The effect of temperature levels on antioxidant activity in chicken eggs

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Abstract. Generally, egg heating aims to inhibit microbial contamination, especially on the surface of eggshells and liquid egg products. The application of heating temperature to eggs was carried out at temperatures below 100ºC. However, the heating temperature can have an impact on antioxidant activity and the characteristics of egg protein consumption. The treatment uses 3 temperature levels i.e., 45ºC, 55º C, and 65ºC, respectively. Each treatment was repeated 5 times, a total of 15 units. Each sample was heated for 3 minutes. Parameters were antioxidant activity, total protein, and dissolved protein. The results showed that the different heating temperatures had a significant effect ($P<0.01$) on antioxidant activity, total protein, and dissolved protein. Value of antioxidant activity and total protein decreased with the increasing of heating temperature. However, increasing the heating temperature can increase the dissolved protein value of chicken eggs. The heating temperature of 45ºC on chicken eggs can optimize the antioxidant activity, total protein, and dissolved protein value.

1. Introduction

Eggs are highly nutritious animal food. Besides, eggs contain bioactive compounds that are beneficial to health called functional foods [1,2]. Functional food is food that provides health effects to its users. The potential of eggs as a functional food is quite high [3], due to the nature of poultry as a bio-convert from feed nutrition to egg nutrition [4,5]. Also, the functional properties of eggs can be obtained through processing mechanisms [6], either by heating or pasteurization [7].

Egg pasteurization is done by heating eggs at a temperature below 100ºC [8]. Pasteurization or heating of eggs aims to inhibit microbial contamination, especially on the surface of eggshells and liquid egg products. The pasteurization temperature used depends on the types of raw material. According to the USDA USDA (1980) that heating the eggs aims for pasteurization [8]. The heating can be done at 60ºC for 3.5 minutes [9]. Meanwhile, according to Cotterill [10], the heating process is 56.7ºC for 3.5 minutes or 55.6ºC for 6.2 minutes. The maximum pasteurization temperature for eggs is 65ºC. The high temperature was reported as a cause of a decrease in egg characteristics [6,11].

Research by Ubba *et al.*, (2018) and Nahariah *et al.*, (2014) were revealed that the heating process increased the antioxidant activity of eggs [12,13]. The temperature and heating time in eggs changed the protein structure of the eggs. Changes in egg protein structure from complex to simple. The change in protein structure resulted in the formation of peptide bonds such as dipeptides or oligopeptides. The ability of oligopeptides to inhibit oxidative reactions is indicated as antioxidants. Research by Nahariah *et al.*, (2014), explained that egg whites that were oven-dried at 45ºC for 39 hours showed antioxidant

activity of 26.85% [13]. The use of an incubation temperature of 38°C in infertile eggs from the hatchery industry can provide an inhibitory action against oxidation of 77.99% [14].

The heating process in eggs has an impact on changes in their antioxidant activity [7,15]. Likewise, heating of eggs can also result in changes in total protein and dissolved protein. However, the studies of this examined are limited. Therefore, the objectives of this study were to examine the effect of optimal heating temperature on consumption eggs on antioxidant activity, total protein, and dissolved protein.

2. Materials and Methods

The materials were one day old chicken eggs, aquades, aquabidistila, alcohol, methanol, phenolic acid, H_2SO_4, ice water, HCl, Folin Ciocalteu, 10% Na_2CO_3, K_2SO_4, HgO, NaOH-thiosulfate, Lowrey reagent, Folin reagent, Calcium Permanganate (PK), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Tricarboxylic Acid (TCA), and Bovine Serum Albumine (BSA). The equipment was beaker glass, waterbaths, test tubes, *Graduated Cylinder*, Kjeldahl flasks, stopwatches, ovens, UV-VIS spectrophotometers, analytical scales, 1000µm micropipettes, vortex, mixer, erlenmeyer flask, distillators and incubators, hot plate, spoits, and centrifuges.

This study used a completely randomized design (CRD) with 3 treatments and 5 replications. The total treatment was 15 units. The treatment of the research was the heating temperature of the eggs i.e., 45ºC, 55ºC, and 65ºC, respectively. Furthermore, all the treatments were heating for 3 minutes. The parameters measured were antioxidant activity, total protein, and dissolved protein. Measurement of the antioxidant activity by DPPH method [16], while measurement of total protein by AOAC (1984) and dissolved protein by Wikandari *et al.,* (2011), respectively [17,18].

3. Results and Discussions

The results showed that the antioxidant activity and characteristics of egg protein changed due to the differences in heating temperature. The results of the study are presented in Table 1.

Table 1. Antioxidant activity and characteristics of egg protein with different heating temperatures.

Description (%)	Temperature (°C)			Mean±SD or SE
	45	55	65	
Antioxidant activity	92.69±1.43^{a}	86.89±1.04^{b}	87.47±1.80^{c}	89.02±3.04
Total protein	12.42±0.13^{a}	11.53±0.09^{b}	11.46±0.54^{b}	11.80±0.54
Dissolved protein	0.24±0.01^{a}	0.24±0.02^{b}	0.31±0.15^{c}	0.27±0.03

Different superscripts on the same line show significant differences ($P < 0.01$).
Data±SD or SE?

3.1. Antioxidant activity in eggs by different heating temperature treatment

The results showed (Table 1), that different heating temperatures had a significant effect ($P < 0.01$) on the antioxidant activity of the eggs.

The LSD test showed that the antioxidant activity was significantly different with the increasing heating temperature. This is probably caused by changes in the protein structure of the eggs due to high temperature. By Miryanti *et al.,* (2011), using high temperatures in food can have an impact on the damage to its antioxidant compounds [19]. Heating of foodstuffs can cause an increase in the chain of initiation and propagation of oxidation reactions, also decreased the antioxidant activity in ingredients [20,21]. High heating temperatures can cause oxidative stress on foodstuffs. The application of high temperatures in the heating process of eggs can cause oxidative stress. Oxidative stress occurs in ingredients or eggs that requires antioxidants to inhibit or reduces the oxidation of ingredients or eggs [7].

Table 1 showed the antioxidant activity of eggs at a heating temperature of 45ºC for 3 minutes at 92.69% and decreased with the increase heating temperature. The value of egg antioxidant activity obtained in this study was higher than the results of previous studies. Research by Nirmalaratne and Wu (2015) found that antioxidants for raw whole eggs were 86.95% [3]. However, high temperatures can also damage antioxidants, so it was necessary to control the temperature to maintain the antioxidant activity of eggs [7,19]. The application of proper heating temperature to eggs has a positive effect on changes in egg protein structure. The proper heating mechanism can inhibit oxidative reactions. However, it is expected to optimize the antioxidant content [1].

3.2. Total protein in eggs by different heating temperature treatment

The results (Table 1) showed that heating temperature had a significant effect ($P < 0.01$) on the total eggs protein.

Heating eggs at a temperature of 45ºC was significantly different ($P < 0.01$) from reducing the protein of the total egg due to the increased heating temperature used in this study. However, total egg protein did not significant difference at heating at 55ºC nor 65ºC. This is probably due to the utilization of temperatures above 45ºC in the egg heating process which has resulted in changes in protein structure. Heating at high temperatures causes protein to decrease [7,22]. Materials that experience high heating result in changes to the secondary, tertiary, and quaternary structures, respectively [23]. These changes result in the denaturation of the protein material [23]. Heating causes the breaking of the hydrogen bonds that support the secondary and tertiary structures of a protein [7,23,24]. This change causes the hydrophobic part of the polypeptide side group to open and release water [1,23].

Relevant research showed that heating eggs using a water bath at 63ºC for 3 minutes and heating by oven at 70ºC for 60 minutes makes egg protein and eggshell membrane clump [25]. Likewise, the research of Novia *et al.,* (2011) showed that the protein content of salted eggs at an oven temperature of 70ºC was 37.54% and decreased to 28.40% at an oven temperature of 80ºC [26].

3.3. Value of dissolved protein in eggs by different heating temperature treatment

The results presented in Table 1 indicated that different heating temperatures have a significant effect ($P<0.01$) on egg dissolved protein.

The LSD??? test showed that the soluble egg protein significantly increased with the increase in heating temperature. This is probably due to the changes in protein structure because the heating breaks down into simpler structures. Eggs are hydrated and their protein structure breaks down and denatures [6,27]. This condition causes the protein to dissolve in solvents such as water [27]. [27] explains that heat-denatured protein material can decrease its water binding ability. Heat energy causes the interruption of non-covalent interactions that exist in the natural structure of proteins. However, the low energy does not break the covalent bonds, namely the peptide bonds [6].

The high heating temperature can be increasing the kinetic energy and it is causing the building blocks of protein to move very fast. This can be resulting in broken protein molecular bonds [28]. Protein components are broken down by heat into simpler compounds and dissolve in water [13]. This is relevant to Tyl *et al.,* (2020), which stated that changes in protein structure can result a decrease in soluble protein [24].

4. Conclusion

There were changes in antioxidant activity and protein characteristics in eggs due to different heating temperatures. Heating eggs at 45ºC for 3 minutes increased the antioxidant activities and protein total. Applying high temperatures to egg heating increase dissolved protein. However, high temperatures decreased the antioxidant activities and protein total.

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