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Increased Physical and Morphological Properties of *Edible Film* Bovine Split Hide Gelatin With The Addition Of SPI And Transglutaminase

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ABSTRACT

Edible films are made of a thin layer of edible material, used as a food coating that to inhibit migration of air and extend the shelf life.The aim of this study was to determine differences in the nature of edible film from bovine split hide gelatin with supplemented soy protein isolate (SPI) and the enzyme transglutaminase. The materials used bovine split hide gelatin (BSHG), Soy protein isolate (SPI), enzyme transglutaminase (TGase) and glycerol. The materials were divided into three treatments T0, T1 and T2 (BSHG + Glycerol 20%, BSHG 80% + SPI 20% + glycerol 20%, BSHG 80% + SPI 20% + TGase 20U + glycerol 20%). Research using the Completely Randomized Design unidirectional pattern. The results showed the treatment effect on elongation and tensile strength and WVTR values edible film. The addition of SPI and transglutaminase enzyme and glycerol caused the film becomes more elastic and more homogeneous film surface and compact.

Key words: bovine split hide, edible film, enzyme transglutaminase, soy protein isolate.

INTRODUCTION

Utilization of bovine split hides is a by-product from tanning industry, during this time as rambak crackers material, handicrafts and glue making. According Suharjito (2007) and Hastutiningrum (2009) that the bovine split hide still contains collagen protein, which when hydrolyzed can produce gelatin. Gelatin is widely used as food ingredients such as gel formers, binders, thickeners, adhesive glue and edible food wrappers (edible film).

Edible film is a thin layer of edible and often used as a food coating, moisture barrier, oxygen and movement of solutes in food (Bourtoom, 2008). The advantage of using edible films for food packaging is to extend the shelf life of products and not to pollute the environment because edible films can be eaten directly with the packaged product. However, poor water vapor resistance of protein films and low mechanical strength compared to synthetic polymers limit the application of edible films as food packaging

Several researches have been done to improve the performance of protein film by modifying tissue polymers with crosslinking by chemical, heating or enzymatic so that cross-linking of amino acids in the functional chains of both proteins (Chambi and Grosso, 2006). Research by Cao *et al.* (2007) made an edible film from combination of B-type bovine with soy protein isolate with the heating process. Chambi and Grosso (2006) were able to create an edible film of a combination of B-type bovine gelatin with casein through an enzymatic process. The enzyme is often used as a cross-linking agent between two proteins is transglutaminase (TGase, protein-glutamine c-glutamyl transferase, EC 2.3.2.13) catalyzed

the acyl transfer reaction between the group γ-carboxiamid group glutamine residue (acyl donor) and e-amino lysine residue (acyl acceptor), resulting in the formation (e- γ -glutamyl) intra-lysine and intermolecular cross-linked proteins (De Jong and Koppelman, 2002).

The effects of Transglutaminase treatment on film properties have been investigated in some proteins. These films show a high elongation value but a lower tensile strength with no significant effect on the moisture permeability of the protein film. Transglutaminase is efficient in reducing WVP gelatin film (Carvalho and Grosso, 2004).

The formation of edible films typically uses plasticizers as a plasticizing agent to the elasticity of the edible film. Glycerol is one of the plasticizers that is widely used in making an edible film. The development of an edible film of animal protein combinations of the bovine split hide gelatin and soy protein isolate protein by cross-linking transglutaminase enzyme with glycerol as a plasticizer and to improve the physic and morphology properties of bovine split hide edible film.

MATERIALS AND METHODS

Material research

Materials of the research were bovine split hide gelatin (BSHG) , soy protein isolate (SPI), and transglutaminase enzyme (TGase) and glycerol. The tools used in the research were analytical scales, water baths, thermometers, oven, Universal Testing Machine and SEM.

Edible film bovine split hide gelatin (BSHG) preparation (T0)

Edible film production of bovine split hide gelatin refers to the method of Sobral et al. (2002) as follows: a 7% (w / v) edible film-forming solution by dissolving 7 g of gelatin by 100 ml dH_2O at 55ºC and stirred for 30 min. It was then added with gliycerol 20% (v / v) and heated 10 minutes. The solution were poured into a tray and dried in an oven at 50ºC to dry.

Edible film BSHG + Soy protein isolate (SPI) preparation (T1)

The method of making edible film refers to Chambi and Grosso (2006) method with modification of soy protein isolate with the ratio (80: 20) with the final concentration of 20% solution (w / v). Bovine split hide gelatin and soy protein isolate each dissolved with dH_2O in water bath at 55º C for 30 min. Both solutions were mixed and added by 20% glycerol (w / v) and stirred with heating 50ºC for 10 min, then dried in oven at 50ºC.

Edible film BSHG + SPI + TGase preparation (T2)

The method of making the edible film refers to Chambi and Grosso (2006) method, same as T2 treatment, after the two mixed solutions were added by 20U transglutaminase enzyme, then heated at 50ºC for 15 min, followed by heating at 85ºC for 10 minutes. Then lower the temperature 50ºC and added with 20% glycerol (w / v), stirred for 10 minutes, then dried in oven at 50ºC.

Tensile Strength Measurement

The tensile strength of edible films was tested using Universal Testing Instrument Lloyd's LRX 5-type machine according to the method of Kim *et al*. (2002). The tensile strength value was calculated by dividing the maximum load by the initial cross-sectional area of the specimen.

Elongation Testing

Elongation is expressed as the maximum force that is given to the film to tear (Newton) divided by the film cross-sectional area (m^2), while the length is calculated as [(Δtmax x test speed) /length films beginning] x 100% according to the method of Kim *et al*. (2002).

Water Vapor Transmission Rate Test (WVTR)

Water vapor transmission rate test with desiccant Gravimetric Method (Xu *et al.*, 2005) water transmission rate is expressed as the slope of the gain edible film (g/h) divided by vast areas of the film tested (m^2).

Film Morphology Observation

Analysis of the edible film morphology was observed by Scanning Electron Microscope (SEM) type JEOL JSM-5310 LV at 4000 times magnification for a flat cross-sectional area.

Experimental Design and Data Analysis

Data were analyzed using completely randomized design each treatment was replicated three times, by treatment of different combinations of edible film material as follows (T0 = bovine split hide gelatin + 20% glycerol , T1 = 80% bovine split hide gelatin + 20% soy protein isolate + 20% glycerol, T2 = 80% bovine split hide + 20% soy protein isolate + 20 U transglutaminase enzyme + 20% glycerol). Mean differences were tested by Duncan's Multiple Range Test according to Steel and Torrie (1993).

RESULTS AND DISCUSSION

Data of research result of mechanical properties of the edible film are presented in Table 1.

Table 1. Average value of tensile strength, elongation and WVTR edible film of research results.

Parameter	T0	T1	T2
Tensile strenght (Mpa)	$0.92\pm0,05^{a}$	0.83 ± 0.14^{b}	1.06 ± 0.05^{b}
Elongation(%)	$51.13\pm1,99^{a}$	66.45 ± 2.73^{b}	60.79 ± 1.46^{c}
WVTR (g.H2O. m^{-2}.jam^{-1})	19.38 ± 0.55^{a}	20.39 ± 0.92^{b}	18.60 ± 0.01^{b}

^{a,b} Different superscripts at the same row indicate significant difference ($P < 0.05$).

Tensile strength

The results of statistical calculations showed that the effect of adding transglutaminase enzyme showed a significant difference ($P < 0.05$). The tensile strength value of T2 film is higher than the tensile strength of T0 and T1 film, this is because soy protein isolate and transglutaminase enzyme act as cross-linking cause molecular chain is longer and molecular weight is bigger, so the tensile strength of the film is also bigger (Chambi and Grosso, 2006).

The value of the tensile strength of the film resulted from 0.83 to 1.06 MPa. However, this result is lower than the result of Sompie's (2014) study using pig leather gelatin that is 2.820 to 5.637 MPa and Said (2011) research result is 1.714 to 5.770 MPa using goat skin gelatin. Cao et al. (2007) states that tensile strength decreases in edible films made using soy protein concentrate, because not all soy protein isolate particles participate in the formation of film.

Elongation

The results of the edible film elongation testing produced from bovine split hide gelatin and soy protein isolate and the addition of transglutaminase enzyme (Table 1) shows a noticeable difference ($P < 0.05$). The value of the T0 film elongation is lower because there is no crosslinking between the bovine split hide gelatin molecules. Cao et al. (2007) reported that the edible elongation of the film formed from bovine hide gelatin and soy protein isolate

increased by 50% along with increased gelatin concentration, as well as the addition of transglutaminase enzymes that act as cross-linking agents that form crosslinks between protein molecules.

The value of elongation between 51.23 to 60.79%, this value is still in the range of research results Said (2011) is 54.051 to 95.117% of goat skin gelatin.

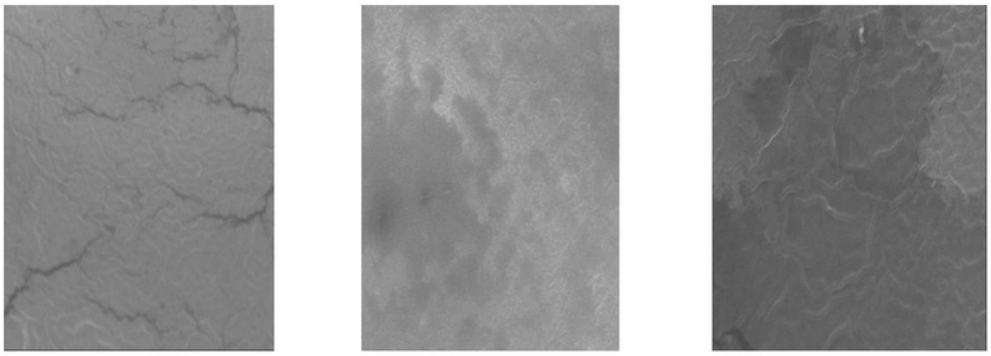
WVTR

The results of statistical calculations (Table 1) showed that the addition of soy protein isolate and transglutaminase enzyme significantly affected the value of WVTR edible film. The addition of soy protein isolate raises the value of WVTR (T1), this is because soy protein isolate is indeed hydrophilic. The combination of these materials has a high water absorption capacity causing an increase in the rate of water vapor transmission (M. Sara, 2015).

The treatment of T2 with the addition of the WVTR transglutaminase enzyme decreased, this is because the addition of transglutaminase enzymes that cross-linked may increase the amount of hydrophobic amino acid on the surface of film (Chen, 2002), while Orliac et al. (2002) observed that the hydrophobic rise of the film surface resulting in an increase in water resistance from the film.

Film Morphology

The edible film morphology can be seen by using scanning electron micrograph (SEM). The result of SEM edible film analysis of the research results can be seen in Figure 1.



T0 T1 T2

Figure 1. Results of scanning electron micrograph edible film of bovine split hide gelatin and soy protein isolate by cross-linking transglutaminase enzyme using glycerol. (T0 = bovine split hide gelatin *with* 20% glycerol, T1 = 80% bovine split hide gelatin+ 20% *soy protein isolate* + 20% glycerol , T2 = 80% bovine split hide gelatin + 20% *soy protein isolate*+ 20 U Enzyme TGase + 20% glycerol).

Figure 1 shows the addition of soy protein isolate (T1) affecting the edible films pores of smaller and compact. Film crack decreases when compared to T0. The mixing of gelatin and soy protein isolate with a 80:20 combination between the two is indicated by the reduced cracking and good edible film structure in which the collagen fibril structure begins to blend with the soy protein isolate particles.

Figure T2 shows bovine split hide gelatin edible film with a combination of soy protein isolate and the addition of transglutaminase enzyme looks more homogeneous and begin to appear film form and structured not many cracks. This is due to the interaction between bovine split hide gelatin and soy protein isolate by cross linking transglutaminase enzyme. This result is consistent with research by Mariniello et al. (2003), that films of soy and pectin

flour with transglutaminase treatment have a finer, compact and higher homogeneity surface than those not treated with transglutaminase.

CONCLUSION

The addition of soy protein isolates and transglutaminase enzymes as cross linking to edible films of bovine split hide gelatin increased tensile strength and film elongation, decreased WVTR and more homogeneous and compact film surfaces.

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