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Antimicrobial activity and the properties of edible hydroxypropyl methylcellulose based films incorporated with encapsulated clove (*Eugenia caryophyllata* Thunb.) oil

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Abstract: Antimicrobial activity and the properties of edible film were studied by incorporating with encapsulated clove (*Eugenia caryophyllata* Thunb.) oil as a natural antibacterial agent. The minimal bactericidal concentration (MBC) of clove oil inhibited the growth of *E. coli* O175:H7, *S. aureus* and *L. monocytogenes* were tested in the stationary phase. The results showed that 1.0, 1.5 and 0.5% of clove oil inhibited about 10^5 cfu/mL of *E. coli* O175:H7, *S. aureus* and *L. monocytogenes in vitro* experiments respectively. Antimicrobial edible films were prepared by incorporating encapsulated clove oil from 1, 2, 3 folds of MBC into 1.5% of hydroxypropyl methylcellulose (HPMC) film solution. The edible HPMC films exhibited antibacterial activity against pathogenic bacteria tested by using agar diffusion assay. The results showed that increasing encapsulated clove oil in the edible HPMC films from 1, 2, 3 folds of MBC yielded a higher inhibition zone from 18.50-26.75, 28.75-39.00 and 16.67-31.00 mm of *E. coli* O175:H7, *S. aureus* and *L. monocytogenes*, respectively. Tensile strength, elongation at break, water vapor permeability, and film solubility were significantly ($p<0.05$) decreased with the incorporation of encapsulated clove oil. The color of edible HPMC films was affected by the addition of encapsulated clove oil; the results showed that increasing the encapsulated clove oil resulted in darker and more yellowish films. The lower transparency of the edible HPMC films was noticed when a greater amount of encapsulated clove oil was incorporated ($p<0.05$). Encapsulated clove oil filled edible HPMC films provided the films with a rougher surface than pure edible films.

Keywords: Antimicrobial film, encapsulation, clove oil, pathogens, mechanical properties

Introduction

Edible films have received considerable attention in recent years because of their advantages over synthetic films. The advantages of edible films over other traditional synthetics are that as they can be consumed with the packaged products. The films can function as carriers for antimicrobial and antioxidant agents. In a similar application they can also be used at the surface of foods to control the diffusion rate of preservative substances from the surface to the interior of the food. This is further improved by films carrying food additives such as antioxidants, antimicrobial, colorants, flavors, fortified nutrients, 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).

Antimicrobial packaging is a promising form of active food packaging. Since microbial contamination of these foods occurs primarily at the surface, due to post-processing handling, attempts have been made to improve safety and delay spoilage by use of antibacterial sprays or dips. However, direct surface application of antibacterial substances on foods has limited benefits because the active substances are neutralized on contact or diffuse rapidly from the surface into the food mass.

On the other hand, the incorporation of bactericidal or bacteriostatic agents into product formulation may result in partial inactivation of the active substances by product constituents. This is therefore expected to have only limited effect on the surface microflora (Quintavalla and Vicni, 2002). Thus the application of antimicrobial packaging could extend the shelf-life of products and provide microbial safety for consumers (Rooney, 1995). It acts to reduce, inhibit, or retard the growth of pathogen microorganisms in packed foods and packaging materials (Vermeiren *et al*., 1999). Antimicrobial agents such as organic acids, bacteriocins and spice oil have been tested for their ability to control food spoilage (Abugroun *et al.*, 1993; Miller *et al*., 1993; Hotchkiss, 1995).

Among the clove (*Eugenia caryophyllata* Thunb) species, clove bud oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties. It is used traditionally as a flavoring agent and as an antimicrobial material in food. The high levels of eugenol contained in clove oil give it strong biological and antimicrobial activity. This phenolic

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compound can denature proteins and reacts with cell membrane phospholipids thus changing their permeability.

Clove oil also has several therapeutic effects, including antiphlogistic, anti-vomiting, analgesic, antispasmodic, anticarminative, kidney reinforcement, antiseptic, and HCMV extracorporeal restraining effects (Gulcin *et al*., 2004; Wenqiang *et al*., 2007). Kim *et al*. (1998) reported that clove oil has been successfully used for asthma and various allergic disorders through oral administration. However, clove oil exhibits sensitivity to light, heat and oxygen, and has a short storage life if not stored properly (Shaikh *et al*., 2006). Hence, clove oil needs to be protected before and during its incorporation in the products.

Encapsulation is the technology used for packing solid, liquid or gaseous materials in miniature sealed capsules for release at controlled rates using desired release triggers. The simplest microcapsule consists of a core surrounded by a wall or barrier. The core is the component requiring protection and may be composed of one or more ingredients. The wall may be single or multi-layered (Pothakamury and Barbosa-Canovas, 1995). This technology is used in foods and beverages for a range of purposes. These are to: control the release of active ingredients; protect ingredients from the environment; reduce flavor loss during the product's shelf-life; extend the flavor perception and mouth-feel over a longer period of time; and enhance the ingredient's bioavailability and efficacy (Berry, 2004).

The use of packaging films containing antimicrobial agents could be made more efficient by the slow migration of the agents from the packaging material to the surface of the product. This helps maintain high concentration where this is needed. If an antimicrobial can be released from the package during an extended period, the activity can also be extended to include the transport and storage phases of food distribution. Antimicrobial substances incorporated into packaging materials may control microbial contamination by reducing the growth rate and maximum growth population, extend the lag-phase of the target microorganism, or inactivate microorganisms by contact. Preliminary tests found that the addition of clove oil into a hydroxylpropyl methylcellulose (HPMC) film solution caused phase separation and heterogeneous films were obtained. In addition, the mechanical and physical properties of the films were not acceptable. Hence, this study was undertaken to improve the antimicrobial efficacy of edible film based on hydroxylpropyl methylcellulose (HPMC) by incorporating encapsulated clove oil. The mechanical and physical properties of edible HPMC films were also identified, and antimicrobial efficacy was assessed against three food pathogenic bacteria.

Materials and methods

Materials

The buds of dried clove (*Eugenia caryophyllata* Thunb.) used were obtained from a herbal drugstore at Songkhla Province. Commercial Hydroxypropyl methylcellulose (HPMC) with an average molecular weight of 162.14 kDa was purchased from High Science Ltd. (Thailand). Commercial grade- chitosan flake (approx. 85% degree of deacetylation) with an average molecular weight of about 75 KDa was obtained from Bona Fides Marketing Co. Ltd. (Thailand). Commercial grade glycerol and maltodextrin (MD) were obtained from Vidyasom Co.Ltd. (Thailand). The emulsifier used was Tween 80 (Fisher Scientific, FairLawn, NJ). Trypticase soy broth (TSB), Trypticase soy agar (TSA), and Yeast extract (YE) were purchased from Hi-media; High Science Ltd., (Thailand). *E coli* O157:H7, *S. aureus* and *L. monocytogenes* were given by the Food Safety Laboratory, 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 the media from a single colony and incubated overnight under the appropriate media and atmospheric conditions.

Methods

Extraction of clove oil

The ground clove bud samples (30 g) were weighed and extracted with 250 mL absolute ethanol for 6 hours (Wenqiang *et al.*, 2007)., This was then filtered through filter paper (Whatman No.1). After extraction, the clove oil was concentrated using a rotary vacuum evaporator (Model 2215; BUCHI, Switzerland) at 40°C. The clove oil was collected and stored in opaque, air tight containers at 4°C awaiting use and further analysis.

Preparation of inoculums

E. coli O175:H7, *S. aureus* and *L. monocytogenes* were cultured into 5 ml of trypticase soy broth supplemented with yeast extract (TSBYE), and incubated in a shaker incubator at 35ºC for 18-24 hours. The optical density (OD) of the bacteria was adjusted to the standard of McFarland NO.0.5 with 0.85-0.90 g sodiumchloride/100 ml sterile solution to achieve a concentration of approximately 10^8

CFU/ml. The final concentration of the cell number to approximately 10^5-10^6 cfu/mL was obtained by diluting 100 times with sterile sodium chloride solution.

Determination of minimal bactericidal concentration (MBC)

Minimum bactericidal concentrations (MBC) of clove oil inhibit the growth of *E coli* O175:H7, *S. aureus* and *L. monocytogenes*. These were analyzed by modification of the method used by Canillac and Mourey (2001). The clove extract was diluted in the range 0% to16% w/v in TSBYE and tested. The method used has been described elsewhere. The medium was inoculated with 0.1 ml of a pre-culture in TSBYE at 37ºC. The cells of the inoculums were at the exponential growth phase after 2-3 h of static incubation or at a stationary phase after 17-19 hours of static incubation. The final concentration of bacteria, determined by the plate count method, was of the order of 3×10^5 CFU/ml. The MBC is the lowest concentration of clove extract for which no growth was detected after 48 h at 37 ºC (Canillac and Mourey, 2001).

Encapsulation of clove oil

The encapsulation of the clove oil was prepared by modifying the method used by Klaypradit and Huang (2008). Chitosan with concentrations of 2% was first dispersed in 1% aqueous acetic acid and continuously stirred at room temperature until the mixture was completely dissolved using visual examination. Clove oil at 20%, previously mixed with Tween 80 (2.5%), was added to the solution until the mixture was completely dissolved. The mixture was emulsified using a homogenizer (OMNI International, Waterbury, CT) at 10000 rpm for 5 min. When total dissolution was obtained the 20% of MD was slowly added. A homogenizer was used at 10000 rpm for 5 mins and the encapsulated clove oil was kept in amber-colored bottles and stored at 4°C until used.

Preparation and casting of antibacterial edible film

Edible films were prepared by modification of the method used by Pranoto *et al*. (2005). Then 1.5% Hydroxypropyl methylcellulose (HPMC) was dissolved into 100 mL of distilled water and rotary shaking was undertaken concurrently for 30 min. As the edible HPMC film was brittle, 30% of glycerol was added to the edible film solution. Subsequently, the encapsulated clove oil at 1, 2, 3 fold of MBC of each tested bacteria was added and mixed for 5 min. After mixing, the mixture was degassed under vacuum conditions and cast on flat, leveled non-stick trays to set. Once set, the trays were held at 50°C for 15 h undisturbed, and then cooled to an ambient temperature before the films were peeled off the plates. Film samples were stored in plastic bags and held in desiccators at 50% RH for further testing. All treatments were made in triplicate.

Antimicrobial activity

Testing of the antimicrobial activity of the edible films was carried out using the agar diffusion method according to Pranoto *et al*. (2005). The edible film were cut in 17 mm diameter discs and then placed on Mueller Hinton agar (Merch, Darmstadt, Germany) plates. These 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 hours. Observations were made of the diameter of the inhibitory zone surrounding film discs and contact area of edible film with agar surface. 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 were conditioned at 50% RH and 27 $\pm$2°C by placing them in desiccators over a saturated solution of $Mg(NO_3)_2$.$6H_2O$ for 72 hours or more. These were used for testing water vapor permeability (WVP), tensile strength (TS), and elongation (E). For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

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 calculating water vapor permeability (WVP) and tensile strength (TS).

Film solubility

A modified method from Jangchud and Chinnan (1999) was used to measure film solubility. Pieces of film, 20 mm x 20 mm, were dried at 70°C in a vacuum oven for 24 h and then weighed to the nearest 0.0001g for the initial dry mass. The films were then immersed in 20 ml of distilled water in 50 ml screw cap tubes containing 0.05 g/100 g sodium benzoate. The tubes were capped and placed in a shaking water bath for 24 h at 25$\pm$2°C. A portion of the solution was removed and set aside for later use for solubility

tests. The remaining solution and pieces of film were poured onto (Whatman NO.1) qualitative filter paper and rinsed with 10 ml distilled water. These were then dried at 70°C in a vacuum oven for 24 h to determine the dry mass of the film. Five measurements were taken for each treatment. The total soluble matter was calculated from the initial gross mass and the final dry mass using the following equation:

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

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 20 g of Silica gel (desiccant) to produce a 0% RH below that of 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 film surface and desiccant.

The water vapor transmission rate (WVTR) of each film was measured at 60±2% RH and 25±2°C. After noting 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). The 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 of 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 film.

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

Tensile strength was measured with a LLOYD Instrument (Model LR30K, LLOYD Instruments Ltd., Hampshire, England) as per the ASTM D882-91 Standard Method (ASTM, 1993b). Ten samples, 2.54 cm x 12 cm, were cut from each film. The initial grip separation and crosshead speed were set at 50 mm and 30mm/minutes, 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:

$$\%\,\varepsilon = 100 \times (d\ \text{after} - d\ \text{before})/d\ \text{before}$$

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 values [(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 (assessed by means of five measurements at different locations on each specimen) was measured on 10 cm x 10 cm segments of film. The total color difference (ΔE_{ab^*}), hue angle (H), and chroma (C) were calculated using the following equation:

$\Delta L^* = L^*$ sample $- L^*$ standard, $\Delta a^* = a^*$ sample $- a^*$ standard, $\Delta b^* = b^*$ sample $- b^*$ 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 hours.

Scanning electron microscopy

Film samples were examined for surface characteristics using a 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 the 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 ASTM D1746. The transparency (T_{600}) was calculated from

the following equation:

$$T_{600} = (\log \%T)/x$$

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

Results and Discussion

Minimal bactericidal concentration (MBC)

The susceptibility of the *S. aureus*, *E. coli* O175:H7 and *L. monocytogenes* to clove oil w as determined by the MBC. The results showed that clove oil inhibited tested microorganisms. The susceptibility of the test microorganisms to clove oil, the MBC of clove oil, was 0.5, 1.0 and 1.5% of *L. monocytogenes*, *E. coli* O175:H7 and *S. aureus* respectively (data not shown). These results indicated that clove oil contained eugenol, carvacrol and thymol which are phenolic compounds that cause antimicrobial activity. Zaika (1988) reported the differences in the antimicrobial activity of clove oil with clove oil exerting a strong inhibitory effect. The essential oil fraction is particularly high in clove and eugenol and accounts for 63.37 of that fraction (Shelef, 1983).

Prindle and Wright (1997) reported that the antimicrobial activity of phenolic compounds was dependent on concentration. This affected enzymatic activity related to energy production at low concentrations and caused protein precipitation at high concentrations. Moreira *et al.* (2005) also reported on the the parameters of antimicrobial activity of the essential oil of cloves on survival and growth of different microorganisms. The exact cause–effect relationship for the mode of action of phenolic compounds has not been determined. Davidson (1993) indicated that they may inactivate essential enzymes, reacting with the cell membrane or disturb material functionality. Cox *et al*. (2000) exposed gram negative bacterium *E. coli* AG100, gram-positive bacterium *Staphylococcus aureus* NCTC 8325 and yeast *Candida albicans* to the MBC of tea tree oil. They reported that this inhibited respiration and increased the permeability of bacterial cytoplasm and yeast plasma membranes.

Our results suggest that clove oil could be put into use as bactericidal and bacteriostatic agents. Using them in the treatment of food will prevent the deterioration of stored foods. They could prevent growth or even decrease the viable cell numbers of food pathogenic bacteria such as *S. aureus*, *E. coli* O175:H7 and *L. monocytogenes*. But the phenolic components of the essential oil are the reasons for the strongest antimicrobial activity being shown (Farag *et al*., 1989).

Antimicrobial activity of edible HPMC films

The antimicrobial effect of the edible HPMC films obtained was tested by using *L. monocytogenes*, *E. coli* O175:H7 and *S. aureus* as test microorganisms. The results of the antimicrobial tests were in close agreement with our previous findings obtained from MBC tests. Table 1 shows the antimicrobial inhibition zones for edible HPMC films incorporated with encapsulated clove oil plotted against microorganisms are. Film without encapsulated clove oil was not effective against any of the microorganisms used in the tests.

The zone of inhibition was observed even at 1 fold of MBC for *S. aureus*, *E. coli* O175:H7 and *L. monocytogenes*. As the concentration of encapsulated clove oil increased, the zone of inhibition increased significantly for all microorganisms tested. The inhibitory zones of *S. aureus*, *E. coli* O175:H7 and *L. monocytogenes* increased from 28.75±0.50 to 39.00±0.82, 18.50±0.58 to 26.75±0.96 and 17.67±0.36 to 31.00±0.52 mm diameter. This was the case when encapsulated clove oil increased from 1 fold of MBC to 3 folds of MBC respectively (Table 1). These results proved that the active compound of encapsulated clove oil could be immobilized in the edible HPMC films and subsequently released, thereby inhibiting the target microorganisms. The existing inhibition zone diameter of tested microorganisms can be attributed to the fact that the mode of action of phenolic compounds, especially eugenol (approximately 63.37%), disintegrates the outer membrane of bacteria. This releases lipopolysaccharides and increases the permeability of the cytoplasmic membrane to ATP (Cosentino *et al*., 1999: Dadalioglu and Evrendilek, 2004: Wenqiang *et al*., 2007). Our results pointed out the potential value of the application of encapsulated clove oil as a natural preservative in food packaging technology.

Table 1. Antimicrobial activity of edible HPMC films incorporated with encapsulated clove oil against *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus*

Bacteria types	Clove oil % (w/v)	Observation at 24 hours	
		Inhibitory zone[A] (mm)	Contact area[B]
Listeria monocytogenes	0 (Control)	0.00±0.00[a]	-
	0.5 (1 fold of MBC)	16.67±0.36[b]	+
	1.0 (2 folds of MBC)	25.00±0.32[c]	+
	1.5 (3 folds of MBC)	31.00±0.52[d]	+
Escherichia coli	0 (Control)	0.00±0.00[a]	-
	1.0 (1 fold of MBC)	18.50±0.58[b]	+
	2.0 (2 folds of MBC)	24.50±0.58[c]	+
	3.0 (3 folds of MBC)	26.75±0.96[d]	+
Staphylococcus aureus	0 (Control)	0.00±0.00[a]	-
	1.5 (1 fold of MBC)	28.75±0.50[b]	+
	3.0 (2 folds of MBC)	31.74±0.50[c]	+
	4.5 (3 folds of MBC)	39.00±0.82[d]	+

+: 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.

Film properties

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 involved includes scientific and also technological and practical aspects (Freddi *et al*., 1995). 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 tensile strength 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 tensile testing. It is expressed as the percentage of change of the original length of the specimen between the grips used to stretch the film (Gontard *et al*., 1992).

The addition of encapsulated clove oil influenced the film's properties. The TS and ε of the edible film incorporated with encapsulated clove oil are depicted in Figure 1. The TS of edible HPMC films was affected by the encapsulated clove oil. The results demonstrated that the TS of edible HPMC films decreased with the addition of encapsulated clove oil, and the maximum TS occurred when no encapsulated clove oil was added (20.38 MPa). The TS of the edible HPMC films decreased as the concentration of encapsulated clove oil increased (Figure 1A). For example, the TS of the films decreased from 20.38 to 7.26 MPa when encapsulated clove oil was added at 0.5 to 3.0 %. This notable decrease in the TS of the edible HPMC films indicated the presence of oil as an additive material of the films.

Gontard *et al*. (1994) showed that an increment of oil and/or lipid content yielded a loss in the mechanical properties for emulsified wheat gluten-based films. In general, lipid films lack the structural integrity of polysaccharide films (Gontard *et al.*, 1995). Therefore, the incorporation of lipids into hydrophilic polysaccharide films in an effort to decrease their water vapor permeability can negatively affect film strength as expressed by TS measurements. The increase in the lipid concentration causes a partial replacement of lipids in the film matrix. Weller, Gennadios and Saraiva (1998) reported that the decrease in Young's modulus of protein film accompanying the increase in lipid concentration was related to the weakening effect of lipid on the protein network. This was due to the lack of structural integrity of the lipid. The interactions between non-polar lipid molecules and between the polar polymer and non-polar lipid molecules are believed to be much lower than those between the polar polymer molecules.

Typically, a strength reduction of edible composite films with the incorporation of lipids has also been reported (Shellhammer and Krochta, 1997; Yang and Paulson, 2000; Bertan *et al*., 2005). However, the results showed no significant differences ($p < 0.05$) occurring in the TS of edible HPMC films between 0.5 and 1.0% of encapsulated clove oil incorporated in edible HPMC films (Figure 3A). Furthermore, the increases of encapsulated clove oil concentrations above 1.5% caused greater reduction of TS. These results were similar to those results of Pranoto *et al*. (2005). They reported that the TS of alginate-based edible film incorporating garlic oil markedly affected the film's TS, as seen in the reduction of TS value with increased amounts of garlic oil. A significant difference ($p<0.05$) in TS was markedly shown after a 0.3% incorporation of garlic oil.

These values were similar to those of Bertan *et al*. (2005) who reported a decrease in the TS of films made from bovine hide gelatin with the addition of hydrophobic substances. The elongation at break (ε) of edible HPMC films increased from 17.4 to 24.5% as the concentration of encapsulated clove oil increased from 0 to 1.5% (Figure 1B). The same effect was found by Shellammer and Krochta (1997). They observed an increase in the ε of milk whey protein with an increase in lipids concentration caused by a plasticizing effect. However, the addition of encapsulated oil above 1.5 % resulted in a marked decrease of ε (Figure 1B). Gallo *et al.* (2000) attributed the reduction of ε to the affinity of lipids with the emulsifier. The water content of films containing lipids compared to films containing only rice starch-chitosan could be due to the decreased elongation generally noticed when lipids are dispersed in the hydrocolloid matrix (Gallo *et al*., 2000). Decreasing the ε of edible HPMC films as the encapsulated clove oil is increased might be due to a lower continuity of the HPMC network in the presence of clove oil.

Water vapor permeability and film solubility

Water vapor permeability (WVP) is proportionality constant and assumed to be independent of the water vapor pressure gradient applied across the films. However, hydrophilic (edible or non-edible) materials, such as protein films, deviate from this ideal behavior due to the interactions of permeating water molecules with polar groups in the films' structure (ASTM, 1991). A main function of an edible films or coatings is often to impede moisture transfer between food and the surrounding atmosphere, or between two components of a heterogeneous food product. Thus the WVP should be low as possible.

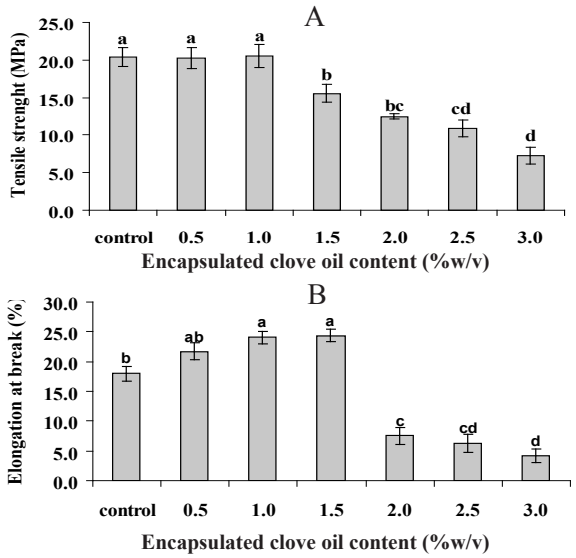

Figure1. Influence of encapsulated clove oil content on the tensile strength (A) and elongation at break (B) of edible HPMC films (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

The incorporation of encapsulated clove oil affected the WVP of the edible HPMC films. A significant difference ($p<0.05$) was shown even after incorporating 0.5% of encapsulated clove oil. This probably occurred due to the hydrophobic property of encapsulated clove oil. In this system, encapsulated clove oil could enter the structural matrix of edible HPMC films; this results in decreased moisture passing through the edible film. Gontard *et al*. (1994) observed that when lipids were used in composite film formulations, large amount of lipids could promote a protective effect on WVP. The WVP value tended to decrease as higher amounts of encapsulated clove oil was incorporated. The WVP value varied from 70.45 to 25.85 g.mm/m^{2}day.kPa (Figure 2A).

It has also been previously reported that the increase of lipid content especially between 0 and 30% allows for the improvement of the barrier's efficiency (Kamper and Fennema, 1984a; Morillon *et al*., 2002). Kamper and Fennema (1984a, b) examined the effect of fatty acids on the WVP of hydroxypropyl methylcellulose (HPMC) films. They indicated that stearic acid and a 1:1 composite of stearic-palmitic acids were the most effective fatty acids in reducing the WVP of the films. Guillén *et al*. (2009) studied the addition of sunflower oil to cod gelatin in proportions of 0%, 0.3%, 0.6%, and 1%. This was done to improve the hydrophobic properties of the resulting films and decrease WVP and the soluble matter content.

Water resistance is an important property of edible films for use in food protection. This is so where water activity is high, or when film must be in contact with water during the processing of coated food, as in avoiding the exudation of fresh or frozen products (Gontard *et al*., 1992). Generally, higher solubility would indicate lower water resistance. However, high solubility may an advantage for some applications (Stuchell and Krochta, 1994). The effect of encapsulated clove oil on film solubility is shown in Figure 2B. Film solubility markedly decreased when the encapsulated clove oil was incorporated. For example, film solubility decreased from 20.25% to 13.67% when encapsulated clove oil was increased from 0.5% to 3.0% (Figure 2B). The loss in solubility was pronounced when greater levels of lipids were applied. Water solubility is indicative of the film's hydrophilicity. The addition of encapsulated clove oil thus increased the hydrophobicity of the edible HPMC films. Similar results were reported by Kim and Ustanol (2001) on lipid-whey protein emulsion films.

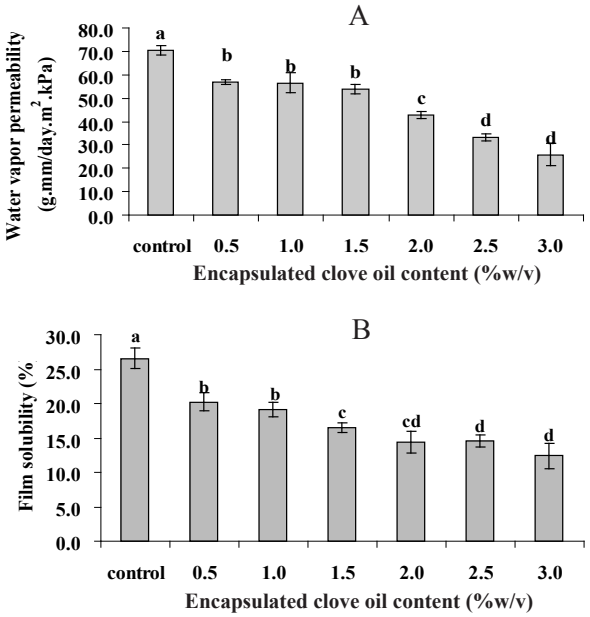

Figure 2. Influence of encapsulated clove oil content on the water vapor permeability (A) and film solubility (B) of edible HPMC films (error bars are standard errors of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

Color

The values of color measurement taken into account were L^*, a^*, b^*, ΔE_{ab^*}, hue angle and chroma. The color performances of encapsulated clove extract incorporated in edible film can be seen in Figure 3. Edible HPMC films without the incorporation of clove oil appeared clear and transparent. The addition of encapsulated clove oil affected the appearance of the color of edible film. The edible HPMC films became lighter yellow as evidenced by the increased L^*, a^*, b^* and chroma values when the concentration of encapsulated clove oil in the HPMC films increased (Figure 3 and 4).

The incorporation of encapsulated clove oil significantly increased the yellowness of film as evidenced by high b^* and chroma values for edible HPMC films (Figure 3). This was due to the encapsulated clove oil and/ or lipids being more yellow than the edible HPMC films. In addition, the clove oil could be oxidized by oxygen during the casting process. This was attributed to the edible HPMC films containing encapsulated clove oil being inherently more yellow compared with edible HPMC films with no encapsulated clove oil.

Total color change was observed by reading the ΔE_{ab^*} values. Experiments showed that the ΔE_{ab^*} of edible HPMC films showed no significant change ($p<0.05$) when the content of encapsulated clove oil increased from 1.0% to 2.5%. However, there were significant changes at higher than 2.5%. This indicated a color change due to the incorporation of encapsulated clove oil. However, the addition and content of encapsulated clove oil in this study did not seem to significantly affect the hue angle of edible HPMC films (Figure 4).

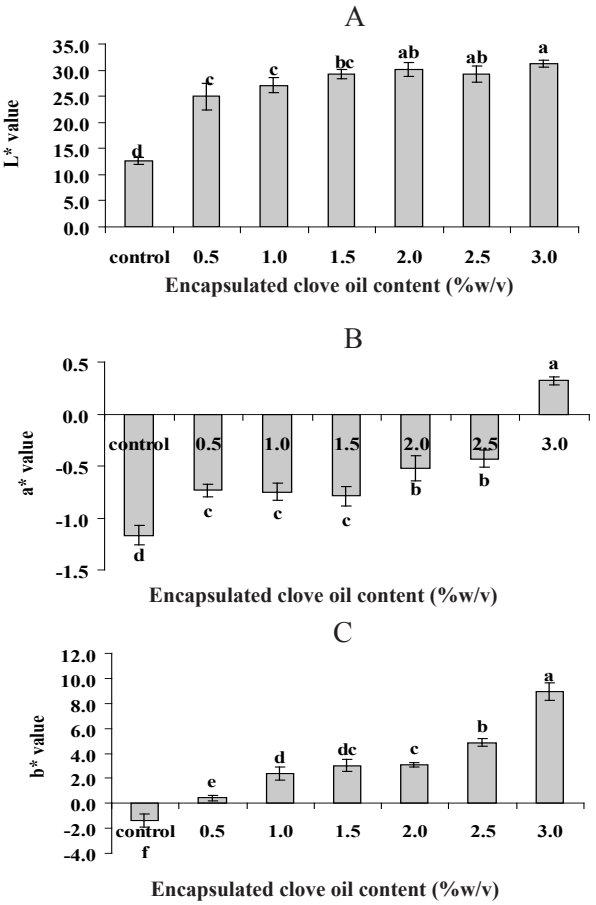

Figure 3. Influence of encapsulated clove oil content on the L^*(A), (B) a^* and b^*(C) of edible HPMC films (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

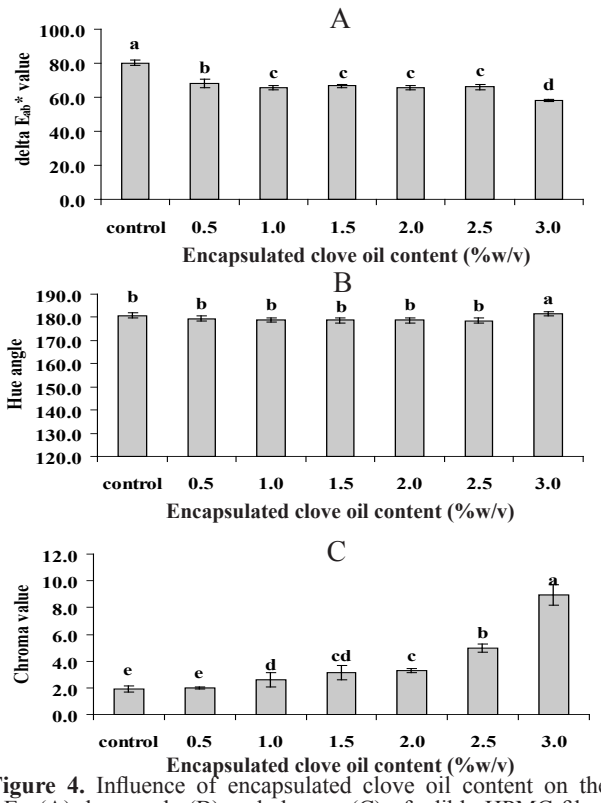

Figure 4. Influence of encapsulated clove oil content on the ΔE_{ab^*}(A), hue angle (B) and chroma (C) of edible HPMC films (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

Transparency

Visually, edible HPMC films were quite transparent, though the films turned darker when encapsulated clove oil was added. This was due to the light-scattering effect of the emulsion that was formed by the composite encapsulated clove oil incorporated in the edible HPMC films. At the same time, film darkness increased with the amount of encapsulated clove oil added. Transparency decreased perceptibly on adding encapsulated clove oil (Figure 5). The addition of lipids generally caused films to lose or reduce their transparency (Yang and Paulson, 2000; Shaw *et al*., 2002; Pommet *et al*., 2003; Bertan *et al*., 2005). The results demonstrated that the transparency of edible HPMC films markedly decreases from 1.32 to 0.68 when the encapsulated clove oil was increased from 0.5% to 3.0% (Figure 5). Bertan *et al*. (2005) also reported increased film opacity with increasing concentrations of hydrophobic substances.

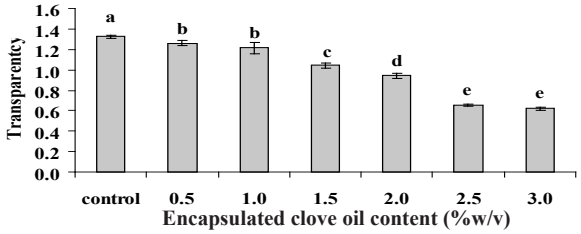

Figure 5. Influence of encapsulated clove oil content on the transparency of edible HPMC films (error bars are standard error of the mean of five measurements from three separate films). Different letters indicate significantly different groups determined by Duncan's test ($p<0.05$).

Microstructure

It was decided to elucidate more clearly the relationship between the surface characteristics and moisture barrier properties of the edible HPMC films incorporated with encapsulated clove oil. A scanning electron microscopy (SEM) was used to determine the surface morphology of edible HPMC films with encapsulated clove oil as well as edible HPMC films without encapsulated clove oil (Figure 6). It was observed that the reference film (without encapsulated clove oil added) was free of air bubbles, smooth and had a continuous surface without a grainy and porous structure (Figure 6). The micrographs show increased surface irregularity with the addition of encapsulated clove oil. Each type of film was made by the same procedure. It is unlikely that the irregular features were due to the encapsulated oil, a droplet being more intense on the surface or the entrapped air bubbles, since each type of film was made by the same procedure.

Figure 6 illustrates the scanning electron micrograph of edible HPMC films containing various concentrations of encapsulated clove oil. The results show that the encapsulated clove oil was more intense on the surface as the concentration of incorporated encapsulated clove oil increased, resulting in a more noticeably irregular surface (Figure 6).

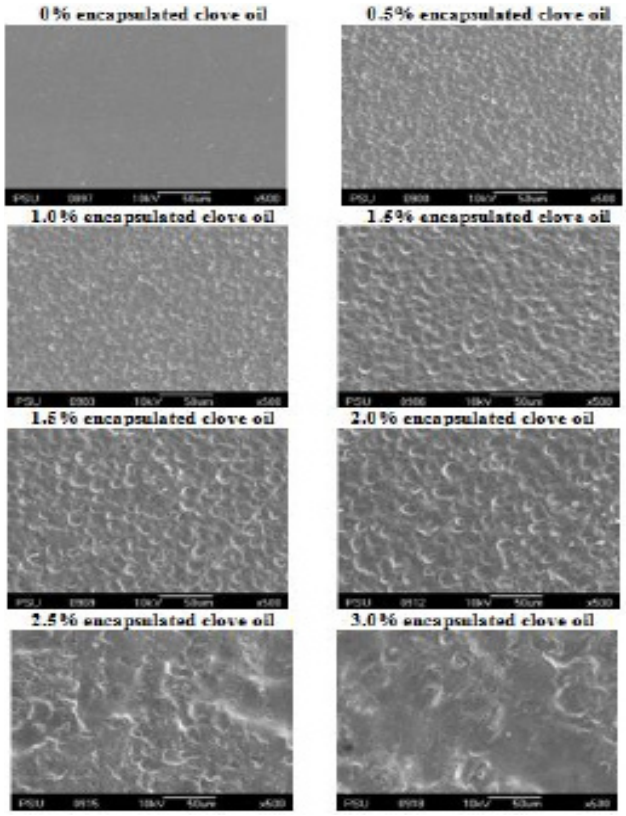

Figure 6. Scanning electron micrograph of edible HPMC films containing various encapsulated clove oil content

Conclusions

The edible HPMC films incorporated with encapsulated clove oil exhibited antibacterial activity against pathogenic bacteria tested by using agar diffusion assay. Increasing the encapsulated clove oil in the edible HPMC films yielded a higher inhibition of tested pathogenic bacteria. TS, ε, WVP, and FS properties were significantly decreased with the incorporation of encapsulated clove oil. The color of edible HPMC films was darker and more yellowish as encapsulated clove oil was increased. A lower transparency of the edible HPMC films was noticed when a greater amount of encapsulated clove oil was incorporated. Encapsulated clove oil incorporated in edible HPMC films provided the films with a rougher surface than that of pure edible HPMC films. Our results pointed out the potential value of the application of encapsulated clove oil as a natural preservative in food packaging technology.

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