Antimicrobial and Mechanical Properties of Jackfruit Seed Starch-based Films Containing Carvacrol

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Abstract

The demand for the development of a biodegradable antimicrobial packaging system from food wastes continues to grow because of food safety issues and the negative environmental impact brought about by synthetic plastics. In this study, a jackfruit seed starch-carrageenan (JSS-CG) blend film containing carvacrol was prepared. The antimicrobial capacity of the films against Staphylococcus aureus BIOTECH 1582 was evaluated by disk diffusion assay. Results revealed that zones of inhibition increased with the increase in carvacrol concentration. The optimum concentration of carvacrol for inhibiting the growth of S. aureus was 10% with a zone of inhibition of 19.67 mm. Furthermore, the film effectively controlled the growth of S. aureus in cheese stored at 28 °C. The microbial count of cheese decreased with storage time. Log reductions of 0.24, 0.56 and 0.66 were recorded after 12, 24 and 36 h of storage, respectively. The film exhibited a tensile strength of 12.03 MPa and an elongation at break of 72.55% based on the mechanical test. This study suggests that JSS-CG film containing carvacrol has potential as a food packaging material with desirable antimicrobial and mechanical properties.

Keywords*:* antimicrobial films, carrageenan, carvacrol, jackfruit seed starch

1. Introduction

Foodborne illness is a growing public health concern. In the Philippines alone, there were 209 cases of foodborne disease outbreaks from 2008 to 2015 (Azanza *et al.*, 2019). Staphylococcal food poisoning is one of the most common causes of foodborne diseases, resulting from the ingestion of toxins produced by enterotoxigenic strains of *Staphylococcus aureus* (Aydin *et al.*, 2011; Strommenger *et al.*, 2018). *S. aureus* is a gram-positive bacterium that can easily contaminate food during handling as it is naturally found in the

human skin and mucous membrane. The enterotoxins that are not destroyed during cooking can lead to severe gastrointestinal illnesses. Milk and other dairy products such as cheese are reported to be contaminated with this bacterium (Normanno *et al.*, 2007; Rall *et al.*, 2008).

One strategy to address this food safety concern is the use of antimicrobial packaging to prevent contamination. Synthetic plastics (e.g., polyethylene and polypropylene) are commonly used due to their excellent barrier properties and durability (Marsh and Bugusu, 2007; Ramos *et al.*, 2016). However, these plastics pose an alarming environmental threat, as they take an average of 500 years to decompose. Consequently, this leads to pollution detrimental to marine and terrestrial ecosystems. Hence, researchers have focused their efforts on developing a biodegradable antimicrobial film designed not only to provide a barrier between the food and the external environment, but to also control the growth of pathogenic bacteria. These films are made from plant- or animal-based polymers incorporated with antimicrobial agents. The polymers serve as a matrix for the controlled release of antimicrobials on the surface of the product (Basch *et al.*, 2013; Abdou and Sorour, 2014; Boonruang *et al.*, 2017).

Starch is a natural polysaccharide derived from plants. It is commonly used in the preparation of films due to its abundance, renewability, and low cost. Jackfruit (*Artocarpus heterophyllus)* is grown in tropical countries and can be used as a starch source. The seeds, which account for 15-18% of the total fruit mass (Ocloo *et al.*, 2010), are discarded as waste. According to Oates and Powell (1996), the seeds contain 12-28% starch which can be used as raw material for the preparation of films. Starch alone produces films with poor mechanical properties. However, blending starch with natural polymers such as carrageenan can improve the properties of the film (Abdou and Sorour, 2014). Carrageenan is a natural polysaccharide derived from red seaweed consisting of sulfated D-galactose and 3,6-dehydro-D-galactose units. It is commonly used as a thickener and stabilizing agent in various food products.

Among the antimicrobials incorporated into the polymeric films are bacteriocins, organic acids, and essential oils (Del Nobile *et al.*, 2008; Cao-Hoang *et al.*, 2010; Basch *et al*., 2013; Ollé Resa *et al.*, 2016; Boonruang *et al*., 2017; Soni *et al.*, 2018). Carvacrol is a phenolic monoterpene present in essential oils of plants that belong to the Labiatae family, such as oregano. It is a transparent yellowish liquid at room temperature and is generally regarded as safe (GRAS) by the United States Food and Drug Administration. Recent

studies have reported the inhibitory effects of the direct use of carvacrol, either alone or in combination with other essential oils and agents, against food pathogens such as *S. aureus*, *Escherichia coli*, *Aeromonas hydrophila, and Vibrio cholera* (Lambert *et al.*, 2001; Friedman *et al.*, 2002; Mathela *et al.*, 2010; Rattanachaikunsopon and Phumkhachorn, 2010; dos Santos Rodrigues *et al.*, 2017; Alkan Tas *et al.*, 2019; Ribes *et al.*, 2019). Velasco and Fundador (2020) reported that carvacrol incorporated in films effectively reduced the growth of *S. aureus* in food stored at refrigerated temperature by 84% after 24 h at 4 °C. The present study aimed to develop an antimicrobial film using jackfruit seed starch and carvacrol to control the growth of *S. aureus* in cheese stored at ambient temperature. The mechanical properties of the films were also evaluated.

2. Methodology

2.1 Materials

Jackfruit seeds were procured from Bankerohan Public Market, Davao City, Philippines. κ-Carrageenan (CG) was obtained from Marstons Food Corporation, Davao City. Carvacrol was purchased from Sigma-Aldrich, United States. *S. aureus* BIOTECH 1582 was acquired from the Philippine National Collection of Microorganisms, BIOTECH, University of the Philippines Los Baños. The Megazyme Total Starch Assay kit was kindly donated by Dr. Juma Novie Alviola from University of the Philippines Mindanao.

2.2 Extraction of Jackfruit Seed Starch (JSS)

The extraction followed the method from Pimpa *et al.* (2012) with slight modifications. Jackfruit seeds were washed with water and the seed coating was removed. The resulting endosperm was ground with an equal amount of 0.5% $NaHSO_3$. The mixture was filtered to remove the seed fibers. The suspension was allowed to stand for 2 h at 4 °C and centrifuged for 15 min. The precipitate was washed twice with distilled water and freeze-dried at -48 ºC for 48 h. The dried sample was ground and sifted through a 100-mesh sieve. The purity of JSS was determined using the Megazyme Total Starch Assay kit.

2.3 Preparation of Carvacrol Emulsion

A volume of carvacrol (30, 60, 90, 120, 150 and 180 µL) was mixed with 200 uL Tween 80 and 1 mL distilled water using a vortex mixer (Labnet, United States) until an emulsion was formed. The emulsions were used in the preparation of films.

2.4 Preparation of JSS-CG/Carvacrol Films

JSS and CG (1.5 g, 70:30) were gelatinized in 35 mL distilled water at 90 °C with constant stirring for 15 min. Glycerol was then added until a final concentration of 30% v/w was achieved. The resulting mixture was cooled to 50 °C, followed by the addition of carvacrol emulsion to obtain the desired concentrations (2, 4, 6, 8, 10 and 12% v/w). The solution was poured into a plastic Petri plate (90 x 15 mm) and dried in the oven for 24 h at 45 °C. The resulting films were peeled off and conditioned in a desiccator for 24 h until further use. Films without carvacrol served as the control.

2.5 Mechanical Testing

Mechanical properties of the JSS-CG films (5 mm x 30 mm) containing 0 and 10% carvacrol were determined using universal testing machine (EZ-SX, Shimadzu, Japan). These were then compared with the mechanical properties of JSS film and commercially available polyethylene film. The test was carried out at room temperature with a load cell of 100 N, crosshead speed of 20 mm/min and a 10 mm distance between grips.

2.6 Antimicrobial Activity Test

2.6.1 Disk Diffusion Assay

Bacterium lawns of *S. aureus* (10^8 CFU/mL) were prepared on Mueller Hinton Agar plates. JSS-CG/carvacrol films were cut into 6 mm disks and surface-sterilized under UV light for 3 min on both sides. The films were placed on the bacterium lawns and the plates were incubated at 37 °C for 18 h. Zones of inhibition were determined by measuring the average zone diameter at two cross-sectional points. The optimized concentration of carvacrol in the films was subjected to actual product testing.

2.6.2 Actual Product Testing

Commercial processed-filled cheese was used to evaluate the antimicrobial activity of the film based on the method described by Barbosa *et al.* (2013) with some modifications. Cheese slices (2 x 2 cm; ca. 1 g) were placed on sterile Petri plates and surface-sterilized under UV light for 3 min on each side. The slices were then inoculated with 20 µL of the *S. aureus* suspension (10^8 CFU/mL). The spike-inoculated samples were covered with JSS-CG films containing 10% carvacrol and stored at room temperature (28 °C). A control treatment was prepared by covering the spike-inoculated sample with the film containing 0% carvacrol.

Analyses of the microbial load of the samples were performed at 0 h (initial) and after 12, 24 and 36 h of storage. The untreated sample served as the control. Samples were homogenized in a 0.1% peptone solution using a vortex mixer. The mixtures were then serially diluted and spread onto the Baird Parker Agar. The plates were incubated for 24-36 h at 37 °C and the results were expressed as log CFU/mL.

2.7 Statistical Analysis

One-way and two-way analyses of variance and Tukey's test were used to determine the significant differences among treatments at $\alpha = 0.05$.

3. Results and Discussion

3.1 Extraction of JSS

Crude starch was extracted from jackfruit seeds using 0.5% $NaHSO_3$. This extraction method yielded 14.62% starch, which agrees with Oates and Powell (1996). The amount of starch present is dependent on the physiological maturity of the fruit. According to Azizur Rahman *et al.* (1999), the starch in the seed is hydrolyzed into simple sugars as the fruit ripens, thus giving a low amount of starch extracted. JSS had a purity of 59.69%, indicating the presence of impurities such as protein, fiber, fat, minerals and ash (Ocloo *et al.*, 2010). The starch was odorless, white and fine powder.

3.2 Mechanical Test

Table 1 shows the comparison of the mechanical properties of the films. JSS films had the lowest tensile strength (1.53 MPa) while JSS-CG films exhibited higher tensile strength (12.16 MPa). This proves that blending starch with CG improves the tensile strength of the films due to the ability of CG to form a strong three-dimensional network (Yuguchi *et al.*, 2002). However, the elongation at break (EAB) decreased with the addition of CG.

Table 1. Mechanical properties of various films

Parameter	JSS	JSS-CG	JSS-CG/carvacrol*	PE**
Tensile strength (MPa)	1.53±0.08^{a}	12.16±0.57^{b}	12.03±0.19^{b}	27.37±0.84^{c}
EAB (%)	117.30±1.80^{c}	65.82±1.82^{a}	72.55±0.74^{b}	467.06±2.18^{d}

Within a row, means that do not share a number are significantly different at $\alpha = 0.05$; *Films containing 10% carvacrol; **polyethylene.

Furthermore, the tensile strength of JSS-CG/carvacrol films was comparable to the JSS-CG films, suggesting the addition of carvacrol did not affect the tensile strength of the film. On the other hand, the EAB increased from 65.82 to 72.55%. This was due to the plasticizing effect of carvacrol which resulted in a decrease in resistance to break. A similar finding was also reported for films containing different essential oils (Tongnuanchan *et al.*, 2014). In comparison to polyethylene films, JSS-CG/carvacrol films had lower TS and EAB. Although the strength of JSS-CG/carvacrol films was not superior compared to polyethylene films, these can be used as a wrapper for candies and other food products which do not require high impact strength material.

3.3 Antimicrobial Activity Test

3.3.1 Disk Diffusion Assay

The antimicrobial capacity of JSS-CG films with different concentrations of carvacrol (0, 2, 4, 6, 8, 10 and 12%) against *S. aureus* was evaluated using disk diffusion assay. As shown in Table 2, the inhibitory effect of the films increased with the increase in the concentration of carvacrol. Films containing 4% carvacrol started to exhibit a zone of inhibition of 6.11 mm. On the other hand, films with 10% carvacrol gave a larger zone of inhibition (19.67 mm). Further increase of the carvacrol concentration to 12% did not significantly enhance the ability of the film to control the growth of *S. aureus.* According

to Burt (2004), the inhibitory effect of carvacrol is caused by the permeabilization of the bacterial membranes, which disrupts ion gradients. Furthermore, carvacrol reduces the cytoplasmic membrane potential of the microorganism by acting as a transmembrane carrier of monovalent cations. This permits the exchange between its hydroxyl proton and potassium (K^+), a monovalent ion. Consequently, this initiates the depletion of the ATP pool and triggers the collapse of the proton motive force, eventually leading to the death of the bacterial cell (Ultee *et al.*, 2002).

Table 2. Antimicrobial activity against *S. aureus* of JSS-CG films with different concentrations of carvacrol

Concentration of Carvacrol (%v/w)	Zone of Inhibition (mm)
0	0^a
2	0^a
4	6.11 ± 1.64^b
6	8.33 ± 2.03^b
8	11.89 ± 0.51^c
10	19.67 ± 0.93^d
12	20.67 ± 0.33^d

Treatment means within a column followed by the same letter are not significantly different at $\alpha = 0.05$.

On the contrary, control films (0% carvacrol) had no inhibitory effect on *S. aureus* indicating that neither JSS nor CG had an antimicrobial property. Hence, the antimicrobial effect can be attributed solely to the presence of carvacrol. In the succeeding experiments, JSS-CG films with 10% carvacrol was used since a higher concentration of carvacrol did not significantly enhance its antimicrobial activity.

3.3.2 Actual Product Testing

The antimicrobial efficacy of the films on *S. aureus*-spiked cheese was investigated by monitoring the microbial count at 0 h (initial) and after 12, 24 and 36 h of storage at room temperature (28 °C). Cheese was chosen as a food model system as *S. aureus* can potentially contaminate dairy products when not properly handled or processed. As shown in Table 3, the microbial count of the samples covered with JSS-CG/carvacrol films significantly decreased with storage time. This suggests the diffusion of carvacrol from the film to the food sample. After 12 h of storage, the microbial count was reduced by 0.24 log cycle (from 6.43 to 6.19 CFU/mL) which corresponds to a 42.06%

reduction. Prolonging the storage time to 36 h resulted in a further decrease in the microbial count by 0.66 log cycle (from 6.36 to 5.70 CFU/mL) or 78.72%.

The microbial counts were slightly lower for the samples covered with JSS-CG films than the initial count. This was due to the direct contact of the film with the microorganism inoculated on the sample's surface. Another factor was the presence of preservatives such as potassium sorbate inherent to the cheese, which inhibited the growth of the bacterial cells. Potassium sorbate is widely used in the food industry to prolong the shelf-life of various food products (Jorge, 2003). Thus, the log reduction was calculated based on the difference between the microbial count of the samples covered with JSS-CG films and samples covered with JSS-CG/carvacrol films. This is to rule-out the two aforementioned factors.

Table 3. *S. aureus* count on cheese covered with JSS-CG and JSS-CG/carvacrol films after different storage times at 28 °C

Treatment	Initial Log CFU/mL	*S. aureus* count (log CFU/mL) Storage Time (h)		
		12	24	36
JSS-CG	6.48 ± 0.02^{W}	6.43 ± 0.00^{bX}	6.40 ± 0.02^{bX}	6.36 ± 0.01^{bY}
JSS-CG/carvacrol*		6.19 ± 0.012^{aX}	5.84 ± 0.01^{aY}	5.70 ± 0.02^{aZ}

Within a column, a-b compares the significant difference between means at $\alpha = 0.05$; within a row, W-Z compares the significant difference among means at $\alpha = 0.05$; *Films containing 10% carvacrol.

4. Conclusion and Recommendation

A biodegradable antimicrobial film was prepared by incorporating carvacrol in the JSS-CG blend. A concentration of 10% carvacrol was considered optimum for inhibiting the growth of *S. aureus* on the film. Moreover, the JSS-CG/carvacrol film reduced the microbial count of *S. aureus*-spiked cheese stored at 28 °C. Log reductions of 0.24, 0.56 and 0.66 were observed after 12, 24 and 36 h of storage, respectively. The presence of carvacrol did not affect the tensile strength of the film. Overall, JSS-CG/carvacrol films have potential as an alternative food packaging material that can control the growth of *S. aureus*. The findings of this study also address the environmental problems brought about the use of non-biodegradable plastics. Lastly, the utilization of jackfruit seeds as raw material for the preparation of films also alleviates food waste management concerns. Further studies are recommended to evaluate the

gas and water vapor permeability, surface morphology and other physical properties of the film to determine its appropriateness as a packaging material. The effectiveness of the film against other pathogenic bacteria can also be explored.

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