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Short Communication

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

Maizura, M., *Fazilah, A., Norziah, M. H. and Karim, A. A.

Food Technology Division, School of Industrial Technology,
University Sains Malaysia, 11800 Penang, Malaysia

Abstract: Antibacterial effect of modified sago starch-alginate edible film incorporating lemongrass oil at various concentrations was studied. Edible films were prepared from a mixture of modified sago starch and alginate. Lemongrass oil (0.1 - 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 antibacterial activity against food pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Staphylococcus aureus*. The edible film exhibited antibacterial activity against *Escherichia coli* O157:H7 and *Salmonella* Enteritidis by using agar diffusion assay method. For films tested against *Escherichia coli* O157:H7, the zone of inhibition increased significantly ($p < 0.05$) with addition of lemongrass oil at all levels both in the presence and absence of glycerol. The films also significantly ($p < 0.05$) inhibited the growth of *Salmonella enteritidis* only with 0.4% lemongrass oil (in the presence and absence of glycerol). However, the films containing lemongrass oil did not show any inhibition effect on *Staphylococcus aureus*.

Keywords: Edible film, sago starch, alginate, lemongrass oil, antibacterial activity

INTRODUCTION

Sago starch is isolated from sago palm (*metroxylon spp.*) which is better known as "rumbia" and it has been distributed throughout South East Asia (Ahmad *et al.,* 1999). According to Abd Aziz (2002), sago palms have great potential for starch production in Malaysia and it is well known as an abundant renewable raw material. Moreover, in Malaysia, sago starch ranks fifth for agricultural revenue after pepper, oil palm, cocoa, and rubber. A mixture of starch and alginate to form edible film has been studied by Wu *et al.* (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).

The use of natural plant extracts is desirable for development of new food products and nutraceuticals as well as new active packaging systems. Antimicrobial packaging is a 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).

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). 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 to study the antimicrobial properties against three food pathogenic bacteria namely, *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Staphylococcus aureus*.

*Corresponding author
E-mail: *fazilah@usm.my*

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.

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).

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 with 95% ethanol and then incorporated into the film solution at different concentrations (0.1%, 0.2%, 0.3% and 0.4%, v/w of 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 casted onto polyacrylic plates (16 x 16 cm) followed by oven drying at 40°C for 24 h.

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 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' on the film discs.

RESULTS AND DISCUSSION

Sago starch was modified enzymatically to obtain a higher percentage of linear fractions. The linear fractions (expressed as apparent amylose content) increased from 28.7% for native sago starch to 52.0% after the debranching process, which formed stronger gels and stiffer film (Maizura *et al.,* 2007).

Antibacterial Activity

The results of the antibacterial activity of films 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 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 zones were observed.

As the concentration of lemongrass oil increased, the inhibition zone of *Escherichia coli* O157:H7 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 for all lemongrass oil concentrations except at 0.4% level. This could be attributed to the increased solubility of lemongrass oil in the matrix and more uniform dispersion of

Table 1: Antibacterial activity of films containing lemongrass oil against *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Staphylococcus aureus*

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)	Inhibitory zone (mm^2)	Inhibitory zone (mm^2)
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.

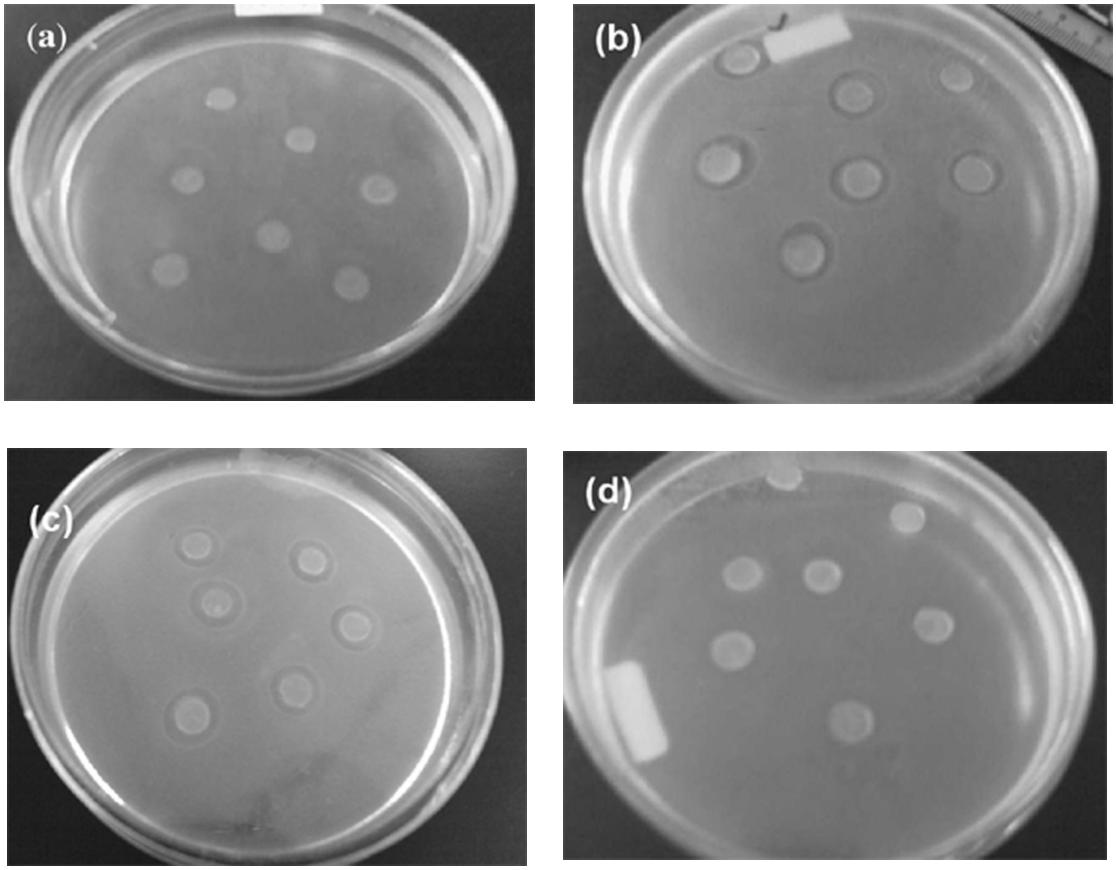

Figure 1: Representative picture of inhibitory effect of modified sago starch-alginate film incorporated with 0.2% (v/w) lemongrass oil against all tested bacteria in comparison with the control. (a) Control, (b) *Escherichia coli* O157:H7, (c) *Salmonella* Enteritidis and (d) *Staphylococcus aureus*

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 (with glycerol) and unplasticized (without glycerol) films were 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).

Films containing lemongrass oil also exhibited inhibitory effect on the growth of *Salmonella* Enteritidis. The inhibitory zone was significantly ($p < 0.05$) increased at 0.4% level for films in the presence and absence of glycerol. The results showed that the presence of active antibacterial compounds improved the inhibitory effect of *Salmonella* Enteritidis after it was incorporated at 0.4% level.

In this study, the results revealed that the film incorporated with lemongrass oil did not show any inhibitory zone on *Staphylococcus aureus*. This is in contrast to results 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 the 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 the 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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