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Evaluation of The Characteristics of Collagen Extract of Broiler's Bone from Chicken Abattoir Waste in Makassar, Province of South Sulawesi, Indonesia

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ABSTRACT
Bone of chicken riched in collagen compounds is a kind of waste produced by the chicken slaughter. Collagen, a hydrocolloid product resulted from partial hydrolysis of animal tissue, is currently used widely in food and pharmaceutical fields. The process of bone demineralization in collagen production process affect its characteristics both quantitatively and qualitatively. The purpose of this study was to evaluate the characteristics of the collagen extract which was manufactured using several types of materials and in different time of demineralization process. The main material was the waste of broiler's bone, CH_3COOH 1 M and HCl 1 M. The design used was completely randomized design (CRD) 2x2 with 3 replications. The first factor was the two types of demineralization material (CH_3COOH and HCl), the second was a two-different time of demineralization process (48 and 96 hours). Data was analyzed by analysis of variance. The parameters observed were yields, gel strength, and viscosity. The results showed that the application of the type of material and time of demineralization was significantly different ($p<0.05$) from the yields, however there was no significant effect on the value of gel strength and viscosity ($p>0.05$). The value of the yields was indicated best at the use of HCl 1M compared to CH_3COOH 1M in both processing time 48 hours and 96 hours.

KEYWORDS: collagen extract; bone; broiler; waste, demineralization.

INTRODUCTION

Bone, one of the slaughter's by-products, has not been optimally utilized recently due to the public's assumption that it was still a livestock waste. In fact, the use of bone from feed industry is still limited as raw materials in animal feed, therefore bone is still categorized as a low economic value by-product. Since poultry is the largest farm population in Indonesia, chicken's bone as a waste is still large enough.

Structurally, bone is rich in bioactive compounds particularly collagen protein that is bounded strongly with minerals such as calcium and phosphorus [1-4]. Therefore, bone as a by-product of livestock has great potential to be used as a raw material source of collagen.

Collagen extract is widely applied in food industry, for instance as a raw material in food supplement for the elders [5-7]. In addition, bone is also widely used as thickeners, and stabilizers in the candy industry, as well as the basic material in drug manufacturing, such as capsule shell [8]. Collagen compounds collagen in animal bones, have similarities with that in human body, in terms of chemical composition, morphology,

distribution, function and pathology of the collagen compound [9].

Some research indicated that collagen extract of bovine bone using weak acid (CH_3COOH) as a demineralization material for 48 and 96 hours respectively produced yields by 2.26% and 2.51% with a gel value of 200.68 g *Bloom* and 297.57 g *Bloom* and viscosity 6.00 cP and 7.50 cP [10]. Collagen extract from pig processed using acetic acid 2%, 4% and 6% at different ages was resulted in average yields 10.92%, 11.55% and 11.99% respectively. The gel strength was 138.71 g *Bloom*, 137.64 g *Bloom* and 137.43 g *Bloom*. Viscosity was 6.97cP, 6.91cP and 6.88 cP [11].

However, some experts have suspected that the current imported collagen extract was produced from pig skin. Evidently, this is a problem for a majority moslem country [12-14]. In order to obtain a collagen extract with the maximum quantity from bone, demineralization with a combination of materials and the right processing time is required. In the demineralization process, there is a process of hydrolysis and solubilization of mineral components as well as protein denaturation in limited bond. This process greatly affects the physical properties and chemical of the collagen produced [15]. The purpose of this study was to evaluate characteristics of collagen extract of broiler's bone from chicken abattoir waste in Makassar, province of South Sulawesi, Indonesia.

MATERIALS AND METHODS

Research Materials:

The raw materials in this study was the waste of broiler's bone from all parts of the body (ex-clude head and legs) obtained from the traditional chicken slaughtering units in Daya area, Makassar.

Raw material, chicken bone is the by-product of a chicken meat boneless process, which was furtherly cut into small pieces sized 2-3 cm. The bone pieces were then homogenizing to form a composite. The demineralization's materials used were weak acid, acetic acid (CH_3COOH 1 M) and strong acid, hydrochloric acid (HCl 1 M).

Research Equipments:

The main equipments used in the production process, namely: water bath (*Memmert Type WNB7-45*), digital oven (*Memmert*), analytical balance (*Sartorius TE 214S*), flask, beaker glass, erlenmeyer, funnel cups, measuring cups and thermometers. Supporting equipment for quality testing process, namely: Universal Testing Machine (*GY-4*), viscometer Brookville and pH meter (*Hanna NI 8520*).

Research Methods:

Preparing a demineralization solution:

Demineralization solution in the form of acetic acid (CH_3COOH) and hydrochloric acid (HCl) is made with the concentration of one molar each.

Research Design:

This study was conducted on the laboratory experiments using basic design in the form of completely randomized design (CRD) 2 x 2 factorial design with three replications. Two factors were applied: Factor I used two types of demineralization materials (CH_3COOH 1 M and 1 M HCl) and factor II used two curing time process (48 hours and 96 hours).

Research Implementation:

Implementation of this research was referred to the collagen extract production process had been modified. A total of 300g of raw material, the composite waste of broiler's bone was inserting into the glass beaker containing a degreasing solution, ethanol 70%. Degreasing process carried out for 24 hours at a room temperature. The bones were then washed under running water for 3 minutes. Next, it was put in demineralized solution according to the appropriate combination of treatments that have been determined until the entire surface of the bone was completely submerged. Raw materials that have undergone bone demineralization process were call *ossein*. After that, it was washed again on running water. Then, it was neutralized with alkaline solution ($Ca(OH)_2$ 20% w/v) for 24 hours with bone ratio: alkaline solution (1: 1.5). The bones samples have neutralized were and add with distilled water in the ratio of bone; distilled water (1: 1). The first extraction process was carried out in a water bath under temperature of 70°C (fraction 1) for 24 hours followed by a second extraction at a temperature of 75^{o}C (fraction 2) for 24 hours. The result of the extraction was an extract liquid collagen. The result of the first and second fraction was then combined, and poured on an aluminum trays that were previously given a layer of clear plastic which then dried in an oven under temperature 55°C for 18-20 hours. A layer of dried collagen extracts that had been dry was then grinded in a blender to form a powder and then weighed to determine the yields value. The next powder packed in plastic clips in order to test the quality.

Data analysis:

Data was analyzed using analysis of variance by factorial design supported with statistical program SPSS (one-way ANOVA). The treatment showed a significant influence, therefore it was continued to a real difference test using Duncan'S Multiple Range Test (DMRT) at the level of 5% [16].

Process of Analysis:

Yields [17]. Bone waste was weighed to determine the initial weight of the raw material (g). After the production process, the dried collagen extract was weighed to determine the weight of the final product (g). The next yields was then calculated. Yields (%) = (weight of the end product/initial weight of the raw material) x 100%.

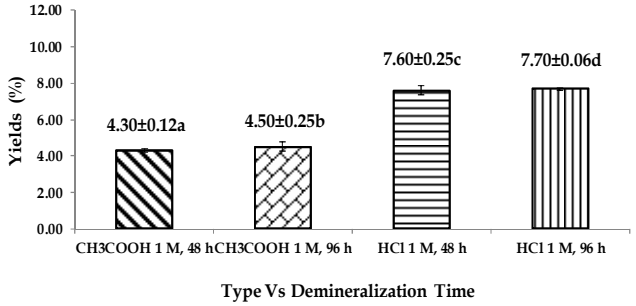
Gel strength (Modification). Gel strength was measured with a Universal Testing Machine (*GY-4*) equipped with a teflon plunger cylinder (diameter 13 mm). Collagen extract solution was prepared with concentration of 6.67% w/v (6.67 g added with distilled water to 100 ml) prior to heating at a temperature of ± 60°C until the extract was completely dissolved. The solution was then put into a container with a diameter of 5 cm and a height of 6 cm, and then stored in a fridge at a temperature of 5°C for 16-18 hours. The sample in the container was placed right at the bottom of the plunger (d = 13 mm) for further testing process. The test was done 3 times to each sample at a temperature of ±10°C. The readings given maximum force on the plunger was F.max gel samples (N/cm2). The plunger speed was penetrated in to gel of 10 mm/minute with the depth of 4 mm. Gel strength (g *Bloom*) = 20 + 2.86.10-3. D, where D = (F/G) x 980. Value F = maximum force (N/cm^2) and G = constant (0.07).

Viscosity [18]. (Modification). The viscosity of the sample was measured with a Brookville viscometer. Collagen solution was prepared at a concentration of 6.67% w/v (6.67 g added with distilled water to 100 ml) prior to heating at a temperature of ±60°C until the collagen particles were completely dissolved. Liquid collagen extract solution was poured into a bowl and then the viscosity measured. The measurement of the value of collagen extract was done at room temperature (28^oC). The result of the measurement was recorded in units of centipoise (cP).

RESULTS AND DISCUSSION

Yields (%):

Yields in principle are a number of products resulted from a number of processed raw materials [19], and influenced by the production process [20-21]. The greater the value of the yields indicates the more efficient of the production process. The comparison of the yields value generated through the application of the combineded treatments is presented in Figure 1.



Note: ^{a,b,c,d} ; The different superscript in each diagram showed significant difference ($p<0.05$)

Fig. 1: Graph of the comparison of yields (%) of collagen extract produced from some combination treatments of demineralization process of broiler's bone

Based on the data analysis of variance showed in Figure 1, the difference in types of materials and time of bone demineralization process in the production is significant ($p<0.05$) on the yields value of the collagen extract. As shown in Figure 1, the use of strong acid as demineralization ingredients produce higher yields than the use of a weak acid. There is the ability of the increased acid concentration to increase the yields value [22].

Concentration has an enormous influence on the solubility of collagen. The increased concentrations of acid causes an increase in the concentration of H^+ ions in solution demineralization, eventually will accelerate the

process of hydrolysis [23]. The faster the rate of hydrolysis, the higher the number of molecules converted into collagen which leads to the increase in the value of the yields.

Gel Strength (g Bloom):

Gel strength is one of the parameters to determine the physical quality of collagen products [24]. The comparison of the gel strength of collagen extract from broiler's bone produced using a combination of different types and processing time is presented completely in Figure 2.

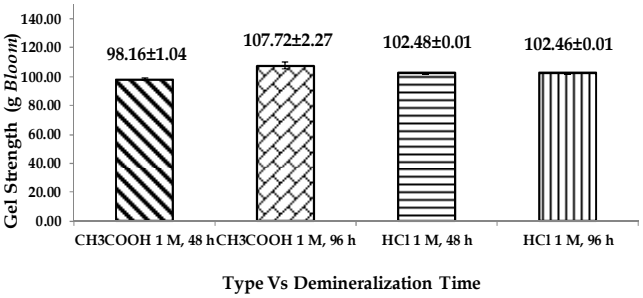


Fig. 2: Graph of the comparison of gel strength (g *Bloom*) of collagen extract produced from some combination treatments of demineralization process of broiler's bone

Figure 2 indicates that the value of gel strength of some combination process is quite varied. The highest value of gel strength of the product extract was produced from a combination of CH_3COOH demineralization with processing time of 96 hours and the lowest value was in the use of HCl with 96 hours of processing time. Gel strength impairment might be caused by the termination of the polymer chain of amino acid in excess with increasing in acid concentration in this case the use of a strong acid, so that the bonds among polymer molecules that construct protein was converted into collagen split into very short monomer chains. Eventually, it would be damaged and would lead to a limited gel formation process. Gel strength is highly dependent on the hydrogen bonds among water molecules with free hydroxyl groups of the amino acid groups, the size of the protein chain, concentration and distribution of molecular weight [25].

Based on the standard Gelatin Manufacturers Institute of America (GMIA) [26], the physical properties such as strength of the resulting gel on the use of acid demineralization still meets the required standards 50-300 g *Bloom* and 75-300 g *Bloom*. Gel strength influenced by hydrogen bonds among water molecules with free hydroxyl groups of the amino acid groups, the size of the protein chain, concentration and molecular weight distribution. The use of high concentrations of materials in the production process of collagen is able to either increase or decrease the value of gel strength, whereas the quality of collagen resulted from a production process is highly dependent on the extraction process performed on the protein molecule.

Viscosity (cP):

Viscosity in principle is the ability of a liquid resist to flow. The flown process of a liquid is affected by the viscosity that occurs due to the adsorption and colloidal development [27]. The comparison of viscosity values of the combined treatments is presented briefly in Figure 3.

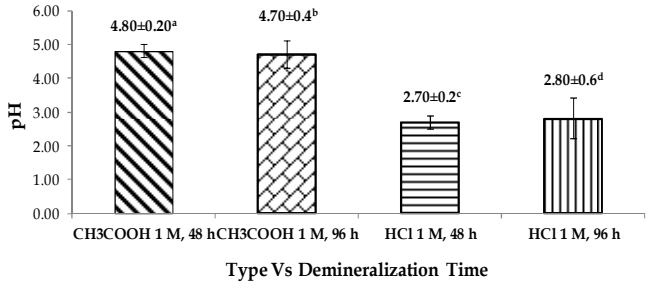


Fig. 3: Graph of the comparison of viscosity (cP) of collagen extract produced from some combination treatments of demineralization process of broiler's bone

Data as viewed in Table 3 represents the viscosity of collagen extract. The statistical tests showed that different types of the materials and time of the demineralization process on broiler's bone indicated no significant effect ($p>0.05$) on the viscosity of collagen. It could be caused by the insufficient time of the process applied in the production process in hydrolysing and breaking the peptide bonds of the protein structure of the skin to the fullest. The increase in viscosity values is basically influenced by the molecular structure of amino acids that make up proteins. The longer composition of amino acids, the higher the viscosity of collagen [28]. As can be interpreted in Figure 3, that the use of the demineralization material in the form of strong acid with the addition of processing time eventually could increase the viscosity of the product. The increased concentrations of materials tends to increase the viscosity if the material is able to "break" the bond of peptide on the proper bonding with longer molecules.

pH:

The value of pH is used to express the degree of acidity or alkalinity of a solution. Comparison of pH of broilers' collagen extract produced using different types and time of demineralization is presented in Figure 4.

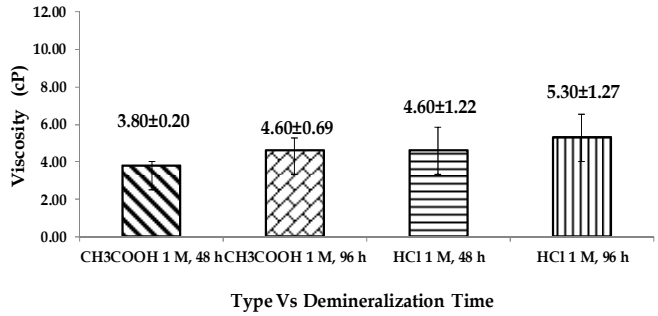


Fig. 4: Graph of the comparison of pH of collagen extract produced from some combination treatments of demineralization process of broiler's bone

Results of analysis of variance in Figure 4 indicates that the application of different types and time of demineralization is highly significant ($p<0.01$) to the pH value of collagen extract of broilers' bone. At the time of acid introduction in the demineralisation process, the existing collagen fibers in bone will be swelled, therefore the internal cohesion properties of the collagen fibers will decrease. This leads to the dilation of the bonding structure of amino acids in the collagen molecule and the trapped of the acidic materials among the bonds. Acid molecules trapped in the bond structure and undissolved at the neutralization process, will directly affect pH of the final product. Evidently, the acid concentration may alter pH of the product. The rate of collagen conversion into gelatin depends on several process parameters (temperature, time and pH) of the raw material and pretreatment.

Conclusion:

The application of the different type of material and time of demineralization process in the production process of collagen extracts with broiler's bone as the raw material significantly effected the value of product yield ($p<0.05$). However, there was no effect on the value of gel strength and viscosity ($p>0.05$). The use of HCl 1M in the process of demineralization resulted averagely a higher collagen extract yield than CH_3COOH 1M on the use of processing time of 48 hours and 96 hours.

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