



# Application of senduduk (*Melastoma malabathricum*) leaf powder as a natural antioxidant in beef sausages: Role of particle size on product quality

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## ABSTRACT

This study evaluated the effects of *Melastoma malabathricum* (senduduk) leaf powder with different particle sizes (30, 40, and 50 mesh) on the quality of beef sausages. Sausages containing 0.75% senduduk powder were compared with a control and a butylated hydroxytoluene (BHT)-treated group. Parameters analyzed included microbial load, lipid oxidation (TBA), pH, cooking loss, moisture, water holding capacity (WHC), emulsion stability, elasticity, tenderness, and sensory properties. BHT showed superior antimicrobial and antioxidant activity, significantly lowering TPC and TBA values. Although senduduk powder did not significantly affect microbial counts, the 50-mesh size improved oxidative stability, WHC, elasticity, and aroma. However, it did not impact tenderness, color, or meaty taste. Overall acceptability of senduduk sausages was lower than BHT and control, possibly due to unfamiliar herbal flavors. These results suggest that while senduduk leaf powder has potential as a natural antioxidant, low inclusion levels limit its effectiveness, and further optimization is required for performance comparable to synthetic antioxidants.

## 1. Introduction

Lipid oxidation and microbial spoilage remain major challenges in the preservation of emulsified meat products such as sausages. These deteriorative processes compromise not only the shelf-life and safety, but also the sensory and nutritional quality of meat products (Dominguez et al., 2019). To mitigate these issues, synthetic antioxidants like butylated hydroxytoluene (BHT) are widely employed due to their proven efficacy in inhibiting oxidative degradation and microbial growth (Zahid et al., 2019). However, growing consumer awareness and regulatory concerns about the potential health risks associated with synthetic additives have driven research toward natural alternatives.

Plant-based antioxidants derived from herbs, fruits, and leaves are gaining popularity as functional ingredients in meat systems due to their abundance

of bioactive compounds, such as polyphenols, flavonoids, and tannins. These compounds exhibit antioxidant and antimicrobial activities by scavenging free radicals, chelating metal ions, and disrupting microbial cell membranes (Rasheed et al., 2024; Tiwari et al., 2023). Among these, *Melastoma malabathricum*—commonly known as senduduk—has emerged as a promising candidate due to its rich phytochemical profile and broad spectrum of biological activities.

Numerous studies have documented the medicinal properties of *M. malabathricum*, including antioxidant, antimicrobial, anti-inflammatory, and wound-healing activities (Isnaini et al., 2023; Tiwari et al., 2023). Its leaves contain significant amounts of phenolic acids (e.g., gallic and ellagic acids), flavonoids (e.g., quercetin and kaempferol), and tannins, which contribute to its radical-scavenging ability and

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microbial inhibition (He et al., 2022; Tiwari et al., 2023; Hasan et al., 2024). In food-related applications, aqueous and ethanolic extracts of senduduk leaf have demonstrated strong ferric-reducing and DPPH-scavenging activity, suggesting potential as natural antioxidants in lipid-rich systems (Zhang et al., 2024). However, the direct incorporation of senduduk leaf powder into meat matrices, particularly in emulsion-type sausages, remains largely unexplored.

In addition, the role of particle size in enhancing the functional efficacy of plant powders is increasingly recognized. Particle size reduction can improve the surface area, dispersion, and release kinetics of bioactive compounds, thus enhancing their interaction with proteins and lipids in the meat matrix (Duguma et al., 2023; Liang et al., 2024). Finer particles can facilitate improved oxidative stability, water-binding, and textural attributes due to more uniform incorporation and increased bioavailability of phenolic constituents (Agamou et al., 2024; Dacanal, 2024).

This study aims to evaluate the effects of *M. malabathricum* (senduduk) leaf powder with different particle sizes (30, 40, and 50 mesh) on the microbiological, physicochemical, and sensory properties of beef sausages. In addition to natural treatments, a comparison with synthetic BHT and a negative control was conducted. Key quality indicators assessed were total plate count (TPC), thiobarbituric acid (TBA) values, pH, cooking loss, water holding capacity (WHC), emulsion stability, elasticity, instrumental

tenderness, and sensory attributes. This investigation not only explores the applicability of *M. malabathricum* as a natural additive, but also provides novel insights into the role of particle size optimization in enhancing its performance in meat products.

2. Materials and Methods

2.1. Powder preparation from leaves

The *Melastoma malabathricum* leaves were collected from shrubs in Bengkulu City. The leaves used were the third leaves from the shoot, in intact condition. The leaves were cleaned from dust and other impurities, then air-dried for 72 hours. Afterward, they were oven-dried at 60 °C for 5 hours. The dried leaves were then blended or ground, then sieved sequentially using a 50-mesh sieve; the residue was further sieved with a 40-mesh sieve, and the remaining portion was sieved with a 30-mesh sieve. The fractions obtained from each mesh size were used as treatments.

2.2. Sausage's preparation and treatment application

Bali beef from the thigh was used for sausage production. The meat was separated from connective tissue and fat, cut into smaller pieces, and then ground. After grinding, salt, one-third of the ice crystals, and the treatment powder were added. Vegetable oil, seasonings, *Melastoma malabathricum* leaf powder/BHT, and another one-third of the ice crystals were

Table 1. Sausage formula and *Melastoma malabathricum* leaf powder treatments per batch

| Ingredients                             | Treatments |                          |       |       |       |
|-----------------------------------------|------------|--------------------------|-------|-------|-------|
|                                         | Control    | Butylated hydroxytoluene | 30M   | 40M   | 50M   |
| Meat (g)                                | 400.0      | 400.0                    | 400.0 | 400.0 | 400.0 |
| Vegetable oil (g)                       | 80.0       | 80.0                     | 80.0  | 80.0  | 80.0  |
| Skim milk powder (g)                    | 24.0       | 24.0                     | 24.0  | 24.0  | 24.0  |
| Tapioca flour (g)                       | 60.0       | 60.0                     | 60.0  | 60.0  | 60.0  |
| Ice crystal (g)                         | 140.0      | 140.0                    | 140.0 | 140.0 | 140.0 |
| Salt (g)                                | 8.0        | 8.0                      | 8.0   | 8.0   | 8.0   |
| Garlic powder (g)                       | 7.0        | 7.0                      | 7.0   | 7.0   | 7.0   |
| Pepper powder (g)                       | 3.0        | 3.0                      | 3.0   | 3.0   | 3.0   |
| Nutmeg powder (g)                       | 1.6        | 1.6                      | 1.6   | 1.6   | 1.6   |
| BHT (g)                                 | -          | 0.12                     | -     | -     | -     |
| <i>M. malabathricum</i> leaf powder (g) | -          | -                        | 3.0   | 3.0   | 3.0   |
| Total (g)                               | 723.6      | 724.0                    | 726.6 | 726.6 | 726.6 |

subsequently incorporated while mixing for several minutes. Skim milk powder and tapioca flour, along with the remaining one-third of the ice crystals, were then added until a homogeneous mixture was obtained. The treatments applied are shown in Table 1. The batter was allowed to rest for 10 minutes, stuffed into non-edible polyamide food-grade casings with a diameter of 16 mm, and steamed at 65 °C for 45 minutes.

### 2.3. Total plate count (TPC)

The total microbial count was determined using the plate count method, following the procedure of BSN (2008). A total of 25 g of sausage sample was aseptically homogenized in 225 mL of sterile buffered peptone water (BPW) as the initial dilution. Serial dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  were then prepared by transferring 1 mL from the previous dilution to 9 mL of sterile BPW. From each dilution, 1 mL was transferred into separate sterile petri dishes, followed by the addition of 15–20 mL of sterile plate count agar (PCA). After the agar solidified, the plates were incubated at 37 °C for 24–48 hours. Colony-forming units (cfu) were then counted.

### 2.4. Thiobarbituric acids (TBA)

TBA assay was adapted from the method published by *Tarladgis et al.* (1960). A total of 10 g of sausage was homogenized with 50 mL of distilled water for 2 minutes. The mixture was transferred into a distillation flask, and 47.5 mL of distilled water was added. Subsequently, 2.5 mL of 4 M HCl was added until the pH reached 1.5, along with boiling chips and an antifoam agent. Distillation was performed under high heat to collect 50 mL of distillate within 10 minutes. The distillate was stirred, and 5 mL was transferred into a test tube and mixed with 5 mL of TBA reagent. The mixture was heated in a boiling water bath for 35 minutes. A blank was prepared using 5 mL of distilled water and 5 mL of TBA reagent. After cooling for 10 minutes, the absorbance was measured at 528 nm, using the blank as a reference. The TBA value was calculated as mg of malondialdehyde per kg of sample using the equation:  $TBA = 7.8 \times D$ .

### 2.5. pH value

The pH of the sausage was determined by homogenizing 1 gram of sausage with 9 mL of distilled water. The mixture was stirred until homogeneous, and the pH was measured using a calibrated pH meter.

### 2.6. Cooking loss

Cooking loss was calculated using the difference in weight before and after steaming. The weight of the batter was recorded before stuffing, and the weight of the sausage was measured after steaming, draining, and cooling to room temperature (with the casing removed). Cooking loss was expressed as a percentage using the following formula:

$$\text{Cooking loss (\%)} = [(\text{weight of batter} - \text{weight of sausage}) / \text{weight of batter}] \times 100$$

### 2.7. Moisture content

Moisture content was determined using the oven-drying method (AOAC, 2005). A 2 g sample of sausage was dried in an oven at 105 °C until a constant weight was achieved. Moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = [(\text{initial weight} - \text{weight after drying}) / \text{initial weight}] \times 100$$

### 2.8. Water Holding Capacity (WHC)

Water holding capacity (WHC) was determined using the centrifugation method (Jung & Joo, 2013) with slight modification. A 5 g portion of homogenized sausage sample was placed into a centrifuge tube and mixed 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 supernatant was discarded, and the remaining pellet was re-incubated at 30 °C for 10 minutes. After the second incubation, the supernatant was again discarded. WHC was calculated using the following formula:

$$\text{WHC (\%)} = [\text{weight of sausage after supernatant removal} / \text{weight of sausage with 10 mL added water}] \times 100$$

### 2.9. Emulsion Stability

The measurement of emulsion stability adopted *Seo et al.* (2016) with minor modification. A 7.5 g sample was placed into a centrifuge tube and heated in a water bath at 70 °C for 30 minutes. The sample was then centrifuged at 1000 rpm for 10 minutes. The released supernatant was collected and weighed. This liquid was then dried in an oven at 105 °C for 16 hours. The residue remaining after drying was weighed and recorded as the fat content. The water content was calculated as the difference between the weight of the liquid and the fat content.

Emulsion stability is indicated by the amount of released water. The lower the amount of released water, the higher the emulsion stability.

The following formulas were used:

Released liquid (%) = (weight of released liquid / weight of sample)  $\times$  100

Released water (%) = (weight of water / weight of sample)  $\times$  100

## 2.10. Folding Test

The folding test was conducted based on the method described by Lanier (1992). The evaluation was performed by 25 semi-trained panelists selected from undergraduate students of the Animal Science Study Program, Faculty of Agriculture, University of Bengkulu, who had completed the Advanced Animal Product Technology course. A central section of the sausage was sliced into pieces measuring 3 mm in thickness and 2.5 cm in length. Each slice was folded using the index finger and thumb, and panelists assigned scores based on the degree of cracking observed during folding. A score of 5 was given if the sausage showed no cracking when folded, 4 if there was no cracking when folded into a half-circle, 3 if slight cracking occurred at the fold in the half-circle position, 2 if cracking appeared rapidly when folded into a half-circle, and 1 if cracking occurred immediately when the fingers applied pressure to fold the sample.

## 2.11. Tenderness

The measurement of sausage tenderness was performed using a universal penetrometer. The penetrometer was prepared by ensuring the indicator needle was at the zero position. A weight was then added to the plunger head of the needle rod. The load (g) was calculated by summing the weight of the weight, plunger head, and the needle. The sample was placed directly under the needle, and the pressure lever was pressed for 10 seconds. The indicator needle scale, which shows the depth of needle penetration into the sample (mm), was then read. Each measurement result was converted to the unit of  $\text{mm g}^{-1} \text{s}^{-1}$ .

## 2.12. Sensorial characteristics

The sensory characteristics were assessed by adapting Meilgaard et al. (1999) with slight modification. Sausages were evaluated by 25 semi-trained panelists selected from fifth-semester students of the Department of Animal Science, Faculty

of Agriculture, University of Bengkulu. The panelists were given adequate briefing and instructions regarding the sensory evaluation procedures and organoleptic attributes to be assessed. The evaluated attributes included color (very dull – very bright), odor (very fishy – not fishy), texture (very coarse – smooth), tenderness (not tender – very tender), and overall acceptability (dislike very much – like very much).

Each evaluation was conducted using a linear scale, with the left end indicating the most negative perception and the right end indicating the most positive. A 7 cm horizontal line was used for each attribute. Panelists marked their personal responses on the line, and the distance (in cm) from the left end to the mark was measured as the hedonic and quality score. Each sample was coded with a three-digit random number, and evaluations between samples were interspersed with palate cleansing using plain water.

## 2.13. Statistical Analysis

The obtained data were analyzed using analysis of variance (ANOVA). For variables that showed significant differences among treatments, further analysis was conducted using Duncan's Multiple Range Test (DMRT). A confidence level of 95% was used in all statistical evaluations.

# 3. Results and Discussion

## 3.1. Total Plate Count (TPC)

TPCs of beef sausages ranged from 3.63 to 4.16  $\log \text{cfu g}^{-1}$  (Table 2). A significantly lower ( $P < 0.05$ ) TPC was observed in the BHT group (3.63  $\log \text{cfu g}^{-1}$ ) compared to the control (4.16  $\log \text{cfu g}^{-1}$ ), confirming the well-documented antimicrobial efficacy of synthetic antioxidants through membrane disruption and enzyme inhibition (Osorio-Olivares et al., 2024).

While sausages treated with senduduk leaf powder (30M, 40M, 50M) showed no statistically significantly lower TPC compared to the control, slightly lower TPCs were noted in the 40M (3.97  $\log \text{cfu g}^{-1}$ ) and 50M (4.02  $\log \text{cfu g}^{-1}$ ) groups relative to 30M (4.14  $\log \text{cfu g}^{-1}$ ). These findings suggest that smaller particle sizes could enhance antimicrobial efficacy, likely due to their greater surface area, facilitating greater release and bioavailability of phenolic compounds (Khoo et al., 2017; Prasedya et al., 2021; Zhang et al., 2024).

**Table 2.** Total plate count (TPC), thiobarbituric acid (TBA) value, and pH of beef sausages with senduduk leaf powder, butylated hydroxytoluene (BHT), and control treatments.

| Variables                      | Treatments             |                         |                        |                         |                        |
|--------------------------------|------------------------|-------------------------|------------------------|-------------------------|------------------------|
|                                | Control                | BHT                     | 30M                    | 40M                     | 50M                    |
| TPC (log cfu g <sup>-1</sup> ) | 4.16±0.12 <sup>a</sup> | 3.63±0.24 <sup>b</sup>  | 4.14±0.08 <sup>a</sup> | 3.97±0.37 <sup>ab</sup> | 4.02±0.28 <sup>a</sup> |
| TBA (mg Kg <sup>-1</sup> )     | 0.07±0.02              | 0.03±0.05               | 0.05±0.03              | 0.05±0.03               | 0.04±0.02              |
| pH                             | 6.00±0.28 <sup>a</sup> | 5.70±0.26 <sup>ab</sup> | 5.40±0.12 <sup>b</sup> | 5.46±0.19 <sup>b</sup>  | 5.43±0.33 <sup>b</sup> |

**Legend:** Means in the same row with different superscript letters differ significantly ( $P < 0.05$ ). 30M, 40M, 50M = particle size of senduduk leaf powder in mesh.

Polyphenols exhibit antimicrobial activity via multiple mechanisms, including membrane disruption, inhibition of DNA and protein synthesis, metal ion chelation, oxidative stress induction, and interference with quorum sensing (Rasheed *et al.*, 2024). However, the relatively modest TPC reductions in senduduk treatments suggest that a 0.75% inclusion level may be insufficient to exert a strong antimicrobial effect, potentially due to limited release or thermal degradation of active compounds during processing. These results indicate that higher concentrations or alternative delivery methods could be required to optimize the antimicrobial potential of senduduk leaf powder in meat matrices.

### 3.2. Thiobarbituric Acid (TBA)

Lipid oxidation levels, determined by the TBA assay, ranged from 0.03 to 0.07 mg MDA kg<sup>-1</sup> (Table 2). As expected, the BHT treatment exhibited the lowest TBA value (0.03 mg kg<sup>-1</sup>), confirming its high oxidative stability. Among the treatments with senduduk leaf powder, the 50M group showed the lowest TBA value (0.04 mg kg<sup>-1</sup>), followed by 30M and 40M (both at 0.05 mg kg<sup>-1</sup>). Although the differences were not statistically significant, the data suggest a trend toward improved oxidative stability with decreasing particle size.

The enhanced antioxidant potential of finer particles may be attributed to their increased surface area, which facilitates the release and diffusion of bioactive compounds. Senduduk leaf is known to be rich in phenolic acids and flavonoids (Hipol *et al.*, 2023), which function as hydrogen donors, scavenging lipid peroxyl radicals and inhibiting the propagation phase of lipid oxidation (Tiwari *et al.*, 2023). The lower TBA values observed in the 50M group

suggest a more efficient antioxidant activity, likely due to greater availability and mobility of phenolic constituents.

These findings are consistent with previous studies indicating that smaller particle sizes enhance antioxidant efficacy in meat systems. Duguma *et al.* (2023) reported that finely ground herbal extracts provided more uniform and effective oxidative protection. Similarly, Agamou *et al.* (2024) and Zhang *et al.* (2024) demonstrated that particle size reduction increases total phenolic content and antioxidant capacity due to cell wall disruption and improved compound release. Liang *et al.* (2024) further emphasized that grinding enhances the bioavailability of antioxidant compounds by mechanically breaking plant cell matrices.

Nevertheless, despite the observed trend, the antioxidant efficacy of senduduk leaf powder remained inferior to that of BHT, possibly due to lower concentration or stability of active compounds during processing. These results suggest that while particle size reduction improves the functional performance of natural antioxidants, optimizing the concentration and protection of phenolics during thermal treatment remains essential for maximizing oxidative stability in meat products.

### 3.3. pH Values

The pH of the sausages ranged from 5.40 (30M) to 6.00 (control), showing significant differences ( $P < 0.05$ ) between 30M/40M/50M and the control (Table 2). The lower pH in the senduduk groups compared with the control could be attributed to the acidic nature of phenolic compounds in senduduk powder. These compounds can contribute to a decrease in pH by interacting with meat proteins and other components, leading to a more acidic

environment (Suharyanto et al., 2020) by donating hydrogen ions, resulting in increased acidity (Suharyanto et al., 2022).

However, the pH of sausage obtained from the addition of senduduk leaf powder with particle sizes of 30 mesh to 50 mesh was similar. This is likely due to comparable particle surface areas and phenolic compound release within this narrow particle size range. The minor variation in particle size did not significantly alter the quantity and efficacy of phenolic compounds interacting with the meat matrix, resulting in uniform pH levels across treatments (Novelli et al., 2014). It is possible that further reduction in particle size could result in a significant decrease in pH.

### 3.4. Cooking Loss

Cooking loss did not differ significantly among treatments ( $P > 0.05$ ), ranging from 1.06% to 1.87% (Table 3), indicating that 0.75% addition of senduduk leaf powder with differing particle sizes or BHT had limited impact on moisture and fat loss during heating. Cooking loss reflects emulsion stability and is governed by the integrity of protein-water-fat interactions during thermal processing (Mazumder et al., 2023; Kawata et al., 2023; Faridah et al., 2023). Although antioxidants can enhance structural stability by mitigating protein oxidation (Oh et al., 2024), their effect could be negligible at low inclusion levels. This is consistent with previous findings where low-dose herbal antioxidants (<1%) did not alter cooking loss despite improving WHC and oxidative stability (Bellucci et al., 2022;

Adeyemi et al., 2025). In this study, improved WHC in some treatments did not translate into lower cooking loss, likely due to the limited influence of internal water-binding changes on fluid release during cooking at low additive concentrations. Thus, higher inclusion levels or alternative strategies could be required to achieve significant improvements in cooking yield.

### 3.5. Moisture Content

The moisture content among treatments (control, BHT, 30M, 40M, and 50M) showed no significant differences ( $P > 0.05$ ), with values ranging from 61.80% to 63.59% (Table 3). This observation shows that the addition of senduduk leaf powder at the 0.75% concentration and different particle sizes (30, 40, and 50 mesh) did not substantially alter the overall moisture content in beef sausage.

Moisture content in processed meat is mainly governed by initial water addition, ingredient water-binding capacity, and processing conditions (Kim et al., 2022). In this study, the low-level addition (0.75%) of senduduk leaf powder did not significantly affect total moisture content, despite notably enhancing WHC. This indicates that senduduk leaf powder primarily modifies protein-water interactions, thus improving water retention, rather than altering overall moisture content. These findings are consistent with previous reports, which suggest that small herbal additions typically influence functional properties, like WHC and protein-water interactions, rather than directly affecting moisture levels (Choe et al., 2011; Zhang et al., 2013).

**Table 3.** Physicochemical properties of beef sausages with senduduk leaf powder, butylated hydroxytoluene (BHT), and control treatments.

| Variables                                        | Treatments              |                         |                         |                          |                         |
|--------------------------------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|
|                                                  | Control                 | BHT                     | 30M                     | 40M                      | 50M                     |
| Cooking Loss (%)                                 | 1.06±0.71               | 1.37±0.52               | 1.87±1.48               | 1.68±0.68                | 1.77±1.01               |
| WHC (%)                                          | 78.16±0.88 <sup>c</sup> | 87.14±1.04 <sup>a</sup> | 64.30±0.83 <sup>c</sup> | 76.03±1.03 <sup>d</sup>  | 85.17±0.99 <sup>b</sup> |
| Moisture Content (%)                             | 63.22±2.13              | 61.80±1.31              | 63.36±2.64              | 62.57±1.87               | 63.59±2.49              |
| Fluid Release (%)                                | 0.34±0.22               | 0.10±0.06               | 0.17±0.06               | 0.18±0.06                | 0.11±0.08               |
| Folding Test                                     | 2.44±1.12 <sup>bc</sup> | 2.40±0.96 <sup>bc</sup> | 1.92±0.76 <sup>c</sup>  | 2.68 ± 0.85 <sup>b</sup> | 3.52±1.00 <sup>a</sup>  |
| Tenderness (mm g <sup>-1</sup> s <sup>-1</sup> ) | 0.48±0.22               | 0.55±0.10               | 0.46±0.06               | 0.45±0.11                | 0.45±0.15               |

**Legend:** Means in the same row with different superscript letters differ significantly ( $P < 0.05$ ). 30M, 40M, 50M = particle size of senduduk leaf powder in mesh.

### 3.6. Water Holding Capacity (WHC)

The WHC showed significant differences among treatments ( $P < 0.05$ ), with BHT-treated sausages exhibiting the highest WHC (87.14%), followed by sausages containing senduduk leaf powder with finer particle sizes (50M; 85.17%) (Table 3). The significant differences in WHC suggest that both the nature of additives and particle size significantly influenced water-protein interactions and consequently affected the ability of the product to retain moisture.

The greater WHC in sausages with BHT likely result from BHT's antioxidant activity, which prevents protein oxidation and maintains protein integrity, thereby preserving their water-binding functionality (Boateng *et al.*, 2022). Protein oxidation negatively alters protein structure, reducing WHC (Liu *et al.*, 2022a; Liu *et al.*, 2022b). Additionally, WHC improved with decreasing particle size of senduduk leaf powder (from 30M to 50M), as finer particles enhance extraction and dispersion of bioactive compounds such as flavonoids and tannins. These compounds interact effectively with meat proteins, stabilizing the emulsion and improving water-binding capacity (Dacanal, 2024). Thus, both antioxidant properties and optimal particle size enhance WHC by preserving protein integrity and strengthening protein-water interactions.

### 3.7. Emulsion Stability

The effects of adding senduduk leaf powder of differing particle sizes on the emulsion stability of sausages are presented in Table 3. Emulsion stability, as indicated by fluid release after physical agitation (vortexing), showed numerical differences among treatments, although these were not statistically significant ( $P > 0.05$ ). Specifically, fluid release values were lowest in sausages containing synthetic antioxidant BHT ( $0.10 \pm 0.06\%$ ), followed closely by sausages formulated with the smallest particle size of senduduk leaf powder (50 mesh) at  $0.11 \pm 0.08\%$ . Sausages with 30 mesh and 40 mesh senduduk powders exhibited slightly higher fluid releases of  $0.17 \pm 0.06\%$  and  $0.18 \pm 0.06\%$ , respectively, whereas the control without antioxidants demonstrated the highest fluid release at  $0.34 \pm 0.22\%$ .

The numerical reduction in fluid release with senduduk leaf powder and BHT could be associated with improved emulsion formation due to the presence of phenolic compounds in plant powders, which interact with meat proteins and lipids

to stabilize the matrix (Jung *et al.*, 2022). Finer particles, such as those in the 50 mesh treatment, likely dispersed more effectively, enhancing these interactions (Albert *et al.*, 2019). However, the lack of significant differences suggests that the 0.75% concentration of senduduk leaf powder was insufficient to produce a measurable stabilizing effect. Lee *et al.* (2020) similarly reported no improvement in emulsion stability at low concentrations of plant-derived antioxidants. Measurement variability could also have contributed to the non-significant findings.

### 3.8. Folding Test

The effects of incorporating senduduk leaf powder with varying particle sizes on sausage elasticity, as measured by folding test scores, are presented in Table 3. A significant effect was observed among treatments ( $P < 0.05$ ). The 50M treatment, containing 0.75% senduduk leaf powder at 50 mesh, yielded the highest folding score ( $3.52 \pm 1.00$ ), indicating superior elasticity compared to all other treatments. Conversely, the lowest elasticity was observed in the 30M group ( $1.92 \pm 0.76$ ), suggesting that larger particle sizes might interfere with the uniform distribution and interaction of bioactive compounds with meat proteins. The 40M treatment showed intermediate elasticity ( $2.68 \pm 0.85$ ), while the control ( $2.44 \pm 1.12$ ) and BHT ( $2.40 \pm 0.96$ ) groups did not differ significantly and demonstrated moderate elasticity.

The improved elasticity observed in the 50M treatment is likely due to the increased surface area of finer particles, which enhances their dispersion and integration within the meat matrix. This facilitates stronger interactions between phenolic compounds and myofibrillar proteins, supporting a more cohesive protein gel network. These findings are consistent with Suharyanto *et al.* (2025), who reported that finer plant powders improve protein binding and water retention in meat products. Conversely, the coarser particles in the 30M treatment may have led to incomplete incorporation into the protein matrix, reducing cohesiveness and gel strength, as similarly noted by Santhi *et al.* (2017). Folding scores of BHT-treated sausages were comparable to the control, indicating that BHT had minimal impact on elasticity. This suggests that the physical form and matrix interaction of antioxidants play a critical role in texture, beyond their chemical properties alone.

### 3.9. Tenderness

No significant differences were observed in sausage tenderness among treatments ( $P > 0.05$ ), with values ranging from 0.45 to 0.55 mm g<sup>-1</sup> s<sup>-1</sup> (Table 3). The addition of 0.75% senduduk leaf powder, regardless of particle size, did not influence texture compared to the control or BHT-treated sausages.

These findings suggest that the concentration used may have been too low to induce structural changes in the meat matrix. Previous studies have similarly reported limited effects of low-level plant-based antioxidants on textural parameters in emulsified meat products (Lee et al., 2020). Additionally, differences in particle size (30–50 mesh) may not have been large enough to alter the dispersion or interaction of the powder with meat proteins.

It is also possible that the bioactive compounds in senduduk powder were insufficiently available to influence myofibrillar protein interactions, a key determinant of meat tenderness. The instrumental method used may also lack sensitivity to detect subtle differences in gel firmness at such low additive levels.

Further research with higher inclusion levels or functional extracts of senduduk leaf, as well as more detailed rheological assessments, are warranted to fully understand its potential impact on textural quality.

### 3.10. Sensory Properties

The sensory evaluation showed no significant differences ( $P > 0.05$ ) in color, texture, tenderness, or meaty flavor of the sausages, but significant differences ( $P < 0.05$ ) were observed in aroma and overall acceptance (Table 4). Lightness scores ranged from 4.80 to 5.56 and did not differ significantly. Adding

0.75% senduduk leaf powder with different particle sizes had little effect on color, likely due to its low pigment content, similar to findings in sausages with low levels of natural antioxidants (Lee et al., 2020).

Aroma scores differed significantly ( $P < 0.05$ ), with BHT (5.50) and 50M (5.53) scoring higher than 30M (4.88), while the control and 40M were intermediate. Finer senduduk powder (50 mesh) likely interacted better with volatile compounds, reducing off-odors, aided by phenolic compounds (Lee et al., 2020). Lower aroma scores in 30M could be due to poor dispersion within the meat matrix.

Although texture scores did not differ significantly, BHT and control groups scored higher (5.50 and 5.27, respectively) than 30M and 40M (4.48 and 4.67, respectively). Coarser particles could have disrupted the emulsion structure, affecting mouthfeel. Uniform particle size is important for maintaining good texture in emulsified products (Santhi et al., 2017). Tenderness scores (5.21–5.84) also showed no significant differences, with all sausages rated moderately tender, supporting instrumental results and confirming that low levels of natural antioxidants do not affect tenderness (Lee et al., 2020). Meaty flavor perception was similar across treatments, although the control and BHT (6.16 and 6.18, respectively) scored slightly higher than senduduk groups. This suggests that senduduk powder at 0.75% does not enhance or impair meat flavor, consistent with earlier studies (Domínguez et al., 2019).

Significant differences ( $P < 0.05$ ) were observed in overall acceptance. BHT-treated sausages had the highest score (6.24), followed by the control (5.78), while senduduk treatments were lower (5.14–5.47). Although 50M improved aroma and elasticity, overall acceptability was not significantly enhanced, possibly due to combined effects

**Table 4.** Sensory properties of beef sausages with senduduk leaf powder, butylated hydroxytoluene (BHT), and control treatments.

| Variables            | Treatments                |                          |                          |                           |                          |
|----------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
|                      | Control                   | BHT                      | 30M                      | 40M                       | 50M                      |
| Color (Lightness)    | 5.56 ± 1.32               | 5.44 ± 1.32              | 4.84 ± 1.19              | 4.80 ± 1.35               | 5.17 ± 1.31              |
| Odor (fishy-unfishy) | 5.43 ± 0.92 <sup>ab</sup> | 5.50 ± 0.97 <sup>a</sup> | 4.88 ± 1.12 <sup>b</sup> | 5.06 ± 0.73 <sup>ab</sup> | 5.53 ± 0.91 <sup>a</sup> |
| Texture              | 5.27 ± 1.19               | 5.50 ± 1.00              | 4.48 ± 1.13              | 4.67 ± 0.90               | 5.07 ± 1.26              |
| Tenderness           | 5.38 ± 1.20               | 5.84 ± 1.24              | 5.45 ± 1.16              | 5.23 ± 1.27               | 5.21 ± 1.63              |
| Meaty Taste          | 6.16 ± 1.08               | 6.18 ± 1.04              | 5.75 ± 1.04              | 5.60 ± 1.40               | 5.82 ± 1.42              |
| Acceptance           | 5.78 ± 1.09 <sup>ab</sup> | 6.24 ± 1.04 <sup>a</sup> | 5.47 ± 1.02 <sup>b</sup> | 5.14 ± 1.18 <sup>b</sup>  | 5.29 ± 0.99 <sup>b</sup> |

**Legend:** Means in the same row with different superscript letters differ significantly ( $P < 0.05$ ). 30M, 40M, 50M = particle size of senduduk leaf powder in mesh.

of color, texture, or unfamiliar herbal notes. Overall liking depends on a balance of multiple sensory traits (Fiorentini et al., 2020).

#### 4. Conclusion

This study demonstrates that adding 0.75% senduduk (*Melastoma malabathricum*) leaf powder to beef sausages had limited effects on microbiological, chemical, physical, and sensory qualities.

Finer particles (50 mesh) slightly improved the WHC, emulsion stability, and elasticity without significantly affecting moisture, tenderness, or cooking loss. Sensory evaluation showed better odor and overall acceptance with finer powder, although other attributes remained largely unchanged. Senduduk leaf powder shows potential as a natural antioxidant, but further optimization of concentration, particle size, and processing is needed to match the efficacy of synthetic antioxidants like BHT.

## Primena praha lista biljke senduduk (*Melastoma malabathricum*) kao prirodnog antioksidansa u goveđim kobasicama: Uloga veličine čestica u kvalitetu proizvoda

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### INFORMACIJE O RADU

#### Ključne reči:

*Melastoma malabathricum*

Goveđe kobasice

Veličina čestica

Antioksidans

Senzorski kvalitet

Prirodni konzervansi

### APSTRAKT

U ovoj studiji ispitivan je uticaj praha lista biljke *Melastoma malabathricum* (senduduk) različitih veličina čestica (30, 40 i 50 meš) na kvalitet goveđih kobasica. Kobasice koje su sadržale 0,75% praha senduduka upoređene su sa kontrolnom grupom i grupom tretiranom butiliranim hidroksitoluolom (BHT). Analizirani parametri obuhvatali su mikrobiološko opterećenje, oksidaciju lipida (TBA), pH vrednost, gubitak pri kuvanju, sadržaj vlage, sposobnost zadržavanja vode (WHC), stabilnost emulzije, elastičnost, mekoću i senzorna svojstva. BHT je pokazao superiorno antimikrobno i antioksidativno delovanje, značajno smanjujući ukupni broj mikroorganizama (TPC) i vrednosti TBA. Iako prah senduduka nije značajno uticao na broj mikroorganizama, veličina čestica od 50 meš poboljšala je oksidativnu stabilnost, WHC, elastičnost i aromu. Međutim, nije zabeležen uticaj na mekoću, boju ili mesnati ukus. Ukupna prihvatljivost kobasica sa dodatkom senduduka bila je niža u poređenju sa BHT i kontrolnom grupom, verovatno usled prisustva nepoznatih biljnih aroma. Dobijeni rezultati ukazuju na to da prah lista senduduka poseduje potencijal kao prirodni antioksidans, ali njegova ograničena efikasnost pri niskim koncentracijama zahteva dodatnu optimizaciju kako bi se postigla uporediva svojstva sa sintetičkim antioksidansima.

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## References

- Adeyemi, K. D., Olatunji, O. S., Atolani, O., Ishola, H., Shittu, R.M., Okukpe, K. M. ... & Kazeem, M. O. (2025). Cholesterol oxides and quality attributes of NaCl-substituted low-fat chicken sausages prepared with different antioxidants. *Heliyon* 11(2), e41796.
- Agamou, A., Djeukeu, A.W., Assiene, O. D. S., Zra, T., Obono Ndong, T. L., Mbango Eke, P., Mboube Ngongang, O., Keubing Feudjio, S. R. & Fongnzossie, E. (2024). Influence of particle size fraction and bioactive compound contents on the biological activities of *Solanum torvum* L. leaf powder extracts. *Journal of Experimental Biology and Agricultural Sciences* 12(2), 203–217.
- Albert, C., Beladjine, M., Tsapis, N., Fattal, E., Agnely, F. & Huang, N. (2019). Pickering emulsions: Preparation processes, key parameters governing their properties and potential for pharmaceutical applications. *Journal of Controlled Release* 309, 302–332.
- AOAC, (2005). Official methods of analysis of AOAC International (18th ed.). Association of Official Analytical Chemists.
- Bellucci, E. R. B., Dos Santos, J. M., Carvalho, L. T., Borgonovi, T. F., & Lorenzo, J. M. & da Silva-Barretto, A.C. (2022). Açai extract powder as natural antioxidant on pork patties during the refrigerated storage. *Meat Science* 184, 108667.
- Boateng, E. F., Yang, Z. & Zhang, W. (2022). Effects of kiwi-fruit peel extract and its antioxidant potential on the quality characteristics of beef sausage. *Antioxidants* 11(8), 1441.
- Choe, J. H., Jang, A., Lee, E. S., Choi, J. H., Choi, Y. S., Han, D. J. ... & Kim, C. J. (2011). Oxidative and color stability of cooked ground pork containing lotus leaf (*Nelumbo nucifera*) and barley leaf (*Hordeum vulgare*) powder during refrigerated storage. *Meat Science* 87(1), 12–18.
- Dacanal, G. C. (2024). Particle size and morphology in food science and technology: a review. *International Journal of Food Science and Technology* 59(10), 6821–6833.
- 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.
- Duguma, H. T., Zhang, L., Ofoedu, C. E., Chacha, J. S. & Agunbiade, A. O. (2023). Potentials of superfine grinding in quality modification of food powders. *CyTA-Journal of Food* 21(1), 530–541.
- Faridah, M. R., Yusoff, M. M., Rozzamri, A., Ibadullah, W. Z. W., Hairi, A. N. A., Daud, N. H. A. ... & Ismail-Fitry, M. R. (2023). Effect of palm-based shortenings of various melting ranges as animal fat replacers on the physicochemical properties and emulsion stability of chicken meat emulsion. *Foods* 12(3), 597.
- Fiorentini, M., Kinchla, A. J. & Nolden, A. A. (2020). Role of sensory evaluation in consumer acceptance of plant-based meat analogs and meat extenders: A scoping review. *Foods* 9(9), 1334.
- Hasan, N. A., Wazir, N. N., Samsudin, M. Y., Sanizam, M. M. S. M., Ahmad, N. M., Hisham, N. A. B. ... & Masdek, N. R. N. (2024). Evaluation of phytoactive contents and antibacterial activities of green synthesised cerium oxide nanoparticles using *Melastoma* sp. leaf extract as the capping agent. *Heliyon* 10(14), e34558.
- He, R. J., Wang, Y. F., Yang, B. Y., Liu, Z. B., Li, D. P., Zou, B. Q. & Huang, Y. L. (2022). Structural characterization and assessment of anti-inflammatory activities of polyphenols and depsidone derivatives from *Melastoma malabathricum* subsp. normale. *Molecules* 27(5), 1521.
- Hipol, R. L., Wayas, H., Hipol, R., Bacuyag, F. M., Cabanlong, J., Daquigan, M. & Pladio, L. (2023). Phytochemical content, thin layer chromatographic profile, and pharmacologic activities of the Philippine native *Melastoma malabathricum* Linn. from Benguet. *Science, Engineering and Health Studies* 23050012–23050012.
- Isnaini, Oktaviyanti, I. K. & Budiarti, L. Y. (2023). Antibacterial and wound healing activity of ethanolic extract *Melastoma malabathricum* L. *Research Journal of Pharmacy and Technology* 16(5), 2210–2214.
- Jung, D. Y., Lee, H. J., Shin, D. J., Kim, C. H. & Jo, C. (2022). Mechanism of improving emulsion stability of emulsion-type sausage with oyster mushroom (*Pleurotus ostreatus*) powder as a phosphate replacement. *Meat Science* 194, 108993.
- 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, 391–401.
- Kawata, K., Giotto, F.M., de Mello, A.S., Kingery, T. & Silva, L.H. (2023). Effect of Beef Silver Skin (Epimysium) Levels on Meat Emulsion Stability, Quality Attributes, and Texture Parameters. *Foods* 12(20), 3775.
- Khoo, H. E., Azlan, A., Tang, S. T. & Lim, S. M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research* 61(1), 1361779.
- Kim, D. H., Kim, Y. J., Shin, D. M., Lee, J. H. & Han, S. G. (2022). Drying characteristics and physicochemical properties of semi-dried restructured sausage depend on initial moisture content. *Food Science of Animal Resources* 42(3), 411.
- Lanier, T.C. (1992). Measurement of surimi composition and functional properties. In Lanier, T.C. & Lee, C.M. (Eds.), *Surimi Technology*. Marcel Dekker, pp. 123–163.
- Lee, S. Y., Lee, D. Y., Kim, O. Y., Kang, H. J., Kim, H. S. & Hur, S. J. (2020). Overview of studies on the use of natural antioxidative materials in meat products. *Food Science of Animal Resources* 40(6), 863–880.
- Liang, K., Liang, S. & Zhu, H. (2024). The physicochemical characteristics and phenolic bioaccessibility of defatted millet bran powder prepared using superfine grinding. *LWT- Food Science and Technology* 201, 116173.
- Liu, J., Liu, D., Zheng, A. & Ma, Q. (2022a). Haem-mediated protein oxidation affects water-holding capacity of beef during refrigerated storage. *Food Chemistry* X 14, 100304.
- Liu, Y., Zhang, L., Gao, S., Bao, Y., Tan, Y., Luo, Y. ... & Hong, H. (2022b). Effect of protein oxidation in meat and exudates on the water holding capacity in bighead carp (*Hypophthalmichthys nobilis*) subjected to frozen storage. *Food Chemistry* 370, 131079.
- Mazumder, M. A. R., Sujintonniti, N., Chaum, P., Ketnawa, S. & Rawdkuen, S. (2023). Developments of plant-based emulsion-type sausage by using grey oyster mushrooms and chickpeas. *Foods* 12(8), 1564.

- Meilgaard, M. C., Carr, B. T. & Civille, G. V. (1999). Sensory evaluation techniques. CRC Press.
- BSN National Standardization Agency of Indonesia. (2008). Methods for testing microbial contamination in meat, eggs, milk, and their processed products (SNI 2897:2008). National Standardization Agency of Indonesia.
- Novelli, E., Fasolato, L., Cardazzo, B., Carraro, L., Taticchi, A. & Balzan, S. (2014). Addition of phenols compounds to meat dough intended for salami manufacture and its antioxidant effect. *Italian Journal of Food Safety* 3(3), 1704.
- Oh, Y. N., Choi, H. Y., Kim, Y. B., Hong, S. G. & Kim, H. Y. (2024). Effect of paprika powder on the antioxidant capacity of emulsion-type sausages. *Food Science of Animal Resources* 44(5), 1126.
- Osorio-Olivares, M.E., Vásquez-Martínez, Y., Díaz, K., Canelo, J., Taborga, L. & Espinoza-Catalán, L. (2024). Antibacterial and antioxidant activity of synthetic polyoxygenated flavonoids. *International Journal of Molecular Sciences* 25(11), 5999.
- Rasheed, H. A., Rehman, A., Karim, A., Al-Asmari, F., Cui, H. & Lin, L. (2024). A comprehensive insight into plant-derived extracts/bioactives: Exploring their antimicrobial mechanisms and potential for high-performance food applications. *Food Bioscience* 104035.
- Santhi, D., Kalaikannan, A. & Sureshkumar, S. (2017). Factors influencing meat emulsion properties and product texture: A review. *Critical Reviews in Food Science and Nutrition* 57(10), 2021–2027.
- Seo, J. K., Yum, H. W., Kim, G. D., Jeong, J. Y., & Yang, H. S. (2016). Properties of frankfurter-type sausages with pork back-fat replaced with bovine heart surimi-like materials. *Korean Journal for Food Science of Animal Resources* 36(4), 523.
- Suharyanto, Nuraini, H., Suryati, T., Arief, I. I. & Sajuthi, D. (2022). Antioxidant, antimicrobial and physicochemical properties of beef sausages enriched with an aqueous extract of Senduduk (*Melastoma malabathricum* L.) leaf. *International Journal of Food Studies* 11(2), 320–334.
- 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.
- Suharyanto, S., Warnoto, W., Banjarnahor, T.L., Timoti, I. & Hajrawati, H. (2025). Improving beef meatball properties by adding senduduk (*Melastoma malabathricum* L.) leaf powder. *International Journal of Agricultural Technology* 21(1), 299–316.
- Tarladgis, B. G., Watts, B. M., Younathan, M. T. & Dugan, L. R. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society* 37(1), 44–48.
- Tiwari, M., Barooah, M. S. & Bhuyan, D. (2023). Phytochemical and bioactive potentialities of *Melastoma malabathricum*. In *Recent Frontiers of Phytochemicals*. Elsevier, pp. 601–615.
- Zhang, W., Xiao, S. & Ahn, D. U. (2013). Protein oxidation: Basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition* 53(11), 1191–1201.
- Zhang, Z., Chen, Y., Wang, S., Tong, Z., Luan, F., Jiang, B. ... & Xu, Z. (2024). Effect of particle size on physical properties, dissolution, in vitro antioxidant activity, and in vivo hepatoprotective properties of *Tetragonia hemsleyana* Diels et Gilg powders. *Pharmaceutics* 16(11), 1352.

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