Production, Properties and Potential Application of Antimicrobial Edible Films Incorporated with Kiam (*Cotylelobium lanceotatum*) and Phayom (*Shorea talura*) Wood Extracts

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ชื่อวิทยานิพนธ์	การผลิต คุณสมบัติ และแนวทางการใช้ฟิล์มบริโภคได้ชนิดยับยั้งจุลินทรีย์ ซึ่งเตรียมได้จากการผสมสารสกัดจากไม้เคี่ยม (*Cotylelobium lanceotatum*) และไม้พยอม (*Shorea talura*)
ผู้เขียน	นางสาวจุฑาภรณ์ ชนะถาวร
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บทคัดย่อ

จากการศึกษาผลของความสามารถในการยับยั้งเชื้อจุลินทรีย์ของสารสกัดจากไม้เคี่ยมและไม้พยอม พบว่าความเข้มข้นของสารสกัดจากไม้เคี่ยมและไม้พยอมที่ 300 มิลลิกรัม/ลิตร เป็นความเข้มข้นต่ำสุด (Minimal bactericidal concentration, MBC) ที่สามารถยับยั้งการเจริญของจุลินทรีย์ได้อย่างมีนัยสำคัญ ($p<0.05$) และผลการทดสอบความสามารถในการยับยั้งเชื้อจุลินทรีย์ 3 ชนิด คือ *E. coli, S. aureus* และ *L. monocytogenes* ของฟิล์มบริโภคได้ซึ่งเตรียมจากไฮดรอกซี่โปรพิลเมทิลเซลลูโลส (hydroxypropyl methylcellulose, HPMC) ที่ผสมสารสกัดจากไม้เคี่ยมและไม้พยอมความเข้มข้น 1-5 เท่าของ MBC พบว่า สามารถยับยั้งการเจริญของ *L. monocytogenes* ได้ดีกว่า *S. aureus* และ *E. coli* ตามลำดับ โดยพบว่าที่ความเข้มข้น 1 และ 2 เท่าของ MBC ไม่สามารถยับยั้งการเจริญของจุลินทรีย์ได้ แต่สามารถยับยั้งการเจริญของจุลินทรีย์ได้ที่ความเข้มข้น 3 4 และ 5 เท่าของ MBC และที่ 5 เท่าของ MBC เกิดวงใสการยับยั้งรอบแผ่นฟิล์มสูงที่สุด เมื่อความเข้มข้นของสารสกัดจากไม้เคี่ยมและไม้พยอมเพิ่มขึ้นส่งผลให้ค่าการทนต่อแรงดึงสูงสุด และค่าการยืดตัวเมื่อขาดของฟิล์มลดลง ($p<0.05$) ขณะที่ค่าการซึมผ่านของไอน้ำและความสามารถในการละลายของฟิล์มเพิ่มขึ้น นอกจากนี้พบว่าฟิล์มมีสีดำแดงเพิ่มขึ้น และมีความโปร่งใสของฟิล์มลดลง ($p<0.05$) เมื่อเติมสารสกัดจากไม้เคี่ยมและไม้พยอม เมื่อใช้กล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด พบว่าการผสมสารสกัดจากไม้เคี่ยมและไม้พยอมในแผ่นฟิล์มส่งผลให้แผ่นฟิล์มมีความหยาบมากกว่าฟิล์มที่ไม่เติมสารสกัดจากไม้เคี่ยมและไม้พยอม

จากการศึกษาผลร่วมของสารสกัดจากไม้เคี่ยมและไม้พยอมในอัตราส่วนต่างๆ ที่เติมลงในสารละลาย HPMC ฟิล์ม ต่อความสามารถในการยับยั้งเชื้อจุลินทรีย์และคุณสมบัติของฟิล์ม พบว่าวงใสของการยับยั้งรอบแผ่นฟิล์มมีค่าเพิ่มขึ้นตามอัตราส่วนของสารสกัดจากไม้เคี่ยมเพิ่มขึ้น อีกทั้งพบว่าค่าการทนต่อแรงดึงสูงสุดและค่าการยืดตัวเมื่อขาดเพิ่มขึ้นด้วย โดยค่าดังกล่าวมีค่าสูงสุดที่อัตราส่วนระหว่างสารสกัดจากไม้เคี่ยมและไม้พยอมเท่ากับ 100:0 เมื่อพิจารณาค่าการซึม

ผ่านของไอน้ำ ความสามารถในการละลายของฟิล์ม ค่า L* a* และ b* และความโปร่งใสของฟิล์ม พบว่ามีค่าลดลงเมื่ออัตราส่วนระหว่างสารสกัดจากไม้เคี่ยมและไม้พยอมเพิ่มขึ้น

เมื่อพิจารณาผลการศึกษาการเพิ่มความสามารถในการยับยั้งเชื้อจุลินทรีย์โดยผสมสารสกัดจากไม้เคี่ยมที่ความเข้มข้น 3 เท่าของ MBC ลงในฟิล์มที่เตรียมจากไคโตแซน พบว่า ฟิล์มไคโตแซนที่เติมสารสกัดจากไม้เคี่ยมมีค่าความสามารถในการยับยั้งเชื้อจุลินทรีย์สูงกว่าฟิล์มจากไคโตแซนที่ไม่เติมสารสกัด เมื่อทดสอบความแข็งแรงของฟิล์ม และพบว่าฟิล์มไคโตแซนมีค่าการทนต่อแรงดึงสูงสุด และค่าการยืดตัวเมื่อขาดของฟิล์มลดลง ($p<0.05$) ในขณะที่ค่าการซึมผ่านของไอน้ำ และความสามารถในการละลายของฟิล์มเพิ่มขึ้น อย่างไรก็ตามฟิล์มมีสีเหลืองเพิ่มขึ้น มีผลทำให้ความโปร่งใสของฟิล์มลดลง ($p<0.05$) เมื่อเติมสารสกัดจากไม้เคียม นอกจากนี้พบว่าฟิล์มไคโตแซนมีลักษณะหยาบขึ้นเมื่อเติมสารสกัดจากไม้เคี่ยม ขณะเดียวกันพบว่าฟิล์มไคโตแซนที่เติมสารสกัดจากไม้เคี่ยมมีค่าความสามารถในการทนต่อความร้อนเพิ่มขึ้น ซึ่งสังเกตจากการเพิ่มขึ้นของค่า glass transition temperature (T_g)

จากการประยุกต์ใช้แผ่นฟิล์มต้านเชื้อจุลินทรีย์ในผลิตภัณฑ์อาหาร 2 ชนิด คือ ปูอัดและแฮม พบว่าผลิตภัณฑ์อาหารที่ห่อด้วยฟิล์มจากไคโตแซนที่ผสมสารสกัดจากไม้เคี่ยมมีค่า TBAR ความชื้น วอเตอร์แอคติวิตี้ และ ค่าสีของผลิตภัณฑ์อาหารเปลี่ยนแปลงช้ากว่าผลิตภัณฑ์อาหารที่ห่อด้วยแผ่นฟิล์มที่เตรียมจาก HPMC ที่ผสมสารสกัดจากเคี่ยม และ PVC ตามลำดับ ($p<0.05$) เมื่อพิจารณาจำนวนจุลินทรีย์ทั้งหมด และโคลิฟอร์มในผลิตภัณฑ์อาหารดังกล่าวระหว่างการเก็บรักษาพบว่าผลิตภัณฑ์อาหารที่ห่อด้วยแผ่นฟิล์มจากไคโตแซนที่ผสมสารสกัดจากไม้เคี่ยมสามารถชะลอการเจริญของ จุลินทรีย์ได้ดีกว่าผลิตภัณฑ์อาหารที่ห่อด้วยแผ่นฟิล์มที่เตรียมจาก HPMC ที่ผสมสารสกัดจากเคี่ยม และ PVC ตามลำดับ จากผลการทดลองดังกล่าวสามารถกล่าวได้ว่าสารสกัดจากไม้เคี่ยมและไม้พยอมเป็นสารยับยั้งเชื้อจากธรรมชาติที่สามารถนำมาประยุกต์ใช้เพื่อยืดอายุการเก็บรักษาของอาหารได้

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ABSTRACT

In this study, the minimum bactericidal concentration (MBC) of Kiam and Phayom wood extracts was established as 300 mg/L was inhibited completely ($p<0.05$). Antimicrobial properties of hydroxylpropyl methyl cellulose (HPMC) films containing 1-5 folds of MBC of Kiam and Phayom wood extracts were tested against *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*. The edible HPMC films containing Kiam and Phayom wood extracts was effective on *L. monocytogenes* than *S. aureus* and *E. coli* , respectively. The use of Kiam and Phayom wood extracts at 1 and 2 folds of MBC incorporated into edible HPMC films did not exhibit any antimicrobial activity whereas inhibitory effect of edible HPMC film containing Kiam and Phayom wood extracts was observed at 3, 4 and 5 folds of MBC. The greatest zone of inhibition was observed at 5 folds of MBC of Kiam and Phayom wood extracts incorporated in edible films. Edible HPMC films incorporating with Kiam wood extracts had higher potential antimicrobial activity than Phayom wood extracts. Tensile strength (TS) and elongation at break (ε) significantly decreased as incorporation of Kiam and Phayom wood extracts concomitantly with increased in water vapor permeability (WVP) and films solubility (FS). However, TS, ε and FS of HPMC films incorporated with Kiam wood extracts were higher than edible HPMC films incorporated with Phayom wood extracts. The color of edible HPMC films was affected by the addition of Kiam and Phayom wood extracts; the results showed that increasing of Kiam and Phayom wood extracts yielded darker and more reddish films. The lower transparency of the edible films was observed when greater amount of Kiam and Phayom wood extracts was incorporated ($p<0.05$).

Incorporation of Kiam and Phayom wood extracts into edible HPMC films resulted in the films with rougher surface than pure edible HPMC films.

Influence of the ratio of Kiam and Phayom wood extracts on the antimicrobial properties and properties of edible HPMC films were investigated. The results showed that, the inhibition zone increase with increasing the ratio of Kiam and Phayom wood extracts. TS and ε of edible HPMC films tended to increase with the ratio Kiam and Phayom woods extracts increased, and the maximum occurred at the Kiam and Phayom woods extract of 100:0. The WVP and FS of edible HPMC films decreased with an increased in content of Kiam wood extracts. Higher ratio of Kiam and Phayom wood extracts resulted in darker and more opacity as indicated by the increase in L*, a*, b* and chroma values and decrease in transparency value, respectively.

Enhancing antimicrobial activity of chitosan films by incorporating Kiam wood extracts were studied. The inhibitions of Kiam wood extracts incorporated into chitosan films were stronger than those of chitosan unfill Kiam wood extracts. TS and ε value of the chitosan films incorporated with Kiam wood extracts was lower than that of chitosan films unfilled Kiam wood extracts. Incorporation of Kiam wood extracts affected the WVP and FS values and tended to increase as Kiam wood extracts were incorporated. The results demonstrated that the chitosan films incorporated with Kiam wood extracts were lighter and darker red-yellowish evidenced by L*, a* and b* and ΔE_{ab}*, chroma and hue angle values. Morphology of the chitosan film unfilled Kiam wood extracts had smoother surface than the chitosan films filled Kiam wood extracts. According to the thermal properties of antimicrobial, chitosan films incorporated with Kiam wood extracts had the highest peak temperature whereas the edible HPMC films had the lowest peak.

Application the antimicrobial films in commercial imitated crab meat and ham wrapped by antimicrobial films was determined. The results showed that, both food products wrapped with chitosan films incorporated with Kiam wood extracts had lower and lesser changed in TBARS, moisture content water activity and color than food products wrapped with edible HPMC films incorporated with Kiam wood extracts and PVC films, respectively. Regarding to the effect of antimicrobial films on sensory scores of food products, the results showed that both imitated crab

meat and ham wrapped with chitosan films incorporated with Kiam wood extracts received a higher score in term of flavor, odor, appearance and overall acceptability than the imitated crab meat and ham wrapped with edible HPMC films incorporated with Kiam wood extracts and PVC wrapped films, respectively. In addition food samples wrapped with chitosan films incorporated with Kiam wood extracts demonstrated lower TVC and coliforms than edible HPMC films incorporated with Kiam wood extracts and PCV films, respectively. Our results pointed that incorporation of Kiam and Phayom wood extracts as a natural antibacterial agents have a potential to prolong the shelf-life of foods.

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CHAPTER 1

INTRODUCTION

The demand for greater stringency in relation to hygiene and safety issues associated with fresh and processed food products, concomitant with ever-increasing demands by retailers for cost effective extensions to product shelf-life and the requirement to meet consumer needs in relation to convenience (easily preparation or ready-to-eat), quality and food safety. Recent food-borne microbial outbreaks are driving force for innovative ways to inhibit microbial growth in the food while maintaining quality, freshness and safety. Using of packaging to provide an increased margin of safety and quality is one of the alternative options. The development of food packaging may include material with antimicrobial properties. These packaging technologies could play a role in extending shelf-life of food to reduce the risk form pathogens (Appendini and Hotchkiss, 2002).

Antimicrobial packaging is a promising form of active food packaging, since microbial contamination of foods occurs primarily at the surface, due to post-processing handling; attempts have been made to improve safety and to delay spoilage by use of antibacterial sprays or dips. However, direct surface application of antibacterial substances onto foods have limited benefits because the active substances are neutralized on contact or diffuse rapidly from the surface into the food mass. On the other hand, incorporation of bactericidal or bacteriostatic agents into product formulations may result in partial inactivation of the active substances by product constituents and is therefore expected to have only limited effect on the surface microflora (Quintavalla and Vicni, 2002). Then application of antimicrobial packaging could extend the shelf-life of product and provides microbial safety for consumers. It acts to reduce, inhibit, or retard the growth of pathogen microorganisms in packed foods and packaging material (Vermeiren *et al*., 1999). Several compounds have been proposed for antimicrobial activity in food packaging, including organic acids, enzymes such as lysozyme, and fungicides such as benomyl, imazalil and natural antimicrobial compounds such as spices (Tharanathan, 2003; Weng and Hotchkiss, 1992). These compounds carry mostly antimicrobial and some antioxidant

properties. In addition, natural compounds, such as nisin and lysozyme, have been studied as potential food preservatives added to the edible films that are safe for human consumption (Cagri *et al*., 2004).

Edible films are defined as thin layer of material which can be eaten by the consumer and provide a barrier to moisture, oxygen and solute movement for the food. The material can complete food coating or can be disposed as a continuous layer between food components (Guilbert, 1986). The edible films have received a consideration attention in the recent years because of their advantage over the synthetic films. The advantages of edible films over other traditional synthetic such as they can be consumed with the package products. The films can function as carriers for antimicrobial and antioxidant agents. In a similar application they also can be used at the surface of food to control the diffusion rate of preservative substances from the surface to the interior of the food. And another possible application for edible films could be their use in multilayer food packaging materials together with non edible films. It is one of the most effective methods of maintaining food quality. This is further improved by film carrying food additives such as antioxidants, antimicrobial, colorants, flavors, fortified nutrient, and spices (Pena and Torres, 1991). In many cases, the agents being carried are slowly released into the food surface and therefore remain at high concentrations for extended periods of time.

The antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed potential as natural agents for food preservation, pharmaceuticals, alternative medicine and natural therapies. In order to prolong the storage stability of foods, synthetic antimicrobial are mainly used in industrial processing. But according to the toxicologists and nutritionists, the side effects of some synthetic antioxidants used in food processing. For this reason, the search for antimicrobials from natural source has received much interesting to replace synthetic ones. Beside, these naturally occurring antimicrobial can be formulated as functional foods and can help to prevent spoilage of food products. Basically, most plants contain a variety of substances called ‘‘phytochemicals” that come from naturally occurring components present in plants. The phytochemical preparations with functionalities in preventing antimicrobial properties have tremendous potential for extending the shelf-life of food products. The extracts of plants are of growing

interest both in the industry and scientific research because of their antibacterial, antifungal, antiviral and anti-parasitical activities that make them useful as natural additives in foods, cosmetic and pharmaceutical industries. Several antimicrobial compounds occur naturally in plants (Banks *et al*., 1986; Walker, 1994) and are known to retard the growth or kill food-borne pathogens (Beuchat and Golden, 1989). Essential oils (Beuchat, 1994) and juices (Beuchat and Doyle, 1995) of plants are known to have antilisterial activity. Antimicrobial properties of Kiam and Phayom wood extracts are well known. The bark of *Cotylelobium lanceotatum* (Kiam) and *Shorea tolura* (Phayom) have been effectively employed in the central and the south of Thailand to retard the growth of microflora which causes spoilage of palm sap. This research was to study antimicrobial efficacy of edible films based on hydroxylpropyl methylcellulose (HPMC) by incorporating Kiam and Phayom wood extracts. Furthermore, improvement of antimicrobial efficacy of edible HPMC films by incorporating with chitosan was also determined. Mechanical and physical properties also were characterized, and antimicrobial efficacy was assessed against three food pathogenic bacteria. The relationship between the structure and their physical properties and antimicrobial activity is also discussed.

Review of Literature

1. Edible film

Edible films are defined as thin layer of material which can be eaten by the consumer and provide a barrier to moisture, oxygen and solute movement for the food. The material can complete food coating or can be disposed as a continuous layer between food components (Guilbert, 1986). Edible films can be formed as food coatings and free-standing films, and have potential to be used with food as gas aroma barrier (Kester and Fennema, 1986). However, the technical information is still needed to develop films for food application (Donhowe and Fennema, 1993). The edible films and coatings have received a consideration attention in the recent years because of their advantage over the synthetic films. The advantages of edible films over other traditional synthetic films are summarized below:

1. They can be consumed with the package products. This is obviously of critical importance since it represents the environmentally ideal package.

2. There is no package to dispose of even if the films are not consumed they could still contribute to the reduction of environmental pollution.

3. The films are produced exclusively from renewable, edible ingredients and therefore are anticipated to degrade more readily than polymeric materials.

4. The films can enhance the organoleptic properties of packaged foods provided that various components (flavorings, colorings, sweeteners)

5. The films can supplement the nutrition value of the foods. This is particular true for films made from proteins.

6. The films can be used for individual packaging of small portion of food, particularly products that currently are not individually packaged for practical reasons such as pears, beans, nuts and strawberries.

7. The films can be applied inside heterogeneous foods at the interfaces between different layers of components. They can be tailored to prevent deteriorative inter-component moisture and solute migration in foods such as pizzas, pies and candies.

8. The films can function as carriers for antimicrobial and antioxidant agents. In a similar application they also can be used at the surface of food to control the diffusion rate of preservative substances from the surface to the interior of the food.

9. The films can be very conveniently used for micro encapsulation of food flavoring and leavening agents to efficiently control their addition and release into the interior of foods.

10. Another possible application for edible films could be their use in multilayer food packaging materials together with non edible films. In this case, the edible films would be the internal layers in direct contact with food materials.

Production of edible films causes less waste and pollution, however, their permeability and mechanical properties are generally poorer than synthetic films (Kester and Fennema, 1986). Extensive research is needed on the development of

new materials, methods of films formation, methods to improve film properties and the potential applications.

1.1 Classification of edible films

Edible films can be produced from materials with film forming ability. During manufacturing, film materials must be dispersed and dissolved in the solvent such as water, alcohol or mixture of water and alcohol or mixture other solvents. Plasticizer, antimicrobial agent, colors or flavor can be added in this process. Adjusting pH and/or heating the solutions may be done for the specific polymer to facilitate the dispersion. Film solution is then casted and dried at desired temperature and relative humidity to obtain free-standing films. In the food application, film solutions could be applied to food by several methods such as dipping, spraying, brushing and panning followed by a drying step. Kester and Fennema (1986) classified the edible films based on the nature of material as polysaccharide, protein, lipid and composite films.

1.1.1 Polysaccharides

Polysaccharides used for edible films or coatings include cellulose and derivatives starch and derivatives pectin, seaweed extracts, exudate gums, microbial fermentation gums, chitosan (Krochta and Mulder-Johnson, 1997). Polysaccharides are very generally very hydrophilic resulting in poor water vapor and gas barrier properties. Although coating by polysaccharide polymers may not provide a good water vapor barrier, these coating can act as sacrificing agent retarding moisture loss from food products (Kester and Fennema, 1986).

1.1.1.1 Cellulose and derivatives

Cellulose is composed of repeating D-glucose units linked through ß-1, 4 glycosidic bonds. In its native state, the hydroxymethyl groups of anhydroglucose residues are alternatively located above and below the plan of the polymer backbone. This results in very tight packing of polymer chains and a highly crystalline structure that resists salvation in aqueous media. Water solubility can be increased by treating cellulose with alkali to swell the structure, followed by reaction with chloroacetic acid, methyl chloride or propylene oxide to yield carboxy methyl cellulose (CMC), methyl cellulose (MC), hydroxy propyl cellulose (HPMC) or hydroxyl propyl

cellulose (HPC). Placement of bulky substituents along the cellulose molecule, in the form of ether linkages at reactive hydroxyls, separates the polymer chains and interferes with formation of the crystalline unit cell, thereby enhancing aqueous solubility (Krumel and Lindsay, 1976). MC, HPMC, HPC and CMC film possess good film-forming characteristic; films are generally odorless and tasteless, flexible and are of moderate strength, transparent, resistance to oil and fats, water-soluble, moderate to moisture and oxygen transmission (Krochta and Mulder-Johnson, 1997). MC is the most resistant to water and it is the lowest hydrophilic cellulose derivatives (Kester and Fennema, 1986) however, the water vapor permeability of cellulose ether film is still relatively high. MC and HPMC have ability to form thermally induced gelatinous coating; they have been used to retard oil absorption in deep frying food product (Kester and Fennema, 1986). MC could be applied as coating on confectionery products as barrier to lipid migration (Nelson and Fennema, 1991). A number of groups have investigated composite films composed of MC or HPMC and various kinds of solids, such as beeswax and fatty acids (Kamper and Fennema, 1985; Greener and Fennema, 1989; Koelsch and Labuza, 1992; Debeaufort *et al*., 1993). Many of these have water vapor permeability as low as low density polyethylene (LDPE). These composite films were all polymer-lipid bilayer formed either in one step from aqueous ethanolic solutions of cellulose ether fatty acids.

Cellulose can also be chemically modified to ether, ethyl cellulose (EC), which is biodegradable but not edible. EC films can either be cast from non-aqueous solutions or extruded. Like the other cellulose ethers. EC films are poor moisture barrier, but they have been reported to be good oil and fat barriers (Park *et al*., 1992).

1.1.1.2 Starch

Starch consists of amylose and amylopectin, the ratio of amylose and amylopectin depends on the type and variety of raw material. Amylose is a linear chain of D-glucose residues linked through α-1,4 glycosidic bonds. Amylopectin is a branched molecule consisting of glucose units connected by α-1,4 and α-1,6 linkages. High amylose starch as cornstarch is a good source for films formation; free-standing films can be produced from aqueous solution of gelatinized amylose and drying. Normal cornstarch consists of approximately 25% amylose and 75% amylopectin.

Mutant varies of corn are produced which contain starch with up to 85% amylose (Debeaufort *et al*., 1993). Wolf *et al*. (1951) produced self-supporting films by casting aqueous solutions of gelatinized amylose, followed by solvent evaporation. The films were transparent and had very low permeability to oxygen at low RH (Rankin *et al*., 1958). Mark *et al*. (1966) reported that films produced from high amylose corn starch (71% amylose) had no detectable oxygen permeability at RH levels less than 100%. This was true for both unplasticized and plasticized (16% glycerol) films. This result is surprising in light of the fact that addition of plasticizers and absorption of water molecules by hydrophilic polymers increase polymer chain mobility and generally lead to increased gas permeability (Banker, 1966). Partial etherification of high-amylose starch with propylene oxide, to yield the hydroxypropylated derivative, improves water solubility. As expected, films produced from hydroxypropylated starch possess virtually no resistance to the passage of water vapor: however, as with the pure amylose films, resistance to oxygen transport is substantial (Jokay *et al*., 1967). Oxygen permeation through plasticized and unplasticized films was not detectable at 25^{o}C and RH up to 78% (Roth and Mehltretter, 1967). At the high RH, films became distorted due to the moisture absorption and were not tested for oxygen permeability; however, it is likely that oxygen transport increased greatly as the film became hydrate. Jokay *et al*. (1967) applied hydroxylpropylated starch films almond nutmeats, and organoleptic evaluation revealed that the film retarded development of oxidative rancidity during storage. Starch hydrolysates (dextrin) of low dextrose equivalent (DE) have been suggested for use as protective coatings. Although hydrophilic in nature, starch hydrolyses do provide a limited resistance to transport of water vapor. Allen *et al*. (1963) evaluated the relative barrier properties of edible film materials by coating them onto a cellulose acetate support. Starch films displayed minimal resistance to water transport, while films of low-DE dextrin and corn syrup were approximately 2- and 3-fold more resistant, respectively. Murray and Luft (1973) coated almond nutmeats with a 50% solution of a 10-DE starch hydrolysate. Sensory evaluation indicated that the coated nuts maintained a more desirable texture than uncoated controls during storage. Presumably, this was attributation to a reduction in the rate of moisture absorption by coated almonds. Films of starch hydrolysates may exhibit

some resistance to oxygen transmission. Dipping of fresh sliced apples in a 40% solution of a 15-DE hydrolysate prior to dehydration prevented browning of the tissue, probably by retarding the entrance of oxygen.

1.1.2 Lipid Films

Lipid compounds utilized as protective coating consist of acetylated monoglycerides, nutural wax, and surfactants. The most effective lipid substances are paraffin wax and beeswax. The primarily function of lipid coating is to block transport of moisture due to their relative low polarity. In contrast, the hydrophobic characteristic of lipid forms thicker and more brittle films. Consequently, they must be associated with film forming agents such as proteins or cellulose derivatives (Park *et al*., 1994). Generally, water vapor permeability decrease when the concentration of hydrophobicity phase increases. Lipid-based films are often supported on a polymer structure matrix, usually a polysaccharide, to provide mechanical strength.

1.1.2.1 Waxes and Paraffin

Paraffin wax is derived from distillate fraction of crude petroleum and consists of mixture of solid hydrocarbon resulting from ethylene catalytic polymerization. Paraffin wax is permitted for use on raw fruit and vegetable and cheese. Carnauba wax is exudates from palm tree leaves (Copoernica cerifera). Beewax (white wax) is produced from honeybees. Candelilla is obtained from candelilla plant. Mineral oil consists of a mixture of liquid paraffin and naphtheric hydrocarbon. Waxes are used as barrier films to gas and moisture (skin on fresh fruits) and to improve the surface appearance of various foods (e.g., the sheen on sweet). Applied in a thick layer, they must be removed before consumption (certain cheese); when used in thin layers, they are considered edible. Waxes (notably paraffin, carnauba, candellila and bee wax) are the most efficient edible compounds providing a humidity barrier (Jokay *et al*., 1967).

1.1.2.2. Acetoglyceride

Acetylation of glycerol monosterate by its reaction with acetic anhydride yields 1-stearodiacetin. This acetylated monoglyceride displays the unique characteristic of solidifying from the molten state into a flexible, wax-like solid (Feuge *et al*., 1953). Most lipids in the solid state can be stretched to only about 102% of their original length before fracturing. Acetylated glycerol monostearate,

however, can be stretch up to 800% of its original length, water vapor permeability of this film is much less than that of polysaccharide film with the exception of methyl cellulose or ethyl cellulose. Acetylated monoglyceride coating have been used on poultry and meat cuts to retard the moisture loss during storage (Kester and Fennema, 1986).

1.1.3 Protein Films

In their native states, proteins generally exist as either fibrous proteins, which are water insoluble and serve as the main structural materials of animal tissues, or globular proteins, which are soluble in water or aqueous solutions of acids bases or salts and function widely in living system. The fibrous proteins are fully extended and associated closely with each other in parallel structures, generally through hydrogen bonding, to form fibers. The globular proteins fold into complicated spherical structures held together by a combination of hydrogen, ionic, hydrophobic and covalent (disulfide) bond. The chemical and physical properties of these proteins depend on the relative amounts of the component amino acid residues and their placement along the protein polymer chain. Of the fibrous proteins, collagen has received the most attention in the production of edible films. Several globular proteins, including wheat gluten, corn zein, soy protein, and whey protein, have been investigated for their film properties. Protein films are generally formed from solutions or dispersions of the protein as the solvent/carrier evaporates. The solvent/carrier is generally limited to water, ethanol or ethanol-water mixtures. Generally, proteins must be denatured by heat, acid, base, and/or solvent in order to form the more extended structures that are required for film formation. Once extended, protein chains can associate through hydrogen, ionic, hydrophobic and covalent bonding. The chain-to-chain interaction that produces a cohesive films are affected by the degree of chain extension and the nature and sequence of amino acid residues. Uniform distribution of polar, hydrophobic, and/or thiol groups along the polymer chain increase the likelihood of the respective interactions. Increased polymer chain-to-chain interaction results in films that are stronger but less flexible and less permeable to gases, vapors and liquids (Kester and Fennema, 1986).

Polymers containing groups that can associate through hydrogen or ionic bonding result in films that are excellent oxygen barrier but that are susceptible

to moisture. Thus, protein films are expected to be good oxygen barriers at low relative humidities. Polymers containing a preponderance of hydrophobic groups are poor oxygen barriers but excellent moisture barriers. The more hydrophobic, water-insoluble proteins. However, the fact that they are not totally hydrophobic and contain predominantly hydrophillic amino acid residues limits their moisture-barrier properties. Creation of protein-versed edible films with low water vapor permeability requires addition of lipid components. This is analogous to the situation with synthetic polymers where moisture- sensitive oxygen-barrier polymers must be either co polymerized with a hydrophobic polymer or sandwiched between hydrophobic polymer layers to limit the ability of water to reduce barrier properties. Various types of protein have been used as edible films. These include collagen, casein, whey protein, corn zein, wheat gluten, soy protein, mung bean protein, and peanut protein (Gennadois *et al*., 1993; Bourtoom, 2008).

Several approaches can be used to form edible films (Kester and Fennema, 1986) as follows:

1. Simple coacervation: A single hydrocolloids is driven from aqueous suspension or caused to undergo a phase change by evaporation of solvent, addition of a water-miscible nonelectrolyte in which the hydrocolloids is not soluble (e.g., alcohol), addition of an electrolyte to cause salting out or crosslinking, or alteration of pH.

2. Complex coacervation: Two solutions of oppositely charged hydrocolloids are combined, causing interaction and precipitation of the polymer complex.

3. Thermal gelation or precipitation: A sol-gel transformation can occur by heating of a protein to cause denaturation followed by gelation (e.g., egg albumin) or precipitation, or simple cooling of a warm hydrocolloid suspension.

2. Active packaging

Active packaging is an innovative concept that can be defined as a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food. Active packaging technologies includes with gas absorbing/emitting packaging, selective

permeable films (Kester and Fennema, 1986), microwave susceptors. Controlled release packaging is a group of technologies that uses a packaging as a delivery system to release active compounds such as antimicrobial, antioxidant, enzyme, flavor, and nutraceuticals. Most attention in this group has been focused on antimicrobial packaging (Appendini and Hotchkiss, 2002) and antioxidant packaging (Kester and Fennema, 1986). Active packaging is one of the innovative food packaging concepts that have been introduced as a response to the continuous changes in current consumer demands and market trends. It has been defined as "a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food". The definition of active packaging was chosen for the European FAIR-project CT 98-4170 (Gennadois *et al*., 1993).

Active packaging can be classified into active-releasing systems (emitters) which actively add compounds to the packaged food such as carbon dioxide, water, antimicrobials aromas or preservatives and active-scavenging systems (absorbers), which remove undesired compounds such as oxygen, radicals, water, ethylene, carbon dioxide, taints and other specific compounds. Ethylene absorbers and oxygen scavengers are the most used and patented of all active packaging technologies (Brody, 2001). Oxygen removers are developed to avoid oxidation process. However, radicals mainly oxo, hydroxyl and superoxide are originated from oxygen and they are the main initiators of oxidation. Thus, a different approach can be considered: by eliminating radicals as soon as they are formed, the propagation of the oxidation reaction cannot take place and consequently, the concentration of molecular oxygen is not important, but the presence of radicals.

In general, active food packaging can provide several functions that do not exist in conventional packaging systems. The active functions may include scavenging of oxygen, Moisture or ethylene, emission of ethanol and flavours, and antimicrobial activity.

Microbial contamination reduces the shelf-life of foods and increases the risk of food bone illness. Traditional methods of preserving foods from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging, and adding antimicrobial agents or salts.

Unfortunately, some of these techniques cannot be applied to some food products, such as fresh meats and ready-to-eat products.

3. Antimicrobial packaging

Antimicrobial packaging is system that can kill or inhibit the growth of microorganisms and thus extend the shelf-life of perishable products and enhance the safety of packaged products. Antimicrobial packaging can kill or inhibit target micro-organisms (Han, 2000). Among many applications such as oxygen-scavenging packaging and moisture-control packaging, antimicrobial packaging is one of the most promising innovations of active packaging technologies. It can be constructed by using antimicrobial packaging materials and/or antimicrobial agents inside the package space or inside foods. Most food packaging systems consist of the food products, the headspace atmosphere and the packaging materials. Any one of these three components of food packaging systems could possess an antimicrobial element to increase antimicrobial efficiency.

Antimicrobial packaging research generally started with the development of antimicrobial packaging materials that contain antimicrobial chemicals in their macromolecular. However, without the use of alternative packaging materials, common packaging materials can be utilized for antimicrobial packaging systems there is antimicrobial activity in packaged foods or in the in-package atmosphere. Edible antimicrobial agents can be incorporated into food ingredients, while antimicrobial resources can interleaved in the in-package headspace in the form of sachets, film, sheets or any in-package supplements, to generate antimicrobial atmospheres.

Besides the use of antimicrobial packaging materials or antimicrobial inserts in the package headspace, gaseous agents have been used to inhibit the growth of microorganisms. Common gases are carbon dioxide for modified atmosphere packaging, sulfur dioxide for berries, and ethanol vapor for confections. These gases are injected into the package headspace or into palletized cases after shrink-wrapping of a unit load on a pallet. Vacuum, nitrogen-flushing and oxygen-scavenging packaging, which were originally designed for preventing the oxidation of packaged foods, also possess antifungal and antimicrobial properties against aerobic bacteria as

a secondary function, since these microorganisms are restrictively aerobic (Brody, 2001). However, these technologies, which control the low oxygen concentration to inhibit the growth of aerobic microorganisms, could cause the onset of anaerobic microbial growth. Controlling anaerobic bacteria in modified atmosphere packaging is a very important issue in maintaining the quality and safety of the products.

3.1 Shapes and compositions of antimicrobial packaging systems

Packaging is s system use to contain and protect enclosed products, which consists of a product, a package, and the in-package atmosphere. Antimicrobial agents may be incorporated in the non-food parts of the packaging system, which are the package or the in-package atmosphere. Antimicrobial agents can be incorporated directly in packaging materials in the form of films, over coating on the film, sheets, trays, and containers, or in the in-package space in the form of inserts, protecting the coated foods from microbial quality degradation (Han, 2000). Figure 1 illustrates the possible forms of antimicrobial packaging systems.

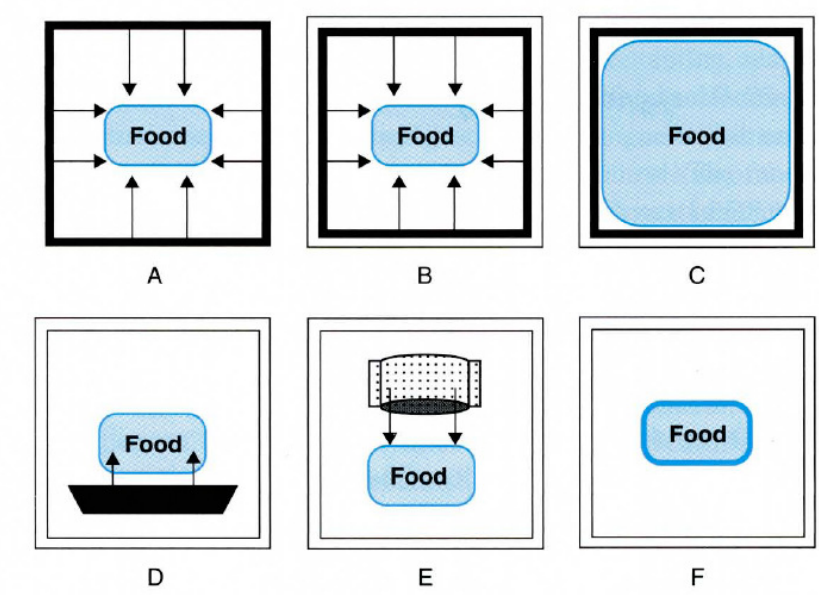


Figure 1. Possible ways to construct antimicrobial food packaging systems, the use of antimicrobial packaging materials (A) antimicrobial coating on conventional packaging materials (B) immobilization of antimicrobial agents in polymeric packaging materials (C) the use of antimicrobial trays or pads (D) the use of sachet/insert containing volatile antimicrobial agents (E) antimicrobial edible coating on foods (F) (adapted from Han, 2000).

3.2 Antimicrobial agents

Various antimicrobial agents could be incorporated into conventional food packaging systems and materials to create new antimicrobial packaging systems. Table 1 shows potential antimicrobial agents and food-grade preservatives. They can generally be classified into three groups; chemical agents, natural agents, and probiotics. For the purpose of food preservation, all packaging ingredients should be food-grade additives. The chemical agents can be mixed with food ingredients, incorporated into packaging additives or inserted into the headspace atmosphere. The antimicrobial agents are in contact with and consumed with the food products in these applications (Han, 2000).

Table 1. Examples of potential antimicrobial agents for antimicrobial food packaging systems.

Classification	Antimicrobial agents
Organic acids	Acetic acid, benzoic acid, lactic acid, citric acid, malic acid, probionic acid, sorbic acid, succinic acid, mixture of organic acids
Acid salts	Potassium sorbate, sodium benzoate
Alcohol	Ethanol
Bacteriocins	Nisin, pediocin, subtillin, lacticin
Fatty acids	Lauric acid, palmitoleic acid
Chelating agents	EDTA, citrate, lactoferrin
Enzymes	Lysozyme, glucose oxidase, Lactoperoxidase
Antioxidants	BHA, BHT, TBHQ, iron salts
Antibiotic	Natamycin
Sanitizing gas	Ozone, chlorine dioxide, carbon monoxide, carbon dioxide
Sanitizers	Cetyl pyridinium chloride, acidified NaCl
Polysaccharide	Chitosan
Phenolics	Catechin, cresol, hydroquinone
Plant volatiles	Allyl isothiocyanate, cinnamaldehyde, eugenol, linalool, terpineol, thymol, cavacrol, pinene
Plant/spice extracts	Grape seed extracts, grapefruit seed extracts, hot beta acid, Brassica erucic

Modified from Han (2000)

3.3 Controlled release technology of antimicrobial packaging

The design of an antimicrobial packaging system requires the balanced consideration of controlled release technology and microbial growth kinetics. When the mass transfer rate of an antimicrobial agent will be faster than the growth rate of the target microorganism, loaded antimicrobial agent will be diluted to less than the effective critical concentration before the expected storage period is complete, and the packaging system will lose its antimicrobial activity because the package food has almost infinite volume compared to the volume of packaging material and the amount of antimicrobial agent. Consequently, the microorganism will start to grow following depletion of the antimicrobial agent. On the contrary, when the migration rate is too slow to maintain the concentration above the minimum inhibitory concentration, the micro-organism can grow instantly, before the antimicrobial agent is released. Therefore, the release rate of the antimicrobial agent from the packaging material to the food must be controlled specifically to match the mass transfer rate with the growth kinetics of the target micro-organism. Controversially, in the case of antimicrobial edible coating systems the mass transfer of antimicrobial agents is not desirable, since the migration of the incorporated antimicrobial agents from the coating layer into the food product dilutes the concentration in the coating layer. Compared to the volume of the coating layer, the coated food has almost infinitive volume. Therefore, the migration will deplete the antimicrobial agent in the coating layer, decrease the concentration below the minimum inhibitory concentration, and thus reduce the antimicrobial activity of the coating system. The migration of incorporated antimicrobial agents contributed to antimicrobial effectiveness in the case of packaging systems; no migration is beneficial in the coating system.

The solubility of the antimicrobial agents in the foods is a critical factor of antimicrobial release. If the antimicrobial agent is highly soluble in the food, the migration profile will follow unconstrained free diffusion, while the very low solubility creates the dissolution-dependent monolithic system. For example, when highly soluble potassium sorbate was incorporated in packaging material (e.g. plastic films or papers) and the antimicrobial packaging materials were used for semi-solid or high-moisture foods, such as paste, yogurt, fruit jelly, soft cheese and sliced ham, the potassium sorbate dissolved in the food immediately after packaging. The potassium

sorbate concentration increased very fast on surface of the foods and the surface concentration decreased slowly as the potassium sorbate diffused into the food. Fast diffusion of the antimicrobial agents in the food decreased the surface concentration rapidly. The maintenance of the surface concentration is highly dependent on the release rate from the packaging materials (diffusivity of packaging materials) and the migration rate of the foods (diffusivity of the foods). Since the flux of the release from the packaging materials decrease as the amounts of antimicrobials in the packaging materials reduces with release time, the period when the surface concentration is maintained above the minimum inhibitory concentration is carefully estimated, considering its rapid decay profile (Figure 2) (Han, 200).

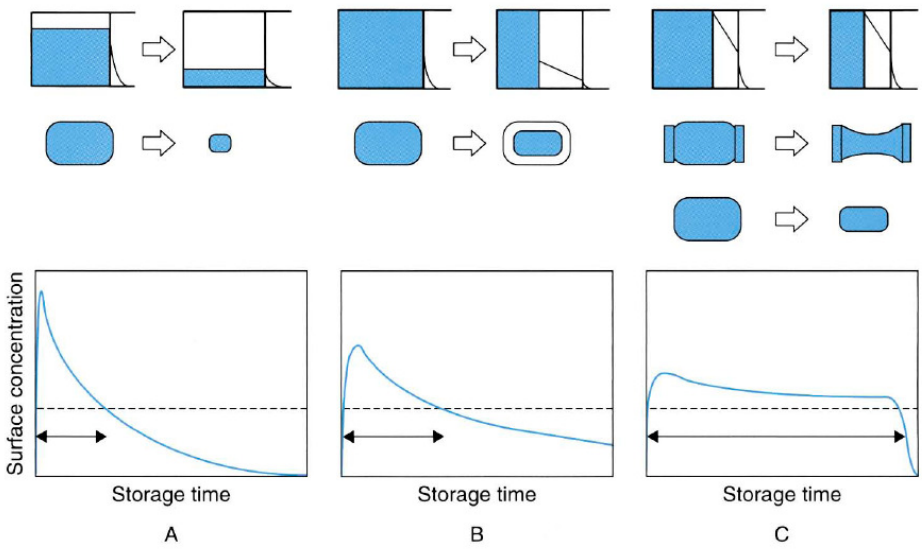


Figure 2. Release profiles of antimicrobial agents from various systems. Unconstrained free diffusion from packaging materials, or fast dissolution from antimicrobial tablets (A) slow diffusion of very low solubility agents from monolithic packaging materials (B) membrane (reservoir) systems with constant flux of permeation, slow dissolution from antimicrobial powder/tablets or gaseous agent release from concentrated antimicrobial sachets/tablets with constant volatility in a closed packaging system (C).

4. Plant extract

Antimicrobial agent can be extracted from various sources such as spices and their essential oil, food plants and antimicrobial peptide produced by bacteria (Beuchat and Doyle, 1995). Plant secondary metabolites, such as essential oils and plant extracts had antimicrobial activities and most essential oils derived from plants are known to possess insecticidal, antifungal, acaricidal, antibacterial and cytotoxic activities (Tepe *et al*., 2004). Therefore, they are intensely screened and applied in the fields of pharmacology, pharmaceutical botany, medical and clinical microbiology, phytopathology and food preservation The antimicrobial activities of the essential oils against the tested bacteria differed, dependind on location and seasonal variations (Wang *et al*., 2006). Kabuki *et al*. (2000) reported that mango seed kernel ethanol extract was composed of 79.5% polyphenol and had a broad spectrum antimicrobial activity. The extract was more active against gram-positive than gram-negative bacteria including some food borne pathogen. The essential oil of *Lippia orgiganoides* inhibits the growth of several microorganisms (bacteria; *S. aureus, L. casei, S. mutans* and fungi; *Candida spp., C. Neoformans*) (Kabuki *et al*., 2000). The inhibitory effect of several terpenoids on microbial oxygen uptake and oxidative phosphorylation has also been demonstrated (Kabuki *et al*., 2000).

Phenolic and non-phenolic alcohols in *L. origanoides* exhibited the strongest inhibitory effects, followed by aldehydes and ketones. The monoterpene hydrocarbons were less active and it has been suggested that this behavior depends on the free hydroxyl group from the alcohols. Wang *et al*. (2006) suggested that basil, clove, garlic, horseradish, marjoram, oregano, rosemary, and thyme exhibited antimicrobial activities. Those herbs can be practical for protecting seafood from the risk of contamination by *V. parahaemolyticus* (marine pathogenic bacteria) and also can be used in hurdle technology with low temperature. Moreover, these spices and herds, as well as essential oils have been well known to have inhibitory effects against a variety of bacteria including Gram-negative bacteria and *E. coli.* Carom seed, ginger, Japanese pepper, sage, spearmint, and turmeric were additionally found to exhibit antimicrobial activities against *V. parahaemolyticus.*

Eugenol, carvacrol and thymol are phenolic compounds in cinnamon, cloves, sage and oregano that present antimicrobial activity. The exact cause-effects relation for the mode of action of phenolic compounds has not been determined, But (Tepe *et al*., 2004b) indicated that they may inactivate essential enzymes, reacting with the cell membrane or disturbing material functionality. Main components of oregano extract, cavacrol and thymol, appear to make the cell membrane permeable, and are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Burt, 2004).

Benkeblia (2004) studied the effect of concentrations (50, 100, 200, 300 and 500 mL/L) of essential oil extracts from three type of onions (green, yell and red) and garlic against two bacteria, *S. aureus*, *S. enteritidis*, and three fungi, *A. niger*, *P. cyclopium* and *F. oxysporum*. The essential oil (EO) extracts of these *Allium* plants (garlic and onions) exhibited antibacterial activity, with garlic showing the highest inhibition and green onions the lowest. Comparatively, 50 and 100 mL/L concentrations of onions extracts were less inhibitory than 200, 300 and 500 mL/L concentrations. However, with garlic extract, high inhibitory activity was observed for all tested concentrations. *S. aureus* showed less sensitivity towards EO extracts inhibition, however *S. enteritidis* was strongly inhibited by red onion and garlic extracts. The fungus *F. oxysporum* showed the lowest sensitivity towards EO extracts, whereas *A. niger* and *P. cyclopium* were significantly inhibited particularly at low concentrations.

Oonmetta-aree *et al*. (2006) studied the antimicrobial activity of the Zingiberaceae family (galangal, ginger, tumeric and krachai) on *S. aureus* 209P and *E. coli* NIHJ JC-2 by using an agar disc diffusion assay. The galangal extract had the strongest inhibitory effect against *S. aureus*. The minimum inhibitory concentration (MIC) of the galangal extracts was 0.325 mg/mL and the minimum bactericidal concentration (MBC) at 1.3 mg/mL using the broth dilution method. Transmission electron microscopy clearly demonstrated that the galangal extract caused both outer and inner membrane damage, and cytoplasm coagulation. The disruption of the cytoplasmic membrane properties was determined by the releasing of cell materials including nucleic acid which absorbed UV/VIS spectrophotometer 260 nm. The major

compound of the extract was D,L-1'acetoxychavicol acetate which was identified by GC-MS and NMR.

Seydim and Sarikus (2006) reported that antimicrobial properties of spice extracts are well known. According to these researched, antimicrobial properties of whey protein isolate (WPI) films containing 1.0-4.0% (w/v) ratio of oregano, rosemary and garlic essential oils were tested against *E. coli* O157:H7 (ATCC 35218), *S. aureus* (ATCC 43300), *S. enteritidis* (ATCC 13076), *L. monocytogenes* (NCTC 2167) and *L. plantarum* (DSM 20174). Ten milliliters of molten hard agar was inoculated by 200 ul of bacteria cultures (colony count of 1×10^8 CFU/mL) grown overnight in appropriate medium. Circular discs of WPI films containing spice extracts, prepared by casting method, were placed on a bacterial lawn. Zones of inhibition were measured after an incubation period. The film containing oregano essential oil was the most effective against these bacteria at 2% level than those containing garlic and rosemary extracts ($p<0.05$).The use of rosemary essential oil incorporated into WPI films did not exhibit any antimicrobial activity whereas inhibitory effect of WPI film containing garlic essential oil observed only at 3% and 4% level ($p<0.05$). The results of this study suggested that the antimicrobial activity of some spice extracts were expressed in a WPI based edible film.

Rojas-Grau, *et al*. (2007) studied the antimicrobial properties of 0.1-0.5% suspensions of essential oils (EOs)/ oil compounds (OCs) against the food borne pathogen *E. coli* O157:H7 in alginate-apple puree edible film oregano oil/carvacrol; cinnamon oil/cinnamaldehyde; and lemongrass oil/citral. The results obtained demonstrate that carvacrol exhibited the strongest antimicrobial activity against *E. coli* O157:H7. The antimicrobial activities were in the following order: carvacrol>oregano oil> citral lemongrass oil> cinnamaldehyde> cinnamon oil.

5. Chitin and Chitosan

Chitin is the second most abundant naturally occurring biopolymer (after cellulose) and it found in the exoskeleton of crustaceans, in fungal cell walls and other biological materials. It is mainly poly (ß-(1-4)-2-acetamide-D-glucose), which is structurally identical to cellulose except that secondary hydroxyl on the second carbon atom of the hexose repeat unit is replaced by an acetamide group.

Chitosan is derived from chitin by deacetylation in the presence of alkali. Therefore, chitosan is a copolymer consisting of (ß-(1-4)-2-acetamido-D-glucose and (ß-(1-4)-2-acetamide-D-glucose units with the latter usually exceeding 80%. Chitosans are described in terms of the degree of deacetylation and average molecular weight and their important resides in their antimicrobial properties in conjunction with their cationicity and their-forming properties (Mi *et al*., 2006). Chitosan can form semi-permeable coatings, which can modify the internal atmosphere, thereby delaying ripening and decreasing transpiration rates in fruits and vegetables. Films from aqueous chitosan are clear, tough, flexible and good oxygen barriers, carbon dioxide permeability could be improved by methylation of polymer. Butler and his cooperators (Ouattara, 2000a) observed that films from chitosan were rather stable and mechanical and barrier properties changed only slightly during storage.

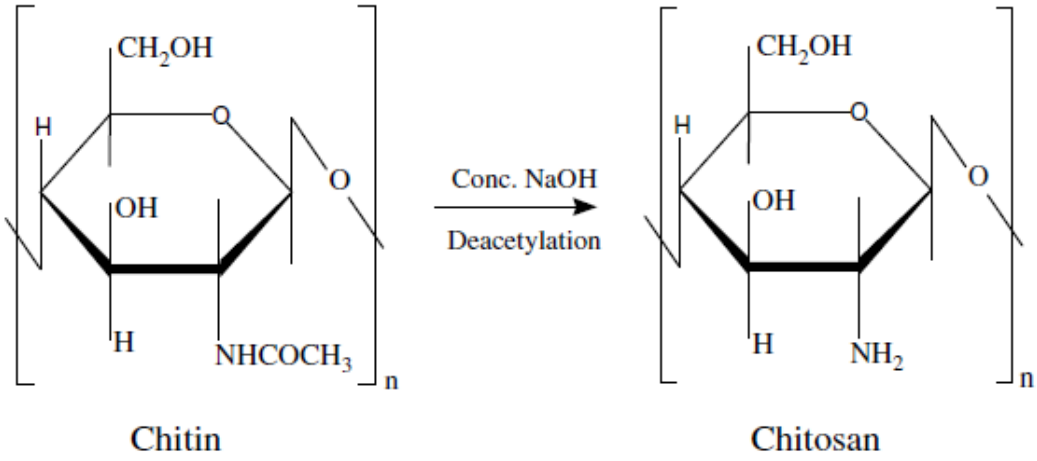


Figure 3. Chitin and Chitosan
Source: Rabea *et al*. (2003)

The positive charge on the C2 of the glucosamine monomer below pH 6, chitosan is more soluble and has a better antimicrobial activity than chitin. The exact mechanism of the antimicrobial action of chitin, chitosan and their derivatives is still imperfectly known, but different mechanisms have been proposed (Rabea *et al*., 2003). One of the reasons for the antimicrobial character of chitosan is its positively charged amino group which interacts with negatively charged. Microbial cell membranes, leading to the leakage of proteinaceous and other intracellular

constituents of the microorganisms (Shahidi *et al*., 1999). Chitosan acted mainly on the outer surface of bacteria. The antimicrobial activity of chitosan was observed against a wide variety of microorganisms including fungi, and some bacteria. The antimicrobial action is influenced by intrinsic factors such as the type of chitosan, the degree of chitosan polymerization, the host, the natural nutrient constituency, the chemical or nutrient composition of the substrates or both, and the environmental conditions (e.g., substrate water activity or moisture or both). The development of complementary methods to inhibit the growth of pathogenic bacteria such as packaging material-associated antimicrobial agents is an active area of research. There has been increasing interest in antimicrobial characteristics of films made from chitosan have been carried out earlier. Among other polymers, chitosan has received a significant attention as antimicrobial film-forming agent for food preservation to the researchers due to its biodegradability, biocompatibility, cytotoxicity, and antimicrobial activity. Chitosan films are easily prepared by evaporation of its dilute acid solutions.

The antimicrobial activity of chitosan was observed against a wide variety of microorganisms including fungi, and some bacteria. The antimicrobial action is influenced by intrinsic factors such as the type of chitosan, the degree of chitosan polymerization, the host, the natural nutrient constituency, the chemical or nutrient composition of the substrates or both, and the environmental conditions (e.g., substrate water activity or moisture or both). The development of complementary methods to inhibit the growth of pathogenic bacteria such as packaging material-associated antimicrobial agents is an active area of research. There has been increasing interest in antimicrobial edible packaging materials. A number of studies on the antimicrobial characteristics of films made from chitosan have been carried out earlier (Shahidi *et al*., 1999)

Chitosan has a great potential for a wide range of applications due to its biodegradability, biocompatibility, antimicrobial activity, non-toxicity and versatile chemical and physical properties. Thus, chitosan based films have proven to be very effective in food preservation. The presence of amino group in C2 position of chitosan provides major functionality towards biotechnological needs, particularly, in food applications. Chitosan based polymeric materials can be formed into fibers,

films, gels, sponges, beads or even nanoparticles. Chitosan films have shown potential to be used as a packaging material for the quality preservation of a variety of food. Besides, chitosan has widely been used in antimicrobial films to provide edible protective coating, in dipping and spraying for the food products due to its antimicrobial properties. Chitosan has exhibited high antimicrobial activity against a wide variety of pathogenic and spoilage microorganisms, including fungi, and Gram-positive and Gram-negative bacteria. The present review aims to highlight various preparative methods and antimicrobial activity including the mechanism of the antimicrobial action of chitosan based films. The optimisation of the biocidic properties of these so called biocomposites films and role of biocatalysts in improvement of quality and shelf life of foods has been discussed.

Fan *et al*. (2008) studied the effects of chitosan coating on quality and shelf lives of silver carp during frozen storage. Fish sample were treated with aqueous solution of 2% chitosan, and then stored at -3°C for 30 days. The control and the treated fish samples were analyzed periodically for microbiological (total viable count), chemical (pH, TBA. TVB,-N, K-value), and sensory characteristics. The results indicated that the effect of chitosan coating on fish samples was retained their good quality characteristics and extend the shelf life during frozen storage, which was supported by the results of microbiological, chemical, and sensory evaluation analyses.

Dutta *et al*. (2008) reported that chitosan has a great potential for a wide range of applications due to its biodegradability, biocompatibility, antimicrobial activity, non-toxicity and versatile chemical and physical properties. Thus, chitosan based films have proven to be very effective in food preservation. The presence of amino group in C2 position of chitosan provides major functionality towards biotechnological needs, particularly, in food applications. Chitosan based polymeric materials can be formed into fibers, films, gels, sponges, beads or even nanoparticles. Chitosan films have shown potential to be used as a packaging material for the quality preservation of a variety of food. Besides, chitosan has widely been used in antimicrobial films to provide edible protective coating, in dipping and spraying for the food products due to its antimicrobial properties. Chitosan has exhibited high

antimicrobial activity against a wide variety of pathogenic and spoilage microorganisms, including fungi, and Gram-positive and Gram-negative bacteria.

Mathew and Abraham, 2008 studied the effect of water soluble chitosan on the survival of *E. coli* and *S. aureus* inoculate on seabass slices stored at 4°C. Water-soluble chitosan pretreatment (1000 ppm) on seabass slices showed the antimicrobial effect on microbiological inhibition as evidenced by the lowered TVC and LAB count, compared with control samples. Microbiological changes of seabass slices inoculated with levels of *E. coli* or *S. aureus* (10^4 CFU/g) were monitored during storage. Chitosan pretreatment reduced colony count of *S. aureus* and extended the lag phase of *E. coli*. Therefore, water soluble chitosan pretreatment not only retarded microbiological deterioration of seebass slices but also reduced or inactivated some pathogenic bacteria to some extent.

6. Kiam (*Cotylelobium lanceolatum* craih.)

Kiam (*Cotylelobium lanceolatum* craih.) is a species of plant in the *Dipterocarpaceae* family. The name is derived from Latin (*lanceolatus* = in the form of a lance) and refers to the shape of the leaf blade. Kiam is a canopy tree, up to 45 m, found in kerangas forests on raised beach terraces and sandstone plateau and on organic soils over limestone. The species is found in Peninsular Thailand, eastern coastal areas of Peninsular Malaysia. Kiam are rich in polyphenolic compounds in herbaceous and woody plants are known to have antimicrobial activity (Scalbert, 1991). Pieces of wood from the Kiam tree (Figure 4) have been traditionally submerged in sugar palm sap in Thailand to prevent or retard microbial fermentation.

Figure 4. Illustrative picture of Kiam wood
Source: Scalbert (1991)

7. Phayom (*Shorea talura* Roxb.)

Phayom is the plant in the *Dipterocarpaceae* family. The science word calls *Shorea talura* Roxb. Phayom spreads in the west of India, Burma, Thailand, Indo-China Peninsular, and Malaysia. We can find it in every part of Thailand which are the forest both dry and moist and tropical forest that high from sea-level 60-1,200 m. It is perennial plant and appear medium to big size, height 15-40 m. Phayom is also the shed leaves plant, has round shape at the top, compact rind brown or gray color. It's rind crack around trunk and compact chips. The rind inside has soft brown mix to yellow color and past by the dark brown line. Trunk circle line is nearly 300 cm.

Characteristics of Phayom leafs, narrow parallel rim wind 3.5-4 cm. and length 8-10 cm, base and tail are curve as short outgrowth. Flesh of leaf is quite dense, glossy smooth. On leafs are including line between 15-20 couples, curve to the rim and small, model same as rung can see wood at the middle of leafs. It rim is bend and curve, stem length 2.0-2.5 cm.

Characteristics of blossom, it have white or mix with yellow color, very fragrance, big bouquet at the tail of bough or above leaf track. The base petal have five it lean but not stuck together. Corolla have five also, it is plait and revolve follow clock hands. Petal under corolla is smooth and dark color, indent at rim,

including 15 stamens, stem length 1.5 cm. Inside oblong ovary divide to 3 holes, each hole have 2 tender eggs (Scalbert, 1991).

Characteristics of yield, it model like a tip shuttle size 1.2×2.0 cm. hide in the bulge, wing base including narrow wings, each one have 10 line, long 3 wings size 1×8 cm and 2 short wings size 3 cm. Period to blossom between December to February, it will shed leafs finished before sprout blossom. Give yields between January to March, wood texture have soft yellow. If abandon it for a long time will become to brown color and always past by dark line that are oil tube or gum, short thorn, rough texture, solid and gummy. It is easy to arrange, plane and saw. Specific gravity around 0.77, solid of wood texture around 643 kg. , strong is around 748 kg. /square cm, natural durable since 1.8 to 20.8 years, average 11.9 years. Steep into to the medicate water is so difficult. Phayom can adjust to side with environment very well.

Benefits from Phayom are as the following;

1. Wood texture use in any constructions, if steep it into medicate water will increase durable. It is accepted to make railway sleeper, pole, beam, block, making dugout, building a boat that can endure the barnacle, making oar, paddle, pole, mast, agriculture tools, mortar, pestle, lever, rollers used in the sugar-cane mill, building component of cart, coach-work , and making wood tile.

2. Benefits about herb; boil wood textiled and rind with water for drink can solve diarrhea and bowel inflamed, blossom; can mix to be heart tonic, nourish heart and decrease fever.

3. Other benefits of rind or splinter; put in fermentation or cylinder that contain sugar use to tan leather and may eat with betel instead areca, and mix with oil to smear wood or caulk up a boat (Scalbert, 1991).

Figure 5. Illustrative picture of Phayom tree
Source: Scalbert (1991)

8. Phenolic compounds

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Scalbert, 1991).

Phenolic compounds composed of one or more aromatic benzene rings with one or more hydroxyl groups (C-OH). This enormous class includes numerous plant compounds that are chemically distinct from terpenes. Although the essential oils are often classified as terpenes, many of these volatile chemicals are actually phenolic compounds, such as eucalyptol from (Eucalytus globulus), citronellal from and clove oil from Syzygium aromaticum. Like the terpenes, many phenolic compounds are attached to sugar molecules and are called glucosides or glycosides,

depending on the type of sugar. Most vanilla flavorings sold in markets are synthetic vanillin containing artificial food coloring and preservatives. Vanillin is a single-ring phenolic compound derived from the breakdown of lignin, a complex phenolic polymer that gives seasoned wood its color, hardness and mass. Natural vanilla flavoring also comes from vanillin plus several other aromatic compounds in the seed capsules of the vanilla orchid (Vanilla fragrans). The double-ring phenolic compounds called coumarin imparts the distinctive sweet smell to newly-mown hay. Coumarin is also an anticoagulant that represses the synthesis of prothrombin, a plasma protein produced in the liver in the presence of vitamin K. Prothrombin is the precursor of the enzyme thrombin which catalyzes the conversion of fibrinogen to fibrin in the clotting process. Threads of fibrin wind around blood platelets in the damaged area of a blood vessel and provide the framework of a blood clot. Coumarin is converted into the anticoagulant dicoumarin during the improper curing of sweet clover hay from species of Melilotus. Hemorrhaging and death may occur in cattle that eat spoiled sweet clover hay, depending on the amount consumed. Dicoumarin and related drugs are used in human medicines as blood thinners and are commonly used in rodent poisons such as Decone, which literally cause rats to bleed to death.

Lignin is a valuable phenolic polymer that gives wood its characteristic brown color, density and mass. It has been estimated that 40 percent of the weight of the world's forests is lignin! Lumber is essentially composed of dead xylem cells that have dried out. The dead tissue is hard and dense because of lignin in the thickened secondary cell walls. In order to make paper, logs and wood chips must be converted into pulp. Several methods are used to convert wood into pulp, including the ground wood process, sulfite process and the sulfate process. In addition to chemically digesting the wood until it is reduced to its component fibers, the lignin must also be removed in fine quality papers. Cardboard containers and supermarket shopping bags (kraft paper) are stiff and brown because they still contain lignin.

Flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease. Ingestion of alcohol-free red wine or a phenolic compound mixture extracted from red wine has been shown to improve the antioxidant status of plasma in humans. Consumption of controlled diets high in fruits and vegetables increased significantly the antioxidant

capacity of plasma, and the increase could not be explained by the increase in the plasma R-tocopherol or carotenoid concentration. Moreover, epidemiological studies have found that there is a significant negative association between the intake of fruits and vegetables and heart disease mortality (Compos *et al*., 2003).

Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs. Flavonoids and other plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks (Campos *et al*., 2003). They are important in the plant for normal growth development and defense against infection and injury. Flavonoids also partly provide plant colors present in flowers, fruits, and leaves. They generally occur as glycosylated derivates in plants, although conjugation with inorganic sulfate or organic acid as well as malonylation are also known (Wang and Weller, 2006). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Campos *et al*., 2003).

Berries and fruits contain a wide range of flavonoids and phenolic acids that show antioxidant activity. Main flavonoid subgroups in berries and fruits are anthocyanins, proanthocyanins, flavonols, and catechins. Phenolic acids present in berries and fruits are hydroxylated derivates of benzoic acid and cinnamic acid. Meikleham and Pizzi, 1994 studies on antioxidative activities of fruit extracts have been focused mainly on grapes, which have been reported to inhibit oxidation of human lowdensity lipoprotein (LDL) at a level comparable to wine. Fresh strawberry extract was reported to have 15 times higher total antioxidant capacity than trolox in an artificial peroxyl radical model system (Wang and Weller, 2006). Extracts of blackberries, black and red currants, blueberries, and black and red raspberries possessed a remarkably high scavenging activity toward chemically generated superoxide radicals (Wang and Weller, 2006). Hydroxycinnamic acids typically present in fruits have been shown to inhibit LDL oxidation in vitro. Also phenolic extracts of berries (blackberries, red raspberries, sweet cherries, blueberries, and

strawberries) were shown to inhibit human low-density lipoprotein (LDL) and liposome oxidation (Meikleham and Pizzi, 1994).

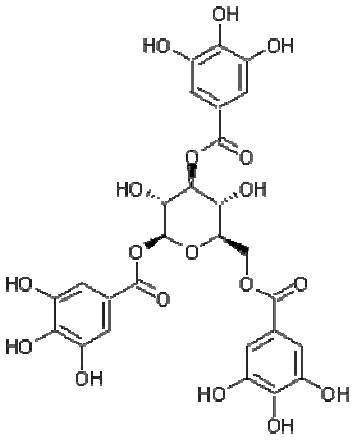
The antioxidant capacities (oxygen radical absorbance capacity, ORAC) and total phenolic contents in extracts of 27 culinary herbs and 12 medicinal herbs were determined. The ORAC values and total phenolic contents for the medicinal herbs ranged from 1.88 to 22.30 micromol of Trolox equivalents (TE)/g of fresh weight and 0.23 to 2.85 mg of gallic acid equivalents (GAE)/g of fresh weight, respectively. *Origanum. x majoricum*, *O. vulgare ssp. hirtum,* and *Poliomintha longiflora* have higher ORAC and phenolic contents as compared to other culinary herbs. The ORAC values and total phenolic content for the culinary herbs ranged from 2.35 to 92.18 micromol of TE/g of fresh weight and 0.26 to 17.51 mg of GAE/g of fresh weight, respectively. These also were much higher than values found in the medicinal herbs. The medicinal herbs with the highest ORAC values were *Catharanthus roseus, Thymus vulgaris, Hypericum perforatum,* and *Artemisia annua.* A linear relationship existed between ORAC values and total phenolic contents of the medicinal herbs (R = 0.919) and culinary herbs (R = 0.986). High-performance liquid chromatography (HPLC) coupled with diode-array detection was used to identify and quantify the phenolic compounds in selected herbs. Among the identified phenolic compounds, rosmarinic acid was the predominant phenolic compound in *Salvia officinalis, Thymus vulgaris, Origanum x majoricum,* and *P. longiflora,* whereas quercetin-3-O-rhamnosyl-(1 --> 2)-rhamnosyl-(1 --> 6)-glucoside and kaempferol-3-O-rhamnosyl-(1 --> 2)-rhamnosyl-(1 --> 6)-glucoside were predominant phenolic compounds in *Ginkgo biloba* leaves (Wang and Weller, 2006).

The antioxidative activity of a total of 92 phenolic extracts from edible and nonedible plant materials (berries, fruits, vegetables, herbs, cereals, tree materials, plant sprouts, and seeds) was examined by autoxidation of methyl linoleate. The content of total phenolics in the extracts was determined spectrometrically according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalents (GAE). Among edible plant materials, remarkable high antioxidant activity and high total phenolic content (GAE > 20 mg/g) were found in berries, especially aronia and crowberry. Apple extracts showed also strong antioxidant activity even though the total phenolic contents were low (GAE < 12.1 mg/g). Among nonedible plant

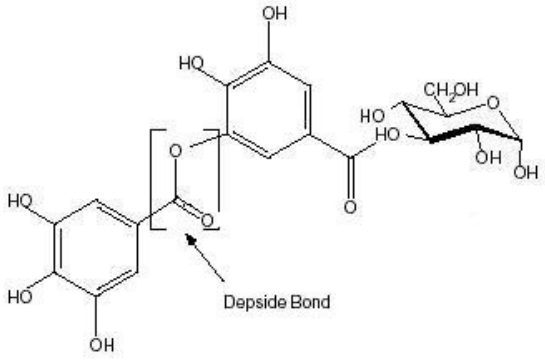
materials, high activities were found in tree materials, especially in willow bark, spruce needles, pine bark and cork, and birch phloem, and in some medicinal plants including heather, bog-rosemary, willow herb, and meadowsweet. In addition, potato peel and beetroot peel extracts showed strong antioxidant effects. To utilize these significant sources of natural antioxidants, further characterization of the phenolic composition is needed (Compos *et al*., 2003).

9. Tannin

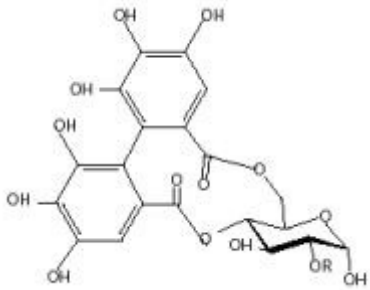
Tannins are high molecular weight polyphenols that can be found in different parts of plants and trees such as seeds, fruits, roots and barks. As tannins contain an abundant amount of adjacent hydroxyl groups in their molecules, they form chelates with metals. They are water soluble and must be immobilized when using them as sorbents. Tannins are divided into two classes of polymers, the hydrolysable tannins and the condensed tannins. Condensed tannins also known as polyflavonoids or proanthocyanidins comprise a group of polyhydroxyflavon-3-ol oligomers and polymers linked by carbon-carbon bonds between flavanol subunits. Quebracho is a condensed tannin that has a polymeric structure containing the flavanoid units. Quebracho is mainly based on combinations of resorcinol, catechol and pyrogallol building blocks. Tannins, natural biomass containing multiple adjacent hydroxyl groups and exhibiting specific affinity to metal ions, can probably be used as alternative, effective and efficient adsorbents for the recovery of metal ions. During the last years, the interest on biomaterials and specifically in tannins was growing. Tannins are an important class of secondary plant metabolites, water-soluble polyphenolic compounds of molecularweight ranged between 500 and some thousands Daltons. There are three kinds: hydrolyzable, condensed and complex tannins. However, tannins are water-soluble compounds, thus when they are used directly as an adsorbent for recovery of metals from aqueous systems, they have the disadvantage of being leached by water. To overcome this disadvantage, attempts have been made to immobilize tannins onto various water-insoluble matrices (Animut, 2007).



Tannin ($C_{27}H_{24}O_{18}$)



Gallotannin hydrolysable



Eallagitannin hydrolysable

Figure 6. Structure of tannin
Source: Swain (1979)

10. Application of Kiam and Phayom woods extracts as antimicrobial and food applications

Chanthachum and Beuchat (1997) studied the effect of water extracts from Kiam (*Cotylelobium lanceotatum* craih.) wood on inhibitory and lethal activity against pathogenic bacteria and microorganisms isolated from sugar palm sap. Bacteria representing six genera *Acetobacter*, *Flavobacterium*, *Lactobacillus*, *Leuconostoc*, *Micrococcus* and *Saccharomyces* isolated from sap were inhibited on agar media when 0.2% (w/v) water extracts of Kiam wood saw dust was applied. Extracts could inhibit the growth of *L. monocytogenes,* toxigenic, *S. aureus* and *B.*

cereus but not *Salmonella*. The behavior of *L. monocytogenes* inoculated onto shredded raw cabbage which was then treated with 0, 0.5 or 5% solutions of wood Kiam extracts and subsequently stored at 5°C for up to 5 days was also determined. Populations of aerobic mesophiles and psychrotrophic microorganisms were monitored. Significantly ($p<0.05$) reduced populations of *L. monocytogenes* were detected in cabbage treated with 0.5 or 5.0% Kiam wood extracts. Treatment with Kiam wood extracts retarded the rate of growth of aerobic mesophiles and psychrotrophic microorganisms naturally present on cabbage. Treatment with 5.0% Kiam extracts had a sustained lethal effect during a 5 days test period.

Faparusi and Bassir (1972) reported the phenolic compounds in woody plants were antimicrobial activity. The *Saccoglottis gabonensis* extracts in bark inhibited with bacteria growth completely ($p>0.05$). The *Saccoglottis gabonensis* and *Shorea disticha*, were found to have antimicrobial properties and employed to inhibit growth of microflora of palm wine and retarded the tendency of the wine to become sour. When tested against yeast to find did not exhibit antimicrobial activity. The phenolic compounds in herbaceous and woody plants have antimicrobial activity. Pieces of wood have been traditionally submerged in sugar palm sap to prevent or retard microbial fermentation. The chemical compounds of the bark responsible for inhibition of microbial growth were reported to be isocoumarin (lactone) and (distichol), respectively.

Thaweerut (1992) studied the potential application of water extracts of *Cotylelobium lanceotatum* (Kiam) and *Shorea talura* (Phayom) on pork quality by spraying. The results showed that the concentration of Kiam and Phayom wood extracts affected to the antioxidant in pork. Increasing concentration of Kiam and Phayom wood extracts reduce the oxidation. When amount of Kiam and Phayom wood extract increase to affected with the flavor of pork.

Thannajetsada (1993) studied the effect of concentrations (100, 50 and 25 g/L) of Kiam and Phayom wood extracts on antimicrobial activity of five microorganisms, *E. coli, Bacillus cereus*, *S. aureus, Saccharomyces cerevisiae* and *Aspergillus niger*. Both Kiam and Phayom wood extracts as 100 50 and 25 g/L affective with *B. cereus* than *S. cerevisiae*, *S. aureus* and *E. coli*. However Kiam wood extracts were inhibit the microorganism than Phayom wood extract as similar

concentration. As 100 g/L concentration of Kiam and Phayom wood extracts were less inhibitory of *A.niger*.

Interapichet and Nukuew (1994) reported the water extracts of *Cotylelobium Lanceotatum* (Kiam) and *Shorea talura* (Phayom) wood on pork and beef cut surfaces. The sprayed meats and control were placed on foam trays and wrapped with polyethylene cling wrap. Meat samples were kept 4°C for a period of 10 days. Aerobic plate counts of the control and treated meat were significantly different ($p<0.05$). Both Kiam and Phayom extracts had relatively similar antimicrobial activities. The maximum reductions in microbial loads were about 1.35 and 1.24 log cycles for pork treated with Kiam and Phayom extracts at 7 days, respectively and about 1.15 and 1.21 log cycles for beef treated with Kiam and Phayom extracts at days 4 and 3, respectively. Development of off-odors was observed. Control samples of both pork and beef became sour after 48 h while treated meat cuts did not display any sign of spoilage till day 5. Strong off-odors were detected at day 7 and were described as sour and ammoniacal odors. In addition, sulfide odors were observed from control meats in the last two days of storage.

OBJECTIVES

1. To prepare antimicrobial edible films from Kiam and Phayom wood extracts.
2. To study the effect of concentration of Kiam and Phayom wood extracts on antimicrobial properties and property of edible HPMC films.
3. To study the effect of the ratio between Kiam and Phayom wood extracts on antimicrobial and property of edible HPMC films.
4. To enhance antimicrobial activity of chitosan films by incorporating Kiam and Phayom wood extracts.
5. To study the potential application on antimicrobial films in food products.

CHAPTER 2

RESEARCH METHODOLOGY

1. Materials

1.1 Raw materials

Kiam (*Cotylelobium lanceotatum*) and Phayom (*Shorea tolura)* woods were obtained from local community in Songkhla province. Commercial Hydroxypropyl methylcellulose (HPMC) with average molecular weight of 162.14 kDa was purchased from High Science Co. Ltd. (Thailand). Comercial grade chitosan flake approx 85% degree of deacetylation with average molecular weight of 75 kDa was purchased from Bona Fides Marketing Co. Ltd. (Thailand). Commercial grade sorbitol was obtained from Vidyasom Co. Ltd. (Thailand). *E. coli, S. aureus* and *L. monocytogenes* were obtained from Food Safety Lab, Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat-Yai, Songkhla. Cultures were streak- plated once a week and cultures for experiments were inoculated into media from a single colony and incubated overnight under the appropriate medias and atmospheric conditions.

1.2 Chemical reagents

- Trypticase Soy Broth from High Science Co. Ltd. Thailand)
- Plate Count Agar (PCA) from High Science Co. Ltd. (Thailand)
- Trypticase Soy Agar (TSA) from High Science Co. Ltd. (Thailand)
- Sodium Chloride (NaCl) from Labscan Asia Co. Ltd.
- Lauryl tryptose (LST) broth from High Science Co. Ltd. (Thailand)
- Brilliant Green Lactose Bile Broth from High Science Co. Ltd. (Thailand)
- Ethanol 95% from Himedia Lab. Pvt. Ltd.
- Sodium benzoate Vidyasom Co. Ltd. (Thailand)
- Trichloroacetic acid (TCA)
- Thiobarbituric acid (TBA)
- Hydrochloric acid (HCl)

2. Instruments

Instruments	Model	Company/City/Country
Magnetic stirrer	RO 10 power	IKA LABORTECHNIK, Stanfen, Germany
stirrer	RW 20n	IKA LABORTECHNIK, Stanfen, Germany
Homogenizer	T 25	Ultra Turrax, Malaysia
Water bath	W 350	Memmert, Schwabach, Germany
Scanning Electron Microscope	JSM-5800 LV	JEOL, Tokyo, Japan
Universal testing machine	LR 30K	Lloyd Instruments Ltd, Hampshire, UK
Spectrophotometer	UV-1601	Shimadzu, Kyoto, Japan
Differential scanning calorimeters	Perkin-Elmer DSC-7	Norwalk, Conn., U.S.
IR spectrometer	Equinox 55	Bruker, Japan
Hunter associates laborator	Inc., VA.	USA

3. Preparation of Kiam and Phayom Wood Extracts

Kiam (*Cotylelobium lanceotatum*) and Phayom (*Shorea tolura)* woods were chopped in small pieces. The pieces of Kiam and Phayom wood was mixed with water in a proportion of 10 g:100 mL and the extraction was performed by continuous stirring in a water bath at 50^{o}C for 24 h, according to the optimized method described by Chanthachum and Beuchat (1997). The extract thus obtained was filtered through Whatman no. 1 filter paper and dried by using hot air oven at 50ºC for 24 h, ground and placed in bottle and at 4ºC until needed.

3.1 Preparation of inoculums

E. coli, *S. aureus* and *L. monocytogenes* were cultured into 5 mL of Tryticase Soy Broth (TSB), and incubated in a shaker incubator at 35ºC for 18-24 h.

The optical density (OD) of the bacteria was adjusted to the standard of McFarland NO. 0.5 with 0.85-0.9 g sodium chloride/100 mL sterile solution to achieve a concentration of approximately 10^8 CFU/mL. The final concentration of the cell number of approximately 10^5-10^6 CFU/mL was obtained by diluting 100-1000 times with sterile sodium chloride solution.

3.2 Minimum bactericidal concentration (MBC)

Minimum bactericidal concentration (MBC) from Kiam and Phayom wood extracts inhibiting the growth of *E. coli*, *S. aureus* and *L. monocytogenes* were analyzed by modification of the method used by Canillac and Mourey (2001). The Kiam and Phayom wood extract was diluted in to 100, 200, 300, 400 and 500 mg/L, in trypticase soy broth (TSB). The medium was inoculated with 0.1 mL of a pre-culture in Trypticase Soy Agar (TSA) at 37ºC. The cells of the inoculum were in exponential growth phase after 2-3 h of static incubation or in stationary phase after 17-19 h of static incubation. The final concentration of bacteria, determined with plate count method was of the order of 10^5 CFU/mL. The MBC is the lowest concentration of Kiam and Phayom wood extract which no growth was detected after 48 h at 37ºC.

4. Preparation of antibacterial edible HPMC films

Edible films were prepared by modification of the method used by Pranoto *et al*. (2005). Hydroxypropyl methylcellulose (HPMC) 1% was dissolved into 100 mL of distilled water and concurrently shaking in rotary shaker. The HPMC film was brittle, 40% of sorbitol was added into the edible films solution. Subsequently, the Kiam and Phayom wood powder at 1, 2, 3, 4 and 5 folds of MBC was added and stirred for 5 min. After mixing, the mixture was degassed under vacuum and cast onto flat, leveled non-stick trays to set. Once set, the trays were held at 50^{o}C for 10 h undisturbed, and then cooled to ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60% RH for further testing. All treatments were made in triplicate.

5. Antimicrobial activity of edible HPMC films

Antimicrobial activity testing of the edible HPMC films according to Pranoto *et al*. (2005). The edible HPMC films were cut 16 mm diameter discs and treated with UV-light for 2 h, then placed on Mueller Hinton agar plates, with had been previously seeded with 0.1 mL of inoculums containing approximately 10^5-10^6 CFU/mL of tested bacteria. The plates were then incubated at 37ºC for 24 h. Observation on the diameter of the inhibitory zone surrounding film discs and contact area of edible HPMC films with agar surface were measured. Experiments were done in triplicate.

6. Determination of edible HPMC film properties

6.1 Conditioning

All films were conditioned prior to subjecting them to permeability and mechanical tests according to the Standard method, D618-61 (ASTM, 1993a). Films used for testing water vapor permeability (WVP), tensile strength (TS), and elongation at break (ε) were conditioned at 60% RH and 27±2^{o}C by placing them in desiccators over a saturated solution of $Mg(NO_3)_26H_2O$ for 72 h or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

6.2 Film thickness

The thickness of the films was measured with a precision digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan) to the nearest 0.0001 mm (±5%) at five random locations on the film. Mean thickness values for each sample were calculated and used in water vapor permeability (WVP) and tensile strength (TS) calculations.

6.3 Film solubility

A modified method from Jangchud and Chinnan (1999) was used to measure film solubility. Film pieces, 20 mm x 20 mm, were dried at 70^{o}C in a vacuum oven for 24 h and then weighed to the nearest 0.0001 g for the initial dry mass. Films were immersed into 20 mL of distilled water in 50 mL screw cap tubes

containing 0.01 g/100 g sodium benzoate. The tubes were capped and placed in a shaking water bath for 24 h at 25±2°C. A portion of the solution was removed and set aside for later use in protein solubility tests. The remaining solution and film piece was poured onto (Whatman no. 1) qualitative filter paper, rinsed with 10 mL distilled water, and dried at 70°C in a vacuum oven for 24 h to determine the dry mass of the films. Five measurements were taken for each treatment. Total soluble matter was calculated from the initial gross mass and the final dry mass using the following equation:

$$\% \text{ FS (db)} = \frac{(\text{film mass before test - film mass after test})}{\text{film mass before test}} \times 100\%$$

6.4 Water vapor permeability (WVP)

The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh, 1993) was used to determine the WVP of films. The test cups were filled with 20 g of Silica gel (desiccant) to produce a 0% RH below the film. A sample was placed in between the cup and the ring cover of each cup coated with silicone sealant (high vacuum grease, Lithelin, Hannau, Germany) and held with four screws around the cup's circumference. The air gap was at approximately 1.5 cm between the films surface and desiccant. The water vapor transmission rate (WVTR) of each film was measured at 60±2% RH and 25±2°C. After taking the initial weight of the test cup, it was placed into a growth chamber with an air velocity rate of 125 m/min (Model KBF115, Contherm Scientific, Lower Hutt, New Zealand). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Sartorious Corp.) every 3 h for 18 h. A plot of weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time (g/h). The WVTR was expressed in gram units, per square meter, per day. Steady state over time (slope) yielded a regression coefficient of 0.99 or greater. Six samples per treatment were tested. The WVP of film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the films.

6.5 Mechanical properties

Tensile strength (TS) was measured with a LLOYD Instrument (Model LR30K, LLOYD Instruments Ltd., Hampshire, England) as per ASTM D882-91 Standard Method (ASTM, 1993b). Ten samples, 2.54 cm x 12 cm, were cut from each films. Initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum force by the initial specimen cross-sectional area, and the percent elongation at break (ε) was calculated as follows:

$$E = 100 \text{ X } (d_{\text{after}} - d_{\text{before}})/d_{\text{before}}$$

Where, d was the distance between grips holding the specimen before or after the break of the specimen.

6.6 Color

A CIE colorimeter (Hunter associates laboratory, Inc., VA. USA) was used to determine the film L*, a*, and b* color value [(L* = 0 (black) to 100 (white); a* = -60 (green) to +60 (red); and b* = -60 (blue) to +60 (yellow)]. The standard plate (calibration plate CX0384, L*= 92.82, a*= -1.24, and b*= 0.5) was used as a standard. Color (means of five measurements at different locations on each specimen) was measured on 10 cm x 10 cm segment of film. Total color difference (ΔE^*_{ab}), hue angle (H), and chroma (C) were calculated using the following equation:

$$\Delta L^* = L^*_{\text{sample}} - L^*_{\text{standard}},\ \Delta a^* = a^*_{\text{sample}} - a^*_{\text{standard}},\ \Delta b^* = b^*_{\text{sample}} - b^*_{\text{standard}}$$

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$$

$$C = [(a^*)^2 + (b^*)^2]^{0.5}$$

$$H = \tan^{-1}(b^*/a^*) \text{ when } a^* > 0 \text{ and } b^* > 0$$

$$H = 180^o + \tan^{-1}(b^*/a^*) \text{ when } a^* < 0$$

$$H = 360^o + \tan^{-1}(b^*/a^*) \text{ when } a^* > 0 \text{ and } b^* < 0$$

Prior to taking color measurements, film specimens were pre-conditioned at 60% RH and 27±2°C for 72 h.

6.7 Transparency

The transparency of films was determined using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The film samples were cut into rectangles and placed on the internal side of the spectrophotometer cell. The transmittance of films was determined at 600 nm as described by Han and Floros (1997). The transparency of the films was calculated as follows:

$$\text{Transparency} = -\log (T_{600}/x)$$

Where T_{600} is the transmittance at 600 nm and x is the film thickness (mm).

6.8 Scanning Electron Microscope (SEM)

Microstructure of the film samples was determined using Scanning Electron Microscopy (SEM). Film samples were examined for surface characteristics using JEOL JSM-5800 LV scanning electron microscope (SEM) (JOEL Ltd., Tokyo, Japan) operated at 10 kV. Five samples were mounted on a bronze stub and sputter-coated (Sputter coater SPI-Module, PA, USA) with a layer of gold prior to imaging.

6.9 Differential scanning calorimeters (DSC)

Thermal properties of native films were analyzed with a Perkin-Elmer DSC-7 (Norwalk, Conn., U.S.A.) equipped with an intra-coolant Thermal Analysis Controller TAC7/DX (Perkin-Elmer). Samples (approx. 20 mg each, db) were weighed into stainless steel pans (Perkin-Elmer) designed to withstand high pressures and suppress the volatilization of sample into the pan using a micro-syringe. The stainless steel pan was sealed with an O-ring, and allowed to reach equilibrium of moisture for overnight. The DSC pan was used for a reference pan. The heating rate was programmed by holding at -20°C for 1 min, followed by ramping the temperature range of -20°C to 180°C at a rate of 20°C/min, and holding at 180°C for 1 min. Measurements were made at least in duplicate for 1 treatment.

7. Effect of the ratio of Kiam and Phayom wood extracts on antimicrobial and properties of edible HPMC films

Edible films from HPMC were prepared (optimum condition selected from 2.0) and the influence of the ratio of Kiam and Phayom wood extracts (0:100, 25:75, 50:50, 75:25 and 100:0) on the antimicrobial properties, mechanical properties, water barrier properties and miscibility of edible HPMC films were investigated. The design of experiment as followed:

Table 2. Experimental of the effect of the ratio of Kiam and Phayom wood extracts on antimicrobial and properties of edible HPMC films

Experiment	Ingredient (100%)	
No.	Kiam wood extracts	Phayom wood extracts
1	100	0
2	25	75
3	50	50
4	75	25
5	0	100

Film samples were prepared according to the selected experiments and tested as followed;

- Antimicrobial activity (application by Pranoto *et al*. (2005)) *
- Tensile strength and elongation at break by ASTM D882-91 (ASTM, 1995)
- Water vapor permeability (application by McHugh *et al*. (1993))
- Film solubility (Jangchud and Chinnan, 1999)
- Film color by Hunter system
- Morphological properties (selected samples)

* Remark, before bring the sample film to test antimicrobial activity, must kill germs by UV at least 2 h.

8. Enhancing antimicrobial activity of chitosan films by incorporating wood Kiam and/or Phayom wood extracts

After received the suitable condition from item 3-4, antimicrobial effect of chitosan edible films (1% of chitosan was dissolved in 1 % acetic acid) incorporating wood extracts was compared between chitosan film fill and unfill Kiam wood extracts. Mechanical and physical properties were characterized, and antimicrobial efficacy was assessed against three food pathogenic bacteria namely *E. coli, S. aureus* and *L. monocytogenes*. The resulted films were tested as followed;

- Antimicrobial activity (application by Pranoto *et al*. (2004))*
- Tensile strength and elongation at break by ASTM D882-91 (ASTM, 1995)
- Water vapor permeability (application by McHugh *et al*. (1993))
- Film solubility (Jangchud and Chinnan, 1999)
- Film color by Hunter system
- Morphological properties (selected samples)

* Remark, before bring the sample film to test antimicrobial activity, must kill germs by UV at least 2 h.

9. Application the antimicrobial films in food products (imitated crab meat and ham)

Commercial imitated crab meat and ham were wrapped in a antimicrobial films (edible HPMC films incorporated with Kiam and/or Phayom wood extracts and chitosan films incorporated with Kiam and/or Phayom wood extracts at selected concentration) and kept at $4^{o}C$ for 14 days. Wrapped both imitated crab meat and ham with polyvinylchloride (PVC) films was used as references. The food products were sampled for testing of chemical quality and microorganism every 2 days as followed;

- a_w value by Thermoconstanter
- Color value in Hunter (JUKI) system
- TBARs value (Buege and Aust, 1978)
- Total viable count by method of Standard Method (Canillac and

Mourey, 2001)

- Coliform bacteria
- Sensory evaluation (using a nine-point hedonic scale)*

The sensory quality of products sample was evaluated by a twenty-five member trained panel from the laboratory staff. Panellists scored for sensory characteristics, such as, odour, appearance and overall acceptability, using a nine-point hedonic scale (1, dislike extremely to 9, like extremely). Acceptability was defined as having a score of>5.

* Remark, before testing of chemical quality, microorganism and sensory quality, the antimicrobial films were completely removed from food products.

10. Experimental design and statistical analysis

A completely randomized experimental design was used in session 2, 3 and 4 to characterization involved in the effect of Kiam and Phayom wood extracts on the antimicrobial and properties of edible HPMC films, effect of combination between Kiam and Phayom wood extracts on the antimicrobial and properties of edible HPMC films and enhancing of antimicrobial activity of edible HPMC films incorporated with Kiam and/or Phayom wood extracts by addition of chitosan. The session 5 was carried out according to randomized blocks design with three replications (3 packaging x 3 replications). Analysis of variance (ANOVA) was used to compare mean differences of the samples. If the differences in mean existed, multiple comparisons were performed using Duncan's Multiple Range Test (DMRT).

CHAPTER 3

RESULTS AND DISCUSSION

1. Characteristics of Kiam and Phayom wood extracts

The Kiam and Phayom wood extracts showed a light brown to yellowish color. They are odorless and contained 85.40 and 32.09 mg/L, of tannic acid, respectively (data not shown). With regard to the antimicrobial activity, the Kiam wood extracts has proved antimicrobial activity (Chanthachum and Beuchat, 1997). These authors also identified the most abundant phenolic compounds in Kiam and Phayom wood that may be responsible for the antimicrobial activity observed four major fractions. One of the four fractions consists of dichloro-1, 2-benzene, cineole-1, 8-nonanol-dimethyl-1, 2-benzene, naphthaline, a-terpeneol, hydrocarbon ses- quiterpenes and hexadeconal.

2. Minimal Bactericidal Concentration (MBC) of Kiam and Phayom wood extracts

The different concentration of Kiam and Phayom wood extracts showed various degrees of growth inhibition against *E. coli*, *S. aureus* and *L. monocytogenes* using the broth dilution method. The growth of *E. coli*, *S. aureus* and *L. monocytogenes* was inhibited by Kiam and Phayom wood extracts at 300 mg/L, which delayed the lag phase and lowered growth rate and final cell concentration of the microorganism. The mechanism of action responsible for antimicrobial activity of phenolic compounds present in herbaceous and woody plants has not been fully defined, although activity has been attributed to inhibition of extracellular enzymes, deprivation of substrates required for growth, inhibition of oxidative phosphorylation or iron deprivation. Scalbert (1991) reported that the antibacterial properties of woody plant extracts are associated with its lipophilic components, leading to change in membrane potential and increase in permeability of the cytoplasm membrane for protons and potassium ions, including depletion of the intracellular ATP pool.

3. Antimicrobial and properties of edible HPMC films incorporated with Kiam wood extracts

3.1 Antimicrobial activity of edible HPMC films incorporated with Kiam wood extracts

The growth inhibition zones measured by using agar diffusion assay. Kiam wood extracts exhibited different inhibition levels against *E. coli*, *S. aureus* and *L. monocytogenes* as shown in Table 3. The inhibitory activity was measured based on clear zone surrounding circular film strips. Measurement of clear zone diameter included diameter of film strips, therefore, the values were always higher than the diameter of film strips (16 mm) whenever clearing zone was present. If there is no clear zone surrounding, we assumed that there is no inhibitory zone, and furthermore, the diameter was valued as zero.

In this study, the inhibition zone increased with increasing concentration of Kiam wood extracts. Edible HPMC films containing Kiam wood extracts at 1 and 2 folds of MBC was not effective against any test microorganisms. However as 2 folds of MBC showed slightly affect on *L. monocytogenes* with demonstrated a meager inhibitory zone. As the concentration of Kiam wood extracts increased higher than 2 folds, the zone of inhibition increased significantly ($p<0.05$) for *E. coli*, *S. aureus* and *L. monocytogenes*. The results showed that, at high concentrations (3 to 5 folds of MBC), Kiam wood extracts exhibited marked inhibition activity against bacteria tested. The results showed that the zone of inhibition subjected *E. coli*, *S. aureus* and *L. monocytogenes* increased from 18.33 ± 0.2 to 23.00 ± 0.30, 20.00 ± 0.35 to 25.33 ± 0.50 and 22.67 ± 0.36 to 29.65 ± 0.58 mm diameter when Kiam wood extract increased from 3 MBC to 5 MBC, respectively (Table 3 and Figure 7). Comparatively, *E. coli* (Gram-negative bacteria) was lesser sensitive to the inhibitory activity of Kiam wood extracts than *L. monocytogenes* and *S. aureus* (Gram-positive bacteria, respectively). Sensitivity to tannins and other phenolic compounds varies greatly among organisms. Some, including *E. coli* (Lewis and Starkey, 1969) and *Pseudomonas fluorescens* (Basarada, 1966), both gram-negative species, are capable of growing on tannins as a source of carbon. Whether the strains of *E. coli* tested in our study are capable of metabolizing

Kiam wood tannins or other component is not known. The gram-negative bacteria investigated in our study appear lesser sensitive to Kiam wood extract, whereas the gram-positive were sensitive, suggesting that differences in sensitivity may be associated with cell wall structure or function. Thus it would seem that from the limited number of microorganism tested, the inhibitory activity of Kiam and wood extracts is restricted to gram-positive species.

Table 3. Antimicrobial activity of edible HPMC films incorporated with Kiam wood extracts against *E. coli*, *S. aureus* and *L. monocytogenes*.

Bacteria types	Kiam wood extracts (mg/L)	Inhibitory zone[A] (mm)
E. coli	0 (Control)	0.00 ± 0.00^{a}
	300 (1 folds of MBC)	0.00 ± 0.00^{a}
	600 (2 folds of MBC)	0.00 ± 0.00^{a}
	900 (3 folds of MBC)	18.33 ± 0.20^{b}
	1200 (4 folds of MBC)	21.33 ± 0.45^{c}
	1500 (5 folds of MBC)	23.00 ± 0.30^{d}
S. aureus	0 (Control)	0.00 ± 0.00^{a}
	300 (1 folds of MBC)	0.00 ± 0.00^{a}
	600 (2 folds of MBC)	0.00 ± 0.00^{a}
	900 (3 folds of MBC)	20.00 ± 0.35^{b}
	1200 (4 folds of MBC)	22.32 ± 0.40^{c}
	1500 (5 folds of MBC)	25.33 ± 0.50^{d}
L. monocytogenes	0 (Control)	0.00 ± 0.00^{a}
	300 (1 folds of MBC)	0.00 ± 0.00^{a}
	600 (2 folds of MBC)	17.50 ± 0.20^{b}
	900 (3 folds of MBC)	22.67 ± 0.36^{c}
	1200 (4 folds of MBC)	26.67 ± 0.54^{d}
	1500 (5 folds of MBC)	29.65 ± 0.58^{e}

[A] Values are measurements of diameter of inhibitory zone and expressed in mm. Values (n=4) with different superscript letters are significantly different ($p<0.05$). The diameter of edible HPMC films discs were 16 mm.

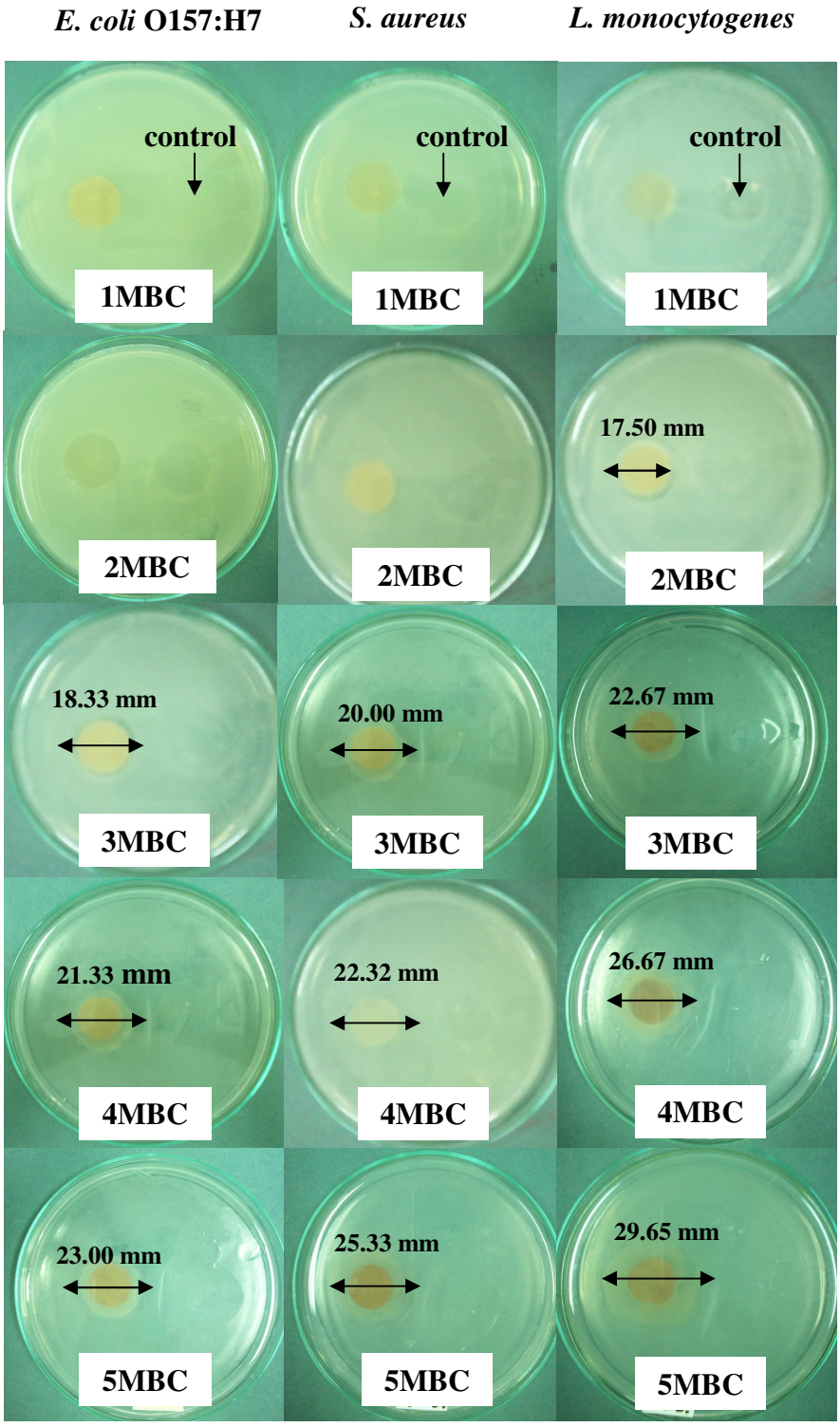


Figure 7. Representative picture of inhibitory zone of edible HPMC films incorporated with Kiam wood extracts at 1-5 folds of MBC against *E. coli* O157:H7, *S. aureus* and *L. monocytogenes*. The diameter of edible HPMC films discs were 16 mm.

3.2 Properties of edible HPMC films incorporated with Kiam wood extracts

3.2.1 Tensile strength (TS) and elongation at break (ε)

An edible film must withstand the normal stress encountered during its application and the subsequent shipping and handling of the food to maintain its integrity and barrier properties. Tensile strength (TS) is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated TS at yield or at break, respectively (ASTM, 1991). High TS is generally required, but deformation values must be adjusted according to the intended application of the films. That is, whether it is undeformable material to provide structural integrity or reinforce structure of the food (Gontard *et al*., 1992). TS and ε of the edible HPMC films incorporated with Kiam wood extracts are summarized in Figure 8. The TS and ε of edible HPMC films gave similar result, as addition of Kiam wood extracts. Incorporation of Kiam wood extracts markedly affected mechanical properties of resulting films, as seen in the reduction of TS and ε. The decreased TS and ε of the edible HPMC films after incorporation with Kiam wood extracts may be associated with the increased film thickness (data not shown). On the other hand, all the tensile properties of the edible HPMC films incorporated with Kiam wood extracts decreased significantly ($p<0.05$). This may also be due to the incomplete dispersion of the Kiam wood extracts into the polymer matrix, which is caused by the incompatibility of Kiam wood extracts and HPMC biopolymer. The results showed that TS and ε decreased from 36.61 to 18.48 MPa and 28.82 to 11.19% when the Kiam wood extracts increased from 300 to 1500 mg/L, respectively. It is reasonable due to the presence of Kiam wood extracts as an additive material.

In this system, Kiam wood extracts might contribute to retarding the intermolecular interaction of the structural carbon in edible HPMC films, and induces the development of a heterogeneous film structure, featuring discontinuities, resulted in the decrease in TS and ε of the films. Besides, Kiam wood extracts could easily fit into HPMC chains and inhibit the bonding between molecules of HPMC. The reduction in mechanical properties as affected by additives was previously investigated for various hydrocolloid-based films (Park and Chinnan, 1990; Gontard

et al., 1993). The mechanical property changes were characterized by decrease in density and reversibility of intermolecular interactions occurring in the antimicrobial edible films have also been reported by Yang and Paulson (2000). As a consequence, the density of intermolecular interaction decrease in material and the free volume between polymer chains increases (Cuq *et al*., 1997). These results agree with Ozdemir and Floros (2004), who reported comparable values for sorbitol-plasticized WPI films under similar conditions. The addition of oregano extracts in the sorbitol-plasticized WPI films resulted in a decrease of ε and σ max with increasing extract concentration. The effect of spice extracts addition in films has been studied and in all the cases significant decreases in TS and elastic modulus have been reported (Rojas-Grau *et al*., 2007).

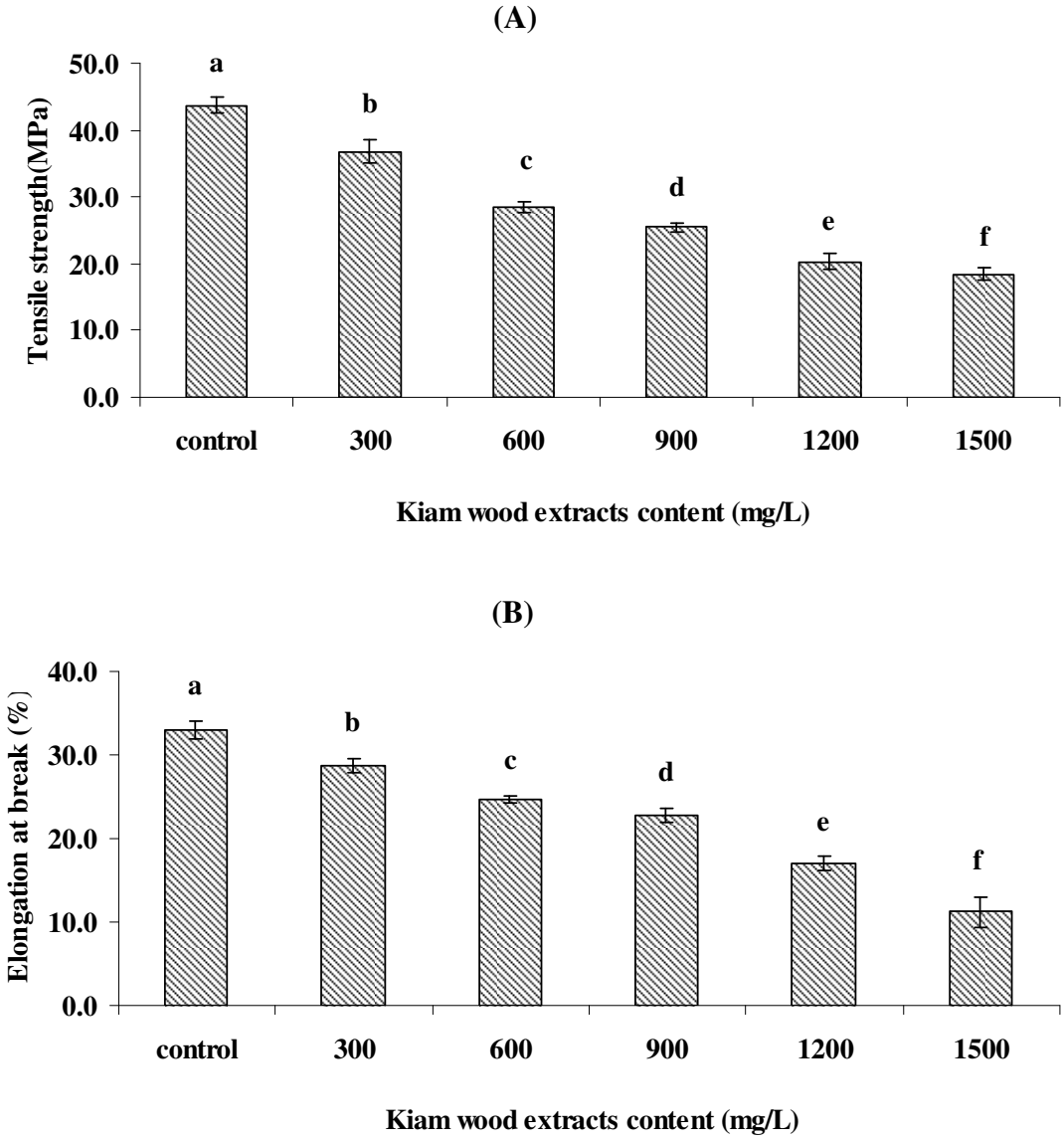


Figure 8. Tensile strength (A) and elongation at break (B) of edible HPMC films as a function of Kiam wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

3.2.2 Water vapor permeability (WVP) and film solubility (FS)0

Water vapor permeability (WVP) is a proportional constant assumed to be independent of the water vapor pressure gradient applied across the films. However, hydrophilic materials, such as protein films, deviate from this ideal behavior due to interactions of permeating water molecules with polar groups in the film's structure (Hagenmaier and Shaw, 1990). Deviation from the ideal behavior can also be induced by the effects of structure on materials (Gontard *et al*., 1992). Since a main function of a biodegradable or edible film is often to impede moisture transfer between food and the surrounding atmosphere, or between two components of a heterogeneous food product, WVP should be as low as possible. Film solubility (FS) is an important property of edible films for applications as food protection where water activity is high, or when the film must be in contact with water during processing of the coated food (e.g. to avoid exudation of fresh or frozen products) (Gontard *et al*., 1992). Generally, higher solubility would indicate lower water resistance. However, a high solubility may an advantage for some applications (Stuchell and Krochta, 1994). In the present study, the WVP properties were affected by the incorporation of Kiam wood extracts. The WVP of edible HPMC films increased from 15.09 to 27.77 g.mm/m^2.d.kPa when the Kiam wood extracts increased from 300 to 1500 mg/L. This tendency could be explained by structural modifications of the HPMC network.

The incorporation of Kiam wood extracts modified the molecular organization of the HPMC network, which increased in free volume. The network became less dense and as a consequence more permeable. Permeability increase with increase in Kiam wood extracts content could be related to hydrophillicity of Kiam wood extracts. Introducing hydrophillic additives, favorable to adsorption and desorption of water molecules, is known to enhance WVP of hydrocolloid-based films (Gontard *et al*., 1993; McHugh, 1994). The increase in WVP with increase in hydrophillicity of additives concentration is also common in edible films (McHugh *et al*., 1994; Cuq *et al*., 1997). The hydrophillicity of the additives will increase the water content of the film, consequently increasing the mobility of the molecules. In addition, increase in the water content could also affect permeate solubility in the film. Stuchell and Krochta (1994) indicated that water vapor transfer generally occurs

through the hydrophilic portion of the film and depends on the hydrophilic-hydrophobic ratio of the film components. The antimicrobial edible HPMC films were clearly not dispersed without visual loss of integrity after a 24 h immersion in water. Irrespective of Kiam wood extracts, an increase in its content led to an increase in FS (Figure 9). It could be hastily concluded that Kiam wood extracts enhance FS in water. The dry matter solubilized in water is likely to be constituted mainly by the Kiam wood extracts and plasticizers. High interaction density and more certainly, the presence of intermolecular covalent bonds is responsible for partial insolubility of these films. This water solubility behavior could not be generalized, and understanding the FS remains a complex subject. A decrease in the polymer network interaction density due to the Kiam wood extracts presence was thus associated with this increase in solubility properties. The lowest FS of edible HPMC films incorporated by 300 mg/L Kiam wood extracts were noticed, while increasing the amount of Kiam wood extracts content showed higher FS (Figure 9). It could be explained that, with higher content of Kiam wood extracts, there are more molecules of Kiam wood extracts untrapped in the cross linked network and able to escape into solution.

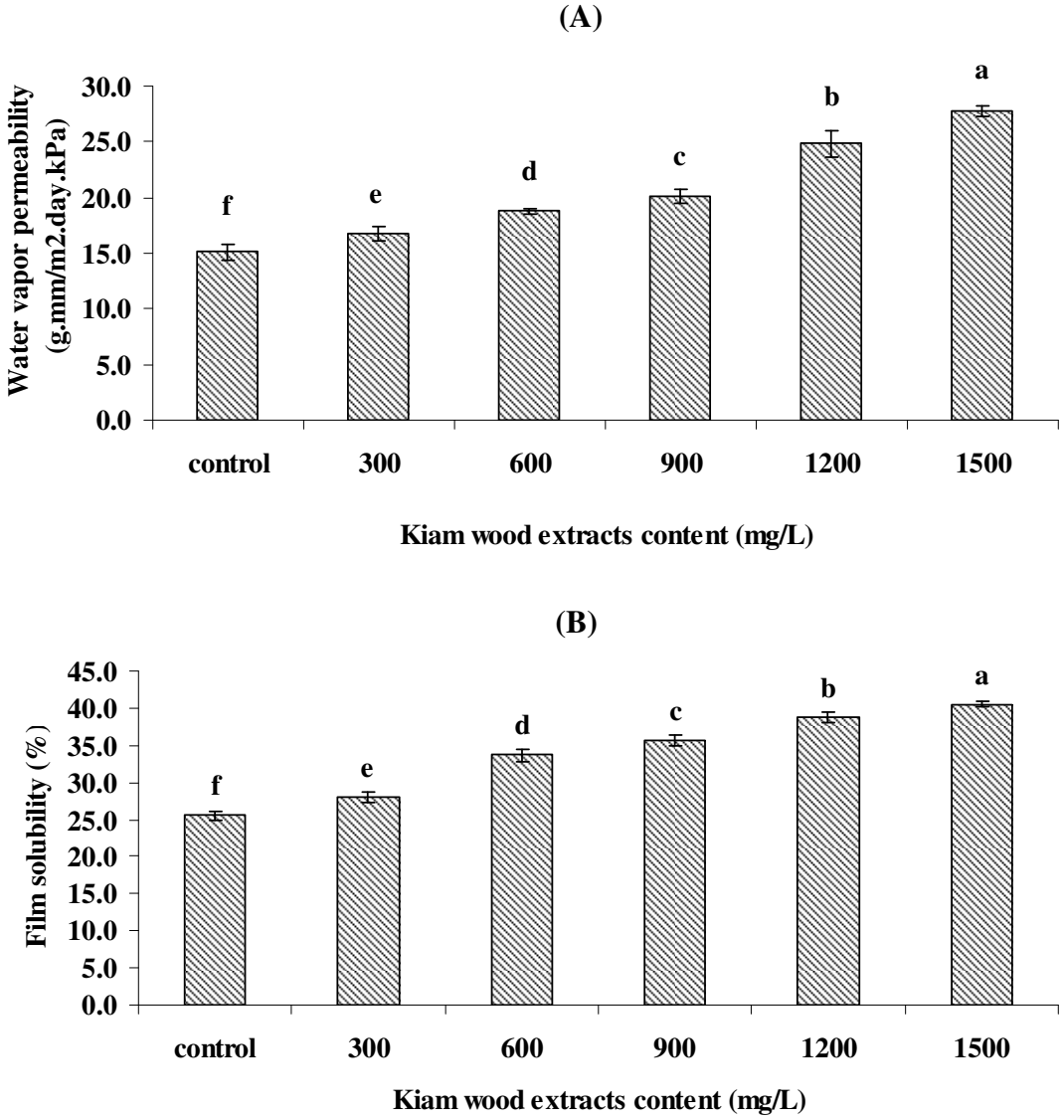


Figure 9. Water vapor permeability (A) and film solubility (B) of edible HPMC films as a function of Kiam wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

3.2.3 Color and Transparency

The values of color measurement taken into account were L*, a*, b* and ΔE_{ab}*, chroma and hue angle. The color performances of Kiam wood extracts incorporated edible HPMC films can be seen in Figure 10 and 11. Addition of Kiam wood extracts affected the appearance of edible HPMC films in both color and transparency. Edible HPMC films unfilled Kiam wood extracts appeared clear and transparent. Edible HPMC films filled Kiam wood extracts was became lighter and more red-yellowish as evidenced by the increased L*, a*, b* and chroma values when the concentration of Kiam wood extracts in the edible HPMC films increased (Figure 10 and 11). A significant ($p<0.05$). Results demonstrated that L*, a* and b*, chroma and hue angle values increased as the content of Kiam wood extracts incorporated increased (Figure 10 and 11). This was due to the Kiam wood extracts being more red-yellowish than the edible HPMC films. Transparency of the edible HPMC films is also importance in some instances, when used as packaging materials. Incorporated of Kiam wood extracts into the edible HPMC films resulted in decrease their transparency. Edible HPMC films unfilled Kiam wood extracts was the highest transparent. However, the lower transparency of the edible HPMC films was noticed when a greater concentration of Kiam wood extracts incorporated (Figure 12). The decrease in transparency could possibly arise from the light scattering from the retarding of light transmission of the edible HPMC films and Kiam incorporated in edible HPMC films.

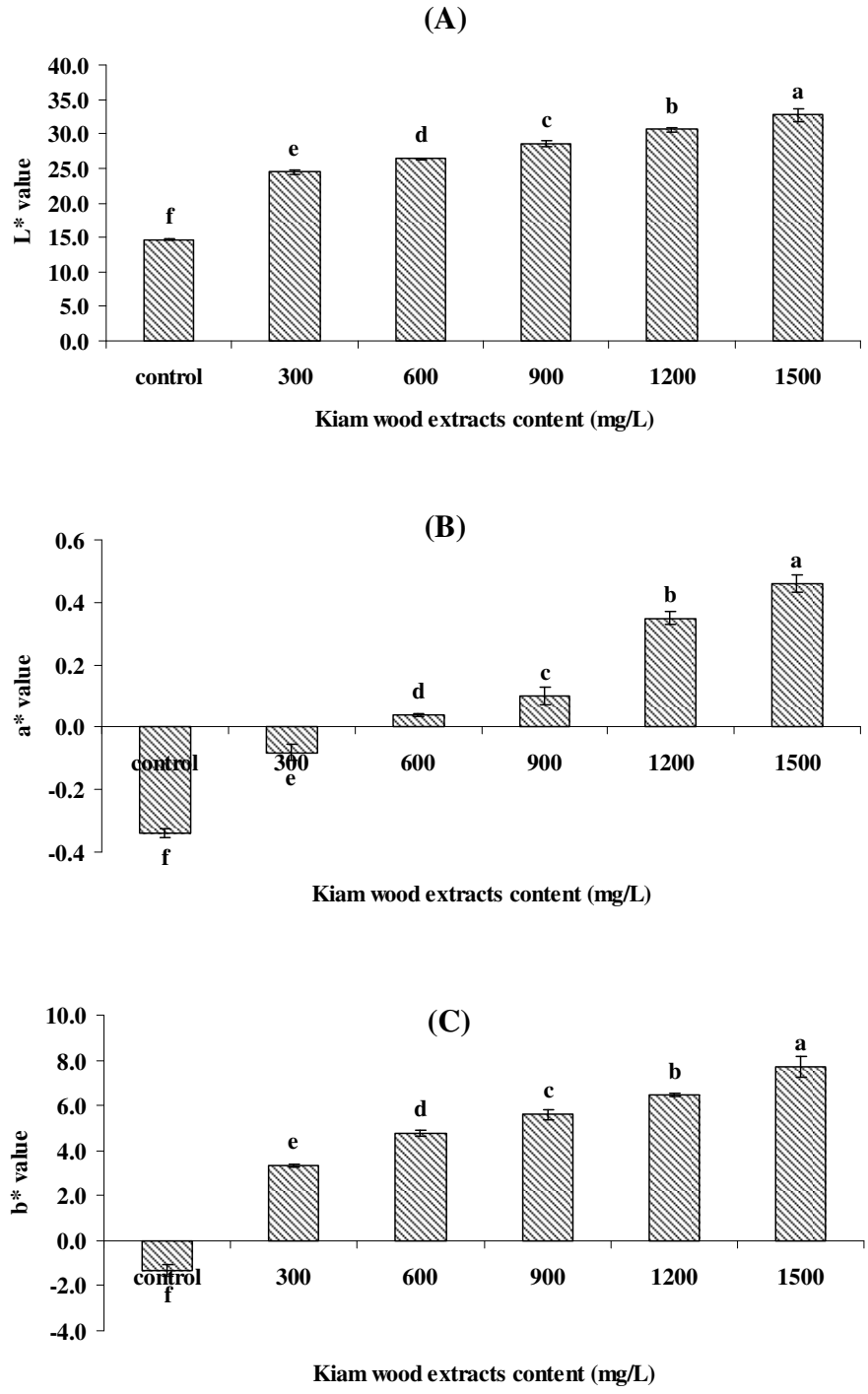


Figure 10. L* (A), a*(B) and b* (B) values of edible HPMC films as a function of Kiam wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

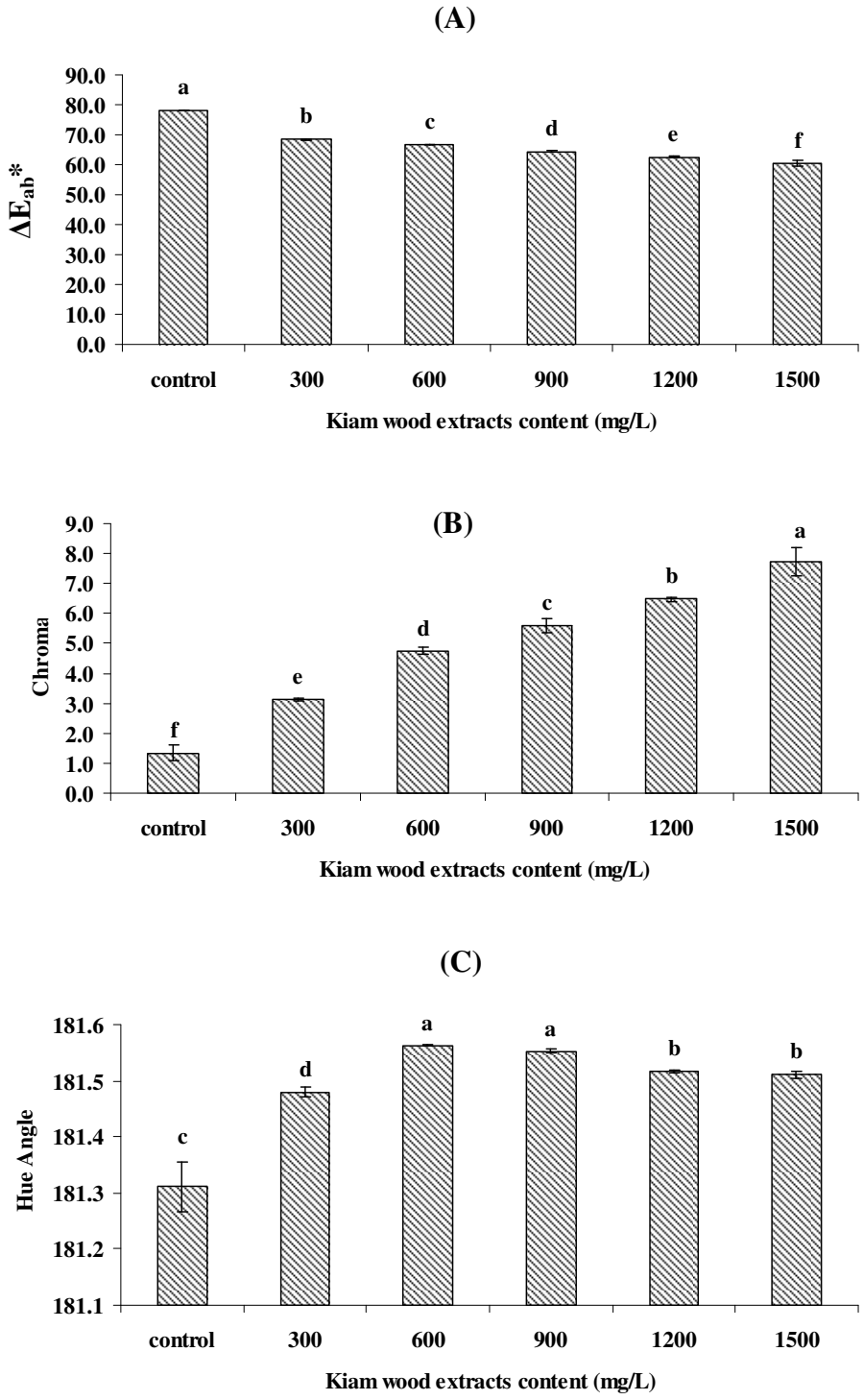


Figure 11. ΔE_{ab}* (A), chroma (B) and hue angle (C) of edible HPMC films as a function of Kiam wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

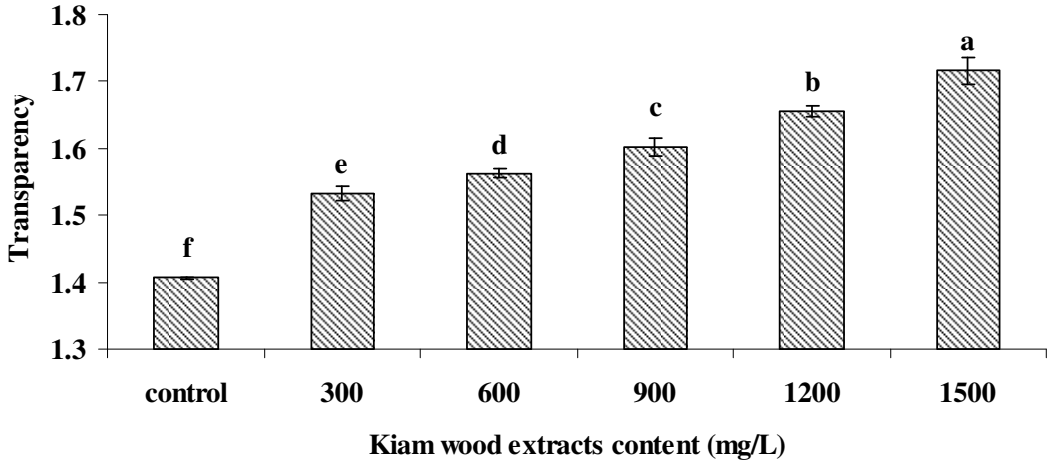


Figure 12. Transparency of edible HPMC films as a function of Kiam wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

3.2.4 Morphology of the films

The morphology of the antimicrobial edible HPMC films incorporated with Kiam wood extracts was identified by Scanning electron microscopy (SEM). Figure 13 shows the surface of antimicrobial edible HPMC films unfilled and filled Kiam wood extracts at 300 to 1500 mg/L. Compared with antimicrobial edible HPMC films, the morphology of the existence of the Kiam wood extracts in the edible HPMC films can be easily observed in the films. However, it is difficult to distinguish the individual Kiam wood extracts filler dispersion due to its homogeneous. Some Kiam wood extracts appear as agglomeration at the surface of the sample (Figure 13). By comparing the distribution of Kiam wood extracts into the antimicrobial edible HPMC films as affected by the Kiam wood extracts content, the results showed that higher addition of Kiam wood extracts are more or less evenly distributed within the antimicrobial edible HPMC films. However some Kiam wood extracts are not fully homogeneous and form small aggregates when 1500 mg/L of Kiam wood extracts was applied (Figure 13).

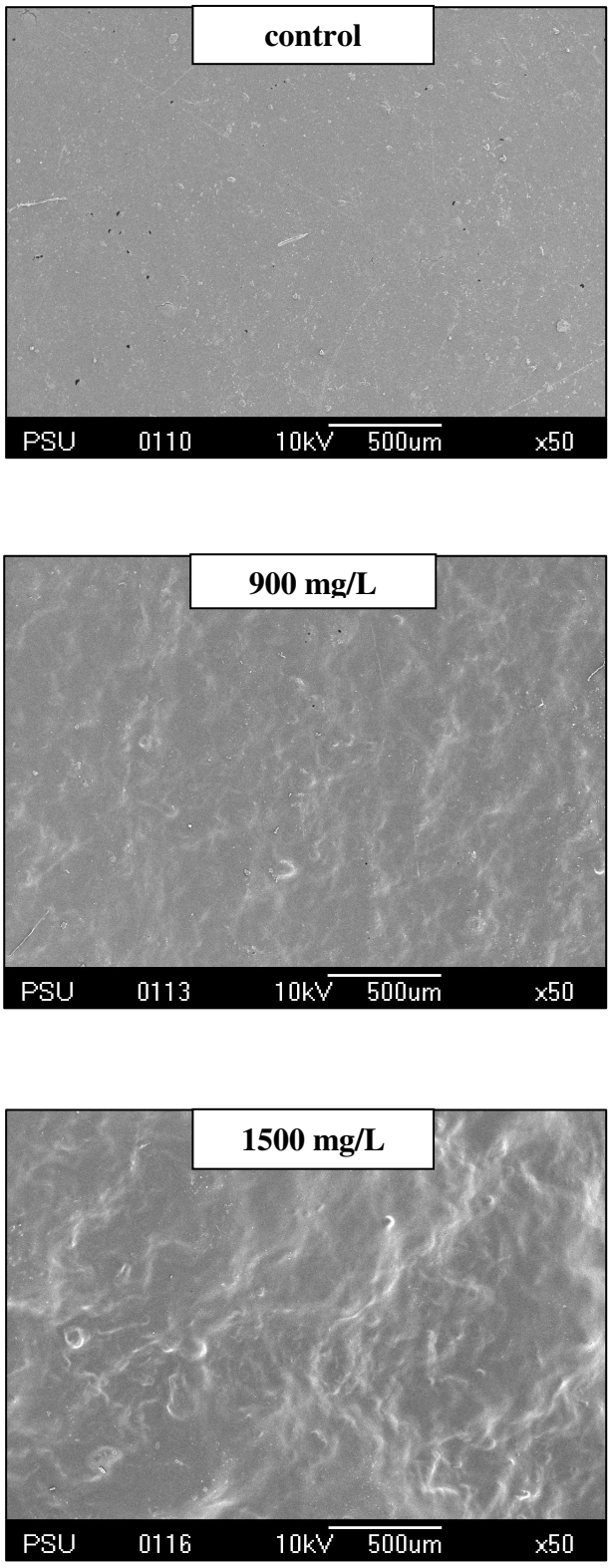


Figure 13. Scanning electron micrograph of edible HPMC films containing 900 mg/L and 1500 mg/L Kiam wood extracts.

4. Antimicrobial and properties of edible HPMC films incorporated with Phayom wood extracts

4.1 Antimicrobial activity of edible HPMC films incorporated with Phayom wood extracts

Increasing concentration of Phayom wood extracts were incorporated to edible HPMC films and were tested against microorganisms for the zone of inhibition area (Table 4). Films containing 1 and 2 folds of MBC of Phayom wood extracts were not effective against any tested microorganisms. The minimum amount of Phayom wood extracts level that showed inhibition was 3 folds of MBC for all tested microorganisms. As the concentration increased, the zone of inhibition also increased significantly for *E. coli, S. aureus* and *L. monocytogenes*. The greatest zone of inhibition was observed at 5 folds of MBC against *E. coli, S. aureus* and *L. monocytogenes* ($p<0.05$). The results demonstrated that the zone of inhibition subjected *E. coli*, *S. aureus* and *L. monocytogenes* increased from 17.33 ± 0.58 to 21.33 ± 0.58, 19.00 ± 0.00 to 22.67 ± 0.58 and 20.33 ± 0.58 to 25.67 ± 0.58 mm diameter when Phayom wood extract increased from 3 MBC to 5 MBC, respectively (Table 4 and Figure 14). Fapasuri and Bassir (1972) reported that *Saccoglottis gabonensis* bark significantly inhibited growth of microflora of palm wine particular of bacterial growth resulting in reduction in rate of souring of palm wine. The chemical compounds of the bark responsible for inhibition of microbial growth were reported to be isocoumarin (lactone) and distichol (polyphenol), respectively (Faparusi and Bassir, 1972).

The mechanism of action responsible for antimicrobial activity of phenolic compounds present in herbarceous and woody plants has not been fully defined, although activity has been attributed to inhibition of extracellular enzymes, deprivation of substrates required for growth, inhibition of oxidative phosphorylation or iron deprivation (Scalbert, 1991). Basarada (1966) reported that the sensitivity to tannins and other phenolic compounds varies greatly among organisms. Some, including *E. coli* and *P. fluorescens*, both gram-negative species, are capable of growing on tannins as a source of carbon. Whether the strains of *E. coli* tested in our study are capable of metabolizing Phayom wood tannins or other component is not

known. Hence, the gram-negative bacteria investigated in our study appear lesser sensitive to Phayom wood extract, whereas the gram-positive were sensitive, suggesting that differences in sensitivity may be associated with cell wall structure or function. Thus it would seem that from the limited number of microorganism tested, the inhibitory activity of Phayom wood extracts is restricted to gram-positive species.

Table 4. Antimicrobial activity of edible HPMC films incorporated with Phayom wood extracts against *E. coli*, *S. aureus* and *L. monocytogenes*.

Bacteria types	Phayom wood extracts (mg/L)	Inhibitory zone[A] (mm)
E. coli	0 (Control)	0.00 ± 0.00^{d}
	300 (1 folds of MBC)	0.00 ± 0.00^{d}
	600 (2 folds of MBC)	0.00 ± 0.00^{d}
	900 (3 folds of MBC)	17.33 ± 0.58^{c}
	1200 (4 folds of MBC)	19.67 ± 0.58^{b}
	1500 (5 folds of MBC)	21.33 ± 0.58^{a}
S. aureus	0 (Control)	0.00 ± 0.00^{d}
	300 (1 folds of MBC)	0.00 ± 0.00^{d}
	600 (2 folds of MBC)	0.00 ± 0.00^{d}
	900 (3 folds of MBC)	19.00 ± 0.00^{c}
	1200 (4 folds of MBC)	21.00 ± 0.00^{b}
	1500 (5 folds of MBC)	22.67 ± 0.58^{a}
L. monocytogenes	0 (Control)	0.00 ± 0.00^{d}
	300 (1 folds of MBC)	0.00 ± 0.00^{d}
	600 (2 folds of MBC)	0.00 ± 0.00^{d}
	900 (3 folds of MBC)	20.33 ± 0.58^{c}
	1200 (4 folds of MBC)	22.33 ± 0.58^{b}
	1500 (5 folds of MBC)	25.67 ± 0.58^{a}

[A] Values are measurements of diameter of inhibitory zone and expressed in mm. Values (n=4) with different superscript letters are significantly different ($p<0.05$). The diameter of edible HPMC films discs were 16 mm.

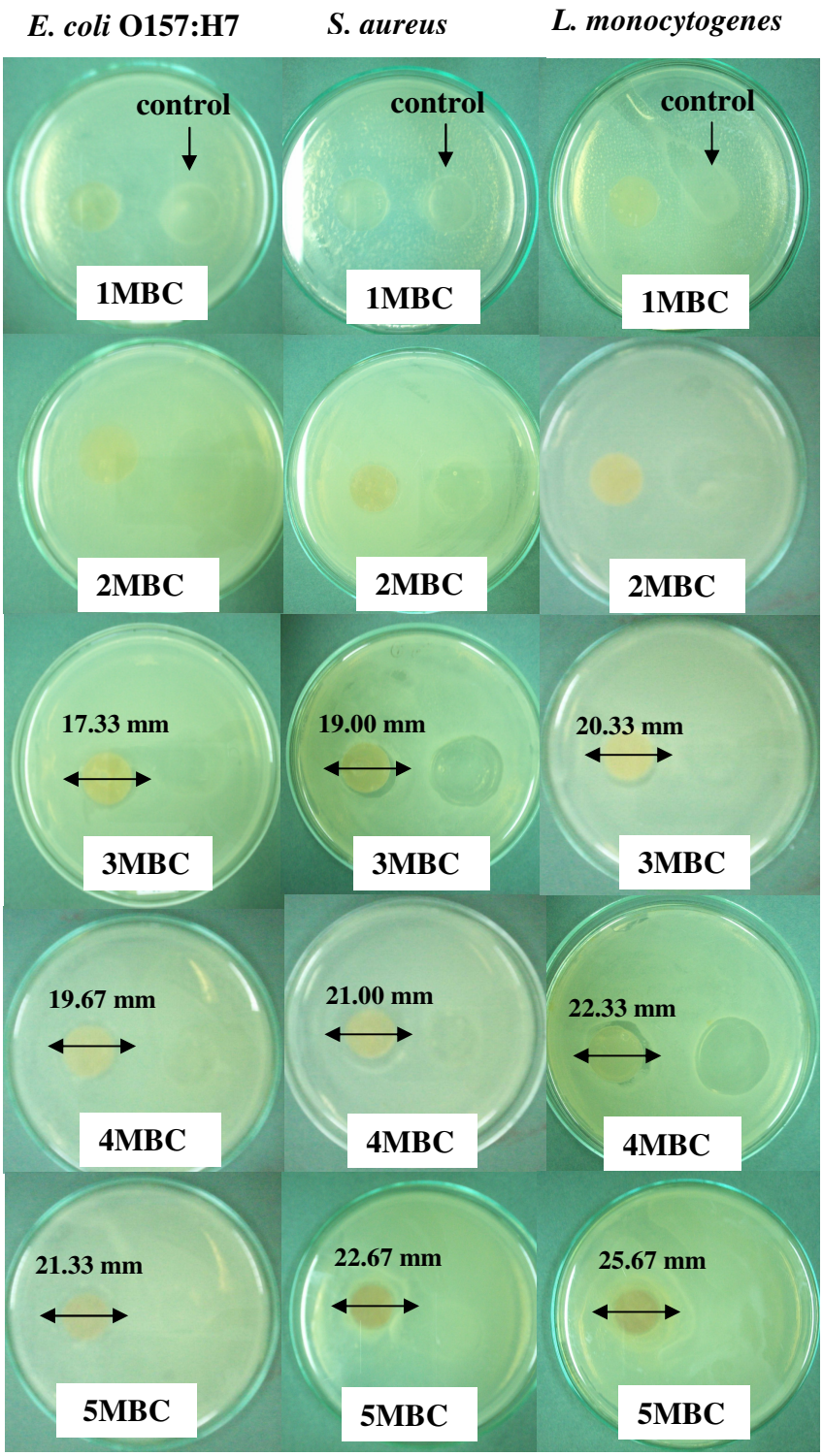


Figure 14. Representative picture of inhibitory zone of edible HPMC films incorporated with Phayom wood extracts at 1-5 folds of MBC against *E. coli* O157:H7, *S. aureus* and *L. monocytogenes*. The diameter of edible HPMC films discs were 16 mm.

4.2 Properties of edible HPMC films incorporated with Phayom wood extracts

4.2.1 Tensile strength (TS) and elongation at break (ε)

Biopolymer materials, such as films, may be subjected to various kinds of stress during use; the determination of the mechanical properties involves not only scientific but also technological and practical aspects (Cagri *et al*., 2001). Tensile strength (TS) is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated TS at yield or at break respectively (ASTM, 1991). Elongation at break (ε) is an indication of a film's flexibility and stretch ability (extensibility). This is determined as the point when the film breaks under FS. It is expressed as the percentage of change of the original length of the specimen between the grips of a film to stretch (extend) (Gontard, 1992). The addition of Phayom wood extracts influenced the film's properties. TS and ε of the edible HPMC films incorporated with Phayom wood extracts are depicted in Figure 15.

The TS of edible HPMC films were affected by the Phayom wood extracts. The results demonstrated that the TS of edible HPMC films decreased with the addition of Phayom wood extracts, and the maximum occurred when no Phayom wood extracts was added (43.75 MPa). The TS of the edible HPMC films decreased from 29.18 to 14.01 MPa when Phayom wood extracts was added at 300 to 500 mg/L. The remarkable decrease in the TS of the edible HPMC films indicated the presence of Phayom wood extracts as an additive material of the films. The changes in mechanical properties of edible HPMC films were characterized by the Phayom wood extracts (as additive) weaken the intermolecular forces between the chains of adjacent macromolecules, increasing the free volume and causing a reduction of mechanical resistant. Thus, the increase in the Phayom wood extracts content causes a reduction of the TS due to the decrease in the intermolecular interactions. Besides, the increase in the Phayom wood extracts content increases the moisture content of the film because of its high hygroscopic character, which also contributes to the reduction of the forces between the adjacent macromolecules. The effect of additive and/or

plasticizer on reduction of the mechanical properties is well known and its explanation is reported by some researchers (Cuq *et al*., 1997).

Elongation at break of edible HPMC films decreased from 26.19 to 9.14% as the concentration of Phayom wood extracts increased from 300 to 500 mg/L (Figure 15B). The same effect was found by Ozdemir and Floros (2004), who reported comparable values for sorbitol-additive WPI films under similar conditions. The addition of oregano extracts in the sorbitol-plasticized WPI films resulted in a decrease of ε with increasing extract concentration. The effect of spice extracts addition in films has been studied and in all the cases significant decreases in elastic modulus have been reported (Rojas-Grau, 2007).

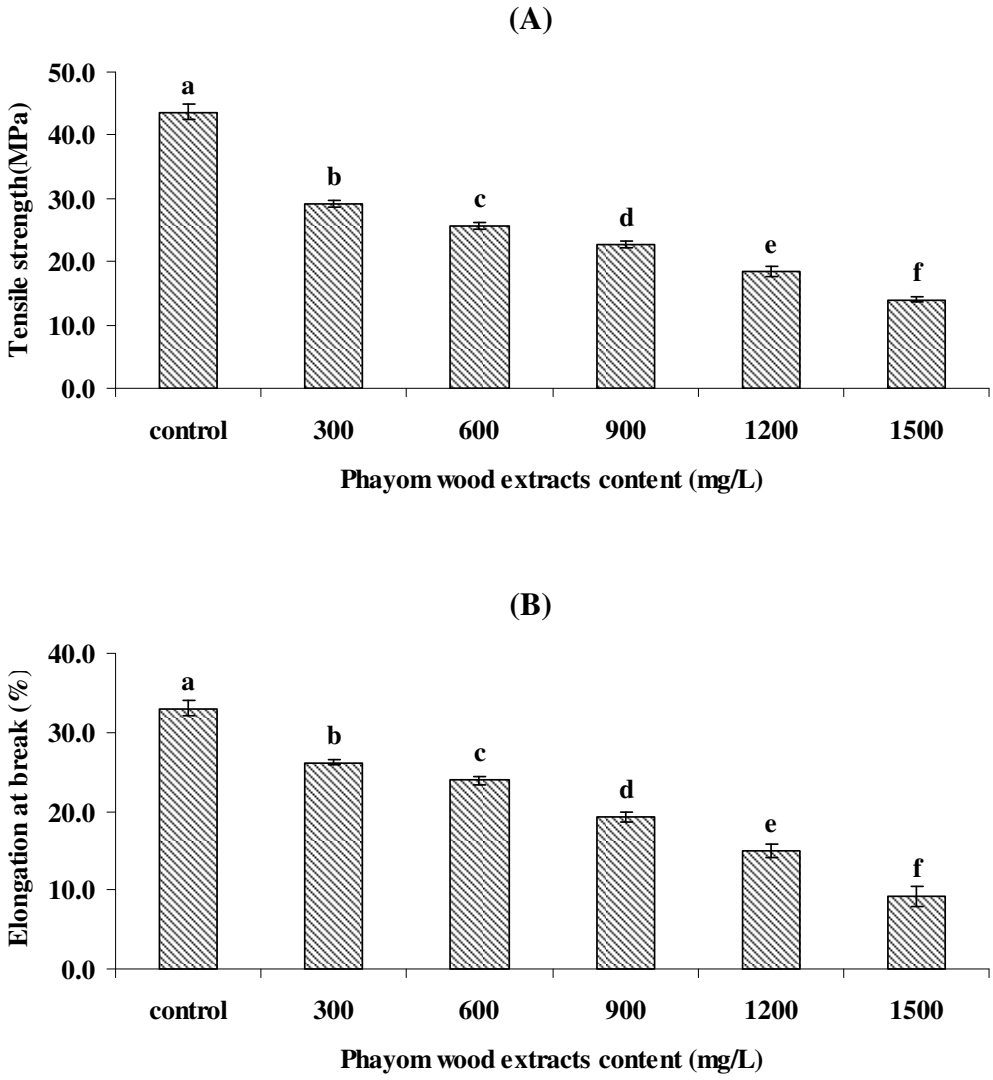


Figure 15. Tensile strength (A) and elongation at break (B) of edible HPMC films as a function of Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

4.2.2 Water vapor permeability (WVP) and film solubility (FS)

Water vapor permeability (WVP) of edible HPMC films with different concentrations of Phayom wood extracts was examined at a vapor pressure difference of 0/60%RH across the film. The WVP of edible HPMC films was affected by the incorporation of Phayom wood extracts. The WVP value varied from 16.82 to 28.97 g.mm/m^2.d.kPa when Phayom wood extracts increased from 300 to 1500 mg/L as depicted in Figure 16A. This tendency could be explained by structural modifications of the polymer network. The network may become less dense because of an increase in the mobility of the polymeric chains and in the free volume of the film. These consequences of the Phayom wood extracts are favorable to the adsorption or desorption of water molecule. Furthermore, the increase of WVP might be related to the hydrophillicity of Phayom wood extracts. In this system, Phayom wood extracts might contribute to extend inter molecular interaction of the structural matrix in edible HPMC films therefore; it enhanced moisture passing through the edible films. The WVP value of film or coating material should be taken into account when applying onto a moist product such as precooked beef.

The films ability to retard moisture loss from the product (Yang and Paulson, 2000) is an important characteristic that affects product quality. Irrespective of Phayom wood extracts, an increase in its content led to an increase in FS (Figure 16B). It could be hastily concluded that Phayom wood extracts enhance FS in water. The dry matter solubilized in water is likely to be constituted mainly by the Phayom wood extracts and plasticizers. High interaction density and more certainly, the presence of intermolecular covalent bonds is responsible for partial insolubility of these films. This water solubility behavior could not be generalized, and understanding the FS remains a complex subject. A decrease in the polymer network interaction density due to the Phayom wood extracts presence was thus associated with this increase in solubility properties. The highest FS of edible HPMC films incorporated by 1500 mg/L Phayom wood extracts were noticed, while increasing the amount of Phayom wood extracts content showed higher FS (Figure 16B). It could be explained that, with higher content of Phayom wood extracts, there are more

molecules of Phayom wood extracts untrapped in the cross linked network and able to escape into solution.

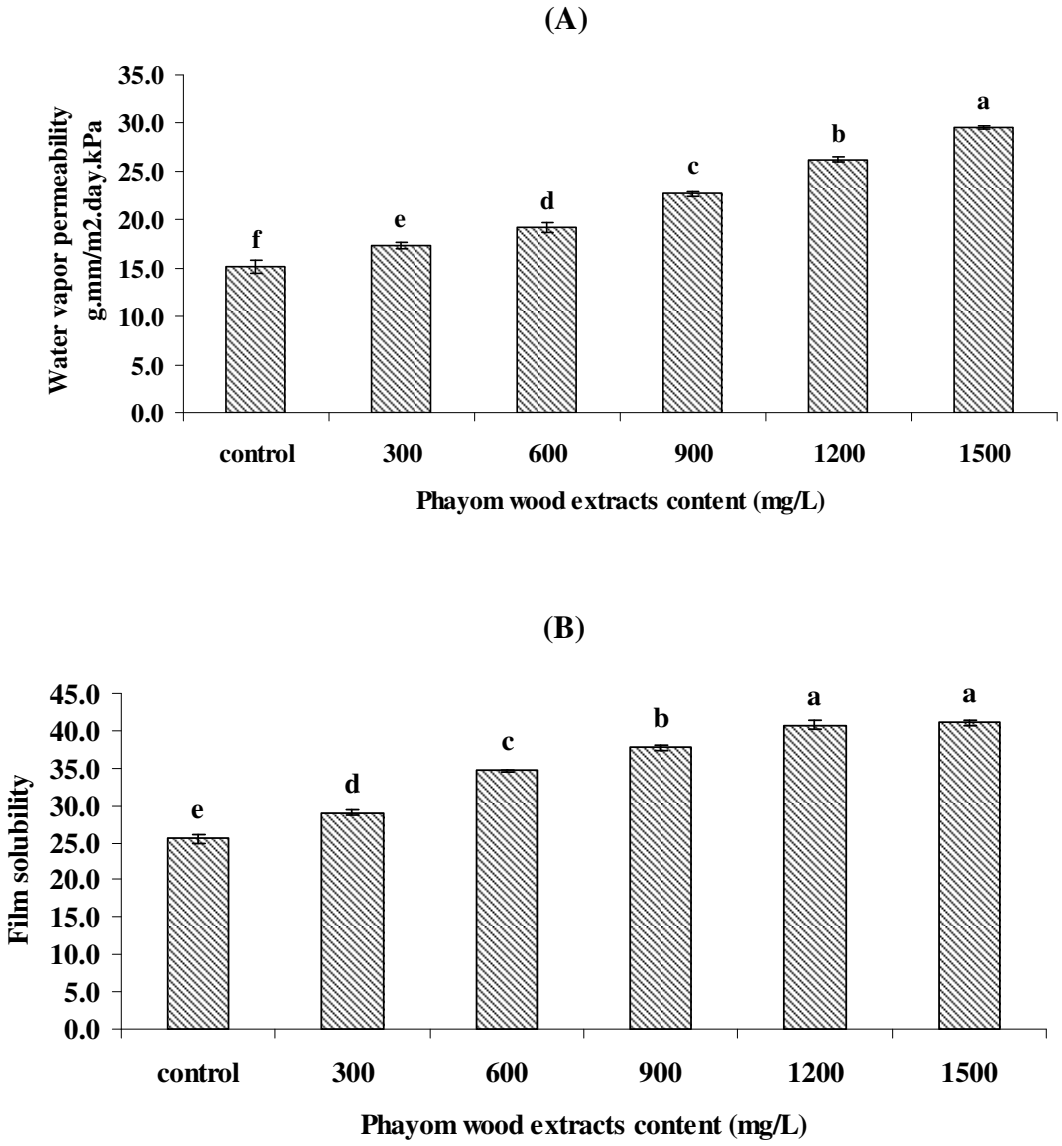


Figure 16. Water vapor permeability (A) and film solubility (B) of edible HPMC films as a function of Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

4.2.3 Color and Transparency

In the present study, color was affected by the incorporation of Phayom wood extracts into the edible HPMC films. Color of films different concentration of Phayom wood extracts incorporated into edible HPMC films expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/blueness), ΔE_{ab}*, chroma and hue angle values are shown in Figure 17 and 18. Addition of Phayom wood extracts affected the appearance of edible HPMC films in both color and transparency. Edible HPMC films unfilled Phayom wood extracts appeared clearing and transparency. Edible HPMC films filled Phayom wood extracts became lighter and red-yellowish as evidenced by the increased L*, a*, b* and chroma values when the concentration of Phayom wood extracts increased (Figure 17 and 18). A significant ($p<0.05$). This was due to the Phayom wood extracts being more dark and red-yellowish than the edible HPMC films. Transparency of the edible HPMC films is also importance in some instances, when used as packaging materials. Incorporated of Phayom wood extracts into the edible HPMC films resulted in decrease their transparency. Edible HPMC films unfilled Phayom wood extracts was the highest transparent. However, the lower transparency of the edible HPMC films was noticed when a greater concentration of Phayom wood extracts incorporated (Figure 19). The decrease in transparency could possibly arise from the light scattering from the retarding of light transmission of the edible HPMC films and Phayom incorporated in edible HPMC films.

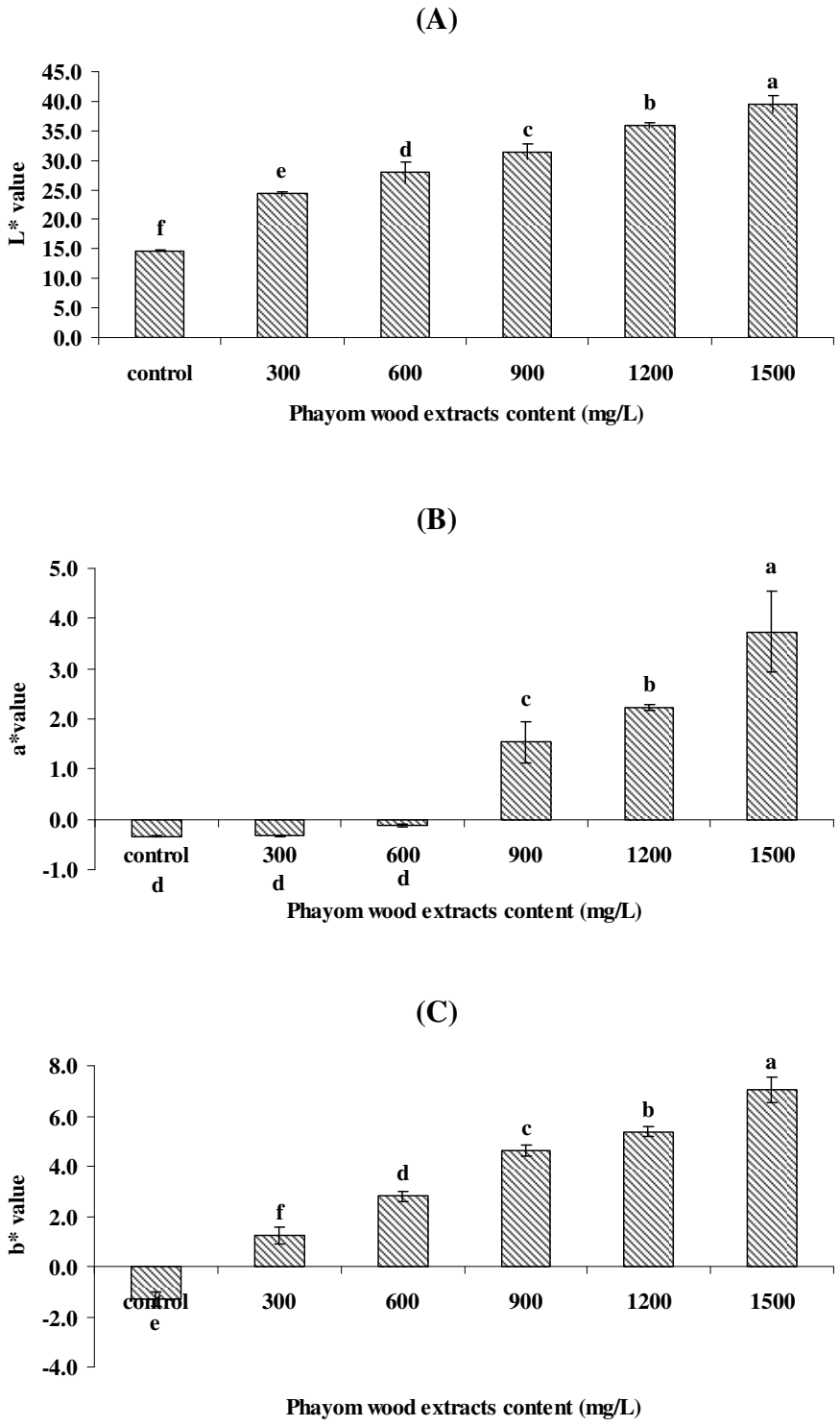


Figure 17. L* (A), a*(B) and b* (B) values of edible HPMC films as a function of Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

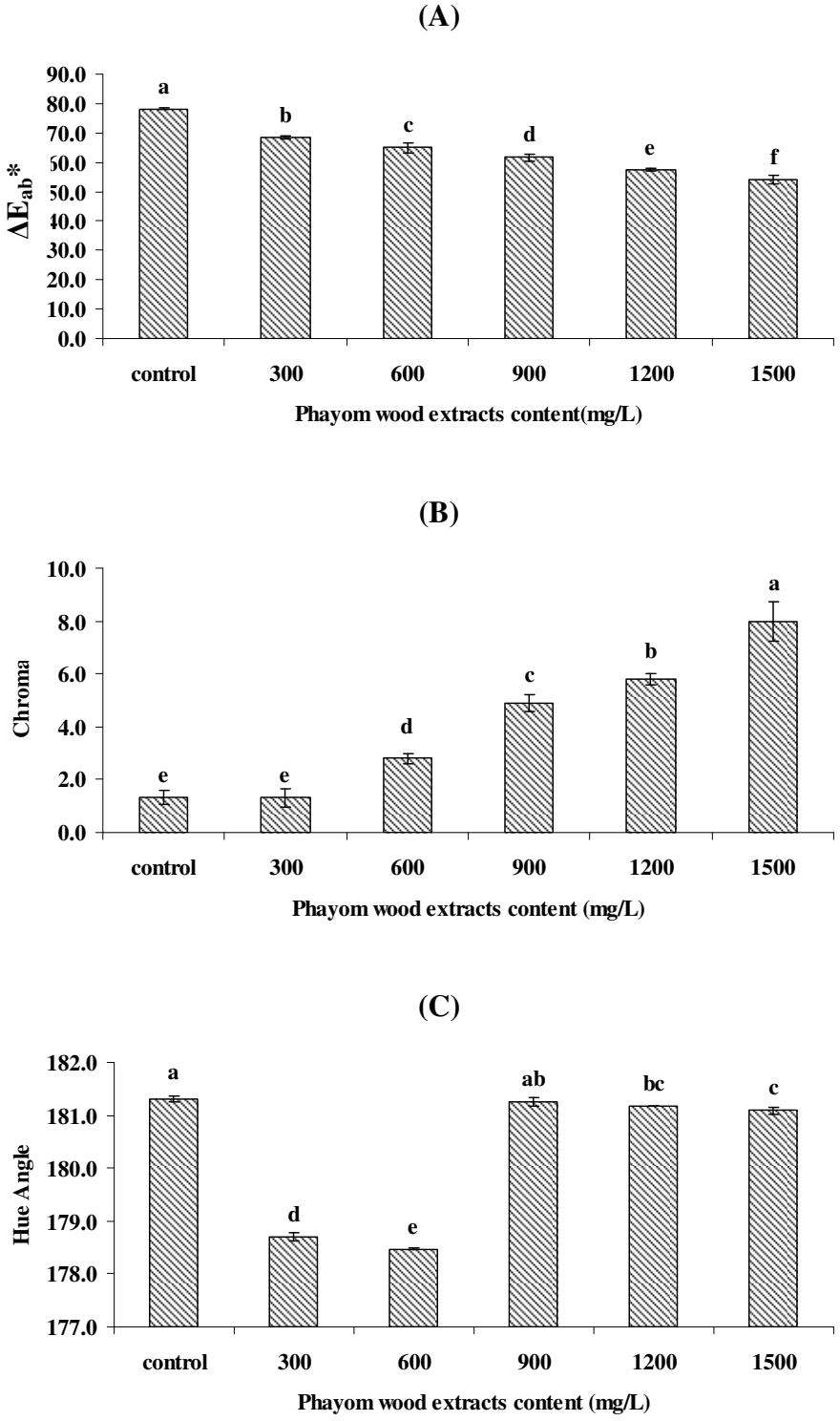


Figure 18. ΔE_{ab}* (A), chroma (B) and hue angle (C) of edible HPMC films as a function of Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

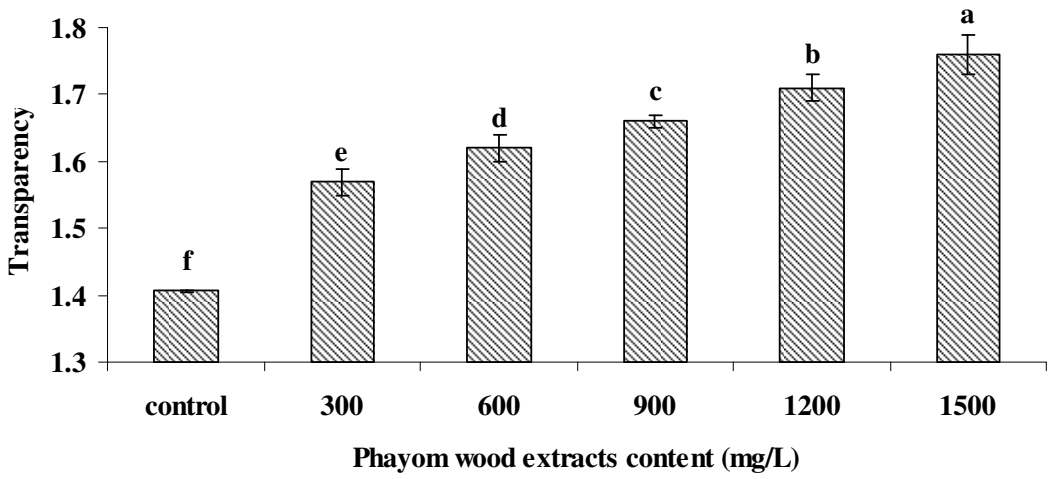


Figure 19. Transparency of edible HPMC films as a function of Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

4.2.4 Morphology of the films

Morphology observed by scanning electron microscope (SEM). SEM micrographs of edible HPMC films incorporated with Phayom wood extracts at different amounts fill and unfill Phayom wood extracts are shown in Figure 20. The control film (unfill Phayom wood extracts) had the smooth and continuous surface without grainy and porous structure. This indicated that film with ordered matrix was formed. With the incorporation of Phayom wood extracts, the surface of film became rougher, especially with increasing Phayom wood extracts content. Edible HPMC films incorporated 900 mg/L of Phayom wood extracts had denser and smoother surface than the edible HPMC films incorporated of 1500 mg/L of Phayom wood extracts.

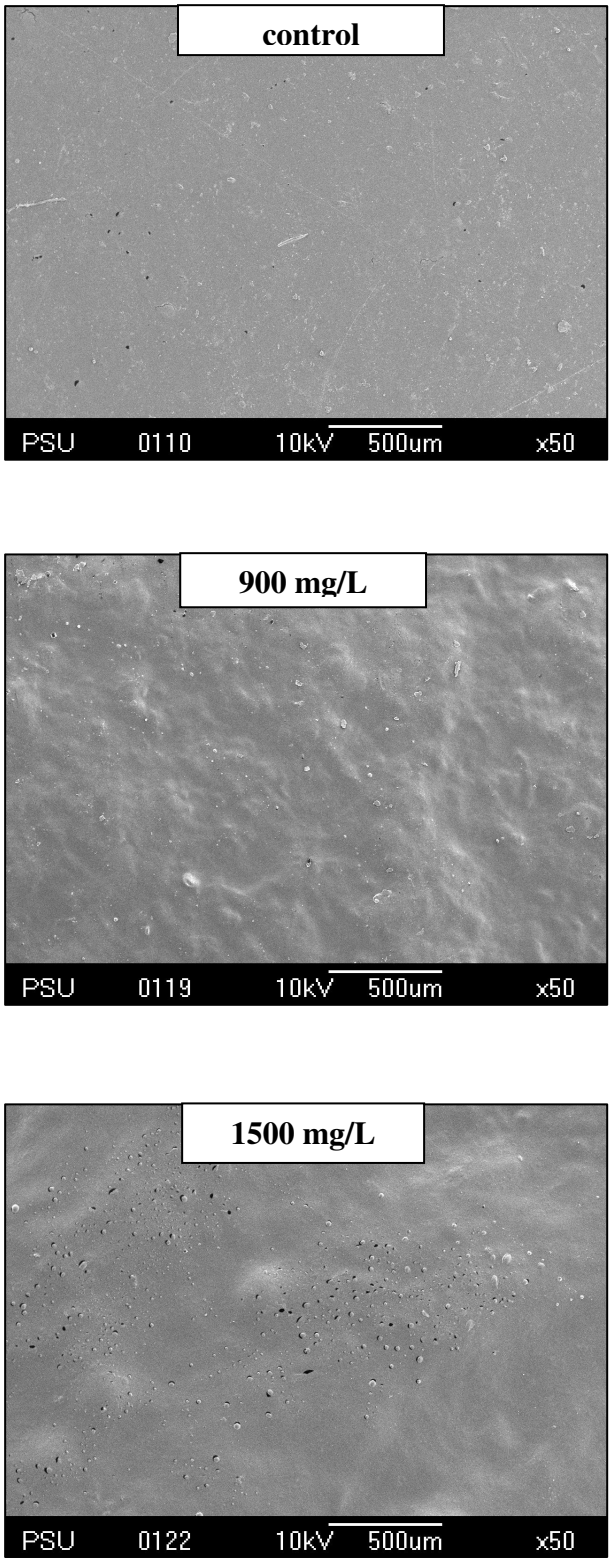


Figure 20. Scanning electron micrograph of edible HPMC films containing 900 mg/L and 1500 mg/L Phayom wood extracts**.**

5. Effect of the ratio between Kiam and Phayom wood extracts on antimicrobial and properties of edible HPMC films

Edible films from HPMC were prepared and the influence of the ratio of Kiam and Phayom wood extracts (0:100, 25:75, 50:50, 75:25 and 100:0) on the antimicrobial properties, mechanical properties, water barrier properties and miscibility of edible HPMC films were investigated. The 3 folds of MBC (900 mg/L) of wood extracts were applied into the edible HPMC films (selected from previous study according to the antimicrobial inhibitory and mechanical property of HPMC films incorporated with Kiam wood extracts compared to Polyvinyl Chloride, PVC).

5.1Effect of the ratio between Kiam and Phayom wood extracts antimicrobial activity of edible HPMC films

Kiam and Phayom wood extracts incorporated into edible HPMC films exhibited different inhibition level against *E. coli, S. aureus and L. monocytogenes* as shown in Table 5. Edible HPMC films unfilled Kiam and Phayom wood extracts were not effective against any tested microorganisms. The results showed that, the inhibition zone increase with increasing the ratio of Kiam and Phayom wood extracts. Low content of Kiam (25:75) wood extracts showed meager inhibition of tested microorganism. The results demonstrated that the zone of inhibition subjected *E. coli*, *S. aureus* and *L. monocytogenes* varied from 17.50$\pm$0.58 to 19.67$\pm$0.58, 16.33$\pm$0.58 to 20.00$\pm$1.00 and 17.00$\pm$0.58 to 22.67$\pm$1.53 mm diameter when Kiam wood extract increased from 25 to 100%. According to these results reveal that edible HPMC films incorporating with Kiam wood extracts has higher potential antimicrobial activity than Phayom wood extracts. It could be resulted from the fact that Kiam wood extracts contained higher tannic acid (85.40 mg/L) than Phayom wood extracts (32.09 mg/L). Comparatively, *E. coli* was less sensitive to the inhibitory activity of the Kiam and Phayom wood extracts incorporated in HPMC edible films than *S. aureus* and *L. monocytogenes* with was lesser inhibited at same content of Kiam and Phayom wood extracts.

Table 5. Effect of the ratio between Kiam and Phayom wood extracts on antimicrobial properties of edible HPMC films against *E. coli*, *S. aureus* and *L. monocytogenes*.

Bacteria types	Kiam: Phayom wood extracts (900 mg/L)	Inhibitory zone[A] (mm)
E. coli	0 (Control)	0.00 ± 0.00^{e}
	0:100	17.33 ± 0.58^{d}
	25:75	17.50 ± 0.50^{cd}
	50:50	18.33 ± 0.58^{bc}
	75:25	18.50 ± 0.50^{b}
	100:0	19.67 ± 0.58^{a}
S. aureus	0 (Control)	0.00 ± 0.00^{d}
	0:100	19.00 ± 0.00^{ab}
	25:75	16.33 ± 0.58^{c}
	50:50	18.67 ± 0.58^{b}
	75:25	19.67 ± 0.58^{ab}
	100:0	20.00 ± 1.00^{a}
L. monocytogenes	0 (Control)	0.00 ± 0.00^{e}
	0:100	20.33 ± 0.58^{bc}
	25:75	17.00 ± 0.58^{d}
	50:50	19.00 ± 1.00^{c}
	75:25	20.67 ± 0.58^{b}
	100:0	22.67 ± 1.53^{a}

[A] Values are measurements of diameter of inhibitory zone and expressed in mm. Values (n=4) with different superscript letters are significantly different ($p<0.05$). The diameter of edible HPMC films discs were 16 mm.

5.2 Effect of the ratio between Kiam and Phayom wood extracts on the properties of edible HPMC films

5.2.1 Tensile strength (TS) and elongation at break (ε)

Tensile strength (TS), elongation at break (ε) are parameter that relate mechanical properties of films to their chemical structure (McHugh and Krochta, 1994b) TS expresses the maximum stress developed in a film during tensile testing (Gennadios, 1994). The TS and ε changes of the edible HPMC films incorporated with Kiam and Phayom wood extracts are summarized in Figure 21. The TS varied from 25.14 to 26.94 MPa. Incorporated of Kiam and Phayom wood extracts markedly affected films TS, as seen in the reduced TS value as addition of Kiam and Phayom wood extracts. It is reasonable due to the presence Kiam and Phayom wood extract as an additive material. Therefore, addition of Kiam and Phayom wood extracts caused a reduction of TS. These results were similar to those reported by Pavlath *et al*. (1999). The results demonstrated that the TS of edible HPMC films trend to increase with the addition Kiam woods extracts, and the maximum occurred at the Kiam and Phayom woods extract of 100:0. The increasing TS values of the edible HPMC films, with the increase of Kiam and Phayom woods extract ratios from 25:75 to 100:0. The content of phenolic compounds (tannic acid) of Kiam wood extracts (85.40 mg/L) contains higher than Phayom wood extracts (32.09 mg/L) are attributable to a higher formation of intermolecular between Kiam wood extracts and HPMC molecules than Phayom woods extracts.

The value of ε was affected by the Kiam and Phayom wood extracts (Figure 21B). The ε value of edible HPMC films incorporated with Kiam and Phayom wood extracts varied from 17.73 to 22.81%. On the other hand, incorporation of Kiam and Phayom wood extracts at certain levels decreased ε. The results showed that the ε of edible HPMC films increased with an increase in the ratio of Kiam and Phayom wood extracts resulted from Kiam wood extracts have a mostly the phenolic compound and tannic acid. These results suggested that tannic acid showed the superior enhancing effect on the film strength most likely due to their multi-dentate mechanism. Haslam (1989) reported that phenolic compounds could react with more than one polymer site and led to cross-links between polymer-polymer. Besides, phenolic compounds could be converted to quinone, a polymer cross- linker, in which

new covalent cross links could be formed (Strauss and Gibson, 2004). According to the results, increasing in both TS and ε was found in edible HPMC film, tannic acid is large molecules with several functional groups, which can polymerize HPMC with a longer chain length. This might contribute to the increase in ε of resulting films.

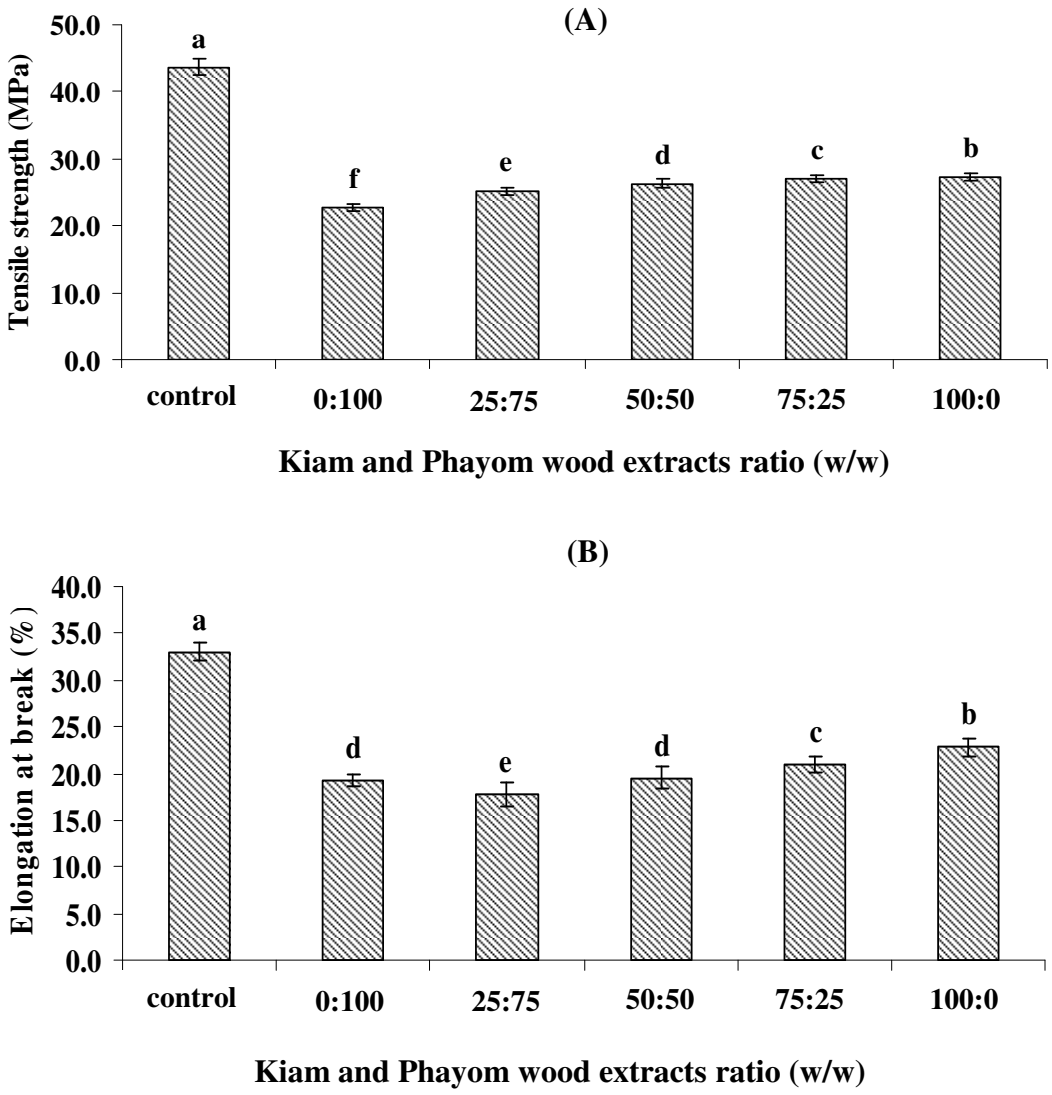


Figure 21. Tensile strength (A) and elongation at break (B) of edible HPMC films as function of Kiam and Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

5.2.2 Water vapor permeability (WVP) and film solubility (FS)

The WVP value of film or coating material should be taken into account when applying onto a moist product such as precooked beef patties. The films ability to retard moisture loss from the product (Wu, 2001) is an important characteristic that affects product quality. In the present study, WVP properties were affected by the incorporation of Kiam and Phayom wood extracts, presumably because these Kiam and Phayom wood extracts consist mostly of tannic acid are high molecular weight polyphenols. In this system, Kiam and Phayom wood extracts might contribute to extend intermolecular interactions of the structural matrix in edible HPMC films; therefore, it enhanced moisture passing through the edible films. Guilbert (1986) indicated that water vapor transfer generally occurs through the hydrophilic portion of the film and depends on the hydrophilic–hydrophobic ratio of the film components. In addition, the addition of cross-linking by tannic acid via the multidentate mechanism (Haslam, 1989) might induce the formation of polymer bundles, which could not form the continuous and fine matrix. This would cause the irregular film, in which water vapor could permeate more easily. Ou *et al*. (2005) reported the presumed reason was that tannin acid had many hydroxy groups, which could combine with water so the apparent WVP did change.

The WVP of edible HPMC films decreased with an increased in content of Kiam wood extracts. The WVP value varied from 23.14 to 20.09 gmm/m^2day.kPa. By the reason of Kiam wood extracts contained higher content of phenolic compounds (tannic acid) than Phayom wood extracts are attributable to a higher formation of intermolecular between Kiam wood extracts and HPMC molecules than Phayom woods extracts. Nuthong *et al*. (2009) reported that types and concentration of phenolic compounds affect the WVP of edible biopolymer films, mostly addition of phenolic compounds results in decrease in WVP. This might be due to phenolic compounds induced the interaction between molecule of resulted films. Cao *et al*. (2007) reported that tannic acid slightly decreased WVP of gelatin films. The differences in WVP of films added with different phenolic compounds were possibly due to the varying film polymer matrixes formed. The edible HPMC films incorporated with Kiam and Phayom wood extracts were clearly not dispersed without visual loss of integrity after a 24 h immersion in water.

Irrespective of the effect of Kiam and Phayom wood extracts, as addition of Kiam and Phayom wood extracts resulted in decreased in FS (Figure 22). It could be hastily concluded that wood extracts enhance FS in water. Low molecular weight of phenolic compounds (i.e. tannic acid) formed during storage of film-forming solution and immobilized in the network (Cuq *et al*., 1995) could thus constitute the HPMC-based materials that solubilize in water. The dry matter solubilized in water is likely to be constituted mainly by the plasticizer (additive). This water solubility behavior could not be generalized, and understanding the FS remains a complex subject. Plasticizer solubilization in water was already observed for films based on wheat gluten or treated soy proteins or produced by transglutaminase catalytic cross-linking of whey protein (Gontard *et al*., 1992). Stuchell and Krochta (1994) have pointed out to the increase in FS in water when the hydrophillic content increased for treated whey protein-and soy protein-based films. A decrease in the polymer network interaction density due to the plasticizer presence was thus associated with this increase in solubility properties. The lowest FS of edible HPMC films plasticized by 75 and 100% of Kiam wood extracts was observed (Figure 22B). It could be explained that, higher content of Kiam wood extracts, there are less molecules of wood extracts untrapped in the cross linked network and unable to escape into solution.

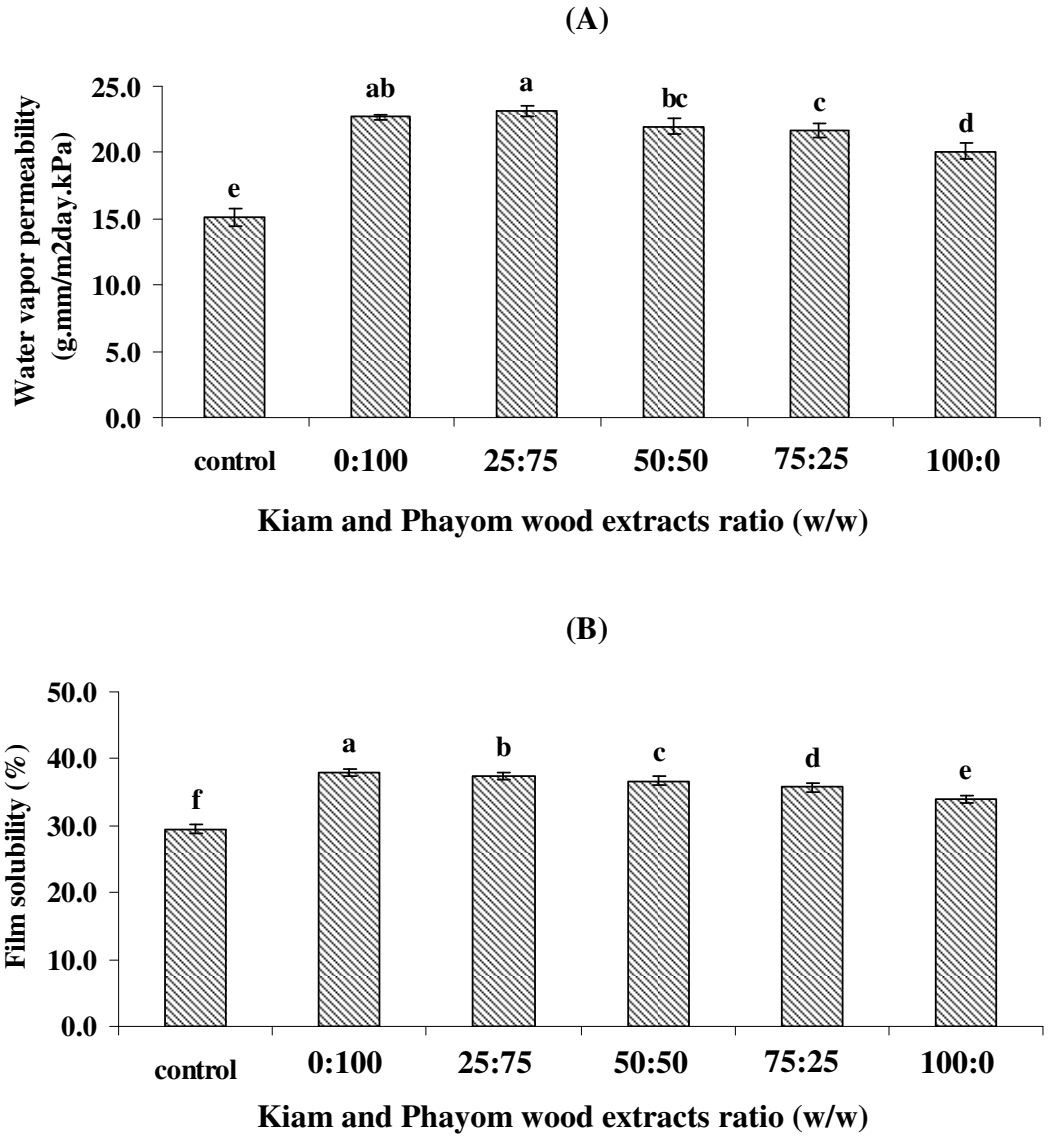


Figure 22. Water vapor permeability (A) and film solubility (B) of edible HPMC films as a function of Kiam and Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

5.2.3 Color and Transparency

The results of the edible HPMC films color measurements were expressed in accordance with the CIELAB system, and the rectangular coordinates (L*, a* and b*) were defined. The color of films was affected by the Kiam and Phayom wood extracts. L*, a* and b* value of edible HPMC films incorporated Kiam and Phayom wood extracts at different ratios are shown in Figure 23. In general, the films became darker and more red yellowish when Kiam and Phayom wood extracts was incorporated as evidenced by the increase in L*, a*, b* and chroma value ($p<0.05$). The results showed that b* and chroma value demonstrated significantly increased ($p<0.05$) as the ratio of Kiam and Phayom wood extracts increased (Figure 23 and 24), while other colors value showed not significantly different ($p>0.05$). It might be the fact that Kiam wood extracts had darker and more red yellowish that Phayom wood extracts. The transparency of edible HPMC films incorporated with Kiam and Phayom wood extracts at different levels in the presence shown in Figure 25. Films incorporated with Kiam and Phayom wood extracts were less transparent as indicated by the greater transparency values. However, marked differences were found among films incorporated with differences level of Kiam and Phayom wood extracts ($p<0.05$). Therefore, an increase in Kiam and Phayom wood extracts levels resulted in more opacity as indicated by the increase in transparency value.

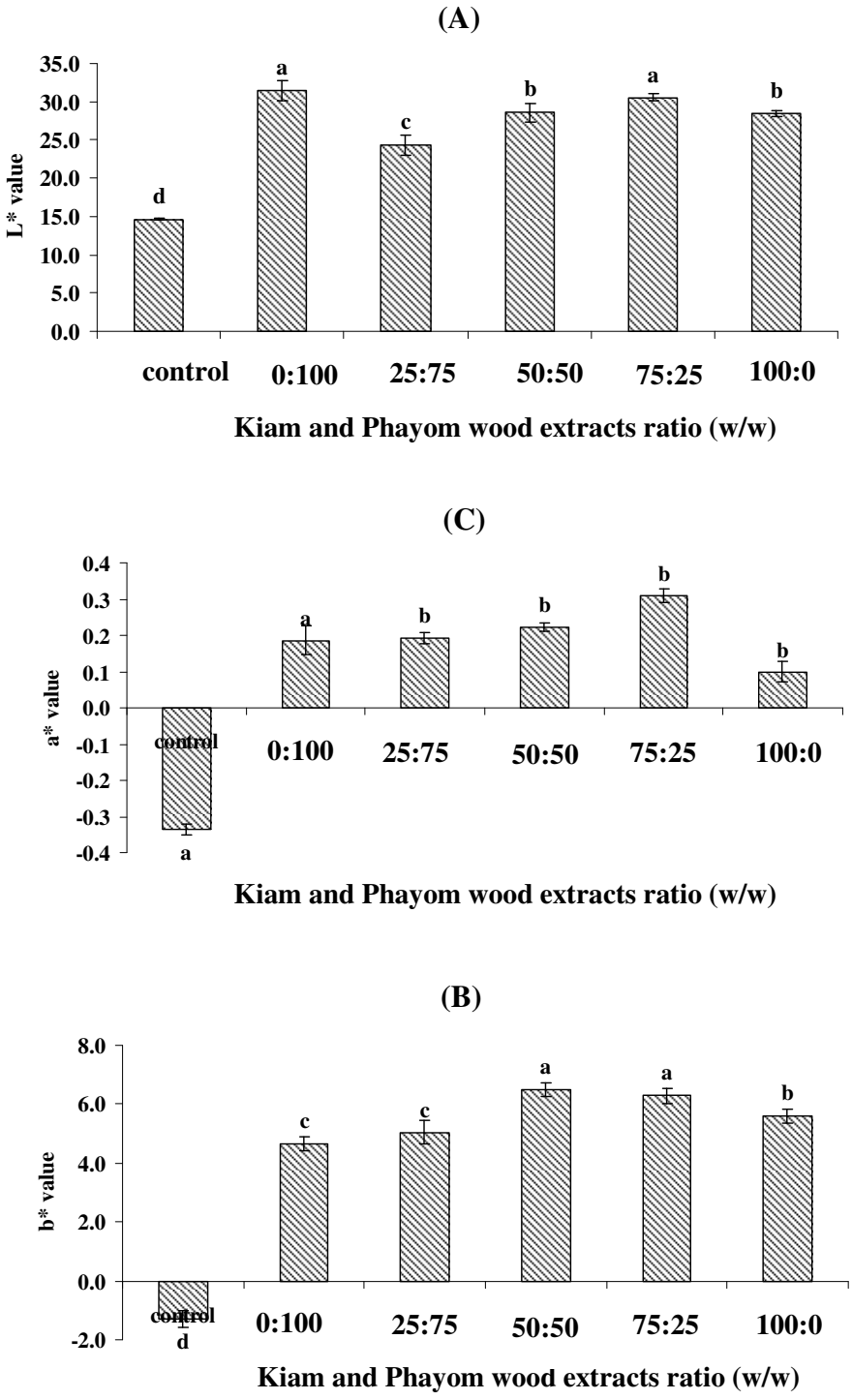


Figure 23. L* (A), a*(B) and b* (B) values of edible HPMC films as a function of Kiam and Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

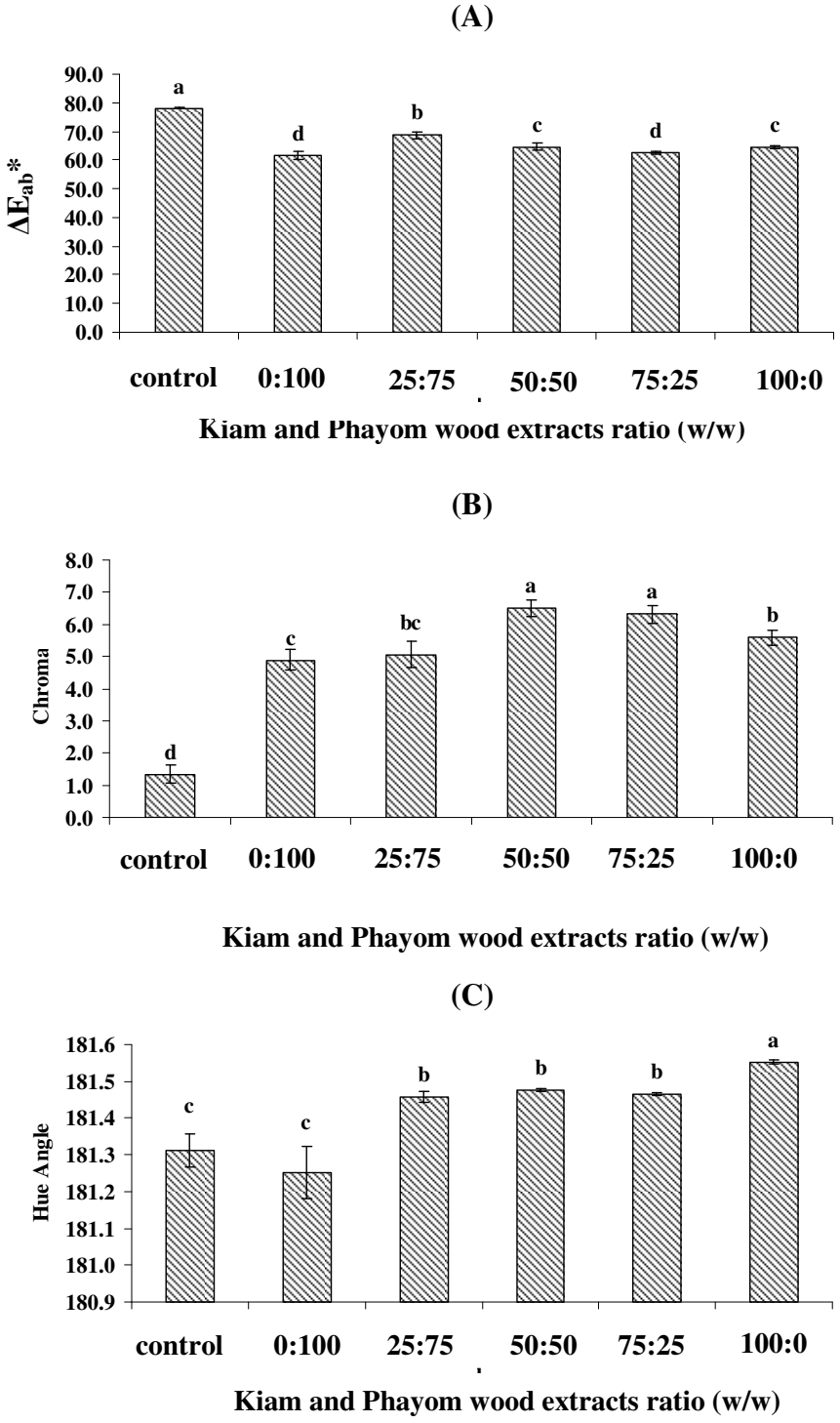


Figure 24. ΔE_{ab}* (A), chroma (B) and hue angle (C) of edible HPMC films as a function of Kiam and Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

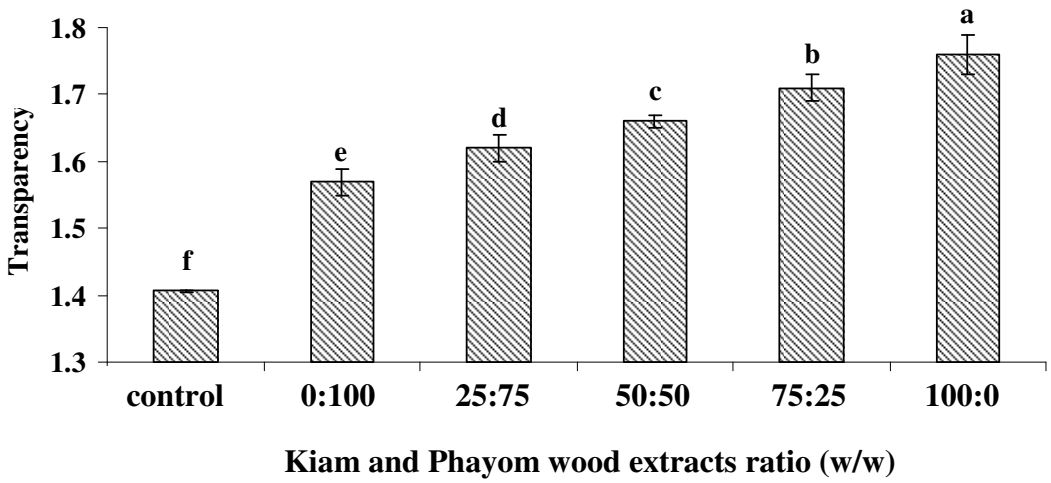


Figure 25. Transparency of edible HPMC films as a function of Kiam and Phayom wood extracts concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

6. Enhancing antimicrobial activity of chitosan films by incorporating Kiam wood extracts

Antimicrobial effect of chitosan edible films incorporating Kiam wood extracts (900 mg/L or 3 MBC selected according to the antimicrobial properties against tested microorganism and mechanical properties of HPMC films) was compared between chitosan films fill and unfill Kiam wood extracts. Mechanical and physical properties were characterized, and antimicrobial efficacy was assessed against three food pathogenic bacteria namely *E. coli, S. aureus* and *L. monocytogenes*.

6.1 Antimicrobial activity of chitosan films incorporated with Kiam wood extracts

The details of antimicrobial activity of chitosan edible films incorporated with Kiam wood extracts exhibited different inhibition levels against *E. coli*, *S. aureus* and *L. monocytogenes* as shown in Table 6. The inhibitory activity was measured based on clear zone surrounding circular film strips. Measurement of clear

zone diameter included diameter of film stripa, therefore, the values were always higher than the diameter of film strips (16 mm) whenever clearing zone was present. If there is no inhibitory zone, and furthermore, the diameter was valued as zero. Contact area was used to evaluate growth inhibition underneath film discs in direct contact with target microorganisms in agar (Pranoto, 2005). In these response study, In terms of surrounding clearing zone, the control film (chitosan films) show inhibitory effect against all tested microorganisms. Incorporating Kiam wood extracts as into chitosan film revealed higher antimicrobial effect. The inhibitions of Kiam wood extracts incorporated into chitosan films were stronger than those of chitosan unfill Kiam wood extracts. Chitosan films incorporated Kiam wood extracts exhibited different inhibition levels against *E. coli*, *S. aureus* and *L. monocytogenes*. Comparatively, *E. coli* was less sensitive than *L. monocytogenes* and *S. aureus*. The results showed that the zone of inhibition subjected *E. coli*, *S. aureus* and *L. monocytogenes* increased from 17.00$\pm$0.5 to 21.17$\pm$0.29, 18.00$\pm$0.50 to 23.50$\pm$0.50 and 19.50$\pm$0.50 to 25.50$\pm$0.50 mm diameter when Kiam wood extract was incorporated ($p<0.05$) shown in Table 6. The higher inhibitory effects of chitosan film contained Kiam wood extracts may be attributed to the main phenolic compounds in the Kiam wood extracts. Therefore, it hindered the release of potassium sorbate to inhibit microorganism surrounding film strips during agar diffusion assay. Among inhibited microorganisms, *L. monocytogenes* was the most sensitive and susceptible to Kiam wood extracts. The antimicrobial agents were obviously more effective against Gram-positive bacteria than the Gram-negative bacteria studied.

This is to be expected as the cell wall structures of these categories of bacteria are different and Gram-positive bacteria are more sensitive to such agents. According to Brody (2001), the antimicrobial effect of chitosan occurred without migration of active agents. As chitosan is in a solid form, therefore, only organisms in direct contact with the active sites of chitosan is inhibited. Chitosan is incapable to diffuse through the adjacent agar media (Coma *et al*., 2002). The agar diffusion test is a method commonly used to examine antimicrobial activity regarding the diffusion of the compound tested through water-containing agar plate. The diffusion itself is dependent on the size, shape and polarity of the diffusing material. The chemical structure and the cross linking level of the films also affect this phenomenon (Cagri *et*

al., 2001). When antimicrobial agents are incorporated, there will be diffusing materials through agar gel, and furthermore, resulting clearing zone on the bacterial growth. Incorporating antimicrobial agents into chitosan edible film thus improves antimicrobial efficacy of chitosan, as diffused antimicrobial actively would add to non migrated antimicrobial potency of chitosan.

Table 6. Antimicrobial activity of edible chitosan films incorporated Kiam wood extracts against *E. coli*, *S. aureus* and *L. monocytogenes*.

Bacteria types	Kiam wood extracts (900 mg/L)	Inhibitory zone[A] (mm)
E. coli	Chitosan films	17.00 ± 0.50^{b}
	Kiam wood extracts + Chitosan films	21.17 ± 0.29^{a}
S. aureus	Chitosan films	18.00 ± 0.50^{b}
	Kiam wood extracts + Chitosan films	23.50 ± 0.50^{a}
L. monocytogenes	Chitosan films	19.50 ± 0.50^{b}
	Kiam wood extracts + Chitosan films	25.50 ± 0.50^{a}

[A] Values are measurements of diameter of inhibitory zone and expressed in mm. Values (n=4) with different superscript letters are significantly different ($p<0.05$). The diameter of edible HPMC films discs were 16 mm.

6.2 Properties of edible chitosan films incorporated with Kiam wood extracts

6.2.1 Tensile strength (TS) and elongation at break (ε)

Tensile strength (TS) and elongation at break (ε) of chitosan films unfill and fill Kiam wood extracts are shown in Figure 26. It shows the effect of Kiam wood extracts incorporated into chitosan films and the resultant change in properties. TS value of the chitosan films incorporated with Kiam wood extracts was 38.93 MPa and much lower than that of chitosan films unfilled Kiam wood extracts (45.68 MPa). A significant ($p<0.05$) reduction of TS was revealed by incorporation of Kiam wood

extracts into chitosan films. This result confirms the outcome of the report by Cagri (2001), who had concluded that incorporation of additives other than cross linking agents generally lowers TS value. The flexibility of the film is indicated by the percentage elongation (ε) value and it was found to be influenced by the Kiam wood extracts. The ε values of Kiam wood extracts incorporated chitosan films are shown in Figure 26B. Comparing with the control films, the ε values of the chitosan films decrease varied from 35.92 to 26.27% with the incorporation of Kiam wood extracts. The reduction in percentage elongation with addition of Kiam wood extracts might be due to the increase in the number of intermolecular cross-links and decrease in the intermolecular distance. The mechanical property changes were characterized by decrease in density and reversibility of intermolecular interactions occurring in the chitosan films network formed. Begi *et al*. (2001) reported that gelatin films yielded lower extensibility when glutaraldehyde was applied in the films.

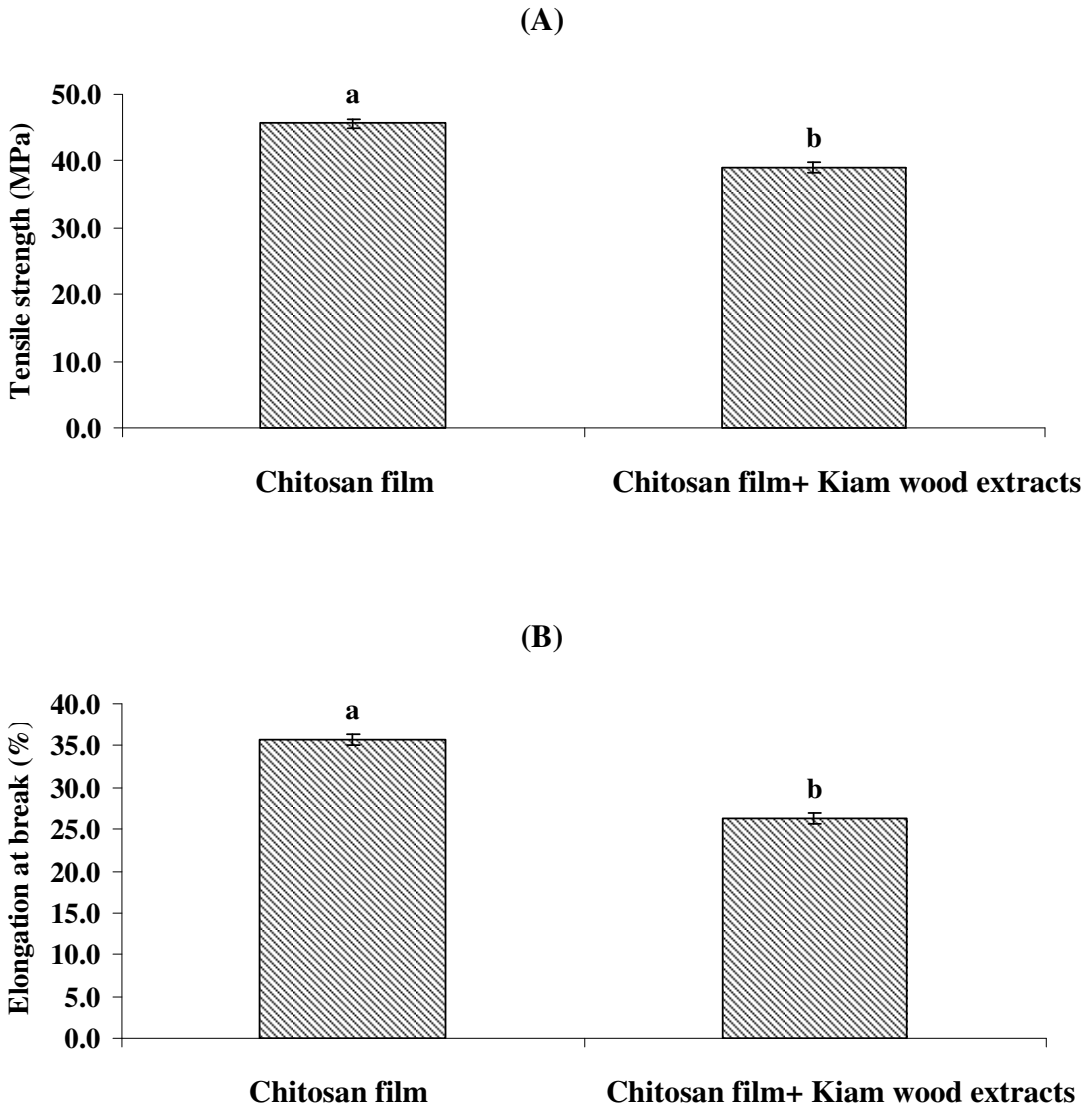


Figure 26. Tensile strength (A) and elongation at break (B) of chitosan films as a function of Kiam wood extracts (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

6.2.2 Water vapor permeability (WVP) and film solubility (FS)

Water vapor permeability (WVP) is a measure of ease of the moisture to penetrate and pass through a material. WVP value of film or coating material should be taken into account when applying onto moist product such as precooked beef patties. The films ability to retard moisture loss from the product (Wu, 2001) is an important characteristic that affects product quality. In the present study, WVP properties of chitosan films were affected by the incorporation of Kiam wood extracts. The WVP value and tended to increase as Kiam wood extracts were incorporated. It is probably occurred due to the hydrophilicity property of Kiam wood extracts. WVP value varied from 8.98 to 11.84 g.mm/m^2.day.kPa (Figure 27A). Hernandez (1994) indicated that water vapor transfer generally occurs through the hydrophilic portion of the film and depends on the hydrophilic-hydrophobic ratio of the film components. Hydrophilic groups in the film material tend to cause poor moisture barrier (Cagri *et al*., 2001). Wu (2001) reported that WVP value of edible protein films increased as antimicrobial agents was added. By the reason of the antimicrobial agents contributed to extend intermolecular interaction and furthermore, loosening the compactness of the structure. This enhanced moisture passing through the edible films and thereby increases WVP values of the films. In this system, Kiam wood extracts might contribute to extend intermolecular interactions of the structural matrix in edible HPMC film, therefore, it enhanced moisture passing through the edible films. Regarding the effect of Kiam wood extracts on the FS of chitosan films, the results demonstrated that the film solubility of chitosan films increased with the addition of Kiam wood extracts. A significant ($p<0.05$) was shown in Figure 27B.

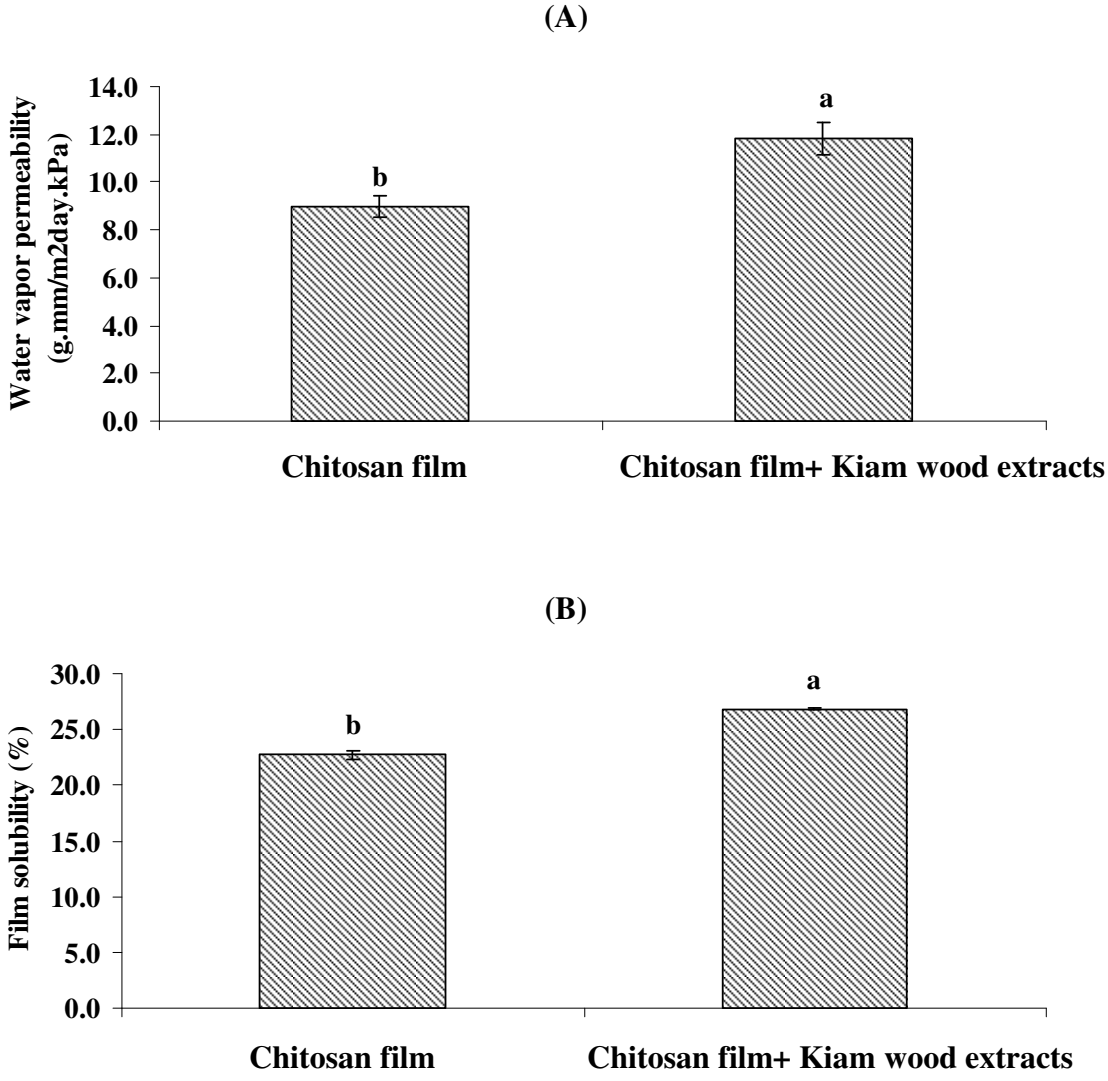


Figure 27. Water vapor permeability (A) and film solubility (B) of chitosan films as a function of Kiam wood extracts (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

6.2.3 Color and Transparency

Total color difference was observed by reading L*, a* and b* and ΔE_{ab}*, chroma and hue angle values. The color of chitosan films and chitosan films incorporated with Kiam wood extracts are shown in Figure 28 and 29. Chitosan films produced was slightly yellow. The results demonstrated that the chitosan films incorporated with Kiam wood extracts were lighter and dark brown-yellowish indicated by increasing L*, a* and b* and ΔE_{ab}*, chroma and hue angle values (Figure 28 and 29). A significant ($p<0.05$). Kiam wood extracts contained a high amount of phenolic compounds (tannin) affects the color of the resulting films. (Chanthachum and Beuchat, 1997). Hence, these pigments most likely contributed to the darker and reddish color of chitosan films. The transparency of chitosan films fill and unfill with Kiam wood extracts is shown in Figure 30. Chitosan films incorporated with Kiam wood extracts was lower transparent than chitosan film unfill Kiam wood extracts as indicated by the high transparency value. Therefore, pigment in the wood extract might affect the transparency of chitosan films ($p<0.05$).

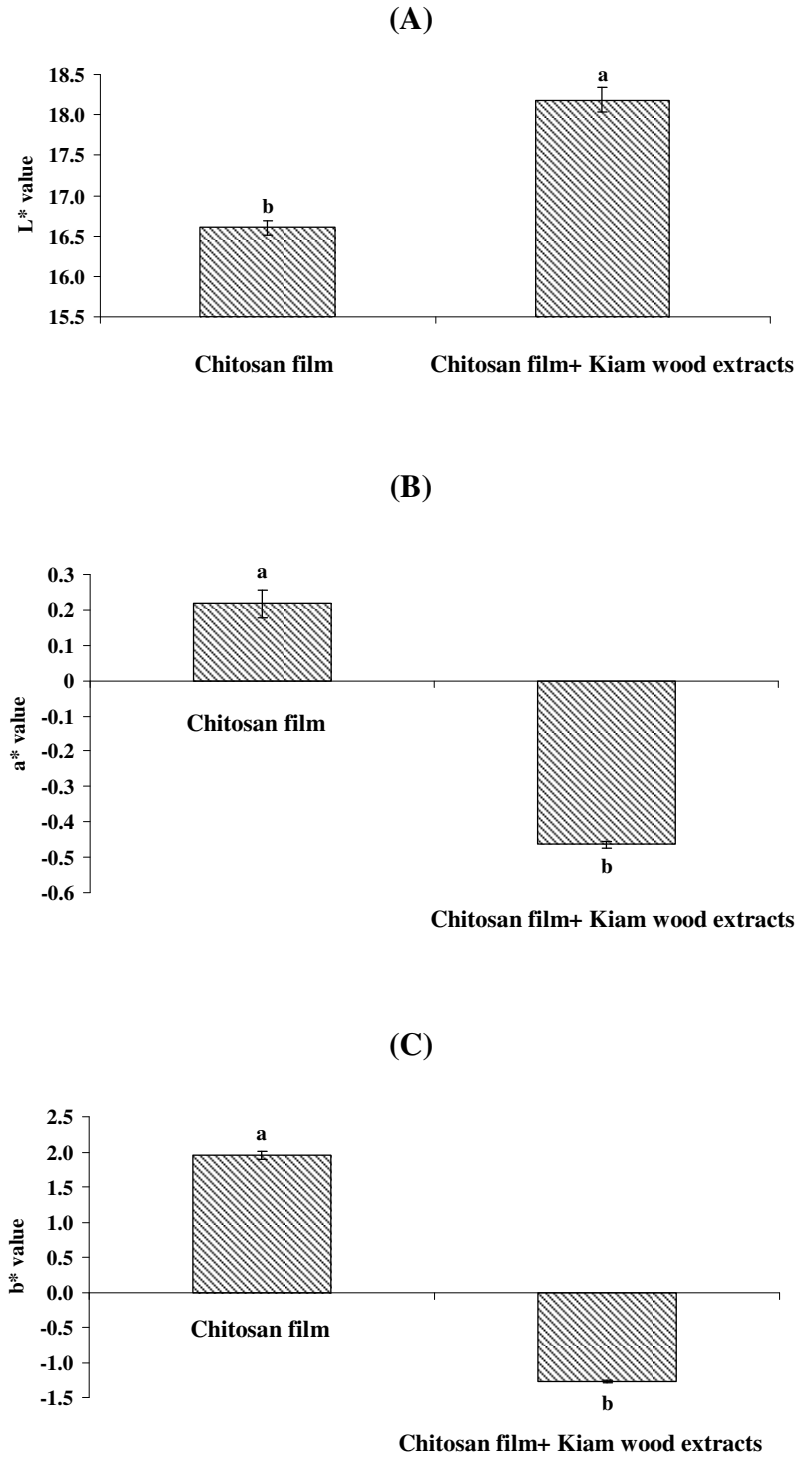


Figure 28. L* (A), a* (B) and b* (B) values of chitosan films as a function of Kiam wood extracts (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

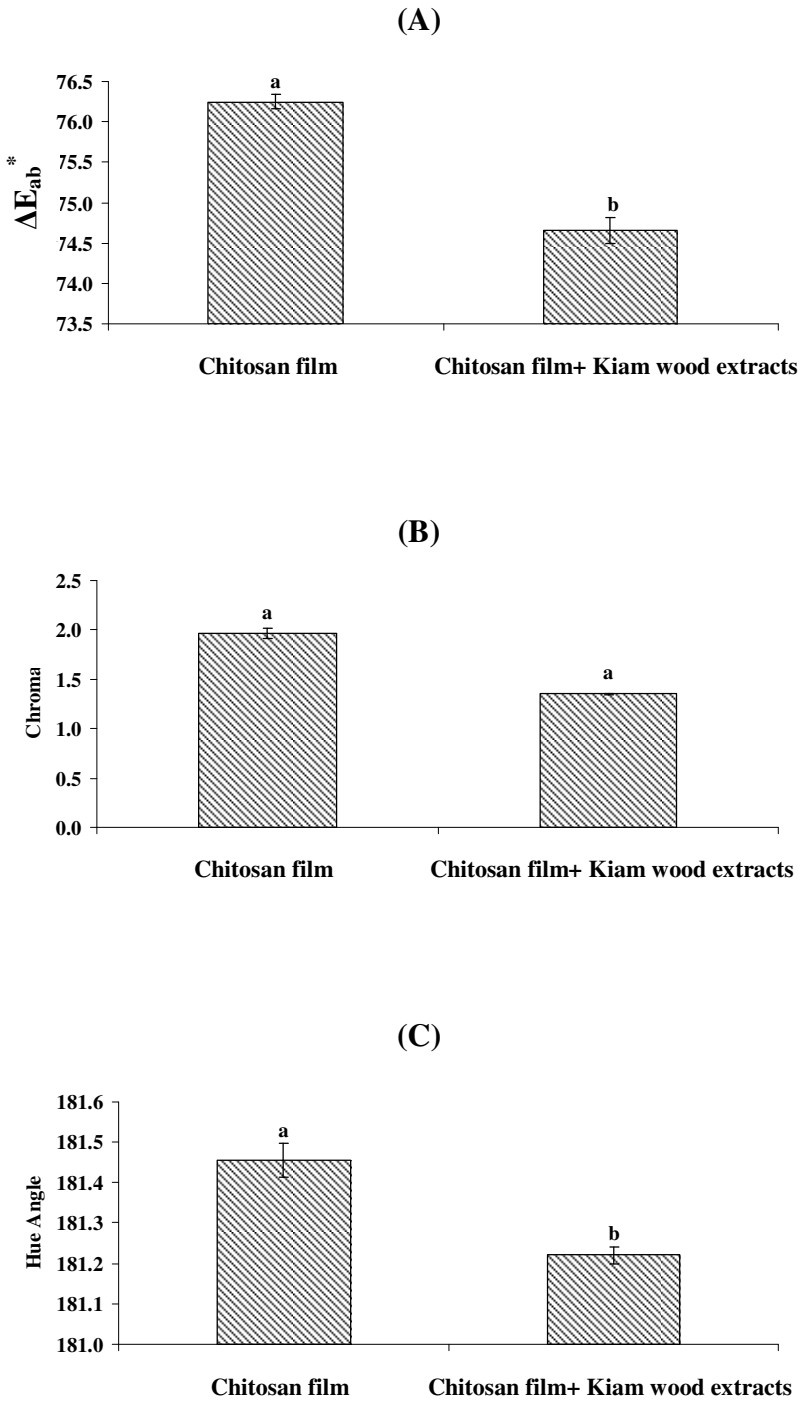


Figure 29. ΔE_{ab}^* (A), chroma (B) and hue angle (C) of chitosan films as a function of Kiam wood extracts (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

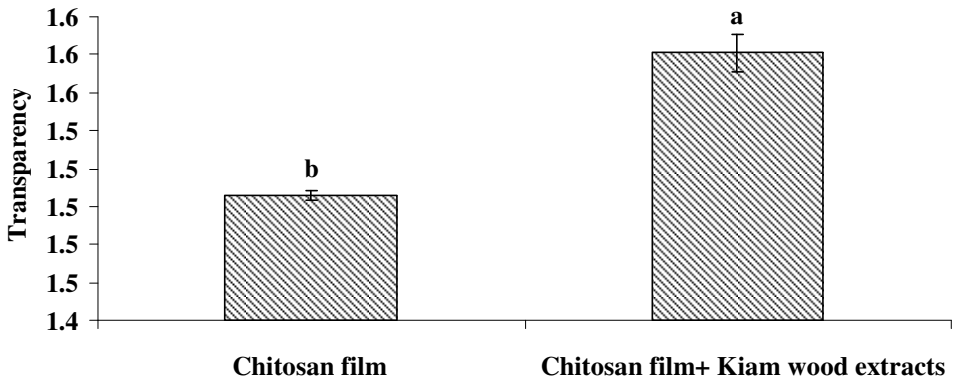


Figure 30. Transparency of chitosan films as a function of Kiam wood extracts (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

6.2 4 Morphology of the films

Morphology of the films observed by scanning electron microscope (SEM) showed that chitosan film unfilled Kiam wood extracts (control films) had smoother surface than the chitosan films filled Kiam wood extracts (Figure 31). The control films had translucent and continuous surface without grainy and porous structure. However, the chitosan films incorporated with Kiam wood extracts were not completely homogeneous. The entanglement of long chains of chitosan together with phenolic compounds would enhance the roughness of film surface. The results showed that Kiam wood extracts dispersed uniformly in chitosan film, however no occurrence of conglomeration (Figure 31).

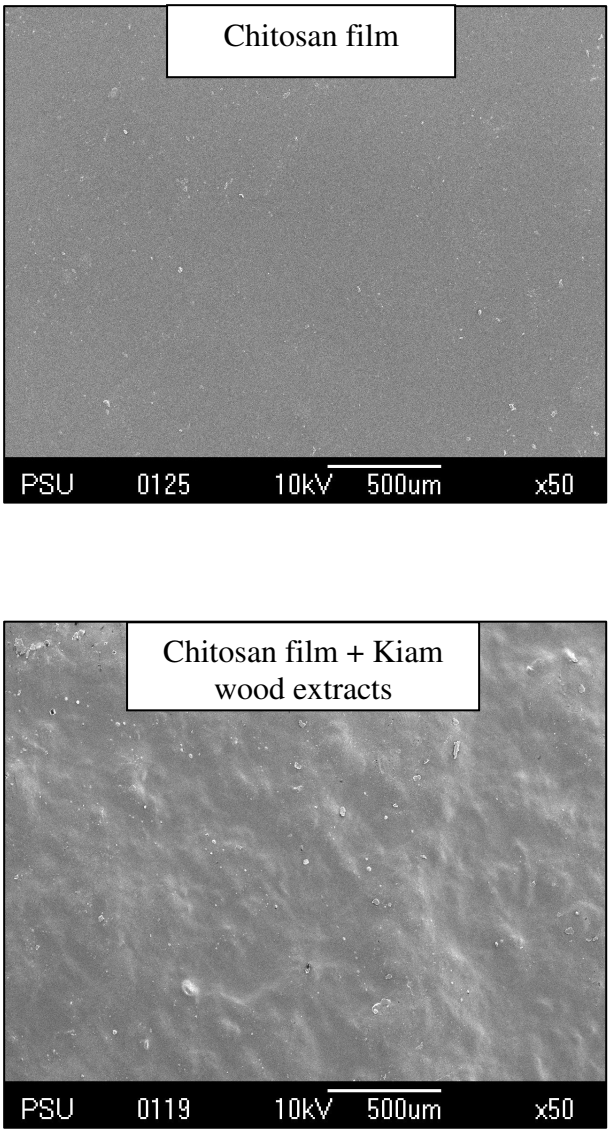


Figure 31. Scanning electron micrograph of chitosan films and chitosan film incorporated with Kiam wood extracts.

7. Differential scanning calorimeters (DSC) of edible HPMC and Chitosan films incorporated with Kiam wood extracts

Typical DSC thermograms of the edible HPMC films, chitosan films and edible HPMC films incorporated with Kiam wood extracts (900 mg/L) are depicted in Figure 32. The abrupt change in the slope of heat flow curves of films reveals their glass transition, Tg. According to the result, Tg of films shifted to the

higher temperature when Kiam wood extracts incorporated in the films. Increase in the Tg associated with the wood extracts addition was observed. Tg of the films can vary depending on the composition in films, plasticizer, lipid and the cross-linking (Audic and Chaufer, 2005). The chitosan films incorporated with Kiam wood extracts had the highest peak temperature (Figure 32) whereas the HPMC films had the lowest endotherms (Figure 32). The results demonstrated that a glass transition temperatures increase from 85.37 to 86.33, 85.37 to 86.50, 83.37 to 84.86°C in HPMC films, HPMC films incorporated with Phayom, HPMC films incorporated with Kiam wood extracts, chitosan films and chitosan films incorporated with Kiam wood extracts, respectively.

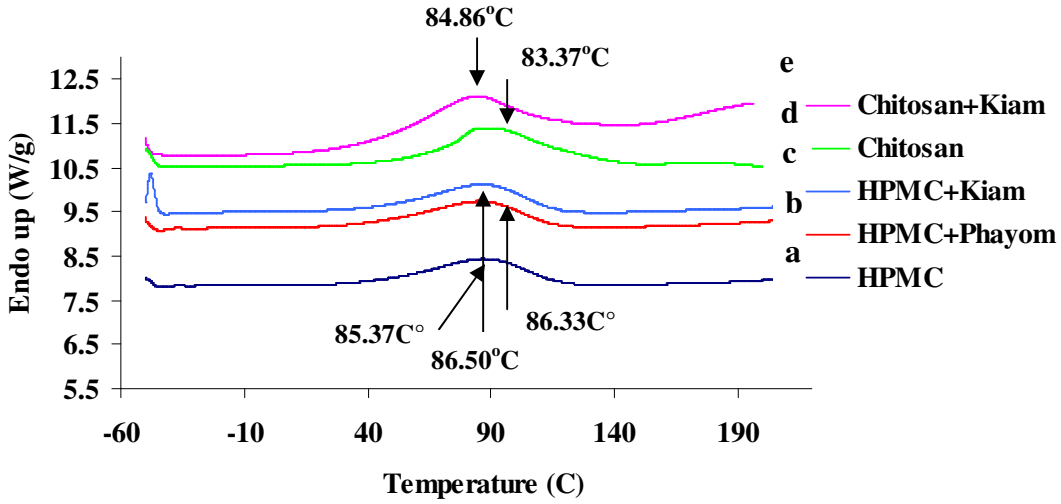


Figure 32. DSC thermogram of the edible HPMC films (a) edible HPMC films incorporated with Phayom wood extracts (b) HPMC films incorporated with Kiam wood extracts (c) chitosan films (d) chitosan films incorporated with Kiam wood extracts.

8. Application the antimicrobial films in food products (imitated crab meat and ham)

According to the effect of Kiam wood extracts presented more inhibitory on tested microorganism and also addition of Kiam wood extracts at 3 folds of MBC (900 mg/L) yielded a good in antimicrobial properties together with excellent

in mechanical properties of resulted films compare to PVC. Hence, addition of 3 folds of MBC of Kiam wood extracts were selected for application in food products.

Commercial imitated crab meat and ham was wrapped in a antimicrobial films (HPMC films incorporated with Kiam wood extracts and chitosan films incorporated with Kiam wood extracts (at 900 mg/L or 3 folds of MBC) and kept at 4^{o}C for 14 days. Wrapped both imitated crab meat and ham with polyvinylchloride (PVC) films was used as references. The food products were sampled for testing of chemical quality and microorganism every 2 days).

8.1 Effect of antimicrobial films on TBARS of food products

TBARS is an index of lipid oxidation. Changes in TBARS values of imitated crab meat and ham wrapped using different films (PVC, HPMC films incorporated with Kiam wood extracts and chitosan films incorporated with Kiam wood extracts) during the storage are shown in Figure 33. TBARS values of all samples increased continuously with increasing storage time ($p<0.05$). The results showed that after 12 day of storage, TBARS value of imitated crab meat wrapped in chitosan films incorporated with Kiam wood extracts, edible HPMC films incorporated with Kiam wood extracts and PVC films were 2.11, 2.87 and 3.31 mg.malonaldehyde/mg, while the TBRARS of ham were 2.61, 3.09 and 3.45 mg.malonaldehyde/mg, respectively. Due to the high content of polyunsaturated fatty acid in the food product (Saunders, 1994). The double bonds in an unsaturated fatty acid are locked into position when oxygen reacts with the methylene group adjacent to the double bonds resulted in lipid oxidation (Porter *et al*., 1995). The results demonstrated that food samples wrapped with chitosan films incorporated with Kiam wood extracts had lower TBARS value than HPMC films incorporated with Kiam wood extracts and PVC film, respectively. This observation indicated the fact that not only Kiam wood extracts inhibited lipid oxidation in both imitated crab meat and ham but chitosan also retarded lipid oxidation. The data revealed that the chitosan films incorporated with Kiam wood extracts was suitable for preservation of imitated crab meat and ham by inhibiting the oxidation of lipid.

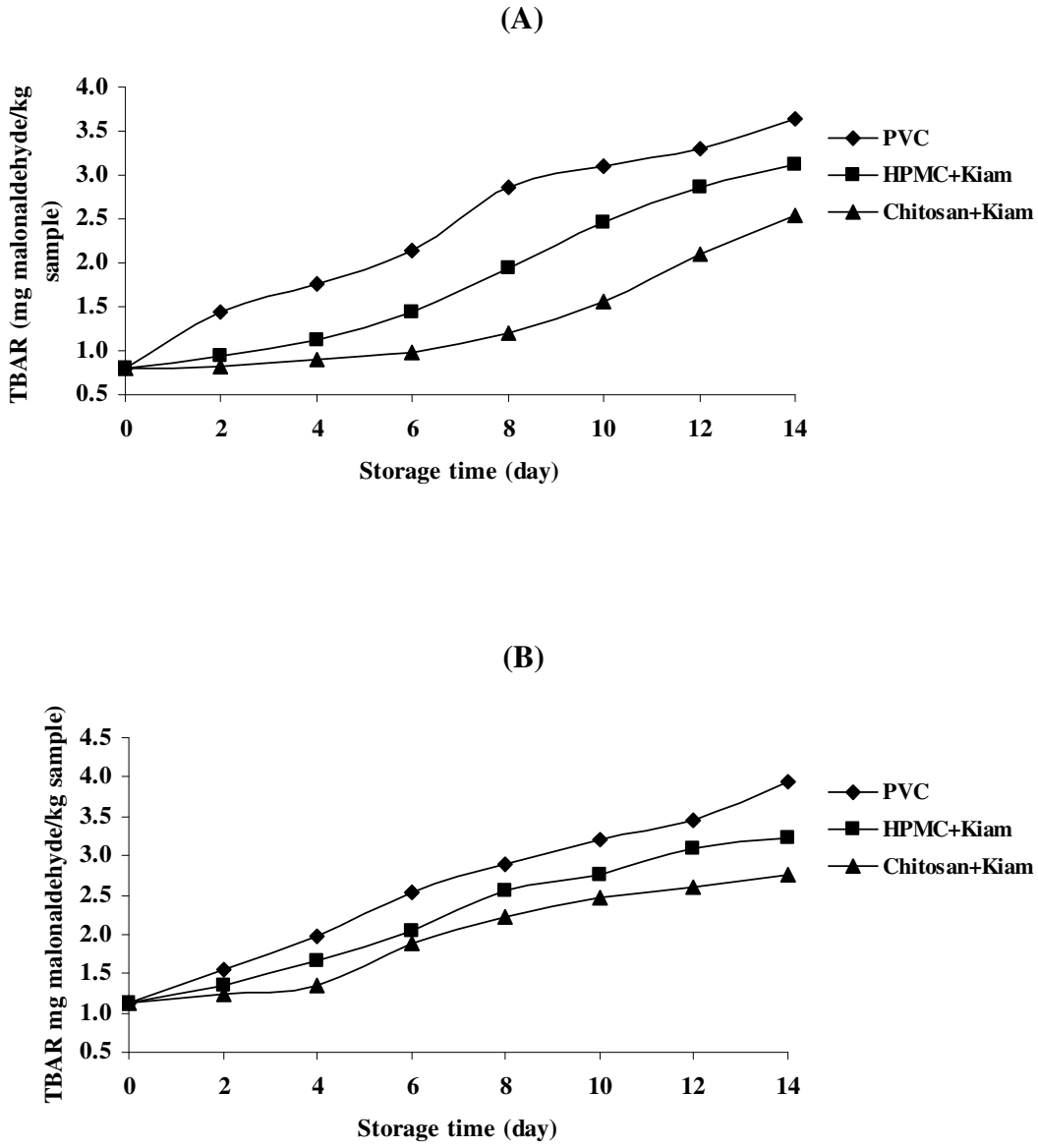


Figure 33. Effect of antimicrobial films on TBARS of imitated crab meat (A) and ham (B) during storage at 4^{o}C for 14 days.

8.2 Effect of antimicrobial films on moisture content of food products

Moisture content of imitated crab meat and ham changed as the storage time increased ($p<0.05$) (Figure 34). The moisture content of the imitated crab meat from all treatments tended to increase over time with the range of 66.32-73.70, 66.32-75.9 and 66.32-79.30% and 69.20-79.80, 69.20-82.24 and 69.20-86.70% for ham when chitosan films incorporated with Kiam wood extracts, edible HPMC films incorporated with Kiam wood extracts and PVC films were applied, respectively. Boo *et al*. (2005) reported that moisture content of dried anchoviella increased during storage with various packaging materials. The results showed that, both food products wrapped with chitosan films incorporated with Kiam wood extracts had lower moisture content than food products wrapped with edible HPMC films incorporated with Kiam wood extracts and food products wrapped with PVC films during storage period, respectively (Figure 34). It could be resulted from the chitosan films and edible HPMC films permit lower water vapor transmittance than PVC films, when food products wrapped in this material has lower moisture content. Moreover, chitosan and edible HPMC could also absorbed the moisture from the food products during storage resulted in lower moisture content of food products wrapped with chitosan films and edible HPMC films incorporated with Kiam wood extracts.

The stability of food and their resistance to oxidation is a function of moisture content. Most biochemical reactions are accelerated by increasing moisture content, but there are other for which the speed of reaction is greatest at low relative humidity. For instance, auto-oxidative processes are particularly favored by very low moisture content. In addition to its activity as a solvent and reactant, water can participate directly in hydrolytic cleavage, the products of which can participate in nonenzymatic browning (Boo *et al*., 2005) According to the result, chitosan films and edible HPMC films incorporated with Kiam wood extracts exhibited the superior water vapor barrier property than PVC films.

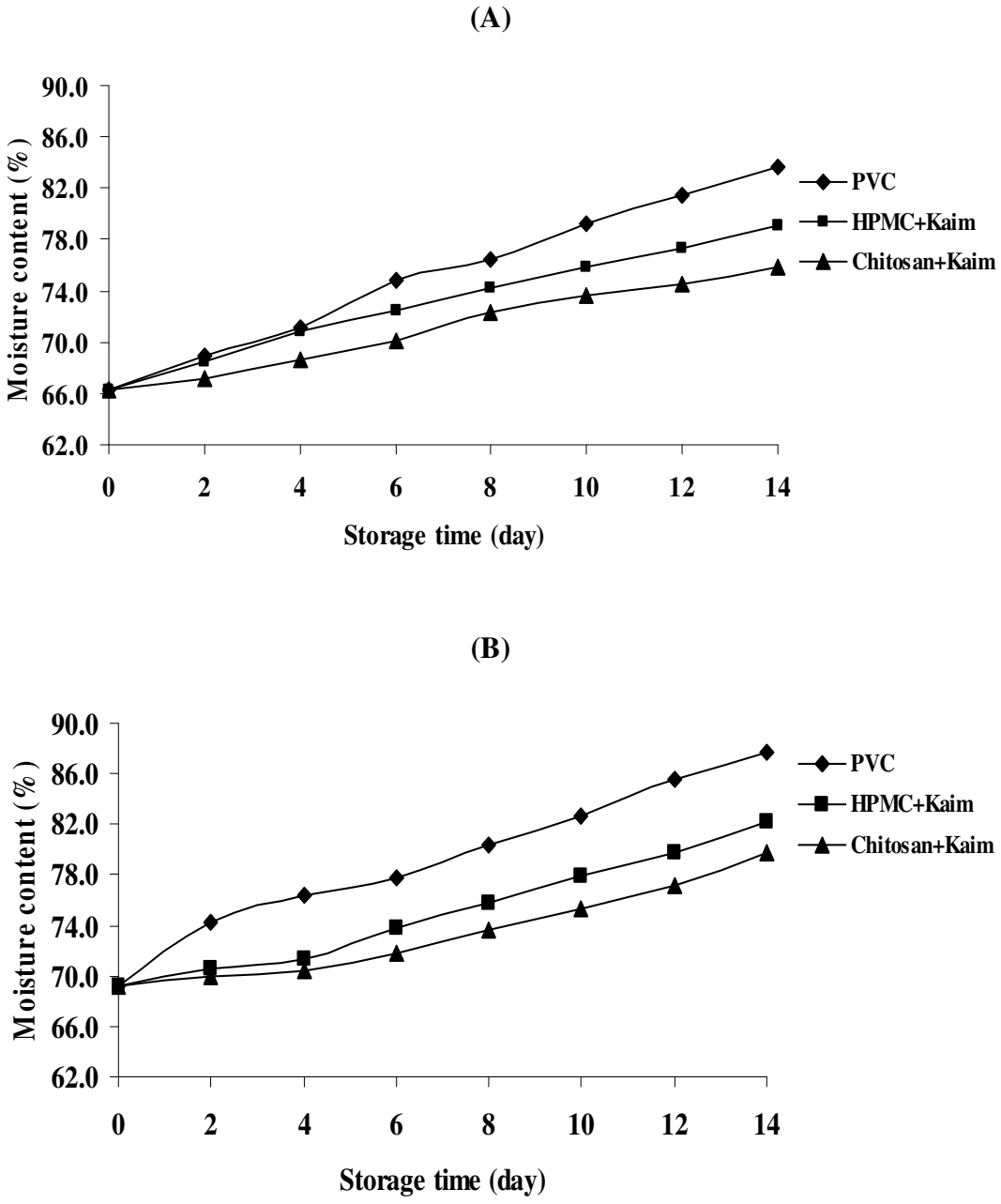


Figure 34. Effect of antimicrobial films on moisture content of imitated crab meat (A) and ham (B) during storage at 4^{o}C for 14 days.

8.3 Effect of antimicrobial films on water activity of food products

As seen from all results, wrapping material type affected the a_w of imitated crab meat and ham significantly ($p<0.05$). The a_w of the imitated crab meat from all treatments tended to increase over time with the range of 0.943- 0.959, 0.943-0.968 and 0.943-0.984% and 0.946-0.971, 0.946-0.979 and 0.946- 0.988% for ham when chitosan films incorporated with Kiam wood extracts, edible HPMC film incorporated with Kiam wood extracts and PVC films were applied, respectively. The results demonstrated that the a_w of the imitated crab meat and ham wrapped with chitosan film incorporated with Kiam wood extracts was lower than those wrapped with edible HPMC films incorporated with Kiam wood extracts and PVC films, respectively (Figure 35). The increase in a_w in PVC films wrapped sample may be due to penetration of water through the wrapping material from surrounding. The longer storage time resulted in higher a_w (Figure 35).

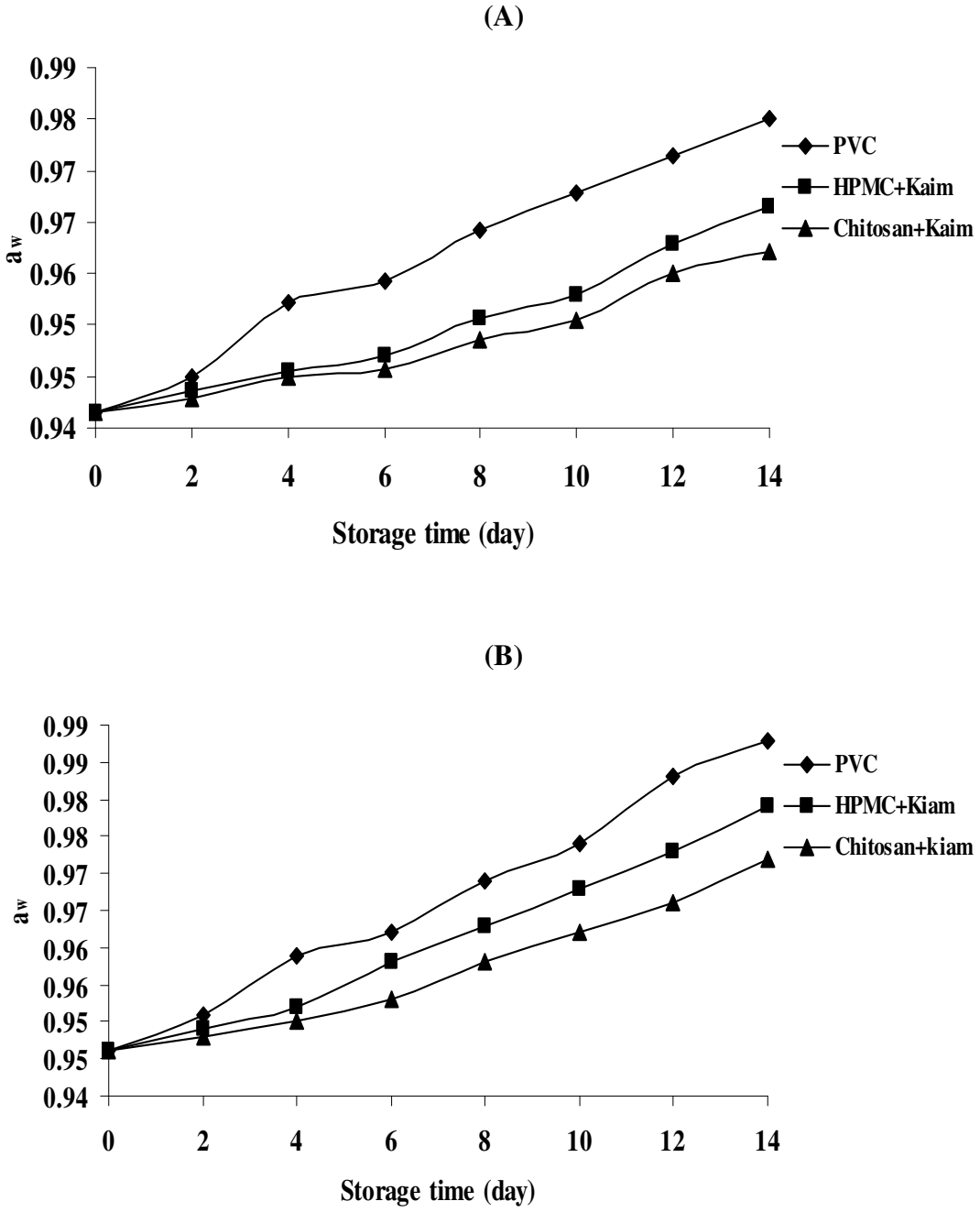


Figure 35. Effect of antimicrobial films on water activity (a_w) of imitated crab meat (A) and ham (B) during storage at 4°C for 14 days.

8.4 Effect of antimicrobial films on color of food products

L*, a* and b* values of imitated crab meat and ham wrapped with three different films are shown in Figure 36 and 37. It was observed that the decrease in L* value and increase in a* and b* values were observed in both imitated crab meat and ham throughout the storage period. According to the results, the increased of a* and b* values indicated the formation of yellowish pigment, especially via maillard reaction, which might be associated with increasing moisture content in food samples. Food samples wrapped with chitosan films incorporated with Kiam wood extracts demonstrated higher lightness but lower redness and yellowness than edible HPMC films incorporated with Kiam wood extracts and PCV films, respectively as indicated by higher L* value and lower a* and b* values, respectively ($p<0.05$). These results pointed out that Kiam wood extracts and chitosan films could retarded the browning reaction, which might be associated with lipid oxidation.

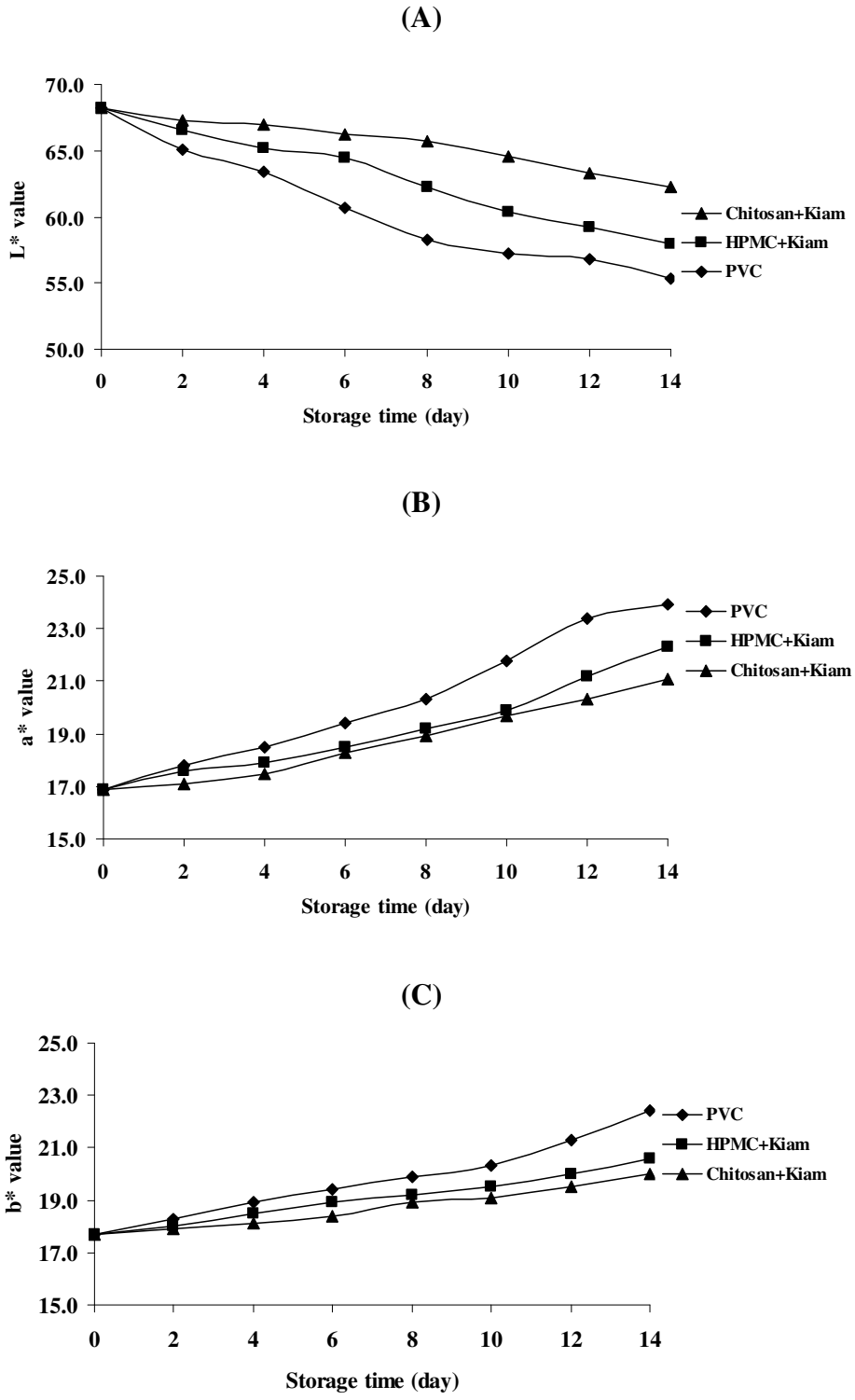


Figure 36. Effect of antimicrobial films on L* (A), a* (B) and b* (B) values of imitated crab meat during storage at 4^{o}C for 14 days.

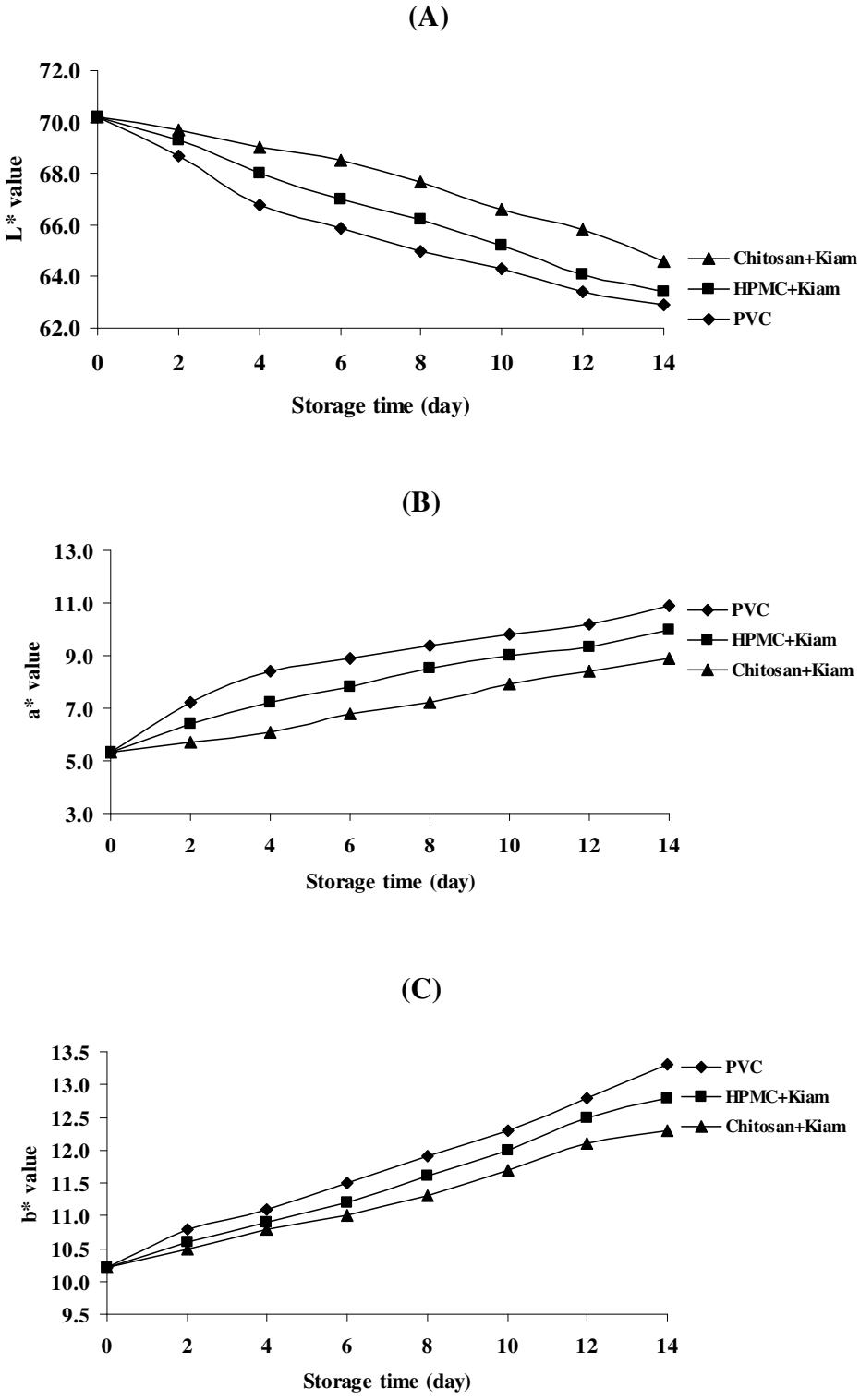


Figure 37. Effect of antimicrobial films on L* (A), a* (B) and b* (B) values of ham during storage at 4°C for 14 days.

8.5 Effect of antimicrobial films on sensory evaluation of food products

The sensory qualities of imitated crab meat and ham samples were evaluated in terms of odour, appearance and overall acceptability, using a nine-point hedonic scale (1, dislike extremely to 9, like extremely). The scale was quickly adopted by the food product for measuring the acceptability of foods. The verbal anchors of the scale were selected so that the psychological distance between successive scale points is approximately equal. This equal-interval property helps justify the practice of analyzing the responses by assigning successive integer values (1, 2, 3, … up to 9) to the scale points and testing differences in average acceptability using parametric statistics.

In this study, the effect of antimicrobial films on sensory evaluation of food products are given in Figure 38-40. The imitated crab meat and ham were considered to be acceptable for human consumption until the sensory score reached 5 (Truelstrup Hansen *et al*., 1995). Sensory scores showed a significant decrease with increasing storage period. Both imitated crab meat and ham wrapped with chitosan films incorporated with Kiam wood extracts received a higher score in term of odour, appearance and overall acceptability than the imitated crab meat and ham wrapped with edible HPMC films incorporated with Kiam wood extracts and PVC wrapped films, respectively (Figure 38-41). It is well known that spoilage gives rise to the subsequent development of strongly rancid and putrid of protein resulted in the rejection of products by panelists. According to the results pointed out that chitosan films incorporated with Kiam wood extracts could prolong shelf-life of both imitated crab meat and ham better than edible HPMC films incorporated with Kiam wood extracts and PVC films. This may be attributed to both chitosan and Kiam wood extracts could act as antioxidant, antimicrobial activity and also together excellent in water and oxygen barrier properties of chitosan and edible HPMC films, resulted in better spoilage retardant of food products.

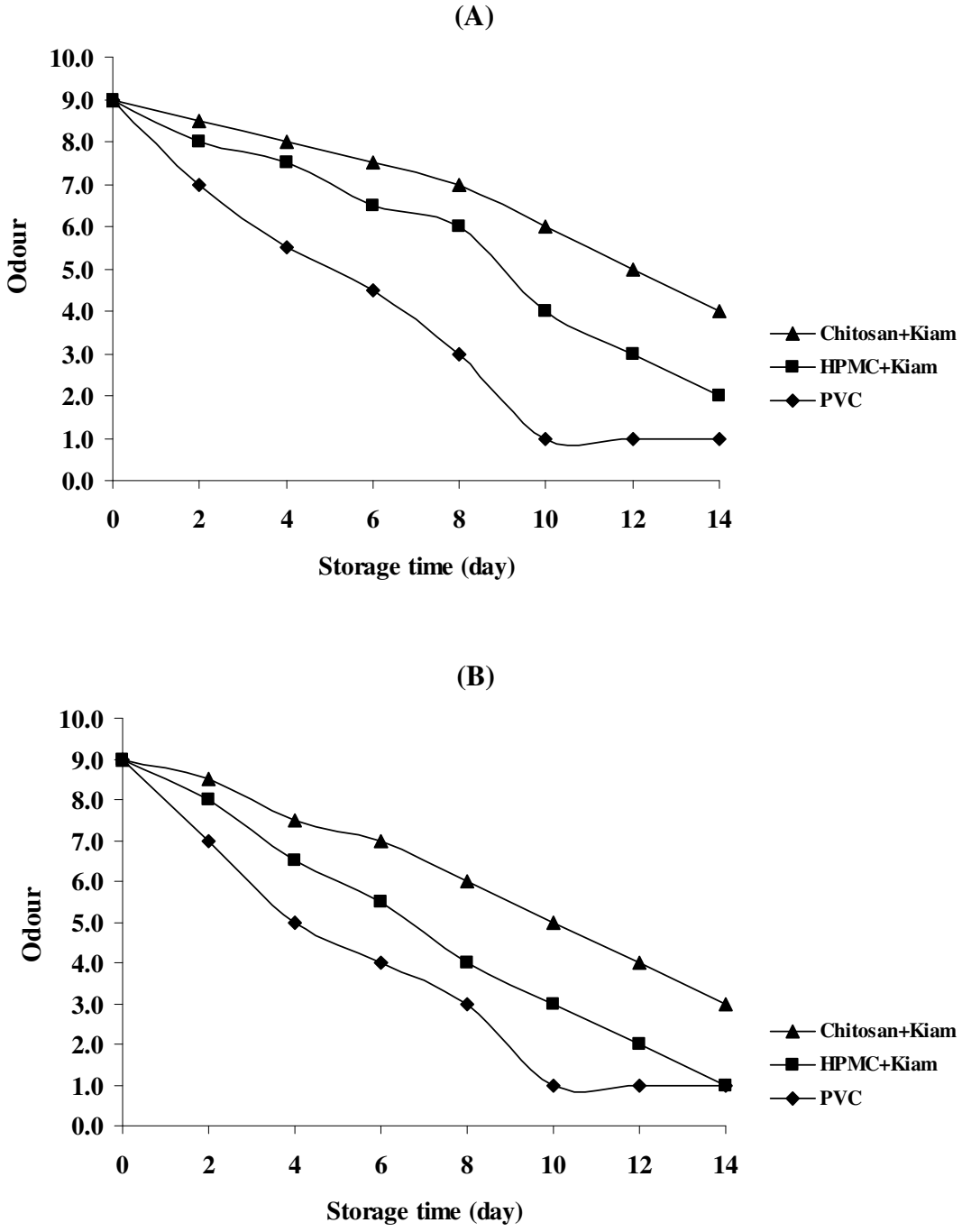


Figure 38. Effect of antimicrobial films on odour of imitated crab meat (A) and ham (B) during storage at 4^{o}C for 14 days.

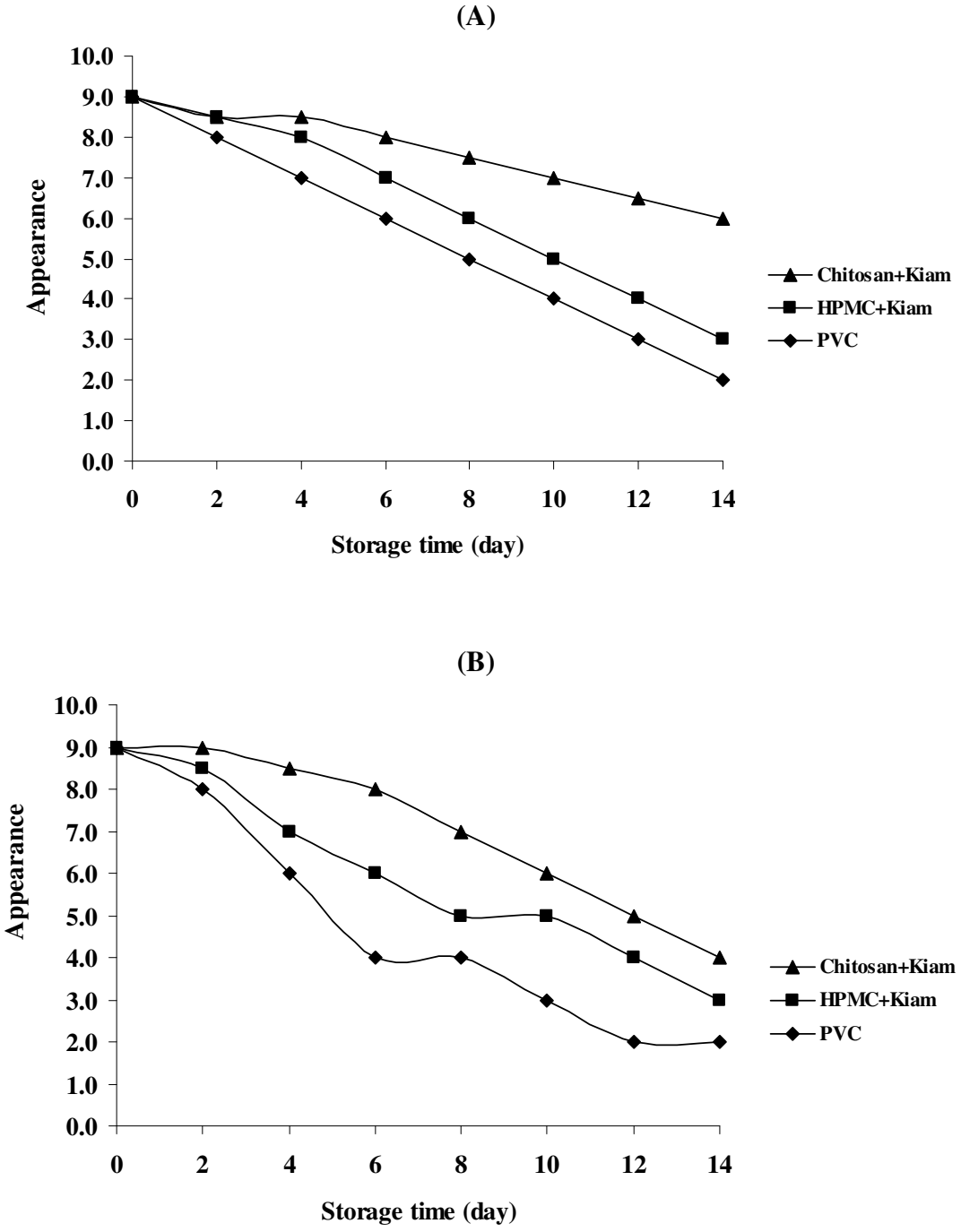


Figure 39. Effect of antimicrobial films on appearance of imitated crab meat (A) and ham (B) during storage at 4^{o}C for 14 days.

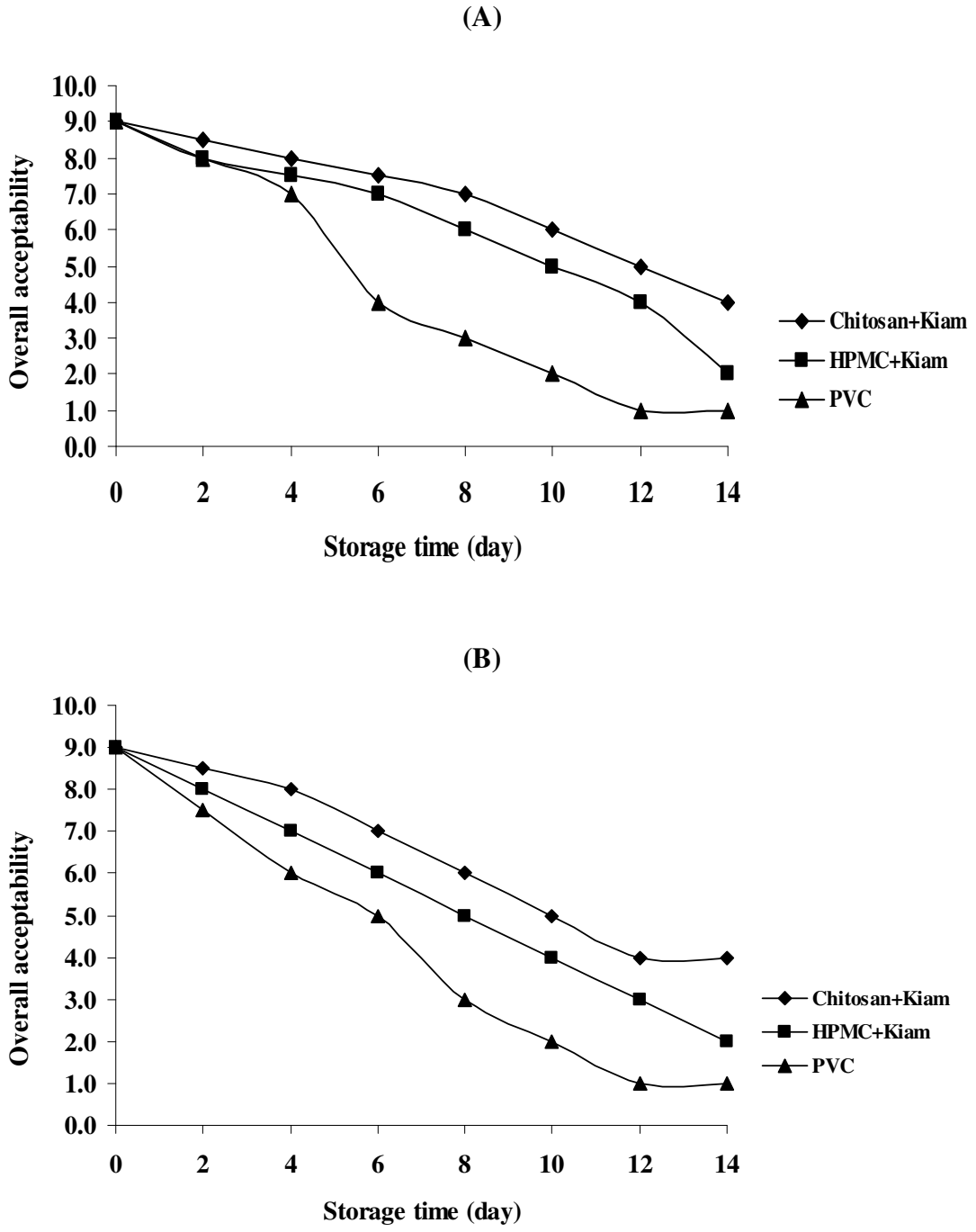


Figure 40. Effect of antimicrobial films on overall acceptability of imitated crab meat (A) and ham (B) during storage at 4^{o}C for 14 days.

8.6 Effect of antimicrobial films on microbiological property of food products

Changes in total viable count (TVC) of imitated crab meat and ham wrapped with different antimicrobial films sample during storage are shown in Figure 42. TVC showed a significant increased with increasing storage period. The results demonstrated that wrapping type plays a significant role as a protection against TVC of both imitated crab meat and ham. In the samples, wrapped with chitosan films incorporated with Kiam wood extracts, the TVC were lower than edible HPMC films incorporated with Kiam wood extracts and PCV films, respectively (Figure 42). This is due to higher antimicrobial properties of chitosan and Kiam wood extracts compared to PVC films. The changes in the coliforms bacteria of imitated crab meat and ham wrapped with PVC plastic films, edible HPMC incorporated with Kiam wood extracts and chitosan incorporated with Kiam wood extracts during storage at 4^{o}C for 14 days are shown in Table 7 and 8. The results showed that the number of coliforms of both imitated crab meat and ham wrapped with chitosan films incorporated with Kiam wood extracts was found to be the lower than imitated crab meat and ham wrapped with edible HPMC films incorporated with Kiam wood extracts and PVC films, respectively (Table 7 and 8). The results revealed that chitosan films incorporated with Kiam wood extracts can be attributed to the inhibitory effect of coliforms bacteria.

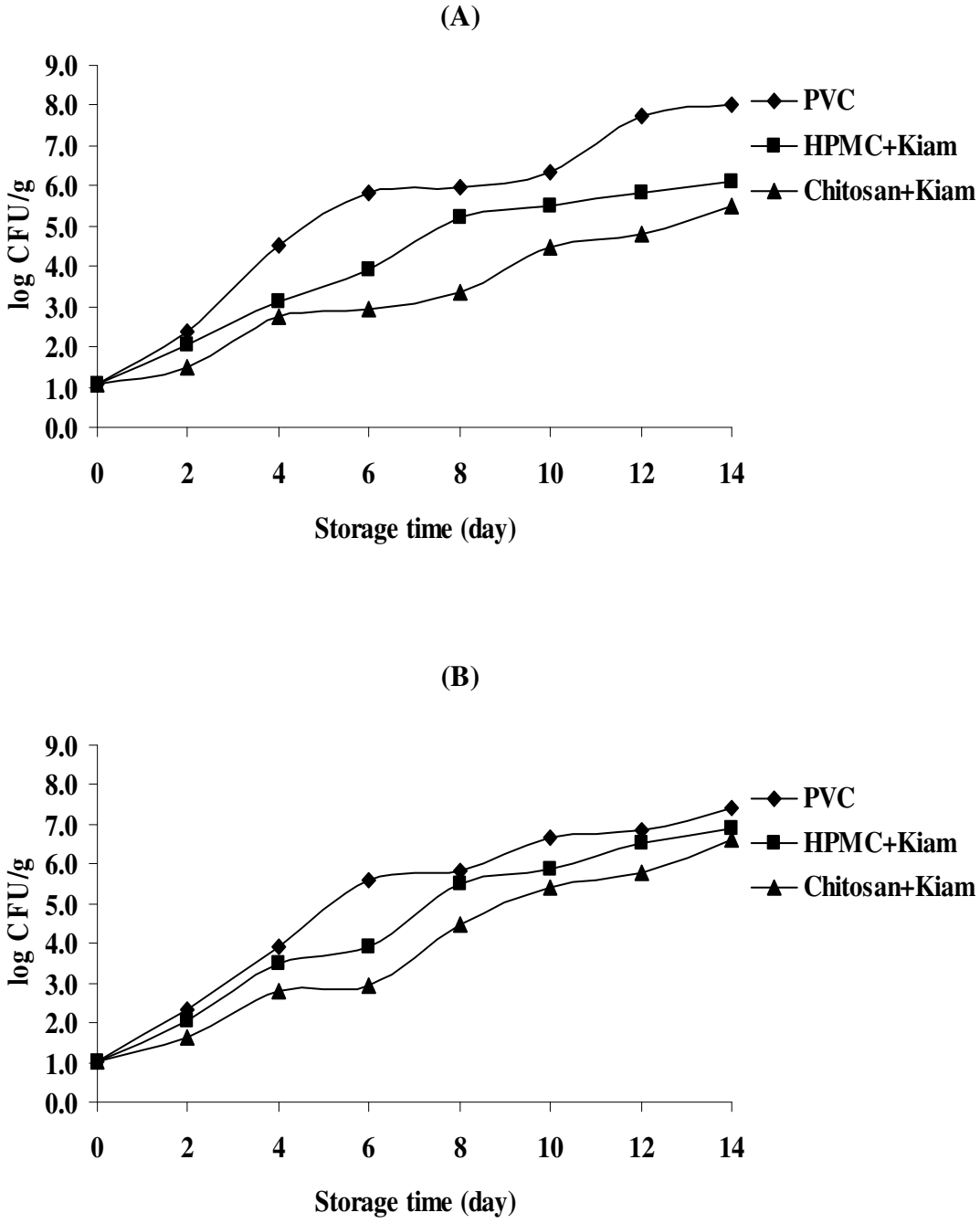


Figure 41. Effect of antimicrobial films on total viable count (TVC) of imitated crab meat (A) and ham (B) during storage at 4^{o}C for 14 days.

Table 7. Effect of antimicrobial films on coliforms number of imitated crab meat during storage at 4°C for 14 days.

Storage time (day)	Coliforms bacteria (MPN)		
	PVC	HPMC+Kiam	Chitosan+Kiam
0	<3	<3	<3
2	23	4	<3
4	240	23	4
6	460	43	7
8	1000	75	21
10	>2400	210	120
12	>2400	1000	150
14	>2400	>2400	1000

Table 8. Effect of antimicrobial films on coliforms number of ham.

Storage time (day)	Coliforms bacteria (MPN)		
	PVC	HPMC+Kiam	Chitosan+Kiam
0	<3	<3	<3
2	28	7	<3
4	120	23	21
6	460	39	28
8	1000	93	75
10	>2400	210	150
12	>2400	1000	460
14	>2400	>2400	>2400

CHAPTER 4

CONCLUSIONS

1. Kiam and Phayom wood extracts affected antimicrobial activity on the three bacteria used in this study. Incorporating of Kiam and Phayom wood extracts into edible HPMC films at levels of more than 300 mg/L led to a significant inhibitory effect on *E. coli*, *S. aureus* and *L. monocytogenes*. Edible HPMC films incorporating with Kiam wood extract has higher potential antimicrobial activity than Phayom wood extracts. The edible HPMC films incorporating Kiam and Phayom wood extracts were more effective on *L. monocytogenes* than *S. aureus* and *E. coli* respectively. The greatest zone of inhibition was observed at 5 folds of MBC. Incorporating Kiam and Phayom wood extracts markedly decreased tensile strength, elongation at break and the transparency of edible HPMC films, while water vapor permeability and film solubility were increased. The color of edible films showed darker and more red-yellowish as Kiam and Phayom wood extract increased. Kiam and Phayom wood extracts incorporated in edible HPMC films provided the films with a rougher surface than did pure edible HPMC films. Our results revealed that incorporating antimicrobial edible HPMC films with Kiam wood extracts has promise and has good potential in many food applications.

2. The results showed that, the inhibition zone increase with increasing the ratio of Kiam and Phayom wood extracts. TS and ε of edible HPMC films tended to increase with the addition Kiam and Phayom woods extracts, and the maximum occurred at the Kiam and Phayom woods extract of 100:0. The WVP and FS of edible HPMC films decreased with an increased in content of Kiam wood extracts. Higher ratio of Kiam and Phayom wood extracts resulted in darker and more opacity

3. The inhibitions of Kiam wood extracts incorporated into chitosan films were stronger than those of chitosan unfill Kiam wood extracts. TS and ε value of the chitosan films incorporated with Kiam wood extracts was lower than that of chitosan films unfilled Kiam wood extracts. Incorporation of Kiam wood extracts affected the WVP and FS values and tended to increase as Kiam wood extracts were incorporated. The results demonstrated that the chitosan films incorporated with Kiam

wood extracts were lighter, darker and more red-yellowish. Morphology of the chitosan film unfilled Kiam wood extracts had smoother surface than the chitosan films filled Kiam wood extracts. Chitosan films incorporated with Kiam wood extracts had the highest peak temperature whereas the edible HPMC films had the lowest peak.

4. The results showed that, both imitated crab meat and ham wrapped with chitosan films incorporated with Kiam wood extracts had lower and lesser changed in lower TBARS, moisture content water activity and color than food products wrapped with edible HPMC films incorporated with Kiam wood extracts and PVC films, respectively. Moreover, the results showed that both food samples wrapped with chitosan films incorporated with Kiam wood extracts received a higher sensory score than the imitated crab meat and ham wrapped with edible HPMC films incorporated with Kiam wood extracts and PVC wrapped films, respectively. In addition, food samples wrapped with chitosan films incorporated with Kiam wood extracts demonstrated lower TVC and coliforms than edible HPMC films incorporated with Kiam wood extracts and PCV films, respectively. These results pointed that incorporation of Kiam wood extracts as natural antibacterial agents have a potential to prolong the shelf-life of foods.

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APPENDIX

ANALYTICAL METHODS

1. Moisture content (AOAC, 1999)

Method

1. Dry the empty dish and lid in the oven at 105°C for 30 min and transfer to desiccator to cool (30 min). Weigh the empty dish and lid.
2. Weigh about 5 g of sample to the dish. Spread the sample with spatula.
3. Place the dish with sample in the oven. Dry for 16 h or overnight at 105°C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried content.

Calculation

$$\% \text{ Moisture} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where W_1 = weight (g) of sample before drying

W_2 = weight (g) of sample after drying

2. Determination of thiobarbituric acid substance (TBARS) (Buege and Aust, 1978)

- TBA solution: 0.375 g of thiobarbituric acid, 15 g of trichloroacetic acid, and 0.875 mL of hydrochloric acid were mixed thoroughly in 100 mL of distilled water.

Method

1. Mix sample (0.5 g) with 2.5 mL of TBA solution.
2. Heat the mixture for 10 min in a boiling water bath (95-100°C) to develop pink color and cool with tap water and centrifuge at 5500 rpm for 25 min.
3. Read the absorbance of the supernatant at 532 mm.
4. Prepare a standard curve with malonaldehyde bis(dimethyl acetal)

(MDA) at concentration ranging from 0-10 ppm.

5. Calculate the quantity of TBARS in each sample using standard curve as mg MDA/kg sample.

3. Conventional Method for coliforms

Method

1. Weigh 50 g food into sterile high-speed blender jar.
2. Add 450 mL of Butterfield's phosphate-buffered water and blend for 2 min. weigh portion that is equivalent to half of the sample and add sufficient volume of sterile diluent to make a 1:10 dilution. The total volume in the blender jar should completely cover the blades.
3. Prepare decimal dilutions with sterile Butterfield's phosphate diluent. Number of dilutions to be prepared depends on anticipated coliform density. Shake all suspensions 25 times in 30 cm arc or vortex mix for 7 s.
4. Transfer 1 mL portions to 3 LST tubes for each dilution for at least 3 consecutive dilutions. Hold pipet at angle so that its lower edge rests against the tube. Let pipet drain 2-3 s. Not more than 15 min should elapse from time the sample is blended until all dilutions are inoculated in appropriate media.
5. Incubate LST tubes at 35°C. Examine tubes and record reactions at 24 ± 2 h for gas, i.e., displacement of medium in fermentation vial or effervescence when tubes are gently agitated. Re-incubate gas-negative tubes for an additional 24 h and examine and record reactions again at 48 ± 2 h. Perform confirmed test on all presumptive positive (gas) tubes.
6. From each gassing LST tube, transfer a loopful of suspension to a tube of BGLB broth, avoiding pellicle if present. Incubate BGLB tubes at 35°C and examine for gas production at 48 ± 2 h. Calculate most probable number (MPN) (see Appendix 2) of coli forms based on proportion of confirmed gassing LST tubes for 3 consecutive dilutions.

4. Total viable count (TVC)

Method

1. Using separate sterile pipets, prepare decimal dilutions of 10-2, 10-3, 10-4, and others as appropriate, of food homogenate for sample preparation) by transferring 10 ml of previous dilution to 90 ml of diluent.

2. Avoid sampling foam. Shake all dilutions 25 times in 30 cm (1 ft) arc within 7 s.

3. Pipet 1 mL of each dilution into separate, duplicate, appropriately marked petri dishes. Reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into petri dish.

4. Add 12-15 mL plate count agar (cooled to 45 ± 1°C) to each plate within 15 min of original dilution. For milk samples, pour an agar control, pour a dilution water control and pipet water for a pipet control.

5. Add agar to the latter two for each series of samples. Add agar immediately to petri dishes when sample diluent contains hygroscopic materials, e.g., flour and starch. Pour agar and dilution water control plates for each series of samples.

6. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface.

7. Let agar solidify. Invert solidified petri dishes, and incubate promptly for 48 ± 2 h at 35°C. Do not stack plates when pouring agar or when agar is solidifying.

5. Table of hedonic Scale (nine-point hedonic scale)

Scale Point Description	Assigned Value
Like Extremely	9
Like very Much	8
Like Moderately	7
Like Slightly	6
Neither Like Nor Dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike Very Much	2
Dislike Extremely	1
Total Responses……	
Mean Rating…….	
Standard aviation…….	
Percentage “Dislike” Responses……...	

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