International Journal of Food Studies | IJFS October 2022 | Volume 11 | pages 320–334

Antioxidant, Antimicrobial and Physicochemical Properties of Beef Sausages Enriched with an Aqueous Extract of Senduduk (*Melastoma malabathricum* L.) Leaf

Suharyanto[a*], Henny Nuraini[b], Tuti Suryati[b], Irma Isnafia Arief[b], and Dondin Sajuthi[c]

[a] Department of Animal Science, Faculty of Agriculture, University of Bengkulu, Jl. WR. Supratman Kandang Limun, Kec. Muara Bangka Hulu, Kota Bengkulu, Bengkulu, Indonesia, 3371A
[b] Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Jl. Agatis, Babakan, Kec. Dramaga, Bogor, Jawa Barat, Indonesia, 16680
[c] Department of Veterinary Clinic Reproduction and Pathology, Faculty of Veterinary, IPB University, Jl. Agatis, Babakan, Kec. Dramaga, Bogor, Jawa Barat, Indonesia, 16680
*Corresponding author
suharyanto@unib.ac.id

Received: 25 August 2021; Published online: 18 October 2022 Check for updates

Abstract

The use of natural products in sausages has become a new trend for health reasons. A natural product that could be incorporated into sausages is an extract of the senduduk (*Melastoma malabathricum* L.) leaf. Senduduk is an abundant shrub herb in Indonesia. This kind of plant is mostly used as a traditional medical remedy and as an ingredient in some culinary recipes. This study was carried out to investigate the effect of an aqueous extract of senduduk leaf (SLE) on the antioxidant, antimicrobial and physicochemical properties of beef sausage. Four treatments were used: ingredients consisting of beef, vegetable oil, skim milk, tapioca, salt, phosphate, ice cubic, garlic, pepper, dan nutmeg as a Control; the Control ingredients plus 0.01% of butylated hydroxytoluene (BHT); the Control ingredients plus 0.83% of SLE (SLE-1), and the Control ingredients plus 1.1% of SLE (SLE-2). All ingredients of each formula were homogenously blended and the sausage mix was cooked. The addition of BHT and SLE affected the proximate composition, with the moisture content decreasing as the duration of chilled storage increased. The addition of SLE lowered the pH and a_w value and both tended to increase during chilled storage. SLE also enhanced the WHC of the sausages which increased in value during chilled storage. The addition of BHT and SLE could increase the antioxidant activity of the sausages as indicated by scavenging DPPH free radicals. SLE in sausages could inhibit microbial growth during chilled storage. It can be summarized that the addition of an aqueous extract of senduduk could improve the physicochemical, antioxidant and antimicrobial properties of beef sausages.

Keywords: Antioxidant; Antibacterial; Beef sausages; *Melastoma malabathricum*; Physicochemical

1 Introduction

The development of the sausage industry in Indonesia has a positive impact for the population by increasing the consumption of animal protein. From a nutritional perspective, sausage is rich in protein with a high biological value (Tran et al., 2020). However, sausages have limitations related to high-fat content and high-water activity (Boeira et al., 2020) which can undergo lipid oxidation and microbial contamination (Domínguez et al., 2019). Lipid oxidation and microbial con-

 10.7455/ijfs/11.2.2022.a6

tamination in the sausage can lead to deterioration in physical and sensorial properties (de Carvalho et al., 2020; Luong et al., 2020).

Several measures have been employed to reduce such deterioration and the most popular application is by use of synthetic agents. Unfortunately, these substances are associated with negative side effects on humans such as carcinogenic (Gultekin et al., 2015), triggering colorectal disease (Herrmann et al., 2015), intestinal and metabolic disorders, and also cardiovascular disease (Partridge et al., 2019). Application of plant extracts has also been extensively employed in meat products for health reasons (de Carvalho et al., 2020; Hung et al., 2016; Pateiro et al., 2021; Tran et al., 2020).

Plants are rich in polyphenols which play essential roles as antioxidants and antimicrobials. One of the potential plants to find greater use for food purposes is senduduk (*Melastoma malabathricum* L.). This plant is a shrub which is abundantly found in Indonesia and used for folk medicinal and food purposes (Susanti et al., 2008; Thatoi et al., 2008). A senduduk leaf extract (SLE) could act as a natural antioxidant and antimicrobial (Alwash et al., 2014; Wong et al., 2012; Zakaria et al., 2011), without causing any toxicity (Alnajar et al., 2012; Alwash et al., 2014; Kamsani et al., 2019).

For food purposes, the extraction process should use a solvent such as water which is not harmful to humans. An aqueous extract of senduduk leaf has antibacterial capability and antioxidant activity (Suharyanto et al., 2019). A SLE could improve the physicochemical properties of a beef sausage mix and replace the use of nitrite in the formulation (Suharyanto et al., 2020). This study aimed to investigate the effect of an extract of senduduk leaf on the physicochemical, antioxidant and antimicrobial properties of beef sausages.

2 Materials and Methods

2.1 Extract preparation

The senduduk leaves were cleaned of undesired materials and then air-dried for 5-6 h at 45 ^{o}C. The leaves were powdered into a 35 mesh. The extraction method was adapted from Doughari and Manzara (2008). Briefly, the powder (40 g) was macerated in distilled water (400 mL) in a 1000 mL Erlenmeyer flask for 24 h. The macerate was filtered using Whatman No. 1 filter paper and evaporated using a rotary evaporator (Heidolph, Antrieb-W-Mikro, Germany) at 40^{o}C. The viscous raw extract was freeze-dried (Snijders Scientific, LY5FME, the Netherlands). The extract of senduduk leaf (SLE) was stored at -25^{o}C until use.

2.2 Preparation of sausages

The Brahman cross round meat, free of connective and fat tissue, was cut into small pieces and then minced using a meat mincer. Ingredients used in the formulation of beef sausages are shown in Table 1. Four treatments were employed in the study: ingredients consisting of beef, vegetable oil, skim milk, tapioca, salt, phosphate, ice cubic, garlic, pepper and nutmeg as a Control; the Control plus 0.01% of BHT (BHT); the Control plus 0.83% of SLE (SLE-1) and the Control plus 1.1% of SLE (SLE-2).

The procedure used to prepare sausages was that of Arief et al. (2014), with slight modification. Briefly, all ingredients were blended homogeneously to form a mix for each treatment. The emulsified mix was filled into casings (food grade polyamide plastic with a diameter of 16 mm). The raw sausages were steamed at 65 ^{o}C for 45 min. The cooked sausages were stored at 4 ^{o}C and observed at 0, 6, 12 and 18 days of storage.

2.3 Proximate composition determination

Proximate composition was determined using AOAC (AOAC, 2005) methods on day 0 of storage. Moisture, crude protein and crude fat contents were determined by oven drying at 105 ^{o}C, the Kjeldahl method and the soxhlet method, respectively. The carbohydrate content was calculated by difference.

Table 1: Formulations of the sausage mixes.

Ingredients	Formula			
	Control	BHT	SLE-1	SLE-2
Beef (g)	500	500	500	500
Vegetable oil (g)	100	100	100	100
Skim milk (g)	30	30	30	30
Tapioca flour (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
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
BHT (g)	**-**	**0.09 (0.01%)***	**-**	**-**
Extract (g)	**-**	**-**	**7.5 (0.83%)***	**10 (1.1%)***

* based on total mass of ingredients in the formulation (908.75 g)

2.4 pH value determination

The pH value was measured using the AOAC (AOAC, 2005) procedure. 10 g of crushed sausage was mixed into 100 mL of distilled water. The solution was filtered and then the pH of the filtrate was measured using a pH meter (Schott Instrument Lab 850).

2.5 Water activity determination

The water activity (a_w) was measured using the Lorenzo et al. (2014). The sufficiently crushed sausage was placed in the container and its a_w value was measured at 25 ^oC using a calibrated a_w-meter (Novasina Ms-1).

2.6 Water holding capacity determination

The water holding capacity (WHC) was determined using the Jung and Joo (2013) procedure, with a minor modification. 2.5 g of the crushed sausage was placed in a centrifugation tube, to which 10 mL of distilled water was added, and then incubated at 30 ^oC for 30 min. The supernatant was removed and the residual crushed sausage reincubated at 30 ^oC for 10 min. Finally, the remaining supernatant was removed. The WHC was calculated by the formula as shown below.

$$WHC(\%) = \frac{Weight\ of\ sample\ without\ supernatant}{Weight\ of\ sample\ with\ water\ added} \times 100 \quad (1)$$

2.7 Total phenolic content

Sample preparation was carried out according to the Sukisman et al. (2014) procedure by dissolving and homogenizing 1 g of crushed sausage in 5 mL of methanol for 24 h. The filtrate of the solution was used to determine the total phenolic content according to the Al-Saeedi and Hossain procedure (Al-Saeedi & Hossain, 2015), with a minor modification. 0.4 mL of the filtrate was mixed with 3 mL of 20% Folin-Ciocalteou solution (Merck KGaA, Germany) and left to stand for 5 min. Then, the mixture was reacted with 3 mL of 10% Na2CO3 and incubated for 60 min in the dark and at room temperature. The absorbance of the mixture was measured using a spectrophotometer (Agilent, UV-Vis 8453, USA) at 760 nm wavelength. An identical technique was employed with several standard gallic acid concentrations (0-16 mg mL^{-1}). A linear regression equation of the gallic acid absorbance was used to calculate the total phenolic content of the sample and expressed in mg equivalent gallic acid [100 g] $^{-1}$ dry matter.

2.8 Antioxidant activity

The antioxidant activity was determined using the Mahmoudi et al. procedure (Mahmoudi et al., 2016). 0.2 ml of the prepared sample, according to the Sukisman et al. procedure (Sukisman et al., 2014), was reacted with 6 $\times 10^{-5}$ mol L^{-1} of 1.8 mL of DPPH solution (Sigma-Aldrich, D9132-1G, Germany), and then shaken gently for 20 s. The solution was left to stand in a dark place and at room temperature for 60 min. The absorbance of the solution was then measured using a spectrophotometer (Agilent, UV-Vis 8453, USA) at a wavelength of 517 nm. Standard butylated hydroxytoluene (BHT) (Himedia, GRM797-500G, India) solutions, at various serial dilutions (0.0-4.5 mg [100 mL]$^{-1}$), were employed using the same technique.
Antioxidant activity was expressed by scavenging percentage and antioxidant capacity. The scavenging activity was calculated by the following formula:

$$Scavenging\ activity(\%) = \frac{Acontrol - Asample}{Acontrol} \times 100 \qquad (2)$$

where Acontrol was the absorbance of the DPPH solution without sample and Asample was the absorbance of the sausage. The antioxidant capacity of the sample was calculated using the linear regression equation of BHT as a standard and expressed as mg equivalent BHT [100]$^{-1}$ g dry matter.

2.9 Thiobarbituric acid reactive substances (TBARS) assay

Malondialdehyde (MDA) was determined using the TBARS assay, according to the Turgut et al. method (Turgut et al., 2016). 5 g of crushed sausage was homogenized in 15 mL of distilled water and then centrifuged at 2000 × g for 15 min. One mL of the supernatant was reacted with 2 mL of 0.25 M HCl, containing 0.375% (w/v) Thiobarbituric acid (TBA) (Merck, KgaA, Germany) and 15% (w/v) Trichloroacetic acid (TCA) (Merck, KgaA, Germany), and then 3 mL of 2% Butylated Hydroxytoluene (BHT) was added. The mixture was vortexed and incubated at 100 C for 15 min. The mixture was cooled at room temperature and then centrifuged at 1000 × g for 10 min. A similar procedure was employed with the various concentrations (2×10^{-6} to 10×10^{-6}) of the 1,1,3,3-tetraethoxypropane (TEP) (Sigma-Alrdrich, Germany) standard. The absorbance of all samples and standard mixtures were measured using a spectrophotometer (Agilent, UV-Vis 8453, USA) at 531 nm wavelength. TBARS were calculated using the TEP standard curve and expressed as mg malondialdehyde (MDA) kg^{-1} of sausage.

2.10 Microbiological activity

Microbiological activity was determined by the pour method according to Arief et al. (2014). Aseptically, 25 g of the crushed sausage was homogenized in 225 mL of sterile buffered peptone water (Oxoid, UK). Serial dilutions of this suspension (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) were then prepared. 1 ml was pipetted into a sterile Petri dish, for each series of the dilution and then 15-20 mL of plate count agar (Oxoid, UK) media was poured to determine the total plate count. In a similar way, in different Petri dishes, 15-20 of Eosin Methylene Blue Agar (Oxoid, UK) was poured to determine *Escherichia coli* and 15-20 of Xylose-Lyxine Deoxycholate Agar (Oxoid, UK) was poured to determine *Salmonella sp.* 100 ul of each dilution was pipetted into sterile Petri dishes and then 15-20 mL of with Baird Parker Agar (Oxoid, UK) was poured to determine *Staphylococcus aureus*. Once set, Petri dishes were incubated at 37 ^{o}C for 24-4 h. Then, the colonies formed were counted.

2.11 Statistical Analysis

A completely randomized experimental design was used and data were analyzed by one-way ANOVA. Tukey's multiple comparison test was used to determine if there were significant differences (P<0.05) between treatments.

3 Results and Discussion

3.1 Proximate composition

The proximate composition of the sausage is presented in Table 2. Addition of BHT and SLE up to 1.1% had no effect on the moisture content (P>0.05), however, the ash content of sausage, with added SLE, was higher (P<0.05) than for the Control and BHT sausages. SLE-2 and Control sausages contained similar fat and protein contents, and both were lower (P<0.05) compared to BHT and SLE-1 sausages. The BHT sausage had the lowest protein content and the SLE-1 sausage had the lowest carbohydrates content. The significant effect of SLE-1 and SLE-2 on some measures of proximate composition of sausage was most probably caused by the plant aqueous extracts.

3.2 Moisture content, pH, a_w and WHC

The addition of plant extract influences the proportion of ingredients in sausages. Therefore, it will affect the physical properties of sausages. The moisture content of sausages during cold storage is presented in Figure 1. There was no interaction (P>0.05) between the storage period and the formulation of sausage. The SLE-2 sausage had lower moisture content than the Control sausage (P<0.05). BHT and SLE-1 sausages were not significantly different from the Control. During cold storage, water vapor from the product surface migrates to the surroundings (El-Nashi et al., 2015). The lower moisture content of SLE-2 sausages was most possibly caused by the increased volume of solids so that the moisture content decreased.

Figure 2 shows that there was a significant interaction (P<0.05) between the chilled storage period and the sausage formulation on pH value. SLE-1 and SLE-2 sausages had a lower pH value (P<0.05) than the Control and BHT sausages. The low pH value of SLE-1 and SLE-2 sausages was most probably due to the SLE containing phenolic compounds (Fernandes et al., 2018; Suharyanto et al., 2019; Susanti et al., 2008; Wang et al., 2015; Wong et al., 2012). The decline in the pH value of SLE-1 and SLE-2 sausages was due to the phenolic compounds in the extract donating hydrogen so that the substance became acidic (Andarwulan & Faradilla, 2012).

The a_w values in this study showed an interaction between storage period and sausage formulation (P<0.05). In Figure 3, the SLE-1 and SLE-2 sausages had lower a_w values (P<0.05) compared to the Control. The a_w values increased on the 12th and 18th days of storage except for SLE-2 sausages. The low values of a_w in SLE-1 and SLE-2 sausages were thought to be due to the content of phenolic compounds in the extracts binding water molecules. Phenolic compounds contain many hydroxyl groups and can form hydrogen bonds with water molecules (Andarwulan & Faradilla, 2012) so that the presence of free water is reduced and results in a decreasing a_w value.

Water holding capacity (WHC) describes the ability of a matrix to bind water in the matrix or added water. The WHC during chilled storage is presented in Figure 4. On day 0, the WHC of SLE-1 and SLE-2 sausages was higher than the Control and BHT sausages. After 6 days of storage, the WHC of SLE-1 and SLE-2 sausages were lower than Control WHC and BHT sausages. SLE-2 sausages continued to degrade up to 18 days of storage. Although it decreased, the WHC value of SLE-2 sausages remained higher than the Control sausages until the 18^{th} day of storage.

3.3 Total phenolic content, radicals scavenging and antioxidant capacity

The total phenolic content of sausages is shown in Figure 5. The Control sausages contained the lowest phenolic content (P<0.05) when compared to sausages enriched with antioxidant agents (BHT, SLE-1, and SLE-2). The BHT sausage contained the highest total phenolic content, while SLE-1 and SLE-2 sausages had equivalent total phenolic contents. All sausages decreased in total phenolic content during storage (P<0.05). The total phenolic content on the 18^{th} day of storage declined by 12.40%, 8.50%, 9.14%

Table 2: Proximate composition of sausages enriched with an antioxidant agent on day 0 of storage

Treatments	Moisture	Ash	Fat	Protein	Carbohydrate
			%		
Control	63.66±0.26[a]	2.95±0.04[b]	5.96±0.22[b]	12.55±0.11[b]	14.88±0.11[a]
BHT	63.54±0.34[a]	2.97±0.02[b]	6.94±0.03[a]	11.88±0.09[c]	14.66±0.40[a]
SLE-1	63.45±0.33[a]	3.23±0.02[a]	7.26±0.06[a]	13.21±0.21[a]	12.85±0.49[b]
SLE-2	63.39±0.18[a]	3.27±0.01[a]	5.80±0.14[b]	12.49±0.33[b]	15.05±0.64[a]

Each value is expressed as mean ± standard deviation (n = 3). The different letter in the same column indicates significantly different (P<0.05).

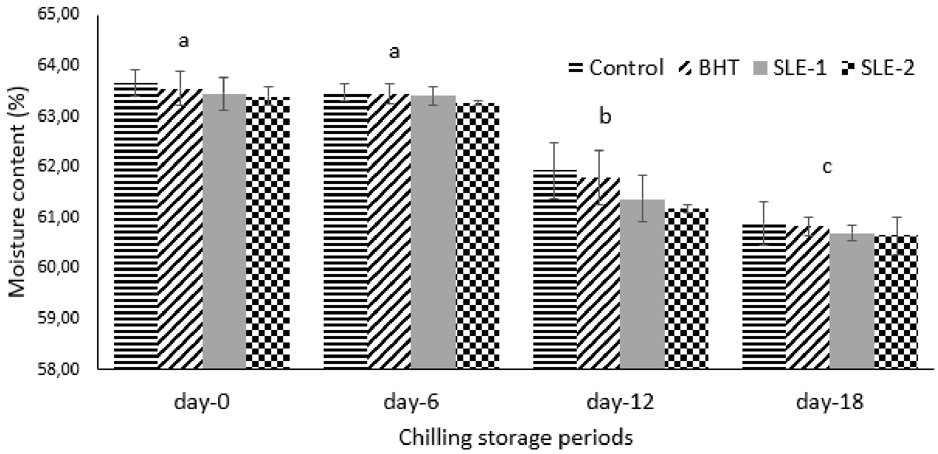

Figure 1: The moisture content of sausages enriched with an antioxidant agent during chilled storage (4 ± 1^{o}C).

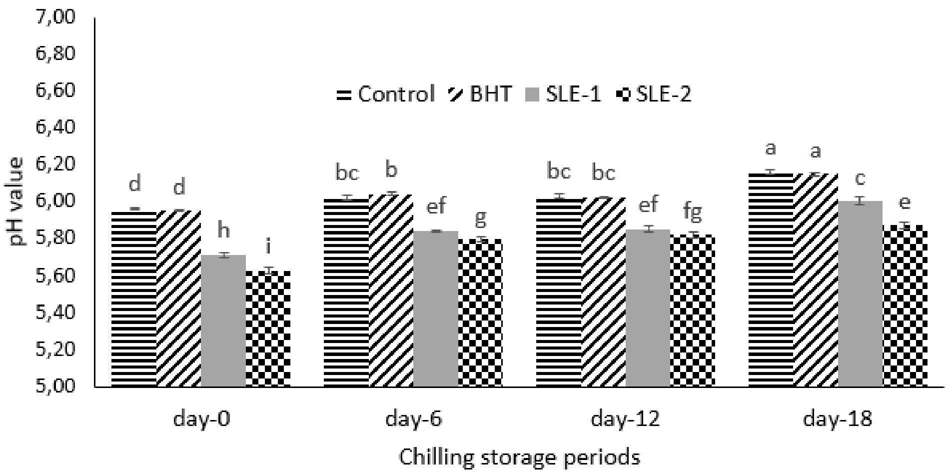

Figure 2: The pH value of sausages enriched with an antioxidant agent during chilled storage (4 ± 1^{o}C).

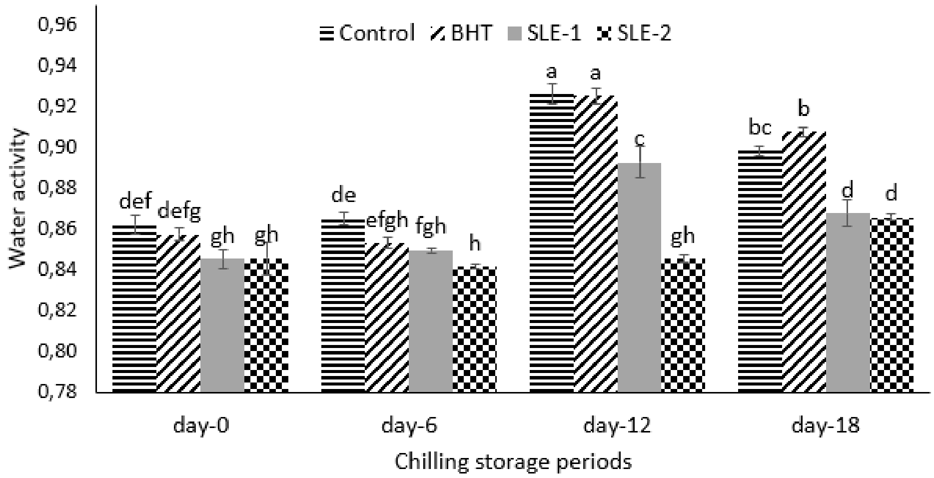

Figure 3: The water activity of sausages enriched with an antioxidant agent during chilled storage (4 ± 1^{o}C).

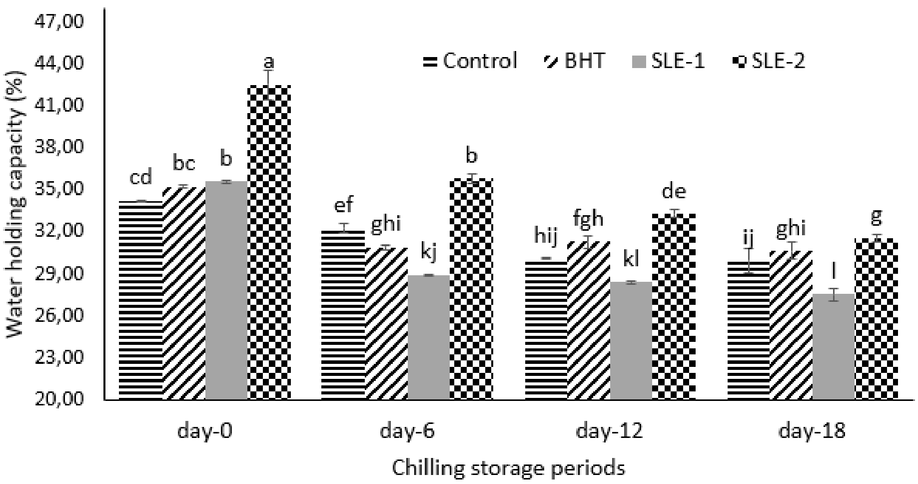

Figure 4: The water holding capacity of sausage enriched with an antioxidant agent during chilled storage (4 ± 1^{o}C).

and 9.05% for the Control, BHT, SLE-1 and SLE-2 sausages, respectively. The phenolic content in the Control sausage probably came from the spices. Spices such as garlic, pepper, nutmeg and others are rich in phenolic compounds (Suryati et al., 2014). The high content of total phenolic in SLE-1 and SLE-2 sausages might be caused by the addition of SLE. This is reasonable because the leaves contain a lot of phenolic compounds (Suharyanto et al., 2019; Susanti et al., 2008; Wong et al., 2012).

This research indicated that the total phenolic content of the sausage influences DPPH radicals scavenging and antioxidant capacity. Figures 6 and 7 show that the Control sausage had the lowest DPPH radical scavenging and antioxidant capacity ($P<0.05$), respectively. The SLE-1 and SLE-2 sausages had comparable DPPH radicals scavenging and antioxidant capacity to BHT sausages. All sausages underwent a decrease in their DPPH radical scavenging ability and antioxidant capacity during chilled storage. The decline was in line with the decrease in the total phenolic content of the sausages during storage. The phenolic compounds worked as antioxidants. A similar pattern of decline was also observed in the antioxidant capacity of sausages (Figure 7). The antioxidant capacity of all sausages decreased during chilled storage ($P<0.05$). Whilst the antioxidant capacity of Control sausages decreased in each storage period, the BHT, SLE-1 and SLE-2 sausages only decreased their antioxidant capacity on the 12^{th} and 18^{th} days of storage. Over 18 days of chilled storage, the antioxidant capacity of the Control, BHT, SLE-1 and SLE-2 sausages reduced by 43.37%, 11.76%, 12.06% and 10.11%, respectively. BHT, SLE-1 and SLE-2 sausages had equivalent antioxidant capacities except for the 6^{th} day of storage, whilst the BHT sausages had higher antioxidant capacities. The antioxidant capacity of the Control sausage was the lowest ($P<0.05$). This phenomenon is confirmed by the total phenolic content in each sausage.

The high percentage of DPPH scavenging and antioxidant capacity in BHT, SLE-1 and SLE-2 sausages was hypothesized to be due to the addition of antioxidant agents to the sausage formulation. BHT is a synthetic compound that contains a phenolic group and has an effective ability as an antioxidant. The SLE also contains phenolic compounds and plays an essential role as an antioxidant (Alwash et al., 2014; Suharyanto et al., 2019; Susanti et al., 2008; Wong et al., 2012).

3.4 Thiobarbituric acid reactive substances (TBARS)

TBARS value indicates the level of oxidation of a product. The lower the TBARS value of a sample, the lower oxidation of a product. Sausages enriched with antioxidant agents (BHT, SLE-1 and SLE-2) showed significantly lower TBARS values ($P<0.05$) than the Control. On day 0 of storage, BHT sausages had the lowest TBARS value. Yet, on days 6 and 12, it was not markedly different from SLE-2 sausages ($P>0.05$) but lower than SLE-1 sausages ($P<0.05$). However, on the 18th day of storage, these sausages had TBARS values that were not notably different. In general, all sausages underwent an increase in TBARS value. It indicates the accumulation of oxidation products in the sausages during storage. Although the TBARS value of the Control sausage was quite high, it was still below the detectable rancidity threshold of 5 mg MDA/kg (Insausti et al., 2001). The low TBARS values in SLE-1 and SLE-2 sausages indicates that the extract acted as an antioxidant (Alnajar et al., 2012; Alwash et al., 2014; Suharyanto et al., 2019; Zakaria et al., 2011). This ability was most likely contributed by the phenolic compounds of the extract (Jin et al., 2015; Kalem et al., 2017; Zhang et al., 2017).

Phenolic compounds are capable of scavenging DPPH radicals and have adequate antioxidant capacity so that they are able to inhibit oxidation characterized by low TBARS values. These capabilities are due to the phenolic compounds which have redox potential to absorb and neutralize free radicals, inhibit singlet oxygen and decompose peroxides (Kalem et al., 2017). This mechanism takes place by transferring the H atom from the OH group of the phenolic compounds to the peroxyl radical chain where the next reaction occurs with the resultant peroxyl (Bendary et al., 2013). Phenolic compounds can also donate hydrogen to react with reactive oxygen and nitrogen species in the termination reaction and play a role in

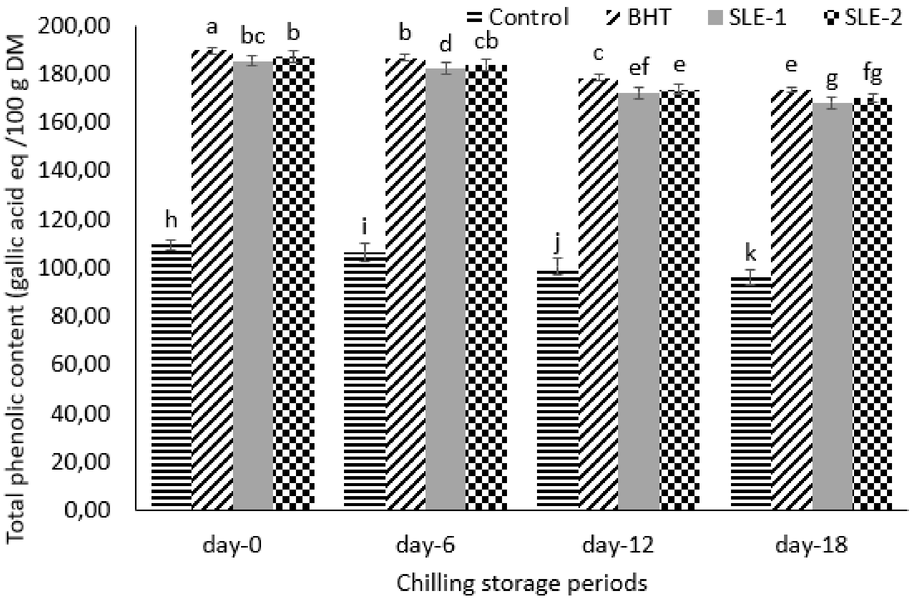

Figure 5: The total phenolic content of sausages enriched with an antioxidant agent during chilled storage (4 ± 1^{o}C).

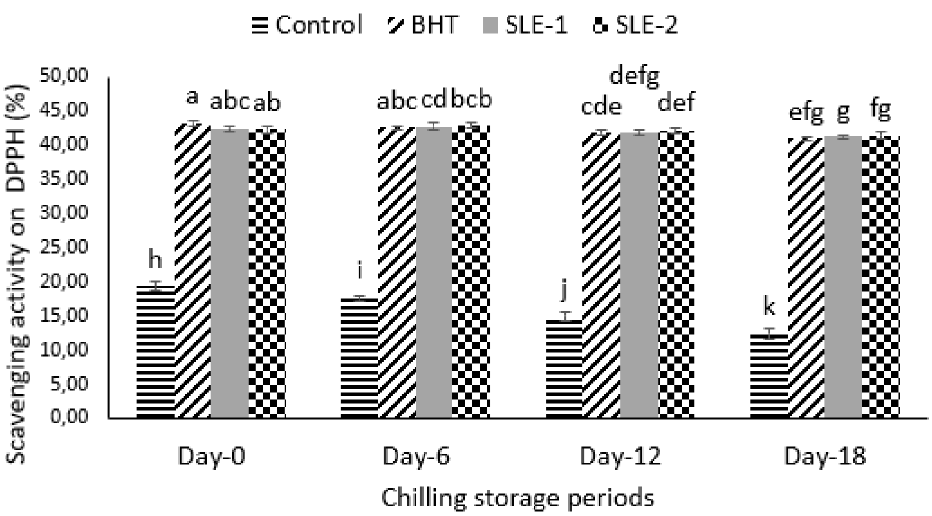

Figure 6: The scavenging activity on DPPH of sausages enriched with an antioxidant agent during chilled storage (4 ± 1^{o}C).

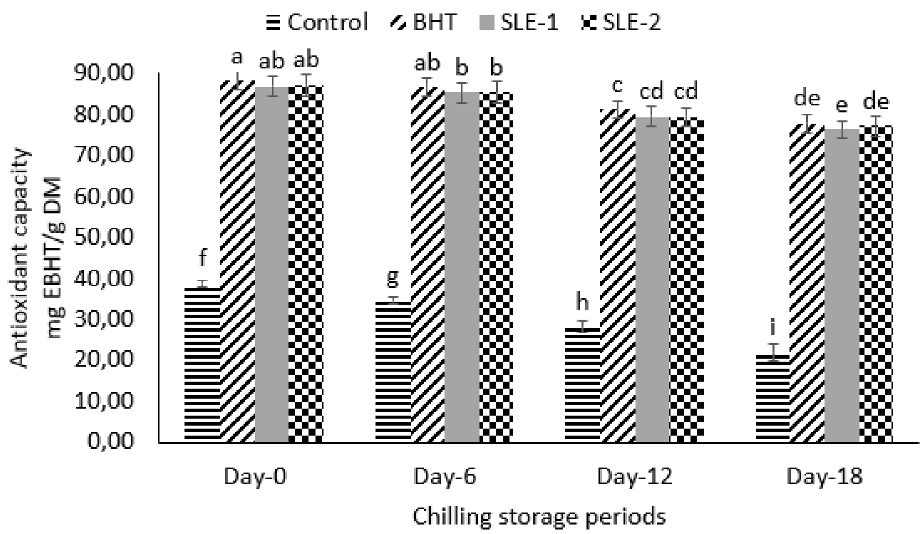

Figure 7: The antioxidant capacity of sausages enriched with an antioxidant agent during chilled storage (4 ± 1^oC).

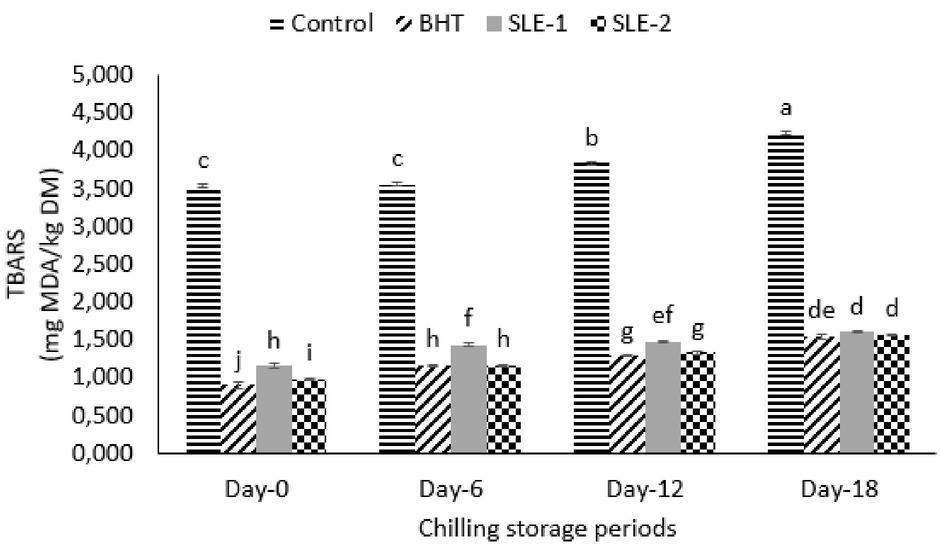

Figure 8: The TBARS of sausages enriched with an antioxidant agent during chilled storage (4 ± 1^oC).

Table 3: Bacterial growth in sausages enriched with an antioxidant agent during chilled storage (4 ± 1^{o}C).

Treatments	Day-0	Day-6	Day-12	Day-18
Total Plate Count (CFU g^{-1}):				
Control	nd	2.7×10^{1}	2.0×10^{2}	3.6×10^{2}
BHT	nd	$<10^{1}$	1.6×10^{2}	3.0×10^{2}
SLE-1	nd	nd	2.0×10^{1}	2.7×10^{1}
SLE-2	nd	nd	10^{1}	2.7×10^{1}
Staphylococcus aureus(CFU g^{-1}):				
Control	nd	nd	$<10^{1}$	2.7×10^{1}
BHT	nd	nd	$<10^{1}$	2.0×10^{1}
SLE-1	nd	nd	$<10^{1}$	2.0×10^{1}
SLE-2	nd	nd	$<10^{1}$	1.7×10^{1}
Salmonella (CFU g^{-1}):				
Control	nd	nd	nd	$<10^{1}$
BHT	nd	nd	nd	$<10^{1}$
SLE-1	nd	nd	nd	nd
SLE-2	nd	nd	nd	nd
E. coli (CFU g^{-1}):				
Control	nd	nd	nd	nd
BHT	nd	nd	nd	nd
SLE-1	nd	nd	nd	nd
SLE-2	nd	nd	nd	nd

CFU – colony forming unit, nd – not detected.

breaking the cycle of new radical formation. The radicals formed from the reaction are more stable than the initial radicals (Pereira et al., 2009).

3.5 Microbiological activity

Microbiological activity shows the extent of SLE's effect on the microbiological quality of sausages. The role of SLE as an antibacterial has been known through exploratory studies (Alnajar et al., 2012; Alwash et al., 2014; Wong et al., 2012). Bacterial growth in sausage enriched with an antioxidant agent during chilled storage (4 ± 1^{o}C) is presented in Table 3. Sausages without the addition of SLE (Control and BHT) grew bacteria (total plate count) on the 6th day of storage, while no microorganisms were detected in sausages with added SLE (SLE-1 and SLE-2). The longer the storage period, the higher the total plate count. On the 18th day of storage, SLE-1 and SLE-2 sausages reached log 1 colonies but the Control and BHT sausages reached log 2 colonies.

On the 12th day of storage, all sausages grew less than 1 log of *Staphylococcus* colonies. This bacterial colony developed up to the 18th day of storage, with a population of about 1 log (Tables 3). *Salmonella sp.* bacteria colonies grew on the Control and BHT sausages on the 18th day of storage with less than one log, while no *Salmonella sp* colonies were detected in SLE-1 and SLE-2 sausages until the end of the observation (Table 3). The results of this study also showed that *E. coli* bacteria were not detected in all sausages in each storage period.

The addition of 0.83% (SLE-1) and 1.1% (SLE-2) was able to inhibit the growth of several pathogenic bacteria in sausages until the 18th day of chilled storage. This was most likely due to phenolic compounds contained in SLE (Susanti et al., 2008; Wong et al., 2012) which can act as antibacterial agent (Alnajar et al., 2012; Alwash

et al., 2014; Zakaria et al., 2011).
In general, all sausages meet the requirements of the Indonesian National Standard (SNI) except for the Control and BHT sausages where less than 10^1 *Salmonella* colonies were detected. Based on the SNI for sausages, the maximum total plate count is 1×10^5 CFU g^{-1}, the maximum *Staphylococcus* is 1×10^2 CFU g^{-1}, *Salmonella* must be negative, and the *E. coli* must be less than 3 MPN (most probable number) g^{-1} (BSN, 2015).

4 Conclusions

The addition of an extract of senduduk leaf up to 1.1% of the total mass of ingredients in the formulation improved the physicochemical properties of sausages, and inhibited oxidation and microbial growth in sausages until the 18th day of chilled storage. The ability to retard oxidation was equivalent to 0.01% BHT.

Acknowledgements

Gratefulness is expressed to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the financial support of the first-named author through a Dissertation Research Grant (Contract no. 061/ADD/SP2H/LT/DRPM/VIII/2017).

References

Alnajar, Z. A. A., Abdulla, M. A., Ali, H. M., Alshawsh, M. A., & Hadi, A. H. A. (2012). Acute Toxicity Evaluation, Antibacterial, Antioxidant and Immunomodulatory Effects of Melastoma malabathricum. *Molecules*, *17*(3), 3547–3559. https://doi.org/10.3390/molecules17033547

Al-Saeedi, A. H., & Hossain, M. A. (2015). Total phenols, total flavonoids contents and free radical scavenging activity of seeds crude extracts of pigeon pea traditionally used in Oman for the treatment of several chronic diseases. *Asian Pacific Journal of Tropical Disease*, *5*(4), 316–321. https://doi.org/10.1016/S2222-1808(14)60790-8

Alwash, M. S. A., Ibrahim, N., Ahmad Yaacob, W., & Bin Din, L. (2014). Antibacterial, Antioxidant and Cytotoxicity Properties of Traditionally Used Melastoma malabathricum Linn Leaves. *Advance Journal of Food Science and Technology*, *6*(1), 6–12. https://doi.org/10.19026/ajfst.6.3022

Andarwulan, N., & Faradilla, R. F. (2012). *Senyawa Fenolik pada Beberapa Sayuran Indigenous dan Indonesia*. SEAFAST Center IPB.

AOAC. (2005). *Official methods of analysis of AOAC International* (18th ed., tech. rep.). Association of Officiating Analytical Chemists. Maryland.

Arief, I. I., Afiyah, D. N., Wardhani, D. P., & Suryati, T. (2014). Physicochemical and organoleptic of beef sausages with teak leaf extract (Tectona grandis) addition as preservative and natural dye. *International Food Research Journal*, *21*(5), 2033–2042. http://repository.ipb.ac.id/handle/123456789/76697

Bendary, E., Francis, R. R., Ali, H. M. G., Sarwat, M. I., & El Hady, S. (2013). Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds. *Annals of Agricultural Sciences*, *58*(2), 173–181. https://doi.org/10.1016/j.aoas.2013.07.002

Boeira, C. P., Piovesan, N., Flores, D. C. B., Soquetta, M. B., Lucas, B. N., Heck, R. T., Alves, J. d. S., Campagnol, P. C. B., dos Santos, D., Flores, E. M. M., da Rosa, C. S., & Terra, N. N. (2020). Phytochemical characterization and antimicrobial activity of Cymbopogon citratus extract for application as natural antioxidant in fresh sausage. *Food Chemistry*, *319*, 126553. https://doi.org/10.1016/j.foodchem.2020.126553

BSN. (2015). *Standar Nasional Indonesia (SNI): Sosis Daging* (tech. rep.). Jakarta.

de Carvalho, F. A. L., Munekata, P. E. S., de Oliveira, A. L., Pateiro, M., Domínguez, R., Trindade, M. A., & Lorenzo, J. M. (2020). Turmeric (Curcuma longa L.)

extract on oxidative stability, physicochemical and sensory properties of fresh lamb sausage with fat replacement by tiger nut (Cyperus esculentus L.) oil. *Food Research International*, *136*, 109487. https://doi.org/10.1016/j.foodres.2020.109487

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants*, *8*(10), 429. https://doi.org/10.3390/antiox8100429

Doughari, J., & Manzara, S. (2008). In vitro antibacterial activity of crude leaf extracts of Mangifera indica Linn. *African Journal of Microbiology Research*, *2*, 67–72. https://academicjournals.org/article/article1380102713_Doughari%20and%20Manzara.pdf

El-Nashi, H. B., Fattah, A. F. A. K. A., Rahman, N. R. A., & El-Razik, M. M. A. (2015). Quality characteristics of beef sausage containing pomegranate peels during refrigerated storage. *Annals of Agricultural Sciences*, *60*(2), 403–412. https://doi.org/10.1016/j.aoas.2015.10.002

Fernandes, R. P. P., Trindade, M. A., Lorenzo, J. M., & de Melo, M. P. (2018). Assessment of the stability of sheep sausages with the addition of different concentrations of Origanum vulgare extract during storage. *Meat Science*, *137*, 244–257. https://doi.org/10.1016/j.meatsci.2017.11.018

Gultekin, F., Yasar, S., Gurbuz, N., & Ceyhan, B. M. (2015). Food Additives of Public Concern for their Carcinogenicity. *Journal of Nutritional Health and Food Science*, *3*(4), 01–06. https://doi.org/10.15226/jnhfs.2015.00149

Herrmann, S. S., Duedahl-Olesen, L., & Granby, K. (2015). Occurrence of volatile and non-volatile N-nitrosamines in processed meat products and the role of heat treatment. *Food Control*, *48*, 163–169. https://doi.org/10.1016/j.foodcont.2014.05.030

Hung, Y., de Kok, T. M., & Verbeke, W. (2016). Consumer attitude and purchase intention towards processed meat products with natural compounds and a reduced level of nitrite. *Meat Science*, *121*, 119–126. https://doi.org/10.1016/j.meatsci.2016.06.002

Insausti, K., Beriain, M. J., Purroy, A., Alberti, P., Gorraiz, C., & Alzueta, M. J. (2001). Shelf life of beef from local Spanish cattle breeds stored under modified atmosphere. *Meat Science*, *57*(3), 273–281. https://doi.org/10.1016/S0309-1740(00)00102-9

Jin, S.-K., Ha, S.-R., & Choi, J.-S. (2015). Effect of Caesalpinia sappan L. extract on physico-chemical properties of emulsion-type pork sausage during cold storage. *Meat Science*, *110*, 245–252. https://doi.org/10.1016/j.meatsci.2015.08.003

Jung, E., & Joo, N. (2013). Roselle (Hibiscus sabdariffa L.) and soybean oil effects on quality characteristics of pork patties studied by response surface methodology. *Meat Science*, *94*(3), 391–401. https://doi.org/10.1016/j.meatsci.2013.02.008

Kalem, I. K., Bhat, Z. F., Kumar, S., & Desai, A. (2017). Terminalia arjuna : A novel natural preservative for improved lipid oxidative stability and storage quality of muscle foods. *Food Science and Human Wellness*, *6*(4), 167–175. https://doi.org/10.1016/j.fshw.2017.08.001

Kamsani, N. E., Zakaria, Z. A., Nasir, N. L. M., Mohtarrudin, N., & Alitheen, N. B. M. (2019). Safety Assessment of Methanol Extract of Melastoma malabathricum L. Leaves following the Subacute and Subchronic Oral Consumptions in Rats and Its Cytotoxic Effect against the HT29 Cancer Cell Line. *Evidence-Based Complementary and Alternative Medicine*, *2019*, 1–14. https://doi.org/10.1155/2019/5207958

Lorenzo, J. M., Gómez, M., & Fonseca, S. (2014). Effect of commercial starter cultures on physicochemical characteristics, microbial counts and free fatty acid composition of dry-cured foal sausage. *Food Con-*

trol, *46*, 382–389. https://doi.org/10.1016/j.foodcont.2014.05.025

Luong, N.-D. M., Jeuge, S., Coroller, L., Feurer, C., Desmonts, M.-H., Moriceau, N., Anthoine, V., Gavignet, S., Rapin, A., Frémaux, B., Robieu, E., Zagorec, M., Membré, J.-M., & Guillou, S. (2020). Spoilage of fresh turkey and pork sausages: Influence of potassium lactate and modified atmosphere packaging. *Food Research International*, *137*, 109501. https://doi.org/10.1016/j.foodres.2020.109501

Mahmoudi, S., Khali, M., Benkhaled, A., Benamirouche, K., & Baiti, I. (2016). Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian Ficus carica L. varieties. *Asian Pacific Journal of Tropical Biomedicine*, *6*(3), 239–245. https://doi.org/10.1016/j.apjtb.2015.12.010

Partridge, D., Lloyd, K. A., Rhodes, J. M., Walker, A. W., Johnstone, A. M., & Campbell, B. J. (2019). Food additives: Assessing the impact of exposure to permitted emulsifiers on bowel and metabolic health – introducing the FADiets study. *Nutrition Bulletin*, *44*(4), 329–349. https://doi.org/10.1111/nbu.12408

Pateiro, M., Gómez-Salazar, J. A., Jaime-Patlán, M., Sosa-Morales, M. E., & M. Lorenzo, J. (2021). Plant Extracts Obtained with Green Solvents as Natural Antioxidants in Fresh Meat Products. *Antioxidants*, *10*(2), 181. https://doi.org/10.3390/antiox10020181

Pereira, D., Valentão, P., Pereira, J., & Andrade, P. (2009). Phenolics: From Chemistry to Biology. *Molecules*, *14*(6), 2202–2211. https://doi.org/10.3390/molecules14062202

Suharyanto, S., Nuraini, H., Suryati, T., Arief, I. I., & Sajuthi, D. (2019). Antioxidant and Antibacterial Properties of Aqueous Extract of Senduduk (Melastoma malabathricum L.) Leaf from Indonesia for Food Additive. *Pakistan Journal of Nutrition*, *18*(4), 391–400. https://doi.org/10.3923/pjn.2019.391.400

Suharyanto, S., Nuraini, H., Suryati, T., Arief, I. I., & Sajuthi, D. (2020). Aqueous Leaf Extract of Senduduk (Melastoma malabathricum L.) Could Improve the Physicochemical Properties of Beef Sausage Dough. *Jurnal Ilmu dan Teknologi Hasil Ternak*, *15*(2), 86–96. https://doi.org/10.21776/ub.jitek.2020.015.02.4

Sukisman, Purnomo, H., Rosyidi, D., & Radiat, L. E. (2014). Quality Properties, Antioxidant Capacity and Total Phenolic Content of Traditional Deep Fried Shredded Meat (Abon) of Palu, Central Sulawesi. *American Journal of Food Technology*, *9*(2), 80–88. https://doi.org/10.3923/ajft.2014.80.88

Suryati, T., Astawan, M., Lioe, H. N., Wresdiyati, T., & Usmiati, S. (2014). Nitrite residue and malonaldehyde reduction in dendeng — Indonesian dried meat — influenced by spices, curing methods and precooking preparation. *Meat Science*, *96*(3), 1403–1408. https://doi.org/10.1016/j.meatsci.2013.11.023

Susanti, D., Sirat, H. M., Ahmad, F., & Ali, R. M. (2008). Bioactive constituents from the leaves of Melastoma malabathricum. *Jurnal Ilmiah Farmasi*, *5*(1), 1–8.

Thatoi, H. N., Panda, S. K., Rath, S. K., & Dutta, S. K. (2008). Antimicrobial Activity and Ethnomedicinal Uses of Some Medicinal Plants from Similipal Biosphere Reserve, Orissa. *Asian Journal of Plant Sciences*, *7*(3), 260–267. https://doi.org/10.3923/ajps.2008.260.267

Tran, T. T. T., Ton, N. M. N., Nguyen, T. T., Le, V. V. M., Sajeev, D., Schilling, M. W., & Dinh, T. T. N. (2020). Application of natural antioxidant extract from guava leaves (Psidium guajava L.) in fresh pork sausage. *Meat Science*, *165*, 108106. https://doi.org/10.1016/j.meatsci.2020.108106

Turgut, S. S., Soyer, A., & Işıkçı, F. (2016). Effect of pomegranate peel extract on lipid and protein oxidation in beef meatballs during refrigerated storage. *Meat Science*, *116*, 126–132. https://doi.org/10.1016/j.meatsci.2016.02.011

Wang, Y., Li, F., Zhuang, H., Chen, X., Li, L., Qiao, W., & Zhang, J. (2015). Effects of plant polyphenols and α-tocopherol on lipid oxidation, residual nitrites, biogenic amines, and N-nitrosamines formation during ripening and storage of dry-cured bacon. *LWT - Food Science and Technology*, *60*(1), 199–206. https://doi.org/10.1016/j.lwt.2014.09.022

Wong, K.-C., Ali, D. M. H., & Boey, P.-L. (2012). Chemical constituents and antibacterial activity of Melastoma malabathricum L. *Natural Product Research*, *26*(7), 609–618. https://doi.org/10.1080/14786419.2010.538395

Zakaria, Z. A., Rofiee, M. S., Mohamed, A. M., Teh, L. K., & Salleh, M. Z. (2011). In Vitro Antiproliferative and Antioxidant Activities and Total Phenolic Contents of the Extracts of Melastoma malabathricum Leaves. *Journal of Acupuncture and Meridian Studies*, *4*(4), 248–256. https://doi.org/10.1016/j.jams.2011.09.016

Zhang, Q. Q., Jiang, M., Rui, X., Li, W., Chen, X. H., & Dong, M. S. (2017). Effect of rose polyphenols on oxidation, biogenic amines and microbial diversity in naturally dry fermented sausages. *Food Control*, *78*, 324–330. https://doi.org/10.1016/j.foodcont.2017.02.054