CHARACTERIZATION OF CARRAGEENAN EDIBLE FILM WITH NATURAL ANTIOXIDANTS FROM *Syzygium cumini* LEAF EXTRACT (SCLE)

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Abstract: Edible film have received considerable attention because of their advantages including their use as edible packaging material over synthetic films. This study aimed to characterize an edible film based on carrageenan and *Syzygium cumini* leaf extract (SCLE) as a natural antioxidant. The addition of SCLE was carried out with various concentrations of 0, 5, 10 and 15%. Characterization was carried out by measuring the physical properties, mechanical properties and antioxidant activities. The result showed that the presence of SCLE gave an increase in thickness and a decrease in solubility and water content compared to the edible film without the addition of SCLE. The addition of 5% SCLE resulted in a slight increase in tensile strength and a reduction in elongation at break. The edible films also showed an increase in antioxidant activity with the addition of SCLE where the highest antioxidant activity was at the addition of 15% SCLE. The incorporation of natural antioxidants in edible films can be a potential strategy to produce promising active packaging to extend product shelf life in the food packaging industry.
Keywords: edible film; carrageenan; *Syzygium cumini.*

Abstrak: Edible film telah mendapat banyak perhatian karena keuntungannya sebagai kemasan yang dapat dimakan dibandingkan film sintetik**.** Tujuan dari penelitian ini adalah untuk mengkarakterisasi edible film berbasis karaginan dan ekstrak daun *Syzygium cumini* (SCLE) sebagai antioksidan alami. Penambahan SCLE dilakukan dengan variasi konsentrasi 0, 5, 10 dan 15%. Karakterisasi dilakukan dengan mengukur sifat fisika, sifat mekanik dan aktivitas antioksidannya. Hasil menunjukkan bahwa keberadaan SCLE memberikan peningkatan pada ketebalan dan penurunan pada kelarutan dan kadar air dibandingkan edibe film tanpa penambahan SCLE. Penambahan SCLE sebanyak 5% menghasilkan sedikit peningkatan pada kuat tarik dan pengurangan pada perpanjangan putus. Edible film juga menunjukkan peningkatan aktivitas antioksidan dengan penambahan SCLE dimana aktivitas antioksidan tertinggi pada penambahan 15% SCLE. Penggabungan antioksidan alami pada edible film dapat menjadi strategi potensial untuk menghasilkan kemasan aktif yang menjanjikan untuk memperpanjang umur simpan produk pada industri kemasan makanan.
Kata kunci: edible film; karaginan; *Syzygium cumini.*

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Introduction

Organoleptic, nutritional and microbiological properties are determinants of food quality, all of which can change dynamically during storage and distribution. These changes are mainly caused by interactions between food and the surrounding environment or migration between various components in food (Debeaufort & Voilley, 2009). The role of packaging is undeniable in the life cycle of any food product (Sohail et al., 2018). Most food products can interact to some extent with their packaging. Migration, permeation and absorption are the mechanisms that determine the extent of these interactions, which are affected by temperature, storage time and other environmental parameters. The main challenge faced by food and packaging manufacturers is to ensure the integrity and safety of their products, so that factors related to packaging including chemical and physical integrity will be controlled, ensuring that products reach consumers in optimal conditions (Mannheim & Passy, 1990).

Innovations continue to emerge in food packaging, aiming to create more efficient quality preservation while increasing the attractiveness and marketability of food (Cerqueira et al., 2011). The use of renewable raw materials to produce bioplastics or edible films that can reduce the problem of plastic waste is an attractive alternative to be developed (Bourtoom, 2008; Gheribi et al., 2020; Hassan et al., 2018; Suput et al., 2015; Tavassoli-kafrani et al., 2015; Dhanapal et al., 2012; Ribeiro & Iberian, 2018). Edible films made from polysaccharides can be used instead of synthetic packaging because they can act as a good barrier to oxygen (Yang & Paulson, 2000)

Carrageenan is a polysaccharide derived from seaweed and has been widely used as a food additive (Bono et al., 2014; Liu et al., 2015; Ega et al., 2016) and is a linear sulfate of D-galactose and 3,6-anhydro D-galactose (Campo et al., 2009; Manuhara et al., 2016). Much research on carrageenan-based edible films and evaluation of their characteristics have been carried out (Herliany et al., 2013; Dwimayasanti, 2016; Saiful et al., 2013). The use of carrageenan on edible films showed an increase in mechanical properties and water vapor permeability (Abdou & Sorour, 2014; Setijawati, 2017).

The new trend in edible films is focusing on functional improvement through the incorporation of active compounds such as antioxidant agents; Eça et al., 2014). The addition of antioxidants to edible films can produce better preservation quality (Kumari et al., 2017). Research on the antioxidant activity of *Syzygium cumin*i leaf extract has been widely reported (Artanti et al., 2019; Marliani et al., 2014). Ruan et al., (2008) showed the presence of phenolic compounds which are responsible for their antioxidant activity. Other studies have also reported that *S. cumini* contains terpenoids, tannins, coumarins, saponins, alkaloids dan cardiac glycosides (Akhtar et al., 2018).

The edible film with the addition of antioxidants is an active packaging that promises to extend the shelf life of the product. In this study, edible films were made with the addition of *S. cumini* leave extract (SCLE) as a natural antioxidant.

Material and methods

Raw materials

Carrageenan, glycerol Merck, aquadest Merck, methanol Merck, ethanol Merck, DPPH (1,1-diphenyl-2-picrylhydrazil), *S. cumini* leave extract (SCLE) is based on the procedure carried out by Sari et al., (2018) by extracting the maceration method using 96% methanol. The *S. cumini* leave was obtained from Aceh, Indonesia.

Film formation

Edible film formation refers to the method of Rusli et al., (2017) using the best concentration of carrageenan and glycerol produced and modifications to the addition of *S. cumini* leave extract (SCLE) as a natural antioxidant. The carrageenan was dissolved according to the concentration in aquadest which had been heated at 95^{o}C for 30 minutes while stirred, and then glycerol was added. The film-forming solution was left at 95°C while stirred for 10 minutes. Furthermore, the solution was divided into 4 parts and each was added SCLE with a concentration of 0; 5; 10; and 15% (w/v) and stirred. Then it was cooled to 80^{o}C and printed on a petri dish. The result of edible film carrageenan (EFC) and edible film carrageenan with SCLE (EFC-SCLE) was tested for functional groups by FTIR (Shimadzu)

Edible film characterization

Film thickness

The thickness test on the edible film was carried out by using a digital micrometer with an accuracy of 1 μm. The measurements were carried out at three different test sites on the film randomly. Afterwards, the thickness values average was calculated.

Water solubility

The solubility test refers to the procedure of Rusli et al., (2017) with solubility calculated by using the formula :

$$\text{Water solubility (\%)} = \frac{\text{initial weight-final weight}}{\text{initial weight}} \times 100\% \quad ....(1)$$

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Water content

The water content test was carried out by heating the edible film using an oven at a temperature of 105^o C. After heating the edible film was remeasured and the water content was calculated by using the formula :

$$\text{Water content (\%)}=\frac{\text{initial weight-final weight}}{\text{initial weight}}\text{x100\%}......................(2)$$

Mechanical properties

The mechanical properties of edible films such as tensile strength and elongation at break were measured using the AND tool with MCT2150 type. The edible film was cut into a piece with a size of 35 mm x 50 mm, and then they were measured. The tensile strength of edible films was calculated by dividing the maximum force to tear the film (F) by the cross-sectional area of the film (A). While the elongation at break was calculated by dividing the increase in the length of the film when it is torn (b) by the initial length of the film before being pulled (a). Tensile strength and elongation at break were calculated by the formula:

$$\text{TS (Mpa)}=\frac{F}{A}\ \ldots\ldots\ldots\ldots\ldots.....(3)$$

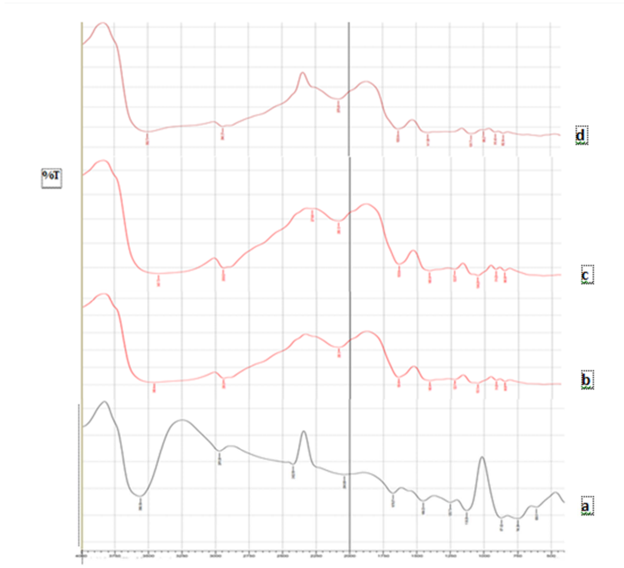
$$\text{EB (\%)}=\ \frac{b\text{-}a}{a}\ x100\%\ ....(4)$$

Antioxidant activity

The test was carried out by adding 1 mL of 0.4 mM DPPH and 5 mL of ethanol to 100 µL samples with various concentrations (50; 100;150 and 200 µg/mL). The mixture was vortexed and left for 30 minutes. The absorbance was measured at a wavelength of 515 nm, and the absorbance of the blank was also measured. The measurement results of antioxidant properties compared with vitamin E.

Result and discussion

The FTIR spectra of edible film carrageenan (EFC) and edible film carrageenan with *S. cumini* leave extract (EFC-SCLE) in different concentrations are shown in Figure 1. The carrageenan film showed characteristic bands at 3560 cm^{-1} (OH stretching), 2970 cm^{-1} (CH stretching), 1678 cm^{-1} (polymer-bound water), 1253 cm^{-1} (O=S=O asymmetric stretching), and 871 cm^{-1} (stretch -O-SO_3- on the C-4 residue of D-galactose) (Table 1). The combination of EFC-SCLE shows a wider band around 3560 cm^{-1} indicating the presence of intermolecular hydrogen bonds between the hydroxyl groups in SCLE and carrageenan. In addition, the edible film carrageenan absorption band at 1253 cm^{-1} shifted to 1222 cm^{-1} when SCLE was coupled. This indicates an intermolecular interaction



formed between SCLE and $–SO_3$- of the carrageenan polymer matrix (Y. Liu et al., 2019).

Figure 1. FTIR spectra of (a) edible film carrageenan (EFC); (b) edible film carrageenan – 5% SCLE (EFC- 5%SCLE); (c) EFC- 10%SCLE; and (d) EFC-15%SCLE**.**

Table 1. Characteristic band of EFC and EFC-SCLE (5%, 10% and 15%)

Functional groups	EFC	EFC + 5% SCLE	EFC + 10% SCLE	EFC+SCLE 15%
OH stretching	3560 cm^{-1}	3456 cm^{-1}	3421 cm^{-1}	3502 cm^{-1}
CH stretching	2970 cm^{-1}	2939 cm^{-1}	2939 cm^{-1}	2943 cm^{-1}
Polymer-bound water	1678 cm^{-1}	1639 cm^{-1}	1635 cm^{-1}	1635 cm^{-1}
O=S=O asymmetric stretching	1253 cm^{-1}	1222 cm^{-1}	1222 cm^{-1}	1091 cm^{-1}
Stretch -O-SO_3- on the C-4 residue of D-galactose	871 cm^{-1}	848 cm^{-1}	848 cm^{-1}	852 cm^{-1}

Thickness

The thickness value of the film can be seen in Table 2. The thickness value ranges from 0.0643-0.0927 µm. The thickness was increased with increasing concentrations of SCLE (5, 10 and 15%) added. Increasing the SCLE concentration will increase the value of the total solid on the film and increase the thickness. The research of Silveira da Rosa et al., (2019) also showed an increase in the thickness of the carrageenan film with the addition of olive leaf extract caused by an increase in solids.

Water solubility

Solubility is a measure of how soluble a film can be when it is consumed and also indicates the integrity of the film in a liquid environment (Fardhyanti & Julianur, 2015). The desired solubility value depends on the application or intended use (Kurt & Kahyaoglu, 2014). Solubility values in this study ranged from 12.5%-75%. The solubility value of edible film decreased with the addition of SCLE. The highest solubility (%) in edible film carrageenan (EFC) with a value of 75%, and the lowest with the addition of 15% SCLE with a value of 12.5% (Table 2).

Water content

The water content analysis aims to find out how much water content is still left behind because it will be related to quality (Fardhyanti & Julianur, 2015). The value of water content in these studies ranged from 60% to 28.6%. The value of the water content in the edible film decreased with the addition of SCLE. The highest water content is found in edible film carrageenan (EFC) with a value of 60%, which is caused by the hydrophilic group in carrageenan which can form intermolecular interactions with moisture (Tavassoli-kafrani et al., 2015). On the other hand, edible films with SCLE (5, 10 and 15%) showed lower water content than edible films with carrageenan. In addition, there was a significant decrease in water content with increasing SCLE concentration. This can be caused by the phenolic group of SCLE forming strong intermolecular interactions with the hydroxyl group of carrageenan so that the interaction between carrageenan and moisture was limited (Liu et al., 2019). The research of Liu et al., (2019) also showed that the addition of mulberry phenolic extract to the -carrageenan film also reduced the water content of the film.

Table 2. Film thickness, water solubility and water content of edible films

Edible film	Thickness (µm)	*Water solubility* (%)	*Water content* (%)
EFC	0.0643	75	60
EFC + 5% SCLE	0.0863	33.3	50
EFC + 10% SCLE	0.0917	28.6	42.9
EFC + 15% SCLE	0.0927	12.5	28.6

Table 3. Mechanical properties of edible films

Edible film	TS (MPa)	EB (%)
EFC	0.8567	147.35
EFC + 5% SCLE	0.9255	112.42
EFC + 10% SCLE	0.7722	121.72
EFC + 15% SCLE	0.7956	131.78

Table 4. Antioxidant activities of edible films

Edible film	IC_{50} (ppm)
EFC	36344.6
EFC + 5% SCLE	736.6
EFC + 10% SCLE	335.5
EFC + 15% SCLE	173.2

Tensile strength and elongation at break properties

Tensile strength and elongation at break were used to describe the mechanism of the properties of edible film materials with their chemical structure properties (Ninnemann, 1968). Tensile properties of edible films such as tensile strength (TS) and elongation at break (EB) can be seen in Table 3. TS increased with the addition of 5% SCLE compared to the carrageenan edible film as a control and decreased with the addition of 10 and 15% SCLE. Meanwhile, with the addition of SCLE (5-15%), there was a decrease in EB value when compared to edible film carrageenan as a control. An increase in TS can be caused by the presence of SCLE which contains phenolic compounds (Ruan et al., 2008) and forms new interactions between polyphenols and polysaccharides such as hydrogen bonds (Dobson et al., 2019; Jancikova et al., 2020). The decrease in EB value may indicate excessive hydrogen bonding between the polyphenol compounds and the carrageenan matrix. This excessive hydrogen bonding can induce polymer aggregation and reduce chain mobility and flexibility (Nguyen et al., 2020). Based on the results obtained, the addition of 2% SCLE is adequate for food packaging applications because of its sufficient resistance.

Antioxidant activity

Antioxidant activity was expressed as the percent absorbance inhibition or as the concentration of antioxidants required to cause a 50% decrease in the initial concentration of DPPH (IC_{50}) (Bonilla et al., 2012). DPPH radicals have been widely used to test the ability of compounds as free radical scavengers (Jouki et al., 2013). In Table 4 it can be seen that the antioxidant activity of the edible film shows that the addition of SCLE (5-15%) could increase the antioxidant activity. The highest antioxidant activity was found in the addition of 15% SCLE with an IC_{50} value of 173.2, while the lowest antioxidant activity was found in the edible film carrageenan as a control with an IC_{50} value of 36344.6. The antioxidant activity of plant extracts was often explained by their total phenolic and flavonoid content with a good correlation. Flavonoids represent the main group of phenolics in the leaves of *Syzygium cumini* (Ruan et al., 2008)

Conclusion

The results of this study showed that the carrageenan edible film was successfully combined with SCLE. The addition of SCLE to the edible film of carrageenan gave an increase in thickness and a decrease in solubility and water content compared to the edible film without the addition of SCLE. The addition of 5% SCLE resulted in a slight increase in tensile strength and a reduction in elongation at break. Edible films also showed an increase in antioxidant activity with the addition of SCLE where the highest antioxidant activity was at the addition of 15% SCLE. Therefore, the edible films enriched with SCLE can be used as a promising antioxidant packaging system to extend the shelf life of products.

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