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Effect of Variation of Moringa Leaf Extract (*Moringa oleifera L.*) on Antioxidant Activity of Edible Film CMC/Chitosan

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Abstract

The edible film is an alternative to using synthetic polymer products because it is non-toxic, biodegradable, increases food safety, and extends food shelf life. The purpose of this study was to see how the effect of adding Moringa leaf extract on the antioxidant activity of CMC/Chitosan edible film. This study consisted of two stages, the first stage was extracting Moringa leaves using ethanol, and the second stage was making edible film CMC/chitosan with the addition of variations of Moringa leaf extract (0.5%, 1%, 1.5%, and 2%). The results of the antioxidant activity test showed that the edible film with the best variation was EE with an IC_{50} value of 4.45 ppm which was categorized as very strong and physical properties such as absorption, solubility, and water vapor transmission were 72.19%, 92.04%, and 6.21 g/m²h.

Keywords: Edible film, antioxidant activity, moringa leaf extract

Introduction

The edible film is a biodegradable packaging that functions as a food wrapper or coating that can be consumed together with the packaged product (Maan et al., 2021; Santi et al., 2020). The basic ingredients of edible films are proteins, polysaccharides, and fats. In the manufacture of edible films, one or a combination of these materials can be used (Saputra et al., 2021). The edible film is an alternative to the use of synthetic polymer products because of its advantages, namely non-toxicity, biodegradation, increasing food safety, and extending the shelf life of food. In addition, edible films also function to protect food from microorganisms, physical damage, moisture, and oil (Homez-Jara et al., 2018). The advantage of edible films compared to synthetic plastics is that they are more environmentally friendly. Applications for the use of edible films include wrapping candy, sausages, instant noodle seasonings, fruit, food, salads, and other food wrappers (Santi et al., 2020).

Carboxymethyl cellulose (CMC) is a polysaccharide derived from cellulose. with non-toxic properties, biodegradability, and biocompatibility, so that it can be used as raw materials for making edible films (Hidayati et al., 2021; Mahendra & Mitarlis, 2017). However, CMC-based edible films have a weakness that is hydrophilic. As a result of the hydrophilic nature of CMC, the edible film produced will have high water absorption properties and low water resistance (Bourbon et al., 2021; Hashmi et al., 2021; Salama et al., 2019; Salama et al., 2019). To correct this weakness, chitosan was added to the CMC edible film to increase the water resistance of the edible film (Bangar et al., 2021; Kalateh-Seifari et al., 2021). Chitosan is one of the developing biomaterials in the food packaging industry (Yusof et al., 2019). Chitosan is a polysaccharide obtained from the deacetylation of chitin with biodegradability, non-toxicity, biocompatibility, and antifungal properties. In addition, chitosan is useful as a stabilizer and emulsifier in the manufacture of edible films (Bangar et al., 2021; Saputra et al., 2021; Tavares et al., 2021).

The combination of CMC and chitosan edible films resulted in poor physical properties (Salama et al., 2019; Saputra et al., 2021). To fix this problem, additives were added to the edible film (Putri et al., 2022). The addition of additives to the edible film mixture will produce edible films with good physical and, mechanical characteristics and low permeability (Behrestaghi et al., 2020; Wang et al., 2019) so it is hoped that with the addition of additives in the form of moringa leaf extract on edible films will produce edible films that have good antioxidant properties.

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Moringa leaves are one the natural antioxidants because they contain compounds such as ascorbic acid, carotenoids, and phenolic substances. Among these bioactive compounds, the flavonoid group in Moringa leaves (eg, quercetin and kaempferol) was found to be the main contributor to its antioxidant activity (Looi et al., 2019). Moringa leaves also contain minerals, essential amino acids, and antioxidants such as vitamin C, and vitamin E, and are rich in other secondary metabolites. The results of the phytochemical test of Moringa leaves show the presence of tannins, alkaloids, flavonoids, anthraquinone saponins, steroids, and triterpenoids that act as antioxidants (Tukiran et al., 2020).

The antioxidant activity of Moringa leaf extract has been widely studied. Previous researchers have researched the antioxidant activity of Moringa leaf extract using various methods such as DPPH, ABTS, and FRAP. The results of the study stated that Moringa leaf extract has great antioxidant value so it has the potential to be used as an antioxidant (Jahan et al., 2018; Nobossé et al., 2018; Tukiran et al., 2020; Xu et al., 2019).

Based on the description above, the researcher wanted to see the effect of adding Moringa leaf extract to the CMC/Chitosan edible film on the antioxidant activity of the resulting edible film so that it can produce an edible film with good antioxidant activity.

Method

Extraction of moringa leaf

The Moringa leaf extraction process is carried out based on the method used (Susanty et al., 2019). Separate the Moringa leaves from the stems and them dry. Moringa leaves that have been dried are crushed and sieved. The Moringa leaf powder produced was put into a maceration bottle and macerated using ethanol for 3 days and filtered. The resulting filtrate is then rotated through an evaporator to produce a concentrated extract. Moringa leaf extract produced is stored in vials.

Edible film cmc/chitosan with the addition of moringa leaf extract

CMC solution is made by dissolving CMC and aquadest. Then homogenized using a stirrer for 60 minutes. After that, the chitosan solution which had been dissolved with CH_3COOH 1% was added and homogenized using a stirrer at 60 °C for 60 minutes. Then, Moringa leaf extract was added with variations of 0.5%, 1%, 1.5%, and 2% and homogenized with a stirrer for 15-30 minutes. After that, it was printed using an elcometer and dried in an oven at 45 °C for 24 hours. The dried edible films were tested for water absorption, solubility, water vapor transmission rate, and antioxidant activity with DPPH.

Nb: E_A = 0%; E_B = 0.5%; E_C = 1%; E_D = 1.5% and E_E = 2%

Characterization of edible film

Assay of antioxidant activities

The antioxidant activities of films were estimated using the 1, 1- diphenyl-2-picrylhydrazyl (DPPH). The antioxidant activities of the edible film were determined using the following equation (1):

$$Inhibition\ (\%) = \frac{Abs\ Blanko - Abs\ Sample}{Abs\ Blanko} \times 100 \quad (1)$$

Water absorption (%)

Water absorption is the ability of a sample to absorb water. The water absorption test is done by measuring the initial mass of the sample to be tested (W_1), then putting it in a container containing aquadest for 5 minutes. Then the sample is taken from a container containing aquadest and the water on the surface of the sample is removed with a tissue (Sariningsih et al., 2019). then the water absorbed by the sample is measured using the following equation (2):

$$Water\ Absorption\ (\%) = \left(\frac{W - W_1}{W_1}\right) \times 100 \quad (2)$$

Solubility (%)

Solubility on the edible film was carried out by cutting the edible film with a size of 2 × 2 cm and each weighed filter paper. Edible film and filter paper were dried in an oven at 105 °C for 24 hours. Weighed each separately and marked as initial weight (W_1). After that, put the edible film wrapped in filter paper and immersed in a vessel containing 50 ml of distilled water for 24 hours while stirring slowly. Then the insoluble filter paper and edible film were dried in an oven at 105 °C for 24 hours, after that the edible film and filter paper was weighed and marked as final weight (W_2) (Arifin et al., 2020). Then the edible film is measured using the following equation (3):

$$Solubility\ (\%) = \left(\frac{W_1 - W_2}{W_1}\right) \times 100 \quad (3)$$

Water vapor transmission rate (WVTR)

The water vapor transmission rate test was measured by the gravimetrically determined cup method. Cut the edible film into the shape of a circle with a diameter according to the surface of the cup. The initial weight of the cup was weighed and 3 grams of silica gel was added. Tied edible film on a cup containing silica gel. Coated with liquid wax on the surface of the edible film. Conditioned at room temperature for 24 hours. Weighed the final weight of the sample and measured using the following equation (4):

$$WVTR = \frac{\Delta W}{24 \times t \times A} \quad (4)$$

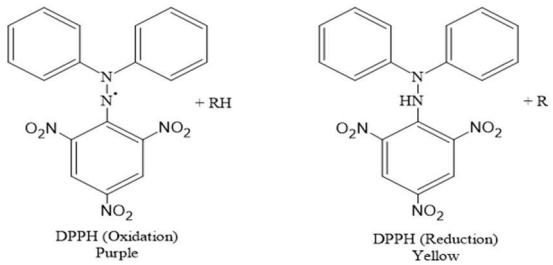
where, ΔW the weight change before and after the test (g); t is the test time (h), and A is the test area (m^2)

Results and Discussion

Antioxidant activities

Antioxidant activity was carried out using the DPPH method. The DPPH method is one of the antioxidant test methods to determine antioxidant activity. This method was chosen because it is a simple, easy, fast, and sensitive method and only requires a small sample to evaluate the antioxidant activity of natural compounds (Molyneux, 2004). The principle of this DPPH method is that there is a change in the intensity of the purple color of the DPPH which is proportional to the concentration of the DPPH solution. DPPH free radicals which have unpaired electrons will give a purple color. The color will turn yellow when the electrons are paired. This change in the intensity of the purple color occurs due to the reduction of free radicals produced by the reaction of the DPPH molecule with the hydrogen atoms released by the sample compound molecules to form DPPH compounds and cause the DPPH color to decay from purple to yellow. This color change will give a change in absorbance at the maximum wavelength of DPPH, which is 517 nm so that the value of free radical scavenging activity will be known which is expressed by the IC_{50} (*Inhibitory concentration*) value. The IC_{50} value is defined as the concentration of the test compound that can reduce free radicals by 50%. The smaller the IC50 value, the higher the free radical scavenging activity (Nuraeni & Sulistijowati, 2021).

Figure 1. Reaction DPPH with antioxidants



(Rizkayanti et al., 2017)

The absorbance value obtained will be used to calculate the percent inhibition by equation (1). The following absorbance values and percent inhibition are presented in **Tables 1-6.**

Table 1. Absorbance and inhibition (%) of edible film E_A

Concentration (ppm)	Absorbance	Inhibition (%)
Blanko	1.47	-
20	1.40	5.21
40	1.32	9.77
60	1.25	14.93
80	1.15	21.62

Table 2. Absorbance and inhibition (%) of edible film E_B

Concentration (ppm)	Absorbance	Inhibition (%)
Blanko	1.11	-
20	0.99	11.35
40	0.89	19.66
60	0.73	34.28
80	0.59	47.07

Table 3. Absorbance and inhibition (%) of edible film E_C

Concentration (ppm)	Absorbance	Inhibition (%)
Blanko	0.99	-
20	0.72	27.67
40	0.64	35.11
60	0.56	43.56
80	0.46	53.03

Table 4. Absorbance and inhibition (%) of edible film E_D

Concentration (ppm)	Absorbance	Inhibition (%)
Blanko	0.61	-
20	0.31	49.03
40	0.29	51.77
60	0.28	53.81
80	0.26	57.16

Table 5. Absorbance and inhibition (%) of edible film E_E

Concentration (ppm)	Absorbance	Inhibition (%)
Blanko	0.25	-
20	0.11	52.76
40	0.11	56.11
60	0.10	59.04
80	0.09	63.15

Table 6. Absorbance and inhibition (%) of vitamin c as a control

Concentration (ppm)	Absorbance	Inhibition (%)
Blanko	0.1097	-
20	0.0470	57.09
40	0.0397	63.81
60	0.0323	70.54
80	0.0223	79.66

After determining the percentage of inhibition, then a figure of the relationship between the concentration of edible film on the X-axis and the percentage of inhibition on the Y-axis is made, which is presented in **Figure 2**.

Based on **Figures 2-7**, a linear regression line equation is generated for each edible film and vitamin C. Through the linear regression line equation, the value (IC_{50}) is calculated by substituting the Y value with a value of 50 so that the IC_{50} value for each edible film is presented in **Table 7**.

Table 7 Inhibitory concentration (IC_{50})

Edible Film	IC_{50}	Category
E_A	186.45	Weak
E_B	85.98	Medium
E_C	74.14	Medium
E_D	27.72	Strong
E_E	4.45	Very Strong

Based on the test results of antioxidant activity, it can be seen that with the increase in the amount of Moringa leaf extract mixed in the edible film solution, the IC_{50} value decreases. The highest IC_{50} value was edible film E_E (without Moringa leaf extract) and the lowest was edible film E_E (2% Moringa leaf extract) with a value of 4.45 ppm. The same opinion was expressed by research by Nuraeni & Sulistijowati (2021) which stated that the addition of antioxidant compounds to edible films was proven to be efficient in increasing antioxidant properties. The same opinion was expressed by research by Rizkayanti et al. (2017), the more concentration of Moringa leaf extract added, the more antioxidant compound particles contained, so the greater the antioxidant activity. In this study, with the addition of Moringa leaf extract to the edible film, the antioxidant properties of the edible film increased, indicated by the decreasing value of IC_{50}. The smaller the IC_{50} value, the stronger the antioxidant activity (Sulistijowati et al., 2019).

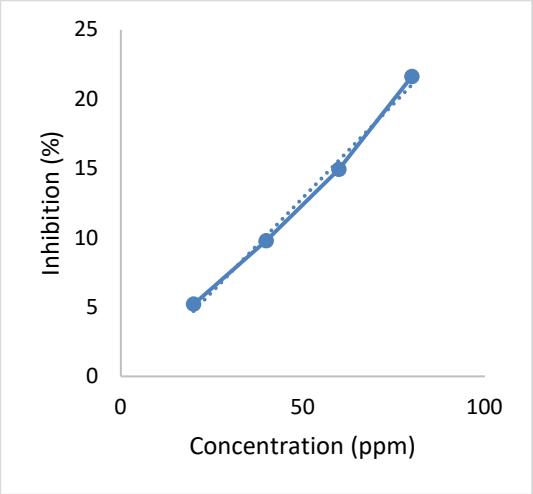


Figure 2. Correlation between edible film E_A concentration and percent inhibition

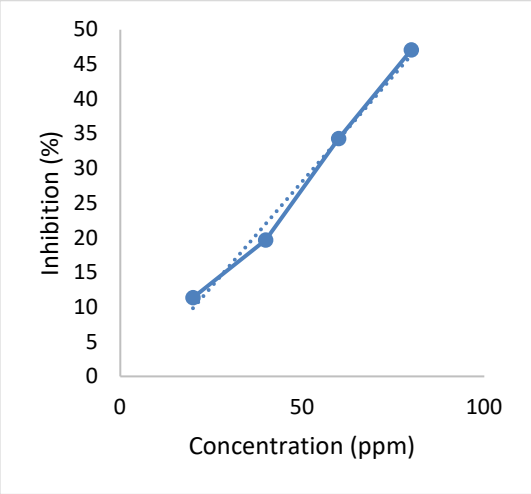


Figure 3. Correlation between edible film E_B concentration and percent inhibition

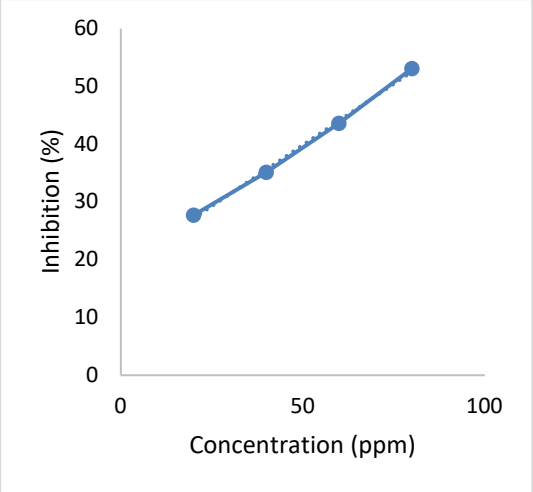


Figure 4. Correlation between edible film E_C concentration and percent inhibition

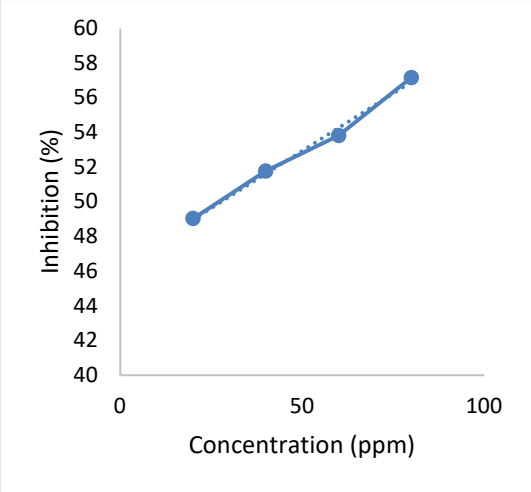


Figure 5. Correlation between edible film E_D concentration and percent inhibition

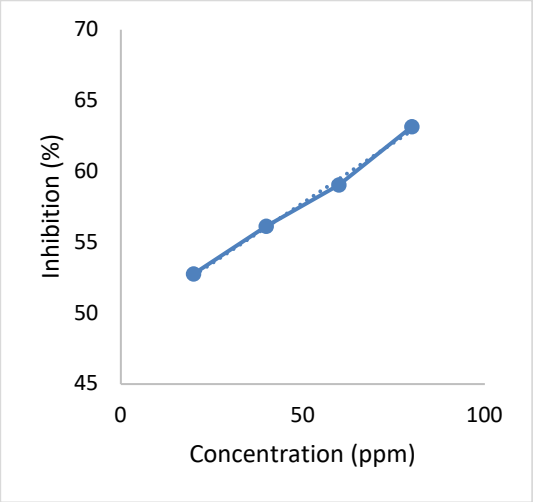


Figure 6. Correlation between edible film E_E concentration and percent inhibition

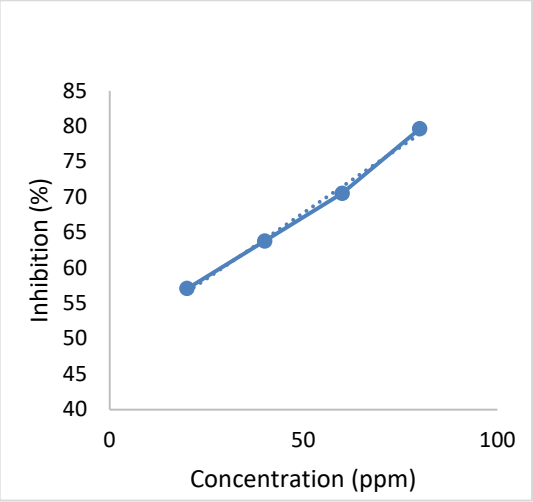


Figure 7. Correlation between vitamin C concentration and percent inhibition

Based on the IC_{50} value, antioxidants are categorized:

Table 8. Category of antioxidants

Inhibitory Concentration (IC_{50})	Category
IC_{50} <10 ppm	Very Strong
IC_{50} 10-50 ppm	Strong
IC_{50} 50-100 ppm	Medium
IC_{50} 100-250 ppm	Weak
IC_{50} >250 ppm	Non Active

(Susanty et al., 2019)

As a comparison, vitamin C was used as a positive control because vitamin C is a strong antioxidant. The IC_{50} value of vitamin C used was 2.243 ppm. Based on IC_{50}, edible film E_E, and vitamin C are categorized as very strong antioxidants.

Water absorption, solubility, and water vapor transmission rate

The test results showed that with the addition of Moringa leaf extract in a mixture of edible film solutions, there was an increase from 59.07% to 72.19% which was owned by E_E. The increase in absorption is due to the hydrophilic nature of the Moringa leaf extract.

Solubility is one of the main parameters in edible films when used as packaging for a product. The results of the solubility test showed that the solubility test on the edible film increased with the increase in the amount of Moringa leaf extract added. The solubility value increased from 78.83% to 92.04% which was owned by the edible film E_E. The solubility value in the results of this study can be said to be quite high compared to other studies. According to (Pitak & Rakshit, 2011), the high solubility of edible films makes it easy for edible films to dissolve in water. Edible films with high solubility are very good for use in ready-to-eat food products because they dissolve easily when consumed. Solubility relates to the physical properties of the edible films' ability to dissolve in water so that when discharged into the environment they can decompose naturally. The higher the concentration of Moringa leaf extract added, the more solubility tends to increase, this is because Moringa leaf extract has properties that can bind to water (hydrophilic).

Table 9. Water absorption, solubility, and water vapor transmission

Sample	Water Absorption (%)	Solubility (%)	Water Vapor Transmission Rate (g/m^2 h)
E_A	59.07	78.,83	3.85
E_B	61.22	80.25	4.54
E_C	65.88	84.36	5.36
E_D	67.43	89.62	5.71
E_E	72.19	92.04	6.21

The water vapor transmission rate test serves to determine the resistance of a film to water and gas in units of area and to find out how long the strength of a film is to withstand the entry of water so that the shelf life of a product can be extended (Dwimayasanti & Kumayanjati, 2019). Good WVP for the packaging of a food product that can absorb small water vapor so that the packaged product can avoid damage caused by air. From the table, edible film E_E has the highest value with a value of 6.21 ($g/m^2.h$). The WVTR value produced is still at a predetermined standard by the Japanese Industrial Standard (1975) (Dwimayasanti & Kumayanjati, 2019) which is a maximum of 10 $g/m^2.h$.

The WVP value obtained depends on the ratio of the material with hydrophilic and hydrophobic properties to the composition of the edible film formulation. Edible films that have a high water vapor transmission value are generally made of protein and polysaccharides. The addition of chitosan was able to restrain the rate of water vapor transmission due to its hydrophobic nature, but because of the hydrophilic nature of Moringa leaf extract. So that the more the amount of Moringa leaf extract added to the WVTR increased.

Conclusions

Edible film CMC/Chitosan with variations in the addition of 2% Moringa leaf extract produced an edible film that has good antioxidant activity with an IC_{50} value of 4.45 ppm which is categorized as a strong antioxidant. The test results of water absorption, solubility, and water vapor transmission rate were 72.19%, 92.04%, and 6.21 ($g/m^2.h$).

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