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The Physical and Chemical Properties of Marshmallow made from Bufallo (*Bubalus bubalis*) Hide Gelatin Compared to Commercial Gelatin

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Abstract

In recent years, there is increasing demand of gelatin application, particularly in confectionary products such as marshmallow. Due to halal issue of predominant porcine gelatin, alternative gelatin from other source, like buffalo hide, is critically needed. This study aimed to analyze physical and chemical properties of marshmallow made from buffalo hide gelatin, which was compared to commercial marshmallow and those made using commercial gelatin. Conducted in two stages, the first part was gelatin extraction from buffalo hide and its quality analyses. The second was marshmallow preparation from the gelatin and its analyses. Concentration of buffalo hide gelatin was 4%, 5%, dan 6%, whereas commercial gelatin was 7%, 8%, and 9%. The experiment was repeated three times and all determination were performed in duplicated. Results showed that marshmallow from buffalo hide gelatin contained 15,5 – 18,40 % (wb) moisture; 0,64 - 1.16% (db) ash content; 43,34 – 48,83 % (db) total sugar; and 24,25 – 28,73 % (db) reducing sugar. Textural analyses indicated that the highest value of marshmallow hardness (129.50 g/mm2) and highest value of springiness (5.70 mJ/mm2) were obtained by marshmallow made using 5% and 6% buffalo hide gelatin, respectively), thus concluded that buffalo hide is potential halal gelatin source to produce marshmallow.

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Introduction

Food industries need more innovations and modifications of the shapes, taste, and material compositionbecause the good prospects of food product increased the demand. However, there are several aspects that must be considered in the product development, one of them is the halal food regulation. Gelatin is one of the food additives that often used by the industry because has many functions. In food applications, gelatin can be functioned as a foaming agent, emulsifier, biodegradable film-forming material, colloid stabilizer and microencapsulating agent (Gómez-Guillén *et al*., 2011).

Marshmallow is one of food confectionary product from gelatin with the soft and compact textured, chewy with a variety of shapes, aroma, flavors and colors (Nakai and Modler, 1999). Most of marshmallow are imported with the gelatin resource do not state explisitly. Karim & Bhat (2009) said most of gelatin in the world obtain from porcine. According to halal food regulation, pig and derivation are forbidden to consume. Therefore, the aimed of this research to extract gelatin from Buffalo hide as alternative gelatin to follow halal food regulation. Buffalo *(Bubalus bubalis)* is a local farm with good the potential to be cultivated. Buffalo hide has a high protein content, is about 27.51±0.05% (Mulyani et al, 2016). It contains high collagen tissue and able to obtained gelatin as the raw material making marshmallow.

The scope of the present paper was to investigate the extraction process buffalo hide with alkali-acid pre-treatment and then applied in making of marshmallow. The physical and chemical properties of marshmallow from buffalo hide were determined and compared to those of marshmallow from commercial gelatin and commercial marshmallow.

Materials and methods

Buffalo hide preparation

Buffalo hide obtained from breeders in Bantul, Yogyakarta. Buffalo hides that used still in wet condition. Sheet of hides were washed and cleaned the hair by soaked in a 2% (w/v) aqueous lime overnight. Then, it was scraped to remove residual fat using fleshing knife (Said et al, 2011; Mulyani et al, 2017).

Gelatin extraction

750 g buffalo hide sheets were cut (1x1 cm), then soaked in 1500 ml 0.5 M NaOH solution for 2 hours. After that, the hides were washed with water to rinsed NaOH with two repetitions. Then, hide soaked with 1500 ml 0.9 M HCl solution for 4 hours and washed to neutralization the pH until in the range 5-6. After the reach the neutral pH, residual water removed and put the buffalo hide in a 1L erlenmeyer with the aquadest addition into the mark. The extraction did in 2 step: at 65°C for 5 hours and 70°C for 5 hours in a waterbath. The filtrate was evaporated by using cabinet dryer with temperature 50°C for 48 hours to evaporated filtrate be gelatin sheets. The sheets were blended to obtained powder gelatin (Mulyani et al., 2017).

Determination of gelatin quality

The yield obtained from the ratio of dry weight powdered gelatin with initial weight of the skin sheets. The amount of yield can be obtained by using the formula (Kim et al., 2012; Ktari et al., 2014) :

$$\text{Yield (\%)} = \frac{weight\ of\ gelatin\ powder}{weight\ of\ buffalo\ skin} \times 100\%$$

The viscosity was determined using *Brookfield Shynchro-Lectric Viscosimeter* (British Standard 757. 1975; Niu et al., 2013). 6.67 grams buffalo skin gelatin was dissolved in 100 ml distilled water and heated in water bath at 50°C for 12 minutes at speed of 60 rpm. The test is continued by measuring the pH using pH meter. PH measurements performed at two different points.

Gel strength analysis used *Texture Analyzer* brand *Brookfield*. Samples of gelatin that has dissolved allowed to stand in a cooling room at 8-10°C for 24 hours. The texture analysed by pressuring in *Texture Analyzer* at two different points. Hardness values expressed in g/mm^3 (Faridah et al., 2006).

The measurement of foaming properties was carried out according to the method described previously (Shahidi, et al., 1995; Jridi et al, 2013). Foaming expansion (FE) is indicated as the foams development in minutes 0, where the calculation by:

$$\text{FE (\%)} = \frac{VT - V0}{Vo} \times 100\%$$

Foaming stability (FS) is calculated as the volume of foam that remains after standing in 30 minutes and 60 minutes, then calculations due to:

$$\text{FS (\%)} = \frac{Vt - V0}{V0} \times 100\%$$

where V_T show the total volume after stirring (ml), V_0 is the volume before stirring, V_t is the total volume after standing at room temperature for 30 minutes and 60 minutes.

Marshmallow processing

Gelatin with 4-9% concentrations soaked in 20 ml water. In other place, put 50 g of sucrose, 50 g of glucose syrup, and 30 ml water in a pan then heated with 105 – 115 °C. The sugar solution pour in gelatin and stirred. After that, mixed the dough until fluffy and soft, then cooled in the room temperature. Finally, sowed the powder to decrese the stickiness, marshmallow process had been done.

Determination of Marshmallow quality

The principle of hardness analysis carried out by pressing the sample. The test used the *Texture Analyzer* brand *Brookfield*. Samples (jelly candy) that have been made at this stage was tested using *Texture Profile Analyzer* then pressurized by the probe at two different points. The measurement results is reading automatically by the software. Hardness values expressed in g/cm^3(Faridah et al., 2006).

Coloring test measured by using chromameter on samples (marshmallow) at two different points (Jamilah and Harvinder, 2002; Pranoto et al, 2007). The tool showed the value of L, a* and b*. L indicated the intensity of the black (-) and white color (+), a * indicated the intensity of the green (-) and red color (+), b * indicated the intensity blue (-) to yellow color (+).

Statistical analysis

The experiment was repeated three times and all determination were performed in duplicated. To compare the means of measurements, analysis of variance (ANOVA) with Turkey's multiple range test, was used ($p<0.05$).

Results and Discussion

Gelatin Quality

The yields of buffalo hide gelatins was found by extracted at 65 and 70 °C for 5 h were 25 – 30.2 % yield higher than gelatin from fish was 1,61 – 3,53% and bovine split hide was 6,46 – 13,11% in Wulandari et al (2016). The higher value in buffalo hide because high collagen hidrolysed. The treatment of extraction also give different effect to.gelatin depended on the processing parameters such as temperature, extraction time, pH, pretreatment conditions and properties of the starting raw material (Karim and Bhat, 2009). Buffalo hide gelatin characterization was shown in Table 1.

The degree of acidity is one of parameters in the testing of functional properties. Bovine skin gelatin was used as a comparison. Measurements using a pH-meter showed buffalo hide gelatin pH in 5.81 while commercial gelatin in 5.18. There were two stages in gelatin treatment: first alkaline process and then acid process. When soaking, collagen absorbs most of the acid solution. Residual acid which is not absorbed will be trapped in the web of hydrolyzed collagen fibrils and participate in the extraction process thus affecting the acidity of the resulting gelatin (Yustika, 2000).

Viscosity is important to describe physical properties of gelatin. Buffalo gelatin has 23.02 cP , while commercial gelatin has 7.95 cP. According to GMIA (2012), gelatin viscosity reference ranges 1.5–7.5 cP. Higher viscosity of buffalo hide gelatin may be due to a higher molecular mass.

Gel strength of gelatin is determined by amino acid composition and ratio of α-chain and the amount of

Table 1. Buffalo hide gelatin characterization compared with commercial gelatin

Parameter	Sample		Reference
	Buffalo hide gelatin	Commercial gelatin	
Yield (%)	25 – 30.2		3.5[1)] – 13.11 [2)]
Viscosity (cPs)	23.02	7.95	1.5 – 7.5 [3)]
Gel strength (*bloom*)	196.00	350.25	75 – 300 (tipe A) [3)] 75 – 276 (tipe B) [3)]
Color			
- L	42.55	35.8	
- a*	1.32	1.75	
- b*	7.08	6.96	
Ph	5.18	5.81	3.8 – 5.5 (tipe A) [3)] 5 – 7.5 (tipe B) [3)]
Foaming properties (%)			
- FE (%)	35.60	81.20	76.5 [4)]
- FS 30min (%)	9.60	27.20	74 [4)]
- FS 60 min (%)	5.20	12.00	
Ash content (%)	0.46	0.37	Maks. 3.25 % [5)]
Water content (%)	9.66	8.48	Maks. 16 % [5)]

Wulandari, *et al.* (2007) [1)], Hasan (2007) [2)], ; GMIA (2012) [3)] ; Hafidz (2011) [4)]; Anonim (1995) [2)]
FE = *Foaming Expansion*
FS = *Foaming Stability*

Table 2. Chemical properties of marshmallow

Chemical Properties (%)	Concentration						Reference (SNI, 1995)
	MBHG			MCG			
	4%	5%	6%	7%	8%	9%	
Moisture	15.5±0.00^{a}	15.95±0.21^{a}	18.25±0.21^{c}	15.50±0.14^{a}	17.15±0.21^{b}	18.40±0.42^{c}	Max 20
Ash content	0.72±0.04^{a}	0.99±0.07^{c}	1.16±0.20^{d}	0.64±0.01^{a}	0.69±0.09^{a}	0.83±1.95^{b}	Max 3
Total sugar	48.83±3.34^{a}	50.15±4.75^{a}	46.73±4.50^{a}	45.65±4.76^{a}	43.65±4.02^{a}	43.34±4.47^{a}	Max 20
Reducing sugar	28.73±1.62^{a}	27. 59±3.24^{a}	27.01±4.05^{a}	25.29±4.85^{a}	24.72±2.42	24. 25±1.62^{a}	

β-component (Balti *et al*., 2011). Gelatin has the ability to form a reversible gel. Gelling is the process of forming hydrogen bonds between the semi-solid gelatin molecules that are bound to the water component (Glicksman, 1969). These molecules form more than one crystal to form a three-dimensional network that binds the fluid and forms a strong crosslink, causing the formation of the gel. Bloom degrees shows the strengthness of gelatin produced from a certain concentration of gelatin solution (Hermanianto et al, 2000). Buffalo hide gelatin has strength value 330 to 335 g bloom, while commercial gelatin has 195 to 200 g bloom. These results indicate that the strength of buffalo hide gelatin corresponds to the standard range specified by GMIA (2012) but the buffalo hide gelatin is higher than the standard. Gel strenght was influenced by pH, viscosity, and amino acid compotition. Fresh buffalo hide gelatin is dominated by glycine, glutamic acid, proline and hydroxyproline. Proline and hydroxyproline of buffalo hide gelatin were higher than bovine (Mulyani et al., 2016). The higher content of the imino acids (Pro+H.Pro) may contribute to its higher viscoelastic properties by promoting triple helix formation and stabilization of gelatin at low temperature (Sarbon, et al, 2013).

Buffalo hide gelatin has 9.66% moisture content and commercial gelatin has 8.48%. Buffalo hide gelatin did not show a good binding water capability than commercial, but they still corresponded to the Indonesian National Standart (SNI) (1995). Mulyani et al. (2017) reported that in her study, buffalo hide gelatin moisture content was 7.05 ± 0.07 to 8.92 ± 0.06%. Alfaro et al. (2014) and standard commercial gelatin was 9-14% (Eastoe and Leach, 1977).

This study showed the ash content of buffalo hide gelatin was 0.46% higher than commercial gelatin 0.37%. According to Mulyani et al. (2017) buffalo hide gelatin had 0.53 ± 0.04 to 1.23 ± 0.09% ash content. These values are appropriate to the GMIA standard, ranged from 0.3 to 2.0% and the maximum value is 2.6% (Muyonga et al. 2004).

Foam formation is generally controlled by transportation, penetration and reorganization of protein molecules at the air-water interface (Koli *et al*., 2012). In this study, foaming properties was conducted both of foaming expansion (FE) and foaming stability (FS). The integrity of foam is measured by foam expansion (FE). Persentage of all gelatin samples decrease with increasing its concentration (Fig. 1). The % FE buffalo hide gelatin is lower than commercial gelatin and pig hide gelatin. However, gelatin foam size was not measured in this study. The % FS shows increrasing foam stability at 30 min and 60 min. % FS in All gelatin decrease by increasing the time (Fig. 2). The weakness of buffalo hide gelatin is lower % FE and % FS value than commercial and reference gelatin.

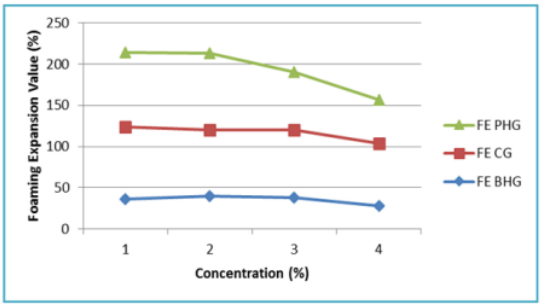

Fig. 1. Foaming Expansion Value (%) at different concentration (1-4% w/v). FE BHG: Foaming expansion buffalo hide gelatin; FECG: Foaming expansion commersial gelatin; and FEPHG: Foaming expansion porcine hide gelatin.

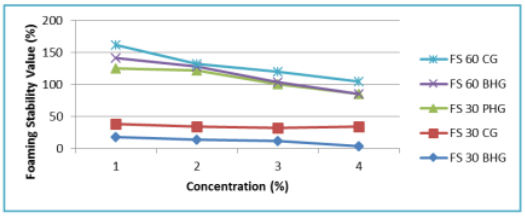

Fig. 2. Foaming Stability Value (%) at different concentration (1-4% w/v). FE BHG: Foaming expansion buffalo hide gelatin; FECG: Foaming expansion commersial gelatin; and FEPHG: Foaming expansion porcine hide gelatin.

Chemical properties of Marshmallow

Chemical properties of marshmellow can be seen in Table 2. The moisture content affects marshmallow appearance, texture, taste, and microbiology properties. In this study marshmellow made from 4, 5, 6% buffalo hide gelatin showed 15.5; 15,95; 18.25% at moisture content, respectively. The 7, 8, 9% commercial gelatin produced 15.5, 17.15, 18.40%, respectively. Standar of moisture in product 15-22% (Periche et al, 2015). Another study stated that marshmallow using gelatin 8% had a moisture content of 14.98-16.73% (Kirtil et al, 2017). Statistically, there was significant difference ($P<0.05$). The higher gelatin concentration used to increase marshmallow moisture. However, using buffalo hide gelatin up to 8% and commercial gelatin up to 9% still produce products with moisture according to the standard. Gelatin is a unique hydrocolloid mixed with water. Gelatin forms dispersions that produce crosslinking in its helix-shaped chains, trapping water in the marshmallow dough and immobilizing the water molecules in the network (Du Toit et al, 2016).

The ash content of marshmallow from 4, 5, 6% buffalo hide gelatin were 0.72, 0.99, and 1.16%, respectively and the commercial gelatin at 7, 8, 9 % showed 0.64, 0.69, 0.83% ash content, respectively. Statistically, there was significant difference ($P<0.05$). Marshmallow ash content is increasing with increasing gelatin concentration, but the value is still lower than the standard set by industry (3%). BHG is extracted through an alkaline-acid process, resulting in a relatively higher ash content than commercial gelatin (Table 1).

Determination sugar content was important on confectionary product. In this study, marshmellow total sugar of 4, 5, 6% buffalo hide gelatin were 48.83, 50.15,

46.73% respectively, and the commercial gelatin in 7, 8, 9 % were 45.65, 43.65, 43.34% respectively. Marshmallow from buffalo gelatin had higher total sugar than commercial, but it was not significantly different (P>0.05). The sugar content is also similar to that of Kirtil et al (2017) marshmallow with gelatin 8% and sugar content of 44.1-56.9%.

Marsmallow reducing sugar from 4, 5, 6% buffalo hide gelatin were 28.73, 27.59, 27.01% respectively, and concentration 7, 8, 9 % of commercial gelatin showed 25.29, 24.72, 24.25% respectively. There was no significant difference (P>0.05) between them. The content of reducing sugar was higher than standar reference (SNI, 1995) because some of sugar was converted to sucrose when heating and acid process.

Physical properties of Marshmallow

Texture atribute of marshmellow which was analyzed such as hardness, springiness, and chewiness. Buffalo hide gelatin marshmellow had the highest value of hardness. It was about 6% (129.5 g/mm^3). The highest of springiness was commersial gelatin marshmallow, it was 9% (5,01 mm). The highest value on chewiness was showed by buffalo hide gelatin marshmallow, it was 6% (5,70 mJ). ANOVA test showed that has no different signifantly in (p>0.05) than Marshmallow commercial.

Table 3. Atribut Texture in Marshmallow

Sample	Atribut		
	Hardness(g/mm^3)	***Springness (mm)***	***Chewiness (mJ)***
MBHG (4%)	98.25± 5.30^a	4.7±0.11^a	4.10±0.42^a
MBHG (5%)	124.25±2.47^a	4.77±0.11^a	5.5 ± 0.35^a
MBHG(6%)	129.50±2.12^a	4.61±0.21^a	5.70±0.14^a
MCG (7%)	84.00±24.04^a	4.64±0,.0^a	3.60±0.51^a
MCG (8%)	102.75±27.9^a	4.09±0.91^a	5.35±0.35^a
MCG (9%)	105.25± 6.68^a	5.01±0.33^a	3.60±1.70^a
M Com.	95.25±27.93^a	4.82±0.18^a	4.50±1.41^a

Keterangan :
MBHG = Marshmallow from Buffalo Hide Gelatin
MCG = Marshmallow from Commercial Gelatin.
M Com. = Commercial Marshmallow

Texture atribute of marshmellow which was analyzed such as hardness, springiness, and chewiness. Buffalo hide gelatin marshmellow had the highest value of hardness. It was about 6% (129.5 g/mm^3). The highest of springiness was commersial gelatin marshmallow, it was 9% (5,01 mm). The highest value on chewiness was showed by buffalo hide gelatin marshmallow, it was 6% (5,70 mJ). ANOVA test showed that has no different signifantly in (p>0.05) than Marshmallow commercial.

High gelatin concentrations increase the hardness and chewiness of marshmallows. In accordance with the result of Periche et al (2015), the higher the percentage of gelatine, the higher the hardness of marshmallow. However, the effect of gelatin on hardness also depends on sugar levels. In this case the sugar content is already defined, i.e 43.65-50.15% (Table 2). Tan and Lim (2008) stated gelatin gel network formation contributes toward hardening in Marshmallow. BHG have high gel strenght (Table 1) so its ability to form a network formation is so high that the concentration of 6% is the highest concentration to be applied to marshmallow.

Gelatin also makes marshmallows chewy by forming a tangled three-dimensional network of polymer chains. Once gelatin is dissolved in warm water, it forms a dispersion, which results in a cross-linking of its helix-shaped chains. The linkage in the gelatin protein network, called 'junction zones' (Du Toit, et al, 2016). Tan and Lim (2008) stated gelatin gel network formation contributes toward hardening in Marshmallow. In this case BHG have high gel strength so its ability to form a network formation is so high that the concentration of 6% is the highest concentration to be applied to marshmallow.

CONCLUSION

The yield of buffalo hide gelatin (BHG) is high (20-30.2% of the fresh hide). In general, the physical properties of buffalo hide gelatin are higher than commercial gelatin, especially gel strength and viscosity, whereas its foaming properties are lower than commercial gelatin. Marshmallow with 6% BHG has the highest hardness and chewiness equivalent to commercial Marshmallows. Buffalo Hide Gelatin (BHG) can use as alternative gelatin to made marshmallow commercial.

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