The antioxidant value of chicken eggs subjected to the fermentation using \textit{Lactobacillus plantarum} at different temperature and incubation time

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Abstract. Eggs contain antioxidants that are very beneficial to the body, but the antioxidants found on egg consumption were still low. Increased antioxidants can be done with the use of fermentation technology with \textit{Lactobacillus plantarum} bacteria. This research used a completely randomized design with a factorial pattern, with the treatment of temperature and time incubation, each with three replications. As many as 243 fresh chicken eggs obtained from a chicken farm, fermented with \textit{Lactobacillus plantarum} FNCC 0027. Parameters measured in the research were bacterial count (Log10 CFU/mL), dissolved protein (%), antioxidant activity (%) and antioxidant concentration (mL GAE/100mL). The research results indicated that an increase in the number of bacteria, dissolved proteins, and antioxidant activity but a decrease of the concentration antioxidants with increasing temperature and time incubation. Antioxidant activity on eggs chicken fermented of optimum at 37°C incubated temperature for 96 hours of incubation time.

1. Introduction

Eggs are livestock products that provide the most considerable contribution to the achievement of society's nutritional adequacy \cite{1}. Eggs are a cheap source of animal protein and easily accessible to the layers of society. Eggs have balanced amino acids, essential fats, some minerals, and vitamins. Eggs also contain antioxidants that are very beneficial to the body. Antioxidants can prevent disease by counteracting free radicals that can damage cells in the body but the antioxidants, that are found in eggs consumption, are still low.

Egg albumen has an antioxidant potential of 20.99\% \cite{2}. Chicken eggs have low antioxidants due to low nutritional adequacy in the body of poultry, especially protein. Laying hens were relatively susceptible to disease problems, physiological stress, and environmental stress. Thus, laying hens need a lot of energy. The amount of protein in the feed and the occurrence of a reshuffle of proteins to produce energy was limited so that the antioxidants were low.

Increased antioxidants can be done with the use of fermentation technology. Several studies had proven that fermentation could increase antioxidants. Fermentation technology in food using microbes had been done, among others, by using \textit{Lactobacillus} bacteria. Species of \textit{Lactobacillus} commonly used in egg fermentation were \textit{Lactobacillus plantarum}. This bacteria could grow at 45°C with an optimum temperature of 30–37°C \cite{2,3}.

Lactic acid bacteria can grow in eggs but there is no known temperature and optimum time of \textit{L. plantarum} to grow in egg consumption to produce antioxidants. Chicken eggs fermented using \textit{L. plantarum} with specific temperature and time are expected to increase the number of lactic acid.
bacteria, thereby increasing lactic acid and dissolved protein, thus optimizing antioxidants in fermented chicken eggs. Thus, the researchers conducted a study about the antioxidant value of chicken eggs through fermentation of *L. plantarum* with different temperatures and incubation time.

2. Materials and methods

Egg samples were cleaned and pasteurized at 60°C for 3.5 minutes [2]. The eggs were separated from their shells and put into a sample bottle. As many as 100 mL samples were homogenized and subsequently sterilized using ultraviolet for 15 minutes. The sterile sample was added to a working culture of 10 mL and further homogenized with a tube shaker [2]. The samples were fermented according to the research treatment of incubated temperature 30°C, 37°C, 44°C and incubated time 0 hour, 48 hours, and 96 hours. The fermented samples were tested for parameters.

2.1. Enumeration of bacteria in the egg medium

Testing the number of microbes done by the method of pouring plate. As many as 1 mL egg sample to be tested was diluted in a 9 mL NaCl 0.86% solution and homogenized using a vortex. Dilution was done from $10^{-1}$ to $10^{-8}$. Next, each dilution was fertilized in a petri dish containing the medium of MRS (Man Rogosa Sharpe) agar and incubated at 37°C for 24 hours. The cup that gave the colony count of 25 - 250 was used as the calculation of the colony [2].

2.2. Modified soluble proteins

The supernatant of the sample was taken as much as 0.1 mL and then added 1.9 mL of aquadest and also added 2.5 mL of Lowrey reagent. The mixture was homogenized and stored at room temperature for 10 minutes. Further, 0.5 mL of Folin reagent was added and incubated at room temperature for 30 minutes until the blue color was formed. Further, the absorbance of the sample on a spectrophotometer was measured with a wavelength of 600 nm, using a standard solution of Bovine Serum Albumin (BSA). The value of dissolved protein had read on the monitor screen [2].

2.3. Antioxidant activity with DPPH (1,1-diphenyl-2-picrylhydrazyl) method

1 mL sample to be tested was diluted into 9 mL of methanol and homogenized using a vortex. Dilution was from $10^{-1}$ to $10^{-5}$. Each dilution was tested as much as 0.2 mL of the sample solution to the test tube and DPPH solution was added as much as 3.8 mL and 0.2 mL of methanol. The sample mixture was homogenized using a vortex and left for 60 minutes in the darkroom. The absorption of the solution was measured using a uv-vis spectrophotometer at 515 nm of wavelength. The amount of antioxidant activity was calculated by the formula [2,4].

2.4. Concentrations of antioxidants

The sample of 1 mL was diluted into a 10 mL bottle of flask and supplied the volume with aquadest to the boundary mark and then took 1 mL of sample solution into a 5 mL flask and each added 100 μL of Folin-Ciocalteu reagent, Na$_2$CO$_3$ 10% and then sufficiently, volume with Iabidistila up to the boundary mark. The absorption was measured at 600 nm of wavelength [2].

2.5 Data analysis

The obtained data were analyzed used a completely randomized design with factorial patterns (3×3) and using SPSS application [2].

3. Results and discussion

3.1. Number of bacteria

The analysis of variance showed that the temperature and time incubation had a significant effect (P<0.01) on the number of bacteria in fermented chicken eggs. The number of bacteria increased as the incubation temperature increases and optimal at 37°C. Increased incubation temperature of 44°C decreases bacterial count. The improvement of the number of bacteria (log10 CFU/mL) was in line with increasing incubation time (0, 48, and 96 hours). The number of bacteria was in the optimum fermented chicken eggs at 37°C incubation temperature for 96 hours incubation time.
Table 1. Number of bacteria (Log 10 CFU/mL) on chicken eggs through fermentation of *L. plantarum* with different temperatures and incubation time.

| Incubation time (hours) | Temperature | Average |
|-------------------------|-------------|---------|
|                         | 30ºC        | 37ºC    | 44ºC    |         |
| 0                       | 6.983       | 6.999   | 7.040   | 7.007\(^a\) |
| 48                      | 9.067       | 9.386   | 9.293   | 9.249\(^b\) |
| 96                      | 9.213       | 9.616   | 9.485   | 9.438\(^c\) |
| Average                 | 8.421\(^a\) | 8.667\(^b\) | 8.606\(^c\) |         |

Note: Different superscripts on the same row and column show highly significant differences (P<0.01).

There was an improvement in the number of bacteria on incubation temperature at 30ºC and 37ºC but decreased at 44ºC. This was due to the influence of temperature on the availability of food needed by bacteria. The high incubation temperature was thought to decrease the nutrients in the bacterial growth medium. Bacteria divided nutrients in the form of proteins and fats that were found in chicken eggs into simpler compounds such as acids and peptides. These compounds used by bacteria to grow and develop. *L. plantarum* also required optimum temperatures to grow rapidly in the egg medium. The incubation temperature of 37ºC had a high bacterial count so it was suspected as the optimum temperature of *L. plantarum* to grow and metabolize rapidly. The temperature was a physical factor that affected the rate of growth through its influence among others on chemical reactions and the stability of the protein molecule structure. Chemical reactions improved increasing temperature because the improvement in temperature causes an increase in reactant kinetic energy. Growth was essentially the result of metabolism, a directional chemical reaction that takes place in cells catalyzed by enzymes. Increased temperatures lead to increased growth until an increase in temperature was not followed by increased growth [2].

The number of bacteria (Log10 CFU/mL) in different chicken eggs was significantly increased in the treatment of different incubation times. The number of bacteria increases with increasing incubation time. The growth of bacteria in the egg medium was related to the growing time of bacteria that can metabolize the nutrients in the chicken egg medium. *L. plantarum* divided proteins into simple compounds to serve as a source of energy for growth and reproduction. Bacterial growth in a medium was thought to be closely related to the bacteria's ability to metabolized existing nutrients, especially the ability to divided proteins. During growth, lactic acid bacteria divided proteins into amino acids and peptides that were used as nitrogen sources for cell growth and multiplication [2]. Fermentation breaks down carbohydrates in food while food containing protein requires a certain type of bacteria, such as *L. plantarum*, that can degrade protein by proteolytic activity to produce amino acids. An increase in total acid in fermented egg albumen during the fermentation process is associated with the possibility of an increase in the number of bacteria that can break down carbohydrate and protein compounds into simpler compounds, including acid and water [2].

3.2. Dissolved protein

Analysis of variance showed that the temperature and time incubation had a significant effect (P<0.01) on the dissolved protein in fermented chicken eggs. The soluble protein increases with increasing incubation temperature and optimum at 37ºC. The improvement of the incubation temperature of 44ºC decreased the dissolved protein. The improvement of soluble protein (%) was in line with increasing incubation time. The dissolved protein content in the optimum fermented chicken eggs at 37ºC incubation temperature during 96 hours incubation time.

Treatment of different incubation temperatures in chicken eggs as the growth medium of *L. plantarum* showed that the dissolved protein increased with increasing incubation temperature but decreased above 37ºC. This is thought to be a large number of lactic acid bacteria during the fermentation process that can increase protease enzymes. The protease enzyme used to break down proteins into simpler compounds so that many of the peptides were produced. A large number of proteins were degraded due to microbial proteolytic properties during the fermentation process so as to degrade proteins into soluble proteins, peptides, and amino acids [2].
Table 2. Dissolved protein (%) on chicken eggs through fermentation of *L. plantarum* with different temperatures and time incubation.

| Incubation time (hours) | Temperature | Average |
|-------------------------|-------------|---------|
|                         | 30°C        | 37°C    | 44°C    |
| 0                       | 80.81       | 81.12   | 81.27   | 81.07<sup>a</sup> |
| 48                      | 91.30       | 94.59   | 93.15   | 93.01<sup>b</sup> |
| 96                      | 91.58       | 96.59   | 95.20   | 94.46<sup>b</sup> |
| **Average**             | **87.90<sup>a</sup>** | **90.77<sup>b</sup>** | **89.87<sup>b</sup>** |

Different superscripts on the same row and column showed significant differences (P<0.05).

The dissolved protein in fermented chicken eggs increases as the incubation time. Protein degradation process done by bacteria caused increased soluble protein. The bacteria produce proteolytic enzymes that break down proteins into simpler particles so that the soluble proteins increase. Increased soluble protein was also suspected because of the process of protein synthesis by a large number of bacteria that produced proteins from these bacteria. The longer the incubation time, the protein content was also higher. The increase in protein levels was caused because during the fermentation of lactic acid bacteria produced proteolytic enzymes. The incubation time caused the *L. plantarum* population to increase, thus making the dissolved protein content also increases. Increasing the amount of protein was also caused by the increase in the number of microorganisms that acted as single cell protein (SCP) which was a protein obtained from microorganisms [2].

3.3. Antioxidant activity

Analysis of variance showed that the temperature and time incubation had a significant effect (P<0.01) on the dissolved protein in fermented chicken eggs. Antioxidant activity increases with increasing incubation temperature and optimal at 37ºC. Increased incubation temperature of 44ºC decreased antioxidant activity. The increase in antioxidant activity (%) was in line with increasing incubation time. Antioxidant activity was in the optimum fermented chicken eggs at 37ºC incubation temperature during 96 hours incubation time.

Table 3. The antioxidant activity (%) on chicken eggs through fermentation of *L. plantarum* with different temperatures and time incubation.

| Incubation time (hours) | Temperature | Average |
|-------------------------|-------------|---------|
|                         | 30°C        | 37°C    | 44°C    |
| 0                       | 23.24       | 23.34   | 23.45   | 23.34<sup>a</sup> |
| 48                      | 47.08       | 69.62   | 59.85   | 58.85<sup>x</sup> |
| 96                      | 57.96       | 73.29   | 70.05   | 67.10<sup>y</sup> |
| **Average**             | **42.76<sup>a</sup>** | **55.42<sup>b</sup>** | **51.12<sup>b</sup>** |

Different superscripts on the same row and column showed significant differences (P<0.05).

Antioxidant activity increases with increasing temperature and optimum at 37ºC. There was no difference in antioxidant activity at 37ºC and 44ºC incubation temperature. This indicated that an increase in antioxidant activity as the number of bacteria increases. *L. plantarum* can increase antioxidant activity. The increase was allegedly due to the overhaul of structural compounds such as carbohydrates, proteins, and fats to be simpler which were caused by the metabolic activity of *L. plantarum* bacteria. *L. plantarum* can generally degrade structural compounds such as proteins and fats. Increased antioxidant activity was in line with the increase in total phenol and flavonoid in the fermented material with *L. plantarum* bacteria [2].

The antioxidant activity of fermented chicken eggs increases with increasing incubation time. The longer the incubation time caused higher antioxidant activity. Increased antioxidant activity may occur presumably due to the activity of lactic acid bacteria in fermented chicken eggs. Lactic acid bacteria...
break down proteins and fats in eggs into simpler compounds such as acids and peptide groups that had antioxidant properties. Fermentation was produced compounds that can raise and stabilize antioxidant activity such as lactic acid, acetic acid, citric acid, succinic acid, malic acid, acetaldehyde, diacetyl, and acetoin. The increased antioxidant activity by increasing incubation time. The incubation time increased the free phenol and flavonoid compounds due to the hydrolysis of sugars by lactic acid bacteria enzymes. This phenomenon corresponds to several studies that showed that the total amount of phenol or total flavonoids contained was related to the effectiveness of antioxidant activity produced [2].

Increased antioxidants were characterized by low protein oxidation in fermented chicken eggs resulting in increased soluble protein. The ability of antioxidants that suppress oxidation activity can stop the process of cell destruction by giving electrons to free radicals that neutralize free radicals. Soluble proteins that had peptide bonds improved the antioxidant properties to protect against oxidative stress either protein oxidation or fat oxidation. The ability of antioxidants as fat peroxihydroxide inhibitors that inhibit fat oxidation in the linoleic acid system [2]. Antioxidants from peptides can increase the intracellular antioxidant (glutathione) and elevated antioxidant enzymes (glutathione reductase, glutathione S-transferase and catalase) [2].

3.4. Concentrations of antioxidants

The analysis of variance showed that temperature and time incubation had a highly significant effect (P <0.01) on antioxidant concentration in fermented chicken eggs. The concentration of antioxidants decreased as the incubation temperature increased and was optimal at 37°C. Increased incubation temperature of 44°C increased the concentration of antioxidants. There was a reduction in the concentration of antioxidants (mL GAE/100mL) in line with increasing incubation time. The antioxidant concentration was in the optimum fermented chicken eggs at 37°C incubation temperature for 96 hours incubation time.

| Incubation time (hours) | Temperature (°C) | Average | 30°C | 37°C | 44°C |
|-------------------------|------------------|---------|------|------|------|
| 0                       |                  |         | 24.97| 24.75| 24.55|
| 48                      |                  |         | 22.77| 22.62| 23.07|
| 96                      |                  |         | 21.68| 20.79| 21.27|
| Average                 |                  |         | 23.14| 22.72| 22.96|

Note: Different superscripts on the same row and column showed significant differences (P<0.05).

The concentration of antioxidants (mL GAE/100mL) in rabid chicken eggs was significantly decreased as the incubation temperature increased. This happens because during the fermentation takes place synthesis of carbohydrates and proteins by lactic acid bacteria resulting in the formation of organic acids such as lactic acid that caused acid conditions. Acid conditions had pH values of 3.9 - 5 lead to the formation of phenol compounds through hydroxycinnamic acid and ferulic acid. The high content of total sugar and protein triggers the growth of lactic acid bacteria more and more because lactic acid bacteria more and more change the protein into primary metabolites (lactic acid) and secondary metabolites (polyphenols). During the fermentation, lactic acid bacteria produce enzymes that can degrade proteins which can liberate phenolic compounds so as to increase the phenol group in flavonoid compounds so as to increase total phenol content, total flavonoids and antioxidant activity of the medium used [2].

The concentration of antioxidants in fermented chicken eggs increased as the incubation time increased. This was thought to be due to increased lactic acid from the bacterial metabolism activity of *L. plantarum*. Changing egg proteins by lactic acid bacteria into lactic acid that gave electrons of H⁺ ions in free radicals so that free radical molecules become less dense. *Lactobacillus plantarum* can break down complex materials in a material. The composition of the material influenced the length of time that the bacteria need in decomposing a material. This was thought to be due to increased lactic
acid from the bacterial metabolism activity of \textit{L. plantarum} [2]. The higher the acid value, the lower of the antioxidant concentration. However, this decline can optimize antioxidant activity [2]. Antioxidants with too high concentrations, especially for phenolic group antioxidants actually result in the pro oxidation or disappearance of antioxidant abilities [2].

The amount of bacteria increases the activity of bacterial metabolism so as to produce a high acid. The bacteria also produce proteolytic enzymes that can break down egg proteins into simple compounds such as peptides that had antioxidant properties. These bacteria can also synthesize proteins from themselves so that the higher dissolved proteins were produced. The number of dissolved proteins that had peptide bonds that are widely identified as antioxidants to obtain high antioxidants, but the concentration of antioxidants to be low due to high acid conditions and inhibit the oxidation process.

4. Conclusion
The optimum antioxidant activity on the fermented chicken eggs was at 37ºC for 96 hours of incubation.

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