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ENHANCEMENT OF PHYSICAL PROPERTIES OF GELATIN-BASED FILM BY BOVINE SERUM ALBUMIN

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ABSTRACT

Biopolymer-based films are created from plant or animal materials such as carbohydrates and proteins. In this study, a biopolymer-based film from gelatin and Bovine Serum Albumin (BSA) was created to form a film through cross-linking interaction of both biopolymers. The synthesized biopolymer film was characterized using Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), and Fourier Transform Infrared (FTIR) as morphological evaluation. Besides, the encapsulation efficiency of BSA-gelatin blend film was also evaluated. The coagulation process was performed using acetone carried out at 35 ºC for 4 hours under stirring. The formed particles were separated from the solution using high centrifugation around 12,000 rpm for 30 min and then kept at -80 ºC.

Based on the FTIR spectra showed that BSA and gelatin have the same functional group such as hydroxyl (OH) and Amina I, II, and III which appear at 3278 cm^{-1}, 1633 cm^{-1}, 1542 cm^{-1}, and 1241 cm^{-1}, respectively. After linkage reaction, the gelatin-BSA interaction created more strength interaction that caused no vibration functional groups seen. It also increased glass transition temperature where the resulted melting point of gelatin-based and 70% BSA/gelatin-based films are 152.2 and 260 °C, respectively. The encapsulated BSA in the blend film reached above 90% in the BSA/gelatin ratio of 70%.

1.Introduction

In the last decade, nanotechnology is a fascinating field that was interested some researchers. The expansion of nanotechnology eventually is considered in this stage because it possesses various advantages and is favorable in some industry fields. Hence, some products were designed via nanotechnology, including nanofibers (Jeong and Park, 2014; Panzalvota et. al., 2014) and nanocomposites (Umamaheswari et. al., 2015; Premlatha and Kothai, 2015; Satapathy et. al, 2017).

One of the raw materials commonly used and interested by the researcher is gelatin. Gelatin has been exploited and developed via nanotechnology to be converted to nanomaterial products, named; gelatin nanoparticles (GNPs). The application of gelatin is mostly used in several products beneficial to drugs delivery system (Foox and Liberman, 2015), as well as delivery of protein including Bovine Serum Albumin (BSA) and Human Serum Albumin (HSA) (Kaintura et al., 2015), and as food

packaging potential (Hanani et al., 2014), and hard and soft capsule (Prasad, 2017).

The application of gelatin as a macromolecule transport, including protein delivery models, produces many advantages because it can protect from protein degradation and control the release time (Thakur et al, 2013). Besides, the application of gelatin nanoparticles also increases the bioavailability of the drug (Azimi et al, 2014). Gelatin applications are also a proficient carrier for large or small molecules (Jahanshahi and Babaei, 2008).

The gelatin application as a protein delivery system has been carried out and reported by several studies. Azimi et al. (2014) reported that gelatin from bovine skin is used as BSA delivery using a two-step desolvation method. The encapsulated BSA into gelatin nanoparticles using the water/oil (w/o) emulsion method has an average particle diameter is 840 nm (Li et al, 1998). The study from Azimi et al. (2014) showed that encapsulated BSA into gelatin nanoparticles using a two-step desolvation method resulted in a particle size around 200-300 nm. This study is not applying gelatin nanoparticles as a delivery system of BSA but it is focused on the evaluation of the biopolymer gelatin-based and BSA/gelatin-based film that is able to support the application of food packaging or hard and soft capsule. A previous study by Panzavolta et al., (2014) created gelatin-based film with a simple method.

2. Materials and methods

2.1. Synthesis of Blend Film from BSA and Gelatin

The synthesis of BSA-gelatin-based film follows the research from Sailaja and Amareshwar (2012) with slight modification. Gelatin nanoparticles were synthesized using acetone as a desolvating agent via a one-step desolvation method. 1 g of hydrolyzed gelatin was dissolved with 10 mL aquades and heated at 35^0C for 5 min. The pH of the gelatin solution was adjusted around pH range 2.5-3 using HCl 0,1 N and then heated at 35^0C for 5 min. After that, 25 mL of the acetone solution was added to the gelatin solution dropwise for 10 min. In the last step, this solution was added with 500 uL glutaraldehyde 8% and stirred for 4 hours until raising the particles. To obtain the gelatin nanoparticles from the solution was separated using centrifugation at 1000 rpm for 30 min. Afterward, the gelatin nanoparticles were dried to get the solid form. The encapsulation process of BSA level into gelatin nanoparticles is performed using a similar method with gelation nanoparticles producing. BSA/gelatin ratio used is between 0-100%.

2.2. The Entrapment Calculation of Bovine Serum Albumin (%) into Blend Film

The entrapped procedure of BSA into gelatin nanoparticles was determined using a spectrophotometer at λ = 278 nm (Azimi et al, 2014). The unloading of BSA was used to calculate the encapsulation efficiency (EE, %) using the equation:
(Abdala et al., 2015)

EE (%) = [(total BSA added – amount of free BSA)/total BSA added] x 100. (1)

2.3. Characterization of gelatin nanoparticles

The characterization of the gelatin nanoparticle products was analyzed using Scanning Electron Microscopy (SEM) (Phenom Pro X) to detect the morphology of the nanoparticles and Differential Scanning Colorimetry (DSC) (1-STARe, Mettler Toledo, Columbus, OH) to characterize the melting point of gelatin nanoparticle resulted. The Fourier Transform Infrared (FTIR) (Perkin Elmer) was used to detect the functional group vibrations of nanoparticle products.

3.Results and discussions

3.1. Identification of Interaction between BSA and Gelatin using FTIR

The gelatin-based film was synthesized using the one-step desolvation method because it is a more simple and convenient method. This method is able to encapsulate biopolymer is approximately 99% (Wang et al., 2018) and can produce stable gelatin nanoparticles in ranging small particle sizes of around 200-300 nm

(Mohanty and Bohidar, 2003; Azimi et. al, 2014). BSA/gelatin-based film is a new material because the previous research was focused on the application of gelatin as a BSA delivery system. Both gelatin and BSA are composed of different protein monomers. The amino acid levels in gelatin are glycine of 33%; Proline of 21%; Hydroxyproline of 10%, and X (other amino acid monomers) of around ~36% (Djabourov, et al., 1988; Yasmin et al., 2017). While the amino acids of BSA are Lysine, Threonine, Valine, Leucine, Isoleucine, Tryptophan, Histidine, Methionine, Methionine + Cysteine, Tyrosine, Phenylalanine, Phenylalanine + Tyrosine, Aspartic acid, Serine, Glutamic acid, Proline, Glycine, Alanine, and Arginine (Prata and Sgarbieri, 2008).

To evaluate the chemical interaction between gelatin and BSA in blend film can be seen in Figure 1. Figure 1 described that BSA possesses the vibration fields in 3278 cm^{-1} as a hydroxyl (OH) stretching or NH_2 stretching vibration (Amide A) (Qiu et al., 2019). The vibration of C-H alkane arises of around 2972 cm^{-1} and the supported other wavenumbers at 1538 cm^{-1} and 1454 cm^{-1} as CH_3 and CH_2 vibration, respectively (Figure 2). Besides, the wavenumber at 2972 cm^{-1} also illustrates the vibration asymmetric of Amide B as free $–NH^{3+}$ from lysine or terminated N (Yang et al., 2019). While the functional group vibration at 1633 cm^{-1}, 1542 cm^{-1}, and 1241 cm^{-1} indicate molecule vibrations from amide groups such as amide I, amide II, and amide III (Kaintura et al., 2015; Yang et al., 2019).

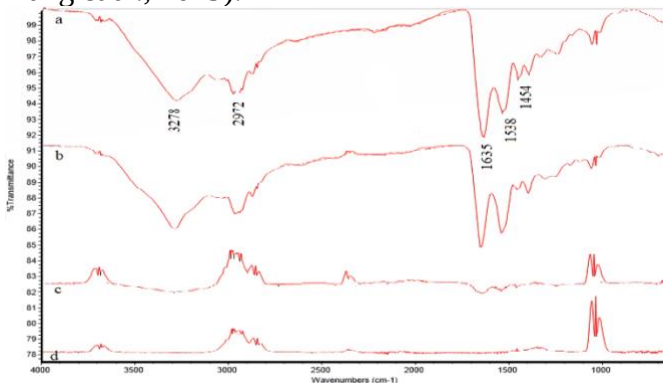

Figure 1. FTIR Spectrum from; a. BSA ; b. Hydrolyzed Gelatin; c. Gelatin-based film; d. 70% BSA/gelatin-based film

The blended gelatin-BSA film and gelatin film have changed the vibrations of functional groups. Previous research from Kaintura et al., (2015) reported that the indication of protein encapsulated into gelatin nanoparticles would change their vibrations and decrease FTIR intensity. However, the gelatin nanoparticles involve intermolecular interaction through hydrogen bonds. It caused the vibration of functional groups in the gelatin structure to be unable to vibrate freely. Therefore, the intensity of the FTIR spectrum of gelatin nanoparticles has decreased significantly compared with gelatin structure (Figure 1). It is also seen in the blended gelatin-BSA as an indication that BSA functional group also interacts with gelatin functional groups forming a rigid molecule.

3.2. Melting point of BSA/Gelatin-based Film

The rigidity of BSA/gelatin-based film had influenced the melting point of the material. DSC analysis also reveals differences in melting point transposition between gelatin nanoparticles and BSA encapsulated into gelatin nanoparticles. Both particles have been observed that the melting point of the gelatin-based film is an exothermic peak of about 152.20°C, while BSA encapsulated into the gelatin-based film is able to increase the melting points which is an exothermic peak of around 201.43°C (Figure 2).

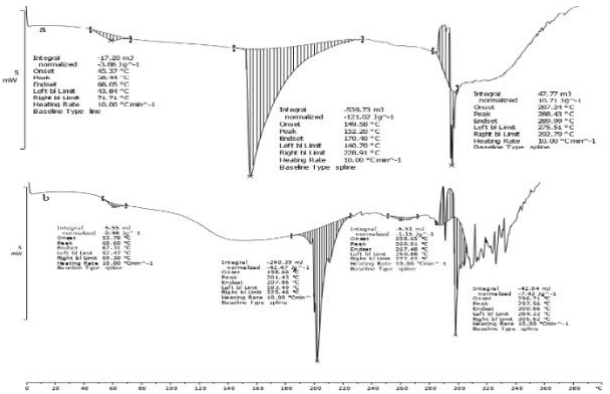

Figure 2. The Result of Differential Scanning Colorimetry (DSC) of nanoparticle; a. Gelatin-based film; b. 70% BSA/gelatin-based film

Based on this result, the interaction of BSA with gelatin is able to build a rigid structure, it can occur through the inclusion of complex interaction between BSA and gelatin causing its melting point to increase significantly. Yasmin et al., (2017) explained that mechanical properties of gelatin nanoparticles covering thermal properties and water swelling ability were significantly affected by the interaction gelatin formation, especially in complex biopolymers. Previous research reported several characteristics of the material applied for edible plasticizer film excipients (Table 1).

Table 1 explained that the development of blended polymers can improve their physical properties and quality. Occasionally, they can be fabricated as phenolic-polymeric hybrids in nutraceutical and drugs delivery systems, edible films, hydrogels, and nanoparticles (Liu et al., 2019). One of the features evaluated for the polymeric-based film is the T_g value (Liang et al., 2019). The T_g value indicates generally to amorphous structure or semicrystalline materials formed (Hoque et al., 2011; Acevedo et al., 2014), while the T_m value is related to the dissociation of hydrogen bond and polymers chains cleavage occur (Cai et al., 2019). The result of this study showed that the gelatin-BSA composite can increase the T_g value indicating both polymers interact strongly. The intermolecular reaction of both polymers will increase of T_g value (Hosseini et al., 2013), indicating that the moisture content in the matrix decreased (Avecedo et al., 2014). This phenomenon will affect other physical and mechanical properties of the materials. The rigidity of this film was also seen in SEM analysis (Figure 3).

Table 1. The development of blended polymeric as edible film material

Polymeric Agent 1	Polymeric Agent 2	Physical Properties	Applications	References
Chitosan	Gelatin	$T_{g\ (gelatin)}$: 50.6°C, T_m: 69.6 °C $T_{g\ (gelatin\text{-}chitosan)}$: 51.1°C, T_m: 75.0°C	Biofilm	Acevedo et al., 2014
Chitosan	Gelatin	$T_{g\ (gelatin\text{-}chitosan)}$: 56.18°C	Edible film	Cai et al., 2019
Chitosan	Fish gelatin	$T_{g\ (gelatin)}$: 29.8°C $T_{g\ (chitosan)}$: 56.1°C $T_{g\ (gelatin\text{-}chitosan)}$: 55.9°C	Edible film	Hosseini, et al., 2013
Chitosan	Cellulose	$T_{g\ (chitosan)}$: 91.03°C $T_{g\ (chitosan\text{-}cellulose)}$: 91.33°C	Biofilm	Liang et al., 2019
Chitosan	HPMC	$T_{g\ (chitosan)}$: 114.06°C $T_{g\ (HPMC)}$: 164.56°C $T_{g\ (chitosan\text{-}HPMC)}$: 196.23°C	Edible film	Rotta et al., 2011
Chitosan	Methyl Cellulose	$T_{m\ (chitosan)}$: 118°C $T_{m\ (methyl\ cellulose)}$: 115°C $T_{m\ (chitosan\text{-}methyl\ cellulose)}$: 110°C	Edible film	Rachtanapun and Wongchaiya, 2012
Gelatin	-	$T_{g\ (gelatin)}$: 60 °C, T_m: 214,02°C	Biofilm	Perkasa et al., 2013
Gelatin	MPI	$T_{g\ (gelatin)}$: 33.70°C $T_{g\ (MPI)}$: 22.83°C $T_{g\ (gelatin\text{-}MPI)}$: 30.08°C	Edible film	Hoque et al., 2011
Gelatin	PVA	$T_{g\ (gelatin\text{-}PVA)}$: 43-53°C, T_m: 98.5-138.9°C	Edible film	Sobral et al., 2011
Gelatin	BSA	$T_{g\ (gelatin)}$: 58.44 °C, T_m: 152.20°C $T_{g\ (gelatin\text{-}BSA)}$: 60.60°C, T_m: 201.43°C	Potential for edible film and hard capsule	This work

PVA: Poly Vinyl Alcohol; HPMC: hydroxypropylmethylcellulose; MPI: mungbean protein isolate
Tg: glass transition temperature, Tm: melting temperature

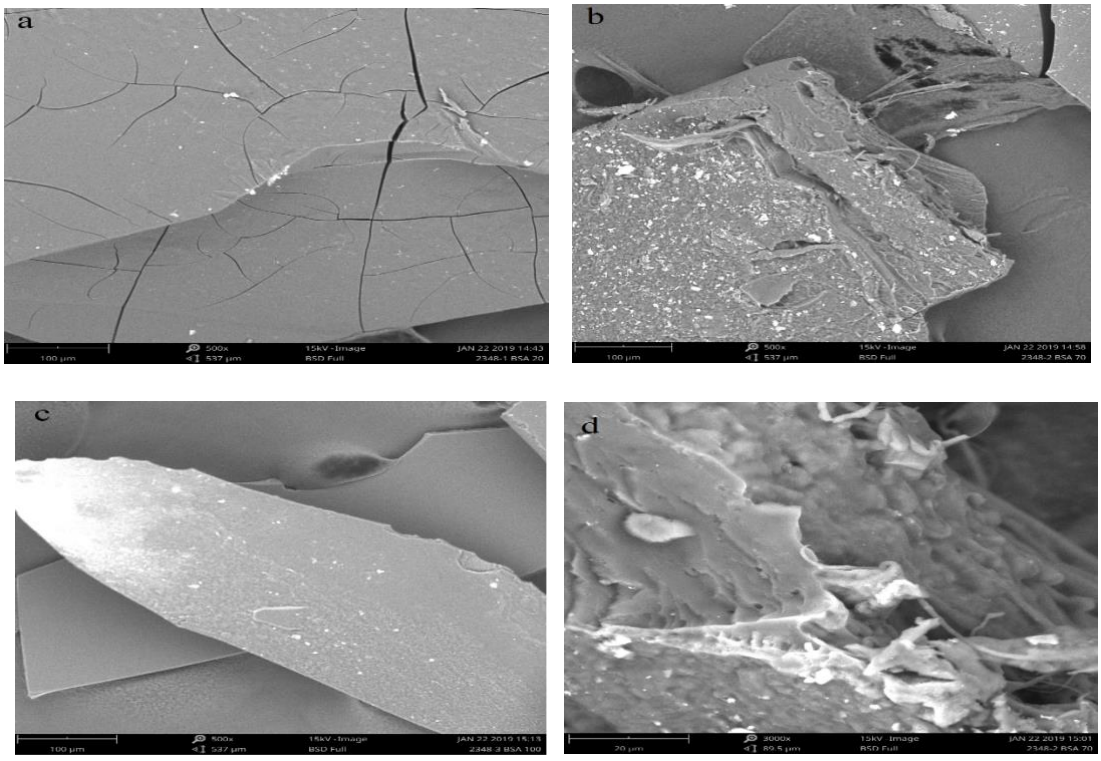

Figure 3. Scanning Electron Microscopy of Different of BSA Concentration loaded into Gelatin-based film; a. 20 % (w/w) BSA; b. 70% (w/w) BSA; c. 100% (w/w) BSA (all scale pictures were seen at 100 µm) and, d. 70% BSA (scale picture was seen at 20 µm)

3.3. Morphology analysis of gelatin-based film using SEM

Based on figure 3. the effect of BSA concentration affected the different morphology of the produced gelatin-based film. The use of 20% BSA loaded into gelatin-based film generate particle properties that have easily cracked. Whereas the 70% BSA and 100% BSA can create a rigid material and a smooth material, respectively. Even the 70% BSA in gelatin-based film produces strong fibers (figure 3b and 3d). This fiber is an aggregate of spherical shape from gelatin nanoparticles because of the temperature conditions in the gelatin-based film processing. Besides, the suggestion of this study is to use a 70% BSA/gelatin ratio in the production of BSA/gelatin-based film because gelatin-based film can encapsulate the optimum of BSA concentration in 50-70% BSA/gelatin ratio up to more than 90% (Figure 4). The encapsulation efficiency (EE) resulted was similar to previous research from Kaintura et al., (2015) reported that the encapsulation efficiency of Bovine Serum Albumin (BSA) and Human Serum Albumin (HSA) into gelatin nanoparticles which is 90% and 80%, respectively. Meanwhile, Azimi et al., (2014) also explained that the two-step desolvation method can produce a particle size range of gelatin nanoparticles of 200-300 nm with entrapment efficiency of BSA reached 87.4%. Based on this result, these physical properties are supporting the use of modified materials as edible plasticizer film excipients or hard capsule materials. However, the blended gelatin-BSA is also applied as a soft capsule or an edible plastic because BSA creates smooth material in a high BSA/gelatin ratio.

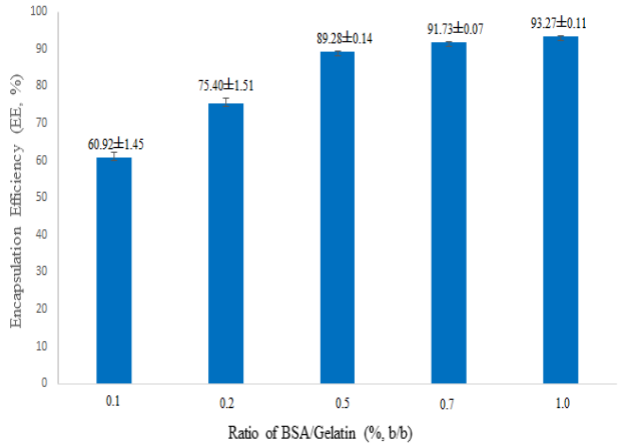

Figure 4. Encapsulation Effeciency (EE) of BSA Loaded into Gelatin-based film

4. Conclusions

The encapsulated BSA into gelatin nanoparticles affects several properties of the material including vibration of functional groups, melting point, and physical properties. The BSA/gelatin ratio of 70% produced a strong fiber that is agglomerating with other fiber become a solid material and the encapsulation efficiency reached above 90%. Based on this research, the effect of the encapsulated BSA into gelatin nanoparticles can improve material properties, including rigid characteristics and melting point. Therefore, it may be applied as edible plasticizer film excipients.

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