

# CHARACTERIZATION OF EDIBLE FILM BASED ON DIFFERENT RATIOS OF K-CARRAGEENAN/GELATINE WITH THE ADDITION OF CANOLA OIL

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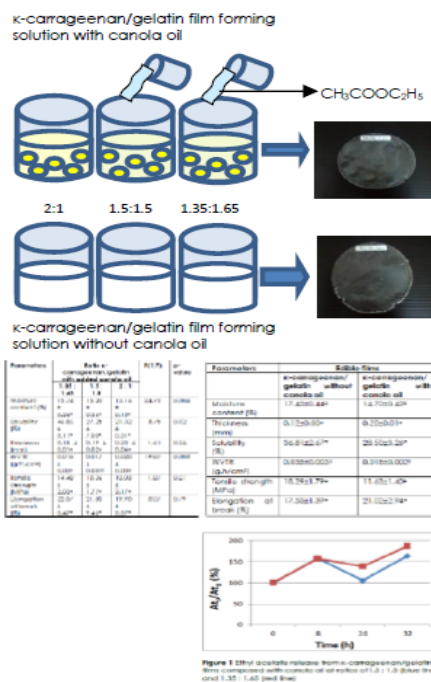
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## Article history

Received  
2 July 2019  
Received in revised form  
22 December 2019  
Accepted  
16 January 2020  
Published online  
27 February 2020

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## Graphical abstract



## Abstract

k-carrageenan/gelatin mixtures and canola oil can form an emulsion film when they are well combined and can mediate the release of volatile substances. The objectives of this study were: 1) to investigate the effect of different ratios of k-carrageenan/gelatin with and without the addition of canola oil on moisture content, solubility, thickness, water vapor transmission rate, tensile strength and percent elongation, 2) to study the release of ethyl acetate with different ratios of k-carrageenan/gelatin with added canola oil. Edible films were prepared with varying ratios of k-carrageenan/gelatin with and without the addition of canola oil. Ethyl acetate release from selected films was also observed. The results showed that different ratios of k-carrageenan/gelatin with canola oil addition induced changes in moisture content, thickness, water vapor transmission rate, and tensile strength ( $p < 0.05$ ). A significant decrease in the water vapor transmission rate of films was induced by canola oil with a ratio of k-carrageenan/gelatin of 1.35:1.65. In addition, k-carrageenan/gelatin at a ratio of 1.35:1.65 with added canola oil triggered a larger release of ethyl acetate. These findings confirmed that different ratios of k-carrageenan/gelatin with canola oil could be used to tailor emulsified films with enhanced barrier properties and to trigger the release of volatile substances.

Keywords: k-carrageenan, gelatin, canola oil, emulsion, ethyl acetate

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## 1.0 INTRODUCTION

The use of emulsion-based films has grown extensively because of their ability to act as a barrier against water vapor permeability (WVP) and gases, and act as carriers of aromatic compounds. These barrier and carrier functions are one of the advantages of emulsion-based films, including their ability to maintain the sensory quality and prolong the shelf-life of food products. Various materials have been used to account for the formula of emulsion-based films. However, the

main challenge is to ensure the compatibility between materials which affect the functional properties of emulsion-based films.

The successful fabrication of an emulsion-based edible film as a barrier and carrier is determined by the right selection of the materials used in the polymer matrix, compatibility and preparation techniques (Galus and Kadzińska, 2015) [1]. Emulsions are thermodynamically unstable systems and are prone to destabilize (e.g., coalescence, creaming) during food processing, transportation or storage (Mao et al., 2013) [2] and emulsified films

tend to have better mechanical properties in comparison with pure lipid layers (Galus and Kadzińska, 2015) [1]. During emulsion film formation, hydrophobic compounds, such as polysaccharides and proteins, must be dispersed into hydrocolloid aqueous solution. Polysaccharides and proteins are usually applied to form films with good mechanical properties and can impart barrier properties to oxygen and carbon dioxide, but their ability to prevent moisture loss is lower. To achieve barrier properties in films, lipids are often added in film-forming solutions.

There have been numerous studies addressing the influence of lipid or oil addition on the physical and mechanical properties and release of volatile compounds from emulsified films. Anker *et al.* (2002) added acetylated monoglyceride to a whey protein film solution. The authors reported a greater reduction in WVP values in bilayers as compared to emulsified films [3]. Also, Quezada-Gallo *et al.* (2000) showed that the nature of the lipid phase not only contributes to the WVP but can also influence the mechanical properties of emulsified films composed of methylcellulose and paraffin oil plus paraffin wax [4]. By studying stearic acid in whey protein films, Yoshida and Antunes (2004) showed that a greater decrease of WVP and solubility of emulsified films was induced by adding stearic acid, but this also weakened their mechanical properties [5].

Moreover, Shokri and Kamkar (2018) reported the ability of films containing oil to increase the shelf life of food products. The authors reported chitosan and WPI-based films containing the essential oil *Mentha pulegiums* had the highest DPPH radical scavenging activity and the greatest inhibitory effects on oxidation of linoleic acid [6]. Concerning the release of volatile compounds, it has been shown that materials used in the matrix of edible films contribute to the release and retention of volatile compounds (Seuvre *et al.*, 2006) [7]. In select cases, iota-carrageenan emulsion based edible films showed better performance in the retention of polar aroma compounds as compared to lipid films (Marcuzzo *et al.*, 2010) [8]. In  $\beta$ -lactoglobulin-pectin stabilized emulsions, lipophilic volatiles could be released at lower rates over wide pH and salt concentration ranges (Benjamin *et al.*, 2012) [9]. Moreover, Evageliou *et al.* (2015) showed that double layered gelatin-gellan films were as effective as aroma barriers [10].

Carrageenan, gelatin, and lipid or oil are usually the components used to produce emulsion-based films.  $\kappa$ -carrageenan consists of repeating units of (1,3)-D-galactopyranose and (1,4)-3,6-anhydro- $\alpha$ -D-galactopyranose, with sulfate groups in a given amount and position (Campo *et al.*, 2009) [11]. It is promising as a film-forming material because it has one negative charge per disaccharide, with a tendency to form excellent gel and film-forming properties (Seol *et al.*, 2009) [12]. The film-forming

potential of proteins such as gelatin is also frequently used because edible films made from gelatin are commonly thin, flexible, and transparent biodegradable materials. The addition of gelatin has been shown to modify the mechanical properties of composite films based on corn starch (Fakhouri *et al.*, 2015) [13]. However, both carrageenan and gelatin have limited impermeability to water vapor since these biopolymers have a highly hydrophilic nature. Attempts to improve the barrier properties of these biopolymers include the addition of oil, such as vegetable oils, in film formulations. Canola oil is characterized by being very low in saturated fat and high in monounsaturated fat and contains significant amounts of omega-3 (alpha-linoleic acid) and omega-6 acids (linoleic acid), both of which are essential for the human diet (Laoretani *et al.*, 2014) [14].  $\kappa$ -carrageenan and gelatin have been known to increase the viscoelasticity of gels (Derkach *et al.*, 2015) [15] and to exhibit good film-forming properties (Pranoto *et al.*, 2006) [16]. However, the properties of emulsified films and the release of ethyl acetate from films with different ratios of  $\kappa$ -carrageenan/gelatin and added canola oil have not been investigated.

The aims of this study were: 1) to investigate the effect of different ratios of  $\kappa$ -carrageenan/gelatin films with and without the addition of canola oil on the moisture content, solubility, thickness, water vapor transmission rate, tensile strength, and percent elongation, and 2) to study the release of ethyl acetate in  $\kappa$ -carrageenan/gelatin films with added canola oil.

## 2.0 METHODOLOGY

### 2.1 Materials

#### 2.1.1 Samples

$\kappa$ -carrageenan (Galic Artabahari, Indonesia) and gelatin (Gelita, China), purchased from Nura Jaya (Surabaya, Indonesia), were used as film-forming components of the continuous hydrophilic phase for emulsion-based films. Canola oil, purchased from the local market, was used as the hydrophobic dispersal phase, and glycerol (technical grade), purchased from Brataco, Indonesia, was added as a plasticizer. Span 80 and tween 80, obtained from Brataco, Indonesia, were employed as emulsifiers. The volatile compound selected for analysis was ethyl acetate (Merck, Germany).

## 2.2 Methods

### 2.2.1 Film Formation

Two films were produced using the casting technique. First,  $\kappa$ -carrageenan/gelatin films without canola oil were prepared by dissolving glycerol (30%) and  $\kappa$ -carrageenan/gelatin (4.5%) in distilled water, with different ratios of  $\kappa$ -carrageenan and gelatin (1.35:1.65; 1.5:1.5; 2:1).  $\kappa$ -carrageenan/gelatin films, with 40% of added canola oil, were also prepared in the same manner as the first films.

For  $\kappa$ -carrageenan/gelatin films, 3 g of gelatin was dissolved in 200 ml of warmed distilled water (65°C) for 15 min using a magnetic stirrer. After the gelatin was completely dissolved, 6 g of  $\kappa$ -carrageenan was successively added under constant stirring for 15 min. Glycerol was added into solution under constant stirring for 15 min. The solution was cooled down to 50°C before being cast in Petri dishes (10 cm diameters). As much as 20 ml of film-forming solutions were then spread onto the Petri dishes and were subsequently dried in an oven at 50°C for 18 h. Finally, dried films were peeled off from the dishes and kept in desiccators at room temperature.

To prepare  $\kappa$ -carrageenan/gelatin films with added canola oil, another step is necessary. This was carried out after glycerol was added into the solutions, but before pouring the film-forming solution, to incorporate blends of emulsifiers and canola oil. Span 80 and tween 80, at the amounts required, were pre-solubilized in 40% canola oil. Then, all of these components were homogenized with an Ultra-Turrax homogenizer (T 25 basic IKA®-WERKE) at 24,000 rpm for 2 min. As much as 20 ml of film-forming solutions were poured onto Petri dishes and dried in an oven at 50°C for 18 h. Finally, dried films were peeled off from the dishes and kept in desiccators at room temperature.

To study the release of ethyl acetate, emulsified films were prepared by applying the amount of pre-solubilized span 80 and tween 80 required, and 2 ml of ethyl acetate in canola oil. Then, all the components were added into the film solution after glycerol was added. The solution was homogenized with Ultra-Turrax at 24,000 rpm for 2 min. As much as 20 ml of film-forming solution was poured onto Petri dishes and dried in an oven at 30°C for 18 h. Finally, dried films were peeled off from the dishes and kept in desiccators at room temperature.

### 2.2.2 Film Characterization

#### Moisture Content

The determination of moisture content was carried out following method by Zahedi *et al.*, 2010 [17]. The moisture content of films was determined by

calculating the loss of film weight after drying in an oven at 105°C for 24 h.

#### Thickness

The thickness of films was measured by using a digital caliper (KRISBOW KW06-422) to the nearest 0.01 mm at five random positions. The values of thickness represent the means of five measurements randomly taken from each sample evaluated.

#### Water Vapor Transmission Rate (WVTR)

The WVTR was determined using a modified gravimetric desiccant method (Poeloengasih and Marseno., 2003) [18]. Small cups with lids were prepared and filled with silica gel. Then, the films were placed on the mouth of the cup and sealed with wax. After sealing, the cups were weighed and then, placed in a desiccator containing NaCl for the time intervals of 0, 8, 24, 32 and 48 h. Then, the weights of the cups were recorded. Data obtained was fitted with a linear regression equation to obtain the slope of the weight of the cups. The WVTR is expressed by the slope of the weight of cup (g/h) divided by the area of the film tested (cm<sup>2</sup>). The mean value of three replications was reported for each sample.

#### Tensile Strength and Percent Elongation at Break (EB)

TS and EB were measured using a Universal Testing Machine (Hung Ta, HT-2010). Film strips (10 cm x 2 cm) were prepared and mounted onto a separate grip of the Universal Testing Machine. Initial grip separation was set at 60 mm and the cross head-speed was set at 30 mm/min. The tensile strength of the specimen was calculated by dividing the maximum load to rupture (F) and the area of films (A). The calculation of tensile strength is represented in eq (1).

$$\text{Tensile Strength} = \frac{F}{A} \quad (1)$$

EB percentage was calculated by dividing the film elongation at the moment the rupture of the specimen occurred (b) by the initial length of the specimen (a) and then multiplying by 100. The calculation of elongation at break is represented in eq (2).

$$\text{EAB} = \frac{b}{a} \times 100 \quad (2)$$

#### Solubility in Water

The solubility of samples was calculated following the method of Ahmad *et al.*, 2012 [19]. It was expressed as the percentage of the film dry mass, which is soluble after 24h immersion in distilled

water. First, 3x2 cm of samples were dried in an oven at 105°C for 24 h and weighed (W1). After 24 h drying, each sample was immersed in a 50 ml tube containing 10 ml of distilled water. After 24 h immersion in distilled water, the solution was filtered using filter paper (Whatman no.1). Finally, samples that remained on the filter paper were oven dried at 105°C for 24 h, after which the samples were weighed to determine the dried remnant of the insoluble mass (W2). Solubility was then calculated using the formula:

$$\text{Solubility (\%)} = \frac{W1 - W2}{W1} \times 100 \quad (3)$$

### 2.2.3 Volatile Release of Emulsified Films

#### Solid Phase Microextraction (SPME) Procedure

To follow the volatile release, the emulsion film containing ethyl acetate was transferred into a 22 ml vial and then sealed with a polytetrafluoroethylene/silicon septum and screw cap. The vial was then heated in a water bath at 30°C for 30 min. The sampling times were 0, 8, 24, and 32 h. All samples were analyzed in duplicate.

#### GC-MS conditions

The volatile compounds present in the headspace of the film were analyzed by a GC equipped with MS and a packed HP-5MS 5% Phenyl Methyl Siloxane (30 m x 250 µm x 0.25 µm). The oven temperature was set 50°C for 5 min, then ramped to 160°C at 3°C/min for 0 min. Helium 0.8 ml/min was used as the carrier gas.

### 2.2.4 Data Analysis

Data analyses were performed using R software (R-3.3.1 release). A T-test was used to determine significant differences among physical and mechanical values calculated in κ-carrageenan/gelatin with and without canola oil addition. To measure the effect of different ratios of κ-carrageenan/gelatin with canola oil on physical and mechanical values, analyses of variance (ANOVA) were performed using the *lm* function in the stats package. Three replications were used to determine physical and mechanical properties. For all data analyses, the effects were considered significant when  $p < 0.05$ .

## 3.0 RESULTS AND DISCUSSION

### 3.1 The Effect of Canola Oil Addition on Physical, Mechanical and Barrier Properties

Because the compatibility between components determines the quality of emulsion-based films, the first aim of this study was to investigate the effect of canola oil addition on moisture content, solubility, thickness, water vapor transmission rate, tensile

strength, and percent elongation of κ-carrageenan/gelatin films. The data obtained for physical, mechanical and barrier properties for both films were analyzed with a t-test (Table 1).

**Table 1** Comparison of the physical, mechanical, and barrier properties of both films

Parameters	Edible films	
	κ-carrageenan/ gelatin without canola oil	κ-carrageenan/ gelatin with canola oil
Moisture content (%)	17.43±0.44 <sup>a</sup>	14.70±0.42 <sup>b</sup>
Thickness (mm)	0.12±0.00 <sup>a</sup>	0.20±0.01 <sup>b</sup>
Solubility (%)	36.81±2.67 <sup>a</sup>	28.50±3.26 <sup>a</sup>
WVTR (g.h/cm <sup>2</sup> )	0.035±0.003 <sup>a</sup>	0.018±0.002 <sup>b</sup>
Tensile strength (MPa)	18.29±1.79 <sup>a</sup>	11.63±1.40 <sup>b</sup>
Elongation at break (%)	17.53±1.39 <sup>a</sup>	21.02±2.94 <sup>a</sup>

Values were mean ± standard error. Values with the same superscript letters between κ-carrageenan/gelatin without canola oil and κ-carrageenan/gelatin with canola oil were not significantly different ( $p > 0.05$ ).

The addition of canola oil into film-forming emulsions affected the thickness of films ( $p < 0.05$ ), as canola oil provided more hydrophobic substances which prevented water evaporation during drying resulting in thicker films, which could in turn influence the moisture content. Greener and Fennema (1989) reported that edible films containing hydrophobic substances such as waxes and oils could form thicker films [20]. This fact is quite important because the oil could bind a little of the water content, as the addition of oil into a hydrocolloidal matrix modifies the equilibrium in water content-relative humidity relationships. With added oil, the more hydrophobic tails, which are easily dispersed and prone to swelling with water, result in greater evaporation during drying. The significant effect of the addition of canola oil on the decrease in moisture content ( $p < 0.05$ ) observed in this study is in accordance with the result of previous studies reported by Soazo *et al.*, 2011 on whey protein emulsion films [21] and Syarifuddin *et al.*, 2017 on sodium alginate/gelatin films [22]. In this study, the authors also reported a lower water solubility of films when canola oil was incorporated (Table 1). These values are similar to other pure films or those containing lipids, such as: 22.4-39.4% of whey protein and beeswax films at 5°C [Soazo *et al.*, 2013] [23], and 27.5-43.1% of cassava starch containing carnauba wax and stearic acid [Chiumarelli and Hubinger, 2014] [24]. Also, there was a significant difference between the WVTR of κ-carrageenan/gelatin with and without canola oil ( $p < 0.05$ ) due to hydrophobicity,



and WVTR was reduced from 0.035 to 0.018 g/h.cm<sup>2</sup>. This could be explained by the fact that some vegetable oils, such as canola oil, with the help of hydrophobic substances that disperse in the films, change the polarity of the components. The superior performance of oil may be attributed to its liquid state, which could promote continuity by increasing resistance to the transfer of water molecules.

Moreover, the addition of canola oil promoted a significant decrease in tensile strength ( $p < 0.05$ ). This result could be due to a decrease in the interaction between water molecules and hydrophilic particles. The presence of oil can reduce the affinity between carrageenan and water. This effect was also reported by other authors when adding oil to skin gelatin [25,26] and could also be attributed to the structural discontinuities provoked by the incorporation of the oil.

### 3.2 The Effect of Different Ratios of K-Carrageenan/Gelatin Films with Canola Oil Addition on Physical, Mechanical and Barrier Properties

Since canola oil-induced changes in the physical, mechanical and barrier properties of films, we also investigated the influence of different ratios of carrageenan/gelatin on the physical and mechanical values of films with added canola oil. A series of ANOVAs were performed on each parameter. The results are summarized in Table 2.

**Table 2** Main factor analysis using the Bonferonni test on  $\kappa$ -carrageenan/gelatin films with added canola oil

Parameters	Ratio $\kappa$ -carrageenan/gelatin with added canola oil			F(1;7)	p-value
	1.35 : 1.65	1.5 : 1.5	2 : 1		
Moisture content (%)	15.74 ± 0.36 <sup>a</sup>	15.20 ± 0.36 <sup>a</sup>	13.16 ± 0.15 <sup>b</sup>	24.73	0.002
Solubility (%)	46.86 ± 8.11 <sup>a</sup>	27.25 ± 7.00 <sup>b</sup>	21.32 ± 0.31 <sup>b</sup>	8.75	0.02
Thickness (mm)	0.18 ± 0.01 <sup>a</sup>	0.19 ± 0.02 <sup>a</sup>	0.23 ± 0.04 <sup>a</sup>	1.61	0.24
WVTR (g/h.cm <sup>2</sup> )	0.016 ± 0.00 <sup>b</sup>	0.017 ± 0.00 <sup>ab</sup>	0.020 ± 0.00 <sup>a</sup>	19.87	0.003
Tensile Strength (MPa)	14.48 ± 2.03 <sup>a</sup>	10.36 ± 1.77 <sup>a</sup>	10.03 ± 3.17 <sup>a</sup>	1.87	0.21
Elongation at break (%)	22.07 ± 0.47 <sup>a</sup>	21.00 ± 9.46 <sup>a</sup>	19.90 ± 5.57 <sup>a</sup>	0.07	0.79

Values were mean ± standard error. Values with the same superscript letters between ratios were not significantly different ( $p > 0.05$ )

The results indicated that different ratios of  $\kappa$ -carrageenan/gelatin did not fully modify the physical and mechanical properties of films. Physical properties are essential parameters, and increasing the  $\kappa$ -carrageenan to gelatin ratio led to a decreased moisture content from 15.74% to 13.16% ( $p < 0.05$ ). Possibly, enriching films with carrageenan leads to cross-linking between the chains of the  $\kappa$ -carrageenan polymer (Watanabe et al., 1996) [27] in which the cross-linking induces a decrease in the availability of hydroxyl groups. This limits polysaccharide-water interactions by hydrogen bonding (Martins et al., 2010) [28]. Notably, these results were also linked with decreased solubility in water ( $p < 0.05$ ). This happen could be due to the lower concentration of gelatin (and a correspondingly higher concentration of carrageenan) leads to the formation of brittle bonds with canola oil, resulting in structural instability. Films with added canola oil were more susceptible to dissolution, and an increase in water solubility as a function of lipid concentration has been previously reported for gelatin-lipid films (Bertan et al., 2005) [29]. Additionally, a significant difference was observed in WVTR values between films with a ratio of 1.35:1.65 and 2:1 ( $p < 0.05$ ). It seems that decreasing amount of  $\kappa$ -carrageenan influences the formation of ionic complexes with higher concentrations of gelatin, and consequently, the compact matrix appeared to be weaker than with the higher amount of carrageenan, resulting in a significant reduction in WVTR values of films. Also, the differences in WVTR values may be attributed to differences in film gel formation and the interaction between  $\kappa$ -carrageenan and gelatin, which was influenced by the amount of carrageenan. As the amount of carrageenan increased, the hydrophilic phase increased, leading to an increase in the WVTR value of films. Other authors observed similar results for mixtures of polysaccharides such as agar, cassava starch and arabinoxylan (Phan The et al., 2009) [30] and  $\kappa$ -carrageenan and locust bean gum (Martins et al., 2012) [31]. It is interesting to note that  $\kappa$ -carrageenan/gelatin films with added canola oil over the respective ratios studied had no significant ( $p > 0.05$ ) effect on tensile strength and percent elongation. This happen because the thicknesses observed in the present study were relatively similar, and the same is true for the amount of oil used. Debeaufort and Voilley, 1995 reported that the mechanical properties of emulsion films were strongly influenced by the presence of lipids and by film thickness [32].

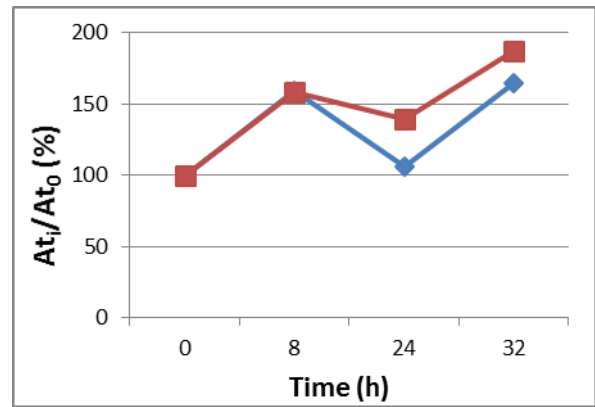
### 3.3 Ethyl Acetate Release of K-Carrageenan/Gelatin with Added Canola Oil Films

Since the emulsified film has potential as a flavor carrier, the second aim of this work was to study the behavior of the release of volatile compounds from films with added canola oil. In the present

study, the area counts ratio was investigated instead of the concentration of the volatile compound, to avoid the influence of the quantity of the volatile compound on the release kinetics (Marcuzo et al., 2010) [8]. Thus, in this way, it possible to compare the release behaviors of ethyl acetate without knowing the quantity of ethyl acetate.

In the present study, films composed of  $\kappa$ -carrageenan/gelatin with added canola oil in the ratio 2:1 were not studied in the ethyl acetate release study since we found that WVTR was the highest in comparing films with ratios of 1.5:1.5 and 1.35:1.65 (Table 2). With carrageenan/gelatin ratios of 1.5:1.5 and 1.35:1.65, significant differences were observed insolubility, but no significant differences were seen in WVTR values. It is interesting to investigate further and understand the release behavior of ethyl acetate since the two emulsified films contain different hydrophilic compounds and the solubility of ethyl acetate in water is higher (121.53 g/L) (Marcuzo et al., 2010) [8]. Ethyl acetate was chosen because it is present in some food products.

The release behavior of ethyl acetate is shown in Figure 1. It is expressed as  $A_{t_i}/A_{t_0}$ , which corresponds to the ratio between area counts at the time  $i$  and area count at time 0. In Figure 1, it is possible to observe ethyl acetate release from carrageenan/gelatin in ratios of 1.5:1.5 (blue line) and 1.35:1.65 (red line). This study was designed to assess whether the matrix of emulsified films composed of different ratios of  $\kappa$ -carrageenan/gelatin could release volatile compounds such as ethyl acetate. In both cases the release is related to the film matrix; in fact, the release rate of ethyl acetate through the matrix of films composed with carrageenan/gelatin with added canola oil ratio 1.5:1.5 exhibited the same behavior as emulsified films composed of a carrageenan/gelatin ratio of 1.35:1.65 during 8 h. Release decreased slightly in the first 24 h, but from that point, the release of ethyl acetate from films composed of a carrageenan/gelatin ratio of 1.35:1.65 increased slightly with increasing time. Similar kinetics seemed to characterize ethyl acetate release from the two films. This could indicate that emulsions with higher carrageenan concentrations have a lower solubility which could inhibit the mass transfer of volatile molecules across the oil-water interface. It has been shown that increasing carrageenan concentrations accelerate gelation and lead to significant increases in the viscoelastic properties of modified gels (Derkach et al., 2015) [15] due to the increase in hydrogen bonds.



**Figure 1** Ethyl acetate release from  $\kappa$ -carrageenan/gelatin films composed with canola oil at ratios of 1.5 : 1.5 (blue line) and 1.35 : 1.65 (red line)

## 4.0 CONCLUSION

To conclude, we showed that in edible film fabrication, materials used such as  $\kappa$ -carrageenan/gelatin modulated by oil addition influence the physical and mechanical properties in a complex way. Moreover, canola oil incorporation into  $\kappa$ -carrageenan/gelatin films affect the release of ethyl acetate, probably due to lower  $\kappa$ -carrageenan concentration.

Further studies should consider the kinetic release of other compounds to understand the role of canola oil and film composition better.

## Acknowledgement

The authors fully acknowledged Hasanuddin University and Direktorat Riset dan Pengabdian Masyarakat, Direktorat Jenderal Penguatan Riset dan Pengembangan Kementerian Riset, Teknologi, dan Pendidikan Tinggi Republik Indonesia for funding this research.

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