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Characterization of antioxidant-antimicrobial κ-carrageenan films containing *Satureja hortensis* essential oil

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ABSTRACT

The present work was aimed at characterizing biodegradable composite kappa-carrageenan films incorporated with *Satureja hortensis* (SEO) in terms of their physical, optical, mechanical, barrier and antioxidant properties. Also, in a comparative study, we sought to evaluate the antimicrobial effectiveness of these films against five pathogens. The films' water vapor barrier properties were found to improve considerably upon the addition of SEO. Carrageenan composite films were less resistant to breakage, more flexible and more opaque with lower gloss than the control film. These results can be explained by the film's microstructure, which was analyzed by atomic force microscopy and scanning electron microscopy. The films incorporating SEO showed good antioxidant properties; this effect was greatly improved when the proportion of added SEO was 3%. Films with SEO effectively inhibited the five microorganisms tested. The results of the present study suggest that SEO as a natural antibacterial agent can potentially be used in packaging a wide range of food products, particularly those that are highly oxidative and microbial sensitive.

1. Introduction

For the past 50 years, synthetic petroleum polymers have been widely used in a variety of packaging materials; however, they have become a major source of waste-disposal problems due to their poor biodegradability. To solve this problem, much research has aimed to obtain an environmentally friendly packaging material [1,2]. Recent decades have seen extensive investigation into biodegradable coatings or films prepared from biopolymers, including proteins, polysaccharides and lipids or their combinations. Edible, biodegradable films, by acting as barriers to control the transfer of moisture, oxygen, lipids and flavors can prevent quality deterioration and increase the shelf life of food products [3]. Moreover, biodegradable film can be used to carry active ingredients, such as antioxidant and antimicrobial agents that provide an extra stress factor against foods' oxidative and microbial deterioration [4,5].

Carrageenans are natural, water-soluble hydrocolloids composed of a linear chain of sulfated galactans and extracted from certain species of red seaweed. They are classified according to the number and position of a sulfated ester on 3,6-anhydro-D-galactose residues. Carrageenans have high potential as a film-forming material. Cooling a hot solution of carrageenan during film casting and drying leads to a transition of random coil to double helix, which results in the formation of a compact and structured film after the dehydration of the solution [6]. In one study, Park [7] reported that κ-carrageenan can produce a clear film with good mechanical and structural properties, including a tensile strength higher than those of ι and γ-carrageenan films.

A number of hydrophobic compounds, such as lipids, are frequently incorporated into hydrocolloid-based films as depressors of water vapor permeability (WVP). The incorporation of plant essential oils into these films represents an interesting alternative to lipids. Their potential health benefits, as well as their

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strong antioxidant-antimicrobial properties, make them possible substitutes for synthetic antioxidant-antimicrobial agents to achieve oxidative and microbial stability, as well as safer food products [8].

Satureja hortensis is an annual, aromatic and medicinal plant belonging to the *Lamiaceae* family, which mainly grows in the Mediterranean region. This plant is used as a seasoning agent and traditional herb in folk medicine [9]. *Satureja hortensis* essential oil's (SEO) antioxidant and antimicrobial characteristics, which result from its high content of phenolic compounds, have been extensively demonstrated [10,11].

The addition of essential oils into a film matrix, instead of applying them directly on food products, could contribute to reducing required doses of essential oils, while keeping their antioxidant-antimicrobial activities [4]. Additionally, although carrageenan-based films have good mechanical and structural properties, they perform poorly as water-vapor barriers due to their hydrophilic nature; this limits their application. The presence in films of hydrophobic essential oils provides a practical solution by reducing its affinity for water [5].

Recently, several reports on the antimicrobial activity of various essential oils incorporated into biodegradable films using direct application (direct contact between microorganisms and antimicrobial agents) have been published [4,12]. Some authors reported that the vapor phase of essential oils (no direct contact between the essential oil and the medium surface) exhibits good inhibitive power against foodborne pathogens and spoilage bacteria, and is even more effective than direct application; this in turn can reduce the organoleptic alteration induced by essential oils [13,14].

To our knowledge, few studies have been carried out to evaluate the effectiveness of biodegradable films containing essential oils in the vapor phase versus direct contact. In addition, there are no reported data on the characteristics of carrageenan composite films containing SEO. This study aimed to develop a new biodegradable film based on carrageenan-SEO composite film through emulsification; to assess the film's antimicrobial effect in both the vapor phase and direct contact, as well as its antioxidant activity; and to explore their impact on the relevant properties to evaluate the films' suitability as food coatings: WVP, mechanical, optical and microstructural properties. These results, currently not present in the literature, but are very important for evaluating these films' possible applications as packaging material.

2. Materials and methods

2.1. Materials

Kappa-carrageenan (Rico Co, Philippine), Essential oil (SEO), supplied by Barij Company, (Kashan, Iran), Tween 80 and glycerol (Fluka, Sigma–Aldrich, MO, USA), were used to prepare film-forming dispersions (FFD). Mueller–Hinton agar (MHA) and Mueller–Hinton Broth (MHB) were bought from Merck Co (Darmstadt, Germany). Folin–Ciocalteu reagent, sodium carbonate, standard gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co (St. Louis, MO). All other reagents used were of analytical grade.

2.2. Bacterial strains

Staphylococcus aureus ATCC 25923; *Bacillus cereus* PTCC 1154, *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Salmonella typhimurium* ATCC 14028 were provided by Iranian Research Organization for Science and Technology (Tehran, Iran). Stock cultures of the studied bacteria were grown in MHB at 30 °C for 24 h before the tests.

2.3. Preparation of films

Kappa-carrageenan based films were prepared by the method of Park [7] with some modifications. A series of preliminary experiments were conducted to determine the appropriate concentration of plasticizer (glycerol) for preparing films. Results showed that filmogenic solutions containing 50% (w/w) glycerol (based on carrageenan weight) were easily removed from the plate. Film solutions were prepared by dissolving κ-carrageenan (1%, w/v), in distilled water under magnetic stirring. Following the addition of glycerol at constant concentration (50% (w/w) based on carrageenan weight), stirring was continued for a further 40 min at 82 °C. The emulsions were obtained by adding SEO to the carrageenan solution to reach final concentrations of 1, 2 and 3% (v/v) and Tween 80 as an emulsifier in quantities proportional to the essential oils (0.1, 0.2 and 0.3%, v/v).

FFDs without any essential oils were also prepared for later comparison. Homogenization was carried out using a rotor-stator homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) at 13,500 rpm for 3 min at 80 °C, and then the emulsions were cooled to 65 °C to remove any air bubbles incorporated during homogenization. The FFDs were casted on the center of a rimmed circular area (177 cm^2) of clean and leveled glass plates, and then dried at 30 °C for 30 h (casting and drying were carried out at 30 °C, which is at temperature below the helix melting point reported for carrageenan polymer [6]). Dried films were peeled off the casting surfaces and stored inside desiccators at 25 °C and 53% relative humidity (RH) until evaluation. Saturated magnesium nitrate solution was used to meet required RH.

2.4. Determination of physical properties of films

2.4.1. Thickness

Film thickness was determined using a manual digital micrometer (Mituto, Tokyo, Japan) to the nearest 0.001 mm. Reported values were average of at least ten random locations for each film sheet.

2.4.2. Moisture content

The films moisture content was determined by drying in an oven at 110 °C until a constant weight was reached (dry sample weight). Three replications of each film treatment were used for calculating the moisture content.

2.4.3. Film solubility in water

The water solubility was determined in triplicate according to method of Ojagh et al. [5]. Briefly, pre-weighed film samples were immersed under constant agitation in 50 ml of distilled water for 6 h at 25 °C. After filtration, undissolved film was dried at 110 °C to constant weight. The initial dry weight was determined by drying at 110 °C to constant weight. The water solubility (%) of the film was calculated according to the equation WS (%) = $((W_o - W_f)/W_o) \times 100$, where W_o is the initial weight of the film expressed as dry matter and W_f is the weight of the desiccated undissolved film.

2.5. Mechanical properties

Mechanical properties, including tensile strength (MPa) and elongation at break (%) of the film samples were measured at 25 °C with a Testometric Machine M350-10CT (Testometric Co., Ltd., Rochdale, Lancs., England) according to ASTM standard method D882 [15]. All of the tested film strips (1.5 cm × 10 cm) equilibrated at 25 °C and 53% RH in desiccators containing $Mg(NO_3)_2$ saturated solutions for 48 h prior to testing. Equilibrated film strips were fixed between the grips with an initial separation of 50 mm, and the cross-head speed was set at 50 mm/min. Tensile strength was