

AQUEOUS LEAF EXTRACT OF SENDUDUK (*Melastoma malabathricum* L.) COULD IMPROVE THE PHYSICOCHEMICAL PROPERTIES OF BEEF SAUSAGE DOUGH

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ABSTRACT

Improving comminuted meat products characteristics using a natural agent, such as phytochemicals, in order to replace the use of nitrite, have become a need due to the health reason. The quality of the sausage is also affected by the initial characteristics of the dough. Therefore, this research was conducted to investigate the effect of aqueous leaf extract of senduduk (*Melastoma malabathricum* L.) on the physicochemical properties of beef sausage dough. Different four formulas as treatment were employed to form the dough: formula A was as a control consisted of beef meat, vegetable oil, skim milk powder, tapioca, salt, phosphate, and seasoning; formula B was control added with extract 0.55%; formula C was control added with sodium nitrite 0.0011%, and formula D was control added with extract 0.55% and sodium nitrite 0.0011%. All ingredients were blended to be the dough. The result of the study denoted that the extract (B and D) significantly decreased ($P<0.05$) pH, and a_w value with no difference in water content among the dough. The total phenolic content of the dough containing extract (B and D) was markedly higher ($P<0.05$) than were others. It increased significantly on antioxidant capacity, scavenging activity, and reduced the thiobarbituric acid reactive substances (TBARS) value of the dough. There was also no nitrite residual detected in all dough. In conclusion, the extract could improve the physicochemical properties of beef sausage dough and replace the use of nitrite in the dough.

Keywords: Antioxidant; nitrite replacement; *Melastoma malabathricum*; physicochemical; sausage

INTRODUCTION

Sausage has been becoming a popular food in Indonesia. It can be found in an abundance of places and levels of society. Sausage has also come to be a daily diet menu of Indonesian. It is a positive circumstance since it can intensify the protein consumption of Indonesia people. Unfortunately, most of the commercial sausages were manufactured by including the synthetic food additive, which has negative side effects. Some are carcinogenic (De Mey *et al.*, 2014), inducing metabolic disorders (Shalaby and Shanab, 2013), and triggering colorectal disease (Herrmann *et al.*, 2015; Zhu *et al.*, 2014).

Recently, the use of natural agents such as phytochemicals as preservative and antioxidant have been rising for food purposes. The increased use of natural agents is due to awareness of the health of consumers. Accordingly, it is critical to shift to a natural agent in which one of them is senduduk (*Melastoma malabathricum* L.) leaf extract (SLE). Traditionally, senduduk leaf is utilized for healing diarrhea, dysentery, wounds, sore legs, and thrush (Susanti *et al.*, 2008). Therefore, the exploration of senduduk leaf is focalized on the pharmacological aspect. Many researchers reported that the SLE contains the phenolic compounds which be able to scavenge free radical and inhibit the oxidation process, as well as anti-bacteria both *in vitro* and *in vivo* assay (Alnajjar *et al.*, 2012; Alwash *et al.*, 2014; Wong *et al.*, 2012; Zakaria *et al.*, 2011). Even, SLE does not cause any acute toxicity and safety (Alnajjar *et al.*, 2012; Alwash *et al.*, 2014; Kamsani *et al.*, 2019). For food purposes, Suharyanto *et al.* (2019) noted that the maceration using water as a solvent also

yielded SLE containing phenolic substances and could inhibit the bacteria growth. This result can promote the intensified efforts of SLE utilization in food and magnify safety and healthy food. The necessity of using aqueous extract is to evade consumers from the negative effects of organic solvent residues in the extract (Anyanwu *et al.*, 2017).

On the other hand, the characteristics of the sausage are also determined by the dough quality. The use of SLE in the dough will in turn influence the final product of the food. Therefore, examining the effect of SLE on the sausage dough is fundamental to provide an impact on the sausage product. The aim of the study was to assess the effect of aqueous leaf extract of senduduk (*Melastoma malabathricum* L.) on the physicochemical properties of beef sausage dough.

MATERIALS AND METHODS

Extract preparation

The senduduk leaf collected from shrub in Bengkulu were purified from undesired materials or substances. The clean leaves were air-dried for 5-6 h at 45°C. The dried leaves were then powdered and sieved into a 35 mesh. The technique of extraction adopted from Doughari and Manzara (2008). The powder (40 g) were macerated using distilled water (400 mL) in 1000 mL Erlenmeyer flask in the dark and room temperature for 24 h. The macerate was filtered using whatman No. 1 filter paper. The filtrate solvent was evaporated using a rotary evaporator (Heidolph, Antrieb-W-Mikro, Germany) at 40°C. The viscous raw extract obtained was freeze-dried (Snijders Scientific, LY5FME, the Netherlands). The extract was stored at -25°C until use.

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Dough preparation

The round meat of Brahman Cross was separated from connective and fat tissue and then was cut into small pieces. The cut meat was minced using a meat mincer and further was formulated with the ingredients as shown in Table 1. Four formulas were employed in the research: formula A as a control consisted of beef, vegetable oil, skim milk powder, tapioca, salt, phosphate, and seasoning; formula B was control added with SLE 0.55%; formula C was control added with sodium nitrite 0.0011%, and formula D was control added with SLE 0.55% and sodium nitrite 0.0011%. All ingredients were blended to be the dough for each formula.

Measurement of pH, a_w , and water content

The dough pH was determined by adopting AOAC (2005) procedure. A total of 10 g of sausage dough was dissolved and

homogenized in 100 mL of distilled water. The solution was filtered using filter paper and the pH filtrate was measured using pH meter (Schott Instrument Lab 850). The measurement of a_w was performed using a_w -meter (Novasina Ms-1) calibrated previously (Lorenzo *et al.*, 2014). The dough was put in the a_w -meter holder and measured. Water content was determined using AOAC (2005) method.

Total phenolic content

Sample preparation was carried out by dissolving 1 g dough into 5 mL of absolute methanol for 24 h (Sukisman *et al.*, 2014). The solution was then filtered and the filtrate was used for determining the total phenolic content by using Al-Saeedi and Hossain (2015) procedure with a slight modification. Briefly, 0.4 mL of the filtrate was reacted with 3 mL of 20% Folin-Ciocalteou (Merck KGaA, Germany) solution and let stand for 5 min.

Table 1. The ingredient of the sausage dough

Materials	Formulas			
	A	B	C	D
Beef meat (g)	500	500	500	500
Vegetable oil (g)	100	100	100	100
Skim milk (g)	30	30	30	30
Tapioca (g)	75	75	75	75
Cubic ice (g)	175	175	175	175
Salt (g)	15	15	15	15
Garlic (g)	8.75	8.75	8.75	8.75
White pepper (g)	1	1	1	1
Nutmeg (g)	2.5	2.5	2.5	2.5
Phosphate (g)	1.5	1.5	1.5	1.5
NaNO₂ (g) (0.0011%)*	-	-	0.01	0.01
Extract (g) (0.55%)*	-	5	-	5

Description: * Based on the total ingredient (908.75 g).

The mixture was added with 3 mL of 10% Na₂CO₃ solution, and then incubated for 60 min in a dark and room temperature. The mixture was absorbed using a spectrophotometer (Agilent, UV-Vis 8453, USA) at a wavelength of 760 nm. A similar procedure was applied to various standard gallic acid concentrations (0-16 mg/mL). The total phenolic content was calculated

using a linear regression equation of the gallic acid absorbance and expressed in mg equivalent gallic acid/100 g dough dry matter.

Antioxidant activity

Sample preparation was employed using Sukisman *et al.* (2014) procedure. One-gram dough was dissolved into 5 mL of

absolute methanol for 24 h. The solution was filtered and the filtrate was used for antioxidant capacity determination (Mahmoudi *et al.*, 2016). As much as 0.2 mL of sample-methanolate solution was mixed with 1.8 mL of DPPH-methanolate solution (Sigma-Aldrich, D9132-1G, Germany) at a concentration of 6×10^{-5} mol/L and shaken for 20 s.

The mixture was incubated in a dark and room temperature for 60 min. It was then measured for absorbance using a spectrophotometer (Agilent, UV-Vis 8453, USA) at a wavelength of 517 nm. In the same way, standard butylated hydroxytoluene (BHT) (Himedia, GRM797-500G, India) solutions in various serial dilutions (0.0-4.5 mg/100 mL) were employed.

Percent scavenging activity was calculated by the formula $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$ where A_{control} was the absorbance of the DPPH solution without dough and A_{sample} was the absorbance of the dough. The antioxidant capacity of the dough was calculated using the linear regression equation of BHT as a standard and expressed as mg equivalent BHT/100 g dry matter.

Thiobarbituric acid reactive substances (TBARS) value

Thiobarbituric acid reactive substances (TBARS) assay was employed for measuring malondialdehyde content by using Turgut *et al.* (2016) procedure. A-5 g dough was homogenized in 15 mL of distilled water and centrifuged at $2000 \times g$ for 15 min. As much as 1 mL of the supernatant of the mixture was added with 2 mL of 0.25 M HCl containing thiobarbituric acid (TBA) (0.375%, w/v) and trichloroacetic acid (TCA) (15%, w/v) and then added with 3 mL of BHT 2%.

The tube was vortexed and incubated at 100°C for 15 min. The mixture was cooled at room temperature and then centrifuged at $1000 \times g$ for 10 min. Finally, it was measured for absorbance using a spectrophotometer (Agilent, UV-Vis 8453,

USA) at a wavelength of 531 nm against the blind. TBARS were calculated using 1, 1, 3, 3-tetraethoxypropane standard curve (concentration 2×10^{-6} to 10×10^{-6} M) and expressed as mg malondialdehyde (MDA)/kg of dough.

Nitrite residual

The nitrite residual of the sausage dough was determined by AOAC (2005) procedure. A-5 g of sausage dough was added with 40 mL of distilled water and heated to reach 80°C. The solution was transferred to a 500 mL volumetric flask and hot distillate water was added until the volume reached 300 mL. The flask containing the solution was put into the steam bath for 2 h and stirring occasionally. The solution was cooled at room temperature and then was filtered.

Twenty-five mL of filtrate was put in the 50 mL volumetric flask, added with 2.5 mL of sulphanolate and stand for 5 min. Then, a reagent of 2.5 mL N- (1-Naphthyl) ethylenediamine (NED) 2 HCl is added and homogenized and reached to the mark. The solution was allowed to stand for 15 min to form pink and then its absorbance was measured using a spectrophotometer (Agilent, UV-Vis 8453, USA) at a wavelength of 540 nm.

An equivalent procedure was applied for blanks of 45 mL of distilled water, 2.5 mL of sulphanilamide solvents, and 2.5 mL of NED solvents. Standard curves from NaNO_2 solution (Sigma-Aldrich, Germany) with a serial concentration of 0.2; 0.4; 0.6; 0.8 $\mu\text{g/mL}$ was applied like the sample. Nitrite content was calculated based on the standard curve regression equation and expressed in mg/kg of dry matter.

Statistical analysis

The experiment was designed by using a completely randomized design with three replications of each treatment. The data presented as mean with standard deviations. The data were analyzed by one-way ANOVA and the differences of treatments were continued with multiple

comparisons Tukey test. The significant difference was set at $p < 0.05$. The statistical analysis using the general linear model of SAS, version 9.3.

RESULTS AND DISCUSSION

Value of pH, a_w , and moisture content

The addition of SLE improved physical characteristics. The data in Table 2 depict that the incorporation of SLE and nitrite in the dough decrease the value of pH, a_w , and water content of the dough. The SLE in the dough was a factor determining the physical properties of the dough. Formula B which is a dough containing SLE had a lower value than control (formula A) in pH and a_w variables. The nitrite addition just only affected on the dough if it was mixed with the SLE (formula D). It was supported by the data Formula C which statistically not different from Formula A. The capability of SLE to lower the pH value of the dough was likely influenced by the phenolic

compounds of the SLE. Aqueous extract of senduduk leaf hold phenolic compounds (Suharyanto *et al.*, 2019) which capable of decreasing pH value of extract (Wang *et al.*, 2015). The lessen of pH value is contributed by the hydroxyl group of phenolic compounds of the extract (Pereira *et al.*, 2009). This result supported other studies of plant extracts that decrease the pH value of food product (Devatkal *et al.*, 2010; Jung and Joo, 2013). It was also confirmed that the SLE pH measured was 4.1 at a concentration 500 mg/mL (data not shown).

The addition with nitrite to formula C also indicated the lower pH than control. Nitrite in meat products dissolves and form HNO_2 (nitrous acid) to produce NO (nitric oxide) and NO_2 (nitrite) (Honikel, 2008). The NO molecule reacts with myoglobin or amino acids, while NO_2 reacts with water to re-form HNO_2 and HNO_3 (nitric acid) (Honikel, 2008). These compounds make the product more acidic (Suryati *et al.*, 2014).

Table 2. The mean of pH, a_w , and water content of the sausage dough added with SLE

Formulas	pH	a_w	Water content (%)
A	5.97±0.08 ^a	0.89±0.005 ^a	63.18±0.77 ^a
B	5.85±0.01 ^b	0.87±0.010 ^{bc}	62.13±0.45 ^{ab}
C	5.89±0.01 ^{ab}	0.88±0.002 ^{ab}	61.67±0.04 ^b
D	5.83±0.02 ^b	0.85±0.004 ^c	62.12±0.14 ^{ab}

Description: Data are shown as mean±standard deviation. Different superscripts in the same column indicate a significant difference ($P < 0.05$).

Water activity (a_w) of the product is one of the essential factors affecting the shelf life. The lower the value of a_w , the better the quality of the food product is obtained. This study elucidated that SLE improved the a_w value of the sausage dough. The inclusion of SLE with or without nitrite combination reduced a_w value of the dough. The control (formula A) gained similar a_w value from nitrite addition (formula C). It indicated that SLE as a determinant of the decline a_w value. The low a_w value of dough added with SLE was due to the addition of extracts. The extract reduced the presence of free water in the dough that most probably caused by phenolic compounds forming

hydrogen bonds with water molecules (Andarwulan and Faradilla, 2012). The water content of the dough added with SLE (Formula B and D) and control (Formula A) had similar value. However, the dough containing SLE also gave similar value to the nitrate-containing dough. Generally, the addition of SLE could not affect the water content of the dough.

In this case, nitrite decreased the water content of the dough. This phenomenon revealed that the quality of the dough added with SLE had better physical quality, which indicated by the lower pH and a_w value. Reducing water content economically will result in the loss of production.

Total phenolic content

The addition of SLE in the sausage dough increased the total phenolic content. The mean of total phenolic content of all dough are shown in Table 3. The result exhibited that formula B reached the highest phenolic content. It is most likely due to the contribution of SLE in the dough. Suharyanto *et al.* (2019) extracted senduduk leaf using water as a solvent and the result exerted phenolic compound in the extract. Other researchers were also found that water extracts of plants yielded plant extract with phenolic compounds held (Anggraini, 2017; Mariem *et al.*, 2014; Nurdiana and Marziana, 2013).

Formula A and C that were not be added with SLE contained comparable phenolic compounds but they were lower than the formula blended with SLE (formula B and D). Although not added with SLE, formula A and C contained phenolic compounds. Those were most likely

contributed by the seasons. Suryati *et al.* (2014) stated that seasons also play a vital role by contributing the phenolic compounds to the product.

The combination of senduduk leaf extract and nitrite reduced precisely extract role in contributing phenolic compounds. The decrease in the total phenolic content of formula D was most probably caused by nitrosation of the phenolic compound (González-Mancebo *et al.*, 2002) and a little water proved to be important in the initial period of the reaction (Ji *et al.*, 2011). Nitrosation might be mediated by the lower pH of the obtained result. González-Mancebo *et al.* (2002) stated that nitrosation could be inhibited by the rising pH value. The study exhibited that the combination of SLE and nitrite exerted the lowest pH value of the dough (as shown in Table 2). This condition promoted the lower of existing phenolic compounds through a nitrosation process.

Table 3. The mean of total phenolic content, scavenging activity, and antioxidant capacity of the sausage dough added with SLE

Formula	Total phenolic content (mg GAE/100 g dry matter)	Scavenging activity (%)	Antioxidant capacity (mg BHTE/100 g dry matter)
A	130.12±10.24 ^c	24.24±2.65 ^b	36.11±2.11 ^b
B	180.65±1.78 ^a	43.19±2.85 ^a	62.90±1.49 ^a
C	124.05±10.70 ^c	25.86±1.44 ^b	37.09±0.45 ^b
D	143.02±10.63 ^b	42.49±1.83 ^a	62.28±0.74 ^a

Description: Data are shown as mean±standard deviation. Different superscripts in the same column indicate a significant difference (P<0.05); GAE = Gallic acid equivalent; BHTE = BHT equivalent.

Antioxidant capacity and scavenging activity

The existence of phenolic compounds in the dough added with SLE affected the antioxidant capacity and scavenging activity of the dough. Antioxidant capacity expresses the concentration of antioxidants in the extract that equivalent to BHT. Scavenging activity shows the percentage of the capability of antioxidant in the extract to scavenge DPPH free radical. These data are shown in Table 3. The pattern of the antioxidant capacity and scavenging activity were similar to those phenolic compounds

of the dough. The higher phenolic compounds held in the dough the higher the antioxidant capacity and scavenging activity of the dough. This study indicated that the addition of SLE significantly enhanced the antioxidant capacity and scavenging activity of the dough as shown by formula B and D. This result revealed that SLE in the dough played a role in increasing antioxidant activity.

This role was acted by phenolic compounds of the SLE. As mentioned above, the SLE contained phenolic compounds and these compounds could

increase the antioxidant activity of plant extract (Alnajjar *et al.*, 2012; Zakaria *et al.*, 2011). This research result also showed that nitrite had a low effect on the antioxidant activity (antioxidant capacity and scavenging activity) of the dough. The antioxidant activity was not different from control (formula A).

Nitrites can actually act as antioxidants (Karwowska *et al.*, 2020). The addition of nitrite can form NO which in the lipid oxidation mechanism will form ROONO (Patel *et al.*, 2000), but the role of these antioxidants is probably inhibited by the presence of phenolic compounds. This is because NO can react with phenolic compounds to form nitroso compounds from phenolic (González-Mancebo *et al.*, 2002). These derived compounds apparently cannot act as antioxidants (Zubillaga *et al.*, 1984).

TBARS value and nitrite residual

The result of the study showed that the TBARS value was significantly different lower of dough added with SLE from not

added. The lower value of TBARS indicating the lower MDA production. MDA is one of lipid oxidation and this is a marker to determine the lipid oxidation. TBARS is indicated by the MDA production and expressed as mg MDA/kg of dough (Table 4).

The low TBARS value of dough added with SLE due to phenolic compounds in SLE acts as an antioxidant by preventing the oxidation process (Alnajjar *et al.*, 2012; Alwash *et al.*, 2014; Zakaria *et al.*, 2011). The TBARS value of the dough added with SLE confirmed that the phenolic compounds of the SLE capable of antioxidants role. The capability of phenolic compounds is due to the hydroxyl group structure of these compounds (Bendary *et al.*, 2013). The phenolic compounds donate hydrogen and react to reactive species in the termination reaction to break down the new radical formation cycle (Pereira *et al.*, 2009). However, TBARS values of all doughs were below the detectable threshold of rancidity, which is 5 mg MDA/kg (Insausti *et al.*, 2001).

Table 4. The mean of TBARS value and nitrite residual of the sausage dough added with SLE

Formula	TBARS (mg MDA/kg dry matter)	Nitrite residual (mg/kg dry matter)
A	3.08±0.075 ^a	Nd
B	1.41±0.018 ^d	Nd
C	1.55±0.046 ^c	Nd
D	1.96±0.060 ^b	Nd

Description: Data are shown as mean±standard deviation. Different superscripts in the same column indicate a significant difference (P<0.05); Nd = not detected.

Any nitrite residues in all doughs were not detected as shown in Table 4. This condition was possible due to the amount added was very little so that it was below the detectable threshold of tools and methods. Honikel (2008) stated that the nitrite concentration will decrease by 65% from the time of incorporation to the heating process finished. In formula D, it was thought to be due to the reduction process by phenolic compounds. Some previous research results suggested that phenolic compounds could reduce nitrites in Dendeng products (Suryati *et al.*, 2014) and dry-cured bacon (Wang *et*

al., 2015). The factors that caused the detection of nitrite residues in this study were quite complex. Detection of nitrite residues did not indicate that this product is safe from the negative effects of nitrites. The maximum limit of nitrite residual is 30 mg/kg product weight (BPOM, 2019).

CONCLUSION

The addition of aqueous extract of senduduk leaf improved the physical and chemical properties of the raw sausage dough. The addition of 0.55% senduduk leaf

extract decreased the pH, a_w , and water content of the dough; enhanced the total phenolic content, antioxidant activity of the dough; and reduced the TBARS value of the dough. The use 0.55% of senduduk leaf extract could replace nitrite 0.0011% in the dough physicochemical characteristics. The good characteristics of the dough will generate a good properties of final product.

CONFLICT OF INTEREST

We declare that there are no conflict of interest in this work.

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