Synthesis of Active Hybrid Films Reinforced with Cellulose Nanofibers as Active Packaging Material

Active packaging films derived from renewable biopolymers with an antioxidant formulation are a promising alternative in prolonging food shelf life. This study aimed to develop active hybrid films from semi-refined carrageenan, plasticized with glycerol, incorporating \( \alpha \)-tocopherol, and enhanced with cellulose nanofibers derived from empty fruit bunch as reinforcing agents for improved film function in active packaging. The active hybrid films were characterized for their properties, and the release of antioxidant \( \alpha \)-tocopherol was observed in food simulant and fresh meat. The application of the active hybrid films reinforced with cellulose nanofibers as active packaging for food products is presented.

Keywords: Antioxidant, Active packaging films, Cellulose nanofibers, Semi-refined carrageenan, \( \alpha \)-Tocopherol

1 Introduction

Single-use and non-biodegradable plastics lead to large amounts of waste in the oceans and landfills, which can be harmful to the environment. Many researchers have highlighted studies on how biodegradable polymers produced from renewable and natural plant components such as polysaccharides can help to improve environmental sustainability [1, 2]. Active and ecological packaging is capable of delaying microbial food degradation and prolonging food shelf life, in addition to replacing single-use plastics [3, 4]. Moreover, new active packaging films, such as those made from polysaccharides, are designed not only to have comparable characteristics to plastics, but also to improve the functionality of packaging by adding antioxidants and/or antimicrobial agents (e.g., tocopherol, essential oils) as natural preservatives for foods. This is because the biopolymeric matrices of the film have no specific migration limits, thus allowing release of the antioxidant additive from the active packaging material to the food product [5, 6].

In Sabah, Malaysia, carrageenan, in the form of linear sulfated polysaccharides, is found primarily in commonly harvested brown-red species of seaweed such as *Kappaphycus alvarezi*. Many studies have reported that semi-refined carrageenan (SRC)\(^ {1)\} \) has shown great binding and gelling properties in film production and may be acquired at a reasonable price [7]. Meanwhile, Farhan et al. [6] revealed that addition of glycerol as a plasticizer to the composition enhances the tensile strength and breaking elongation of SRC films, lowering brittleness and increasing flexibility and transparency.

1) List of symbols at the end of the paper.

Natural antioxidants such as \( \alpha \)-tocopherol (Tp) in film formulations have the ability to protect and inhibit lipid oxidation during food storage. Tp is known as a non-hazardous natural antioxidant, shows positive effects toward human health, especially as an anticancer agent, and it lessens the risk of cardiovascular diseases [8]. During polymer processing, adding Tp as an antioxidant coating in packaging is advantageous due to its acting as an excellent stabilizer with high solubility [7, 9]. Abd Hamid [5] \(^ {\text{\textregistered}}\) name and ref. no. do not correspond.\(^ {\text{\textregistered}}\) demonstrated an increase in film strength and barrier properties as well as a protective effect in food storage on addition of 0.4 % v/v Tp as an active and hydrophobic compound in film formulation. The carrageenan-based film is a hydrophilic biopolymer, and previous research has shown that the active film made from SRC has high water solubility (48.60 %) [13], which results in a less efficient film with low water absorption, brittleness, and reduced resistance when in contact with water.

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One strategy to overcome this problem is to introduce nanofillers as reinforcing agents, such as cellulose nanocrystal reinforced polyhydroxyalkanoate-type polymer [10] and cellulose nanofibers (CNFs) [11]. CNFs have a large interfacial area between the matrix and the filler that can be used to develop the mechanical properties of biopolymers [12]. Starch-based films reinforced with CNF have applications as packaging with excellent optical clarity and increased tensile qualities, but polymer nanocomposites containing only nanocellulose are not suitable for active packaging applications [14]. Our previous studies showed that the addition of CNFs at 10 % v/v improved significantly the physical and mechanical properties and film solubility of active hybrid films [15]; however, there are limited studies showing the effect of reinforcing agents such as CNFs synthesized from empty fruit bunch (EFB) in the active packaging application. Thus, the development of active hybrid films containing SRC (2% w/v), plasticized with glycerol (G; 0.9 % v/v), incorporating Tp (0.4 % v/v), and reinforced with various concentrations of CNFs (2, 5, 7, and 10 % v/v) to achieve adequate film characteristics while prolonging antioxidant release in food simulant and fresh meat has not been studied. As a result, the goal of this research was to determine the activity of hybrid films reinforced with various concentrations of CNFs (2, 5, 7, and 10 % v/v) in the release of antioxidant agents from films formulated for food products (meat patties).

2 Methods

2.1 Preparation of Active Hybrid SRC/G/Tp with CNFs

All procedures are described in the Supporting Information.

3 Results and Discussion

3.1 Field-Emission Scanning Electron Microscopy of CNFs Derived from Empty Fruit Bunch

The morphology and average diameter of the CNFs derived from EFB were investigated by field-emission scanning electron microscopy (FE-SEM). In Fig. 1, the size range of the CNFs is 0–50 nm in diameter. The FE-SEM image showed that CNFs stack on top of each other forming an interconnected network of fibers of average diameter 25–50 nm. This finding was supported by Zhang, Barhoum, and Bechelany [16], who found that elementary fibrils or nanofibers produced from woods and tunicates by a mechanical treatment (refining) process were a few micrometers in length and less than 50 nm in diameter. Thus, these results have confirmed the diameter of CNFs.

3.2 Functional Groups of CNF and SRC by Fourier Transform Infrared (FTIR) Spectroscopy

These results are described in the Supporting Information.

3.3 Functional Groups of SRC/G/Tp/CNF Active Hybrid Films with Different Concentrations of CNFs by FTIR Spectroscopy

These results are described in the Supporting Information.

3.4 Thermal Stability of SRC/G/Tp/CNF Active Hybrid Films with Different Concentrations of CNFs

Thermogravimetric analysis (TGA) was conducted to determine the thermal degradation behavior of SRC/G/Tp/CNF active hybrid films and the interaction between carrageenan, CNF, G, and Tp. As shown in Fig. 2a, on heating to 600 °C, two stages were observed for the control film, while three stages of weight loss were seen for films incorporating CNF, G, and Tp. The control curve shows the primary mass loss at 71.40 °C (weight loss: 13.85 %), attributed to the loss of water adsorbed in the film sample. It was suggested by a prior study that control carrageenan shows a weight loss at 80 °C [17]. Meanwhile, the thermal stabilities of SRC films incorporating additives (CNFs, G, and Tp) showed no significant differences. The first stage for active hybrid films was observed at an average temperature of 60–150 °C (weight loss: 14–18 %), which can be attributed to the breakdown of intramolecular and intermolecular hydrogen bonds of water to the polysaccharide structure [18, 19]. In comparison, the decomposition temperatures of
SRC and SRC/G/Tp/10% CNF at the first stage were 71.44 and 83.38 °C, respectively, showing that CNF addition enhanced the thermal stability of the hybrid film samples. This behavior of SRC/CNF/G hybrid films could be attributed to strong cross-linking and bonding with polysaccharide, carrageenan, and CNF molecules, causing increased strength of the film network and resulting in stiffer, more compact films.

The second weight loss stage observed at 140–230 °C (weight loss: 19–23 %) is due to degradation related to the loss of water and glycerol bond structure [7, 18, 20, 21]. The decomposition temperature increased with increasing glycerol content, suggesting that the plasticizer had stabilized the polymer to some extent. In addition, the presence of glycerol facilitates dispersion and leads to a better interaction between polymer and matrix. Furthermore, depolymerization and dehydration of the saccharide rings in the film sample are linked to the loss of weight range found in the second stage [21].

The third degradation stage at 230 °C was attributed to the deterioration of polysaccharides in SRC and CNF in Fig. 2a. This observation of weight loss was linked to the hydrogen bonding via polar interactions to the carboxylate (CNF) and sulfo groups (SRC) [19]. The temperatures of the derivative thermogravimetry (DTG) peaks corresponded to the maximal weight loss of the TGA curve (Fig. 2b). The greatest weight loss for SRC film alone was found at 216.0 °C. The addition of additives in SRC/G/10 % CNF, SRC/G/0.4 % v/v Tp incorporating 2, 5, 7, and 10 % v/v CNF increased the decomposition temperature compared to SRC to 246.67, 243.41, 240.39, 246.77, and 246.15 °C, respectively. These findings revealed that adding CNF to active hybrid films increases their thermal stability. The higher thermal stability is caused by the higher degree of crystallinity of CNF, which requires greater enthalpy (energy) to break down the crystalline structure. Thus, the decomposition temperatures of active hybrid films were higher than that of the control film. Similar findings were reported by Soni et al. [22], who found that the inclusion of CNF enhanced the thermal stability of chitosan film.

3.5 Antioxidant Activity of SRC/G/Tp/CNF Active Hybrid Films

These results are described in the Supporting Information.

3.6 Lipid Degradation Measurements on Meat Patties Wrapped with SRC/G/Tp/CNF Active Hybrid Film

The secondary phase of lipid degradation can be evaluated by thiobarbituric acid reactive substances (TBARS) as milligrams of malondialdehyde (MDA) per kilogram of sample (MDA kg⁻¹). Secondary products of polyunsaturated fatty acids, such as MDA, can be found during oxidation [23], whereby it contributes a rancid scent, modifies the flavor, and produces an unpleasant food taste [24]. The TBARS value for lipid degradation was selected at 1.0 MDA kg⁻¹ as the acceptable limit [25]. Fig. 3 shows the TBARS values for samples wrapped with active packaging film containing different concentrations of CNF over 16 d of storage. Compared to all active films containing Tp,
non-wrapped samples showed the highest rate of oxidation \((p < 0.05)\), followed by samples in film without the inclusion of natural antioxidant, after 4 d of meat storage. Meanwhile, there were no significant differences in TBARS value between active films reinforced with 2, 5, 7, and 10 % v/v of CNF. During 8 d of storage onwards, degradation of the samples without Tp increased rapidly, since there was no protective effect on the food model. In comparison, active packaging film reinforced with CNF exhibited a slow rate of lipid oxidation due to the antioxidant effect on the food model.

A previous study showed that the most commonly used natural antioxidant in diets of ruminants and monogastric animals is Tp. High concentrations of Tp in the cellular membranes results in neutralization of the unstable free radicals produced during storage [26]. The role of Tp is to generate stable molecules that do not degrade once oxidized. This process blocks further oxidative degradation, forming, e.g., MDA, which can be determined by the TBARS method [7]. However, in comparison to other samples, SRC/G/Tp/10 % CNF showed the lowest TBARS value at 16 d of storage. This is because CNF from EFB contains a small portion of oil, and it is currently thought that some of the phytonutrients contained in the oil are maintained in the EFB [27]. A previous study has shown that EFB extracts are also rich in the phenolic compound 4-hydroxybenzoic acid, which displays antioxidant activity; 2,2-diphenyl-1-picrylhydrazyl (DPPH) analytical studies on sunflower oil reported extended shelf life [28]. Since CNF synthesized from EFB contains a small amount of phenolic compound 4-hydroxybenzoic acid (antioxidant), CNF reinforced \(\alpha\)-tocopherol by a synergic effect of antioxidant release to the food model. This is the reason why the active film with 10 % CNF shows good antioxidant effects towards the food model, as measured by TBARS and metmyoglobin analysis. The results here suggested that SRC/G/Tp/10 % CNF exhibited the lowest amount of lipid oxidation by TBARS. On the other hand, non-wrapped and SRC showed no significant difference \((p > 0.05)\) with the highest TBARS value of > 1 MDA kg\(^{-1}\), resulting in the rancidity that is responsible for the unacceptable taste and off-flavors that are important factors in rejection by the consumer.

### 3.7 Brown Color Development of Meat Patties Wrapped with Active Hybrid SRC/G/Tp Having Different Concentrations of CNF

The red color of meat is the main trait indicating the freshness of the meat patties to consumers. Previous research found that the change in red color is directly proportional to the rate of oxidation determined by TBARS [29]. Three major types of myoglobin, namely deoxymyoglobin, oxymyoglobin, and metmyoglobin, are responsible for the characteristic color of fresh meat [30]. Metmyoglobin accumulation is considered to be the cause of meat discoloration to a brownish color. Therefore, the formation of brown color is analyzed by percentage metmyoglobin, which indicates the correlation of oxidation rate to the formation of metmyoglobin in the meat patties during storage [31].

In this study, all samples experienced a decrease in percentage metmyoglobin throughout storage, as illustrated in Fig. 4 \((p < 0.05)\). Non-wrapped patties showed the highest percentage metmyoglobin compared to wrapped samples \((p < 0.05)\). During day 4, all SRC-film-wrapped samples showed no significant differences in percentage metmyoglobin and there was no substantial difference in color between the samples \((p > 0.05)\). After 4 d of storage, metmyoglobin formation increased significantly \((p < 0.05)\). Control and non-wrapped SRC showed higher percentages of metmyoglobin formation compared to wrapped samples, whereby the metmyoglobin concentration had risen to almost 30 % over the next 8 d of storage. Throughout the 8 d of meat storage, meat patties wrapped with 0.4 % (v/v) Tp active film showed a low degree of brown coloration \((p < 0.05)\). At 16 d of storage, non-wrapped patties exhibited the highest metmyoglobin formation, with a value of 46.8 %, while SRC/G/Tp/10 % CNF displayed the least brown color (39.91%), for which the metmyoglobin content was less than 40 % \((p < 0.05)\). This result was due to the synergic antioxidant effect between 10 % CNF and Tp, which delayed lipid oxidation and maintained the color of the meat patties [32].

![Figure 4. Percentage metmyoglobin assays of meat patties wrapped with SRC/G/Tp/10 % CNF for 16 d of storage at 4 ± 1 °C.](image)

Besides, incorporation of CNF in the active film showed that CNF itself did not prevent release of antioxidants to the food model, as the SRC-CNf film showed significantly \((p < 0.05)\) lower metmyoglobin formation \((39.61–41.76 \%)\) compared to SRC \((43.51 \%)\) and SRC/G \((42.94 \%)\). Consumers deem meat samples acceptable if the metmyoglobin percentage is less than 40 %, according to Borzi et al. [31]. Several authors have corroborated this finding, observing that the decreased color of muscle tissue is influenced by the decreased amount of metmyoglobin during preservation [33]. Lipid oxidation in meat products generates free radicals, which are capable of initiating the oxidation reaction of oxymyoglobin (reddish) to metmyoglobin (brownish) and resulting in meat discoloration during storage. Antioxidant coating can mitigate metmyoglobin oxidation by scavenging hydroxyl free radicals generated in the
oxidation of oxymyoglobin and decreasing the percentage met-myoglobin value analyzed in meat during storage. A previous study showed that chitosan incorporating green tea extract was able to delay myoglobin oxidation in meat products, as green tea extract contains phenolic compounds that are capable of reducing lipid radicals [34].

4 Conclusion

The interaction between SRC, glycerol, α-tocopherol, and CNFs was successfully demonstrated by FTIR spectroscopy. The incorporation of CNF into SRC film greatly affected the thermal stability of the film. Therefore, the results show that the incorporation of CNF, α-tocopherol, and glycerol could influence the thermal stability of the developed film. The addition of α-tocopherol to the matrix led to release of antioxidant during 31 d of storage and could be used in food packaging applications to prolong food shelf-life and hinder the oxidation process. The addition of various concentration CNFs to the active films showed no significant effect on the release of antioxidant into food simulant. As a result, the SRC-based film incorporating 0.4% α-tocopherol with 10% CNF and plasticized with 0.9% glycerol exhibited good film properties while optimizing the physical and mechanical characteristics, hence offering a potential alternative for degradable packaging to provide continuous release of antioxidant agents for food protection.

Supporting Information

Supporting Information for this article can be found under DOI: 10.1002/ceat.202100366. This section includes additional references to primary literature relevant for this research [35–48].

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Abbreviations

- CNF: cellulose nanofiber
- DPPH: 2,2-diphenyl-1-picrylhydrazyl
- DTG: derivative thermogravimetry
- EFB: empty fruit bunch
- FE-SEM: field-emission scanning electron microscopy
- FTIR: Fourier transform infrared
- G: glycerol
- MDA: malondialdehyde
- SRC: semi-refined carrageenan
- TBARS: thiobarbituric acid reactive substances
- TGA: thermogravimetric analysis
- Tp: α-tocopherol

References

Research Article: Active food packaging was developed by reinforcing films made from semi-refined carrageenan derived from seaweed with cellulose nanofibers made from empty fruit bunch to improve thermal properties and incorporating α-tocopherol as antioxidant. Release of the antioxidant was studied in a food simulant and fresh meat and found to delay lipid oxidation of the food product during 16 d of refrigeration.

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