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Comparison of Rendement, Viscosity, and Degree of Acidity of Bone Gelatin of Bali Cattle Fed with Lamtoro at Different Extraction Temperatures

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Abstract: The increase in Bali cattle bone waste due to increased slaughter has become a severe societal problem because it will pollute the environment. If Bali cattle bone waste is appropriately processed, it will produce products with high nutritional and economic value, including gelatin. This research aimed to compare the yield, viscosity, and degree of acidity (pH) of bone gelatin from Bali cattle fed with lamtoro at different extraction temperatures. The gelatin from Bali cattle bone was produced under the influence of different extraction temperatures of 50; 60; and 70° C and each was repeated 4 times. The research method used was laboratory experimental. Data were analyzed using a Completely Randomized Design (CRD). The parameters observed were yield, pH, and viscosity. The results show that different extraction temperatures (50, 60, 70° C) had a significant effect ($P < 0.05$) on viscosity while a very significant effect ($P < 0.01$) on the degree of acidity (pH) and no significant effect ($P > 0.05$) to the yield. It can be concluded that a higher extraction temperature (70° C) will be accompanied by an increase in gelatin yield, but the extraction temperature of 60° C showed the maximum viscosity and acidity (pH) values.

Keywords: Cattle bones; Extraction; Gelatin; Lamtoro feed

Introduction

Slaughtering Bali cattle can be a problem if the by-products, without further processing, become waste, even though the by-products of Bali cattle, in this case, the scapula bone, Endang et al. (2020) state still contain protein that can be processed into gelatin products which are beneficial for health and have economic value and can be used as an alternative source ingredient for making halal gelatin. Gelatin is obtained from animal connective tissue such as bones and skin through a hydrocolloid process resulting from protein extraction of collagen fragments (triple helix) into gelatin (single helix) (Aisman et al., 2022). Likewise, Yang et al (2024) stated that gelatin be extracted from different tissue sources (e.g., bone and skin). There are two ways to make gelatin, namely the process using the method acid and base (Syahputra et al., 2022). The amino acid composition, especially hydroxyproline, has an influence on changes in the stability of the triple helix structure (Hasma et al., 2020). Gelatin has unique properties that can melt at body temperature (Nurilmala et al., 2022) and to change shape reversibly between sol and gel or otherwise (Herliana et al., 2023). The nucleic acids contents (dsDNA, ssDNA and miRNA) from commercially-available gelatins (Kuramata et al., 2022).

According to Cahyaningrum et al. (2021) gelatin is an additional ingredient in food in the form of proteins or polysaccharides, which function as thickeners and stabilizers, gelatin's ability to form food texture greatly determines its functionality. Gelatin can be used as an emulsifier, foam-forming, gelling, and edible film

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(Nurilmala et al., 2023). Though gelatine concentration was varied (Hes et al., 2023). Gelatin powder is widely recognized for its hygroscopic nature due to its propensity to absorb moisture from the surrounding environment (Fikry et al., 2024). And gelatin use the injectable bone substitute (IBS) based on hydroxyapatite gelatin was synthesized with the addition of alendronate (Putra et al., 2019).

Bone waste contains a lot protein, such as collagen is quite high which if processed, then it has the potential to produce gelatin products (Hasma, 2018). The main component of cattle bone is proteins composed of, among other things, the amino acids glycine-proline and hydroxyproline-arginine-glycine as the main component of collagen protein (Retno, 2012). Bone waste contains a lot protein, such as collagen is quite high which if processed, then it has the potential to produce gelatin products (Hasma et al., 2020). The strong dehydration of gelatin facilitated the folding of the polymer chains into helix bundles (Wang et al., 2021). Generally, the 3D structure of gelatin can be enzymatically hydrolyzed (Liu et al., 2022).

Meanwhile, the lamtoro plant has several chemical contents in lamtoro leaves, including protein, fat, phosphorus and iron, calcium, and vitamins (A, B1, and C). Lamtoro gung seeds contain protein, mimosine, leucanin, and leucanol (Dilaga et al., 2021). According to Laconi & Widiyastuti (2010), the compounds contained in lomtoro leaves include 40% carbohydrates, 25.9% protein, 4% tannin, 2.36% calcium, 7.17% mimosine, 4.2% nitrogen, phosphorus 0.23%, and nitrogen 4.2%. One of the essential processes in making gelatin is extraction. Extraction is a denaturation process to change collagen fibers that are insoluble in water by adding hydrogen bond-breaking compounds heated to a temperature range of 50-90^{o} C (Said et al., 2011). Extraction is one of the crucial stages in making gelatin, because during this process, collagen fibers become denatured to gelatin (Sompie & Siswosubroto, 2020). There has not been much research using different extraction temperature variations on cattle bones fed lamtoro. Therefore, research has been conducted on making Balinese cattle bone gelatin by feeding lamtoro through different extraction methods.

Method

The research method used was laboratory experimental. For the first stage, the bones were cleaned, reduced in size to 2 cm^2, soaked using sunlight to clean the remaining dirt attached to the bones for 24 hours, then soaked in acetic acid (CH_3COOH) with a concentration of 6% for 48 hours. Bones that have been given acetic acid pretreatment were washed using running water and then neutralized by soaking in CaCO3 for 24 hours, then washed clean and the bones mixed with sterile distilled water in a ratio of 1:1, then extracted using a water bath at a temperature of 50, 60 and 70^{o}C for 24 hours. The extraction results were then placed in a baking sheet mold covered with heat-resistant plastic and dried in an oven for 24 hours. The dried gelatin was then blended until it formed gelatin powder and was ready for analysis.

Data were analyzed using a Completely Randomized Design (CRD), with 50 different extraction treatments, 60 and 70^{o} C, and repeated four times. The data were processed using the SPSS version 21 program. The parameters observed were yield, pH, and viscosity.

Rendement

The data was obtained by dividing the dry weight of gelatin divided by the weight of the raw material times 100% using the Formula 1.

$$\text{Rendement} = \frac{\text{Dry weight of gelatin}}{\text{Bone weight}} \times 100\% \quad (1)$$

Viscosity

Each beef bone sample was weighed at 6.67g and then dissolved in distilled water to a volume of 100 ml. Then it was cooled to a temperature of 20°C, and the viscosity was measured using an Ostwald viscometer (Endang et al., 2020).

$$\text{Viscosity} = \frac{viscosity\ value\ x\ average\ lap\ time\ (seconds)}{average\ calibration\ time\ (seconds)} \times 100\% \quad (2)$$

pH

A gelatin solution with a concentration of 6.67% (w/w) was prepared with distilled water solution to a volume of 100ml. The sample solution was heated at 70^{o}C and homogenized with a magnetic stirrer for 10 minutes, the degree of acidity was measured at room temperature with a pH meter (Aisman et al., 2022).

Result and Discussion

The research results can be seen in Figure 1. Yield is the amount of dry gelatin produced from a number of fresh bone raw materials in clean condition through the extraction process (Aisman et al., 2022). According to Hasma et al. (2020), yield is an essential. After further analysis using the Duncan test, there was a difference between the extraction temperature of 50^{o}C and the extraction temperature of 70^{o}C, but the temperature of 60^{o}C showed no difference. The difference in extraction temperature will affect the H+ ions, which hydrolyze collagen from triple helix chains to increasingly single helix chains (Aisman et al., 2022). an increase in H+ ions speeds up the rate of collagen hydrolysis, resulting in more triple helix being fragmented into α, ß, and γ chains (Lestari et al., 2024). High concentrations and long soaking times are thought to reduce the amount of gelatin yield produced. This is because, in this treatment, the resulting ossein becomes very soft and destroyed, causing much of the ossein to be lost during the neutralization process (Febriansyah et al., 2019). According to Mega et al. (2018), the soaking process is the most effective for producing gelatin with a high yield. Gelatin can be a medium the growth of microbes that plays a role in it reaction or decomposition (Febriana et al., 2021). Gelatin influenced by the movement of air inside protein network structure (Suryati et al., 2015). Proteins experience denaturation, causing the chemical bonds of proteins damaged so it doesn't have a strong bond with minerals (Capriyanda & Mujiburohman, 2021).

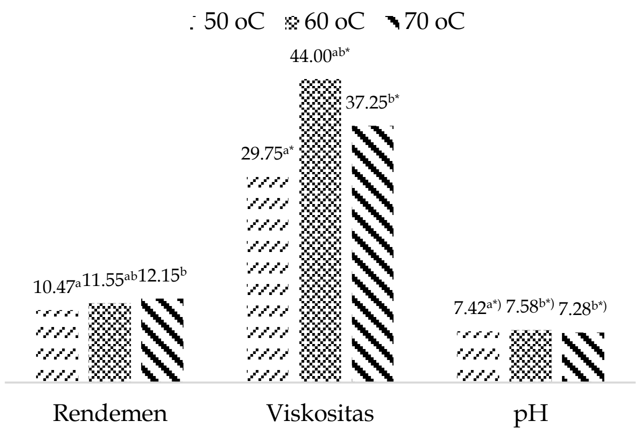

Figure 1. abExtraction temperature shows differences but does not significantly affect the yield (*Rendemen*). ab* extraction temperature showed a difference and significantly affected viscosity (*Viskositas*) ($P < 0.05$). ab*) extraction temperature showed a difference and had a very significant effect ($P < 0.01$) on pH.

The viscosity is a measure of a fluid's internal resistance to flow and shear under the force of gravity. In the case of gelatin, it is determined by molecular weight as well as the polydispersity of the gelatin polypeptides or amino acids (Ashrafi et al., 2023). The higher the gelatin gel strength, the higher the viscosity, which is attributed to the higher proportion of crosslinking components (Ruan et al., 2023). Viscosity is the viscosity of a solution. Viscosity is also defined as the flow power of the molecules of a solution (Hasma, 2018). Gelatin viscosity is the hydrodynamic interaction between gelatin molecules in solution. The colloid system in solution can increase by thickening the liquid so that absorption and development of the colloid occur (Rahmawati & Hasdar, 2017).

The variance analysis shows that Bali cattle bone gelatin's extraction temperature significantly affects viscosity ($P < 0.05$). The viscosity value of gelatin (Figure 1) is highest at an extraction temperature of 60°C, namely 44.00 cP, while the lowest value is at an extraction temperature of 50°C namely 29.75 cP. This shows that every increase in extraction temperature at 60° C has a maximum viscosity level compared to an extraction temperature of 70° C. The higher the extraction temperature, the more open the structure of the amino acid chain will be and will cause the chain to become shorter and viscosity to decrease. The higher extraction temperature causes the triple helical structure of the collagen to transform into a single chain structure. Changes in the collagen chain structure leads to a decrease in the molecular weight of gelatin (Pradarameswari et al., 2018).

The viscosity value or thickness of the gelatin solution is very closely related to the water content of the dry gelatin. The lower the water content of dry gelatin, the higher its ability to bind water (to form a gel). The greater the amount of water bound by gelatin, the thicker the gel will become, and this directly influences the higher viscosity value measured (Hido et al., 2021). The high or low viscosity value is greatly influenced by the distribution of gelatin peptide molecules in solution as well as the molecular weight of the gelatin peptide (Prasetya et al., 2022). The greater the molecular weight of gelatin, the slower the distribution of gelatin molecules in the solution, resulting in a high viscosity value (Rahmawati & Hasdar, 2017). Excessive drying will break the gelatin protein bonds, resulting in low viscosity quality, and highwater content will cause the gel to become difficult to thicken (Yu et al., 2023).

The degree of acidity (pH) is a parameter in determining gelatin quality standards. The pH value of gelatin can be shown in Figure 1. The variance analysis shows Bali cattle bone gelatin's extraction temperature significantly affects pH ($P < 0.01$). The highest gelatin pH value was at an extraction temperature of 60° C, namely 7.58, while the lowest was at 70° C, namely 7.28. The concentration of acetic acid showed a significant effect on pH ($P < 0.01$). The resulting average pH value shows neutral because it is around 7. Gelatin with a neutral pH tends to be preferred, so the neutralization process has a vital role in neutralizing the remaining acid after soaking (Oktaviani et al., 2022). According to Aprilyani (2013), the neutrality of the resulting pH indicates that gelatin is safe for human consumption. The pH value was measured using a pH meter because the pH of the solution affects other gelatin properties, such as viscosity as well as the application of gelatin in products (Mega et al., 2018). The washing process played an important role in neutralizing the pH (Fatimah et al., 2023). The pH value in the neutral range indicated that the process of neutralizing extraction went perfectly (Hasma et al., 2019). The pH value neutral is able to keep the helix chain from breaking easily thereby increasing the quality of gelatin (Ristyanti et al., 2022).

Conclusion

The higher the extraction temperature (70° C), the better the characteristics of cattle bone gelatin with fed lamtoro.

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Author Contributions
Each of the authors in this article contributed to the research. conceptualization, H.H, D, M.A; data analysis, H.H, M.S; methodology, H.H, W.W, R.U.F, N.H, and T.V.M; validation H.H, D, M.A; formal analysis, W.W, D; investigation, W.W, M.S; resources H.H, M.A and D.; data curation, R.U.F.: writing—original draft preparation, M.S and H.H; writing—review and editing, H.H, N.H.: visualization, and R.U.F and T.V.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare there is no conflict of interest with the funders. All research results obtained are correct and carried out collaboratively. The funders had no role in the study's design, in the collection, analysis, or interpretation of data, in script writing; or in the decision to publish the results of this research.

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