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Microbiological activities in fermented egg whites with different level of milk and fermentation times

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Abstract. Microbiological processing of eggs is a process involving microbes in producing egg products. The research objective was to study the microbiological activity of fermented egg whites using different levels of milk and fermentation time. The studies used a completely randomized factorial pattern design, 4 x 5 treatments with 4 replications. The research materials were eggs, full cream milk powder and mixed LAB (*L. bulgaricus*, *L. acidophilus*, and *Streptococcus thermophilus*). The parameters measured were microbial activity, among others, total microbes, total acid and the pH value of fermented egg whites. The treatment of the study was the addition of milk level (%) including, 0, 2, 4 and 6. Fermentation times were 0, 12, 24, 36, and 48 h. The results showed that milk level and fermentation times had a significant effect ($P < 0.01$) and there was an interaction between the two to total microbes and pH values. But both did not show a significant effect on the total acid in the fermentation egg whites. Adding 4% of milk with 12 h of fermentation can increase the number of microbes and reduces the pH value of fermented egg whites. The total acid in the egg white does not change during the fermentation process and the addition of milk levels.

1. Introduction

Processing of eggs includes physical, chemical, biochemical and microbiological engineering. Microbiological processing of eggs is a process involving microbes in producing egg products [1]. The general use of microbes in the egg process is still limited. Fermentation of egg whites using *L. plantarum* on egg whites was carried out and showed changes in microbial activity but not optimal [2]. The glucose content in eggs is only 0.4-0.9% [3] so that the availability of energy for the growth of microbial lactic acid bacteria is limited.

Energy addition needs to be done to optimize microbial work. Milk is one alternative as a source of energy for the growth of LAB [4]. In addition to energy requirements, microbial growth is also largely determined by the length of fermentation[5,6]. The research objective was to study the microbiological activity of fermented egg whites using different levels of milk and fermentation time. The addition of milk powder and the appropriate length of fermentation are expected to increase microbial activity.



2. Materials and methods

This study used main ingredients in the form of egg whites and full cream milk powder. LAB, bacteria mixed consisted of *L. bulgaricus*, *L. achidopillus*, and *Streptococcus thermophilus*. This study used a completely randomized factorial pattern (factorial CRD) 4 x 5 with 4 replications. Each treatment unit used 100 ml of egg whites. The research treatments included the addition of different level milk powder (%) 0; 2; 4; 6 and fermentation times 0, 12, 24, 36, and 48 h, respectively. Parameter measurements include total microbes [1], pH values [7], and total acids [6].

3. Result and discussion

3.1. Total count bacteria

The results of the variance analysis showed that the fermentation time and milk level used had a significant effect ($p < 0.01$) on total bacteria that grew on egg whites. There is an interaction between the two against the total bacteria produced.

Table 1. Total microbes in Fermented Egg Whites with Different Levels of Milk and Fermentation Times

Milk Levels (%)	Fermentation Time (Hour)					Mean
	0	12	24	36	48	
0	4.03±0.52	5.75±0.64	4.40±0.57	5.96±0.58	3.98±0.50	4.83±1.01 ^{ab}
2	3.47±0.61	5.98±0.52	4.32±0.51	4.97±0.49	3.58±0.61	4.47±1.08 ^a
4	4.9±0.51	6.75±0.52	4.45±1.21	5.05±0.44	3.44±0.50	4.93±1.26 ^b
6	5.17±0.09	7.81±0.63	5.14±0.79	5.44±0.44	3.64±0.61	5.44±1.46 ^c
Mean	4.40±0.82 ^a	6.57±0.98 ^b	4.58±0.81 ^a	5.36±0.59 ^c	3.66±0.54 ^d	4.91±1.24

Description: Different superscripts in the same row and column show significant differences ($p < 0.05$)

The addition of fermentation time resulted in an increase in the number of microbes that grew on the egg white and optimal at 12 hours of fermentation. Addition of fermentation time of more than 12 hours resulted in a decrease in the number of microbes produced. This is probably caused by energy sources still available in egg whites until 12 hours of fermentation. Adequacy of energy is used for the development of microbial cells. Addition of fermentation time can reduce the amount of energy available. So that it can inhibit developing microbial cells. This is in accordance with [8], the addition of fermentation time will reduce the number of microbial cells formed despite sufficient nutritional conditions. LAB microbes will remain normal activities but no cell division or addition occurs [8,1].

The results showed that adding milk powder can also increase the number of microbes. This is probably due to the availability of nutrients that can be used by bacteria for the growth and development of cells. LAB bacteria can break down milk sugar into preparations of C, H, and O to fulfill their daily needs [8]. The sugar content in milk is lactose. Lactose can be decomposed by LAB activity into a simpler structure, galactose, and glucose. Galactose and glucose will break down into smaller compounds such as C, H and O. This component is needed for the development and growth of bacteria. Bacteria, especially LAB *L. bulgaricus*, *L. achidopillus*, and *Streptococcus thermophilus* is a type of lactic acid bacteria that use a lot of sugar for the development and growth of its cells.

3.2. The pH value

The results of variance analysis showed that the addition of different milk levels and fermentation times had a significant effect ($p < 0.01$) on the pH value of fermented egg white and there were interactions between the two. The addition of fermentation time results in a decrease in the pH value of fermented egg whites. The pH value decreases in egg whites fermented for 24 hours. However, the value of OH is not significantly different from the fermentation of 36 and 48 hours. This shows that

the availability of energy, especially glucose in egg whites, is still quite available for use by all three LAB, *L. bulgaricus*, *L. achidopillus* and *Streptococcus thermophilus*. Glucose will be broken down into lactic acid compounds so that the pH value decreases [2,9,10].

Table 2. The pH value in fermented egg whites with different levels of milk and fermentation times

Milk levels (%)	Fermentation time (Hour)					Mean
	0	12	24	36	48	
0	6.18±1.86	6.87±0.55	6.57±0.75	6.00±0.87	5.17±0.29	6.16±1.08 ^a
2	7.17±0.45	6.00±0.24	5.28±0.17	4.66±0.47	4.27±0.04	5.47±1.09 ^b
4	6.15±0.95	6.64±0.62	5.23±0.35	4.97±0.55	4.47±0.36	5.49±0.98 ^b
6	7.73±1.26	5.75±0.09	4.90±0.29	4.56±0.36	4.34±0.25	5.46±1.38 ^b
Mean	6.80±1.31 ^a	6.31±0.61 ^b	5.49±0.77 ^c	5.05±0.79 ^{cd}	4.57±0.44 ^d	5.65±1.16

Description: Different superscripts in the same line and column show real differences ($p < 0.01$)

Addition of milk level to fermented egg whites using three LAB bacteria can reduce the pH value of the product. This is because the sugar content in milk is a type of sugar in the form of galactose and glucose. Both types of sugar if broken down can produce lactic acid. Decomposition of sugar can be through the catabolic process of 2 hexose monomers [11]. Lactic acid production can result in a decreased pH value [1,12]. There is a decrease in pH value in line with increasing fermentation time and the level of milk used.

3.3. The total acid

Variance analysis showed that the fermentation time and the addition of milk levels did not show a significant effect on the total acidity of fermented egg white. This shows that the fermentation time and the level of milk added to egg white do not contribute to the formation of total acidic fermented egg whites. This is probably due to the low ability of LAB bacteria that are used to break down the components in egg whites.

Table 3. Total acid in fermented egg whites (%) with different levels of milk and fermentation times

Milk Levels (%)	Fermentation Time (Hour)					Mean
	0	12	24	36	48	
0	0.17±0.05	0.06±0.04	0.11±0.02	0.07±0.03	0.08±0.02	0.09±0.05
2	0.06±0.03	0.09±0.01	0.08±0.02	0.07±0.02	0.10±0.05	0.08±0.03
4	0.09±0.05	0.07±0.03	0.08±0.03	0.07±0.03	0.09±0.01	0.08±0.03
6	0.07±0.02	0.07±0.02	0.06±0.04	0.08±0.06	0.08±0.02	0.07±0.03
Mean	0.10±0.05	0.07±0.02	0.08±0.03	0.07±0.03	0.09±0.03	0.08±0.03

The lactic acid bacteria used in this study were *L. bulgaricus*, *L. achidopillus*, and *Streptococcus thermophilus*. The three types of bacteria use energy derived from sugar derivatives. The LAB will break down sugar into lactic acid [11,13]. Egg whites were food that has a fairly low carbohydrate content of only about 0.3% [3]. The addition of milk levels is expected to increase the availability of sugar in egg whites. But the results of this study indicate that the amount of available energy is only sufficient for the growth and development of microbial cells. So, even though the amount of microbes increases but has not been able to break down the material into lactic acid.

Fermentation in egg whites using *L. plantarum* significant increased total acidity in fermented egg whites [1,13]. Likewise in the study of Mangalisu et al. [6], using *L. Plantarum* in the egg fermentation process, the remainder of the hatchery industry results in an increase in the percentage of total acid

produced. This is probably due to the ability of *L. Plantarum* to break down carbohydrates and proteins. *L. Plantarum* is proteolytic which can break down protein into an energy source [14,15]. If bacteria have sufficient energy, the growth and development of microbial cells can be stimulated. In addition, bacteria that have sufficient energy can degrade materials well and optimize their metabolic results, including lactic acid.

4. Conclusion

Adding 4% of milk with 12 h of fermentation can increase the number of microbes and reduces the pH value of fermented egg whites. The total acid in the egg white does not change during the fermentation process and the addition of milk levels.

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