Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 11(1), 1-7, 2023

Antioxidant Profiles of Chitosan-Alginate Films with Addition of *Moringa oleifera* Leaf Extract for Active Packaging

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Received: Februari 2023
Received in revised: Februari
Accepted: March 2023
Available online: April 2023

Abstract

This research aims to study the antioxidant activity of chitosan-alginate films by adding *Moringa oleifera* leaf extract. These films will be applied as active packaging for food. This research was initiated by the extraction of *Moringa oleifera* leaves. The *Moringa oleifera* leaves extract was prepared maceration and soxhletation techniques using distilled water as a solvent. The maceration method is resulting the yield 6.97%, while the yield of extract from the soxhletation method was 8.57%. After the extraction process, screening phytochemicals of *Moringa oleifera* leaves extract was tested to identify secondary metabolite compounds. Screening phytochemicals of the extract showed that *Moringa oleifera* extract contains alkaloids, flavonoids, tannins, and saponins. Synthesizing of films was conducted at room temperature through a homogenization technique using a hot plate stirrer. The variations of final concentrations of extract were 0% (film A0), 0.50% (film A1), 0.75% (film A2), and 1.00% (film A3). Based on the antioxidant activity test using DPPH, the % RSA value of films with the addition of extract from soxhletation method was higher than films with the addition of extract from the maceration technique. The highest percentage of RSA value was 43.65% from A3 film with the extract's final concentration of 1.00%.

Keywords: Alginate, antioxidant, chitosan, Moringa oleifera, packaging

INTRODUCTION

Food sanitation is an effort to create and maintain food conditions from bacteria, free radicals, and both chemical and biological contaminations. Food sanitation is one of the food sector's efforts to maintain food quality relating to human health. One of the problems decreasing food quality is lipid oxidation. Lipid oxidation is a reaction between a food product and free radicals, causing the food product's quality degradation, including changes in taste, aroma, and color.

Along with technological advances, consumer demand for healthy food products with a long shelf life is also increasing. The development of active packaging technology has become an alternative packaging system that can protect the quality and extend the shelf life of food (Ria Barleany et al., 2020). Antioxidant packaging technology is one of the active packaging technologies that have been widely developed. This technique uses an active substance, known as either an antioxidant or antibacterial activity, to be incorporated into the film matrix to increase the stability of food products and to prevent the food from oxidation the lipid process and disrupting microorganisms and bacteria (Majid et al., 2018);

The manufacturing of active packaging using synthetic antioxidants is now being avoided because they are considered dangerous, toxic, and can potentially have carcinogenic properties. The use of natural antioxidants such as extracts from plants, fruits, and essential oils, is the right solution and is considered safer to be developed (Vilela et al., 2018). The use of a natural active agent as an antioxidant such as tea extracts in chitosan composite films by Peng et al., (2013) showed good results in their antioxidant activity. The addition of tea extract, grape seed extract, ginger extract, and gingko leaf extract in the gelatin film by Li et al., (2014) also showed good antioxidant activity and potential to be developed for food packaging.

The use of biodegradable materials in the manufacturing of food packaging is now starting to focus on reducing the environmental impact. Some biodegradable polymers usually used to manufacture film are chitosan and alginate. Chitosan is known to have biocompatible and non-toxic properties, so it is safe for humans (Priyadarshi & Rhim, 2020).

Alginate is also a natural polymer that has anionic groups ($–COO^-$) in its structure so that it can form a polyelectrolyte complex with chitosan, which has cationic groups ($–NH_3^+$) (Latupeirissa et al., 2022; Sunardi et al., 2021). The previous research from Kulig et al., (2017) showed that the chitosan-alginate polyelectrolyte complex film has better mechanical strength than films made from only one type of polymer. Therefore, films made from the combination of chitosan and alginate have the potential to be used as a film matrix for active packaging.

Moringa oleifera is a plant known as a miracle plant in Indonesian society. *Moringa oleifera* has believed to have many benefits for the health of the body including the treatment of inflammation, tumors, infectious diseases, and others. *Moringa oleifera* contains glucosinolate compounds, isothiocyanate compounds, and minerals that benefit the body. A previous study from Sreelatha & Padma, (2009) showed that water extract from *Moringa oleifera* leaves has vigorous antioxidant activity with IC_{50} value of 18.15 µg/ml for mature leaves and 19.12 µg/ml for tender leaves. Several studies using *Moringa oleifera* leaf extract in developing food packaging were conducted by Mandala et al., (2020) and Ju et al., (2019). Mandala et al., (2020) created bioplastics using polyvinyl alcohol (PVA)-starch with the addition of *Moringa oleifera* leaf extract to determine the antibacterial activity of these bioplastics. Meanwhile, Ju et al, (2019) developed active packaging in the form of antioxidant packaging made from khorasan flour with the addition of *Moringa oleifera* leaf extract as an antioxidant agent. The results of their research indicated that the presence of moringa leaf extract resulted in good antioxidant activity, so it has the potential to be developed as an antioxidant agent in an active packaging system, called antioxidant packaging.

METHODOLOGY

Materials and Instrumentals

In this study, the instruments used were beakers, petri dishes, hot plate stirrers, magnetic stirrers, stirring rods, spatula, test tubes, analytical balances, vials, filter paper, aluminum foil, and UV-Vis spectrophotometer type Genesis 150.

The materials used in this study were chitosan powder (DD=0.94) obtained from Phy Edumedia-East Java, alginate powder from Buana Chem-West Java, 2% acetic acid, chloroform, ammonia, sulfuric acid, Mayer, Wagner, and Dragendorff reagents, glacial acetic acid, Mg powder, $FeCl_3$ 1%, *Moringa oleifera* leaves obtained from Central Lombok, radical DPPH powder, ethanol 96%, and distilled water.

Methods

Preparation of *Moringa oleifera* leaves

Moringa oleifera leaves are washed with water and dried in the sun for ± 4 hours from 07.00 am to 11.00 am. The drying process was carried out until the Moringa leaves were dry to minimize the water content of *Moringa oleifera* leaves. After drying, the leaves are crushed using a mortar into powders. Then, the powder leaves were stored in a dry place at room temperature (± 25°C).

Extraction of *Moringa oleifera* leaves Using Maceration Technique

Dried Moringa leaves were extracted using the maceration technique based on Susanty et al., (2019) and Gus Mahardika & Fajri (2023) with some modifications. About 20 g of *Moringa oleifera* leaves powder were weighed and extracted using 100 mL water as solvent. Moringa leaves powder was immersed for 24 hours. After 24 hours, samples were stirred and filtered using filter paper. Moringa leaves were soaked again with 50 mL water with the same ratio for 24 hours. The treatments were conducted with three replications to collect moringa macerate. All macerate obtained was then collected to remove the solvent, and the yield percentage was calculated.

Extraction of *Moringa oleifera* leaves Using Soxhlet Technique

Extraction of *Moringa oleifera* leaves using the soxhletation was conducted according to method from Sreelatha & Padma (2009) with some modifications. 20 g of *Moringa leaves* were put into a boiling flask, and distilled water as solvent was added. Extraction was carried out by heating the sample for ± 7 hours at a temperature of 70 ºC. The macerate obtained was collected to calculate the percentage of yield.

Phytochemical Testing of Moringa oleifera Extract

The phytochemical testing of *Moringa oleifera* leaves extract was performed to identify metabolites secondary of the extract, including alkaloid, tannin, flavonoid, terpenoid, and saponin (Dali et al., 2022; Hasti et al., 2022)

Alkaloid test

About 0.1 g of *Moringa oleifera* extract was added by acidified alcohol (± 10 mL), then heated and filtered using filter paper. Then, 0.4 mL of ammonia and 1.0 mL of chloroform were added to 1 mL of the

filtrate, and shaken for 3 minutes. About 2 mL of acetic acid was added to the mixture to obtain the chloroform layer. The next layer was divided into two tubes, and three drops of both Mayer's and Dragendorff's reagents were added to each tube. The extract will positively contain alkaloids if it produces a white precipitate (Mayer's reagent) or a reddish-brown precipitate (Dragendorff's reagent).

Tannin test

Moringa extract (0.1 g) was heated using DMSO (±2 mL) and filtered using filter paper. The filtrate added a few drops of 0.1% $FeCl_3$. The extract will positively contain tannin if the filtrate forms a brownish-green or blue-black coloration.

Flavonoid test

About 0.2 g of the extract was put into a test tube and dissolved using ethanol (± 3 mL), then heated for 5 minutes. A few drops of concentrated hydrochloric acid were added to the mixture, and about 0.1 g of magnesium powder was added. The extract will positively contain flavonoids if the mixture produces a dark red color.

Terpenoid test

About 0.1 g extract was added to chloroform, and then mixed with concentrated sulfuric acid. The formation of reddish-brown coloration indicates extract positively contains terpenoids.

Saponin test

About 0.1 g was put into a test tube and added by distilled water until all samples sank. The sample is then boiled for ± 3 minutes. The formation of emulsion on vigorous shaking was regarded as positive for saponin.

Synthesize of film chitosan-alginate

Films were produced using the method from Riyandari et al., (2022). The chitosan-alginate film was synthesized by dissolving the chitosan powder using 2% acetic acid and stirring for 30 minutes until a homogeneous chitosan solution was formed. Alginate powder was dissolved using distilled water and homogenized on a hot plate stirrer for 20 minutes. Then, the chitosan solution and the alginate solution by stirring for ± 1 hour on a hot plate stirrer with a temperature of 25 ºC. After stirring, the extract solution (yield of extract/50 mL) was added to the chitosan-alginate film solution. Stirring continued for ± 24 hours, and the film solution was cast into a petri dish. Drying of the film solution was carried out at room temperature by allowing the film solution for ± 7 days. The film formed was then taken from the petri dish and stored in a dry place before being tested for antioxidant activity. The film compositions were shown in Table 1 below.

Table 1. Compositions of films

Film code	Chitosan (g)	Alginate (g)	Glacial acetic acid (mL)	Distilled water (mL)	Moringa extract (mL)
A0	0.020	0.005	16.00	4.00	0.00
A1	0.020	0.005	16.00	4.00	0.10
A2	0.020	0.005	16.00	4.00	0.15
A3	0.020	0.005	16.00	4.00	0.20

Assessment of Antioxidant Activity

The determination of the antioxidant activity of films was performed using the methods of Carnaval et al., (2022). Analysis was conducted to determine the effectiveness of *Moringa oleifera* leaf extract becoming an antioxidant agent in chitosan-alginate films. An antioxidant activity test was performed using the fixed reaction time method. Films added by *Moringa oleifera* leaf extract were soaked in 20 mL 96% (v/v) ethanol. DPPH solution containing 75 µM was prepared by dissolving the DPPH powder in 96% (v/v) ethanol. About 0.5 mL of the test solution was put into a vial, and 3.5 mL of DPPH radical was added. The mixture solution was then stirred using a stir bar and placed in the dark for ± 30 minutes at room temperature. Next, the absorbance of the tested film solution was measured at 1, 4, 24, 28, 48, and 72 hours. The absorbance of the test solution was measured using a UV-Vis spectrophotometer at a wavelength of 518 nm with three replications. The control solution was also prepared by dissolving 0.5 mL of ethanol 96% (v/v) and mixing it with 3.5 mL of DPPH solution. The antioxidant activity is calculated by the following equation 1.

$$\%\text{RSA} = (1 - \frac{A_{sample}}{A_{control}}) \times 100\% \quad (1)$$

Data Analysis

In this study, the design method used was a completely randomized design (CRD) with one independent variable: variations in the concentration of moringa leaf extract. Data analysis was carried out using the analysis of variance method at a 95% confidence level (α=0.05) using Microsoft Excel to determine the effect of the addition of moringa leaf extract on the antioxidant activity of the chitosan-alginate film. Furthermore, a significant difference

test (LSD) was carried out to see the significance between the two values.

RESULTS AND DISCUSSION

Phytochemical Screening of Extract

Based on phytochemical screening, it was known that the water extract of moringa oleifera leaves contains some secondary metabolites including alkaloids, tannins, flavonoids, and saponins. The result of phytochemical screening were shown in Table 2.

Table 2. The result of phytochemical screening

Secondary metabolite	Water extract (maceration)	Water extract (soxhletation)
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Terpenoids	-	-
Saponins	+	+

Note: (+): positive, (-): negative

According to the phytochemical test, identical results were obtained to the previous study by Oba et al., (2019) where water extract of moringa seeds contained alkaloids, tannins, saponins, flavones, steroids, terpenoids, and anthraquinones. Saputra et al., (2020) also stated that moringa leaves have several secondary metabolites, including tannins, saponins, steroids, alkaloids, and flavonoids. Some secondary metabolites found in moringa leaf extract are known to have the potency as an antioxidant compound for food packaging.

Synthesize of Chitosan-Alginate Films with the Addition of *Moringa oleifera* Extract

The appearance of the chitosan-alginate films with the addition of extract using maceration can be seen in Figure 1, while the film made from the soxhletation method moringa leaf extract is shown in Figure 2. Based on the physical appearance in Figure 1, the film with the addition of macerated Moringa leaf extract shows slightly yellowish coloration, especially at the edges of the films. However, in the center of the film, the film looks transparent. Figure 2 showed that the film with the addition of moringa leaf extract using the soxhletation method has a yellower color than the film with the addition of macerated Moringa leaf extract. In contrast, films without the addition of the extract on the A0 film were colorless and looked transparent.

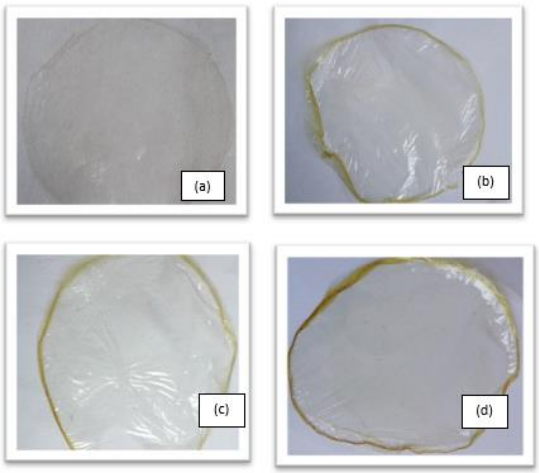

Figure 1. The film with the addition of moringa leaf extract using the maceration technique. Film A0 without extract (a), film A1 with final concentration extract of 0.50% (b), film A2 with final concentration extract of 0.75% (c), and film A3 with final concentration extract of 1.00% (d).

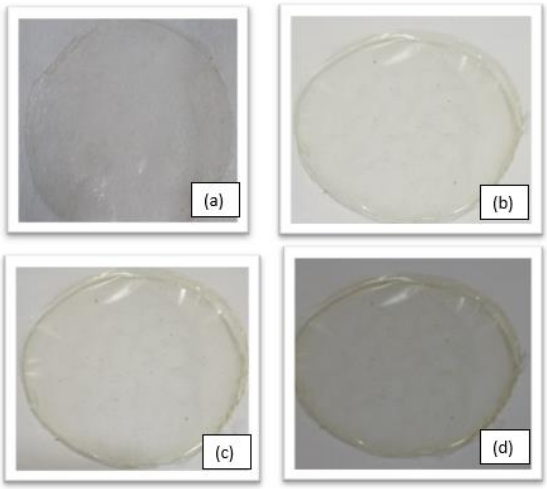

Figure 2. A film with the addition of moringa leaf extract using soxhletation technique. Film A0 without extract (a), film A2 with final concentration extract of 0.50% (b), film A2 with final concentration extract of 0.75% (c), and film A2 with final concentration extract of 1.00% (d).

Assessment of Antioxidant Activity

Determination of the antioxidant activity of the films was carried out using a DPPH solution. Active compounds in moringa leaf extract released from the films will react with DPPH radicals.. The antioxidant activity of the film is expressed in %RSA (radical scavenging activity) indicating the amount of radical captured by the active compound released from the films. The higher of %RSA value, the higher concentration of active components in counteracting

free radicals. The antioxidant profiles of the chitosan-alginate film were shown in Figure 3, Figure 4, and Figure 5.

The results in Figure 3, Figure 4, and Figure 5 showed that the %RSA value of the films added by the extract from soxhletation method was higher than the film with the addition of extract from the maceration method. It indicated that the concentration of the active compounds in the soxhletation moringa leaf extract is higher than the macerated extract. It can be seen from the yield of the extract obtained from the soxhletation method, which is greater than the yield from maceration.

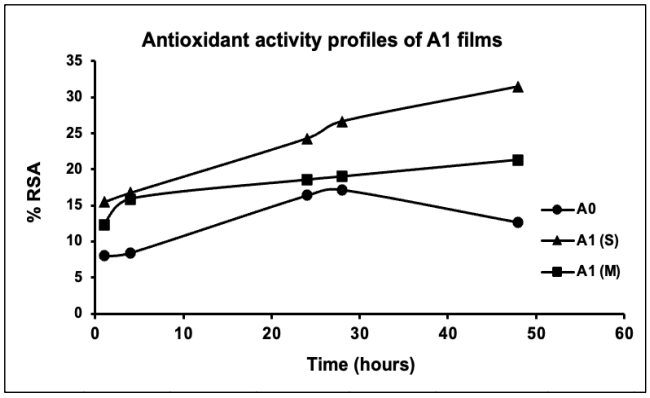

Figure 3. Antioxidant activity profile of A1 film with a final concentration of 0.50% moringa leaf extract where A0: a film without extract, A1(S): A1 film with extract using soxhletation method. A1 (M): A1 film with extract using maceration method.

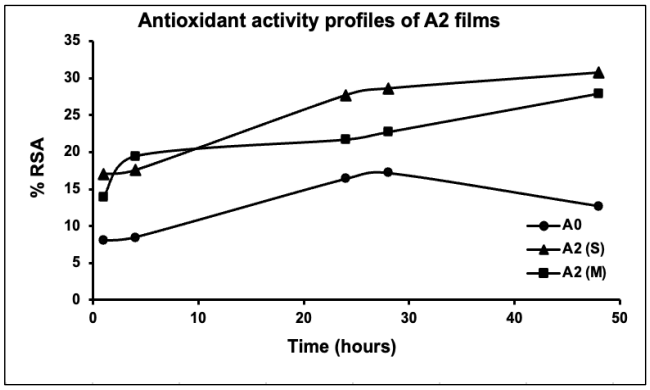

Figure 4. Antioxidant activity profile of A2 film with a final concentration of 0.50% moringa leaf extract where A0: a film without extract, A2 (S): A2 film with extract using soxhletation method. A2 (M): A2 film with extract using the maceration method.

Antioxidant activity profiles on films A0, A1, A2, and A3 showed an increase of %RSA values per unit of time. At 48 hours, the highest %RSA value occurred in A3 film, where the % RSA value for A3 film resulting from the addition of macerated extract reached 38.32%, and A3 film with soxhletation extract reached 43.65% RSA.

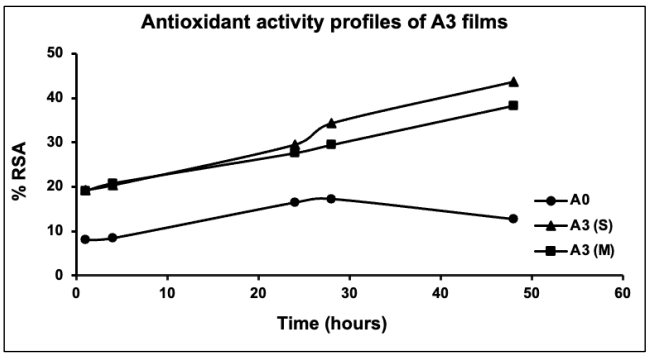

Figure 5. Antioxidant activity profile of A3 film with a final concentration of 0.50% moringa leaf extract where A0: a film without extract, A3 (S): A3 film with extract using soxhletation method. A3 (M): A3 film with extract using the maceration method.

Increasing the concentration level of moringa leaf extract in films A1, A2, and A3 also increased the % RSA value. It confirmed that the higher the concentration of moringa extract added, the higher the antioxidant activity of the films. Similar results were also obtained in the study of Ju et al., (2019), who added moringa leaf extract as an antioxidant agent to the khorasan flour film. The antioxidant test results showed that the addition of a 1% concentration of moringa leaf extract produced % an RSA value of up to 37.89%.

Table 3. % RSA value of chitosan-alginate film at 48 hours with the addition of Moringa leaf extract from extraction by maceration and soxhletation methods

Treatments	RSA value with maceration method (%)	RSA value with soxhlation method (%)
A0 (0%)	$12,69 \pm 0,72^{a}$	$12,69 \pm 0,72^{a}$
A1 (0,50%)	$21,32 \pm 0,72^{b}$	$31,47 \pm 1,44^{b}$
A2 (0,75%)	$27,92 \pm 1,44^{c}$	$34,26 \pm 1,08^{bc}$
A3 (1,00%)	$38,33 \pm 1,79^{d}$	$43,65 \pm 0,72^{d}$

Note:

- Data are shown as mean ± standard deviation
- Different superscript notations in the same column show significantly different results at a value of α=0.05
- A0, A1, A2, and A3: films with the addition of moringa leaf extract with final concentrations of 0%, 0.50%, 0.75%, and 1.00%

Another study conducted by Peng et al., (2013) showed that the addition of tea extract could also

increase the chitosan film's antioxidant activity. The research results of Peng et al, (2013) showed that the addition of 1% green tea extract to the chitosan film resulted in % an RSA of 60% in 48 hours. Meanwhile, the addition of black tea extract in 48 hours produced an RSA value of only 32%.

To determine the effect of moringa leaf extract on the antioxidant activity of the film was tested using a single classification analysis of variance (one-way ANOVA) at a significance level (α) <0.05. The antioxidant activity data used was the %RSA value of the film at 48 hours. The results of ANOVA testing showed that there was an effect of the addition of moringa leaf extract on antioxidant activity. The %RSA value for 48 hours of films can be seen in Table 3.

CONCLUSION

The addition of moringa leaf extract to the chitosan-alginate films showed good antioxidant activity. Moringa leaf extract obtained from soxhletation showed higher antioxidant activity than macerated moringa leaf extract. Chitosan-alginate films added by moringa leaf extract can be developed and has the potency to be applied directly to food.

ACKNOWLEDGMENT

The author acknowledges to Institute for Research and Community Service (LP2M) of Universitas Islam Negeri Mataram for all support until this article was published.

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