ANTIMICROBIAL ACTIVITY AND PHYSICAL PROPERTIES OF SAGO STARCH – ALGINATE EDIBLE FILM INCORPORATE WITH LEMON GRASS ESSENTIAL OIL

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The increase of consumers demand for higher quality and longer shelf-life in foods, while reducing disposable packaging materials, has encouraged further research into edible films and coatings from natural polymer such as polysaccharides. The sago starch –alginate (SA) ratio (4: 1) was produced with addition of 20% glycerol as plasticizer. Further, lemon grass (LG) essential oil was added at different percent (0 (control), 0.1, 0.2, 0.3 and 0.4%) v/v of edible film solution. The films were characterized for Antimicrobial activity, Water vapor permeability (WVP), tensile strength (TS), percent elongation (%E) and water solubility. The antimicrobial properties of the films containing 0.1 to 0.4% (v/v) ratios of lemon grass essential oil were tested against *Escherichia coli* 0157: H7 (E-coli). Zones of inhibition were measure after an incubation period (24hr). The films containing (LG) essential oil was effective against E-coli at all levels. The film containing 0.1% (LG) essential oil was significantly ($p<0.05$) different to films containing 0.2%, 0.3% and 0.4% level respectively for the zone of inhibition area. Tensile strength of the films were significantly ($p<0.05$) different by incorporation of (LG) essential oil at 0.1% level (15.96 Mpa) to 0.2% level and 0.3% level which is 14.43Mpa and 13.42Mpa respectively. In general, the tensile strength of films containing (LG) essential oil decreased as the oil content increased. The percent elongation values were found to be increase with the increase percent of oil content. The percent elongation of the films were significantly ($p<0.05$) different by incorporation of (LG) essential oil at 0% level (3.71%) to 0.1% level (5.13%), 0.2% level (5.77%), 0.3% level (8.67%) and 0.4% level (13.21%) respectively. The water permeability value varied from 2.345 to 4.04 g.m/m^2.s.Pa. The water vapor permeability values tended to increase as higher amounts of (LG) essential oil were incorporated. Percent water solubility of the films was also found to increase as amount of (LG) essential oil increased. These results revealed that lemon grass essential oil has a good potential to be incorporated into sago starch- alginate to make antimicrobial edible film or coating for various food application.

Antibacterial Activity and Mechanical Properties of Partially Hydrolyzed Sago Starch–Alginate Edible Film Containing Lemongrass Oil

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ABSTRACT: Edible films were prepared from a mixture of partially hydrolyzed sago starch and alginate (SA). Lemongrass oil (0.1% to 0.4%, v/w) and glycerol (0% and 20%, w/w) were incorporated in the films to act as natural antimicrobial agent and plasticizer, respectively. The films were characterized for antimicrobial activity, water vapor permeability (WVP), tensile strength (TS), percent elongation at break (%E), and water solubility (WS). Fourier transform infrared (FTIR) spectroscopy was conducted to determine functional group interactions between the matrix and lemongrass oil. The zone of inhibition was increased significantly ($P < 0.05$) by addition of lemongrass oil at all levels in the presence and the absence of glycerol. This indicates that the film containing lemongrass oil was effective against *Escherichia coli* O157:H7 (*E. coli*) at all levels. In the absence of glycerol, the tensile strength of film decreased as the oil content increased, but there was no significant ($P > 0.05$) difference in percent elongation. The percent elongation at break and WVP values for film with 20% glycerol was found to be increased significantly ($P <$ 0.05) with an increase in lemongrass oil content. Addition of lemongrass oil did not have any interaction with the functional groups of films as measured by FTIR.

Keywords: antibacterial activity, edible film, *Escherichia coli* o157:h7, glycerol, lemongrass oil, mechanical properties, sago starch, water vapor permeability

Introduction

Edible films and coatings prepared from polysaccharides, proteins, and lipids have a variety of advantages such as biodegradability, edibility, biocompatibility, esthetic appearance, and barrier properties against oxygen and physical stress. With regard to biodegradable packaging, starch is the commonly used agricultural raw material because it is a renewable source, widely available, relatively easy to handle, and inexpensive (Lourdin and others 1995).

Starch is a mixture of 2 polymers, the linear D-glucan amylose and the highly branched amylopectin. These 2 polymers exhibit different behaviors with regard to gelation and development of crystallinity. In gel formation, amylose and amylopectin form inter and intramolecular physical crosslinks to produce a macromolecular network (Miles and others 1985a, 1985b). Physical crosslinkages in the macromolecular network of starch are formed mainly by microcrystalline domains of amylose, which contributes to the higher tensile strength of films (Rindlav-Westling and others 1998). Several studies have been done to analyze starch-based film properties (Arvanitoyannis and Biliaderis 1998, 1999; Rindlay-Westling and others 1998; Mali and others 2002; Ryu and others 2002; Rodriguez and others 2006; Talja and others 2007). Starch produces films that have good mechanical properties and coverings that are efficient barriers against low-polarity compounds (Kester and Fennema 1986).

Enzymatic production of linear long-chain dextrin from sago starch has been studied by Wong and others (2005). The pullulanase enzyme is able to split off α-1,6 glucosidic bonds in amylopectin and increase the linear long-chain dextrin content of sago starch. The linear long-chain dextrin of starch and high-amylose starch, composed of at least 40% amylose, has the ability to form strong gels and films as reported by Zallie (1994).

Sodium alginate, which is the sodium salt of the alginic acid, is a polyelectrolyte. It is a copolymer consisting of D-mannuronic and L-guluronic acid monomers (Borchard and others 2005). Edible films prepared from hydrocolloids like alginate form strong films and exhibit poor water resistance because of their hydrophilic nature (Guilbert 1986; Kester and Fennema 1986). A mixture of starch and alginate to form edible film has been studied by Wu and others (2001). Alginate has a potential to form biopolymer film or coating component because of its unique colloidal properties, which includes thickening, stabilizing, suspending, film forming, gel producing, and emulsion stabilizing (King 1982). Alginate is able to form a strong film (Guilbert 1986; Kester and Fennema 1986); therefore, the mixture of these 2 biopolymers is desirable to improve the mechanical properties of film.

The most commonly used plasticizers in starch-based films are polyols, such as sorbitol and glycerol. Glycerol is often used to modify the mechanical properties of hydrophilic films. According to Gontard and others (1993), it improves film extensibility but reduces film elasticity. Glycerol is compatible with amylose and improves mechanical properties of films by decreasing intermolecular attraction and interfering with the amylose packing (Donhowe and Fennema 1993). They avoid cracking of film during handling and storage (Gontard and others 1993). A Plasticizer, such as glycerol, is often used to modify the mechanical properties of film (Gaudin and others 1999; Myllarinen and others 2002). It is a low-molecular-weight nonvolatile substance and addition into film reduces internal hydrogen bonding between polymer chains while increasing

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molecular volume, resulting in an improvement of film flexibility (Mali and others 2006).

Antimicrobial edible films have received attention as a potential pathogen intervention strategy for various muscle foods. The combining of antimicrobials with packaging films to control the growth of microorganism in food could have a significant impact on shelf-life extension and food quality (Quintavalla and Vicini 2002). Addition of an antimicrobial agent into packaging may affect the physical properties of packaging materials. Various antimicrobial compounds incorporated into edible films have been investigated. Incorporation of natural derivatives such as oregano, rosemary, and garlic oils into edible film to inhibit the growth of microorganisms have been successfully demonstrated (Quattara and others 2000; Pranoto and others 2005; Seydim and Sarikus 2006). However, no publications in the literature are available regarding antibacterial property of lemongrass oil incorporated into edible film. Therefore, it is important to investigate the possibility of producing antibacterial edible film by incorporating lemongrass oil.

Generally, the essential oils possessing the strongest antibacterial properties against foodborne pathogens that contain higher concentrations of phenolic compounds, such as carvacrol, eugenol, and thymol, exhibit a wide range of biological effects including antioxidant and antimicrobial properties. The mode of action is considered to be the disturbance of the cytoplasmic membrance, disrupting the proton motive force, electron flow, and active transport, and/or coagulation of bacteria cell contents (Burt 2004). According to Onawunmi and others (1984), lemongrass oil has shown antibacterial activity against *Escherichia coli* O157:H7, *Bacillus subtilis*, and *Staphylococcus aureus*. In their study, drops of lemongrass oil were placed on filter paper before it was tested on specific bacteria. They found that the antimicrobial activity of lemongrass oil is related to high amounts of 1,8-cincole (>30%), geranial (>30%), and neral (>20%). However, citral isomers (neral, 32.3%, geranial, 41.28%) are the most abundant compounds in lemongrass oil as reported by Choi and others (2000). The presence of minor components such as nerol, borneol, linalool, cinnamaldehyde, carvacrol, geraniol, myrtenal, and eugenol are also known to exhibit antimicrobial activity (Hinou and others 1989). The objective of this research was to develop a partially hydrolyzed sago starch–alginate edible film base with incorporated lemongrass oil and glycerol. The physical, mechanical, and antimicrobial properties of these films were then investigated.

Materials

Sago starch (*Metroxylon sagu*) was obtained from Nitsei Sago Industries Sdn. Bhd. Penang, Malaysia. The other chemicals used in this analysis were alginate from (Acros Organic, Fairlawn, N.J., U.S.A.), glycerol from (Qrec, New Zealand), pullulanase enzyme (EC 3.2.1.4.1, pullulan 6-glucanohydrolase) from Novo Nordisk (Bagsvaerd, Denmark), and broth and agar were purchased from Merck, (Darmstadt, Germany).

Analysis of amylose content

Apparent amylose contents was determined by iodine-binding method described by Jarvis & Walker (1993). Amylose (Type III from potato, amylopectin free), and amylopection from potato both from Fluka, (Buchs, Switzerland) were used as standards. Iodine reagent was prepared from I_2 (0.2) dissolved in 100 mL of 2% (w/v) KI. Starch samples were homogenized in 80% (v/v) ethanol to remove sugar free lipids, centrifuged and dried in an air oven at 40 °C for 4 h. Amylose, and amylopectin standards, and starch samples were dissolved in 5 mL of 1 M KOH solution and 5 mL of distilled water was added. Next, 1 mL of this solution was neutralized with 5 mL of 0.1 M HCL, iodine reagent (0.5 mL) was added, and the volume was made up to 50 mL with distilled water. Absorbance values were determined at 6 wavelengths: 504, 548, 580, 630,700, and 800 nm with the Shimadzu UV-160 A UV-visible recording spectrophotometer (Tokyo, Japan). For the multicomponent analysis to determine the amount of amylose and amylopection, pairs of simultaneous equations based on Beer's Law ($A = E_0 \times C \times 1$) were used:

$$Abs_1 = E_0\text{am}_1 \times C_{\text{am}} + E_0\text{ap}_1 \times C_{\text{ap}}$$

$$Abs_2 = E_0\text{am}_2 \times C_{\text{am}} + E_0\text{ap}_2 \times C_{\text{ap}}$$

where Abs_1 is the sample absorbance at the 1st wavelength; Abs_2 is the sample absorbance at the 2nd wavelength; $E_0\text{am}_1$ is the absorptivity of amylose at the 1st wavelength; $E_0\text{am}_2$ is the absorptivity of amylose at the 2nd wavelength; C_{am} is the amylose concentration and C_{ap} is the amylopectin concentration.

Preparation of raw material

Partially debranched starch was prepared by enzymatic hydrolysis. Sago starch (200 g) was suspended in 1000 mL sodium acetate buffer, pH 5.0 (20% w/v, starch slurry). Pullulanase enzyme (20% v/w of starch) was added and the starch suspensions were incubated at 58 °C and agitated at 200 rpm in an orbital incubator shaker (Certomat® *SII*; B. Braun Biotech Intl., Melsungen, Germany) for 20 h. The enzyme was deactivated at 75 °C for 15 min followed by centrifugation at 3000 × *g* for 15 min and the precipitate was collected and dried in an oven at 40 °C.

Organisms and preparation of cultures

E. coli O157:H7 culture was obtained from the Microbiology Laboratory (School of Industrial Technology, USM, Malaysia). The bacterial culture was grown on nutrient agar slants and kept at 4 °C. Subculturing was carried out every month to maintain bacterial viability.

Preparation of lemongrass oil

Fresh lemongrass (*Cymbopogon citratus*) was purchased from a local market in Penang. The lemongrass oil was obtained by using conventional steam distillation for 7 h according to the method of Dadalioglu and Evrendilek (2004). The oil was collected, dried with anhydrous sodium sulfate, and stored in airtight glass vials at 4 °C until used.

Preparation of partially hydrolyzed sago starch–alginate film

Film forming solutions were prepared from a mixture of partially hydrolyzed sago starch and sodium alginate (4:1) based on total weight basis (5 g) including 20% glycerol in 200 mL distilled water. Lemongrass oil was initially diluted to 10% concentration using ethanol (95%) and then incorporated into the edible film solution at various concentrations (0.1%, 0.2%, 0.3%, and 0.4% v/w) of edible film forming solution. The mixture was heated to 85 °C with continuous stirring for 45 min before it was cooled to room temperature. The solutions (95 g) were cast onto polyacrylic plates (16 × 16 cm) followed by oven drying at 40 °C for 24 h. The dry films obtained were peeled off and stored for further analysis.

Film thickness

Film thickness was measured using a micrometer (dial thickness gauge 7301; Mitutoyo Co., Tokyo, Japan) to the nearest 0.01-mm accuracy at 5 locations, and the average value was calculated.

Scanning electron microscopy (SEM)

Films were observed using SEM. Dried film samples were placed on double-stick tape mounted on a specimen holder, coated with 100 to 200 thickness of gold and photographed using SEM apparatus (Leo Supra 50vp, Oberkochen, Germany).

Antimicrobial activity of films

Antimicrobial activity test on films was carried out using the agar diffusion method according to Chen and others (1996). The zone of inhibition assay on solid media was used for determination of the antimicrobial effects of films against *E. coli* O157:H7. The edible films were cut into 6-mm-diameter discs and then placed on Mueller Hinton agar (Merk, Darmstadt, Germany) plates, which had been previously seeded with 0.2 mL of inoculums containing approximately 10^5 to 10^6 CFU/mL of *E. coli*. The plates were then incubated at 37 °C for 24 h. After that, the plates were examined for 'zone of inhibition' of the film discs. The contact area was used to evaluate growth inhibition underneath the film disc in direct contact with target microorganisms in the agar. The area of the whole zone was calculated and then subtracted from the film disc area, and this difference area was reported as zone of inhibition.

Mechanical properties

Tensile strength and elongation at break of films were tested using a TA-TX2 texture analyzer (Stable Micro System, Goldaming, Surrey, U.K.) in accordance with ASTM D-882 (1981b). Five sample strips (14.0 × 2.0 cm) of each formulation were cut and clamped between tensile grips. Each film strip was placed in pneumatic grips (25 psi) and stretched at 0.5 mm s^{-1} and with an initial distance between the grips of 100 mm. The parameters determined were tensile stress (Mpa) and elongation at break (%E). Five replicates were tested.

Water vapor permeability

Water vapor permeability (WVP) tests were conducted following ASTM (1981a) Method E96–80. The test film was sealed as a patch onto a glass permeation cell containing silica gel (RVP = 0) with 1.5-cm-deep headspace. The glass permeation cells were 4.5 cm in diameter and 2.8-cm high. The cell was then placed in a desiccator maintained at 30 °C and RVP = 0.52 using a saturated salt solution of magnesium nitrate. The cell was weighed daily over a 6-d period. The water vapor transmission rate (WVTR) was calculated from the slope of the straight line divided by the test area. The samples were measured in 3 replicates. WVP (g Pa^{-1} s^{-1} m^{-1}) was calculated as WVP = $[WVTR / S (R_1 - R_2)] \times d$, where S = saturation vapor pressure (Pa) of water at test temperature, R_1 = RVP in the desiccator, R_2 = RVP in the permeation cell, and d = film thickness (m).

Water solubility

Water solubility of the films was determined according to Romero-Bestida and others (2005). Pieces of film (2 × 3 cm) were cut from each film and were stored in a desiccator with silica gel (0% RH) for 7 d. Samples were weighed to the nearest 0.0001 g and placed into beakers with 80 mL deionized water. The samples were maintained under constant agitation for 1 h at room temperature (approximately 25 °C).The remaining pieces of film after soaking were filtered through filter paper (Whatman no.1), followed by oven drying at 60 °C to constant weight. Samples were measured in 3 replicates and the percentage of total soluble matter (% solubility) was calculated as follows:

$$\% \text{ solubility} = \frac{(\text{initial dry weight} - \text{final dry weight})}{\text{initial dry weight}} \times 100$$

FTIR analysis

The spectra of films (0% and 20% glycerol incorporated with 0.1%, 0.2%, 0.3%, and 0.4% v/w lemongrass oil) were recorded by Fourier transform infrared (FTIR) spectrometry (System 2000, Perkin Elmer Wellesly, Md., U.S.A). The light source of transmittance was 650 to 4000 cm^{-1}. The spectra obtained were used to determine possible interactions of functional groups between partially hydrolyzed sago starch–alginate with lemongrass oil.

Statistical analysis

Experimental data were analyzed using Excel (Microsoft Inc.) and SAS software (SAS 1998). Duncan's multiple-range test ($P < 0.05$) was used to determine significant differences between means.

Results and Discussion

All films produced were easy to handle and transparent. They were also easily removed from the casting plate surface and had good flexibility and a smooth surface (based on visual observation). The average thickness of films was 0.066 ± 0.005 mm. Sago starch was modified enzymatically to obtain a slightly different form of starch with a higher percentage of linear fractions. The linear fraction content increased from 28.7% for native sago starch to 52.0% after the debranching process. The linear chains present in solution interact through hydrogen bonds, and consequently, form stronger gels and stiffer film (Rindlav-Westling and others 1998).

Antimicrobial activity

Inhibitory activity was measured based on the clear zone surrounding a circular film disc. If there is no clear zone, it is assumed that there is no inhibition. The results showed that the films containing lemongrass oil were effective against *E. coli* O157:H7. As the concentration of lemongrass oil increased, the zone of inhibition also increased significantly ($P < 0.05$) at all levels. The results of the antimicrobial assessment of edible film with incorporated lemongrass oil against *E. coli* O157:H7 are presented in Table 1. The presence of glycerol in the film markedly affects the inhibitory effect of the lemongrass oil in the film. The results showed that lemongrass oil exhibited significantly ($P < 0.05$) higher antimicrobial activity in the presence of glycerol, as evidenced by bigger inhibitory zones at all lemongrass oil concentrations except 0.4%. This could be attributed to the increased solubility of lemongrass oil in the matrix and more uniform dispersion of the oil in the film. However, there was no significant difference ($P > 0.05$) in the inhibitory zone for plasticized and unplasticized films at 0.4% addition of lemongrass

Table 1 – Antimicrobial activity of films incorporated with lemongrass oil against *Escherichia coli* O157:H7

Concentration (%wt/wt) of glycerol	Concentration (%v/w) of lemongrass oil	Inhibitory zone (mm^2)	Contact area
0	0 (Control	–	–
	0.1	$29.5^g \pm 2.8$	+
	0.2	$39.0^f \pm 2.6$	+
	0.3	$62.6^d \pm 3.3$	+
	0.4	$92.9^a \pm 2.6$	+
20	0 (Control)	–	–
	0.1	$50.0^e \pm 3.2$	+
	0.2	$69.9^c \pm 2.5$	+
	0.3	$78.9^b \pm 2.6$	+
	0.4	$94.0^a \pm 2.0$	+

Means ± standard deviation ($n = 5$) with different superscript letters are significantly different at ($P < 0.05$).
+ represents an inhibitory effect; and – represents no inhibitory effect.

oil. By increasing the concentration of lemongrass oil to 0.4%, it was observed that the capability of the oil to dissolve and disperse in both plasticized and unplasticized films was almost the same.

In this study, it seemed that lemongrass oil still exhibited similar antimicrobial activity as reported by Onawunmi and others (1984) when incorporated in the film. This suggests that the concentrations of active antimicrobial components in the oil present in the film are able to inhibit the growth of *E. coli*.

Mechanical properties

The addition of plasticizing agent to edible films is required to overcome film brittleness caused by extensive intermolecular forces. It also reduces these forces and increases the mobility of polymer chains, besides improving flexibility and extensibility of the film. Tensile strength represents the maximal force per original cross-sectional area that the film could sustain before breaking, while elongation reflects the extensibility of the material (Lim and others 1999).

The results given in Table 2 indicate that in the absence of glycerol the tensile strength of films was decreased significantly at all lemongrass oil concentrations. This is consistent with the results of [illegible]anoto and others (2005), who reported that increasing concentration of garlic oil in the film also decreased the tensile strength. It is suggested that the presence of lemongrass oil in the film had changed the tensile strength of the film by acting as a plasticizer, which increased the flexibility of the polymer chains. The elongation at break, however, did not change significantly as the level of lemongrass oil in the film was increased. The most likely reason could be due to the glass transition (T_g) of the film being very close to room temperature (testing temperature). Under this condition, the film was quite rigid and showed insignificant change in the elongation at break. However, in this study, T_g was not determined and therefore this statement remains to be substantiated. Leohakunjit and Noomhorn (2004) stated that as film structure softens, the tensile strength decreases and elongation increases and higher elongation indicates that the film is more flexible when subjected to tension and mechanical stress.

The results in Table 2 also clearly indicate that in the presence of 20% glycerol, the tensile strength of the film was reduced by more than 50% compared to films without glycerol. This could be attributed to the typical plasticizing effect of glycerol as reported by many researchers (Beeler and Finney 1983; Leohakunjit and Noomhorn 2004; Mali and others 2006). The effect of increasing levels of lemongrass oil on tensile strength was less obvious, due to the dominant plasticizing effect of glycerol. It is also interesting to note that the elongation at break showed significant increase with lemongrass oil concentration. It is evident that the glycerol has markedly exerted its plasticizing effect, thus making the films more rubbery.

Water vapor permeability

The effect of lemongrass oil on WVP of films in the presence and absence of glycerol is shown in Table 3. Generally, the WVP of plasticized films increases as plasticizer content of the film increases (Arvanitoyannis and others 1996). However, in the absence of plasticizer, the film made form starch is brittle (Koskinen and others 1996). Table 3 shows that film in the absence of glycerol had higher WVP compared to film plasticized with glycerol. This observation was also made by Talja and others (2007); the higher WVP of film lacking glycerol could be caused by microcracks occurring in the film. The formation of micro-cracks enhances moisture penetration through the edible film and thereby increases its WVP value. Figure 1 and 2 are the SEM of films in the presence and absence of glycerol content, respectively. From the micrographs, there seem to be no microcracks at the surface of the films in the presence of glycerol. However, microcracks were observed at the surface of films that lacked glycerol, which probably contributed to the increases in WVP of these films. The results also show that incorporation of lemongrass oil affects the WVP of films. The increase of WVP is related to the modification of film structure by the lemongrass oil. The addition of lemongrass oil into film caused a greater flexibility in polymeric structure and contributed to the increased water absorption in the film. As a result, in the presence of glycerol, the WVP value increased significantly after addition of 0.2% v/w lemongrass oil.

Water solubility

Solubility of edible films indicates their integrity in an aqueous environment. Higher solubility would indicate lower water resistance. Film solubility is an important factor that determines biodegradability of films when used as packaging wrap (Gnanasambadam and others 1997). Although a lower solubility of edible films is required during storage, a high solubility of edible film will be advantageous during cooking food products coated with edible films (Laohakunjit and Noomhorm 2004). Figure 3 shows that percent water solubility was higher for all plasticized films. Incorporation of glycerol into the film solution contributed to less retrogradation of starch in the film and thereby decreased the formation of crystalline aggregates in the starch gels. The crystallinity in a polymer sample is dependent on the ability of the chains to form crystals as well as the mobility of the chains

Table 2–Effect of lemongrass oil on tensile strength and percentage elongation of film in the presence and absence of glycerol

Concentration (%wt/wt) of glycerol)	Concentration (%v/w) of lemongrass oil	Tensile strength (Mpa)	Elongation at break (%)
[illegible]	0	$41.6^a \pm 0.9$	$1.7^f \pm 0.2$
	0.1	$38.6^b \pm 1.8$	$2.0^f \pm 0.2$
	0.2	$35.9^c \pm 0.8$	$2.0^f \pm 0.1$
	0.3	$34.4^d \pm 0.7$	$1.9^f \pm 0.0$
	0.4	$32.3^e \pm 0.7$	$2.0^f \pm 0.2$
	0	$16.0^f \pm 0.1$	$3.7^e \pm 0.2$
	0.1	$16.0^f \pm 1.1$	$5.1^d \pm 0.5$
	0.2	$14.4^g \pm 0.6$	$5.8^c \pm 0.3$
	0.3	$13.4^{hg} \pm 0.4$	$8.7^b \pm 0.4$
	0.4	$12.9^h \pm 0.4$	$13.2^a \pm 0.7$

[illegible] ± standard deviation ($n = 5$) with different superscript letters are [illegible]cantly different at ($P < 0.05$).

Table 3–Effect of lemongrass oil on water vapor permeability of film in the presence and absence of glycerol

Concentration (%wt/wt) of glycerol	Concentration (%v/w) of lemongrass oil	Water vapor permeability (g. m/m^{-2}. s. Pa) × 10^{-10}
0	0	$5.1^a \pm 0.2$
	0.1	$4.1^b \pm 0.3$
	0.2	$4.9^a \pm 0.1$
	0.3	$4.9^a \pm 0.2$
	0.4	$5.1^a \pm 0.2$
20	0	$2.4^d \pm 0.2$
	0.1	$2.5^d \pm 0.3$
	0.2	$3.3^c \pm 0.1$
	0.3	$3.3^c \pm 0.3$
	0.4	$4.0^b \pm 0.3$

Means ± standard deviation ($n = 3$) with different superscript letters are significantly different at ($P < 0.05$).

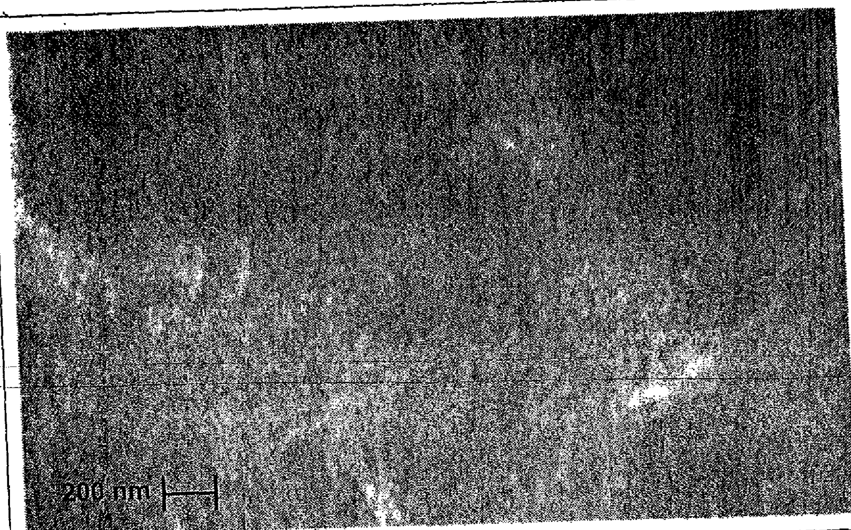


Figure 1 – Scanning electron micrograph of film in the presence of glycerol (magnification 20.00 k ×)

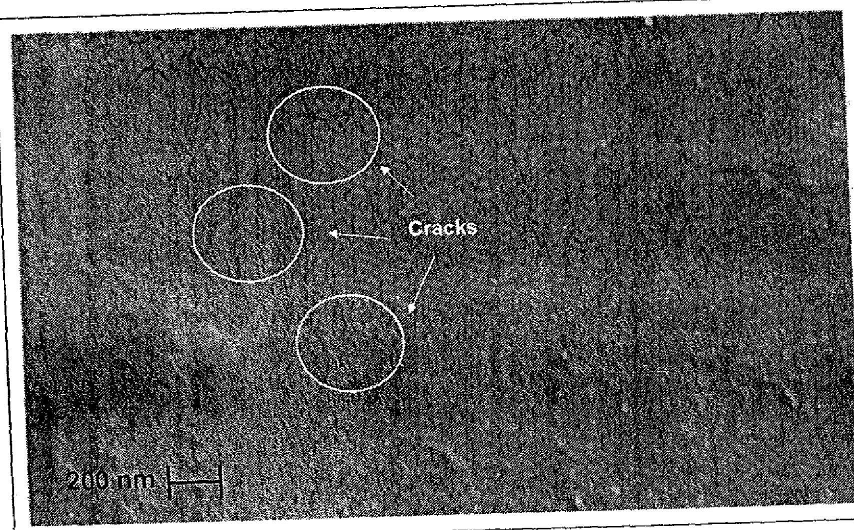


Figure 2 – Scanning electron micrograph of film in the absence of glycerol (magnification 20.00 k ×). Cracks in the film are indicated by arrows.

during the crystallization process (Rindlav-Westling and others 1998). Less formation of crystallites in the films render it to swell in water easily and to disintegrate. Therefore, film solubility in water will be higher. In addition, incorporation of glycerol into film also increased the solubility of film due to their hydrophilic properties that cause it to strongly interact with water and easily incorporate into a network of hydrogen bonds as reported by Laohakunjit and Noomhorm (2004). The results show that water solubility of the films in the absence of glycerol did not increase significantly ($P > 0.05$) as the concentration of lemongrass oil increased. At 0.4% of lemongrass oil with 20% glycerol, it interfered with the arrangement of the polymer chains and the hydrogen bonding and thereby less interaction between the starch molecules. The increased of percent water solubility could be due to the leaching of amylose from starch component in the film.

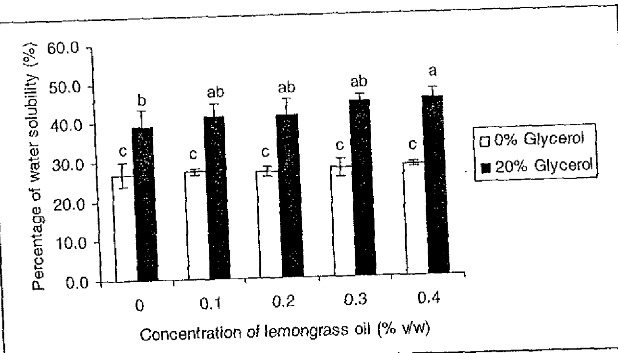
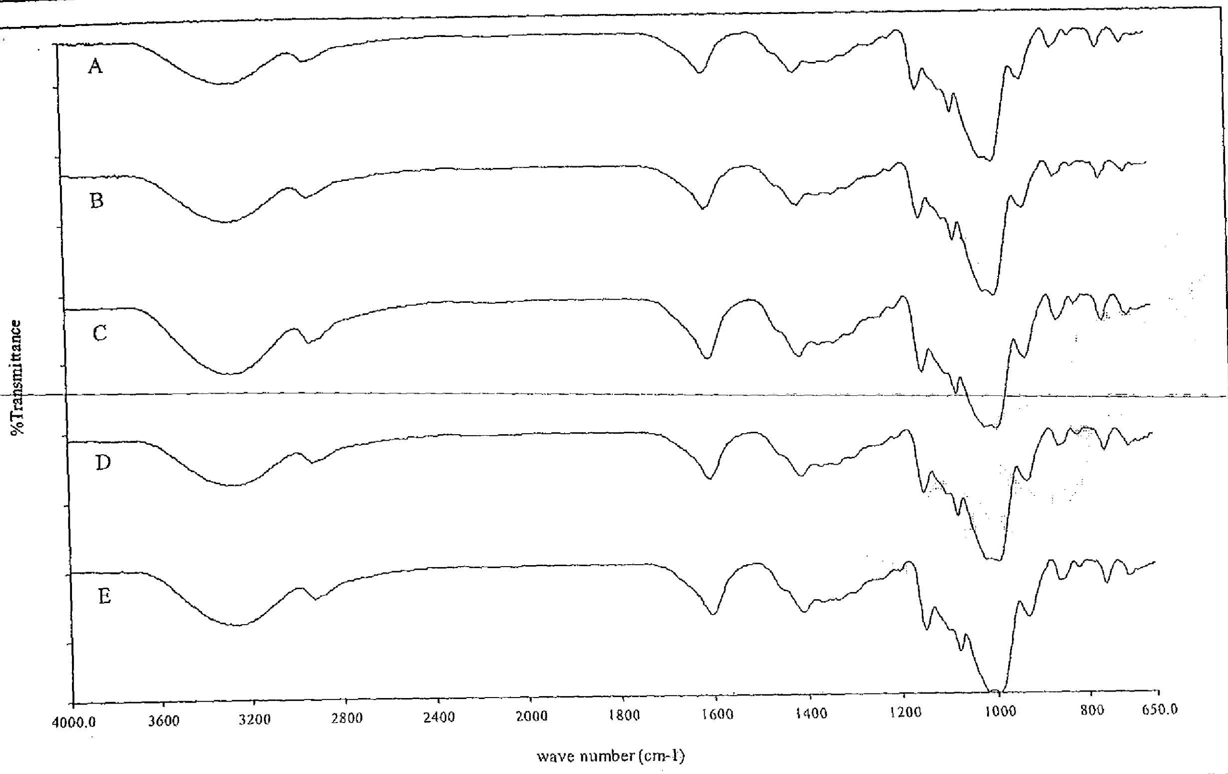


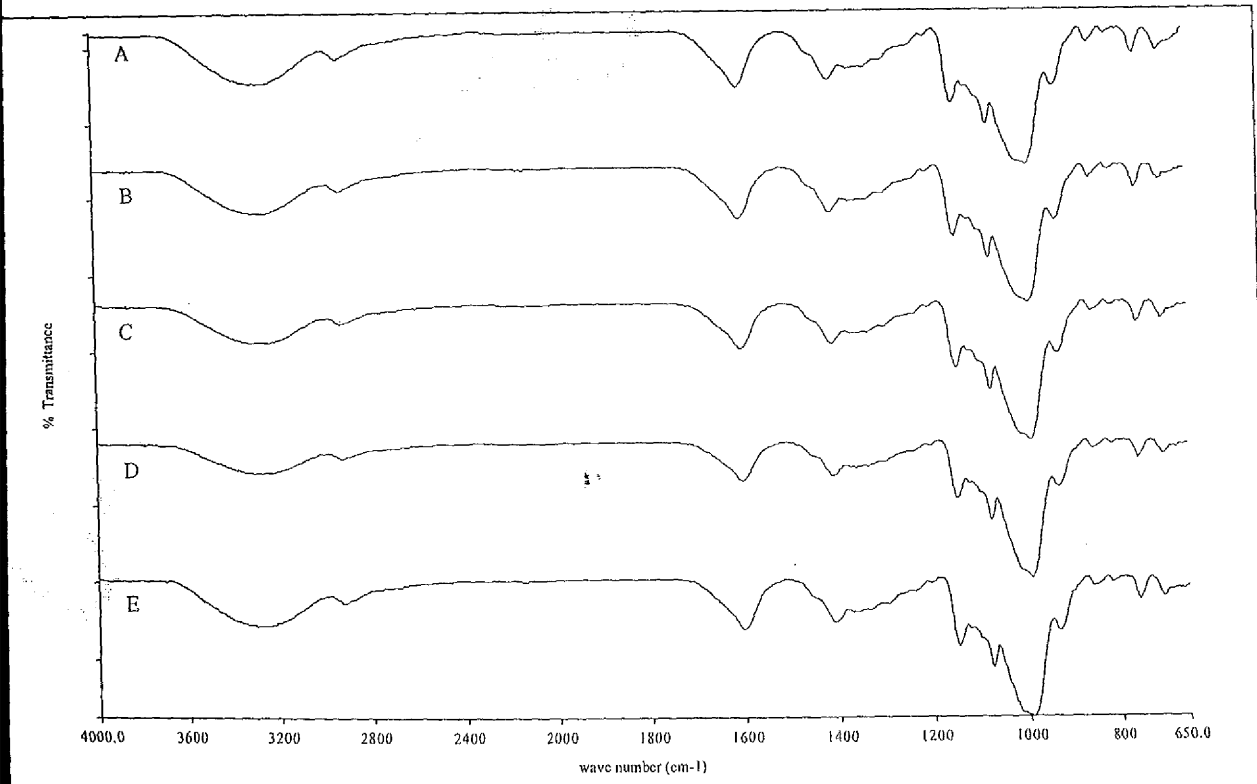
Figure 3 – Effect of lemongrass oil on water solubility of film in the presence and absence of glycerol. The vertical bars are the standard deviations of the means ($n = 3$) and different letters are significantly different at ($P < 0.05$).

FTIR analysis

FTIR microscopy was used to study the interaction between film matrix and lemongrass oil. The spectra of the films in the presence and absence of incorporated glycerol with different concentrations of lemongrass oil are presented in Figure 4 and 5, respectively. All spectra show similar IR absorbance patterns. From the results, it appears that there was no structural change for both films in the presence and absence of glycerol after incorporation of lemongrass oil at all levels. This indicates that there was no interaction between active compounds of lemongrass oil with the functional groups of the starch–alginate film. Therefore, the active compounds of lemongrass oil were free to inhibit the *E. coli* cells. As the concentration of lemongrass oil increased, the zones of



ure 4—Spectra of Fourier transform infrared (FTIR) spectroscopy of partially hydrolyzed sago starch-alginate ble films with glycerol: (A) SA film, (B) SA film + 0.1% lemongrass oil, (C) SA film + 0.2% lemongrass oil, (D) SA h + 0.3% lemongrass oil, and (E) SA film + 0.4% lemongrass oil.



gure 5—Spectra of Fourier transform infrared (FTIR) spectroscopy of partially hydrolyzed sago starch-alginate ible films without glycerol: (A) SA film, (B) SA film + 0.1% lemongrass oil, (C) SA film + 0.2% lemongrass oil, (D) SA m + 0.3% lemongrass oil, and (E) SA film + 0.4% lemongrass oil.

inhibition also increased significantly at all levels. However, the addition of lemongrass oil into the film changed its mechanical properties of film by acting as plasticizer, which improves its flexibility and extensibility.

Conclusion

The results obtained demonstrate that the films containing lemongrass oil are effective in inhibiting the growth of *E. coli* O157:H7 based on the zone of inhibition assay. The antimicrobial effect was enhanced in the presence of glycerol compared to film in the absence of glycerol. The mechanical properties of the film were affected by the presence of lemongrass oil. The WVP and %E increased and TS decreased with the addition of different concentrations of lemongrass oil to the polymer matrix. The plasticizing effect of glycerol was evident and it provides the edible film with flexibility as well as mechanical strength. Films with glycerol showed surfaces without pores or microcracks compared to films without glycerol.

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Antibacterial Activity of Modified Sago Starch-Alginate Based Edible Film Incorporated with Lemongrass (*Cymbopogon Citratus*) Oil

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Key words: Edible film; Glycerol; Lemongrass oil; Antibacterial activity

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Introduction

The use of natural plant extracts is desirable for development of new food products and nutraceuticals as well as new active packaging system. Antimicrobial packaging is the packaging system that is able to reduce, inhibit, or retard the growth of pathogenic microorganisms in packed foods and packaging material (Vermeiren *et al.,* 1999). Several attempts have been made in developing active packaging systems in which antimicrobial agents are incorporated into the polymeric material and are slowly released on the food surface (Devlieghere *et al.,* 2004; Quintavalla and Vicini, 2002; Vermeiren *et al.,* 2002).

Onawunmi *et al.* (1984) reported that the antimicrobial activity of lemongrass oil is related to high amounts of 1,8-cincole (>30%), geranial (>30%), and neral (>20%). However, citral isomers (neral, 32.2%, geranial, 41.28%) are the most abundant compounds in lemongrass oil as reported by (Choi *et al.,* 2000; Paranagama *et al.,* 2003). These components individually showed antibacterial action on gram-negative and gram-positive organisms (Onawunmi *et al.*, 1984).

The objectives of this research were to develop modified sago starch-alginate edible film based with incorporated lemongrass oil and study on the antimicrobial properties against three food pathogenic bacteria namely, *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Staphylococcus aureus.*

Materials and methods

Materials

Sago starch (*Metroxylon sagu*) was obtained from Nitsei Sago Industries Sdn. Bhd. Penang, Malaysia. The other chemicals used in this study were alginate from Acros Organic (New Jersey, USA), glycerol from Qrec (Auckland, New Zealand), pullulanase enzyme (EC 3.2.1.4.1, pullulan 6-glucanohydrolase) from Novo Nordisk (Bagsvaerd, Denmark) and broth and agar were purchased from Merck, (Darmstadt, Germany)

Preparation of raw material

Modified starch was prepared by enzymatic hydrolysis. Sago starch (200 g) was suspended in 1000 mL sodium acetate buffer, pH 5.0 (20% w/v, starch slurry). Pullulanase enzyme (20% v/w of starch) was added and the starch suspensions were incubated at 58 °C and agitated at 200 rpm in an orbital incubator shaker (Certomat ® *SII*; B. Braun Biotech International, Melsungen, Germany) for 20 h. The enzyme was deactivated at 75 °C for 15 min followed by centrifugation at 3000 x g for 15 min and the precipitate was collected and dried in an oven at 40 °C. The apparent amylose contents were determined in triplicate by iodine-binding using the method described by Jarvis and Walker (1993)

Organisms and preparation of cultures

Escherichia coli O157:H7, *Salmonella enteritidis* and *Staphylococcus aureus* cultures were obtained from the Microbiology Laboratory (School of Industrial Technology, USM, Malaysia). The bacterial culture was grown on nutrient agar slants and kept at 4 °C. Subculturing was carried out every month to maintain bacterial viability.

Preparation of lemongrass oil

Fresh lemongrass (*Cymbopogon citratus*) was purchased from a local market in Penang. The lemongrass oil was obtained by using conventional steam-distillation for 7 h according to the method of Dadalioglu and Evrendilek (2004). The oil was collected, dried with anhydrous sodium sulfate, and stored in airtight glass vials at 4 °C until further use.

Preparation of modified sago starch-alginate film

Film forming solutions were prepared from a mixture of modified sago starch and sodium alginate (4:1) based on total weight basis (5 g) including 20% glycerol in 200 mL distilled water. Lemongrass oil was initially diluted to 10% concentration using ethanol (95%) and then incorporated into the edible film solution at different concentrations (0.1%, 0.2%, 0.3% and 0.4%, v/w of edible film forming solution). The mixture was heated to 85 °C with continuous stirring for 45 min before it was cooled to room temperature. The solutions (95 g) were cast onto polyacrylic plates (16×16 cm) followed by oven drying at 40 °C for 24 h. The dry films obtained were peeled off and stored for further analysis.

Antibacterial activity of films

Antibacterial activity test on films was carried out using the agar diffusion method according to Chen et al. (1996). The zone of inhibition assay on solid media was used for determination of the antibacterial effects of films against *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Staphylococcus aureus*. The edible films were cut into 6-mm-diameter discs and then placed on Mueller Hinton agar (Merk, Darmstadt, Germany) plates, which had been previously seeded with 0.2 mL of inoculums containing approximately 10^5 - 10^6 CFU/mL of tested bacteria. The plates

were then incubated at 37 °C for 24 h. After that, the plates were examined for 'zone of inhibition' of the film discs. Inhibitory activity was measured based on the clear zone surrounding a circular film disc. The area of the whole zone was calculated and then subtracted from the film disc area, and this difference area was reported as zone of inhibition. If there is no clear zone, it is assumed that there is no inhibition. Contact area was used to evaluate growth inhibition underneath the film disc in direct contact with target bacteria in the agar.

Results and discussions

The average thickness of films was 0.07 ± 0.01 mm. Sago starch was modified enzymatically to obtain a higher percentage of linear fractions. The linear fractions increased from 28.7% for native sago starch to 52.0% after the debranching process, which formed stronger gels and stiffer film. Based on visual observation, the films produced were transparent and had a smooth surface.

Antibacterial activity

The results of the antibacterial assessment of edible film containing lemongrass oil against *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Staphylococcus aureus* are presented in Table 1. Figure 1 shows the inhibitory effect of modified sago starch-alginate films incorporated with 0.2% lemongrass oil against all tested bacteria in comparison with the control. The control modified sago starch-alginate edible film did not show inhibitory effect against all tested bacteria. The results showed that *Escherichia coli* O157:H7 was the most sensitive bacteria against lemongrass oil incorporated films, followed by *Salmonella enteritidis*. However, the films were not effective against *Staphylococcus aureus*, as no inhibitory zone were observed.

As the concentration of lemongrass oil added increased, the inhibition zone of *Escherichia coli* O157:H7 was increased significantly ($p<0.05$) at all levels for films in the presence and absence of glycerol. The results showed that, films incorporated with lemongrass oil exhibited significantly ($p<0.05$) higher antibacterial activity in the presence of glycerol, as evident by larger inhibitory zones at all lemongrass oil concentration except at 0.4% level. This could be attributed to the increased solubility of lemongrass oil in the matrix and more uniform dispersion of the oil in the film. By increasing the concentration of lemongrass oil to 0.4%, it seemed that the capability of the oil to dissolve and disperse in both plasticized and unplasticized films was almost the same. In this study, it was clearly shown that lemongrass oil incorporated in the film exhibited antibacterial activity against *Escherichia coli* O157:H7 as was also reported by Onawunmi et al. 1984 and Rojas-Grau et al. 2007.

From the results obtained, increasing lemongrass oil level up to 0.3% did not significantly improve the antibacterial activity on the tested bacteria. However, the inhibitory zone was significantly ($p<0.05$) increased at 0.4% level for both films in presence and absence of glycerol. The results showed that, the presence of active antibacterial compounds improved the inhibitory effect of *Salmonella enteritidis* after t was incorporated at 0.4% level.

In this study, it seemed that the film incorporated with lemongrass oil did not show any inhibitory zone on *Staphylococcus aureus.* This result was however in contrast to result obtained by Onawunmi et al. (1984) who reported that the antibacterial activity of lemongrass oil against *Staphylococcus aureus* in liquid media was more sensitive compared to *Escherichia coli* O157:H7. This is probably due to loss of certain active compounds during film forming process that are more effective in inhibiting the growth of these bacteria.

Conclusion

The results showed that the lemongrass oil incorporated in the films had antibacterial activity on *Escherichia coli* O157:H7 and *Salmonella enteritidis* based on clear inhibition zone exhibited. Antibacterial effect was enhanced in the presence of glycerol for all levels of *Escherichia coli* O157:H7 and at 0.4% for *Salmonella enteritidis* compared to film in the absence of glycerol. The results obtained can serve as a guide for selection of suitable levels of lemongrass oil that can be incorporated into film in order to have an effective inhibition.

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Table 1: Antimicrobial activity of films incorporated with lemongrass oil against pathogenic bacteria

Concentration (% w/w) of glycerol	Concentration (% v/w) of lemongrass oil	*Escherichia coli* O157:H7 Gram (-)		*Salmonella enteritidis* Gram (-)		*Staphylococcus aureus* Gram (+)	
		Inhibitory Zone(mm^2)	Contact area	Inhibitory Zone (mm^2)	Contact area	Inhibitory Zone (mm^2)	Contact area
0	0 (control)	0^{h}	-	0^{e}	-	0	-
	0.1	$29.5^{g} \pm 2.8$	+	$45.7^{cd} \pm 3.9$	+	0	-
	0.2	$39.0^{f} \pm 2.6$	+	$44.2^{d} \pm 4.0$	+	0	-
	0.3	$62.6^{d} \pm 3.3$	+	$46.1^{cd} \pm 2.8$	+	0	-
	0.4	$92.9^{a} \pm 2.6$	+	$53.1^{b} \pm 3.4$	+	0	-
20	0 (control)	0^{h}	-	0^{e}	-	0	-
	0.1	$50.0^{e} \pm 3.2$	+	$49.1^{c} \pm 2.2$	+	0	-
	0.2	$69.9^{c} \pm 2.5$	+	$47.9^{cd} \pm 1.6$	+	0	-
	0.3	$78.9^{b} \pm 2.6$	+	$48.7^{c} \pm 3.2$	+	0	-
	0.4	$94.0^{a} \pm 2.0$	+	$61.7^{a} \pm 2.4$	+	0	-

Means ± standard deviation (n=5) with different superscript letters are significantly different at ($p<0.05$).
+ represents inhibitory effect; - represents no inhibitory effect.

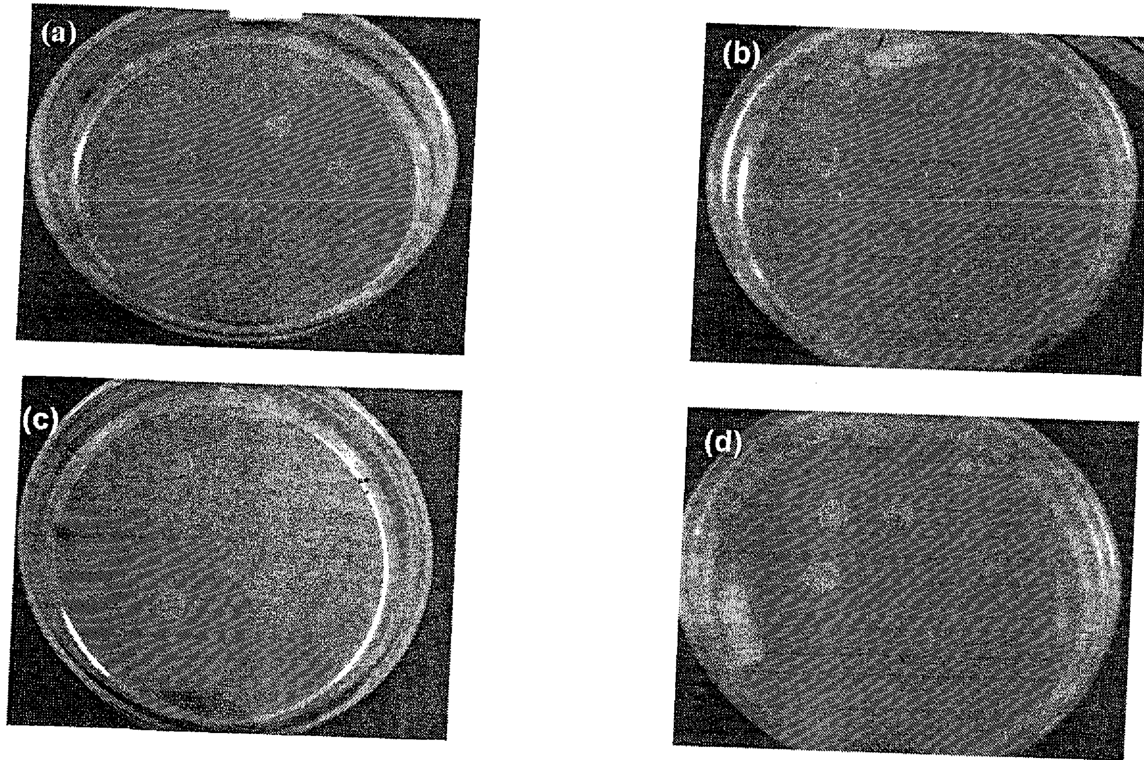

Figure 1: Representative picture of inhibitory zones of modified sago starch-alginate film with 0.2% (v/w) lemongrass oil compared to control against tested bacteria. (a) Control, (b) *Escherichia coli* O157:H7, (c) *Salmonella enteritidis* and (d) *Staphylococcus aureus*.