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Enhancing Tensile Strength, Thermal Stability, and Antioxidant Characteristics of Transparent Kappa Carrageenan Films Using Grapefruit Essential Oil for Food Packaging Applications

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Cite This: *ACS Omega* 2024, 9, 9003–9012

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ABSTRACT: The trends in food packaging technologies are shifting toward utilizing natural and environmentally friendly materials prepared from biopolymers such as kappa carrageenan to replace synthetic polymers. In the current study, varying amounts (0.1, 0.2, and 0.3%) of grapefruit essential oil (GFO) were incorporated in kappa carrageenan-based edible films to improve their physicochemical properties. The developed film samples were characterized for their barrier, mechanical, morphological, optical, thermal, antioxidant, and biodegradable properties. The results obtained showed that the tensile strength of the carrageenan films enhanced significantly from 65.20 ± 4.71 to 98.21 ± 6.35 MPa with the incorporation of GFO in a concentration-dependent manner. FTIR and SEM analysis confirmed the intermolecular bonding between carrageenan and GFO, resulting in the formation of compact films. Incorporating GFO significantly enhanced the thermal resistance of oil-loaded films, as confirmed by TGA, DSC, and DTG analysis. The addition of GFO led to a substantial increase in the radical scavenging activity of the films, as evidenced by the DPPH and ABTS assays. Furthermore, the developed films were biodegradable in soil and seawater environments, indicating their potential as a sustainable alternative to traditional plastics. Findings demonstrated that GFO can be used as a natural antioxidant agent in kappa carrageenan-based films for potential applications in food packaging.

Fabrication of Kappa Carrageenan Films for Food Packaging Applications
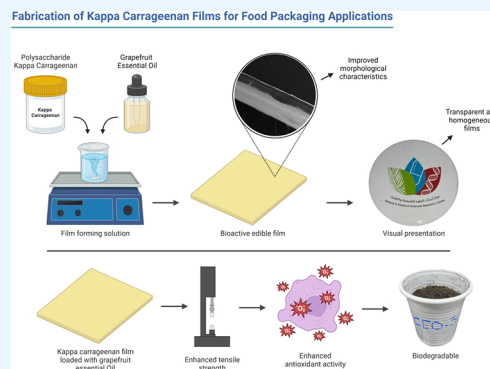
1. INTRODUCTION

Due to mounting concerns related to the deleterious effect of synthetic plastics on the environment, health, and food safety, nowadays, biodegradable, nontoxic, and plastic-free packaging material are more preferred. Carrageenans are natural water-soluble linear polysaccharides derived from red seaweed.[1] Based on the position and number of ester sulfate groups, these polysaccharides are divided into iota, kappa, lambda, mu, nu, and theta carrageenans. These hydrophilic sulfated galactans contain repeating sequences of alternate disaccharide units of D-galactose and 3,6-anhydro-D-galactose linked by alpha-1,3 and beta-1,4-glycosidic linkages. Kappa carrageenan is known for its high film-forming potential to produce a transparent film with excellent mechanical and structural properties with relatively higher TS than films from other carrageenan subtypes.[2] However, kappa carrageenan possesses a poor water vapor barrier and is incapable of protecting food against microbial- and oxidation-induced spoilage.

The incorporation of plant-based hydrophobic compounds, especially essential oils, can improve the water vapor permeability, antioxidant, and antimicrobial properties of edible films. Furthermore, essential oils offer several health benefits and act as possible alternatives to synthetic antioxidant/antimicrobial agents to safeguard a different range of food products. Past reports suggest that the plasticization effect of essential oil over edible films is by decreasing the crystallinity and promoting plastic deformation by interfering with aggregation of chains and supporting slipping of the polymeric chains. These results showed the development of flexible regions in the polymeric matrix, which could lead to a drop in the TS. However, reversible effects are rarely reported where essential oil caused an increase in the crystallinity and TS. TS and thermal stability of the film are the crucial parameters as most of the biopolymer films are delicate and thus cannot withstand high mechanical shock during the transportation of packed food.

Higher TS is mostly favored especially while packing heavier food to ensure a better seal as films with higher TS can prevent

damage caused during transportation. Synthetic and semi-synthetic plastics such as cellophane (120 MPa), polystyrene film (3 MPa), polypropylene (35 MPa), and polyethylene (18.2 MPa) possess respective TS which also varies depending upon the composition and the method of preparation.[3−6]

Grapefruit essential oil (GFO) derived from *Citrus paradisi* is known for its antimicrobial and antioxidant effects. Like other citrus oils, their main constituents are terpenes and terpene oxides. This oil has been documented for various biological activities, including its potential inhibitory effects against food-borne pathogenic microorganisms. Additionally, several essential oils have been reported for their significant impact on mechanical, thermal, and barrier attributes of edible films. Hence, GFO exhibits promising effects as a potential bioactive agent to develop active food packaging material that facilitate the suppression of microorganisms and oxidative reactions as well as improve physical and chemical attributes of film.[7] This study was designed to fabricate an edible film utilizing kappa carrageenan loaded with grapefruit oil and to evaluate its physicochemical attributes and antioxidant characteristics.

2. MATERIALS AND METHODS

2.1. Biopolymer and Additives Procurement. Carrageenan (kappa) variety was acquired from Sisco Research Laboratories Pvt Ltd. located in Mumbai, India. The GFO (batch no. NNIGFEO/107/0821) utilized in the study was provided by Nature Natural India. Glycerol, with a purity of 99%, was sourced from BDH Laboratory Supplies based in London, UK.

2.2. Development of Films. Edible films were fabricated using the casting technique outlined in our prior investigation.[8] A filmogenic solution containing carrageenan was prepared by combining 2% (w/v) carrageenan with 100 mL of distilled water. Subsequently, the mixture was subjected to agitation at 800 rpm, utilizing a magnetic stirrer (Daihan Scientific, Seoul, Korea), for a duration of 20 min, maintaining a temperature of 60 °C. As a plasticizing agent, glycerol was incorporated into the prepared solution at a concentration of 0.5% (v/v). Subsequently, the solution containing both carrageenan and glycerol was subjected to stirring at 1100 rpm for 10 min. The resulting solution was divided into four labeled beakers designated as CEO1−CEO4. Different quantities of GFO (details outlined in Table 1) were introduced into the beakers

Table 1. Constituents of the Film-Forming Solution at Different Concentration Levels

codes	carrageenan (w/v)	glycerol (v/v)	grape fruit essential Oil (v/v)
CEO1	2%	0.5%	blank
CEO2	2%	0.5%	0.1%
CEO3	2%	0.5%	0.2%
CEO4	2%	0.5%	0.3%

labeled as CEO2, CEO3, and CEO4, while CEO1 was kept as the control. The resultant solution containing GFO was mixed at 1100 rpm for 30 min and poured into sterile polystyrene Petri plates (90 × 14 mm) marked as CEO2, CEO3, and CEO4. Subsequently, the films were air-dried at 25 °C for 24 h, carefully detached from the Petri plates' surface, cut into desired shapes, and conditioned under 58% relative humidity at 25 °C for a period of 48 h. The fabricated films were

subjected to a comprehensive examination of their diverse properties. The composition of the prepared film samples (CEO1−CEO4) is shown in Table 1.

2.3. Thickness. The assessment of the thickness of CEO edible films was conducted employing a Mitutoyo digital micrometer 2046F, manufactured by Kawasaki, Japan. This micrometer exhibited a notable degree of precision, enabling the measurement of film samples with an accuracy of 0.01 mm. To ascertain the mean thickness, measurements were acquired from five distinct positions on each individual film.

2.4. Mechanical Attributes. The mechanical characteristics of the film samples were evaluated following the procedure outlined by Bhatia et al.[8] The experimental approach followed the ASTM D882 standard method guidelines using a texture analysis (TA. XT plus, Stable Micro Systems, England) with a 5 kg load cell. The evaluation of both the elongation at break (EAB) percentage and the tensile strength (TS) in MPa was calculated directly from the stress−strain curves using the software Exponent Connect.

2.5. Water Permeation. The water vapor permeability (WVP) of the CEO films containing GFO was assessed through gravimetric analysis following the protocol elucidated by Erdem et al. The experimentation involved the utilization of glass test cups characterized by an internal diameter of 5 cm and a depth of 3 cm. Prior to testing, the films were conditioned in a desiccator set at 50% relative humidity (RH). To create varying RH levels, water (RH = 100%) and silica gel (RH = 0%) were used. The control and GFO-loaded edible films were tightly wrapped over cups filled with silica gel. The cups were periodically weighed (every 1 h) for a 24 h period to monitor the weight gain. The WVP was then determined using a specific formula:

$$\text{WVP} = \frac{\Delta m}{\Delta t \times \Delta P \times A} \times d \tag{1}$$

In eq 1, the rate of moisture uptake ($\Delta m/\Delta t$) is characterized by the mass of moisture gained over a specific time interval (g/d), where A signifies the surface area of the film (m^2), ΔP represents the disparity in water vapor pressure across the film (kPa), and d corresponds to the thickness of the film (mm).

2.6. Moisture Percentage. The gravimetric method was utilized to measure the moisture content (MC) of the GFO-incorporated CEO films. Strips of the film, with dimensions of 3 × 4 cm, were carefully cut and weighed to obtain their initial weight ($W1$). Subsequently, these films were subjected to drying at a temperature of 105 °C until their weight reached uniformity. After drying, the final weight was recorded as $W2$. Using the following formula, we calculated the MC of the CEO films:

$$\text{MC} = \frac{W1 - W2}{W1} \times 100 \tag{2}$$

2.7. Swelling Index. The method employed to determine the swelling index of GFO-loaded CEO film samples was as described by Erdem et al.[9] Initially, film strips measuring 2 × 2 cm^2 were prepared and weighed ($W1$). Subsequently, these edible films were submerged in deionized water for a period of 2 min at 25 °C. After removing excess water from the films using a filter paper, they were weighed again ($W2$). The swelling index of the film samples was calculated using the following formula:

$$\text{Swelling Index} = \frac{W2 - W1}{W1} \times 100 \qquad (3)$$

2.8. Transparency and Color Analysis. To evaluate the transparency characteristics of the edible films, a spectrophotometric methodology was applied utilizing an ONDA-Vis spectrophotometer (V-10 Plus, Italy). This approach followed the procedural guidelines as followed in 10. The color properties of the films were determined by following the methodology described by Rhim et al.[11] A Minolta colorimeter CR-300 from Minolta Camera Co., Osaka, Japan was employed for this experiment. The color properties (L = lightness, a = green-red color, and b = blue-yellow color) were determined. The color difference (ΔE) was then calculated based on the obtained values of L^*, a^*, and b^* using the following formula.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \qquad (4)$$

In eq 4, ΔL^*, Δa^*, and Δb^* represent color differences among the film samples and the standard (L^* = 95.9, a^* = −0.09, and b^* = 0.1).

2.9. Biodegradability. In the current study, the method by Dalev et al.[12] was used with a few modifications. The evaluation of GFO-loaded CEO film biodegradability was conducted by employing vegetable compost. Plastic cups were utilized, and vegetable compost was deposited to a depth of roughly 40 mm. Each sample, measuring 20 mm × 20 mm, was weighed and then buried around 20 mm deep within the compost. The plastic cups were maintained at room temperature, with moisture levels upheld through daily water spraying. The retrieval of samples from the compost occurred after 10 days. Subsequently, the samples were meticulously retrieved, subjected to drying within a vacuum oven at 50 °C, and then weighed using the subsequent formula.

The degradation of film samples in seawater was assessed by weighing and submerging the film segments measuring 2 × 2 cm^2 in labeled conical flasks containing 30 mL of seawater. These flasks were subsequently placed under ambient room temperature conditions. The film strips, once submerged, were periodically examined for signs of degradation with a 5 day interval between observations.

$$\text{Biodegradability}(\%) = \frac{W2 - W1}{W1} \times 100 \qquad (5)$$

2.10. Morphological Properties. The images were acquired by utilizing a scanning electron microscope (JSM6510LA, Jeol, Japan) set to a voltage of 20 kV. Sample preparation involved securing the films onto aluminum stubs using double-sided adhesive tape. Following this, a fine layer of gold was sputter-coated onto the films, enhancing the surface visibility during the imaging procedure.

2.11. X-ray Diffraction. The X-ray diffraction (XRD) analysis was carried out using a Bruker D8 Discover instrument. The instrument was operated at 40 kV and measured the 2-theta (2θ) angle in the range of 5 to 55°. Data collection occurred at a scan rate of 0.500 s per data point. The experiment utilized copper ($K\alpha$) radiation with a wavelength of 1.5418 Å.

2.12. FTIR. To assess the chemical interactions within the fabricated films, FTIR spectrometry analysis was performed. The FTIR spectra of the films were captured employing an FTIR spectrometer (InfraRed Bruker Tensor 37, Ettlingen, Germany), spanning a wavenumber range of 4000−400 cm^{-1}.

The experimental procedure entailed data collection at a resolution of 4 cm^{-1}.

2.13. Thermal Resistance. Thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC) and differential thermogravimetric analysis (DTG) was executed using a TA Instruments SDTQ600 thermal analyzer from New Castle, DE, USA. Each measurement involved encapsulating 5−10 mg of the CEO film within sealed aluminum capsules, which were then placed in the equipment. The film underwent temperature variation from 25 to 600 °C, with a gradual heating rate of 10 °C/min, all while being enveloped by a nitrogen-rich atmosphere. Calibration was achieved using zinc as the metallic standard.

2.14. Antioxidant Properties. As per the approach outlined by Re et al.,[13] the assessment of the ABTS cation radical scavenging capacity of films was conducted with certain adaptations. The absorbance was measured at 734 nm over a duration of 6 min. The execution of this procedure involved subjecting a vortexed solution (for 30 s) comprising the film (30 mg) and 1.9 mL of a 7 mmol/L ABTS radical solution was prepared utilizing a potassium persulfate solution (2.45 mM).

The evaluation of DPPH free radical scavenging activity adhered to the procedure detailed by Brand-Williams et al.[14] Initially, 150 mg of the film sample was mixed via vortexing with 1.95 mL of DPPH solution for 30 s. Subsequently, the mixture was subjected to a 30 min incubation period within a dark room environment. The mean of three measurements was calculated for both the blank and film sample absorbance at 517 nm. The obtained outcomes were represented as the percentage of absorbance inhibition. This entire process was repeated three times for each test. The computation of the percentage inhibition was executed utilizing the following formula:

$$\%\text{inhibition} = (\text{Ac} - \text{At})/(\text{Ac}) \times 100 \qquad (6)$$

where Ac = absorbance of the blank solution and At = absorbance of the test solution.

2.15. Statistical Analysis. To conduct statistical analysis, the mean values, accompanied by their corresponding standard errors, were extracted from three independent replicates. One-way analysis of variance (ANOVA) was executed to ascertain the significance of variations among the mean values. Following the ANOVA, Duncan's test was applied for post hoc analysis, aimed at pinpointing specific pairwise distinctions. This post hoc test was conducted with a significance level established at 5% and a confidence level of 95%.

3. RESULTS AND DISCUSSION

3.1. Visual Assessment. Carrageenan-based film samples loaded with GFO, namely, CEO2, CEO3, and CEO4, were examined visually and compared to the control sample CEO1 (depicted in Figure 1). All film samples displayed a homogeneous and fine structure with no oil droplets visible on the surface. However, the film containing varying amounts of GFO exhibited a minor reduction in transparency as compared to the control. There was a slight change in the color appearance between the GFO-loaded-films and the control and this can be attributed to the higher concentrations of essential oil present in the films. Films incorporated with GFO were easily detached from the Petri dish and exhibited no visible particles or surface cracks. While the overall visual appearance of the film remained unaffected by different GFO concentrations, however, there was a slight difference in color,

Figure 1. Visual analysis of the carrageenan-based film samples loaded with and without GFO.

potentially arising from interactions within the polymer matrix of the film.

3.2. Thickness. Carrageenan-based film samples exhibited thickness values varying from 0.044 to 0.064 mm. This could be due to the increase in the number of solid contents after incorporation of GFO. Films with thickness equivalent to 0.050 mm are usually considered as thin and cohesive films.[15] Previous report suggested that the thickness of the films made up of polymer ideally must be less than ≤0.25 mm.[16] Based on these reports, the present films can be considered as thin films.

3.3. Mechanical Attributes. The TS and EAB of kappa carrageenan with and without GFO are summarized in Table 2. The film without GFO had a TS of 65.20 MPa which is

Table 2. Thickness and Mechanical Properties[a,b]

film samples	thickness (mm)	EAB (%)	TS (MPa)
CEO1	0.044 ± 0.005[a]	11.02 ± 1.03[a]	65.20 ± 4.71[a]
CEO2	0.045 ± 0.006[ab]	10.42 ± 1.30[a]	93.02 ± 6.66[b]
CEO3	0.055 ± 0.006[bc]	5.58 ± 0.54[b]	94.20 ± 7.57[b]
CEO4	0.064 ± 0.005[c]	5.73 ± 1.78[b]	98.21 ± 6.35[b]

[a]Mean values of carrageenan-based film samples (CEO1 blank, CEO2 containing 0.1% of GFO, CEO3 containing 0.2% of GFO, and CEO4 containing 0.3% of GFO). The ± sign means standard deviations. [b]The letters (a, b, c, and d) assigned to distinct values within a given column denote statistically significant differences ($p < 0.05$).

higher than that reported in previous studies (22−32 MPa).[17,18] The incorporation of esssential oil in kappa carrageenan films increased the TS from 65.20 ± 4.71 to 98.21 ± 6.35 MPa; however EABs decreased (11.02 ± 1.03 to 5.73 ± 1.78%) significantly ($p < 0.05$) in a concentration-dependent manner. The kappa carrageenan film without oil exhibited the lowest TS (65.20 MPa) and the highest EAB (11.02%). The increase in TS could be attributed to the presence of low-molecular-weight substances such as aldehyde, ketone, and phenolic components in GFO and their cross-linking effects on the polymeric network to improve the interaction between chains as mentioned in the previous

studies.[19,20] These results are also supported with XRD findings, where incorporation of GFO increased the crystallinity of the films in a concentration-dependent manner.

In accordance with prior findings, the incorporation of *P. maackii* juice rich in polyphenols exhibited a beneficial impact on the TS of films composed of kappa carrageenan and hydroxypropyl methylcellulose. Conversely, the introduction of *L. ruthenicum* extract led to a reduction in both the TS and EAB of kappa carrageenan films. Correspondingly, the supplementation of kappa carrageenan films with satureja hortensis essential oil yielded a comparable outcome of decreased TS and EAB.[18] The reduction in EAB percentages, from 11.02 to 5.73%, is likely attributed to the partial substitution of strong polymer−polymer interactions with weak polymer−oil interactions within the film matrix, in accordance with previous findings.[21] The incorporation of essential oil in the polymeric matrix often provides variable results as it solely depends on the type, proportion of ingredients used, and conditions in which films were prepared.[22]

3.4. Water Permeation. The WVP of composite films with and without EO at different concentrations is presented in Table 3. Previous report showed that blank chitosan showed

Table 3. WVP, MC, and Swelling Index Mean Values of Carrageenan-Based Film Samples (CEO1 Blank, CEO2 Containing 0.1% of GFO, CEO3 Containing 0.2% of GFO, and CEO4 Containing 0.3% of GFO)[a,b]

film samples	WVP ((g·mm)/(m²·h·kPa))	MC (%)	swelling index (%)
CEO1	0.383 ± 0.015a	13.62 ± 0.014a	804.5 ± 32.3a
CEO2	0.397 ± 0.016a	16.24 ± 0.39b	748.4 ± 26.2a
CEO3	0.471 ± 0.005b	16.79 ± 0.48bc	659.1 ± 15.2b
CEO4	0.532 ± 0.015c	17.59 ± 0.55c	635.0 ± 28.5b

[a]The ± sign means standard deviations. [b]The letters (a, b, c, and d) assigned to distinct values within a given column denote statistically significant differences ($p < 0.05$).

WVP 2.18 (g mm/m² h kPa); however composite films without BPEO addition showed a lower value of 0.315 ± 0.001 g mm/m² h kPa. Blending of chitosan with sodium caseinate significantly ($p < 0.05$) reduced the WVP values when compared with chitosan blank as reported in previous study.[23] The WVP measurements obtained in this current study show close similarity to those of synthetic cellophane films (0.248 g mm/kPa h m²), although the values are significantly greater than WVP of LDPE films (0.0072 g mm/kPa h m²).[24]

Comparable findings were reported in prior research studies wherein essential oils were used in polymeric films.[25] As per the previous report, this could be due to the incoherence initiated in the film matrix by oil droplets in causing inconsistency in the film matrix resulting in an increase in water vapor transmission.[25] Another report suggested that interaction between oil components and certain hydrophilic protein regions may cause a drop in the hydrophobicity of the film.[26] Moreover, the ratio of hydrophilic−hydrophobic components of the film also impacts the WVP of the films[27]

It is not always certain that addition of essential oil in the film matrix decreases the WVP values of the films due to its hydrophobic nature because oil incorporation also causes inconsistency in the matrix, thereby impacting the micro-

Table 4. Transparency and Color Analysis Mean Values of Carragenin-Based Film Samples (CEO1 Blank, CEO2 Containing 0.1% of GFO, CEO3 Containing 0.2% of GFO, and CEO4 Containing 0.3% of GFO)[a,b]

film samples	transparency	L	a*	b*	ΔE
CEO1	88.40 ± 7.31a	96.00 ± 0.01a	−0.02 ± 0.01a	0.65 ± 0.04bc	0.56 ± 0.04ab
CEO2	76.13 ± 3.14ab	96.08 ± 0.04a	−0.01 ± 0.00a	0.58 ± 0.04b	0.51 ± 0.03a
CEO3	70.59 ± 2.74bc	96.06 ± 0.05a	−0.01 ± 0.01a	0.71 ± 0.04c	0.63 ± 0.03b
CEO4	67.10 ± 1.56c	96.28 ± 0.02b	0.04 ± 0.01b	0.46 ± 0.01a	0.54 ± 0.02a

[a]The ± sign means standard deviations. [b]The letters (a, b, c, and d) assigned to distinct values within a given column denote statistically significant differences ($p < 0.05$). L: lightness, a^*: green-red color, b^*: blue-yellow color, and ΔE^*: overall color variation.

structure of the film.[28,29] A study was conducted that investigated the impact of varying concentrations of thyme and basil essential oils on the water permeability of chitosan films. Another research revealed a notable increase in water permeation as the essential oil concentrations increased.[25] The authors concluded that the enhanced diffusivity of water molecules could be ascribed to disruption of the structural network of the film, induced by the incorporation of essential oils.[18]

It is widely acknowledged that tortuosity holds a significant role in the process of water vapor transfer, as indicated by prior research.[26] The incorporation of GFO as a hydrophobic dispersed phase into hydrophilic films based on carrageenan leads to an increase in the tortuosity factor. This increase subsequently results in a decrease in the rate of water vapor transmission.

3.5. Moisture Percentage and Swelling Index. Natural MC modifiers or absorbers that can either absorb the moisture or displace the moisture from the polymeric matrix are in huge demand. Kappa carrageenan demonstrated 13.62% MC, which was due to the hydrophilic nature of glycerol and k-carrageenan.[30] Table 3 shows the effect of loading GFO on the MC and swelling index of the carrageenan films. It was found that MC of the films increased from 13.62 to 17.59% with increasing concentration of GFO. A similar behavior was noticed by Lee et al. where a significant rise in the MC of hydroxypropyl methylcellulose films was observed due to the inclusion of oregano EO nanoemulsion.[31] Therefore, it cannot be assumed that an increase in oil content always decreased the MC in the films. Another study suggested that addition of nanoemulsions with oil to the gelatin−chitosan film did not considerably change the MC of the films due to the presence of low oil content.[31] This variation in the results could be attributed to the type, the amount of ingredients used in the films, and the intermolecular interactions between them.[9] However, these outcomes of our work were in contrast with those obtained by Shojaee-Aliabadi et al.[32]

The swelling index values of the prepared films varied from 804.5 to 635.0% due to the hydrophilic nature of κ-carrageenan, and thus absorb more water and swell rapidly.[32] Swelling index of the films decreased significantly from 804.5 to 635% after incorporation of the GFO. This phenomenon could be attributed to the inherent hydrophobic characteristics and cross-linking effect of polyphenolic components present in GFO that also caused an increase in the crystallinity and TS of the films, resulting in a low swelling ratio. A similar behavior was observed for other biodegradable films incorporated with hydrophobic substances.[33,34]

3.6. Transparency and Color Analysis. Optical attributes of food packaging materials must comply with customer requirements. Films with high transparency are more preferred, and it can effectively disclose the present state of the packed

food. Carrageenan films devoid of GFO exhibited clear and translucent attributes. However, films incorporating GFO exhibited a slight yellow color (validated through visual inspection, as depicted in Figure 1), indicated by change in the values of b and delta E (outlined in Table 4). This could be due to the presence of phenolic components of GFO, which could have shown light absorption at low wavelengths as reported earlier.[18] Transparency of films decreased with an increase in the GFO concentration significantly ($P < 0.05$) (Table 4). Films with the highest concentration of GFO showed less transparency probably due to the less-miscible phase formed by the essential oil, increase in the GFO content, and light scattering caused by the oil droplets distributed in the film network.[35,36]

3.7. Biodegradability. The film samples underwent assessment of their biodegradability within a commercial compost-based soil environment and when submerged in seawater conditions (Figure 2). In the compost soil environ-
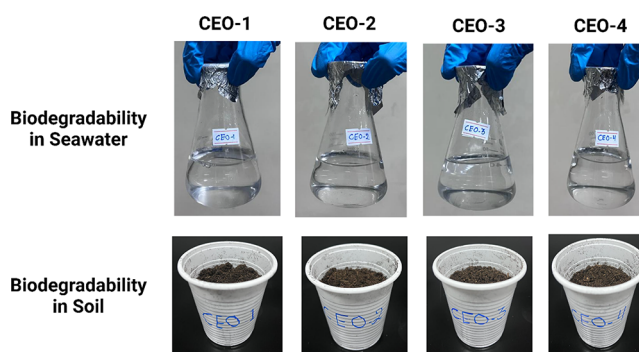

Figure 2. Biodegradability analysis of carrageenan edible films loaded with and without GFO.

ment, the CEO1 film sample exhibited a weight reduction of 25% over a 20 day monitoring period. Conversely, film samples incorporated with GFO such as CEO2, CEO3, and CEO4 experienced weight losses of 22, 23, and 20%, respectively, after the same 20 day period. The observed decline in weight after the 20 day evaluation interval can be attributed to the synergistic effect of microorganisms and the dissolution of soluble compounds originating from the films, facilitated by the presence of water introduced into the soil.[37] Carrageenan-based film samples were subjected to degradation evaluation within the seawater environment. The film strips were immersed in a conical flask containing seawater. It was observed after 30 days that the film strips underwent fragmentation into smaller pieces, thereby exhibiting their biodegradable characteristics. Overall, the findings demonstrated that the films were biodegradable in both soil and seawater environments, indicating their potential as a sustainable alternative to traditional plastics. This observation

aligned with prior reports indicating that films derived from carrageenan exhibit favorable biodegradable properties.[38,39] Furthermore, the biodegradability of a biopolymer-based film can be affected by the chemical structure, molecular weight, and concentration of the biopolymer used as well as the environmental conditions such as temperature, moisture, and microbial activity.

3.8. Morphological Attributes. Figure 3 shows the morphological characteristics, encompassing surface and
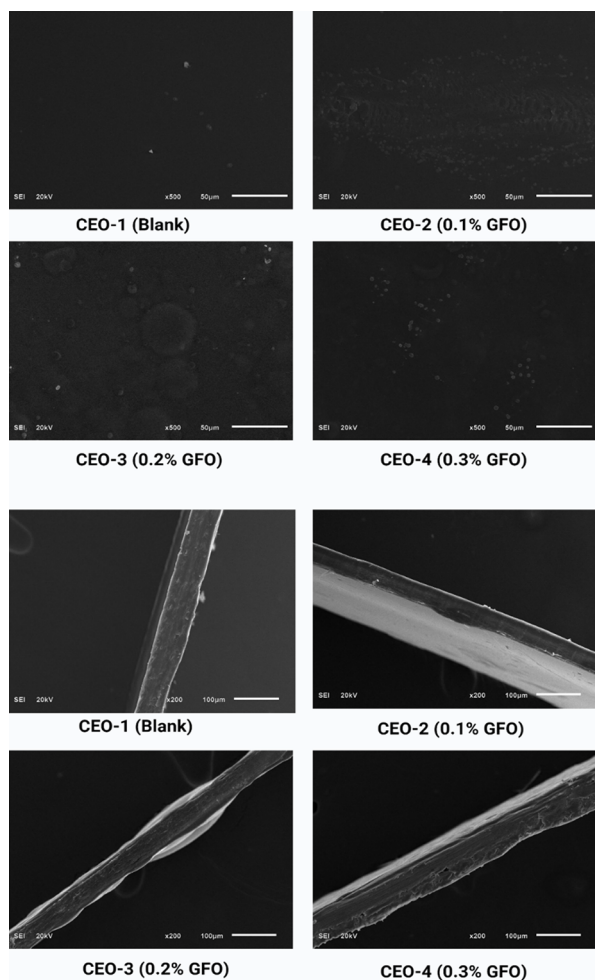

Figure 3. SEM analysis (surface and cross-sectional) of the carrageenan-based film samples loaded with and without GFO.

cross-sectional micrographs, of edible films composed of carrageenan and infused with varying quantities of GFO. The surface characteristics of the developed film samples exhibited a uniform and even microstructural composition. Films containing (GFO) additives at concentrations of 0.1 and 0.2% exhibited relatively uneven textures in comparison to the control film. The CEO1 and CEO3 film samples showed some particles on the surface. However, as shown in Figure 3, increasing the amount of GFO resulted in a more homogeneous and smooth structure, making films more compact. In the CEO3 film surface, the oil droplets are visible, indicating that the GFO was not properly dispersed in the film matrix. In the cross-sectional micrographs, the observed film samples were compact and smooth due to the intermolecular interaction between the polymer and GFO. The CEO3 film sample showed agglomerates and some cracks at different

points on the film surface compared to the other samples.[40] Incorporation of essential oils into films composed of carrageenan caused surface heterogenicity and further concluded that addition of an emulsifier, particularly Tween 20, can reduce the surface heterogenicity and further improve the texture of the films. Furthermore, it can be observed that the heterogenicity in a film sample is inversely proportional to the transparency, as shown in Table 4. Overall, the SEM analysis demonstrated that the intermolecular interaction between carrageenan and GFO resulted in the production of compact films with desirable mechanical properties.

3.9. Crystallinity of the Films. The XRD diffractograms of all samples are shown in Figure 4. Due to the amorphous
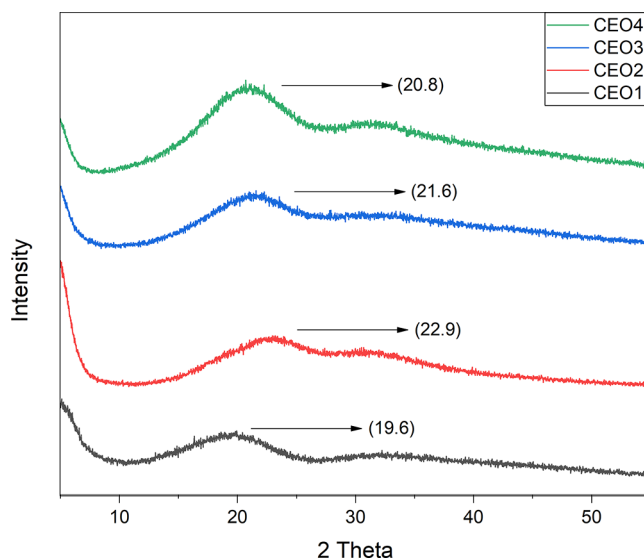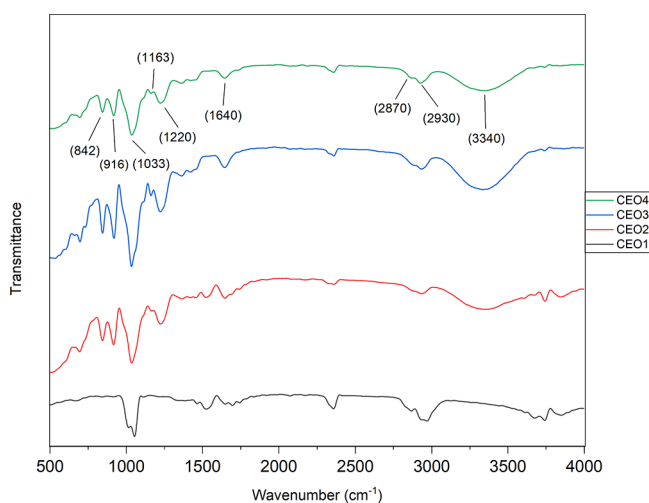

Figure 4. XRD graph of carrageenan-based film samples (CEO1 blank, CEO2 containing 0.1% GFO, CEO3 containing 0.2% GFO, and CEO4 containing 0.3% of GFO).

nature, the kappa carrageenan film did not show any noticeable crystalline peak as reported in the previous study.[41] The XRD patterns of the kappa carrageenan film with and without oil displayed broad diffraction peaks between 19° and 22°. A similar diffraction peak of blank kappa carrageenan film was observed in the previous study.[42] X-ray scattering shows the κ-carrageenan disorder-to-order transition, leading to helices aggregation and amorphous state.[43]

All films demonstrated almost similar diffraction patterns with slight variations in the intensities which could be due to the addition of GFO. Addition of GFO into kappa carrageenan films slightly changed the peak positions in the XRD pattern; however, it caused variations in the peak intensities. The increase in peak intensities after addition of GFO could be due to the increase in new hydrogen bond formation, increase of regular domains, hence increased compactness, and film crystallinity. A similar behavior was observed in the previous studies.[43−45] Overall, the XRD analysis suggested good compatibility among the polymer, plasticizer, and oil. In previous studies, a decrease in crystallinity has also been observed after the addition of oil, where the diffractogram of oil-loaded samples showed shifting and the disappearance of the peaks. This could be due to the plasticization effect of oil, resulting in the loss of original crystalline structure of the polymer.[46−48]

3.10. FTIR. FTIR analysis was carried out to the study the chemical interaction in the polymer matrix). Figure 5 illustrates

Figure 5. FTIR spectrum of carrageenan-based film samples (CEO1 blank, CEO2 containing 0.1% of GFO, CEO3 containing 0.2% of GFO, and CEO4 containing 0.3% of GFO).

the following distinct peaks observed in the spectrum: 842, 916, 1033, 1163, 1220, 1640, 2870, 2930, and 3340 cm^{-1}. A broad absorption peak was observed in the range of 3300–3400 cm^{-1}, which is due to vibrational stretching of the O−H bonds present in the components of CEO. Additionally, the absorbance peak observed at 1640 cm^{-1} confirms the bending of water molecules present in the film matrix. The spectral peaks observed at 2870 and 2930 cm^{-1} are due to the asymmetric and symmetric C−H stretching vibrations of methylene groups present in carrageenan. The sharp spectral peak observed at 916 cm^{-1} is due to C−O stretching in 3,6-anhydro-D-galactose. Carrageenan also shows characteristic peaks for sulfate-associated vibration observed in the FTIR spectra. The peak observed at 842 cm^{-1} represents D-galactose-4-sulfate, and the peak at 1220 cm^{-1} indicates the asymmetric stretching of sulfate ester (O=S=O). The peak observed at 1035 cm^{-1} indicates C−O present in glycosidic linkage, whereas the spectral peak at 1159 cm^{-1} represents asymmetric stretching of C−O−C.[40,43,49,50] Overall, the FTIR spectrum indicates the interaction among the film-forming components.

3.11. Thermal Analysis. The inclusion of GFO in the films caused variation in the thermal stability of the films as presented by TGA, DSC, and DTG thermograms. All kappa carrageenan-based films showed several thermal decomposition regions as reported in previous studies.[51] The initial thermal decomposition phase was found between 37 and 102 °C due to the hydrophilic nature of carrageenan, leading to water evaporation and chemisorbed water via hydrogen bonds. The next thermal decomposition phase was observed between 120 and 230 °C due to glycerol degradation.[52] The third thermal decomposition phase appeared at 240−270 °C (maximum peak in the DTG curve), principally due to the thermal degradation of the carrageenan matrix.[51] A similar behavior has been reported in a previous study where κ-carrageenan and a locust bean gum composite material were studied.[43]

After the third degradation stage, all films confirmed a progressive reduction in weight up to 500 °C. This could be related to further breakdown of the polymer end products produced during the third phase (Figure 6a). A similar behavior was reported in the previous work.[43] The TGA and DTG thermograms showed that addition of GFO increased the final residue remaining at 600 °C as reported in the previous study.[43]

Overall, thermograms (Figure 6a−c) of all films showed that GFO addition delayed the thermal degradation of the films in a concentration-dependent manner. Increase in the thermal stability of the films could be due to the increase in the crystallinity and TS of the films (as confirmed by XRD and mechanical results), after incorporation of GFO. This could be related to the cross-linking effect of polyphenolic components present in GFO resulting in the formation of a compact and dense structure.

DSC was performed to study the miscibility and compatibility between polymers and essential oil. The thermogram of the films is demonstrated in Figure 6b. The glass transition temperature (T_g) of kappa carrageenan was 90 °C and that of composite films are in the range of 92−101 °C. However, the glass transition temperature of the kappa carrageenan reported in the previous study is 138.1 °C.[53] The thermogram of the oil-loaded films shows that the incorporation of GFO affected the T_g of the films. On inclusion of GFO (0.1−0.3 v/v %) into the kappa carrageenan, T_g of the composite films increases with the percentage of oil increased.
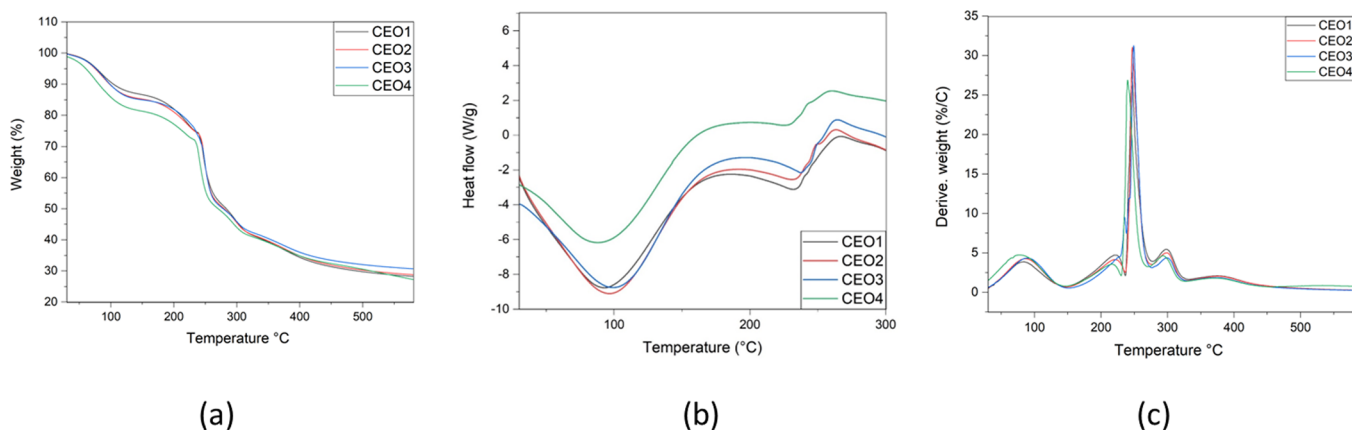
Figure 6. TGA (a), DSC (b), and DTG (c) analyses of carrageenan-based film samples (CEO1 blank, CEO2 containing 0.1% of GFO, CEO3 containing 0.2% of GFO, and CEO4 containing 0.3% of GFO).

This could be related to the cross-linking effect of polyphenolic components present in the GFO that made the material dense, compact, and relatively more crystalline by increasing the hydrogen bonding. This resulted in a reduction in the free volume and therefore a reduction in the mobility of the chains.[54]

3.12. Antioxidant Properties. Oxidative reactions in the food impact the color, flavor, nutritional value, and overall appearance of foodstuff by the development of rancidity. Synthetic antioxidants such as butylated hydroxy anisole and butylated hydroxytoluene often cause toxicity via migration from films to food products. Due to their safe profile, edible nature, and comparable antioxidant and antimicrobial effects, essential oil has been considered as a good replacement to synthetic antioxidants. Several reports evidenced the high use of EOs in the production of active edible films. These natural preservatives contain multicomponent components, and they often act synergistically against free radicals and microorganisms to prevent food spoilage and oxidation.

Like citrus EOs, GEFO mainly contains terpenes and terpene oxides. Terpene oxides mainly contribute to the aroma of the oil, while terpenes contribute less to the aroma. GFO has been reported for antioxidant and antimicrobial effects.[7]

In the present study, the antioxidant potential of the GFO-loaded films were determined using DPPH and ABTS cation radical scavenging assays. Table 5 shows the antioxidant

Table 5. Free Radical Scavenging Activity of Carrageenan-Based Film Samples (CEO1 Blank, CEO2 Containing 0.1% of GFO, CEO3 Containing 0.2% of GFO, and CEO4 Containing 0.3% of GFO)[a,b]

film samples	DPPH (% inhibition)	ABTS (% inhibition)
CEO1	$5.00 \pm 0.73a$	$18.81 \pm 1.23a$
CEO2	$9.46 \pm 0.25b$	$22.21 \pm 0.10b$
CEO3	$10.12 \pm 0.22c$	$26.56 \pm 0.51c$
CEO4	$11.36 \pm 0.46d$	$30.73 \pm 0.73d$

[a]The $\pm$ sign means standard deviations. [b]The letters (a, b, c, and d) assigned to distinct values within a given column denote statistically significant differences ($p < 0.05$).

activities of blank and different GFO-loaded carrageenan films. The control film containing kappa carrageenan showed low antioxidant effects as reported earlier.[18,42] The results demonstrated that the DPPH radical and ABTS cation radical scavenging activities of the samples considerably raised ($P < 0.05$) with the increase in the GFO amount, as presented in Table 5. Gómez-Estaca et al.[55] reported that the antioxidant potential of polymeric films is usually related to the proportion of antioxidant incorporated. The findings obtained from our study are in line with this statement. This showed that GFO (0.3% v/v)-loaded films showed the highest antioxidant activity (11.36 against DPPH radical and 30.73% against ABTS cation radical).

4. CONCLUSIONS

In conclusion, this study demonstrates the promising potential of GFO as a valuable additive for kappa carrageenan-based edible films intended for future food packaging applications. The incorporation of GFO in various concentrations led to notable improvements in the properties of the films, enhancing their TS, compactness, thermal resistance, and antioxidant properties. Additionally, the biodegradability of the developed films in both soil and seawater environments highlights their potential as sustainable alternatives to conventional plastics, aligning with the current shift toward environmentally friendly packaging solutions. Findings of the current study are of immense value not only for further scientific exploration but also for the potential practical application of edible packaging in various industries.

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Notes

The authors declare no competing financial interest.

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