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The Functional Properties of Buffalo skin Gelatin Extracted Using Crude Acid Protease from Cow's Abomasum

Sri Mulyani[1*], Umar Santoso[2], Yudi Pranoto[2], Francis M.C. Setyabudi[2], Anang Mohamad Legowo[1]

[1]Department of Food Technology, Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia
[2]Faculty of Agricultural Technology, Gadjah Mada University, Indonesia

*Corresponding author (ully_tht@yahoo.com)

Abstract

The study was investigated the functional properties of buffalo skin gelatin. Gelatin was extracted from swamp buffalo skin using crude acid protease from cow's abomasum (CAPC) in concentration variation of 0; 2.5; 5; and 7.5 U/mg. The temperature to hydrolysis was set to 28, 37, and 40°C with three repetitions. The emulsion activity index (EAI), emulsion stability index (ESI), foaming expansion (FE), and foaming stability (FS) were investigated. The interaction between CAPC concentration and hydrolysis temperature had a significant effect ($p<0.05$) on EAI, ESI, FE and FS. The highest EAI was obtained in CAPC concentration of 5 U/mg, hydrolysis temperature of 40°C, which was 12.04 m^2/g. The higher the concentration of CAPC decreased the ESI. The hydrolysis temperature 40°C produces higher FE compared to hydrolysis temperature at 28 or 37°C. The highest FE was obtained at CAPC 5 U/mg with 37°C, which was 102.93%. The FS values ranged from 44.91 to 55.00%. This value was higher than commercial gelatin which was 34.90%. As conclusion, buffalo skin gelatin with the best functional properties was obtained using CAPC 5 U/mg with the hydrolysis temperature of 40°C.

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Introduction

Buffalo skin has a thick and strong structure indicating the high density of collagen bundles. The density of collagen bundles on the skin of cattle is correlated positively with the amino acid content of hydroxyproline (Mulyani *et al.,* 2016) and the complexity of collagen crosslinking. The presence of amino acid hydroxyproline containing pyrrolidine groups causes high thermal stability of collagen (Haugh and Draget, 2009).

Pretreatment is a preliminary process on the extraction of gelatin from the skin using curing materials of bases, acids or proteases. Conventionally, the gelatin extraction from the skin usually uses a base process, but the process takes a long time and produces low purity gelatin. Therefore it is necessary to modify the process of pretreatment, such as using protease from cow's abomasum. Acid proteases can also break peptide bonds in collagen telopeptide areas containing intramolecular and intermolecular covalent cross-interactions (Lassoed *et al.*, 2014; Sila *et al.*, 2015) thereby the extraction efficiency and α-chain proportions may be increased. If the peptide bonds in the area of the telopeptide and the amino acid residues mentioned above are broken due to acid proteases, then during the thermal extraction the structures of tropocollagen will more easily decompose into monomer α-chain and the secondary structure of collagen becomes unstable. The secondary structural changes are expected to result in more hydrophilic and hydrophobic sides were exposed, resulting in the improvement of emulsifying properties (Jridi *et al.*, 2015). The limited study about functional properties of buffalo skin gelatin using acid protease was found, therefore this study was investigated to explore functional properties gelatin from buffalo skin that was extracted using acid protease.

Materials and method

Skin was obtained from CV. Panji Jaya, Yogyakarta, Indonesia from 2-3 years old male buffalos. Cow's abomasum was obtained from slaughtering house in Solo, Indonesia. Ammonium sulphate, glycine base, tris base, and NaOH were purchased from Merck. Bovine skin gelatin, bovine blood hemoglobin, and

trichloroacetic acid were obtained from Sigma Aldrich.

Gelatin was extracted from swamp buffalo skin using crude acid protease from cow's abomasum (CAPC) in concentration 0; 2.5; 5; and 7.5 U/mg. The hydrolysis temperature was set to 28, 37, and 40°C. The emulsion activity index (EAI), Emulsion stability index (ESI), foaming expansion (FE) and foaming stability (FS) were investigated.

Preparation and partial purification

Frozen cow's abomasum was thawed and cut into pieces in thickness of 1-1.5 cm and then homogenized for one minute with 10 mM of Tris-HCl buffer, pH of 7.5, and at a ratio of 1:2 (w/v). The homogenate was centrifuged at 10,000 x g for 45 minutes at 4ºC using Sigma Sartorius of 3-30 K centrifuge. The supernatant was collected to be adjusted to a pH of 2.0 with 1 M HCl and allowed to stand at 25ºC for 30 minutes. The suspension was centrifuged at 5,000 x g for thirty minutes at 4ºC. The supernatant was then fractionated with ammonium sulphate (50-80% of saturation) then centrifuged at 10,000 x g for ten minutes at 4ºC. The residue was suspended in 50 mM of Glycine-HCl buffer (pH of 2.0) and dialyzed for 24 hours at 4ºC using the MWCO dialysis sack of 12 kDa. The dialysate was used as a crude acid protease extract from cow's abomasum (Balti *et al.*, 2011; Hasna *et al.*, 2019)

Buffalo skin gelatin extracted using CAPC

Buffalo skin was cut into small pieces (1×1 cm) and then soaked in 0.05 M NaOH (1:4, w/v) for 2 h (Mulyani *et al.*, 2017). The alkaline treated skin was then washed with distilled water until a neutral pH. The alkaline-treated skin was soaked in 100 mM glycine–HCl buffer, pH 3.0 with a solid/solvent ratio of 1:4 (w/v) and subjected to partial hydrolysis with a crude acidic protease (CAPC) at 0; 2,5; 5 and 7,5 U/g of skin. The mixtures were stirred for 4 h at 37°C. The pH of the mixtures was then raised to 7.0 using 10 M NaOH. In order to inactivate proteases, the mixtures were stirred gently for one hour at 4°C then washed with distilled water. The treated skin mixtures were then extracted at 60-75˚C for 4 hours, and the filtrates were cabinet-dried at 50-55˚C for 48 h. The gelatin extracted from buffalo skin and pretreated with CAPC per g of skin was referred to as GCAPC.

Determination of Emulsion Activity

Soybean oil (10 ml) 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˚C. Emulsion (50 µl) 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 (Thermo Scientific Genesis IOS) at wavelength 500 nm. Emulsion activity index (EAI) and emulsion stability index (ESI) were calculated by formula that was provided by previous researcher (Ktari *et al*, 2014)

Determination of Foaming Activity

Foaming Expansion (FE) dan Foaming Stability (FS) was determined according to the previous method by other researcher. The gelatin solution (80 ml), 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 (Duan *et al*, 2018).

Result and Discussion

The Emulsifying Properties

Emulsifying properties were observed through the EAI and ESI. The mechanism of emulsion formation was related to the ability of gelatin peptide adsorption on the surface of oil droplets formed during the homogenization process, then becomes a protective membrane that prevents the incorporation of oil droplets into large fat globules (Dickinson and Lorient, 1994).

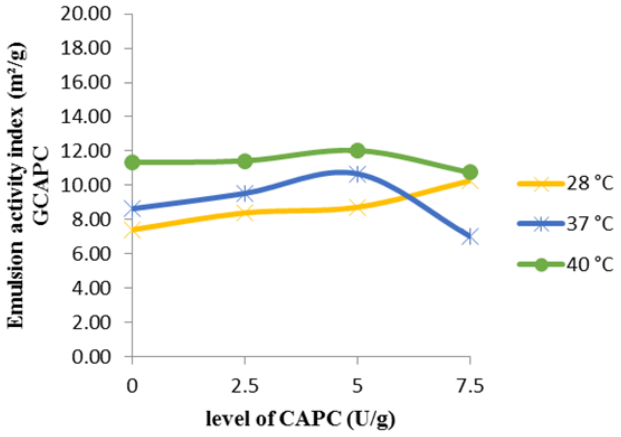

Figure 1. The emulsion activity index (EAI) of buffalo skin gelatin extracted using crude acid protease

Interaction between CAPC concentration and hydrolysis temperature was significantly affected ($p<0.05$) to emulsion activity index of buffalo skin gelatin (Figure 1). Gelatin with the highest EAI value was obtained at the treatment of CAPC 5 U/g at hydrolysis temperature of 40ºC, which was 12.04 m²/g. Higher CAPC might reduce EAI value. The higher CAPC level and hydrolysis temperature tended to increase EAI. CAPC contains aspartic protease, including pepsin. Pepsin break to the telopeptide area in collagen, so that solubility may increase (Jridi *et al.*, 2013). Specifically, pepsin hydrolyze peptide bonds in aromatic amino acid residues, such as phenylalanine, tryptophan and tyrosine. Pepsin eliminates inter-molecular crosslink that form non-helical structural bonds in the terminal N area of polypeptides (Ofory, 1999), thus allowing changes in protein conformation and amino acid composition in gelatin which ultimately affect the emulsion properties (Bkhairia *et al.*, 2016). The differences of EAI can be caused by differences in intrinsic characteristics, size, composition and conformation of proteins or peptides (Jridi.*et al.*, 2013). Gelatin protein which was degraded at extraction using CAPC produced peptide sizes with short chains. The higher level of CAPC, the more gelatin produced contains more short-chain peptides, so that they can migrate more effectively and quickly to the surface of droplets than long-chain peptides (Kaewruang *et al.*, 2013).

Hydrolysis temperature of 40ºC produced highest EAI because this was the optimum temperature for CAPC activity as investigation from previous research that CAP of a smooth stomach hound and European eel bellies have optimum activity at 40 ° C (Castillo-Yanez *et al*., 2004; Bougatef *et al*., 2008; Wu *et al*., 2009).

The interaction between level of CAPC and hydrolysis temperature was significantly affected (p<0.05.) the ESI. Figure 2 shows that the higher level of CAP and hydrolysis temperature, reduced the ESI.

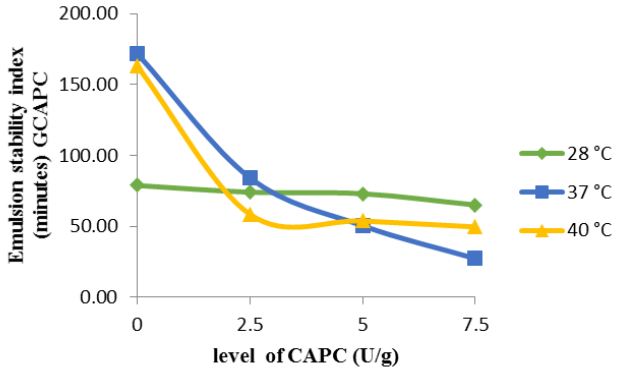

Figure 2. The emulsion stability index (ESI) of buffalo skin gelatin extracted using crude acid protease from bovine abomasum

The higher proteolytic activity of CAPC causes more protein chain breaks, especially in the residues of aromatic and hydrophobic amino acids which increase the exposed hydrophobic amino acids, so the ability of gelatin to bind oil tends to increase. The affinity of gelatin for water is reduced, as a result the gel strength and viscosity were lower so that the gelatin is unable to form a protective film layer of thick and strong oil droplets. This is especially the case if the gelatin protein molecular weight distribution is in the same range. Surh *et al*. (2006) state that oil-in-water emulsions made with high molecular weight fish skin gelatin (~120 kDa) were more stable than those made with low molecular weight fish gelatin (~50 kDa). Emulsion stability is related to the large molecular weight of the peptide and the number of hydrophobic peptides. Strong and thick oil droplet protective membranes are closely related to the stability of gelatin emulsions (Duan *et al*., 2018). In addition, smaller peptide chains provide many charged ends of the peptide. However, too much tensile force can inhibit the formation of elastic membranes on the droplet surface, resulting in low emulsion stability (Aeswiri and Benjakul, 2009). The results of the commercial gelatin emulsion (BSG) emulsion showed that the EAI value was 13.87 m²/g and the ESI was 120.48 minutes, while the buffalo skin gelatin from the acid alkali process (GAA) had an EAI value of 13.91 m²/g and ESI amounting to 107.07 minutes. Therefore, buffalo skin gelatin extracted using CAPC 5 U/g and hydrolysis temperature 4ºC was the best treatment. GCAPC had EAI 12.03 m²/g and ESI 50.52 minutes.

The Foaming Properties

The ability to form foam is one of the important properties of gelatin, due to its potential application in the food system (Bkhairia *et al*., 2016). This ability can be observed from the variable foaming expansion (FE) and foaming stability (FS). The interaction between CAPC concentration and hydrolysis temperature significantly affected (p<0.05) the FE value of buffalo skin gelatin. Figure 3 shows that the FE value of buffalo skin gelatin was higher with a greater CAPC concentration. Hydrolysis temperature 37ºC produced the highest FE value compared to 28 and 37ºC in CAPC treatment. Using CAPC of 5 U/mg, hydrolysis temperature 37ºC produces buffalo skin gelatin with the highest FE, which is 102.93%. The FE value was greater than the value of commercial gelatin FE (BSG), which was 75.75%.

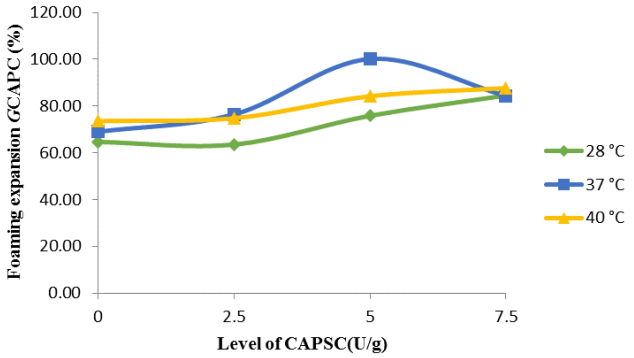

Figure 3. The foaming expansion (FE) of buffalo skin gelatin extracted using crude acid protease

Proteins which had faster adsorption ability on the surface of the air-water phase showed better foaming ability and not biodegradable adsorption ability on the surface phase. At the time of adsorption at the surface of the air-water phase, the ends of the protein or peptide chain must be hydrophobic charged. Peptides with hydrophobic ends may form large hydrophobic circles, so that the adsorption of polypeptides on the surface of the air-water phase is faster (Mutilangi *et al*., 1996). Particularly in gelatin, the amino acid content with hydrophobic sides such as alanine, valine, isoleucine, leucine, proline, methionine, phenylalanine, tyrosine and tryptophan are high causing a large foam capacity.

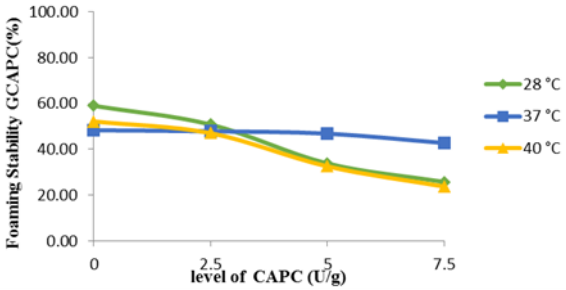

Figure 4. The foaming stability (FS) of buffalo skin gelatin extracted using crude acid protease

The interaction between CAPC concentration and hydrolysis temperature significantly affected (p<0.05) the foaming stability of buffalo skin gelatin. As shown in Figure 4, the higher CAPC concentration decreased the froth stability of buffalo skin gelatin. The difference in hydrolysis temperature did not have a major effect on changes in buffalo skin gel FS, which was between

44.91-55.00 %. This value was still relatively higher than the value of commercial FS gelatin (BSG), which was 34.90 %. The stability of the froth from a protein solution generally correlates positively with the molecular weight of the peptide (Jongjaroenrak *et al*., 2005). Gelatin with larger molecular weight tends to form a thicker layer of interface film, so that the froth becomes more stable. However, at the same molecular weight distribution between gelatin. The stability of the froth is related to protein conformation, for example changes in the hydrophilic and hydrophobic side of gelatin. Increased openness of hydrophobic amino acids increases the affinity of gelatin for foam and air. Lassoed *et al.* (2014) states that the nature of protein froth is influenced by protein sources, protein intrinsic factors, protein composition and conformation when in a solution or air / water interface.

Conclusion

Buffalo skin gelatin with the best functional properties was obtained using CAPC 5 U/mg and the hydrolysis temperature treatment of 40°C.

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