Journal of Current Science and Technology, September - December 2023 Vol. 13 No. 3, pp. 762-773
Copyright ©2018-2023, Rangsit University
ISSN 2630-0656 (Online)

Cite this article: Praseptiangga, D., Afrida, B., Mufida, N., & Widyaastuti, D. (2023). Effects of stearic acid and zein incorporation on refined kappa carrageenan-based composite edible film properties. *Journal of Current Science and Technology*, *13*(3), 762-773. https://doi.org/10.59796/jcst.V13N3.2023.1324

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Effects of Stearic Acid and Zein Incorporation on Refined Kappa Carrageenan-Based Composite Edible Film Properties

Danar Praseptiangga[1,2*], Beta Afrida[1], Nuha Mufida[1] and Dea Widyaastuti[1]

[1]Department of Food Science and Technology, Faculty of Agriculture, Universitas Sebelas Maret (UNS), Surakarta 57126, Indonesia
[2]Functional Nano Powder University Center of Excellence, Universitas Padjadjaran, Jatinangor 45363, Indonesia

*Corresponding author; Email: dpraseptiangga@staff.uns.ac.id

Received 27 May, 2023; Revised 25 June, 2023; Accepted 4 August, 2023;
Published online 30 August, 2023

Abstract

Incorporating hydrophobic materials into a polysaccharide-based film to form a composite edible film has been considered an effective way to strengthen the film properties, especially the water vapor resistance. Fatty acids, such as stearic acid, with long-chain and straight structures, exhibit strong hydrophobic performance to prevent water vapor diffusion through the film surface. Meanwhile, zein has been revealed as an encouraging material due to its compactness, less allergic, and gas barrier properties. The investigation of kappa-carrageenan/zein/stearic acid-based green composite edible film has been limited. Thus, this study aims to examine the effect of increasing stearic acid and zein concentrations on improving the moisture barrier and mechanical properties of kappa carrageenan-based composite edible film. Different concentrations of stearic acid (5, 10, and 15% w/w carrageenan) and zein (2.5, 5, and 7.5% w/w carrageenan) were applied to the composite edible film prepared using the solution casting method. The fabricated films have a thickness of 0.092–0.122 mm. The results indicated that increasing the concentration of stearic acid enhances the water vapor barrier and tensile strength of the edible film ($p < 0.05$). However, the increased zein concentration slightly weakened the water vapor barrier properties. Then, the elongation of the manufactured films was quite improved by the increment of stearic acid proportion, but neither by the increment of zein proportion nor the combination of these two substances. However, the incorporation of stearic acid and zein into refined-kappa carrageenan-based film remarkably improved the tensile strength, elongation, and water vapor barrier properties by 12–18%, 23–27%, and 43–44%, respectively, in comparison to the neat film. Based on the analysis result, the manufactured film which consists of 10% stearic acid and 2.5% zein is considered as the best film formula. This study, therefore, revealed the potentiality of stearic acid enforcement in food packaging applications.

Keywords: *carrageenan; biopolymer; edible film; fatty acid; physico-mechanical properties; stearic acid, zein*

1. Introduction

Research on edible film packaging has been carried out to solve the devastating effect caused by undegradable conventional packaging waste. Moreover, researchers are focusing on this topic to develop edible films with enhanced characteristics that the food industry demands. Edible film should protect the food from microorganism contamination, oxygen, water vapor interaction and maintain food quality (Mohamed et al., 2020). On the other hand, it is safe to be eaten along with packed food (Hassan et al., 2018). Besides can be ingested, edible film also acts as primary packaging without any adverse effect on food product properties and/or consumer acceptability (Hassan et al., 2018). Since mechanical properties of edible film are designed to maintain integrity and barrier properties of food product, high tensile strength is generally required but deformation values must be adjusted in accordance with the intended application of the film. For instance, in terms of wrapping film application, high film elasticity is a desirable characteristic to keep the material in a deformed state, which promotes edible film to overwrap the food product (Gontard et al., 1992).

Different types of polysaccharides have been applied as renewable materials to produce edible film due to their excellent film-forming ability and abundance at affordable prices, such as cellulose, chitosan, gums, pectin, pullulan, starch, and seaweed extract (Sánchez-Ortega et al., 2014). Among them, carrageenan, extracted from marine red algae, exhibited remarkable film-forming ability due to its gelling power (Hanani, 2017). However, the hydrophilic properties of carrageenan limit its performance in food packaging applications regarding the low moisture barrier properties (Sedayu et al., 2019).

To enhance the edible film's performance, other materials were incorporated to produce the composite edible film. In the case of the edible film made of carrageenan, adding hydrophobic material, such as lipids and oils, is expected to strengthen its moisture barrier properties (Thakur et al., 2016). The incorporation of various plant oils, such as essential oils and fatty acids, notably improved the water vapor permeability of kappa carrageenan-based film due to its hydrophobic nature (Hanani, 2017). Past studies also revealed that incorporation of essential oils, such as cinnamon and *Kaempferia galanga* L. essential oils, provided good antimicrobial and antioxidant activities on semi-refined carrageenan-based films regarding the content of bioactive substances (Praseptiangga et al., 2016; Praseptiangga et al., 2022; Praseptiangga et al., 2021c). Meanwhile, fatty acids such as oleic acid, palmitic acid, and stearic acid also have been applied to edible films regarding their properties' improvement (Fakhouri et al., 2018; Hashemi Gahruie et al., 2020; Thakur et al., 2017). Moreover, previous studies have observed that stearic acid displayed the most hydrophobic performance in comparison to oleic and palmitic acid (Amini & Razavi, 2020) and superior moisture barrier property (Chen et al., 2022) on the surface of edible films as a result of its long chain and saturated fatty acid (Seyedi et al., 2015).

Another substance, such as zein, is listed as a potential material to increase edible film properties in other studies (Praseptiangga et al., 2021a; Sanchez-Garcia et al., 2010; Teklehaimanot et al., 2020; Wang et al., 2017). Zein is an industrial protein extracted from solid residues of corn flour production. As the main protein of corn, zein contains large amounts of hydrophobic chain and neutral amino acids and some sulfur-containing amino acids that uniquely support the film-forming capability (Luís et al., 2019). Crosslinking reactions between starch and zein-induced compact structures exhibit better oxygen barrier properties, potentially preventing the packed food from oxidation (Agarwal, 2021). In addition, the protein-based film also may provide nutrients, particularly zein which is considerably less allergic compared with other commonly used proteins (such as casein, soy protein, and wheat gluten) (Luís et al., 2019). The mechanical properties of zein-based film also exhibit excellent features compared with other plant protein-based film such as soy protein isolate and wheat gluten protein (Fu et al., 2022). However, the zein-based film exhibited brittle film and stiffer in the mixture. Hence, adding fatty acids as an amphiphilic plasticizer emulsified not only as zein-based film blends but also as a cross-linking agent, which facilitates the improvement of the mechanical and water barrier properties (Hassan et al., 2018). The specific study about the combination of refined-kappa carrageenan, stearic acid, and zein for manufacturing edible film has been limited. Therefore, the present study investigated the effect of the mixture to produce desirable film properties. Furthermore, the addition of zein as film material has potential to be one of alternatives to utilize zein as by-products of the corn flour industry. Then, the application of food grade substances in the present study would ensure food safety of the fabricated edible film as well as biodegradability and environmentally friendly food packaging.

2. Objectives

The aim of this study was utilization of stearic acid and zein as additional substances in kappa-carrageenan edible film and investigation of the effect of increasing the stearic acid and zein concentrations on the moisture barrier and mechanical properties of kappa-carrageenan-based composite edible film.

3. Materials and methods

3.1 Materials

The primary material to produce the edible film was refined kappa carrageenan powder extracted from dried red algae (*Kappaphycus alvarezii*), which was acquired from Karimun Jawa Islands, Central Java, Indonesia. The algae were harvested after 45 days of planting. The requirements for extracting refined kappa carrageenan involved $Ca(OH)_2$, 96% ethanol, KCl powder (Brataco Co.Ltd., Bekasi, Indonesia), and HCl (EMSURE® Merck KGaA, Darmstadt, Germany). Then, the plasticizer used was glycerol (Brataco, Co., Ltd., Bekasi, Indonesia). The incorporated materials were stearic acid powder pro-analysis (Honeywell Riedel-de Haën, Seelze, Germany) and zein powder obtained from the Department of Food and Agricultural Products Technology, Universitas Gadjah Mada (Yogyakarta, Indonesia).

3.2 Extraction of refined kappa carrageenan

In the present study, refined kappa carrageenan was extracted using a hot alkaline solution method previously described by Manuhara et al. (2016b). Briefly, dried red algae were washed and soaked in 3 L water for 24 h; the size was reduced using scissors and a blender to obtain the algae pulp. Afterward, the pulp was dissolved in water with a ratio of 1:80 (v/v) under alkaline conditions by using a $Ca(OH)_2$ solution. The extraction was conducted by heating the mixture at 90 °C while continuously stirring for 2 hrs. Subsequently, the filtrate was separated from the solid part and neutralized using 1% HCl solution to reach pH 7 and then reheated for 30 min at 60 °C. By adding 2.5% KCl solution (1:1 v/v) and stirring continuously for 15 min, the filtrate was coagulated and separated carrageenan gel and water by filtration. The obtained carrageenan gel was soaked into 96% alcohol solution by continuously stirring for 1 hrs and filtered to separate alcohol and water. Lastly, the drying process of the kappa carrageenan was conducted at 70 °C for 24 h with a cabinet dryer and then was milled to an 80-mesh powder.

3.3 Films preparation

The production of composite edible films was referred to by Praseptiangga et al. (2017). It started with mixing 2 g of refined kappa carrageenan into 100 ml of distilled water and then heating to 60 ˚C. In addition, 1% (v/v) glycerol was added to the mixture and stirred continuously for 10 min at a temperature of 60 ˚C. The amount of refined kappa carrageenan and glycerol was based on preliminary study (not published). In a separate arrangement, zein powder (2.5%, 5%, and 7.5% w/w carrageenan) was diluted in 10 ml ethanol, followed by 10 min stirring, before blending with stearic acid (5%, 10%, and 15% w/w carrageenan) and carefully agitated for 20 min. Subsequently, the resulting mixture solution was introduced into the prepared film solution and heated to 80 ˚C for 5 min, under continuous stirring. This sample was then poured onto a plastic plate (23x15x2 cm) and dried in a drying oven (type MOV-112, Sanyo, Japan) at 60 ˚C for about 12 hrs. The dried film was eventually peeled off from the plate for further characterization. A similar procedure was conducted to produce control film, which consisted of refined kappa carrageenan without zein and stearic acid addition.

3.4 Films characterization

The manufactured films were characterized by thickness, mechanical properties, and water vapor transmission rate (WVTR).

3.4.1 Thickness

The thickness of the film sheet was measured using a digital micrometer (type KW06-85, KRISBOW, Indonesia) at five different points, and the average value was obtained (Praseptiangga et al., 2021b).

3.4.2 Mechanical properties

The mechanical properties were evaluated with modification according to ASTM D 882-02 (ASTM, 2002). The measurement comprised tensile strength (MPa) and elongation at break (%) test using a universal testing machine (type Z0.5, zwickiLine, Germany). Initially, the film specimen was cut into an arch-shaped strip with a dimension size of 10 cm x 2.5 cm. The film layer was attached to the grips of the testing machine and operated at a test speed of 10 mm/min with an initial grip separation of 50 mm. As the obtained data, the maximum load (N) was divided by the initial cross-sectional area (m^2) to determine the tensile strength value. Then the elongation at break was calculated by dividing the elongated length at break by the initial length of the film which is described in percentage value (%) (Praseptiangga et al., 2021a).

3.4.3 Water vapor transmission rate

The WVTR of the films was determined gravimetrically based on ASTM E96-92 with modifications as conducted in past study (Manuhara et al., 2016a). Concisely, the procedure was conducted by cutting the film into a circular shape with a length diameter of 7.5 cm. Subsequently, 10 g of silica gel (desiccant) was filled into the test cup which was covered and well-sealed with the film sample and then weighed to obtain the initial weight. Afterward, the test cup was placed into a glass desiccator under set-up conditions at 70% RH and 28 ± 2 ˚C by filling the desiccator with saturated NaCl solution. The weight change of the sample was observed by weighing the test cup every hour for 8 hrs. The collected data were plotted to figure out the slope of linear regression which represented the quantity of water vapor at steady state permeating through the film surface per unit time (g/hr). The WVTR was calculated by dividing the slope (g/hr) by the transfer area (m^2) (Praseptiangga et al., 2021a).

3.5 Statistical analysis

This experiment used a 3x3 factorial design with two independent variables: stearic acid and zein concentration. Univariate analysis of variance (Two-way ANOVA) was used to analyze the statistical difference among the obtained data at a 5% level of probability ($p < 0.05$). Then Duncan's multiple range test was performed to evaluate the significant difference.

4. Results

4.1 Thickness

The thickness of the refined kappa carrageenan-based composite edible film with the addition of stearic acid and zein in different concentrations, as presented in Table 1, was in the range of 0.092 – 0.122 mm. The result was comparable with the previous study result, which utilized palmitic acid for manufacturing refined kappa carrageenan-based film (0.095 – 0.109 mm) (Praseptiangga et al., 2021a). Even though these values were higher than those of the kappa carrageenan-based films without stearic acid and zein addition which have a thickness of 0.032 – 0.056 mm for kappa carrageenan film with sorbitol as the plasticizer (Balqis et al., 2017) and 0.044 – 0.053 mm for semi-refined kappa carrageenan-based edible film (Manuhara et al., 2016a). The average thickness of the fabricated films was 0.098 mm that still could be considered as thin film. In general, edible film is described as a thin layer with a thickness of less than 0.1 – 0.3 mm (ASTM, 1989). In this limit, the edible film as the packaging material is expected to keep the appearance as well as the sensory properties of the food products when the packaged product is eaten together with the edible film (Jancikova et al., 2020). The film thickness also determines other film characteristics, such as elongation, tensile strength, and water vapor permeability, which is dependent on film composition and processing parameters (Arham et al., 2016).

Based on the two-way ANOVA results in Table 2, the thickness was significantly influenced by the increasing concentration of stearic acid but neither by zein concentration nor the interaction of those two factors. Increasing the amount of stearic acid to 15 % significantly increased the thickness of the films, as presented in Table 3. Meanwhile, an increase in zein concentration did not significantly impact the composite film thickness. However, the addition of either stearic acid or zein remarkably produced lower thickness edible films compared with native film thickness (Table 1 and Table 3). Thus, the thickness of fabricated films tended to decrease by the addition of stearic acid and/or zein powder and increase by increasing concentration of stearic acid. The incorporation of sucrose fatty acid ester, butyric acid, and stearic acid also exhibited lower thickness values than the native film, rice starch/iota-carrageenan-based film (Thakur et al., 2017).

Previous works reported that incorporating certain substances into edible film, particularly stearic acid, will contribute to more dissolved solids loaded in the film, forming a film thickness (Roy & Rhim, 2019). Rising stearic acid concentration significantly increased the thickness of iota carrageenan-based films (Thakur et al., 2016) and *Salvia macrosiphon* gum-based edible films (Amini & Razavi, 2020). The introduction of other lipid substances, such as plant oils, also exhibited thicker kappa carrageenan-based film (Hanani, 2017). The phenomenon was related to conformational changes that occur due to the transition from an ordered polymer structure to an unordered form due to the presence of fatty acids that may be responsible for the increment (Amini & Razavi, 2020).

Table 1 Thickness, tensile strength (TS), elongation at break (EAB), and water vapor transmission rate (WVTR) of the edible films*

Stearic acid (%)	Zein (%)	Thickness (mm)	TS (MPa)	EAB (%)	WVTR (g/hr.m^2)
0	0	0.122±0.011	2.52±0.18	15.16±3.72	23.82±3.12
5	2.5	0.096±0.003	2.64±0.25	17.99±2.83	13.88±0.05
5	5.0	0.094±0.001	2.87±0.16	17.94±2.14	13.95±0.02
5	7.5	0.094±0.003	2.67±0.32	18.64±2.65	13.95±0.05
10	2.5	0.092±0.001	3.12±0.22	18.34±0.91	13.80±0.10
10	5.0	0.093±0.002	2.91±0.16	17.24±1.14	13.84±0.02
10	7.5	0.092±0.003	2.87±0.12	16.76±1.42	13.92±0.02
15	2.5	0.099±0.003	2.72±0.20	18.96±1.72	13.32±0.03
15	5.0	0.097±0.001	2.62±0.32	18.33±0.47	13.39±0.04
15	7.5	0.100±0.002	2.69±0.22	20.38±0.31	13.51±0.00

*Values are presented as mean ± SD (n=4).

Table 2 Two-way ANOVA of stearic acid and zein concentration based on the properties of edible films

Variable	Source	Mean square	F	Sig.
Thickness	Stearic Acid	0.000	7.064	0.003
	Zein	5.333	0.324	0.726
	Stearic Acid* Zein	5.542	0.337	0.851
TS	Stearic Acid	0.287	5.715	0.008
	Zein	0.022	0.444	0.646
	Stearic Acid* Zein	0.060	1.188	0.336
EAB	Stearic Acid	9.542	2.340	0.114
	Zein	1.934	0.474	0.627
	Stearic Acid* Zein	2.879	0.706	0.594
WVTR	Stearic Acid	0.961	0.985	0.385
	Zein	0.051	0.052	0.949
	Stearic Acid* Zein	0.005	0.005	1.000

Table 3 Main factor analysis using Duncan's multiple range test*

Factor	Concentration (%)	Thickness (mm)	TS (MPa)	EAB (%)	WVTR (g/hrs.m^2)
Stearic acid	0	0.122^c	2.520^a	15.116^a	23.824^b
	5	0.095^{ab}	2.729^a	18.188^b	13.929^a
	10	0.092^a	2.967^b	17.448^b	13.854^a
	15	0.098^b	2.677^a	19.224^b	13.406^a
Zein	0	0.122^B	2.520^A	15.116^A	23.824^B
	2.5	0.096^A	2.828^B	18.432^B	13.666^A
	5.0	0.094^A	2.801^B	17.833^B	13.728^A
	7.5	0.095^A	2.743^{AB}	18.596^B	13.795^A

*Different letters in the same column within a factor indicate significantly different ($p < 0.05$).

The film thickness is also influenced by the preparation process, such as film homogeneity and drying condition (Praseptiangga et al., 2022). Film homogeneity plays a crucial role in advancing the film's mechanical properties, which are indicated by well-distributed thickness. For producing homogeneous and good coverage film, the stirring speed of the emulsion contributes to better dispersion of the ingredients in the film matrix, particularly the distribution of stearic acid, diminishing the formation of pores and regions by fatty acid agglomeration (Schmidt et al., 2013). On the other hand, thinner film is provided by increasing the temperature and time of drying, which can highly promote more water to evaporate (Bagheri et al., 2019; Praseptiangga et al., 2016).

4.2 Mechanical properties

Mechanical properties indicate packaging material integrity which determines and protect packed product quality during supply chain distribution (Luís et al., 2019). Table 1 shows the mechanical properties of the fabricated composites films. The tensile strength varied between 2.52 - 3.12 MPa, and the elongation varied between 15.16 – 20.38 %. Moreover, the results were slightly higher than the tensile strength of kappa-carrageenan incorporated with palmitic acid and zein, which the range of 2.02 – 2.54 MPa (Praseptiangga et al., 2021a), and the elongation of the fabricated films was higher than the elongation of the semi-refined kappa-carrageenan-based film with the addition of cinnamon oil (Praseptiangga et al., 2016), palmitic acid (Praseptiangga et al., 2018), and semi-refined iota-carrageenan-based film with cinnamon oil (Praseptiangga et al., 2021c) which the range in 4.09 – 6.06 %, 9.86 – 9.89 %, and 9.50 – 17.16 %, correspondingly.

Based on Table 2, the result of Two-way ANOVA analysis for the film tensile strength had a similar pattern with the film thickness analysis result. The increasing concentration of stearic acid significantly influenced the film's tensile strength. Still, neither the zein concentration nor the combination of the two substances affected the film strength significantly. The tensile strength was enhanced by increasing stearic acid concentration. However, it declined as the stearic acid concentration increased to 15% (Table 3). Previous study reported that the highest tensile strength of semi-refined iota carrageenan-based film was obtained by 20% palmitic acid increment as the critical concentration due to the tensile strength was declined by increasing palmitic acid concentration (Praseptiangga et al., 2017).

A similar trend also occurred in another study, in which the tensile strength of the starch films was enhanced by adding more stearic acid but decreased when a higher concentration was applied. The authors suggested that the increase was related to the enhanced network resistance due to a uniformly incorporated stearic acid in the matrix. At higher concentrations, however, the stearic acid may be unable to disperse homogeneously and form an emulsion with low stability, decreasing the tensile strength (Schmidt et al., 2013). The report was in line with adding a small amount of stearic acid (4% w/w) that significantly improved the tensile strength of soy protein isolate/ sodium alginate-based edible film. In contrast, the tensile strength became lower by increasing the stearic acid proportion (Chen et al., 2022). The weakened network structure correlates with the lipid cohesion issue in the polymer matrix (Schmidt et al., 2013), which allows the replacement of stronger polymer-polymer bonding with weaker polymer-oil bonding by fatty acid addition (Hanani, 2017). Moreover, the saturated fatty acid tends to crystallize at ambient temperature, forming voids and cracks in the film matrix and lowering the tensile strength (Amini & Razavi, 2020).

On the other hand, an insignificant decrease occurred when the amount of zein was increased (Table 3). Another study suggested that the decline in tensile strength film was probably because the zein solution could not distribute homogeneously within the film solution, which created molecular space in the film structure (Zuo et al., 2019). The zein-based film indicates brittle material that performs stiffly when mixed with other materials. Otherwise, zein can also provide higher structural integrity due to intermolecular cross-linking (Teklehaimanot et al., 2020). Furthermore, a past study also reported that the addition of zein by more than 10% decreased the mechanical properties of konjac glucomannan-based film, which interrupted the intermolecular interaction and compatibility between the matrix film and zein (Wang et al., 2017).

Based on the two-way ANOVA result, the elongation at break of the film in this study did not change significantly by increasing the stearic acid or zein concentration (Table 2). However, lifting the amount of stearic acid from 10 % to 15 % slightly increased the elongation value (Table 3), which may be associated with the plasticizing effect of the fatty acid addition (Seyedi et al., 2015). An appropriate plasticizer at an adequate concentration influence to generate extensive and flexible film (Balqis et al., 2017) and simultaneously could decrease the tensile strength which previous studies have presented. The tensile strength of cassava starch-based film declined, and the elongation at break was enhanced by increasing glycerol concentration. The increment of plasticizer addition lowers the interfacial interaction between adjacent polymer bonds, thus weakening the tensile strength of the film (Schmidt et al., 2013; Yokesahachart & Pajareon, 2020). The plasticizing effect also was described by incorporating plant oils (corn oil, soybean oil, olive oil, and sunflower oil) that significantly improved elongation at break of kappa carrageenan-based film (Hanani, 2017). A similar trend is also represented by the mechanical properties of corn/wheat starch-based film incorporated with zein (Zuo et al., 2019).

Furthermore, the incorporation of stearic acid and zein significantly improved the mechanical properties of composite films compared with the control film (Table 3). The tensile strength was enhanced by 18 % and 12 % with the addition of 10 % stearic acid and 2.5 % zein, respectively. Then, the elongation at break was also upgraded by 27 % and 23 % with the introduction of 15 % stearic acid and 7.5 % zein, respectively. The enhancement of mechanical properties indicated the compatibility between refined-kappa carrageenan as the base material and stearic acid and zein as supporting materials. The positive effect of fatty acids as the hydrophobic plasticizer on the rice starch/iota-carrageenan film also improved the film mechanical properties (Thakur et al., 2017). Since zein is a partially hydrophobic protein (amphiphilic protein) and carrageenan is hydrophilic, aggregation would induce due to low affinity between these two polymers during the drying process. However, previous study revealed that the composite film could provide better dispersion due to the affinity improvement by unfolding zein's structure at higher temperature (such as 321 K), which enables it to expose hydrophilic groups on the polypeptide (Cheng & Jones, 2019). Moreover, the use of heat treatment facilitates protein denaturation and complex formation between the electrostatic interaction of carrageenan and zein, and then allows the formation of hydrophobic or disulfide bonds (Bealer et al., 2020). Meanwhile, past studies also reported that zein esterification using fatty acid exhibited zein film with excellent elasticity regarding effective plasticization of long aliphatic chains, which increased the mobility of zein molecules (Tadele et al., 2023).

4.3 Water vapor transmission rate

The water vapor barrier of the film in this study was presented as the water vapor transmission rate (WVTR). Edible film has a major role to control moisture transfer between the food and the surrounding atmosphere, or between two substances of a heterogeneous foodstuff, thus WVTR value should often be as minimum as possible (Gontard et al., 1992; Thakur et al., 2016). Based on Table 1, the WVTR of fabricated composite films was in the range of 13.32–13.95 g/hrs.m^2 and the average WVTR of control film was 23.82 g/hrs.m^2. The obtained result was comparable with previous studies in which the WVTR of the kappa-carrageenan-based film with palmitic acid and zein varied between 13.79–14.43 g/hrs.m^2 (Praseptiangga et al., 2021a). On the other hand, the higher result observed by past studies were as follows: semi-refined kappa-carrageenan (Praseptiangga et al., 2016) and semi-refined iota-carrageenan-based films (Praseptiangga et al., 2018) with the incorporation of cinnamon oil varied between 22.72–23.52 g/hrs.m^2 and 21.45–23.61 g/hrs.m^2, respectively. The results inferred that combining refined carrageenan-based film with fatty acid and zein could be more effective control film WVTR than semi-refined carrageenan and essential oil. Previous study revealed that the effectiveness of fatty acids or essential oils in reducing WVTR related to the more hydrophobic nature of those components, which are less polar than other components (Aliheidari et al., 2013).

Statistically, increasing the concentration of stearic acid, zein, or the combination did not significantly affect the WVTR result (Table 2). However, as shown in Table 3, the WVTR of the refined kappa carrageenan-based film tended to decline gradually to 44% as the amount of stearic acid was raised. It may be explained by the presence of stearic acid's hydrophobic carbon chains, which add to the film's hydrophobicity (Liu et al., 2021). This hydrophobic material prevents water vapor molecules from passing through the film (Seyedi et al., 2015). Considering that the straight shape molecule of stearic acid, as a saturated fatty acid, may produce a denser and more aligned arrangement, thus improving the manufactured film's water resistance (Hanani, 2017). The tendency was analogous to the WVTR of semi-refined iota carrageenan-based film that declined by incorporating 30% palmitic acid due to its hydrophobic nature (Praseptiangga et al., 2017). Increasing the addition of palmitic acid up to 15% into semi-refined kappa carrageenan also decreased the WVTR value and significantly prevented weight loss from wrapped minimally processed chicken breast fillets (Praseptiangga et al., 2018). Other studies also reported a similar trend that increasing stearic acid concentration lowered the water vapor permeability of hydrocolloid-based edible film, such as *Salvia macrosiphon* gum-based film (Amini & Razavi, 2020), *Lepidium perfoliatum* seed gum-based film (Seyedi et al., 2015), and basil seed-based film (Amini et al., 2015). Furthermore, better moisture barrier property is also provided, along with the increasing chain length and degree of saturation of lipids. However, it also depends on the presence of a tortuous path and steric hindrance in the water molecules' diffusion (Seyedi et al., 2015).

Based on Table 3, incorporation of zein into edible films provided lower WVTR result in comparison to the control film. Other studies revealed that the addition of zein could decrease the film's water vapor permeability due to its hydrophobic characteristic (Teklehaimanot et al., 2020; Zuo et al., 2019). Zein has a cylindrical dowel-shaped structure with hydrophobic lateral sides, which is constructed by α-helices, β-pleated sheets, and random coils and is responsible for building a stronger network. Thus, it prevents water adsorption through the film surface and improves the film water barrier property (He et al., 2020). Past studies also investigated that the addition of the zein/carrageenan complex improved the water permeability of gelatin film. The interaction forms a cross-linked network that enables water molecules to pass through a twisted path, and then stopping water vapor outside the foodstuff (Cheng et al., 2022).

4.4 Determination of The Best Film Formula

The best edible film formula was determined by using a non-dimensional scaling method (Sullivan et al., 2015), which was described by Praseptiangga et al. (2021b). The physicochemical parameters of composite films, such as tensile strength, elongation at break, and water vapor transmission rate were used to decide the best film formula. The mutual relevance of film quality was indicated with variable weight (VW) of 1. Then, the calculation result of the overall score on each edible film in terms of these parameters is shown in Table 4. Based on the data, the combination of 10 % stearic acid and 2.5 % zein produced the highest score of edible film formula, which was considered as the best film formula.

5. Conclusion

The effect of increasing stearic acid and zein levels on improving the water vapor barrier and mechanical properties of kappa carrageenan-based composite edible film was studied. The thickness was significantly influenced by the increasing concentration of stearic acid. The addition of both stearic acid and zein significantly produced lower film thickness in comparison to the control film. Then, increasing the amount of stearic acid to 15% significantly increased the thickness of the films. The tensile strength was notably enhanced by increasing the stearic acid to 10 %. Nevertheless, it declined as the stearic acid concentration increased to 15%. The elongation at break of the film did not change significantly with an increase in either stearic acid or zein concentration. On the other hand, the mechanical properties were improved by incorporating stearic acid and zein. Concurrently, raising the concentration of stearic acid enhanced the water vapor barrier. Yet, the increase in zein concentration weakened the water vapor barrier properties. The analysis using a non-dimensional scaling method presented that the addition of 10 % stearic acid and 2.5% zein provided the best film performance.

Table 4 Determination of the best stearic acid and zein concentration using non-dimensional scaling method

Stearic acid (%)	**Zein (%)**	**Variable score**			Total score
		TS	**EAB**	**WVTR**	
0	0	0	0	0	0
5	2.5	0.07	0.18	0.32	0.56
5	5.0	0.19	0.18	0.31	0.69
5	7.5	0.08	0.22	0.31	0.62
10	2.5	0.33	0.20	0.32	0.85
10	5.0	0.22	0.13	0.32	0.67
10	7.5	0.19	0.10	0.31	0.61
15	2.5	0.11	0.24	0.33	0.69
15	5.0	0.06	0.20	0.33	0.59
15	7.5	0.09	0.33	0.33	0.76

The result of this study revealed the potentiality of stearic acid enforcement in food packaging applications. In addition, further study related to oxygen barrier properties, thermal property, and microstructure analysis of the designed edible films is still needed to comprehensively understand the film characteristics.

6. Acknowledgements

The authors are grateful to the Ministry of Education, Culture, Research, and Technology, the Republic of Indonesia, for the Matching Fund (Kedaireka) Program 2022, and Universitas Sebelas Maret for the research grant of Penelitian Hibah Grup Riset (HGR-UNS) A 2023.

7. References

Agarwal, S. (2021). Major factors affecting the characteristics of starch based biopolymer films. *European Polymer Journal*, *160*, Article 110788. https://doi.org/10.1016/j.eurpolymj.2021.110788

Aliheidari, N., Fazaeli, M., Ahmadi, R., Ghasemlou, M., & Emam-Djomeh, Z. (2013). Comparative evaluation on fatty acid and Matricaria recutita essential oil incorporated into casein-based film. *International Journal of Biological Macromolecules*, *56*, 69–75. https://doi.org/10.1016/j.ijbiomac.2013.02.007

Amini, A. M., & Razavi, S. M. A. (2020). Physicochemical characterisation of Salvia macrosiphon gum based edible films incorporated with various fatty acids. *International Journal of Biological Macromolecules*, *162*, 1494–1499. https://doi.org/10.1016/j.ijbiomac.2020.08.034

Amini, A. M., Razavi, S. M. A., & Zahedi, Y. (2015). The influence of different plasticisers and fatty acids on functional properties of basil seed gum edible film. *International Journal of Food Science and Technology, 50*(5), 1137–1143. https://doi.org/10.1111/ijfs.12765

Arham, R., Mulyati, M. T., Metusalach, M., & Salengke, S. (2016). Physical and Mechanical Properties of Agar Based Edible Film with Glycerol Plasticizer. *International Food Research Journal*, *23*(4), 1669–1675.

ASTM. (1989). Standard Test Method for Tensile Properties of Thin Plastic Sheeting 1 D 882-02, ASTM International. *Astm International-14*. US: ASTM International.

ASTM. (2002). Standard Test Method for Tensile Properties of Thin Plastic Sheeting, ASTM International. *Astm International*, *14*(C), 1–12. https://doi.org/10.1520/D0882-09

Bagheri, F., Radi, M., & Amiri, S. (2019). Drying conditions highly influence the characteristics of glycerol-plasticized alginate films. *Food Hydrocolloids, 90*, 162–171. https://doi.org/10.1016/j.foodhyd.2018.12.001

Balqis, A. I., Khaizura, M. N., Russly, A. R., & Hanani, Z. N. (2017). Effects of plasticizers on the physicochemical properties of kappa-carrageenan films extracted from Eucheuma cottonii. *International Journal of Biological Macromolecules*, *103*, 721–732. https://doi.org/10.1016/j.ijbiomac.2017.05.105

Bealer, E. J., Onissema-Karimu, S., Rivera-Galletti, A., Francis, M., Wilkowski, J., Salas-de la Cruz, D., & Hu, X. (2020). Protein–Polysaccharide Composite Materials: Fabrication and Applications. *Polymers*, *12*(2), Article 464. https://doi.org/10.3390/polym12020464

Chen, H., Wu, C., Feng, X., He, M., Zhu, X., Li, Y., & Teng, F. (2022). Effects of two fatty acids on soy protein isolate/sodium alginate edible films: Structures and properties. *LWT - Food Science and Technology, 159*, Article 113221. https://doi.org/10.1016/j.lwt.2022.113221

Cheng, C. J., & Jones, O. G. (2019). Effect of drying temperature and extent of particle dispersion on composite films of methylcellulose and zein nanoparticles. *Journal of Food Engineering*, *250*, 26–32. https://doi.org/10.1016/j.jfoodeng.2019.01.012

Cheng, C., Chen, S., Su, J., Zhu, M., Zhou, M., Chen, T., & Han, Y. (2022). Recent advances in carrageenan-based films for food packaging applications. *Frontiers in Nutrition*, *9*(7), Article 1004588. https://doi.org/10.3389/fnut.2022.1004588

Fakhouri, F. M., Martelli, S. M., Caon, T., Velasco, J. I., Buontempo, R. C., Bilck, A. P., & Innocentini Mei, L. H. (2018). The effect of fatty acids on the physicochemical properties of edible films composed of gelatin and gluten proteins. *LWT*, *87*, 293–300. https://doi.org/10.1016/j.lwt.2017.08.056

Fu, M., Cao, M., Duan, J., Zhou, Q., Dong, M., Zhang, T., Liu, X., & Duan, X. (2022). Research on the Properties of Zein, Soy Protein Isolate, and Wheat Gluten Protein-Based Films Containing Cellulose Nanocrystals. *Foods*, *11*(19), Article 3010. https://doi.org/10.3390/foods11193010

Gontard, N., Guilbert, S., & Cuq, J. (1992). Edible Wheat Gluten Films : Influence of the Main Process Variables on Film Properties using Response Surface Methodology. *Journal of Food Science*, *57*(1), 190–199. https://doi.org/10.1111/j.1365-2621.1992.tb05453.x

Hanani, Z. N. (2017). Physicochemical characterization of kappa-carrageenan (Euchema cottoni) based films incorporated with various plant oils. *Carbohydrate Polymers*, *157*, 1479–1487. https://doi.org/10.1016/j.carbpol.2016.11.026

Hashemi Gahruie, H., Mostaghimi, M., Ghiasi, F., Tavakoli, S., Naseri, M., & Hosseini, S. M. H. (2020). The effects of fatty acids chain length on the techno-functional properties of basil seed gum-based edible films. *International Journal of Biological Macromolecules*, *160*, 245–251. https://doi.org/10.1016/j.ijbiomac.2020.05.136

Hassan, B., Chatha, S. A. S., Hussain, A. I., Zia, K. M., & Akhtar, N. (2018). Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. *International Journal of Biological Macromolecules*, *109*, 1095–1107. https://doi.org/10.1016/j.ijbiomac.2017.11.097

He, F., Kong, Q., Jin, Z., & Mou, H. (2020). Developing a unidirectionally permeable edible film based on κ-carrageenan and gelatin for visually detecting the freshness of grass carp fillets. *Carbohydrate Polymers*, *241*, Article 116336. https://doi.org/10.1016/j.carbpol.2020.116336

Jancikova, S., Dordevic, D., Jamroz, E., Behalova, H., & Tremlova, B. (2020). Chemical and physical characteristics of edible films, based on κ- and ι-carrageenans with the addition of lapacho tea extract. *Foods*, *9*(3), Article 357. https://doi.org/10.3390/foods9030357

Liu, P., Gao, W., Zhang, X., Wang, B., Zou, F., Yu, B., ... & Cui, B. (2021). Effects of ultrasonication on the properties of maize starch/stearic acid/ sodium carboxymethyl cellulose composite film. *Ultrasonics Sonochemistry*, *72*, Article 105447. https://doi.org/10.1016/j.ultsonch.2020.105447

Luís, Â., Domingues, F., & Ramos, A. (2019). Production of Hydrophobic Zein-Based Films Bioinspired by The Lotus Leaf Surface: Characterization and Bioactive Properties. *Microorganisms*, *7*(8), Article 267. https://doi.org/10.3390/microorganisms7080267

Manuhara, G. J., Praseptiangga, D., Muhammad, D. R. A., & Maimuni, B. H. (2016a). Preparation and characterization of semi-refined kappa carrageenan-based edible film for nano coating application on minimally processed food. *6th Nanoscience and Nanotechnology Symposium (NNS2015), AIP Conference Proceedings*, *1710*(1), 030043. https://doi.org/10.1063/1.4941509

Manuhara, G. J., Praseptiangga, D., & Riyanto, R. A. (2016b). Extraction and Characterization of Refined K-carrageenan of Red Algae [Kappaphycus Alvarezii (Doty ex P.C. Silva, 1996)] Originated from Karimun Jawa Islands. *Aquatic Procedia*, *7*, 106–111. https://doi.org/10.1016/j.aqpro.2016.07.014

Mohamed, S. A. A., El-Sakhawy, M., & El-Sakhawy, M. A. M. (2020). Polysaccharides, Protein and Lipid -Based Natural Edible Films in Food Packaging: A Review. *Carbohydrate Polymers*, *238*, Article 116178. https://doi.org/10.1016/j.carbpol.2020.116178

Praseptiangga, D., Afrida, B., & Mufida, N. (2021a). Mechanical and barrier properties of refined kappa carrageenan-based edible film incorporating palmitic acid and zein. *Asia-Pacific Journal of Science and Technology*, *27*(4), 1–8.

Praseptiangga, D., Fatmala, N., Manuhara, G. J., Utami, R., & Khasanah, L. U. (2016). Preparation and preliminary characterization of semi refined kappa carrageenan-based edible film incorporated with cinnamon essential oil. *The 2016 Conference on Fundamental and Applied Science for Advanced Technology (CONFAST 2016),* *AIP Conference Proceedings*, *1746*, 020036. https://doi.org/10.1063/1.4953961

Praseptiangga, D., Ferichani, I. P., & Mufida, N. (2022). Development and Characterization of Bioactive Edible Films Based on Semi-Refined Kappa Carrageenan Incorporated with Honey and Kaempferia galanga L. Essential Oil. *Trends in Sciences*, *19*(17), Article 5761. https://doi.org/10.48048/tis.2022.5761

Praseptiangga, D., Giovani, S., Manuhara, G. J., & Muhammad, D. R. A. (2017). Formulation and characterization of novel composite semi-refined iota carrageenan-based edible film incorporating palmitic acid. *The 2nd International Conference on Energy Engineering and Smart Materials (ICEESM 2017), AIP Conference Proceedings*, *1884*, 030006. https://doi.org/10.1063/1.5002516

Praseptiangga, D., Maimuni, B. H., Manuhara, G. J., & Muhammad, D. R. A. (2018). Mechanical and Barrier Properties of Semi Refined Kappa Carrageenan-based Composite Edible Film and Its Application on Minimally Processed Chicken Breast Fillet. *International Conference on Advanced Materials for Better Future, IOP Conference Series: Materials Science and Engineering*, *333*(1), 012086. https://doi.org/10.1088/1757-899X/333/1/012086

Praseptiangga, D., Mufida, N., Panatarani, C., & Joni, I. M. (2021b). Enhanced multi functionality of semi-refined iota carrageenan as food packaging material by incorporating SiO_2 and ZnO nanoparticles. *Heliyon*, *7*(5), Article e06963. https://doi.org/10.1016/j.heliyon.2021.e06963

Praseptiangga, D., Rahmawati, A., Manuhara, G. J., Khasanah, L. U., & Utami, R. (2021c). Effects of Plasticizer and Cinnamon Essential Oil Incorporation on Mechanical and Water Barrier Properties of Semirefined Iota-Carrageenan-based Edible Film. *3rd International Conference on Food Science and Engineering, IOP Conference Series: Earth and Environmental Science*, *828*(1), 012034. https://doi.org/10.1088/1755-1315/828/1/012034

Roy, S., & Rhim, J. (2019). Carrageenan-based antimicrobial bionanocomposite films incorporated with ZnO nanoparticles stabilized by melanin. *Food Hydrocolloids*, *90*, 500–507. https://doi.org/10.1016/j.foodhyd.2018.12.056

Sanchez-Garcia, M. D., Hilliou, L., & Lagaron, J. M. (2010). Nanobiocomposites of Carrageenan, Zein, and Mica of Interest in Food Packaging and Coating Applications. *Journal of Agricultural and Food Chemistry*, *58*(11), 6884–6894. https://doi.org/10.1021/jf1007659

Sánchez-Ortega, I., García-Almendárez, B. E., Santos-López, E. M., Amaro-Reyes, A., Barboza-Corona, J. E., & Regalado, C. (2014). Antimicrobial Edible Films and Coatings for Meat and Meat Products Preservation. *The Scientific World Journal*, *2014*, 1–18. https://doi.org/10.1155/2014/248935

Schmidt, V. C. R., Porto, L. M., Laurindo, J. B., & Menegalli, F. C. (2013). Water vapor barrier and mechanical properties of starch films containing stearic acid. *Industrial Crops and Products*, *41*(1), 227–234. https://doi.org/10.1016/j.indcrop.2012.04.038

Sedayu, B. B., Cran, M. J., & Bigger, S. W. (2019). A Review of Property Enhancement Techniques for Carrageenan-based Films and Coatings. *Carbohydrate Polymers*, *216*, 287–302. https://doi.org/10.1016/j.carbpol.2019.04.021

Seyedi, S., Koocheki, A., Mohebbi, M., & Zahedi, Y. (2015). Improving the physical and moisture barrier properties of Lepidium perfoliatum seed gum biodegradable film with stearic and palmitic acids. *International Journal of Biological Macromolecules*, *77*, 151–158. https://doi.org/10.1016/j.ijbiomac.2015.03.005

Sullivan, W. G., Wicks, E. M., & Koelling, C. P. (2015). *Engineering Economy* (16th ed.). New York: Pearson.

Tadele, D. T., Shorey, R., & Mekonnen, T. H. (2023). Fatty acid modified zein films: Effect of fatty acid chain length on the processability and thermomechanical properties of modified zein films. *Industrial Crops and Products*, *192*, Article 116028. https://doi.org/10.1016/j.indcrop.2022.116028

Teklehaimanot, W. H., Ray, S. S., & Emmambux, M. N. (2020). Characterization of pre-gelatinized maize starch-zein blend films produced at alkaline pH. *Journal of Cereal*

Science, *95*, Article 103083. https://doi.org/10.1016/j.jcs.2020.103083

Thakur, R., Pristijono, P., Golding, J. B., Stathopoulos, C. E., Scarlett, C. J., Bowyer, M., ... & Vuong, Q. V. (2017). Amylose-lipid complex as a measure of variations in physical, mechanical and barrier attributes of rice starch- ι -carrageenan biodegradable edible film. *Food Packaging and Shelf Life*, *14*, 108–115. https://doi.org/10.1016/j.fpsl.2017.10.002

Thakur, R., Saberi, B., Pristijono, P., Golding, J., Stathopoulos, C., Scarlett, C., ... & Vuong, Q. (2016). Characterization of rice starch-ι-carrageenan biodegradable edible film. Effect of stearic acid on the film properties. *International Journal of Biological Macromolecules*, *93*, 952–960. https://doi.org/10.1016/j.ijbiomac.2016.09.053

Wang, K., Wu, K., Xiao, M., Kuang, Y., Corke, H., Ni, X., & Jiang, F. (2017). Structural characterization and properties of konjac glucomannan and zein blend films. *International Journal of Biological Macromolecules*, *105*, 1096–1104. https://doi.org/10.1016/j.ijbiomac.2017.07.127

Yokesahachart, C., & Pajareon, S. (2020). Comparative study of physico-mechanical properties, thermal stability and water absorption of biodegradable films prepared from commercial oxidized and cross-linked cassava starches. *Journal of Current Science and Technology*, *10*(2), 121–129. https://doi.org/10.14456/jcst.2020.12

Zuo, G., Song, X., Chen, F., & Shen, Z. (2019). Physical and structural characterization of edible bilayer films made with zein and corn-wheat starch. *Journal of the Saudi Society of Agricultural Sciences*, *18*(3), 324–331. https://doi.org/10.1016/j.jssas.2017.09.005