Cellulose Nanofiber and Carrageenan Films Infused Eugenol for Food Preservation

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Abstract. This study explores the physicochemical and antioxidant properties of bio-based polymer films made from semi-refined carrageenan (SRC), plasticized with glycerol, and enhanced with eugenol to inhibit lipid degradation in meat patties. The active films were developed using 2% w/w SRC, 0.9% v/v glycerol, 10% v/v cellulose nanofiber (CNF) as a reinforcing agent, and 0.2% and 0.4% v/v eugenol (Eu). The wettability of the films was evaluated using contact angle analysis, while weight loss was assessed through soil burial degradation over a period of four weeks. Additional tests examined the films' swelling and transparency properties. Lipid degradation in meat patties was measured using Thiobarbituric Acid Reactive Substances (TBARS). The CNG-0.4%Eu films showed a contact angle of 109.01º and a swelling rate of 93.44%. The antioxidant films successfully delayed lipid oxidation in meat patties, with final TBARS values of 0.768–0.844 mg malondialdehyde per kg of sample. Therefore, the CNG-0.4%Eu formulation demonstrates potential as an alternative food packaging material to extend shelf life.

1 Introduction

In recent years, synthetic plastic packaging known for being lightweight, durable, and flexible has become the top choice for food packaging worldwide. However, the widespread use of synthetic plastics leads to extensive pollution when they are disposed. These plastics not only contaminate soil and rivers but also release toxic chemicals into the atmosphere when incinerated outdoors [1]. Consequently, a growing interest has emerged in making unique packaging films from natural materials to tackle the rising problem of plastic pollution. This shift towards biodegradable alternatives not only addresses environmental concerns but also presents new economic opportunities by harnessing the potential of

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antioxidants in various industrial applications. Moreover, these innovative active packaging films, aside from being environmentally responsible, offer the added advantage of extending the shelf life of perishable foods through the reduction of oxidation reactions [2, 3].

Among the natural polysaccharides, carrageenan is the most abundant polymer derivative obtained from certain species of red algae. Carrageenan exhibits biocompatibility, excellent film-forming properties and the ability to produce biodegradable films. Carrageenan can be extracted using two different method, refined carrageenan (RC) and semi-refined carrageenan (SRC). SRC, the cheaper option has better binding and gelling properties and suitable material for film formation compared to RC [4]. However, the high hydrophilicity of carrageenan makes it unsuitable for the stand-alone production of films due to its low water barrier and low water content. Glycerol (G) is used as plasticizer to improve the properties of the active films. It improves the flexibility and stretchability of the resulting films, making them more resistant to cracking and improving their overall performance as a food packaging material[2]. To increase the mechanical strength of the active films, cellulose nanofibers (CNF) are also added. Cellulose nanofibers, which are derived from renewable sources such as cellulose and palm waste, have a high aspect ratio and excellent mechanical properties. Their integration into the biopolymer matrix creates a dense and interconnected network of reinforcing fibers. This network structure increases the tensile strength, tear resistance and overall durability of the active films, making them better suited to withstand various packaging challenges [5]. The inclusion of natural antioxidants, such as essential oils or plant extracts, represents an active ingredient that retards oxidation reactions and enhances the shelf life of food products [6]. Previous studies has demonstrated the preservation benefits of adding antioxidants like avocado oil to food product such as pork burgers[7]. Eugenol(Eu), derived from cloves has also emerged as a natural antioxidant capable of forming stable compounds that impede oxidation, preserving the color and freshness of meat patties [8]. Additionally, research involving eugenol-loaded chitosan coatings has shown promising results in safeguarding meat colour [9]. Despite these promising findings, the active film formulations using SRC as a biopolymer CNF as a nanofiller and the incorporation of varying concentrations of eugenol on the rate of degradability, swelling and shelf life of fresh meat has not been fully studied yet. This research aims to fill this knowledge gap by developing, analyzing, and comparing the antioxidative effects of 0.2% and 0.4% (v/v) eugenol incorporated into SRC-based active films. The study will assess their wettability, degradability rate, swelling properties, efficacy in prolonging the shelf life of food products by evaluating parameters such as degradation over 14-day period at 4 ± 1°C.

2 Materials and Method

2.1 Materials

Semi-refined carrageenan range <200 μm powder size were obtained from TACARA Sdn. Bhd. and cellulose nanofiber (CNF) was purchased from UPM Biomass Centre, Malaysia. Glycerol (99%), Eugenol (96%), Thiobarbituric acid, phosphate saline tablet, acetic acid were supplied by Sigma-Aldrich, Gillingham, England.

2.2 Preparation of CNG-Eu of Active Film

Semi-refined carrageenan, SRC (2% w/v) was dissolved in 100 ml distilled water and heated to 60 °C with continuous magnetic stirring. Glycerol, G (0.9 % v/v) was then added and the temperature was maintained for 5 minutes. Then 10% (v/v) cellulose nanofibers (CNF)were added to the solution. The solution was brought to a temperature of 80 °C and held for 15

minutes. Then antioxidant, eugenol, Eu were added at a temperature of 40 °C. Finally, the active film (CNG-Eu) were peeled off the cast plates.

2.3 Contact Angle

The wettability of the samples were assessed by the values of the static contact angle, and the evaluations were performed at constant room temperature and humidity. For this purpose, the sessile drop technique was used with a CAM -101 contact angle measurement system from KSV Instruments Ltd. of Helsinki, Finland. The system consisted of a liquid dispenser, a CCD camera, and drop shape analysis software. To determine the contact angles, 1 µL drops of the test liquids (MilliQ water) were carefully placed on the surface of each sample. A series of images taken at 0.016 second intervals were analyzed using the appropriate software. Measurements were performed in triplicate, and results were reported as mean ± standard deviation. The averaged static contact angle values were then determined by Laplace-Young curve fitting based on the imaged water drop profile.

2.4 Swelling Activity of the CNG-Eu Active Films

Swelling studies of films were carried out in water at room temperature. Samples were immersed in petri dish filled with 40 ml of water solution. At each 60 minutes intervals, films were moved out, and the surface water of films was wiped using filter paper and weighed. The swelling percentage of films is then calculated:

$$Swelling\ (\%) = \left[\frac{Wt - Wo}{Wo}\right] \times 100\% \quad (1)$$

Where W_0 was the initial weight of the films and W_t was the final weight of the swollen films at time. Tests were conducted in duplicate to minimize error and were reported as a mean value.

2.5 Degradability in Soil Burial Degradation

Test were carried out at 25 ± 0.1 °C under moisture-controlled conditions. The films were cut into 3 × 3 cm pieces. They were weighed to estimate their initial weight (W_o), and buried 5 cm deep in the soil. The biodegradation test was carried out for 1 month where the samples were removed from the soil every week, softy brushed and weighed to determine the final weight (W_t). The soil temperature was around 25 °C and the soil was regularly watered to maintain the moisture. The weight loss of the film was calculated according to equation below [10].

$$Weight\ loss\ (\%) = \frac{W_O - W_t}{W_O} \quad (2)$$

2.6 Determination of Antioxidant Activity through Thiorbarbituric Acid Reactive Substance (TBARS)

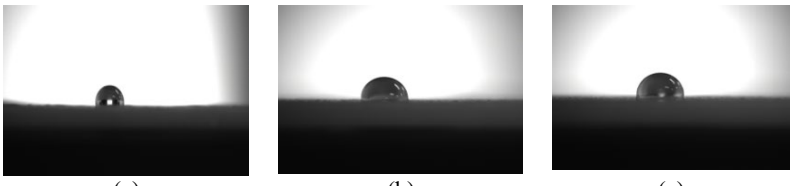
TBARS method was used to determine the extent of lipid oxidation of meat over period of storage. The analysis method is referred from Xu et al. Firstly, 1g of each sample was weighed and put into a test tube to be mixed with 3 g/L of aqueous EDTA. Sample was then

immediately mixed with 5mL of thiorbarbituric acid (TBA) by using homogenizer (Ultra Turrax) for 2 ± 1 minutes with 40,000 rpm speed. Next, the mixture was incubated in 97 ± 1°C of hot water for 10 minutes while vigorously shaken to get a homogenous mixture. Then, mixture is cooled for 10 minutes before centrifuged by using high-speed freezing centrifuge (Eppendorf-refrigerated centrifuge 5810) at 4,000rpm for 10 minutes at 4°C. After liquid sample were recovered, each sample absorbance value was measured by using spectrophotometer at 532nm. The TBARS value were determine by using standard curve from MDA and linear regression method. Sample results is then obtained in mg malonaldehyde per kg sample (mg malonaldehyde/kg sample). All measurement were also carried out in duplicate.

3 Results

3.1 Contact Angle

Fig. 1 shows contact angle images for different types of films. The contact angle is a crucial parameter in assessing the wettability and surface characteristics of a material. Film CNG shows a significantly higher contact angle of 176°, suggesting the liquid barely wets the surface and forms nearly spherical droplets. The introduction of Eugenol (Eu) into film CNG affects the contact angle. As the Eu concentration increases from 0.2% to 0.4%, the contact angle values fluctuate. The contact angle exhibit 91.30° for film CNG-0.2%Eu meanwhile film CNG-0.4%Eu displays a contact angle of 109.01°. This is because Eu consists phenolic hydroxyl group (–OH), which contributes to its polar character. The presence of polar functional groups can influence the intermolecular interactions at the solid-liquid interface. The hydroxyl group in Eu is capable of forming hydrogen bonds with other molecules. In active film, these hydrogen bonds can affect the surface energy and affinity of the film for a liquid. At lower concentrations, the hydroxyl groups could improve wetting by promoting better interaction with the liquid, resulting in a lower contact angle. However, as the concentration of eugenol increases, an excess of hydroxyl groups can lead to the formation of a more polar and less wettable surface. This excess could increase the surface tension of the film, making it less wettable and increasing the contact angle. According Salarbashi et al., 2014 [11], active edible film from soluble soybean polysaccharide (SSPS) incorporated with different concentrations of Zataria multiflora Boiss (ZEO) and Mentha pulegium (MEO) essential oils were developed and its wettability generally increases as well (29.55-79.60°) that make as promising candidate as bioactive film. Besides, a lower contact angle indicates higher wettability, which enhances the film's interaction with the meat surface and improves its barrier properties. This reduction in moisture and lipid migration is essential for controlling lipid degradation, which contributes to rancidity and spoilage. Additionally, eugenol's antimicrobial properties further support the film's effectiveness in preserving meat quality. Thus, optimizing the contact angle helps ensure that the packaging effectively extends the shelf life and maintains the quality of meat products.



(a) (b) (c)

Fig. 1. Contact angle of a)CNG b)CNG-0.2%Eu c)CNG-0.4%Eu

3.2 Swelling

Swelling activity is crucial for film degradability, influencing its breakdown over time. Films with higher swelling rates absorb moisture effectively when in soil, contributing essential nutrients, aligning with environmental sustainability goals. Based on Fig. 2, over time, all films showed increased swelling percentages, with the incorporation of Eugenol into the CNG emphasizing this trend. Incorporating Eugenol into CNG films at various concentrations generally increases the swelling rate compared to the control film. However, higher concentrations (0.4%) result in a decrease in swelling rate due to eugenol's hydrophobic nature, derived from its molecular structure. Lower Eugenol concentrations (0.2%) exhibit a dominance of Eugenol's hydrophilic properties, encouraging water absorption and facilitating increased swelling in the film structure. However, as the concentration of Eugenol increases beyond a certain threshold (0.4%), an interesting phenomenon occurs. The film matrix begins to reach a state of saturation with Eugenol, where the hydrophobic Eugenol molecules become increasingly prevalent within the matrix. This higher concentration of hydrophobic molecules creates a water-repellent barrier within the film, essentially acting as a hydrophobic shield that obstructs the entry of additional water molecules. This hydrophobic barrier effect becomes more pronounced with increasing Eugenol concentration, and as a consequence, it hinders the film's ability to swell further. Water molecules encountering this barrier face resistance when attempting to infiltrate the film matrix, resulting in the observed reduction in swelling rate[12]. In summary, the decrease in swelling rate with higher Eugenol concentrations is a consequence of Eugenol's hydrophobic nature and its capacity to create a hydrophobic barrier within the film matrix. As Eugenol concentration rises, this barrier becomes more prominent, impeding the film's propensity to absorb water and consequently reducing its swelling rate. Addition of plasticizer such as oil into carrageenan polymer lead to significant decrease of films solubility and it has been confirmed[13]. In summary, the data shows that the incorporation of Eugenol into CNG films has an influence on their swelling behavior. Lower concentrations of Eugenol appear to enhance the films' water absorption properties, while higher concentrations may start to counteract this effect, possibly due to Eugenol's hydrophobic characteristics. But since the aim of this experiment is to determine the degradability side of the films, swelling analysis is done to prove if the films swell, since it aids in degradability rate.

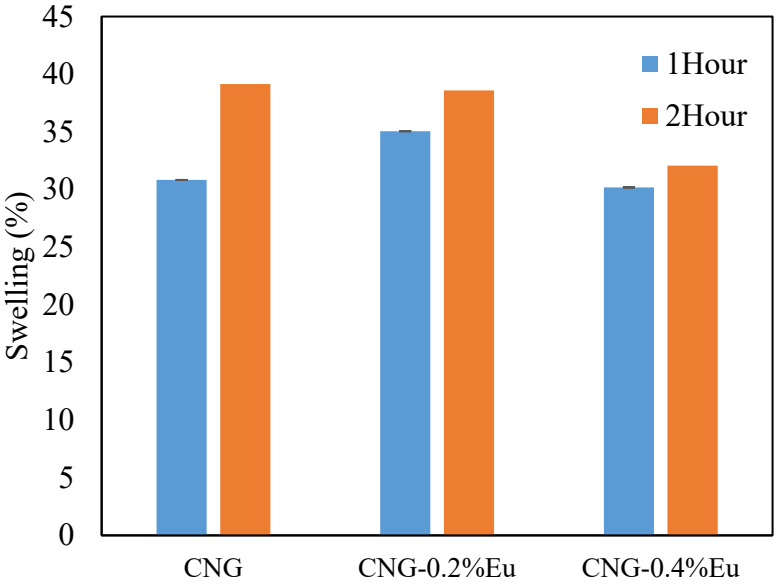
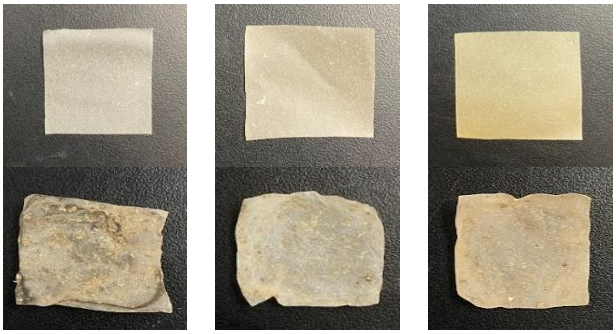


Fig. 2. Swelling of different CNG-Eu active films.

3.3 Degradability in Soil Burial Degradation

The biodegradability test was conducted to assess the degradation of the active packaging film as an indicator of its environmental friendliness. Fig. 3 shows the condition of the formulated films after being buried in control soil for 4 weeks. Due to the natural composition of the films, which are exposed to mechanical degradation (from wind and abrasion), light (photodegradation), and microbiological degradation (from bacteria and fungi), mass loss occurred. Initially, at day 0, the films were 100% intact. By day 28, approximately 48.94% of the original film mass remained, indicating moderate degradability in the soil. Similarly, CNG-0.2% Eu films also showed gradual degradation, with about 47.32% of the original mass remaining by day 28, reflecting a slightly faster degradability rate compared to CNG films. CNG-0.4% Eu films exhibited a relatively faster degradability rate, with around 45.05% of the original mass remaining by day 28. This enhanced degradability is due to the incorporation of eugenol, which has hydrophilic and hydrophobic functional groups that promote water absorption, microbial activity, and chemical reactivity. Semi-refined carrageenan, glycerol, and cellulose nanofibers contribute to degradability through their inherent biodegradable properties. Semi-refined carrageenan, a sulfated polysaccharide, undergoes enzymatic hydrolysis, while glycerol, a polyol, can act as a carbon source for microorganisms. Cellulose nanofibers, composed of repeating glucose units, are degraded enzymatically by cellulases. Together, these components work synergistically in the film matrix, creating a structure that balances water absorption, microbial growth, and chemical interactions, facilitating efficient degradation into environmentally friendly byproducts. According to Carissimi et al. (2018), films with high solubility tend to biodegrade rapidly because their sensitivity to water increases the availability of film structure components for microbial metabolism [14]. This behavior is similar to some agro-based matrices, such as gelatin films, which were reported by Martucci & Ruseckaite (2009) to biodegrade within 10 days.



(a) (b) (c)

Fig. 3. Degradability in soil burial degradation of a)CNG b) CNG-0.2%Eu c) CNG-0.4%Eu during Day 1 and Day 28.

3.4 Lipid Degradation of Meat Patties

Lipid oxidation in meat patties is a natural and intricate chemical process that occurs when fats and lipids in the meat are exposed to oxygen, heat, and other oxidative factors. This process results in the formation of various oxidation products, with malondialdehyde (MDA) being a key byproduct. The amount of MDA can be measured using the thiobarbituric acid reactive substances (TBARS) method, with an acceptable TBARS value set at 1 mg malondialdehyde/kg sample. Fig. 4 illustrates the impact of lipid oxidation in meat patties wrapped in different films over a 14-day storage period at 4 ± 1°C. Lipid oxidation increases over time, with TBARS values rising in the following order at the end of storage: CNG-0.4% Eu < CNG-0.2% Eu < CNG < Plastic < No wrapping. The TBARS value of unwrapped meat patties peaks on the 6th day of storage, surpassing the values of all samples wrapped in the developed films. From day 6 onward, unwrapped meat samples exceed the acceptable TBARS limit, while all other samples encased in the developed films consistently remain below 1 mg malondialdehyde/kg sample, indicating superior lipid preservation. The antioxidant films effectively delay lipid oxidation, with final TBARS values ranging from 0.844 to 0.786 mg malondialdehyde/kg sample, as the antioxidant migrates into the meat, reducing lipid degradation. Eugenol, a phenolic compound, combats the formation of MDA and other oxidative byproducts by acting as an antioxidant. Its phenolic structure, featuring a hydroxyl group (OH) attached to a benzene ring, enables it to donate electrons to neutralize free radicals generated during lipid oxidation. When eugenol encounters free radicals, it donates an electron from its hydroxyl group, stabilizing the radical by pairing its unpaired electron. This electron transfer quenches the radical and prevents further oxidative damage, thus extending the shelf life of meat patties. This is supported by Cheng et al. (2019), which demonstrated that eugenol incorporated with chitosan positively impacts meat shelf life.[15].

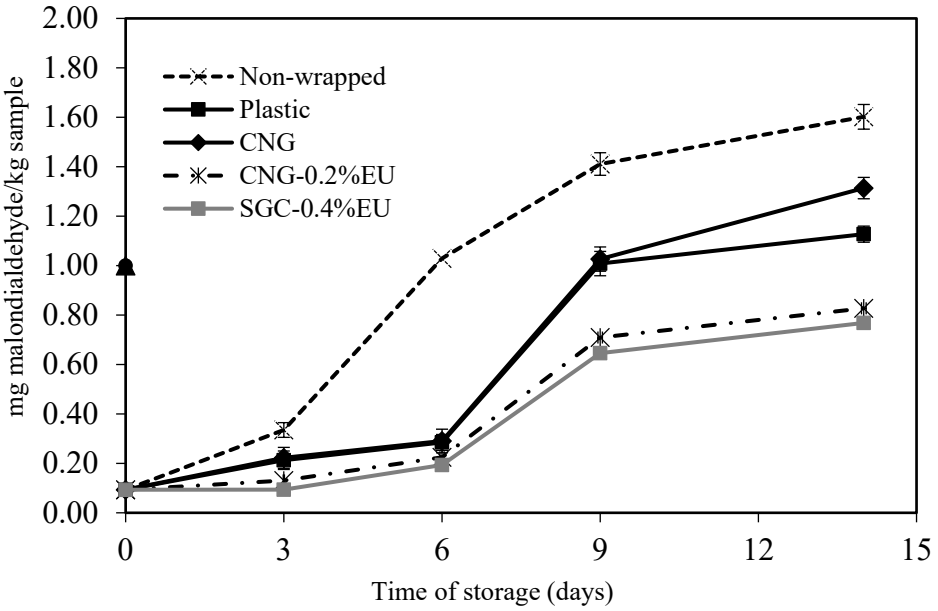


Fig. 4. Lipid oxidation of meat patties that wrapped with CNG with different concentration of eugenol throughout 14 days of storage at 4°C.

4 Conclusion

The inclusion of 0.4% eugenol (Eu) in semi-refined carrageenan films (CNG-0.4%Eu) has demonstrated significant potential as an effective active packaging solution. These films, formulated with 2% w/v CNG, 0.9% v/v glycerol, and 10% v/v cellulose nanofiber (CNF), were thoroughly assessed for properties such as wettability, weight loss, swelling, and lipid oxidation in meat patties. The CNG-0.4%Eu films displayed a favorable contact angle of 126.87º and a swelling rate of 92.81%. Additionally, these films successfully inhibited lipid oxidation in meat patties during storage, as indicated by reduced thiobarbituric reactive substance (TBARS) levels (ranging from 0.646 to 0.782 mg malondialdehyde/kg sample) and less than 50% brown color development over a 14-day storage period. These findings suggest that the CNG-0.4%Eu formulation is a promising alternative for extending the shelf life of food products, particularly in preventing lipid degradation and maintaining the quality of meat patties.

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