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Properties of Edible Film Produced using Combination of Collagen Extracts of Bligon Goatskin with Glycerol

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Abstract: Edible films are one of packaging products that environmental friendly. Edible films can produce from the by-products of the slaughtering of livestock locally. Edible films used as materials packaging of foodstuff to protect food products of contamination of air, light, lipid, dissolved substances and microorganisms. One of the main advantages of edible film packaging is a natural character, so it can consume together with such food products. The purpose of this study was to evaluate the properties of edible film that produced from a combination of collagen extracts from glycerol as a plasticizer. The study was using collagen extract produced from materials of goatskin, Bligon types and ages 2-2.5 years as the main material and glycerol as ingredients plasticizer. Methods of this study were using experiment design with completely randomized pattern unidirectional the design. Three concentrations of glycerol applied, namely: 80%; 90% and 100% (of the total extract collagen in the mix) combined with 10% (w/v) extract collagen. The results showed that the difference concentration of glycerol that used in the mix effect in the thickness, tensile strength and elongation, but no significant on the solubility, water vapor transmission rate (WVTR) as well as a_w values of edible films. The study implication was the application F_1 formula (10 g (10%) of extract collagen solid + 8 g of glycerol (80%) + 100 mL of distilled water) generating properties of edible films better.

Keywords: Edible Film, Collagen Extract, Plasticizer, Glycerol, Goatskin

Introduction

Bligon goat is one of the local livestock resources in Indonesia. One by-product of slaughtering the goat is the skin. Information sources related to the utilization of goatskin as a raw material for food packaging are still lacking. One of type of food packaging that environmental friendly was edible film. One of the advantages of edible film as the packaging material is can be consumed along with the product, so that no waste. Edible films need to be developing as an effort to create an environment zero waste.

In the formation, process of edible film requires a precise formulation in order to form a mass that is able to function properly. Edible film formation influenced by the type and composition of the constituent materials. One of using collagen as an edible film today is as a packaging in the meat industry (Wang *et al*., 2015). The use of whey protein combined with glycerol also been developed as a packaging material (Perez *et al*., 2016). During this time, the manufacture of edible film comes from of starch, whereas, the basic ingredient of the protein has not been widely used. Edible films of three types of material (glycerol, starch and gum nuts) developed as a composite packaging material for fruit products (Saberi *et al*., 2016). The use of essential oils incorporated to carrageenan as material for edible film developed by Soni *et al*. (2015) as the packaging material in the chicken patties product.

In principle, the edible film serves to inhibit the process of mass transfer. In this case the factors related with: (1) moisture, (2) oxygen, (3) carbon dioxide, (4) aroma, (5) lipid and (6) other solutes. This is doing to protect the food from the invasion of water vapor and oxygen (Liu and Han, 2005). One of the benefits of edible film is environmentally friendly (Kim and Ustunol, 2001; Simelane and Ustunol, 2005). One of protein source material that be used is collagen potentially. Collagen has some important functional properties, namely: (1) the gel strength, (2) time of gelling, (3) the melting temperature, (4) viscosity, (5) viscosity, (6) texture and (7) water content. Some properties of surface tension owned by the of collagen, namely: (1) the shape and stability, (2) emulsion, (3) protection of colloids, (4) the shape and stability of the foam, (5) form of films and (6) adhesion and cohesion. The properties of edible film related to the nature and formulation of the materials used. The combination of protein extract collagen with plasticizer (glycerol) allows undertaken produce the right combination. The use of plasticizer considered to improve the properties of edible film. This is doing through interaction process between the polymer chains making up the protein with a plasticizer (Brody, 2005). The addition of plasticizer in a film is very important. Plasticizer has very small volatile molecules. When added to the polymer material, it will form intermolecular attractive force. This will increase the volume of free bonds (Kokoszka *et al*., 2010).

Plasticizer plays an important role as a "barrier" interactions between molecules and increase the number of molecules that are free (Mali *et al*., 2004) as well as weaken the strength of intermolecular bonds in the polymer chain in the opposite (Gounga *et al*., 2007). Edible film is a thin layer formed from materials suitable for consumption (Krochta and Johnson, 1997). To prevent the loss of water molecules during the storage process, the required packaging material with properties better (Krochta *et al*., 1994). Some previous researchers had been incorporate of materials such as proteins, polysaccharides or fat (wax) in making edible film formulation (Caner *et al*., 1998). So, the use of materials of protein considered. This is because the properties of the resulting edible film showed better results (Klahorst, 1999).

Extract collagen from the group of proteins combined with glycerol from the group of fatty accordance exact formulation is very important to improve the quality and properties of edible film. The study aimed to evaluate the properties of edible film that produced using collagen extract combinated with glycerol as a plasticizer.

Materials and Methods

Research Materials

This study was using the main ingredient of goatskin, male, type of Bligon, age of 1.5-2 years, glycerol (*brataco chemika*) as a plasticizer, distilled water, aluminum foil, clip plastic, solution of NaCl 40% (w/v) and silica gel. The equipment was used in this study, namely: teflon plate round with diameter 22 cm, analytical balance (*Sartorius TE 214S*), digital waterbath (*Memmert WNB7-45*), digital oven (*Memmert*), glass beaker, beakers, funnel, glass stirrer, thermometer, cutter knives, scissors and pipette of volume 100 mL (*Pyrex*), tube acrylic with diameter of 45 mm and height of 21 mm and a desiccator.

Research Methods

Preparations Process of Making Collagen Extract

A total of three skin sheet of goat of Bligon types, male, prepared to be processed further became collagen extract. Methods of hydrolysis of extract collagen performed in stages (Ockerman and Hansen, 2000). Goatskin weighed and washed, for further immersion in a solution of lime ($CaCO_3$) for 2 h. Goatskin further neutralized with HCOOH solution to achieve a pH of 7-7.5. After the neutralization process followed by cutting into a size of 3×3 cm. Skin pieces are then composited until homogeneous and then stored in a refrigerator at a temperature of frozen (-18°C) to prevent bacterial contamination.

A total of 400 g of goat skin as raw materials put in CH_3COOH 0.5M solution for 96 h. at a temperature of 5-10°C. Goatskin transferred into a 500 mL tube erlenmeyer then added with distilled water on the ratio 1: 1. The extraction process was doing in a water bath for 9 h at a temperature of 55-70°C. The extraction process consists of three phases, the first phase (3 h) at a temperature of 55-60°C. The second phase (3 h) used the temperature of 60-65°C and the third phase (3 h) at a temperature of 65-70°C. The results of the product of three stages of the process and then done the screening process (filtration) and homogenized. Results of filtration product obtained liquid extract collagen. The collagen extract dried on 70°C for 2 h and then cooled in a refrigerator on temperature of ± 5-10°C for 30 min. Further, the results of the fraction dried in an oven at a temperature of 55°C for 18-20 h to obtain solid collagen extract products. Extract collagen packed in plastic packaging.

Preparation Process of Edible Film Solution

Edible film formulation done by using a combination of extracts collagen solid (protein) with glycerol (fat). The total of 10 g of solid collagen extract dissolved in 100 mL of distilled water (10% w/v) and then homogenized. The use of glycerol applied three levels, namely: F1 = 80%; F2 = 90% and F3 = 100%, where, percentage of glycerol based on the amount of extract collagen used in the mix, namely (F1 = 80%×10 g = 8 g of glycerol); (F2 = 90%×10 g = 9 g of glycerol) and (F3 =

100%×10 g = 10 g of glycerol). Based on these calculations means that three levels mixed formulations, namely:

- F_1 = 10 g of extract collagen solid + 8 g of glycerol + 100 mL of distilled water
- F_2 = 10 g of extract collagen solid + 9 g of glycerol + 100 mL of distilled water
- F_3 = 10 g of extract collagen solid + 10 g of glycerol + 100 mL of distilled water

Process of Making Edible Film

Three beaker glass sizes 250 mL were prepared. Three formula of solution (F1, F2 and F3) made in the glass beaker. The process of making edible film was using casting techniques (Carvalho *et al*., 2007; Sobral *et al*., 2001). Each glass beaker containing a solution of edible film (F1, F2 and F3) then put into a water bath and heated at 70°C for 45 min while stirring. The particles of solid collagen extract and glycerol homogenized. Edible film solution poured on the cast in hot conditions. Teflon dried in oven at a temperature of 55°C for 18-20 h until forming a thin layer on the cast. Teflon removed from the oven and placed at room temperature for approximately 10 min. Gradually, the film opened using a blunt knife to prevent damage. The film wrapped in klip plastic and put in a desiccator to be tested.

Method of Analysis

Thickness (mm). The value of the film thickness was measure by using a micrometer (Digimetic Micrometer Mitutoya) (Kim *et al*., 2002). Film placed between clamps of micrometer and subsequently measured at random at 5-7 different places. The average value of the measurement results later counted.

Tensile strength (MPa). Tensile strength values measured using Universal Mechanical Testing Machine (Zwick/Z 0.5) (Kim *et al*., 2002). Model of test samples prepared as in Fig. 1. The assay samples using the width of 5 mm, while a thickness of the film is determined from the average thickness of the measurement results. Speed of testing machine is 10 mm/min with a distance between clamps is 50 mm. Tensile strength values = F_{max} /A, where F_{max} is the maximum pull force of edible film sample until to break up (N); A = wide of film area (mm^2) ((length (l) × width (w)).

Elongation at break (%). Elongation measurements performed in conjunction with a tensile strength test using Mechanical Universal Testing Machine (Zwick/Z 0.5). The test sample made as in Fig. 1 (Kim *et al*., 2002). Elongation is the length of the length of the film early. Elongation at break (%). = Lc-Lo/Lo×100%, where Lo = initial length of the film (mm); Lc = length of the samples edible film after being drawn up dropping out (mm).

Solubility (%). Solubility is one of the physical properties of edible film that shows the percentage of the dry weight of the dissolved after immersion in water for 24 h (Gontard *et al*., 1992; Fakhouri *et al*., 2004). Film to be analyzed cut to the size of 2×2 cm and then wrapped with filter paper. Film samples and filter paper dried at a temperature of 105°C for 24 h. Film samples and filter paper weighed separately (for the determination of the weight of the dry film (W1). Soaking done 24 h at a temperature of 28°C and during the immersion process occasional stirring. The sheets of film and filter paper dried at a temperature of 105°C for 24 h to determine the weight of the film is not soluble in water (W2). Solubility = W_1-W_2/W_1×100%, where W_1 = weight of dry film initially (g); W_2 = weight of dry film after immersion (g).

Water Vapor Transmission Rate ($g.H_2O.m^{-2}.h^{-1}$). This value determined by gravimetric method (Xu *et al*., 2005). A acrylic tube with diameter 45 mm = 4.5 cm (r = 2.25 cm = 0.0225 m) and height 21 mm was used as a measuring tool. Film sample to be tested was placed on the top of tube containing 10 g of silica gel blue (RH = 0%). The acrylic tube containing a sample of the film then put in a desiccator. A container was placed in a desiccator containing a saturated salt solution (NaCl) 40% (w/v) (RH = 75%) at a temperature of 25°C. The water vapor diffused through the film absorbed by the silica gel so will increase the weight of the gel silica. Acrylic tubes weighed every hour for 7 h. Finally, the data were then calculated using linear regression equation (slope should be calculated). WVTR = Slope of weight gain tube (g/h) (S)/Total of Area Film (TAF) (m^2) (A), where: S is the value of b (slope) and a (intercept) the regression equation (y = a + bx); Total Area Film (TAF) (m^2) (A) = ($\pi.r^2$) = 3.14×$(0.0225)^2$ = 0.00159 m^2, where r = diameter of film = 0.0225 m.

Activity water. The determination of the water activity (a_w) was conducted by using the tool a_w meter (*decagon Divice Pullman WA 99163, USA*). A total of 1 g of film samples placed on the cylindrical container. The container is then placed on the bottom of a_w meter finally, value of a_w can be determined.

Design of Study and Data Analysis

The experiment conducted experimentally using a Completely Randomized Design (CRD) One-way pattern as the basic design. The parameters observed, namely: Thickness, tensile strength, elongation, Water Vapor Transmission Rate (WVTR) and activity water (a_w) value. Data were analyzed using analysis of variance with the help of statistical program SPSS version 15.0. The experiment showed the real effect and further performed the real difference test with Duncan'S Multiple Range Test (DMRT) at the level of 5% (Steel and Torrie, 1991).

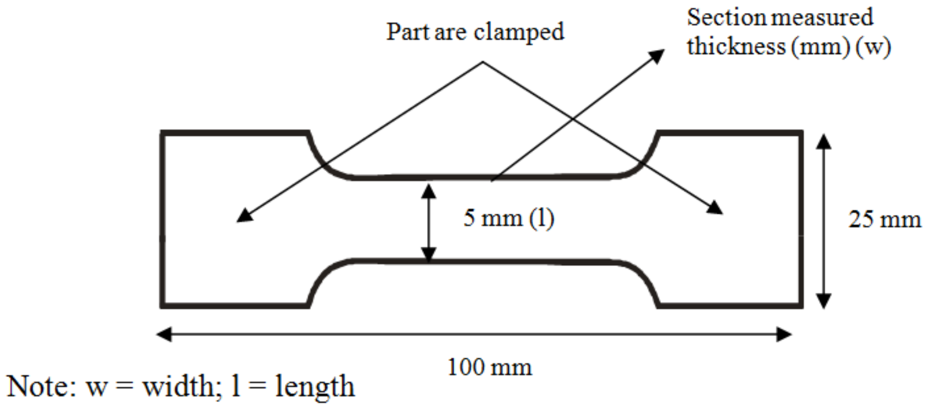

Fig. 1. Model of sample pieces of edible film to test the tensile strength and elongation at break

Results and Discussion

Thickness

The film thickness were affects the properties of edible film. Differences in concentrations of materials was used in the mixture will produce edible film with a thickness that varies. Results of analysis of variance of data in Fig. 2 shows that the differences in the level of glycerol was applied in combination collagen-glycerol extract showed significant effect to the properties of edible film thickness.

The number increased glycerol concentration in the mixture shows the real effect in increasing the thickness of the edible film. This happens because of the addition of the amount of polymer in the composition of edible film (Garcia *et al*., 2000; Tapia-Blacido *et al*., 2005). The concentration of dissolved solids in the mixture affects the thickness of edible film. This happened because of the greater concentration of solids, the structure of the edible film be thicker as well. Moreover, in the composition of the collagen extract and glycerol also contains high organic compounds (Talja *et al*., 2007).

The molecular structure of the collagen extract and glycerol can undergo a process of interaction. This is because the glycerol molecule is hydrophilic molecules with smaller form (Gontard *et al*., 1993). This molecule is very easy to enter in between the protein chains that make up the structure of collagen. Furthermore, these molecules will bind to form hydrogen bonds between the amide groups on the protein structure. The thickness of the edible film made in the range of 0.253 to 0.315 mm. Edible film thickness increased with increasing use of glycerol as a plasticizer in combination collagen extract-glycerol (Zhang and Han, 2006b).

Tensile Strength

Tensile strength is one of the physical properties of edible film closely related to the chemical structure of edible film. These properties determined by the type of edible film forming materials that will affect the nature of the structural cohesion edible film (Gontard *et al*., 1993).

Results of analysis of variance of data in Fig. 3 shows that, the difference in concentration of glycerol, that was apply show significant effect on the value of tensile strength of edible film. Increased of collagen concentration in the solution be causes reduction of tensile strength value of edible film. This is caused by the film matrix have destabilize as consequent increasing of concentration in the structure of the protein component hydrophilic of edible film. Glycerol has hydrophilic properties, which can cause the bending properties of edible film. Therefore, it can affect the value of tensile strength edible film (Gontard *et al*., 1993).

The tensile strength of edible film is the range from 2.277 to 5.265 MPa. Increased use of plasticizer can lower tensile strength edible film. Glycerol is one of plasticizer have applied widely to the manufacture of edible film because plasticizer has hydrophilic properties (McHugh and Krochta, 1994; Gennadios *et al*., 1998).

Elongation at Break

Elongation at break is the change in length of the edible film after given the maximum tensile force to break up edible film compared to the length at first. Results of analysis of variance of data in Fig. 3 shows that increased levels of glycerol show a significant influence on increasing the elongation properties of edible film. Edible film structure is a matrix formed by the interaction ties, such as hydrophobicity, hydrogen and disulfide. Edible film formed with a collagen concentration is higher, will increase the interaction between the ties. This causes the protein molecular bond stronger (Gennadios *et al*., 1998). Enhancement of the amount of plasticizer glycerol will increase the elongation and elastic modulus of the edible film. Plasticizer glycerol has a textural effect that can increase the flexibility of the protein matrix. This causes elongation of the edible film also increased (Reed *et al*., 1998). In addition, the plasticizer has the plasticity effect to produce high elongation (Lim *et al*., 1999).

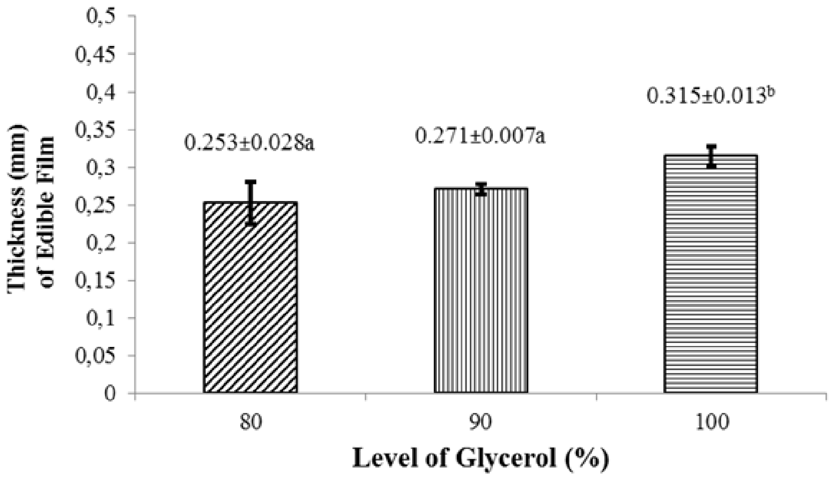

Fig. 2. Thickness value (mm) of edible film between collagen extract combined with glycerol on the different concentrations

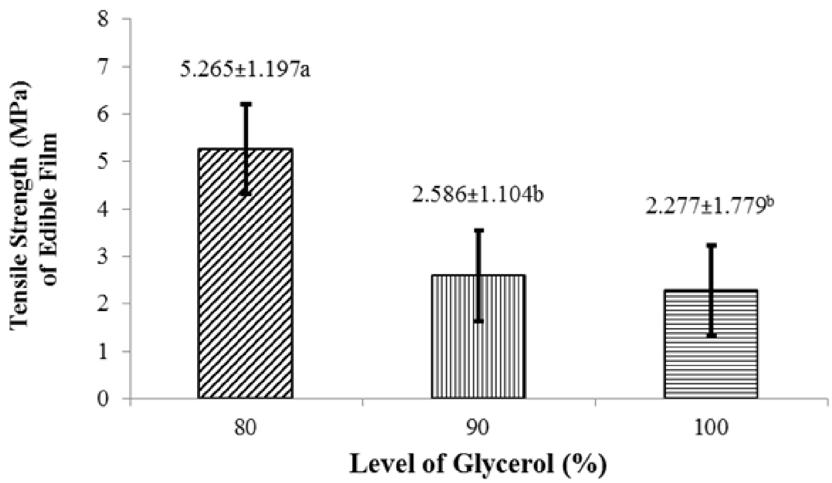

Fig. 3. Tensile strength value (MPa) of edible film between collagen extract combined with glycerol on the different concentrations

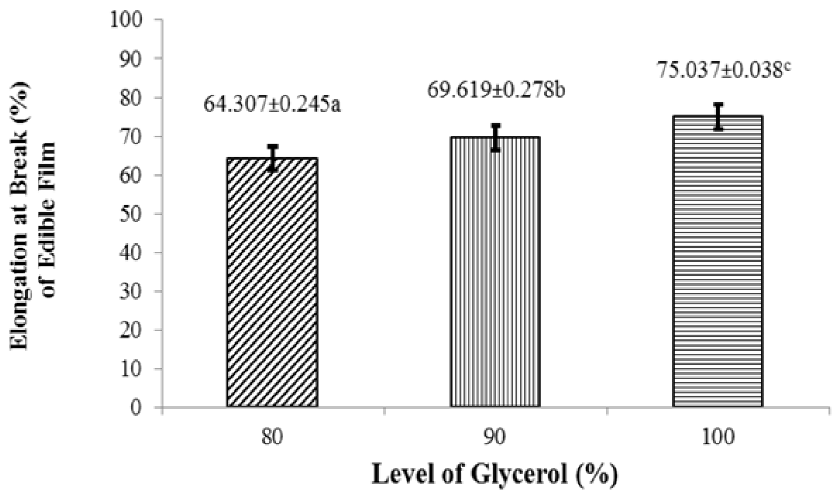

Fig. 4. Elongation at break (%) value of edible film between collagen extract combined with glycerol on the different concentrations

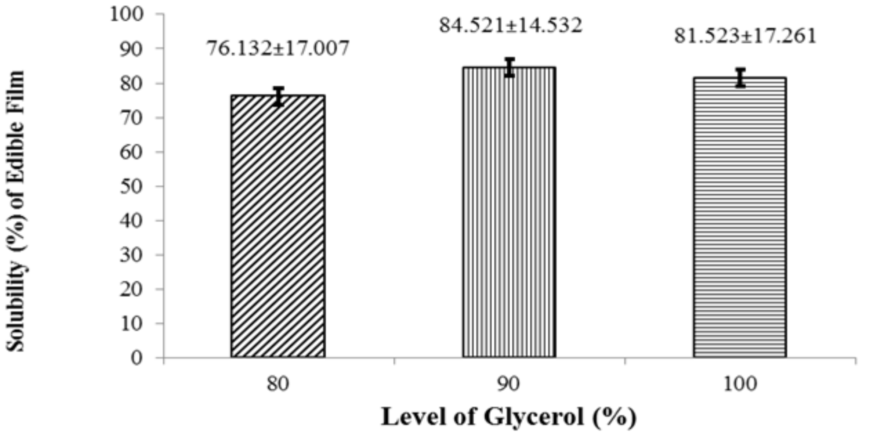

Fig. 5. Solubility (%) value of edible film between collagen extract combined with glycerol on the different concentrations

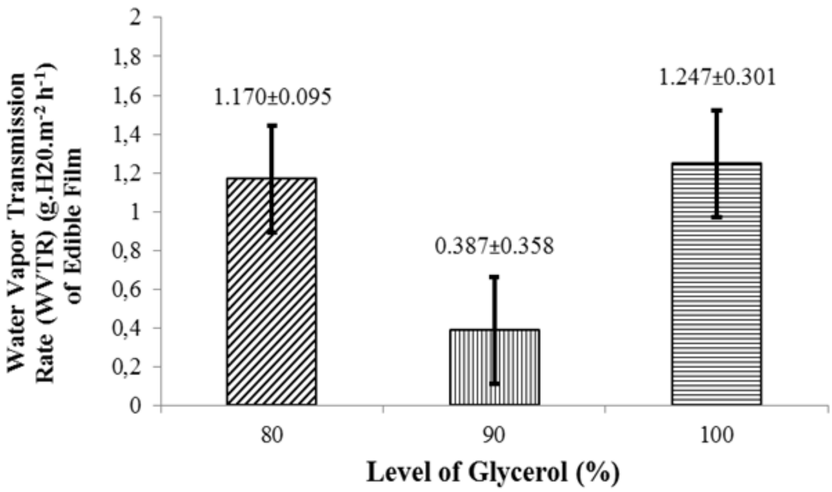

Fig. 6. Water Vapor Transmission ate (WVTR) ($g.H_2O.m^{-2}.h^{-1}$) value of edible film between collagen extract combined with glycerol on the different concentrations

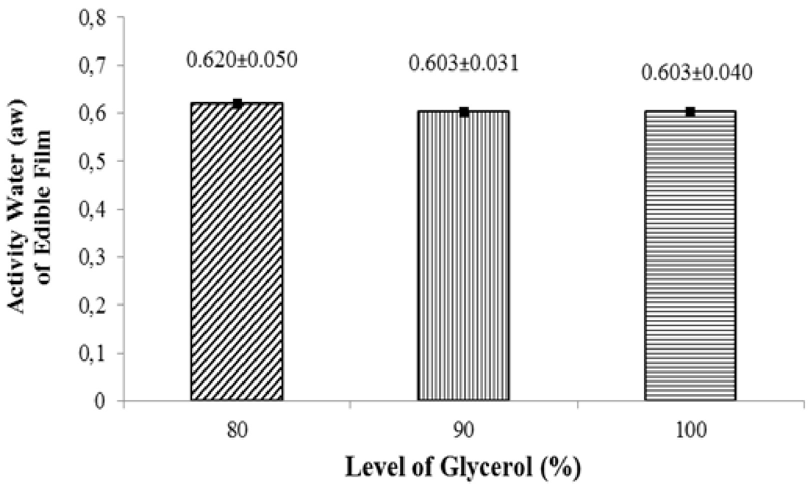

Fig. 7. Activity water (a_w) value of edible film between collagen extract combined with glycerol on the different concentrations

Solubility

Solubility is one of the physical properties of edible film that shows the percentage of the dry weight of the dissolved after immersion in water for 24 h (Gontard *et al*., 1992; Fakhouri *et al*., 2004). The results of the data in Fig. 4 show that, increased concentration of glycerol in the manufacture of solution of edible film no significant on solubility values of edible film. The addition of plasticizer in the solution of edible film will be cause decrease bond of intermolecular and intermolecular drastically. Increased concentrations of plasticizer will give the effect of space between the molecules that widening (Bozdemir and Tutas, 2003; Mendieta-Taboada *et al*., 2007). This can lead to increased solubility of edible film, including the disulfide bond (S-S) (Perez-Gago and Krochta, 1999).

Water Vapor Transmission Rate

One of the most important functional properties of the edible film is its ability to control the mass transfer, mechanical protection and related to the sensory value. The results of the data in Fig. 5 show that, increased levels of glycerol showed no significant effect on the Water Vapor Transmission Rate (WVTR) of the edible film. Several previous studies have pointed out the similarities of properties between the thickness with the permeability on the hydrophilic edible film (Liu and Han, 2005).

Increasing concentrations of plasticizer of glycerol can be increasing the flexibility of edible film and the Water Vapor Transmission Rate (WVTR) (Navarro-Tarazaga *et al*., 2008; Bourtoom *et al*., 2006). The use of plasticizer lead to changes in the physical and functional properties of edible film, namely: (1) increase the flexibility, sensitivity and moisture and then (2) the functional properties. Plasticizer reduction will be affect: (1) biopolymer chains adjacent to each other, (2) sensitivity to happen outside of water and (3) the flexibility of the material (Bergo and Sobral, 2007).

Activity Water (a_w)

Water activity (a_w) plays an important role in the process of packaging of food products. This measurement aims to determine the stability of the product of edible film during the storage process. As packaging materials, edible film expected to protect the packaged product.

Results of analysis of variance of data in Fig. 6 and 7 show that increased of glycerol concentration was not significant on the properties of edible film. According to Nelson and Cox (2000), collagen has a number of hydrophilic amino acids contained in the protein component. Glycine is one of the hydrophilic amino acids in the collagen structure which has the largest composition of the molecular bonds (approximately 35%). value of a_w indicates a measure that states the status of the amount of water in an energy system, in which water molecules can interact directly with component materials of protein (Bell and Labuza, 2000).

Conclusion

Differences in concentrations of glycerol that combined with the extract from collagen in the production process of edible film have significant effect on the properties of edible film such as: thickness, tensile strength and elongation at break, but no significant effect on the solubility, WVTR and activity water (a_w) value. The combination formula for F_1 (10 g (10%) of extract collagen solid + 8 g of glycerol (80%) + 100 mL of distilled water showed the properties of edible film better F_2 and F_3 related to the thickness, tensile strength and elongation at break parameter. The properties of edible film was using the formula F_1 same with F2 and F3 for the solubility, WVTR and a_w parameters. The use of collagen extracts, as a source of protein will improve the properties of edible film, so, the combination of the use of the extract from collagen with glycerol as plasticizer considered to improve the properties of edible film.

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Author's Contributions

Muhammad Irfan Said: Wrote the manuscript and designed the experiments.

Yuny Erwanto: Wrote the manuscript (reseach methods, discussion, grammar and spelling checking).

Effendi Abustam: Designed the experiments and assisted in data analyzed.

Ethics

This article is original and has not been published or presented elsewhere. All the authors have approved the manuscript and agree with submission to this journal. There are no conflict interest to be declare.

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