The fortification effect of duck eggshell nano-calcium on the physical quality of beef sausage

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Abstract. This research was aimed the fortification effect of duck eggshell nano-calcium on the physical quality of beef sausage. The materials include beef, duck eggshell nano-calcium, tapioca, soy protein isolate, palm oil, salt, pepper, garlic, onion, shallot, coriander, nutmeg, sugar, frankfurter, sodium tripolyphosphate, monosodium glutamate, and ice. The treatments for fortification of duck eggshell nano-calcium were 0; 0.15; 0.3; 0.45; and 0.6% of the total dough. The parameters tested were pH value, water holding capacity, tenderness, and water activity. The physical quality data were analyzed by analysis of variance and if there was a significant difference (P<0.05) then further tested with Duncan's New Multiple Range Test. The results showed that the fortification of duck eggshell nano-calcium had a significant effect (P<0.05) on the pH value, water holding capacity, tenderness, and water activity of beef sausage. Fortification of duck eggshell nano calcium up to 0.6% can increase the value of pH, tenderness, water activity, and decrease water holding capacity.

1. Introduction

Duck eggshells are one of the wastes from duck farming which are abundant with a high calcium content. The utilization of by-products from duck eggshells [1] can be used as a source of dietary calcium. Duck eggshells contain about 94-97% calcium carbonate [2]. The calcium content of eggshells in the form of flour is as much as 50.75% [1]. The function and economic value of duck eggshells can be increased by the application of nanotechnology to form nanoparticles. High energy ball milling is nanotechnology that can be applied to change the size of eggshell particles into nano. The process of applying nanotechnology with high energy ball milling can change the particle size of duck eggshells from 13,229 nm to 347 nm in the form of calcium oxide [3] with a calcium content of around 54.36-59.27% [4]. Particles in nanosize have the advantage of being easy to dissolve and high absorption in the intestine so that they are more efficient when compared to micro-sized calcium [5].

The performance of nano-sized particles is better due to an increase in a surface area [6]. One type of metal oxide that has been widely applied is nano calcium oxide (NCaO). Nano calcium oxide can be used as an antibacterial agent [7], catalyst [8], food additive [9–13], drug delivery system [14] which can increase absorption [15] so that it can be absorbed almost 100% by the body [16]. Nano calcium duck eggshell as a source of food is very good compared to other sources of calcium as a component that is functional and has a positive impact on body health. The results of research...
conducted by the International Osteoporosis Foundation (IOF) show that 1 in 4 Indonesian women with an age range of 50 to 80 years has a risk of osteoporosis so that Indonesian women have a risk of osteoporosis 4 times higher than men at risk of osteoporosis [17]. Men up to 55 years of age and after 55 years of age have a higher risk of osteoporosis than women. Low intake of calcium into the body causes the risk of osteoporosis [18].

One of the things that the body needs to meet bone health is calcium as a macrominer. Fulfillment of body calcium obtained from broccoli, spinach, soybeans, milk, and dairy products. Fulfillment of body calcium can also be met in other ways such as consuming calcium in the form of supplements. Whereas there is another way that is safer and healthier, namely by consuming food products fortified with calcium. Eggshell nano-calcium as a natural source of calcium has been developed as a fortified material for functional food products. Fortified sausage is one of the most popular forms of food in Indonesia. Sausage is one of the comminution products of meat which is in the process of being made with or without the addition of other food ingredients that are inserted into the sausage casing with or without a cooking process [19]. Sausage fortified with eggshell calcium can be used as a functional food [20]. Sausage fortified with eggshell calcium has been studied [21] and has the potential to prevent osteoporosis for those who consume it [22]. So far, there has been no supporting research on the effect of nano-calcium fortification on the physical quality of sausages. The physical quality of sausage is one of the variables used to evaluate the quality of sausage products that are tested objectively. Therefore, this study was conducted to determine the effect of duck eggshells nano-calcium fortification of on the physical quality of beef sausage and its potential as a functional food.

2. Materials and Methods

2.1. Materials
The materials used in this study consisted of beef, duck eggshell nano-calcium, tapioca, palm oil, salt, garlic, pepper, monosodium glutamate (MSG), sodium tripolyphosphate (STPP), soybean protein isolate, frankfurter, ice, and collagen casing.

2.2. Methods
This research consists of several steps. This research started from the preparation of duck eggshell nano-calcium, sausage processing, physical quality test, and statistical analysis.

2.2.1. Preparation of duck eggshell nano-calcium. Eggshell nano-calcium are made using high energy ball milling [3]. Duck eggshells were soaked in hot water for 10 minutes, the eggshell membranes were cleaned, dried at 105°C for 12 hours, then mashed using a sample mill. Eggshell flour was calcined at 1000°C for 2 hours to produce calcium oxide. Eggshell calcium oxide was further processed using high energy ball milling for 60 minutes to produce nano calcium oxide powder.

2.2.2. Preparation of ingredients. The sausage dough formulations that will be used in this study are beef (50%), tapioca (16.5%), soybean protein isolate (2.5%), palm oil (10.5%), salt (1.2 %), STPP (0.5%), MSG (1%), pepper (0.2%), garlic (1.2%), shallot (2%), onion (2%), coriander (0.2%), nutmeg (0.2%), sugar (0.5%), frankfurter (1%), and ice (10.5%).

2.2.3. Sausage processing. The level of fortification of eggshell nano-calcium in this study were: 0; 0.15; 0.3; 0.45; and 0.6% of the total sausage dough. Beef cut into small pieces, cleaned of connective tissue, then ground. Ground meat and oil were mixed, added salt, sodium tripolyphosphate, eggshell nano-calcium treatment, and half of the ice. All spices are ground, and oil is added to the dough, soy protein isolate, tapioca, and the remaining half of the ice are mixed until smooth. Sausage dough is inserted into the collagen casing. Raw sausage is then boiled for 45 minutes at a temperature of 60-70°C and after being cooked the sausage is cooled and then tested for physical quality.
2.3. Physical Quality Test
The fortified beef sausage with duck eggshell nano-calcium tested physical quality, namely the pH value [23], water holding capacity [24], tenderness [25], and water activity [26].

2.4. Statistic Analysis
The data physical quality were analyzed by analysis of variance using completely randomized design and if there was significantly different (P<0.05), then tested further by the Duncan's New Multiple Range Test [27].

3. Results and Discussion
The physical quality is a parameter of meat quality consisting of pH value, water holding capacity, tenderness, and water activity of meat products that were tested objectively [28]. The results of the physical quality test of sausage fortified with duck eggshell nano-calcium were presented in Table 1.

Table 1. The results of the physical quality test of sausage fortified with duck eggshell nano-calcium

| Variable                          | Fortification Level |
|-----------------------------------|---------------------|
|                                   | 0%                  | 0.15%               | 0.30%               | 0.45%               | 0.60%               |
| Value of pH                       | 6.38<sup>a</sup>    | 6.68<sup>a</sup>    | 7.38<sup>b</sup>    | 8.43<sup>c</sup>    | 8.93<sup>d</sup>    |
| Water holding capacity (%)        | 93.96<sup>a</sup>   | 92.70<sup>b</sup>   | 85.80<sup>c</sup>   | 79.30<sup>d</sup>   | 74.41<sup>e</sup>   |
| Tenderness (mm/g/ 5 second)       | 8.30<sup>ab</sup>   | 8.50<sup>bc</sup>   | 8.45<sup>b</sup>    | 8.00<sup>c</sup>    | 8.90<sup>e</sup>    |
| Water activity                    | 0.68<sup>ab</sup>   | 0.78<sup>ab</sup>   | 0.78<sup>ab</sup>   | 0.75<sup>ab</sup>   | 0.83<sup>b</sup>    |

<sup>abcd</sup> Different superscripts at the same row indicate significant differences (P<0.05)

3.1. Value of pH
The results showed that different levels of fortified duck eggshell nano-calcium had a significant effect (P<0.05) on the pH value of beef sausage. The pH value of beef sausage produced ranged from 6.38-8.93. The higher the level of fortified duck eggshell nano-calcium, the pH value of beef sausage increased. This is following the research of Suryanto et al. [12] that fortification of eggshell calcium can increase the pH value of processed meat products. Eggshell nano-calcium fortified in a beef sausage in the form of calcium oxide which decomposes into Ca<sup>2+</sup> and O<sup>-2</sup> [1]. Ca<sup>2+</sup> is alkaline and O<sup>-2</sup> is acidic so oxygen will evaporate into gas and beef sausage fortified with duck eggshell nano-calcium has higher levels of alkaline calcium, which causes the pH value of beef sausage to increase.

3.2. Water holding capacity
The results showed that different levels of fortified duck eggshell nano-calcium had a significant effect (P<0.05) on the water holding capacity of beef sausage. The water holding capacity of beef sausage produced ranges from 74.41-93-96%. The higher the level of fortified duck eggshell nano-calcium, the water holding capacity of beef sausage decreases. The decreasing water holding capacity of sausages is followed by the increasing pH value. The water holding capacity of sausages is strongly influenced by the pH value [1]. Increasing the pH value causes changes in the protein content of meat [29] and with the presence of fortified duck eggshell nano-calcium in sausage processing, the ability of meat proteins to bind water decreases as a result, the water holding capacity of sausages decreases.

3.3. Tenderness
The results showed that different levels of fortified duck eggshell nano-calcium had a significant effect (P<0.05) on the tenderness of beef sausage. The tenderness value of beef sausage produced ranges from 8.00-8.90 mm/kg/5 second. The higher the level of eggshell nano-calcium duck fortification, the tenderness value of beef sausage increased. The increase in the value of sausage tenderness indicates that the resulting sausage becomes more tender. This can be influenced by the water holding capacity of the sausage which is decreasing so that the texture of the sausage is getting more open as a result the sausage becomes more tender. In addition, the increasing pH value of sausages affects the binding
capacity of sausage water (Table 1). This is in accordance with the research of Prayitno et al. [30] that tenderness has a relationship with the pH value and water holding capacity.

3.4. Water activity
The results showed that different levels of fortified duck eggshell nano-calcium had a significant effect (P<0.05) on the water activity of beef sausage. The water activity value of beef sausage produced ranged from 0.68-0.83. The higher the level of fortified duck eggshell nano-calcium, the water activity value of beef sausage increased. The increase in water activity indicates that the free water content in the sausage is getting higher. This can be influenced by the decreasing water holding capacity so that the ability of meat protein to bind the free water content decreases as a result the water activity value of sausages increases. In addition, the increase in water activity can also be affected by the meat processing process. This is in accordance with Novasina [31] that the water activity value of meat products decreases during the processing where the water activity value of sausages ranges from 0.97-0.96 so that the sausages fortified with duck eggshell nano-calcium in this research still meet the standards for water activity value.

4. Conclusion
The results showed that the fortification of duck eggshell nano-calcium had a significant effect on the pH value, water holding capacity, tenderness, and water activity of beef sausage. Fortification of duck eggshell nano-calcium up to 0.6% can increase the value of pH, tenderness, water activity, and decrease water holding capacity.

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