JURNAL AGROINDUSTRI
https://ejournal.unib.ac.id/index.php/agroindustri
pISSN: 20885369 eISSN: 26139952
DOI: 10.31186/jagroindustri.15.2.150-161

MINIMIZING OXIDATION AND MICROBIAL CONTENT IN BEEF SAUSAGES THROUGH THE INCLUSION OF SENDUDUK (*Melastoma malabathricum* L) LEAF POWDER

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Received 18-01-2024, revised 23-09-2025, approved 08-11-2025

ABSTRACT

Sausages are a popular food item in Indonesia, often sold by vendors along the roadside or in wheelbarrows. However, this selling method carries a risk of contamination, which can lead to spoilage. While synthetic preservatives can help, they may also pose health risks to consumers. The addition of natural ingredients, such as senduduk (Melastoma malabathricum L.) leaf powder (SLP), into sausage production, can be an alternative solution. This study aimed to evaluate the effects of SLP on the oxidation, microbial content, and physical properties of beef sausages. This study used a Completely Randomized Design with varying concentrations of SLP (0%, 0.5%, 1%, and 1.5% w/w) across four replications. The Thiobarbituric Acid (TBA) number, Total Plate Count (TPC), pH, cooking yield, water holding capacity (WHC), tenderness, and folding test were determined. The results demonstrated that adding SLP effectively reduced both the TBA number and the total plate count in the sausages at all tested concentrations. It also lowered the pH on the first day of storage and decreased the water holding capacity (WHC) when added at a concentration of 1.5%. Additionally, it influenced the results of the folding test. However, no significant differences were observed in the cooking yield and tenderness of the sausages. In conclusion, the addition of SLP into sausage production can effectively reduce oxidation and microbial content in beef sausages.

Keywords: beef meat, melastoma malabathricum, microbial contamination, oxidation, sausage

INTRODUCTION

Sausages have become a beloved food item for nearly all of Indonesia's population. Sausages are readily available in supermarkets, roadside stalls, pushcarts, and street food vendors. This development has positively contributed to the increased consumption of animal protein among Indonesians. However, the sales techniques, cooking methods, and serving practices tend to be unpackaged and often neglect hygiene considerations. This has consequently given rise to a new challenge in food safety.

On the other hand, a primary concern in the production of sausages pertains to the occurrence of lipid oxidation (Lavado & Cava, 2023) and microbial contamination (Zhu et al., 2022). Both of which can lead to a decline in nutritional quality, food safety, and shelf life (Olvera-Aguirre et al., 2023). This issue is exacerbated by unhygienic sales and serving practices, as outlined above. Conventionally, the aforementioned challenges are addressed through the use of synthetic preservatives and antioxidants. However, in tandem with the growing consumer awareness of food health and safety, many food products are now turning to natural preservatives and antioxidants, particularly those derived from plants or herbs (Olvera-Aguirre et al., 2023).

One plant with significant potential as an antioxidant and antimicrobial agent is Senduduk (*Melastoma malabathricum* L.). Senduduk is a shrub belonging to the Melastomataceae tribe and is widely found in South and Southeast Asia. (Paulpriya, 2019; Tiwari et al., 2023; Zheng et al., 2021). Almost all parts of this plant hold medicinal value in traditional medicine, addressing a range of health concerns such as wound healing, diarrhea, dysentery, hemorrhoids, skin conditions, toothache, and jaundice. (Tiwari et al., 2023).

Scientifically, the biological activities of Senduduk leaves, particularly their antibacterial and antioxidant properties, have been studied (Zheng et al., 2021). The pharmacological properties of Senduduk leaves are attributed to the presence of compounds such as flavonoids, tannins, and organic acids (Zheng et al., 2021). Extraction results using organic solvents like ethanol and methanol have revealed the presence of phenolic compounds in Senduduk leaves (Amalia et al., 2019). Additionally, water extracts of Senduduk leaves have also shown their ability to act as antioxidants and antimicrobials, which can be attributed to the presence of phenolic compounds (Lestari et al., 2022; Suharyanto et al., 2019).

The use of the water extract of Senduduk leaves has been explored in sausage production (Suharyanto et al., Suharyanto et al., 2019; Suharyanto et al., 2020, 2022; 2023). Incorporating aqueous extract of Senduduk leaves at a concentration of 0.55% of the total sausage mixture has been shown to preserve the quality of sausages for up to 12 days when stored at a cold temperature (4 ± 1°C) (Suharyanto et al., 2019). Incorporating a Senduduk leaf extract at a concentration of up to 1.1% of the total ingredient mass in the sausage formulation led to enhancements in the sausages' physicochemical characteristics while effectively preventing oxidation and microbial growth for a duration of up to 18 days during refrigerated storage. (Suharyanto et al., 2022). Furthermore, the addition of 1% aqueous extract of Senduduk leaves by the meat's weight could suppress oxidation for up to 10 hours under room temperature conditions (Suharyanto et al., 2023). These research findings that Senduduk leaves can be applied to sausage products as antioxidants and antimicrobial agents. However, the use of Senduduk leaf powder is expected to offer greater practicality. To date, there has been no exploration of using Senduduk leaf powder in sausages. Therefore, the objective of this study was to assess the impact of Senduduk leaf powder on oxidation, total microbial count, and the physicochemical characteristics of sausages under room temperature conditions.

MATERIALS AND METHODS

Powder Preparation

The Senduduk leaves were obtained from Srikuncoro village, district of Pondok Kelapa, Central Bengkulu Regency, Bengkulu Province, Indonesia. Subsequently, the leaves were air-dried for 72 hours and was dried in an oven (Memmert UM300, 2 shelves) at a temperature of 45°C for 5 hours. The dried leaves were then processed into a powder using a blender (FRITSCH P-11 Knife Mill) and sieved through a 35-mesh sieve, resulting in senduduk leaves powder (SLP), whichas utilized for the research.

Sausage Preparation

The Bali beef was obtained from the local market in Bengkulu city, Indonesia. After the removal of connective tissues and fat, the beef was minced using a meat grinder. minced using a meat grinder (Panasonic MK-MG1360/1300w). The beef sausages were prepared with the following treatments. The components used in crafting the beef sausages are detailed in Table 1. Four different treatments were employed in the experiment: a base mix comprising beef, vegetable oil, skim milk, tapioca, salt, phosphate, ice cubes, garlic, pepper, and nutmeg, labeled as the Control (T0); the Control with an addition 0.5% of SLP (T1); the Control with 0.1% of SLP; and Control with 1.5% of SLP (T3). The percentage of SLP addition was based on the meat weight.

The sausage preparation procedure closely followed that of Suharyanto et al. (2022) minor adjustments. Essentially, all the ingredients were thoroughly mixed to create a uniform blend for each treatment. This emulsified mixture was then stuffed into casings made of food-grade polyamide plastic, with a 16 mm diameter. The raw sausages were steam-cooked at 65°C for 45 minutes. The cooked sausages were stored at room temperature and observed at 0, 5, and 10 hours.

Cooking Yield

The cooking yield value was assessing the difference in weight before and after cooking the samples and calculated by the formula below. (Kang et al., 2022).

$$Yield\ (\%) = \frac{Cooked\ sausage\ weight}{Uncooked\ sausage\ weight} \times 100$$

Water Holding Capacity (WHC)

The WHC value was determined according to the method Jung and Joo (2013), with slight adjustments. A total of 5 g of mashed sausage samples was placed into a centrifuge tube, which was then filled with 10 ml of distilled water. The mixture was incubated at 30°C for 30 minutes. After incubation, the mixture was centrifuged at 3000 rpm for 30 minutes. The resulting supernatant was discarded, followed by another 10-minute incubation at 30°C, and the supernatant was removed. The WHC was calculated using the formula as follows:

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

Table 1. Formulation of Sausages Based on Each Treatment

Ingredients	Treatments			
	T0	T1	T2	T3
Meat (g)	400	400	400	400
Vegetable oil (g)	80	80	80	80
Powder skim milk (g)	24	24	24	24
Tapioca flour (g)	60	60	60	60
Cubic ice (g)	140	140	140	140
Salt (g)	15	15	15	15
Garlic (g)	7	7	7	7
White pepper powder (g)	4	4	4	4
Nutmeg (g)	2	2	2	2
Senduduk leaves powder (% w/w of meat)*	0.00	0.5	0.10	1.50

Tenderness

The measurement of sausage tenderness was carried out using a universal penetrometer (Fitri, 2025). The penetrometer was prepared by ensuring that the indicator needle was positioned at zero. We add weights to the plunger head of the needle rod. The load (in grams) was determined by summing the weight of the weights, plunger head, and needle rod. The sample was precisely positioned beneath the needle. The plunging lever was depressed for 10 seconds. Read the scale on the indicator needle, which indicates the depth of penetration into the sample (in mm). The sausage hardness was expressed in mm/g/second.

Folding Test

The folding test was determined according to the Kamani et al. method (Kamani et al., 2019). This measurement is an important functional test used to evaluate the elasticity and structural integrity of the gel network in sausage products. The center part of the sausage was cut and sliced to a thickness of 3 mm and a length of 2.5 cm. The sausage was folded using the index finger and thumb with the following scoring criteria: a score of 5 if the sausage does not crack when folded, a score of 4 if the sausage does not crack when folded halfway, a score of 3 if the sausage slowly cracks at the halfway fold, a score of 2 if the sausage quickly shows cracks at the halfway fold, and a score of 1 if the sausage exhibits cracking when the thumb and index finger press to fold the sample.

pH Value

The pH value of sausages was measured by dissolving 1 gram of sausage and mixing it with 9 mL of distilled water until it became homogeneous. The mixture of sausage in distilled water was then measured for its pH using a calibrated pH meter (AZ Instrument 86505) (Savadkoohi et al., 2014).

Thiobarbituric Acids (TBA)

The TBA number reffered to Apriyantono et al. (1989). The process involved weighing 10 grams of meatballs carefully and placing them into a blending bag. Then, 50 mL of distilled water was added, and the mixture was blended for 2 minutes. The resulting mixture was quantitatively transferred into a distillation flask while being rinsed with 47.5 mL of distilled water. Approximately 2.5 mL of HCl was added to adjust the pH to 1.5. Boiling chips and an anti-foaming agent were added as needed, and the distillation flask was set up. An "electric mantle heater" was used if available. The distillation was carried out with high heat to obtain 50 mL of distillate over 10 minutes of heating. The distillate was thoroughly mixed, and 5 mL of the distillate was pipetted into a closed reaction tube. Then, 5 ml of the TBA reagent was added. The tube was sealed, mixed thoroughly, and heated for 35 minutes in boiling water. A blank solution was created using 5 mL of distilled water and 5 mL of the TBA reagent, following the same procedure as for the sample. The reaction tubes were cooled with a cooling bath for approximately 10 minutes, and then the absorbance (D) was measured at a wavelength of 528 nm, using the blank solution as the zero point. Calculate the TBA value, expressed in milligrams of malondialdehyde per kilogram of the sample. The formula used was $TBA = 7.8 \times D$.

Total Microorganism

Total microorganisms were determined using the total plate count according to the method of Suharyanto et al. (2019). A total of 25 grams of sausage sample was aseptically ground and homogenized in 225 mL of sterile Buffered Peptone Water (BPW) as the initial dilution. This initial dilution suspension was then serially diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} by pipetting 1 mL from the previous dilution to the next in sterile BPW media. For each dilution series, 1 mL was transferred to separate sterile petri dishes and then overlaid with 15-20 mL of sterile Plate Count Agar (PCA) to determine the total microbial count. After all the media solidified, they were incubated at 37°C for 24-48 hours, and colony counts were determined. The preparation of BPW and PCA solutions followed the protocols of the brand used.

Data Analysis

This study employed a completely randomized design (CRD) with four treatments and four replications. The storage duration was not considered as a treatment, but rather as the observation time point for the measured variables. The data were analyzed using Analysis of Variance (ANOVA) and the difference among treatments were compared with Duncan Multiple Range Test (DMRT) at a significance level of 0.05.

RESULTS AND DISCUSSION

Cooking Yield, Water Holding Capacity, Tenderness, Folding Test

The term cooking yield refers to the amount of product obtained after the cooking process. The higher the cooking yield, the better the production quality. The result of this study indicated that there was no significant difference in cooking yield among the treatments. The data are shown in Table 2. This suggests that the addition of SLP up to 1.5% of the meat weight did not lead to changes in factors influencing cooking yield. Some of the factors affecting cooking yield include temperature, pH, viscosity, myofibril protein functionality, and fat globules in the product (Vaskoska et al., 2020). In other words, adding 1.5% of 35-mesh SLP did not result in changes in viscosity, the functionality of meat protein in sausages, or fat globules.

The findings of this study are consistent with several other studies that have employed plant-based powders. The addition of 1% lotus root powder (Ham et al., 2017) 3% buckwheat powder (Lee et al., 2018) could improve the cooking yield of sausages, and the inclusion of 1% water spinach flour could enhance the cooking yield in meatballs (Vebrianty et al., 2021).

Table 2 depicts the results of the WHC of the sausages. The WHC was affected by the addition of SLP during storage at room temperature at hours 0 and hours 5 ($P<0.05$), but at hour 10 of room temperature storage, WHC did not significantly different ($P>0.05$). At hour 0, the WHC value with the addition of 1.5% SLP (T3) was the lowest, and at hour 5, the water-binding ability of sausages with the addition of 1% SLP (T2) started to decrease, so it was not significantly different from T3. After 10 hours of storage, all sausages had the same WHC value. This phenomenon indicated that adding SLP up to 1% during 5 hours of room temperature storage did not result in a change in the WHC of the sausages. However, when 1.5% (T3) was added, it caused a decrease in the water-binding ability of the sausages.

The decrease in WHC of T3 sausages is most probably due to the addition of 1.5% SLP, which is a substantial amount, affecting the sausage's ability to bind water. This is because the addition of SLP reduces the proportion of ingredients with water-binding capabilities. However, research findings on the addition of leaf-derived powders in processed meat products vary. In the study by Vebrianty et al. (2021), the addition of water spinach powder up to 1.5% resulted in a higher water-binding capacity in meatballs compared to no addition of water spinach powder. The addition of tea leaf powder produced water-binding capacity in sausages that was not significantly different from that without the addition (Purnamayanti et al., 2020).

Adding Albizia leaf powder up to 1.5% also did not result in a significant difference in WHC in beef meatballs compared to the control group (Hajrawati et al., 2021). However, in this study, a 10-hour storage period caused no significant difference in WHC among treatments. This suggests that prolonged storage for up to 10 hours leads to a decline in the water-binding ability of sausages without the addition of SLP.

The tenderness of the sausages in this study indicated non-significant differences among all treatments (P>0.05) at each observation time. The tenderness values of these sausages ranged from 5.18 to 6.70 mm/g/second. The addition of SLP up to 1.5% resulted in sausages with tenderness that did not differ from those without SLP addition. These findings align with Purnamayanti et al (2020), where the addition of green tea flour up to 2% resulted in lamb sausage tenderness that did not significantly different from the control. The tenderness of sausages is influenced by several factors, including the composition and ingredients used. Therefore, the addition of SLP up to 1.5% did not cause aggregation of different components change.

The folding test scores exhibited a decrease with increasing additions of SLP (P<0.05), as depicted in Table 2. Reduction in the folding test scores occurred during storage for 0, 5, and 10 hours. The folding test scores indicate the elasticity and compactness of an emulsion product like sausage. Higher folding test scores denote greater elasticity and compactness, with a score of 5 indicating no breakage or cracks when folded into a circle, while a score of 1 indicates cracking when the sausage is folded.

The addition of SLP resulted in increased non-ingredient particles, thus influencing the compactness and elasticity of an emulsion product. The elasticity level of the sausage is closely related to the protein gel formation process during cooking, where myofibrillar meat proteins play a crucial role in this gel formation (Kamani et al., 2019). Adding SLP beyond the ingredient proportion disrupts the process of myofibrillar meat protein gel formation. Kamani et al. (2019) reported that substituting meat protein with plant protein led to a decrease in gel-forming ability, a phenomenon believed to be similar to the decrease caused by the addition of SLP. This study's addition of 0.5 – 1.5% has resulted in a decrease in the sausage's gel-forming ability.

The pH Value, TBA Number, and Total Plate Count

The findings of this research indicate that the addition of SLP significantly influences the pH value of sausages at 0 hours (P<0.05) and has no significant effect after 5 and 10 hours of storage (P>0.05), as observed in Table 2. Variations in the pH value at 0 hours were evident between sausages without SLP addition (T0) and those with added SLP at 0.5%, 1%, and 1.5% (T1, T2, and T3). However, there were no significant differences observed among sausages with varying levels of SLP. This demonstrates that the addition of 0.5%, 1%, and 1.5% SLP did not cause a notable pH change in the sausages, whereas the addition of 0.5% led to a decrease in pH.

The storage duration of 5 to 10 hours resulted in similar pH values for all sausages. This occurred because the pH of sausages without SLP decreased over the storage duration, while the SLP-added sausages maintained their pH levels. Consequently, sausages supplemented with SLP exhibited greater pH stability.

The lower pH values at 0 hours in sausages employed with SLP are presumed to be due to the presence of polyphenolic compounds in SLP. These compounds are known to cause lower pH levels in products compared to those without polyphenols (Fernandes et al., 2018; Suharyanto et al., 2019). The addition of SLP to sausages caused the powder to donate hydrogen, rendering the product more acidic (Ibrahim et al, 2022).

The use of SLP in sausages has demonstrated a reduction in the level of sausage oxidation, as indicated by the decreasing TBA values corresponding to higher levels of SLP usage (Figure 1). Each addition of 0.5% resulted in a significant decrease in the TBA value of the sausages. This reduction pattern was observed at the 0^{th}, 5^{th}, and 10^{th} hour during room temperature (25-28 °C) storage. The substantial decrease in TBA values is strongly believed to be due to the bioactive compounds such as polyphenolic compounds present in the added SLP, which function as antioxidants (Apridamayanti et al., 2021; Suharyanto et al., 2019). The bioactive compounds, presumed to act as antioxidants, are phenolic compounds found in SLP (Lestari et al., 2022; Paulpriya, 2019; Suharyanto et al., 2019).

Table 2. Cooking Yield, Water Holding Capacity, Tenderness, Folding Test, and pH of Sausages

Parameters	Treatments			
	T0	T1	T2	T3
Cooking Yield (%):				
- Hour-0	98.55±0.13	98.43±0.16	98.37±0.12	98.40±0.13
- Hours-5	98.38±0.23	98.31±0.14	98.39±0.17	98.42±0.10
- Hours-10	98.22±0.15	98.29±0.21	98.39±0.16	98.29±0.10
Water Holding Capacity (%):				
- Hour-0	72.75±2.57^{ab}	73.60±1.25^{a}	73.21±1.76^{a}	69.75±2.13^{b}
- Hours-5	74.09±1.62^{a}	73.84±1.30^{a}	72.39±1.15^{ab}	71.10±0.92^{b}
- Hours-10	71.31±1.36	72.44±0.51	70.15±2.42	71.84±2.53
Tenderness (mm/g/s):				
- Hour-0	5.97±0.42	5.43±0.35	5.18±1.68	5.93±0.49
- Hours-5	6.70±0.72	5.45±0.77	5.57±0.67	6.31±0.99
- Hours-10	6.42±0.21	6.42±0.53	5.80±0.74	6.21±0.73
Folding Test:				
- Hour-0	4.58±0.50^{a}	3.92±0.78^{b}	3.92±0.88^{b}	1.29±0.55^{c}
- Hours-5	4.50±0.51^{a}	3.67±0.48^{b}	3.54±0.51^{b}	2.96±0.62^{c}
- Hours-10	4.58±0.50^{a}	3.63±0.58^{b}	3.50±0.83^{b}	3.33±0.64^{b}
pH:				
- Hour-0	6.42±0.31^{a}	6.03±0.05^{b}	6.01±0.07^{b}	5.96±0.08^{b}
- Hours-5	6.12±0.31	6.02±0.10	5.92±0.09	5.85±0.09
- Hours-10	6.04±0.15	5.95±0.15	5.87±0.10	5.84±0.10

Description: Different superscripts at the same row indicate significantly different ($P<0.05$)

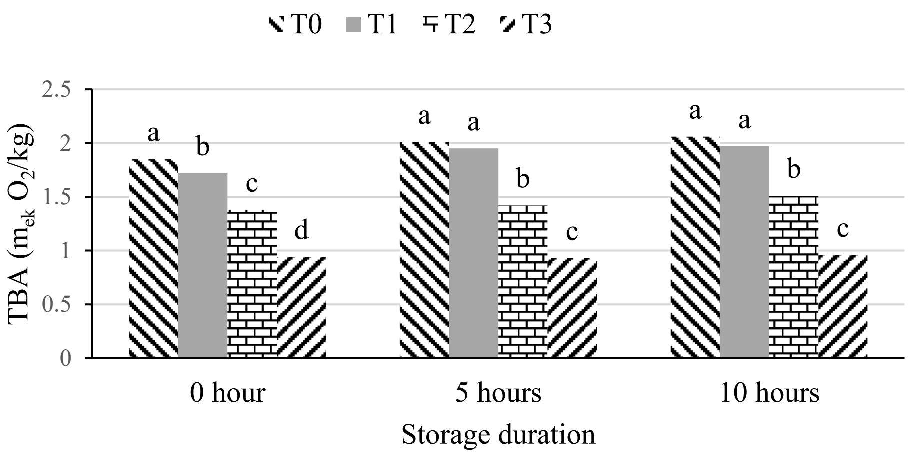
The use of SLP in sausages has demonstrated a reduction in the level of sausage oxidation, as indicated by the decreasing TBA values corresponding to higher levels of SLP usage (Figure 1). Each addition of 0.5% resulted in a significant decrease in the TBA value of the sausages. This reduction pattern was observed at the 0^{th}, 5^{th}, and 10^{th} hour during room temperature (25-28 °C) storage. The substantial decrease in TBA values is strongly believed to be due to the bioactive compounds such as polyphenolic compounds present in the added SLP, which function as antioxidants (Apridamayanti et al., 2021; Suharyanto et al., 2019). The bioactive compounds, presumed to act as antioxidants, are phenolic compounds found in SLP (Lestari et al., 2022; Paulpriya, 2019; Suharyanto et al., 2019).

Phenolic compounds are known to counteract free radicals and prevent oxidation (8). According to Kalem et al.

(Kalem et al., 2017). The ability of phenolic compounds to act as antioxidants stems from their redox potential to absorb and neutralize free radicals, inhibit singlet oxygen, and decompose peroxides (Parcheta et al, 2021). Parcheta et al. (2013) suggested that this mechanism occurs by the transfer of an H atom from the OH group of phenolic compounds to the peroxyl radical chain, thereby interrupting subsequent peroxyl radical chain reactions.

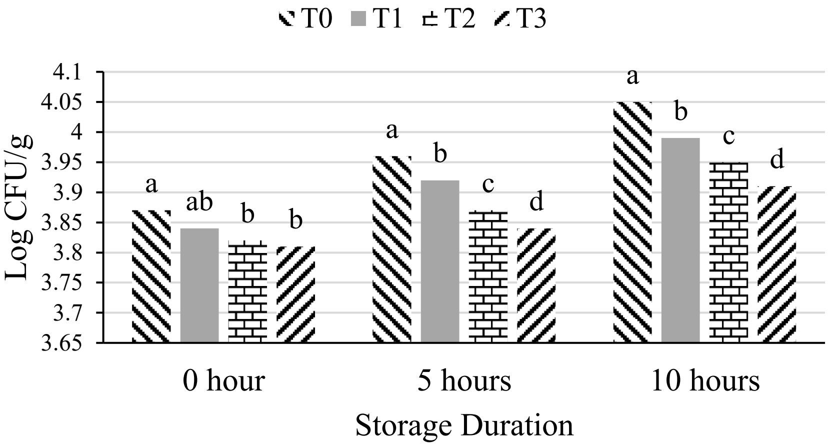
The total plate count indicates the overall microbial presence within sausages. The addition of SLP significantly suppresses the growth of beef sausage microbes (P<0.05), as presented in Figure 2. At the 0-hour storage, it was observed that the addition of 0.5% SLP did not effectively curb the microbial count, while additions of 1% and 1.5% exhibited reductions in microbial counts. Meanwhile, at the 5th and 10th hour storage intervals, significant differences in microbial counts were evident between sausages without SLP and those supplemented with 0.5% SLP. The shift in significance from the 0-hour to the 5th and 10th-hour intervals is believed to be due to an increase in microbial counts in SLP-absent sausages during storage. Conversely, sausages with added SLP maintained relatively stable microbial counts throughout the 10-hour storage, causing a significant difference after 5 hours or more.

The data in Figure 2 indicate that the addition of SLP effectively maintains microbial counts from significant proliferation. This capability to restrain microbial proliferation is presumed to be due to the presence of phenolic compounds in the SLP (Suharyanto et al., 2019; Suharyanto et al., 2022), known for their antimicrobial properties (Alwash et al., 2014; Suharyanto et al., 2022). The antimicrobial mechanism of phenolic compounds can occur through various pathways (Apridamayanti et al., 2021), including the formation of complexes with extracellular bacterial proteins via hydrogen bonding. This action causes bacterial cell membranes to function less effectively and become damaged, leading to the release of many intracellular compounds. Another mechanism involves inhibiting energy metabolism by impeding bacterial oxygen utilization, achieved through protein denaturation, protoplasmic coagulation, and impeding energy metabolism (Apridamayanti et al., 2021).



Description: different letters above the bar indicate a significant difference(P<0.05)

Figure 1. TBA Number of Sausages as Affected by The Addition of Senduduk Leaf Powder Level.



Description: different letters above the bar indicate significant different (P<0.05)

Figure 2. Total Plate Count (TPC) value of Sausages as Affected by The Addition of Senduduk Leaf Powder Level.

CONCLUSION

The addition of senduduk leaf powder (SLP) up to 1.5% in beef sausages has demonstrated a reduction in oxidation levels and total microbial count without exerting an influence on the cooking yield and tenderness of the sausages. The incorporation of SLP resulted in an initial decrease in the pH of the sausages during the initial stages of production; however, the pH levels eventually equalized after a 5-hour storage period. Optimal results were observed with the addition of 0.5% SLP in beef sausages. Nevertheless, the introduction of SLP has led to a reduction in sausage elasticity.

ACKNOWLEDGEMENTS

The author expresses gratitude to the Faculty of Agriculture, University of Bengkulu, for funding this research through the Faculty of Agriculture's Outstanding Research Grant of the University of Bengkulu in 2021.

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