# Influence of Duck Eggshell Nano-Calcium Fortification on the Chemical Quality of Beef Sausage

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(received 19-02-2022; revised 10-06-2022; accepted 05-07-2022)

#### ABSTRAK

Prayitno AH, Rukmi DL, Widiyawati A, Prasetyo B. 2022. Pengaruh fortifikasi nano kalsium kerabang telur itik terhadap kualitas kimia sosis sapi. JITV 27(2):100-106. DOI:http://dx.doi.org/10.14334/jitv.v27i2.3009.

Kerabang telur itik merupakan salah satu *bio-waste* dari industri peternakan unggas dan rumah tangga yang dibuang ke tempat sampah. Kerabang telur itik mengandung kalsium tinggi yang dapat diaplikasikan sebagai alternatif sumber kalsium harian tubuh. Kalsium kerabang telur itik berstruktur nano dapat digunakan sebagai bahan tambahan pangan dalam pengolahan sosis sapi. Tujuan penelitian ini adalah untuk mengetahui pengaruh fortifikasi nano kalsium kerabang telur itik terhadap kualitas kimia sosis sapi. Materi penelitian ini terdiri atas daging sapi, tepung tapioka, isolat protein soya, minyak sawit, garam, lada, bawang putih, bawang merah, bawang bombay, ketumbar, pala, gula, *frankfurter*, sodium tripolifosfat, monosodium glutamat, es, dan kerabang telur itik berstruktur nano. Perlakuan fortifikasi nano kalsium kerabang telur itik yaitu 0; 0,15; 0,3; 0,45; dan 0,6% dari total adonan. Parameter yang diuji yaitu kadar air, protein, lemak, karbohidrat, serat, abu, gula, kalsium, natrium, dan energi. Setiap perlakuan terdiri dari 5 replikasi. Data hasil uji kualitas kimia dianalisis dengan analisis variansi pola searah dan jika terdapat perbedaan yang signifikan (P<0,01) diuji lanjut dengan uji *Duncan's New Multiple Range Test*. Hasil penelitian menunjukan bahwa fortifikasi nano kalsium kerebang telur itik yang berbeda berpengaruh sangat nyata (P<0,01) terhadap kadar protein, lemak, abu, gula, kalsium, dan natrium, tetapi tidak mempengaruhi kadar air, karbohidrat, serat, dan energi sosis sapi. Forifikasi nano kalsium kerabang telur itik sampai level 0,6% dapat meningkatkan kadar protein, abu, dan kalsium tetapi menurunkan kadar lemak, gula, dan natrium sosis sapi.

Kata Kunci: Sosis Sapi, Kualitas Kimia, Kerabang Telur Itik, Fortifikasi, Nano Kalsium

## ABSTRACT

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Duck eggshells are one of bio-wastes from poultry industry and household that have been disposed. Duck eggshells contain high calcium which can be applied as an alternative source of daily calcium for the body. Nanostructured duck eggshell calcium can be used as a food additive in beef sausage processing. This study was conducted to determine the chemical quality of beef sausage fortified by duck eggshell nano-calcium. The materials include beef, soy protein isolate, palm oil, garlic, salt, pepper, shallot, onion, tapioca, monosodium glutamate, sodium tripolyphosphate, nutmeg, coriander, frankfurter, sugar, duck eggshell nano-calcium, ice, and nano-structured duck eggshell. Treatments for fortification of duck eggshell nano-calcium were 0; 0.15; 0.3; 0.45; and 0.6% of the total dough. Parameters tested were moisture, protein, fat, carbohydrate, fiber, ash, sugar, calcium, sodium, and energy of the sausage. Each treatment consisted of 5 replications. Data collected was analyzed by analysis of variance using completely randomized design and if there was significant different (P<0.01) then further tested by the Duncan's New Multiple Range Test. Results showed that the fortification of duck eggshell nano-calcium had a highly significant effect (P<0.01) on protein, fat, ash, sugar, calcium, and sodium, but did not affect moisture, carbohydrate, fiber, and energy of beef sausage. Fortification of duck eggshell nano-calcium but decreased fat, sugar, and sodium of beef sausage.

Key Words: Beef Sausage, Chemical Quality, Duck Eggshell, Fortification, Nano-calcium

# INTRODUCTION

Duck eggshell is one of bio-wastes from duck farming which is abundant with high calcium content (Prayitno et al. 2022) and a lot of it is produced from household waste. Utilization of bio-waste from duck eggshells can be used as a source of dietary calcium.

Duck eggshells contain calcium carbonate as much as 94-97% (Nurlaela et al. 2014), while eggshells in the form of flour contain calcium around 50.75% (Prayitno et al. 2016). The properties and added value of duck eggshells can be enhanced by the application of nanotechnology to produce eggshell nanoparticles. High energy ball milling (HEM) is a nanotechnology that can

be applied to convert particles from eggshells to nanosized ones. The application of HEM technology can change duck eggshell particles from 13,229 nm to 347 nm (Prasetyo & Prayitno 2020) with a calcium content of 54.36-59.27% (Prayitno et al. 2020).

The performance of nano-sized particles is better due to an increase in surface area (Habte et al. 2019). Shabnam et al. (2020) stated that nano-sized minerals addition to food have a better and faster absorption rate in stomach than micro-sized minerals. Nano-calcium oxide (NCaO) is one type of metal oxide that is widely applied to food products such as meat products. Nano-calcium oxide can be applied as an antibacterial (Roy et al. 2013), food additives (Suryanto et al. 2014; Prasetyo & Prayitno 2021), catalyst (Gopalappa et al. 2012), a drug delivery system (Balaganesh et al. 2018) that can increase absorption (Jampilek et al. 2019) so that it can be absorbed almost 100% by the body (Suptijah et al. 2012).

The International Osteoporosis Foundation (IOF) reports that prevalence of osteoporosis in women in Indonesia at the age of 50-70 years and over 70 years is 23% and 53% (Pusdatin 2020). Men after 55 years of age have a higher risk of osteoporosis than women (Jahari & Prihatini 2014). Premenopausal women have a risk of developing osteoporosis with a higher risk of 21.7% than men with a risk of osteoporosis of 14.8% (Mansoben et al. 2021). Low intake of calcium into the body can cause the risk of osteoporosis. Nurmaliza et al. (2021) stated that one of the risk factors that cause osteoporosis in women is calcium intake. Nano calcium in duck eggshells can be used as an excellent food additive compared to other sources of calcium as a mineral that is functional and has a positive impact on health

One of the macro minerals that the body needs to meet bone health is calcium. The body's calcium is usually used to be met from spinach, broccoli, soybeans, milk, and processed products. Fulfillment of body calcium can be met in another way, is by consuming calcium-fortified foods. Micro-sized calcium is only absorbed by the body about 50% which can lead to deficiency (Pravitno et al. 2021). Eggshell nano-calcium as a natural source of calcium has been developed as a fortification material for functional food products. Sausage is the most consumed processed meat products in Indonesia. Sausage is one of the processed meat products which is processed with or without addition of other food additives that are inserted into casing with or without cooked (SNI 2015).

Foods fortified with eggshell calcium can be used as a functional food to reduce the risk of osteoporosis, especially in the elderly (Arnold et al. 2021). Calciumfortified foods can increase calcium intake (Palacios et al. 2021) one of which is sausage products. Effect of eggshell nano-calcium fortification on beef sausage has

been investigated on sensory quality (Prasetyo & Prayitno 2021) and physical quality (Prayitno et al. 2022). Sausage is a processed meat product that is relatively low in calcium (Engeloug et al. 2017; Huang et al. 2021). People of all ages who are starting to worry about their bone health have prompted the food industry to respond by fortifying calcium in foods (Cormick et al. 2021). Supporting research on duck eggshells nanocalcium fortification on the chemical quality of beef sausage has not been investigated. This research was designed to develop new healthier sausages fortified with nano-calcium and study its effect on the chemical quality of sausages taking into beneficial to the calcium intake. Chemical quality of sausages is one of the important variables in determining the quality of sausage products that are objectively tested. Therefore, this study aimed to determine the chemical quality of beef sausage fortified with duck eggshell nano-calcium.

#### MATERIALS AND METHODS

#### Materials

The materials used in this research include beef, tapioca flour, soy protein isolate, palm oil, garlic, pepper, salt, shallot, onion, monosodium glutamate, coriander, nutmeg, sugar, frankfurter, sodium tripolyphosphate, ice, nano-structured duck eggshell, and collagen casing.

## Methods

This research started from the preparation of duck eggshell nano-calcium, preparation of formulations and ingredients, sausage processing, chemical quality test, and statistical analysis.

# Preparation of duck eggshell nano-calcium

Duck eggshell nano-calcium were made using highenergy ball milling (Prasetyo & Prayitno 2020). Duck eggshells were soaked in hot water for 10 minutes, dirt and eggshell membranes were cleaned, dried at 105 $\Box$ C for 12 hours, and mashed. Eggshell flour was calcined at 1,000 $\Box$ C for 2 hours and further processed using HEM for 60 minutes to produce a duck eggshell nanocalcium powder.

# Preparation of formulations and ingredients

Beef sausage formulation, ingredients, and beef sausage processing were made according to Prasetyo & Prayitno (2021). The formulation and ingredients of

beef sausage fortified with duck eggshell nano-calcium was presented in Table 1.

# Preparation and sausage processing

Treatmentin in this research was according to Prasetyo & Prayitno (2021), with level of duck eggshell nano-calcium fortification as follow: 0; 0.15; 0.3; 0.45; and 0.6% of the total dough. The beef was cleaned of connective tissue, cut into small pieces, then ground. The ground beef and oil were mixed, and salt, sodium tripolyphosphate, duck eggshell nano-calcium, and ice were added. All spices were ground, soy protein isolate, tapioca, oil, and ice were mixed until smooth. Sausage dough was inserted into the collagen casings. The raw sausage was then boiled for 45 minutes at a temperature of 60-70°C and cooled at room temperature, and then a chemical quality test was carried out.

**Table 1.** The formulation and ingredients of beef sausage fortified with duck eggshell nanocalcium

No.	Ingredients	Percentage (%)		
1.	Beef	50		
2.	Tapioca flour	16.5		
3.	Soy protein isolate	2.5		
4.	Palm oil	10.5		
5.	Salt	1.2		
6.	Sodium tripolyphosphate	0.5		
7.	Monosodium glutamate	1		
8.	Pepper	0.2		
9.	Garlic	1.2		
10.	Shallot	2		
11.	Onion	2		
12.	Coriander	0.2		
13.	Nutmeg	0.2		
14.	Sugar	0.5		
15.	Frankfurter	1		
16.	Ice	10.5		

# Chemical quality test

The fortified sausage was analyzed for chemical quality including moisture, protein, fat, carbohydrate, fiber, ash, sugar, calcium, sodium, and energy (AOAC 2019).

# Statistical analysis

Chemical quality data was analyzed by analysis of variance using completely randomized design and if

there was significantly different (P<0.01) then further tested by the Duncan's New Multiple Range Test (Riadi 2014).

#### RESULTS AND DISCUSSION

# Moisture

Results showed that the fortification did not significantly affect (P>0.01) moisture content of beef sausage. The moisture content ranged from 41.42-41.70%. This result was lower than the results of Cunningham et al. (2015) the moisture ranged between 56.8-67%. El-Nashi et al. (2015) reported beef sausage has a moisture content around 50.24-61.89%, and Prayitno et al. (2021) stated that moisture content was around 49.65-50.69%. Moisture content of beef sausage from this study still meets the standard for sausage moisture content, which was a maximum of 67% (SNI 2015).

There was no difference in moisture between nanocalcium fortified sausages and without nano-calcium fortification. This result showed that moisture of beef sausage is not affected by nano-calcium fortification and moisture of beef sausage from this study is good quality. Whereas in other processed meat products, namely meatballs, the presence of calcium fortification of eggshells can increase moisture content of the product (Suryanto et al. 2014).

## **Protein**

Results showed that the fortification with different levels had a highly significant effect (P<0.01) on protein content of sausage. Protein content from this study ranged from 15.09-15.41%. Average protein content of sausage from this study was almost the same as the results of Cunningham et al. (2015) that ranges from 13.5-17.4% and El-Nashi et al. (2015) reported that the protein content between 12.72-16.32%. Protein content of beef sausage from this study still meets the standard for sausage protein content, which was at least 13% (SNI 2015).

Prayitno & Rahman (2020) stated that protein content of processed meat products could be influenced by the ingredients used. The protein content from this study showed an increasing value as the level of duck eggshell nano-calcium fortification increased. This can be caused by the higher level of fortification of duck eggshell nano-calcium, the higher the protein that can bind to duck eggshell nano-calcium. Calcium in nanoparticle size has high solubility and reaction ability and calcium ion (Ca<sup>2+</sup>) as a cation has the ability to interact with meat protein so as to increase the proportion of protein in the product (Prayitno et al. 2016).

#### Fat

Results showed that the fortification with different levels had a highly significant effect (P<0.01) on fat content of beef sausage. Fat content of beef sausage produced from this study ranged from 11.53-11.86%. This result was almost the same as the results of Cunningham et al. (2015) which ranged from 10 to18.8%, but it was still lower when compared to the results of El-Nashi et al. (2015) which ranged from 16.23 to17.943%. The fat content from this study still meets the standard for sausage fat content, which was a maximum of 20% (SNI 2015).

The fat content in this study showed a decreasing value along with the increasing level of duck eggshell nano-calcium fortification. The fat content showed a value that was inversely proportional to the protein content of beef sausage produced in this study (Table 2). The higher the protein content, the lower the fat content. This was because the higher the level of fortification of duck eggshell nano-calcium, the higher the protein that can bind to duck eggshell nano-calcium (Prayitno et al. 2016). The bond between protein and duck eggshell nano-calcium was strong, it will bind more water than fat during sausage processing so that the fat content of beef sausage in this study was lower with higher protein content of beef sausage, although the moisture content of beef sausage in this study for each treatment did not differ.

# Carbohydrate

Results showed that the fortification with different levels did not significantly affect (P>0.01) carbohydrate content of beef sausage. The carbohydrate from this study ranged from 28.31 to 28.48%. The average carbohydrate from this study was higher than the results

of El-Nashi et al. (2015) which ranged from 3.29 to 14.97%, while according to Leonard et al. (2019) the carbohydrate content was around 9.62-19.31%. Manihuruk et al. (2017) found that the carbohydrate content ranged between 19.82-21.47%. The absence of a significant difference from each treatment to the carbohydrate content of beef sausage can be caused by the ingredients used in beef sausageformulation in this study have almost the same carbohydrate and have a higher carbohydrate content composition when compared to the research conducted by El-Nashi et al. (2015) and Leonard et al. (2019).

The treatments of fortification in this study did not significantly affect carbohydrate content of beef sausage. The absence of differences in the carbohydrate content can be attributed to the absence of starch content of duck eggshell nano-calcium fortification materials used in the processing of beef sausage.

#### Fiber

Results showed that duck eggshell nano-calcium fortification with different levels did not significantly affect (P>0.01) fiber content of beef sausage. The fiber content in this study ranged from 0.23-0.27%. This result was lower than that of Cunningham et al. (2015) which ranged from 0.4-0.7%, while according to Sánchez-Zapata et al. (2013) sausage fiber content was around 1.46%.

The absence of a significant difference from each treatment to the fiber content of beef sausage can be caused by the ingredients used in this study have almost the same levels and have a lower fiber composition when compared to the research conducted by Cunningham et al. (2015) dan Sánchez-Zapata et al. (2013). The increasing level of duck eggshell nanocalcium fortification in this study could not affect significantly on fiber content of beef sausage.

Table 2. Chemical quality of fortified beef sausage duck eggshell nano-calcium

Variable	Fortification level				
v arrable	0%	0.15%	0.3%	0.45%	0.6%
Moisture (%) <sup>ns</sup>	41.70	41.64	41.42	41.34	41.42
Protein (%)	15.09 <sup>a</sup>	15.19 <sup>b</sup>	15.29 <sup>c</sup>	15.32°	15.41 <sup>d</sup>
Fat (%)	11.86 <sup>d</sup>	11.77 <sup>cd</sup>	11.66 <sup>bc</sup>	11.63 <sup>ab</sup>	11.53 <sup>a</sup>
Carbohydrate (%) <sup>ns</sup>	28.40	28.37	28.48	28.47	28.31
Fiber (%) <sup>ns</sup>	0.23	0.24	0.27	0.27	0.27
Ash (%)	$2.96^{a}$	3.04 <sup>b</sup>	3.14 <sup>c</sup>	$3.24^{d}$	3.35 <sup>e</sup>
Sugar (%)	3.33 <sup>b</sup>	3.29 <sup>b</sup>	$3.22^{a}$	$3.20^{a}$	$3.19^{a}$
Calcium (mg/100 g)	25.00 <sup>a</sup>	305.50 <sup>b</sup>	619.00 <sup>c</sup>	921.50 <sup>d</sup>	1.165.00 <sup>e</sup>
Sodium (mg/100 g)	$26.00^{c}$	$23.00^{b}$	21.50 <sup>ab</sup>	$21.00^{a}$	$20.00^{a}$
Energy (kcal/100 g) <sup>ns</sup>	281.00	280.00	280.00	280.00	279.00

ns Not significant, a-d Different superscripts at the same row indicate highly significant differences (P<0.01)

# Ash

Results showed that the fortification with different levels did highly significantly affect (P<0.01) ash content of beef sausage. The ash content in this study ranged from 2.96-3.35%. This result was higher than the results of Cunningham et al. (2015) which ranged from 2.2-2.7% but still lower than the results of the Sánchez-Zapata et al. (2013) which around 4.93%. Gad EL Rab et al. (2019) study which found that the ash content of beef sausage ranges from 6.74-7.20%. The ash content of beef sausage from this study still meets the standard for sausage ash content, which is a maximum of 3% (SNI 2015).

The ash content in this study showed an increasing value along with the increasing level of duck eggshell nano-calcium fortification (Table 2). The increase in ash content from this study could be due to the higher the level of fortification of nano-calcium duck eggshell, the higher the increase in mineral content sourced from duck eggshell nano-calcium. Prasetyo & Prayitno (2020) found that the minerals contained in duck eggshell nano-calcium were phosphorus 0.70%; magnesium 0.41%; sodium 0.35%; and 59.27% calcium.

# Sugar

Results showed that the fortification of duck eggshell nano-calcium with different levels had a highly significant effect (P<0.01) on the sugar content of beef sausage. The sugar content in his study ranged from 3.19-3.33%. This result was higher than that of Hadipernata et al. (2016) which ranged between 0.5-1.71% and Coloretti et al. (2019) reported that the sugar content of sausages was around 0.75%.

The sugar content of beef sausage from the results of this study showed a decreasing value along with the increasing level of duck eggshell nano-calcium fortification. This could be seen based on the research results obtained on the sugar content of beef sausage (Table 2). The decrease in sugar content in beef sausage from this study was inversely proportional to the calcium content of beef sausage which increased with increasing levels of duck eggshell nano-calcium fortification.

### Calcium

Results showed that the fortification of duck eggshell nano-calcium with different levels did highly significantly affect (P<0.01) calcium level of beef sausage. The calcium content ranged from 25-1.165 mg/100 g. The average calcium content was higher than the results of Irshad et al. (2016) which ranged from

6.48-203 mg/100 g and Gad EL Rab et al. (2019) which ranged from 146.61-246.85 mg/100 g. Processed meat products generally have lower levels of calcium content when compared to processed meat products fortified with calcium materials (Prayitno et al. 2016).

The calcium content showed an increasing value along with the increasing level of duck eggshell nano-calcium fortification (Table 2). The increase in calcium levels from this study could be caused by the higher the level of fortification of duck eggshell nano-calcium, the higher the increase in calcium levels sourced from duck eggshell nano-calcium. The calcium level contained in the duck eggshell nano-calcium was 59.27% calcium (Prayitno, et al. 2020).

#### Sodium

Results showed that the duck eggshell nano-calcium fortification with different levels did highly significantly affect (P<0.01) sodium content of beef sausage. The sodium content ranged from 20-26 mg/100 g. The average sodium content from this study was lower than the results of Carraro et al. (2012) which ranged from 680.19-998.15 mg/100 g and Stanley et al. (2017) which ranged from 597.3-908.8 mg/100 g. This lower sodium content indicated that the beef sausage from this study has a healthier appeal. Reducing sodium intake in processed meat products can be seen as an effort to reduce risk factors for hypertension and heart disease (Carraro et al. 2012).

The sodium content from this study showed a decreasing value along with the increasing level of the fortification (Table 2). Duck eggshell nano-calcium has a sodium content of 0.35% (Prasetyo & Prayitno 2020). The sodium content in the duck eggshell nano-calcium does not increase the sodium content of the product. The decrease in sodium content in this study was inversely proportional to the calcium content of beef sausage which increased with increasing levels of the fortification fortification.

The absence of a significant difference from each treatment to the energy of beef sausage can be caused by the ingredients used in the formulation of beef sausage in this study have almost the same energy. The increasing level of duck eggshell nano-calcium fortification in this study could not have a significant impact on the energy of beef sausage. Beef sausage carbohydrate has no difference between treatments, so it does not have a significant impact on energy for each beef sausage treatment in this study.

Result showed that the fortification of duck eggshell nano-calcium did highly significantly affect protein, fat, sugar, calcium, sodium, and ash, but did not affect moisture, carbohydrate, fiber, and energy of beef sausage. Fortification of duck eggshell nano-calcium up

to 0.6% could increase the protein, ash, and calcium content but decrease fat, sugar, and sodium content of beef sausage.

#### **CONCLUSION**

The results showed that the fortification of duck eggshell nano-calcium had a highly significant effect on protein, fat, sugar, calcium, sodium, and ash, but did not affect on moisture, carbohydrate, fiber, and energy of beef sausage. Fortification of duck eggshell nano-calcium up to 0.6% could increase the protein, ash, and calcium content but decrease fat, sugar, and sodium content of beef sausage.

#### **ACKNOWLEGMENT**

This paper was supported by research funds of Pusat Penelitian dan Pengabdian Kepada Masyarakat (P3M) of Politeknik Negeri Jember.

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