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Identification of Proximate Composition of Fermented Chicken Eggs by Using *Lactobacillus plantarum* with Different Incubation Times

Identifikasi Komposisi Proksimat Telur Ayam Ras Fermentasi Menggunakan Lactobacillus plantarum dengan Waktu Inkubasi yang Berbeda

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ABSTRACT

Eggs that have a balanced amino acid content can fullfill protein that needs in humans, However, eggs have a low shelf life so they were easily damaged. Fermentation technology on foodstuffs by using microbes has been widely carried out, among others using *Lactobacillus* bacteria. The type of *Lactobacillus* bacteria commonly used in egg fermentation is *Lactobacillus plantarum*. This study was conducted experimentally by using a completely randomized design (CRD) with 3 treatments and 3 replications each. The treatment was carried out by fermentation with an incubation temperature of 37 ^{o}C with different incubation times of 0, 48, and 96 hours with research parameters water content, crude fat, crude fiber, BETN and ash content. The results showed that different incubation time treatments on fermented chicken eggs had a significant effect (P<0.05) on water content, crude fat, crude fiber, BETN and ash content. The nutritional composition of fermented eggs by using *L. plantarum* could be seen from the decrease in water content, crude fiber and BETN and an increase in crude fat and ash content with increasing incubation time. The value of water content, crude fat, crude fiber, BETN and optimum ash content at an incubation temperature of 37 ^{o}C for 96 hours of incubation time.

Keywords: eggs, fermentation, *L. plantarum*, proximate analysis

ABSTRAK

Telur yang memiliki kandungan asam amino yang seimbang dapat memenuhi kebutuhan protein pada manusia, namun telur memiliki daya simpan yang rendah sehingga mudah mengalami kerusakan. Teknologi fermentasi pada bahan pangan dengan menggunakan mikroba telah banyak dilakukan antara lain dengan menggunakan bakteri jenis *Lactobacillus*. Bakteri jenis *Lactobacillus* yang umum digunakan pada fermentasi telur adalah *Lactobacillus plantarum*. Penelitian ini dilakukan secara eksperimental dengan menggunakan Rancangan Acak Lengkap (RAL) dengan 3 perlakuan dengan masing – masing 3 kali ulangan. Perlakuan tersebut dilakukan fermentasi dengan suhu inkubasi 37 ^{o}C dengan waktu inkubasi yang berbeda yaitu 0, 48, dan 96 jam dengan parameter penelitian kadar air, lemak kasar, serat kasar, BETN dan kadar abu. Hasil penelitian menunjukkan perlakuan waktu inkubasi yang berbeda pada telur ayam fermentasi memberikan pengaruh yang nyata (P<0,05) terhadap kadar air, lemak kasar, serat kasar, BETN dan kadar abu. Komposisi Nutrisi Telur Fermentasi menggunakan *L. plantarum* dapat diketahui dari penurunan kadar air, serat kasar dan BETN dan terjadi peningkatan lemak kasar dan kadar abu seiring meningkatnya waktu inkubasi. Nilai kadar air, lemak kasar, serat kasar, BETN dan kadar abu optimum pada suhu inkubasi 37 ^{o}C selama 96 jam waktu inkubasi.

Kata kunci: analisis proksimat, fermentasi, *L. plantarum,* telur

INTRODUCTION

Eggs provide the largest contribution of animal protein to the community because apart from relatively cheap prices and easy availability of eggs. Eggs that have a balanced amino acid content can fullfil protein that needs in humans, but the presence of abundant eggs causes side effects for the eggs themselves. Eggs will be damaged if the shelf life is too long. There are various ways that can be done so that the shelf life of eggs can be extended, one of them is fermentation.

Fermentation technology is carried out to obtain benefits as functional food that is good for health, facilitates digestive absorption and extends product shelf life. Fermentation technology in foodstuffs by using microbes has been widely carried out, among others, using *Lactobacillu*s bacteria. *Lactobacillus* type bacteria commonly used in egg fermentation is *Lactobacillus plantarum*. These bacteria can grow by requiring the nutritional value contained in the growth medium.

L. plantarum bacteria are proteolytic bacteria that can convert protein compounds into simpler compounds (Nahariah *et al.* 2015). *L. plantarum* bacteria grows in one medium if the ability to metabolize nutrients is well developed and during growth the ability to break down proteins into amino acids is maintained for cell proliferation (Nisa *et al.* 2008). During the fermentation process, *L. plantarum* will produce metabolites such as lactic acid, hydrogen peroxide, and bacteriocins that function as antibacterial compounds (Mahon *et al.* 2015). Bacteriocins are peptides or protein compounds that are released into the extracellular by lactic acid bacteria and have a bactericidal effect towards harmful bacteria that are closely related phylogenetically (Urnemi *et al.* 2011). The presence of this antibacterial activity can be used to prevent the development of pathogenic bacteria.

Bacteriocins have long been identified by researchers and are considered natural products in the form of proteins or peptides from bacteria in fermentation products. *Lactobacillus plantarum* is one of the lactic acid bacteria that has ability to produce lactic acid and degrade the nutritional content of eggs thus, it has the ability to produce bacteriocins which also have antibacterial properties. The purpose of this study was to identify the proximate contents of fermented chicken eggs by using *L. plantarum* bacteria.

MATERIALS AND METHODS

Material

The equipment used 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 (Thermo Genesys 20). The 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 conducted experimentally by using a completely randomized design (CRD) with 3 treatments and 3 replications each. The treatment was carried out by fermentation with an incubation temperature of 37 °C with different incubation times of 0, 48, and 96 hours.

Methods

Culture Propagation

Lactobacillus plantarum was stored on *De Man Ragosa Sharpe (MRS)* agar. Propagation of culture by making sub-cultures. Sub-culture was made by transferring the culture stock into liquid medium of *MRS broth* (OXOID CM0359) to which 20% tomato juice was added and incubated for 24 hours (Pramono *et al.* 2003). Cultures that had been stored in *MRS broth* media were inoculated as much as 10% into egg whites containing 20% tomato juice to produce working cultures (Nahariah *et al*. 2013).

Sample Preparation

Egg samples were cleaned using clean water. The eggs were then fumigated by using Calcium Permanganate (CP) powder and formalin in a closed room for 5 minutes 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 minutes (Froning *et al*. 2002) then separated from the shell and then put into a sample bottle. The sample bottles were first cleaned by using warm water and sterilized. 100 ml sample was homogenized and then sterilized by using ultraviolet by placing it in a *PCR Hood* for 15 minutes. The sterile sample was added with 10 ml of working culture (10^6 CFU/ml) and then homogenized with a *tube shaker*, the sample was then fermented according to the research treatment (Nahariah *et al.* 2015).

Tested Parameters

Water Content

Prepare a porcelain dish that had been cleaned in an oven at a temperature of 105 °C for 2 hours. Then it was cooled in a desiccator for hour and then weighed (a gram). Put the sample into a porcelain cup and weigh ± 1 gram of the sample (b gram). Next, to bake at 105 °C for 8 hours or leave overnight. Remove from oven and cool in desiccator for ½ hour then weigh (c gram) with the following calculation:

$$\% \text{ Dry Ingredients} = \frac{C - A}{B} \times 100\%$$

$$\%\text{Water} = 100\% - \% \text{ Dry Ingredients}$$

Crude Fat

Weigh ± 1 gram of the sample then put it into a 15 ml test tube and added chloroform to a 10 ml scale and after that shaked it and let it cool overnight. Squeeze up to 10 ml with chloroform and shake again. Filtered with filter paper into a test tube. Pinch 5 ml into a cup of known weight (a gram) and oven at 100 °C for 4 hours. After that, took it out from the oven and cold it in a desiccator for ½ hour then weigh it (b gram). The calculation is as follows:

$$\% \text{ Crude Fat} = \frac{P \times (b - a)}{\text{Sample Weight}} \times 100\%$$

P = Dilution (10/5)

Crude Fiber

Weigh ± 0.3 sample into a 50 ml test tube and added 30 ml of 0.3 N H_2SO_4 and heat for 30 minutes. After that added 15 NaOH 1.5 N and heat for 30 minutes. Strain into sintered glass No. 1 while sucked by using a vacuum pump. Washed successively with 40 ml of hot water, 40 ml of 0.3 N H_2SO_4, 40 ml of hot water and 10 ml of acetone. Then dried in the oven for 8 hours or leave overnight. After that, cold in desiccator for hour then weigh (a gram). After that, the sample was ashed into an electric furnace for 3 hours at a temperature of 500 °C. Let the sample cool a bit then put it in the desiccator for ½ hour then weigh it (b gram). The calculation is as follows:

$$\% \text{ Coarse Fiber} = \frac{A - B}{\text{Sample Weight}} \times 100\%$$

BETN

The way to get the value of the Nitrogen-Free Extract (BETN) is with the formula:

BETN= [100-(Ash Content + Crude Fiber Content + Crude Fat Content + Crude Protein Content) 100%]

Ash Content

Porcelain dish and the sample in determining the water content, it was inserted into the electric furnace. The temperature of the kiln was set to 600 °C, then left for 3 hours to turn into ashes (to speed up the ashing process, once the kiln was opened). Cold the porcelain dish into the desiccator for ½ hour then weigh (d gram). Calculation as follows:

$$\% \text{ Ash} = \frac{D - A}{B - A} \times 100\%$$

RESULTS AND DISCUSSION

The proximate composition of fermented chicken eggs by using different incubation times can be seen in Table 1.

Water Content

Table 1 showed that the proximate composition of fermented chicken eggs with different incubation times had a significant (P<0.05) effect on water content. Moisture content decreased with increasing incubation time. This was presumably because the metabolic products of *L. plantarum* including water decreased with incubation time. Different incubation time treatments for fermented chicken eggs resulted in the lowest water content value at 96 hours treatment, which was 80.32%, while the highest at 0 hours treatment was 81.61%. This showed that the longer the incubation time, the lower the amount of water content produced. Moisture content was water content which was the result of metabolism resulting from the fermentation process, the more *L. plantarum* grows, the higher the metabolic results (Purnomo *et al.* 2003). Several studies on fermentation with *L. plantarum* were carried out by (Syahrir 2002) on cow's milk curd which produced a water content of 73.02% during 48 hours of fermentation. The use of *L. plantarum* bacteria in the fermentation of milkfish paste produced a water content of 73.01% after 5 days of fermentation (Zummah *et al*. 2013).

The high water content at incubation time of 0 hours was thought to be due to the metabolic activity of *L. plantarum*. Incubation time would produce amylolytic activity of lactic acid bacteria due to an increase in the number of *L. plantarum* bacteria. Amylolytic activity would be able to hydrolyze simple proteins, with protein hydrolysis, more glucose and other sugars would be produced, then the glucose and sugar would be converted into pyruvate by liberating water molecules, so that the water content was also higher (Zummah *et al.* 2013).

Crude Fat

The results of the proximate analysis (Table 1) showed that the incubation time treatment had a significant effect (P<0.05) on crude fat. Further test results showed that the higher the incubation time the amount of crude fat also increased. This was presumably due to a decrease in water content at the time of increasing incubation time indicating that the bacteria were able to utilize the protein present in the egg so that it did not remodel the crude fat reserves in the egg. The results showed that the longer the incubation time, the higher the crude fat value. This was in line with the opinion of Sitio (2019), that crude fat content would increase along with the increase in fermentation time.

Coarse Fiber

The results showed a significant effect (P<0.05) on the crude fiber of fermented chicken eggs. The amount of crude fiber at 0 hours of incubation showed the highest value of 0.5% and decreased with increasing incubation time of 96 hours giving a value of 0.2%. The decrease in the number along with the increase in incubation time was thought to be due to the fermentation process that occured in chicken eggs causing a decrease in the amount of crude fiber. This was in

Table 1. The proximate composition of fermented chicken eggs with different incubation times

Incubation Time (hours)	Proximate Composition (%)				
	Water content	Crude Fat	Crude Fiber	BETN	Ash Content
0	81.61 ± 0.31c	4.33 ± 0.05a	0.50 ± 0.00c	4.17 ± 0.12b	0.72 ± 0.01a
48	80.94 ± 0.03b	5.51 ± 0.12b	0.33 ± 0.06b	4.03 ± 0.23b	0.73 ± 0.00b
96	80.32 ± 0.09a	5.93 ± 0.01c	0.20 ± 0.00a	2.37 ± 0.23a	0.75 ± 0.00c

Description : Different superscripts in the same column showed significant differences (P<0.05).

line with the opinion of Sitio (2019), that the fermentation process caused the microorganisms contained in probiotic supplements to be able to break down long-chain carbohydrates, proteins, and fats. The breakdown of long chains of carbohydrates, proteins and fats made complex molecule meals simple.

BETN

The results of the proximate analysis showed that the different incubation time of the Nitrogen-Free Extract (BETN) had a significant effect (P<0.05). The highest number of BETNs was found at 0 hours of incubation with a value of 4.17%, while the lowest number of BETN was found in 96 hours of treatment, which was 2.37%. The decrease at different incubation times was due to the longer incubation time, the lower the BETN value.

The decrease in BETN levels at 96% incubation time occurred because the crude fiber of fermented eggs also decreased. This was in accordance with the opinion of Tillman *et al.* (1991) that a decrease in the crude fiber content of a material would reduce the BETN content. Inversely proportional to the incubation time of 0 hours giving a high amount of BETN in line with the high amount of crude fiber in the treatment of 0 hours so it would give a high BETN value as well.

Ash Content

Table 1 showed the results of the proximate analysis of fermented chicken eggs with different incubation times that significantly affected (P<0.05) on the amount of ash content. Further test results showed that the higher the incubation time, the higher the amount of ash content. This was presumably because the metabolic activity of *L. plantarum* bacteria in eggs had not been maximized, resulting in an increase in ash content. This was in line with the decrease in water content in this study so that it would have a side effect of increasing the amount of ash content in fermented eggs. The work of *L. plantarum* bacteria decreased with the length of incubation time because the longer the incubation time, *L. plantarum* bacteria were not able to metabolize optimally. This was in accordance with the opinion of Sitio (2019), that the change in the amount of ash content was caused by bacterial activity during the fermentation process.

CONCLUSION

The eggs fermentation cause some changes in nutritional composition such as decreasing water content, crude fiber and BETN, and increasing crude fat and ash content during the increasing incubation time. The value of water content, crude fat, crude fiber, BETN and optimum ash content at an incubation temperature of 37 °C for 96 hours of incubation time.

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