Changes in protein properties of pasteurized liquid whole egg with added sugar

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Changes in Protein Properties of Pasteurized Liquid Whole Egg with Added Sugar

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Abstract. Liquid Whole Egg (LWE) is a product with complete nutrition containing protein, fat, carbohydrates, water, essential amino acids, vitamins and minerals. LWE also has functional properties such as the ability to coagulate and the ability to form foam, therefore LWE is widely used in the food processing industry because it has advantages such as ease of storage, ease of transportation and ease of application. To get LWE that is safe from contamination by pathogenic microbes, it is necessary to do a heating process first, for example pasteurization. To prevent damage to protein and functional properties of LWE due to pasteurization, other additives such as sucrose can be added. This study aims to determine the effect of adding a certain concentration of sucrose to changes in protein in pasteurized whole egg liquid. The materials used were 1 day old eggs with 58-62 grams in weight from ISA Brown strain chickens aged 48 weeks and sucrose. The experimental design used was a completely randomized design (CRD) with 4 treatments and 5 replications. The treatment given was the addition of sucrose with concentrations of 0%, 5%, 10% and 15%. Pasteurization was done at 65 °C for 3.5 minutes. Parameters observed were protein content, level of coagulation, foaming property and protein electrophoresis pattern. Data from protein content, coagulation rate and foaming power were analyzed using Analysis of Variance (ANOVA) at a significance level of 5% while electrophoresis patterns were analyzed descriptively. The results showed that the addition of sucrose had no significant effect ($p>0.05$) on the foaming power and the rate of coagulation, while the addition of sucrose had a significant effect ($p<0.05$) on the protein content of pasteurized liquid whole egg, where the protein content decreased as the concentration of added sucrose increases. Electrophoresis pattern using SDS-PAGE method showed the protein profile of liquid whole egg, which could be identified as ovomucin (223.23 kDa), ovomucin (121.54 kDa), ovotransferrin (82.14 kDa), ovalbumin (42.72 kDa) , ovomucoid (28.85 kDa), lysozyme (13.55 kDa), and apoprotein (10.89 kDa). The addition of sucrose did not change the protein profile of the pasteurized LWE.

INTRODUCTIONS

Liquid whole egg (LWE) is a food product that is a mixture of egg yolk and white which has been widely used by the food industry as the main ingredient and supporting material. The use of eggs in the food industry is not only because eggs have high nutritional value but also because the protein in eggs has functional properties that other types of protein do not have. One egg has a composition consisting of 73.7% water, 12.9% protein, 11.2% fat and 0.9% carbohydrates [1]. The use of LWE in the food industry has several advantages including making it easier to transport and store and easier to apply [2]. LWE has a high level of food safety, this is because LWE products have gone through a pasteurization process that can prevent the presence of pathogenic bacteria in the product. However, the pasteurization process can cause damage to several types of protein that are sensitive to heat and can reduce the

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functional properties of the egg protein. Therefore, it is necessary to add other additives that can reduce protein damage due to the pasteurization process.

Sugar (sucrose) is a commonly added additive in food products. The addition of sucrose to pasteurized LWE can prolong the coagulation time so that the protein is not easily agglomerated or damaged [3], the addition of sucrose can also minimize the damage from its functional properties and improve the taste of pasteurized LWE products into products with high profit value in their livestock industry [4]. The use of LWE as a beverage product has been widely carried out in various countries, for example the addition of egg whites in Peruvian pisco sour drink products, where the addition of egg whites is intended to get a creamy texture from the foam formed after shaking [5]. In addition, in countries such as America, Germany, Japan, England and France there is a drink called egg nog which is made from a mixture of whole eggs with the addition of milk, sugar and spices [6]. This can be an idea for LWE product innovation as a ready-to-drink egg product in Indonesia. This study aims to determine the effect of adding sugar on protein and its properties of pasteurized LWE.

MATERIALS AND METHODS

The research was carried out in April 2021 at the Laboratory of Food Chemistry and Nutrition, the Laboratory of Food and Agricultural Products Engineering, Faculty of Animal and Agricultural Sciences, and the Integrated Laboratory of Diponegoro University, Semarang

Materials

The materials used were one day old fresh eggs with 58-62 grams in weight from ISA Brown strain laying hens aged 48 weeks, and sucrose. The materials used in analysis consist of distilled water, selenium reagent mixture (Merck) catalyst, 45% NaOH 4% boric acid, BCG-MR, 0.1 N sulfuric acid (H_2SO_4), SDS sample buffer, mercaptetanol, Coo dye. -Masie Briliant Blue (CBB), CBB staining solution, acetic acid and alcohol. The tools used include a homogenizer (IKA T25 digital ultra turrax), analytical balance (Sigma), filter, water bath (Memmert), thermometer, kjeldhal flask, measuring cup, erlenmeyer, destruction tool, distillation tool, burette, stative, mixer, polyacrilamide gel and electrophoresis device (BIO-RAD).

Method

Preparations of Liquid Whole Egg

Preparation of liquid whole egg was referring to [7] with some modifications. Eggs were cleaned with water then broken and the contents were separated. The contents of the eggs (yolk and white) were then homogenized at a speed of 10,000-12,000 rpm then filtered and divided into 4 different containers for further addition of sucrose with a concentration (w/w) of T_0= 0%, T_1= 5%, T_2= 10%, and T_3= 15% . LWE pasteurization refers to [8], dipping the LWE into the water bath until the center temperature reaches 63ºC along 3 minutes, after completion the LWE was cooled at room temperature until the temperature reaches 35-40ºC.

Protein content analysis

Analysis of protein content was carried out by the Kjeldhal method [9]. As amount of 0.5 g of LWE sample was put into a Kjeldahl flask and added with 10 ml of H_2SO_4 and 0.5 g of selenium reagent mixture (Merck) as catalyst, then destructed for 1-1.5 hours until the solution becomes clear and allowed to cool. The solution was diluted with 50 ml of distilled water, then 10 ml was taken for the distillation process and 10 ml of 45% NaOH was added. The resulting distillate was accommodated with Erlenmeyer which already contained 5 ml of 4% boric acid + BCG-MR (a mixture of bromocresol green and methyl red). The distillate was titrated with 0.1 N sulfuric acid until it changed color and the volume of sulfuric acid used was recorded. Protein content is calculated by the formula:

$$PC = \frac{0{,}01\ H_2SO_4\ x\ titration\ number\ x\ 0{,}014\ x\ 6{,}25\ x\ 5}{grams\ samples}\ x\ 100\%$$

Coagulation rate analysis

Testing the coagulation level of pasteurized LWE was carried out with the principle of separating the coagulant from the LWE which was still in the form of a liquid. The method used is a modification of [10]. LWE was filtered using a sieve with a size of 100 mesh. The clumps left on the sieve were LWE coagulation. The LWE coagulation rate is determined using the formula:

$$Coagulation\ Level = \frac{coagulated\ LWE\ weight\ (g)}{initial\ LWE\ weight\ (g)}$$

Foaming Power Analysis

The foaming power test was carried out using the method referred to with modifications. LWE as much as 100 ml (V1) was beaten using a mixer for 90 seconds at low speed in a plastic container with a scale. Then the mixer is lifted and the volume increase (V2) is observed. The foaming power can be determined using the following equation:

$$Foaming\ Power = \frac{V2-V1}{V1}\ x\ 100\%$$

Electrophoretic pattern Analysis

Electrophoretic pattern analysis was carried out using the method referred to [, using sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE). A sample of 15 µL was mixed with 15 µL of SDS sample buffer, and 3 µL of mercaptoethanol, then the sample was put into boiling water for 2 minutes. Samples were put into gel wells as much as 15 µL for each well and the electrophoresis device was connected to an electric current of 60V 12 mA for 6 hours. The gel was removed and stained with Coomassie Briliant Blue (CBB) for 60 minutes and then washed with *CBB* staining solution consisting of acetic acid and alcohol to obtain bands on the gel.

Data Analysis

Data of protein content, level of coagulation and foaming power were analyzed using the Analysis of Variance (ANOVA). If ANOVA showed a significant effect ($p < 0.05$) of treatment, then continued with Duncan's Multiple Range Test to determine the differences among treatments, while the result of electrophoretic patterns were analyzed descriptively.

RESULTS AND DISCUSSION

The results of the protein content, coagulation rate and foaming power of pasteurized LWE with the addition of sucrose can be seen in Table 1.

TABLE 1. Pasteurized LWE Protein Content, Coagulation Level and Foaming Power with Added Sucrose

Adding sucrose	Protein Content (%)	Coagulation Level (%)^{ns}	Foaming Power (%)^{ns}
0%	12.051 ± 0.281^{a}	5.84±1.05	110±20.74
5%	11.376 ± 0.389^{b}	4.87±0.92	100±31.02
10%	11.302 ± 0.291^{bc}	3.75±0.35	99±43.07
15%	10.770 ± 0.574^{c}	4.75±1.81	98.4±27,80

$^{a-c}$ values with different superscript in the same column showed significant differences ($p<0.05$)
^{ns} nonsignificant difference ($p> 0.05$)

Based on Table 1, it is known that the protein content of pasteurized LWE with the addition of sucrose ranged from 10.770% to 12.051%. The highest protein content of 12.051% was LWE pasteurized without added sucrose (0%) and the lowest protein content was 10.770% owned by LWE with 15% added sucrose. Pasteurized LWE

protein content showed a decreasing trend, this could be due to a change in the proportion of the sucrose fraction and protein fraction [13] as a result of the increasing concentration of added sucrose. The decrease in protein content can also be caused by the formation of complex compounds between amino acids in eggs and sucrose. It is known that sucrose is a type of non-reducing sugar, but in the presence of heat it can cause sucrose to undergo an inversion so that it breaks the glycoside bonds to form the monosaccharides glucose and fructose which are types of reducing sugars [14]. The presence of reducing sugars in LWE can cause the Maillard reaction and form more complex compounds.

The Maillard reaction can cause a decrease in nutrients and eliminate amino acids, especially essential amino acids [15] so that protein content in LWE decrease. The Maillard reaction that occurs in addition to involving the monosaccharide fraction resulting from the inversion of sucrose can also be caused by the carbohydrate content in the egg itself. It is known that eggs have a carbohydrate percentage of 0.9% consisting of glucose and some others are galactose, mannose, glucosamine and sialic acid [16]. This also causes a decrease in protein levels in LWE without the addition of sucrose when compared to protein levels in fresh eggs, which is 12.4% [17].

The coagulation level of pasteurized LWE with the addition of sucrose ranged from 3.75% to 5.84%. The ANOVA results showed that the addition of sucrose had no significant effect ($p>0.05$) on the level of coagulation of pasteurized LWE. The degree of coagulation of eggs depends on the sensitivity of the specific protein to heat and its concentration. Egg white begins to coagulate at 62°C, while egg yolk begins to coagulate at 65°C [18]. Several types of protein began to coagulate the lowest at 57°C and maximum coagulation at 73°C [19]. Recent research by [20] showed that sucrose inversion has occurred at a heating temperature of 65°C, this also does not rule out the possibility that there has been an inversion of sucrose in pasteurized LWE with a pasteurization temperature of 63°C. The inversion reaction will produce the monosaccharides glucose and fructose which are types of reducing sugars. The presence of reducing sugars in LWE can cause the Maillard reaction and produce more complex compounds. This resulted in the value of the level of coagulation LWE was not different because the sugar had been bound to the protein molecule first.

The foaming power of pasteurized LWE with the addition of different sucrose concentrations has a foaming power ranging from 98.4% to 110%. The ANOVA results showed that the addition of sucrose had no significant effect ($p>0.05$) on the foaming power of pasteurized LWE. The foaming power of pasteurized LWE can be said to be low because it only has a maximum volume increase of 110%, while the foaming power of fresh eggs can increase up to 600% [21]. The low foaming power of pasteurized LWE is due to the addition of sucrose which can increase the viscosity of LWE so that less air can be trapped and form foam [22]. Sucrose is also thought to be able to form hydrogen bonds with ovalbumin protein thereby increasing its hydrophilic properties and reducing surface activity.

The foam in the egg beating process is caused by the presence of ovalbumin, ovomucin, globulin, ovotransferrin, lysozyme and ovomucoid where these types of proteins are the dominant proteins in eggs [23]. The inversion reaction that occurs in sucrose due to the pasteurization process produces reducing sugars in the form of glucose and fructose [20] causing a Maillard reaction with amino acids in LWE. This reaction can form more complex compounds involving the type of protein that can affect the formation of foam, so that there is no difference in the froth content of pasteurized LWE with the addition of sucrose. The formation of froth on beating eggs is influenced by various factors, including the length of shaking, pH, temperature, and the addition of chemicals or other additives [24]. Too much foam in pasteurized LWE can make it difficult for liquid products to be consumed, therefore the presence of foam in pasteurized LWE must be minimized.

Figure 1 shows various types of proteins contained in pasteurized LWE with added sucrose in the molecular weight (MW) range of 10-250 kDa. Lanes T0, T1, T2, and T3 are the results of electrophoretic patterns for pasteurized LWE without added sucrose (0%), added sucrose 5%, 10% and 15%, respectively. Determination of the MW of protein is done by entering formula from the equation of the line in Figure 2, namely $y=-0.1014x + 2.729$, where x is the migration distance of each band.

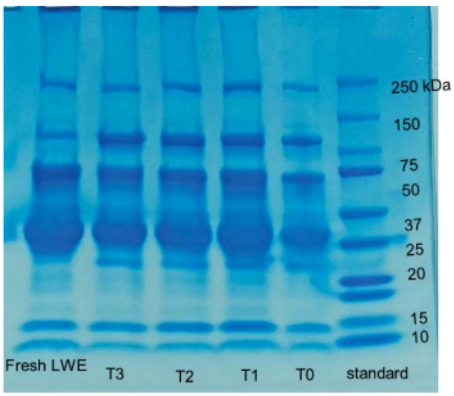

FIGURE 1. Pasteurized LWE Electrophoresis Pattern with Added Sucrose

In this study the band suspected of being lysozyme appeared around the 15 kDa band, with MW for T0, T1, T2, and T3 respectively, 13.36; 13.97; 13.27 and 13.61 kDa. Theoretically, lysozyme has a MW of about 14.4 kDa [25]. The suspected lysozyme band also appeared in fresh LWE without added sucrose and without pasteurization with a MW of 14 kDa. Furthermore, a band that is around 10 kDa also appears, this band can be identified as the apoprotein fraction which has a low MW. Apoprotein is a mixture of various types of proteins that bind to lipids contained in egg yolks. Apoprotein has a low MW, therefore it is difficult to separate its fractions, but the dominant component is apovitelinin which is a high-lipid low-density-lipoprotein [26]. The MW apoproteins identified in this study were 10.68 kDa, respectively; T0, T1, T2, and T3 treatments; 10.93 kDa; 10.93 kDa; and 11.03 kDa and the apoprotein MW in fresh LWE was 10.93 kDa.

Ovomucoid protein appeared in the range of 25-37 kDa, this band was present in all treatments but the band was not present in fresh LWE without pasteurization. The theoretical MW of the ovomucoid is 28 kDa [16]. In addition to the ovomucoid band that appears in this range, it can also be assumed to be a flavoprotein because it has almost the same MW, which is 29.2 kDa [27]. In this study, the MW of ovomucoid at treatment T0, T1, T2, and T3 was 28.2; 28.6; 28.6 and 29.9 kDa.

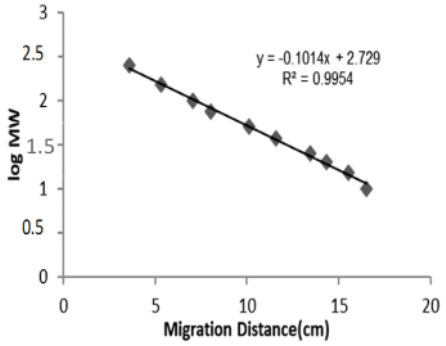

FIGURE 2. SDS-PAGE Standard Curve

Next is ovalbumin, the suspected ovalbumin band appears in the range of 37-50 kDa, this band is present in all treatments including fresh LWE. Theoretically the MW of ovalbumin is about 45 kDa [25]. Ovalbumin MW for each treatment T0, T1, T2, and T3 was 42.24; 42.44; 42.44; and 43.74 kDa while in fresh LWE ovalbumin has a MW of 47.47 kDa. Ovalbumin is the most abundant protein in eggs with a percentage of 54% [12]. The suspected ovotransferrin band also appeared in all treatments with a MW of about 76 kDa [25]. The MW of ovotransferrin in treatment T0, T1, T2, and T3 was 77.33; 82.17; 82.94; and 86.1 kDa, and in fresh LWE it has a MW of 85.9 kDa.

Ovomucin protein was also identified in pasteurized LWE with added sucrose. The band presumed to be ovomucin appears in the 150-250 kDa range. Theoretically ovomucin has a MW of about 220 kDa [28]. The ovomucin band was present in all treatments including fresh LWE. The MW of ovomucin for each treatment T0, T1, T2, and T3 was 223.22; 222.7; 223.75; and 223.22 kDa. Ovomucin in fresh LWE has a MW of 234.44 kDa. [28] and [29] also mentioned that ovomucin has MW 120 kDa which is a derivative form of the β-ovomucin subunit. In this study, bands that were thought to be a derivative form of β-ovomucin appeared in the range of 100-150 kDa with MW in each treatment T0, T1, T2, and T3 was 121.08; 123.17; 123.94; and 117.18 kDa. Meanwhile, the MW of β-ovomucin in fresh LWE was 131.08 kDa. Based on the description of the electrophoretic pattern of LWE protein that has been described, the addition of sucrose did not cause a change in the type of protein in pasteurized LWE.

CONCLUSION

The addition of sugar to LWE did not cause changes in foaming power and coagulation level of pasteurized LWE, but cause changes in protein content, where there was a decreasing trend in protein content along with the added sucrose concentration. Pasteurized LWE protein profiles by SDS-PAGE method could be identified as ovomucin (223.23 kDa), β ovomucin (121.54 kDa), ovotransferrin (82.14 kDa), ovalbumin (42.72 kDa), ovomucoid (28.85 kDa), lysozyme (13.55 kDa) and apoprotein (10.89 kDa). Addition of sugar did not change the type of protein in pasteurized LWE.

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