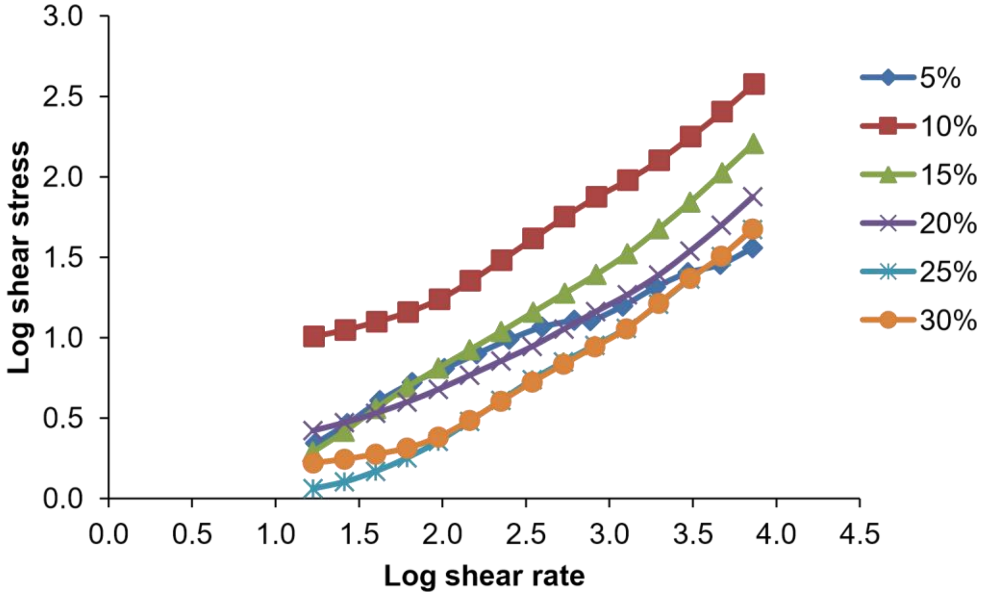

Figure 3.15 Shear stress against shear rate on log-log plot of 4% (w/v) CMC etherified with different NaOH concentrations.

3.2.3.2 Shear viscosity of 4% w/v CMC

Previously, all CMC samples produced in this study demonstrated shear thinning behaviour. Another significance parameter that has been investigated in this study is shear viscosity. Shear viscosity is an important parameter in order to study the correlation between the effects of NaOH concentrations on viscosity of CMC. Figure 3.16 shows the graph of shear viscosity against shear rate of CMC etherified with 5% to 30% (w/v) NaOH at 4% (w/v) CMC concentration. All sample shows the decreased of shear viscosity over shear rate. This was proven by the calculation of flow behaviour index (n) obtained from log-log plot of shear stress and shear rate data which was previously discussed in section 3.2.3.1. As all samples exhibited pseudoplastic behaviour, increased in shear rate resulted in decreased in viscosity. This situation can be observed in Figure 3.16 as all samples displayed a reduction of viscosity when shear rate increased. CMC etherified with 10% and 15% (w/v) of NaOH demonstrated higher viscosity compared to other samples. This revealed that higher DS value obtained by both CMCs is responsible in the increment of shear viscosity. Increase in DS value enhances the ability of CMC to immobilise water in a system. This is due to higher charge from carboxymethyl group thus increase the amount of swelling of the molecules due to the charge group repels with each other. This situation also results in swollen of CMC chain as well as CMC become more soluble. This elaboration is in accordance with study reported by Rachtanapun et al., in 2012 who mentioned that higher DS value increased the viscosity of sample due to more carboxymethyl groups substituted the hydroxyl groups of cellulose backbone.

Compared with 10% and 15% (w/v) NaOH, CMC etherified with 5%, 20%, 25% and 30% (w/v) NaOH displayed slightly lower DS values with only 0.61 and below thus demonstrated lower viscosity of CMC solutions. The results obtained in this experiment are in agreement with a study conducted by Adinugraha et al., (2005) on CMC produced from cavendish banana pseudo stem. In this study, he stated that increased of NaOH will increase the formation of sodium glycolate and sodium chloride thus lowering the DS value due to the degradation of CMC polymer and formed a dilute solution with low viscosity. CMC etherified with 10% and 15% (w/v) NaOH have exhibited higher shear stress compared to other samples. As can be seen in Figure 3.16, higher shear viscosity was demonstrated by CMC etherified with 10% (w/v) NaOH which recorded the value of 152.5 mPas at the lowest shear rate. However, this value declined and reached a stable state at 51.27 mPas when shear rate increased. Similar curved lines were also been displayed by other CMC samples.

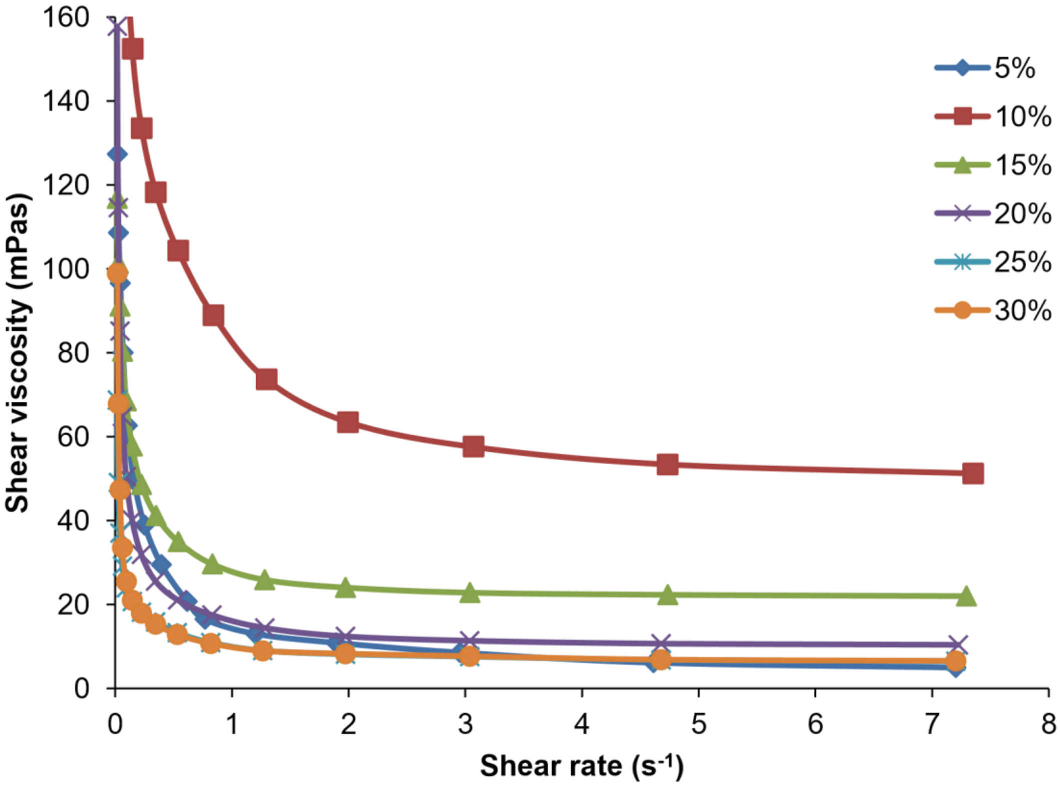

Figure 3.16 Shear viscosity against shear rate of 4% (w/v) CMC etherified at different NaOH concentrations

Distinct differences of shear viscosity can be detected between 10% and 15% (w/v) NaOH in Figure 3.16. However, for CMC etherified with 5%, 20%, 25% and 30% (w/v) NaOH, identical curved lines were formed and stable state of shear viscosity were recorded between 5.02 to 10.40 mPas. The findings obtained in this study was in agreement with the results obtained by Adinughraha et al., (2005) who proved that higher DS value was obtained at lower NaOH concentration thus resulting in higher value of viscosity. The results obtained in my study also in accordance with other published studies. In 1997, Almedia and Dias have reported that the decreased in viscosity with increasing shear rate is mainly related to the disentanglement of macromolecular chains under shear field and breaking of possible structure in solution that attributed to the break of macromolecular aggregates. Karatas and Arslan (2016) indicated that at low shear rates, apparent viscosity reduced more sharply while at higher shear rates, apparent viscosity reduced more slowly. This is due to the less resistance flow in high shear rates caused by breaking of aggregate. However, the results obtained in my study was not in concordance with another study demonstrated by Rachtanapun and Rattanapanone (2011) on viscosity of CMC produced from *Mimosa pigra* who found that viscosity of CMC increased when concentration of NaOH increased. These authors explained that even though DS value decreased when the concentration of NaOH increased, the viscosity of CMC still increased due to the aggregation of CMC produced.

On the other hand, the value of viscosity for all CMC sample reported in my work are correlated with the value obtained for molecular weight in section 3.2.2.6. From the results, higher molecular weights were recorded for CMC etherified with 10% and 15% (w/v) NaOH with the value of 61672 Da and 43012 Da respectively. Increased in molecular weight was also due to the size of polymer formed during etherification process as this could be due to the greater number of carboxymethyl groups substituted the hydroxyl groups at the cellulose backbone.

3.2.4 Morphology of CMC from wheat bran

The effect on different concentration of NaOH used during etherification process on CMC morphology can be investigated using Scanning Electron Microscopy (SEM). SEM is an imaging technique that has been used to characterise samples to a resolution of nanometres with image magnification that ranged from approximately 10 to 300, 000 nm. Through SEM, it can provide the information on surface topography, crystalline structure, and the orientation of materials composing the sample as well as chemical composition of sample observed. SEM is a type of electron microscope that uses an electron gun to emit electron and form a focused beam of high energy electrons. The sample surface needs to be coated with a thin layer of metal such as gold or platinum. This step is very important in order to avoid a repulsive reaction of electron beam. Electrons are expedited to energy values that range of a few hundred eV to 50 KeV and scanned or rastered over the surface of the sample. The interaction between electron and sample surfaces results in the emission of electrons and photons from the samples. Thus, these electrons carry a substantial amount of kinetic energy and this energy is scattered as a variety of signals produced by electron sample interactions.

A secondary electron detector is a standard equipment in all scanning electron microscope and is the most common mode of detection used. Secondary electron is a detection of interaction between atom and electrons that emitted by the electron beam. According to Gianfrancesco (2017), the number of secondary electrons is a function of the angle between the surface and the beam. By scanning the sample and detecting the secondary electrons, an image displaying the tilt of the surface is created. The effect of NaOH concentration used during etherification process on characteristic of CMC produced can be investigated through surface morphology of sample.

In this experiment, surface morphology of alkaline cellulose and carboxymethyl cellulose from wheat bran etherified at different concentrations of NaOH were observed at 500x and 3000x magnification. Figure 3.17 presents the image on surface morphology of alkaline cellulose. As can be seen in the picture, at 500x magnification, alkaline cellulose contained a mixture of bulky granules and rod-like shape structure. The surfaces of these mixtures were smooth due to the addition of 3% (w/v) of NaOH during mercerisation process. Hence, the structures of alkaline cellulose has become soft as hydroxyl ions from NaOH swell the cellulose structure and disrupt the intermolecular hydrogen bonds between cellulose and hemicellulose (Bian *et al.,* 2012). Due to this reaction, NaOH solubilises hemicelluloses and produces residue enriched with cellulose. The image acquired in Figure 3.17 was similar with the morphology reported by Mondal et al., (2015) on cellulose structure prepared from cornhusk that exhibited a mixture of rough granules and rod like structures.

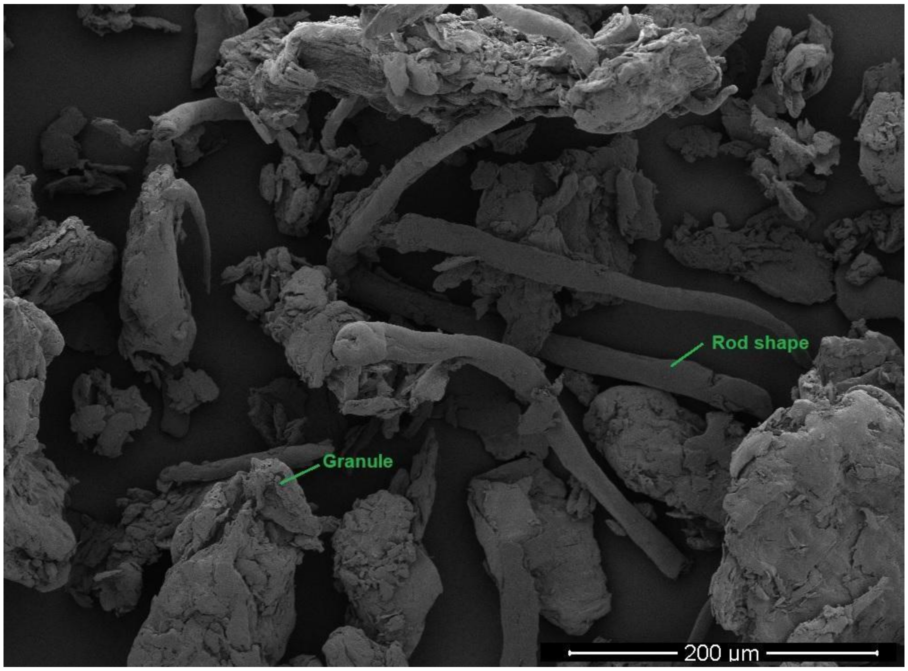

Figure 3.17 Morphology of alkaline cellulose at 500x magnifications

Figure 3.18 exhibits the surface morphology of CMC etherified using 5%, 10% and 15% (w/v) NaOH at magnifications of 500x and 3000x respectively. At 5% (w/v) NaOH concentration, sample exhibited smoother bulky surfaces compared to alkaline cellulose. However, there are no obvious changes that can be detected on CMC surface. At this stage, alkaline cellulose has been etherified with sodium monochloroacetate and NaOH in order to produce CMC. Due to the substitution of carboxymethyl group at cellulose backbone, a serene structure has been recorded as in Figure 3.18 (b) at 3000x magnifications. Even though NaOH has been used during etherification, the level of 5% (w/v) NaOH concentration can be considered as quit low as it does not really change the surfaces of CMC. However compared to 10% (w/v) NaOH, the surface of CMC granules have shifted to become smoother under

500x magnifications with irregular shape. To observe any details changes that occurred on CMC surface, the magnification was increased to 3000x. As can be seen in Figure 3.18 (d), a rough structure can be detected on rod like structure surfaces. At this stage, increased in NaOH concentration will influence the morphology of CMC by producing more coarse structure.

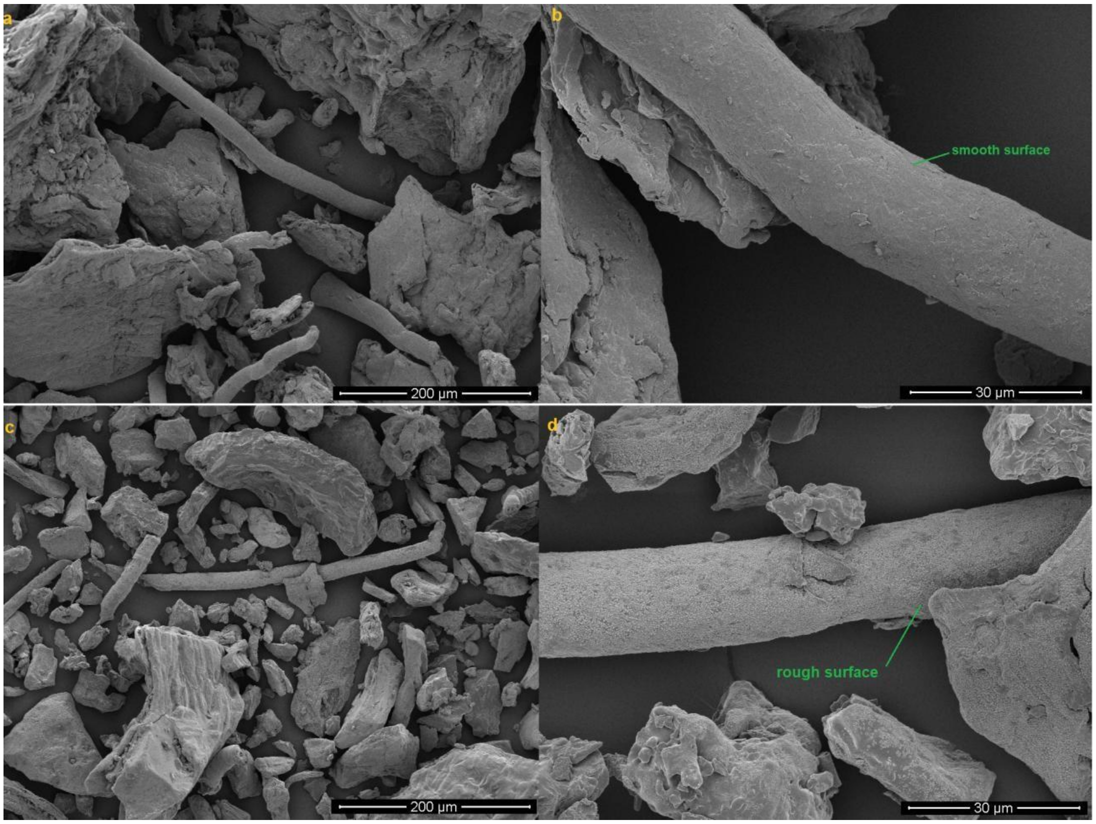

Figure 3.18 Morphology of (a) CMC5 at 500x, (b) CMC5 at 3000x, (c) CMC10 at 500x and (d) CMC10 at 3000x magnifications.

Compared with Figure 3.19 (a), an analogous structure was also demonstrated by CMC etherified with 15% (w/v) NaOH. It can be seen that the morphology of CMC surfaces in Figure 3.19 (b) was very much alike with Figure 3.18 (d) which representing CMC etherified with 10% (w/v) NaOH. Compared to 10% NaOH, 15% (w/v) NaOH exhibited more harsh surfaces. This could be due to the formation of sodium chloride and sodium glycolate as by-products in CMC production. A clear formation of particle which believed to be sodium chloride and sodium glycolate can be detected in Figure 3.19 (d). When the concentration of NaOH reached 20% (w/v), the surface of granules becomes rougher and irregular structure. During etherification, carboxymethyl group will attach to cellulose backbone and caused the formation of by-product.

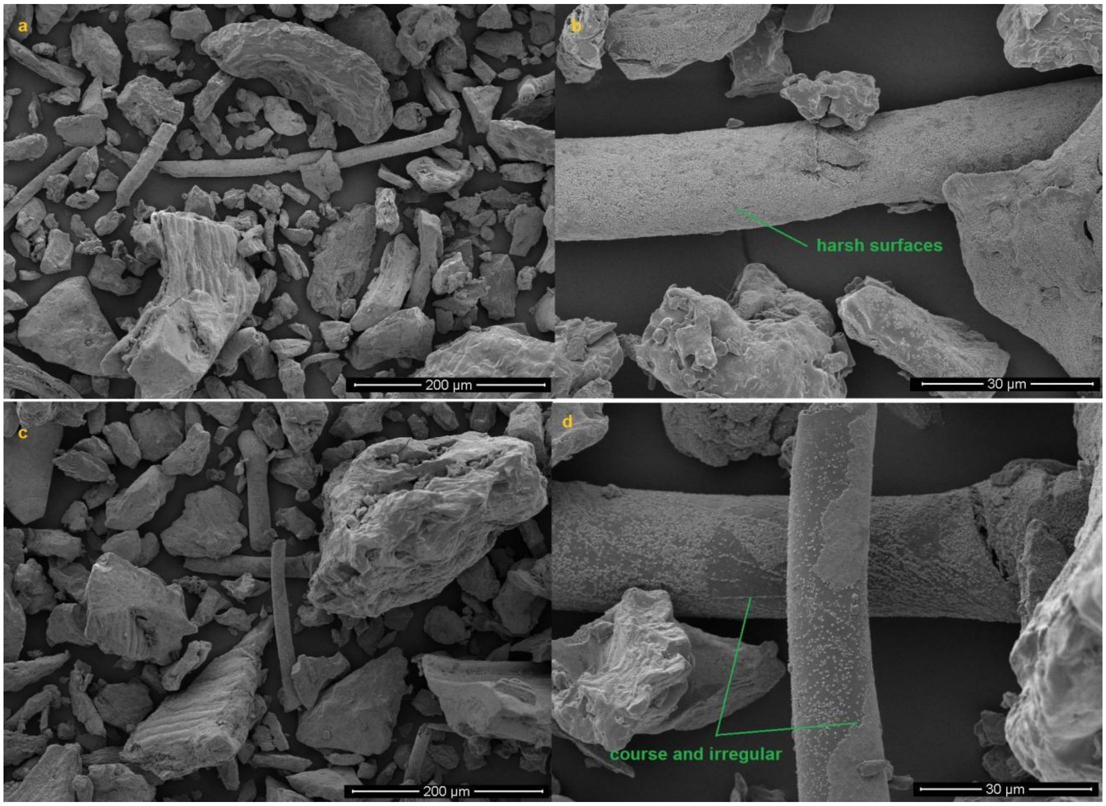

Figure 3.19 Morphology of (a) CMC15 at 500x, (b) CMC15 at 3000x, (c) CMC20 at 500x and (d) CMC20 at 3000x magnifications.

Santos et al. (2015) in their published paper on carboxymethylation of cellulose extracted from brewer’s spent grain reported that carboxymethyl groups formed a bulky structure on fibre surface after carboxymethylation. Thus, increased in NaOH concentrations result in the formation of coarse and harsh structures on the surface of CMC. Besides carboxymethyl groups, the formation of sodium chloride and sodium glycolate also affects the morphology of CMC produced. As in Figure 3.20 (b) and (d), 25% and 30% (w/v) NaOH has demonstrated a course and harsh surface on bulky granules and rod shape structure. This might be due to the formation of by-products namely sodium chloride and sodium glycolate. The findings on morphology of CMC presented in this work was similar with the morphology reported by Mondal et al., (2015) on structure of CMC prepared from corn husk and CMC from durian husk by Rachtanapun and Suriyatem (2009) who claimed that CMC molecules are in rod like shape.

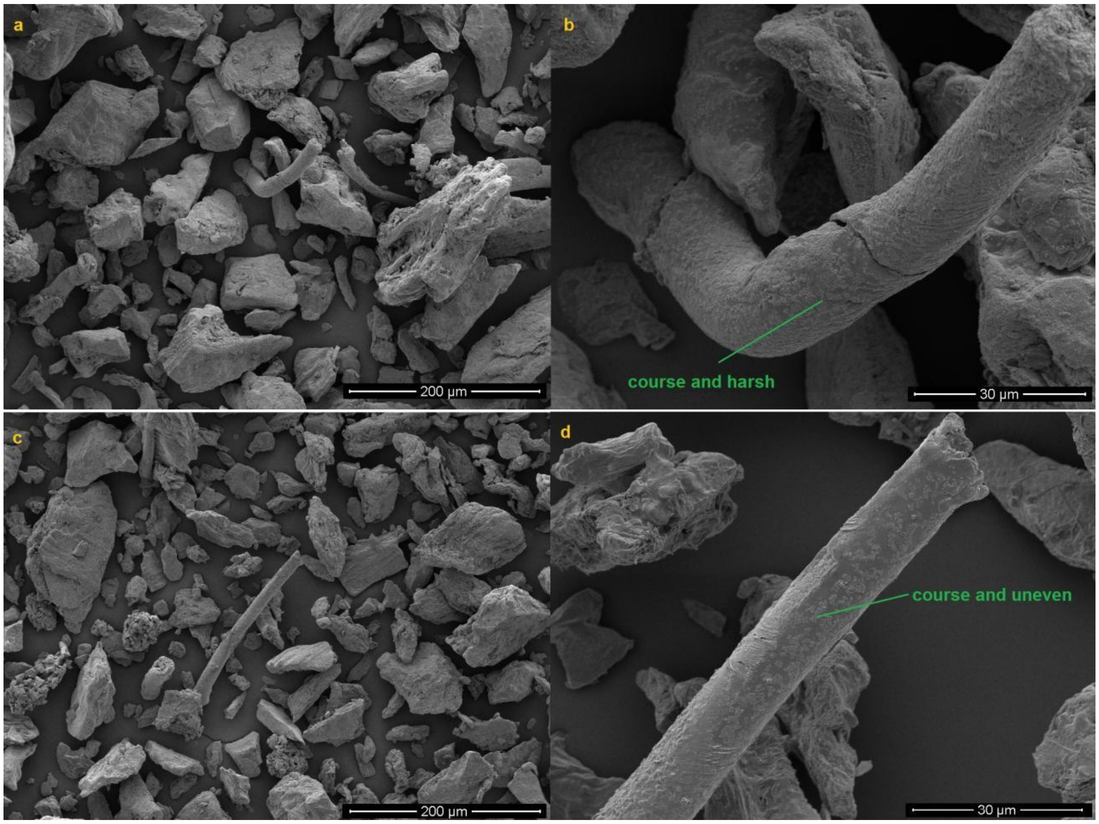

Figure 3.20 Morphology of (a) CMC25 at 500x, (b) CMC25 at 3000x, (c) CMC30 at 500x and (d) CMC30 at 3000x magnifications.

3.3 Conclusions

Alkaline cellulose and CMC were successfully extracted from wheat bran through macerisation and etherification process. The yield of CMC increased from 1.6 g/g at 5% (w/v) NaOH to 1.99 g/g at 0% (w/v) NaOH and decreased to 1.79 g/g at 30% (w/v) NaOH concentration. At higher concentrations of

NaOH, sodium glycolate and sodium chloride were formed and affect the purity of CMC wheat bran. In this study, DS value demonstrated by all samples ranged from 0.3 to 0.84. CMC extracted with 10% (w/v) NaOH exhibited the highest DS value. However, when the concentration of NaOH reached 20% (w/v), the DS value and viscosity of CMC started to decrease. On the other hand, the value for DS correlates with molecular weight and viscosity of CMC. High DS value results in higher molecular weight and viscosity. These characteristics were demonstrated by CMC extracted using 10% and 15% (w/v) NaOH that recorded amongst the highest DS value, molecular weight as well as viscosity. Overall, all CMC exhibited a brighter, reddish and yellowish in colour compared to alkaline cellulose with total colour differences were below 7.90 while yellowness index ranged between 9.09 to 41.10.

In this work, all CMC samples have been proven to demonstrate a pseudoplastic or shear thinning behaviour by exhibiting the reduction in viscosity with increased of shear rate. Different concentration of NaOH also affects the morphology of CMC produced. All CMC samples produced in this study demonstrated the mixture of bulky granules and rod like shape structure. However, increased in NaOH concentration resulted in rough and coarse surfaces which could be due to the substitution of carboxymethyl group and formation of sodium glycolate and sodium chloride as by-products. The findings obtained in this work have shown significant implications for the understanding on how different concentration used during etherification process affects the characteristics of CMC produced. Besides, these data may complement those of earlier studies and serve as a base for future studies. CMC wheat bran obtained in this work can be used as a new source of biopolymer synthesised from agricultural waste. Besides it can minimise the agro-industry waste, this wheat bran can be fully utilised by converting it to a new source of cellulose derivatives. Not only that, this cellulose gum is potential to be used as food ingredients in different industry such as food product, cosmetic and pharmaceutical. Due to its behaviour which can form a gum, it can also be used to produce film or coating in order to improve mechanical and barrier properties of packaging material.

CHAPTER 4

PHYSICAL AND MECHANICAL PROPERTIES OF PE/PET/CMC FILMS

4.1 Introduction

CMC is an example of polysaccharides that has been utilised in a wide range of application such as drug delivery, biosensor, coating and film (Huang and Zhou, 2014). According to Olatunji (2015), naturally derived polymers could represent a self-renewed bio-based source, environmentally safe and cost effective materials. Due to their abundant, low cost, easily available and excellent biodegradability, the used of CMC as a film or coating has been extensively studied. In recent years, there has been an increasing interest on CMC as an element used in the development of film. Several authors have demonstrated the formation of CMC film extracted from papaya peel, (Rachtanapun, 2009), durian rind (Rachtanapun et al. 2012), rice stubble (Rodsamran and Sothornvit, 2017) and bleached bagasse pulp (Kamthai and Magaraphan, 2017). Not only that, CMC also been used as an element in producing blend films such as chitosan/CMC (Hu et al., 2016) and gelatine/CMC/xanthan gum (Nur Hazirah et al., 2016).

Data from several studies suggest that multilayer films could be developed from synthetic polymers and biopolymer layers. A study conducted by Bugnicourt et al. (2013) stated that multi-layered PET/whey protein/PE film exhibited similar results for water vapour transmission rate. Besides, Kurek (2012) found that multi-layered PET/chitosan/PE film demonstrated similar performance of oxygen permeability to that of PE/EVOH/PE film. In order to develop a multilayer film made from synthetic polymers and biopolymer, layer-by-layer method can be applied. According to Decher (1997), this method is a versatile and a straightforward approach to build up multi-layered films. Yoshida et al. (2016) stated that layer-by-layer films composed of deposition of synthetic polymers and biopolymers including protein and polysaccharides on the surface of solid substrates.

To the best of our knowledge, the used of biopolymer such as CMC to produce multilayer film using layer-by-layer technique is still limited. To date, there is no study reported on the use of CMC extracted from plant waste as a multi-layered film. Therefore, the aim of this study was to apply CMC as a coating on PE/PET film using layer-by-layer method. Besides that, the purpose of this study was to investigate the best conditions of CMC volume, drying temperature and drying time during the development of PE/PET/CMC multi-layered film. This study was conducted as to measure physical properties of PE/PET/CMC film such as film thickness, moisture content of films, film solubility, moisture absorption and colour of films produced. The effect of different CMC layers on tensile strength and elongation at break were also been analysed as to study the mechanical properties of films produced.

4.2 Results and discussions

4.2.1 Preparation of PE/PET/CMC films with single and double layer coating

PE/PET/CMC films with single or double layer CMC coatings were successfully prepared using method described in section 2.4.1.2. The results obtained in this study showed that CMC layer attached well with PE/PET film after drying process was performed at 27^{o}C for 12 hours. There was no separation between CMC and PE/PET film observed. Figure 4.1 shows the formation of CMC layer on PE/PET film. At 27^{o}C, water in CMC solution slowly evaporates and formed a CMC layer on PE/PET film.

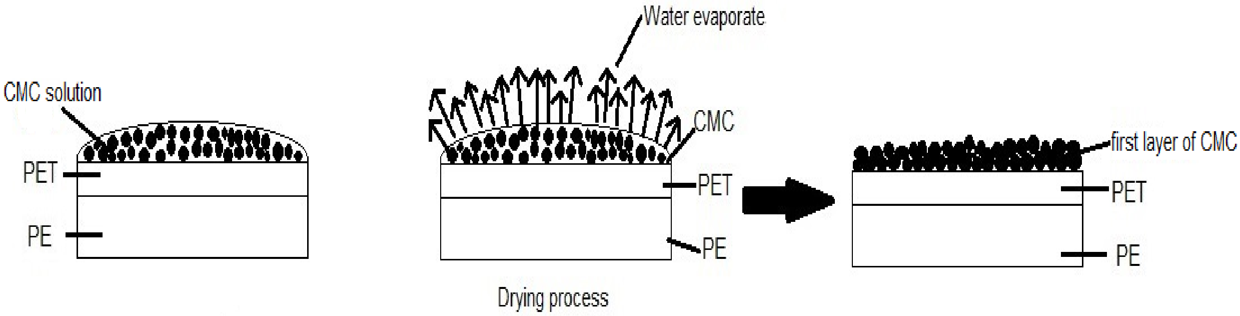

Figure 4.1 Drying process of PE/PET/CMC film with single coating.

Meanwhile for PE/PET/CMC film with double layer, 35 ml of CMC solution was added on PE/PET/CMC with single coating and dried for another 12 hours at 27°C. Similar as in Figure 4.1, water that contained in CMC solution will evaporate and create a new layer on PE/PET/CMC film with single coating. The new film that formed from second drying process is known as PE/PET/CMC with double layer film. Figure 4.2 displays the formation of PE/PET/CMC film with double layer coating.

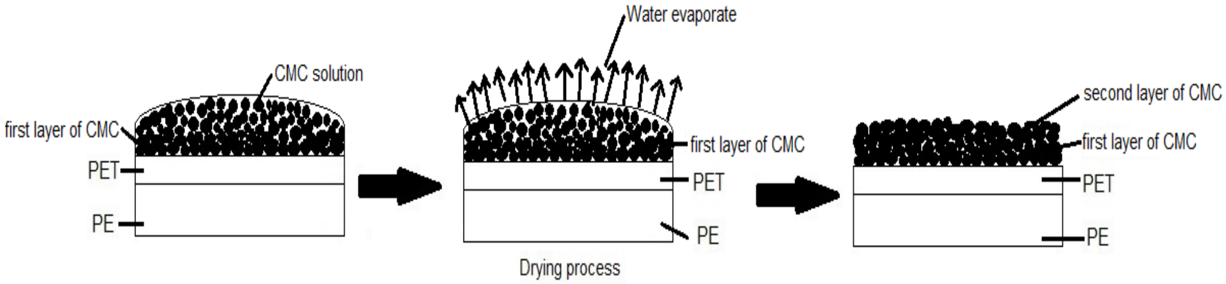

Figure 4.2 Drying process of PET/PE/CMC film with double coating.

4.2.2 Comparison on physical properties of PE/PET/CMC films

4.2.2.1 Film thickness

Film thickness is one of the characteristics that need to be determined in measuring the physical properties of film produced. Besides, it is also used to calculate other characteristic such as mechanical, barrier and optical properties of films (Galus and Lenart, 2013). Figure 4.3 shows the graph of film thicknesses for PE/PET and PE/PET/CMC films with single and double layer coating. In this experiment, all measurements were conducted in quintuplicate. The findings showed that the thickness of PE/PET film without any CMC coating was recorded at 0.065 mm. From the technical data provided by Tecno Pack (2015), the thickness of PET and PE films were 12 and 50 μm respective with another 2 μm was contributed by adhesive used to attach the films. In my study, PE/PET/CMC15 exhibited the highest film thickness for both single and double layer coating with 0.089 mm and 0.113 mm respectively. PE/PET/CMC30 with single and double layer coating exhibited the lowest values with 0.075 mm and 0.094 mm respectively.

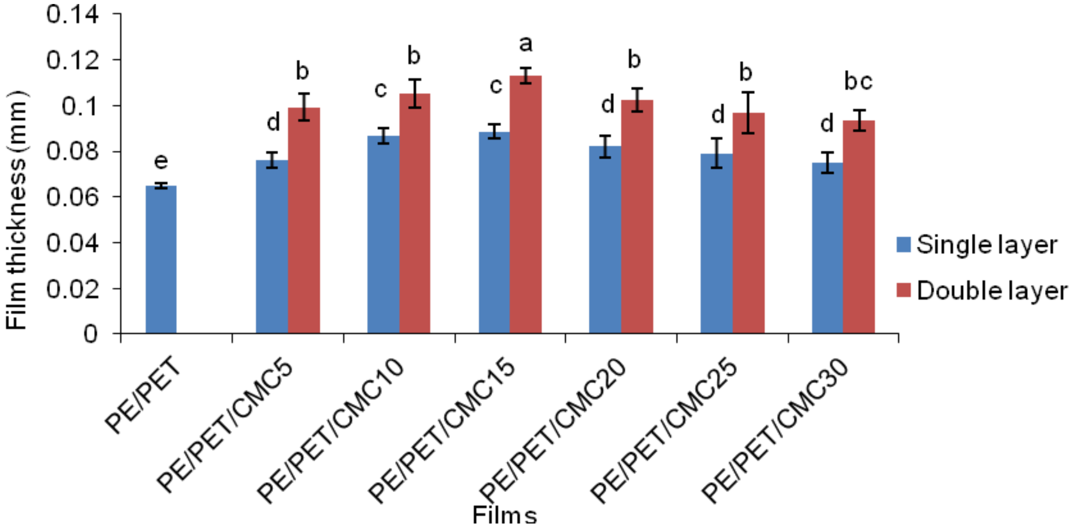

Figure 4.3 Thickness of PE/PET and PE/PET/CMC films with CMC coating. (Notes: Data represent means of n=5 measurements while error bars represent the standard deviation. Different letter (a-e) represent significant different with ($p<0.05$). Film thickness was statistically analysed using Bonferroni).

A possible explanation for increased or decreased in film thicknesses was due to DS value exhibited by each CMC which has been discussed in section 3.2.2.2. According to Pushpamalar et al. (2006), cellulose hydroxyl and sodium chloroacetate used during etherification in the presence of NaOH produce CMC as a main product. Higher substitution of carboxymethyl group at cellulose backbone results in the increment of DS value. DS value also influence the viscosity of CMC. Thus, increased in DS value will also increase the viscosity of CMC. In addition, glycerol which was used as a plasticiser acts as a cross linking agent (Chung et al., 2009) and could reduce the intermolecular forces of polymer (Ghamsemlou et al., 2011). During drying process of film, water that bound with hydroxyl group from CMC and glycerol evaporates and thus creates a linkages between CMC and glycerol. This condition caused the upturn of intermolecular interactions (Jahit et al., 2016) between CMC and glycerol and therefore creates a thin layer of CMC coating on PE/PET film.

From Figure 4.3, the values obtained for PE/PET/CMC films with single layer coating were between 0.075 mm to 0.089 mm. Overall, the thickness of CMC coating formed on PE/PET film for all samples were between 0.010 to 0.024 mm. Meanwhile for double layer coating, another 35 ml of CMC solution was deposited on PE/PET/CMC film with single layer coating and dried for another 12 hours. During this process, water from the second coating evaporates and new intermolecular interactions were produced between CMC and glycerol thus creates a new layer of CMC coating. Films with double layer coating have higher concentration of CMC and glycerol thus increased the thickness of films. Compared to PE/PET/CMC films with double layer coating, the film thicknesses were between 0.094 mm to 0.113 mm with the increment of coating thickness ranged from 0.029 mm to 0.048 mm. From the result, there was a significance differences between PE/PET film and other samples with $p<0.05$. For single layer coating, there was no significant different between PE/PET/CMC10 and PE/PET/CMC15 due to higher DS value demonstrated by each CMC. However, these samples were significantly different with PE/PET/CMC5, PE/PET/CMC20, PE/PET/CMC25 and PE/PET/CMC30. Compared to PE/PET with double layer CMC coating, only PE/PET/CMC15 was significantly difference with other samples due to higher value of film thickness recorded. Overall, there was a significant different between single and double layer coating for all samples.

4.2.2.2 Moisture content

Study on moisture content of film was conducted to measure the effect of CMC coating applied on PE/PET film. The value for moisture content of film should be kept as minimum as possible as it influenced other film's characteristics such as moisture absorption and water vapour permeability. CMC is a hydrophilic material (Mondal et al., 2015) and contains a hydroxyl group that can impart hydrophilicity of the film. Figure 4.4 shows the graph of moisture content for PE/PET films and PE/PET/CMC films with single or double layer coating. The values obtained were based on triplicate sample. From the findings, the value of moisture content for all samples ranged between 0.59 ± 0.2% to 7.55 ± 0.31%. PE/PET films without CMC coating showed the lowest moisture content value while PE/PET/CMC15 with double layer coating displayed the highest value of moisture content. From this study, it shows that when the concentration of NaOH used to extract CMC wheat bran increased to 15% (w/v), the value for moisture content in the sample also increased. However, when the concentration of NaOH reached 20% (w/v) and above, the value for moisture content started to decrease. For single layer CMC coating, the value for moisture content increased from 3.45 ± 0.20% to 6.15 ± 0.35% for PE/PET/CMC5 and PE/PET/CMC15 respectively. On the other hand, the value for moisture content decreased to 3.23 ± 0.19% and 3.17 ± 0.25% for PE/PET/CMC20 and PE/PET/CMC30 respectively.

In chapter 3 section 3.2.2.2, CMC10 and CMC15 have demonstrated higher DS value compared to other sample with 0.83 and 0.84 respectively. Higher DS values indicate higher number of carboxymethyl group substituted at cellulose backbone (Adinughara et al., 2005). This carboxymethyl group is hydrophilic thus; the number of possible hydrogen bond to interact with water molecules is higher. In comparison with PE/PET/CMC20, PE/PET/CMC25 and PE/PET/CMC30, the value for moisture content were slightly lower due to the CMC produced using 20%, 25% and 30% (w/v) NaOH exhibited lower DS value that ranged from 0.36 to 0.40. The substitution of carboxymethyl group at cellulose backbone during etherification process in the production of CMC wheat bran will affect the hydrophilicity of CMC produced. As a result, carboxymethyl group tends to absorb and diffuse water when expose to environment (Ghanbarzadeh et al., 2011) and creates CMC-glycerol and CMC-water interactions thus affecting the moisture content of CMC films. Higher degree of substitution of CMC15 demonstrated in previous chapter of this study proved that PE/PET film coated with single or double layer of CMC would have higher moisture content compared to another sample. Marvdashti et al. (2017) in her study reported that increase in the availability of hydroxyl group in film matrix would also increase the possibility of hydrogen bond to interact with water molecules thus resulting in higher moisture content of film.

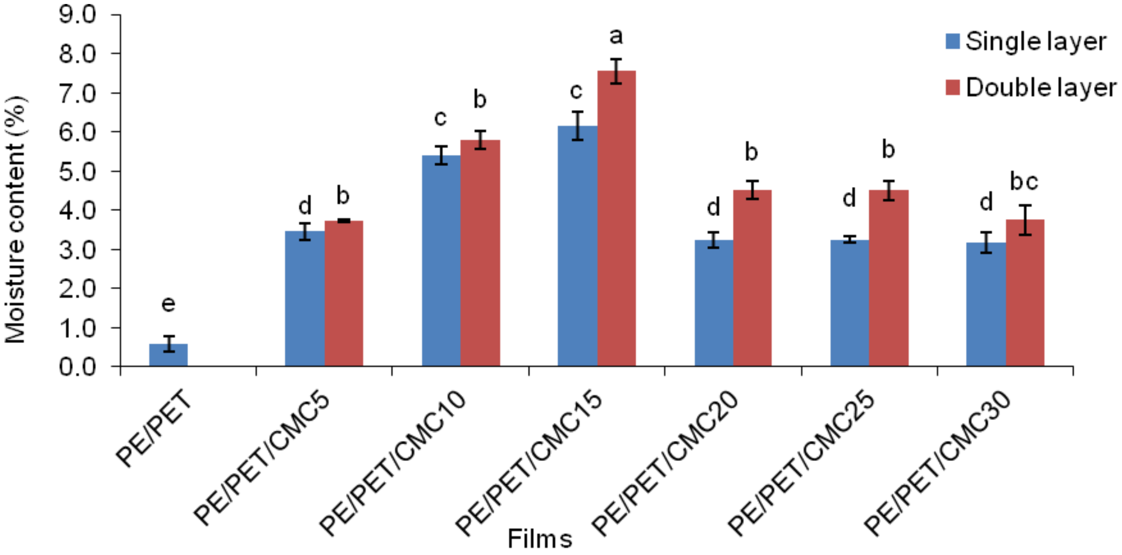

Figure 4.4 Moisture content of PE/PET and PE/PET/CMC films with single or double layer coating.
(Notes: Data represent means of n=3 measurements while error bars represent the standard deviation. Different letter (a-e) represent significant different with ($p<0.05$). Moisture content was statistically analysed using Bonferroni).

In this study, the concentration of glycerol added into CMC wheat bran solution was 20% (v/v). The moisture content recorded for PE/PET/CMC5 was 3.73 ± 0.03%. This value rose to 7.55 ± 0.31% for PE/PET/CMC15 but decreased to 4.51 ± 0.23% and 4.50 ± 0.24% for PE/PET/CMC20 and

PE/PET/CMC25 respectively. The value for moisture content was further reduced to 3.75 ± 0.37% for PE/PET/CMC30. The result of film's moisture content obtained in this study was in accordance with study conducted by Ahmadi *et al.,* (2012), who reported that the amount of moisture content occurred in films was due to the water holding capacity of glycerol. This was due to hydroxyl groups that available in glycerol. These authors also stated that if the concentration of glycerol is more than 10%, the value of moisture content will be increased. When glycerol increased to 10% or higher, film properties would change due to the formation of glycerol-CMC and glycerol-water interaction.

Higher moisture content was found in PE/PET/CMC film with double layer coating compared to single layer coating due to the addition of more glycerol and CMC molecules. According to Cerquira *et al.,* (2012) when the concentration of glycerol increased, moisture content also increased due to the water holding capacity of glycerol. Therefore, it increased the availability of hydroxyl group in glycerol. This situation results in the raise of possible hydrogen bond to interact with water causing the hydroxyl group to hold more water molecules. This statement is also supported by study done by Cho and Rhee (2002) stated that increased absorption of moisture by the films was caused by the increment of plasticiser concentration. From Figure 4.4, similar trend for moisture content can be observed with PE/PET/CMC15 shows the highest value of moisture content with 6.15 ± 0.35% and 7.55 ± 0.31% for single and double layer coating respectively. Mali et al. (2005) reported that higher moisture content will affect film permeability to water and gas thus the film cannot act as a good barrier against water and gases. From Figure 4.4, there was a significant different between PE/PET film and other PE/PET samples coated with single or double layer coating with $p<0.05$. Generally, PE/PET/CMC10 and PE/PET/CMC15 were significantly difference between PE/PET/CMC5, PE/PET/CMC20, PE/PET/CMC25 and PE/PET/CMC30 for single and double layer coating. Compared to other samples, PE/PET/CMCM15 displayed a significant different between single and double layer coating.

4.2.2.3 Film solubility

Film solubility is an important variable to determine the nature of material used towards moisture resistant. According to Perez-Gago and Krochta (1999), low films solubility is needed for edible film to enhance product integrity and water resistance compared to high film solubility that cannot protect the product from humidity and water loss (Gontard et al., 1993). In my study, film solubility was measured as to determine the effect of different NaOH concentrations used during etherification process on CMC produced. Figure 4.5 shows the graph of film solubility percentages for PE/PET/CMC film with single and double layer coatings. All mean values obtained in this study were based on triplicate samples. Solubility for PE/PET film without any coating also been analysed. The value of film solubility for PE/PET film was recorded at 1.43 ± 0.30%, the lowest value obtained compared to another sample. Among single layer CMC coating, PE/PET/CMC10 showed the highest film solubility with 32.92 ± 0.80% followed by PE/PET/CMC15, PE/PET/CMC5 and PE/PET/CMC20 with 27.98 ± 0.86%, 24.16 ± 0.40% and 23.49 ± 0.62% respectively.

PE/PET/CMC25 exhibited lower percentage of film solubility with 16.25 ± 0.78% while PE/PET/CMC30 displayed the lowest value with 12.13 ± 0.65%. From this result, it shows that increased in NaOH concentration during CMC etherification will decrease the solubility of PE/PET/CMC film. This is because higher concentrations of NaOH exhibited lower degree of substitution and thus reduce the number of carboxyl group attached to the cellulose backbone (Adinughara et al., 2005). To date, there are only few studies that reported values for film solubility using CMC in combination with other biopolymers. These studies included a research conducted by Ghanbarzadeh et al., (2011) who demonstrated that starch/CMC film containing 40% (v/w) glycerol exhibited film solubility of 21.33%. Besides, another study by Tongdeesoontorn et al., (2011) stated that cassava starch/CMC film recorded a value of 73% while Jouki et al., (2013) reported that film produced using cress seed gum and glycerol demonstrated film solubility between 48.30 to 54.12%. Lower degree of substitution will reduce the ability of CMC molecules to bind with water due to lower substitution of carboxymethyl group at cellulose backbone. As a result, CMC tend to be less hydrophilic. Karoosi et al. (2013) in his study reported that hydrophilic compound would increase film solubility while hydrophobic compound will decrease the solubility.

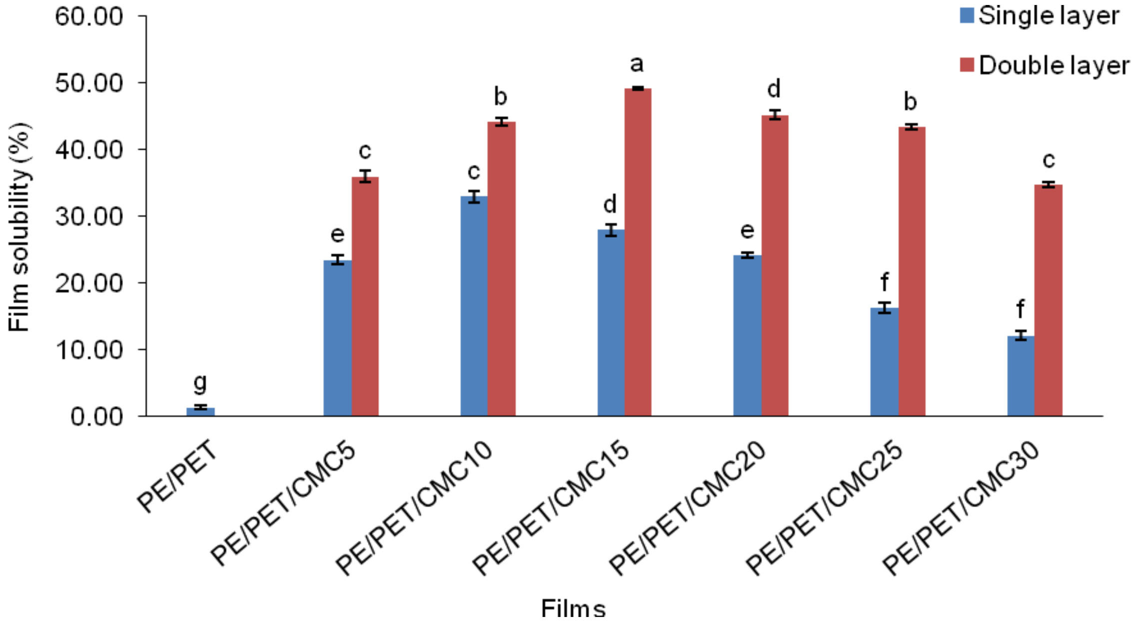

Figure 4.5 Percentages of film solubility for PE/PET and PE/PET films with single and double layer coating.
(Notes: Data represent means of n=3 measurements while error bars represent the standard deviation. Different letter (a-g) represent significant different with ($p<0.05$). Film solubility was statistically analysed using Bonferroni).

McHugh and Krochta, (1994) reported that solubility is related to the load of free hydroxyl group in polymeric matrix that will permit the formation of hydrogen interactions between film and water. Glycerol and CMC are hydrophilic materials that contained a hydroxyl group. Based on the degree of substitution of CMC wheat bran, CMC10 and CMC15 exhibited the highest DS value compared to another sample. This result can be seen in Figure 3.11 in chapter 3 where CMC10 and CMC15 recorded DS values of 0.84 and 0.83 respectively. The result obtained for DS value was correlated with the percentages of film solubility showed by PE/PET/CMC10 and PE/PET/CMC15 in this chapter. Higher DS values indicate that, the number of carboxylmethyl group available in the CMC structure are higher hence, it increases the solubility and hydrophilicity of the film.

For PE/PET/CMC films with double layer coating, similar trend for percentages of film solubility were observed. From the results, PE/PET/CMC15 film showed the highest percentage of film solubility with 49.06 ± 0.18%. The increment in volume of coating solution added on PE/PET increased the amount of glycerol and CMC in the coating and thus, resulted in the increment of hydroxyl and carboxymethyl group present in the coating. Due to the increased in proportion of hydrophilic compound arising from glycerol and CMC, these materials tend to bind with more water and as a result increased film solubility. This finding agreed with study done by Dick et al., (2015) on the effect of glycerol concentration in the production of chia seed mucilage edible film. In his study, when the concentration of glycerol increased from 25% to 75% (v/w), the percentage of film solubility increased from 52.74 ± 0.96% to 84.50 ± 0.74%.

Compared to PE/PET/CMC15, other samples coated with double layer coating showed lower percentages of film solubility due to lower DS value recorded by each CMC before. On the other hand, the values obtained for all double layer coating samples were higher compared to single layer coating. This is due to the increment of CMC and glycerol volumes added on PE/PET film thus increase the film thickness. In Figure 4.5, the results obtained for PE/PET/CMC20, PE/PET/CMC10 and PE/PET/CMC25 were 45.15 ± 0.71%, 44.13 ± 0.59% and 43.38 ± 0.40% respectively. In contrast, PE/PET/CMC30 recorded the lowest value of film solubility with 34.72 ± 0.45%. PE/PET/CMC30 with single and double layer coating exhibited the lowest values amongst other samples. This is due to the lowest degree of substitution recorded in CMC30 before with only 0.32.

Besides CMC, the increment of film solubility in double layer coating was due to the existence of hydroxyl group from glycerol. In correspond to study carried out by Cuq et al., (1997) and Ghasemlou et al., (2011), the occurrence of glycerol in film could diminish interactions between biopolymer molecules. This situation would increase the solubility of film due to hydrophilic nature of glycerol. This condition will cause more water to engage into the polymer matrix and formed more regions that are mobile. From Figure 4.5, there were significant differences between each sample for PE/PET with single layer CMC coating except between PE/PET/CMC25 and PE/PET/CMC30. Compared to PE/PET/CMC with double layer coating, there were no significant differences between PE/PET/CMC5 and PE/PET/CMC30 as well as PE/PET/CMC10 and PE/PET/CMC25 with $p<0.05$. From this finding, increased in DS values and volume of hydrophilic material such as CMC and glycerol could increase the solubility of film. Therefore, CMC films with higher solubility are potential to be used and applied as an edible film.

4.2.2.4 Colour measurement

Colour is an important characteristic in food packaging as it indicates the appearance of food product inside the package. Lightness value (L) measures the colour of films with 0 value indicates black colour while 100 indicates for white colour. Table 4.1 shows the value of lightness (L), redness (a) and yellowness (b) for PE/PET/CMC films. The (L) value for PE/PET film was recorded at 95.64 ± 0.01. Overall, the (L) values for PE/PET/CMC with single layer coating were between 95.77 ± 0.01 to 96.28 ± 0.01. The highest (L) value was demonstrated by PE/PET/CMC5 while the lowest value was displayed by PE/PET/CMC15. Compared to PE/PET/CMC with double layer coating, there were slightly small reductions in (L) value for all samples.

However, these reductions are not significant. For PE/PET/CMC with double layer coating, the (L) values range between 95.63 ± 0.04 to 96.08 ± 0.08. In this study, PE/PET/CMC5 exhibited the highest value similar with single layer coating, while PE/PET/CMC25 showed the lowest (L) value.

Decreased in (L) value for all PE/PET/CMC films with double layer coating were due to the formation of intermolecular forces between CMC with CMC and CMC with glycerol (Ghamsemlou et al., 2011). During drying process in single layer coating, water that initially bound with hydroxyl and carboxymethyl groups were evaporated and creates a linkage between polymer-polymer and polymer-glycerol interactions. When another 35 ml of CMC solution was poured on dried PE/PET/CMC film with single layer coating, these polymers and glycerol attracted and bound with water molecules from coating solution to form CMC-water and glycerol-water interactions. Once water was evaporated during second time of drying process, these interactions would change back to CMC-CMC and CMC-glycerol interactions. This finding is also supported by Ghanbarzadeh et al., (2010), who reported that increasing the glycerol content would change the colour index because of new bond formation. When the concentration of CMC and glycerol increased, the lightness of the film reduced due to the formation of new layer of CMC coating. Increased in glycerol content also resulted in decreased of lightness. In this study, films produced using single layer coatings were transparent and could be seen through. This feature is favourable in food packaging, as it will affect consumer acceptability (Acevedo-Fani et al., 2015). From Table 4.1, the (L) values for PE/PET/CMC5S, PE/PET/CMC5D, PE/PET/CMC20S and PE/PET/CMC25S were significantly difference as compared to other samples with $p<0.05$.

Table 4.1 also shows the redness-greenness value (a) of PE/PET/CMC films produced using single and double layer coating. Positive (a) value measures the degree of redness while negative (a) value relates with the degree of greenness of sample. Among all sample, PE/PET film without any CMC coating displayed -0.005 ± 0.001 for (a) value. Compared to PE/PET/CMC with single layer coating, the lowest (a) value was shown by PE/PET/CMC10 film with -0.090 ± 0.002, while the highest (a) value was displayed by PE/PET/CMC25 with -0.015 ± 0.002. This is followed by PE/PET/CMC30 and PE/PET/CMC5 with both of these films recorded at -0.021 ± 0.007 and -0.026 ± 0.003 respectively. In this analysis, PE/PET/CMC20 and PE/PET/CMC15 exhibited lower (a) values with -0.032 ± 0.02 and -0.073 ± 0.005. Negative (a) value obtained in this study indicates that all films displayed a slightly green in colour. When the concentration of NaOH used to extract CMC increased from 5% to 10% (w/v), the (a) value also increased. However, this value decreased when concentration of NaOH increased to 25% (w/v) and the level of this effect cannot be observed with the naked eye.

For PE/PET/CMC with double layer coating, all samples showed the increment in (a) value. From the result, the value obtained for (a) values were between -0.325 ± 0.040 to -0.052 ± 0.002. The lowest value was observed at PE/PET/CMC25 while the highest value was demonstrated by PE/PET/CMC5. Similar result was observed between PE/PET/CMC10 and PE/PET/CMC15 with both samples showed -0.144 ± 0.005 and -0.146 ± 0.003 respectively. When the concentration of NaOH used to extract CMC wheat bran increased to 25% (w/v) the (a) value reduced to -0.247 ± 0.060. This value later declined to -0.325 ± 0.040 resulting in the lowest (a) value among all samples. The raise of (a) value in all samples with double layer coating was due to the amount of CMC and glycerol added on single layer coating thus creating a network with current layer of CMC coating. Previously, the colour of CMC obtained in this study has been reported under section 3.2.2.5. Due to the carboxymethylation reaction (Rachtanapun et al., 2012), the (a) value of CMC wheat bran increased.

Table 4.1 Lightness (L), redness-greenness (a) and blueness-yellowness (b) values for PE/PET and PE/PET/CMC films with single and double layer coating.

Films	Lightness value (L)	(a) value	(b) value
PE/PET	95.636 ± 0.013^{b}	-0.005 ± 0.001^{c}	0.464 ± 0.031^{d}
PE/PET/CMC5S	96.280 ± 0.009^{a}	-0.026 ± 0.003^{c}	0.585 ± 0.003^{d}
PE/PET/CMC10S	95.867 ± 0.022^{b}	-0.090 ± 0.002^{c}	1.075 ± 0.012^{c}
PE/PET/CMC15S	95.769 ± 0.005^{b}	-0.073 ± 0.005^{c}	1.353 ± 0.005^{b}
PE/PET/CMC20S	96.009 ± 0.131^{ab}	-0.032 ± 0.023^{c}	0.539 ± 0.053^{d}
PE/PET/CMC25S	95.950 ± 0.138^{ab}	-0.011 ± 0.005^{c}	0.451 ± 0.010^{d}
PE/PET/CMC30S	95.876 ± 0.055^{b}	-0.021 ± 0.007^{c}	0.642 ± 0.037^{d}
PE/PET/CMC5D	96.084 ± 0.085^{a}	-0.052 ± 0.023^{c}	0.699 ± 0.140^{d}
PE/PET/CMC10D	95.756 ± 0.041^{b}	-0.144 ± 0.004^{bc}	1.248 ± 0.020^{b}
PE/PET/CMC15D	95.661 ± 0.031^{b}	-0.146 ± 0.003^{bc}	1.550 ± 0.027^{a}
PE/PET/CMC20D	95.824 ± 0.058^{b}	-0.247 ± 0.064^{b}	1.784 ± 0.195^{a}
PE/PET/CMC25D	95.632 ± 0.041^{b}	-0.325 ± 0.044^{a}	1.719 ± 0.121^{a}
PE/PET/CMC30D	95.676 ± 0.013^{b}	-0.182 ± 0.008^{b}	1.611 ± 0.050^{a}

(Notes: Data represent means of n=3 measurements while error bars represent the standard deviation. Different letter (a-d) represent significant different with ($p<0.05$). L, a and b values were statistically analysed using Bonferroni).

As described in section 2.4.1.2, film produced using double layer CMC coating contained another 35 ml of CMC solution. This situation consequently increased the amount of CMC and glycerol in the coating deposited on PE/PET film. In previous chapter, CMC powder produced from wheat bran exhibited reddish and yellowish in colour. However, when CMC powder dissolved in water, it created a yellowish solution. With the addition of glycerol, the colour of the solution becomes lighter and thus reduces the reddish colour of CMC powder resulting to the greener films. This situation is caused by the increment of CMC and glycerol concentration added onto PE/PET films. In this study, there was no significant difference between PE/PET film and other PE/PET/CMC with single layer coating for (a) value. Compared to

PE/PET/CMC with double layer coating, PE/PET/CMC25S was significantly difference with another sample. There was no significant difference observed between PE/PET/CMC10D and PE/PET/CMC15D as well as PE/PET/CMC20D and PE/PET/CMC30D. (b) value indicates the degree of blueness and yellowness of films. Positive (b) value measures the yellowness while negative (b) value measures the blueness of the sample.

Based on Table 4.2, (b) value for PE/PET films was 0.464 ± 0.031. For PE/PET coated with single layer of CMC coating, (b) value increased from 0.584 ± 0.003 to 1.075 ± 0.012 for PE/PET/CMC5 and PE/PET/CMC10 respectively. When the concentration of NaOH used to etherify CMC increased to 15% (w/v), (b) value increased to 1.353 ± 0.005 for PE/PET/CMC15. However, (b) value started to decrease to 0.539 ± 0.053 for PE/PET/CMC20 when NaOH concentration reached up to 20% (w/v) and further reduced to 0.451 ± 0.011 for PE/PET/CMC25 at 25% (w/v) NaOH concentration. Despite of these reductions, the (b) value for PE/PET/CMC30 rose slightly to 0.643 ± 0.037. Previously in section 3.2.2.5, (b) values for CMC wheat bran extracted using different concentrations of NaOH ranged between 15.92 ± 0.20 to 22.01 ± 0.54 thus produced yellowish colour of CMC. This finding suggests that colour of film produced can be affected by the initial colour of material used. Because of that, the colours of PE/PET/CMC films are a bit yellowish.

Compared with PE/PET/CMC with single layer coating, there was an increased in (b) value for all PE/PET/CMC films with double layer coating. Among all double layer samples, PE/PET/CMC5 exhibited the lowest (b) value while PE/PET/CMC25 demonstrated the highest (b) value with 0.699 ± 0.140 and 1.653 ± 0.036 each. In contrast with single layer sample, PE/PET/CMC films with double layer coating showed an increment in (b) values when concentration of NaOH used in etherification process increased from 5% to 30% (w/v). Higher (b) value in double layer coating was due to the presence of more CMC and glycerol molecules in the coating solution added on PE/PET films. The (b) value for all CMC powder ranged from 17.08 ± 0.27 to 22.01 ± 0.54. This finding has been discussed under section 3.2.2.5. Higher value obtained was due to the carboxymethylation during CMC production (Rachtanapun et al., 2012). CMC solution added on single layer coating will then create a network or linkages between CMC-CMC and CMC-glycerol interactions once the coating dried (Ghamsemlou et al., 2011). Therefore, the films produced will become thicker and yellower due to more linkages created by CMC and glycerol on the surface of the film. Film colour of food package is very important criterion that may affect consumer acceptability as it can display general appearance of food product. From the result, PE/PET/CMC10S and PE/PET/CMC15S were significantly difference with other single layer coating samples and there was no significant difference between PE/PET/CMC15D, PE/PET/CMC20D, PE/PET/CMC25D and PE/PET/CMC30D. However, these samples were significantly difference with other samples.

Table 4.2 also shows the yellowness index (YI) for PE/PET films coated with single and double layer of CMC. Based on ASTM E 313 (2005), yellowness index is the number calculated from spectrophotometric data that describes the changes in colour of test sample from white to yellow. According to Table 4.2, the value for yellowness index demonstrated by PE/PET film was 0.693 ± 0.046. For single layer coating, the highest yellowness index was displayed by PE/PET/CMC15 followed by PE/PET/CMC10 with 2.019 ± 0.008 and 1.603 ± 0.019. PE/PET film coated with CMC25 showed the lowest value of yellowness index with only 0.671 ± 0.016. Other than that, PE/PET film coated with CMC20, CMC5 and CMC30 recorded 0.802 ± 0.079, 0.867 ± 0.004 and 0.957 ± 0.056 respectively. From these data, it can be expressed that (b) value affects the yellowness index of the sample. Higher (b) value would contribute to higher value of yellowness index. Similar trend can be observed between (b) and yellowness index value. As PE/PET/CMC10 and

PE/PET/CMC15 showed higher (b) value, PE/PET/CMC10 and PE/PET/CMC15 also demonstrated higher (b) values in yellowness index.

Table 4.2 Yellow index, total colour differences and transparency of PE/PET and PE/PET/CMC films with single and double layer coating.

Films	Yellowness Index (YI)	Total colour different (E)	Transparency
PET/PE	0.693 ± 0.046^{c}	4.388 ± 0.012^{b}	3.138
PET/PE/CMC5S	0.867 ± 0.004^{c}	3.765 ± 0.008^{d}	3.077
PET/PE/CMC10S	1.603 ± 0.019^{b}	4.271 ± 0.025^{bc}	3.015
PET/PE/CMC15S	2.019 ± 0.008^{a}	4.443 ± 0.004^{b}	3.003
PET/PE/CMC20S	0.802 ± 0.079^{c}	4.027 ± 0.013^{c}	3.041
PET/PE/CMC25S	0.671 ± 0.016^{c}	4.075 ± 0.013^{c}	3.057
PET/PE/CMC30S	0.957 ± 0.056^{c}	4.174 ± 0.058^{c}	3.079
PET/PE/CMC5D	1.040 ± 0.029^{c}	3.979 ± 0.010^{c}	2.960
PET/PE/CMC10D	1.862 ± 0.029^{a}	4.426 ± 0.034^{b}	2.929
PET/PE/CMC15D	2.315 ± 0.041^{a}	4.609 ± 0.029^{a}	2.899
PET/PE/CMC20D	2.430 ± 0.024^{a}	4.490 ± 0.057^{ab}	2.944
PET/PE/CMC25D	2.470 ± 0.053^{a}	4.682 ± 0.032^{a}	2.965
PET/PE/CMC30D	2.406 ± 0.075^{a}	4.618 ± 0.005^{a}	2.982

Notes: Data represent means of n=3 measurements while error bars represent the standard deviation. Different letter (a-d) represent significant different with ($p<0.05$). Yellow index, total colour different and transparency were statistically analysed using Bonferroni).

Compared to single layer coating, all PE/PET coated with double layer coating showed an upturn of yellowness index. From Table 4.2, the values for yellowness index were between 1.040 ± 0.029 to 2.470 ± 0.053. From the data obtained, increased in NaOH concentration results in higher yellowness index. This can be observed at PE/PET/CMC5 that exhibited the lowest value for yellowness index while the highest value was shown by PE/PET/CMC25. When PE/PET films were coated with double layer CMC10 and CMC15 solution, the yellowness value increased from 1.862 ± 0.029 to 2.315 ± 0.041 respectively. This was followed by PE/PET coated with CMC30 and CMC20 coating solutions with each represent the value of 2.406 ± 0.075 and 2.430 ± 0.024. In comparison with PE/PET with single layer CMC coating, the yellowness index of double layer coating seems to increase even though the concentration of NaOH has reached 20%, 25% and 30% (w/v). In this case, (b) values obtained for all PE/PET films coated with double layer coating were also increased towards the increment of NaOH concentrations.

On the other hand, film thickness also influences the yellowness index. As discussed in section 4.2.2.1 earlier, higher film thicknesses were observed in PE/PET/CMC with double layer coating to that of single layer coating. This is due to the increased in intermolecular interaction (Jahit *et al*., 2016) between CMC and glycerol that formed on PE/PET films thus creates a thin layer of CMC-glycerol linkages on PE/PET film. Hence, these linkages will significantly increase the (b) values and yellowness index. Jouki *et al.,* (2013) stated that addition of plasticiser might improve optical properties of the films. This is true for PE/PET/CMC film with double layer coating that contained more glycerol and CMC. From Table 4.2, there were significant differences between PE/PET/CMC10 and PE/PET/CMC15S with other single layer coating samples. PE/PET/CMC5 showed a significant difference ($p<0.05$) with other double layer coating samples. Compared between single and double layer coating, there were a significant different for all samples except for PE/PET/CMC5 and PE/PET/CMC15.

Besides yellowness index, delta E or total colour differences (ΔE) for all PE/PET/CMC films were also been examined in this study. Total colour difference measures the changes in variation of (L), (a) and (b) values of the sample as compared to white colour. In this analysis, the values for (ΔE) ranged from 3.765 to 4.682. When the value of (ΔE) were between 3 to 6, colours are perceptible at a glance and considered as acceptable number. A total colour difference for PE/PET film without coating was reported at 4.388 ± 0.012. For PE/PET/CMC with single layer coating, the values ranged from 3.765 ±0.008 to 4.443 ± 0.004. The highest value was shown by PE/PET/CMC15 while the lowest value was displayed by PE/PET/CMC5. Compared to PE/PET film without any coating, the value obtained for total colour difference was within the ranged. Total colour different started to increase to 4.271 ± 0.025 for PE/PET/CMC10S and to 4.443 ± 0.004 for PE/PET/CMC15S. However, these values were slowly reduced to 4.174 ± 0.058, 4.075 ± 0.0137 and 4.027 ± 0.0135 for PE/PET/CMC30S, PE/PET/CMC25S and PE/PET/CMC20S respectively. When the concentration of NaOH used to extract CMC were higher than 15% (w/v), total colour differences were diminished.

This is due to the value of (a) and (b) obtained previously which declined after 15% (w/v) NaOH concentration. Overall, the value of total colour difference was influenced by lightness, redness or greenness as well as yellowness or blueness of films. There were increments of total colour difference in all PE/PET/CMC films with double layer coatings compared to single layer coating. From the data shown in Table 4.2, PE/PET/CMC25D exhibited the highest value with 4.682 ± 0.032 followed by PE/PET/CMC30D with 4.618 ± 0.005, PE/PET/CMC15D with 4.609 ± 0.029 and PE/PET/CMC20D with 4.490 ± 0.057. Amongst all samples, PE/PET/CMC10D and PE/PET/CMC5D displayed lower value with 4.426 ± 0.034 and 3.979 ± 0.010 each. Su *et al.,* (2010) in their study on blended films of soy protein isolate and carboxymethyl cellulose reported that factors such as pH, cross linking degree, amount of plasticiser, thermal treatment and weight content of blends capable to greatly affect film colours.

Besides yellowness index and total colour different, transparency of film also been analysed. Marvdashi *et al.,* (2017) reported that film transparency provides information about its ability to protect packaged material from disadvantages of light. According to Xu *et al.,* (2007), transparency of films is an indicator of the miscibility of polymer blends films. This statement supports the results obtained in this study as more linkages of polymer-polymer and polymer-plasticiser were formed in PE/PET/CMC with double layer coating film thus reducing the transmittance of light through films. The highest value of transparency was demonstrated by PE/PET film without any coating with 3.138. PE/PET/CMC films with single layer coating exhibited transparency values of 3.003 to 3.079 while PE/PET/CMC films with double layer coating displayed values between 2.899 to 2.982, slightly lower compared to single layer coating. Lower values of transparency in PE/PET/CMC with double layer coating were due to the thickness of film produced. According to Sun *et al*., (2013), film thickness and colour have direct effect on transparency. Besides higher values in film thickness, (L), (a) and (b) values would also influence the transparency of the films. Kanatt *et al*., (2012) in his study confirmed that transparency and opacity are reversely related, as high in transparency would lead to low opacity.

4.2.2.5 Contact angle measurement

Jayasekara *et al.,* (2004) reported that contact angle measurement could be used to study the changes in surface composition, migration of functional group in polymers, hydrophobicity, hydrophilicity and surface homogeneity of film. It is also correlates to the surface feature like smoothness and roughness of films as increased in smoothness would decrease in contact angle. Bai *et al.,* (2013) reported that, surface contact angle is an angle between film surface and tangent at the droplet-film intersection. Higher value of contact angle exhibits that the surface of film is hydrophobic while lower value of contact angle indicates that the surface of film is hydrophilic. Table 4.3 shows the contact angles for PE/PET/CMC films with single and double layer coating. From the results, PE/PET film showed the highest contact angle with 64.49 ±

2.53°. However, after 60 seconds the angle reduced to be 58.96 ± 0.97°. For single layer coating samples, contact angle for PE/PET film coated with CMC5 was demonstrated at 27.50 ± 2.34°. The angle increased up to 35.29 ± 1.50° when film was coated with CMC10. This value however declined to 26.60 ± 2.31° for PE/PET/CMC15S. Increased in NaOH concentration resulted in decreased of contact angle. This can be proved by results obtained for PE/PET/CMC20S, PE/PET/CMC25S and PE/PET/CMC30S with each of the films displayed the values of 23.70 ± 2.42°, 23.77 ± 1.75° and 18.98 ± 1.01°. Overall, PE/PET/CMC10S exhibited the highest contact angle while PE/PET/CMC30S showed the lowest contact angle. From the results, the differences of contact angle values at 0 and after 60 seconds were between 1.14° to 5.90°.

Higher number of carboxylmethyl group that substituted the cellulose backbone caused higher contact angles at PE/PET/CMC5S, PE/PET/CMC10S and PE/PET/CMC15S. Previously in section 3.2.2.3, the degree of substitution for CMC5, CMC10 and CMC15 were recorded at 0.60 to 0.88. Increased in carboxymethyl group would lead to the formation of viscous cellulose gum. As CMC is a hydrophilic material, it attracts water. Therefore, when water was dropped on the coating surface, CMC will absorb water and the contact angle formed on the surface would become smaller. Besides that, Ghasemlou *et al.,* (2011) stated that higher hydrophlicity of the samples is also attributable to the water binding capacity of the glycerol. Compared to CMC20, CMC25 and CMC30, the values for degree of substitution ranged from 0.32 to 0.40. This situation results in lower substitution of carboxymethyl group (Barai *et al.,* 1997) and thus reduce the contact angle formed on the coating. In addition, the formation of sodium chloride in CMC during etherification process also contributes to the lower contact angle measurement due to the polymer degradation (Barai *et al.,* 1997). From Table 4.3, the value of contact angle for PE/PET was significantly difference compared to other samples. There were no significant differences observed between PE/PET/CMC10S and

PE/PET/CMC10D as well as PE/PET/CMC30S and PE/PET/CMC30D with $p<0.05$.

When higher concentration of sodium hydroxide was used to extract CMC from wheat bran, more sodium chloride was produced as a by-product. This situation caused higher amount of sodium chloride formed in CMC coating. Due to the nature of sodium chloride and CMC that is hygroscopic and hydrophilic, CMC coating would absorb the dropped water and swollen, thus increased the spread ability of water on coating and decreased the contact angle of film. Besides, sodium chloride could act as a surfactant in CMC solution and reducing the surface tension of CMC coating. Therefore, the occurrence of carboxyl group in CMC and the formation of sodium chloride in CMC coating enhanced the wettability of films and increased films solubility. This condition is in agreement with statement done by Marvdashti et al., (2017) explained that different in nature and chemical structure of film forming materials will diverse the contact angle. According to Phan et al., (2005), water droplet spreads out more rapidly on the surface of a soluble film compared to insoluble one.

On the other hand, increased in the volume of CMC coating solution will result in decreased of contact angle values. This situation can be noticed in all PE/PET/CMC films with double layer coating that showed a reduction of contact angle values compared to PE/PET/CMC film with single layer coating. From Table 4.3, it can be seen that PE/PET/CMC5D displayed the value of 26.36 ± 2.17^{o}. All samples with double layer coating exhibited similar trend with films coated with single layer coating. The highest contact angle value was demonstrated by PE/PET/CMC10D with 31.99 ± 0.99^{o} while the lowest was 15.68 ± 1.55^{o} shown by PE/PET/CMC30D. From the results, all samples with double layer coating show a reduction in contact angle values after 60 seconds. The values obtained for all samples were between 13.81 ± 1.46^{o} to 28.46 ± 1.24^{o}. This situation was likely due to the higher volume of carboxymethyl and hydroxyl groups from CMC and glycerol molecules present in the coating. Latey (1969) reported that the contact angle is also dependent on the liquid surface tension as lower contact angle was due to lower surface tension. This statement has been supported by Cerqueira et al., (2009), who mentioned in his study that the interaction between liquid and surfaces is caused by the dispersion forces that can influence the effectiveness of coating spreading on the surface. As in Table 4.3, no significant different was recorded between 0 and after 60 seconds for all films.

Table 4.3 Contact angle of PE/PET and PE/PET/CMC films at 0 and 60 seconds.

Films	0 second	60 seconds
PE/PET	64.49 ± 2.53^a	58.96 ± 0.97^a
PE/PET/CMC5S	27.50 ± 2.34^a	22.79 ± 2.42^a
PE/PET/CMC10S	35.29 ± 1.50^a	30.76 ± 2.24^a
PE/PET/CMC15S	26.60 ± 2.31^a	20.70 ± 1.55^a
PE/PET/CMC20S	23.70 ± 2.42^a	19.25 ± 2.72^a
PE/PET/CMC25S	23.77 ± 1.75^a	18.27 ± 0.98^a
PE/PET/CMC30S	18.98 ± 1.01^a	15.91 ± 0.96^a
PE/PET/CMC5D	26.36 ± 2.07^a	22.29 ± 2.32^a
PE/PET/CMC10D	31.99 ± 0.99^a	28.46 ± 1.24^a
PE/PET/CMC15D	21.32 ± 0.73^a	17.20 ± 0.71^a
PE/PET/CMC20D	21.41 ± 1.98^a	18.29 ± 1.54^a
PE/PET/CMC25D	20.96 ± 1.35^a	16.99 ± 1.58^a
PE/PET/CMC30D	15.68 ± 1.55^a	13.81 ± 1.46^a

(Notes: Data represent means of n=6 measurements while error bars represent the standard deviation. Small letter (a) represent no significant different with ($p<0.05$). Contact angle was statistically analysed using paired sample T-test).

The differences in contact angle values for PE/PET/CMC with double layer coating at 0 and after 60 seconds were in a range of 1.86 to 4.11°. Carneiro-da-Cunha *et al.,* (2010) reported that the most wettable surfaces present low values ($\theta<20^o$) and the hydrophobic surfaces show higher value ($\theta>70^o$). In my study, higher amount of CMC and glycerol will affect the wettability. Carboxyl and hydroxyl groups would bind with water and thus reduced the wettability of coating. Fu et al., (2005) has reported that the properties of surfaces were influenced by the interpenetration of layers. As in Table 4.3, PE/PET/CMC with double layer coating showed lower contact angle compared to single layer film. When contact angle was recorded more than 90^o, the film was hydrophobic while less than 90^o, the film formed was hydrophilic. In my study, all films demonstrated hydrophilicity as the contact angles were less than 90^o. Therefore, CMC coating demonstrated higher wettability.

4.3.2 Comparison between single and double layer CMC coatings on mechanical properties of PE/PET/CMC film

The mechanical properties of PE/PET/CMC films were investigated to understand the effect of stresses in film structures and deformation of film (Nix, 1988). In this study, tensile strength, elongation at break and Young Modulus of films were evaluated. Tensile strength measures the maximum force applied to the film without film fracture during stretching process while elongation at break measure the ratio of final length of film before rupture to films' original length. Further elaborations and discussions on these three parameters are provided in section 4.3.2.1 and 4.3.2.2.

4.3.2.1 Tensile strength

Tensile strength or also known as tensile stress is the maximum force applied to the film when being stretched without fracture divided by cross sectional area of the film (ASTM D882, 2002). Figure 4.6 shows the mean value for tensile strength of PE/PET/CMC with single coating. Means were calculated based on quintuplicate data. Among all samples, PE/PET film exhibited the highest value with 42.28 ± 3.74 mPa followed by PE/PET/CMC5S with 41.09 ± 2.28 mPa. Compared to PE/PET/CMC10S and PE/PET/CMC15S, the values for tensile strength were slightly lower for both samples with 32.31 ± 2.75 mPa and 33.33 ± 3.68 mPa respectively.

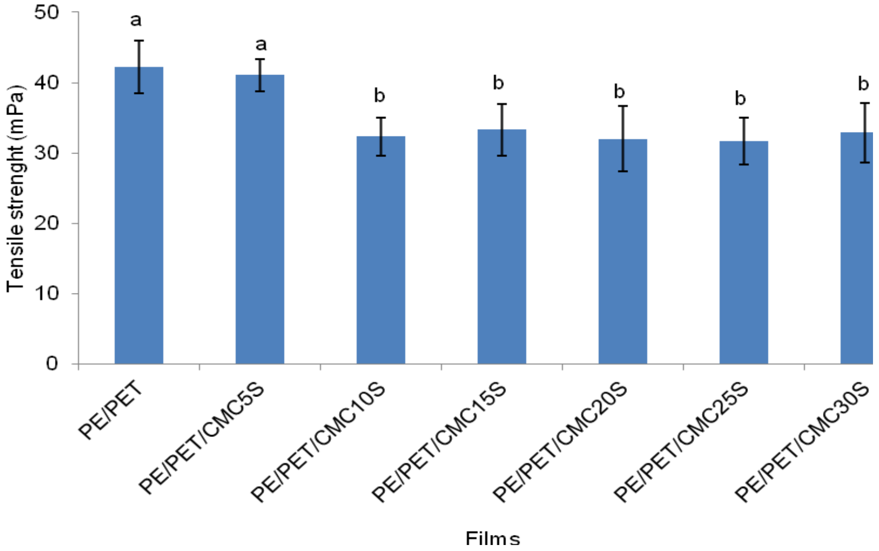

Figure 4.6 Tensile strength of PE/PET and PE/PET/CMCS films.

(Note: Data represent means of n=5 measurements while error bars represent the standard deviation. Different letter (a-b) represent significant different with ($p<0.05$). Tensile strength was statistically analysed using Tukey HSD)

From the result, it can be seen that when the concentration of NaOH used in etherification process increased, the tensile strength for all samples were reduced. This statement is true for PE/PET/CMC20S, PE/PET/CMC25S and PE/PET/CMC30S that demonstrated the value between 31.69 ± 3.35 mPa to 32.90 ± 4.24 mPa. Adinughara et al., (2005) reported that higher NaOH concentration cause the formation of sodium glycolate thus lowering the tensile strength of the film. From Figure 4.6, there was no significant difference between PE/PET and PE/PET/CMC5S. However, the mean values for tensile strength demonstrated by these two samples were significantly differences with other PET/PE/CMCS.

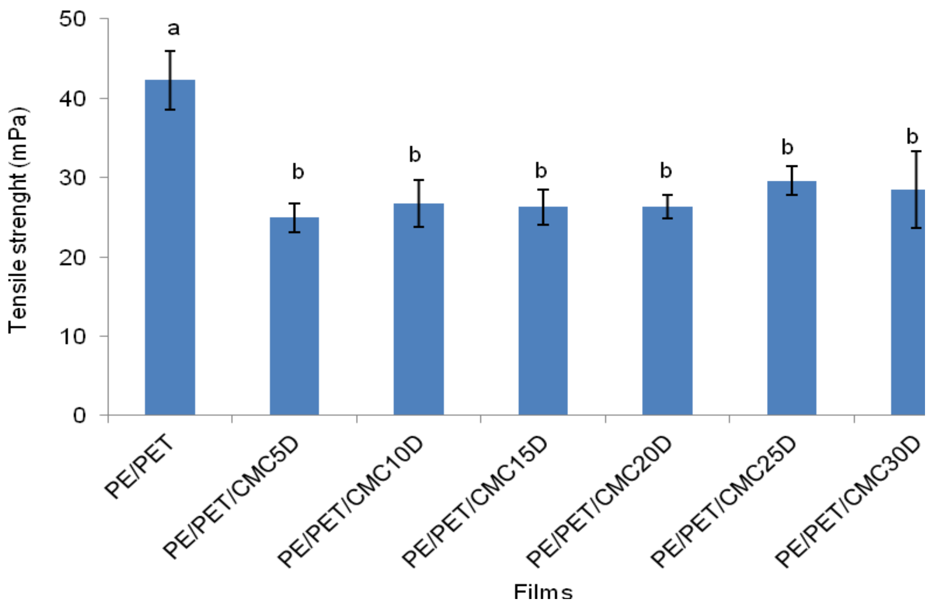

Figure 4.7 Tensile strength of PET/PE and PE/PET//CMCD films.

(Note: Data represent means of n=5 measurements while error bars represent the standard deviation. Different letter (a-b) represent significant different with ($p<0.05$). Tensile strength was statistically analysed using Tukey HSD).

Compared to PE/PET/CMC films with single layer coating, films with double layer coating exhibited the increment in tensile strength value when the concentration of NaOH increased. As shown in Figure 4.7, PE/PET/CMC5D displayed the lowest value of tensile strength with 24.95 ± 1.78 mPa. The mean value increased slightly to 26.76 ± 2.97 mPa at PET/PE/CMC10 and recorded the values of 26.30 ± 2.20 mPa and 26.29 ± 1.48 mPa at PE/PET/CMC15D and PE/PET/CMC20D respectively. The highest value of tensile strength for double layer coating was showed by PE/PET/CMC25D with 29.60 ± 1.84 mPa. However, the value reduced a bit to 28.47 ± 4.81 mPa for PE/PET/CMC30D. Lower tensile strength were observed in all double layer coating samples due to the higher film thicknesses of PE/PET/CMC films. This is due to the addition of another 35 ml of CMC solution on the surface of PE/PET/CMC film with single layer coating therefore increased the amount of CMC and glycerol in the coating.

As reported by Jouki *et al.,* (2013), the raise of glycerol concentration in film can improve film extensibility and reduce its resistance. This is due to the modification of plasticiser towards the functional properties of biopolymer films by reducing intermolecular forces and increasing the mobility of polymer chains as reported by Piermaria *et al.,* (2011). Figure 4.7 also demonstrated that there were significant differences between PE/PET and other PE/PET/CMCD films. However, no significant different were detected amongst PE/PET/CMCD films.

4.3.2.2 Elongation at break (EB)

Elongation at break (EB) is also known as tensile strain. EB measures the ratio of final length of film before rupture to film's original length (ASTM D822, 2002). Figure 4.8 shows the graph of elongation at break (EB) for PE/PET and PE/PET/CMCS films. From the graph, the value of EB for all sample ranged from 56.92% to 72.74%. PE/PET without any CMC coatings exhibited the value of 63.85%. Among single layer coating samples, the highest EB value was shown by PE/PET/CMC5S with 72.74%. For PE/PET/CMC10S, the value diminished to 56.92% but increased to 63.35% at PE/PET/CMC15S. The increased or decreased of EB values is caused by the strength of film towards the stress applied. According to ASTM D822, (2002), a material that stretches prior to the break point is called a ductile material while one that breaks after only small degree of stretching is called brittle. From the graph, when the concentration of NaOH increased to 20% (w/v), the EB value obtained by PE/PET/CMC20S was 60.80%. The value rose up to 67.67% for PE/PET/CMC25S but reduced to 60.40% for PE/PET/CMC30S. From Figure 4.8, there were significant differences between PE/PET with other PE/PET/CMCS except for PE/PET/CMC15. No significant different was observed between PE/PET/CMC20S and PE/PET/CMC30S.

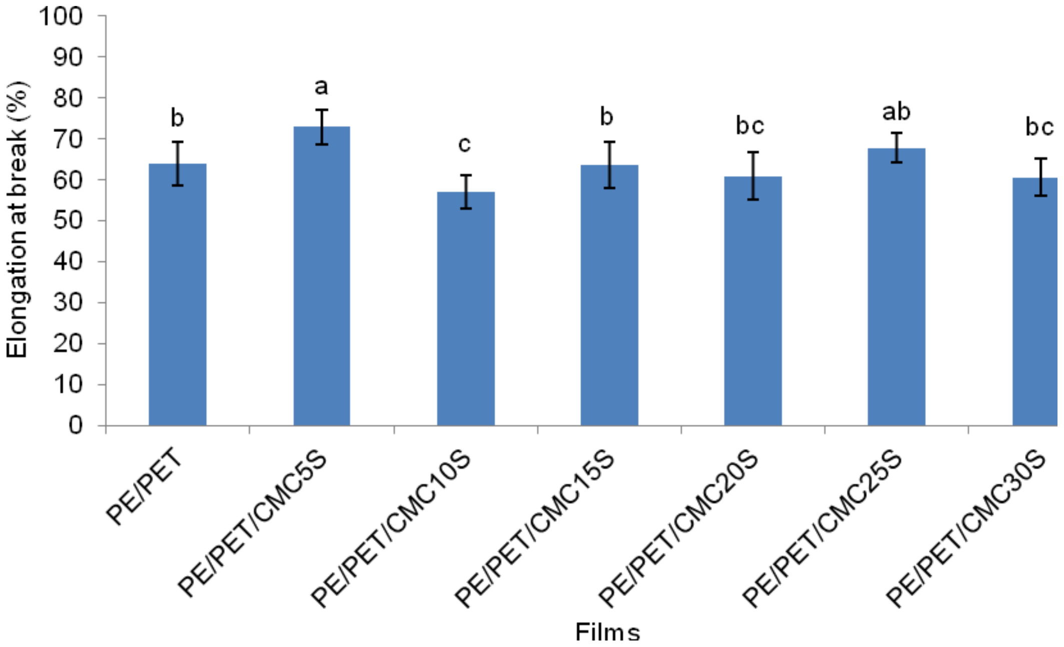

Figure 4.8 Elongation at break of PET/PE and PE/PET/CMCS films.

(Note: Data represent means of n=5 measurements while error bars represent the standard deviation. Different letter (a-c) represent significant different with ($p<0.05$). Elongation at break was statistically analysed using Tukey HSD).

On the other hand, higher EB values were observed in all PE/PET/CMCD. Garcia *et al.,* (2006) reported that higher elongation at break indicates that film is more flexible when subjected to tension and mechanical stresses. Therefore, film that exhibits higher EB value is more flexible and has the ability to stretch. In Figure 4.9, the highest EB value for double layer coating film was demonstrated by PE/PET/CMC25 followed by PE/PET/CMC5 with 76.28% and 73.82% each. This value was continued by PE/PET/CMC15, PE/PET/CMC30 and PE/PET/CMC20 with the values of 72.13%, 71.76% and 71.63% respectively. Amongst all sample, the lowest EB value was exhibited by PE/PET/CMC10 with 55.51%. From the result, the differences in moisture content of films might cause the variations in film flexibility. According to Zhang and Han (2006), water molecules could increase the free volume of biomaterials and thus improves film flexibility. Besides, the formation of hydrogen bonds between hydroxyl group of glycerol and carboxymethyl group of CMC capable to form a uniform and homogenous network or linkages (Ghamsemlou *et al.,* 2011). Therefore, it will enhance the elongation at break of the film. As in Figure 4.9, no significant differences were observed between PE/PET/CMC5D and PE/PET/CMC25D as well as between PE/PET/CMC15D, PE/PET/CMC20D and PE/PET/CMC30D. However, PE/PET was significantly different with other PE/PET/CMCD films.

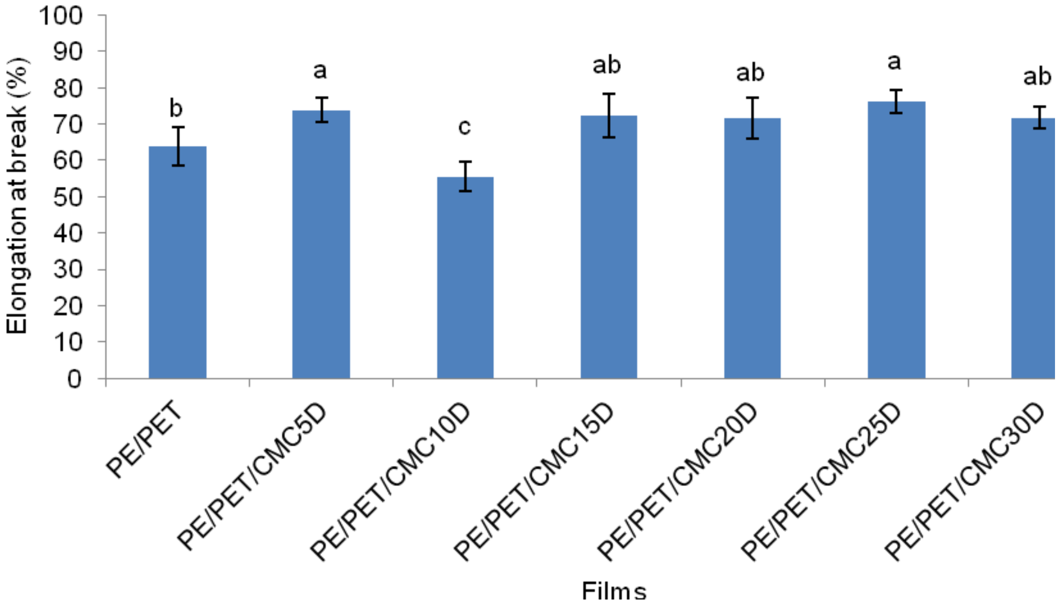

Figure 4.9 Elongation at break of PET/PE and PE/PET/CMCD films.

(Note: Data represent means of n=5 measurements while error bars represent the standard deviation. Different letter (a-c) represent significant different with ($p<0.05$). Elongation at break was statistically analysed using Tukey HSD).

As reported by Ahmadi *et al.,* (2012), increasing of glycerol content will decrease tensile strength values and thus increasing elongation break value due to the penetration of small molecular glycerol in polymer matrix and reduction of CMC-CMC interactions that alter the mechanical properties of films. This is so true for all EB values indicated by all PE/PET/CMCD. Ghamselou *et al.,* (2011) in their study on edible film made from kefiran reported that small quantity of plasticiser could be easily inserted between polymer chains and producing a cross linker effect that decreases the free volume of polymer, improved the extensibility of films and diminished the mechanical properties of films.

4.4 Conclusion

PE/PET/CMC films with single and double layer coating were successfully developed at drying temperature of 27^oC after 12 and 24 hours. In this study, PE/PET/CMC films produced using single layer coating exhibited film thickness that ranged from 0.075 mm to 0.089 mm while higher film thickness were observed at PE/PET/CMC films with double layer coating with the value of 0.094 mm to 0.113 mm. All films produced in this study demonstrated the value below than 7.55% for moisture content. Overall, all samples displayed lighter, greener and yellowish film colour. In addition, increased in CMC and glycerol amount added on PE/PET film resulted in increments in yellowness index and total colour differences. However, the value for transparency reduced as compared to PE/PET/CMC films produced with single layer coating. The differences of film properties were affected by difference characteristics of CMC produced. Increased in NaOH concentrations during etherification resulted in the production of by product such as sodium glycolate and sodium chloride that are hygroscopic and could absorb more moisture. Besides, due to the hydrophilic nature, increase in CMC and glycerol content also results in the formation of more hydrophilic film. This condition was proved by the value obtained through contact angle measurement that demonstrated the value of lower than 70^o and thus confirmed a hydrophilic characteristic of film. In term of mechanical properties, increased in NaOH concentrations used during etherification demonstrated lower tensile strength. Compared to PE/PET/CMC films with single layer coating, PE/PET/CMC with double layer coating demonstrated lower tensile strength but higher in elongation at break. In general, PE/PET/CMC15 with double layer coating displayed higher values in film thickness, moisture content, film solubility and contact angle. Amongst all sample, PE/PET/CMC5 with single layer coating displayed similar tensile strength and higher elongation at break compared to PE/PET bilayer film.

CHAPTER 5

WATER VAPOUR PERMEABILITY AND FILM MORPHOLOGY OF PE/PET/CMC FILMS

5.1 Introduction

Biopolymer derived from agricultural waste has the ability and competence to be used as ingredients in film making. Debeufort *et al.,* (1998) in his study reported that polysaccharides such as starches, cellulose derivatives, plant gums and protein such as gelatine have been employed in film and coating making as food packaging. However, the use of single biopolymer in the production of film will result in the paucity of structural integrity and characteristic functionally. In 2003, Thanathan reported that polysaccharides, protein and lipids are generally used in the preparation of biodegradable packaging or composites films. These biopolymers have been used in combination with other ingredients such as plasticisers and additives to improve its properties. The corporation of these plasticisers such as glycerine, glycerol and sorbitol into biopolymer material could promote and provide the flexibility of film (Garcia *et al.,* 2000). In another study, Tomasula (2009) stated that biopolymer films or coatings have the potential to replace one or more polymeric film layers in multilayer packaging systems.

Permeability of film towards water vapour is an important criterion for food packages in order to preserve the quality of food products. Previous study reported that CMC is very efficient towards oxygen, carbon dioxide and lipid (Ghanbarzadeh and Almasi, 2009). However due to its hydrophilic nature, CMC has poor resistance to water vapour transmission. On the other hand, the combination of PE/PET film could produce a transparent film with good barrier properties and a good chemical resistance (Flight Plastic UK, 2017).

Therefore in this chapter, the effect of CMC applied on PE/PET film on water vapour permeability was investigated. Moreover, results on topography that provides information on the surface features of film and morphology characteristics that stipulates data on the shape and arrangement of the particles was evaluated. This study was conducted as to reveal information of the surface, near the surface and in atomic scale using Scanning Electron Microscopy (SEM). To date, research on the effect of CMC on permeability of film, surface features and morphological characteristics of multilayer films are still limited. Therefore, this study is expected to grant the understanding on the use of biopolymer such as CMC on the permeability and morphology of PE/PET/CMC films.

5.2 Results and discussion

In this chapter, the effect of moisture absorption on PE/PET/CMC films was also investigated as it relates with water vapour permeability of film.

5.2.1 Moisture absorption

Moisture absorption measures the capability of film to absorb moisture from the environment. It is a crucial parameter that must be determined in the development of coating or film as it has a great influence on permeability of water through the film. Higher moisture absorption indicates that moisture from the environment could affect the quality of product inside the package. Figure 5.1 and 5.2 show the percentages of moisture absorption for PE/PET/CMC produced with single or double layer coating during 120 hours of storage at 25^{o}C. All measurements were conducted in triplicate. According to Figure 5.1 and 5.2, the percentages of moisture absorption for all samples started to increase after 0 hour. At this point, CMC coating on PE/PET film started to swell due to the nature of CMC and glycerol that present in the coating which are hydrophilic. As a result, it started to absorb water from the environment.

From Figure 5.1 (a), PE/PET film shows the lowest percentage of moisture absorption uptake that ranged from 0.06% to 0.47% after 24 hours and 120 hours respectively compared to other samples. The main reason of PE/PET film shows the smallest percentage is that it does not have any CMC coating on it. Amongst all single layer samples, PE/PET/CMC15 exhibited the highest percentage of moisture absorption with 10.55% after 24 hours and increased to 10.60% after 120 hours of storage. The lowest percentage was recorded by PE/PET/CMC5 with 0.56% and 1.54% after 24 hours and 120 hours respectively. For PE/PET/CMC10, PE/PET/CMC20, PE/PET/CMC25 and PE/PET/CMC30 films, the value for moisture absorption ranged from 4.88% to 5.79% after 24 hours and increased slightly to 6.15% to 7.29%.

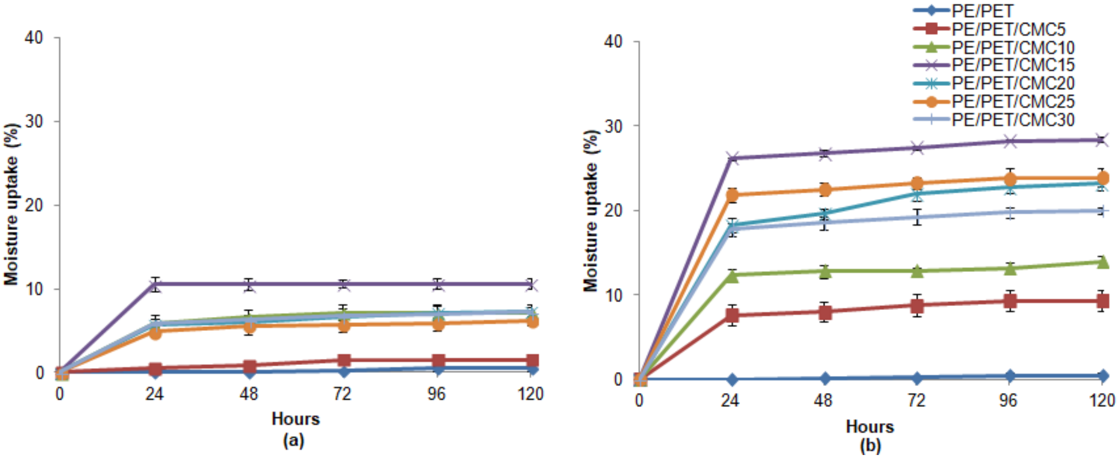

Figure 5.1 Percentages of moisture uptakes for PE/PET and PE/PET/CMC films with (a) single layer and (b) double layer coating during 120 hours storage period.
(Note: Data represent means of n=3 measurements while error bars represent the standard deviation).

Marvdashti et al. (2017) in her study stated that the amount of water absorbed by the films would increase slowly due to the presence of hydrophilic groups in the coatings. This justification is in agreement with the result obtained in my study as shown in Figure 5.1, where the trend for moisture uptake by the film seems to increase slowly. The increment of moisture absorption percentage also can be caused by the amount of sodium chloride produced in CMC wheat bran during etherification process. Sodium chloride is a hygroscopic material that tends to attract and hold water molecules from surrounding. Due to this, sodium chloride that formed on dried CMC coating layer will start to absorb water during storage period at 25°C. In previous chapter, the amounts of sodium chloride in CMC wheat bran ranging from 2.67 ± 0.53% to 28.36 ± 1.03%. According to Figure 5.1, it can be seen that the percentages of moisture absorption for all sample increased over time.

The trends of moisture absorption for PE/PET/CMC with double layer coating were shown in Figure 5.1 (b). From the graph, PE/PET/CMC5 shows the lowest percentage of moisture absorption with only 7.64% after 24 hours of storage and this value increased to 9.34% after 120 hours of storage. Compared to other samples, PE/PET/CMC10 and PE/PET/CMC30 films recorded the value of 12.32% and 17.76% respectively after 24 hours. From the results, there were increments of moisture uptake after 120 hours with both sample exhibited the values of 13.95% and 19.98% respectively. The highest percentage of moisture absorption was shown by PE/PET/CMC15 with 26.11% after 24 hours and this value increased to 28.36% after 120 hours of storage. The increments of moisture uptake were also demonstrated by PE/PET/CMC20 and PE/PET/CMC25 with the value of 18.26% and 21.80% after 24 hours and 23.20% to 23.89% after 120 hours of storage.

Compared to PE/PET/CMC with single layer coating, higher percentages of moisture absorption coating were observed in PE/PET/CMC with double layer CMC coating. This is due to higher volume of CMC coating added on PE/PET film. CMC coating solutions contained CMC and glycerol. Both substances are hydrophilic materials due to carboxymethyl and hydroxyl group from CMC and glycerol that are responsible to absorb moisture from the environment. Ghanbarzadeh et al., (2011) in his study on emulsified films based on carboxymethyl cellulose and oleic acid stated that glycerol is hydrophilic substance that could become immobilised between CMC chains and interact with water molecules. Thus, increased the amount of coating solution in double layer coating will result in higher CMC-glycerol interactions. This is also supported by Ghasemlou et al. (2011) who reported that increasing of glycerol would change the molecular structure through reduction of crosslink between biopolymer molecules and enhance the CMC solubility.

Besides carboxymethyl and hydroxyl groups, another factor that contributes to the increments of moisture absorption is the purity of CMC solution used. Purity of CMC content is affected by the formation of sodium chloride. Due to the formation of sodium chloride in CMC wheat bran, the absorption of moisture by the films also increased. Addition of more CMC solution in double layer coating also deposited more sodium chloride on PE/PET/CMC films once the coatings dried. In this study, sodium chloride content in CMC15 was reported to be 20.47 ± 0.27%. From the result, moisture absorption for all PE/PET/CMC films with double layer coating samples demonstrated between 1.9 to 6 times higher compared to PE/PET/CMC with single layer coating.

5.2.2 Water vapour permeability

Water vapour permeability is an essential criterion that must be measured as to determine whether a developed food packaging hold a good resistance towards moisture. Lower value of water vapour permeability indicates that the film use as a packaging material could retain moisture in food product by minimizing the diffusion of moisture through film to the environment. In addition, the diffusion of moisture from the environment to food product through film should be restrained. Both characteristics are very crucial for food product in the process of maintaining a good quality food product. To measure water vapour permeability, water vapour transmission rate for each of the films need to be determined first. In this study, water vapour transmission rate was measured based on the calculation of moisture uptake by silica gel that was placed in permeability cup covered with PE/PET or PE/PET/CMC films and stored for 120 hours in incubator at 25°C. Detailed procedures on determination of film permeability have been elaborated previously in section 2.7.

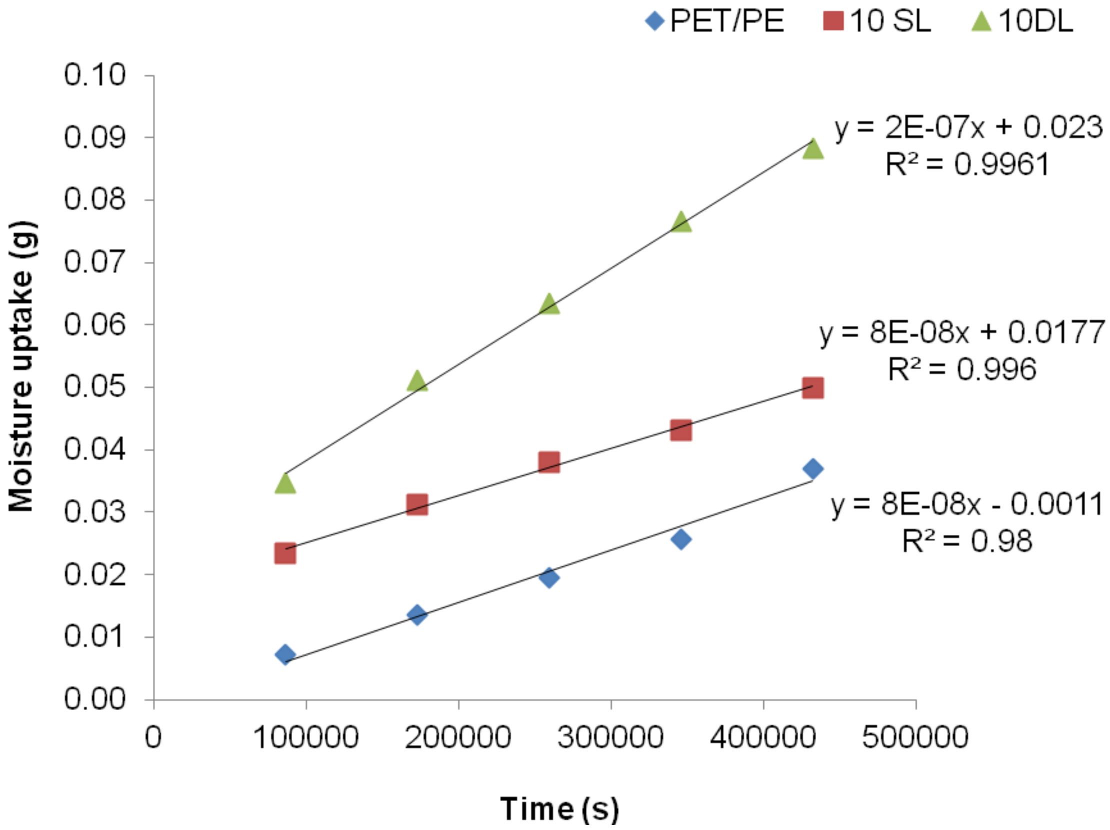

Figure 5.2 Moisture uptakes of PE/PET, PE/PET/CMC10S and PE/PET/CMC10D films against time.

Figure 5.2 shows the graphs of moisture uptake for PE/PET, PE/PET/CMC10S and PE/PET/CMC10D films towards time. Moisture uptakes for all samples increased when the storage time increased. The slope obtained from these straight lines can be used to calculate water vapour transmission rate (WVTR) of films by dividing the value obtained with the area of film. WVTR measures the transmission rate of water vapour through a tested film (Rendon-Villalobos, 2017) while water vapour permeability (WVP) measures the passage of water vapour through the film. In this study, the area of film exposed to the environment was $0.001 m^2$. Based on Table 5.1, all

PE/PET/CMCS films exhibited lower water vapour transmission rate compared to PE/PET film. Water vapour transmission rate for PE/PET film was recorded at 1.37 x 10^{10} $gs^{-1}m^{-2}$. Among all PE/PET/CMC samples with single layer coating, the values of water vapour transmission rate ranged from 4.45 x 10^{9} $gs^{-1}m^{-2}$ to 9.50 x 10^{9} $gs^{-1}m^{-2}$ with the lowest value was displayed by PE/PET/CMC25 and the highest value was exhibited by PE/PET/CMC5. When the concentration of NaOH increased from 10%, to 20% (w/v), the value of water vapour transmission rate decreased from 8.60 x 10^{9} $gs^{-1}m^{-2}$ to 6.79 x 10^{9} $gs^{-1}m^{-2}$.

These values were corresponded to the degree of substitution of CMC produced which measures the availability of carboxymethyl group in CMC coating that tend to bound with water. Besides, the occurrence of hydroxyl group from glycerol also contributes to the hydrophilicity of film. The WVTR value reduced to 4.45 x 10^{9} $gs^{-1}m^{-2}$ for PE/PET/CMC25 and slightly increased to 5.79 x 10^{9} $gs^{-1}m^{-2}$ when concentration of NaOH increased to 30% (w/v). The used of glycerol as a plasticiser in this study caused the inclusion of glycerol molecules between the polymer chains. As a result, it increased the water vapour diffusivity through film and accelerate water vapour transmission rate. This statement is also supported by Pearoval et al., (2002), who mentioned that water vapour transmission rate of film depends on the diffusivity and solubility of water molecules in the film matrix.

Data obtained from water vapour transmission rate can be used to calculate water vapour permeability (WVP). From this study, PE/PET film displayed the highest value of WVP with 3.76 x 10^{2} $gs^{-1}m^{-1}Pa^{-1}$. When PE/PET film was coated with single layer of CMC, the value of WVP ranges from 1.82 x 10^{2} $gs^{-1}m^{-1}Pa^{-1}$ to 3.15 x 10^{2} $gs^{-1}m^{-1}Pa^{-1}$. Besides moisture uptake of film, time of storage and area of exposed film, film thicknesses and differences in pressure are also other factors that could affect the permeability of film. In this study, film thickness for single layer coating ranged from 0.000075 m to 0.000089 m.

Several authors reported that WVP is directly related to the hydrophilic nature of the film as more hydrophilic film will have greater WVTR (Bourtoom and Chinnana, 2008; Ferreira *et al.,* 2009). Even though PE/PET film does not have any CMC coating on it, the film still permeates moisture from the environment.

Table 5.1 Water vapour transmission rate (WVTR) and water vapour permeability (WVP) of PE/PET and PE/PET/CMCS films

Films	WVTR ($gs^{-1}m^{-2}$)	WVP ($gs^{-1}m^{-1}Pa^{-1}$)
PE/PET	1.37×10^{10a}	3.76×10^{2a}
PE/PET/CMC5S	9.50×10^{9ab}	3.03×10^{2ab}
PE/PET/CMC10S	8.60×10^{9ab}	3.15×10^{2ab}
PE/PET/CMC15S	6.54×10^{9b}	2.45×10^{2ab}
PE/PET/CMC20S	6.79×10^{9b}	2.34×10^{2ab}
PE/PET/CMC25S	4.45×10^{9b}	1.48×10^{2b}
PE/PET/CMC30S	5.79×10^{9b}	1.82×10^{2b}

(Notes: Data represent means of n=3 measurements. Different letter (a-b) represent significant different with ($p<0.05$). WVTR and WVP were statistically analysed using Tukey HSD).

Table 5.2 presents the values for WVTR and WVP for PE/PET and PE/PET/CMCD films. In PE/PET/CMCD samples, the values for water vapour transmission rate were between 4.27×10^9 $gs^{-1}m^{-2}$ to 8.37×10^9 $gs^{-1}m^{-2}$ with PE/PET/CMC25 demonstrated the highest value while PE/PET/CMC15 with the lowest value. Increased in NaOH concentrations from 20% to 25% (w/v) also increased the value of water vapour transmission rate from 4.28×10^9 $gs^{-1}m^{-2}$ to 8.37×10^9 $gs^{-1}m^{-2}$ respectively. The formation of sodium chloride as by-product in CMC produced at 20% and 25% (w/v) NaOH concentration also affect the transmission rate of film. This is due to more moisture was absorbed by sodium chloride besides CMC and glycerol molecules. Rachtanapun et al., (2012) revealed that hydrophilic nature of CMC films could also be related to the increasing amount of carboxymethyl group present in CMC produced.

Table 5.2 Water vapour transmission rate (WVTR) and water vapour permeability (WVP) of PE/PET and PE/PET/CMCD films

Films	WVTR ($gs^{-1}m^{-2}$)	WVP ($gs^{-1}m^{-1}Pa^{-1}$)
PE/PET	1.37×10^{10a}	3.76×10^{2a}
PE/PET/CMC5D	6.42×10^{9ab}	2.67×10^{2ab}
PE/PET/CMC10D	5.06×10^{9ab}	2.23×10^{2ab}
PE/PET/CMC15D	4.27×10^{9b}	2.03×10^{2b}
PE/PET/CMC20D	4.28×10^{9b}	1.84×10^{2b}
PE/PET/CMC25D	8.37×10^{9ab}	3.42×10^{2a}
PE/PET/CMC30D	4.79×10^{9b}	1.89×10^{2b}

(Note: Data represent means of n=3 measurements. Different letter (a-b) represent significant different with ($p<0.05$). WVTR and WVP were statistically analysed using Dunnett T3).

Compared to single layer coating, all films with double layer coating exhibited lower WVP except for PE/PET/CMC25D and PE/PET/CMC30D with 3.42×10^2 $gs^{-1}m^{-1}Pa^{-1}$ and 1.89×10^2 $gs^{-1}m^{-1}Pa^{-1}$ respectively. The variation of these results could be due to the differences of film thickness. Overall, all films demonstrated lower WVP compared to PE/PET film. Films with double layer coating exhibited better water vapour permeability compared to single layer coating. This might be due to the linkages created by CMC-CMC and CMC-glycerol on PE/PET film thus minimising the permeability of moisture to the film. WVTR and WVP of PE/PET/CMC films depend on several factors such as mobility in polymer chain and interaction between the functional groups of the polymer matrix. Souza *et al.,* (2010) reported that WVP can be altered by modifies the integrity of film, hydrophilic-hydrophobic ratio and water diffusivity rate.

5.2.3 Film morphology and structure of PE/PET/CMC from wheat bran

Film morphology could be used to elucidate film structure characteristics such as its resistance to water vapour transmission (Li *et al.,* 2015), opacity (Paula *et al.,* 2015) and mechanical properties (Saurabh *et al.,* 2015). In this study, film morphology of PE/PET film and PE/PET/CMC with single and double layer coating were observed using Scanning Electron Microscope. Figure 5.3 (a) shows the cross section image of PE/PET film at 500x and 3000x magnifications. Previously, PE/PET film exhibited the film thickness of 0.065 mm. From the images, there are two layers of films which are laminates of Polyethylene (PE) and Polyethylene terephthalate (PET). These layers can be seen clearly at 3000x magnification with wavelength of 30 nm as in Figure 5.3 (a). For PE film's surface as in Figure 5.3 (c), smooth structure with some slightly bubbly surfaces can be observed on PE/PET film. This is could be due to the process of lamination between PE and PET film.

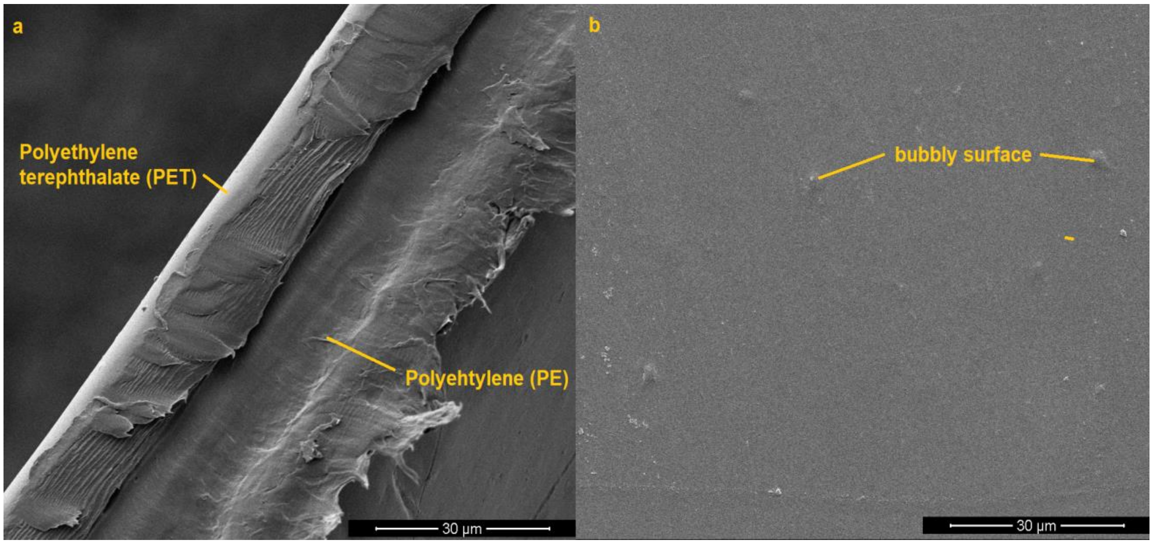

Figure 5.3 Film morphology of PE/PET film, a) cross section image of PE/PET film and b) PE film's surface

Figure 5.4 (a) and (b) shows the cross section structure of PE/PET/CMC5 with single and double layer coating at 3000x magnification. The formation of CMC layer on PE/PET film can be clearly seen in Figure 5.4 (a) and (b) that represent PE/PET/CMC5S and PE/PET/CMC5D respectively. Total film thickness for PE/PET/CMC5S was 0.076 mm while PE/PET/CMC5D demonstrated the value of 0.099 mm. The thickness of CMC5S layer formed on PE/PET film was only 0.011mm whereas the thickness of CMC5D layer on PE/PET film was 0.034mm that is three times higher compared to CMC5S. Overall through the cross section structure, both films are made up of three layers. Figure 5.4 (c) and (d) represents the surface structure of PE/PET/CMC5S and PE/PET/CMC5D. At 3000x magnification, it can be seen that the surface of PE/PET/CMC5S was a bit harsh and rough compared to PE/PET/CMC5D. Both films show no sign of cracks or pores. This is due to the amount of glycerol present on PE/PET/CMC films. The finding obtained in this study was in agreement with Jouki et al., (2013) who reported that glycerol plasticised film capable to show smooth and uniform surface morphology without cracks, breaks or opening on the surfaces.

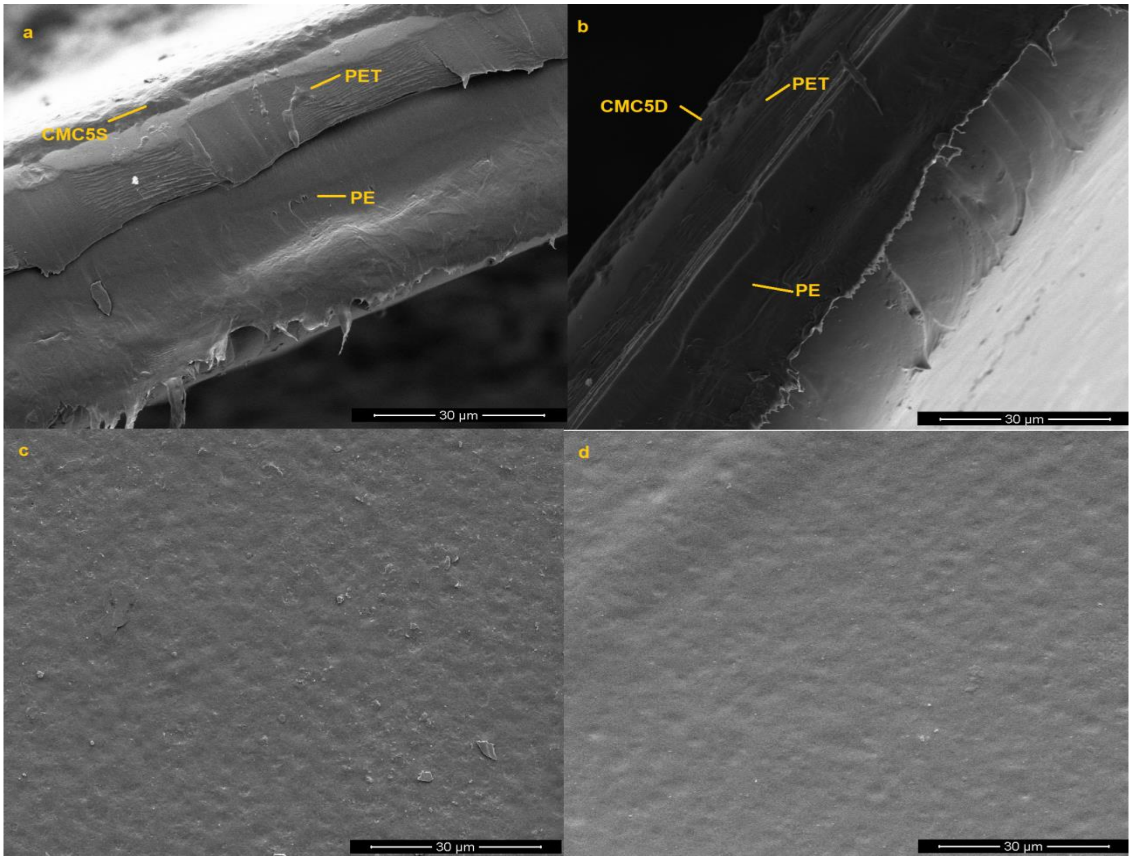

Figure 5.4 Cross section images of (a) PE/PET/CMC5S film and (b) PE/PET/CMC5D, and surface morphology of (c) PE/PET/CMC5S film and (d) PE/PET/CMC5D at 3000x magnification.

Figure 5.5 (a) and (b) display the image of cross sectional structure of PET/PE/CMC10S and PE/PET/CMC10D at 3000x magnification. Thicker CMC layer was observed in PET/PE/CMC10D film compared to PET/PE/CMC10S. This is due to higher total film thickness exhibited by PET/PE/CMC10D with 0.105 mm compared to PET/PE/CMC10S with only 0.087 mm. The thickness of CMC10D formed on PE/PET film was recorded at 0.040 mm compared to CMC10S with only 0.022 mm. Compared to CMC10S, CMC10D exhibited thicker and denser CMC layer. The formation of PE/PET/CMC layers for both samples can be seen clearly in these figures.

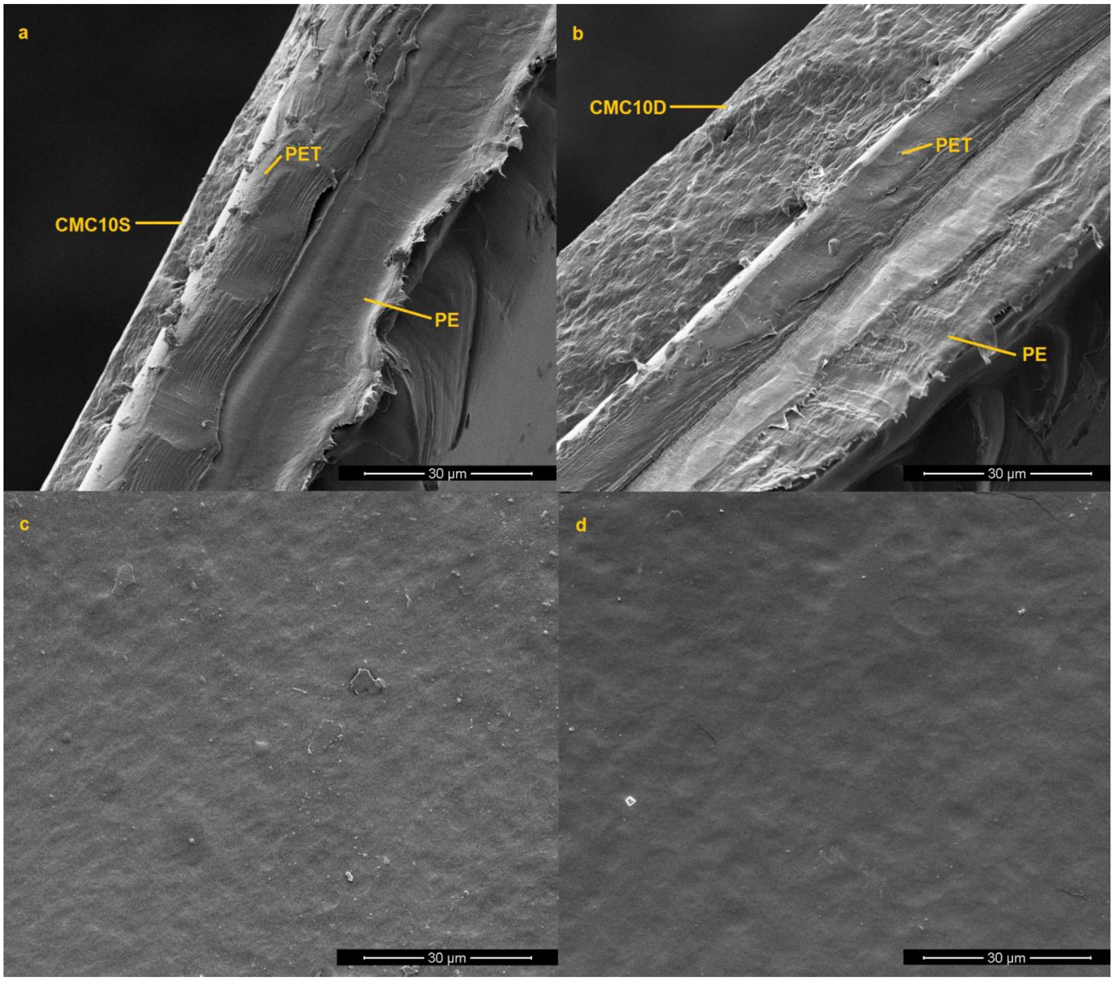

Figure 5.5 Cross section images of (a) PE/PET/CMC10S film and (b) PE/PET/CMC10D, and surface morphology of (c) PE/PET/CMC10S film and (d) PE/PET/CMC10D at 3000x magnification.

The images of surface structure for PET/PE/CMC10S and PE/PET/CMC10D films were shown in Figure 5.5 (c) and (d). At 3000x magnification, both films demonstrated uneven surface structures with CMC10S exhibited rougher surface morphology compared to CMC10D. This could be due to the properties of CMC10 that demonstrated higher degree of substitution previously. Besides, the addition of glycerol in CMC solution creates linkages of CMC-CMC and CMC-glycerol molecules in the coating (Ghasemlou et al., 2011). Higher amount of CMC and glycerol will result in denser networks produced in the coating. This finding also supported by Marvdashti et al., (2017) who suggested that film that displayed smooth surfaces was caused by a homogeneous structure where polysaccharides chains aggregated to form a continuous and dense network.

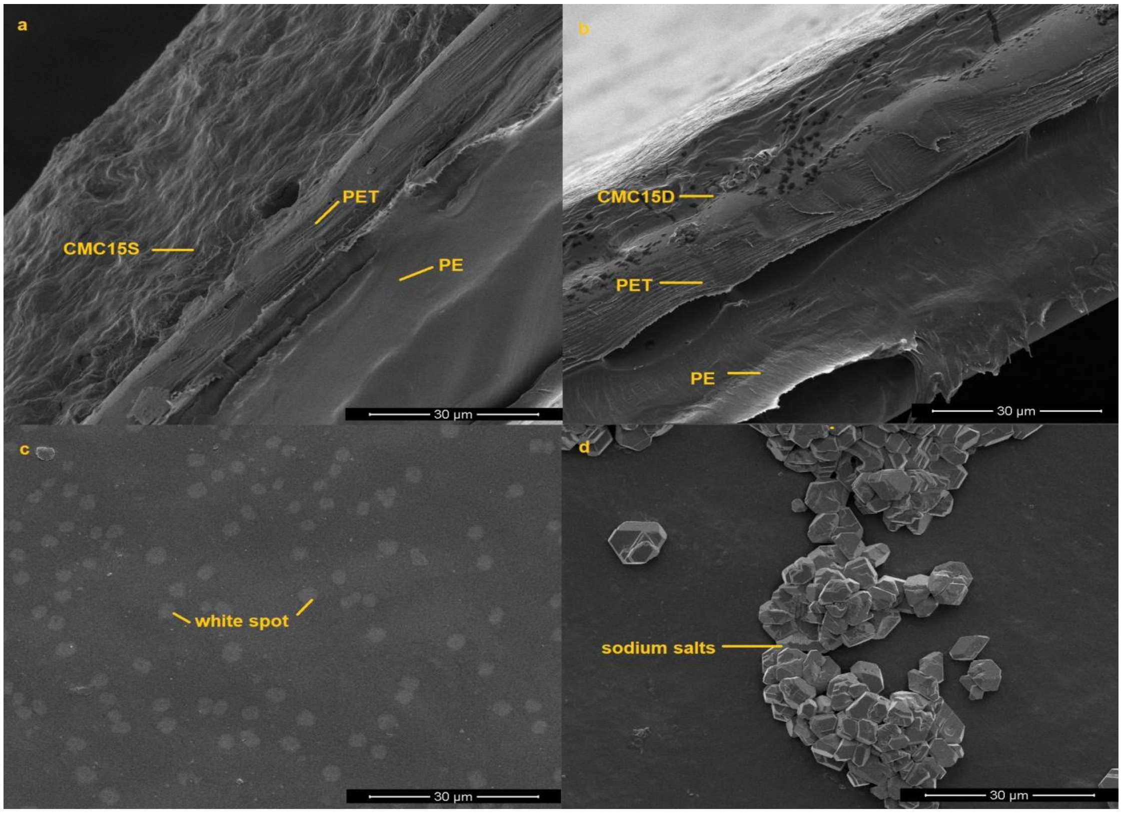

Figure 5.6 Cross section images of (a) PE/PET/CMC15S film and (b) PE/PET/CMC15D, and surface morphology of (c) PE/PET/CMC15S film and (d) PE/PET/CMC15D at 3000x magnification.

Figure 5.6 (a) and (b) shows the picture of cross sectional structure of PE/PET/CMC15S and PE/PET/CMC15D at 3000x magnification. From the images, the cross sectional structure of PE/PET/CMC15 films is similar with PE/PET/CMC10 where the formation of three layers structure can be seen clearly. However, the thickness of CMC15S and CMC15D layer formed on PE/PET film were a bit higher compared to CMC10 previously. Total film thickness of PE/PET/CMC15S and PE/PET/CMC15D were recorded at 0.089 mm and 0.113 mm respectively. The thickness of CMC15D layer was reported at 0.048 mm while CMC15S recorded the value of 0.024 mm which is half than CMC15D. Similar with PE/PET/CMC10 films, different layers between CMC coating and PE/PET film can be distinctly seen in the images.

CMC15S and CMC15D displayed a homogenous structure without obvious layer separation. However, unsmooth cross section surface of CMC15 layer can be seen as in Figure 5.6 (a) and (b). The agglomeration of CMC and glycerol caused the CMC layer to become a dense network. On the other side, this factor will affect the moisture uptake and WVP of film due to hydrophilic nature of CMC and glycerol as reported by Ghanbarzdeh et al. (2011). The morphology of films surface can be viewed in Figure 5.6 (c) and (d). For CMC15D, the formation of crystal can be observed on film surface. This might be due to the present of sodium chloride as a by-product in CMC (Barai et al., 1997) that affect the purity of CMC solution. During drying process, sodium chloride tends to form a crystal on the film surface. Unlike CMC15S, a smooth surface can be seen on CMC15D. However, PE/PET/CMC15S indicated the appearance of white spot on its surface. This could be due to the presence of some sodium chloride in the CMC15S layer affected by the drying process.

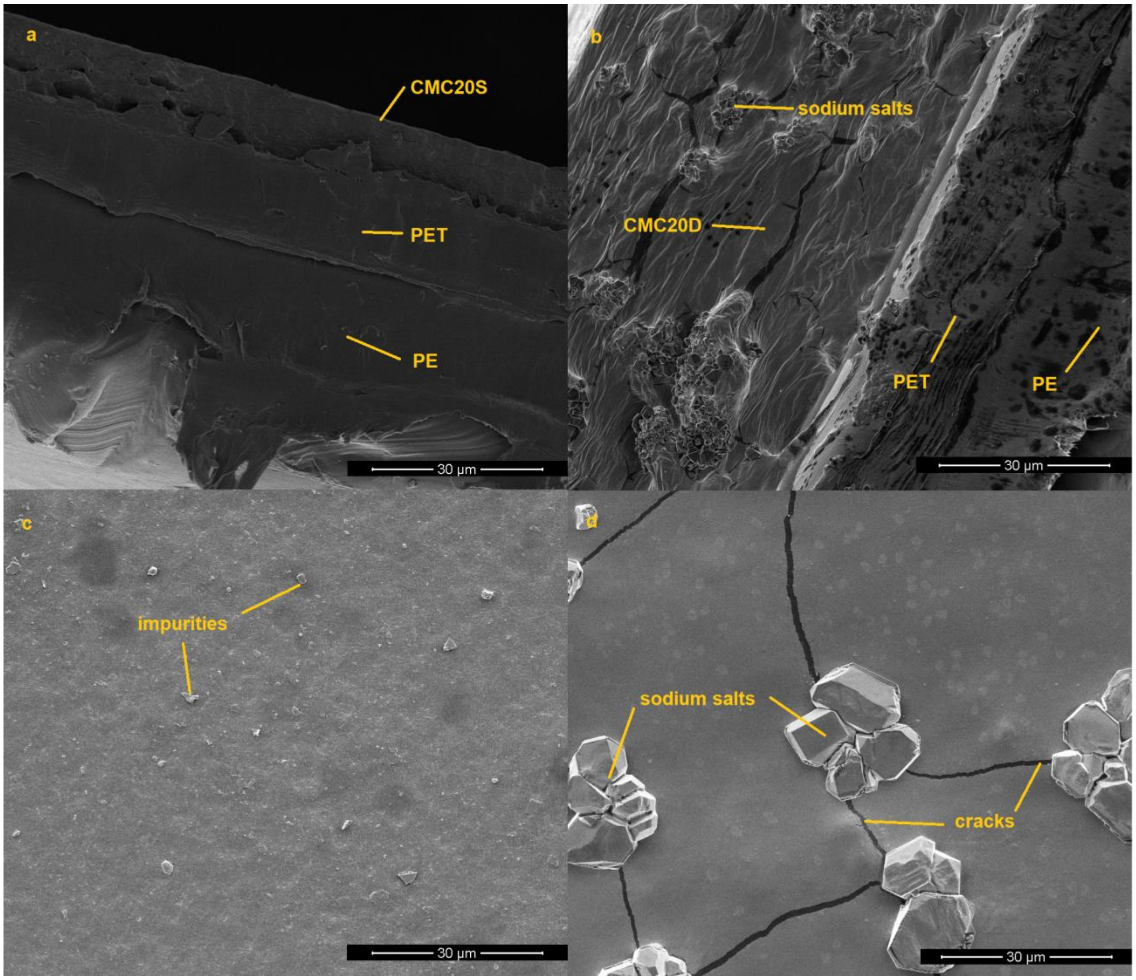

Figure 5.7 Cross section images of (a) PE/PET/CMC20S film and (b) PE/PET/CMC20D, and surface morphology of (c) PE/PET/CMC20S film and (d) PE/PET/CMC20D at 3000x magnification.

For PE/PET/CMC20, the cross section and surface morphology of single and double layer coating were presented in Figure 5.7. Cross sectional images of CMC20S and CMC20D can be observed in Figure 5.7 (a) and (b). Through these images, obvious differences in thickness layer can be observed. Previously, the thickness of PE/PET/CMC20D was recorded at 0.102 mm while PE/PET/CMC20S demonstrated the value of 0.082 mm. CMC20D formed a thicker layer with 0.037 mm compared to CMC20S with only 0.017 mm. Compared to CMC20S, CMC20D exhibited brittle and uneven structure. Through these images, both layers are well attached with PE/PET film without any opening observed. Cross section image also shows the agglomeration structure thus makes CMC20D to become irregular. The existence of cracks at CMC20D layer can be examined at 3000x magnification. This is due to the characteristic of CMC20 that has lower carboxymethyl group which has been discussed previously in section 3.2.2.6. Lower carboxymethyl group indicates that CMC contained lower DS value and therefore reduce the intermolecular interactions between CMC and glycerol (Ghasemlou *et al.* 2011).

Surface morphology of PE/PET/CMC20S and PE/PET/CMC20D films were displayed in Figure 5.7 (c) and (d). CMC20S shows a smooth cross sectional surface while CMC20D layer formed an obvious lump structure with the formation of sodium salts in the layer. Between these two films, CMC20S exhibited smooth structure. However, the presence of impurities on CMC20S could be due to the purity of CMC20 used which exhibited lower DS value. Besides, there are no cracks observed on CMC20S's film surface. Compared to CMC20D, the surface structure was unsmooth and irregular. In addition, this film exhibited the formation of sodium salts and displayed a few cracks. The appearance of cracks could affect the permeability of film towards water.

Figure 5.8 shows the cross section and surface images of PE/PET/CMC25S and PE/PET/CMC25D films. A conspicuous image of cross section for both films can be observed in Figure 5.8 (a) and (b). As in Figure 5.8 (a), a thin layer of CMC25S was formed on PE/PET film. The total thickness for this film was recorded at 0.079 mm while PE/PET/CMC25D film demonstrated the value of 0.097 mm. These differences resulted in thicker CMC25D layer as displayed by Figure 5.8 (b). In this study, the thickness of CMC25D layer was demonstrated at 0.032 mm, twice higher than the thickness of CMC25S layer with only 0.014 mm. Compared to CMC25S, CMC25D layer revealed a clear view of polymer agglomeration. Similar with PE/PET/CMC20D, some cracks or breaks in CMC25D layer can be noticed from the image. On the other hand, these cracks could also due to the used of platinum during coating process in sample preparation with sample coater. With the occurrence of CMC aggregation in the layer, the present of sodium salts can also be seen. A homogenous coating structure without any cracks or breaks was found at cross section image of CMC25S. However, an irregular and rough surface structure as in Figure 5.8 (c) was observed on CMC25S at 3000x magnification. Compared to CMC20S previously, the surface of CMC25S is much rougher and coarser. The formation of sodium salts can also be detected on PE/PET/CMC25D film with some sign of cracks on the film's surface as in Figure 5.8 (d).

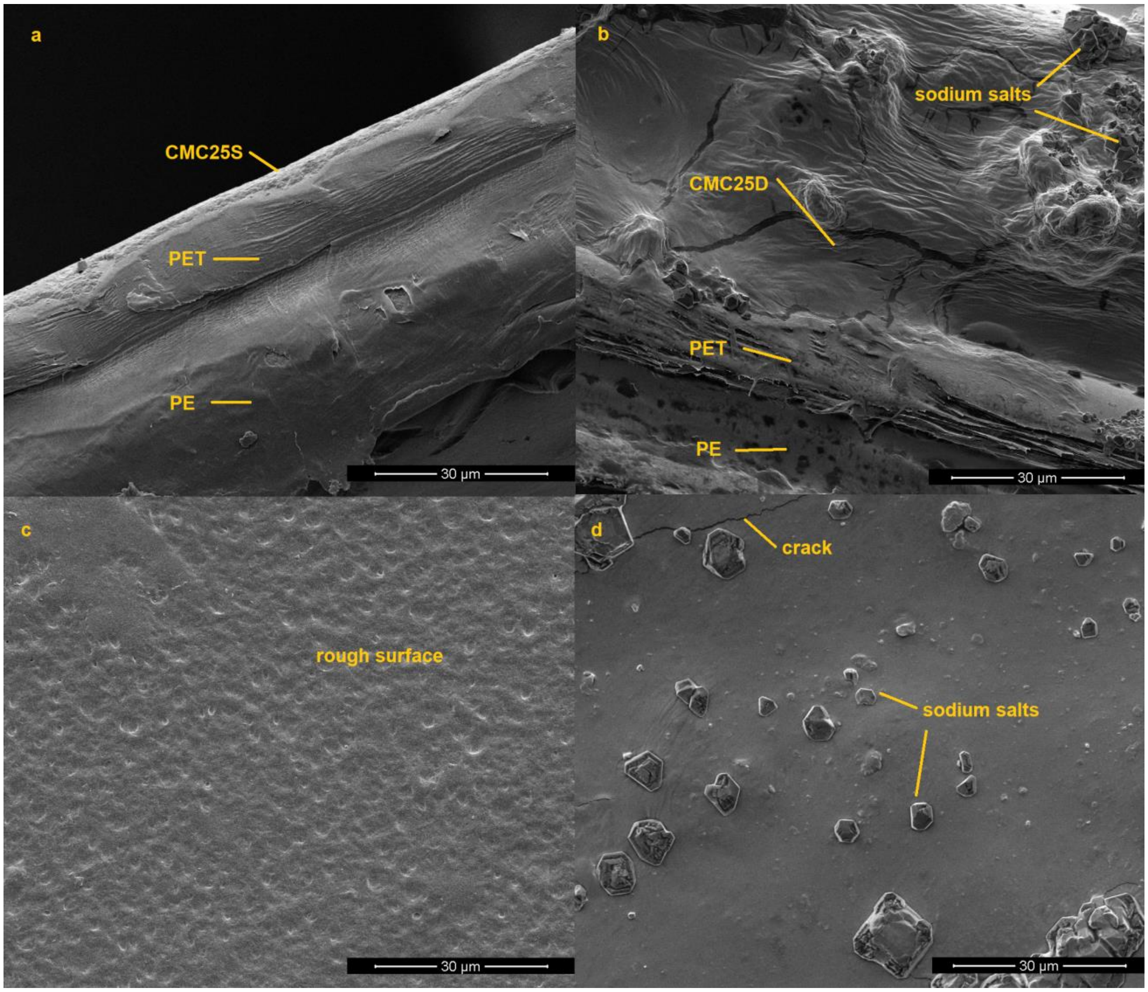

Figure 5.8 Cross section images of (a) PE/PET/CMC25S film and (b) PE/PET/CMC25D, and surface morphology of (c) PE/PET/CMC25S film and (d) PE/PET/CMC25D at 3000x magnification.

Figure 5.9 shows the cross section and surface morphology images of PE/PET/CMC30S and PE/PET/CMC30D at 3000x magnification. The total film thicknesses for PE/PET/CMC30S and PE/PET/CMC30D films were 0.0075 mm and 0.0094 mm respectively. In Figure 5.9 (a), the cross section morphology of PE/PET/CMC30S film was similar with PE/PET film. Two layers of PE/PET films can be seen clearly while CMC30S layer turns to be a very thin layer on PE/PET. The difference of thickness between PE/PET and PE/PET/CMC30S films was recorded at 0.010 mm. Compared to CMC30D, an obvious image of CMC layer coating can be clearly seen in Figure 5.9 (b). This is due to the thickness of CMC30D layer that was determined at 0.029 mm. This value was nearly three times higher compared to CMC30S.

Figure 5.9 (c) and (d) represents the surface morphology of CMC30S and CMC30D. A similar characteristic with PE/PET/CMC25S and PE/PET/CMC25D films on surface morphology were also observed in PE/PET/CMC30 films. As in Figure 5.9 (c) and (d), the formation of sodium salts can be observed on CMC30S and CMC30D surfaces respectively. No crack was observed on CMC30S surface but the formation of sodium salts can be detected on the film. CMC30D also displayed the presence of sodium salts with the addition of surface cracks. The appearance of these salts could be due to the amount of sodium chloride content present in CMC during etherification process which already been discussed in section 3.2.2.4 previously. The occurrence of sodium salts and cracks on film surface will increase the water transmission rate through the film thus will raise the water vapor permeability of films.

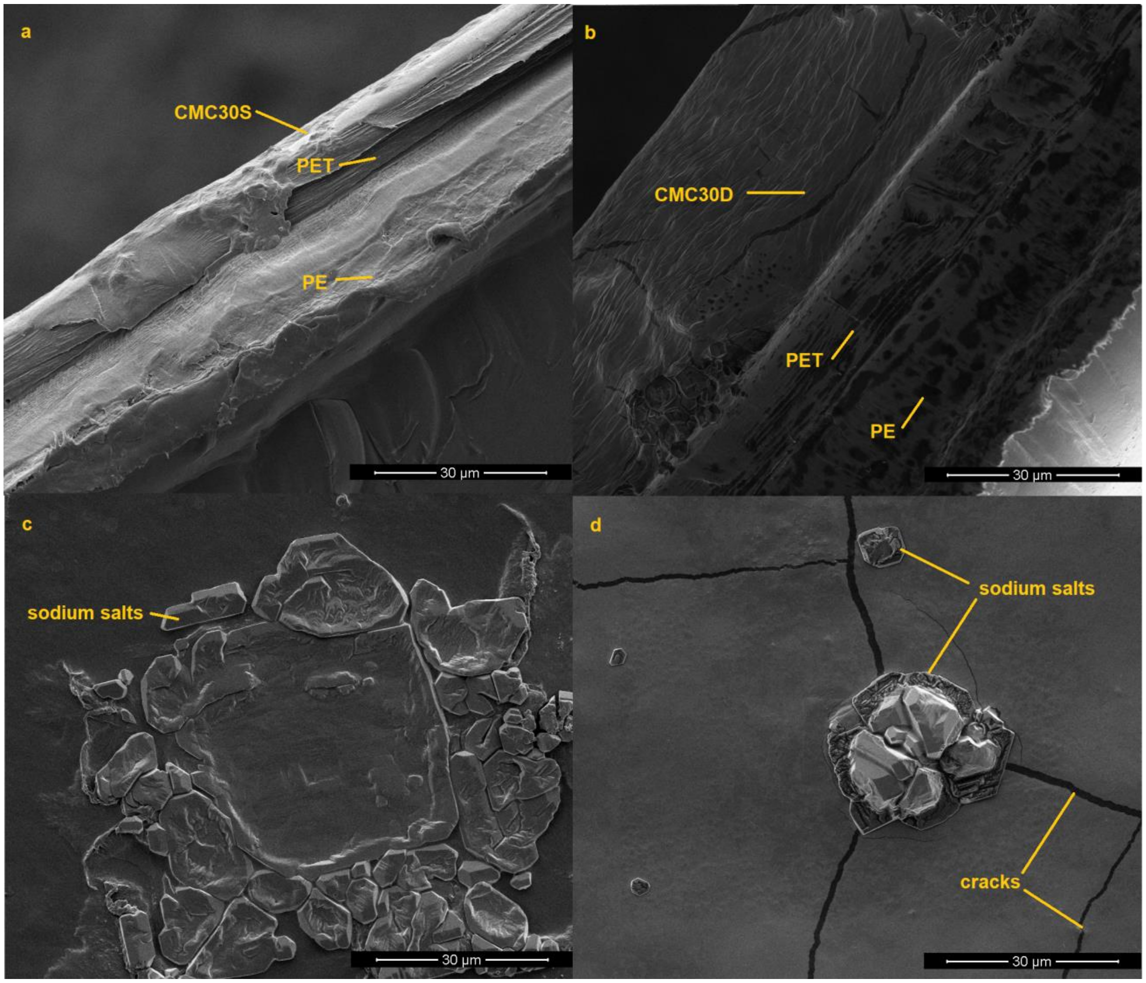

Figure 5.9 Cross section images of (a) PE/PET/CMC30S film and (b) PE/PET/CMC30D, and surface morphology of (c) PE/PET/CMC30S film and (d) PE/PET/CMC30D at 3000x magnification.

5.3 Conclusion

The differences of film properties were affected by difference characteristics of CMC produced, the amount of CMC and glycerol added in the coating. Increase in CMC and glycerol content will result in the formation of more hydrophilic film. Besides, the formation of sodium salts will enhance the film to absorb moisture from surrounding. In this study, PE/PET/CMCD exhibited higher moisture uptake compared to PE/PET/CMCS. This situation resulted in higher value of WVTR and WVP of films. PE/PET/CMCS films demonstrated higher WVP compared to PE/PET/CMCD films except for PE/PET/CMC25D and PE/PET/CMC30D. The result obtained in this study shows that there was a significant difference recorded between PE/PET film and PE/PET/CMCS and PE/PET/CMCD. However, no significant difference was observed between PE/PET and PE/PET/CMC25D. This might be due to the formation of sodium salts on the surface of film that increase the WVP of film. Comparing with PE/PET film, all PE/PET/CMCS and PE/PET/CMCD showed lower WVP. Besides, PE/PET/CMCS displayed smooth surface structures compared to PE/PET/CMCD which exhibited the formation of sodium salts as well as the sign of cracks. Therefore, the findings obtained in this study proved that CMC extracted from wheat bran could be used as a new source or ingredient in developing new bio plastic or multi-layer films due to its characteristics investigated in this study. This study also contributes a good finding into food packaging field as the use of polysaccharides on film such as CMC wheat bran on PE/PET could reduce the WVP of the film which favored by certain food product.

CHAPTER 6

GENERAL DISCUSSION

In this study, new biopolymer such as CMC can be produced from agro industry waste such as wheat bran. The aims of this study were: 1) to synthesis alkali cellulose from wheat bran and converts it to carboxymethyl cellulose, 2) to investigate the effect of NaOH used during etherification on characteristic of CMC produces, 3) to produce multi-layer films by applying CMC wheat bran on PE/PET films, 4) to measures the physical and mechanical properties of PE/PET/CMC films and 5) to study water vapour permeability and film morphology of PE/PET/CMC films.

6.1 Ideal conditions for CMC preparation.

In this study, alkaline cellulose was synthesis from wheat bran using 3% (w/w) NaOH concentration during macerisation process. 2% (v/v) of H_2O_2 and 0.2% (w/v) of TAED were used in bleaching process in order to whiten the colour of wheat bran pulp. In this study, the ideal condition to synthesis CMC from alkaline cellulose during etherification process was using 10% (w/v) NaOH. From the finding, this concentration exhibited the highest DS value with 0.84. Besides, CMC10 also demonstrated purity content of 89.14% with 7.83% and 3.03% of sodium glycolate and sodium chloride respectively. It also displayed higher molecular weight of 61672 Da. Not only that the value recorded for shear viscosity was 51.27 mPas, the highest value recorded compared to other samples. Therefore, based on these data, 10% NaOH concentration can be recommended to be used by other researcher as references in etherification process.

In this study, all CMC produced from wheat bran are potential to be used in consumer goods and industrial application. According to ICIS (2019), CMC can be categorised according to its purity. Technical grades CMC demonstrated between 90 to 95% purity and can be used as adhesives, coatings, in paper and textile industries. On the other hand, industrial grades CMC exhibit the purity between 50 to 90%. From the results obtained in this study, the purity of CMC produced was between 63.40 to 92.30%. All CMC can be categorised as industrial grades except for CMC5 that recorded the highest purity. These CMC can be used as an ingredient in detergent, mining and petroleum industries. However, industrial grades CMC can be purified to technical grades thus can be used in wider application such as food and beverages, pharmaceutical and cosmetic industries. In term of industrial scale production, CMC can be produced using appropriate equipment such as digester tank for mercerisation and etherification process, high pressure bleaching tank and vacuum dryer that is more effective in bulk scale.

6.2 Effect of NaOH concentrations on characterisation of CMC.

The characteristics of CMC produced from wheat bran was investigated according to the purity of CMC, moisture content, colour measurement, degree of substitution, molecular weight, rheological measurement and morphology of CMC. Purity of CMC was affected by sodium glycolate and sodium chloride content. At lower NaOH concentrations such as 5% and 10% (w/v), the purity of CMC was higher. However, the purity of CMC tends to decrease with the increased of NaOH. On the other hand, the amount of sodium glycolate in CMC was slightly higher as compared to NaCl at higher NaOH concentrations. This finding proved that at higher NaOH concentrations, the formation of by products such as sodium glycolate and NaCl increased as the reaction between sodium chloroacetate and alkaline cellulose tended to shift to the formation of by-product (Barai et al., 1997). However, at 20% (w/v) of NaOH concentration, the highest NaCl content was demonstrated and thus lower the purity of CMC itself. This is due to the lower substitution of carboxymethyl group at cellulose backbone thus lowering the DS value of CMC produced.

The presence of these by-products will affect the properties of CMC such as lowering the DS value and molecular weight of polymer. Besides that, it also reduces the viscosity of CMC and forming a dilute CMC solution. Another problem associated with the presence of by-product is that the formation of sodium salt on CMC film and thus make the film to become more hydrophilic as the sodium salts tend to attract water from the environment. In order to overcome these problems in CMC, purification process must be conducted. Purification involves the process of washing CMC with 96% (v/v) of ethanol for a few times (Saputra et al., 2015) before drying process takes place. In addition, low concentration of NaOH that ranged from 5% to 10% is preferred to be used during etherification process. This process could reduce the impurities in the CMC. Other than that, DS value of CMC can be increased and the characteristics of CMC such as purity and viscosity can be improved.

Overall, the percentage of moisture content displayed by all CMC produced in this study were below than 8%. This value was in accordance with the standard from International Cenological Codex (2009) which stated that the moisture content of CMC must not contain more than 12%. Different NaOH concentrations used during etherification process do show an effect on colour of CMC. Generally, all sample demonstrated a light, yellowish and reddish colour of CMC powder. The lightness of CMC powder was due to the bleached process that was conducted during synthesis of alkaline cellulose. At this stage, H_2O_2 was used to brighten the pulp of wheat bran as it improves the delignification process and effluent colour of the samples (Sundara, 1998). The yellowish colour could be due to the effect of delignification process where wheat bran pulp was bleached in order to remove the lignin. Thus, the pulp produced was brighter compared to the unbleached pulp. Besides, higher (a) value in all samples was due to the substitution of carboxymethyl group at cellulose backbone during etherification process (Rachtanapun et al., 2012). Rachtanapun et al., (2012) also demonstrated similar findings on CMC synthesised from durian rind that are yellowish and reddish in colour.

Therefore, the results obtained in my study exhibited some similarity and in accordance with a study done by Rachtanapun et al., (2012).

On the other hand, the used of NaOH as a solvent during etherification process affect the degree of substitution of CMC produced. From the findings, DS value tended to increase at lower concentration of NaOH. However, at higher concentration such as 20% to 30% (w/v), the value for DS tended to decrease to 0.36. In 1997, Barai et al. reported that at high concentration of NaOH, polymer degradation occurred and thus lowering the DS values. This is caused by the reaction between alkaline cellulose, sodium chloroacetate and NaOH that would produce CMC together with sodium glycolate and sodium chloride and affect the purity of CMC produced (Pushpamalar et al., 2006).

In addition, DS value also had been influenced by molecular weight of CMC. DS value measures the substitution of carboxymethyl group at cellulose backbone at C2, C3 and C6. Increased in DS value resulted in the increment of polymer size and therefore produces a higher molecular weight of the polymer. Due to higher DS values recorded by CMC extracted with 10% and 15% (w/v) NaOH, similar trend of graft can also be observed for molecular weight with the values of 61672 Da and 43012 Da respectively. However, the molecular weights of CMC were decreased at higher NaOH concentrations. Until now, no previous study has been reported on the investigation of different NaOH concentrations on molecular weight of CMC. Therefore, the findings obtained in this research are expected to complete current references.

The effect of different concentrations of NaOH can be investigated in rheological properties of CMC solution. The formation of viscous or dilute solution depended on DS value of CMC produced. Higher DS value caused CMC to form a viscous solution compared to CMC with lower DS value which displayed a dilute solution. Amongst all sample, higher shear stress was demonstrated by CMC extracted with 10% and 15% (w/v) of NaOH. At higher concentrations such as 20%, 25% and 30% (w/v) NaOH, a dilute solution was formed hence lower shear stress were recorded. This situation was due to the formation of sodium glycolate and sodium chloride in CMC content that affect the purity of CMC. Higher amount of sodium glycolate and sodium chloride resulted in dilute CMC solution when mixed with water and therefore reduced the shear stress of the solution.

All CMC produced in this study demonstrated shear thinning behaviour which is the result of slower increases of shear stress towards the increases of shear rate. Shear thinning or pseudoplastic behaviour appears due to the long chain of CMC molecules that tend to orient themselves in the direction of flow. Besides, increased in DS value enhances the ability of CMC to immobilise water in a system therefore increased the shear viscosity of solution. For these reasons, all CMC solutions produced in this study were categorised as non - Newtonian fluids. These results are in accordance with the previous findings reported by Togrul and Arslan (2003), Yasar et al., (2007) and Karatas and Arslan (2016) on rheological behaviour of CMC produced from plant waste.

In this study, alkaline cellulose demonstrated the morphology of rough granules and rod like structures. The used of NaOH during etherification also results in the formation of bulky granules. Compared to lower concentration of NaOH, the morphology of CMCs was rougher, coursers with irregular surface structure. The formation of crystal particles all over CMC surface could be due to the formation of sodium chloride and sodium glycolate. These findings also in agreement with Santos et al. (2015) who mentioned that carboxymethyl groups formed a bulky structure on fibre surface after carboxymethylation.

6.3 Effect of CMC on physical and mechanical properties of PE/PET/CMC films

PE/PET/CMCS and PE/PET/CMCD films were successfully prepared using 1% (w/v) CMC solution with 0.2% (v/v) glycerol and dried at 27°C for 12 hours 24 hours respectively. Thirty five millilitre of CMC solution was used to cover 150 cm^2 of film dimension. All films formed using these conditions produced a transparent film without obvious appearance of white dots on the surface of CMC layer and no separation of film was observed in between the films.

The results obtained in this study revealed that CMC wheat bran that exhibited higher DS value produced a thicker film compared to CMC with lower DS value. During drying, water that bound with carboxymethyl group from CMC and hydroxyl group from glycerol evaporates. This reaction removes water from CMC layer and formed a cross linkage between CMC and glycerol molecules. PE/PET/CMCD films demonstrated higher film thickness compared to PE/PET/CMCS films. This is due to higher concentration of CMC and glycerol on double layer coating thus resulting in higher formation of cross-linking between CMC and glycerol molecules after drying process.

Higher DS values also affect the moisture content of PE/PET/CMC films. Increased in DS values is a result of higher substitution of carboxymethyl group at cellulose backbone. Due to its nature as hydrophilic materials, carboxymethyl group are able to hold water molecules. Not only that, the addition of glycerol in CMC layer also influenced the moisture content of film produced. A study conducted by Cerquira et al. (2012) reported that the addition of glycerol could enhance the water holding capacity because of increased in the availability of hydroxyl group in glycerol. In this study, all PE/PET/CMCS and PE/PET/CMCD films demonstrated the moisture content of below than 7.55%.

Besides moisture content, DS value also influence the solubility of film. In this study, film produced using CMC that demonstrated higher DS value such as CMC extracted with 10% and 15% (w/v) NaOH exhibited higher film solubility compared to other samples. Higher DS value increased the ability of CMC molecules to bind with water while lower DS value reduced the capability of CMC molecules to bind with water. This condition caused CMC to be less hydrophilic and reduce film solubility. This finding was in agreement with a study conducted by Karoosi et al., (2013) who reported that hydrophilic compound could increase film solubility while hydrophobic compound could decrease film solubility. In my study, PE/PET/CMCD films exhibited higher film solubility. Not only it is caused by carboxymethyl group presents in the coating, but it also been affected by glycerol and presence of sodium salts that available in CMC layer. Therefore, these factors increased the hydrophilicity of films.

Another characteristic that affect physical properties of film is moisture absorption. From the findings obtained in this study, DS value also influenced the moisture absorption of film. Besides this variable, the purity of CMC produced is another significant factor that influenced the moisture uptake of film. The formation of sodium chloride in CMC produced during etherification will affect the film to absorb and hold more water molecules and later enhance the hydrophilicity of film. As been discussed previously, carboxymethyl group and hydroxyl group from CMC and glycerol are hydrophilic materials and thus responsible in absorbing moisture from the environment. For that reasons, the increment of moisture uptake can be seen in all films except PE/PET film during 120 hours of storage period. In this study, higher moisture uptake was demonstrated by PE/PET/CMCD compared to PE/PET/CMCS films.

Overall, all films produced in this study using single or double layer CMC coating were transparent. Compared to PE/PET/CMCS, the lightness (L) of PE/PET/CMCD films was decreased. However, PE/PET/CMCD films produced were a bit greenish and yellowish in colour due to the initial colour of CMC produced in this study which is yellowish. Besides, Ghanbarzadeh et al., (2010) in his study stated that the addition of glycerol content changed the colour index of film produced.

Increased in NaOH concentrations during etherification also increased the wettability of film. The findings obtained in section 4.2.2.5 demonstrated that all films exhibited a reduction of contact angle after 60 seconds thus indicates that films produced in this study were hydrophilic. However, compared to the coating produced using CMC10, CMC20, CMC25 and CMC30 recorded lower contact angle and therefore proved that the surface of film produced using these CMCs were wettable. Increased in wettability was due to the formation of by-products such as sodium chloride. Besides carboxymethyl group and glycerol, sodium chloride also increased the wettability of film surface, as this material is a hygroscopic. According to Carneiro-da-Cunha et al., (2010), the most wettable surfaces present lower values with contact angle of less than 20^o therefore in my study, films produced using CMC extracted with higher NaOH concentrations with double layer coating exhibited the most wettable surfaces.

Mechanical properties of films were analysed based on tensile strength and elongation at break of films. The findings from this study shows that increased in NaOH concentrations results in lower tensile strength of films. This can be seen in all PE/PET/CMCS films. However, PE/PET/CMCD films exhibited the increment in tensile strength when the concentration of NaOH increased. These results are in accordance with Adinughara et al., (2005) in his published paper who revealed that higher NaOH caused the formation of sodium glycolate in CMC hence reducing the tensile strength of films. Another factor that contributes to the differences of tensile strength between single layer and double layer coating was film thickness. Previously, all PE/PET/CMCD films exhibited higher film thicknesses. This is due to the addition of CMC and glycerol on the coating. The results obtained in this study was conformance with a study produced by Jouki et al., (2013) who reported that the raise of glycerol concentration in film capable to improve film extensibility and reduce its resistance.

On the other hand, all PE/PET/CMCD films displayed higher EB values compared to PE/PET/CMCS due to higher amount of CMC and glycerol occurred in the coating. Ahmadi et al. (2012) supported this statement and mentioned that the addition of glycerol content reduces the value of tensile strength, and therefore increased the value of EB. The increments of EB value was due to the infiltration glycerol molecular in polymer matrix and reduce the interaction between CMC-CMC molecules thus modify the mechanical properties of films. In this study, PE/PET/CMCS films exhibited better rigidity and flexible compared to PE/PET/CMCD films.

6.4 Water vapour properties and morphology of PE/PET/CMC films.

In this study, increased in NaOH concentrations during etherification process influenced the water vapour properties of film produced. In general, PE/PET/CMCS films exhibited higher WVP values compared to PE/PET/CMCD films. Both types of PE/PET/CMC also demonstrated lower WVP compared to PE/PET film. The addition of hydrophilic materials such as carboxylmethyl and hydroxyl group from CMC and glycerol contributes to the absorption of moisture from surrounding and increased the hydrophilicity of film. This finding correlates with the results obtained for moisture uptake of film which displayed higher moisture uptake in PE/PET/CMCD films. This result is supported by Rachtanapun et al., (2012) who described that hydrophilic nature of CMC enhances the uptake of moisture and thus decreased the water vapour properties of films. Therefore, the application of CMC as a film could improve the value of WVP

PE/PET consists of two bilayer films, which are Polyethylene (PE) and Polyethylene terephthalate (PET), laminated together and can be clearly seen at 3000x magnifications. This film displayed a smooth surface structure with some bubbly surfaces. A distinct image of PE/PET/CMCS and PE/PET/CMCD films that consists of three films layers can be seen clearly under SEM. Compared to PE/PET/CMCS films, PE/PET/CMCD films exhibited thicker layer due to the addition of higher volume of CMC coating. For double layer coating, CMC coating still formed as a one film on PE/PET bilayer even though it involved twice of drying process. In general, either film produced with single or double layer CMC attached well with PE/PET film and did not displayed any layer separation.

Overall, PE/PET/CMCS films displayed smooth surface structure compared to PE/PET/CMCD films which exhibited the formation of particles as well as the sign of crack. Besides, films produced using CMC10 and CMC15 demonstrated thicker and denser layer of CMC films. The application of double layer CMC coating however could enhance the surface structure by producing a smoother structure with a dense network. This result was in accordance with Marvdashti et al., (2017), who suggested that smooth film surfaces was influenced by a homogeneous structure between glycerol and CMC thus forming a continuous and dense network.

This finding also been supported by Jouki et al., (2013) stated that glycerol can perform a smooth and uniform surface morphology without cracks, breaks or opening on the surfaces. However, films produced using higher NaOH concentrations exhibited poor surface structure of films. This effect can be observed at PE/PET/CMC20D, PE/PET/CMC25D and PE/PET/CMC30D films that demonstrated an irregular and rough surface structure with some sign of cracks and formation of salt crystals. This could be due to the amount of NaCl that present in CMC as a by-product during etherification process. Therefore, in this study, PE/PET/CMC5D and PE/PET/CMC10D have been identified to display good surface structure and morphology compared to other samples.

6.5 CMC as a source of biopolymer and its applications as PE/PET/CMC film.

From this study, it is proved that wheat bran is potential to be used as a source of biopolymer. The abundant production of wheat bran as agro-industrial waste produced from milling process of flour can be reduced and converted into CMC. This situation could help in utilising wheat bran into new hydrocolloid apart of being used as animal feed or even burned at the landfill. The used of wheat bran also may help to solve the environmental issue such as CO_2 emission as a result of agro-industrial waste being burned in the open field.

CMC that derived from wheat bran has a wide range of application in food industries, oil drilling, pharmaceutical, paper manufacturing and textiles. It can be used as viscosity modifier, thickening and stabilising agent as well as polymer to develop biodegradable food packaging. In term of its production, CMC is very cost effective as it requires 5% to 15% (w/v) NaOH concentrations, sodium chloroacetate as etherification agent, isopropanol and hydrogen peroxide as basic ingredients for CMC synthesis. Besides, the process such as mercerisation and etherification are not complicated, and CMC can be produced in a short time. Besides wheat bran, other agro-industrial waste that is potential to be used in extracting the CMC are nutshell of tree nut, wheat and paddy straw, and coconut fibre which are always available and abundant.

On the other hand, modified PE/PET/CMC film produced in this study can be used as a new packaging film. This is due to properties of CMC layer that reduce the permeation of moisture from the environment or vice versa. The

CMC layer formed on PE/PET film can act as inner or outer layer of the main packaging. Using an extrusion process, this film can be transformed into laminate and can be used as a plastic sheet to pack fresh cut fruits or even bakery products. This is due to the properties of film that could absorb and retain moisture and therefore could maintain the freshness of food products. The presence of CMC layer on PE/PET film could aid water retention in the package and limit the transmission of water through PE/PET film. However, several modifications on the package such as modified atmosphere packaging and incorporation with antimicrobial substances on CMC layer could enhance the performance of PE/PET/CMC films. Another possible application of this CMC layer obtained in this study is that it also can act as an adhesive to laminate other materials such as plastic, paper and aluminum or even can be used on its own to develop biodegradable packaging.

CHAPTER 7

CONCLUSION, LIMITATION AND FUTURE WORKS

7.1 Summary of the thesis

The aim of the present research was to utilise wheat bran as agro-industry waste into carboxymethyl cellulose (CMC) by determining the effect of NaOH used during etherification. In this project, CMC that produced from wheat bran was used as a coating on PE/PET film to form a multi-layer film. Therefore, the second aim of this study was to apply CMC coating on PE/PET film and investigate the physical, mechanical, water vapour permeability and film morphology of PE/PET/CMC film.

In **Chapter 1**, a review on wheat bran as agro-industrial waste, structure of cellulose, processes involved in cellulose extraction, the production of CMC from plant sources and the importance and application of multilayer films in food industries were presented. This chapter also highlighted the aims, research objectives and research question of this research.

Chapter 2 elaborated the materials and methods used in this research. It comprised three parts of study: 1) Synthesis of alkali cellulose and CMC using different concentration of NaOH, 2) Characterisation of CMC produced from wheat bran and 3) The development and characterisation of PE/PET/CMC multilayers film. Besides, this chapter also described the method used to determine normality and homogeneity of data obtained.

As discussed in **Chapter 3**, the effect of different NaOH concentrations on yield, physicochemical, rheological properties and microscopic analysis of CMC wheat bran were investigated. From the findings, the yield of CMC produced for all sample were more than 1.0 g/g due to the etherification of sodium cloroacetate. Amongst all sample, CMC extracted with 10% NaOH exhibited the highest degree of substitution value. However, at higher concentrations of NaOH, sodium glycolate and sodium chloride were formed and affect the purity of CMC produced. The value for degree of substitution (DS) correlates with molecular weight and viscosity of CMC. Thus, higher DS value results in higher molecular weight and viscosity. From the results, all CMC exhibited a brighter, reddish and yellowish in colour compared to alkaline cellulose. Not only that, all CMC samples has been proven to demonstrate a pseudoplastic or shear thinning behaviour and exhibited the morphology of bulky granules and rod like shape structure.

The development and characterisation of PE/PET/CMC films were investigated in **Chapter 4**. From the findings, PE/PET/CMC films with single and double layer coating were successfully developed at drying temperature of 27^{o}C and dried for 12 and 24 hours respectively. The results of this investigation show that the differences in film properties were affected by difference characteristics of CMC wheat bran produced.

The permeability towards water vapour and film morphology of PE/PET/CMC film were studied in **Chapter 5**. In this chapter, the addition of CMC on PE/PET film could decrease WVTR and WVP of films. Compared to PE/PET/CMC film with single layer coating, PE/PET/CMC with double layer coating exhibited lower WVTR and WVP. In term of film morphology, PE/PET/CMC with single layer coating exhibited smooth surfaces. However, at higher NaOH concentration such as 30% (w/v), the formation of particle on the surface could be observed. Double layer coating displayed the formation of sodium salts on the surface of film. The sign of cracks on the film also can be seen.

Chapter 6 highlights the general discussion that elaborate on the effect of NaOH used during CMC synthesis and the consequences of different NaOH concentrations on the characterisation of CMC produced. Besides, this chapter also explains and correlates the characteristic of CMC with physical, mechanical, water vapour properties and morphology of single or double layer PE/PET/CMC films.

7.2 Research limitations

The generalisability of these results is subject to certain limitation such as:

Literature reviews: Reviews on multilayer film was limited due to only a few published works that has been reported before. There is a gap in the knowledge on the application of biopolymer such as CMC as a coating on multilayer film. Many studies have focused on the development of single film using biopolymers therefore the development and characterisation of multilayer film using biopolymer is still limited.

Data collection: In Chapter 3, the information on functional groups such as carboxymethyl and hydroxyl group in CMC produced is limited due to the absence of Fourier-Transform Infrared Spectroscopy (FTIR) in School of Food Science and Nutrition, University of Leeds. It would be appropriate if this analysis can be carried out to complement the information on characterisation of CMC and thus act as additional reference for future works.

Longitudinal effect: This research was sponsored by Ministry of Higher Education, Malaysia for only three years. Therefore, all the experiments design and analysis involved in this research such as synthesis of CMC from wheat bran, characterisation of CMC produced, development and characterisation of PE/PET/CMC film were outlined to be finished within these three years.

7.3 Implication and contribution to the research development

The novelty of this study is the utilisation of wheat bran as agro-industrial waste into cellulose derivative such as CMC. This cellulose derivative can be used as a new source of biopolymer. The findings obtained in this study could provide new alternatives in reducing agro-industry waste produced by milling industry. Additionally, the uniqueness of this study is mainly due to the use of CMC extracted from wheat bran as a coating material on PE/PET film to form a multi-layered film. The findings gathered in this study in term of PE/PET/CMC film characteristics could build a platform to the knowledge and provide references to other researchers. Besides, CMC itself can be used as a bio plastic to produce biodegradable film in the future. Hence, the findings obtained in this study is hoped to provide and contribute a new knowledge on new biopolymers synthesis from agro-industry waste which would benefit the society along with its potential to be innovated as food packaging material.

7.4 Future works

This research has demonstrated the utilisation of wheat bran as a new source of biopolymer such as CMC and the used of CMC as a material in film making. Based on the findings obtained in this study, further studies should be initiated to explore and scrutinise the potential of this CMC in bio plastic application. This can be undertaken by considering the following:

- it is recommended to study the effects of sodium hydroxide and sodium chloroacetate on functional groups in CMC, as it will provide the understanding on CMC characteristics related to its gel properties.

- it would be interesting to further investigate the use of different hydrocolloids or biopolymer such as polysaccharides, protein and lipid in developing multi-layered films that is potential to be used as biodegradable film.

- it is suggested to cooperate other substances such as natural antimicrobial into biopolymer film to improve its functional properties as active packaging.

- there is a need to assess and study the water sorption kinetics of film produced as to calculate and predict the diffusivity of water through the film.

- more research is required to determine the application and effectiveness of multilayer film produces using biopolymer by evaluating the shelf life of food products through kinetic reaction approach to study the deterioration process as function of time.

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