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Antioxidant activity and chemical characteristics in egg albumen fermented by lactic acid bacteria

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ABSTRAK

Kajian waktu fermentasi menggunakan mikrobia dengan penambahan susu bubuk pada albumen telur masih terbatas. Tujuan penelitian ini untuk mengevaluasi aktivitas antioksidan dan karakteristik albumen telur fermentasi yang menggunakan level susu bubuk full cream dan waktu fermentasi mikrobia yang berbeda. Penelitian ini menggunakan rancangan acak lengkap pola faktorial 4 x 5 perlakuan dengan 4 ulangan. Bahan penelitian antara lain telur, susu bubuk full cream dan tiga jenis Bakteri Asam Laktat (*L. bulgaricus*, *L. acidophilus*, dan *Streptococcus thermophilus*). Perlakuan penelitian yaitu penambahan level susu bubuk (%) meliputi, 0, 2, 4 dan 6. Waktu fermentasi adalah 0, 12, 24, 36, dan 48 jam. Hasil penelitian menunjukkan bahwa penambahan level susu bubuk dan waktu fermentasi pada albumen telur tidak berpengaruh nyata terhadap aktivitas antioksidan. Penambahan level susu bubuk tidak berpengaruh nyata terhadap kandungan glukosa, dan total protein, namun berpengaruh sangat nyata ($P < 0,01$) terhadap kadar air. Waktu fermentasi berpengaruh sangat nyata ($P < 0,01$) terhadap glukosa, total protein namun tidak berpengaruh nyata terhadap kadar air. Aktivitas antioksidan tidak mengalami perubahan selama penambahan level susu bubuk dan fermentasi berlangsung. Waktu fermentasi 24 jam dapat mengurangi total protein dan kadar glukosa. Penambahan 2% susu bubuk dapat mengurangi kadar air pada albumen telur fermentasi.

Kata kunci : Fermentasi, bakteri asam laktat, aktivitas antioksidan, albumen telur

ABSTRACT

Research on fermentation time and the addition of milk powder to egg albumen is still limited. The purpose of this study was to determine the antioxidant activity and chemical characteristics of fermented egg albumen using different levels of full cream milk powder and different microbial fermentation times. This study used a completely randomized factorial pattern design, 4 x 5 treatments with 4 replicates. Research materials include egg albumen, full cream milk powder and mixed Lactic Acid Bacteria (*L. bulgaricus*, *L. acidophilus*, and *Streptococcus thermophilus*). The research treatments were the addition of powdered milk in different level (%) including, 0, 2, 4 and 6. Fermentation times were 0, 12, 24, 36, and 48 h, respectively. The results showed that the addition of powdered milk in different level and fermentation time had no significant effect on the antioxidant activity. The addition of different-level powdered milk was not significant on the glucose content and total protein, but it was very significant ($P < 0.01$) on water content. The fermentation time had a very significant effect ($P < 0.01$) on glucose levels and total protein, but it had no significant effect on the water content of albumen

fermentation. Antioxidant activity did not change during the fermentation time and the addition of different-level milk powder. The 24 h fermentation time could reduce the total protein and glucose levels of egg albumen. Adding 2% milk powder could reduce the water content of egg albumen fermentation.

Keywords: fermentation, lactic acid bacteria, antioxidant activity, egg albumen

INTRODUCTION

Eggs are foods rich in antioxidants, vitamins, calcium, unsaturated fatty acids, and polyunsaturated fatty acids (Kuang *et al.*, 2018; Miranda *et al.*, 2015). Eggs are also food which contains high protein, especially in the albumen part. However, the utilization of egg albumen is still limited so that some efforts need to be made to diversify the food. One of the efforts to increase the utilization of albumen egg is processing the albumen egg into drinks. Egg albumen processed as a beverage product are constrained by the thick characteristics of the albumen proteins (Kuang *et al.*, 2018; Miranda *et al.*, 2015). This is because egg albumen has 2 types of proteins including simple proteins and conjugate proteins. Simple protein consists of ovalbumin by 75%, ovoconalbumin by 3%, and ovoglobulin by 2% of the total egg albumen (Kuang *et al.*, 2018; Abeyrathne *et al.*, 2014). Another protein, which is conjugate protein, is glycoprotein consisting of ovomucoid by 13% and ovomucin by 7%, (Kuang *et al.*, 2018; Miranda *et al.*, 2015). Complex proteins or conjugations are generally bound to carbohydrates and have a thicker characteristic.

In general, the protein content of egg albumen is 12.58% (Kuang *et al.*, 2018). Several studies have been conducted to break down egg albumen proteins. One of which is the method of fermentation using microbes (Adebowale and Maliki, 2011). Fermentation is carried out with the aim of breaking down complex proteins into simpler proteins. A research of Nahariah *et al.* (2018) shows that fermentation using mixed LAB which includes *L.bulgaricus*, *L.achidopillus*, and *Streptococcus thermophilus* can increase the dissolved protein of fermented egg albumen by 5.34 mg/mL.

Egg albumen fermentation is a fermented egg product. Egg albumen fermentation is expected to be a fermented beverage. Egg albumen fermentation has already been developed, but the results have not been maximized. Lactic acid bacteria (LAB) can be used in egg albumen are very limited, so that can inhibit their activity and growth. Microbes which

growth is inhibited will have a low metabolic outcome. Microbes need energy to carry out activities and growth. Energy can be obtained from the results of its metabolism. Eggs, especially the albumen part, are very suitable to function as the additional nutrients from the external environment. Some sources of glucose include milk. Milk can be in either liquid or powder form. Milk contains various nutrients, including lactose which is quite high. Lactose content is one of the energy sources for microbial growth, especially Lactic Acid Bacteria.

Maula *et al.* (2016) explained that the addition of liquid milk to fermented egg albumen can reduce the pH and moisture content of the fermented egg albumen product. The addition of liquid milk can also give a whiter color with a thinner consistency. Chicken egg albumen are food ingredient that contains antioxidants (Wawrzykow and Kokofer, 2011; Nahariah *et al.*, 2014). However, antioxidant activity in egg albumen is still limited. One of the efforts to increase the antioxidants is using the fermentation method. Egg albumen as fermented beverage products are being developed and show changes in the characteristics and the increased antioxidant activity (Nahariah *et al.*, 2018). The addition of 2% full cream liquid milk on the egg albumen fermentation process can increase dissolved protein and maintain total microbial and antioxidant activity (Nahariah *et al.*, 2018). The addition of full cream milk powder is expected to be a source of energy for microbial growth. Moreover, the fermentation time can also play a role in the final metabolism of the fermentation process. This studies aim to examine the effect of fermentation time and the addition of different-level milk powder on the chemical characteristics and antioxidant activity of fermented egg albumen

MATERIALS AND METHODS

This studies used main ingredients in the form of egg albumen and full cream milk powder. Eggs were obtained form chicken farms in Maros Regency, South Sulawesi. The milk powder was a commercial product obtained from a food store.

Lactobacillus sp (LAB) mixed to consisted

of *L. bulgaricus*, *L. acidophilus*, and *Streptococcus thermophilus*. The types of bacteria was derived from commercial yogurt. Yogurt product has been developed at the Teaching Industry, Hasanuddin University. This studies used a completely randomized factorial pattern (factorial CRD) 4 x 5 with 4 replications (Steel and Torrie, 1991). Each treatment unit used 100 ml of egg albumen. 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. The research procedures were: (1) Culture propagation and (2) Sample preparation. In culture propagation, the parent culture dilution was carried out by mixing as much as 20 g of parent culture into a sample bottle containing 300 mL of milk liquid (Maulana *et al.*, 2016). The material mixture was then homogenized and fermented at 37°C for 24 h. Making starter culture (further called *biang*) was done by taking 100 ml of starter culture and mixing with 100 mL of egg albumen. It then homogenized then fermented at 37°C for 24 h (Nahariah *et al.*, 2015). Sample preparation, chicken eggs were sterilized by using non-thermal heating. After that, the egg shell were broken, then the egg albumen and yolk were separated using an egg separator. A total of 100 mL of egg albumen were homogenized using a stirrer without forming foam. Furthermore, 10 mL of starter sub culture and full cream milk powder in different level were added based on the research treatment. Egg sample mixture was homogenized and fermented at 37°C with fermentation time based on the treatment. The testing process was in accordance with each test parameter. Measurement of antioxidant activity using the DPPH method (1,1-diphenyl-2-picrylhydrazyl) were slightly modified. A total of 3.9 mL of DPPH solution (0.1 mM DPPH radical in methanol) was mixed with 0.1 mL of sample (the sample had also been extracted with methanol). The DPPH reagen and sample were mixed and shaken. And then the sample mixture was allowed to stand at a temperature in the dark for 3 h. The absorbance of the sample was measured with a UV-VIS spectrophotometer (Shimadzu brand) at 517 nm. DPPH Radical Scavenging Effect (%) = [(A DPPH-A Sample)/A DPPH] x 100, A DPPH is the absorbance of DPPH, A Sample is the absorbance of the sample (Chaves *et al.*, 2020; Gasic *et al.*, 2014). Measurement of total protein value (Horwitz and Latimer, 2010), a sample of 2

g was poured into the Kjeldahl flask along with 20 g of K₂SO₄, 1g HgO and 25 mL of concentrated H₂SO₄. The kjeldahl flask was allowed to cool and slowly add 200 mL of distilled water until the temperature reaches 250°C. The next step was add the NaOH-thiosulfate solution carefully to prevent sudden mixing. The kjeldahl flask was connected to the distillation apparatus and heated until all ammonia was distilled. The remaining standard acid solution titrated with standard NaOH solution: $N(g/kg) = (V \times 1,401) / W$, N = Nitrogen amount, V= volume, W = sampel weight (1 mL acid 0.1 N = 1.401 mg N), glucose contents and water content (Horwitz and Latimer, 2010).

RESULTS

Antioxidant Activity

Antioxidant activity in fermented egg albumen with different levels of milk powder and fermentation time is presented in Table 1. The results of variance analysis showed that there was no significant effect on the addition of powdered milk in different levels with different fermentation times. Antioxidant activity in fermented egg albumen tended to decrease in the addition of different-level milk powder. This showed that there was a potential for adding different milk powder to reduce antioxidant activity. This was different with the previous studies that the tendency of antioxidant activity to increase in line with the addition of additional levels of full cream liquid milk (Nahariah *et al.*, 2018).

Total Protein Value

The total protein value in fermented egg albumen with different levels of milk powder and fermentation time are presented in Table 2. The fermentation time showed a significant effect ($P < 0.05$) on the protein of the fermented egg albumen products. Further test results showed that the total protein was significantly different decreases with increasing fermentation time. The total protein in egg albumen did not changes before and after 12 h fermentation. However, the optimal time to reduce the total protein value of fermented egg albumen was 24 h fermentation. There was no difference on the protein reduction at 24 h, 36 and 48 h fermentation time.

The results of variance analysis showed that the addition of different milk powder has no significant effect on the total protein value of fermented egg albumen. This suggested that the

Table 1. Antioxidant Activity (%) of Fermented Egg Albumen in Addition to Different Levels of Milk Powder and Fermentation Time

Milk Powder Addition (%)	Time of Fermentation (Hour)					Mean
	0	12	24	36	48	
0	5.72±3.51	6.48±4.27	4.11±1.16	2.82±0.62	2.45±1.67	4.31±2.85
2	3.71±2.03	3.73±3.21	3.92±0.56	2.53±1.21	2.11±1.49	3.20±1.86
4	3.55±4.42	3.96±2.96	3.79±0.81	2.45±0.96	2.21±1.29	3.19±2.35
6	2.15±0.73	2.06±0.70	3.05±0.26	2.97±0.27	2.31±0.39	2.51±0.63
Mean	3.78±3.00	4.06±3.19	3.72±0.81	2.69±0.76	2.27±1.18	3.30±2.15

Table 2. The Total Protein Value (g) of Fermented Egg Albumen with the Addition of Different Levels of Milk Powder and Fermentation Time

Milk Powder Addition (%)	Time of Fermentation (Hour)					Mean
	0	12	24	36	48	
0	8.07±1.37	8.18±1.29	6.53±0.29	7.14±0.48	6.98±0.26	7.38±0.94
2	7.95±1.36	7.78±1.59	6.43±0.16	6.48±1.10	6.90±0.82	7.10±1.08
4	7.81±1.23	7.78±1.36	6.23±0.01	7.17±1.55	6.93±1.71	7.18±1.16
6	8.06±1.61	8.07±1.48	6.07±1.17	6.36±1.05	6.36±1.15	6.98±1.35
Mean	7.97±1.07 ^a	7.95±1.10 ^a	6.31±0.49 ^b	6.78±0.93 ^{ab}	6.79±0.89 ^{ab}	7.16±1.11

Different superscripts in the same row indicate highly significant differences (P<0.01)

addition of milk powder did not contribute to the total protein changes, although it showed the tendency of protein values to decrease in line with the level of milk powder added.

Glucose Content

Glucose contents in fermented chicken egg albumen with different levels of milk powder and fermentation time are presented in Table 3. The results of variance analysis showed that the fermentation time has a very significant effect (P<0.01) on the glucose contents of fermented egg albumen. Duncan Test further showed that glucose contents decreased with the increasing fermentation times. Although there was no difference between pre and post 12 h fermentation, the 12 h fermentation showed

significantly different decrease during 24 h, 36 h, and 48 h fermentation time. The fermentation of egg albumen for 24 h did not show significant differences with the increasing fermentation time of 36 h and 48 h. This showed that the optimal fermentation time was 24 h in which it could reduce glucose contents in fermented egg albumen.

The results of variance analysis showed that the addition of different milk powder had no significant effect on glucose contents of fermented egg albumen. This showed that the addition of milk powder did not give a change in the glucose contents of fermented egg albumen. However, it showed the tendency of glucose contents to decrease in line with the level of added milk powder.

Table 3. Glucose Contents of Fermented Egg Albumen (%) in Addition to Different Levels of Milk Powder and Fermentation Time

Milk Powder Addition (%)	Time of Fermentation (Hour)					Mean
	0	12	24	36	48	
0	3.53±2.23	3.31±2.28	1.73±0.16	0.68±0.52	0.94±0.88	2.03±1.67
2	3.26±2.31	3.24±2.26	1.17±0.55	0.65±0.24	0.73±0.49	1.81±1.67
4	3.21±2.21	3.05±2.17	1.27±0.50	0.67±0.23	0.81±0.47	1.80±1.57
6	3.42±2.53	3.46±2.67	1.67±0.17	0.97±0.59	0.87±0.67	2.08±1.75
Mean	3.35±1.76 ^a	3.26±1.78 ^a	1.46±0.39 ^b	0.74±0.35 ^b	0.84±0.50 ^b	1.93±1.61

Different superscripts in the same row indicate highly significant differences (P<0.01)

Water Content

Water content in fermented egg albumen with the addition of different levels of milk powder and fermentation time are presented in Table 4. The results of variance analysis showed that the fermentation time did not significantly affect the water content of the fermented egg albumen. This showed that the fermentation time did not change the water content of the fermented egg albumen. However, there was a tendency in decreasing water content, in line with the addition of fermentation time to the fermented egg albumen.

The results of variance analysis showed that the addition of different-level milk powder had a very significant effect (P <0.01) on the water content of fermented egg albumen produced. Duncan's follow-up test showed that there was a marked difference in the decrease with increasing milk powder although there was no significant difference between with or without addition of 2% and 4%. However, water content to egg albumen showed a significant difference with the addition of 6% milk powder. This was due to the characteristic of powder from milk powder which can bind water to egg albumen.

DISCUSSION

Table 1 shows the tendency of antioxidants to work optimally to inhibit the oxidation process, especially protein oxidation which occurred in fermented egg albumen. This was characterized by the decreasing antioxidant activity during fermentation. The fermentation process in the egg albumen was likely to produce high antioxidants.

Research by Fastawa *et al.* (2016) shows an increase in the antioxidant activity, in line with the increasing fermentation times in infertile eggs which are left over from the hatchery industry. Fermentation can increase antioxidants and reduce rancidity (Ledesma *et al.*, 2005; Torino *et al.*, 2013).

Antioxidants are bioactive compounds which naturally exist in food ingredients, including eggs (Nahariah *et al.*, 2014; Nys *et al.*, 2011). Antioxidants generally exist in food which contains high protein. Antioxidants are the functional compounds composed of peptide sequences which are derived from decomposed proteins (Fastawa *et al.*, 2016; Nahariah *et al.*, 2015; Majumder *et al.*, 2010). Antioxidant compounds are needed in food ingredients to reduce or inhibit oxidative reactions due to the oxidation process (Wawrzykow and Kankofer, 2011; Chaves *et al.*, 2020). The oxidation process in food ingredients can occur due to the fact that food ingredients experience storage, processing, and post-processing handling (Pengseng *et al.*, 2010).

Antioxidants are bioactive compounds which have the ability to inhibit the oxidation process (Nahariah *et al.*, 2018). Oxidation of food ingredients is related to the presence of oxygen in the air which interacts with the food ingredients (Chaves *et al.*, 2020; Domínguez *et al.*, 2020). The oxidation process in the food ingredients will have an impact on the decline of the food quality (Domínguez *et al.*, 2020). The fermentation process is thought to be the result of oxidative stress which is probably caused by the oxidation of proteins that occur. Although protein oxidation

Table 4. Water Content of Fermented Egg Albumen (%) in the Addition of Different Levels of Milk Powder and Fermentation Time

Milk Powder Addition (%)	Time of Fermentation (Hour)					Mean
	0	12	24	36	48	
0	80.86±0.73	79.18±5.31	79.22±1.97	80.11±0.94	80.09±1.84	79.89±2.49 ^a
2	78.73±2.33	80.24±0.54	79.67±2.42	78.76±1.11	76.42±5.45	78.76±2.92 ^{ab}
4	79.09±0.89	77.62±3.09	78.72±0.90	78.72±0.90	78.16±0.38	78.49±1.61 ^{ab}
6	77.48±2.11	78.00±3.74	75.61±3.56	77.04±1.33	77.79±1.66	77.19±2.53 ^b
Mean	79.04±1.95	78.76±3.39	78.34±2.80	78.66±1.49	78.12±3.01	78.58±2.58

Different superscripts in the same row indicate highly significant differences (P<0.01)

only involves singlet oxygen which has no unpaired electron, it is not free radicals (Domínguez *et al.*, 2020). Therefore, the addition of different levels of milk powder and fermentation time did not show a significant difference in the antioxidant activity of the fermented egg albumen (Table 1). Though the antioxidant potential of product was found to be low. However, this study has provided information about the antioxidant activity of egg albumen fermentation using lactic acid bacteria. Future studies are expected to measure the concentration of antioxidants or IC₅₀ in the product. The IC₅₀ value is the concentration of antioxidants needed to provide 50% inhibition of the free radicals tested. A low IC₅₀ value indicates a high antioxidant capacity.

In general, the optimal growth curve of microbial, especially lactic acid bacteria (LAB), has a growth time of 24 h of fermentation. This was likely related to the ability of microbial to grow and the availability of nutrients during the fermentation process. During the fermentation process, microbial are able to break down proteins and utilize nitrogen as their energy source (Xu *et al.*, 2010). This results in decreasing protein content.

Protein values which decrease in fermented egg albumen may be related to the ability of microbial to break down proteins into dissolved proteins (Table 2). Nahariah *et al.* (2018) showed an increase in soluble protein due to fermentation of egg albumen using liquid milk. The fermentation process in egg albumen results in the breakdown of protein to produce dissolved protein and small peptide (Xu *et al.*, 2010; Nahariah *et al.*

2015; Wikandari *et al.*, 2011).

The glucose contents decrease in the fermentation process of egg albumen (Table 3). This is likely due to the activity of microbial which break down the glucose as energy sources (Mishra *et al.*, 2019). The microbial used in this study are a mixture of LAB types consisting of *L. bulgaricus*, *L. acidophilus*, and *Streptococcus thermophilus*. In general, LAB microbial obtain energy from the glucose content in food ingredients (Mishra *et al.*, 2019; Nahariah *et al.*, 2015). Glucose content in fermented egg albumen before added by milk powder is 3.5% or 0.5% higher than the glucose content in albumen by 3% (Uang *et al.*, 2018). The addition of milk powder is expected to increase carbohydrate content as a source of energy for microbial growth.

Powder milk is a product which has low water content of about 5% due to the drying process carried out (Kalyankar *et al.*, 2016). Low water content causes powder product to have hygroscopic properties. Hygroscopic properties result in powder products which are easily absorbing water. The absorbed water will bind strongly to the material, so that the material-free water content becomes lower. This causes the addition of milk powder in fermented egg albumen will have an impact on the increasing amount of water absorbed and bonded to the material.

Milk is a product which is rich in protein, and if it is dried, the protein becomes porous and binds to water (Kalyankar *et al.*, 2016). This condition resulted in water being trapped and strongly bound to the material-protein matrix (Kalyankar *et al.*, 2016; Landfeld *et al.*, 2008) In

addition, one of the uniqueness of proteins is the presence of amino acid which have hydrophilic properties. It is the bonds will bind water to the surface of the material (Legowo and Hayakawa, 2012; Kuang *et al.*, 2018). Carboxyl bonds in proteins are a combination of carbonil groups and hydroxyl groups. This will result in water absorption properties (Kalyankar *et al.*, 2016). Hydroxyl bonds having -OH ions can trigger protein oxidation (Chaves *et al.*, 2020; Dominguez *et al.*, 2020). Singlet oxygen in protein is not free radicals because it does not have free electrons (Domínguez *et al.*, 2020). Any absorbed water can reduces free water, and water is limited in materials, so the total water content of the material decreases. The water content is important in the storage of food product. If the water content is low, the product can be stored longer. Eggs have a water content of 80-88%. This research can provide information that the application of fermentation using lactic acid bacteria in egg albumen could reduces water content by 2%.

CONCLUSION

Antioxidant activity does not change during the fermentation time and the addition of different level milk powder. The 24 h fermentation time can reduce the total protein and glucose content of fermented egg albumen. Adding 2% milk powder can reduce the water content of fermented egg albumen

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