Identification of Protein Levels as Production of Bacteriosin from *Lactobacillus Plantarum* in Fermented Chicken Eggs

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Abstract. *Lactobacillus plantarum* is a lactic acid bacterium that can produce lactic acid as well as degrade proteins into soluble proteins, peptides, and amino acids. *L. plantarum* can also produce bacteriocin, which has antibacterial properties. The goal of this study was to determine the protein levels in fermented chicken eggs that could increase bacteriocin production during the fermentation process using *L. plantarum* bacteria. This study was carried out experimentally using a completely randomized design (CRD) with three treatments and three replications. Fermentation was used for the treatment, with incubation temperatures of 37 °C and incubation times of 0, 48, and 96 h. The findings revealed that incubation time had a significant ($P < 0.05$) effect on the total protein levels of fermented chicken eggs. The *Least Significant Difference* (LSD) further test results revealed that the total protein level was significantly different ($P < 0.05$) decreased in each incubation time treatment. ANOVA revealed that incubation time had a significant ($P < 0.05$) effect on dissolved protein levels in fermented chicken eggs. The *Least Significant Difference* (LSD) further test results revealed that soluble protein levels were significantly different ($P < 0.05$) increased in each incubation time treatment. The decrease in total protein levels may increase the levels of dissolved protein in fermented chicken eggs. The decrease in total protein levels and increase in dissolved protein levels in optimum fermented chicken eggs incubated at 37 °C for 96 h so that it could be used as a reference in the production of bacteriocins.

Keywords: Bacteriocin · Protein · L. plantarum · Incubation time · Fermentation

1 Introduction

Eggs are a low-cost, easy-to-acquire source of animal protein for the community. Eggs contain a good balance of amino acids, essential fats, minerals, and vitamins. Eggs have a relatively short shelf life. Fermentation technology is one method for extending the

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shelf life of eggs. Fermentation technology was used to obtain benefits such as functional food that is good for health, aids digestion, and extends product shelf life.

Fermentation is an aerobic or anaerobic process that produces various products involving microbial activity. Lactic acid bacteria will hydrolyze lactose in milk, into a variety of simpler carbohydrate compounds. The fermentation process results in increased microbial activity, decreased pH, and increased acid levels in fermented products [1]. The ability of microorganisms to grow and stay alive is important in a food ecosystem. Some of the main factors that affect the growth of microorganisms include nutrient supply, time, temperature and pH [2].

Fermentation technology on foods using microbes has been widely used, including the use of *Lactobacillus* bacteria. *Lactobacillus plantarum* was the most commonly used *Lactobacillus* bacteria in egg fermentation. These bacteria could multiply by consuming the nutritional value of the growth medium [3].

L. plantarum can grow at a temperature of 45 °C but optimum at a temperature of 30–37 °C. Fermentation of broiler eggs using *L. plantarum* with a certain temperature and time can increase the number of lactic acid bacteria so that it increases lactic acid levels and lowers the pH value. Fermented eggs also increase soluble protein which can optimize antioxidants in fermented chicken eggs so that it is expected to be a fermented product that is good for health because it has a complete source of nutrients and high antioxidants [4].

L. plantarum is a proteolytic bacterium that can degrade protein compounds into simpler compounds. These compounds generate the energy that bacteria require to survive. Sufficient nutrients are available in a suitable environment to increase bacterial productivity during a specific fermentation time [5]. Bacterial growth in a medium is closely related to the bacteria's ability to metabolize existing nutrients, particularly the ability to break down proteins. Lactic acid bacteria degrade proteins into amino acids and peptides, which are then used as a nitrogen source for cell growth and multiplication [6].

During the fermentation process, *L. plantarum* produces antibacterial metabolites such as lactic acid, hydrogen peroxide, and bacteriocins [7]. Bacteriocins are peptides or protein compounds released into the extracellular space by lactic acid bacteria that have a bactericidal effect on harmful bacteria that are phylogenetically related [8]. This antibacterial activity can be used to keep pathogenic bacteria.

Bacteriocins have long been recognized by researchers as natural products in the form of proteins or peptides derived from bacteria in fermentation products. Bacteriocins have the potential to be used as natural food preservatives that are safe for consumption, because the active substances which are contained in bacteriocins are proteins that can be degraded by proteolytic enzymes [9].

Lactobacillus plantarum is a lactic acid bacterium that can produce lactic acid and degrade proteins into soluble proteins, peptides, and amino acids, allowing it to produce bacteriocins with antibacterial properties. The goal of this study was to determine the protein levels in fermented chicken eggs that could increase bacteriocin production during the fermentation process using *L. plantarum* bacteria.

2 Materials and Methods

The used equipment in this study were sample tubes, erlenmeyer, micropipette, tip, syringe, analytical balance, measuring cup, incubator, spatula, autoclave, magnetic stirrer, vortex, lamina air flow, hot plate, khedjal flask, fume hood, measuring flask, distillation flask, centrifuge, spectrophotometer. The used materials were mass chicken eggs, *Lactobacillus plantarum* bacterial culture, MRS (Man Rogosa Sharpe) broth, aluminum foil, tomato juice, distilled water, alcohol, H_2SO_4, distilled water, H_3BO_3, NaOH, TCA, lowry reagent, folin reagent, BSA solution.

This study was carried out experimentally using a completely randomized design (CRD) with three treatments and three replications. Fermentation was used for the treatment, with incubation temperatures of 37 °C and incubation times of 0, 48, and 96 h.

2.1 Experimental Design

2.1.1 Culture Propagation

De Man Ragosa Sharpe (MRS) agar was used to store *Lactobacillus plantarum.* Subcultures are used to spread culture. Sub-culture was made by transferring the *culture stock* into liquid medium *MRS broth* (OXOID CM0359) to which 20% tomato extract was added and incubated for 24 h [10]. Cultures that had been stored in *MRS broth* media are inoculated as much as 10% into egg whites containing 20% tomato extract to produce working cultures [11].

2.1.2 Sample Preparation

Chicken egg samples were cleaned by using clean water. The chicken eggs were fumigated by using Calcium Permanganate (CP) powder and formalin in a closed room for 5 min and successively cleaned by using a wet cloth, chlorine solution and wiped with alcohol by using a cotton swab. Eggs were wrapped in aluminum foil and pasteurized at 60 °C for 3.5 min [12] then separated from the shell and then put into a sample bottle. The sample bottles were first cleaned by using warm water and sterilized. The 100 ml sample was homogenized before being sterilized with ultraviolet light in a *PCR* Hood for 15 min. The sterile sample was mixed with 10 ml of working culture before being homogenized with a tube shaker and fermented according to the research protocol [5].

2.2 The Tested Parameters

2.2.1 Measurement of Total Protein Levels

A sample of 2 ml was put into a Kjeldahl flask along with 2 g of a mixture of selenium and 20 ml of concentrated H_2SO_4. The flask was boiled until a clear solution was formed and continued for 30 min. The flask was allowed to cool and slowly added 100 ml of distilled water until the temperature reached 25 °C. Prepared a reservoir consisting of 10 ml of 2% H_3BO_3 + 4 drops of mixed indicator solution in an Erlenmeyer then put 5 ml of the sample solution into a distillation flask. Added 10 ml of 30% NaOH and

100 ml of distilled water, then distilled until the volume of the reservoir became ± 50 ml. Rinsed the tip of the distiller with distilled water and then the container with its contents was titrated with a 0.0171 N H_2SO_4 solution.

2.2.2 Measurement of Dissolved Protein Levels

Put 1.5 g of the sample into a graduated tube, then add 7.5 ml of distilled water. A vortex was used to homogenize the mixture. After centrifuging the mixture for 15 min, the precipitate and supernatant were separated. On a hotplate, the supernatant was boiled. For 15 min, the samples were centrifuged. For the final test, 2 ml of supernatant was taken and 1 ml of 10% TCA solution was added, then the solution and precipitate were separated by centrifuging for 15 min. 0.1 mL of TCA sample extract was mixed with 1.9 mL of distilled water, followed by 2.5 mL of Lowry's reagent. After homogenizing the mixture, it was stored at room temperature for 10 min. After that, 0.5 ml of Folin reagent was added and incubated at room temperature for 30 min, until a blue color was formed. Furthermore, the sample's absorbance was measured on a spectrophotometer with a wavelength of 600 nm using a standard solution of Bovine Serum Albumine (BSA).

2.2.3 Statistical Analysis

The data from the research were tested for normality to find out the residual value was normally distributed if the significance value ($P > 0.05$) then the residual value was normally distributed. Furthermore, homogeneity test was conducted to find out that two or more groups of sample data came from populations that had the same variance, if the significance value ($P > 0.05$) then the data distribution was homogeneous. Furthermore, the data is processed used to analysis of variance (ANOVA). If the results of the analysis of treatment variance revealed a significant effect ($P < 0.05$), the LSD multiple comparison test was used. The data for testing was processed using the SPSS 20 application.

3 Results and Discussion

3.1 Total Protein

Total Protein levels (%) of fermented chicken eggs with different incubation times could be seen in Table 1.

Table 1. Showed that increasing the incubation time had a very significant ($P < 0.01$) effect on total protein levels. Total protein (percentage) decreased as incubation time increased. The LSD further test results revealed that the total protein level was significantly different ($P < 0.05$) in each incubation time treatment.

This was due to the high number of bacteria that broke down protein during fermentation, resulting in a decrease in total protein [13]. The decrease in total protein occurred as a result of microbes converting protein into amino acids and peptides (simple compounds) during fermentation. The amino acid and peptide content of the protein in the fermentation medium could influence the decrease in total protein during fermentation.

Table 1. Total protein (%) and dissolved protein levels (%) of fermented chicken eggs with different incubation time

Incubation Time (hours)	Total Protein (%)	Dissolved Protein (%)
0	10,34 ± 0,34c	79,00 ± 0,36a
48	9,5 ± 0,17b	81,25 ± 0,23b
96	8,52 ± 0,01a	81,79 ± 0,05c

Description: Different superscripts on the same graph showed significant differences ($P < 0.05$).

[14] The decrease in total protein was accelerated as the total number of lactic acid bacteria increased.

Protein levels were influenced by the number of viable bacterial cells, with an increase in the number of enzymes used to break down proteins (proteolytic activity) and increased protein degradation, including protein-breaking enzymes (proteases). In this case the protein would be broken down into peptides and would be further hydrolyzed into amino acids. The results of this breakdown act as precursors in enzymatic reactions and chemical reactions to form flavors [15].

3.2 Dissolved Protein

Tabel 1. Showed that different incubation time treatments for fermented eggs had a very significant effect ($P < 0.05$) on dissolved protein levels. The results of the LSD further test showed that the percentage of dissolved protein level was significantly different ($P < 0.05$) increased in each incubation time treatment. The increase in dissolved protein was caused by a protein degradation process carried out by bacteria.

Bacteria produced proteolytic enzymes, which broke down proteins into smaller particles, increasing the amount of dissolved protein. Dissolved protein increased as a result of the protein synthesis process carried out by a large number of bacteria, allowing it to produce protein from these bacteria. [16] The higher the protein content, the longer the incubation time. The increase in protein level was caused by the production of proteolytic enzymes by lactic acid bacteria during fermentation. The incubation time increased the population of *L. plantarum*, which increased the dissolved protein levels. The increase in protein was also caused by an increase in the number of microorganisms that act as *Single Cell Protein* (SCP), a protein obtained from microorganisms.

The dissolved protein from this treatment was at its best after 96 h of incubation at 37 °C. This occurred because the appropriate number of bacteria were found in the treatment, resulting in proteolytic activity to produce dissolved protein. Protein decomposition was also influenced by incubation time, as *L. plantarum* required time to adapt to egg media and break down the nutrients contained in eggs, resulting in an increase in dissolved protein with increasing incubation time [5]. An increase in the number of bacteria was followed by an increase in the concentration of dissolved protein for 6 to 18 h. This indicated that *L. plantarum* ability to soluble protein was related due to the exponential growth of *L. plantarum's* during fermentation [17]. *Lactobacillus* would

live and reproduce by utilizing the nitrogen and carbon sources found in the fermentation medium. Because most of the components of bacteria were protein, the higher the protein levels in the fermented product, the more bacteria there were.

The decrease in total protein levels and increase in dissolved protein levels in fermented chicken eggs were optimum at 37 °C for 96 h of incubation time, allowing it to be used as a reference in the production of bacteriocins.

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