Improving beef meatball properties by adding senduduk (*Melastoma malabathricum* L.) leaf powder

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Abstract The research findings indicated that incorporating 0.5-1.5% senduduk leaf powder effectively inhibited the oxidation rate of meatballs. Notably, the inclusion of 1.5% demonstrated the ability to prevent oxidation at a level comparable to 0.01% BHT. While it was not discernible alterations observed in the pH of the meatballs, the presence of senduduk leaf powder proved to be effective in suppressing microbial growth. Despite maintaining the water holding capacity and elasticity of the meatballs, the addition of senduduk leaf powder showed the impact tenderness, texture, color, odor, and overall acceptability among the panelists. The addition of senduduk leaf powder is improved the beef meatballs properties in term of reducing oxidation and total microbes of the beef meatball with no any effect on certein physicochemical and sensorial properties.

Keywords: Antimicrobial, Antioxidant, Meatball, Melastoma malabathricum, Physicochemical, Sensory

Introduction

Meatballs, locally known as "bakso," are a beloved and widely consumed food in Indonesia. They are versatile, often enjoyed in soup or as grilled, baked, or other variations. The popularity of meatballs has led to their availability in various outlets, including street vendors, minimarkets, and food carts. However, the diverse methods of preparation and sales have raised concerns about potential contamination, such as contamination with *Salmonella*, *E. coli*, and other pathogenic bacteria (Ali and Alsayeqh, 2022) and exposure to undesired substances. Ensuring microbiological safety is critical to maintaining consumer trust and protecting public health.

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Furthermore, commercial meatballs often contain synthetic preservatives, which may have negative health implications (Fadhil, 2023). One such preservative is butylated hydroxytoluene (BHT), commonly used to prevent oxidation in meat products. However, according to international food safety standards such as Codex Alimentarius, the maximum allowable concentration of BHT in food products is 200 mg/kg. Excessive use of BHT has been associated with potential health risks, including carcinogenicity, when consumed in high doses over prolonged periods (WHO, 2021). The improper or excessive use of synthetic additives in meatballs remains a critical issue, highlighting the need for safer, natural alternatives.

Consumer preferences are shifting towards natural and healthier food options, driving the demand for food products made with natural additives. Senduduk (Melastoma malabathricum), is a plant whose leaves are known to possess various health benefits due to their rich bioactive compounds (Ismail et al., 2022). The plant has been found to contain phytochemical constituents such as polyphenols, tannins, saponin, flavonoids, triterpenes, flavan-3-ols, anthocianins, and steroids (Tiwari et al., 2023). Studies have shown that senduduk leaves contain antioxidants and antimicrobial compounds, which can help prevent oxidation and microbial growth in food products (Lestari et al., 2022; Mayasari et al., 2022). The essential oil extracted from Cinnamomum malabatrum, a species belonging to the same family as Melastoma malabathricum, has been shown to have antioxidant properties and antibacterial activity (Kuttithodi et al., 2023). Water extracts of senduduk leaves have also exhibited antioxidant activity, with compounds such as 4-O-caffeoylquinic acid, quercimeritin, digiprolactone, 3-O-trans-coumaroylquinic acid, norbergenin, and artemisinin identified in the extract (Kuttithodi et al., 2023). Additionally, the methanol extract of senduduk leaves has been found to have antioxidant and anticarcinogenic activities, with various phenolics identified in the extract (Zakaria et al., 2022) and shown antioxidant and antidiabetic properties, inhibiting α -glucosidase and α -amylase enzymes, and reducing blood glucose levels in rats (Zakaria et al., 2022).

The application of senduduk leaf to food has also been studied. Addition of senduduk leaf extract 0.5-1.0% improved certain physicochemical properties of sausages and inhibited oxidation and microbial growth in sausages during cold storage (Suharyanto *et al.*, 2022) and meatballs exposed to room temperature (Suharyanto *et al.*, 2023). However, the use of senduduk leaves in powdered form as a food additive is relatively unexplored. Powdered forms of senduduk leaves offer practical advantages over extracts or other forms, making them easier to incorporate into food products. The addition of senduduk leaf powder to

meatballs could serve as a natural alternative to synthetic preservatives, providing health benefits without compromising taste or texture.

This study aimed to investigate the effects of adding senduduk (*Melastoma malabathricum*) leaf powder to beef meatballs at various concentrations on oxidation, total plate count, physicochemical properties, and sensory attributes. Understanding the effects of senduduk leaf powder addition to beef meatballs can provide valuable insights into the development of natural food additives and contribute to the overall improvement of meatball quality and safety.

Materials and methods

Senduduk leaf powder preparation

Senduduk leaves were obtained from shrubs in Bengkulu City, Indonesia. The leaves were selected at the mature stage, not the tip or the bottom part of the stem. They were cleaned of any impurities, air-dried for 72 hours, and then ovendried at 60 °C for 5 hours. The dried leaves were then blended into powder and sieved through an 80-mesh sieve. The sieved powder was used for the experiment.

Meatballs and treatments preparation

Bali beef thigh meat was cut into small pieces, ground, pounded, and mixed with salt and 1/3-part ice. The mixture was then added with another 1/3-part ice, senduduk leaf powder or *Butyllated Hydroxytoluene* (according to treatments), tapioca, and pounded for 2 minutes. Subsequently, another 1/3-part ice, garlic, and pepper were added to the mixture, pounded until smooth (3 minutes). The mixture was left to rest for 10 minutes, shaped into round balls with a diameter of 5 cm, boiled in boiling water until they float. The meatballs were drained and stored at room temperature, then observed at 0, 5, and 10 hours. The experimental design was a completely randomized design with each treatment repeated 4 times. The composition of each treatment is presented in Table 1.

TBA determination

The determination of the TBA number was based on the method described by Apriyantono *et al.* (1989). Initially, 10 grams of samples were precisely weighed and placed in a blending bag. Subsequently, 50 ml of distilled water was added, and the mixture was blended for 2 minutes. The resulting blend was quantitatively transferred into a distillation flask, rinsing the blending bag with 47.5 ml of distilled water. The pH was adjusted to 1.5 by adding approximately 2.5 ml of HCl. Boiling chips and an anti-foaming agent were added as necessary, and the distillation flask was prepared, utilizing an electric mantle heater if accessible. The distillation process was conducted at 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 sealed reaction tube. Subsequently, 5 ml of the TBA reagent was added to the tube, followed by thorough mixing. The tube was then heated for 35 minutes in boiling water. A blank solution was prepared using 5 ml of distilled water and 5 ml of the TBA reagent, following the same procedure as for the sample. After cooling the reaction tubes for approximately 10 minutes in a cooling bath, the absorbance (D) was measured at a wavelength of 528 nm, utilizing the blank solution as the zero point. The TBA value was calculated in milligrams of malondialdehyde per kilogram of the sample using the formula TBA = $7.8 \times D$.

Ingredients		Treatments				
ingreatents	Control	BHT	0.5%	1%	1.5%	
Beef meat (g)	400	400	400	400	400	
Tapioca (g)	200	200	200	200	200	
Cubic ice (g)	104	104	104	104	104	
Salt (g)	12	12	12	12	12	
Garlic powder (g)	4	4	4	4	4	
Pepper powder (g)	4	4	4	4	4	
BHT (%)*	-	0,01	-	-	-	
Senduduk leaf powder (%)*	-	-	0,5	1	1,5	

Table 1. Composition of meatballs based on the treatment

* Percentage based on the meat basis.

Total plate count determination

The total plate count was assessed using method as outlined by Suharyanto *et al.* (2019a). Initially, 25 grams of sample were aseptically ground and homogenized in 225 ml of sterile Buffered Peptone Water (BPW) to create the initial dilution. This initial dilution was then serially diluted to 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, and 10⁻⁶ by transferring 1 ml from each previous dilution to the next in sterile BPW media. Subsequently, 1 ml from each dilution series was transferred to separate sterile petri dishes and overlaid with 15-20 ml of sterile Plate Count Agar (PCA) to determine the total microbial count. Following solidification of the media, the petri dishes were incubated at 37°C for 24-48 hours, after which colony counts were determined. The preparation of BPW and PCA solutions adhered to the manufacturer's protocols.

pH determination

The pH of meatballs was determined by blending 1 gram of sample with 9 ml of distilled water until a homogeneous mixture was obtained. The sample-water mixture was then measured for pH using a calibrated pH meter (Savadkoohi *et al.*, 2014).

Determination of moisture content (%)

The moisture content was determined using the oven drying method Horwitz and Latimer (2005). A total of 2 g of meatball sample was dried in an oven at 105 °C until a constant weight was obtained. Measurements were taken at 0, 5, and 10 hours of storage. The moisture content was calculated using the following formula.

Moisture Content (%) = $\frac{\text{initial weight} - \text{dried weight}}{\text{initial weight}} \times 100$

Water holding capacity

The evaluation of water holding capacity (WHC) was carried out following the procedure described by Jung dan Joo (2013), with minor modifications. A quantity of 5 g of mashed meatball samples was placed in a centrifuge tube and filled with 10 ml of distilled water. The mixture was then kept at 30°C for 30 minutes. Subsequently, the mixture was centrifuged at 3000 rpm for 30 minutes. The resulting liquid above the sediment was discarded, and the tube was subjected to another 10-minute incubation at 30°C, followed by removal of the supernatant. The WHC was determined using the following formula:

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

Tenderness determination

The evaluation of meatball tenderness was conducted utilizing a universal penetrometer as outlined by Sumarmono (2012). To begin, the penetrometer was calibrated to ensure the indicator needle rested at zero. Subsequently, weights were affixed to the plunger head of the needle rod, with the total load (measured in grams) determined by aggregating the weight of the weights, plunger head, and needle rod. The sample was then accurately positioned below the needle, following which the plunging lever was depressed for a duration of 10 seconds. The scale on the indicator needle was then read to ascertain the depth of

penetration into the sample, expressed in millimeters. Meatball hardness was quantified as millimeters per gram per second.

Emulsion stability

The measurement referred to Yum *et al.* (2018). A total of 7.5 g of the sample was placed into a centrifuge tube, then heated in a water bath at 70°C for 30 minutes. The sample was centrifuged at 1000 rpm for 10 minutes. The resulting supernatant liquid was poured off and weighed. This liquid was then dried using an oven at 105°C for 16 hours. The remaining dried residue was weighed as the fat content. The water content was determined as the difference between the weight of the liquid and the weight of the fat. Measurements were taken at 0, 5, and 10 hours of storage.

Released fluid (%) =
$$\frac{\text{fluid weight}}{\text{sample weight}} \times 100$$

Released water (%) = $\frac{\text{water weight}}{\text{sample weight}} \times 100$

Folding test

The fold test procedure was conducted in accordance with Zhou *et al.* (2022). A 3 mm thick and 2.5 cm long slice was cut from the center of the meatball. The meatball slice was then folded using the index finger and thumb, and scored based on the following criteria: a score of 5 if there was no cracking observed when folded, a score of 4 if there was no cracking observed when folded, a score of 4 if there was no cracking observed when folded halfway, a score of 3 if the meatball slowly cracked at the halfway fold, a score of 2 if the meatball quickly showed cracks at the halfway fold, and a score of 1 if the meatball exhibited cracking when pressure was applied by the thumb and index finger to fold the sample.

Statistical analysis

The data were analyzed using Analysis of Variance (ANOVA) and the defferences of treatments were continued with pos-hoc comparison Duncan Multiple Range Test (DMRT) at a significance level of 0.05.

Results

TBA number of meatballs

The effects of Senduduk leaf powder (SLP) and storage duration on the oxidative stability of beef meatballs were investigated, as indicated by the Thiobarbituric Acid (TBA) number, and the results are presented in Table 2. No interaction was observed between the type of antioxidant and storage duration (P>0.05), suggesting that they independently influence oxidation. The type of antioxidant (BHT and SLP) significantly affects the TBA number (P<0.05) as shown in Table 2. The Butylated Hydroxytoluene (BHT) and SLP at 1.5% concentration were similarly effective in inhibiting oxidation of meatballs as indicated by lower TBA number than other meatballs, while lower concentrations of SLP (0.5 and 1%) were less effective to retard lipid oxidation.

Meatballs	Storage (hours)				
wieatbails	o o		10	Average	
Control	$0.47{\pm}0.10$	0.63 ± 0.09	$0.79{\pm}0.07$	$0.63{\pm}0.16^{a}$	
BHT	0.33 ± 0.04	0.37 ± 0.11	$0.46{\pm}0.07$	$0.39{\pm}0.09^{d}$	
0.5	$0.39{\pm}0.05$	0.57 ± 0.04	$0.59{\pm}0.01$	$0.52{\pm}0.10^{b}$	
1.0	$0.34{\pm}0.02$	$0.42{\pm}0.02$	$0.56{\pm}0.02$	$0.44{\pm}0.10^{\circ}$	
1.5	$0.30{\pm}0.01$	$0.36{\pm}0.02$	0.43 ± 0.01	$0.36{\pm}0.06^{d}$	
Average	$0.37{\pm}0.08^{a}$	$0.47{\pm}0.13^{b}$	$0.57{\pm}0.14^{\circ}$		

Table 2. Thiobarbituric acids (TBA) (mEq/kg) number of beef meatballs

Note: means within a column or row with different letters are significantly different (P<0.05)

Total plate count

The study findings also revealed that there was no notable interaction effect between the concentration of SLP and the length of storage time on the total plate count (TPC) (P>0.05). It indicated that these two variables independently affect the TPC. The concentration factor of SLP had a significant effect (P<0.05) on TPC, while no significant difference was observed between the control and BHT, indicating that BHT did not provide better antimicrobial effects compared to the absence of preservatives. Both lower concentrations of SLP (0.5 and SLP 1%) showed higher efficacy in reducing TPC compared to control and BHT. Meanwhile, SLP 1.5 exhibited the strongest antimicrobial effect with the lowest TPC (Table 3).

Physicochemical properties

In this study, notably, there was no significant interaction between the type of antioxidant used and storage duration, indicating that the effect of antioxidants on pH was consistent regardless of storage time. Interestingly, the type of antioxidant, including SLP at various concentrations, did not significantly impact the pH of the meatballs. The lack of significant change in pH across different antioxidant treatments, including various concentrations of SLP, suggests that these additives do not significantly alter the natural pH buffering capacity of the meatballs. This stability in pH regardless of antioxidant type could be due to the inherent properties of the meat components that resist pH change. The results of the research on the pH value of meatballs are displayed in Table 4.

Meatballs	Storage (hours)				
MeatDails	0	5	10	Average	
Control	4.2±0.08	4.3±0.09	4.3±0.10	4.3±0.10 ^a	
BHT	4.2±0.05	4.2±0.06	4.3±0.07	4.3 ± 0.07^{a}	
0.5	4.0 ± 0.07	$4.0{\pm}0.09$	4.1±0.07	$4.0{\pm}0.08^{b}$	
1.0	4.0±0.05	$4.0{\pm}0.05$	4.1±0.03	$4.0{\pm}0.08^{b}$	
1.5	3.9±0.04	3.9±0.03	4.0±0.02	3.9±0.07°	
Average	$4.0{\pm}0.15^{a}$	4.1 ± 0.14^{b}	4.2±0.14°		

Table 3. Total plate count (log cfu/g) of beef meatballs

Note: means within a column or row with different letters are significantly different (P<0.05)

	Storage (hours)	
0	5	10	Average
5.6±0.18	5.5±0.24	5.3±0.15	5.5±0.19
5.7±0.38	5.4±0.10	5.3 ± 0.08	5.5 ± 0.28
5.7±0.14	5.6 ± 0.08	5.4±0.13	5.6±0.15
5.5±0.19	5.4±0.17	5.3±0.32	5.4±0.22
5.7±0.19	5.5±0.14	5.3±0.20	5.5±0.22
5.6±0.21ª	5.5±0.14 ^b	5.3±0.17°	
	5.6 ± 0.18 5.7 ±0.38 5.7 ±0.14 5.5 ±0.19 5.7 ±0.19 5.7 ±0.19 5.6 $\pm0.21^{a}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table 4. The pH value of beef meatballs

Note: means within a row with different letters are significantly different (P<0.05)

The moisture content of beef meatballs with SLP and BHT as antioxidants, key findings was observed (Table 5). The antioxidants and storage duration had independent effects on the moisture content, as there was no interaction between the two factors (P>0.05). Samples treated with BHT exhibited lower moisture levels compared to those with SLP and the control samples.

The study revealed no significant interaction between the antioxidant type and storage duration, indicating independent effects on WHC. Notably, neither the type of antioxidant (including BHT and various concentrations of SLP) nor the storage duration significantly influenced the WHC. This suggests that factors such as the meat's inherent properties and the meatball formulation play a more critical role in determining WHC than the examined variables. The findings highlighted the complexity of factors influencing physical properties in food products and the specific role of antioxidants in this context. The result is presented in Table 6.

Meatballs –	Storage (hours)				
MeatDans	0	5	10	Average	
Control	66.9±1.5	65.7±0.5	63.8±2.7	63.8 ± 2.1^{b}	
BHT	65.5 ± 0.7	62.8±3.2	59.4±2.8	$59.4 \pm 3.4^{\circ}$	
0.5	68.1±0.9	66.8±1.1	64.1±2.3	64.1 ± 2.2^{ab}	
1.0	68.5±1.3	68.4±1.4	66.8±1.0	66.8 ± 1.4^{a}	
1.5	68.9±1.1	67.9±0.8	64.3±1.9	64.3 ± 2.2^{ab}	
Average	67.6 ± 1.6^{a}	66.3 ± 2.5^{b}	63.7±3.1°		

 Table 5. The moisture content (%) of beef meatballs

Note: means within a column or row with different letters are significantly different (P < 0.05)

Storage (hours)				
0	5	10	Average	
51.1±4.05	51.1±3.03	46.0±3.19	49.4±3.93	
45.9±6.02	42.2±3.87	49.2±4.90	45.8±5.31	
45.5±3.99	52.1±4.33	51.1±3.47	49.6±4.60	
49.4±4.87	49.1±2.14	49.8 ± 2.40	49.4±2.94	
52.6±3.14	50.8±3.62	49.4±4.69	51.0±3.63	
48.9 ± 4.80	49.1±4.74	49.1±3.70		
	51.1±4.05 45.9±6.02 45.5±3.99 49.4±4.87 52.6±3.14	0 5 51.1±4.05 51.1±3.03 45.9±6.02 42.2±3.87 45.5±3.99 52.1±4.33 49.4±4.87 49.1±2.14 52.6±3.14 50.8±3.62	051051.1±4.0551.1±3.0346.0±3.1945.9±6.0242.2±3.8749.2±4.9045.5±3.9952.1±4.3351.1±3.4749.4±4.8749.1±2.1449.8±2.4052.6±3.1450.8±3.6249.4±4.69	

Table 6. The water holding capacity (%) of beef meatballs

The study showed that different antioxidant treatments, including SLP and BHT, affected meatball tenderness variably (Table 7). Specifically, higher concentrations of SLP (1.5%) resulted in less tender meatballs, while BHT and lower SLP concentrations were similar to the control in terms of tenderness. Additionally, meatball tenderness decreased as storage time increased that the products become tough, indicating changes in texture over time. This study sheds light on how both natural and synthetic additives impact the texture of meat products, particularly in the context of storage conditions.

The emulsion stability response of meatballs is characterized by the amount of released fluid and separated water, as seen in Table 8. In this study, the Emulsion Stability (ES) of beef meatballs with senduduk leaf powder (SLP) and BHT, the findings reveal intricate effects of antioxidants and storage duration. For the released fluid, there was no interaction between antioxidants

and storage time, showing that these factors independently influenced fluid stability. However, in released water, an interaction was observed, suggesting that the combination of antioxidants and storage time impacts water stability in the emulsion. Despite these nuances, the overall storage duration did not significantly affect either released fluid or released water. This suggests a complex relationship between antioxidant type, storage duration, and emulsion stability, highlighting the importance of selecting suitable additives to maintain the desired qualities of meat products over time.

Meatballs				
wieatballs	0	5	10	Average
Control	$0.47{\pm}0.01$	0.41±0.01	0.41±0.01	0.43 ± 0.03^{bc}
BHT	$0.44{\pm}0.01$	0.41 ± 0.01	0.40 ± 0.00	$0.42 \pm 0.02^{\circ}$
0.5	0.45 ± 0.00	$0.44{\pm}0.00$	0.43±0.01	$0.44{\pm}0.01^{ab}$
1.0	$0.50{\pm}0.02$	$0.44{\pm}0.05$	0.43 ± 0.03	$0.46{\pm}0.05^{a}$
1.5	0.41 ± 0.02	$0.40{\pm}0.01$	0.37±0.01	$0.39{\pm}0.02^{d}$
Average	$0.45{\pm}0.034^{a}$	$0.42{\pm}0.028^{b}$	$0.41 \pm 0.026^{\circ}$	

Table 7. The tenderness (mm/g/s) of meatballs

Note: means within a column or row with different letters are significantly different (P<0.05)

Meatballs		Storage ((hours)	
wicatballs	0	5	10	Average
	Emulsion Stability	y: released fluid (9	%)	
Control	1.37 ± 0.22	2.21 ± 0.42	2.61±0.33	$2.61{\pm}0.62^{a}$
BHT	2.18±0.53	2.00 ± 0.36	2.45 ± 0.24	$2.45{\pm}0.39^{a}$
0.5	1.77 ± 0.63	$2.09{\pm}0.59$	2.46 ± 0.28	$2.46{\pm}0.54^{\mathrm{a}}$
1.0	1.53 ± 0.66	1.38 ± 0.47	1.70 ± 0.59	$1.70{\pm}0.52^{b}$
1.5	1.71 ± 0.20	1.36 ± 0.37	1.23 ± 0.22	1.23 ± 0.32^{b}
Average	1.71 ± 0.50	1.81±0.53	2.09±0.63	
	Emulsion Stability	v: released water (%)	
Control	1.31 ± 0.27	2.14 ± 0.39	2.47 ± 0.17	$1.97{\pm}0.57^{\rm a}$
BHT	2.15±0.52	1.93 ± 0.31	2.39 ± 0.23	$2.16{\pm}0.38^{\text{a}}$
0.5	1.65 ± 0.46	$2.00{\pm}0.49$	$2.39{\pm}0.24$	$2.01{\pm}0.48^{\rm a}$
1.0	1.45 ± 0.65	$1.34{\pm}0.47$	$1.60{\pm}0.48$	$1.46{\pm}0.48^{b}$
1.5	1.60 ± 0.11	1.34 ± 0.36	1.13 ± 0.15	1.36 ± 0.29^{b}
Average	1.63 ± 0.48	1.75 ± 0.49	$2.00{\pm}0.60$	

Table 8. The emulsion stability (%) of meatballs

Note: means within a column or row with different letters are significantly different (P<0.05)

The data showed that both in released fluid and released water, the duration of storage at room temperature was not significantly affected the emulsion stability (P>0.05), at least up to 10 hours (Table 8). However, the increase of SLP percentage added (1 and 1.5%) lesding to lower the fluid and water released from the meatball. These phenomena indicated that the emulsion stability is shown to be higher than other type of meatballs (Control, BHT, and SLP 0.5%).

The folding test indicated the elasticity of the meatball. The result exerted that the addition of SLP were not different from the control, whereas the addition of BHT decreased the folding test of meatballs (P<0.05) (Table 9). The elasticity of the meatballs was decreased as over time (P<0.05), and there was no interaction between treatments and duration of storage (P<0.05).

Meatballs -		Storage (hours)				
WieatDalls	0 5 10		Average			
Control	4.56±0.58	3.96±0.68	3.88 ± 0.88	4.13 ± 0.87^{b}		
BHT	4.44 ± 0.82	3.64±0.81	3.36±0.81	3.81±0.82°		
0.5	4.40 ± 0.82	4.00±0.71	3.56 ± 0.87	3.99 ± 0.83^{bc}		
1.0	4.36±0.76	4.12±0.73	3.88±1.01	4.12 ± 1.03^{b}		
1.5	4.64 ± 0.49	4.56±0.82	4.04 ± 0.93	4.41±0.95 ^a		
Average	$4.48{\pm}0.55^{a}$	4.06 ± 0.99^{b}	$3.74{\pm}0.92^{\circ}$			

Table 9. Folding test score of beef meatballs

Note: means within a column or row with different letters are significantly different (P<0.05)

Discussion

TBA number of meatballs

The less effective of lower concentrations of SLP (0.5 and 1%) in retarding lipid oxidation could be due to the differences in the antioxidant compounds present in BHT and SLP. BHT is a well-known antioxidant that has shown strong antioxidant effects and is used in various industries (Ramírez-Rojo *et al.*, 2019). On the other hand, SLP is a natural antioxidant that contains phytochemicals such as polyphenols, alkaloids, and terpenoids (Suharyanto *et al.*, 2019b; Suharyanto *et al.*, 2020). The effectiveness of an antioxidant in inhibiting lipid oxidation depends on its ability to scavenge free radicals and prevent the formation of reactive oxygen species (Bayram and Decker, 2023). Therefore, the higher concentration of SLP may have provided a greater amount of antioxidant compounds, resulting in better inhibition of lipid oxidation in the meatballs.

Significantly, oxidation increased with storage time (P < 0.05), as presented in Table 2, a typical trend in meat products due to extended oxygen exposure.

The types of antioxidant and storage conditions play a crucial role in managing food oxidation (Estévez *et al.*, 2022). Natural antioxidants, such as SLP, have shown potential in reducing oxidation when used at specific concentration (Suharyanto *et al.*, 2019a, 2022; Suharyanto *et al.*, 2023).

Antioxidants can inhibit lipid oxidation through various mechanisms, including the regeneration of oxidized antioxidants by other compounds, differences in antioxidant partitioning in homogeneous and heterogeneous systems, the combination of free radical scavenging and metal chelating activities, and the formation of additional antioxidant compounds upon oxidation (Bayram and Decker, 2023). The hydroperoxyl radical (HOO•) also plays a role in the propagation and termination of lipid peroxidation, and its interaction with antioxidants can increase their effectiveness in inhibiting lipid oxidation (Baschieri *et al.*, 2023).

Total microbial

The antimicrobial efficacy of SLP can be attributed to its natural compounds, likely phenolics, flavonoids, or tannins, known for their antimicrobial properties (Suharyanto *et al.*, 2019b). The phenolic compounds inhibit the growth of microorganisms by altering the structure and function of the cytoplasmic membrane, interfering with DNA/RNA synthesis and intermediary metabolism, disrupting the movement of protons leading to ion leakage, inhibiting enzyme synthesis, and interfering in cell communication (Charlene *et al.*, 2022). These compounds disrupt microbial growth, enhancing food preservation. The study observed a positive correlation between the concentration of SLP and the reduction in TPC. Higher concentrations resulted in greater antimicrobial activity, showcasing a typical dose-response relationship.

The study also revealed that storage duration significantly affected TPC, with values increasing over time. This aligns with the general understanding that microbial growth in meat products increases over time (Rovira *et al.*, 2023), especially at room temperature conducive to microbe proliferation (Ramirez-Arcos *et al.*, 2017). The increase in TPC from 0 to 10 hours underscores the necessity of effective preservatives in extending the shelf life of meat products.

Physicochemical properties

However, storage duration showed a significant effect, with a notable decrease in pH as the storage period progressed. This result is in line with the research of Indiarto *et al.* (2020) which stated that the longer the storage of

meatballs, the lower the pH value become. Indiarto *et al.* (2020) described that as meatballs are stored, microorganisms present can proliferate, leading to fermentation processes and the processes often produce acidic by-products, such as lactic acid, which lower the pH. The decline in pH is a common indicator of such microbial activity and is a crucial factor in assessing the spoilage and shelf life of meat products (Indiarto *et al.*, 2020). The study highlights the complexity of factors influencing pH in meat products and underscores the need for further investigation into the role of natural antioxidants in food preservation. While SLP did not significantly affect the pH, it might still contribute positively to the meatballs' quality by other means, such as inhibiting microbial growth or oxidative processes, which were not directly measured by pH.

The moisture content of meatballs treated with BHT is lower compared to those added with leaf powder most probably due to the different properties of these additives. BHT is a synthetic antioxidant that is commonly used to preserve the quality of food products. It has the ability to reduce moisture loss and maintain the moisture content in meatballs, resulting in lower moisture content compared to untreated meatballs (Rasak *et al.*, 2021). On the other hand, leaf powders such as *Melastoma malabathricum*, *Moringa oleifera*, and water spinach are natural agents that are rich in bioactive compounds and antioxidants. These leaf powders have been found to enhance the antioxidant activity of meatballs and improve their physicochemical properties, such as water holding capacity and cooking loss (Vebrianty *et al.*, 2021). However, they may not have the same moisture-retaining properties as BHT, leading to a higher moisture content in meatballs treated with leaf powders (Suharyanto *et al.*, 2020; Vebrianty *et al.*, 2021).

Over time, all meatball variants experienced a decrease in moisture content (P<0.05). It was likely due to water loss from evaporation or structural changes in the meatballs (Kamble *et al.*, 2023). These results underscore the nuanced impact of antioxidant types and storage conditions on the moisture retention of meat products.

Room temperature storage has been found to have varying effects on the water holding capacity of processed meat products such as meatballs, sausages, and nuggets. In the study by Ismail *et al.* (2016), different temperature storage did not have a significant effect on the water holding capacity of beef meatballs. Herbal powders might not alter these key determinants of WHC in meat products significantly. Their primary function might be more related to flavoring, antioxidative properties, or microbial inhibition, rather than directly influencing the meat's ability to retain water.

The results suggested that the water retention properties of meatballs are more likely governed by the inherent characteristics of the meat and the formulation of the meatballs rather than the type of antioxidant or the storage duration. This highlights the complex nature of food matrices and the multiple factors that can influence their physical properties.

The softness of processed meat products decreased with longer room temperature storage due to several factors. One factor is found to affect thr storage time on the physical quality of the meat products. As the storage time increased, the pH value of the meatballs decreased, which can contribute to a firmer texture (Rahmania *et al.*, 2022). Additionally, the storage time can also increase the hygroscopicity of the meat products, making them more prone to moisture absorption and resulting in a drier and tougher texture (Li *et al.*, 2021).

These findings highlighted the importance of antioxidant type and concentration, as well as storage duration, in maintaining the desired textural qualities of meat products. The study contributed to understanding how natural additives like SLP influenced food texture, especially in comparison to synthetic antioxidants like BHT.

The improvement of the stability of meatball emulsion by SLP was the most probably due to its high phenolic compound content and strong antioxidant activity of senduduk leaf (Mita *et al.*, 2020). Other report showed that emulsion and gelling formation in meat products containing senduduk leaf extract indicated senduduk leaf had a positive potential and was composed of double spheroidal polymer amphiphilic anisotropic powder, which contributed to the stability of the emulsion (Jagtap *et al.*, 2020). The hydrophilic-lipophilic balance (HLB) of proteins in senduduk leaf extract and nonmeat proteins used in sausage making to improve the emulsion stability (Suharyanto *et al.*, 2019a).

The addition of 1.5% senduduk leaf powder with a particle size of 80 mesh to meatballs resulted a better elasticity most probably caused by several factors. Firstly, the phytochemical components of *Melastoma malabathricum*, such as tannins, flavonoids, terpenoids, and steroids, contribute to the structural integrity and binding properties of the meatball matrix, enhancing its elasticity (Hajrawati et al., 2021). Additionally, the incorporation of plant-based powders like senduduk can increase the crude fiber content, which has been shown to improve the textural properties of meat products, as seen with other plant powders like cemba and Moringa oleifera (Rasak et al., 2021). The presence of glucomannan in plant-based additives, similar to the effects observed with porang flour, also contributes to water retention and gel formation, which are crucial for maintaining meatball elasticity (Suteky, 2023). Furthermore, the addition of natural gums and fibers, such as those found in senduduk, can enhance the waterholding capacity (WHC) and reduce cooking loss, leading to a firmer and more elastic texture (Dewi and Widjanarko, 2015). The substitution of traditional binders with plant-based powders has been shown to improve the physical

properties of meatballs, as evidenced by studies on taro flour and Centella leaf extracts, which also resulted in increased elasticity and improved textural attributes (Akter *et al.*, 2022).

The addition of 1.5% Senduduk (*Melastoma malabathricum* L.) leaf powder effectively reduced oxidation and microbial counts in beef meatballs, comparable to BHT, while maintaining pH, water-holding capacity, and emulsion stability. Despite some effects on texture and sensory properties, Senduduk leaf powder is a promising natural alternative to synthetic preservatives for improving meatball quality and safety.

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