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FULL PAPER

The quality properties, thiobarbituric acid (TBA) values and microstructure of chicken sausage with local red beetroot powder

[1,*]Swastike, W., [2]Suryanto, E., [2]Rusman, [3]Hanim, C., [2]Jamhari, [2]Erwanto, Y. and [4]Jumeri

[1]*Department of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia.*
[2]*Deparment of Animal Products Technology, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.*
[3]*Deparment of Animal Feed and Nutrition, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.*
[4]*Department of Technology Agricultural Industry , Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia.*

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Abstract

This research was aimed to determine the quality properties, the microstructure of chicken sausage and Thiobarbituric acid (TBA) values with locally Indonesia red beetroot powder. The main ingredients of chicken sausage-making in this research were broiler chicken, filler, binder, beetroot powder, and spices. Red beetroot powder function as a filler was substituted tapioca starch in chicken sausage batter in three different levels. The combination of red beetroot powder with level 0, 1.0, 2.0 and 3.0% of total batter and shelf life at room temperature for 0, 1, 2 and 3 days. Each treatment consisted of five replications. The variables observed using quality properties (moisture, ash, fat, protein, crude fiber and calorie), microstructure and peroxide value of chicken sausage. The data of quality properties and peroxide value were analyzed by using one-way analysis (ANOVA) of Completely Randomized Design. The differences between means were analyzed by Duncan's New Multiple Ranges Test. The data of microstructure was analyzed by descriptive analyses. The moisture, protein, fat and ash contents for chicken sausages were significantly different ($p<0.05$). The chicken sausage with 2% substitution of beet powder produced chicken sausages with a high protein content of 14.77±0.02% while a low-fat content is 0.42±0.01%. Thiobarbituric acid (TBA) values of chicken sausages increased throughout the three days of room temperature storage (38°C). Chicken sausage formulated with red beetroot powder showed a significantly lower TBA value compared to the samples without red beetroot powder ($p<0.05$). In conclusion, a higher level of beetroot powder will improve the quality of chicken sausage and also the microstructure. The best level of beetroot powder addition was 2.0%. The addition of beetroot powder able to maintain fresh sausage conditions up to 2 days of storage at room temperature.

1. Introduction

Chicken meat provides high protein and low fat, and chicken lipids are characterized by relatively high levels of unsaturated fatty acids, which are considered to be positive and healthy by consumers (Hwang *et al*., 2011). Chicken sausage is a precious product that is widely used as an alternative as a source of protein. The high content of protein and fat made a sausage became a perishable food. Oxidation of lipid and auto-oxidation is one of the major causes of quality deterioration and reduction of shelf life of meat products (Min and Ahn, 2005). This may produce changes in meat quality parameters such as color, texture and even nutritional value (Fernandez *et al*., 1997; Lima *et al*., 2013). Synthetic antioxidants such as Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been used extensively to prevent the sausage from oxidation. The concentration of synthetic antioxidants allowed in food is limited to 0.01% of fat content (when used individually). Nowadays, the acceptability of synthetic additives by consumers is low since certain toxicity and carcinogenicity have been identified in some studies (Faine *et al*., 2006). For these reasons, the interest of the

*Corresponding author.
Email: *winny@staff.uns.ac.id*

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meat industry in using natural antioxidants has increased considerably (Kumar *et al*., 2015). Recent studies have implicated them to have toxic effects (Lindenschmidt *et al*., 1986; Shahidi *et al*., 1992), hyperactivity in children, allergies to cancer.

Since the last few decades, public awareness of health and preservation of the global environment has had an impact on people's behavior to consume healthy foods product (Laroche *et al*., 2001). The increased public awareness of health was expected to have a positive impact on the increase in demand for food natural products. It is because these products are produced using a natural product that produces healthier and safer foods for consumption (Suharjo *et al*., 2016). The antioxidant is intended to prevent the presumed deleterious effects of free radicals in the human body, and as prevention to the deterioration of fats and other constituents of the food products (Azmi *et al.,* 2019).

Composite flour as an innovative flour that has attracted much attention in research as well as food product development (Hasmadi *et al.,* 2014; Suresh *et al.,* 2015; Gbenga-Fabusiwa *et al.,* 2018; Jafari *et al.,* 2018; Hasmadi *et al.,* 2018; Nyembwe *et al.,* 2018; Emmanuel *et al.,* 2019). Composite flour defined as a mixture of flours obtained from tubers rich in starch (Hasmadi *et al.,* 2020), with or without wheat flour that created to satisfy specific functional characteristics and nutrient composition (Noorfarahzilah *et al.,* 2014).

Red beetroot is a type of tuber plant that is cultivated in highlands, especially in central Java, Indonesia. They used it only as a dressing salad or as consumed in juice. Beetroot is highly acceptable for its rich nutrient contents viz. dietary fiber, mineral contents such as iron, potassium, calcium, zinc, and sodium, and vitamin contents such as vitamin A, B6, folic acid, niacin, biotin and C (Pinki, 2014). Beetroot contains both betaine and nitrate. Betaine is a trimethyl derivative of amino acid glycine which promotes muscular endurance, strength, and power (Hoffman and Ratamess, 2009). Beetroot is a source of natural antioxidant and also natural coloring (Georgiev *et al*., 2010) because red beetroot contains flavonoid and pigment that brings a lot of function. Consumption of red beet, which is rich in phenolic acids and has a high antioxidant capacity, can help protect against age-related diseases (Ravichandran *et al*., 2013). some natural compounds have higher antioxidant capacity than synthetic compounds and some also have other positive effects on the sensory properties of meat products (Kumar *et al*., 2015). It also can be applied to processing food and or to be food ingredients, even pure beetroot, extract, and or powder. Red beetroot powder can be fortified and or substituted with a filler or binder commonly used in the processing of sausage. Some studies have been carried out on the use of various beetroot for attaining these properties in emulsified type sausages (Jin *et al.,* 2014; Yildiz *et al.,* 2016), and also as a filler (Swastike *et al*., 2020).

Fillers are starch, which is a carbohydrate polymer derivated from plant-based, able to absorb large quantities of water, but they are not suitable for emulsifiers. The emulsification has shown in the microstructural of the sausage. On the other hand, starches can act as fillers that bind water and fat (Heinz and Hautzinger, 2007). The functionality of different types of starches depends on granular structure, amylose, and amylopectin. Amylose contributed to gel strength and amylopectin in a viscoelastic (Mahmood *et al*., 2017). The interaction of water, muscle protein, fat globules, and types of starch is the primary factor responsible for the emulsion of meat products (Peraire *et al*., 2019).

Thus, this study aimed to explore red beetroot powder as a filler and as a natural antioxidant in the quality properties, and microstructure and also Thiobarbituric acid (TBA) values in-room storage of chicken sausage

2. Materials and methods

The ingredients used in this experiment were breast chicken meat, beetroot powder, tapioca flour, Isolated Soy Protein (ISP), spices (salt, fresh Garlic, pepper powder), sodium tripolyphosphate (STTP), cooking oil, and ice water.

The material used in this experiment were a grinder, meat chopper, penetrometer, digital electric scale, analytical scales, filter paper, cooking glove, stove, blender, pH meter, stainless steel pan and knife, thermometer, sausage casing, 35 kg barbell, millimeter block paper, permanent markers, and stopwatch.

2.1 Preparation of red beetroot powder

Red beet (Beta vulgaris L. var. conditiva) roots were bought at Cepogo, Central Java, Indonesia. The red beetroots were washed, peeled, and chopped into small pieces. Chopped red beet was subsequently placed into a freeze dryer. The dried product was grounded, and the final moisture content of the red beet powder was 3.5±0.5%.

2.2 Preparation of chicken sausage

The breast chicken meat, spices, tapioca and beetroot powder in different level were prepared and pondered. Red beetroot powdered that we used was prepared in the

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different level to substituted tapioca flour. The skinless breast meat was purchased from a local retailer. Subcutaneous and excessive connective tissues were removed from the meat and minced through. Four batches were prepared for each treatment; The basic recipe consisted of 65% meat, tapioca flour 18%, 10% ice water and spices (Garlic, pepper, salt) were added to the completed batter until 100%. Minced meat was ground for 1 min using a bowl cutter. Mixed all ingredient for 2 mins and continuous with mixer for 15 mins. The other batter had decreased percentages of tapioca flour (1, 2 and 3%) and replaced with substituted red beetroot powder. The batter was then stuffed casings using a stuffer. The stuffed samples were cooked in a heating chamber to an internal temperature of 80°C. The chicken sausages were then cooled and ready to be evaluated.

2.3 Chemical analysis

Moisture, protein (total nitrogen × 6.25), ash and fat contents of differently prepared beef sausage samples were determined according to AOAC (2012). Carbohydrates (nitrogen-free extract) were determined by differences.

2.4 Thiobarbituric acid value (TBA) analysis

TBA values in chicken sausages were measured following the procedure by Salih *et al*. (1987) with slight modifications. Exactly 5.0 g samples (accurate to 0.01 g) were mixed with 15.0 mL of 20 g/mL trichloroacetic acid solution and homogenized at 10000 r/min for 3 mins in an ice bath, deproteinized for 2 hrs, centrifuged at 2000×*g* for 10 mins at 4°C, the supernatant was filtered with double slow filter paper, and diluted to 50 mL with double-distilled water. A 5.0 mL aliquot of the filtrate was mixed with 5.0 mL 0.02 mol/L 2-Thiobarbituric acid solution, allowed to react at 90°C for 30 mins, cooled to room temperature immediately, and absorbance was read at 532 nm. Results were expressed as mg malondialdehyde (MDA)/kg sample.

3. Result and discussion

3.1 Chemical quality properties of chicken sausage

The chemical quality properties of the chicken sausages were formulated with different levels of red beetroot powder shown in Table 1.

3.1.1 Moisture

The moisture content of the chicken sausages containing red beetroot powder was higher than without red beetroot powder ($p<0.05$). the increase in moisture content might be due to higher moisture present in red beetroot powder. Thus, the increase in the substitution of red beetroot powder caused an increase in the water content of chicken sausage. On the other hand, the substitution of red beetroot powder or replacement of tapioca starch started with the concentration 1, 2, 3% will affect significantly the water content ($P<0.05$). The higher water content also found in the other meat product substituted with texturized vegetable protein (Hidayat *et al.,* 2018). The latter result likely reflected Zargar *et al*. (2014) also reported an increase in the moisture content in chicken sausage with pumpkin, Suradkar *et al*. (2013) and Das *et al*. (2013) also reported an increase in the moisture content of chicken nuggets containing bread crumbs and chicken nuggets containing fermented bamboo shoot, respectively. This was in agreement with the findings of Verma *et al*. (2010) who also observed a similar finding in designer chicken nuggets incorporated with apple pulp. Similar results were also observed by Nazni and Karuna Thara (2011) where ash and fibre contents were increased with the increment of beetroot powder percentages in the cake.

3.1.2 Crude protein

Table 1 shows that the crude protein content of chicken sausages decreasing significantly ($P<0.05$), however, the substitution in 2% level of red beetroot powder almost the same protein with the chicken sausage without red beetroot powder. The probable reasons for the decreased protein content may be attributed to the comparatively lower protein contents of red beetroot powder. Suradkar *et al*. (2013) also reported a decrease in the protein content of chicken nuggets containing breadcrumbs. Verma *et al*. (2013) also observed a decrease in the protein content of sheep meat nuggets on the incorporation of guava powder. Taludkar and Sharma (2009) observed a decrease in the protein content of chicken meat patties incorporated with wheat and oat

Table 1. The chemical quality properties of chicken sausage with different substitution level of red beetroot powder

Parameter	Different level Substitution of red beetroot powder (%)			
(%)	0	1	2	3
Moisture	$64.21±0.04^{a}$	$65.55±0.02^{c}$	$65.44±0.19^{b}$	$66.16±0.28^{d}$
Protein	$14.88±0.01^{c}$	$14.65±0.07^{a}$	$14.77±0.02^{b}$	$15.15±0.02^{d}$
Fat	$0.50±0.013^{c}$	$0.47±0.07^{b}$	$0.42±0.01^{a}$	$0.40±0.01^{d}$
Crude fiber	$0.12±0.005^{a}$	$0.15±0.005^{b}$	$0.18±0,005^{c}$	$0.24±0.008^{d}$

Values are presented as mean±SD, $n = 5$. Values with different superscript within the same row are significantly different ($P < 0.05$).

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bran.

3.1.3 Fat content

The fat content of chicken sausage was gradually decreased significantly (P<0.05). The fat content was getting lower compared to other substitution levels of red beetroot powder. Verma *et al*. (2013) also observed a decrease in the fat content of sheep meat nuggets on the incorporation of guava powder. Suradkar *et al*. (2013), Verma *et al*. (2012a) and (2012b) also reported similar results in different meat products. Taludkar and Sharma (2009) observed a decrease in the fat content of chicken meat patties incorporated with wheat and oat bran. Similar results were reported by Valeria *et al*. (2008) for dry fermented sausages prepared by the incorporation of carrot dietary fibers and Aleson-carbonell *et al*. (2004) for non-fermented dry-cured sausages formulated with lemon albedo. And also, the same with Lucky *et al*. (2020) observed that fat content and energy decreased respectively, as the percentage of beetroot powder increased from 0 to 20%.

3.1.4 Crude fiber

The crude fiber content increased significantly (P < 0.05) with increasing levels of substitution of red beetroot powder. This increase in crude fiber might be due to the high fiber level present in red beetroot powder. A similar increase in the fiber content was also observed by Zargar *et al*. (2014), Verma *et al*. (2013), Das *et al*. (2013), Verma *et al*. (2012a), (2012b) and Taludkar and Sharma (2009) in various meat products.

3.2 Thiobarbituric acid value of chicken sausage

Chicken sausage products contain protein and fat that can cause damage to the product due to oxidation. Oxidation is an indication that the product is under the same or off-flavor, and this condition can result in discoloration, nutritional value and taste. The 2-thiobarbituric acid (TBA) is used to measure secondary products of lipid oxidation primarily derived from PUFA) and indicate a preoccupation (Chen *et al*., 2014). According to Eneke *et al.* (2018), TBA values decreased within the first 2 months suggesting that conditions for initiation of auto-oxidation were lacking or that TBA reacting substances progressively formed complexes with other compounds. The TBA number on the sausage was shown in Table 2.

Table 2 shows that TBA was higher slightly up until the 2nd day of storage and then increased exponentially on the 3rd day of storage. TBARS content was higher in chicken sausages without substitution red beetroot powder. The chicken sausage with substitution red beetroot powder succeeded inhibit the increase of TBARS Value until 2 days in room storage. This result was found due to the presence of antioxidants component of red beetroot powder. It is also similar to Khan and Saghir (2015) that the antioxidant content of carrot powder can reduce the degree of fat oxidation.

Table 2. TBA value of chicken sausage with different substitution level of red beetroot powder

Days-n	Different level Substitution of red beetroot powder			
	0	1	2	3
0	$0.45±0.01^a$	$0.52±0.02^b$	$0.58±0.02^c$	$0.67±0.01^d$
1	$1.82±0.80^a$	$1.7±0.14^b$	$1.18±0.09^b$	$1.79±0.02^b$
2	$2.49±0.02^c$	$2.14±0.04^b$	$1.82±0.07^a$	$2.92±0.04^d$
3	$4.5±0.83^d$	$3.11±0.74^b$	$3.16±0.07^a$	$3.62±0.32^c$

Values are presented as mean±SD, *n* = 5. Values with different superscript within the same row are significantly different (*P* < 0.05).

The TBA value was also increasing gradually in patties with BHT 0.1 g/kg in the refrigerator from the 11th day to the 14th day. The increasing TBARS value was 0.5 mg/kg into 3.6 mg/kg (Sabranek *et al*., 2005). The maximum use of BHA on USDA permitted fresh sausages (2000) is 0.1 g/kg. TBA levels permitted based on SNI no. 2352-1991 on TBA Determination state that the maximum product limit is 3 mg/kg sample, and the maximum peroxide is 10 mg eq/kg.

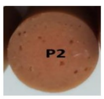
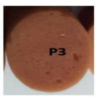
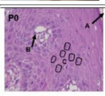
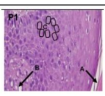
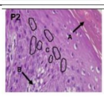
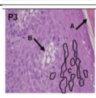
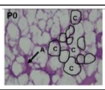
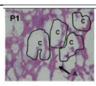
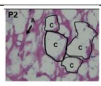
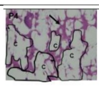
3.3 Microstructure of chicken sausage

The microstructure of chicken sausage is shown in Table 3. Pre-emulsification is the process of producing an oil-in-water emulsion (in the meat batter system) stabilized with an emulsifier (Kang *et al*., 2016). This process could enhance emulsion stability and make ingredients more evenly disperse in the sausage. It was reported that pre-emulsifying oil with a non-meat protein made more meat proteins available for gel structure formation in the continuous phase of the meat batters (Youssef and Barbut, 2011)

The color of the purplish spot is an interpretation of the balance of amylose and amylopectin found in chicken sausage fillers. So, it can be seen that the substitution level that has a purple spot is more evenly distributed (Table 3). Based on Table 3, then the most ideal homogeneity in a row is chicken sausage with a level of substitution red beetroot powder 0>1>2>3. The best microstructure was shown to be tighter than the prepared cavity (Aryanti, 2006) while Barbut (2002) explained that proteins can form different gels depending on pH concentration, ion strength, and temperature.

The factor that affects microstructure is moisture content. The higher water content of the sausage has a positive impact that made the sausage softer than control. If the water content is low it will be harder for the sample. Increasing the level of bits will increase the moisture content (Table 1). The porosity of the material can also occur in samples cooked with sudden pressure changes, resulting in sudden puffing that causes the sample to become porous and have cavities (Rahayoe, 2009). Protein believed to be mostly responsible for functional properties, such as foaming, emulsification, nitrogen solubility, oil, and water absorption (Kinsella, 1979). These properties are affected by the intrinsic factors of protein, such as molecular structure and size, and many environmental factors, including the method of protein separation or production (Yu *et al.,* 2007). According to Kinsella (1979), functional properties are the significant physicochemical properties that are determining the complex interaction between the composition, structure, and molecular conformation.

Table 3. The microstructure of chicken sausage with enlarge 10 times and 100 times

Variable	Different level Substitution of red beetroot powder (%)			
	0	1	2	3
Chicken sausage				
Microstructure with enlarge 10 times				
Microstructure with enlarge 100 times				

A: protein matrix; B: air doplet; C: Emulsified of protein and starch; Purple Spot: starch from flour and or red beetroot powder as a filler. P0 (substitution red beetroot powder 0%), P1 (substitution red beetroot powder 1%), P2 (substitution red beetroot powder 2%), and P3 (substitution red beetroot powder 3%).

4. Conclusion

Based on this study, chicken sausage with the substitution of 2% red beetroot powder not only act as a filler that can improve its chemical quality but can also be a source of natural antioxidants and can inhibit the rate of damage to products due to oxidation during storage. The addition of beetroot powder able to maintain fresh sausage conditions up to 2 days of storage at room temperature.

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