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Semi refined carrageenan-nanocomposite film incorporated with Bentong ginger extracts for active food packaging: Synthesis and characterization

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ABSTRACT

Bentong Ginger (BG), Malaysia patented ginger consist of various bioactive compounds such as gingerols which account for the various pharmacological benefits in human including antioxidant, antimicrobial, and anticancer. The objective of this work was to analyze the effectiveness of BG extracts as an antioxidant additive in plasticized semi refined carrageenan-nanocellulose (CN) to produce active packaging films at concentrations ranging from 5 to 20 % (v/v). The bioactive constituents in BG extracts were discovered using Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry (LC-QTOF/MS) and the possible interaction between the semi refined carrageenan-nanocellulose film network and BG were revealed using Fourier-Transform Infrared (FTIR) Spectroscopy. The effects of BG extracts formulated in CN film on the antioxidant release, physical and mechanical properties of the films were studied. The CN film with 20% BG improved the most, exhibiting excellent mechanical and physical properties as well as antioxidant release tests that revealed the highest release of antioxidant and total phenolic content in 95 % food simulants up to 28 days storages. This study validated that incorporation of BG extract can a promising natural additive for active packaging materials.

1. Introduction

Plastic pollution become critical issue of environmental problem due to non-degradable properties which caused over accumulation in landfill. The problem affected to many countries that have low recycling management system and incompetent on the waste collection and disposal [1]. Souza et al., (2017) [2] stated that the food sector was the predominant client of the packaging industry, the amount of food is still lost or wasted each year is around 1.3 billion tons in part due to inadequate packaging and the heap of single used food packaging. Thus, bio-based active packaging systems derived from renewable sources are consider as alternative to non-degradable petroleum-based packaging with improves features of maintaining food quality, microbial growth and prolong its shelf life [2,3].

Previous literatures reported plant polysaccharides such as carrageenan, starch, pectin, cellulose, chitosan, and alginate exhibited film forming, thickening and gelling-like properties in biopolymer packaging film development [4,5]. Carrageenan is a linear sulfated polysaccharides derived from red seaweeds species of *Eucheuma cottonii* (*Kappaphycus alvarezii*) and it is a potential biopolymer component because of its favorable gelling and binding properties for the production of films [6]. In this study, the biopolymer used to create the active packaging film was semi-refined carrageenan (C). Alkali treatment of the seaweed *(Kappaphycus alvarezii)* yields semi-refined carrageenan (C), while further processing such as filtration and purification leads to carrageenan production. As a result of fewer processing steps, semi refined carrageenan (C) can be obtained at a lower cost than carrageenan. Furthermore, semi refined carrageenan is widely used in food packaging applications because it does not require a high level of refinement [7]. Previous studies represent C derived packaging film plasticized with glycerol (G) increases the flexibility by reducing the brittleness [8] and showed excellent physical and mechanical properties of biopolymer when C were reinforced with cellulose nanofiber [9,10].

In recent years, researchers proposed the development of food active packaging is by incorporating natural plant extracts as antioxidant agent in the biopolymer formulation. Natural antioxidants such as pomegranate peels [11], apple pomace [12], and *Persicaria minor* [13] extracts represented as food additives to help preserve food by preventing rancidity, deterioration, and discoloration caused on by oxidation. Due to well-endowed with bioactive compounds and nutrient such as

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gingerols and shogaols, ginger extracts reported to exhibit various medicinal properties such as such as antioxidant activity [13–15], antimicrobial [16], anti-cancer [16,17], anti-inflammatory [19] and analgesic effects [20]. Irawan et al., (2019) [21] reported the addition of ginger extracts as an antioxidant agent in active film formulation is not only prevents food spoilage and increases shelf life, but it also adds some medicinal benefits in foods. Bentong ginger (BG) shared scientific name with common ginger, is a well-known Malaysia patented ginger believed to have high nutrition and unique taste compare to other common ginger, due to the present gingerols was significantly higher than in local ginger [22]. Therefore, incorporation of BG extract could become a great alternative to improves semi refined carrageenan-nanocomposite film as well considered as potential alternatives to synthetic additives in active food packaging function. However, there is yet little information about their possible application as active components in biopolymer films.

Hence, this study concentrates on the synthesis and characterization of semi refined carrageenan-nanocomposite (CN) films incorporating BG extracts at varying concentrations (5, 10, and 20% v/v) as an antioxidant agent in the development of active packaging. Therefore, the purpose of this research is to determine the bioactive compounds in the BG extracts through Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry (*LC-QTOF/MS*) and to depict CN-based film incorporated with BG extract using Fourier-Transform Infrared (FTIR) Spectroscopy as well as mechanical, physical and antioxidant properties of CN film with BG extracts. Antioxidant release of BG from CN film to food simulant were analyzed using total phenolic content assays and DPPH inhibition for 28 days of storages.

2. Materials and methods

2.1. Materials

Fresh Bentong ginger (BG) was obtained from the Pahang Agriculture Department, Malaysia. TACARA Sdn. Bhd., Tawau, Sabah, Malaysia, supplied semi-refined carrageenan powder (C) (TA150) which extracted from red seaweeds species of *Eucheuma cottonii* (*Kappaphycus alvarezii*) harvested in Tawau, Sabah and cellulose nanofiber (N) was supplied and characterized according to method of Mohd Azman et al., (2022) [9]. All of the chemicals and reagent used were commercial grade and glycerol (G) was food grade supplied by Sigma-Aldrich.

2.2. Preparation of Bentong ginger (BG) extracts

The peel of fresh Bentong ginger was peeled off after it had been cleaned under running water to get rid of any dirt or debris. The ginger pulp was then diced into uniform dimensions of 1 mm × 1 mm × 1 mm each before the samples were oven dried for 24 h at 40 °C. Afterwards, the samples were grinded with a blender which turns it into a powdered form. The process was repeated for a few times until it is really in a fine powder form. 5 g powdered ginger was homogeneously mixed in 100 mL of 80% ethanol solvent. Magnetic stirrer was used to mix the solutions at ambient conditions for 24 h, then separated using refrigerated centrifuge (Eppendorf 5810r, USA) at 4000 × g for 30 min at 20 ℃ and the supernatants were separated and kept at −4 ℃ until the analysis.

2.3. Preparation of active CN films

The technique outlined by Farhan & Hani, (2017) [8] was slightly modified in order to prepare CN-based films. 100 g (w/w) CN film formulation consist of: semi refined carrageenan (C) powder (2 g) was dissolved in distilled water (2 % [w/w]), plasticized with glycerol (G) (0.9 % [w/w]), reinforced with 10 % (w/w) cellulose nanofiber (N) under continuous stirring at 70 ℃ for 10 min. BG extracts (v/v) was added at concentration of 5 % (CN5%BG), 10 % (CN10%BG) and 20 % (CN20%BG) and control was prepared without any addition of BG then, the film solution were homogenized (Ultra Turrax, IKA, Germany) at 1000 rpm for 10 min. The film-forming solution was poured on a 20 cm by 3.5 cm acrylic plate and then dried for a day and kept in a desiccator before use.

2.4. Identification of BG extract bioactive compounds through liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS)

The column used was ACQUITY CSH C18 (1.7 µm, 2.1 × 100 mm) with temperature of 40.0 °C. The mobile phases for solvents A and B were water and acetonitrile mixed with 0.1% formic acid, respectively with 1 µL injection volume and a flow rate of 0.5 mL/min. The gradient consisted of solvent B concentrations ranging from 2 to 30% over 7 min, to 100% at 8 min, and held until 10 min. The spectral channel resolution was set at 1.2 nm, and the UV detection wavelength was set at 254 nm.

2.5. Fourier-Transform Infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectrometer was used to identify the functional groups of the films (Thermo Scientific Nicolet iS5 FTIR Spectrometer, Massachusetts, USA). OMNIC software was used to determine the FTIR spectra in the wavelength range of 500 to 4,000 cm^{-1} at a resolution of 4 cm^{-1}.

2.6. Tensile strength (TS) and elongation at break (EAB)

Tensile strength (TS) and Elongation at Break (EAB) of films were evaluated using universal testing machine (AG-Xplus Series, Shimadzu, Japan). Rectangular film strips with dimension of 1.5 cm × 10 cm were prepared. Then, the samples were tested using the testing machine and measured with a deformation rate of 50 mm/min [23]. Tensile strength (TS) result will be denoted in term of Pa and elongation at break (EAB) in term of percentage (%).

$$TS\,(Pa) = \frac{F_{max}}{\varnothing} \tag{1}$$

where F_{max} represent the maximum weight and ∅ represent the cross-sectional area. EAB of the film samples were calculated as equation below:

$$EAB = \left(\frac{\Delta l}{l_0}\right) \times 100\,\% \tag{2}$$

where Δl refers to extension of film and l_0 stands for sample film's initial length.

2.7. Physical properties of CN active films

2.7.1. Thickness measurement

Thickness measurements will be taken at several spots around the film samples using Vernier calliper, and mean values will be computed. The mean value of thickness was used in the calculations.

2.7.2. Opacity measurement

The opacity of the film was measured using UV–vis spectrophotometer (Thermoscientific Genesys 50, Singapore). Rectangular film strips with dimension of 3 cm × 0.3 cm were prepared and positioned in the cuvette. According to the method mentioned by Harini et al., (2018) [24], the opacity of the film samples was determined using Eq.3 below at 600 nm.

$$Opacity = \frac{Abs600}{b} \tag{3}$$

where Abs 600 represented the absorbance at 600 nm and b was the thickness of film (mm).

2.7.3. Solubility in water

Water solubility of the film samples was prepared with slight method modification outlined by Harini et al., 2018 [24]. Square film strips with dimension of 2 cm × 2 cm were prepared, dried for 24 h at 100 °C and weighed to find their initial dry weight. Film samples were immersed in 0.03 L of distilled water and kept in laboratory water bath with constant shaking at 25 °C for 24 h. The final dry weight of the undissolved films was determined by filtering them through Whatman No. 1 filter paper and drying them for 24 h at 100 °C. The following equation was utilized to determine the percentage of water solubility (WS %):

$$WS = \frac{W_o - W_f}{W_o} \times 100\,\% \quad (4)$$

where W_o = initial dry weight of the film; W_f = final weight of the dried undissolved film.

2.7.4. Moisture content

The moisture content of the film was performed to identify the water content in the active films. The weight loss of the films (4 cm × 4 cm) after drying for 24 h at 105 ℃ over four days was used to determine the moisture content of the films. The moisture content (in percentage) was calculated by using Eq.5 below:

$$MC = \frac{W_i - W_{ff}}{W_i} \times 100\,\% \quad (5)$$

where W_i = initial weight of the film; W_{ff} = final weight of the film.

2.8. Release test of active compounds

Migration test of BG in active packaging polymer into 95% ethanol as a food simulant was conducted as described by L. nan Sun et al., (2021) [25] slight modification. Rectangular film strips pieces with dimension area of 2 cm × 3 cm will be immersed in 10 mL of 95 % of ethanol contained in 30 mL amber glass vials. The vials will be hermetically sealed with a cap and secured with aluminium foil to protect from light and kept at room temperature. Released tests were analyzed for 28 days of storage with 4 days intervals based on polyphenol content (TPC) measured using Folin-Ciocalteu and antioxidant capacity based on DPPH radical scavenging activity, followed the procedure outlined by Yahaya et al., (2020) [23]. The release test will be conducted at least in triplicate.

2.9. Statistical analysis

All of the analyses were done in triplicate in this study, and the results were expressed as mean value ± standard deviation (SD). Microsoft Excel were used to conduct the statistical analysis.

3. Results and discussion

3.1. LC-QTOF/MS analysis of BG extract bioactive compound

BG extract was analyzed using LC-QTOF/MS to determine and verified the presence of bioactive compound that can acts as antioxidant agent in order to produce active packaging film. Based on the data obtained, the components detected from BG extract that having the mass error smaller than 2 ppm and with the highest observed retention time was summarized in Table 1. The biomarker compounds in those extracts as demonstrated in Table 1 were selected based on the findings of prior studies on ginger family. Single chromatograms from LC-QTOF/MS of each compound listed in Table 1 were presented in Fig. 1.

Ginger constituents such as monoterpenes (cineole, citral, limonene, and α/β-pinenes), sesquiterpenes (β-elemene, farnesene, and zerumbone), phenolics (gingerols, 6-shogaol, 6-paradol, and zingerone), and diarylheptanoids (curcumin) have been shown to have biological effects [26]. 6-gingerol is the most abundant gingerol homolog found in ginger rhizome whereas zingerone compound was formed from gingerols by reverse aldol condensation reaction when ginger is dried or roasted, and were reported to have a variety of effects and activities, such as anti- inflammatory, antidiabetic, antidiarrheic, antispasmodic, anticancer, antiemetic, appetite simulant, anxiolytic, antithrombotic, radiation protective and antimicrobial activities [18,27–31]. Curcumin and related compounds such as cyclocurcumin, dihydrocurcumin and octahydrocurcumin are classified as curcuminoids, which are linear, diarylheptanoid molecules. Curcuminoids are natural phenolic compounds found in turmeric, but they also appear as a major constituent in ginger because turmeric is a member of the ginger family (Zingiberaceae). This is because both curcuminoids and gingerol-related compounds produced from same base compound called as L-phenylalanine an essential amino acid, through different biosynthetic pathways [31]. Curcuminoids have diverse pharmacological activities, particularly antioxidant potential, due to their unique chemical structure with diketo-enol configuration [32].

Table 1
Observed *m/z* of BG extract compounds detected in LC-QTOF/MS.

Compound	Observed M/Z	Compound	Observed M/Z
6-gingerol	293.1782	4,5-O-Dicaffeoylquinic acid	515.1227
Zingerone	193.0867	Eugenol	209.0813
Cyclocurcumin	367.1182	Kukoamine A	529.3028
Dihydrocurcumin	369.1338	Xanthohumol	399.1450
Octahydrocurcumin	375.1811	Yakuchinone B	355.1539

4,5-O-Dicaffeoylquinic acid is one of main bioactive metabolites that can be identified in Chinese herb *Flos Lonicerae Japonicae*. According to Tang et al., (2008) [33], 4,5-O-Dicaffeoylquinic acid is a naturally occurring antioxidant compound with anti-inflammatory properties that is linked to free radicals. Eugenol is a natural aromatic compound that can be found in cloves, bay laurel, and cinnamon bark. The modification of the allylic group, hydroxyl group, and substitution of functional groups such as hydroxyl, amine, chloride, and nitro in the aromatic ring of eugenol has been reported to be responsible for a wide range of biological activities [34]. Kukoamine A is a bioactive spermine alkaloid found naturally in the root back of *Lycium chinense*. Previous research has shown that Kukoamine A has antihypertensive, anti-inflammatory, antisepsis, antioxidant, anticancer, and neuroprotective properties [35]. Xanthohumol and yakuchinone B are polyphenol chalcone present in *Humulus lupulus* [36] and *Alpinia oxyphylla* [37], respectively has been found to possess antioxidant and neuroprotective activities [38]. To conclude, bioactive compounds identified in BG extracts is beneficial for human consumption and suitable to be used as active ingredient in food packaging application.

3.2. Fourier-transform infrared (FTIR) spectroscopy analysis

Fig. 3 depicts the functional groups between semi refined carrageenan (C), glycerol (G), cellulose nanofiber (N), and Bentong ginger extract in the wavenumber range of 4000–500 cm^{-1}. The broad band ranging between 3700 and 3000 cm^{-1} represent the O–H stretching caused by the hydroxyl group of carrageenan, water [8] and also confirming the presence of phenols and alcohol with a free O–H group [39]. This spectrum has a broad range because of the abundance of these groups and the diverse chemical nature [40]. Moreover, Anisa Aris & Morad, (2014) [41] demonstrated the presence of aliphatic O–H groups from gingerol in the range of 3600 to 3000 cm^{-1} before degradation to shogaol, despite the fact that shogaol lacks an aliphatic O–H group. The peak observed at 2922 cm^{-1} in all the film formulation represent C–H stretching [7,26,42]. Abd Hamid et al., (2019) [43] claimed that the absorption band in between 3100 and 2800 appeared after the addition of G referred to its alkane groups. The peaks observed at 1643 cm^{-1} are in agreement with the range mentioned by Ili Balqis et al., (2017) [44]

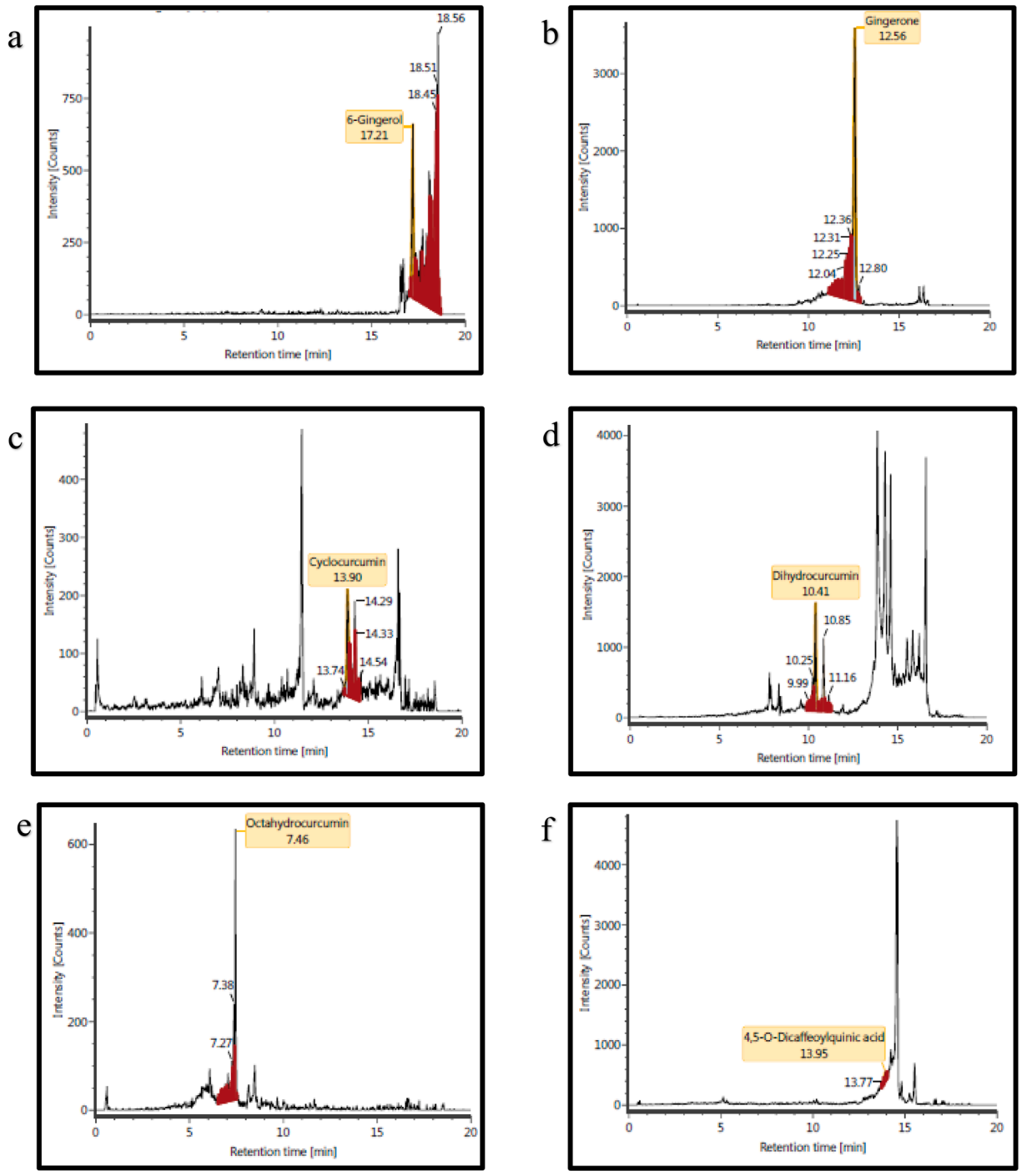

Fig. 1. Chromatograms of each bioactive compounds detected in LC-QTOF/MS; a) 6-gingerol, b) zingerone, c) cyclocurcumin, d) dihydrocurcumin, e) octahydrocurcumin, f) 4,5-O-Dicaffeoylquinic acid, g) eugenol, h) kukoamine A, i) xanthohumol and j) yakuchinone B.

refers to the carbonyl groups (C = O) of D-galactose, monomer of κ-carrageenan. On the other hand, Abd Hamid et al., (2019) [43] and Khojah, (2020) [11] claimed that the peak observed in between 1800 and 1500 cm^{-1} represent the stretching vibration of amide I group (–NH). The sulphate ester (O = S = O), 3,6-anhydrogalactose (C-O-C), and galactose-4-sulfate (C-O-S) are responsible for the peaks at 1228, 929, and 840 cm^{-1}, respectively [44]. The small peak observed in between 1480 and 1380 cm^{-1} and the sharp peak at 1035 cm^{-1} represent an aromatic C = C functional group [27,32] and glycosidic linkages [8], respectively according to previous literature. The spectra bands at 1151 cm^{-1} revealed the occurrence of alkynes, N–C, and N = C groups in R–N = C = S structure and the presence of carboxylic acid groups [39,45]. Addition of BG extracts into CN film show no difference change compared to CN-control film. Similarly, Yahaya et al., (2020) [23] found no difference in functional group between SG-based films with *Persicaria minor* extracts and the SG-control film due to the homogeneity of the film solution. In this study, the CN film formulations were homogenized at high speed to bind the polymer matrix, plasticizer, reinforcing agents, and BG extracts. Furthermore, Saberi et al., (2017) [46] stated that the incorporation of active compounds did not alter the pea starch and guar

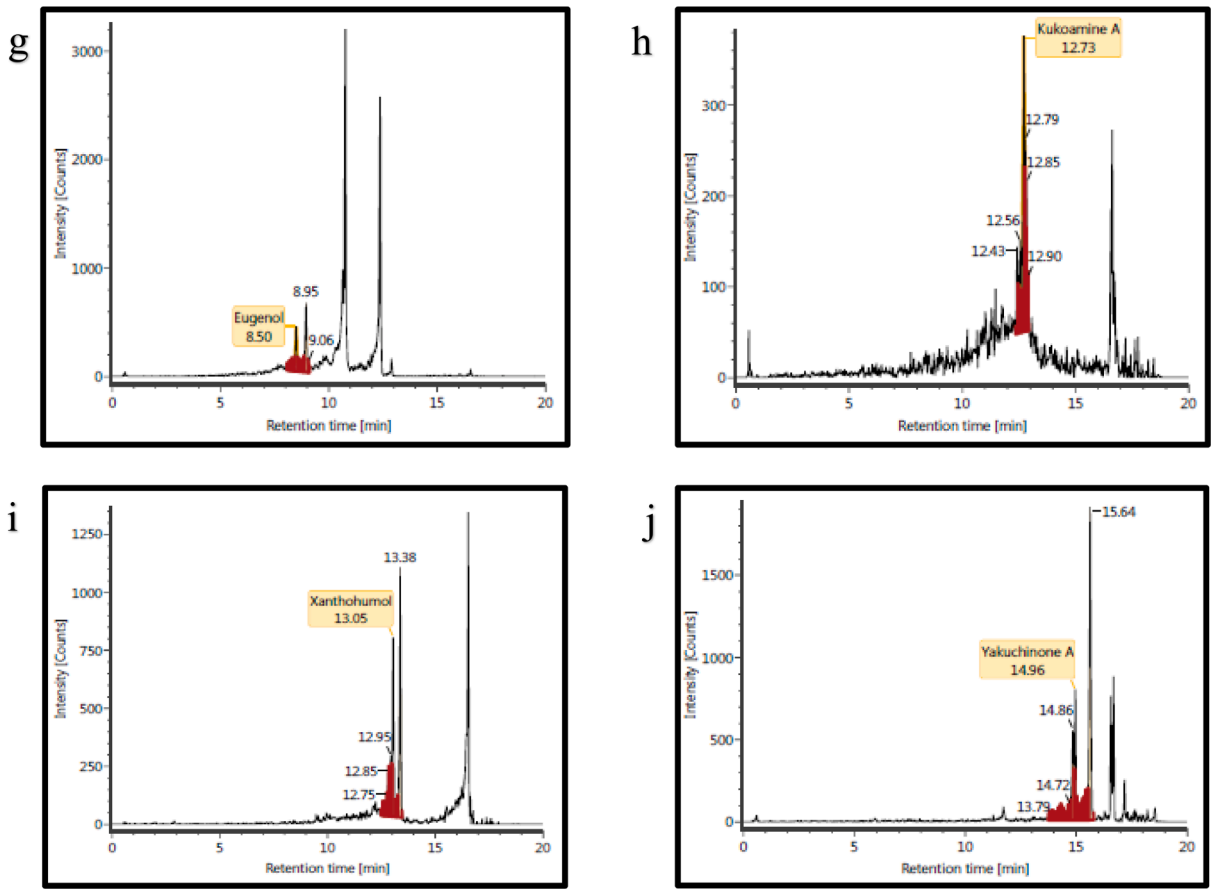

Fig. 1. (*continued*).

gum (PSGG) film, indicating that no significant changes in the polymer backbone were observed, indicating that PSGG are miscible and compatible with natural active compounds. Fig. 2 shows that the CN-films incorporated with BG extracts are homogeneous and uniform, with the color of the film darkening as the BG concentration increases. Hence, this demonstrates that BG extracts are incompatible with the polymer matrix of CN.

3.3. *Mechanical properties of active packaging film*

Mechanical characteristics represents elongation at break (EAB) measurement showed film's ability to stretch and flexible, whereas tensile strength is the maximum strength measuring the resistance of the film [47]. Table 2 summarize the tensile strength and elongation at break of sample films. Mechanical properties of films may attribute to the hydrogen bonds formed between the semi refined carrageenan (C) matrix and glycerol (G) as plasticizer, reduced the intermolecular forces and increase the mobility of the biopolymer chains by creating space within the matrix. Cellulose Nanofiber (N) was added as an additional enhancement to fill the space created by the plasticizers demonstrated high TS and film integrity and solubility [48]. The value reported in this works showed the addition of BG extracts improved TS and EAB, indicates that plant extracts acts as reinforcing agents because of phenolic-rich compounds interact with polysaccharide-based film matrix through hydrogen bonds and electrostatic interactions, improving the mechanical properties of composite films [49]. CN film with 20% BG extracts demonstrated the highest TS and EAB in accordance with the targeted biopolymer properties in the tensile strength range value of 10–100 MPa with elongation at break of > 10% [23]. These outcomes were in line with those of other authors who discovered that adding plant extracts like pomegranate peel [49], *Persicaria minor* [23] and *Prunus maackii* juice [50] improves the overall mechanical properties. However, few literatures showed decreasing of TS value when incorporating active extracts may depend on synergic polymeric matrix interactions between different components [51].

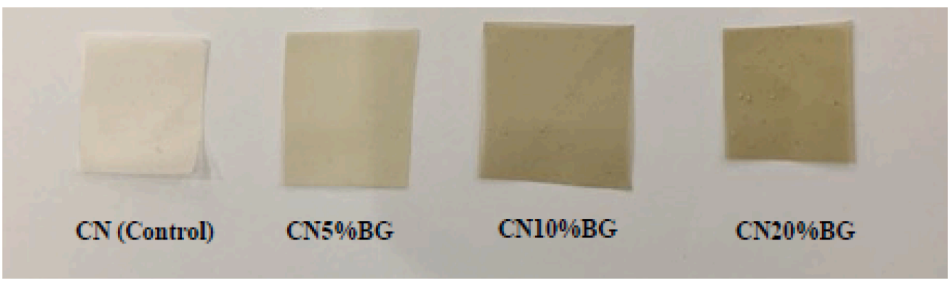

Fig. 2. CN-films physical appearance.

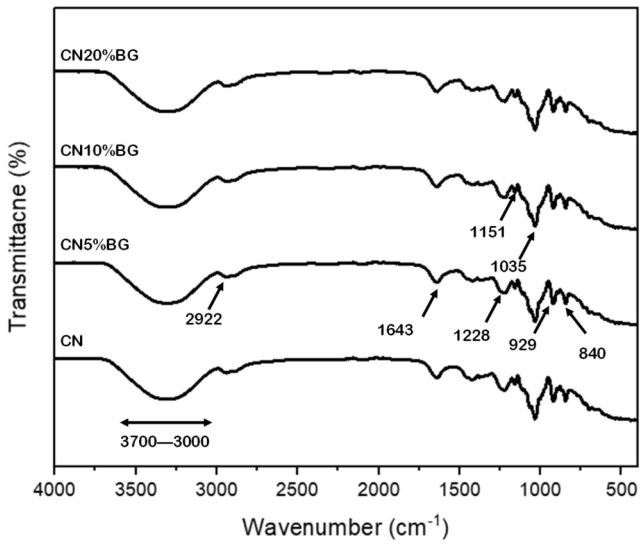

Fig. 3. Fourier Transform Infrared (FTIR) spectra of CN-based film.

Table 2
Result on the mechanical properties of the films.

Sample	Mechanical Properties	
	TS (MPa)	EAB (%)
CN	19.11 ± 1.63[a]	3.05 ± 0.19[a]
CN5%BG	21.07 ± 0.71[b]	9.17 ± 0.25[a]
CN10%BG	22.10 ± 0.76[c]	10.35 ± 0.21[b]
CN20%BG	23.11 ± 0.83[d]	15.03 ± 0.25[c]

Values are mean ± standard deviation. Different letters in the same column indicate significantly different ($p < 0.05$).

3.4. Physical properties of CN active films

In active food packaging applications, physical characteristics of biopolymer-based films including thickness, opacity, water solubility and moisture content plays significant impact to the application on the food quality applied as well representation for consumer choices [2,51]. Table 3 summarizes the findings of CN with BG active film's physical characteristics. The thickness of CN film increases along with the concentration of BG extracts; however, addition of BG extracts exhibits no difference of the thickness between samples ($p > 0.05$). This phenomenon occurs due to the increase in solid content per surface unit of the composite film [53]. Similar findings was reported by Rosa et al., (2020) [54], where thickness of plasticized carrageenan film increases from 0.097 to 0.162 mm when the concentration of olive leaf extracts added increases.

Table 3
The result of the evaluation of physical properties of CN-film.

Sample	Physical Properties			
	Thickness (mm)	Opacity	Water Solubility (%)	Moisture Content (%)
CN	0.096 ± 0.001[a]	6.28 ± 0.05[a]	14.23 ± 1.28[a]	85.01 ± 1.24[a]
CN5%BG	0.106 ± 0.001[b]	3.89 ± 0.01[b]	13.76 ± 1.32[b]	84.89 ± 1.05[b]
CN10% BG	0.126 ± 0.003[b]	4.92 ± 0.01[c]	13.73 ± 0.88[c]	83.68 ± 0.87[c]
CN20% BG	0.134 ± 0.002[c]	5.36 ± 0.02[d]	13.70 ± 0. 95[d]	82.77 ± 0.94[b]

Values are mean ± standard deviation. Different letters in the same column indicate significantly different ($p < 0.05$).

The lower the opacity value, the more transparent the film, making it more appealing and clearer. Highest opacity value been observed for the CN control film compared to other film samples although the differences were not significant ($p > 0.05$). This is due to the presence of cellulose nanofiber (N) as fillers in plasticized biopolymer matrix, which results in lower light scattering and higher transmission in the films due to the strong interaction between N and biopolymer matrix [55]. In contrary, the opacity value of CN5%BG decreases despite the addition of N to the film forming solution. This could be because BG extracts disrupt the polymetric matrix, resulting in a more open structure. The opacity of the film increases as the concentration of BG extracts increases due to the natural yellowish-brown color of the extracts, which inhibits light penetration [2].

Film solubility is used as an indicator to gauge the quality of the films' biodegradability, water resistance, and integrity when used as packaging materials. To improve product integrity and water resistance in some applications, water insolubility is necessary. Incorporation of BG extracts into the film formulation caused the water solubility of the film to decrease. This is due to the strong intermolecular interactions between the CN polymetric matrix and the bioactive compounds in the BG extract, as well as the hydrophobic nature of the extract [52]. Avila et al., (2022) [56] and Souza et al., (2017) [2] mentioned that polyphenol compounds found in natural extracts could bind to the reactive groups of carrageenan and form cross-links via hydrogen bonds or hydrophobic interactions, thereby reducing solubility. De Carli et al., (2022) [52] reported a similar trend for chitosan-based films incorporated with propolis extracts, demonstrating a decrease in solubility as extract concentration increased.

The CN control film recorded highest moisture content and decreases upon the addition of BG extracts. It is possible that the decrease in the moisture content of the CN-active film is due to the cross-linking matrix of carrageenan with the phenolic compound in extracts, which causes a decrease in the polysaccharide polymer's affinity for water molecules and produces films with low moisture content and low solubility in water. This is a crucial feature of edible films used for food protection because it improves the physical properties [57]. Furthermore, it is reported for the first time the use of BG extracts as an antioxidant agent.

3.5. Release test of active compounds in food simulant

Referring to Commission Regulation (EU) No. 10/2011, 95% ethanol food simulant was allocated for food, oil and lipophilic fatty foods. The release test analyzed by the DPPH inhibition and total phenolic content in food simulant for 28 days were illustrated in Fig. 4 a) and b), represent insight antioxidant release manner from active film samples to the food model throughout storages. CN film with 20 %BG demonstrated the highest TPC value and DPPH inhibition during the 28 days followed by lower concentration extracts. Control films without BG extract shows no phenolic released into the food simulant due to the absence of BG as an antioxidant agent in the film, yet there is still a response for TPC and DPPH analysis. This was explained by Rafiquzzaman et al., (2016) [58], who discovered that carrageenan exhibited DPPH scavenging activity due to its hydrogen-donating ability which is dependent on the polysaccharide structure, sulphate (chelating group) and protein content. At Day 0, TPC and DPPH values for all film samples were initially higher due to the presence of phenolic compound embedded at the film's surface. The highest release of phenolic compound was occurred at day 8 and DPPH inhibition at Day 12 for all concentrations and started to decline afterwards. The DPPH inhibition results are actually in contrast to the TPC values at Day 12. According to Munteanu & Apetrei (2021) [59], the antioxidant capacity of phenolic compound depends on several factors such as its chemical structure, the oxidation-prone characteristics, concentration as well as the synergistic effect between the phenolic compounds and the reaction kinetics. The chemical structure of a phenolic compound determines its intrinsic reactivity to free radicals which eventually influences its antioxidant capacity. Table 1 results

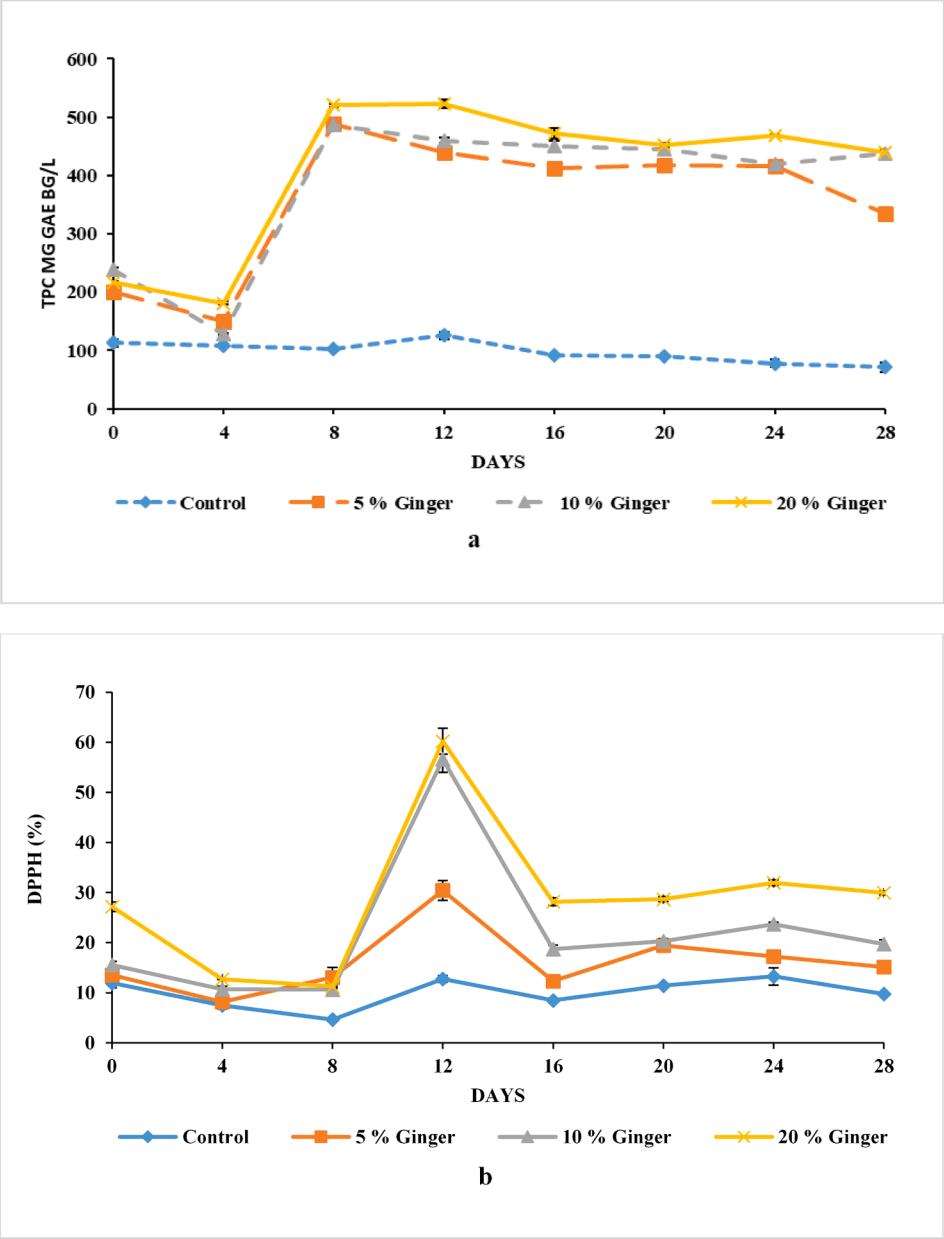

Fig. 4. Changes in a) TPC and b) DPPH values of produced films during 28 days of storages.

shows the present of active compounds in BG extracts are potential agent for its antioxidant activity and presence of phenolic compound. The value reported for this work showed potential active CN with BG extract release in the food simulant, donating the hydrogen atoms to free radicals and reduces the purple DPPH to the yellow colored diphenyl picrylhydrazine. Thus, release of BG extract in CN film indicated throughout storages that caused controlled release manner of active compounds from film matrix due to possible cross-linking between bioactive material and polymer matrix. Luzi et al., (2019) [60] and Hamid et al., (2018) [61], observed similar TPC and DPPH trends for incorporation of gallic acid and quercetin into poly (vinyl alcohol) PVA film formulations over 21 days of storage and incorporation of α-tocopherol into plasticized carrageenan film over 30 days, respectively. Moreover, Ribeiro-Santos et al., (2017) [62] demonstrated quantification of *trans*-cinnamaldehyde and eugenol incorporated in whey protein film that migrated into 95 % food simulants throughout 30 days represent similar trends as this findings. To the best of our knowledge, this is the first report determining the synthesis and characterization of BG extracts to be used as active compounds in active packaging formulation.

4. Conclusion

Identification of bioactive compound in BG extracts through LC-QTOF/MS revealed the presence of 6-gingerol, zingerone, cyclocurcumin, dihydrocurcumin, octahydrocurcumin, 3,4-O-Dicaffeoylquinic acid, eugenol, kukoamine A, xanthohumol and yakuchinone B. The FTIR spectra revealed peaks for –OH and aromatic C = C functional group indicate the integration of bioactive compound into the CN polymer matrix. Mechanical properties (TS and EAB) and physical characteristics including thickness, opacity, water solubility, and moisture content were improved after being treated with BG extract. Additionally, the migration test revealed the release of phenolic compound from film into food simulants and its antioxidant capacity were evaluated through DPPH free radical scavenging activity. Hence, the use of BG extracts as antioxidant additives could be a cutting-edge area for the

development of active food packaging materials that not only to avoid the direct contact of synthetic preservative on to food products but also can prolong its shelf life. Future studies should be conducted on the incorporation of BG extract with other biopolymer material to evaluate its versatility to be classified as a commercial antioxidant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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