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Green Packaging Solutions for Extending the Shelf Life of Fish Fillet: Development and Evaluation of Cinnamon Essential Oil-Infused Cassava Starch and Fish Gelatin Edible Films

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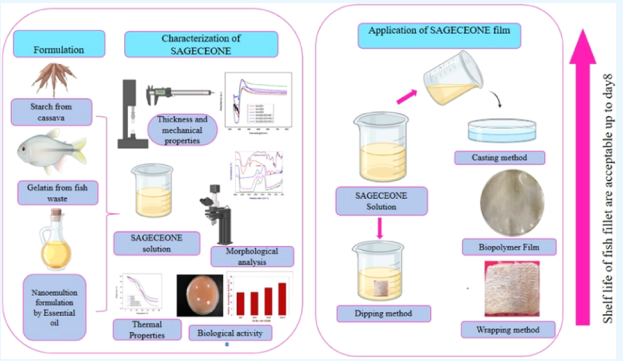
ABSTRACT: Edible, eco-friendly films made from cassava starch, cinnamon essential oil (CEO), and fish gelatin have been shown to extend the shelf life of fish fillets. These biodegradable films offer an environmentally friendly alternative to conventional plastic packaging. This study explores the production of edible films using cassava starch, fish gelatin from processing waste, and CEO nanoemulsion (CEON), focusing on their physical, mechanical, antioxidant, and antibacterial properties. The optimal film blend, consisting of 5% cassava starch and a 1:3 ratio of fish gelatin with 10% CEON (SGCEON3), demonstrated excellent antioxidant and antibacterial properties, extending the fish fillet shelf life to 10 days. These films were light brown with increased thickness (0.19 ± 0.001 mm), tensile strength (20.15 MPa), and elongation at break (270.50%). The TGA analysis showed a consistent mass loss from 30 to 600 °C, and AFM results indicated an average height deviation of 39.925 nm, a roughness of 54.439 nm, a surface symmetry skewness of 0.860, and a kurtosis of 1.77. The FE-SEM images and FTIR spectra confirmed compatibility between fish gelatin and CEON. The migration assay observed a more gradual and constant release of the CEO from the SG films, and the SGCEON3 film is suitable as an antimicrobial packaging material. This study highlights the potential of biopolymer packaging infused with essential oils to extend the shelf life of perishable foods effectively.



1. INTRODUCTION

Advancements in food preservation technology have led to the development of biopolymer bioactive packaging materials. Developers increasingly favor natural biopolymers, waste-derived products, and bioactive plant components. By incorporating nanoemulsified plant essential oils into eco-friendly packaging, improvements in the quality of perishable foods, extension of their shelf life, and enhanced marketability can be achieved. This innovative method guarantees both safety and freshness and represents significant potential for the future of food preservation. They can regulate oxidation, facilitate gas exchange, and manage moisture transfer to prolong shelf life while preserving nutritional value.[1,2] Edible films and coatings containing antioxidants, flavorings, and antibacterial agents are popular for packaging perishable food products. They enhance consumer appeal, simplify supply chain monitoring, and enable long-distance transportation. The growing interest in biodegradable and renewable biopolymer packaging is driven by the need to replace petroleum-based packaging and address environmental concerns. Food engineers are exploring using sustainable biomass to create biodegradable plastic-like materials to reduce the environmental footprint of the plastic industry. Bioplastics are biodegradable and are made from biobased resources, offering a sustainable alternative to nonrenewable petrochemical polymers. Biopolymer packaging from natural compounds replaces synthetic plastics with food-safe films and coatings. Essential oil-derived bioactive compounds with antibacterial and antioxidant properties are commonly incorporated into edible films for food preservation. These compounds have also become significant ingredients in fruit and vegetable coatings.[3,4]

With its semicrystalline structure and 17% amylopectin amylose content, cassava starch is suitable for film formulation. However, its elevated glass transition temperature (189.7 °C) and melting point (256 °C) hinder its processability and thermoformability, resulting in weak and brittle films. In addition to glycerol, other plasticizers, such as propylene glycol, sorbitol, and polyethylene glycol, did not significantly improve strength.[5] Despite these limitations, starch-based films remain

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Table 1. SG and SGCEON Composition

Formulation code	Cassava powder: gelatin	Cassava powder (g)	Gelatin (g)	CEON (%)	Glycerol (%)	Water (ml)
SG1	1:1	5	5	_	10	100
SG2	1:2	5	10	_	10	100
SG3	1:3	5	15	_	10	100
SGCEON1	1:3	5	15	1	10	100
SGCEON2	1:3	5	15	5	10	100
SGCEON3	1:3	5	15	10	10	100

interesting because they are renewable, cost-effective, biodegradable, and edible. High-quality films with improved barrier and mechanical properties can be developed by incorporating plasticizers, emulsifiers, active nanoparticles, antioxidants, and antimicrobial compounds into film-forming solutions.[6] Protein-based films are effective barriers against aromatic compounds, carbon dioxide, and oxygen, but they have limited mechanical strength. Hence, blending gelatin-like proteins with starch can enhance their mechanical properties owing to their good miscibility and cross-linking potential.[7] Gelatin is often used in the food industry as a stabilizer. It can be combined with other hydrocolloids such as hydroxypropyl starches, pectin, acacia (gum arabic), alginate, and pectate esters to improve its properties. Research on blending gelatin with various starches, such as soluble and corn starch, has been well-documented. However, limited information is available about cassava starch films with gelatin. Adding gelatin to a cassava starch film-making solution enhances the properties of the resulting packaging materials and decreases their water solubility.[5] Gelatins derived from animals, such as cowhides and pig hides, are considered true because of their chemical composition, whereas plant-based gelatins, such as seaweed gelatins, are not. Interest in nonmammalian gelatin sources such as fish and insects is increasing due to religious restrictions and concerns about diseases such as bovine spongiform encephalopathy. Fish gelatin, obtained from by-products, such as bones and skin, is a promising alternative to mammalian gelatin. It offers economic benefits by utilizing fish waste and reducing waste in the seafood industry.[8]

Plant-based essential oils are good sources of natural volatile compounds like aldehydes and phenols, which help minimize food spoilage and pathogens.[9] Cinnamon essential oil (CEO) is commonly used in food flavoring, medicine, and cosmetics. Moreover, the CEO has a significant role as a food preservative and can postpone the spoiling of a variety of foods by having a strong inhibitory impact on the majority of foodborne germs.[7] The CEO components, including cinnamaldehyde, eugenol, linalool, and cinnamic acid, are responsible for antibacterial, anti-inflammatory, antidiabetic, antioxidant, and anticancer agents.[10] Several studies have discussed that Tween 20 is a nonionic, low-molecular-weight surfactant to avoid the coalescence of the oil droplets and provide stability. It is also considered as a food-grade emulsifier.[11] Gorjian et al.[12] described that Tween 20 was used as the surfactant in nanoemulsion because it has more stability and is nontoxic, so it was employed in the CEON formulation. Packaging is critical for protecting the quality and extending the shelf life of perishable food goods, particularly in the seafood-fishing industry. Encapsulated CEOs reduce microbial growth and lipid oxidation, thereby extending the shelf life of fish and other foods. Recently, Raj et al.[13] showed that low-energy spontaneously emulsified CEONs could restrict the growth of seafood spoilage microbes, thus extending shelf life. It has been suggested that developing an edible film for packaging fish fillets using gelatin derived from food processing waste, plant essential oils such as CEO, and natural polymers such as cassava starch can enhance preservation and protection and offer additional benefits with consumer acceptance.[10] Hence, the current study aimed to investigate the impact of different compositions of gelatin derived from fish waste with CEON on the edible starch-gelatin biopolymer film packing compatibility properties and their ability to maintain the freshness of fish fillets.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Other Ingredients. Fresh cassava (*Manihot esculenta*) tubers from the local market in Tirunelveli and gelatin extracted from fish processing waste skin obtained from a private seafood processing unit in Tuticorin were used to develop a natural biopolymer bioactive edible film solution. The plasticizers glycerol and polysorbate 20 from Sisco Research Laboratories in Mumbai, CEO from Cyprus Enterprises in Chennai, and double-distilled water (IQ 7003, Milli-Q, Merck KGaA, Germany) were used in all experiments conducted in this study.

2.2. Starch Separation from Cassava Tubers. Fresh cassava tubers weighing 2 kg, with an average length (cm) of 30 ± 7 cm and a width (cm) of 8 ± 3 cm, were procured from a local vegetable market in Tirunelveli. The cassava was peeled and cut into 5 cm pieces. These pieces were soaked in distilled water for 3 days to remove hydrogen cyanide. After fermentation, the pieces were blended and sieved, and the starch was allowed to settle. The starch was dried under sunlight after decanting the liquid and was stored in an airtight container for further use.[14]

2.3. Fish Gelatin Extraction. Waste fish skin from a fish processing plant in Tuticorin was collected. Gelatin was extracted using a hot water hydrolysis technique, as outlined by Hanjabam et al.[15] The process involves cleaning the skin with tap water, cutting it into 5−6 cm pieces, soaking it in a 0.1 M NaOH solution for two hrs, washing it thoroughly, treating it with H_3PO_4, and then washing it again. Gelatin extraction was performed by immersing the treated skin in distilled water at 40−50 °C for 4−12 h, followed by filtration, vacuum drying, and concentration by hot oven drying at 80 °C.

2.4. Starch-Gelatin Film Solution Preparation. Current edible starch-gelatin biopolymer films were fabricated by using a common casting method. First, 100 g of the cassava starch-gelatin biopolymer film-forming solution was prepared. Film-forming solutions were prepared by mixing 5% cassava starch with different concentrations of gelatin (1:1 (SG1), 1:2 (SG2), and 1:3 (SG3)), glycerol (10%), and distilled water to obtain a total volume of 100 ml described in Table 1. Cassava starch was dissolved in distilled water with magnetic stirring at room temperature for 10 min. Gelatin and glycerol were added, and the solution was heated to 80 °C for 30 min with constant

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stirring. The film solution was dried in a 92 cm Petri dish at 50 °C for 24 h.[16]

2.4.1. Preparation and Incorporation of the CEON. The CEON was prepared by combining water, CEO, and Tween 20 in a 1:3 ratio, using a previously described spontaneous emulsification method.[13] 5% CEO and 16.7% Tween 20 were mixed with distilled water for 30 min using a magnetic stirrer at 750 rpm. Water was added at a rate of 4 mL/min while maintaining 50 °C temperature for two hrs. The resulting nanoemulsion underwent stirring at 800 rpm for 60 min. Subsequently, its size, size distribution, surface charge, physical characteristics, and turbidity were assessed to validate its formation. The formulated CEON was incorporated into an appropriately mixed solution of SG3 film formulation, which was selected for further research after the initial screening due to its more appropriate physicochemical properties, which were achieved with a starch-to-gelatin ratio of 1:3. CEON was added to the SG3 film solution at concentrations of 1%, 5%, and 10% (SGCEON1, SGCEON2, and SGCEON3), respectively and mixed for 10 min. The mixture underwent the same procedure as that used to form the CEON-free starch-gelatin biopolymer edible film. The film was analyzed for its thickness, optical properties, water vapor permeability, antioxidant capacity, and mechanical features using the specified method for comparison.[9]

2.4.2. Film Characterization. The uniform color, insoluble gelatin-free particles, and flexible, homogeneous edible films without cracks or weak zones were chosen based on a visual and tactile examination, as previously described.[17] Standard methods also examined their microstructural, physical, and mechanical characteristics. The thickness of the films in five different regions was determined with a digital micrometer (Yuzuki, China).[18] The fabricated films' tensile strength (TS) and elongation percentage were calculated using a Zwick Roell instrument. The behavior of 14 × 4 mm rectangular strips under uniaxial tensile loading at a compression speed of 1 mm/s and a temperature of 21 ± 2 °C was measured by ASTM D 882-12.[19] All film samples' water vapor permeability test was conducted using the ASTM E96 standard method.[20] The cut pieces of SG and SGCEON films were tested using a UV−visible spectrophotometer (UV1650PC; Shimadzu, Tokyo, Japan) in the 200 to 800 nm wavelength range.[21] The SG film SGCEON color was analyzed by a colorimeter (ZE6000, Japan). Each film was cut into 4 × 3 cm^2 and placed inside the cell of the colorimeter, and the lightness (L), red (a), and yellowness (b) of the film were determined.[22] The thermal stability of the films was tested using a simultaneous thermal analyzer (SDT Q600, TA Instruments, USA) with TGA in a nitrogen gas environment from 30 to 600 °C. To remove excess moisture, film samples were heated from room temperature to 120 °C at a rate of 10 °C/min before the experiment. The melting enthalpy (ΔH_m) and glass transition temperature (T_g) were determined.[9] The FTIR spectra of starch-gelatin biopolymer edible films, cassava starch powder, and gelatin functional groups were analyzed in the 4000−400 cm^{-1} range using a TENSOR 37 Spectrophotometer with OPUS 6.0 software.[23]

2.4.3. Morphology Analysis. The SG3 and SGCEON3 film sample's surface topography was analyzed using an MFP-3D atomic force microscope (Asylum Research, Goleta, USA). The data from film samples on an aluminum surface were visualized as a 3D image and analyzed using MFP-3D Origin (Asylum Research, Santa Barbara, CA, USA) software.[18] The surface microstructure of the SGCEON3 film sample was examined by using a SUPRA55VP FESEM (Carl Zeiss, Germany) instrument equipped with an X-MAX (20 mm^2) energy-dispersive system (Oxford Instruments).

2.5. Antioxidant Activity. The antioxidant effects of the SG3 film and CEON incorporated into the SG3 film were analyzed using the DPPH assay (1,1-diphenyl-2-picrylhydrazyl) as described by Rinaldi et al.[24] The film samples (SG3, SGCEON1, SGCEON2, and SGCEON3) were dissolved in a methanol solution of DPPH. Methanol with a DPPH reagent was used as a control. The film mixture was vortexed at 2500 rpm for 1 min and incubated in a dark room for 30 min, and then, its absorbance was measured at 517 nm. The percentage of free radical scavenging is determined using the following equation:

Free radical (%) = ((Abs control − Abs sample)/(Abs control)) × 100 control))100

2.6. Antibacterial Activity. The SG and SGCEON film solutions' antibacterial activity against the spoilage-causing organism *Serratia rubidaea* (MT039483) was determined using the Kirby−Bauer disk diffusion assay. The filter paper was cut into small 6 mm pieces, sterilized, saturated in SG and SGCEON film solutions, dried for 5 min, positioned appropriately on the inoculated plate, and incubated at 37 °C for 24 h.[25]

2.7. Releasing Properties of CEO in Food Materials. The migration testing was used to ascertain the candidate SGCEON3 film CEO release into the food matrix as described by.[26] In brief, the SGCEON3 film was cut into equal sizes (20 mm × 20 mm) and placed in 30 mL of a 95% ethanol solution vial, which was kept in the dark at two different temperatures (4 and 37 °C) for 3 days while being shaken twice a day. A 1 mL solution was taken to analyze the CEO release at different time intervals using UV Spectrophotometry at 280 nm. Each measurement was carried out three times.

2.8. Effect of SGCEON on Fish Fillet Storage. *Lethrinus* sp. fish was sourced from the Inigo Nagar fish landing site in Thoothukudi and transported to the laboratory on ice in under 30 min. Twenty-two fish with an average weight of 250 ± 14 g was divided into three groups. The first group served as the untreated control. The second group was treated with a bioactive cassava starch-fish gelatin-based edible film solution for 1 min and then refrigerated at 4 °C for 30 min. The third group was wrapped in an edible film of cassava starch and fish gelatin. All groups were individually packed in ziplock bags and chilled with crushed ice in a styrofoam box. To reduce temperature fluctuations, the box was placed in a chiller at 4 °C. The sensory characteristics and microbiological and biochemical parameters of the control dipped and wrapped fish fillets were studied for up to 10 days using an alternate-day sampling technique, i.e., 0, 2, 4, 6, 8, and 10 days.[27]

The freshness changes of the stored fish were assessed by measuring the total volatile base nitrogen (TVBN) content using the EC method (1995) with a Kjeldahl distillation assembly (Kelplus Pelican Equipment), as outlined by Vyncke.[28] Fat degradation products in stored fish were assessed using the thiobarbituric acid reactive substances test, as described.[29] The pH of the fish fillet was analyzed according to the AOAC 981 (2016) standard protocol. The AOAC 950.46 method was followed to determine the moisture content. Microbial enumeration was performed following the ISO 4833-1:2013 method, and the number of aerobic organisms capable of forming colonies on solid media at 30 °C was reported as colony-forming units/g.

2.9. Sensory Evaluation of Stored Fish Fillets. Six trained members (4 males and two females) from the

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Thoothukudi Fisheries College and Research Institute, Department of Fish Quality Assurance and Management, conducted a sensory evaluation using the quality index method (QIM) on days 0, 2, 4, 6, 8, and 10 to assess the changes in the outer appearance attributes of the fish fillets stored under different treatments. The sensory evaluation used a 0−3-point scale: 0 for unacceptable, 1 for moderate, 2 for high, and 3 for exceptional acceptability. Characteristics such as texture (firmness and softness), odor (sour and off-odor), color (grayish, yellow, red, and milky), and gaping were assessed.[30]

3. RESULTS AND DISCUSSION

This study investigated the production of bioactive edible packaging using natural polymers, specifically 5% cassava starch and varying concentrations of fish gelatin, along with different amounts of cinnamon essential oil nanoemulsions (CEONs). The casting method has produced sustainable storage packaging for perishable foods such as fish fillets. The brittle, flexible, and less strong cassava starch films, as revealed by the preliminary studies, were overcome with gelatin, which improved the film's physical, mechanical, stability, and water vapor permeability, as observed.[5] The formulated cassava starch-fish gelatin edible films were visually homogeneous and could be easily peeled off from the Petri dish. Initially, the edible cassava starch film solution was clear. When gelatin was added, the color slightly changed to a more yellowish hue as the concentration increased. The resulting cassava starch-gelatin films appeared light brown. This study further examined the various properties of the film, including its physicochemical, mechanical, structural, morphological, and biological characteristics, to determine the most effective combination of natural polymers and nanoemulsions for preserving fish fillets. The formulated SG film's thickness (mm) ranged from 0.007 to 0.13 (SG1:0.07 ± 0.001, SG2:0.1 ± 0.002, and SG3:0.15 ± 0.002). The SGCEON film's thicknesses (mm) ranged from 0.17 to 0.19 (SGCEON1:0.17 ± 0.001; SGCEON2:0.18 ± 0.001; SGCEON3:0.19 ± 0.001). The addition of gelatin significantly increased the film thickness in a concentration-dependent manner. The incorporation of CEON did not significantly affect the film thickness. The thickness of the packaging materials is critical for film formation because it affects the strength, water vapor permeability, clarity, and gas transmission rate.[31] Generally, edible films with thicknesses ranging from 0.159 to 0.384 mm are utilized as packaging materials.[27] The Indian Government recommends a minimum plastic bag thickness of 0.050 mm. The current SG films with a 0.15 mm thickness were found to be suitable for use as carry bags. The thickness of the films increased with an increase in the gelatin concentration.[32] Sancakli et al.[33] revealed the correlation between increased protein concentration and glycerol content with film thickness. Previously, Marichelvam et al.[34] reported preparing bioplastic packaging materials using corn and rice starch with a thickness of 0.25 mm (250 μm).

The SG and SGCEON films exhibited improved mechanical properties, with the addition of CEON significantly increasing the TS and elongation. The TS values of the SG films were as follows: SG1:4.79 MPa, SG2:6.59 MPa, and SG3:6.97 MPa, with corresponding elongations at break (% E) of 150.35%, 258.49%, and 235.5%, respectively. The inclusion of CEON enhanced the TS of SGCEON films (SGCEON1:7.98 MPa, SGCEON2:14.35 MPa, and SGCEON3:20.15 MPa), with % E values of 223.74, 442.324, and 270.50, respectively. This increase in the TS is essential for the durability and resistance of the film to external forces. Acosta et al.[35] described that cassava starch with 50% gelatin films showed increased hardness and good stretchability, which are the requisite food packing or coating properties. CEON may account for the slightly higher spectra of the SGCEON films. Oliveira Filho et al.[23] reported that incorporating nanoemulsions into films is more effective than incorporating microemulsions. A nanoemulsion with smaller droplets increases the surface area interaction with the matrix, whereas larger droplets decrease the film cohesiveness. A higher gelatin concentration in the film enhanced its resistance to external forces and prevented rupture. Tongdeesoontorn et al.[5] reported that higher strength properties of cassava starch-based films were achieved by increasing gelatin concentration. The increase in gelatin and cassava starch concentrations in the film enhances the TS by promoting the formation of intermolecular hydrogen bonds between the OH groups. Additionally, the study found that incorporating CEON into SG3 films increased the TS as the concentration increased, similar to the observations made by Bhatia et al.[36] on kappa carrageenan films with grapefruit EO.

The WVP results showed that adding CEON to the SG films lowered the water solubility in the SGCEON films compared with that in the SG films. The WVP values for the SG films were as follows: SG1, 0.053 ± 0.005 g/m^2; SG2, 0.13 ± 0.001 g/m^2; SG3, 0.15 ± 0.007 g/m^2; and SGCEON films, SGCEON1, 0.22 ± 0.080 g/m^2; SGCEON2, 0.25 ± 0.002 g/m^2; and SGCEON3, 0.29 ± 0.001 g/m^2. Loo and Sarbon[37] found that a film with chicken skin gelatin and 5% tropical starch had low water vapor permeability. As the content of tropical starch gradually increased, the permeability value also increased. Since food packaging is often used to prevent or reduce moisture migration between food and the packaging environment or between two components within a heterogeneous food product, the WVP of the edible films should be as low as possible.[38] Pelissari et al.[39] reported that adding essential oil to the film will decrease water absorption because of hydrophobicity. Similarly, the water vapor permeability will reduce when the hydrophobic compound percentage rises due to water vapor transfer taking place through the hydrophilic section of the film. Thus, the result of WVP is dependent on the ratio of hydrophilic to hydrophobic components in the film. Lee et al.[40] described that the formation of hydrogen bonds between zinc oxide, tapioca starch, and chicken skin gelatin was the cause of the bio-nanocomposite film's decreased WVP. The film matrix's compact can reduce interchain gaps. Water from the surroundings diffused through the bio-nanocomposite film less because of its compact structure. However, it was shown that WVP dramatically increased at a 5% zinc oxide concentration. Similarly, the results revealed that the addition of CEON concentration infused with cassava starch and gelatin film's water vapor permeability was gradually increased.

Incorporating hydrophobic lipids into protein-based films can improve water vapor permeability, prevent dryness and brittleness, and offer protection against microbes. UV radiation can cause food product deterioration, highlighting the significance of the UV barrier properties in packaging materials. The UV absorption ranges observed for the SG and SGCEON films (300−350 nm) were not significantly different, indicating good transparency to visible light (Figure 1). This research is consistent with previous studies that have also reported similar findings.[41−44] Figure 2 illustrates the lightness (L) and redness (a) color index; results showed that the high concentration of CEON in the film decreased, respectively. However, the addition of CEON resulted in SG films significantly increasing

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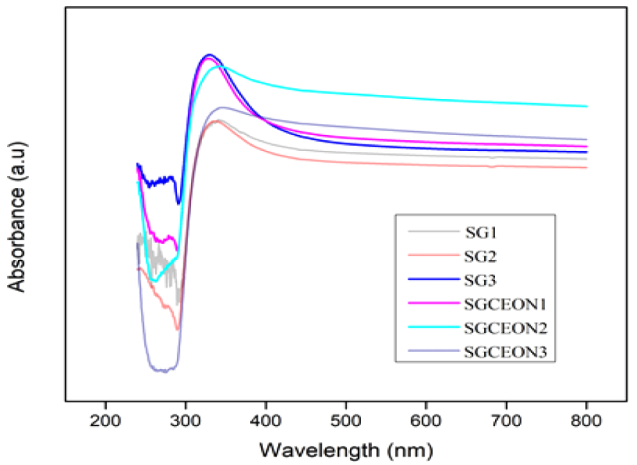

Figure 1. UV−vis spectra of cassava starch films with varying concentrations of fish gelatin (SG) and SG films with different levels of CEON.

yellowness (b). Therefore, the film sample's observed light transmittance increased with increases in CEON. This result implies that the concentration of CEON can affect the film's color and light transmittance. The color may assess the packaging material from oxidative spoilage due to light and UV radiation, which can cause discoloration, flavor off, and nutritional loss.[45]

The TGA analysis of the SG and SGCEON films showed consistent mass loss behavior between 30 and 600 °C, with three distinct stages of deterioration observed for each film. The first stage, occurring at approximately 100 °C, may be due to the evaporation of free water and volatile components. During the next stage, which occurs between 150 and 260 °C, the volatile and nonvolatile compounds of the polymer matrix might undergo decomposition. During the final stage (260−320 °C), cassava starch and gelatin macromolecules may undergo degradation. Castro et al.[46] reported that the degradation process most contributed to the interaction between the carbonyl groups of starch and the amino groups of gelatins. Increasing the gelatin concentration improved the thermal stability, as observed for the SG2 and SG3 films. However, SG1 exhibited a slightly weaker thermal stability. Overall, TGA results indicated that higher concentrations of gelatin and CEON improved the stability of the edible film (Figure 3). The observed physical characteristics of gelatin and essential oil treatments align with those reported in previous studies.[47]

FTIR analysis identified functional groups in the cassava, gelatin, and SG composite films. Cassava starch exhibited characteristic peaks at 3394 cm^{-1} for the OH group stretching vibrations and at 2935 cm^{-1} for the C−H stretching vibrations.

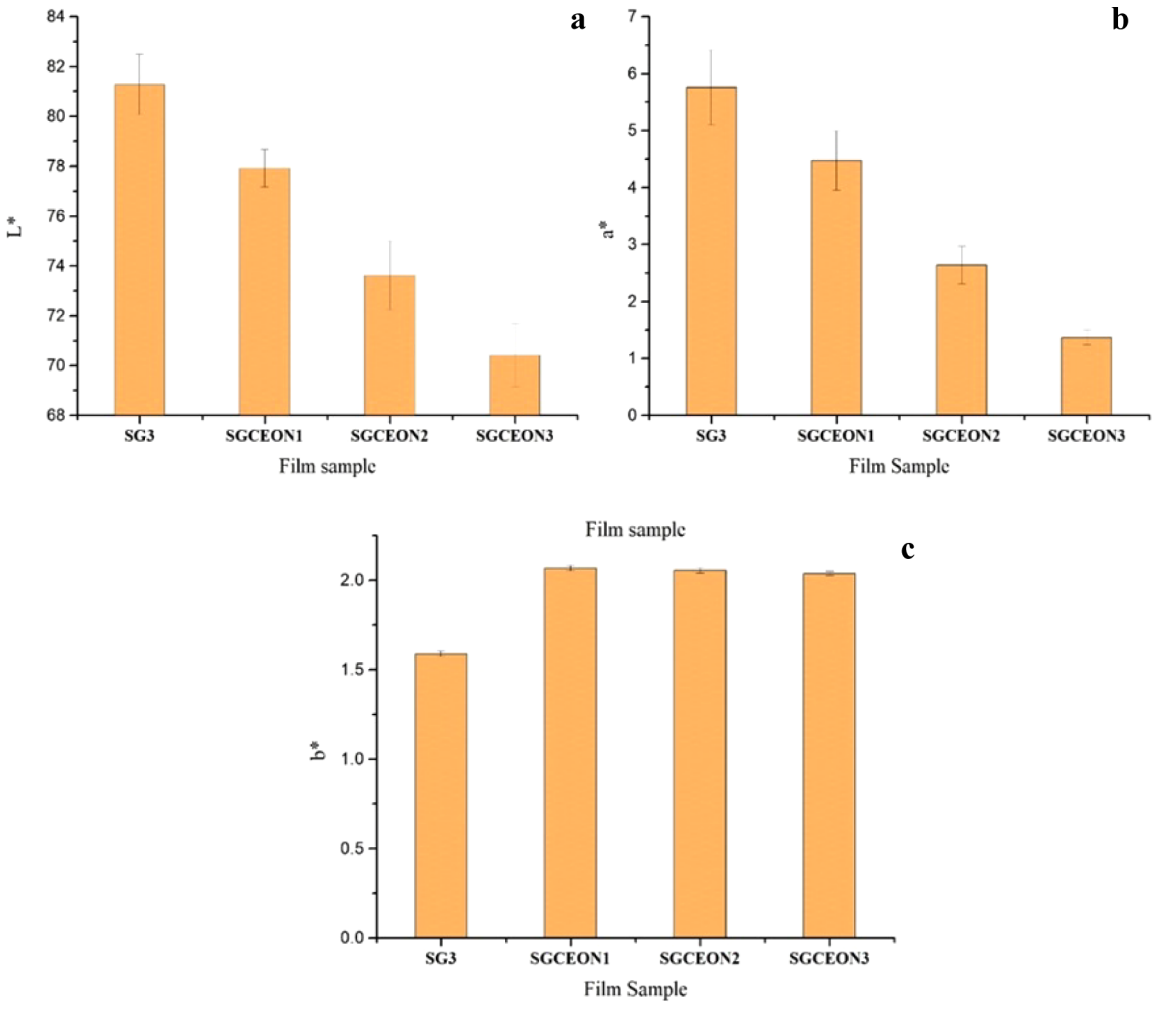

Figure 2. SG3 and SGCEON films color index: (a) L *light, (b) a *redness, and (c) b *yellowness.

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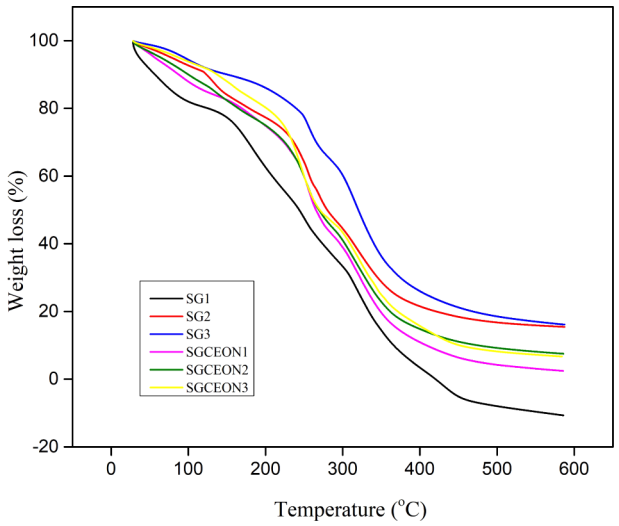

Figure 3. SG and SGCEON TGA thermal analysis curves showing the key events for fish gelatin and the CEON.

With increasing gelatin content, the C−H groups decreased and the broad peak at 2136 cm^{-1} indicated C═C stretching vibrations in the composite films (Figure 3 and 4). The stretching vibrations of the C−O−C and C−O groups were revealed by the 1175 and 1027 cm^{-1} peaks. The decreased C−O−C bands in the starch were due to increased gelatinization. The bands in the range of 1630−1660 cm^{-1} correspond to C═O stretching (amide I), with a peak at 1645 cm^{-1} indicating the presence of the C═O carbonyl group. The OH- groups of cassava starch and gelatin shifted to 3394, 3432, 3666, 3617, and 3653 cm^{-1}, respectively. The amino group of gelatins shifted from 1645, 1651, 1713, 1690, and 1722 cm^{-1} with an increasing gelatin content. The shift in the amide I band with increasing protein (gelatin) content suggests a significant interaction between the starch and gelatin chains, likely due to increased hydrogen bonding. The new peaks were observed at 1082 and 1619 due to the carboxyl and amine groups in SGCEON3. These same peaks were identified in CEON at 1632 and 1084, previously described by Raj et al.[13] Thus, the results confirm the infusion of CEON into the film matrix. Wang et al.[48] demonstrated the presence of weak hydrogen-bonding interactions between starch and gelatin in maize starches incorporated into gelatin films using FTIR spectra (Figure 4).

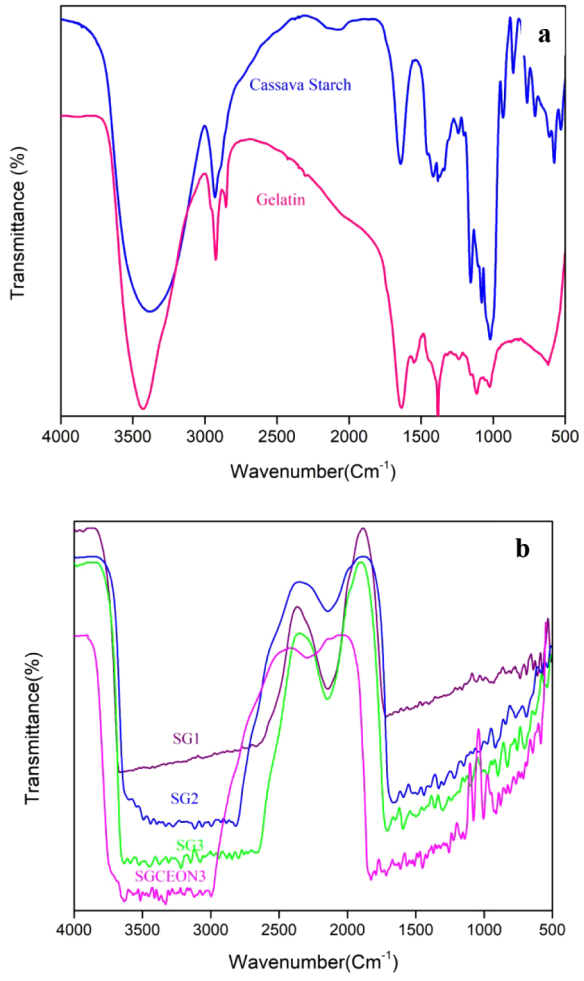

Figure 4. Fourier transform infrared spectra of (a) gelatin and cassava starch and (b) SG1, SG2, SG3, and SGCEON3 films.

Figure 5 shows the morphology and roughness of the SG3 and SGCEON3 edible films determined using AFM. The incorporation of CEON amplified the surface roughness compared with that of the control film, with evenly distributed spherical droplets in a bread-like structure within a continuous biopolymer matrix. FESEM analysis also showed similar results for the SGCEON3 film, as shown in Figure 6. Previous studies by Akbas et al.[49] and Flores-Martinez et al.[50] also revealed surface morphology and roughness changes when essential oils were incorporated into edible films. The AFM results for the SG3 film with CEON showed an average height deviation (R_a) of 39.925 nm, an average deviation (R_q) of 54.439 nm, a skewness (R_{sk}) of 0.86, and a kurtosis (R_{ku}) of 1.77.

In contrast, the average R_a, R_q, R_{sk}, and R_{ku} of the SG3 film without CEON were 22.921 nm, 84.355 nm, −0.00643, and −0.000404, respectively. Hosseini et al.[51] also observed droplets on biobased films containing essential oils, leading to matrix irregularities. FESEM analysis also showed similar results for the SGCEON3 film, as shown in Figure 6. The SGCEON3 film exhibited small oil droplets changing like an uneven microstructure or cavity-like structures on the surface area. Similar results were observed by Frank et al.[52] who, using alginate film incorporated CEON, found that increasing the concentration of oil droplets in the film shows large pores or holes and forms a heterogeneous matrix between alginate and lipophilic compounds, which may reduce the chain−chain interaction.

Figure 7 illustrates the antioxidant activities of SG3, SGCEON1, SGCEON2, and SGCEON3. Higher concentrations of nanoemulsions, particularly SGCEON3, exhibited more potent antioxidant effects than lower concentrations. Unalan et al.[53] reported that cinnamon oil contains natural antioxidant compounds like phenolics and monoterpenes. The maximum inhibition of the film was 17.65%, and increasing the concentration of CEON (1, 5, and 10%) gradually enhanced the antioxidant effect.

The CEON-free SG film did not inhibit the food-spoilage-causing bacterium *S. rubidaea* (MT039483). Increasing concentrations of CEON (SGCEON1, SGCEON2, and SGCEON3) resulted in the largest inhibition zone for SGCEON3 (21 ± 1.0 mm) on MH agar media compared with SGCEON1 and SGCEON2. Begrem et al.[54] studied 47 *Serratia* sp. from raw seafood and found the compounds responsible for fish muscle degradation that causes spoiling. The antimicrobial agents incorporated into the edible films were

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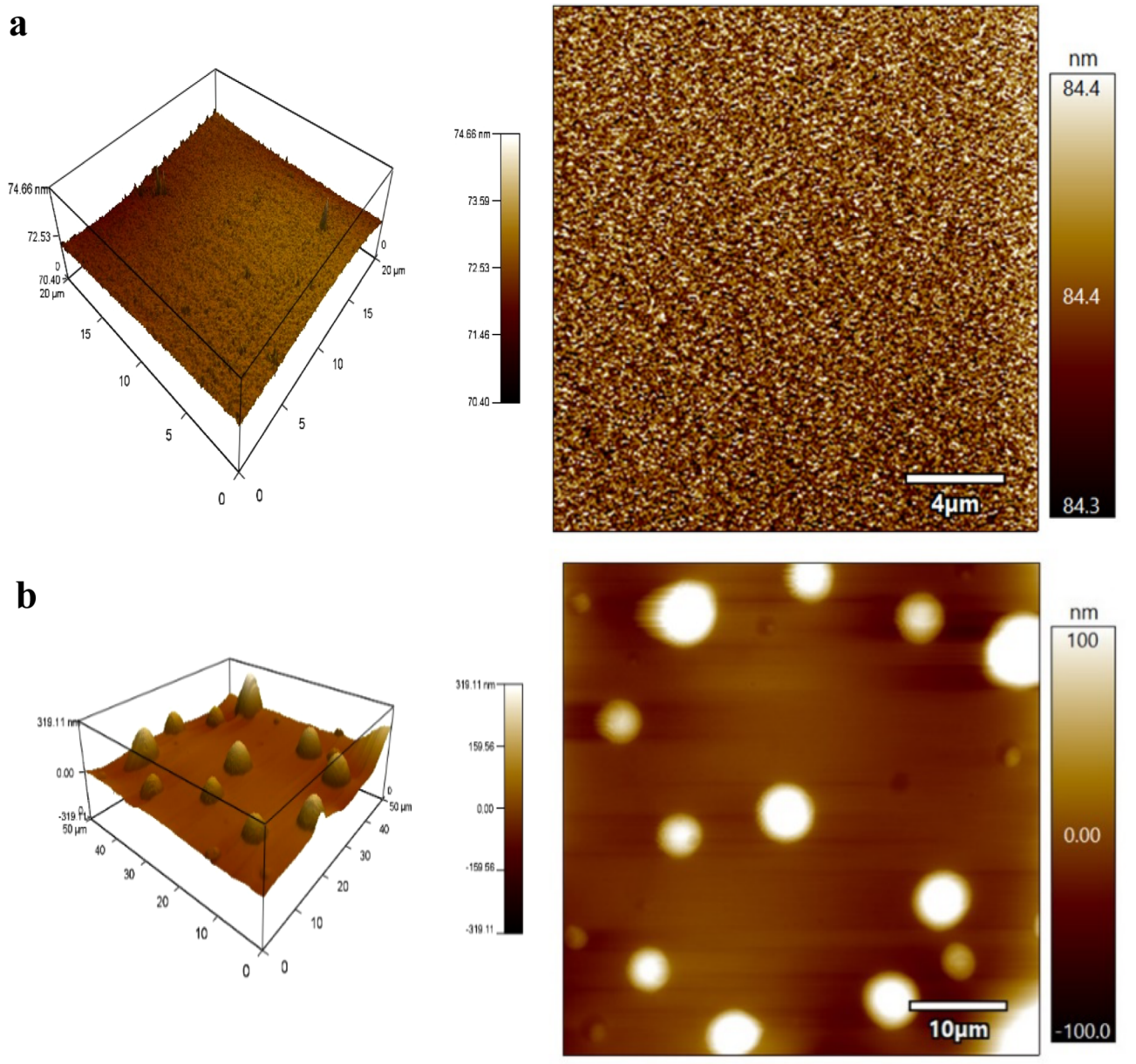

Figure 5. AFM: (a) 2-dimensional and (b) 3-dimensional images of SGCEON3 films.

released sequentially from the biopolymer matrix, thereby ensuring prolonged inhibition of microbial growth.[55] EOs rich in phytochemicals, such as phenolic compounds, can disrupt cell membranes and surface structures. The concentration of oil in the film can reduce sensory attributes while enhancing stability.[56,57] These findings lend support to Lucas-González et al.[41] ideas of using cinnamon bark and leaf oils as antimicrobial agents in food packaging to improve barrier, thermal, and mechanical qualities.[58]

Figure 8 describes the CEON releasing properties of the SGCEON films at differing storage temperatures of 4 and 37 °C. The obtained release curve indicates that the SG film incorporated with the CEO nanoemulsion showed a controlled release of antimicrobial components over time. The results show that the release of CEON from all of the studied SG film samples was continuous and constant and increased with temperature and incubation periods. At 37 °C, during 32 and 72 h of incubation, 26% and 53% of the CEON were released from the SGCEON3 film, respectively. The release was significantly slower at 4 °C compared to 37 °C. The different concentrations of CEON did not have a noticeable effect on the sustained release, suggesting that the sustained release is not directly related to CEON loading. These results suggest that the SGCEON3 film is suitable as an antimicrobial packaging material. Shen et al.[26] described that the free EO's active compounds rapidly transit through the film. Overcoming this issue by encapsulating EO into the film can improve the good compatibility with the film matrix and increase the film activity. Xu et al.[9] observed that the gradual release of CEO enhanced shelf life and antibacterial activity.

The initial TVBN indicator value, representing volatile nitrogenous compounds released during protein breakdown and bacterial decomposition, was 8.69 ± 1.0 mg/100 g. In the control group, fillets' maximum volatile base accumulation rose to 29.12 ± 1.3 mg/100 g on day 8 and 35.24 ± 1.9 mg/100 g on the 10th day. Wrapped and dipped fish fillets in the treated

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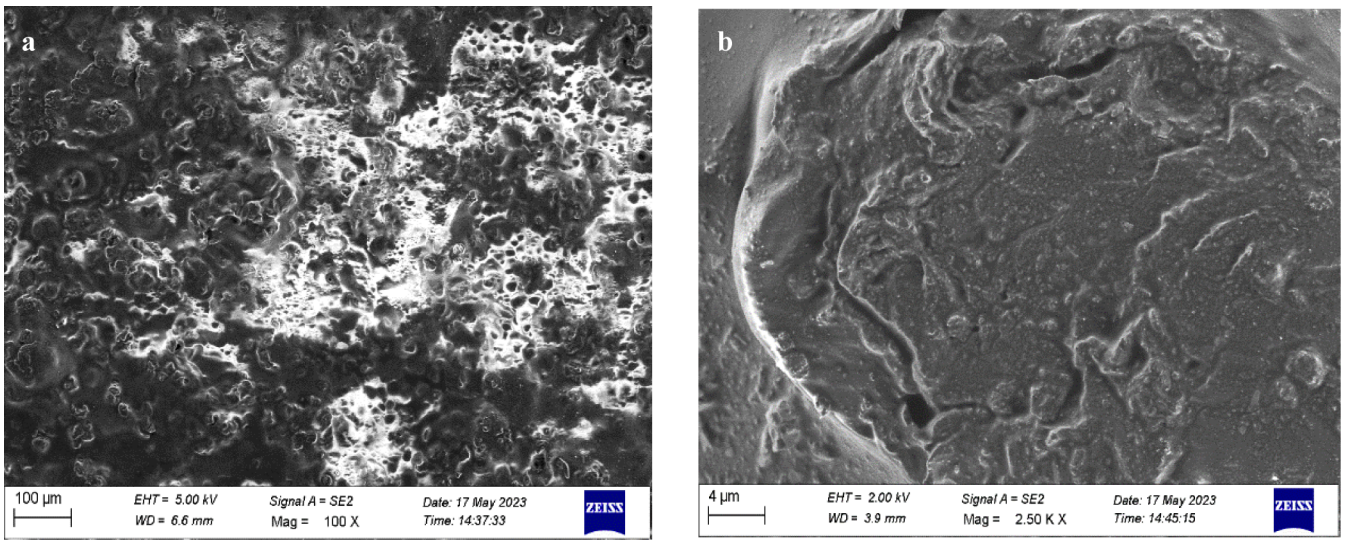

Figure 6. FESEM micrographs of the surface of the CEON3-incorporated SG film (SGCEON3): (a) 100× and (b) 25000×.

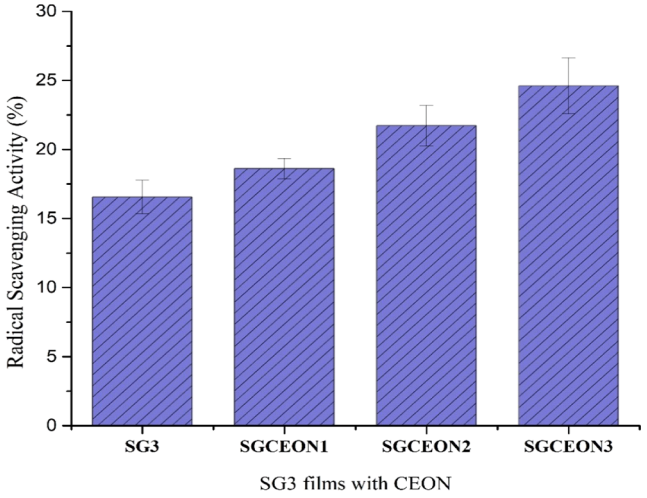

Figure 7. DPPH antioxidant activity of SG3, SGCEON1, SGCEON2, and SGCEON3

samples gradually increased to 29.67 ± 0.9 and 30.73 ± 1.0 mg/100 g, respectively, on day 10 (Table 2). The maximum allowable TVBN level for marine fish species is 30−35 mg TVBN/100 g (CEC, 1995). The SGCEON3 film-coated fillets effectively reduced nitrogen base production caused by microbial contamination until day 10 of chilled storage. Higher TVBN levels indicate endogenous enzyme activity and microbial contamination, which can lead to spoilage during storage.[59] Zhao et al.[60] showed that similar nanocomposite edible films can preserve fish fillets for up to 12 days when stored under chilled conditions. The TBARS assay measures malondialdehyde (MDA) levels in fish fillet samples to assess deterioration during storage, which can affect the odor, flavor, color, and texture.[61] The initial TBARS level on day 0 was 0.03 ± 0.0057 mg MDA/kg. By day 10, the MDA content of the untreated (control) and treated (dipped and wrapped) fillets had increased significantly to 0.3 ± 0.07, 0.1 ± 0.005, and 0.11 ± 0.005 mg MDA/kg, respectively (Table 2). High-antioxidant films are more effective than synthetic alternatives in controlling lipid oxidation and preserving fish products.[62] CEO, rich in polyphenolic and phenolic compounds, can inhibit chemical and sensory changes

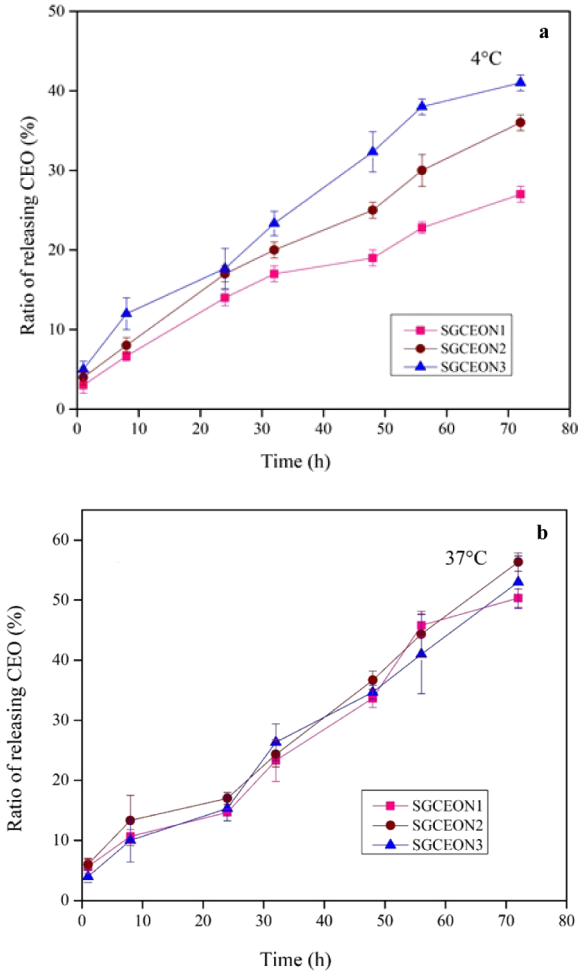

Figure 8. Releasing rate of CEO at different temperatures: (a) 4 °C and (b) 37 °C.

during refrigerated storage.[63] TBA levels were influenced by the coating materials used. Compared to control coatings, polymer coatings can reduce oxygen contact, thereby regulating lipid oxidation and inhibiting ketone and aldehyde production.[15] Coated fish fillets show reduced bacterial populations, indicating

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Table 2. Impact of the SGCEON3 Film Coating on the TVBN, TBA-RS, TPC, pH, and Moisture of the Fillets during Chilled Storage

Days	TVBN (mg/100 g)			TBARS (mg MDA/kg)			TPC (CFU/g)			pH			Moisture (%)		
	C*	D	W	C	D	W	C	D	W	C	D	W	C	D	W
0	8.69 ± 1.0			0.03 ± 0.057			2.3×10^4			6.43 ± 0.057			75.57 ± 0.001		
2	11.55 ± 1.2	7.71 ± 0.5	8.92 ± 1.7	0.04 ± 0.057	0.03 ± 0.057	0.003 ± 0.057	3.3×10^4	1.9×10^4	1.8×10^4	6.43 ± 0.057	6.43 ± 0.057	6.43 ± 0.057	75.20 ± 0.045	74.82 ± 0.041	73.4 ± 0.9
4	17.31 ± 1.9	16.00 ± 2.4	16.4 ± 1.5	0.05 ± 0.057	0.04 ± 0.057	0.04 ± 0.057	4.6×10^5	2.1×10^4	2.7×10^4	6.53 ± 0.057	6.43 ± 0.057	6.43 ± 0.057	76.14 ± 0.037	76.6 ± 0.28	68.71 ± 1.2
6	23.11 ± 2.8	20.5 ± 1.0	21.9 ± 1.9	0.08 ± 0.057	0.05 ± 0.057	0.05 ± 0.057	5.5×10^5	5.2×10^5	4.6×10^5	6.66 ± 0.057	6.46 ± 0.057	6.46 ± 0.057	73.84 ± 0.057	76.52 ± 0.13	60.3 ± 0.22
8	29.12 ± 1.3	25.06 ± 1.5	26.36 ± 1.9	0.5 ± 0.57	0.075 ± 0.057	0.06 ± 0.057	3.7×10^6	1.2×10^5	3.3×10^5	6.76 ± 0.057	6.5 ± 0.057	6.53 ± 0.057	73.76 ± 0.1	73.71 ± 0.10	58.18 ± 2.43
10	35.24 ± 0.9	29.67 ± 0.9	30.73 ± 1.5	0.3 ± 0.47	0.1 ± 0.0057	0.11 ± 0.0057	4.2×10^8	2.2×10^6	1.2×10^6	7.03 ± 0.11	6.7 ± 0.057	6.66 ± 0.057	70.07 ± 1.1	72.66 ± 1.38	50.18 ± 1.0

lower microbial counts and decreased breakdown of substances such as peptides, trimethylamine oxide, and amino acids.[64]

Fresh fish fillets had an initial bacterial count of 2.3 log CFU/g on day 0. In the control group, the bacterial count increased from 3.3 to 7.2 over the storage period. Fish fillets wrapped in starch and protein films containing cinnamon essential oil had bacterial counts of 2.5, 2.9, 3.4, 4.0, 5.7, and 6.0, whereas those dipped in CEO had counts of 2.7, 3.2, 3.8, 4.4, 5.3, and 6.3 in chilled storage (Table 2). Previous research has found that cinnamaldehyde, benzaldehyde, and eugenol are the main volatile compounds in CEO, known for their antimicrobial and antioxidant properties. A study by Ojagh et al.[65] using rainbow trout revealed that cinnamon oil, which contains compounds such as trans-cinnamaldehyde, eugenol, linalool, and other phenolic compounds, inhibited the growth of native flora compared to chitosan coating.

Microbial contamination can produce alkaline compounds, such as trimethylamine (TMA) and ammonia, which can slightly increase the pH. The pH of fresh fish varies depending on the season, species, and catch location, among other factors.[66] In this study, the initial pH on the first day was 6.43 ± 0.05, with the control sample showing a pH range of 6.43 ± 0.05 to 7.03 ± 0.11. The pH of the dipped fillet and wrapped fillet gradually increased from 6.43 ± 0.05 to 6.7 ± 0.05 and from 6.43 ± 0.05 to 6.66 ± 0.05, respectively, over a 10-day storage period. Autolytic protein breakdown and spoilage microbe activity increase pH due to the production of ammonia and trimethylamine. Rasulu et al.[67] showed that adding nanoparticles to chitosan coatings can control lactic acid bacteria and preserve the quality of coconut crab and tuna-smoked fish during storage. The initial moisture content of the fish fillets was 75.57%. Further measurements showed that the moisture content of fish fillets wrapped in edible films ranged from 73.4 to 50.18%, while that of dipped fillets ranged from 74.82 to 72.66%. The moisture content ranged from 75.57 to 70.07% between days 2 and 10. Previous studies have reported that the moisture content ranges from 75.22 to 80.47%. The study found that fish fillets wrapped in edible films lost moisture quickly, whereas dipped fillets maintained stable moisture levels. The control fillets also experienced a gradual decrease in moisture content. Incorporating nanoemulsions into edible films can improve water retention in dipped fish fillets. Studies on edible coatings for fruit storage have shown a reduction in moisture content compared with that of uncoated fruit. Experiments with gelatin-based edible coatings on fish fillets have decreased moisture loss. Adding CEO to gelatin films enhances the antibacterial properties and extends the shelf life of food packaging. The freshness of the three groups of fillets (control, dipping, and wrapping) was assessed from days 2 to 10. Sensory attributes (texture, odor, color, and gaping) were evaluated using the Torry scoring system, ranging from 0 to 6 for borderline freshness 7−9 for acceptable consumption, and >10 for unfit consumption. As shown in Table 3, fish fillets wrapped and dipped in a new edible food package made of cassava starch and fish gelatin with CEON had a shelf life of 8 days compared to the control fillets, which decayed after 6 days. Mezhoudi et al.[68] developed the fish gelatin with *Moringa oleifera* extract coating, increasing the shelf life of fish fillets until 6 days under chill conditions. Bazargani and Pajohi[69] prepared the resveratrol incorporated with sodium alginate-based coating on the trout fillets for 15 days of storage at 4 °C. Zhao et al.[70] found that anthocyanidin plant essential oil compound infused with the chitosan-based film coated on red sea beam fillets was extended until 8 days compared to the

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Table 3. Changes in the Texture, Odor, Color, and Gaping Qualities of the Fish Fillets Were Assessed during SGCEON3 Refrigerated Storage

			Freshness quality parameters change during SGCEON3 treatment					
Parameters	QIM quality index	Score	Sample	Day2	Day4	Day6	Day8	Day10
Texture	Firm and stiff texture, no wateriness	0	Control	0	-	-	-	-
			Dipped	0	0	-	-	-
			Wrapped	0	0	-	-	-
	Slightly soft, initial wateriness	1	Control	-	1	-	-	-
			Dipped		-	-	1	-
			Wrapped		-	-	1	-
	Soft, wateriness noticeable	2	Control	-	-	2	-	-
			Dipped		-	-	-	2
			Wrapped		-	-	-	2
	Very soft and pronounced wateriness	3	Control	-	-	-	3	3
			Dipped		-	-	-	-
			Wrapped		-	-	-	-
Odor	Neutral	0	Control	0	-	-	-	-
			Dipped	0	0	0	-	-
			Wrapped	0	0	0	0	-
	Slightly sour, off-color	1	Control	-	1	1	1	-
			Dipped	-	-	-	1	-
			Wrapped	-	-	-	-	-
	Very sour off odor	2	Control	-	-	-	-	2
			Dipped	-	-	-	-	2
			Wrapped	-	-	-	-	2
Colors	Plain white	0	Control	0	0	-	-	-
			Dipped	0	0	-	-	-
			Wrapped	0	0	-	-	-
	Grayish	1	Control	-	-	1	-	-
			Dipped	-	-	1	1	-
			Wrapped	-	-	1	1	-
	Gray, starting yellow may be slightly red	2	Control	-	-	-	2	-
			Dipped	-	- -	- -	- -	2 2
			Wrapped	-				
	Either yellow or very red, milky surface	3	Control	-	-	-	-	3
			Dipped	-	-	-	-	-
			Wrapped	-	-	-	-	-
Gaping	No gaping, coherent	0	Control	1	-	-	-	-
			Dipped	1	-	-	-	-
			Wrapped	1	-	-	-	-
	Slight gaping but still coherent	1	Control	-	-	-	-	-
			Dipped	-	1	1	-	-
			Wrapped	-	1	1	-	-
	Gaping noticeable, disrupted	2	Control	-	2	2	-	-
			Dipped	-	-	-	2	2
			Wrapped	-	-	-	2	2
	Gaping pronounced, disrupted	3	Control	-	-	-	3	3
			Dipped	-	-	-	-	-
			Wrapped	-	-	-	-	-
Overall score		11	Control	1	4	7	10	11
			Dipped	1	1	4	6	8
			Wrapped	1	1	4	6	8

control fillets. This study explored the potential of using this innovative packaging to extend the shelf life of perishable items, such as fish fillets.

4. CONCLUSIONS

This study used cassava starch and fish gelatin with CEON to develop eco-friendly food packaging to preserve fish fillets. The packaging enhanced the mechanical, physical, thermal, and bioactive qualities, prolonging fish fillets' shelf life under cold conditions. It is also evident that the limitations of pure starch films could be addressed by utilizing fish gelatin and CEON, thereby improving the film performance. Blending cassava starch and fish gelatin, which have good miscibility and cross-linking potential, can enhance bioactivity by incorporating plant essential oil nanoemulsions. This approach improves food packaging performance; however, the limitations of preparation methods, costs, and research gaps revealing film-forming

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intermolecular interactions should be addressed before their use in commercial applications.

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J.S.A.R. contributed to data curation and writing of the draft. M.K. contributed to conceptualization, supervision, and project administration. P.K. contributed to reviewing, validation, and editing. A.H. contributed to reviewing and editing.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

(% E)	elongation percentage
AOAC	Association of Official Analytical Chemists
CEON	cinnamon essential oil nanoemulsion
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	European Commission
F	breaking force
FESEM	field emission scanning electron microscopy
FTIR	Fourier transform infrared
H_3P_4	phosphoric acid
MDA	malondialdehyde
MH agar	Mueller−Hinton Agar
MPa:	megapascals
NaOH	sodium hydroxide
QIM	quality index method
R_a	average height deviation
R_{sk}	skewness
R_{ku}	kurtosis
SG	starch gelatin
SGCEON	starch gelatin cinnamon essential oil nanoemulsion
TBARS	thiobarbituric acid reactive substance
T_g	glass transition temperature
TGA	thermogravimetric analysis
TMAO	trimethylamine oxide
TS	tensile strength
TVBN	total volatile basic nitrogen
ΔH_m	melting enthalpy

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