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Antimicrobial activity of encapsulated spent coffee ground extract-incorporated chitosan films

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Abstract

This work aimed to compare antimicrobial activity of chitosan films containing encapsulated (eSCGE-CH film) and non-encapsulated spent coffee ground aqueous extracts (SCGE-CH film). Chitosan films with eSCGE and SCGE at SCGE equivalent concentrations of 0, 0.5, 0.75 and 1.0% (w/v) were prepared. The antimicrobial activity of eSCGE-CH and SCGE-CH films were evaluated by a drop-test method against some potent food pathogens, including Gram-positive bacteria. (*Staphylococcus aureus* TISTR2329 and *Bacillus subtilis* TISTR1984) and Gram-negative bacteria (*Escherichia coli* TISTR527 and *Salmonella typhimurium* TISTR2519). The eSCGE-CH film showed a higher inhibitory effect against *S. aureus B. subtilis* and *S. typhimurium* than the SCGE-CH film, with *S. typhimurium* being the most susceptible bacteria compared to the others ($p \leq 0.05$). These results indicate that encapsulation could enhance the antimicrobial activity of the SCGE in the CH film suggesting the potential feasibility of the eSCGE-CH film as an antimicrobial packaging for food applications.

Keywords: Active packaging, Encapsulation, Coffee ground, Foodborne pathogens

1. Introduction

Spent coffee ground (SCG) is the main by-product from instant coffee and coffee brewing production, accounting for approximately 65% of the coffee powder [1]. The SCG has been reported as a potential source of various bioactive compounds, such as phenolic compounds, flavonoids, carotenoids, chlorogenic acid and caffeine [2]. These compounds exhibit various biological activities, such as antioxidant activity and inhibitory effects against a variety of foodborne pathogens, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* [2,3]. The microbial inhibitory effect of SCG is paving the way for applying the SCG extract (SCGE) as a natural antimicrobial agent in the food matrix for the recent health concerns of consumers. However, direct addition of antimicrobial agents to food has several limitations. For instance, the extract may interact with the food composition, consequently reducing the effectiveness of the antimicrobials or impairing the food qualities [4].

Incorporating antimicrobial agents into packaging polymers is a potential method to avoid these limitations. Moreover, this method could manipulate the control-release of the antimicrobials into the food matrix [5]. Chitosan (CH), among the biopolymers, is a potential polysaccharide bio-based polymer with superior properties over many others. These properties include biocompatibility, biodegradability, non-toxicity along with its inherent antimicrobial activity and been approved as generally recognized as safe (GRAS) by the FDA and EU. CH is widely applied as an edible antimicrobial film for food matrixes as a result of its superior film forming property with its additional lipid and gas barrier properties [6]. Incorporation of active organic and inorganic compounds into the CH matrix are also applied in order to improve some properties of the CH film, including water vapor permeability, water solubility, mechanical properties as well as the antimicrobial activities [7].

Even though, incorporation of active compounds into the polymer matrix could help reduce the interactions between the active compounds and the food components, their activities may be impaired as a result of the film processing, particularly when high temperature is applied. Encapsulation is generally applied in order to protect

a bioactive core substance within a shell or wall material. It could prevent direct contact of the food and the core bioactive compound, that may impair the food quality or the antimicrobial activity [8]. In addition, it can also manipulate control-release of the active core material to enhance its functionality [9]. Films or coatings containing encapsulated antimicrobial compounds have shown to provide the higher antimicrobial activity and consequently extended the shelf life of various foods compared to films or coatings with non-encapsulated antimicrobial agents [7,10].

The drying technique and the type wall material significantly affect the preservation capacity of the encapsulated compounds. One of the most preferable drying techniques is freeze drying due to its better preservation, particularly heat-sensitive compounds [11]. Maltodextrin (MD) is a good wall material with low viscosity and high solubility at high concentration, good thermal, freeze and acidic stability, resistance to oxidation and relatively low cost [12]. In addition, MD with freeze-drying technique was reported to provide the best encapsulation efficiency [11]. However, MD exhibits low emulsifying capacity, emulsion stability and surfactant properties [13,14]. Consequently, other types of wall materials, such as gum Arabic, modified starch, protein, etc., are added in order to overcome those drawbacks [14].

Chitosan (CH) has been widely used as a wall material in combination with MD to improve the emulsion stability and to enhance the antioxidant and antimicrobial activity of the core bioactive compounds [15]. The encapsulation of various core materials within a CH/MD complex shell provided higher bioactivity than that of the non-encapsulated ones [16]. Our previous work also revealed that CH/MD encapsulation of SCGE exhibited superior antimicrobial activity to non-encapsulated SCGE and the inhibitory effect of the eSCGE could be retained up to 90 days after storage at 10 and -20°C [17].

Until now, there is no report on the incorporation of an encapsulated SCGE (eSCGE) in chitosan films as an antimicrobial food packaging. Therefore, this research aimed to compare the antimicrobial activity of SCGE and eSCGE-incorporated chitosan films in order to explore the possibility of using eSCGE-CH films as an alternative antimicrobial packaging for food application.

2. Materials and methods

2.1 Preparation of spent coffee ground extract (SCGE)

Spent coffee ground (SCG) used in the experiments was obtained from Starbucks coffee shop, Laemthong branch, Long Had Bangsaen Road. Muang Chonburi District, Chonburi Province and stored at -20°C until used. The SCG extract (SCGE) was prepared according to [18] Briefly, the SCG was dried at 60°C until the moisture content of the SCG reached 13%. The dried SCG was mixed with distilled water at a ratio of 1:10 (w/v) and heated to 90°C and held for 5 min and then filtered through Whatman filter paper No. 4. The filtrate was centrifuged at 4500 rpm for 5 min. The SCG supernatant was collected and evaporated using a rotary evaporator under reduced pressure for 3 h to obtain a crude extract. The extract was then freeze-dried in a SCANVAC Coolsafe 110-4 freeze-dryer to obtain SCGE powder and stored at -20°C for further analysis.

2.2 Encapsulation of the SCGE

Encapsulated SCGE (e-SCGE) was prepared according to [17] using chitosan and maltodextrin as wall materials. Chitosan and maltodextrin stock solutions (2% w/v) were prepared in 100 mmol acetate buffer (pH = 4.0) and distilled water, respectively. The chitosan and maltodextrin stock solutions were mixed at the ratio of 86:14 as a wall material solution and subsequently mixed with the SCG supernatants obtained from section 2.1 above before evaporation, at the ratio of 14:86 by weight. The mixture was homogenized for 5 min and evaporated in a rotary evaporator under reduced pressure for 3 h. Then, it was freeze-dried to obtain encapsulated spent coffee grounds extract (eschew), which was subsequently stored at -20°C until used.

2.3 Preparation of chitosan films containing SCGE and eSCGE

Chitosan films containing SCGE (SCGE-CH) and eSCGE (eSCGE-CH) were prepared according to [19] with some modifications. The SCGE in both SCGE-CH and eSCGE-CH films was prepared at an equivalent amount of SCGE of 0, 0.5, 0.75, and 1.0% w/v. The SCGE or eSCGE at the desired amount was dissolved in 150 mL of acetic acid solution (1% w/v) and stirred for 30 min. Chitosan powder (low MW, 90% DE, 3 g) was added to the mixture and stirred for one hour or until a clear solution was obtained. Then, glycerol (0.90 g) was added and stirred for another 30 min. The mixture (115 mL) was poured onto a silicone mold (19 ×19 cm^2) and dried in a hot air oven at 40°C for 24 h. The film was peeled off and stored at 25°C, 50±5 % RH for at least 24 h.

2.4 Bacterial suspension preparation

Microorganisms used in the antimicrobial activity testing were Gram-positive bacteria (*S. aureus* TISTR2329 and *B. subtilis* TISTR1984) and Gram-negative bacteria (*Escherichia coli* TISTR527 and *Salmonella typhimurium*

TISTR2519) from the Thailand Institute of Scientific and Technological Research. The microorganisms were activated by transferring the microbes to Mueller Hinton Agar (MHA) and incubated at 37°C for 24 h. Cell suspensions were prepared by suspending the active culture in 0.85% normal saline solution to obtain the 0.5 McFarland standards turbidity (1.5×10^8 CFU/mL).

2.5 Antimicrobial activity determination by disc diffusion method

The disc diffusion method was conducted according to the Clinical and Laboratory Standards Institute [20]. A sterile cotton swab was dipped into the suspension and evenly swabbed on Mueller Hinton Agar (MHA). The SCGE-CH or eSCGE-CH film was cut into 6 mm diameter and placed onto the MHA surface. Penicillin (10 µg/disc) and ampicillin (10 µg/disc) were applied as positive controls for Gram-positive bacteria and Gram-negative bacteria, respectively, while sterile distilled water disc was used as a negative control. The plates were incubated at 37°C for 18-24 h. The diameter of the inhibition zone was determined and recorded (mm).

2.6 Antimicrobial activity determination by drop test method

The drop test method was modified from [21] The SCGE-CH or eSCGE-CH film or the CH control film was cut into square of 1 × 1 cm. The film was sterilized under UV for 30 min and placed on a sterile glass slide in a sterile plate. The cell suspension (10 µL) was dropped onto the film surface and covered with a UV-sterilized polypropylene film. Subsequently, the plate was incubated at 37°C for 24 h. After incubation, the film was aseptically transferred to a micro-centrifuge tube containing 990 µL of 0.85% sterile normal saline solution and vortexed. Serial dilution was performed until an appropriate dilution was obtained, then the suspension was subjected to colonies counting by a drop plate technique [22].

2.7 Statistical analysis

All tests were performed in triplicates and reported as the mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA) with Tukey's comparison test. The analyses were performed using the Minitab® 18 software at a significant level of $p \leq 0.05$.

3. Results and discussion

The color of the SCGE-CH and eSCGE-CH films is shown in Figure 1. The color of the films containing both SCGE and eSCGE were darker than the pure CH film and increased with increasing SCGE concentration.

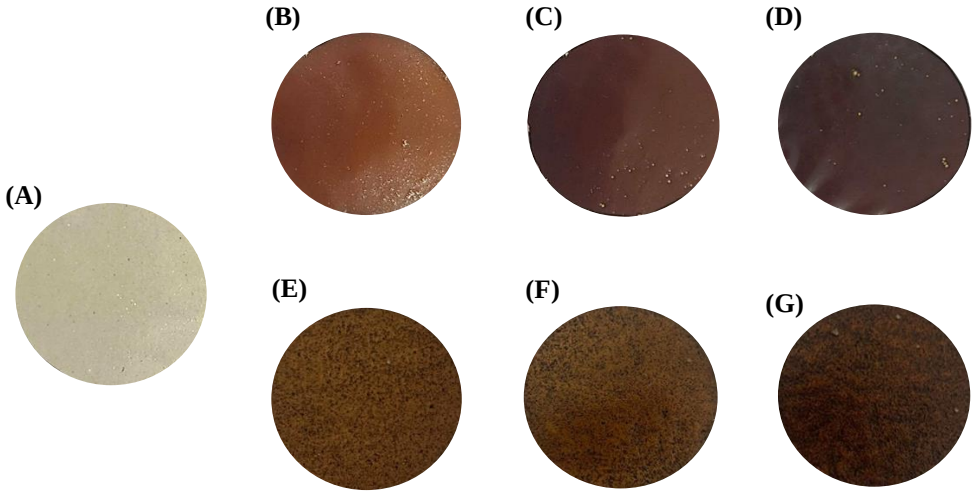

Figure 1 Chitosan films containing SCGE at (A) 0%, (B) 0.50, (C) 0.75%, (D) 1.0% w/v and containing eSCGE at (E) 0.50%, (F) 0.75%, (G) 1.0% w/v.

3.1 Antimicrobial activity of the SCGE and eSCGE-CH films by the disc diffusion method

There was no inhibitory effect of the SCGE-CH and eSCGE-CH films observed for all the tested bacteria (Data not shown). These results may due to hydrogen bonding between the hydroxyl group of the phenolic compounds in SCGE and the amine group of chitosan, reducing the free phenolic compounds in the matrix.

Consequently, the active components in the film were unable to diffuse from the film to inhibit the microorganisms. The antimicrobial effect has been observed at only the higher concentrations, where free bioactive compounds are sufficiently available to diffuse through the solid media to exhibit the antimicrobial effect by the disc diffusion method [19,23].

3.2 Antimicrobial activity of the SCGE and eSCGE-CH films by the drop test method

Table 1 shows the antimicrobial activity of the films against some potent pathogens, including Gram-positive bacteria; *S. aureus* TISTR2329 and *B. subtilis* TISTR1984, and Gram-negative bacteria; *E. coli* TISTR527 and *S. typhimurium* TISTR2519 obtained using the drop test method. The results showed that the SCGE-CH and eSCGE-CH films significantly inhibited all the tested bacteria ($p \leq 0.05$). These results may due to positive charge of carboxylic group of chlorogenic acid, the main antimicrobial agent in the SCGE, which could freely interact with the negative charge of the microbial cell wall which was suspended in the aqueous phase [24]. This reaction could also enhance the chitosan ability to bind to the negative charge of the bacterial cell wall, altering the cell wall structure, consequently decreasing the cell wall permeability properties. As a result, the cell wall allows the chitosan to penetrate into the cell to attach to the microbial DNA, ceasing DNA replication and resulting in cell death [25,26].

In addition, it was also found that the antimicrobial activity of the SCGE-CH and eSCGE-CH films increased with increasing concentration of SCGE and eSCGE in the film ($p \leq 0.05$). These results are consistent with [27] who reported that when the concentration of the SCGE in the gelatin film increased, the antimicrobial activity of the film significantly increased as a result of the higher concentration of the active compounds.

In addition, the results also suggested that the eSCGE-CH film significantly exhibited superior inhibitory effect than the SCGE-CH film against all tested bacteria, except *E. coli* ($p \leq 0.05$). This is indicated by the lower bacterial survivability observed at the equivalent SCGE concentration in the eSCGE-CH film (Table 1). The superior activity may result from the interaction between the hydroxyl and carboxyl groups of the phenolic compounds in the SCGE with the amine functionality of the chitosan molecule *via* H-bonding, thus stabilizing and gradually releasing the active compounds to the aqueous phase to attack the microbes cell wall [28]. In addition, MD has no electrical charge, consequently it contributes as a carrier rather than being involved in the reaction [12]. The superior antimicrobial effect of the eSCGE-CH film, therefore, implies the action of the chitosan in the wall material that may contribute to those synergistic antimicrobial effect of the CH film rather than the effect of encapsulation. These results are consistent with [29] in which the superior inhibitory effect of an encapsulated cinnamon essential oil (CEO) was observed over the non-encapsulated CEO as a result of the synergized antimicrobial activity of the chitosan wall material.

Among all the tested bacteria, *S. typhimurium* exhibited the most sensitive to the eSCGE-CH film compared to the others and was also more susceptible to the eSCGE-CH film than the *E. coli* (Table 1). These trends are indicated by the lowest survivability (1.10×10^7 CFU/mL) at the lowest eSCGE concentration (0.50% w/v) in the film ($p \leq 0.05$). These outcomes may be ascribed to the negative charge of the cell membrane of the Gram-negative bacteria and the more negative charge of the *S. typhimurium* cell membrane compared to that of *E. coli.* The more negative charge at the *S. typhimurium* cell membrane allows a higher interaction with the positive charges of chlorogenic acid (the main bioactive component in the SCGE) and the chitosan, resulting in the higher inhibitory effect of the eSCGE-CH film against *S. typhimurium* over the other bacteria [30].

Table 1 Survivability of the from the drop test method.

Treatment	Survival of microorganisms ($\times 10^7$ CFU/mL)			
	S.aureus	*B. subtilis*	*E. coli*	*S. typhimurium*
SCGE 0 (Control)	6.00 ± 1.41^{a}	1.38 ± 0.20^{a}	8.40 ± 0.56^{a}	10.30 ± 0.14^{a}
SCGE 0.50	5.00 ± 0.28^{a}	1.15 ± 0.04^{a}	2.90 ± 0.14^{b}	4.70 ± 0.14^{b}
SCGE 0.75	4.50 ± 0.71^{a}	0.79 ± 0.04^{b}	1.35 ± 0.07^{c}	4.50 ± 0.14^{b}
SCGE 1.00	0.92 ± 0.08^{b}	0.71 ± 0.04^{b}	0.80 ± 0.06^{c}	1.60 ± 0.21^{c}
eSCGE 0.50	6.40 ± 1.13^{a}	0.78 ± 0.08^{b}	2.50 ± 0.14^{b}	1.10 ± 0.10^{cd}
eSCGE 0.75	1.44 ± 0.14^{b}	0.34 ± 0.00^{c}	1.35 ± 0.01^{c}	1.00 ± 0.01^{d}
eSCGE 1.00	0.78 ± 0.11^{b}	0.24 ± 0.03^{c}	1.10 ± 0.06^{c}	0.70 ± 0.17^{d}

*Different superscript letters in the same column indicate statistically significant differences ($p \leq 0.05$).

4. Conclusion

The SCGE-CH and eSCGE-CH films exhibited inhibitory effects against all the tested pathogens. The antimicrobial activity of the SCGE-CH and eSCGE-CH film increased with the increasing concentration of SCGE and eSCGE in the film. These results indicate that the eSCGE-CH film exhibited superior antimicrobial activity than the SCGE-CH film, with *S. typhimurium* as the most susceptible to the eSCGE-CH compared to the other

pathogens. It can be concluded that the eSCGE-CH film has the potential to be applied as an antimicrobial film in food industry. However, higher amounts of coffee ground extract should be applied to broaden its antimicrobial effect.

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