Effect of pH and Stirring Speed on the Collagenous Protein Extraction from Chicken Bone Waste in a Well Agitated Extraction System

Andri Cahyo Kumoro, Beatricx L. M. Tanjung, Fadilla H. Utami, Diah Susetyo Retnowati and Catarina Sri Budiyati

Department of Chemical Engineering, Faculty of Engineering, Diponegoro University,
Jl. Prof. H. Soedarto, SH -Tembalang, Semarang INDONESIA 50275
***E-mail**: andrewkomoro@yahoo.com*

Abstract :

Chicken bones waste is commonly used for the production of animal feed and fertilizer. Fortunately, chicken bones are also rich in collagenous protein that possesses various functional properties. Considering this fact, the aims of this work are to determine the effect of pH and stirring speed on the alkaline extraction of collagenous protein from chicken bone. The experiments were carried out in a standard three necked flask equipped with heat supply, condensing and agitation facilities to perform isothermic well mixed extraction system. The extraction was conducted using caustic soda solution as solvent, extraction time (1 hour), temperature (80°C), the ratio of chicken bones:solvent (g/mL) = 1 : 4, and bone powders particle size (425 µm). The studied variables are pH: 9, 10, 11 and stirring speed: 100, 200, 300 rpm. The results showed that higher stirring speed and alkalinity of the extraction process resulted in higher protein yield. However, at very high stirring speed (300 rpm) and too alkaline (pH 11) extraction condition, the extraction lost its efficiency. The highest protein yield was achieved when the extraction was performed at pH 10 and stirring speed of 200 rpm for 60 minutes.

Keywords: chicken bones, collagenous proteins, extraction, pH, stirring speed

1. Introduction

According to Central Java Statistic Bureau, poultry production in this province reached 67.915.076 in the year of 2008 **[1]**. The increase in chicken meat consumption as a source of protein has caused a significant increase in chicken bone waste production. Abundant chicken bone waste has become one of the main triggering factors of environmental problems as chicken bone does not offer high economic value that result in limited utilization of chicken bone in various part of human life. However, this fact also suggests the possibility of enhancing chicken bones utilization by extracting and producing a highly functional, low fat, light-colour protein extract **[2]**.

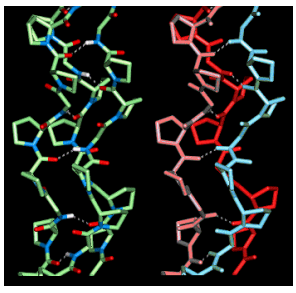

Figure 1. Molecular structure of collagenous protein.

Chicken bone contains organic and inorganic compounds with various benefits, especially for human health and well being. The inorganic compounds are calcium, phosphor, bicarbonates, citrates, magnesium, sodium, etc., while the main organic compounds are proteins. There are two types of protein contained in the bones, namely collagenous and non-collagenous proteins **[3]**. Overall, the portions of collagenous proteins are more than the non-collagenous ones. Fortunately, the collagenous proteins have higher market value due to their flexible usages in cosmetics, gelatin and adhesives (**[4]** as well as partial organs **[5]**. On the other hand, the non-collagenous proteins are very important in the mineralization process. Therefore, this research was aimed to extract the collagenous protein from chicken bone. While there are about 29 types of collagenous protein found in

the nature, 90% of those proteins are encountered in the animal body in which collagenous protein type I remains in the animal bone. The molecular structure of collagenous protein is presented in Figure 1.

Bone proteins can be extracted through solid-liquid extraction process. The extraction can be conducted under acidic or basic condition. However, the acidic extraction of bone protein is more costly as compared to the basic extraction. This is because acidic extraction can only be performed under cryogenic conditions (4^{o}C), which requires extensive cooling by refrigeration systems. From technical and economical considerations, investigation in chicken bone protein extraction under basic condition is preferred. Kumoro *et al*. **[6]** reported the alkaline extraction of collagenous protein from chicken bone under basic condition (pH 10.5) and found that highest extraction yield was obtained at 80^{o}C using chicken bone particle of 250μm in size. To obtain the best extraction environment condition, the effect of pH on the collagenous protein extraction efficiency was investigated in this work. Agitation is another factor that influences the turbulence of the system and the intensity of bone particles – solvent interactions. As far as literatures survey has been conducted, none of them reported the agitation speed used in chicken bone protein extraction. Fortunately, Board *et al*. **[7]** reported that highest yield was obtained in cow bones protein extraction using 200 rpm agitation speed, which can be used as a guide to study the effect of stirring speed on the chicken bone protein extraction efficiency.

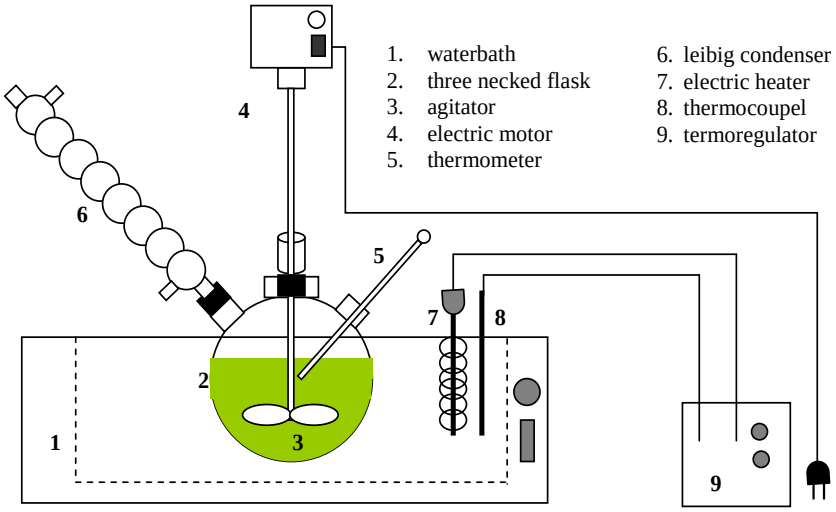

Figure 2. Experimental Set-up

2. Materials and Methods

The chicken bones waste used in this study were obtained from McDonald Outlet Jl. Setia Budi, Semarang-Indonesia. Proximate analysis using the method suggested by Aerssens *et al* **[8]** reported that the dry bone powder contains 7% (w/w) of collagenous protein. Pretreatment was employed to the chicken bone, included drying and size reduction to obtain 425μm bode powders, and inorganic chemicals removal. All chemicals used in this study were of analytical grade and purchased from Merck- Germany.

The experimental apparatus set up is shown in Figure 2. The alkaline solutions were prepared by dissolving NaOH crystal pellets in distilled water. Three hundreds millilitres of the solution was then introduced into a 500 mL capacity of three necked flask, equipped with Leibig condenser, thermometer, mechanical agitator and a sampling device (Figure 2). The system was then heated to 80^{o}C by immersing the flask in a water bath heater. Once the desired temperature was achieved, 75 grams of chicken bone particles was then added into the warm NaOH solution and the agitation was started. Extraction was done at three different pHs (9, 10 and 11) and at three different stirring speeds (100, 200 and 300 rpm). Suspension samples were taken at every 10 minutes interval, and the extraction process was stopped after 1 hour. After extraction, the sample was filtered with a double layer of gauze and the filtrate was centrifuged at 8000×*g* for 10 min. The supernatant was then lyophilized and the resulting extract was used for protein content analysis using Lowry- Folin method **[9]**. Collagen peptide reagent (Wako Pure Chemical Industries Ltd., Osaka) was used as the standard.

3. Results and Discussion

The results of obtained from the extraction of protein from chicken bones are illustrated in Figure 3 and 4.

3.1. Effect of pH

The effect of pH on the extraction of chicken bone protein is presented in Figure 3. It is obvious that the highest extraction yield was achieved when extraction was conducted at pH 10. This fact is indicated by high protein content in the liquid samples. The extraction was slower at pH 9 as the extraction condition is not alkaline enough causing the NaOH solution has lower extracting power than at pH 10. However, increasing the pH value of the NaOH solutions to 11 also reduced the extraction yield. Similar condition was reported by Betti & Fletchert, 2005 from their study on the extraction of protein from Broiler chicken meat at pH 8-12. They came out with the conclusion that the extraction was best at pH 9.5, and increasing pH of the extraction system reduced the extraction yield.

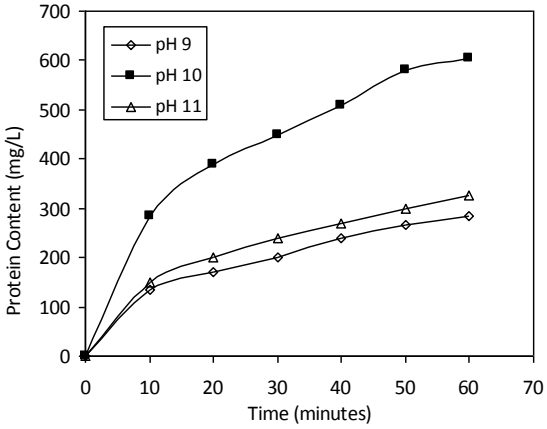

Figure 3. Profile of the protein content in the extraction process at chicken bone particles – solvent ratio (v/w) = 4, stirring speed 200 rpm and 80^{o}C obtained at different pH.

The reduction of extraction yield when extraction is conducted at high alkalinity condition may be associated by protein degradation and denaturation **[2]**. In general, proteins are only marginally stable and are highly susceptible to degradation, both chemical and physical **[10-11]**. Chemical degradation refers to modifications involving covalent bonds, such as deamidation, oxidation, and disulfide bond shuffling. Physical degradation includes protein unfolding, undesirable adsorption to surfaces, and aggregation **[12]**.These phenomena can be proven through conformation changes in the secondary, tertiary and quaternary positions after oxidation and deamidation. Chemical degradations often induce changes in protein conformation and thus influence protein activity and protein stability in the solutions **[13]**. Degradation of protein can be triggered by extreme (acid or basic) condition, heating at high temperature, presence of cationic metal ions, and addition of saturated salt **[14]**.

3.2. Effect of stirring speed

Figure 4 presents the profile of protein content in the liquid samples as a function of stirring speed and time. In the beginning, the protein content in liquid samples increased sharply at all the stirring speeds studied. Lengthening the extraction time gradually increased the protein content in liquid samples to some extent, and finally reached almost constant values depending on the stirring speed applied. In general, extraction efficiency enhances with increasing stirring rate because of the accelerated diffusion of the solute in solution **[15]**. In contrast, the extraction efficiency decrease when stirring rate was more than 200 rpm. Therefore, the use of stirring speed of 200 rpm was found to be the most efficient for the extraction of chicken bone protein compared to the other studied stirring speeds. Similar result was reported by Hou *et al*. [15] when they study the extraction behavior of C16-MCM-41with C8 bonded silica and MCM-41 without modification.

When stirring is applied, the chicken bone particles are in direct contact with NaOH solution as a solvent. Stirring also facilitates extraction because it causes shear stress to the chicken bone particles **[16]**. Although excessive fluid shear can be detrimental, moderate fluid shear can lead to increased chicken bone particles

permeability **[17]**. In addition, when the stirring speed is too high, the chicken bone particles moved together with the NaOH solution at a very close angular velocity value. Since the relative velocity between the chicken bone particle and NaOH solution was then very low, the physical and chemical interactions between the two substances became less effective. This explanation describes well the fact that the protein content in the liquid samples obtained from extraction using 300 rpm was lower than that of 200 rpm. From this phenomenon, another conclusion can also be drawn that the extraction was controlled by film diffusion. When the extraction is controlled by diffusion, the rate of extraction increases with the increase in the stirring rate, whereas if there is no effect on the extraction rate when it is governed by chemical reaction **[18]**.

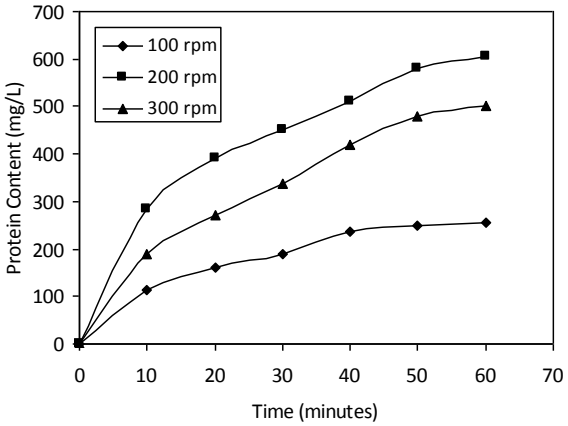

Figure 4. Profile of the protein content in the the extraction process at solvent- chicken bone particle ratio (v/w) = 4, pH 10 and 80^{o}C obtained at different stirring speeds.

4. Conclusions

The extraction of collagenous protein from chicken bone using NaOH solution has been conducted. Both pH and stirring speed influenced extraction efficiency from which optimum pH and stirring speed are present. The best extraction condition was achieved when the extraction was performed at pH 10 and stirring speed of 200 rpm for 60 minutes.

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