Functional properties comparison of hide buffalo gelatin and commercial bovine gelatin as clarifying agent for the tropical fruit juice

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Abstract. The extraction process and skin type would be affected on functional properties of gelatin, particularly on the interaction between oil and water. The present study reports the functional properties comparison of hide buffalo (bubalus bubalis) gelatin (HBG) and commercial bovine gelatin (CBG) as clarifying agent for the tropical fruit juice. Hide gelatin had been extracted for 4 hours using 0.5 M NaOH and 0.9M HCL and then sieved and dried at 50-55°C for 48 hours. The analyzed of water holding capacity (WHC), oil holding capacity (OHC) and hidrophilic lipophilic balance were then applied into HBG and CBG. The tropical fruit juice which was using those HBG and CBG as clarifying agent were then applied for turbidity, pH and Total solid analysis for physical properties. There were no significant differences for WHC and OHC properties between HBG and CBG application of clarifying agent in tropical juice. Furthermore, for the Hydrophilic-Lipophilic Balance result of HBG (14.28±0.13) was revealed higher (P<0.05) than CBG (10.55±0.61). It can be concluded that hide buffalo gelatin has a proper ability as a clarifying agent in apple and star fruit juice to increase the purity or clarity of fruit juice without reducing its main nutrients.

1. Introduction

Gelatin is a protein resulting from partially hydrolysis and denaturation of collagen through thermal processes, mainly derived from skin and bone [1, 2, 3]. Until now, the demand of collagen and its derivatives always increases along with the development of processed food products and industries that require this hydrocolloid. Buffalo hide is one of Indonesia’s local source which can be used as a raw material for making halal gelatin. Wet buffalo hide when compared to other animal skins is generally thicker between 6-8 mm. When compared to cowhide, it is almost twice as thick as cowhide [4]. Extraction of buffalo hide using alkali hydrolysis (NaOH) followed by 0.9 M hydrochloric acid resulted in a yield of 29.3% [5] with physicochemical characteristics according to GMIA standards [6]. In the process of thermal attachment using alkaline-acid pretreatment, tropocholagen is easier to break down its structure into monomers, namely the α chain and the secondary structure becomes unstable. The secondary structure is increasingly unstable, the proportion of α chains and short-chain peptides increases (molecular weight 50-100Kda) [7]. This will affect the functional properties of gelatin such as emulsion activity index (EAI), emulsion stability index (ESI), foaming properties, WHC, OHC, and Hydrophilic Lipophilic Balance (HLB).

In food industry gelatin is widely used as a clarifying agent in beverages, a thickener in desserts, a texturing agent in confectionery and a stabilizer in ice cream, cream cheese and cottage cheese. Emulsifier is a compound that has surface active agent so that it reduces the surface tension between air-liquid and liquids in a system. The ability to reduce surface tension can be seen from the chemical structure that is able to unite two compounds with different polarity [8]. Gelatin has the ability to support emulsion because it has surfactant-like activity, namely the ability to reduce surface tension.
between hydrophobic and hydrophilic components. Emulsion activity is influenced by the molecular weight of gelatin [9]. Buffalo hide gelatin using an alkali acid process has foaming and emulsifying properties better than bovine skin gelatin (commercial gelatin) [10].

This study aims to determine the functional properties of buffalo skin gelatin compared to commercial bovine gelatin and its application as a clarifying agent in tropical fruit juices. These functional properties include Oil Holding Capacity (OHC), Water Holding Capacity (WHC) and hydrophilic lipophilic balance (HLB).

2. Material and Methods
The material used were buffalo hides which obtained from CV. Panji Jaya, Bantul, Yogyakarta. The tropical fruits (apples and starfruits) were purchased from supermarket, Semarang (Superindo). The tools used to test the functional properties were the spectrophotometer (Spectroquant Pharo 300, Merck, Germany), the ULTRA TURAX homogenizer, the vortex (Thermolyne Type 37680 Mixer, USA).

The research procedures included the extraction of buffalo hide gelatin using an alkaline acid pre-treatment, determination of the functional properties of buffalo hide gelatin and application of buffalo hide gelatin in tropical fruit juices.

2.1. Buffalo Hide Gelatin Extraction
A total of 100g buffalo hide was soaked in 400 mL of 0.5 M NaOH (1: 4 w / v) for two hours, drained for 5 min and washed until clean. The skin was immersed in a solution of 0.9 M HCl for 4 hours. After that the skin is drained and washed until it reaches pH value of 5-6, the skin was extracted in a water bath at 65-70°C for 5 hours with a ratio of skin and distilled water 1: 4 (w / v). The extraction results were then filtered using a filter cloth. The solution containing gelatin was dried using a cabinet drier at temperature of 50-55°C for 48 hours [6].

2.2. Determination of water holding capacity (WHC) and oil holding capacity (OHC)
WHC and OHC values were observed based on the centrifugation method ([11]). Duplo gelatin samples of 0.5 g each were dissolved in 20 ml of distilled water (WHC) or palm oil (OHC) in a 25 ml centrifuge tube. The mixture is pulverized for 30 seconds. The dispersion is allowed to stand for 6 hours at room temperature. The next step was centrifuged at 2800 g for 30 minutes. The supernatant was filtered using Whatman filter paper no. 1. Determination of WHC and OHC values can be calculated using formula.

2.3. Determination of Hydrophilic Lipophilic Balance (HLB) ([12])
Determination of the HLB value begins with making standard HLB values from various ratios of tween 80 and span 20 with a final volume of 20 ml. Distilled water was added to each concentration up to a volume of 10 ml and 5 ml of palm oil then homogenized at a speed of 2500 rpm for 30 seconds. The mixture was added with 5 ml of palm oil, then homogenized at 4500 rpm for 90 seconds. Then the emulsion was centrifuged at a speed of 3000 g for 20 minutes and the volume of non-emulsion formed was measured. For the determination of the HLB sample, the same procedure was carried out as the standard HLB, with 10 ml of 1% concentration of gelatin solution as an emulsifier. Each test was repeated three times. Calculation of the hydrophilic lipophilic balance (HLB) value is as follows:

$$\text{Nilai Hidrophil Lipophilic Balance (HLB)} = \frac{\text{volume fase non emulsi}}{\text{volume total}} \times 100\%$$

The HLB value calculated based on the non-emulsion volume in the standard formulation is correlated with the HLB standard through an equation. Furthermore, this equation is used as a reference for calculating the sample HLB.
2.4. Application of buffalo skin gelatin as a clarifying agent in fruit juice

The best treated gelatin was applied to fruit juice with a concentration of 0%; 0.3%; 0.6%; and 0.9% (w/v). Then observed the level of clarity (% T), pH, and levels of total solids (refractometer).

3. Result and Discussion

3.1. Functional Properties of Buffalo Gelatin

The functional properties of gelatin are often related to its application in the food industry. This article is closely related to its function as an emulsifier, pesticide, foaming agent in processed food products and as a clarifying agent in beverage. The functional properties of buffalo skin gelatin compared to commercial cow gelatin can be seen in Table 1.

Table 1. Functional properties of buffalo skin gelatin with alkaline-acid extraction process compared to commercial gelatin bovine skin gelatin

| Functional Properties of Gelatin | Buffalo hide gelatin (BHG) | Commercial gelatin (CBG) |
|---------------------------------|-----------------------------|--------------------------|
| Water holding capacity (WHC)(mg/g) | 11.59±1.90a | 13.37±0.34a |
| Oil Holding Capacity (OHC)(mg/g) | 4.71±0.54a | 5.29±0.35a |
| Hidrophilic Lipophilic Balance (HLB) | 14.28±0.13b | 10.55±0.61a |

a,b Different letters in the same row indicate statistically significant differences (P < 0.05).

3.1.1. Water Holding Capacity.

Water holding capacity (WHC) and oil holding capacity (OHC) are functional properties that are closely related to texture due to the interaction between water, oil and other components. The WHC and OHC values of gelatin from the alkali acid and commercial gelatin are shown in Table 1. Water holding capacity (WHC) is the ability of proteins to absorb water and resist gravity on the protein matrix [11]. The WHC value of acid alkali buffalo hide (BHG) gelatin was similar to that of commercial bovine gelatin (CBG) (Table 1). The WHC value in gelatin is influenced by the amount of hydrophilic amino acid in gelatin [13,3].

3.1.2. Oil Holding Capacity.

Oil holding capacity (OHC) is a functional property related to texture due to the interaction between oil components and other components. OHC buffalo hide gelatin is similar to commercial gelatin from bovine. This is related to the hydrophobic amino acid composition of the gelatin. [13] stated that the high and low OHC values depend on the level of hydrophobic amino acid exposure to gelatin. The OHC value is caused by the presence of non-covalent bonds such as hydrophobic, electrostatic and hydrogen bonds which involve lipid-protein interactions [14]. The OHC value is an important characteristic because it affects the emulsifying capacity. A high OHC value is more suitable for product characteristics such as mayonnaise. The difference in OHC is possible due to differences in conformation, surface hydrophobicity, lipophilic groups and protein degeneration [15].

3.1.3. Hydrophilic Lipophilic Balance (HLB).

The value of HLB (Hydrophilic-Lipophilic balance) is the main parameter in determining the type of emulsion from a food additive that can function as an emulsifier [16]. The HLB value can be related to the activity value and emulsion stability. Gelatin from buffalo hide with alkali-acid pretreatment (HGP) has emulsion activity and stability of 13.91 m²/g, 107.07 minutes, whereas commercial cowhide (CBG) is 12.57 ± 0.24 m²/g, 77, 83 ± 3.19 minutes [10].

The activity and stability of the emulsion are influenced by the amino acid composition of the
gelatin molecule. The balance of the number of hydrophilic and hydrophobic amino acids can determine the solubility level of gelatin in the droplet phase or the continuous phase in an emulsion system. Amino acids that are polar have a tendency to dissolve in water, so they can function as a binder or stabilizer that can cause viscous properties. Buffalo hide gelatin (HBG) had a higher HLB value than CBG (table 1). HBG has a greater hydrophilic amino acid composition than hydrophobic amino acids, so it tends to dissolve in water. Meanwhile, the content of hydrophobic amino acids is also large, including alanine, proline, phenylalanine, leucine and isoleucine. These amino acids have aliphatic and aromatic groups with non-polar sides so that they can interact with the oil phase and form polymer networks on the droplet surface. However, both gelatins are classified as emulsifiers in the oil in water (o/w) emulsion system and can act as stabilizers (HLB> 8). Research by [16] reported that gelatin from tilapia skin with molecular weight distribution of 13.6-140 kDa and 107.5 - 142.23 kDa had HLB values of 8.28 and 8.15 also included in the emulsifier range in the oil emulsion system in water (o/w). Emulsifiers with low HLB values (3-6) are classified as hydrophobic (lipophilic), while emulsifiers with high HLB values (8-18) are hydrophilic and dissolved in the water phase. Therefore, the buffalo hide gelatin from this study is more suitable to be applied in an emulsion system (o/w), such as ice cream, mayonnaise, yogurt, biscuits and cakes [17].

3.2 Application in tropical fruit juices
The Buffalo hide gelatin from the alkali acid process was applied to the fruit juice of the apple and starfruit at a concentration of 0: 0.3; 0.6; and 0.9% (w / v). From the experimental results obtained the optimum results for apple juice at a concentration of 0.6% (w / v), while for star fruit juice at a concentration of 0.3% (w / v). Then at each of these optimum concentrations, differences in the percentage of transmittance (% T), total solids (%), and pH were observed with the data as in Table 2.

| observation conditions | %T  | pH  | Total solid (%) |
|------------------------|-----|-----|-----------------|
| Apple Juice            |     |     |                 |
| Before using gelatin   | 35.14<sup>a</sup> | 4.3<sup>a</sup> | 81.19<sup>a</sup> |
| After using gelatin (0.6%) | 78.23<sup>b</sup> | 4.4<sup>a</sup> | 80.91<sup>a</sup> |
| StarFruit Juice        |     |     |                 |
| Before using gelatin   | 39.23<sup>a</sup> | 4.5<sup>a</sup> | 79.56<sup>a</sup> |
| After using gelatin (0.3%) | 81.12<sup>b</sup> | 4.6<sup>a</sup> | 78.67<sup>a</sup> |

<sup>a,b</sup> Different letters in the same row indicate statistically significant differences (P < 0.05).

Clarifying agents are added to beverage products with the aim of obtaining optimal taste, aroma and color and clarity (Lassoed et al., 2014). Gelatin is used in the production of fruit juice not only for the clarification and precipitation of substances that cause turbidity, but also for reducing the concentration of polyphenols such as tannins and anthocyanogens. The effect is as shown in Table 2, buffalo hide gelatin is able to act as a clarifying agent in apple juice and star fruit juice as indicated by an increase in the percentage of fruit juice transmittation after the addition of gelatin. To determine the nutrient changes in fruit juice, the pH and total solids were observed. Table 2 shows that both the pH value and total solids tended to decrease after the addition of gelatin in both apple juice and starfruit. However, the value was not significant. So, it can be concluded that buffalo hide gelatin can be used as a clarifying agent in the juice without significantly affecting its nutritional value. The positive charge of the gelatin molecule reacts with the negative charge of the polyphenol and anthocyanogen groups, forming hydrogen bonds through electrostatic interactions, causing a complex of compounds that are insoluble (precipitate) in fruit juice. Fruit juices become clearer
19, 20].

4. Conclusion

It can be concluded that the buffalo hide gelatin extracted with alkali acid possesses similar functional properties as commercial gelatin. The buffalo hide gelatin also can be used as clarifying agent in apple juice and starfruit juice to increase the clarity of fruit juice without reducing the main nutrients.

5. References

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