International Food Research Journal 18: 39-54 (2011)

Antimicrobial activity and characteristics of edible films incorporated with Phayom wood (*Shorea tolura*) extract

[1]Jutaporn, C. T., [2]Suphitchaya, C. and [1,]*Thawien, W.

[1]Department of Material Product Technology, [2]Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand

Abstract: The antimicrobial effect against *Escherichia coli* O175:H7, *Staphylococcus aureus* and *Listeria monocytogenes* of edible film containing phayom wood (*Shorea tolura*) extract was investigated. In this study, the minimum bactericidal concentration (MBC) of phayom wood (*Shorea tolura*) extract was established as 300 mg/L at which bacterial growth was completely inhibited. Antimicrobial properties of hydroxylpropyl methyl cellulose (HPMC) films containing 1-5 folds of MBC of phayom wood extract were tested. The edible films containing phayom wood extract exhibited more effective impact on the growth of *L. monocytogenes* than *S. aureus* and *E. coli* ($p<0.05$). The use of phayom wood extract at 1 fold of MBC incorporated into edible HPMC films did not exhibit any antimicrobial activity. However, the inhibitory effect of edible HPMC films containing phayom wood extract was observed only at 3, 4 and 5 folds of MBC. The greatest zone of inhibition was observed at 5 folds of MBC incorporated in edible HPMC films. Tensile strength and elongation at break significantly decreased with incorporation of phayom wood extract concomitantly with increased in water vapor permeability and films solubility. The color of edible films was affected by the addition of phayom wood extract; the results showed that increasing phayom wood extract yielded darker and more red-yellowish of resulted films. A lower transparency of the edible films was observed when a greater amount of phayom wood extract was incorporated ($p<0.05$). Phayom wood extract incorporated in edible films provided the films with a rougher surface than pure edible films.

Keywords: antimicrobial film, phayom wood (Shorea tolura) extract, edible film, pathogens, film properties

Introduction

The demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products, globalization of food trade, and distribution from centralized processing pose major challenges for food safety and quality. Recent food-borne microbial outbreaks are driving a search for innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness, and safety. One option is to use packaging to provide an increased margin of safety and quality. The next generation of food packaging may include materials with antimicrobial properties. These packaging technologies could play a role in extending shelf-life of foods and reduce the risk from pathogens. Antimicrobial polymers may find use in other food contact applications as well (Rooney, 1995). 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 packaging that are safe for human consumption (Cagri et al., 2004). The antimicrobial activity of plant extract has formed the basis of many applications, including their raw and processed potential as natural agents for food preservation, pharmaceuticals, alternative medicine and natural therapies (Cosentino et al., 1999; Bakkali et al., 2008). In order to prolong the storage stability of foods, synthetic antimicrobials are mainly used in industrial processing. However, according to toxicologists and nutritionists, there are side effects to using some synthetic antimicrobials in food processing. For this reason, the search

*Corresponding author.
Email: *thawean.b@psu.ac.th*
Tel: +(66) 74446731, Fax: +(66) 74212889

for antimicrobials from natural sources to replace synthetic ones has received much attention. Furthermore, these naturally occurring antimicrobials can be formulated as functional foods and can help to prevent the 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 functions to prevent antimicrobial properties have tremendous potential for extending the shelf life of food products (Archana et al., 2005; Kamel et al., 2007; Bakkali et al., 2008). The extracts of plants are of growing interest both in industry and scientific research because of their antibacterial, antifungal, antiviral and anti-parasitical activities. These make them useful as natural additives in foods, cosmetic and pharmaceutical industries (Kamel et al., 2007; Bakkali et al., 2008). Several antimicrobial compounds occur naturally in plants (Banks et al, 1986; Nychas, 1994; Walker, 1994) and are known to retard the growth of 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.

Edible films are defined as thin layers of material which can be eaten by the consumer and provide a barrier to moisture, oxygen and solute movement in the food. The material can a complete food coating or it can be disposed as a continuous layer between food components (Guilbert, 1986). Edible films have received consideration attention in recent years because of their advantage over synthetic films. The advantages of edible films over other traditional synthetic films are that they can be consumed with the packaged 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. Another possible application for edible films could be their use in multilayer food packaging materials together with non edible films. This is one of the most effective methods of maintaining food quality. It 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 (Coma et al., 2001).

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 is the forest both dry and moist and tropical forest that high from sea-level 60-1,200 meter. It is perennial plant and appear medium to big size, height 15-40 meter. 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. Phayom are rich in polyphenolic compounds which in herbaceous and woody plants are known to have antimicrobialactivity (Scalbert, 1991). Pieces of wood from phayom wood have been traditionally submerged in sugar palm sap in Thailand to prevent or retard microbial fermentation. This present study was done to improve the antimicrobial efficacy of edible film based on hydroxylpropyl methylcellulose (HPMC) by incorporating phayom wood extract. The 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.

Materials and Methods

Materials

Phayom woods (*Shorea talura)* used was obtained from the local community in Songkhla province. Commercial Hydroxypropyl methylcellulose (HPMC) with an average molecular weight of 162.14 kDa was purchased from High Science Co. Ltd. (Thailand). Commercial grade sorbitol was obtained from Vidyasom Co. Ltd. (Thailand). *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes* were obtained from the 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 the experiments were inoculated into the media from a single colony and incubated overnight in the appropriate media and atmospheric conditions.

Methods

Preparation of Phayom wood extract

Phayom woods (*Shorea talura)* were chopped into small pieces. The pieces of phayom wood were mixed with water in a proportion of 1g: 100mL. The extraction was performed by continuous stirring in a water bath at 50°C for 24 hours, 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 a hot air oven at 50°C for 24 hours, ground and placed in bottle and at 4°C until needed.

Preparation of inoculums

E. coli O157:H7, S. aureus and *L. monocytogenes* were cultured into 5 ml of TSB, and incubated in a shaker incubator at 35°C for 18-24 hours. 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 108 CFU/ml. The final concentration of the cell numbers of approximately 105-106 CFU/mL was obtained by diluting 100 times with sterile sodium chloride solution.

Minimum bactericidal concentration (MBC)

Minimum bactericidal concentration (MBC) from phayom wood extract inhibits the growth of *E coli* O157:H7, *S. aureus* and *L. monocytogenes*. These were analyzed by modification of the method used by Canillac and Mourey (2001). The phayom wood extract was diluted into 100 200 300 400 500 mg/L, in trypticase soy broth (TSB) for the test. The medium was inoculated with 0.1 ml of a pre-culture in TSB at 37°C. The cells of the inoculums were in an exponential growth phase after 2-3 hours of static incubation or in a stationary phase after 17-19 hours of static incubation. The final concentration of bacteria, determined with the plate count method, was of the order of 3×105 CFU/ml. The MBC is the lowest concentration of phayom wood extract for which no growth was detected after 48 hours at 37°C (Canillac and Mourey, 2001).

Preparation of antibacterial edible HPMC films

Edible HPMC films were prepared by modification of the method used by Pranoto et al. (2005). Hydroxypropyl methylcellulose of 1g was dissolved into 100 mL of distilled water and rotary shaking was done concurrently. As the edible HPMC films were brittle, 40g/100g of sorbitol was added to the edible film solution. Subsequently, the 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 the vacuum process and cast onto flat, leveled non-stick trays to set. Once set, the trays were held at 50°C for 10 hours 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.

Antimicrobial activity

Antimicrobial activity testing of the edible HPMC films was carried out using the agar diffusion method according to Pranoto et al. (2005). The edible film were cut into 16 mm diameter discs and then placed on Mueller Hinton agar plates (Merch, Darmstadt, Germany). These had been previously seeded with 0.1 ml of inoculums containing approximately 105-106 CFU/mL of tested bacteria. The plates were then incubated at 37°C for 24 hours. Observation on the diameter of the inhibitory zone surrounding film discs and contact area of edible film with agar surface were made. Experiments were done in triplicate.

Determination of film properties

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 (E) were conditioned at 60% RH and 27+2°C. This was done by placing them in desiccators over a saturated solution of $Mg(NO_3)_2.6H^2O$ for 72 hours or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

Film Thickness

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 the calculation of water vapor permeability (WVP) and tensile strength (TS).

Film Solubility

A modified method from Jangchud and Chinnan (1999) was used to measure film solubility. Film pieces, 20mm x 20mm, were dried at 70°C in a vacuum oven for 24 hours and then weighed to the nearest 0.0001g for the initial dry mass. Films were immersed in 20ml of distilled water in 50ml screw cap tubes containing 0.01g/100g sodium benzoate. The tubes were capped and placed in a shaking water bath for 24 hours 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 #1) qualitative filter paper, rinsed with 10ml distilled water This was dried at 70°C in a vacuum oven for 24 hours to determine the dry mass of the film. 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:

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

Water Vapor Permeability (WVP)

The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh et al, 1993) was used to determine the WVP of films. The test cups were filled with 20g 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 1.5cm between the film 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 in 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 0.0001g with an electronic scale (Sartorious Corp.) every 3 hours for 18 hours. 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 of time (g/hours). 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 film.

Tensile Strength and Elongation at the Break (TS and E)

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

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

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

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 a 10cm x 10cm segment of film. Total color difference (ΔE*ab), hue angle (H), and chroma (C) were calculated using the following equation:

ΔL* = L* sample – L* standard, Δa* = a* sample - a* standard, Δb* = b* sample - b* standard
ΔEab* = [(ΔL*) 2 + (Δa*) 2 + (Δb*) 2]0.5
C = [(a*) 2 + (b*) 2] 0.5
H = tan-1 (b*/a*) when a* > 0 and b* > 0
H = 180o + tan-1 (b*/a*) when a* < 0
H = 360 o + tan-1 (b*/a*) when a* > 0 and b* < 0

Prior to taking color measurements, film specimens were pre-conditioned at 60% RH and 27+2oC for 72 hours.

Scanning electron microscopy

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.

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:

$$\text{Transparency} = \log (T600/x)$$

Where T600 is the transmittance at 600 nm and x is the film thickness (mm).

Statistical analysis

A factorial design was used to characterize the composite films. 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).

Results and Discussions

Characteristics of phayom wood extract

The phayom wood extract showed a light brown to yellowish color and odorless and contained 32.09 mg/L, of tannic acid (data not shown). With regard to the antimicrobial activity, the phayom wood extract has proved antimicrobial activity (Chanthachum and Beuchat, 1997). These authors also identified the most abundant phenolic compounds in Phayom wood extract 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.

Minimal Bactericidal Concentration (MBC) of phayom wood extract

The different concentration of phayom wood extract test 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 phayom wood extract 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). Sikkema et al. (1995) reported that the antibacterial properties of woody plant extract are associated with its lipophilic components, leading to change in membrane potential and increase in permeability of the cytoplasm membrane for protons and potassium

Table 1. Antimicrobial activity of edible HPMC films incorporated with phayom wood extract against *E. coli*, *S. aureus* and *L. monocytogenes*

Bacteria types area[B]	Phayom wood extract (mg/L)	Observation at 24 h	
		Inhibitory zone[A] (mm)	Contact
Escherichia 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}	+
Staphylococcus 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}	+
Listeria 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}	+

+: represents an inhibitory; -: represent no inhibitory effect, [A] Values are measurements of diameter of inhibitory zone and expressed in mm. Values (n=4) with different superscript letters are significantly different ($p<0.05$)

[B] Contact area is the part of agar on Petri dish directly underneath film pieces.

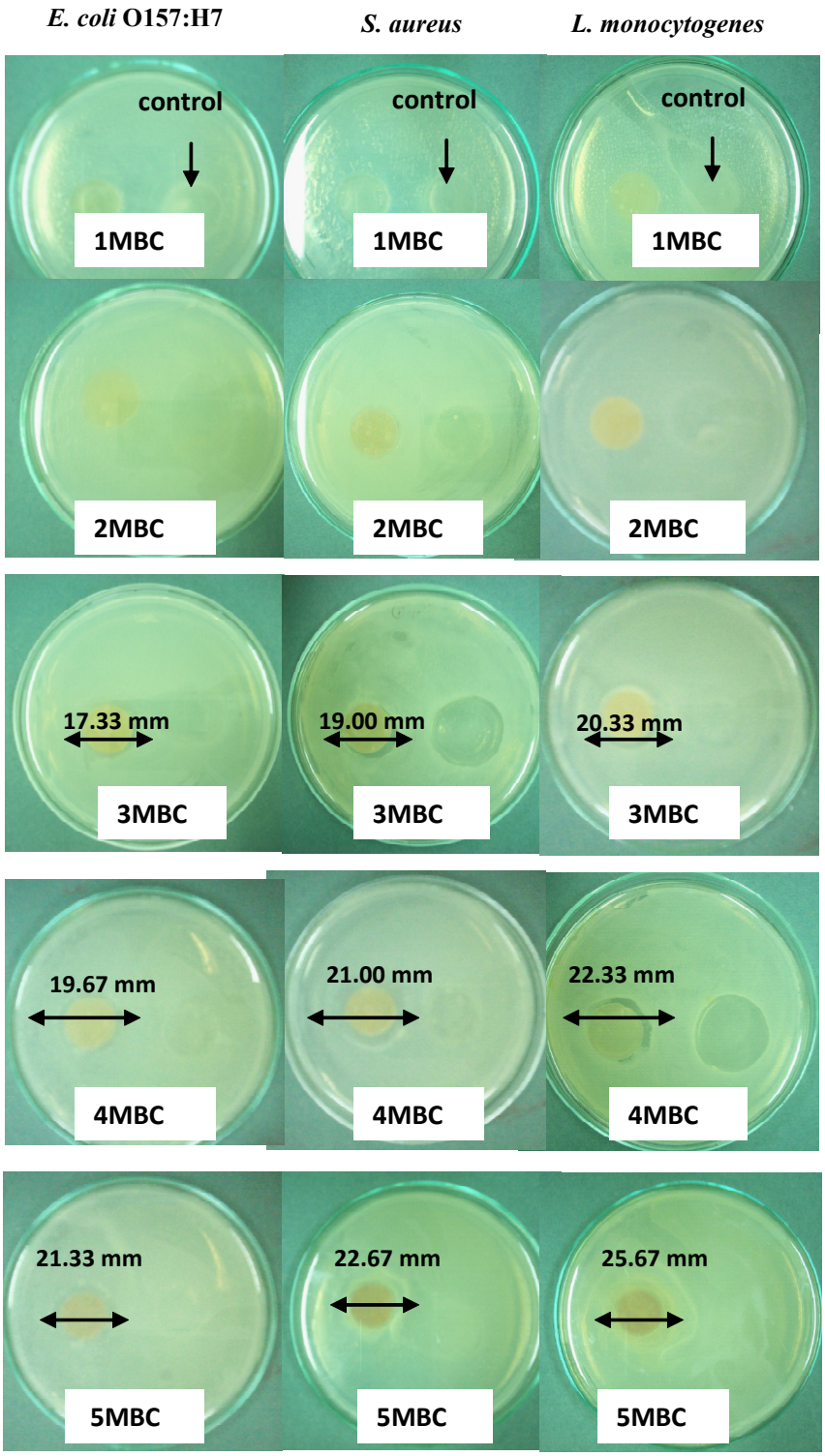


Figure 1. Representative picture of inhibitory zone of edible HPMC films incorporated with phayom wood extract at 1-5 folds of MBC against *E. coli O157:H7, S. aureus* and *L. monocytogenes*.

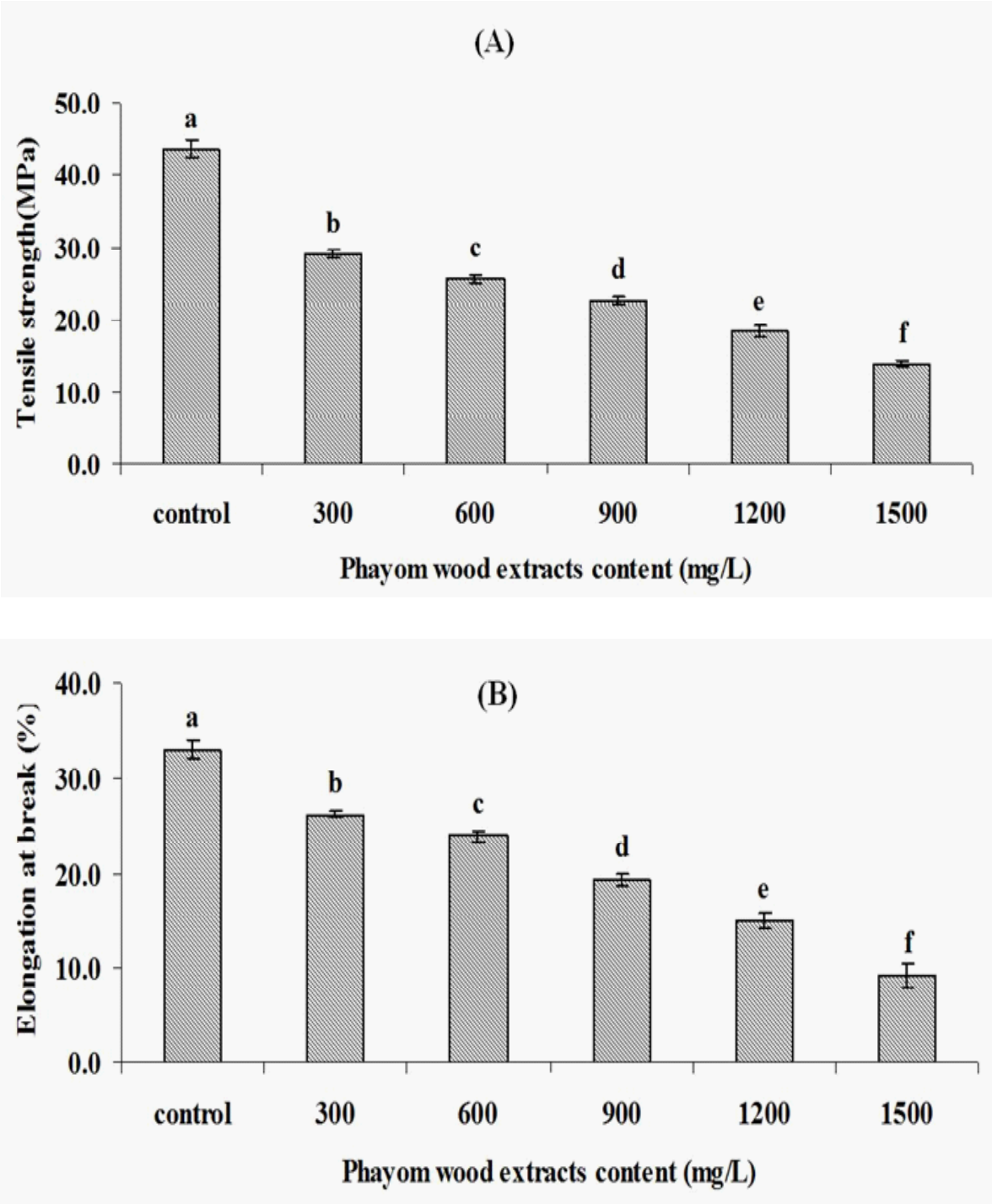
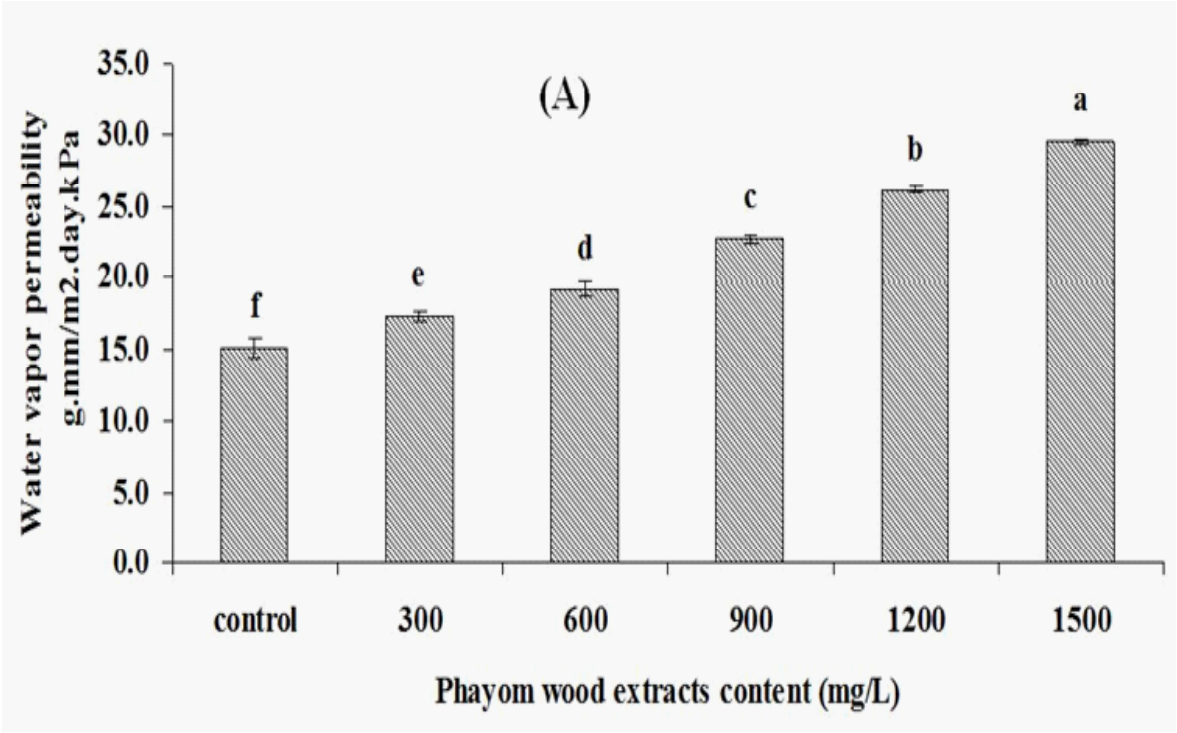


Figure 2. Tensile strength (A) and elongation at break (B) of HPMC films as a function of phayom wood extract concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).



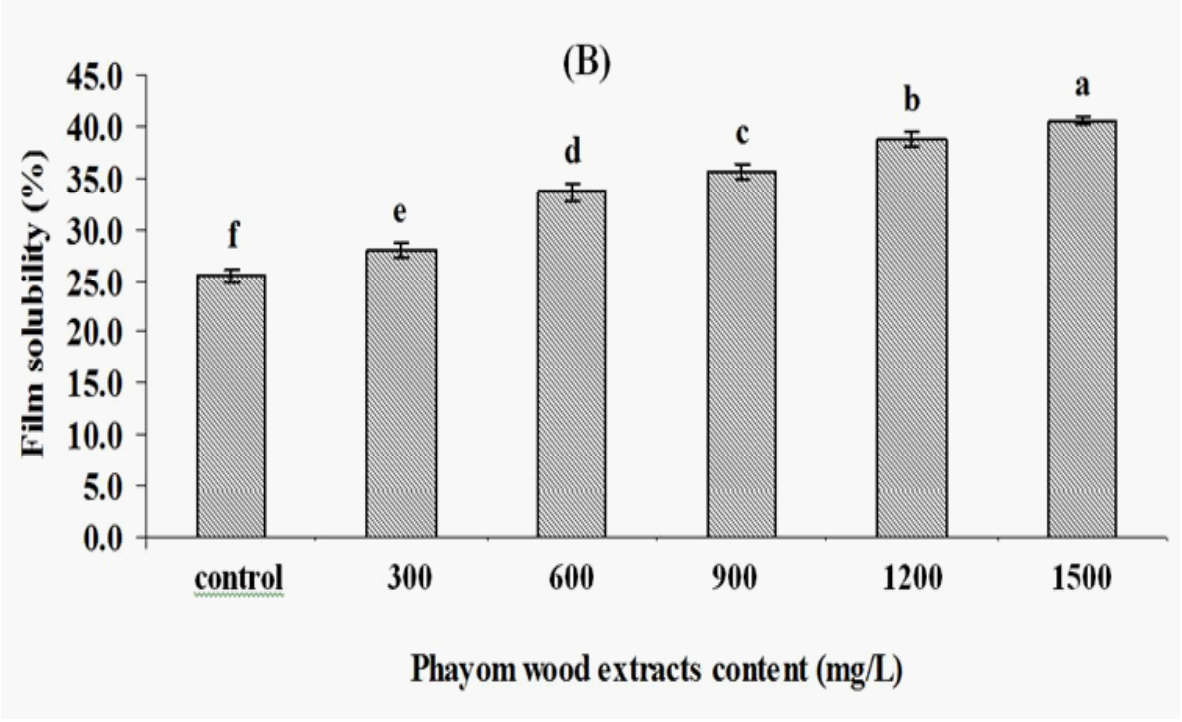
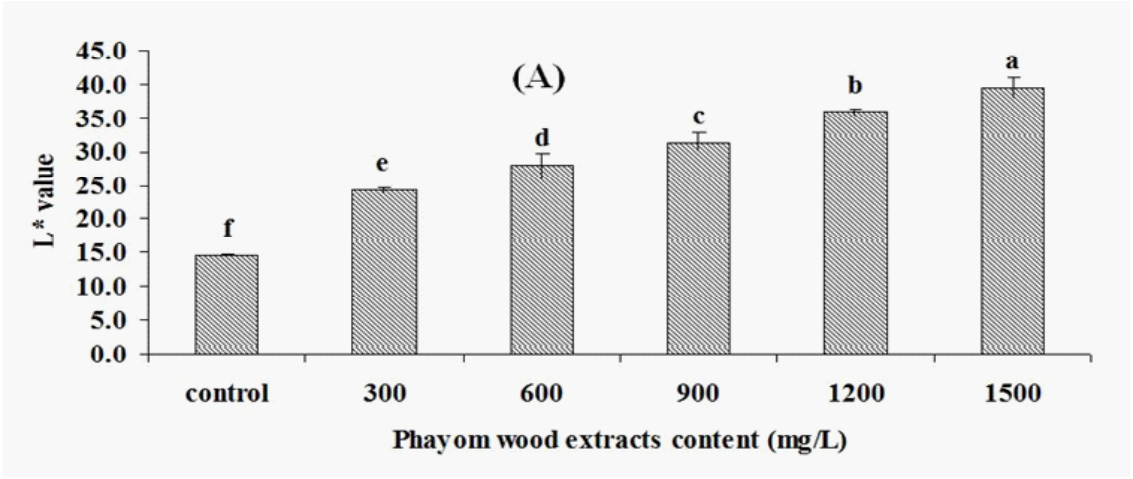
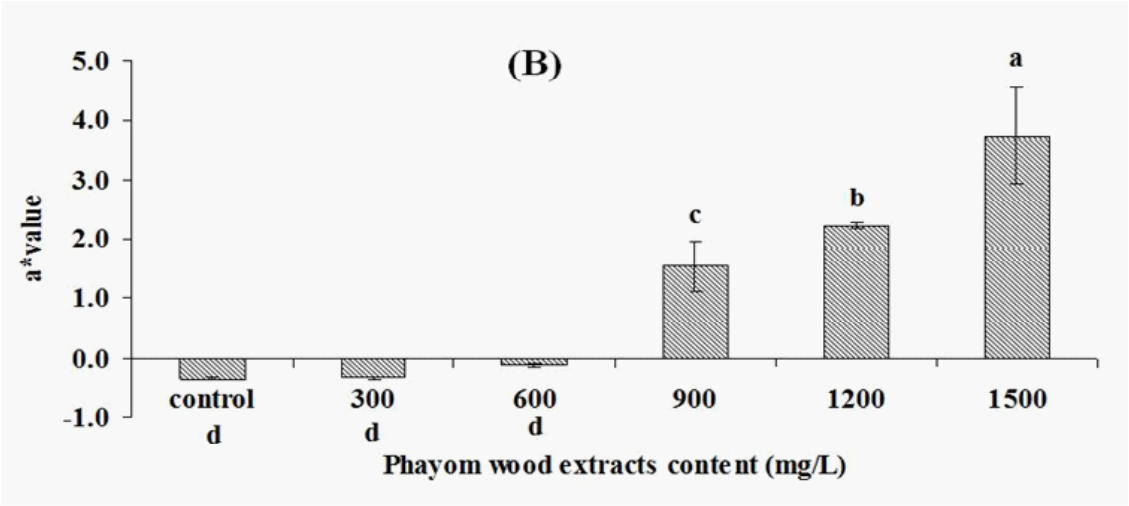


Figure 3. Water vapor permeability (A) and film solubility (B) of edible HPMC films as a function of phayom wood extract concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).





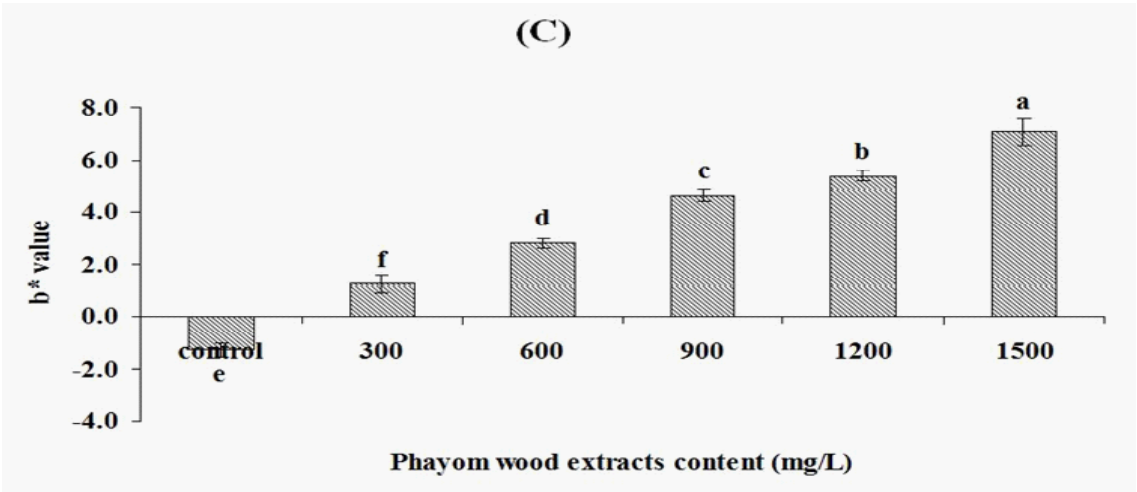


Figure 4. L* (A), a*(B) and b* (B) values of edible HPMC films as a function of phayom wood extract concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

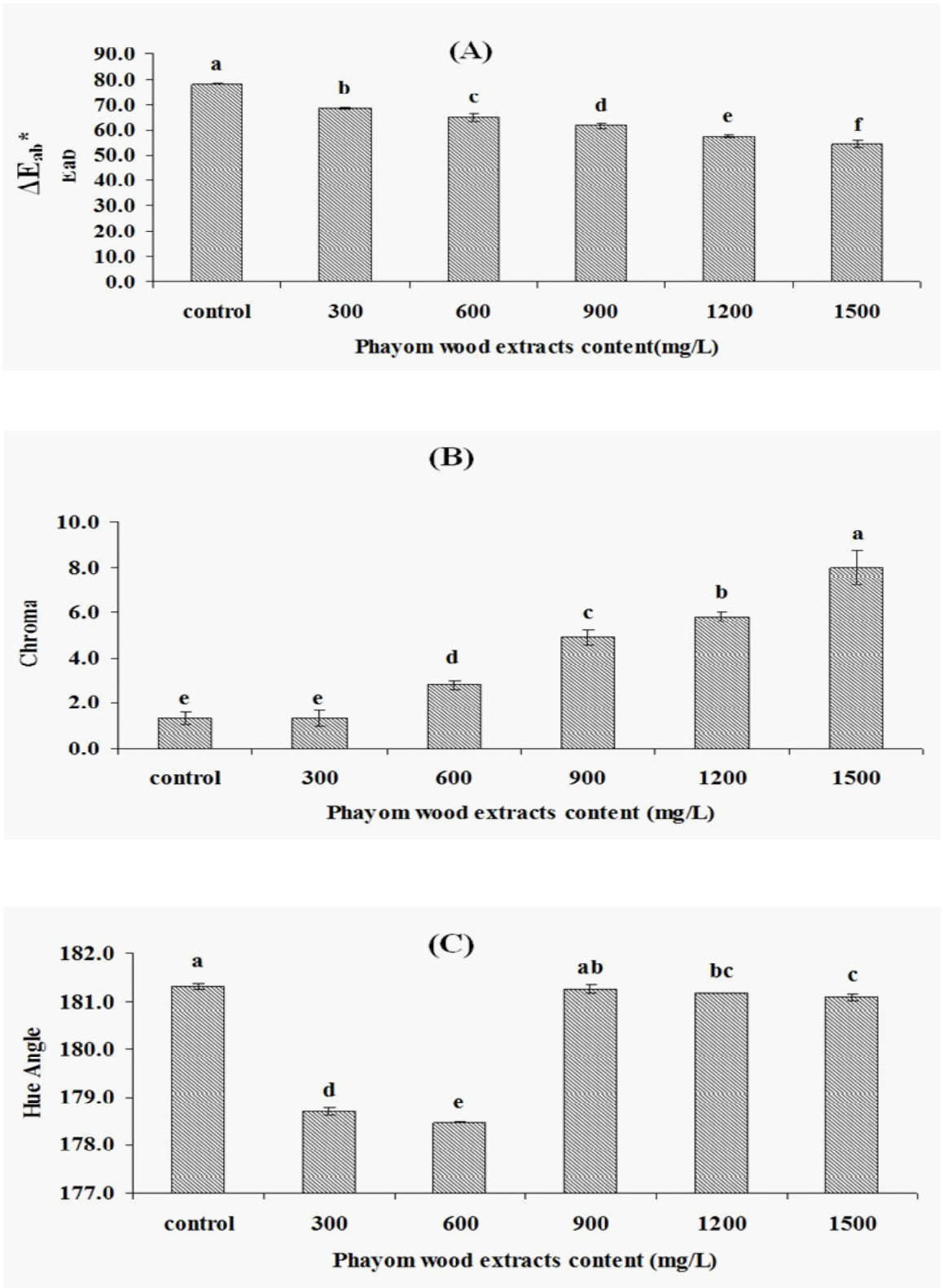


Figure 5. ΔE_{ab}* (A), chroma (B) and hue angle (C) of edible HPMC films as a function of phayom wood extract concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

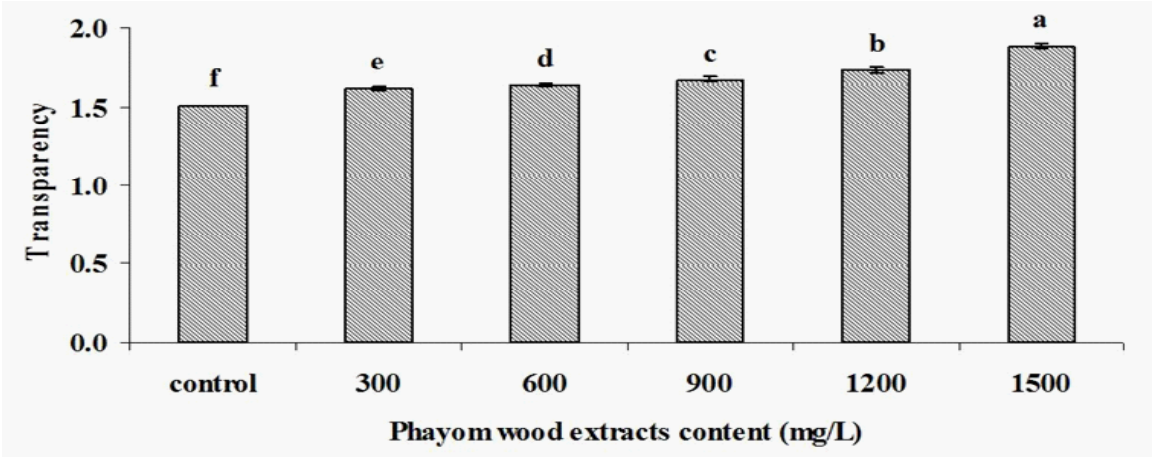


Figure 6. Transparency of edible HPMC films as a function of phayom wood extract concentration (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

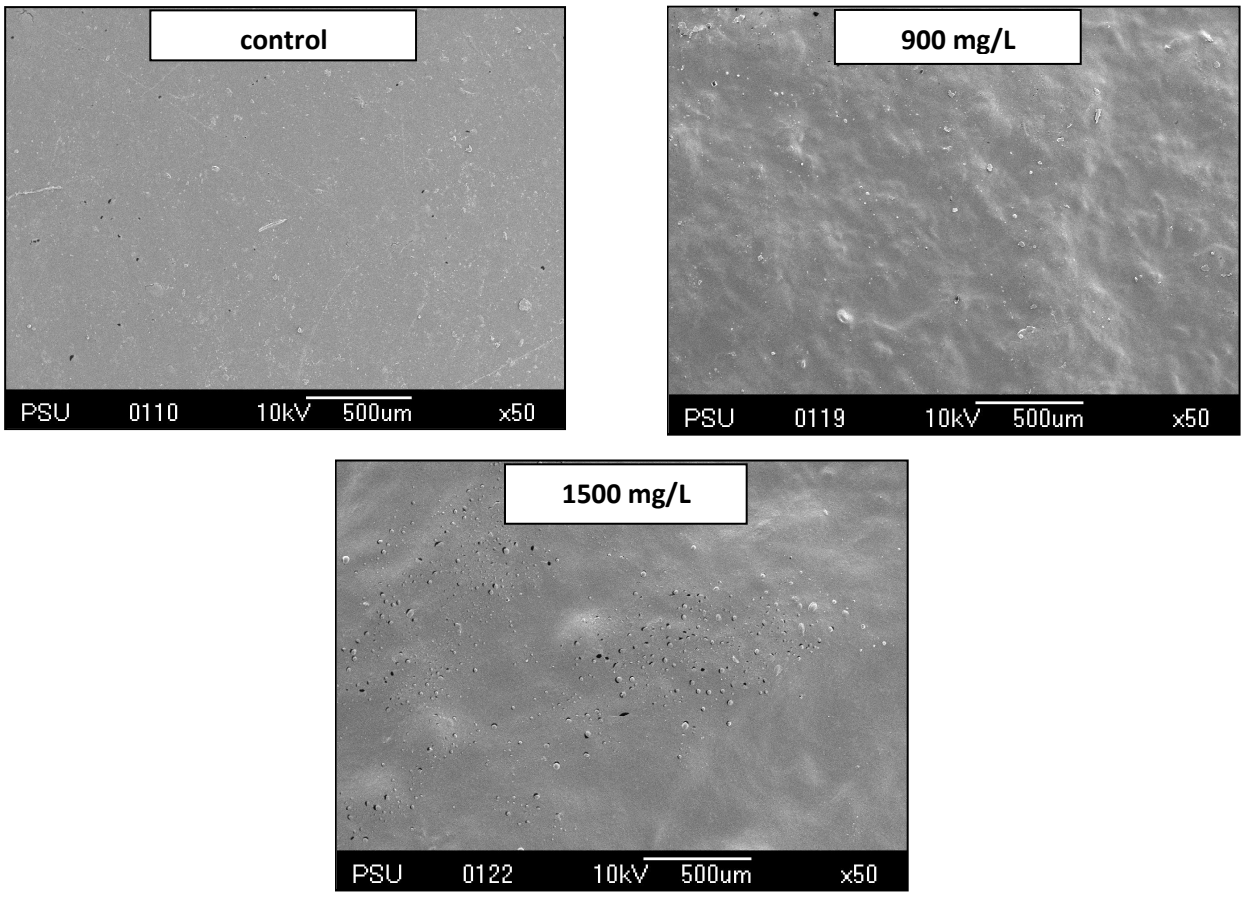


Figure 7. Scanning electron micrograph of edible HPMC films containing 900 mg/L and 1500 mg/L phayom wood extract.

ions, including depletion of the intracellular ATP pool.

Antimicrobial activity of edible HPMC films incorporated with phayom wood extract

Increasing concentration of phayom wood extract were incorporated to edible HPMC films and were tested against microorganisms for the zone of inhibition area (Table 1). Films containing 1 and 2 folds of MBC of phayom wood extract were not effective against any tested microorganisms. The minimum amount of phayom wood extract 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 1 and Figure 1). Fapasuri and Bassir (1972) reported that Saccoglottis gabonensis bark significantly inhibited growth of micro flora 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 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 extract is restricted to gram-positive species.

Properties of edible HPMC films incorporated with phayom wood extract

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). 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 extract influenced the film's properties. TS and ε of the edible HPMC films incorporated with phayom wood extract are depicted in Figure 2. The TS of edible HPMC films were affected by the phayom wood extract. The results demonstrated that the TS of edible HPMC films decreased with the addition of phayom wood extract, and the maximum occurred when no phayom wood extract was added (43.75 MPa). For example, the TS of the edible HPMC films decreased from 29.18 to 14.01 MPa when phayom wood extract was added at 300 to 1500 mg/L (Figure 2A). The remarkable decrease in the TS of the edible HPMC films indicated the presence of phayom wood extract as an additive material of the films. The changes in mechanical properties of edible HPMC films were characterized by the phayom wood extract (as additive) weaken the intermolecular forces between the chains of adjacent macromolecules, increasing the free volume and causing a reduction of mechanical resistant (Sobral et al., 2001). Thus, the increase in the phayom wood extract content causes a reduction of the TS due to the decrease in the intermolecular interactions. Besides, the increase in the phayom wood extract 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 (Sobral et al., 2001). 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 extract increased from 300 to 1500 mg/L (Figure 2B). The same effect was found by Ozdemir and Floros (2004), who reported comparable

values for sorbitol-plasticized WPI films under similar conditions. The addition of oregano extract in the sorbitol-plasticized WPI films resulted in a decrease of ε with increasing extract concentration. The effect of spice extract addition in films has been studied and in all the cases significant decreases in elastic modulus have been reported (Rojas-Grau, 2007).

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

Water vapor permeability (WVP) of edible HPMC films with different concentrations of phayom wood extract 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 extract. The WVP value varied from 16.82 to 27.77 g.mm/m2.d.kPa when phayom wood extract increased from 300 to 1500 mg/L as depicted in Figure 3A. 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 extract are favorable to the adsorption or desorption of water molecule. Furthermore, the increase of WVP might be related to the hydrophillicity of Phayom wood extract. In this system, phayom wood extract 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 extract, an increase in its content led to an increase in film solubility (FS) (Figure 3B). It could be hastily concluded that phayom wood extract enhance FS in water. The dry matter solubilized in water is likely to be constituted mainly by the phayom wood extract 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 extract presence was thus associated with this increase in solubility properties. The highest FS of edible HPMC films incorporated by 1500 mg/L phayom wood extract were noticed, while increasing the amount of phayom wood extract content showed higher FS (Figure 3B). It could be explained that, with higher content of phayom wood extract, there are more molecules of phayom wood extract untrapped in the cross linked network and able to escape into solution.

Color and Transparency

In the present study, color was affected by the incorporation of phayom wood extract into the edible HPMC films. Color of films different concentration of phayom wood extract incorporated into edible HPMC films expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/blueness), ΔEab*, chroma and hue angle values are shown in Figure 4 and 5. Addition of phayom wood extract affected the appearance of edible HPMC films in both color and transparency. Edible HPMC films unfilled phayom wood extract appeared clear and transparent. Edible HPMC films filled phayom wood extract was became lighter and red-yellowish as evidenced by the increased L*, a*, b* and chroma values when the concentration of phayom wood extract in the film increased (Figure 4 and 5). Results demonstrated that L*, a* b*, chroma and hue angle values increased as the content of phayom wood extract incorporated increased (Figure 4 and 5). This was due to the phayom wood extract being more 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 extract into the edible HPMC films resulted in decrease their transparency. Edible HPMC films unfilled phayom wood extract was the highest transparent. However, the lower transparency of the edible HPMC films was noticed when a greater concentration of phayom wood extract incorporated (Figure 6). 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.

Morphology of the films

Morphology observed by scanning electron microscope (SEM). SEM micrographs of edible HPMC films incorporated with phayom wood extract at different amounts fill and unfill phayom wood extract are shown in Figure 7. The control film (unfill phayom wood extract) 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 extract, the surface of film became rougher, especially with increasing phayom wood extract content. Edible HPMC films incorporated 900 mg/L of phayom wood extract had denser and smoother surface than

the edible HPMC films incorporated of 1500 mg/L of phayom wood extract.

Conclusions

Phayom wood extract affected antimicrobial activity on the three bacteria used in this study. Incorporating of phayom wood extract 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*. The edible HPMC films incorporating phayom wood extract 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 phayom wood extract 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 phayom wood extract increased. Phayom wood extract incorporated in edible HPMC films provided the films with a rougher surface than did pure edible HPMC films. Our results pointed that incorporating antimicrobial edible HPMC films with phayom wood extract has promise and has good potential in many food applications.

Acknowledgements

This work was financed by the Thai Research Fund (TRF) (Grant: TRG5280009). Also, the authors would like to thank Assoc. Prof. Dr. Aran H-Kittikul for his guidance and invaluable suggestions. Equipments and facilities were provided by the Department of Material Product Technology, Food Safety Lab. and Starch and Plant Fiber Research Unit (SPF-RU).

References

Archana, B., Dasgupta, N. and De, B. 2005. In vitro study of antioxidant activity of Syzygium cumini fruit. Food Chemistry 90(4): 727-733.

American Society for Testing and Materials (ASTM) 1991. Standard test method for tensile properties of plastics. D638. In: ASTM. Annual book of American Standard Testing Methods ,Vol. 15.09. Philadephia, PA., pp. 159-171.

American Society for Testing and Materials (ASTM). 1993a. Standard practice for conditioning plastics and electrical insulating materials for testing: D618-61 (Reproved 1990). In: ASTM. Annual book of American Standard Testing Methods ,Vol. 8.01. Philadelphia, PA., pp. 146-148.

American Society for Testing and Materials (ASTM).1993b. Standard test method for water vapor transmission rate through plastic film and sheeting using a modulated infrared sensor: D1249-90. In: ASTM. Annual book of American Standard Testing Methods (Vol. 15.09. Philadelphia, PA., pp. 1168-1172.

Bakkali, F., Averbeck, S ., Averbeck, D. and Idaomar, M. 2008. Biological effects of essential oils- A review. Food and Chemical Toxicology 46(2): 446-475.

Banks, J. G., Board, R. G. and Sparks, N. H. C. 1986. Natural antimicrobial systems and their potential in food preservation of the future. Biotechnology Application Biochemistry 8: 122-147.

Basarada, J. 1966. Effects of vegetable tannins on glucose oxidation by various microorganisms. Journal of Microbiology 12: 787-794.

Beuchat, L. R. 1994. Antimicrobial Properties of Spices and Their Essential Oils. Wallingford (UK): CAB International.

Beuchat, L. R. and Doyle, M. P. 1995. Survival and growth of *Listeria monocytogenes* in foods treated or supplemented with carrot juice. Food Microbiology 12: 73-80.

Beuchat, L. R. and Golden, D. A. 1989. Antimicrobials occurring naturally in foods. Food Technology 43(1): 134-142.

Cagri, A., Ustunal, Z. and Ryser, E. T. 2001. Antimicrobial, mechanical and moisture barrier properties of low pH whey protein-based edible films containing sorbic acid. Journal of Food Science 67(4): 833-848.

Cagri, A., Ustunal, Z. and Ryser, E. T. 2004. Antimicrobial edible films and coatings. Journal of Food Protection 67(4): 833-848.

Canillac, N. and Mourey, A. 2001. Antibacterial activity of the oils of *Picea excelsa* on *Listeria monocytogenes, Staphylococcus aureus* and coliform bacteria. Food Microbiology 18: 261-268.

Chanthachum, S. and Beuchat L. R. 1997. Inhibitory effect of kiam (cotylelobium lanceotatum craih). Wood extract on gram-positive food borne pathogens and spoilage micro-organisms. Food Microbiology14: 603-608.

Coma, V, Sebti, I., Pardon, P., Deschamps, A. and Pichavant, R H. 2001. Antimicrobial edible packaging based on cellulosic ethers, fatty acids, and nisin incorporation To Inhibit *Listeria innocua* and *Staphylococcus aureus*. Journal of Food Protection 64: 470-475.

Cosentino, S., Tuberoso, C.I.G., Pisana, B., Satta, M., Mascia, V., Aezedi, E. and Palmas, F. 1999. In vitro antimicrobial activity and chemical composition of *Sardinian thymus* essential oils.Letters in Applied Microbiology 29: 130-135.

Cuq, B., Gontard, N., Aymard, C. and Guilburt, S. 1997. Relative humidity and temperature effects on mechanical and water vapor barrier properties of myofibrillar protein-based films. Polymer Gels and Network 5: 1-15.

Faparusi, S. I. and Bassir, O. 1972. Effect of extracts of the bark of *Saccoglottis gabonensis* on the microflora of palm wine. Applied Microbiology 24: 853-856.

Gontard, N., Guilbert, S. and Cuq, J. L. 1992. Edible wheat gluten film: influence of the main process variable on film properties using response surface methodology. Journal of Food Science 57: 190-199.

Guilbert S. 1986. Technology and application of edible protective films. In: M. Mathlouthi. Food packaging and preservation: Theory and practice (pp. 371-393). London: Elsevier Applied Science Publishing Co.

Han, J. H. and Floros, J. D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. Journal Plastic Film Sheeting 13: 287-298.

Jangchud, A. and Chinnan, M. S.1999. Peanut protein film as affected by drying temperature and pH of film forming solution. Journal of Food Science 64(1): 153-157.

Kamel, M., Hosni, K., Taarit, M. B., Chahed, T., Kchouk, M. E. and Marzouk, B. 2007. Changes on essential oil composition of coriander (*Coriandrum sativum* L.) fruits during three stages of maturity. Food Chemistry 102(4): 1131-1134.

McHugh, T. H., Avena-Bustillos, R. and Krochta, J. M. 1993. Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. Journal of Food Science 5: 899-903.

Nychas, G. J. 1994. Natural antimicrobials from plants. In G. W. Gould. New Methods of Food Preservation (pp. 58-89). London, Blackie Academic and Professional.

Ozdemir, M. and Floros, J. D. 2004. Active food packaging technologies. Critical Reviews in Food Science and Nutrition 44: 185-193.

Pena, D. C. R. and Torres, J.A. 1991. Sorbic acid and potassium sorbate permeability of an edible methylcellulose-palmitic acid films: Water activity and PH effects. Journal of Food Science 56(2): 497-499.

Pranoto, Y., Salokhe, V. and Rakshit, K. S. 2005. Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. Food Research International 38: 267-272.

Rojas-Graü, M. A., Avena-Bustillos, R. J., Olsen, C., Friedman, M., Henika, P. R. and Martín-Belloso, O. 2007. Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films. Journal of Food Engineering 81: 634-641.

Rooney, M. L. 1995. Active packaging in polymer films. In: M. L. Rooney. Active Food Packaging (pp.74-110). Glasgow, UK: Blackie Academic & Professional.

Scalbert, A. 1991. Antimicrobial properties of tannins. Photochemistry 30: 3875-3883.

Sikkema, J., De Bont, J. A. and Poolman, B. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiological Reviews 59(2): 201-222.

Sorbal, P. J. A., Menegalli, F. C., Hubinger, M. D. and Roques, M. A. 2001. Mechanical, water vapor barrier and thermal properties of gelatin based edible film. Food Hydrocolloids 15: 423-432.

Tharanathan, R. N. 2003. Biodegradable films and composite coatings: past, present and future. Trends in Food Science Technology 14: 71-78.

Vermeiren, L., Devlieghere, F., Beest, M., de Kruijf, N. and Debevere, J. 1999. Developments in the active packaging of foods. Trends in Food Science and Technology 10: 77-86.

Walker, J. R. L. 1994. Antimicrobial compounds in food plants. In V. M. Dillon & R. G. Board. Natural Antimicrobial Systems and Food Preservation (pp.181-204).Wallingford, UK: CAB International.

Weng, Y. M. and Hotchkiss, J. H. 1992. Inhibition of surface molds on cheese by polyethylene containing the antimycotic imazalil. Journal of Food Protection 55: 367-369.

Yang, L. and Paulson, A. T. 2000. Effects of lipids on mechanical and moisture barrier properties of edible gellan film. Food Research International 33: 571-578.