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Development of Antioxidant Edible Films Based on Chitosan Enriched with Dragon Fruit (Polyrhizus Hyloroceus) Peel Extract

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Abstract. Formulation and characterization of chitosan-based edible film with the fortification of dragon fruit peel extract (DFPE) were investigated. The effect of the addition of DFPE on the characteristics of edible films was observed through the parameters of antioxidant activity, thickness, water vapor transmission rate, mechanical properties, FTIR, and biodegradation. The antioxidant activity of edible films was tested by the DPPH radical inhibition method. The results showed that increasing the concentration of DFPE increased the value, the rate of water vapor transmission, antioxidant activity, and the rate of biodegradation but decreased the tensile strength. The elongation of the DFPE edible film has a better value (maximum 22,385 ± 7,196%) compared to the control of edible film (14,545 ± 1,208). This study shows that the addition of DFPE into chitosan-based edible films can be developed as active packaging.

Introduction

The development of food packaging with eco-friendly materials is one of the alternatives to reduce negative impacts of synthetic packaging that contributed to ecological problems. Research data shows that the waste from plastic food packaging, especially thin packaging is estimated to reach 22 million tons in 2015 and will continue to increase (Ebnesajjad, 2017). One type of packaging that extensively studies and continues to develop is edible film.

Edible films are thin layers of material coated or wrapped around food, that are made from edible biopolymers and additional food materials (Falguera et al., 2018). The advantages of edible films as alternative packaging are protection of food products from contaminants, safe for consumption, and biodegradable (Boutoom, 2008). Edible film can be synthesized from several biopolymers such as protein, polysaccharide, lipids, and composites (Boutoom, 2008). Polysaccharide that are mostly used as the main material to produce edible films are cellulose-derivative, pectin-derivate, seaweed extract, starch and chitosan (Krochta and Mulder-Johnson, 1997; Mehdizadeh et al., 2012).

Chitosan are natural linear polysaccharide compound derived from chitin. Chitosan is known as a versatile biomaterial because it has several characteristics including nontoxic, low allergenicity, biocompatibility and biodegradability (Cheung et al., 2015). Films that are made from chitosan have strong, durable, and flexible characteristics (Butler et al., 1996), but the use of a single ingredient in edible films such as chitosan only has drawbacks, such as brittle and stiff. Therefore, additional materials are needed like plasticizers and a combination of polymers such as starch, which can increase the homogeneity of the film matrix so that it can improve the mechanical characteristics of edible films (Elsabee and Abdou, 2013). The use of plasticizers as additional material in film can increase the permeability, tensile strength, and resistance of the film (Garcia et al., 2010; Souza et al., 2012).

The addition of antioxidant compounds into edible films has been widely studied because oxidation is one of the problems that affect food quality (Siripatrawan and Harte, 2010). The continuous addition of synthetic antioxidant compounds has health risks, therefore natural source materials are needed which tend to be safer for consumption. Alternative natural ingredients can be obtained from extracts derived from plants such as fruits, vegetables, seeds, stems and roots (Lim et al., 2007).

Dragon fruit is a popular fruit in Indonesia and widely cultivated (Muas et al., 2019). Dragon fruit is only consumed

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by the flesh, while the skin of the fruit which makes up about 30-35% of this fruit is not further utilized and becomes waste (Lou et al., 2014). Nurliyana et al. (2010), reported comparison of the antioxidant activity between the flesh and the skin of the dragon fruit, the results showed that the phenolic content of the skin of the dragon fruit was higher than that of the flesh. Red dragon fruit peel has antioxidant activity because it has flavonoids, phytoalbumin, thiamine, niacin, pyridoxine, cobalamin, phenolic, betacyanin, polyphenol and carotene (Jaafar et al., 2009). Application of waste from dragon fruit has advantages not only as an alternative to natural antioxidants but also from an ecological perspective.

Experimental

Materials and Method

Materials used in this study consisted of chitosan with degree of deacetylation of 70-80%, dragon fruit peel, maize starch, ethanol, ascorbic acid, DPPH, glycerol, KBr, HCl p.a, dragendorff reagent, distilled water, H_2SO_4, , $FeCl_3$.

Preparation, Extraction and Phytochemical Screening

Dragon fruit peels were prepared by washing and cut into small pieces. After drying, the peels were ground using a blender to get fine powder. Dragon fruit peel powder then macerates using two kinds of solvents that are distilled water and 70% ethanol in 1:15 (w/v) ratio for sample and solvents. The mixture was kept in the dark for 5 days, with string every 12 hours. The solution was filtered and evaporated in rotary evaporator. The extraction process was doing remaceration 3 times. The extract was stored in a desiccator and preserved in dark condition for further usage (Lourith and Kanlayavattanakul, 2013). The extracts then analyzed the phytochemical contents for flavonoid, alkaloid, saponin, terpenoid, steroid and tannin (Mujeeb et al., 2014).

Antioxidant Activity of Dragon Fruit Peel Extract (DFPE)

Dragon fruit peel extract was prepared in concentrations of 40, 80, 160, 320 and 640 ppm, then 1 mL DPPH solution was added and methanol added to 5 mL total volume. As standard, ascorbic acid was used with serial concentration 0,25; 0,5; 1; 2; and 4 ppm. The solution then mixed using vortex and incubated in dark condition for 30 minutes. Absorption (A) measured at a wavelength of 516 nm using a UV-Vis spectrophotometer. The percentage of free radical scavengers is calculated using the formula in equation (1).

$$\%\ Inhibition = \frac{blank\ absorbance - sample\ absorbance}{blank\ absorbance} x\ 100\% \quad (1)$$

Concentration of the sample and percentage of inhibition were plotted on the sample absorbance curve and plotted on the x and y axes, respectively, in the linear regression equation. The equation is used to determine the IC_{50} of each sample.

Synthesis of Edible Film

Chitosan solution was made by dissolving 2 grams of chitosan in 100 mL of wt1% acetic acid solution. The solution was magnetically stirred for 4 hours. The mixture of starch-plasticizer was prepared by diluted 3 grams of starch and 3 grams of glycerol in distilled water until 100 ml. Chitosan solution is then added with starch-plasticizer in ratio 7:3 and mixed thoroughly. Glycerol was used as a secondary plasticizer to create films with less brittleness. This mixture was homogenised after DFPE was added in different ratios of 0 wt%, 3 wt%, 5 wt%, and 7 wt% based on the chitosan weight. The solution was poured into polystyrene petri dishes (150x25 mm) in a volume of 30 ml. The solution was dried at 50 ^oC for 24 h. Cast dope without DFPE was used to obtain the control film. Before use, the films were peeled from the petri dishes and stored in a desiccator at 25 ^oC. (Arifin et al., 2016; Rambabu et al., 2018).

Antioxidant activity of Edible Film Assessment

Antioxidant activity of edible film was measured by diluted 0,25 grams of film with 50 ml distilled water. 3.2 ml film extract mixed with 1 ml DPPH solution then added with methanol until the volume reaches 5 ml. The solution was incubated for 30 minutes before being measured for absorption at 516 nm using a UV-vis spectrophotometer.

Characterization of Edible Film

Film Thickness

The thickness of the films was measured using a digital micrometre with a resolution of 0.01 mm. Each film's thickness was measured at 9 different spots, and the average thickness of each film was reported (Siripatrawan and Harte., 2010).

Water Vapour Transmission Rate

Glasses containing 50 ml of distilled water were covered with respective film samples and sealed with double tape to prevent leakage. All components have been measured for the weight. These glasses were attached to a desiccator with silica gel. The water vapor will diffuse through the film and be absorbed by silica gel. The weight difference of the glass before and after 1 h for 7 h was noted (Pokatong and Decyree, 2018).

Mechanical characteristics

Tensile strength and elongation of the films were determined through rupture tests on film samples performed in MesdanLab strength type Tensolab 5000. (Ernita et al.,

2020).

FTIR Analysis

The FTIR spectrum analysis of the samples was carried out using the SHIMADZU Fourier transform infrared spectroscopy FTIR Spectrometers. Measurements were made in the spectral region of 4000-500 cm^{-1} , the data obtained were analyzed using Omnic 8.1 software (Hromis et al., 2015).

Biodegradability Test

The biodegradability analysis of edible film was carried out under aerobic conditions with the help of bacteria and fungi found in the soil. The method used is the soil burrier test method. The edible film was cut with a size of 3 x 3 cm and then buried in the soil with a depth of 2 cm and observed every week for 12 weeks. Biodegradation analysis was carried out through visual observation (Ginting et al., 2018).

Result and Discussion

Extraction and Phytochemical Screening

Dragon fruit peel samples were extracted using distilled water and 70% ethanol as a solvent. The results of the extraction using distilled water, obtained a thick dark brown extract with a yield value 9.81%. The results of the extraction with 70% ethanol obtained a thick extract with a light brown color and a higher yield value 11.69%. The higher yield value of ethanol extract indicates that ethanol can extract more polar and nonpolar components contained in dragon fruit peel.

Phytochemical screening was conducted to determine the content of secondary metabolites in DFPE. Phytochemical test of DFPE using distilled water and ethanol as a solvent contains several secondary metabolites that have the potential to have antioxidant activity. Phytochemical tests on DFPE showed positive results for the presence of alkaloids, flavonoids, saponins, and tannins in both extracts, while terpenoids and steroids were not detected. Similar results were also obtained in research conducted by Kylanel et al., (2018).

Both extracts showed a very strong positive reaction for the tannin test and moderate for the flavonoid test. Saponins were detected in both extracts, but for water extract the intensity was greater than that of the ethanol extract. On the other hand, alkaloid compounds in the ethanol extract were greater than the water extract. Through this phytochemical screening test, it is estimated that the water and ethanolic DFPE contain high amounts of tannin compounds as well as moderate or small amounts of alkaloids, flavonoids, and saponins. These compounds have the potential to produce antioxidant activity especially for flavonoid compounds. The results of the phytochemical test of dragon fruit peel can be seen in Table 1.

Table 1. The results of the phytochemical test of DFPE

Compounds	Water extract	Ethanol extract
Alkaloid	+	++
Flavonoid	++	++
Saponin	++	+
Tannin	+++	+++
Terpenoid	-	-
Steroid	-	-

Note: +++ = Very strong positive reaction
++ = Strong positive reaction
+ = Low positive reaction
- = Negative reaction

Antioxidant Activity of DFPE

The antioxidant activity of dragon fruit peel extract was analyzed by DPPH radical inhibition test, with ascorbic acid as standard. The parameter used to show the antioxidant activity of the extract is inhibitory concentration (IC_{50}), which is the concentration required to inhibit 50% of DPPH radicals. The lower the IC50 value obtained, the higher the antioxidant activity (Lim et al., 2007). Measurement of antioxidant activity was carried out at 516.0 nm the maximum wavelength that has been obtained.

Antioxidant activity of ascorbic acid showed the IC_{50} value 2.44 ± 0.02 ppm, while the water extract and ethanol extract were 753.77 ± 11.25 and 315.25 ± 19.27 ppm, respectively. This result is higher than that obtained by Indrianigsih et al. (2020), as well as Lourith and Kanlayavattanakul (2013), where the ethanol extract of dragon fruit peel obtained IC_{50} values of 698 and 823 ppm, respectively.

Table 2. Antioxidant activity of DFPE

Sample	IC_{50} (ppm)	Category
Ascorbic acid	2,44 ± 0,02	Very strong
Water extract	753,77 ± 11,25	Very weak
Ethanol extract	315,25 ± 19,27	Weak

Note: IC_{50} value in average ± standard deviation

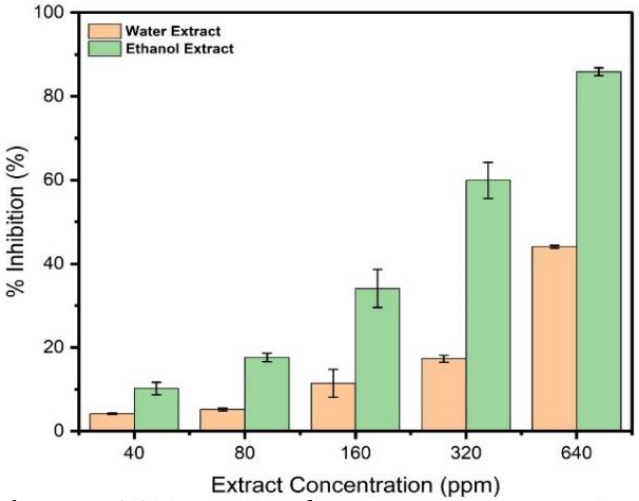

Figure 1. Inhibition activity of DFPE at various concentrations

A substance that has an IC_{50} value ranging from 200-1000 ppm is less active but still has the potential as an antioxidant substance (Fatmawaty et al., 2019). The activity of the two samples was lower than that of the positive control, nevertheless both of the extract still had antioxidant activity as shown in Figure 1 where at a concentration of 640 ppm aquadest extract and ethanol extract could inhibit 44% and 86% of DPPH radicals, respectively.

Alkaloids, flavonoids, saponins, and tannins are the active compounds contained in DFPE. Flavonoids in DFPE are compounds that provide antioxidant activity (Manihuruk et al., 2017). Dragon fruit peel also contains betacyanins and betaxanthins which give red-violet pigments (Lourith and Kanlayavattanakul, 2013). Isolation of red dragon fruit peel betacyanin by Ramdonah et al. (2017), obtained betacyanin levels of 36.67 mg/100 g. Research Yao et al. (2021), showed that betacyanins have very strong antioxidant activity but when degraded, their properties become less active.

Edible Film Synthesized

The synthesis of edible films begins with the chitosan solution in 1 wt% acetic acid. glycerol solutions are then added as plasticizers by weakening the interactions between polymers, thus the film becomes more flexible (Priyadarshi et al., 2018). The addition of starch intended to increase the homogeneity of the film matrix in order to improve the mechanical characteristics of the edible film (Elsabee and Abdou, 2013). Films were made with four variations of the addition of DFPE, 0, 3, 5, and 7 wt% based on the weight of chitosan. The edible film formed in various concentrations of DFPE can be seen in Figure 2.

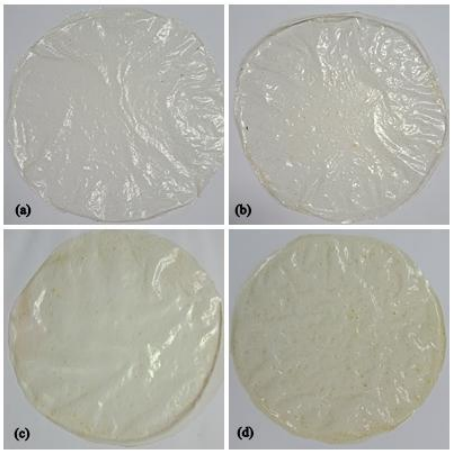

Figure 2. Appearance of the edible film in various concentration of DFPE (a) DFPE 0 wt% (control) (b) DFPE 3 wt% (c) DFPE wt% and (d) DFPE 7 wt%

Antioxidant Activity of Edible Film

The antioxidant activity of edible film was analyzed by DPPH radical inhibition test. Determination of antioxidant activity was measured from the value of percentage of inhibition DPPH radical. Edible film was made in concentration 3200 ppm, then the absorbance was measured at a wavelength of 516 nm. The antioxidant activity of edible films can be seen in Figure 3.

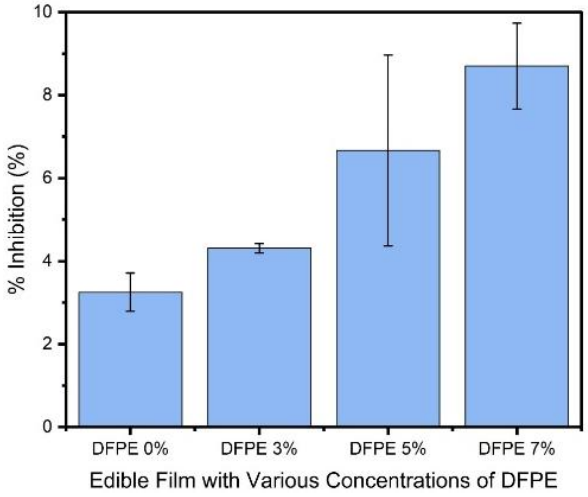

Figure 3. Antioxidant activity of edible film

Edible film with DFPE 0, 3, 5, and 7 wt% produced antioxidant activity with inhibitory value of 3.252 ± 0.459, respectively; 4.390 ± 0.114; 6,667 ± 2,299; and 8.699 ± 1.035 %. Antioxidant activity increased with increasing DFPE concentration. The edible film with 7 wt% DFPE had almost three times greater antioxidant activity than the control edible film. Similar results were also reported by Tongnuanchan et al. (2012), where the edible film of fish skin gelatin with the addition of essential oils of bergamot and lime has antioxidant activity 2 and 3 times greater than the control, respectively. The physical characteristics of edible films are measured in several parameters, that is thickness, water vapor transmission rate (WVTR), tensile strength and elongation. Table 4 shows the physical characteristics of edible films and the value of JIS (Japanese Industrial Standard) in Tanjung et al. (2020), for edible films.

Thickness

The thickness of the edible film increased with increasing levels of DFPE as shown in Table 4. Edible film with 7 wt% DFPE increased by 24% compared to control edible film. The increase in film thickness is caused by the amount of total solids in the solution (Rambabu, 2018). These results are similar with those reported in the study of Crizel et al. (2018), there was an increase of 16% in thickness from the highest addition concentration film with the control film. Based on the JIS standard, the maximum thickness of packaging material is 0.25 mm. All variations of Edible film meet the standard because the thickness value is less than 0.25 mm.

Table 4. Physical characteristic of edible film

Edible film	Thickness (mm)	WVTR (g/m²/day)	Tensile Strength (MPa)	Elongation (%)
0 wt% DFPE	0.082 ± 0,009	15.817 ± 0.372	9.483 ± 2.657	14.545 ± 1.208
3 wt% DFPE	0.094 ± 0,015	19.699 ± 0.770	9.106 ± 2.001	17.143 ± 2.947
5 wt% DFPE	0.096 ± 0,020	19.728 ± 1.820	4.086 ± 0.056	22.385 ± 7.196
7 wt% DFPE	0.102 ± 0,019	21.204 ± 0.717	2.181 ± 0.192	8.669 ± 0.564
JIS	Max. 0.25	Max. 7	Min. 0.3	Min. 10

Note : Value in average ± standard deviation.

Water Vapour Transmission Rate (WVTR)

The shelf life of food is directly related to the interaction of water from the product and the external environment. Packaging must reduce the transfer of water from the environment to the product and otherwise (Crizel et al., 2018). One of the methods to measure these interactions between packaging and the environment is by testing the water vapor transmission rate. The smaller the value of WVTR means smaller the transfer of water vapor between the product and the external environment. The value of WVRT in edible film showed an increase with increasing DFPE concentration as shown in Figure 4b.

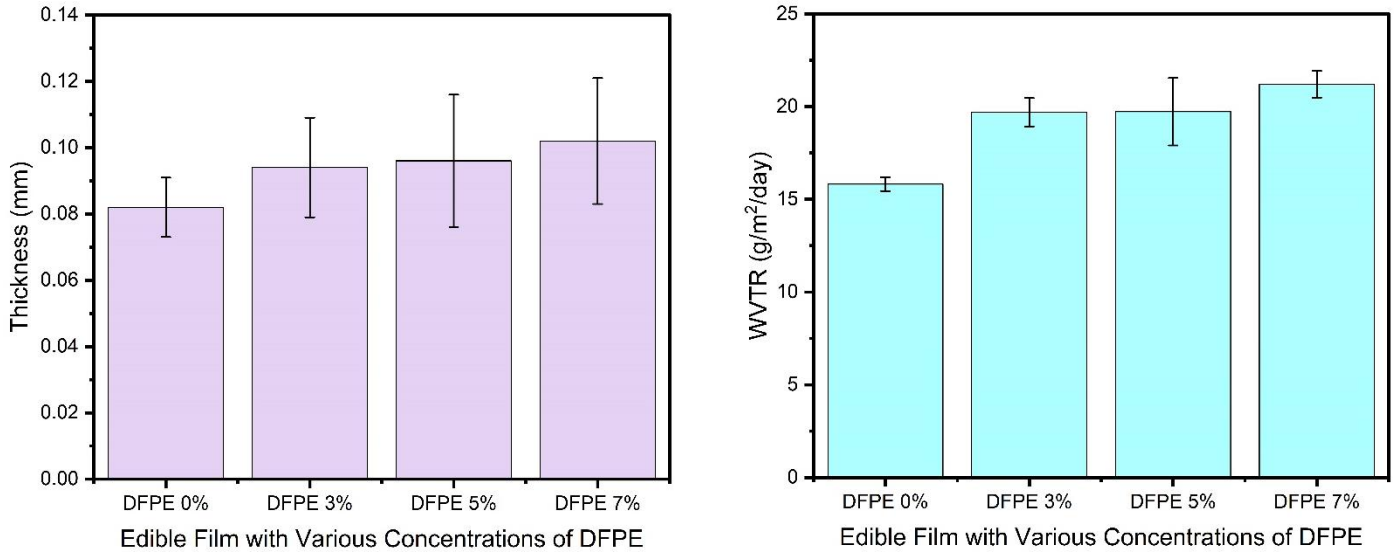

Figure 4. Effect DFPE concentration through (a) thickness and (b) WVTR of edible films.

Mechanical Properties

Mechanical properties are an important characteristic of food packaging to protect food from physical damage such as indentations, shards and scratches (Rawdkuen, 2019). Mechanical properties are usually expressed in tensile strength and elongation. Tensile strength indicates the maximum strength that the film can withstand, while elongation is a measure of the ability of film to stretch. The addition of DFPE into the film affects the tensile strength and elongation of the edible film. There was no significant difference in the tensile strength value of the control and 3 wt% DFPE edible film. Meanwhile, increasing concentration of DFPE 5 wt% and 7 wt% in edible films causes a drastic decrease in the tensile strength value, where the 5% DFPE edible film has decreased by almost 57% while the 7 wt% DFPE edible film decreases the value reaching 77% of the tensile strength value of control film. The elongation in edible film control to DFPE 5% increased as the concentration of DFPE increased. Otherwise, in the 7 wt% DFPE edible film the elongation decreased and was lower than the control edible film. The graph of changes of the tensile strength and elongation can be seen in Figure 5.

In the study of Rubilar et al. (2013), and Moradi et al. (2012), reported that the addition of antioxidant extracts in chitosan-based films would reduce the tensile strength and elongation, while in the Siripatrawan and Harte (2010) study, the addition of 0 - 5% green tea extracts in chitosan films did not change the tensile strength and elongation significantly, but there was an increase when green tea extract at a concentration of 5 - 20%. This is related with the interaction of chitosan matrix and polyphenol extract compounds. The relative change of tensile strength and elongation values can

be caused by intramolecular bonds of the matrix film with the addition of extracts (Souza et al., 2015).

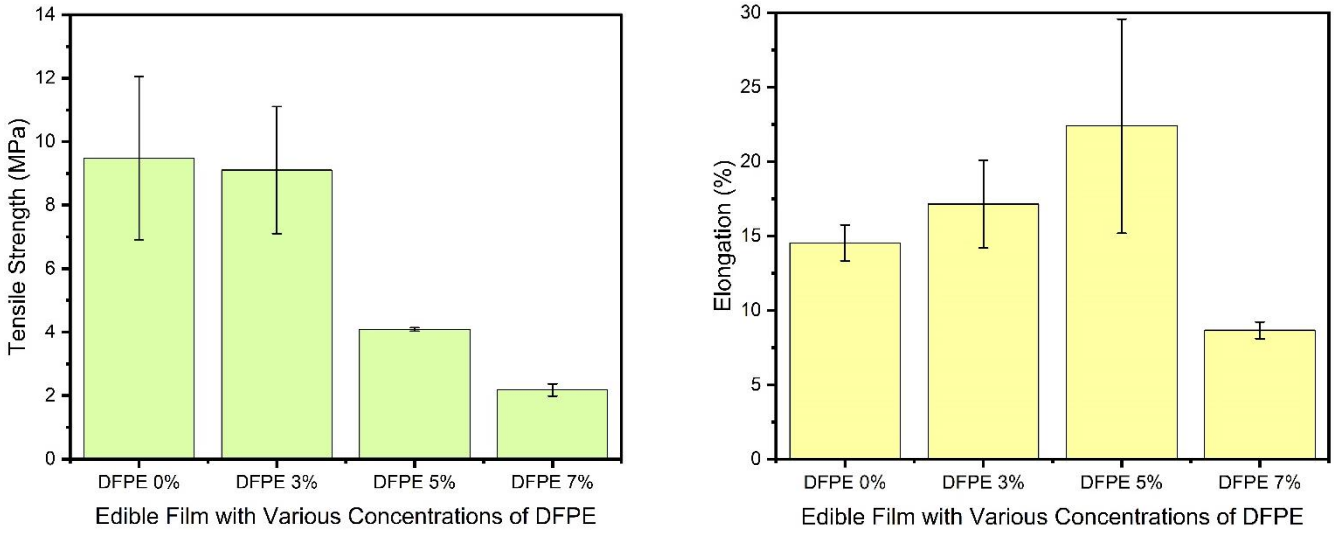

Figure 5. Mechanical properties of edible film (a) tensile strength (b) elongation

The decreased tensile strength value is associated with the interaction of phenolic compounds with the amine groups of the polymer chains which causes cross-linking between polymer chains. This bond causes the film to become stiff and extracts that are linked in the film matrix can weaken the bonds between polymers. The increase in elongation value was caused by the cross-linking of chitosan polysaccharide chains by hydrogen bonds from the extract (Cheng et al., 2017). A simple illustration of the cross-linking interaction of chitosan-starch polysaccharides with DFPE can be seen in Figure 7. Excessive cross-linking of this polymer chain can weaken the plasticizer effect so that the mobility of the film is reduced, resulting in a decrease in elongation value, in 7% Edible film DFPE.

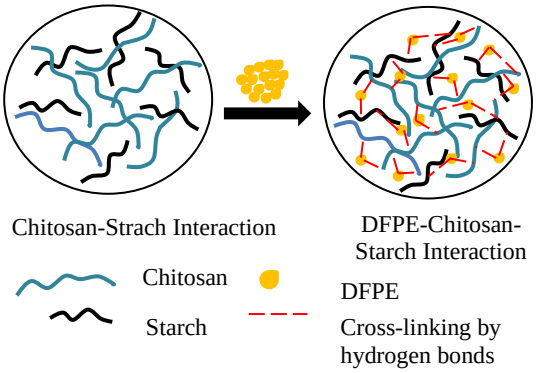

Figure 6. Ilustration of chitosan-starch polysaccharide cross -linking interaction with DFPE (Rambabu, 2018).

FTIR Spectra

Dragon fruit peel extract was characterized by using an FT-IR spectrophotometer. The spectrum (Figure 8) shows absorption at a wave number of 3414 cm^{-1} originating from O-H (phenol or carboxylic acid). The absorption at wavenumber 2926 cm^{-1} forms a strong absorption indicating the areas of aliphatic C-H stretching groups (alkane). The C=O stretching group is supported by the presence of an absorption band at wavenumber 1712 cm^{-1} which indicates the presence of a carbonyl group. The 1598 and 1406 cm^{-1} region indicates the presence of a carbon-carbon double bond (C=C) due to alkene. The peak of C-OH stretching absorption at wave number 1047 cm^{-1} indicates carboxylic acid groups.

Table 5. FTIR analysis result of DFPE

Functional Group	References (cm^{-1}) (Luo et al., 2014)	Present Study (cm^{-1})
O–H	3200 - 3640	3414
C–H stretch	2850 - 2960	2926
C=O stretch	1741	1712
C = C stretch	1600-1627	1598
	1409-1428	1406
C-OH stretch	1022-1103	1047

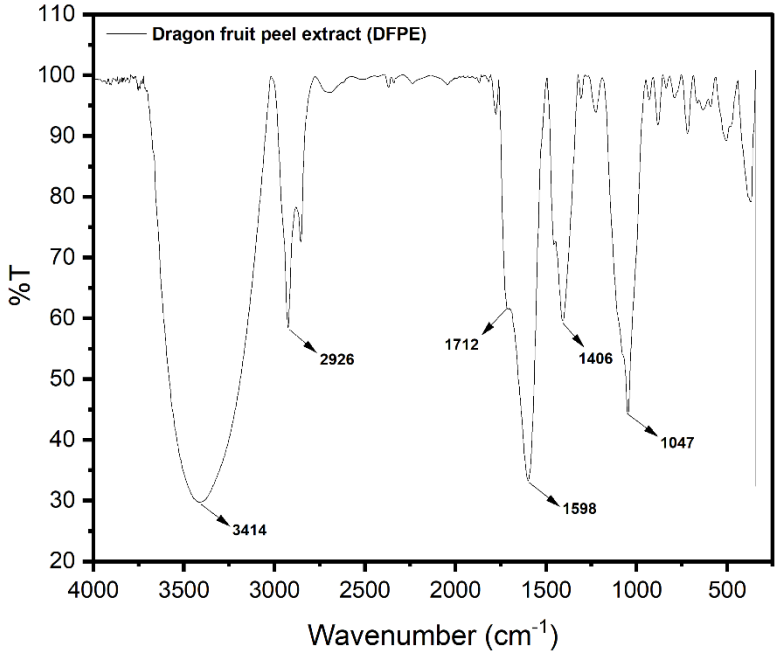

Figure 8. FTIR spectra of dragon fruit peel ethanol extract.

Based on the FTIR spectrophotometer analysis, DFPE was compatible with the existing group in the betalain structure, as well as several phenol/polyphenolic compounds or flavonoid compounds having O-H groups and some aromatic C=C groups. The absorption bands that exist in the spectrum can be briefly seen in Table 5.

FTIR spectroscopy was used to analyze the interaction between chitosan, starch and DFPE films. The FTIR spectrum of the chitosan starch film is shown in Figure 9. There was no significant difference between the control and DFPE edible film spectra because no new absorption peaks were formed in the DFPE edible film spectrum. The addition of DFPE causes a change in wavenumber.

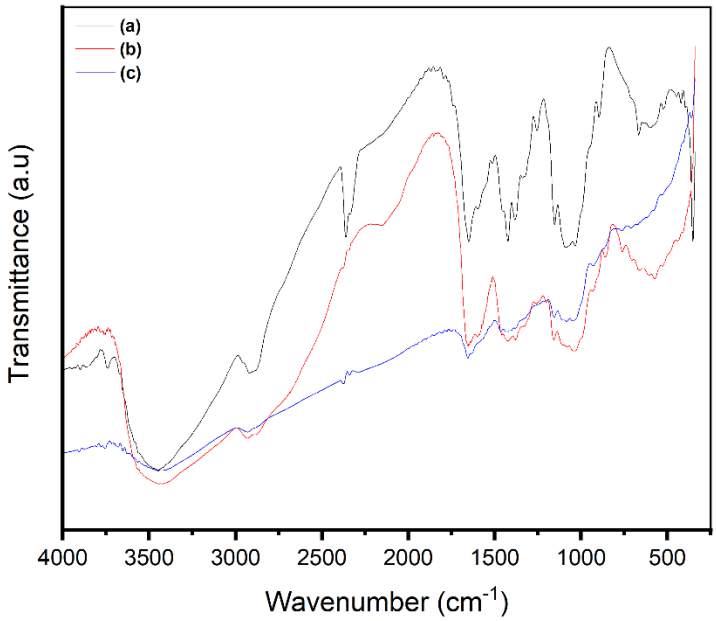

Figure 9. FTIR spectra **(a)** Chitosan **(b)** edible film without DFPE and **(c)** Edible film with DFPE

Biodegradability of Edible film

A biodegradability test was conducted to determine the ability of edible films to be degraded in the environment. The soil burrier test method is used to measure the degree of

degradation of edible films after exposure to decomposition conditions by soil microorganisms (Chie and Wahab, 2019). Observation of biodegradation was carried out by visual observation, which was carried out every week until the edible film was completely decomposed. The process of biodegradation of edible film every week can be seen in Table 6.

Table 6. Edible film biodegradation test results

Week	0 wt% DFPE	3 wt% DFPE	5 wt% DFPE	7 wt% DFPE
1	+++++	+++++	+++++	+++++
2	+++++	+++++	+++++	+++++
3	+++++	+++++	++++	++++
4	+++++	+++++	++++	++++
5	+++++	+++++	+++	+++
6	+++++	++++	+++	++
7	+++	+++	++	++
8	+++	+++	++	++
9	+++	++	+	+
10	++	+	-	-
11	+	-	-	-
12	-	-	-	-

Note:

+++++	= whole Sample	++	= half decomposed
++++	= start decomposed	+	= almost completely decomposed
+++	= decomposed slightly	-	= completely decomposed

Visual observations of edible films for 12 weeks showed that edible films with the addition of DFPE increased the decomposition process. The addition of DFPE has an effect on the degradability of edible films by increasing the decomposition process. These results are supported by the research of Sewing et al. (2016), reporting that increasing OH groups increased the rate of degradation of gelatin, carboxymethyl cellulose and chitosan films. Edible films with active compounds have better biodegradability than control edible films. Therefore the edible films with DFPE are more beneficial in terms of the environment (Suriyatem et al., 2018).

The biodegradability test of chitosan-starch edible films in the research of Rachmawati et al. (2015) takes 74 – 101 days to be completely relegated. Different results were obtained by Anita (2018), where the time required for the chitosan-starch edible film to completely decompose was 58 days. The difference time for this degradation is caused by several factors, including differences in sample composition and soil conditions. Soil conditions such as soil type, fertility, water content, oxygen, organic carbon, and the presence of heavy metals affect the degradation rate of edible films (Oberlintner et al., 2020).

Conclusion

The results of the overall edible film characterization showed that the edible film with the addition of 3% extract gave the best edible film character. This is supported by the value of the mechanical properties, where these properties greatly affect the quality of the edible film. The tensile strength value at the addition of 3% extract did not experience a significant decrease, but the addition of increasing extract composition at compositions 5 and 7% made the tensile strength value decrease drastically. This was also seen in the elongation value, which increased at 3 and 5% extract concentrations but decreased drastically at the addition of 7% extract. The best edible film formulation was added with 3% dragon fruit peel extract with a thickness value of 0.094 ± 0.015 mm, tensile strength 9.106 ± 2.01 MPa, elongation 17.143 ± 2.947%, water vapor transmission rate 19.699 ± 0.770 g/m2/day, and antioxidant activity % inhibition of 4.390 ± 0114%.

Conflict of Interest

The authors declare that there is no conflict of interest.

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