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Effect of Edible Coating Based on Chitosan as Polyelectrolyte Complex Film on Vitamin C Loss at Pineapple Fruit During Storage

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Abstract. The high consumption of fresh fruit encourages the development of studies on safe food coatings, such as hydrocolloid-based edible coatings. In this study, a complex polyelectrolyte based on chitosan (chi) was synthesized and applied as a coating for fresh pineapple. As a cationic polymer, chitosan interacts with anionic polymers, such as carrageenan (crg) and alginate (alg), to form complex polyelectrolyte films without involving crosslinkers. The effect of film coating on the loss of vitamin C and the weight of sliced pineapple at various storage periods was determined. Biodegradability assays and FTIR analysis of films were also studied. The FTIR spectra of the films confirmed the formation of alg-chi and crg-chi complex polyelectrolytes through the interaction of carboxylic groups (alg) and sulfate (chi) with protonated amines from chitosan, at pH 5.28. The alg-chi and crg-chi edible films could maintain the stability of the vitamin C levels and inhibit the weight loss rate in sliced pineapple. The biodegradability test showed that both films had a high % biodegradability. The alg-chi film is more accessible to decompose than the crg-chi film due to the more hydrophilic alg-chi film. Therefore, chitosan-based polyelectrolyte film is an excellent candidate for application in the food coating industry, especially for fresh fruit products.

Keywords: edible coatings · chitosan · film · vitamin C · pineapple

1 Introduction

Pineapple is a type of fruit from the *Bromeliaceae* family with the scientific name *Ananas Comosus Merr*, an exotic tropical fruit because it has a distinctive colour, taste, and aroma and is widely consumed as fresh as cut fruit. In general, pineapple is in demand for fresh consumption with a combination of sweet and slightly sour tastes, which can maintain the vitamin and enzyme content well. In addition, it can be processed through grading, washing, peeling, slicing, and packaging, reducing vitamin levels in pineapples [1], especially vitamin C. The most widely used commercial method is storage at low temperatures, this can reduce the impact of vitamin C loss [2], but this method cannot be

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applied to itinerant vendors selling their wares with refrigerators. The wax coating has been widely applied to fruits and vegetables to solve this problem; the coating is usually made from natural ingredients (non-petroleum-based) and is safe to use for all types of food with GRAS (Generally Recognized as Safe) status. According to the BPOM (Badan Pengawas Obat & Makanan) and the FDA (Food & Drugs Administration), the ingredients used are bee wax, candelilla wax, carnauba wax, shellac, and microcrystalline wax, but most people are hesitant to consume the fruit or vegetable coated with the wax directly. Based on these considerations, hydrocolloid-based edible coatings have been developed.

Hydrocolloids can be used as additives to improve the quality of food products and are implemented in the manufacture of edible coating; these have advantages in mechanical properties and an excellent ability to protect products by inhibiting the transfer of oxygen and water vapour [3]. Chitosan (chi) is a hydrocolloid known as a biodegradable film and food preservative, which has antibacterial properties due to the polymer structure of chi having a positively charged amine group. It is also elastic and edible, extends fruit shelf life, reduces aroma loss, and slows discolouration, gas transfer, and vitamin C degradation. However, chi is weak in washing under high acid and water absorption conditions. The formation of a complex polyelectrolyte film can overcome this weakness.

In this work, chi-based polyelectrolyte complex film (as polycationic) was applied as an edible coating [4, 5], where this film was made using alginate (alg) and carrageenan (crg) as polyanionic. Polyelectrolyte complex, which is formed from chi-alg or chi-crg utilizes electrostatic interactions between these ionic polymers so that the use of crosslinking agents that may be toxic can be avoided [6, 7]. Polyelectrolyte complex films synthesized without other toxic materials meet the criteria for edible coatings; this is supported by the dense structure of the film, which can inhibit the damage process by blocking inhalation or reaction with oxygen. The simple and easy technique of making complex polyelectrolyte films is suitable for application in large-scale companies. Chi-alg [8] or chi-crg [9] film produced not only functions as a barrier (protective) but also has antimicrobial substances that function as inhibitors of microbial activity, maintain fruit freshness during storage, and keep vitamin C from degradation (oxidation). In this study, we will determine the effect of coating a chi-alg or chi-crg on vitamin C loss and weight loss of sliced pineapple at various storage periods, biodegradability, and FTIR analysis of the film.

2 Materials and Methods

A. *Materials*

Films were prepared from chi from crab shells (72 mPa.s with 95% degree deacetylation) and sodium alg from brown algae (45.3 mPa.s) or crg from red seaweed (6.4 mPa.s) obtained from Sigma- Aldrich (UK). Ascorbic acid, glacial acetic acid, sulfuric acid, hydrochloric acid, sodium hydroxide, and ammonium molybdate are received from Merck (UK). The solution was prepared with deionized water. Pineapple was obtained from the local bazaar at Mataram, Indonesia.

B. *Synthesize chi-crg Edible Film*

Chi-crg films were synthesized following a previous study by Ismillayli et al. [7] by mixing the two polymers at a 1:1 mass ratio. Initially, it was to make chi hydrosol and crg hydrosol for chi hydrosol (0.5 g of chi in 12.5 mL of deionized water and 2.5 mL of 10% v/v acetic acid). In comparison, crg hydrosol (0.5 g crg in 12.5 mL deionized water by heating to 60 °C) was prepared under stirring conditions at 500 rpm (for 24 h). Into a 1 L Pyrex media bottle (Fisher Scientific, UK), put the crg hydrosol while stirring at 500 rpm, then add chi hydrosol dropwise (approximately 14 ml/min). Then, the mixture of the two hydrosols is cooled using a water bath at 25 °C for 15 min, followed by measuring pH using a pH meter (TOA Electronic Ltd model IM -20E).

C. *Synthesize chi-alg Edible Film*

The chi-alg film was synthesized following a previous study [6]. Briefly, chi hydrosol (0.5 g of chi in 12.5 mL of deionized water and 2.5 mL of 10% v/v acetic acid), while alg hydrosol (0.5 g of sodium alg in 12.5 mL of deionized water) was prepared under stirring conditions at 500 rpm (for 12 h). Then, the alg and chi hydrosol was left overnight (12 h) to remove air bubbles. Into a 1 L Pyrex media bottle (Fisher Scientific, UK), put the alg hydrosol while stirring at 500 rpm, then add the chi hydrosol and add 1 mL of HCL 32% (w/v) at 25 °C, homogenized using a homogenizer (IKAT18Ultra Turrax, Germany) for 90 s, then added NaOH 10% (w/v) until the pH of the suspension was raised to 5.28 and homogenized again for 90 s.

D. *Coat Sliced Pineapple Using Edible Film*

The mixture of chit hydrosol with both crg or alg was coated on pineapple pieces by dipping fresh pineapple pieces measuring 1 × 1 × 1 cm into both hydrosols for 30 s and dried using a fan. The fruit pieces that have been coated are placed on styrofoam and covered with plastic wrap. All samples were stored at the chiller for 6 days. At the same time, the stability test was carried out by keeping the sample at 4 °C for 1, 5, 24, 72, and 144 h.

E. *Determination of Vitamin C Levels in Sliced Pineapples*

Samples of chopped pineapple were weighed and crushed to form a slurry, then put into a 100 mL volumetric flask added, deionized water was to the mark, and shaken until homogeneous. The pineapple extract solution was pipetted 1 mL and put into a 10 mL volumetric flask. Then add 4 mL of 5% sulfuric acid and 5% ammonium molybdate to the mark and shake until homogeneous, after which the solution was incubated for 30 min [10]. The absorbance of the solution was measured at a maximum wavelength of 660 nm using a visible spectrophotometer (Faithful instrument (Hebei) Co. Ltd). Replication was carried out three times. Vitamin C levels were calculated using the standard curve of ascorbic acid.

F. *Determination of Weight Loss in Sliced Pineapples*

Weight loss of sliced pineapple fruit coated with the edible film was determined by comparing % weight loss of sliced pineapple fruit coated with edible film with variations in storage time and temperature with the weight loss of sliced pineapple fruit without being coated. Weight loss was calculated from the difference in the initial weight (W_0) of sliced pineapple on day 0 with the final weight (W_t) of sliced pineapple after storage using Eq. 1

$$\%\text{weightloss} = \frac{\text{Wt} - W_0}{W_0} \times 100\% \quad (1)$$

G. *Biodegradation Test and FTIR Spectra*

The biodegradability test was carried out by burying the film in the soil (Eq. 2) film had been prepared previously on a 10 × 10 cm^2 glass plate and dried at room temperature overnight for 2 × 24 h. W_0 is the initial weight, and W_t is the final weight of the film.

$$\%\text{biodegredability} = \frac{\text{Wt} - W_0}{W_0} \times 100\% \quad (2)$$

FTIR Characterization of Edible Film using an FTIR spectrophotometer (Shimadzu Prestige-21). The functional groups on the edible film were characterized using an FTIR spectrophotometer with the pellet method KBr at a compression pressure of 2500 Ib/m^2.

3 Results and Discussion

A. *Edible Film Synthesis*

For this purpose, the edible coating based on chi as a polyelectrolyte complex film is formed from a mixture of chi hydrosol with alg or crg. Chi is insoluble in distilled water but can be dissolved in an acidic environment. A carboxyl group in the acid (acetic acid used) will facilitate the dissolution of chi due to hydrogen interactions between the carboxyl group and the amino group of chi [11]. The amine group in chi accepts H^+ released by acetic acid so that the amine becomes positively charged (NH_3^+).

The optimal mixing of chi hydrosol and alg hydrosol was at a pH of about 5.28. It is because alg cannot dissociate at low pH and will not form polyanions due to the lack of distorted carboxylate anion (COO-) [12]. At pH 5.28, the carboxylate group of alg was present in the form of a carboxylate ion. In contrast, the NH_2 group of chi was protonated to form an edible film of chi-alg. The edible film produced at pH 5.28 is more stable and robust (not easily torn), as the results of previous studies [12, 13]. At the same time, chi hydrosol and crg hydrosol are optimal at a pH of around 5. It is because crg cannot dissociate at low pH and will not form polyanions due to the lack of distorted sulfate anion (-OSO_3^-). At pH 5, the sulfate group of crg was present in sulfate ions, while the NH_2 group of chi was protonated to form a chi-crg edible film [15]. The edible

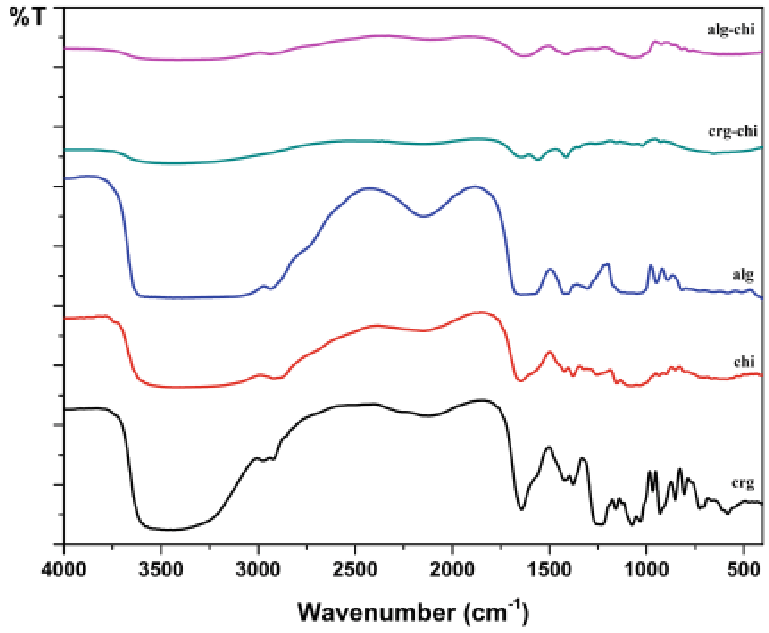

Fig. 1. The FTIR spectra of chi, crg, alg, crg-chi, and alg-chi film

film produced at pH 5 is more stable and has excellent physicomechanical properties, as was the result of previous studies [7].

Figure 1 shows the FTIR results of the alg, chi, and alg-chi films. The absorbance at 3564.4 cm^{-1} indicates the presence of O-H groups from alg. The chi spectrum shows that at wavenumber 3467.08 cm^{-1}, it means an O-H stretching vibration that overlaps with N-H and is strengthened by the appearance of a wavenumber at 1555.39 cm^{-1}. At wavenumber 1639.05 cm^{-1}, it can be indicated as C=O vibration on chi. The spectrum of the alg-chi edible film showed absorption at wave number 3354 cm^{-1}, indicating the presence of OH groups from alg and NH groups from chi. Absorption in the 1639.2 cm^{-1} regions indicates the presence of C=C and C=O groups. The absorption loss at a wavenumber of 1157 cm^{-1} indicates that the amine functional group in chi has protonated and interacted with the hydroxyl group of alg. It is indicated by the appearance of absorption at 1559.9 cm^{-1}, which is the vibration of the protonated amine group (NH_3^+). Absorption at wave number 1346.08 cm^{-1} indicates the presence of carboxylic groups and the occurrence of electrostatic interactions to form alg-chi edible films. It is to previous studies [12, 13], which stated that protonated amine groups and COO- groups in alg-chi edible films appeared in areas 1550 and 1398 cm^{-1}.

Figure 1 shows the FTIR results of the crg, chi, and crg-chi films. The presence of the O=S=O sulfate ester group at wavenumber 1232.28 cm^{-1} gives the characteristics of the formation of a crg film. This is consistent with Paula [16], stating that films containing crg provide a characteristic band in the wave number region of 1215 cm^{-1} associated with O=S=O asymmetric stretching. Wavenumber 1028.59; 933; and 851.22 cm^{-1} indicate the presence of a glycosidic bond (C-O-C); 3,6–anhydrogalactose; and galactose 4 sulfate. Meanwhile, the chi film shows three peak characteristics at wavenumber 1639.05; 1423.88; and 1071.41 cm^{-1}, indicating the presence of C=O stretching, N-H bending (N-acetylation), and the characteristic C-O stretching wavenumber for chi. To prove the

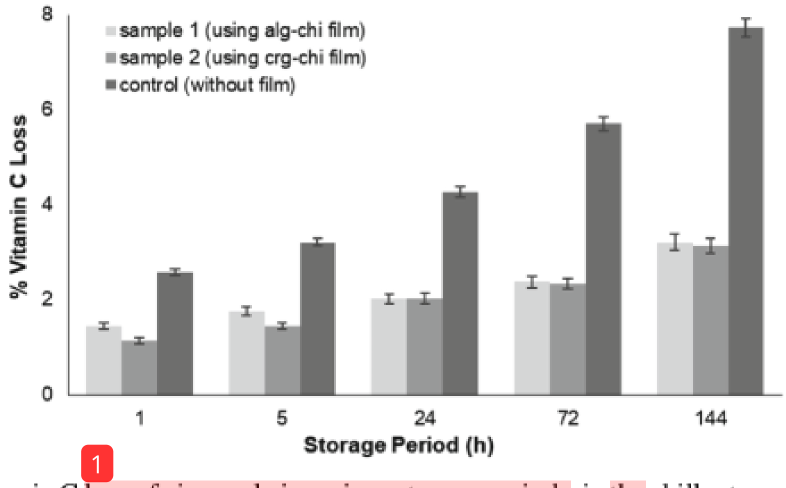

Fig. 2. % Vitamin C loss of pineapple in various storage periods, in the chiller temperature, ranges from 2 to 6 °C.

existence of ionization to form a crg-chi complex polyelectrolyte, namely, the presence of a typical chi absorption peak at wavenumber 1641.25 cm^{-1} indicates the amino functional group, and the crg peak 1414.40 cm^{-1} indicates the -OSO3- functional group. The wave number of 1559.97 cm^{-1} indicates an electrostatic interaction between the sulfate ion group from crg and the amino group from chi. Absorption of 1641.25 cm^{-1} is the combined vigorous intensity of crg-chi. The formation of the edible film crg-chi at 1251.84 cm^{-1} and the loss of the absorption band at 1148.7 cm^{-1} indicated that the amine group was protonated from chi and interacted with the sulfate group from crg. The appearance of a peak in the 1556.1 cm^{-1} area indicates the presence of NH_3^+. The electrostatic crg-chi interaction can be seen from the absorption in the area 1208.2 cm^{-1} [7, 16].

B. *Determination of Vitamin C Loss in Sliced Pineapples*

Alg-chi and crg-chi films that have been made are used to coat the cut pineapple to protect the levels of vitamin C contained therein. Determination of vitamin C levels of pineapple was carried out on pineapples coated with edible film (sample) and those not coated (control) to determine the effect of the edible film alg-chi and crg-chi on pineapple vitamin C levels. The results of determining the levels of vitamin C in pineapple can be seen in Fig. 2.

Based on Fig. 2 shows that the reduction in vitamin C levels of pineapple samples during storage periods of 1, 5, 24, 72, and 144 h experienced a not-too-significant increase. In comparison, the reduction in vitamin C levels of control pineapples increased and was far different from the reduction in vitamin C levels in the sample. It indicates that the alg-chi and crg-chi edible films could maintain the stability of the levels of vitamin C in sliced pineapple. The alg-chi (sample 1) and crg-chi (sample 2) films used to control the loss of vitamin C from sliced pineapple showed that the two films were not much different. Both films could inhibit the rate of reduction of vitamin C levels in fruit. Both edible films can block the oxidation process from outside air and light [2, 17] and keep the fruit from releasing water. Here the chiller temperature also has an effect. Vitamin

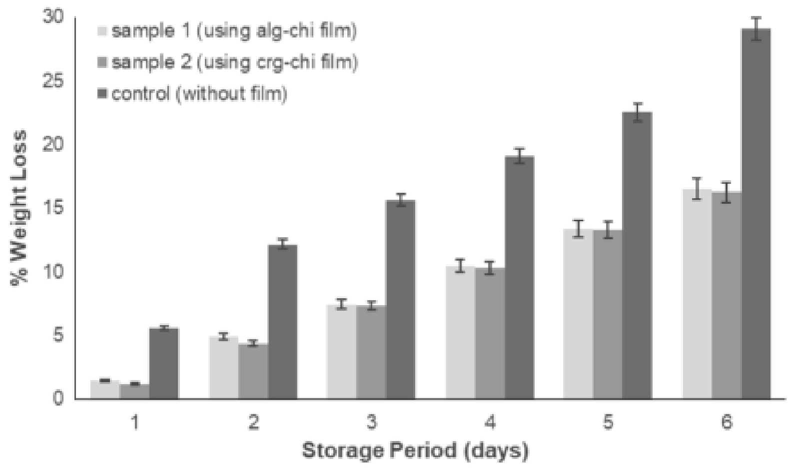

Fig. 3. % Weight loss of pineapple in various storage periods in the chiller temperature ranges from 2 to 6 °C.

C dissolves easily in water, so maintaining water loss will inhibit the rate of reduction of vitamin C levels in fruit. The density of the film is responsible for this. Both alg-chi film and crg-chi film, the two films in forming a film through electrostatic interaction allow the two films to have tiny pores, and application to fresh fruit can be considered.

C. Determination of Weight Loss in Sliced Pineapples

Alg-chi and crg-chi films that have been made are used to coat the pineapple slices to protect against weight loss. Determination of % weight loss of pineapple was carried out on pineapples coated with edible film (sample) and those not coated (control) to determine the effect of edible film alg-chi and crg-chi on pineapple weight loss. The results of determining the weight loss in pineapples can be seen in Fig. 3.

Based on Fig. 3, it can see that the decrease in pineapple weight loss (sample) during the 1st, 2nd, 3rd, 4th, 5th, and 6th days of storage experienced a not-too-significant increase. In contrast, the decrease in pineapple weight loss (control) increased, and the longer the storage time, there decrease in weight loss, even more, and much different from the decrease in weight loss in pineapple (sample). It shows that the alg-chi and crg-chi edible films can maintain the stability of the weight of the pineapple slices. The alg-chi (sample 1) and crg-chi (sample 2) films used to control the weight loss of pineapple slices showed that the two films were not much different. Both films were able to inhibit the rate of weight loss in fruit. Weight loss during fruit storage is predominantly caused by respiration and water loss due to transpiration, dehydration, and metabolic activity [19]; it can be prevented by covering the fruit with edible film. Here the chiller temperature also matters, whereby a lower percentage of weight loss is achieved at cooler temperatures due to lower transpiration rates and reduced moisture loss. The longer fruit storage period consequently increases % weight loss. The density of the film is responsible for this. Both the alg-chi film and the crg-chi film, the two films form a film through electrostatic interaction; this allows the two films to have tiny pores, increases the hydrophobicity of the film, and enhances the barrier effect against moisture loss [20]; thus, its application in fresh fruit can be considered.

Table 1. Edible film biodegradability test results

Burial time (days)	*% Biodegradability*	
	Film Alg-Chi	*Film Crg-Chi*
7	57.87	36.19
14	69.00	59.52
21	75.20	67.14
28	83.00	76.19

D. *Biodegradability*

The biodegradability test of edible film alg-chi and crg-chi was evaluated by determining the weight loss through the film being buried in the soil for 7, 14, 21, and 28 days, as shown in Table 1.

The amount of degraded film increased with increasing burial time, as shown in Table 1. The highest % of biodegradability was 83% at 28 days for alg-chi films and 76.19% at 28 days for crg-chi films. Although both films have a high % biodegradability, the alg-chi film has higher % biodegradability than the crg-chi film due to the more hydrophilic alg-chi film. The high % biodegradability is caused by the loss of protonated amines, carboxylic groups, and =CN functional groups [6, 7]. It indicates that the functional groups containing oxygen and nitrogen degrade well through the buried soil, so these groups are responsible for the degradation of the film.

4 Conclusion

The alg-chi and crg-chi complex polyelectrolytes have been successfully synthesized by mixing cationic polymers (chi) and anionic polymers (alg and crg) at pH 5.28. FTIR analysis showed the interaction of protonated amine-carboxylic groups and protonated amine-sulfated groups as prominent linkages in both polyelectrolyte complexes. Alg-chi and crg-chi film coatings maintained vitamin C levels, and the weight of pineapple slices was better than without coating until the sixth day of storage. In addition, the two polyelectrolyte complex films showed easy decomposition with a higher % biodegradability of the alg-chi film than the crg-chi film due to the more hydrophilic alg-chi film. Hence, both films can be applied in the large-scale fresh fruit coating industry.

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PAGE 4

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PAGE 7

PAGE 8

PAGE 9

PAGE 10