COMPARATIVE STUDIES ON THE CHARACTERIZATION, ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF ALGINATE-BASED EDIBLE BIODEGRADABLE PACKAGING FILMS LOADED WITH ASCORBIC ACID AND CINNAMON ESSENTIAL OIL

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ABSTRACT

Currently, there is a demand for developing innovative green packaging materials instead of synthetic packaging using novel biodegradable materials to reduce the environmental pollution caused by their accumulation, improve safety, and extend the shelf life of foods. This study aimed to develop seaweed-based active edible packaging films from alginate (Alg) containing different concentrations (0.5%, 1%, and 1.5%) of antioxidants such as ascorbic acid (AA) and cinnamon essential oil (CEO) using $CaCl_2$ as the crosslinking agent employing the casting method. The films were characterized and compared for their physical, mechanical, thermal, optical, antioxidant and antimicrobial properties, and biodegradability, considering the effect of antioxidant concentration. Increasing the concentration of AA and CEO resulted in increased film thickness and decreased moisture content, which ranged from 0.14 mm to 0.27 mm and 9.6% to 29.5%, respectively. Alginate-based films containing CEO (Alg+CEO) demonstrated a significantly higher water resistance ($p<0.05$) compared to alginate-based films containing AA (Alg+AA), which was attributed to the hydrophobicity of CEO. The biodegradability of Alg+AA films increased with increasing AA concentration, while Alg+CEO films demonstrated a decrease with increasing CEO concentration. All films demonstrated a soil biodegradability rate of over 70% within 28 days. There was a decrease in tensile strength with increasing AA and LEO concentrations, while the addition of CEO led to increased elongation at break. Both films showed a significantly increased ($p<0.05$) total colour difference (ΔE) with increasing antioxidant concentration. The Alg+CEO films exhibited better antimicrobial activity compared to Alg+AA films against Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacteria, and the incorporation of 1% AA and 1% CEO were the most effective concentrations against Gram-positive and Gram-negative bacteria. The DPPH free radical scavenging activities of 1.5% Alg+AA films (IC_{50} = 0.06 mg/mL) were higher than those of the 1.5% Alg+CEO films (IC_{50} = 0.15 mg/mL). These results proved the potential of AA and CEO-incorporated alginate-based films, which could be used as sustainable green packaging due to their excellent antioxidant and antimicrobial properties.

Keywords: alginate films, antimicrobial, antioxidant, ascorbic acid, cinnamon essential oil

EXTENDED ABSTRACT

INTRODUCTION

Food packaging protects food products from physical, chemical, and biological influences. However, these non-renewable, non-biodegradable, synthetic packaging materials have serious environmental drawbacks, mainly due to their accumulation, leading to huge environmental pollution. Currently, edible and biodegradable films are a fast-emerging technology with increased attention among researchers and consumers, which acts as alternatives to synthetic food packaging. Edible packaging is synthesized utilizing various biopolymers such as alginate, agar, chitosan, carrageenan, lipids, etc.

Alginate is a hydrophilic water-soluble polysaccharide, which is non-toxic, relatively inexpensive, biocompatible, and extracted from brown seaweeds. It can selectively bind to multivalent cations resulting in films with rigid structures (Baek et al., 2018).

Active packaging can be made by fabricating active materials within the packaging materials instead of incorporating them into the food to extend the shelf-life of food products and preserve and improve the organoleptic features of their contents. Therefore, new active packaging solutions that contain natural active materials are beneficial substitutions for conventional packaging techniques.

In order to synthesize active packaging, organic acids and essential oils (EOs) can be used as natural active ingredients. Ascorbic acid (AA), a natural water-soluble vitamin delays the ripening and mold development of fruits and vegetables. The sustainable release of ascorbic acid from fabricated matrices will enhance the quality of fresh produce. Among EOs, cinnamon (*Cinnamomum zeylanicum*) essential oil (CEO) mainly contains monoterpenes such as trans-cinnamaldehyde and eugenol (Baek et al., 2018). AA and CEO can be incorporated into alginate-based films as natural active materials to produce active packaging films with antioxidant and antimicrobial properties.

Therefore, the present study aimed to assess the effect of the addition of various concentrations of AA and CEO on the physical, optical, mechanical, thermal, antimicrobial, and antioxidant properties as well as the biodegradability of alginate composite films.

METHODOLOGY

Based on our previous study, an optimized alginate-based edible film formulation was used. Alginate (2.5% w/v) stock solutions were prepared, and glycerol was added as a plasticizer (Li et al., 2015). The AA was added to the film-forming solution at various concentrations (0.5%, 1%, and 1.5% w/v). All formulations were mixed in a homogenizer to form a homogenous solution and then degassed under the vacuum. The formulations were transferred to glass moulds and dried at ambient conditions. The films were cross-linked with a 1% (w/v) $CaCl_2$ solution using immersion. The dried films were then removed from the Petri dishes and conditioned at 50 ± 5% relative humidity and 25±2 °C until analysis.

The same procedure was used to synthesize alginate-based films incorporating CEO, where Tween 80, which is a surfactant, was added (2% v/v of essential oil) to the film-forming solution.

The physical properties such as thickness, moisture content, swelling degree, optical properties such as surface colour difference (ΔE) and whiteness index, and mechanical properties such as tensile strength and elongation at break (ASTM D882-10) of the films were analyzed. Biodegradability was tested using soil degradation (Kaya et al., 2018).

The antioxidant activity of the samples was evaluated using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay (Chen *et al*., 2021).

A quantitative assay of the antimicrobial activity of the films was done using the agar disk diffusion method (Zhang *et al.,* 2015). The antimicrobial activities of alginate-based films

were tested against four food-borne pathogenic bacteria (Gram-positive: *Staphylococcus aureus*, *Bacillus cereus*, Gram-negative: *Klebsiella pneumoniae*, *Escherichia coli*) isolated in the lab from spoiled fruits and vegetables.

The properties of alginate-based films incorporated with ascorbic acid (Alg+AA) were compared with the alginate-based films incorporated with cinnamon essential oil (Alg+CEO).

RESULTS AND DISCUSSION

Analysis of physical properties

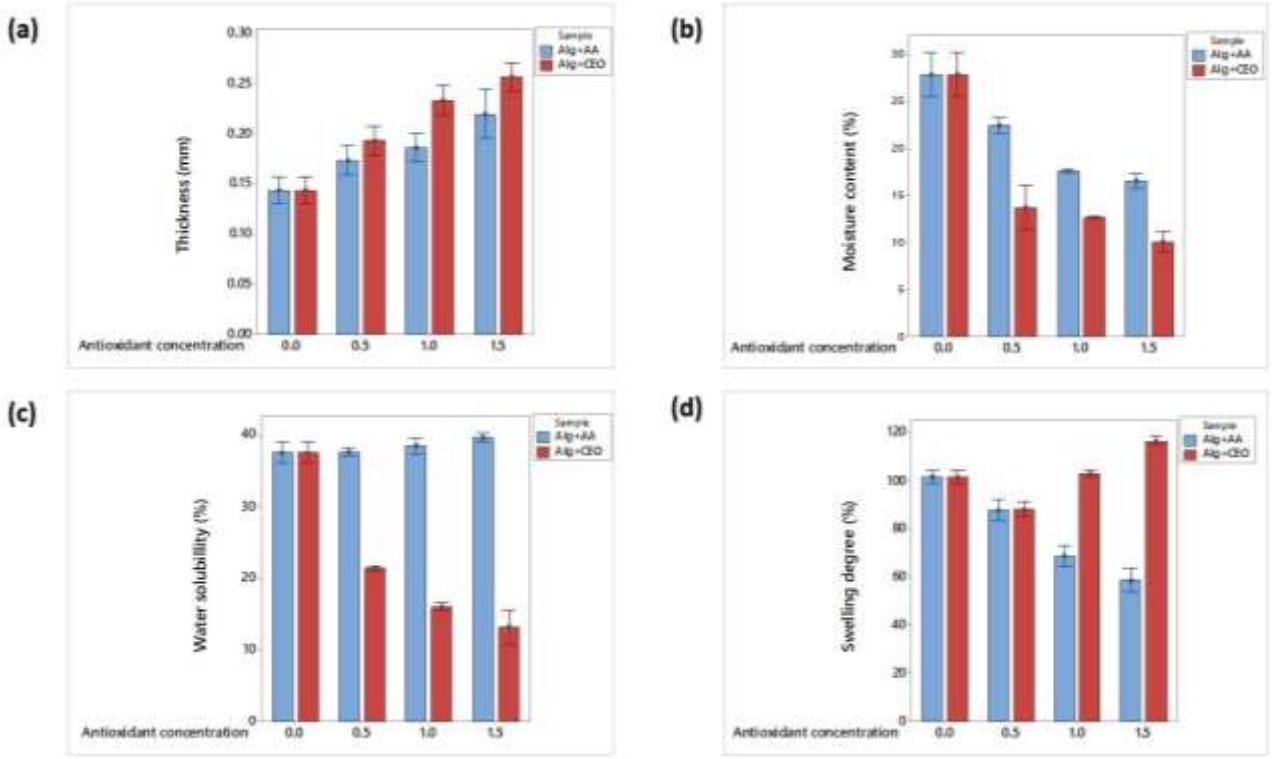

Figure 1: (a) Thickness, (b) moisture content (%), (c) water solubility (%), and (d) swelling degree (%) variations of Alg+AA and Alg+CEO films

The addition of AA and CEO increased the film thickness and decreased the moisture content (MC) with increasing antioxidant concentration, which ranged between 0.14 mm - 0.26 mm, and 9.55% - 29.51%, respectively (Figure 1). The reduction of MC was greater for Alg+CEO films. As alginate is highly hydrophilic, the control films (with no antioxidants) exhibited the highest MC due to the higher molecular entanglement and viscosity in pure alginate solutions, leading to higher retention of water molecules during film drying. The covalent bonding between AA or CEO and the functional groups of the polymeric matrix decreased the polysaccharide-water interactions by hydrogen bonding, ultimately leading to a decrease in the MC of films (Han Lyn and Nur Hanani, 2020).

The solubility declined significantly ($p<0.05$) with increasing CEO and decreasing AA concentrations. This suggests that the water resistance of the films was improved more in Alg+CEO films compared to Alg+AA films due to the increase in hydrophobicity of the films caused by the possible addition of CEO (Benavides et al., 2012).

A significant decrease ($p<0.05$) in the swelling degree (SD) is detected in Alg+AA films when increasing AA concentration, and SD increases with increasing CEO concentration.

This increase could be attributed to the increase in the availability of free EO, causing the formation of a heterogeneous matrix.

Analysis of optical properties

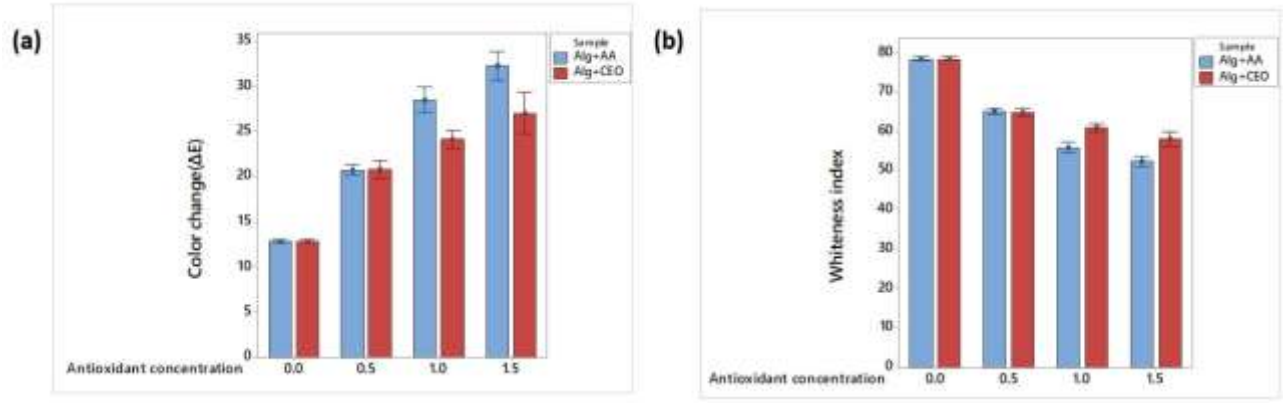

Figure 2: **(**a) Colour change (ΔE) and (b) whiteness index variations of Alg+AA and Alg+CEO films

Both films showed a total colour difference (ΔE) significantly greater ($p<0.05$) than the control film, which increased with the increasing antioxidant concentration (Figure 2). The addition of AA and CEO into alginate-based films significantly decreased ($p<0.05$) the film whiteness index, where the decrease was higher for Alg+AA films.

Analysis of mechanical properties

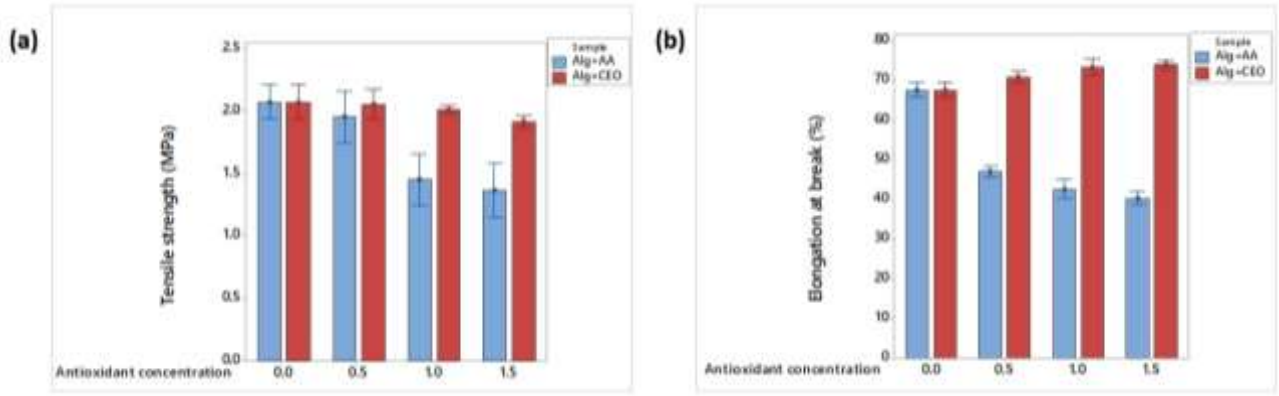

Figure 3: **(**a) Tensile strength (MPa) and (b) elongation at break (%) variations of Alg+AA and Alg+CEO films

The tensile strength (TS) decreased with increasing AA and CEO concentrations, whereas elongation at break (EAB) values of Alg+AA films decreased and Alg+CEO films increased with increasing AA and CEO concentrations, respectively (Figure 3). This may be due to the strong plasticizing effect of the CEO, which increased the flexibility of Alg+CEO films, resulting increase in the EAB values. The decrease in EAB in AA-incorporated films could be due to the stronger destructive capacity of AA to the crystalline structure (Bhatia et al., 2022).

Analysis of antimicrobial properties

According to Figure 4, the control films showed no inhibitory effect against the studied bacteria. With increasing antioxidant concentration, both types of films showed significant inhibition ($p<0.05$) towards both Gram-positive and negative bacteria. Alg+CEO films showed the highest inhibition of *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Escherichia coli* at the optimum antioxidant concentration, compared to Alg+AA films confirming their effective antimicrobial activity.

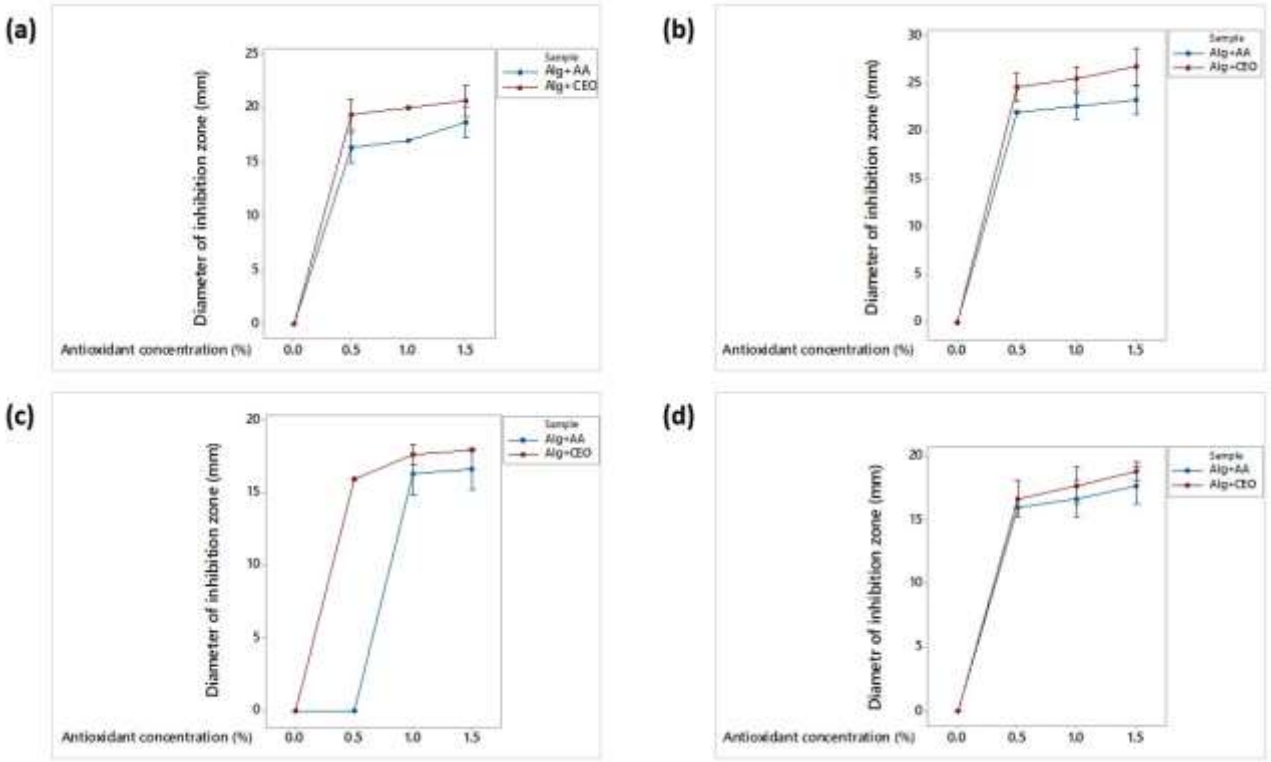

Figure 4: Diameter of inhibition zones of Alg+AA and Alg+CEO films against (a) *Staphylococcus aureus*, (b) *Bacillus cereus*, (c) *Klebsiella pneumoniae*, (d) *Escherichia coli*

DPPH-free radical scavenging assay

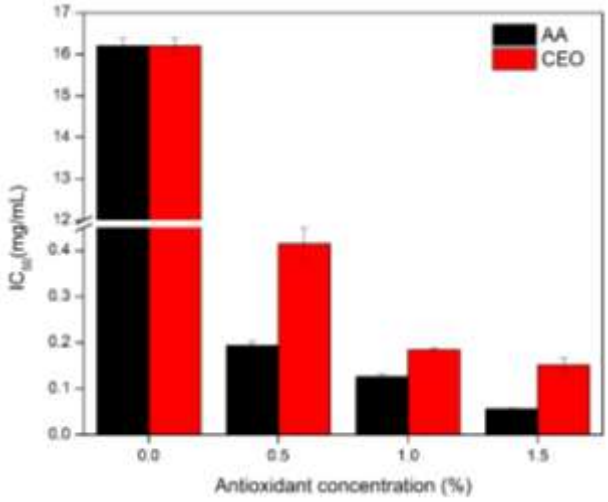

Figure 5: Antioxidant activity (IC_{50} in µg/mL) of Alg+AA and Alg+CEO films

Control films (Alg) showed the highest IC_{50}, confirming the lowest antioxidant activity. The Ca-crosslinking may result in their positive antioxidant activity (Figure 5). The DPPH scavenging activity for all Alg+CEO samples remains lower than those values for Alg+AA, whereas the highest antioxidant activity was obtained by 1.5% AA incorporation.

CONCLUSIONS/RECOMMENDATIONS

The alginate-based films loaded with different concentrations of antioxidants such as AA and CEO were developed and characterized. As the content of antioxidants increased, the film's TS was decreased, whereas EAB was improved by the CEO addition, which can be attributed to the plasticizer action of the CEO. The AA and CEO presented a significant inhibitory effect against food-born pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Escherichia coli*. Furthermore, the incorporation of CEO is more effective than AA, due to their higher antimicrobial activity. The Alg+1.5%AA films showed stronger antioxidant activities than Alg+CEO films, demonstrating their possibility to be applied as new active food packaging materials. Additional work is in progress to study the water vapour and air barrier properties of these films.

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