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The Emulsifying and Foaming Properties of Buffalo (*Bubalus bubalis*) Hide Gelatin Extracted Using Alkali Acid Compared to Commercial Gelatin

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Abstract: ***The present study reports the emulsifying and foaming properties of buffalo (Bubalus bubalis) extracted using alkali and acid compared with bovine skin gelatin. Gelatin from buffalo hide extracted using 0.5 M NaOH and then HCl 0.9M for 4 hours, sieved and dried using at 50-55ºC for 48 hours. Buffalo hide gelatin (BHG) and commercial gelatin (BSG) were tested for emulsifying and foaming properties (emulsion activity index (EAI), emulsion stability index (ESI), foaming expansion (FE) and foaming stability (FS). The results show that the Emulsion Activity Index (EAI) of buffalo hide gelatin was better than commercial gelatin. The EAI results of BHG and BSG are 14.57 ± 0.38 m2/g and 12.57 ± 0.24 m2/g, respectively. The foam expansion (FE) of BHG was 222.91 ± 12,5% and 191.67 ± 14.43%, respectively. The FS value of BHG and BSG is 77.29 ± 5.36% and 48.54 ± 2.4%, respectively. In conclusion, buffalo hide gelatin extracted using alkali_acid process has emulsifying and foaming properties better than commercial gelatin.***

Keywords: alkali-acid, buffalo hide gelatin, emulsifying, foaming

1. Introduction

Gelatin is a protein that is obtained by partial hydrolysis and denaturation of collagen through a thermal process, mainly derived from the skin and bones [1; 2; 3]. In 2017, Indonesia had imported more than 3.000 tons of gelatin from France, Japan, India, Brazil, China, Argentina, and Australia [4]. Global gelatin production is still dominated by Europe with the proportion of 46% from pork skin, 29.4% from cow skin, 23.1% from cow bone and 1.5% from other sources including fish and poultry. Gelatin production in Europe in 2016 had reached 171,300 tons [5]. It is very important to ensure the halal aspect of the gelatin that used for consumption needs, especially for the food and pharmaceutical industries. Therefore, further exploration of resources and technology are needed to provide good quality and halal gelatin.

Buffalo skin has a high content of amino acid proline and hydroxyproline [6]. The chemical structure of amino acid hydroxyproline contains a pyrrolidine group which is causing a high in thermal stability of collagen [7]. The alkaline-acid pretreatment process can improve the gelatin yield because of the unstable triple helix collagen structure due to the break of cross-linking in telopeptide area and amide chains such as intra-intermolecular or noncovalent that caused by acidic so that the collagen solubility is increasing [8; 9; 10].The gelatin yield of buffalo hide obtained by using hydrochloric acid 0.9 is 29,17% [10]. Alkaline-acid pretreatment can produce skin gelatin with physicochemical characteristics that fulfill the requirements of commercial gelatin [11]. However, the functional properties of buffalo hide gelatin that is extracted by the alkali-acid pretreatment method still unknown. Its functional properties have a role in the food or pharmacy industry process. This study aimed to know the functional properties found in buffalo hide gelatin extracted using an alkaline-acid pretreatment method compared to commercial gelatin from cow-hide

2. Material and Methods

2.1 Materials

Buffalo hides were purchaced from CV. Panji, Yogyakarta, Indonesia. Hydrocloric acid and NaOH purchased from Merck. *Bovine skin gelatin* (BSG) were obtained from Sigma Aldrich. The instrument used to test the emulsion and foaming properties was spectrophotometer UV-Vis (Thermo Scientific Genesis IOS), ULTRA TURAX homogenizer, vortex (Thermolyne Type 37680 Mixer, USA).

Extraction of Buffalo hide Gelatin (BHG) Using Alkali Acid

The pieces of the buffalo hide were soaked in 0.5M NaOH (1:4;w/v) for 2 hours.Subsequently, the hide was soaked HCl at 0.9M for 4 hours. The hide was extracted with distillate water (1:4 ; w/v) at 65° C in water bath for 5 hours and then followed in similar manner at 70ºC for the second step ectraction. The extracting result was sieved and dried using at 50-55ºC for 48 hours. This gelatin referred to as Buffalo hide gelatin (HBG)[10]

Determination for emulsifying activity

Buffalo hide gelatin (BHG) and commercial gelatin (BSG) were tested for emulsion activity using the following procedure. Soybean oil (10ml) and gelatin solution (2%, 10 ml) were homogenized (IKA T 50 Ultra-turrax, Germany) at speed 10,000 rpm for 1 min at 25±10℃. Emulsion (50µl) was pipetted and diluted with 5 ml of 0.1% SDS. The mixture was mixed thoroughly for 10 s, and measured, using a spectrophotometer UV-Vis at wavelength 500 nm [12]. Emulsion activity index (EAI) and emulsion stability index (ESI) were calculated by formula that was provided by

previous researcher [13]

Determination for foaming activity

Buffalo hide gelatin (BHG) and commercial gelatin (BSG) were tested for foaming activity using the following procedure. Foaming Expansion (FE) dan Foaming Stability (FS) was determined according to the previous method [12]. The gelatin solution (80ml), at the concentration of 1% (w/v) was prepared and homogenized at a speed of 10,000 rpm for 1 min. Then the whipped sample was immediately transferred into 25 ml cylinders and stood for 0 and 30 min.

3. Result and Discussion

Emulsion activity

The emulsifying properties of buffalo hide gelatin extracted using alkaline-acid pretreatment was observed through the parameters of the Emulsion Activity Index (EAI) and Emulsion Stability Index (ESI). The mechanism of emulsion formation in this test relates to the ability of gelatin peptide adsorption on the surface of oil droplets formed during the homogenization process, then becomes a shield membrane that prevents the merger of oil droplets into a larger fat globule [14].

An emulsion is a thermodynamically unstable system that contains at least two liquid phases which are not mixed, one of which is dispersed as globules in the other liquid phase (Martin, 1993). Emulsions are a major component of several food products. The character of the emulsion (the capacity and stability) has an important role in the formulation of food products. The Emulsion Activity Index (EAI) and Emulsion Stability Index (ESI) for Buffalo Hide Gelatin (BHG) and commercial cow-hide gelatin (BSG) are shown in Table 1.

Table 1. The Emulsifying Properties of Buffalo Hide Gelatin with Alkali-Acid Extraction Process Compared with commercial gelatin (Bovine Skin Gelatin)

Emulsifying activity	Buffalo Hide Gelatin	Commercial Gelatin (BSG)
Emulsion activity index (EAI)(m^2/g)	14.57 ± 0.38^b	$12.57\pm0,24^a$
Emulsion stability Index (ESI)(menit)	95.96 ± 1.84^b	77.83 ± 3.19^a

Different notations of small letter superscript in the same row shows that there are real effects/differences ($p \geq 0.05$)

Emulsion Activity Index (EAI) is determined by the turbidity of the emulsion at a wavelength of 500 nm. EAI test is related to the ability of the protein to cover a surface of two different liquid phases (Pearce & Kinsella, 1978). EAI value is based on the size of the surface area that is stabilized by the protein per unit weight of protein (m2/g). The results show that the Emulsion Activity Index (EAI) of buffalo hide gelatin was better than commercial gelatin. The EAI results of BHG and BSG are 14.57 ± 0.38 m2/g and 12.57 ± 0.24 m2/g, respectively. The difference in the value of gelatin emulsion capacity can be caused by the difference in intrinsic characteristics, size, composition and conformation of proteins or peptides [16]. The protein contained in gelatin is degraded due to the extraction process. This degradation produces a peptide with a simpler size and shorter chain. The gelatin which have a large number of short-chain peptides that enable the migrating to the surface effectively and are localized around oil droplets at a faster rate compared to the long-chain peptides. Besides, gelatin may have more peptides such as amines or carboxyl groups, so that they have the ability in maintaining the stability of oil droplets with electrostatic forces. High protein solubility in the dispersed phase increases the efficiency of the emulsifier because the protein must be able to migrate to the surface of the oil droplet quickly [17].

The emulsion stability index (ESI) is related to the stability of the peptide adsorption ability to cover a surface of two different liquid phases at any given time without experiencing coalescence, flocculation and creaming. ESI is determined based on the turbidity of the emulsion at a wavelength of 500 nm at the 0th minute and the 10th minute after the emulsion is formed through a homogenization process per 10 minute observation period. So the ESI value is expressed in minutes. The results showed that the ESI value of buffalo hide gelatin was 95.96 ± 1,83 minutes and the ESI value of commercial cow-hide gelatin was 77.83 ± 3.19 minutes. The ESI value of commercial cow-hide gelatin (BHG) is higher when compared to cow skin gelatin (BSG). Therefore, BHG emulsion activity is more stable than BSG. Emulsion stability is affected by large peptide molecular weights and large amounts of hydrophobic peptides. The longer the amino acid chain, the thicker and stronger the layer formed that can cover the oil droplet. Shorter peptide chains also provide many ends of charged peptides. Electrostatic force plays an important role in the stabilization of oil shield membranes. However, the tensile force that is too strong can inhibit the formation of elastic membranes outside the oil droplet which results in low emulsion stability [18; 19]. The difference between EAI and ESI in buffalo hide gelatin (BHG) and commercial cow-hide gelatin (BSG) can be caused by differences in the polypeptide composition of the two gelatins. The hydrophobic polypeptide in BHG is more effective in forming a shield membrane of oil droplets in water.

Foaming Activity

Foam is a colloidal gas with the gas as the dispersed phase and the liquid as the dispersing medium. Foam can be formed when the colloidal gelatin is shaken up. The foam characteristic is an important attribute of gelatin. Foaming activity is generally controlled by the movement, penetration and changes in the composition of molecular protein in the gas-liquid phase. Protein molecules must be able to migrate quickly in the gas-liquid phases, and then decompose and change its molecule composition between phases to produce a good foamability.

Foam is a gas that is trapped by a thin layer of liquid that contains some protein molecules. Globular protein diffuses into the surface of the gas-liquid phase and decreases the surface's tension. Afterward, the protein bond will be broken down on the surface. The hydrophobic group would bind the gas phase while the hydrophilic group would bind the liquid phase. When the bubbles come out of the liquid phase, the bubbles will be coated by a film that contains some surfactant molecules with an interface orientation. Gelatin is included as a surfactant.

Table 2. The Foaming activity of Buffalo Hide Gelatin with Alkali-Acid Extraction Process Compared with commercial gelatin (Bovine Skin Gelatin)

Foaming Activity	Buffalo hide Gelatin (BHG)	Commercial Gelatin (BSG)
Foaming expansion (FE) (%)	222.91±12.5[b]	191.67±14.43[a]
Foaming Stability (FS) (%)	77.99±5.36[b]	48,54±2.40[a]

Different notation of small letter superscript in the same row shows there are real effects/differences ($p > 0.05$)

Foaming expansion (FE) of buffalo hide gelatin (BHG) and commercial cow hide gelatin (BSG) are presented in Table 2. The foam expansion (FE) of BHG was 222.91 ± 12.5% and 191.67 ± 14.33%, respectively. This study shows that there are differences between BHG and BSG foam expansion. Foam expansion (FE) is one of the foam properties found in gelatin. The foam which contains a higher amount of protein concentration, has more dense and stable layers or films formed in a gas-liquid phase. In general, a protein that quickly diffuses into the surface of the gas-liquid phase performs a better foaming ability than the one that diffuses at the slow rate and unable to be broken down [20].

During the diffusion process into the surface of the gas-liquid phase, the protein or peptide end of the chain must be hydrophobic [21]. The protein or peptide with the hydrophobic end of the chain will form a larger hydrophobic chain that effected the diffusion of polypeptides into the surface of the gas-liquid phase becoming faster. Therefore, a protein that contains amino acid with the hydrophobic end of the chain (alanine, valine, isoleucine, leucine, proline, methionine, phenylalanine, tyrosine and faster tryptophan) can perform a higher percent of foaming activity of gelatin.

This is suitable with the previous research [11], which showed that the total content of amino acids with the hydrophobic end of the chain owned by buffalo hide gelatin (272.58 mg/g) was higher than the commercial cow-hide gelatin (209.58 mg/g). As of the foaming activity of buffalo hide gelatin is better than the commercial cow-hide gelatin. The FS value of BHG and BSG is 77.29 ± 5.36% and 48.54 ± 2.4%, respectively. The foam stability formed by protein solutions is positively in line with the molecular weight of peptides [22]. The stability of the foam also depends on various parameters, such as the rate of equilibrium surface pressure, the viscosity of the material and its surface, the steric stability and the electrostatic force between the two sides of the foam layer..

Gelatin is often used as a stabilizer to increase the viscosity and form a layer around the air surface. The viscosity of buffalo hide gelatin (BHG) is higher compared to the commercial cow-hide gelatin [10], this is one of the factors that caused the buffalo hide gelatin (BHG) to form the stable foam better than the BSG. The high value of foam stability from buffalo hide gelatin (BHG) is being affected by the cohesive combination between the two sides of the foam due to the hydrophobic peptide group and the prevention of the liquid being escaped out of the gas bubble. This cohesiveness can be performed due to the gelatin that forms layers or films and binds the surface of the gas-liquid phase with a long hydrophobic chain. The result of this study indicates that buffalo hide gelatin has better foaming ability and stability. It is due to several factors such as the presence of hydrophobic peptide and peptide chain length that affecting the foam characteristic. Therefore, buffalo hide gelatin can be applied in the emulsion for food production. One of the food products that require food addition to forming an emulsion in the production process is ice cream. Buffalo hide gelatin has also been proven to be applicable in making marshmallows with quality marshmallows that can be liked by panelists [23]

4. Conclusion

In general, buffalo hide gelatin extracted using alkali_acid process has emulsifying and foaming properties better than commercial gelatin.

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