SYNTHESIS, CHARACTERISATION AND APPLICATION OF GELATIN-ADDITIVE FILMS FOR FOOD PRESERVATION

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ABSTRACT

This study aims to evaluate potential of gelatin-chitosan (Gel-Cs) and gelatin-carboxymethyl cellulose (Gel-CMC) films to preserve perishable food products. Three main studies, namely synthesis, characterisation and preservation have been conducted. Biopolymer films were prepared by using solvent casting method with three volume per volume (Gelatin:Additive) ratios, namely 75:25, 50:50 and 25:75. In this study, chitosan and CMC were used as additives in gelatin-based film forming solutions. The characterisation study was carried out by using Fourier Transform Infrared (FTIR) Spectrometer, Ultraviolet-Visible (UV-Vis) Spectrophotometer, Universal Testing Machine, Thermogravimetric Analyser (TGA) and Scanning Electron Microscope (SEM). The antimicrobial tests were conducted by using two common food pathogens, namely *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative). The preservation study was performed for 12 days in order to evaluate effectiveness of gelatin-additive films to extend shelf life of local agricultural products, namely cherry tomatoes (*Solanum lycopersicum var. cerasiforme*) and grapes *(Vitis vinifera).* Gel-Cs and Gel-CMC films reached their maximum stress at 45.36 MPa and 37.54 MPa, respectively, as compared to gelatin film (35.98 MPa). Meanwhile, 25Gel:75CMC film exhibited the most flexible characteristic with elongation at break (EAB) value of 4.41%. The gelatin-additive films has low light transmission values in both UV (200 nm - 350 nm) and visible (400 nm - 800 nm) regions, which indicated excellent light barrier property of transparent film. Based on antimicrobial activity study, the inhibitory effects of 50Gel:50Cs film was the best against *S. aureus,* while 75Gel:25CMC film exhibited the maximum inhibition zone against *E. coli.* Gelatin-additive films were able to reduce the percentage of weight loss and browning index of cherry tomatoes and grapes. In conclusion, Gel-Cs and Gel-CMC films showed an improvement in physicochemical characteristics and antibacterial protection that potentially able to sustain the quality and prolong the shelf life a variety of food products. In implication, the utilisation of biodegradable gelatin-additive films as alternatives to petroleum-based films for food preservation could create a greener environment.

SINTESIS, PENCIRIAN DAN PENGGUNAAN FILEM GELATIN-BAHAN TAMBAH UNTUK PENGAWETAN MAKANAN

ABSTRAK

Kajian ini bertujuan untuk menilai potensi filem gelatin-kitosan (Gel-Cs) dan gelatin-karboksimetil selulosa (Gel-CMC) untuk mengawet produk makanan yang mudah rosak. Tiga kajian utama iaitu sintesis, pencirian dan pengawetan telah dilaksanakan. Filem biopolimer telah disediakan menggunakan kaedah pelarut tipisan tuang dengan tiga nisbah isipadu per isipadu (Gelatin:Bahan tambah), iaitu 75:25, 50:50 dan 25:75. Dalam kajian ini, kitosan dan CMC telah digunakan sebagai bahan tambah dalam larutan pembentukan filem berasaskan gelatin. Kajian pencirian telah dijalankan menggunakan Spektrometer Inframerah Transformasi Fourier (FTIR), Spektrofotometer Cahaya Ultralembayung-Nampak (UV-Vis), Mesin Ujian Mekanikal, Penganalisis Thermogravimetri (TGA) dan Mikroskop Imbasan Elektron (SEM). Ujian antimikrob telah dilaksanakan dengan menggunakan dua patogen makanan yang lazim, iaitu *Staphylococcus aureus* (Gram positif) dan *Escherichia coli* (Gram negatif). Kajian pengawetan telah dilakukan selama 12 hari untuk menilai keberkesanan filem gelatin-bahan tambah bagi memanjangkan jangka hayat produk pertanian tempatan, iaitu tomato ceri *(Solanum lycopersicum var. cerasiforme*) dan anggur *(Vitis vinifera).* Filem Gel-Cs dan Gel-CMC mencapai tegasan maksimum pada 45.36 MPa and 37.54 MPa, masing-masing berbanding dengan filem gelatin (35.98 MPa). Manakala, filem 25Gel:75CMC mempamerkan ciri paling terlentur dengan nilai pemanjangan pada takat putus (EAB) 4.41%. Filem gelatin-bahan tambah mempunyai nilai penghantaran cahaya yang rendah dalam kawasan UV (200 nm - 350 nm) dan kawasan nampak (400 nm - 800 nm), di mana ia menunjukkan sifat penghalang cahaya yang baik bagi filem lutsinar. Berdasarkan kajian aktiviti antimikrob, kesan rencatan filem 50Gel:50Cs adalah yang terbaik terhadap *S. aureus*, manakala filem 75Gel:25CMC mempamerkan zon perencatan yang maksimum terhadap *E. coli*. Filem gelatin-bahan tambah mampu mengurangkan peratusan kehilangan berat dan indeks keperangan bagi tomato ceri dan anggur. Kesimpulannya, filem Gel-Cs dan Gel-CMC menunjukkan peningkatan dalam sifat-sifat fisikokimia dan perlindungan antibakteria yang berpotensi untuk mengekalkan kualiti dan memanjangkan jangka hayat pelbagai produk makanan. Implikasinya, penggunaan filem gelatin-bahan tambah yang terbiodegradasi sebagai alternatif kepada filem berasaskan petroleum untuk pengawetan makanan dapat mewujudkan persekitaran yang lebih hijau.

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LIST OF ABBREVIATION

CMC	Carboxymethylcellulose
Cs	Chitosan
EAB	Percentage Elongation at Break
FAMA	Federal Agricultural Marketing Authority
FAO	Food Agricultural Organization
FTIR	Fourier Transform Infrared
GDP	Gross Domestic Product
Gel	Gelatin
IMP3	Third Industrial Master Plan
LDPE	Low Density Polyethylene
MITI	Ministry of International Trade and Industry
OP	Oxygen Permeability
PTGE	1, 2, 3-propanetriol-diglycidylether
PVA	Polyvinyl Alcohol
RH	Relative Humidity
SEM	Scanning Electron Microscope
TGA	Thermogravimetric Analyser
TS	Tensile Strength
WHO	World Health Organization
WVP	Water Vapour Permeability
WVTR	Water Vapour Transmission Rate

CHAPTER 1

INTRODUCTION

1.1 Food Processing Industry

Food can be defined as any products or substances that are safe to be consumed and mostly contain essential nutrients such as carbohydrates, proteins, vitamin and mineral for maintaining good health (Abdulmumeen, Risikat, & Sururah, 2012). Nutritious foods are considered as the most basic physical needs, maintain our focus and supplied energy for human activities to ensure a healthy lifestyle. In order to ensure that all nutrients are absorbed by body, quality, freshness and safety of various food products must be

maintained. According to United Nation (2017), the total worldwide population is around 1.7 billion people and expected to increase to 9.5 billion in 2050, exceeded 11.2 billion in 2100. Meanwhile, Ringler et al., (2016) reported that the production of agricultural food products is increasing about 85% by 2050. However, most of consumers do not practice appropriate steps to handle the foods especially in undeveloped countries and therefore may lead to various food-borne illnesses.

Inspite of providing nutrients and energy, food processing has become one of the important sources for economic growth in developing countries. The food industry has been an important sector which is a major contributor to the economic growth of Malaysia. Ministry of International Trade and Industry (MITI) has set the investment for food processing industry at about RM 24.6 billion on 2012 based on Third Industrial Master Plan (IMP3) (Ayupp, 2013).

Recently, production of food products has expanded about 0.4% (January to July 2013: 8.2%) on 2014 with increasing in production of refined palm oil (18.2%), rice (17.6%) and biscuits or cookies (8.7%). Furthermore, Malaysia has been one of potential food producers with increasing product sales by 2.5%, (RM 20.6 billion) from January to July 2013:0.1%; RM20.1 billion and food export has increased rapidly to RM 9.3 billion from RM 8.1 billion in January to July 2013. Table 1.1 presents index of manufacturing production in 2013 and 2014. From Table 1.1, it is clear that the industry of food products has been growing extensively during these two consecutive years thus maintained the positive economic growth. According to 11th Malaysia Plan, the Gross Domestic Product

(GDP) for these sector was almost RM 93.2 billion in January 2016 and expected to increase by 19% by 2020 (The Star Online, 2016).

Table 1.1

Index of Manufacturing Production in 2013 and 2014

Domestic industry	Index		Changes (%)		Part from sum (%)	
	2013	2014	2013	2014	2013	2014
Food products	113.3	123.0	8.2	8.6	5.8	5.9
Drinks	95.2	113.5	-6.5	19.3	0.8	0.9
Basic metal	100.2	102.1	2.9	1.9	3.6	3.4
Transportation equipment	121.7	149.6	9.8	22.9	5.9	6.8

Note. Department of Statistics Malaysia 2015

Agricultural based products are main contributor for food processing industry. The processed food has become most essential part for agro-based industries. With increasing awareness towards healthier and nutritious products, consumers are more demand for minimally processed foods that comes from nature, without addition of synthetic chemicals. Several agricultural products that involved in processing industry are fresh fish and meat, fruits and vegetables. In July 2014, Pahang State Farmers Organization (PASFA) has been targeted to export 100 tan metric season fruits per month to Hong Kong with total profit about RM 20 million per year (Mstar Online, 10 July 2014). Recently, Federal Agricultural Marketing Authority (FAMA) Kelantan are exporting 500 tan metric of

Casaba melon to Thailand and Singapore which has been cultivated by 29 participant of Ladang Kontrak Agrotourism in Kampung Banggol since 2002 (Utusan Online, 2017).

1.2 Food Spoilage and Deterioration

In recent decades, demands toward food quality are increasing as public communities are more concern about its nutritional value and safety. High quality and safety of food ensured satisfactory protection from various pathogenic bacteria, toxicity and dangerous health risk. Food Agricultural Organization of the United Nations (FAO) in 2002 reported that foods are considered as one of the carrier in health problems whether through biological, chemical, physical or nutritional contamination (Amine et al., 2002). The quality of the product depends on level of deterioration and spoilage of food in a period of time. Deterioration and spoilage occur when the food undergoes some changes such as lose its physical appearance, generates bad odour and undergoes off-flavour during processing, washing or handling process.

1.2.1 Food Borne Diseases

Activities of microorganisms are main factor for food spoilage. There are many microorganisms that can lead to food spoilage such as *Escherichia Coli* (*E. coli*), *Salmonella*, *Campylobacter jejuni* (*C. jejuni*), *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*) (Dutta, Tripathi, Mehrotra, & Dutta, 2009). These food

pathogenic bacteria not only make food unsuitable to be eaten but can also affect human life as it can causes severe food borne diseases such as diarrhoeal disease, dysentery and thyphoid (WHO, 2014). Based on a World Health Organization (WHO) summary document that was reported on 2008, diarrhoeal disease was a worldwide killer to 1.9 million children especially in undeveloped countries mostly due to contaminated foods (WHO, 2010). Whereas, a summary document of WHO in 2009 states that *Salmonella* and *E. Coli* O157:H7 are the most dangerous food borne microorganism spreading in the United States (US) that may lead to dysentery and typhoid diseases (WHO, 2010).

Impacts of these microorganisms are being discussed in one of Food Borne Disease Stakeholder Meeting was organized by WHO in 2008. Based on the report, foodborne diseases are categorised as growing interest problems that normally affects human life especially in poor countries. These diseases can lead to premature death and increasing medical costs. Instead, based on the summary report, *C. jejuni* microorganism was regarded as the main cause of Guillain-Barre syndrome. Guillain-Barre syndrome gave long-term effects of neurological symptoms such as acute paralysis. Meanwhile, *E. coli* O157 contamination might trigger haemolytic uraemic syndrome, which is severe kidney failure and can lead to death (Stein, 2008).

Dysentery, thyphoid and food poisoning are most popular diseases caused by food pathogenic bacteria in Malaysia. Based on Health Facts by Ministry of Health Malaysia that are reported every year, total Malaysians affected by dysentery per 100,000 populations increased rapidly from 0.15 to 0.29 cases compared to thypoid that decreasing

rate from 0.75 to 0.73 in 2012 and 2013, respectively. Highest number of cases was reported for food poisoning diseases in 2011 at 56.25 per 100,000 cases. Table 1.2 shows number of food borne diseases per 100,000 populations for four consecutive years respectively.

Table 1.2

Number of Food Diseases per 100,000 Populations for Four Consecutive Years

Food borne diseases	Year			
	2010	2011	2012	2013
Dysentery	0.37	0.15	0.29	0.28
Typhoid	0.74	0.84	0.75	0.73
Food poisoning	44.18	56.25	44.93	47.79

Note. Number of cases per 100,000 populations

1.2.2 Chemical and Physical Changes of Food

Other factors that contribute to food spoilage are presence of enzymes, air and respiration rate that leads to physical changes of food then it cannot be eaten. Good air and moisture rate provide a suitable environment for bacteria and microorganism growth (Richardson & Finley, 2012). Enzymes are natural substances present in varieties of fruits and foods. The presence of these oxidizing enzymes will increase the ripening rates thus shortens the shelf-life of the food. It helps in softening of the fruits due to the over ripening rate. Meanwhile,

oxygen migration can cause deterioration on physical surface of food as it can increase the respiration and oxidation rate that can lead to the off-flavour, browning surface or anaerobic fermentation. Eventually, this situation makes the food unsuitable to be consumed and may affects human health as it offers a good condition for microbial attack.

Most of foods are originally comes from nature such as wheat, fruits, vegetables, fish and fresh meat. In fact, some foods need to be eaten directly after harvested and cannot stay longer than 24 hours under room temperature because microbial attack may ruins the food flavour (Pavlath & Orts, 2009). Moreover, some fruits and vegetables have their own harvest season throughout the year. Seasonal fruits are abundantly available in large quantities throughout the season and need to be consumed directly. However, these agricultural products are rare to be found outside of their harvest season and can be easily damaged during long term storage if inappropriate handling methods are being used. Due to these reasons, handling and storage technique has been modified in order to protect and keep the quality of these agricultural products thus makes it possible to be consumed out of their harvest time (Singh, 2009).

Furthermore, growing technological advances and transportation field makes it possible for these perishable products to be preserved and protected. Increasing interest in global distribution systems enabled the food to be consumed outside of their original sources and makes them possible to be exported and imported (Pavlath & Orts, 2009). However, some goodness of the products such as nutritional value, physical appearance and flavour might be lost during washing, cleaning and storage process. Foods that are not

protected are easily exposed to microorganism attack, oxidation process, moisture and humidity that can lead to deterioration and spoilage of the food itself. Therefore, before these products can be found in consumer's storage, some safety and protection steps need to be taken in order to keep the quality and freshness of the food consumed (Pavlath & Orts, 2009).

1.3 Food Preservation

Preservation of perishable food plays an important role to maintain the freshness and quality varieties of food. Rahman (2009) defined food preservation as an action taken in order to protect and control the quality of foods preserved for a period of time. In other words, food preservation is an essential step in order to retain the freshness, flavour, nutritional value and physical appearance of perishable products after being harvested or taken out from its natural sources (Grandison, 2006; Rahman, 2009).

The advancement in transportation system provides more convenient shipping, particularly export and import of food products. However, some perishable products such as vegetables, fruits, fish and poultry meat cannot withstand to room temperature for a long time as they might spoilt and degraded. Consumers that continually consume foods that have been contaminated are susceptible to various diseases. Therefore, by preserving the foods, people are able to consume foods that are not naturally grown in their places. Before that, there is a need to do some modification to the products, whether physical or chemical

in order to preserve the food and provide additional protection so they can last longer and safe to be consumed.

1.4 Traditional Preservation Techniques

Traditional preservation techniques have been used for centuries in order to protect and preserve foods, whether from physical or chemical changes. Some of these techniques are still being used by consumers because of their low cost, easy and simple application.

1.4.1 Salting

Salting process is a process of which salt is being added directly to the foods in order to removed water content in it. Salt has the potential to remove moisture from the food thus inhibit the formation of microorganisms (Sun, 2012). Microorganism and bacteria cannot survive in high concentrated salty environment. This method is preferred by consumers as it is low cost and requires simple technique. There are two types of salting namely wet salting and dry salting. Wet salting requires food to be rubbed with salt, before soaked in brine (concentrated salty solution). In dry salting method, salt is rubbed throughout the food before storage. Compared to dry salting, foods that were kept in brine solution are preserved longer. Fish is one of common perishable products that normally preserved through wet salting.

1.4.2 Drying

Drying method is also called dehydration method. It is a process in order to remove water from the foods to prevent humid atmosphere that suitable for microorganism growth (Singh, 2009). This process depends mostly on sunlight and climate changes (air and wind). The oldest method for drying is by using sunlight. It requires a constant dry and hot climate from atmosphere in order to remove all the water content from the food surface. Usually, food products will be spread in a tray before being exposed in direct sunlight. This drying process normally takes a few hours or may even a few days to complete. Inspite of microorganism, drying process may also slow down enzymatic process as it is not active without the presence of water. It is one of the most common traditional techniques used in order to increase the shelf life of foods since ancient history.

1.4.3 Freezing

Instead of drying, freezing is another simple and common method to be used to increase shelf life of perishable foods such as vegetables, fruits, fish and meats. Freezing stops activity of microorganisms but does not kill them (Sun, 2012). Foods are kept below 0 °C because microorganisms cannot grow in low temperature surroundings (Tucker, 2016). However, freezing does not change appearance, flavour and texture of food products compared to smoking and salting (Vaclavik & Christian, 2014). Therefore, foods kept in the freezer or refrigerator must be in good condition so that the freshness of the products can be retained.

1.4.4 Smoking

Smoking is categorised as traditional method and has been used widely to preserve particularly fish and meat. The process requires application of smoke to the foods and similar to drying process, it removes moisture, dehydrates the products and also kills some microorganism that can lead to food spoilage (Edelstein, 2014). A few days are needed for the process to be completed at certain temperature range are around 41 to 70 °C. It is interesting to note that smoking enhances the flavour of the food with its browning texture thus increase its commercial value. However, smoking process are found to be toxic to human health as the smoke wood may contain some polycyclic hydrocarbons such as formaldehyde that may causes cancer (Edelstein, 2014).

1.4.5 Canning

Another promising preservation technique is by canning. Foods are being kept in a sterile can before heated to high temperature (241 °F to 250 °F) in order to destroy any microorganism and enzyme present that contribute to food spoilage. This technique was established around 1809 by Nicholas Appert, a French confectioner when he served Napoleon's soldier (Vaclavik & Christian, 2014). Metal or glass containers can be used for this purpose. Canned foods are commercialised in food processing industry as they have longer shelf life (almost two years and above) and can be kept in room temperature without any spoilage and addition of chemical additives. High temperatures are needed to make sure no microorganism left and foods are totally sterilised. Sometimes, calcium and vitamin