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Physicochemical Properties of Sodium Alginate Edible Film Incorporated with Mulberry (*Morus australis*) Leaf Extract

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ABSTRACT

In this study, sodium alginate film incorporated with mulberry leaf extract [0-4 % (v/v)] were evaluated in terms of its physicochemical properties. Results showed that with the increase of mulberry leaf extract concentration, the thickness of the film increased (from 0.07 mm to 0.11 mm), while the color of film produced increased in its green and yellow intensity. In terms of mechanical properties, with the increase of mulberry leaf extract concentration, a significant increase in the tensile strength but a significant decrease in the elongation at break of the film were observed, while no significant effect (p>0.05) on the puncture force was observed. Similarly, no significant effect (p>0.05) on moisture content, water solubility, and water activity of sodium alginate films were found, with the increase of mulberry leaf extract. An increase in the total phenolic content from 8.92 mg GAE/100 g to 41.88 mg GAE/100 g was observed when 4 % (v/v) of mulberry leaf extract was added.

Keywords: Mulberry leaf extract, physicochemical properties, sodium alginate, total phenolic content

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INTRODUCTION

The edible film can be defined as the coating of food material which can be consumed together with the food product itself (Pavlath & Orts, 2009). An increase in the demand from consumers towards natural and safe product leads to the work for the development of edible film, where it can serve as alternative coatings for plastic material coatings and packaging (Janjarasskul & Krochta, 2010; Shit & Shah, 2014).

Edible film base materials can be categorized into three groups, namely polysaccharide, protein, or lipid (Vieira et al., 2011). With the application of edible film, the shelf life of food can be extended by minimizing its contact with air, thus reducing prolong lipid oxidation (Cirillo & Spizzirri, 2015). In addition, the edible film can be incorporated with antimicrobial and antioxidant sources such as herbs and spices (Emiroğlu et al., 2010).

Among the different edible film base, alginate is one of the common polysaccharides used for the development of the edible film (Ferreira et al., 2016). It is extracted from seaweed and structured by 1-4b-D-mannuronic acid (M) and a-L-guluronic acid (G) (Kim, 2013). Its fibrous structure can form films, while its hydrophilic properties can reduce lipid oxidation when applied to foods (Pavlath & Orts, 2009; Varela & Fiszman, 2011).

For film formation, glycerol is commonly used as a plasticizer to enhance flexibility, while calcium chloride forms crosslinking between its calcium ions and sodium alginate, improving the film's water resistance (Rhim, 2004; Vieira et al., 2011). In addition, herbs and spices that have an antimicrobial effect such as oregano, rosemary essential oil, thyme essential oil, *Melastoma malabathricum* extract, and 'asam keping' were also incorporated into film (Chan et al., in press; Choong et al., 2019; Jouki et al., 2014a, b; Seydim & Sarikus, 2006; Zaman et al., 2018).

Luís et al. (2019) incorporated licorice essential oil into carboxymethyl xylan film. They found that the film with the presence of licorice essential oil exhibited positive antimicrobial effect against *Enterococcus faecalis* and *Listeria monocytogenes* while Abdollahi et al. (2019) who added summer savory essential oil into carboxymethyl cellulose-agar biocomposite film reported on the high antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *and Listeria monocytogenes* with essential oil at 1.5% (v/v). Also, rosemary and *Aloe vera* essential oil were added into cellulose acetate in which it had high antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* in the study of El Fawal et al. (2019).

Mulberry leaf is a heart shape or mitten-shaped leaf belongs to Moraceae family (Rahman & Khanom, 2013; Srichaikul et al., 2011). It is used as silkworms' foods, papermaking, Chinese medicine, diabetes, and blood pressure treatment (Chen et al., 2007; Vichasilp et al., 2012). Mulberry leaf was reported to have antimicrobial

and antioxidant properties, in which flavonoid such as quercetin-3-glucoside, kaempferol-3-glucoside, and quercetin-3-(6-malonylglucoside) was found (Islam et al., 2008). To the best of our knowledge, the information on mulberry leaf extract based edible film were scarce. Hence, this work aimed to study the effect of mulberry leaf extract on the physical, mechanical, chemical, antimicrobial, and antioxidant properties of alginate edible film.

MATERIALS AND METHODS

Preparation of Mulberry Leaf Extract

Mulberry leaf was obtained from roadside bushes near Puchong, Selangor, Malaysia. It was identified by the red and black mulberry fruit produced from the tree. It was cut and dried overnight at 40 °C and ground using a grinder (Sharp, EM-11, Malaysia).

The extraction process was performed according to Sarbadhikary et al. (2015) with slight modification. The mulberry powder (10 g) was added with ethanol (100 mL) and stirred for 24 hours in a rotary shaker (Infors AG, Switzerland) at 150 rpm before filtration with filter paper (Whatman No. 3, Filters Fiorini, Sweden) and centrifuged (Centrifuge 5810 R, Eppendorf, Germany) for 15 minutes at 9,860 x *g*. The extract was then subjected to the rotary evaporator (R-000, BÜCHI, Switzerland) under vacuum at 40 °C and the extract (50 mg/mL concentration of mulberry leaf extract, with 5 g extract dissolved 100mL solvent) was then kept at 4 °C for further analysis.

Analysis of Mulberry Leaf Extract

Antimicrobial Properties. The disc diffusion method was carried out with *Staphylococcus aureus* and *Escherichia coli* on Mueller-Hinton Agar (Sarbadhikary et al., 2015). Antibiotic chloramphenicol disc (Oxoid, UK, 30 µg) was used as positive control and a paper disc (6 mm diameter) infused with 50 µL of ethanol served as the negative control. The inhibition zone was measured with a micrometer.

Antioxidant Properties of Mulberry Leaf Extract. The antioxidant property of mulberry leaf extract was performed by utilizing DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay (Wong et al., 2014). Mulberry leaf extract (0.1 mL) was added with 3.9 mL of 0.004% ethanolic DPPH solution into a test tube (wrapped with aluminum foil). The mixture was vortexed and left to stand in the dark for 30 minutes before measuring its absorbance value at 517 nm, using a UV-Vis Spectrophotometer (Uviline 9400, Secomam, France). Percentage of DPPH scavenging activity was calculated by using Equation 1:

$$\text{DPPH Scavenging activity (\%)} = \frac{Abs\ of\ DPPH - Abs\ of\ sample}{Abs\ of\ DPPH} \times 100 \quad (1)$$

where Abs of DPPH is the absorbance value of 0.004% ethanolic DPPH solution and the Abs of sample is referring to the absorbance value of the extract at 517 nm.

Total Phenolic Content of Mulberry Leaf Extract. Folin-Ciocalteu's reagent (1.5 mL) was pre-diluted 10 times with distilled water and mixed with mulberry leaf extract (0.3 mL) and sodium carbonate (1.2 mL). The mixture was mixed and left for 30 minutes in dark, before measuring the absorbance at 765 nm with a UV-Vis spectrophotometer (Uviline 9400, Secomam, France). A standard curve was generated by using gallic acid solution (0- 0.1 mg/g), with regression equation of $y = 0.0119x + 0.0124$ and $R^2 = 0.9991$ (Wong et al., 2014).

Production of Edible Film Incorporated with Mulberry Leaf Extract

About 1.5% (w/v) of sodium alginate powder (Synertec, Malaysia) was added with 200 mL of distilled water and stirred for 30 minutes at 70 °C. Glycerol 0.75% (v/v) was added, followed by stirring for another 15 minutes. Mulberry leaf extract with concentration at 0, 1, 2, 3, and 4% (v/v) was added into film solution, respectively. A preliminary study shows with the addition of 5%, the film formed is sticky and not smooth.

The mixture (25 g) was poured on a petri dish (90 mm × 15 mm) and dried at 40 °C for 24 hours in an oven (Memmert, Germany) (Benavides et al., 2012). The sodium alginate film formed was dipped into the calcium chloride solution (45 mL) and dried again for 1 minute. The films formed peeled using forceps and kept in desiccators.

Analysis of Film

Thickness. Micrometer (JY, China) is used to measure the thickness of the film, by placing it at 5 different locations of the film (Garsuch & Breitkreutz, 2009).

Moisture Content and Water Activity. The initial film weight was measured using an analytical balance (XT 220 A, Precisa, Switzerland). After measuring the initial weight, the film was dried in an oven (Memmert, Germany) at 90 °C for 24 hours, before measuring the dried film weight again (Choong et al., 2019). The moisture content was calculated according to Equation 2:

$$\text{Moisture content (\%)} = (M_i - M_0/M_i) \times 100 \quad (2)$$

where M_0 is defined as the initial mass of the film and M_i is defined as the final mass of the film.

On the other hand, the water activity of the film was determined using a water activity meter (AquaLab Pre, METER Group, USA).

Water Solubility. Determination of water solubility was carried out according to the work of Ma et al. (2016) with slight modifications. The film cut into 2 cm × 2 cm and dried in an oven (60 °C) for 24 hours, after which the weight for the dehydrated film (W_1) was measured. The film was then immersed with distilled water (25 mL) for 2 hours at room temperature in a covered condition, after which the film was then dried again for 24 hours at 60 °C). The second weight measured was the final mass (W_2). Water solubility was calculated according to Equation 3:

$$\text{Water solubility} = \frac{W_1 - W_2}{W_1} \times 100\% \quad (3)$$

where W_1 is interpreted as the initial mass of the film and W_2 is interpreted as the final mass of the film.

Color Profile. The color of the film was determined using a colorimeter (ColorFlex Ez, Hunter lab, US) (Zaman et al., 2018). The colorimeter was a spectrophotometer with Pulsed Xenon Lamp as a light source, coupled with a 256-element diode array and a high- resolution concave holographic grating. The colorimeter was calibrated with white and black tile standards before the sample test. The films were then placed against the sample cup to measure the color.

The color was expressed as L* (lightness-darkness), a* (red-green) and b* (yellow-blue) values, while the total color differences (ΔE) for the films was calculated according to the Equation 4:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}, \quad (4)$$

where ΔL*, Δa*, and Δb* represent the difference of readings as the parameter values compared the sodium alginate with mulberry leaf extract film to the pure sodium alginate film.

Tensile Strength and Elongation at Break. The tensile analysis was performed using the tensile testing machine (TA-XT Plus, Surrey, UK) (Remya et al., 2015). Films strips (50 mm x 20 mm) were placed on the grip, with separation of the initial grip fixed at 50 mm. The speed was set at 25 mm/min for the crosshead. Calculations for the tensile strength and elongation at break were proceeded by using Equations 5 and 6, respectively:

$$\text{Tensile strength} = \frac{Peak\ load\ (N)}{\text{Cross} - \text{sectional area (mm}^2\text{)}} \quad (5)$$

$$\text{Elongation at break} = \frac{Final\ length\ of\ film\ ruptured\ (mm)}{\text{Initial grip length (mm)}} \times 100\% \quad (6)$$

Puncture Force. Puncture force was determined using a texture analyzer, with the needle probe (2 mm diameter) moving at a constant rate of 1 mm/s (Muppalla et al., 2014). The puncture force was calculated using Eq. 7:

$$\text{Puncture force} = \frac{Maximum\ force\ at\ break\ (N)}{Thickness\ at\ broken\ area\ (mm)} \quad (7)$$

Fourier Transform Infrared Spectroscopy (FTIR). Fourier transform infrared spectroscopy (FTIR) spectrometer (Nicolet iS5, Thermo Fischer Scientific, USA) equipped with OMNIC Spectra Software in transmission mode was used to measure the spectra absorbance of the film (Pranoto et al., 2005). The resolution was set as 4 cm^{-1} with an average of 20 scans for wavenumbers in between the range of 500 cm^{-1} to 4000 cm^{-1} against a background spectrum from an empty cell.

Antioxidant and Antimicrobial Properties of Film. The antioxidant properties (free scavenging activity and total phenolic content) were performed following the method of Wong et al. (2014), while antimicrobial properties were conducted according to Fernández-Pan et al. (2012).

Statistical Analysis

Data were represented as a mean value ± standard deviation and were analyzed using analysis of variance (one-way ANOVA) and Tukey's post hoc test was used for analyzing the significant difference among the film properties (SPSS version 23). The *p*-value of ≤0.05 was defined as statically significant.

RESULTS AND DISCUSSION

Antimicrobial Properties of Mulberry Leaf Extract

Mulberry leaf extract (50 mg/mL) was found to have an antibacterial effect against *E. coli* with the minimal inhibition zone of 1.46 ± 0.34 mm while having no antimicrobial activity from the mulberry leaf extract against the *S. aureus*. This result is in agreement with the study done by Thabti et al. (2014), who showed that mulberry leaf extract exhibited antibacterial activity against *E. coli*. Besides that, the result of the antimicrobial activity against *S. aureus* is in agreement with the study done by Manjula and Shubha (2011), who reported that there was no antimicrobial activity against the *S. aureus* (concentration of mulberry leaf extract concentration at 25 µL/L).

Antioxidant Properties and Total Phenolic Content of Mulberry Leaf Extract

It was found that the mulberry leaf extract (50 mg/mL) had free radical scavenging activity of 49.88 ± 1.23%. On the other hand, the total phenolic content of mulberry leaf extract was found to be 553.93 mg GAE/100g. These findings are consistent with results shown by the study of Memon et al. (2010) who found similar free radical scavenging activity in mulberry leaf extract (48.13 ± 1.20%). The antioxidant activity and total phenolic content were contributed by flavonoid compounds such as quercetin and rutin (Katsube et al., 2009). According to Katsube et al. (2006), quercetin is the most abundant flavonoid compound present in mulberry leaf thus, contributing to the antioxidant activity of mulberry leaf.

Thickness

Figure 1 (a) shows the physical properties of sodium alginate incorporated with different concentrations of mulberry leaf extract. With the increase of the mulberry leaf extract, it was found that the thickness of the edible films increased after 3% (v/v) incorporation of mulberry leaf extract (from 0.07 mm to 0.10 mm). Further incorporation of the leaf extract (4% v/v) resulted in no significant difference (p>0.05) with those with 3% (v/v) leaf extract. The increase of thickness was also observed in the work of Benavides et al. (2012) in the film added with oregano essential oil, where the thickness increase was due to the increase of total solids in the film-forming solution. As reported by Utami

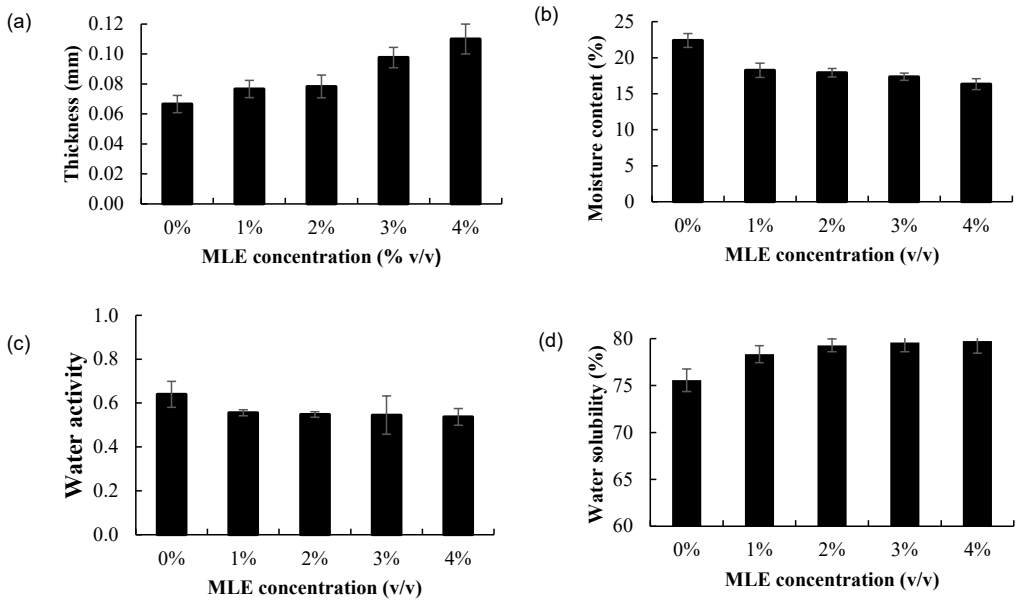

Figure 1. Physical properties including (a) thickness, (b) moisture content, (c) water activity, and (d) water solubility of sodium alginate edible film incorporated with different concentration of mulberry leaf extract (MLE)

et al. (2019), the increase in cinnamon essential oil caused an increase in the total solids of the film-forming solution thus, increasing the film thickness.

Moisture Content and Water Activity

From Figure 1 (b) it was observed the moisture content was decreased with the addition of mulberry leaf extract concentration, with no significance between film added with different extract concentrations (p>0.05). This may be explained by the concentration of mulberry leaf extract at 1% (v/v) that had reached a sufficient level to occupy the microstructural network space of sodium alginate (DeMan et al., 2018). Adding on to that, the decrease in moisture content might be due to the decreased water affinity of films containing more hydrophobic mulberry leaf extract (Han et al., 2018). This is in agreement with the result where an increase of mulberry leaf extract had no significant effect (p>0.05) on the water activity of film produced (Figure 1 c). Low water activity is favored for edible film because it inhibits the growth of organisms and extends shelf life (Ijabadeniyi & Pillay, 2017). Moisture content and water activity of the film influence its moisture absorption behavior, which in turn affects the water solubility (Gennadios & Weller, 1994).

Water Solubility

The result in Figure 1 (d) shows that the sodium alginate without mulberry leaf extract has 75.57% water solubility. Low

water solubility is desirable for edible film, to avoid the complete dissolution of the film (Ozdemir & Floros, 2008). With the addition of mulberry leaf extract [1% - 4% (v/v)], the water solubility of the film was reported to be in the range of 78.35-79.74%. There was no significant difference (p>0.05) between the water solubility of the film when 1% to 4% (v/v) concentration of the mulberry leaf extract was added into the film.

The findings from this analysis are in agreement with the study done by Jutaporn et al. (2011), where the water solubility of the film increased when phayom wood extract was added. The increase in water solubility may be contributed by the decreased polymer network interaction density due to the presence of mulberry leaf extract. Furthermore, alginate film is highly water-soluble, due to its hydrophilic nature (Shit & Shah, 2014).

Color Profile

The color of the film is important as it influences customer acceptance towards a product (Galus & Lenart, 2013). For the films, it is most favorable that the films are light in color or transparent so that it would not affect the food color (Acevedo-Fani et al., 2015). According to Table 1, with an increase in mulberry leaf extract, the sodium alginate film has a decrease in L* value, a* value, while an increase in its b* value and total color change (ΔE). This indicates that the film is darker, greener, and yellower with an increase of extract due to the dark

Table 1

The color profile of sodium alginate edible films with mulberry leaf extract

The concentration of mulberry leaf extract incorporated (%)	Color			
	L*	a*	b*	ΔE
0	25.32 ± 0.77^{c}	-0.26 ± 0.05^{b}	0.27 ± 0.01^{a}	0.00 ± 0.00^{a}
1	21.43 ± 0.38^{b}	0.11 ± 0.04^{c}	2.58 ± 0.13^{b}	4.54 ± 0.25^{a}
2	20.86 ± 0.30^{b}	-0.32 ± 0.03^{b}	4.08 ± 0.16^{c}	5.87 ± 0.21^{a}
3	19.28 ± 1.47^{a}	-0.49 ± 0.03^{a}	4.91 ± 0.17^{d}	7.62 ± 1.33^{b}
4	18.55 ± 0.78^{a}	-0.52 ± 0.05^{a}	5.31 ± 0.15^{e}	8.44 ± 0.74^{c}

Note. $^{a-e}$ Mean ± standard deviations followed by different superscript letters within the same column are significantly different at $p \leq 0.05$ according to Tukey's test

color of mulberry leaf extract. The increase in yellowish and greenish color of the film was from the natural color of the mulberry leaf. Du et al. (2009) reported that the color of the films was influenced by essential oils added, which result in a decrease of L* and a* with the concentration cinnamon essential oil. The result is also in agreement with the study done by Mehdizadeh et al. (2012), who found that there was a decrease in L* and a* with increased *Thymus kotschyanus* essential oil.

Tensile Strength and Elongation at Break

Tensile strength is the maximum amount of stress that a sample can withstand during tensile testing (Benavides et al., 2012). According to Figure 2 (a), the tensile strength increases from 12.91 to 23.45 MPa when the concentration of mulberry leaf extract increased from 1% (v/v) to 3% (v/v). The decrease of tensile strength at high mulberry leaf extract concentrations may be attributed to the formation of discontinuities creating stressed regions in the film matrix, leading to decreased tensile strength (Frank et al., 2018). The decrease of tensile strength at high concentration was also observed by Han et al. (2018) when sodium alginate/carboxymethyl cellulose films were incorporated with cinnamon essential oil.

In terms of elongation at break, the value decreases with the increase of mulberry leaf extract concentration from 0% (v/v) to 4% (v/v), which showed a reduction from 35.60% to 5.76% (Figure 2 b). This result correlates with the findings of Sánchez-González et al. (2010) who found that the addition of tea tree essential oil to chitosan films caused a decrease in elongation at break of the films. This may be due to the increase in compound concentration generating a cross-linking effect through the interaction between polymers with the compound, leading to the reduction of the flexibility for the molecular of the polymer (Ojagh et al., 2010).

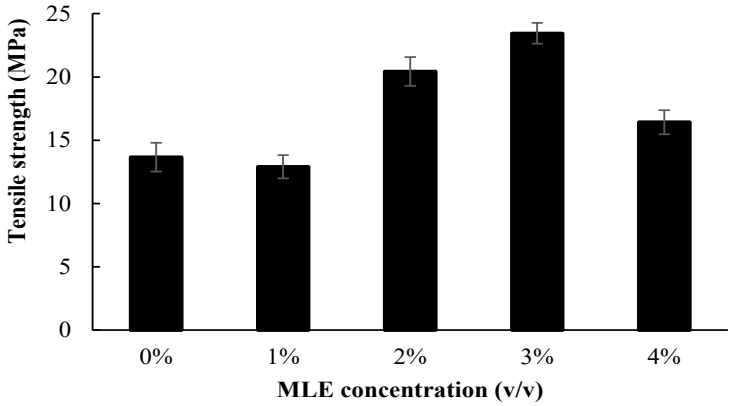

Figure 2. Mechanical properties: (a) tensile strength

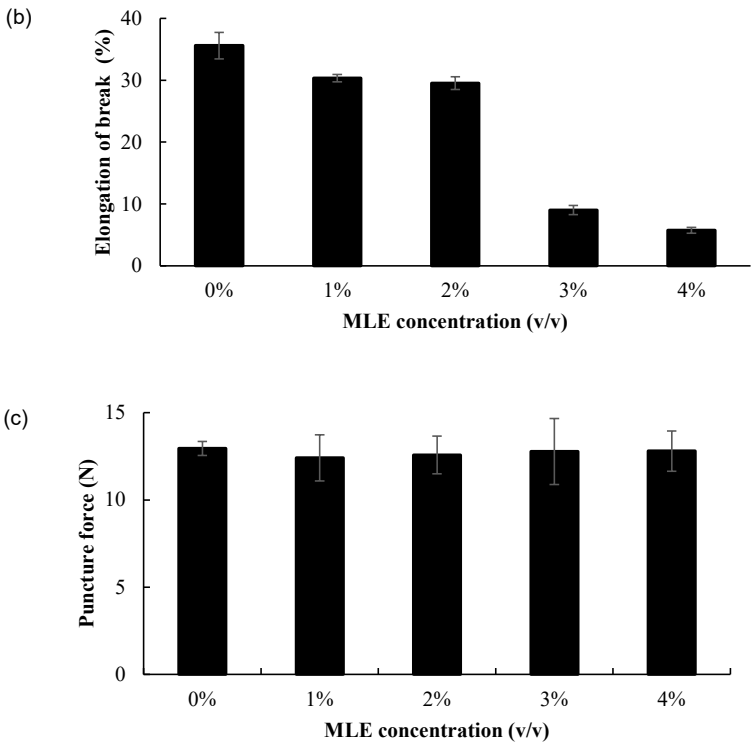

Figure 2 (Continued). Mechanical properties: (b) elongation at break, and (c) puncture force of sodium alginate edible films with mulberry leaf extract

Puncture Force

From Figure 2 (c), it is observed that the puncture force of films ranges from 12.41-12.95 N, with no significant difference (p>0.05) among the films. This result is consistent with the study from Gómez-Estaca et al. (2009), with no significant difference (p>0.05) among the bovine-hide gelatin film incorporated with a low and high concentration of oregano extract. The puncture force did not decrease with an increase in the extract. This is favorable for film properties. Adding on, the same result was observed by Aguirre et al. (2013), who found that there was no significant difference (p>0.05) on the puncture force of triticale protein films incorporated with different oregano essential oil concentrations.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was conducted to identify and analyze the functional group of present and the FTIR spectra can be observed in Figure 3. The film samples had similar broadband around 3279 cm^{-1} to 3286 cm^{-1}, where the absorbance peak was the intensity of O-H stretching (Ba et al., 2010). The appearance of an absorption band for O-H stretching was broad as it was

influenced by the intermolecular hydrogen bonding between the O-H groups. A strong hydrogen bond causes a long O-H bond, and this allows the O-H bond to be stretched easily (Burrows et al., 2017). It can be observed that the amplitude of the band decreased when higher concentrations of mulberry leaf extract were added. This means that the interaction between the bonds was lower at high concentrations. This can explain the lower tensile strength observed when a higher concentration of mulberry leaf extract was added (Tongnuanchan et al., 2013).

The presence of the peak around at 1149 cm^{-1} on the film sample incorporated with 3% (v/v) and 4% (v/v) mulberry leaf extract was associated with the epoxy C-O stretching vibration and glycosidic linkage C-O-C stretching (Čopíková et al., 2013; Hanifah et al., 2015).

The epoxy C-O and glycosidic linkage C-O-C stretching indicate the presence of polysaccharide compound from the mulberry leaf extract. There is also a peak around at 1600 cm^{-1} which indicate the presence of phenolic compound such as chlorogenic acid in the mulberry leaf extract (Liang et al., 2016). However, there is no peak observed around 1600 cm^{-1} for film added with 1% (v/v) and 2% (v/v) mulberry leaf extract, this may be due to the insufficient amount of the phenolic compound from the mulberry leaf extract.

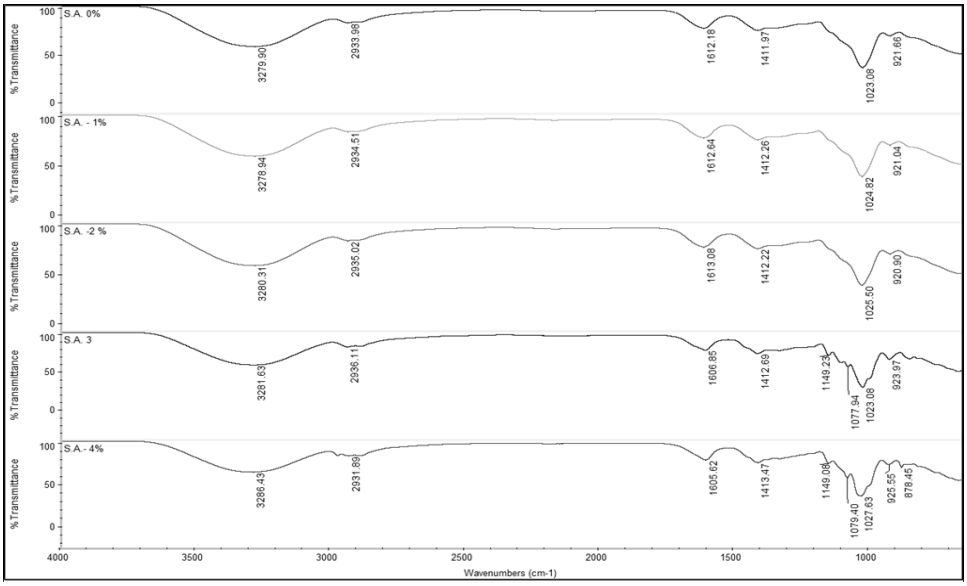

Figure 3. Spectra of Fourier transform infrared spectroscopy (FTIR) of SA-MLE edible film (a) sodium alginate + 0% MLE, (b) sodium alginate + 1% MLE, (c) sodium alginate + 2% MLE, (d) sodium alginate + 3% MLE, and (e) sodium alginate + 4%

Antioxidant Properties and Total Phenolic Content of Sodium Alginate Edible Films with Mulberry Leaf Extract

According to Figure 4 (a), sodium alginate film with no mulberry leaf extract have scavenging activity at 2.48% as the alginate polymer can provide scavenging activities (Ueno & Oda, 2014). With the addition of mulberry leaf extract (from 1%- 4% v/v), the scavenging activity in the film increased from 5.67% to 9.98%. Increasing scavenging activity was due to an increase of mulberry leaf extract concentration (Mehdizadeh et al., 2012).

Similarly, the total phenolic content of the film samples increased from 8.92 mg GAE/100g to 41.88 mg GAE/100g when the concentration of mulberry leaf extract range at 0% (v/v) to 4% (v/v) (Figure 4 b). This is to the addition of mulberry extract which has the total phenolic content (Jouki et al., 2014b). It was also found that there is much reduction of DPPH scavenging activity and total phenolic content, which has decreased 80% and 92%, respectively when the mulberry leave extract (100%) was added into the film in 4% (v/v) concentration.

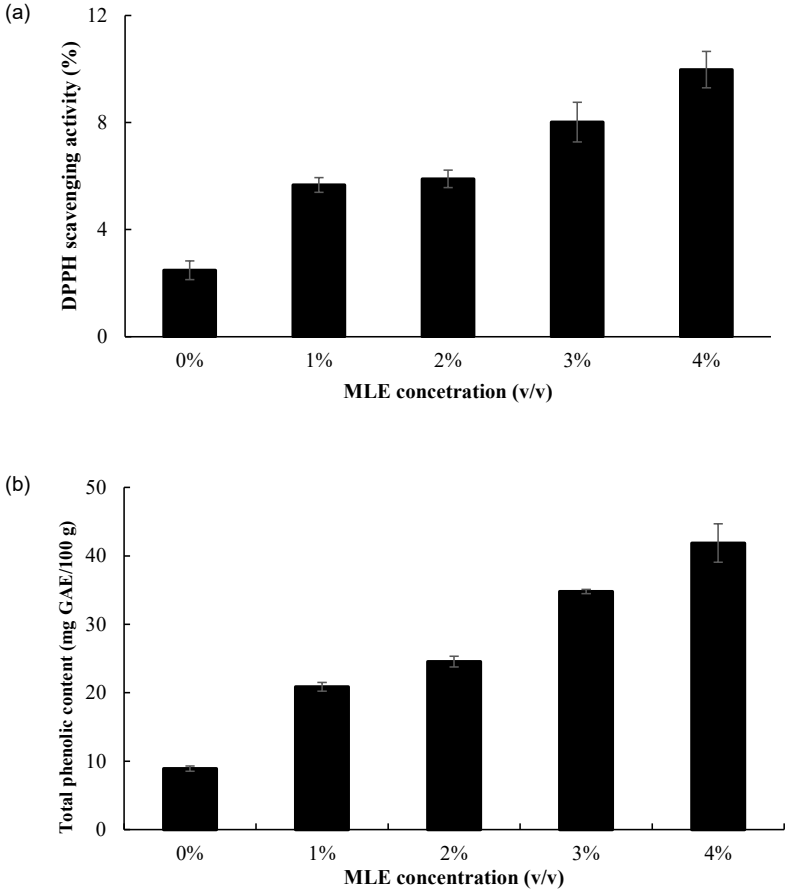

Figure 4. (a) DPPH free radical scavenging activity and (b) total phenolic content of sodium alginate edible films with mulberry leaf extract

Antimicrobial Activity of Sodium Alginate Edible Films with Mulberry Leaf Extract

There was no antibacterial activity found for the sodium alginate edible films with mulberry leaf extract. However, antimicrobial activity was found in the mulberry leaf extract, this may be due to the lesser amount of mulberry leaf extract incorporated into the films as compared to the extract on its own (with 1.46 mm inhibition zone).

This was in agreement with the study of Benavides et al. (2012), where a low concentration of oregano essential oil incorporated into edible film showed no antimicrobial activity. In addition, Chan et al. (2020) also found no antimicrobial acidity in *Melastoma malabathricum* incorporated film, despite the inhibition zone found in the extract.

CONCLUSIONS

With a higher concentration of mulberry leaf extract incorporated, the sodium alginate film shows an increase in thickness which is important for the mechanical aspect of the film. Increased mulberry leaf extract concentration produces films that are darker, greenish, and yellowish. This may decrease consumer acceptance. Besides, the increase of mulberry leaf extract produced a film that is low in tensile strength, a decrease in elongation at the break while not affecting its puncture force. Mechanical properties of the film when added with extract is still weak, hence further study on improving mechanical strength is suggested, by optimizing the concentration of glycerol. The films with mulberry leaf extract contain higher scavenging activity and total phenolic content compared with the film without extract. As the sodium alginate edible film with 3% (v/v) of mulberry leaf extract had higher tensile strength, it was considered as the best formulation among the 4 concentration, although overall the tensile strength is still quite low. The 3% mulberry leaf extract film can be further improved to be a potential film wrapping the foods for enhancing the antioxidant properties and total phenolic content.

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