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Carrageenan-gelatin polyelectrolyte complex film with lemon grass essential oil: Synthesis and characterization for active food packaging applications

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ABSTRACT

Recent developments on packaging innovations have shown growing interest in active packaging films designed to enhance shelf-life and maintain food quality while ensuring biodegradability to address the emerging problem of overproduction and consumption of single-use plastics globally. In this study, active films based on carrageenan-gelatin (CRG-GEL) polyelectrolyte complex (PEC) with the incorporation of lemon grass essential oil (LGEO) through emulsification were synthesized and evaluated. Generally, the addition of LGEO resulted in an improved film thickness, moisture content, water solubility, and hydrophobicity, which, however, decreased the soil biodegradation rate. A decreased tensile strength and increased elongation at break characterize the mechanical properties of the films, which may be caused by the partial replacement of the strong interaction between the polymers with the weaker polymer-oil interaction, as confirmed by the digital micrographs showing discontinuity and heterogeneity in the film surface layers. Moreover, FTIR analysis revealed a narrowing of the spectral bands in the -OH functional group region upon the direct addition of LGEO and through emulsions with increasing concentrations. However, structural changes were not observed in the films upon the LGEO loading at increasing concentration. Meanwhile, the antifungal effect of the sample films was also determined based on the fungal growth proliferation on white bread samples packaged with the sample films of varied treatments and a control group. Overall, the CRG-GEL-PEC films have retarded the fungal growth on the bread surface, and films with higher LGEO concentrations have inhibited mold formation within the 14-day experimentation period.

Keywords: active packaging film, carrageenan, gelatin, lemon grass essential oil, soy lecithin encapsulation

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1. Introduction

The advancements in the field of packaging technology prompt the development of food package designs that are optimal for use as storage and for the protection and preservation of the food product. However, problems arise with the environmental impacts of these packaging materials as they are usually single-use, thrown-away wastes that are largely generated around the world. With this challenge, investigations on the use of "active packaging" films arise where biodegradable alternatives are explored, and bioactive compounds are introduced to infuse properties that can enhance food safety, quality, and shelf life.

Biocomposite materials, based on polyelectrolyte complex (PEC), have been notable for their potential as edible films and as an alternative to the synthetic plastics which are widely used today. Good mechanical properties, such as high strength and flexibility, are among the many properties of an efficient biopolymer film. Without these, biopolymer films will not achieve structural cohesion and will be prone to premature failure and breakage during fabrication, storage, and when used. Proteins and polysaccharides are the most widely utilized biopolymers because they can produce transparent, odorless, and isotropic films.

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Carrageenans are water-soluble, sulfated polysaccharides that are extracted from red seaweeds. Mainly, there are three types of carrageenan: κappa, iota, and lambda. The difference between the three lies in the number of sulfonic groups present in their structures. Whereas κappa has one, iota has two, and lambda has three sulfonic groups. Of these three carrageenan types, the κappa and iota ones form gel while the lambda does not. The film-forming properties of the former are traced to the sulfonic groups in their structure, which facilitates the formation of the films through the self-aggregation of its helical structure [1].

Meanwhile, gelatin is a weak polyampholyte protein that is obtained from the partial hydrolysis of collagen. As elaborated by [2], a mixture of α-chains (single polymer chain), β-chains (two α-chains that are cross-linked covalently), and γ-chains (three α-chains that are covalently cross-linked) comprises the structural composition of gelatin, and its amino composition, on the other hand, is characterized by Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-structure.

The inclusion of essential oils in biopolymer films has garnered significant interest due to their potential antioxidant and antimicrobial properties. These properties may help maintain food quality, ensure food safety, and extend the shelf life of food products. Lemon grass *(Cymbopogon citratus)*, a common flavor enhancer for several dishes, has also turned on the interests of many because of the

antimicrobial property associated with its essential oil. In the study of [3], they have characterized some phytochemicals in the LGEO that are known to be effective antimicrobial compounds, namely geranial, geraniol, and neral.

While loaded essential oils can enhance the quality of the biopolymer films in various ways, its volatile nature, however, creates a drawback as it reduces its effectiveness in dried films. Whereas, some volatile compounds present in it tend to evaporate from the film-forming dispersion during the drying process. It is the goal of this study to provide a way to mitigate the loss of these volatile compounds through the encapsulation of the essential oils using soy lecithin, which could enhance the retention of the volatile compounds during film formation by casting.

Generally, this study aims to synthesize biodegradable, active food packaging films based on carrageenan–gelatin polyelectrolyte complex infused with LGEO and evaluate their physical, mechanical, and degradation properties, assess their ability to inhibit mold growth on white bread samples, and characterize any structural transformations in the biopolymer films.

2. Materials and methods

2.1. Essential oil encapsulation

The emulsion solution was prepared by incorporating 5 mL LGEO through a 15-min continuous stirring, forming a (5% w/v) solution. To facilitate the formation of a stable emulsion, a milk frother was used as an alternative to a sonicator, wherein the LGEO is the dispersed phase and water is the continuous phase. All in all, two emulsions were obtained: one that is a non-loaded emulsion, and the other is loaded with LGEO.

2.2. Film fabrication

In preparing the CRG-GEL PEC solution, carrageenan and gelatin powders were separately dissolved in distilled water and were allowed to swell for 15 min while continuously stirring. Afterward, the temperatures for each solution were then raised to 70°C and 40°C, respectively, before mixing the two solutions, forming a 0.9 (w/v) % carrageenan and 4.0 (w/v) % gelatin solution. At 55°C, two treatments of LGEO were slowly incorporated into the CRG-GEL PEC solution. The first treatment is pure LGEO dissolved in ethanol and mixed with the prepared polymer solution, making 0.05, 0.10, and 0.15% v/v solutions. Meanwhile, the second treatment is the previously prepared emulsions that are used to formulate similar 0.05, 0.10, and 0.15% v/v content solutions. The solutions were kept on continuous stirring for 2 h to ensure a uniformly mixed solution.

After cooling down to 45°C under continuous stirring, the solutions were then cast on non-stick casting plates with a 20 cm diameter. A constant equivalent volume of 40-mL solution was used for each plate. A control group was also prepared for comparative analysis using the same procedure, but was not added with LGEO and the prepared emulsion.

In drying the sample films, the cast solutions were first allowed to sit for 24 h before being subjected to a steam drying process for 1 h and another hour for cooling down before peeling them off in the casting plates. Generated sample films were then individually packed in aluminum foil and stored in zip locks until the time of analysis.

2.3. Physical and mechanical characterization

2.3.1. Film thickness

The thickness of the fabricated films was measured using a micrometer. The measurements were done at three distinct points in the film, and the mean value was reported as the estimated thickness of each film.

2.3.2. Moisture content

The moisture content of the films was evaluated based on the methods described by [4] with slight modification. A uniformly cut films at 3x3 cm were weighed initially before subjecting it to an oven at a 110ºC temperature until a constant weight was achieved and was recorded as the final weight. The calculation of the moisture content was given by:

$$Moisture\ content\ (\%) = \frac{(W_i - W_f)}{W_i} \times 100\%\,, \qquad (1)$$

where W_i = initial weight, and W_f = final weight.

2.3.3. Film solubility

The procedure for determining the solubility of the films in water was adapted from the study of [4]. The film samples were cut into 3x3 cm square sections, and their initial weights were determined. Each film sample was then immersed separately in 100 mL of distilled water for 20 h at room temperature. After the specified time, the undissolved portions of the films were filtered using a filter paper and dried in an oven at 110°C until completely dry. The water solubility percentage was then calculated as follows:

$$Water\ solubility\ (\%) = \frac{(W_i - W_f)}{W_i} \times 100\%\,, \qquad (2)$$

where W_i = initial weight, and W_f = final weight.

2.3.4. Surface hydrophobicity

The surface hydrophobicity of the films was estimated using the sessile drop method, which is based on the optical contact angle (°). The methodology was based on the study of [5] with a few modifications. A 0.5 µL of pure water was carefully dropped using a micropipette onto the air-exposed surface of the films, which had faced upward during the drying process. The images of the drops were taken using a digital microscope, and the optical contact angles were further estimated using the ImageJ free software, based on three replicates for each measurement.

2.3.5. Tensile strength and elongation at break

The tensile strengths (TS) and elongations at break (EAB) of the synthesized films were determined using a Brookfield Texture Analyzer operated based on the methods described by [6] with a few modifications. Measurements were done with a load cell of 10000 g, and the initial grip separation and crosshead speed were set at 30 mm and 1 mm/s, respectively. Three different films were prepared for

each film treatment, and the data obtained were used in the calculation of the TS and EAB. For the tensile strength, the calculation was done using the following equation.

$$TS = \frac{F_{max}}{x \bullet d} \quad (3)$$

where F_{max} = maximum force at break, g, x = width of the film (mm), and d = thickness of the film (mm).

Meanwhile, the elongation at break was determined using the following equation:

$$EAB = \frac{h \bullet t}{l} \quad (4)$$

where h = head speed (1 mm/s), t = time for film extension until break(s), and l = initial length of the film (mm).

2.4. Visual characterization of the film

The surface morphology of the emulsion formed in fabricated films was analyzed using a digital microscope at 1600x magnification.

2.5. Soil burial degradation test

The rate of degradation in soil of the CRG-GEL PEC films was estimated using the method described by [7] with minimal modifications. The initial masses of the previously cut 3x3 cm films were recorded before burying them in sandy loam type of soil. The soil containers were stored in a secluded area for 30 days. After this period, the weights of the dry film samples without soil remnants were measured and used to quantify the films' weight loss using the following equation:

$$Weight\ loss = \frac{w_1 - w_2}{w_1} \times 100\% \quad (5)$$

where W_1 and W_2 are the weights of the films before and after treatment.

2.6. FTIR examination

Fourier transform infrared (FTIR) spectroscopy analysis was used to determine the structural transformation of the fabricated films and the possible interactions of functional groups of the LGEO and the biopolymers. The attenuated total reflectance (ATR) of the Thermo Nicolet Avatar 330 was used to obtain the FTIR spectra of the LGEO, the carrageenan, gelatin, and the fabricated sample films. The percent reflectance scanned at 4 cm^{-1} resolution at a wavelength range of 4000 to 650 cm^{-1} by taking 32 scans per sample was plotted in the generated spectra.

2.7. Fungal growth evaluation on white bread samples

The antifungal property of the fabricated carrageenan-gelatin polyelectrolyte complex films loaded with LGEO and LGEO emulsion was tested on white bread samples. A total of nine bread samples were prepared and packaged using the fabricated films and a control group. One CRG-GEL PEC film without LGEO, three polymer films loaded with LGEO of 0.05, 0.10, and 0.15% v/v concentration, one film with non -loaded emulsion, three polymer films with emulsified LGEO of 0.05, 0.10, and 0.15% v/v LGEO concentration, and one control group are the breakdown of the treatments. Physical observations based on the proliferated fungi on the surface of the bread samples were recorded every day for 14 days.

2.8. Statistical analysis

Statistical tests were performed using the Analysis Toolpak. One-way analysis of variance (ANOVA) was used to characterize the differences between treatment means at a $p \leq 0.05$ level of significance, and the post-hoc analysis used was Tukey's honestly significant difference (HSD) test. The obtained data were reported as mean ± SD (standard deviation).

3. Results and discussion

3.1. Physical properties of the CRG-GEL PEC films

3.1.1. Physical observations

The effects of infusing the LGEO into the carrageenan-gelatin polyelectrolyte complex films through direct loading and emulsification were determined by assessing the properties of the films. These two ways of loading the LGEO characterize the two film treatments prepared in the study – Treatment A is through direct loading, and Treatment B is via emulsification. A total of four films were produced per treatment in one trial with varying concentrations of LGEO (0%, 0.05%, 0.10%, and 0.15% v/v). The fabricated films are shown in Figure 1, and generally, the common properties observed from the films include being firm, transparent, and easy to peel off from the casting plate.

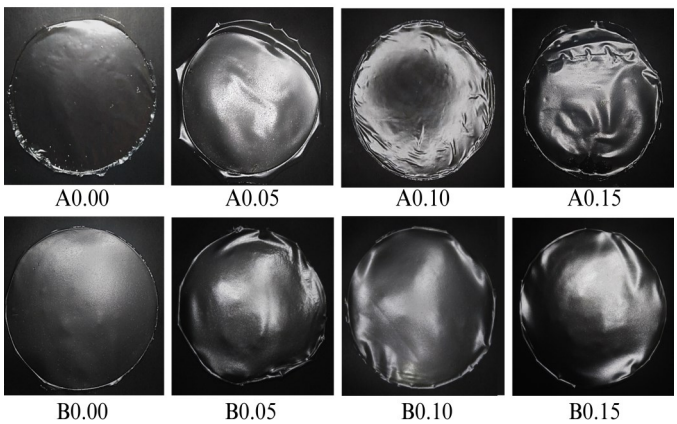

Figure 1. Generated CRG-GEL PEC films prepared using Treatment A (A0.00, A0.05, A0.10, A0.15) and Treatment B (B0.00, B0.05, B0.10, B0.15) with indicated percent concentrations of LGEO.

In terms of the effects of the LGEO addition, for both treatments, the aromatic odor is more prominent for films with higher LGEO (0.10% and 0.15%) concentrations. Moreover, slightly crumpled film products are also observed as the percent concentrations of LGEO are increased for both treatments, especially on the film edges, as a result of its self-detachment starting from the edges during the drying method. This effect is possibly caused by the weakened structure of the polymer networks upon the addition of the hydrophobic essential oil through the interruption and substitution of stronger intermolecular interactions between the biopolymers with weaker interactions of polymer and LGEO [8].

3.1.2. Barrier properties of the films (thickness, water solubility, moisture content, and contact angle)

To test the capacity of the films to act as a barrier, measurements of thickness, moisture content, solubility, and hydrophobicity in terms of water contact angle were conducted, and the corresponding results are summarized in Table 1.

Table 1. Thickness (mm), water solubility (%), moisture content (%), and contact angle (°) of the CRG-GEL PEC films with loaded LGEO and LGEO emulsions

Sample	Thickness (mm)	Water Solubility (%)	Moisture Content (%)	Contact angle (°)
A0.00	0.13 ± 0.02^{a}	68.26 ± 0.99^{a}	24.51 ± 0.80^{a}	62.79 ± 4.07^{a}
A0.05	0.15 ± 0.01^{a}	64.71 ± 0.82^{b}	$22.40 \pm 1.30^{a,b}$	72.36 ± 4.35^{b}
A0.10	0.16 ± 0.02^{a}	63.06 ± 1.97^{b}	$22.77 \pm 0.36^{a,b}$	76.17 ± 1.09^{b}
A0.15	0.21 ± 0.03^{b}	63.73 ± 0.93^{b}	21.44 ± 0.60^{b}	75.11 ± 2.29^{b}
B0.00	0.12 ± 0.03^{A}	70.32 ± 1.55^{A}	23.53 ± 0.70^{A}	55.85 ± 2.64^{A}
B0.05	0.17 ± 0.02^{A}	$67.43 \pm 0.15^{A,B}$	23.18 ± 2.25^{A}	57.14 ± 3.59^{A}
B0.10	0.17 ± 0.02^{A}	63.43 ± 0.31^{B}	24.08 ± 1.21^{A}	59.08 ± 3.57^{A}
B0.15	0.18 ± 0.03^{A}	64.19 ± 2.78^{B}	21.62 ± 0.38^{A}	64.62 ± 3.53^{A}

Note: Different superscript lowercase and uppercase letters within the same column indicate significantly different means of the observed data in Treatment A and Treatment B, respectively, (at $p < 0.05$).

The measured thickness of the synthesized CRG-GEL PEC films varies from 0.12 to 0.21 mm. Generally, the addition of LGEO in both treatments has slightly increased the thickness measurements, but they are found to be not significantly different ($p < 0.05$) except for the A0.15 films as compared to the rest of the films in Treatment A. This observation simply shows the effect of the additional material occupying space in the film matrix. A similar observation was accounted for in the reports of [9,10] upon the addition of varying essential oils to the film-forming solutions.

The solubility and moisture content of active films are two essential parameters for examining their functionality, structural stability, and efficiency, particularly in applications as food packaging or coating. Measurement of film solubility, in particular, can be used in assessing the suitability of the films for packaging applications and predicting the rate of biodegradability and structural durability. In this study, CRG-GEL PEC films exhibited moderate water solubility ranging from 63.06% to 70.36% regardless of the treatment. The general incorporation of LGEO has significantly reduced film solubility due to the hydrophobic nature of the essential oil. Meanwhile, between the different amounts of added LGEO, no significant difference in solubility was observed. A similar phenomenon was achieved by [11] when curcumin extract with LGEO was incorporated into the triple-layered films based on furcellaran/chitosan/gelatin hydrolysates, and explained that such observation is due to the interaction of the functional groups present on the polymer and the hydrophobic nature of the extract with essential oil. In contrast, an increase in solubility was attained by [9] when cinnamon, citronella, pink clove, and thyme essential oils were separately infused into a cassava and gelatin blend film. Such observation was accordingly traced to the establishment of a polymer-oil interaction after the weakening of the interaction between the two polymers.

Moreover, the moisture content of the films is another important measure of the films' performance in terms of withstanding daily life conditions, to determine the type of products it can package, and to assess its initial potential to prevent microbial growth. In this study, a moderate amount of moisture was also found to be contained in the films, with percentages ranging from 21.44 to 24.51. Although there is a common trend of moisture reduction on increasing LGEO concentration, the effect is found to be not statistically significant except when comparing the control films (A0.00) and the films with the highest LGEO percentage (A0.15) of Treatment A. Such a decrease in moisture content was also reported by [12] when starch-based edible films were separately incorporated with increasing concentrations of oregano and black cumin essential oils, proving the influence of the hydrophobic compounds present in the oils on the overall hydrophobic character of the films.

Surface hydrophobicity of the films was also characterized in terms of the optical water contact angles. This is to measure the film's barrier efficiency, compatibility with moist products, and also its potential to prevent microbial growth. Generally, slightly hydrophobic films were produced with average contact angles measured at a 76.17° to 55.85° range. The effect of directly loading LGEO has significantly increased the contact angle, hence also increasing the film hydrophobicity. For LGEO loading through emulsification, the effect is not significantly different. However, an increasing trend in the contact angle measurements is observed upon the increase of the LGEO amount for both treatments. This result affirms the observations of [13] when rosemary essential oil was added to the film matrix of chitosan, sodium caseinate, and the blend film of the two. Overall, these results align with the observed trends in film solubility and moisture content, collectively highlighting the proportional impact of the essential oil's hydrophobic nature.

3.2. Soil burial degradation test

Biodegradation rates of biopolymer films are of important consideration when aiming to seek alternatives to

generally non-biodegradable synthetic plastics. In this study, a good degradation result of 42.63 ± 0.08% was derived for the CRG-GEL PEC films without LGEO. The incorporation of LGEO via direct loading and emulsification resulted in a 3.40% and 6.15% decrease in the rate of degradation and was further found to be statistically significant (at $p < 0.05$). A similar reduction in degradation rate was reported by [14] when pectin films were infused with copaiba oil nano-emulsion, wherein the interference of the said hydrophobic oil on the soil degradation pattern of the film was quantified through a gradual reduction in CO_2 production as the copaiba oil concentration is increased.

Moreover, it is worth emphasizing that the reduction in biodegradation rate aligns with the observed decreases in film thickness, water solubility, and moisture content, alongside increased contact angle following the addition of LGEO. These findings reinforce the hydrophobic nature imparted by LGEO, highlighting its significant influence on the overall physical properties of the films. This correlation underscores the critical role of LGEO in modifying the structural and interactional characteristics of the biopolymer matrix.

3.3. Mechanical properties (TS and EAB)

Several important considerations in assessing the films' level of use as food packaging include strength, durability, and their capacity to withstand external conditions. The primary characterization of these mechanical properties is done mainly by assessing the tensile strength and elongation at break. Tensile strength (TS) quantifies the amount of stress or tension that materials can withstand, while elongations at break (EAB) report the stretchability of the films before breaking. The assessment of the CRG-GEL PEC films based on these parameters is summarized in Table 2.

Table 2. Tensile strength (MPa) and elongation at break (%) of the CRG-GEL PEC films with loaded LGEO and LGEO emulsions

Sample	Tensile Strength (MPa)	Elongation at Break (%)
A0.00	12.94 ± 2.41^{a}	19.42 ± 2.36^{a}
A0.05	11.73 ± 0.93^{a}	4.62 ± 0.72^{b}
A0.10	9.56 ± 2.24^{a}	$22.89 \pm 2.36^{a,c}$
A0.15	9.19 ± 1.59^{a}	27.59 ± 1.54^{c}
B0.00	12.94 ± 2.41^{A}	19.42 ± 2.36^{A}
B0.05	8.23 ± 0.31^{A}	2.71 ± 0.92^{B}
B0.10	9.99 ± 2.51^{A}	28.83 ± 2.01^{C}
B0.15	8.15 ± 1.52^{A}	28.00 ± 1.66^{C}

Note: Different superscript lowercase and uppercase letters within the same column indicate significantly different means of the observed data in Treatment A and Treatment B, respectively, (at $p < 0.05$).

The effect of loading LGEO for both treatment films was found to have no significant effect ($p < 0.05$) in the TS, while a significant variation was observed in terms of EAB. A slight decrease in TS is observed among the treatments when concentrations of LGEO are increased, and in contrast, a significant increase is observed for films with at least 0.10 % (w/w) LGEO. This inverse relationship observed between TS and EAB was also achieved and reported in [15] when oregano oil is infused into whey protein isolate films, explaining that lipid addition induces heterogeneity in the film structures characterized by discontinuities in the film network, causing TS reduction and an elevated stretchability (EAB). To elaborate, these observations are potentially due to the partial replacement of the stronger interactions between polymers with that of a weaker polymer-oil interaction similar in the observations of [16] wherein the interaction of gelatin towards other gelatin molecules has been reduced when citrus essential oil was introduced in the film matrix.

3.4. Visual characterization

A visual check-up of the surface morphology of the fabricated films using a digital microscope at 1600x magnification was conducted on random parts in the films to observe the effects of the incorporation of LGEO on the structure of the films and to characterize the formation of oil emulsions in the sample films. Shown in Figure 2 are the digital micrographs of the generated CRG-GEL PEC films loaded with LGEO and LGEO emulsions.

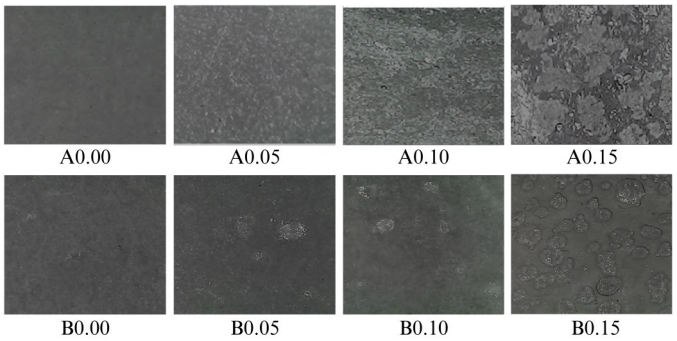

Figure 2. Digital micrographs of the generated CRG-GEL PEC films loaded with LGEO and LGEO emulsion at increasing concentrations.

The carrageenan-gelatin PEC film without LGEO added, as shown in Figure 2 (A0.00) has generated a homogeneous, smooth, continuous, and transparent surface, and did not exhibit any pores or fractures, indicating the high compatibility and an ordered matrix of the two biopolymers used. Meanwhile, increasing concentrations of LGEO added directly to the polymer solutions of 0.05%, 0.10%, and 0.15% v/v concentration as shown in Figure 2 (A0.05, A0.10, A0.15) have shown heterogeneous and discontinuous surfaces. This can be traced to the possible disruption of the weak polymer-oil interaction that partially replaced the strong polymer-polymer interaction during and/or after the drying process. This reduction of smoothness and regularity of surfaces was also observed by [17] in their similar fabrication of Gelatin, κ -Carrageenan, and thymol-loaded zein nanoparticle films. Moreover, these observed changes in the surface structure of the films further confirm and explain the reduction of TS and

increased EAB as presented previously.

Moreover, the digital micrograph of the fabricated film with non-loaded emulsion, as shown in Figure 2 (B0.00), has also generated a smooth, continuous, compact, and transparent surface, indicating a minimal effect of the lecithin emulsifier on the polymer-polymer interaction. Meanwhile, the films loaded with 0.5 and 1.0 v/v % LGEO emulsions as shown in Figure 2 (B0.05, B0.10) have minimal oil droplets that are long distances away from each other. On the other hand, the polymer film with 1.5 v/v % LGEO emulsion (Figure 2 (B0.15)) has shown the most droplets of oil embedded in irregularly-shaped globules that are quite close to each other. These globules found on the LGEO-loaded emulsion films create heterogeneous layers characterized by non-continuous layers, and several dense zones are developed on the films. Such observation was also noticed by [18] as *Bunium alpinum* and *Bunium incrassatum* are incorporated into the gelatin-based films, and as their concentrations are increased. They explained that these most densely built-up zones contain the highest amounts of essential oils, which modify the transverse distribution of the polymers inside the film's matrix, promoting the roughness of the film because water molecules cannot travel through the film network.

Furthermore, the relatively large size distribution of oil emulsions in the second treatment, as shown in Figures 2 (B0.05, B0.10, B0.15) is primarily caused by the modified method of creating emulsions using a milk frother. This makes the emulsified LGEO in films potentially release at a faster rate. As studied by [19], the stability of the emulsions is found to be inversely proportional to the size of the emulsions, whereas relatively smaller particle sizes of emulsions are more stable than the larger ones. In addition, the authors specifically recorded that nano-emulsions were stable even after 30 days.

3.5. FTIR analysis

The FTIR spectra of LGEO and the fabricated CRG-GEL PEC films loaded with LGEO and LGEO emulsion are shown in Figure 3. These spectra were obtained to analyze whether component/s of LGEO had been loaded into the generated films.

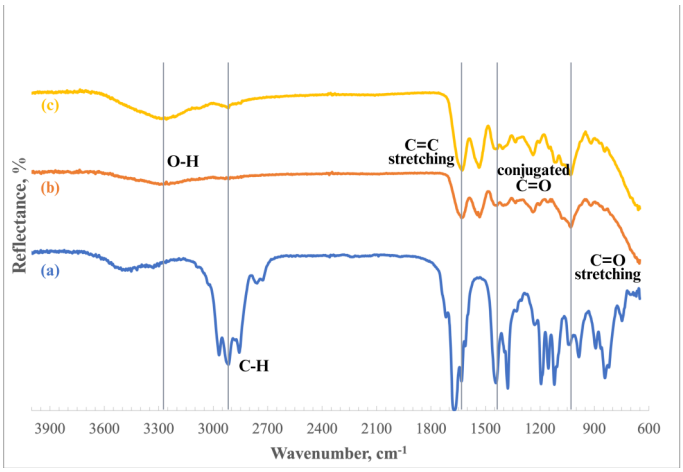

Figure 3. FTIR spectra of (a) LGEO, (b) CRG-GEL PEC with LGEO, and (c) CRG-GEL PEC with LGEO emulsion.

The ATR-FTIR absorption spectra of the LGEO present characteristic bands at 3453, 2916, 2856, 2759, 1672, 1632, and 1443 cm^{-1}. The 3453 cm^{-1} band corresponds to the weak OH stretching, while 2916, 2856, and 2759 cm^{-1} refer to the weak pair of aldehyde C-H stretches. Moreover, the band at 1672 cm^{-1} corresponds to the unsaturated conjugated C=O vibration of citral, while the bands 1632 and 1443 cm^{-1} show the C=C stretching [20].

The spectra of the CRG-GEL PEC film with LGEO emulsion are relatively similar to CRG-GEL PEC film with LGEO, but the former has a slightly higher intensity in the O-H vibrations. With regards to the characteristic bands of LGEO, the C-H stretches at 2916, 2856, and 2759 cm^{-1} wavenumbers were not visible in both sample films, as well as the intense C=O peak at 1672 cm^{-1}. These prominent absorption bands were not reflected in the spectra because of possible volatilization of a significant amount of essential oil, or the oil emulsions might not be embedded in the surface of the sample films and the orientation of the functional groups might be hidden or masked from the surface, and lastly, because the ATR technique employed has a very low limit of detection and is a surface-sensitive measure of reflectance and not a total transmission of the film samples.

The spectra of carrageenan, gelatin, and the carrageenan-gelatin polyelectrolyte complex films are shown in Figure 4. The spectrum of carrageenan showed absorption bands at 3267, 1248, 1018, and 845 cm^{-1}. These absorption bands correspond to the stretching vibration of OH groups, the vibration of the sulfate group, glycosidic linkage, and D-galactose-4-sulfate group vibration, respectively. Meanwhile, the notable absorption bands for gelatin appeared at 3259 cm^{-1} (NH-stretching), 1628 cm^{-1} (Amide I, CO, and CN stretching), 1520 cm^{-1} (Amide II), and 1240 cm^{-1} (Amide III). As stated in the study of [21], the Amide I band between 1600 and 1700 cm^{-1} is the most important and useful peak among the absorption bands for IR analysis of protein's secondary structures, such as gelatin. In reference to the IR spectra obtained from the polyelectrolyte complex of carrageenan and gelatin, a slight shift was observed in the Amide I peak from 1628 to 1626 cm^{-1}. This tiny shift can be traced to the possible association of the carrageenan's negatively charged sulfate ester groups with the positively charged residue in the gelatin structure. This observation was also noted in the study of [22], where the Amide I peak has shifted to a lower wavenumber.

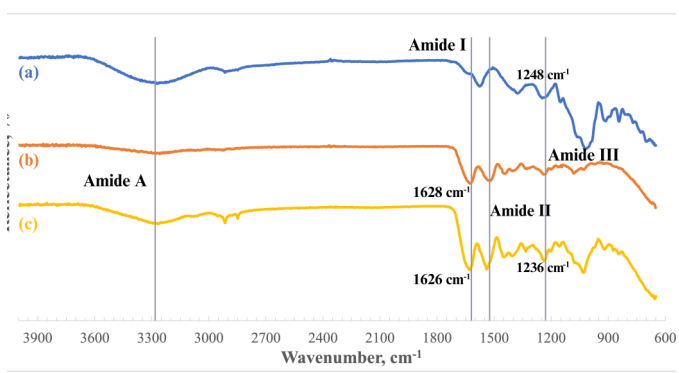

Figure 4. FTIR spectra of (a) carrageenan, (b) gelatin, and (c) CRG-GEL PEC films.

Meanwhile, the vibration of the sulfate group that appears from 1270 to 1230 cm^{-1}, in the complex film formed, the peak has shifted from 1248 to 1236 $cm^{-1,}$ which was also observed in the study of [23] upon the complex formation of gelatin and κ-carrageenan for the design of extended-release pellets. Moreover, they discussed such a shift as a result of the loss of one oxygen in the complex formation. These observed alterations on the FTIR spectra of CRG-GEL PEC film confirm the strong intermolecular interaction between the polypeptide chains of gelatin to the sulfonic groups of carrageenan polysaccharide during the formation of polyelectrolyte complexes [24].

To characterize the interaction of LGEO addition to the complex formation of carrageenan and gelatin, the FTIR spectra of the CRG-GEL PEC films with increasing concentrations of LGEO are presented in Figure 5. Similar IR patterns are observed among the films with no LGEO added and the films with loaded LGEO as it increases in concentration. Thus, a structural change in the films is not observed upon the loading of LGEO regardless of concentration. This also shows the absence of interaction between the functional groups of the CRG-GEL PEC films to the active components of the LGEO.

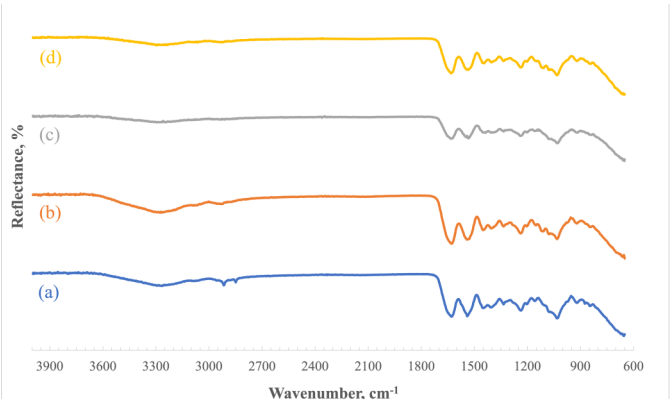

Figure 5. FTIR spectra of CRG-GEL PEC with (a) 0.00% v/v LGEO, (b) 0.05% v/v LGEO, (c) 0.10% v/v LGEO, and (d) 0.15% v/v LGEO

Meanwhile, the superimposition of the spectra in Figure 6 exhibits a notable narrowing of the stretching intensities in the O-H groups upon the increase in the concentration of LGEO, which can be rooted in the similar reduction of hydrogen bonding of the O-H group of carrageenan-gelatin PEC films. Such a result agrees with the study of [25] as they increase the concentrations of eucalyptus and cinnamon essential oils in (Poly(lactide)/poly(butylene adipate-co-terephthalate) blend film. To account, the authors explained that absorption peaks become shorter with increasing concentrations of essential oil because its components are susceptible to chemical transformation, oxidation, and polymerization.

Figure 6 shows the FTIR spectra of the CRG-GEL PEC films with increasing concentrations of LGEO emulsions. Like the observations in Figure 6, the IR patterns are similar among the films with non-loaded emulsion, the films with loaded LGEO emulsion, and as it increases in concentration. Therefore, a structural change in the films is also not observed upon the increase of the incorporated LGEO emulsion in the films. This also shows the absence of interaction between the functional groups of the CRG-GEL PEC films to the active components of the emulsified LGEO.

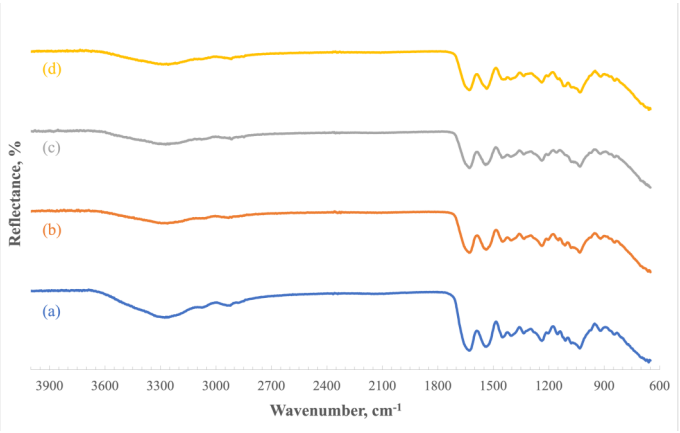

Figure 6. FTIR spectra of CRG-GEL PEC with (a) 0.00% v/v LGEO emulsion, (b) 0.5% v/v LGEO emulsion, (c) 1.0% v/v LGEO emulsion, and (d) 1.5% v/v LGEO emulsion.

There is a broken trend in the narrowing of the peaks in O-H groups upon the addition of the LGEO emulsion in increasing concentration because the absorption band in the film with 1.5% v/v LGEO emulsion is slightly more intense than the film with 1.0% v/v LGEO emulsion. However, it can still be observed that all the fabricated films that are incorporated with the emulsified LGEO have narrowed peaks in the said O-H group vibrations. This still manifests the decrease of hydrogen bonding of the O-H group of carrageenan-gelatin PEC films when LGEO emulsion is incorporated.

3.6. Evaluation of antifungal property on white bread samples

To evaluate the ability of the synthesized films to act as active packaging, their anti-fungal property was tested by using them to package white bread samples in direct contact for 14 days. The control group used is a bread sample that was stored on its original packaging. The testing parameter is characterized by the physical observations based on the proliferated fungi on the surface of the bread samples, recorded starting from the first day until the 14th day of storage. Overall, the general observation shows a delay of fungal growth on the surfaces of the bread samples packed with the fabricated CRG-GEL PEC film,s and especially with loaded LGEO and LGEO emulsion.

Figure 7 shows the incidence of fungal growth on the surface of the bread samples packaged with the experimental sample films. Recorded observations of the experimental setup show the anti-fungal activity of the carrageenan-gelatin polyelectrolyte complex films that increases with increasing concentrations of LGEO, both added directly to the films and through emulsion. For the first treatment, two bread samples have shown surface fungal growth, and these are the ones packed with 0.0% and 0.05% v/v LGEO. On the 9th day of the experiment, fungal growth was observed on the former while the latter exhibited fungal growth on the 11th day and are shown in Figures 7 (b) and (c), respectively. Meanwhile, only the fabricated film with non-loaded LGEO emulsion has proliferated bread molds for treatment B, and it was observed on the 10th day as shown in Figure 7 (d).

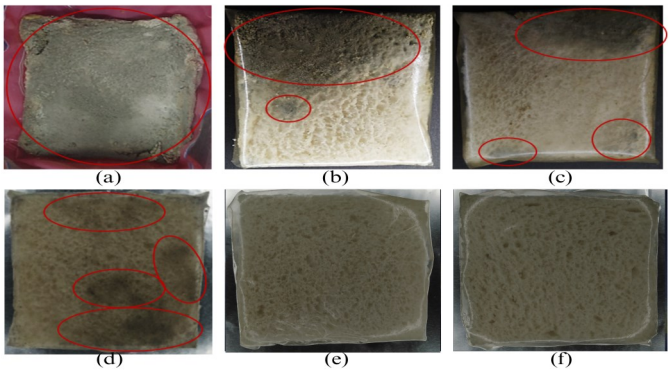

Figure 7. Fungal growth incidence on the white bread samples at (a) day 12 for control group, (b) day 9 for CRG-GEL PEC film with 0.00% v/v LGEO, (c) day 11 for 0.05% v/v LGEO, (d) day 10 for 0.0% v/v LGEO emulsion, (e) day 14 for 0.15% v/v LGEO, and (f) day 14 for 1.5% v/v LGEO emulsion.

The summary of the daily physical observations on the surface growth of fungi in bread samples is presented in Table 3. It can be noticed that for the control group, grayish-green molds started to grow on the sixth day at a 10-40% range, and after a few days, the molds started to spread and completely affected the entire bread surface on the 12th day. Using the control group as a basis for comparison, it can be noticed that the experimental samples were able to cause retardation in the growth of fungi on the surfaces of the packed white bread samples. To account for films with the sample codes A0.00, A0.05, and B0.00, the growth of molds was delayed for 3-5 days with respect to the day of the initial fungal growth of the control group.

Table 3. Daily observations of the test for antifungal activity of the fabricated CRG-GEL PEC films

Sample Code	Daily Fungal Growth Observations													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	-	-	-	-	-	x	x	x	xx	xx	xx	xxx	xxx	xxx
A0.00	-	-	-	-	-	-	-	-	x	x	x	xx	xx	xxx
A0.05	-	-	-	-	-	-	-	-	-	-	x	x	x	x
A0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B0.00	-	-	-	-	-	-	-	-	-	x	x	x	x	x
B0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: Sample code A refers to Treatment A (CRG-GEL PEC films with LGEO through direct loading), and sample code B refers to Treatment B (CRG-GEL PEC films with LGEO loaded through emulsification).
Legend: (-) – no growth; (x) – 10-40% surface growth; (xx) – 40-70% surface growth; and (xxx) – 70-100% surface growth.

Meanwhile, the sample films with 0.10% and 0.15% v/v LGEO in Treatment A, and all the films loaded with LGEO emulsion in Treatment B were able to inhibit the growth of bread molds. Thus, carrageenan-gelatin PEC films loaded with both LGEO and LGEO emulsion are generally capable of slowing down fungal growth on white bread samples. The same observation was reported in the study of [26] when a similar evaluation was performed using polysaccharide-based films with cinnamon essential oil. A significant delay in mold growth was observed for breads packed at higher cinnamon oil concentration, accounting for higher possible retention of the oils in the film matrix. [27] has further elaborated that a controlled release of essential oils from the films is more effective than direct addition, as they retain longer on the

bread surface throughout the shelf-life. It is also achieved in this study as all films with emulsified LGEO in Treatment B have generally inhibited mold growth during the 14-day experimentation period.

4. Conclusions

The experiment successfully incorporated lemon grass essential oil (LGEO) into the carrageenan-gelatin biopolymer film matrix through soy-lecithin emulsification, resulting to the production of active film products. The addition of LGEO, particularly at higher concentrations, caused the films to develop crumpled edges. In terms of the films' barrier properties, increasing LGEO concentration resulted to an improved thickness, moisture content, water solubility, and hydrophobicity, which, however, decreased the soil biodegradation rate. Meanwhile, a decreased tensile strength and increased elongation at break characterize the mechanical properties of the films which may have caused by the partial replacement of the strong interaction between the polymers with the weaker polymer-oil interaction. This aligns with the discontinuity and heterogeneity in film surface layers exhibited on the digital micrographs of the films.

Additionally, FTIR analysis revealed that the direct addition of LGEO and through emulsification has narrowed the spectral bands in the OH functional group region. However, there was no significant structural transformation that was observed in the films upon the loading LGEO at an increasing concentration. Finally, the fabricated films were able cause retardation of fungal growth on the surfaces of the bread samples packed with the CRG-GEL polyelectrolyte complex (PEC) films loaded with LGEO and LGEO emulsion. Further investigations are recommended in incorporating oil nano-emulsions into the films and the optimization of additives that would further enhance the physical, mechanical, and degradation properties of the films.

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