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Comparative analysis of biofilms in the meat and poultry processing industry: taxonomy and interactions

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ABSTRACT

This study aimed to compare the prevalence, taxonomic structure, and interactions of microbial communities in biofilms formed on surfaces in a meat processing plant (MPP) and a poultry processing plant (PPP). Using transmission electron microscopy and high-throughput sequencing of the 16S rRNA V3-V4 region, biofilms were detected in 25% of MPP scrape samples (2/8) and in 85.7% of PPP scrape samples (6/7). Taxonomic analysis revealed the dominance of *Proteobacteria*, *Bacteroidota*, *Actinobacteria*, and *Firmicutes* in biofilms from both facilities, with marked heterogeneity in the MPP (Chao1 index: 336.8–697.8). Network association of microorganisms has identified a synergy between the genera *Flavobacterium*, *Acinetobacter*, and *Psychrobacter*, and an antagonism between the *Marisediminicola* and *Pseudomonas/Acinetobacter*, which highlights the complexity of inter-microbial interactions in industrial settings.

1. Introduction

The microbial ecology of industrial food processing environments is a critical determinant of product safety and quality. Biofilms formed on equipment and surfaces in meat and poultry processing plants serve as reservoirs for spoilage microorganisms and potential pathogens, posing significant risks to both product shelf life and consumer health (Giaouris *et al.*, 2014). Despite advances in sanitation practices, persistent microbial contamination remains a challenge, particularly in facilities with complex workflows and varying environmental conditions. Understanding the composition, diversity, and interactions of microbial communities in these environments is essential for developing targeted disinfection strategies and mitigating contamination risks (Simões *et al.*, 2010).

Meat and poultry processing plants are characterized by unique environmental niches, including low temperatures, high humidity, and organic residues,

which favor the proliferation of psychrotrophic and biofilm-forming bacteria (Sofos, 2008). These microorganisms, such as *Pseudomonas*, *Psychrobacter*, and *Brochothrix*, are well-known agents of food spoilage, capable of degrading proteins and lipids even under refrigeration (Remenant *et al.*, 2015). Additionally, biofilms protect embedded microbes from routine sanitation, enabling recurrent contamination (Bridier *et al.*, 2015). While traditional microbiological methods have provided foundational insights, modern molecular approaches, such as 16S rRNA amplicon sequencing, offer unprecedented resolution for mapping microbial diversity and ecological dynamics in these complex systems (De Filippis *et al.*, 2017).

Previous studies have focused on specific pathogens (e.g., *Listeria monocytogenes*, *Salmonella* spp.) or spoilage organisms in food matrices (Carpentier & Cerf, 2011; Walia *et al.*, 2017), but fewer have systematically analyzed biofilm communities

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on industrial surfaces (Silva et al., 2024). Furthermore, comparative analyses between meat and poultry processing environments are scarce, despite differences in raw materials, processing stages, and sanitation protocols (Stellato et al., 2016). This study addresses these gaps by investigating the taxonomic composition, diversity, and co-occurrence patterns of microbial communities in two distinct processing facilities: a meat processing plant (MPP) and a poultry processing plant (PPP) in the Moscow region.

2. Materials and Methods

2.1. Sampling

In this work, we studied samples of biofilms collected from the surfaces of industrial premises and technological equipment in various areas of a meat processing plant (MPP) and a poultry processing plant (PPP)

in the Moscow region. Biofilm samples were obtained by scraping surfaces with a metal spatula before the daily routine disinfection procedure (Table 1).

The scrapings were immediately placed in sterile saline solution for molecular biological and microbiological studies. The presence of biofilms in the sampled scrapings was proved by studying the structural organization using transmission electron microscopy (TEM) of ultrathin sections of the samples.

2.2. Taxonomic analysis of biofilms. DNA isolation, amplification, and sequencing of 16S rRNA gene fragments.

Total DNA from the samples was isolated using Power Soil kits (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer’s protocols. The variable V3-V4 region

Table 1. Samples taken for the study and conditions at the sampling sites

Sample No.	Zone	Sampled surface
Meat Processing Plant		
1	Carcass storage. Ambient temperature 1.8 °C	The wall of the sewer ladder (drain) in the floor
2		Condensation from the underside of the meat crate release mechanism (elevator)
3		Conveyor lubrication
4		The wall of the sewer ladder (drain) in the floor
5		Wet piece of sealant coming off the wall
6		Surface of the frame on the crate transport trolley (interior)
7		Trolley wheels
8	Accumulator for chopped raw materials	Ice with dirt from the floor
Poultry Processing Plant		
1	Evisceration zone	Ceiling
2	Poultry carcass disinfection zone	Underside of walkways near the Morris Bath
3		The ceiling above Morris’ bathroom
4	Cooling tunnel. Ambient temperature 0 °C	Wall in the carcass cooling tunnel
5		Plastic conveyor roller (top) in the cooling tunnel
6	Packaging zone	Trolley wheels (plastic) at the beginning of the packaging room
7		Trolley wheels (plastic) at the end of the packaging room

of the 16S rRNA gene was amplified using universal primers 341F (5'-CCT AYG GGD BGC WSC AG-3') and 806R (5'-GGA CTA CNV GGG THT CTA AT-3'). The resulting PCR fragments were purified using Agencourt AMPure magnetic beads (Beckman Coulter, Brea, CA, USA) and concentration was measured using Qubit dsDNA HS Assay Kits (Invitrogen, Carlsbad, CA, USA). The PCR fragments were sequenced on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) in the format of 2×300 nucleotide pair-end reads.

2.3. Bioinformatic analysis

Paired readings were combined using the FLASH v.1.2.11 program (Magoč and Salzberg, 2011). After merging, low-quality reads, singletons, and chimeras were excluded. To determine the proportion of operational taxonomic units (OTUs) in each of the samples, original reads (including low-quality and singletons) were superimposed on representative OTU sequences with a minimum identity of 97% over the entire length of the reading. To perform all these procedures, the USEARCH v.11 software package (Edgar, 2010) was used. Taxonomic identification of microorganisms by 16S rRNA gene sequences was performed using the VSEARCH v.2.14.1 algorithm in the Silva v.138 database (Rognes *et al.*, 2016). The analysis of co-presence (absence) networks was carried out based on the Sperman correlation matrix (Langfelder *et al.*, 2012) and constructed using only significant correlation coefficients (Barberán *et al.*, 2014). The threshold for correlation coefficients was set at 0.7 and the threshold for adjusted p values was 0.001. The analysis included only OTUs, the relative content of which was at least 5.0% in at least one sample. Alpha diversity was assessed using the Chao1 and Shannon E indices. The calculations were performed using the Usearch v11 package. Visualization and statistical analysis of diversity data were performed in QIIME. Visualization of the co-occurrence network was performed using the software package Cytoscape v.3.8.2 (Shannon *et al.* 2003; Faust & Raes, 2016). All raw data obtained of 16S rRNA gene fragments were deposited in the NCBI database and are available within the BioProject PRJNA850912 project.

2.4 Microscopic analysis of biofilms

The structural organization of material in the scrapings was studied using transmission electron microscopy (TEM) of their ultrathin sections. Each material was immediately placed in a 2.5% solution of glutaraldehyde in cacodylate buffer (0.05 M sodium cacodylate solution, pH 7.0–7.5) and kept at 4 °C for a day. Then, it was washed three times with the same buffer solution for 5 min and fixed in a solution of OsO₄ (1% OsO₄ 0.7% solution of ruthenium red in cacodylate buffer) for 1.5 h at 4 °C. After fixation, the materials were placed in 2% agar-agar and sequentially kept in a 3% solution of uranyl acetate in 30% ethanol for 4 h, then in 70% ethanol for 12 h at 4 °C. The material was dehydrated in 96% ethanol (2 times for 15 min), then in absolute acetone (3 times for 10 min). Dehydrated materials were then soaked with EPON-812 resin (Epoxy Embedding Medium Epon® 812, Sigma-Aldrich, USA) kept in a mixture of resin: acetone in a ratio of 1:1 for 1 h, then in a mixture of resin: acetone in a ratio of 2:1 in for 1 hour. The resulting material was poured into resin capsules and polymerized at a temperature of 37 °C for a day, then at 60 °C for a day. Ultrathin sections were obtained on an LKB-III microtome (LKB, Sweden) and contrasted in an aqueous solution of 3% uranyl acetate (30 min), then in an aqueous solution of 4% lead citrate (30 min). To detect acid mucopolysaccharides in biofilms, ruthenium red dye (Sigma, USA) was used, when it was added in an amount of 0.7% together with OsO₄, with which it interacted. Using ruthenium red, the presence of extracellular polysaccharides in biofilms of a number of bacteria was shown (Smirnova *et al.*, 2010). The resulting preparations were analyzed using a JEM 100SHP electron microscope (JEOL, Japan) at an accelerating voltage of 80 kV and an operating magnification of 5000–50000×. Photo documentation of the materials was performed using a Morada G2 digital optical imaging system.

3. Results

All the material scrapings obtained were evaluated for the presence of biofilms by studying the structural organization using TEM of ultrathin sections of the samples. The material scrapings were identified as biofilms when the presence of a polysaccharide matrix and a characteristic cluster of cells was visible. At the MPP, out of eight samples taken, the presence of biofilm was proven in only two samples (Table 1 and 2). Biofilms were found in sample 2, taken from the

bottom surface (with condensate) during the delivery of meat boxes to the raw material area and in sample 7, taken from the wheels of a jack-cart trolley. Of the seven samples from the PPP, six contained biofilms of varying maturity (Tables 1 and 2).

3.1 Composition of microflora of biofilms

The taxonomic composition of fifteen selected samples for MPP and PPP was determined based on the analysis of the V3-V4 variable region of the 16S rRNA gene. In total, 435,101 sequences of

variable V3-V4 fragments of the 16S rRNA gene were determined in all samples of surface biofilm contamination (Table 2). The results of clustering the obtained sequences showed the presence of 14 archaeal and 2619 bacterial OTUs with 97% sequence identity.

In the MPP, biofilms from the two locations differed in the taxonomic diversity of their microbial communities (Table 2). The condensate sample from the lower surface of the elevator for the boxes (2MPP, Chao1 — 336.8) showed lower taxonomic diversity compared to the biofilm material collected

Table 2. Chao1 and Shannon diversity indices of the studied surface biofilms obtained from the meat processing plant (MPP) and poutry processing plant (PPP)

Biofilm (refer to Table 1 for the equivalent sample locations)	Diversity indices		Number of readings (included OTUs)
	Chao1	Shannon_E	
Meat processing plant			
2MPP	336.8	3.19	65091
7MPP	697.8	4.44	42882
Poultry processing plant			
1PPP	298.6	3.82	7528
2PPP	265.3	3.80	10669
3PPP	364.8	4.05	6911
4PPP	77.1	1.68	5373
6PPP	172.3	3.32	6617
7PPP	289.8	3.77	5638

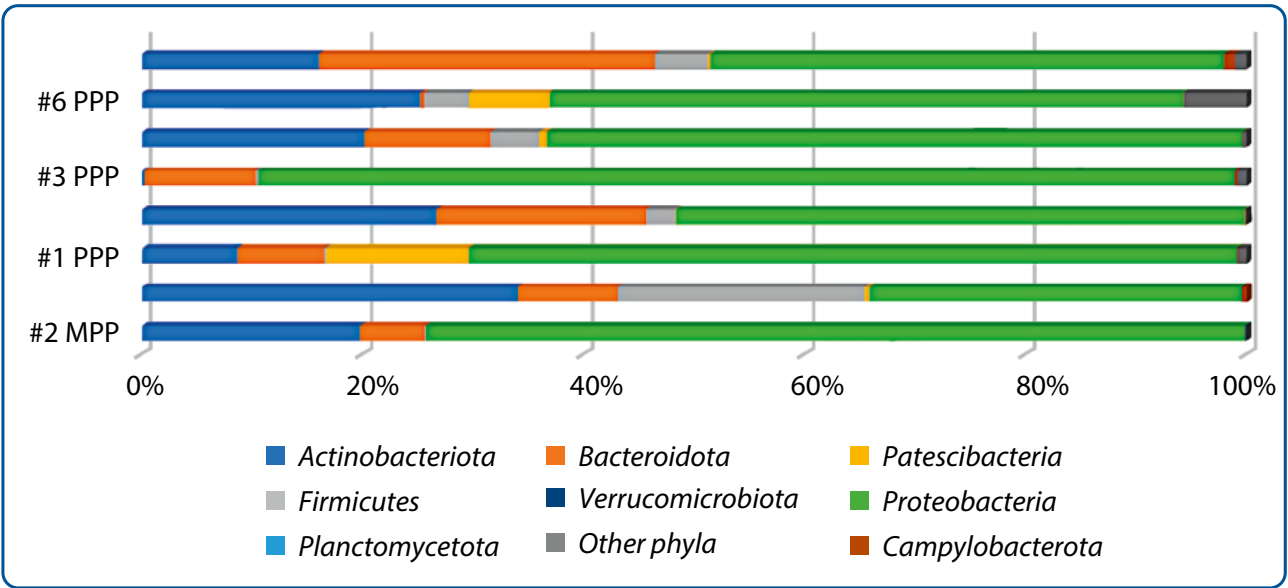


Figure 1. Distribution by class of microorganisms identified in biofilms collected from the studied meat processing plant (MPP) and poultry processing plant (PPP)

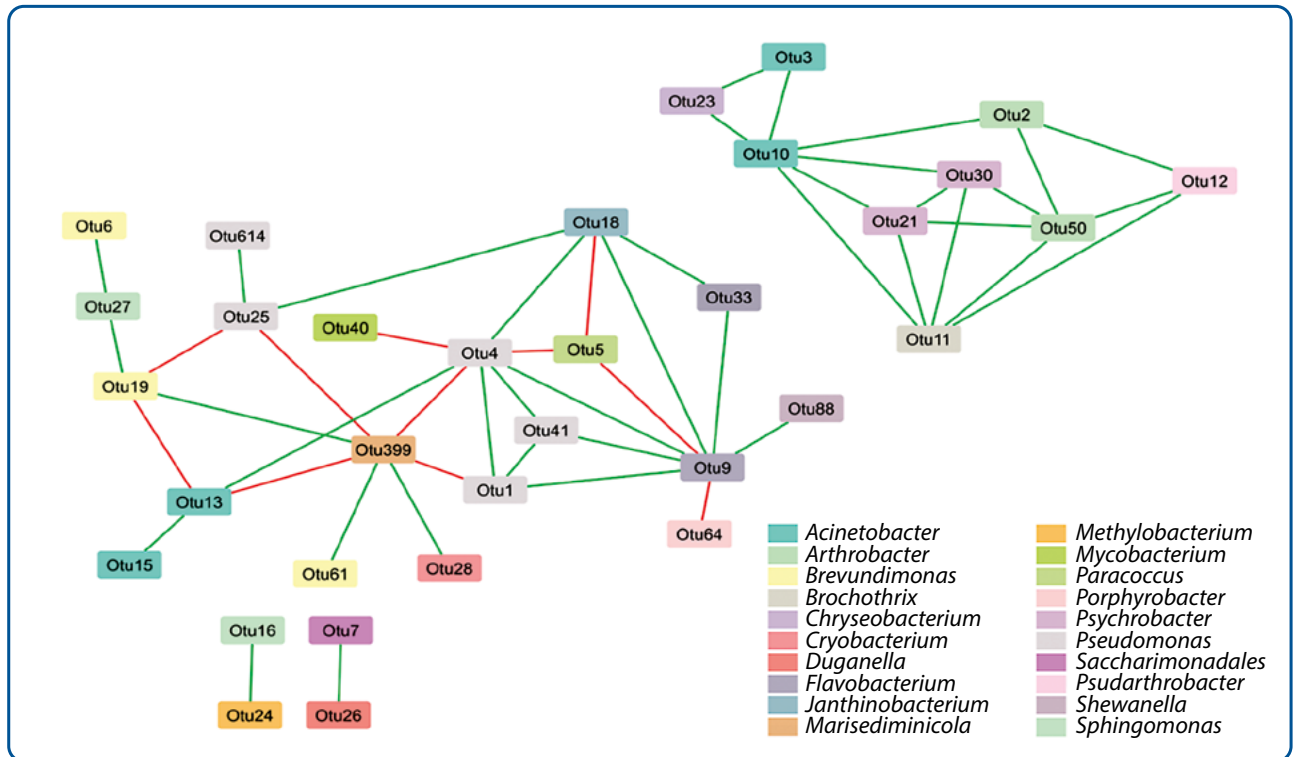


Figure 2. Network analysis of microbial interactions based on correlation analysis. Green indicates a positive interaction between operational taxonomic units (OTUs), while red indicates a mutual exclusion of OTUs.

from a trolley wheel (7MPP, Chao1 — 697.8). The taxonomic diversity of microbial communities in the biofilms from the PPP was less pronounced, as the greatest diversity was observed in biofilm 3PPP (the ceiling near the Morris bath), with a Chao1 diversity index of 364.8. The lowest taxonomic diversity was noted in biofilm 4PPP, where the Chao1 diversity index was only 77.1.

Among the bacteria detected in the biofilms, representatives of the phyla *Actinobacteriota*, *Bacteroidota*, *Proteobacteria*, *Firmicutes*, *Patescibacteria* were dominant (Figure 1).

The microbial composition of the two biofilms from the MPP differed (Figure 1). The microbial composition of biofilm 7MPP was more diverse than in biofilm 2MPP and contained a large number of classes of microorganisms. Representatives of the *Actinobacteriota* class were almost twice as numerous in the biofilm on the cart wheel (7MPP) as in biofilm 2MPP, taken from meat crate condensate. In biofilm 2MPP, most of the microbial community was occupied by a representative of the *Proteobacteria* class. At the same time, no representatives of the *Firmicutes* class were found in biofilm 2MPP, but this contrasted with biofilm 7MPP, in which they made up 22.34% of the total biofilm microorganisms detected.

The biofilms from the SPP contained representatives of *Actinobacteria*, *Bacteroidota*, *Proteobacteria*, *Firmicutes* and *Proteobacteria* in different proportions (Figure 1). The microbial community was least diverse in biofilm 3PPP (ceiling near the Morris bath), where the bulk of the microbial community was *Proteobacteria* (88.36%) (Table 2). The most diverse microbial compositions were in biofilm 6PPP (trolley wheels (plastic) at the beginning of the workshop) and biofilm 4PPP (wall in the carcass cooling tunnel).

3.2. Network analysis of interactions of microorganisms in the studied samples

Network analysis showed the largest number of positive relationships were found for representatives of the dominant genera (Figure 2). Therefore, OTU 9 (*Flavobacterium*) and OTU 10 (*Acinetobacter*) each had six possible associations with representatives of the *Psychrobacter*, *Pseudomonas*, *Brochothrix*, *Chryseobacterium*, *Arthrobacter*, and *Shewanella* genera.

The largest number of mutual exclusion relationships was found for OTU 399, belonging to the genus *Marisediminicola* (a soil bacterium) with three representatives of *Pseudomonas* (OTU 25, OTU 4, OTU 1) and one representative of *Acinetobacter* (OTU 13) (Figure 2).

4. Discussion

The study made it possible to characterize in detail the composition of microbial communities in biofilms formed on the surfaces of a MPP and a PPP. The data obtained demonstrate significant differences in taxonomic diversity and community structure between the two types of industries, which is consistent with the previously described features of microbiomes in the food industry (for example, Bokulich et al., 2016). The Chao1 and Shannon indices, reflecting alpha diversity, were significantly higher in biofilm materials from the MPP than in those from the PPP, which is likely due to more the heterogeneous environmental conditions in the MPP, including temperature fluctuations and the presence of organic substrates in the areas of cutting and storage of raw materials. On the contrary, the lower diversity in PPP can also be explained by strict temperature conditions (for example, a cooling tunnel kept at 0 °C) and regular disinfection procedures that limit the growth of some microorganisms. The dominant phyla (*Actinobacteriota*, *Bacteroidota*, *Proteobacteria*, *Firmicutes*) are typical of biofilms that form in food production plants, which is confirmed by numerous studies (Abdallah et al., 2014; Nikolaev et al., 2022; Fagerlund et al., 2021). However, unique features were observed in individual biofilms in the current study. For example, the high proportion of *Firmicutes* and *Actinobacteriota* in biofilm 7MPP (up to 22.34% and 34.03%, respectively) could be related to their resistance to drying and disinfectants, which was previously noted for industrial surfaces (Alonso et al., 2024).

DNA sequencing allowed for a detailed analysis of the microbial composition of the biofilm materials, which provides valuable information for assessing the risks associated with possible sources of contamination and the spread of pathogenic microorganisms. A key result revealed by taxonomic analysis was that biofilms at both types of meat processing plant were dominated by *Proteobacteria*, *Bacteroidota*, *Actinobacteria*, and *Firmicutes*—phyla that contain foodborne pathogens and spoilage organisms. These four phyla made up a significant part of the total microbial populations at both enterprises. It is important to note that these groups include both non-pathogenic and

potentially pathogenic species. For example, representatives of the genus *Escherichia* are classified in the phylum *Proteobacteria*, including *Escherichia coli*, one of the main causative agents of foodborne infections. The prominence of *Proteobacteria* in biofilm 2MPP (condensate) correlates with findings by Barcenilla et al. (2024), confirming that in meat processing conditions with low temperatures and high humidity, *Proteobacteria* (especially the genera *Pseudomonas*, *Acinetobacter*, and *Enterobacteriaceae*) become key components of biofilms on equipment surfaces. Notably, *Firmicutes* (which made up 22.34% of biofilm 7MPP) include the genera *Listeria* and *Staphylococcus*, which thrive on equipment surfaces (Fagerlund et al., 2017). The absence of *Firmicutes* in biofilm 2MPP could indicate localized antimicrobial interventions or nutrient limitations, warranting further investigation into site-specific microbial selection pressures.

The striking diversity disparity between the two biofilms from the MPP (Chao1: 336.8 vs. 697.8) underscores microenvironmental heterogeneity. The high diversity in biofilm 7MPP (trolley wheel) likely reflects cross-contamination from diverse sources, including soil and organic debris. In the PPP, the dominance of *Proteobacteria* (88.36%) in biofilm 3PPP (ceiling near Morris bathtub) suggests aerosolized contamination from water sources, a phenomenon documented by Elafify et al. (2024). Conversely, the low diversity in biofilm 4PPP (Chao1: 77.1) could indicate selective pressure from disinfectants, favoring resilient taxa like *Pseudomonas*.

Network analysis of microbial communities makes it possible to identify patterns of joint representation of different members of the studied communities. The network analysis conducted in the current study revealed cooperative interactions among *Flavobacterium*, *Acinetobacter*, and *Psychrobacter*—genera known for synergistic biofilm matrix production (Machado et al., 2020). These mutualistic relationships could enhance biofilm resilience, complicating eradication efforts. Conversely, the antagonism between *Marisediminicola* (soil-associated) and *Pseudomonas/Acinetobacter* suggests niche exclusion, possibly due to competition for iron or antimicrobial metabolite production (Coyte et al., 2015). However, we hypothesize that *Marisediminicola* (OTU 399) was the most unadapted (of all the organisms we detected) to the conditions of the food plants, but was not actually an antagonist of *Pseudomonas* or *Arthrobacteria*. This finding contrasts with typical biofilm synergy, highlighting the complexity of microbial interactions in industrial settings.

5. Conclusion

Based on the conducted research of microbial biofilms at meat processing and poultry processing enterprises, the following key conclusions can be drawn. Firstly, the prevalence and diversity of biofilms varied significantly between enterprises: biofilms were detected in only 2 out of 8 samples (25%) in MPP, while in 6 out of 7 samples (86%) in PPP, which indicates more favorable conditions for their formation in PPP. Secondly, microbial diversity (estimated by Chao1 and Shannon indices) was higher in MPP biofilms, especially on the surface of cart wheels (sample 7MPP, Chao1 = 697.8), which is explained by the transfer of microorganisms from soil and raw materials, while in MPP the greatest diversity was observed on the ceiling near the Morris tub (3PP, Chao1 = 364.8), and the

minimum is in the cooling tunnel (4PPP, Chao1 = 77.1) due to low temperatures (0 °C) and disinfection. Thirdly, the taxonomic composition of the dominant phylum (*Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, *Firmicutes*) is consistent with typical meat industry communities, but their distribution depended on location: *Proteobacteria* (88.36%) prevailed in the condensate of the raw material elevator (2MPP), while as on trolley wheels (7MPP), *Firmicutes* (22.34%) and *Actinobacteriota* (34.03%). Fourth, a network association of microorganisms has identified a synergy between the genera *Flavobacterium*, *Acinetobacter*, and *Psychrobacter*, and an antagonism between the *Marisediminicola* and *Pseudomonas/Acinetobacter*, which highlights the complexity of inter-microbial interactions in industrial settings.

Uporedna analiza biofilmova u industriji prerade mesa i živine: taksonomija i interakcije

Yulia Yushina, Elena Zaiko, Andrey Mardanov, Yury Nikolaev, Evgeniy Gruzdev, Ekaterina Tikhonova, Anastasia Semenova, Anzhelika Makhova, Maria Grudistova and Dagmara Bataeva

INFORMACIJE O RADU

Ključne reči:

Mikrobna kontaminacija površina
Biofilmovi
Sekvenciranje visoke propusnosti

APSTRAKT

Apstrakt: Cilj ove studije bio je da se uporedi zastupljenost, taksonomska struktura i međusobne interakcije mikrobnih zajednica unutar biofilmova formiranih na površinama u pogonima za preradu mesa (MPP) i živine (PPP). Korišćenjem transmisijne elektronske mikroskopije i sekvenciranja visoke propusnosti regije V3–V4 16S rRNA gena, biofilmovi su otkriveni u 25% uzoraka iz MPP postrojenja (2/8) i u 85,7% uzoraka iz PPP postrojenja (6/7), što ukazuje na značaj uticaja faktora sredine (npr. vlažnost, higijenska praksa) na formiranje biofilmova. Taksonomska analiza pokazala je dominaciju bakterijskih koljena *Proteobacteria*, *Bacteroidota*, *Actinobacteria* i *Firmicutes* u biofilmima oba pogona, uz izraženu heterogenost u MPP uzorcima (Chao1 indeksi: 336,8–697,8). Analiza mreža interakcija otkrila je kooperativne odnose između rodova *Flavobacterium*, *Acinetobacter* i *Psychrobacter*, koji doprinose otpornosti biofilmova, kao i antagonizam između bakterija iz zemljišta roda *Marisediminicola* i autohtonih *Pseudomonas*. Dobijeni rezultati naglašavaju potrebu za specifično prilagođenim higijenskim protokolima usmerenim na zone sa visokom vlagom i mobilnu opremu, kao i značaj narušavanja mikrobnih interakcija u cilju efikasne kontrole formiranja biofilmova.

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A non-destructive method for evaluating camel meat during freeze-thaw cycles using bio-impedance analysis

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ABSTRACT

Camel meat has a high nutrient profile and is susceptible to deterioration when stored, primarily due to temperature fluctuation in the cold chain. In this study, we utilized electrical impedance to monitor the quality of camel meat following repeated freeze-thaw cycles (two, four and six). Meat properties monitored were colour, pH, and electrical conductivity. Conductivity and pH of camel meat increased, and all colour indices (L^*), (a^*), and (b^*) also underwent significant changes when the meat was subjected to freeze-thaw cycles. The results suggested a reduction in electrical impedance with each freeze-thaw cycle, indicating increased cell membrane permeability and ice crystal-associated structural damage. This study recognizes the potential of electrical impedance to define camel meat quality as a method of further interpreting quality faults associated with freeze-thaw treatment.

1. Introduction

Camel meat, although less common than chicken or beef, is a source of protein. It is distinguished by its mild taste and high nutritional value, including low cholesterol and fat content (Kadim *et al.*, 2022; Baba *et al.*, 2021). Moreover, it contains minerals, essential amino acids, vitamins, biologically active substances, and essential fatty acids such as omega-3 (Mohammed *et al.*, 2020). Camel meat consists of 76–78% water, 19–22% protein, 2.9–3.2% fat, and 1.2% ash (Abdelhadi *et al.*, 2012). It is traditionally used to treat several diseases, for example respiratory diseases, hypertension, cardiovascular diseases, and pneumonia (Si *et al.*, 2022).

In Algeria, where camel meat is very well-regarded for its nutritional composition, this meat is largely produced in the country's southern regions, whereas consumption is dispersed across the country (Djenane *et al.*, 2020). However, like

any other meat, it is highly prone to spoilage during storage and, hence, needs the use of proper preservation methods to ensure its shelf life (Lakehal *et al.*, 2021). Freezing remains one of the most extensive technologies in the meat industry to maintain quality and acts by slowing down microbial growth and minimizing nutrient losses (Daszkiewicz *et al.*, 2018; Medić *et al.*, 2018). On the other hand, during transportation, storage and delivery, camel meat is affected by repeated, fluctuating temperature changes.

Changes in storage temperature have a significant effect on meat's physicochemical properties, which compromise its overall quality (Rahman *et al.*, 2014; Cheng *et al.* 2019; Qi *et al.*, 2012). Monitoring and detecting such changes are, therefore, necessary to maintain product safety and consumer trust. One monitoring means is electrical impedance, now becoming a powerful means for evaluating the quality of biological tissues like meat (Caicedo-Eraso *et al.*, 2020; Zhao *et al.*, 2017). The technique can

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quantify variations in electrical properties resulting from physiological changes in meat during freezing and thawing (Fuentes et al., 2013).

Previous studies have established that electrical impedance is capable of differentiating fresh from thawed beef, chicken, pork, and fish meats (Bai et al., 2018; Wei et al., 2016; Dell'Osa et al., 2021; Chen et al., 2017). There are limited studies on camel meat, however. This study aimed to fill this gap by introducing a new method for detecting camel meat freeze-thaw cycles through bioimpedance measurement. Through the investigation of electrical impedance change, this research aimed to provide the first fundamental data for considering bioimpedance as a tool for camel meat quality monitoring during storage, thus improving camel meat cold chain management.

2. Materials and Methods

Eleven camel (*Camelus dromedarius*) meat samples were obtained from an Algerian municipal slaughterhouse in El Ouadi. Sections of biceps femoris were selected 48 hours post-mortem. Each section was cut into pieces of approximately equal weight, individually vacuum-packed, and stored frozen at -20°C in a freezer (CRF-NT64GF40, Condor, Algeria). The freezing temperature was monitored using an infrared thermometer (TIA 101, China). The meat samples were then subjected to a series of freezing and thawing cycles (two, four and six) at controlled temperatures to mimic the temperature fluctuations of the cold chain. In each cycle, the meat samples were frozen for one week at -20°C and thawed at a refrigerator temperature of 4°C . A group of meat samples was also used as a control group, i.e., they were not subjected to freeze-thawing and were measured directly in the fresh state.

2.1 pH measurement

The pH of each meat sample was measured in a mixture resulting from homogenizing 10 g of meat in 90 mL of distilled water (Zhu et al., 2019). The pH meter (INOLAB) used was previously calibrated before inserting the electrode into the homogenate.

2.2 Electrical conductivity

Electrical conductivity was assessed using a conductivity meter (WTW Lab 540). Briefly, 9 g of each meat sample was homogenized with 90 mL of

distilled water and stirred for 20 min. Electrical conductivity levels were then measured.

2.3 Colour

Before the colorimetric analysis, camel meat samples, approximately 1.5 cm thick were prepared. Images were captured using a computer vision system consisting of a custom-designed enclosure equipped with two adjustable lamps positioned 50 cm above the samples at a 45° angle to achieve consistent illumination. A digital camera (Canon DS126621, China) was fixed vertically at a distance of 30 cm from the sample surface. To reduce external light interference, the interior of the enclosure was covered with light-absorbing black fabric (Lakehal and Lakehal, 2023). Digital analysis of colourimetric parameters, including Lab, HSI, and RGB, was performed using Adobe Photoshop CS3 software.

2.4 Impedance measurement

The electrical impedance measurement was performed using a portable LCR meter (LCR meter BR 2832, China) equipped with a two-needle system, each needle having two Kelvin electrodes. The device utilizes colour-coded cables with gold-plated contacts and reinforced protection. The needles, with dimensions of 10 mm in length, 2.5 mm in width, and 2 mm in height, were placed on the surface of the meat sample to complete the circuit. Measurements were taken at four different frequencies: 10 kHz, 1 kHz, 120 Hz and 100 Hz at four different regions of the meat. For each meat sample, both needles were inserted parallel to the direction of the muscle fibres to a depth of 13 mm, with a 2 cm distance between them. All measurements were conducted at a temperature of $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and repeated four times at each frequency, both on fresh meat and after each freeze-thaw cycle.

2.5 Statistical analysis

The data were statistically analysed using analysis of variance (ANOVA), which was done with IBM SPSS Statistics version 26. The means were compared with Tukey's post-hoc test. A difference was regarded significant if the probability was $p < 0.05$.

3. Results and Discussion

3.1 pH

The pH of camel meat remained almost constant after the second freeze-thaw cycle (Table 1). However, it significantly increased to 5.41 ± 0.07 after the fourth cycle, compared to fresh meat (pH 5.16). By the end of the sixth cycle, the pH measured was 5.54 ± 0.04 . This result corroborates the findings of *Qi et al.* (2012), who observed an increase in pH after five freeze-thaw cycles of beef ($p < 0.05$). This increase in pH after a series of cycles, measured in the current study, could be linked to the release of free amino acids and dipeptides resulting from protein hydrolysis (*Hou et al.*, 2020). Additionally, *Ali et al.* (2015) reported a significant reduction in pH after the sixth cycle. In our previous research (*Lakehal et al.* 2023), we concluded that the variation in meat pH relative to its initial value was affected by oxidation rates, microbial growth, and exudate. This phenomenon may result from the loss of minerals and small protein compounds in the form of exudates, leading to an ionic imbalance in the meat (*Baygar and Alparslan*, 2015).

3.2 Electrical conductivity

Table 1, which shows the influence of freeze-thaw cycles on electrical conductivity, demonstrates a progressive increase with each cycle, with values rising from 1426 ± 23 to 1993 ± 22 . It is important to note that there was no significant difference between fresh meat and the second cycles (C2). However, starting from cycle 4 (C4), a notable difference emerges, marked by a sharp increase in conductivity, reaching 1893, then 1993 in cycle 4. Indeed, each cycle causes cell membrane degradation, leading to the release

of intracellular fluids, which increases conductivity (*Leng et al.* 2020). Thus, this increase reflects the deterioration of meat quality. Electrical conductivity is commonly used to assess the freshness of frozen meat, serving as a reliable indicator of product quality (*Leng et al.*, 2024). Electrical conductivity is a useful parameter for assessing the impact of freezing on meat (*Lakehal et al.* 2021).

3.3 Colour

The evolution of the colour parameters is presented according to the number of freeze-thaw cycles (Table 1). From the first thawing cycle, the lightness of the fresh meat was significantly lower than that of the meat that underwent the freeze-thaw process. The increase in the lightness of red meat after freeze-thaw cycles was observed by *Xia et al.* (2009) in a study on pork. This increase was attributed to the greater light reflection caused by an increased amount of free water, a consequence of heightened protein denaturation (*Holman et al.*, 2017; *Seong et al.* 2017), or by an increase in intramuscular fat content (*Pinheiro et al.* 2019).

The redness (a^*) values of the meat revealed a decrease following the first cycle, but a significant difference ($P < 0.05$) was only detected in the last freeze-thaw cycle (C6) compared to the others (Table 1). These results are similar to those observed by *Qi et al.* (2012), who also noted a decreasing trend in redness with an increasing number of freeze-thaw cycles in lamb meat. The colour changes in the meat during the freeze-thaw cycles can be attributed to the reduction of myoglobin in its chemical form (*Shang et al.* 2020). Indeed, the free radicals generated during lipid oxidation can alter the structure of the haeme group and trigger myoglobin oxidation, leading to a loss of meat colour, which manifests as a decrease in redness intensity. (*Xia et al.*, 2009; *Utrera et al.* 2014).

Table 1. Physicochemical properties of camel meat subjected to multiple freeze-thaw cycles

Physico-chemical parameters	C0	C2	C4	C6
pH	5.165 ± 0.01^c	5.2375 ± 0.06^c	5.4175 ± 0.07^b	5.545 ± 0.04^a
Electrical conductivity ($\mu\text{S}/\text{cm}$)	1426 ± 23.3^b	1504.5 ± 35.32^b	1893 ± 45.32^a	1993 ± 21.59^a
L* value (lightness)	31.14 ± 3.21^b	34.28 ± 4.23^a	33.85 ± 3.07^a	34 ± 3.02^a
a^* value (redness)	13 ± 1.73^a	10.28 ± 0.98^a	10.14 ± 1.56^{ab}	9.42 ± 2.07^b
b^* value (yellowness)	8.71 ± 2.98^b	10.42 ± 1.85^{ab}	11.71 ± 1.45^{ab}	14.14 ± 1.52^a

Legend: C0: fresh meat; C2: two freeze-thaw cycles; C4: four freeze-thaw cycles, C6: six freeze-thaw cycles. Data are given as mean values $\pm$ standard deviation. ^{a, b} different letters show a statistically significant difference ($p < 0.05$).

The yellowness (b^*) values of camel meat showed an increase from the first freeze-thaw cycle, reaching their maximum at the sixth cycle (Table 1). This evolution could be attributed to the denaturation of myoglobin, the accumulation of metmyoglobin, and an increase in lipid oxidation (Rahman et al., 2014; Pinheiro et al., 2019). In contrast, Jeong et al. (2011) observed a decrease in the b^* value after two freeze-thaw cycles, where the meat was stored at -65°C for 12 hours, followed by thawing at 4°C for 12 hours.

3.4 Evolution of meat impedance during freeze-thaw cycles

The meat impedance measured in on four different regions of the meat sample and at various frequencies are presented in Figure 1. In the fresh muscle, the impedance measured in region 4 was the highest ($570.9 \pm 1.20 \Omega$), while region 3 showed the lowest impedance ($501.1 \pm 0.80 \Omega$) among the four

initial measurement locations. These observed variations likely reflect differences in tissue composition or materials measured, particularly regarding the amount of lipids present (Fan et al., 2021; Li et al., 2019). As the excitation frequency increased, the impedance of camel muscle decreased. This was probably due to the high capacitive reactance of the cell membrane at low frequency, where the current flows mainly through the extracellular fluid (Dell’Osa et al., 2021). However, at higher frequencies, the capacitive reactance of the cell membrane decreases, allowing the current to pass through the intracellular fluid as well (Zavadlav et al., 2016).

Impedance values gradually decreased with the repeating of freeze-thaw cycles (Figure 1), suggesting that this procedure enhanced the permeability of cell membranes (Leng et al., 2024). Major structural and chemical changes occur in frozen muscle meat as a result of primary and secondary nucleation and crystal formation during freezing-thawing (Dang et al., 2021). Significant osmotic pressure across the sarcolemma

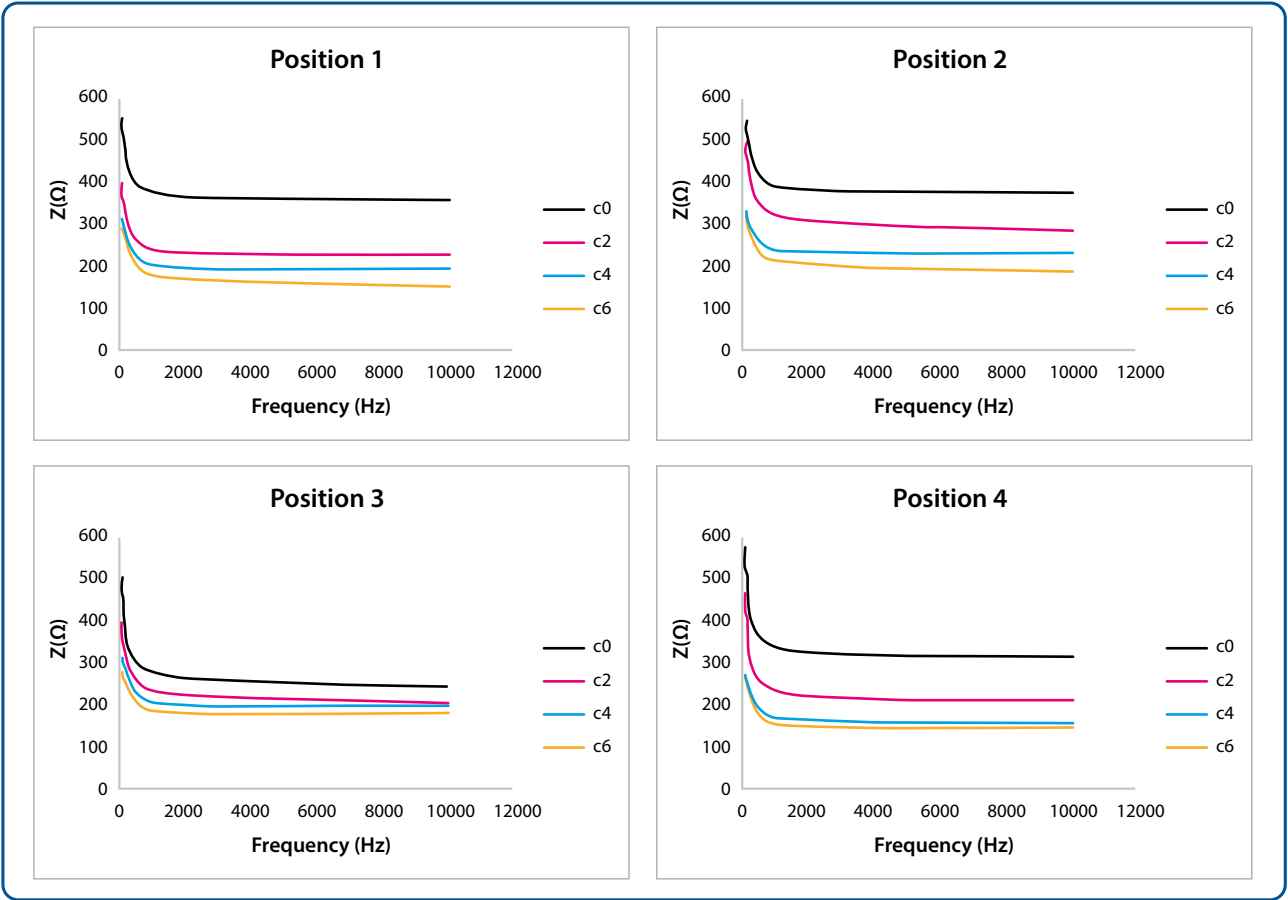


Figure 1. Changes of impedance (Z , Ω) of camel meat at various frequencies and different positions subjected to multiple freeze-thaw cycles.
Legend: C0: fresh meat; C2: two freeze–thaw cycles; C4: four freeze–thaw cycles, C6: six freeze–thaw cycles. Data are given as mean values $\pm$ standard deviation

is created when ice crystals develop, which increases the deformation of muscle microstructures and causes membrane rupture that allows cell contents to leak out (Zhang *et al.*, 2023). Enzymatic activity and oxidation are both accelerated by this process, which can have an impact on several aspects of meat quality.

4. Conclusion

The present study showed that electrical impedance is an effective method of monitoring the quality of camel meat during freeze-thaw processes

. Impedances became lower with an increasing number of freeze-thaw cycles. This was presumed to be a consequence of a breakdown of the cell membrane induced by formation of ice crystals. The findings indicate that electrical impedance is a non-invasive method for detecting certain aspects of quality change in camel meat subjected to multiple freeze-thaw cycles.

Having realized the influence of freezing and thawing cycles on camel meat, electrical impedance spectroscopy could be a valuable tool in monitoring the quality of meat in industrial scale.

Nedestruktivna metoda za procenu kvaliteta mesa kamile tokom ciklusa zamrzavanja i odtapanja korišćenjem analize bioimpedanse

Saliha Lakehal i Brahim Lakehal

INFORMACIJE O RADU

Ključne reči:

Električna impedansa

Meso kamile

Kvalitet mesa

Ciklusi zamrzavanja i odmrzavanja

APSTRAKT

Meso kamile karakteriše visok nutritivni kvalitet, ali je podložno degradaciji tokom skladištenja, pre svega usled temperaturnih fluktuacija u hladnom lancu. U ovoj studiji korišćena je električna impedansa za praćenje kvaliteta mesa kamile nakon ponovljenih ciklusa zamrzavanja i odmrzavanja (dva, četiri i šest ciklusa). Praćene su sledeće osobine mesa: boja, pH vrednost i električna provodljivost. Utvrđeno je da se pH vrednost i električna provodljivost mesa kamile povećavaju, dok su svi parametri boje (L*, a*, b*) značajno promenjeni nakon izlaganja ciklusima zamrzavanja i odmrzavanja. Rezultati su ukazali na smanjenje električne impedanse sa svakim dodatnim ciklusom, što sugerise povećanu propustljivost ćelijskih membrana i strukturna oštećenja izazvana formiranjem kristala leda. Ova studija ukazuje na potencijal primene električne impedanse kao metode za određivanje kvaliteta mesa kamile i bolju interpretaciju oštećenja kvaliteta povezanih sa tretmanima zamrzavanja i odmrzavanja.

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The effects of dry ageing period and subsequent culinary methods on the oral processing parameters of beef

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ABSTRACT

This study investigates the impact of dry aging duration and subsequent cooking methods on the oral processing parameters of beef. Dry aging is a traditional technique that enhances beef by controlled environmental and enzymatic changes. Two dry aging periods (21 and 42 days) were combined with two cooking methods (grilling and sous vide). A sensory panel evaluated the beef samples, assessing parameters such as particle size, chewing rate, and eating rate. The breakdown of food structures during oral processing influences the sensory perception of texture, flavor, and taste, thereby determining a food's overall acceptability and palatability. Results showed that shorter dry aging duration and cooking method significantly affected only some of the oral processing parameters. Shorter dry aging period (21 days) generally led to increased total number and share of smaller particle sizes. In these terms, sous vide cooking resulted in improved oral processing profiles compared to grilling. The longer period (42 days) of dry ageing was without an effect on beef oral processing, irrespective of culinary method applied. These findings contribute to a better understanding of how dry aging and cooking methods influence the sensory experience of beef, potentially aiding in the development of products tailored to consumer preferences.

1. Introduction

Dry aging is a traditional method that enhances beef by exposing it to controlled environmental and enzymatic changes. This technique improves tenderness, deepens flavor, and imparts distinctive texture characteristics. It involves storing large beef cuts, such as ribeye or strip loin, in a tightly regulated setting—typically maintained at low temperatures (2–4 °C), around 85% humidity, and with consistent airflow (Khan Muhammad *et al.*, 2016). Over a period of several weeks to months, natural enzymes in the meat, like calpains and cathepsins, break down complex proteins into smaller peptides and amino

acids. This enzymatic activity softens the meat and contributes to the development of rich, umami flavours (O'Quinn *et al.*, 2018).

The length of the aging period plays a crucial role in determining the final flavor and texture. Generally, longer aging enhances flavour intensity, but the ideal duration depends on factors such as the beef's original quality, the specific aging conditions, and the desired flavor profile (Cenci-Goga *et al.*, 2020). If not carefully monitored, the aging environment—particularly its heat and air circulation—can cause excessive water loss and protein denaturation, leading to a drier texture (Jadhav *et al.*, 2021). Sous vide—meaning “under vacuum”—uses a water bath

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to cook meat at precisely controlled low temperatures, usually between 50 °C and 85 °C (Gómez and Beriain, 2019). This method ensures even heat distribution throughout the meat, minimizing temperature differences and allowing for accurate control of internal temperature and protein denaturation (Przybylski *et al.*, 2021).

Oral processing, a multifaceted and intricate initial stage of digestion, encompasses a series of actions within the oral cavity that prepare food for swallowing and subsequent breakdown in the gastrointestinal tract. This process integrates muscle activity, jaw movements, and tongue movements, all contributing to the transformation of food structure into a bolus suitable for ingestion (Koç *et al.*, 2013). The breakdown of food structures during oral processing directly influences the sensory perception of texture, flavor, and taste, thereby determining a food's overall acceptability and palatability (Foegeding *et al.*, 2015). The parameters of oral processing, including chewing rate, bolus formation time, and salivary flow rate, are highly variable and are influenced by an array of factors, such as individual physiology, food properties, and cognitive expectations (Campbell *et al.*, 2017). The way that food undergoes changes in the mouth has a big impact on how we experience its texture, which is a core feature that affects how much we enjoy eating (Devezeaux De Lavergne *et al.*, 2021). The idea of this research was to examine the effects of dry ageing duration (21 and 42 days) and subsequent culinary methods (grill and sous vide) on the oral processing parameters of beef.

2. Materials and Methods

2.1. Meat samples and ageing process

Beef hindlegs (shank and chump off, $n = 12$) were collected from six beef carcasses on the day of slaughter. Paired legs (left and right) from the same animal were randomly assigned to two ageing treatments: in-bag dry-ageing (21 days) or in-bag dry-ageing (42 days). Briefly, in-bag dry-ageing was carried out in water permeable ageing bags (TUBLIN® 10, 50 µm thick, polyamide mix with water vapor transmission rate 920 g/50 µm²/24 h at 7 °C, 50% RH, and oxygen transmission rate 660 g/m²/24 h at 7 °C, 50% RH, TUB-EX ApS, Denmark) at 2 ± 0.5 °C, 0.5 m.s⁻¹ air velocity and relative humidity of 75 ± 5 %. Samples without a period of dry ageing were investigated as control samples.

2.2. Food oral processing panel

The food oral processing (FOP) panel consisted of eight panellists (four male and four female members, normal body mass index 18–25 kg/m²) with previous experience in similar studies. They all confirmed two main pre-conditions for performing this type of research—good general health condition and no dental problems (Forde *et al.*, 2013). Before performing the planned FOP study, one 2-hour training session was organized to get the panellists familiar with the methods to be employed (Djekic *et al.*, 2021). During this initial session, all panellists signed written consent to participate voluntarily.

2.3. Oral processing analysis

The first task for the panellists was to consume cubical meat samples (2×2×2 cm). The chewing process was recorded using a digital video camera that was placed 30 cm from each panellist (Forde *et al.*, 2013). Video recordings were analysed using stopwatch. This enabled counting of the number of chews and total oral consumption time (Hennequin *et al.*, 2005). In parallel, panellists raised their hand when swallowing to enable counting of the number of swallows per sample (Djekic *et al.*, 2021). The mass of each sample before consumption was measured using a technical balance of 0.01 g accuracy. Based on recorded data, food oral processing parameters were calculated (Aguayo-Mendoza *et al.*, 2019). All panellists received two samples from each ageing period and another two samples from each culinary method.

2.4. Particle size analysis

To analyse particle distribution of the boluses, the same types of meat samples (2×2×2 cm) were collected from each panellist at the moment before swallowing by expectorating (Djekic *et al.*, 2021). Upon collection, they were: (i) rinsed using distilled water on filter paper; (ii) spread out on white plates and, (iii) photographed with a digital camera. The spreading of boluses was performed with care to prevent damage of the particles. Image analysis was conducted using ImageJ software. This enabled counting of the number of particles and calculation of their surface area (Rizo *et al.*, 2019). Bolus analysis was performed in two replicates.

2.5. Statistical analysis

Data from the texture profile analysis and food oral processing study were subjected to analysis of variance as follows: one-way ANOVA was employed for the effects of culinary method (C), aging days (D), and for the number of particles. The statistical significance of the factors C and D was determined using the Tukey HSD test ($p < 0.05$). The chi-square test for association was used in analysing possible relationships between particle size fragmentation and aging of meat ($p < 0.05$).

3. Results and Discussion

Understanding the relationship between the structure of solid foods and their oral processing is paramount for enhancing features such as texture and taste (Guo, 2021). The number of particles in food oral processing relates to the degree of food breakdown during chewing and bolus formation (van der Bilt, 2009). The “ideal” number of particles depends on the desired sensory experience and functionality of the food product and there isn’t a universally “better” option between a larger or smaller number of particles (Tyle et al., 1990). Our results clearly

demonstrated that the sous-vide cooking technique resulted in a notably larger number of particles compared to grilling the meat, both without and with the period of dry ageing (Figure 1). However, no significant differences were found in the number of particles between the samples dry aged for 21 and 42 days, irrespective of the cooking technique applied.

The oral processing of food involves the breakdown of food structure into a bolus suitable for swallowing, with particle size being a key characteristic of this bolus (Koç et al., 2013). Food particle size plays a critical role in food oral processing, influencing texture perception, taste, and digestion (Devezeaux De Lavergne et al., 2021). Smaller particle sizes within the bolus create a larger surface area for digestive enzymes to act upon. This increased surface area facilitates more efficient enzymatic breakdown of carbohydrates, proteins, and fats (Ramírez et al., 2019).

Our results demonstrate that the percentage of small particles in the boluses significantly decreased (by 18.41% in grilled and 11.75% in sous-vide beef samples) after the aging period of 21 days compared to day 0, irrespective of the culinary technique applied. Further ageing for the subsequent three weeks increased the number of small particles by only a relatively insignificant margin of only 2.4% on average for

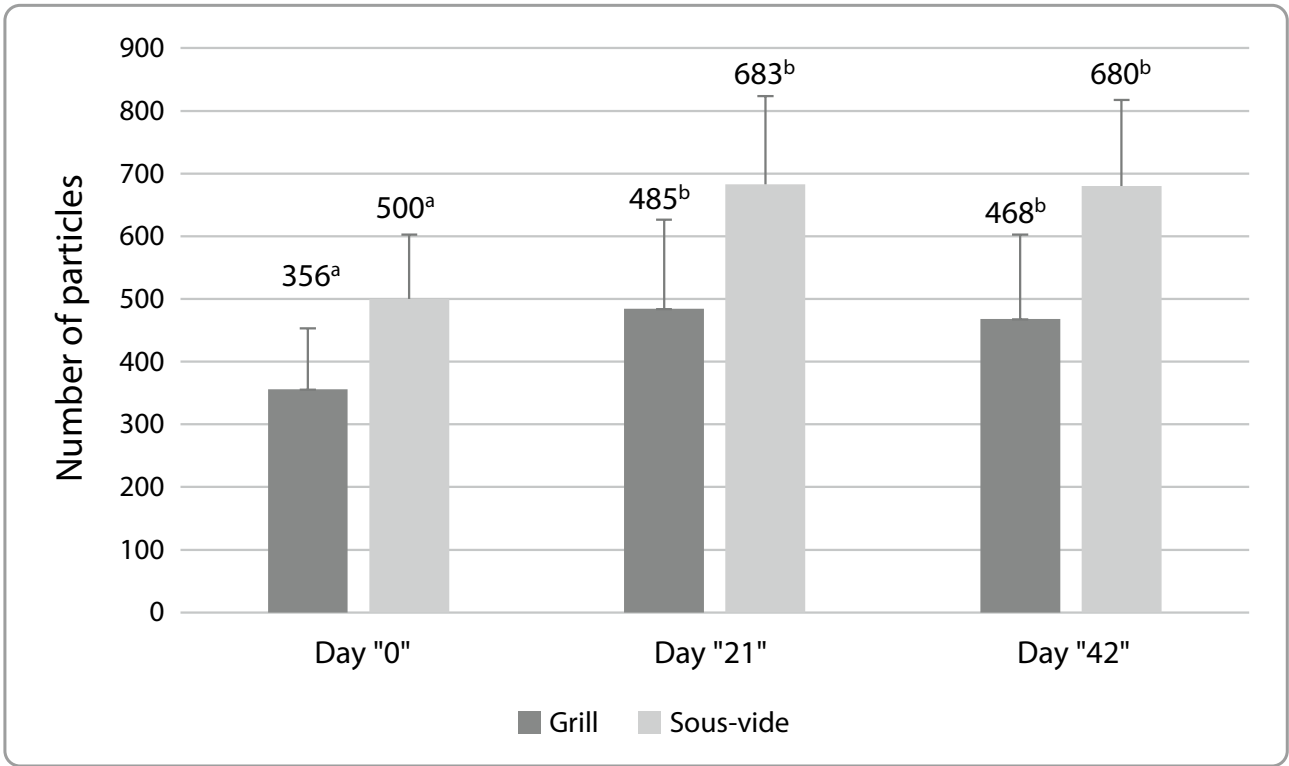


Figure 1. Number of particles of samples obtained in vivo during the aging time for grilled samples (dark grey bars) and sous-vide (light grey bars). Different letters indicate significant differences according to Tukey’s test ($\alpha=0.05$)

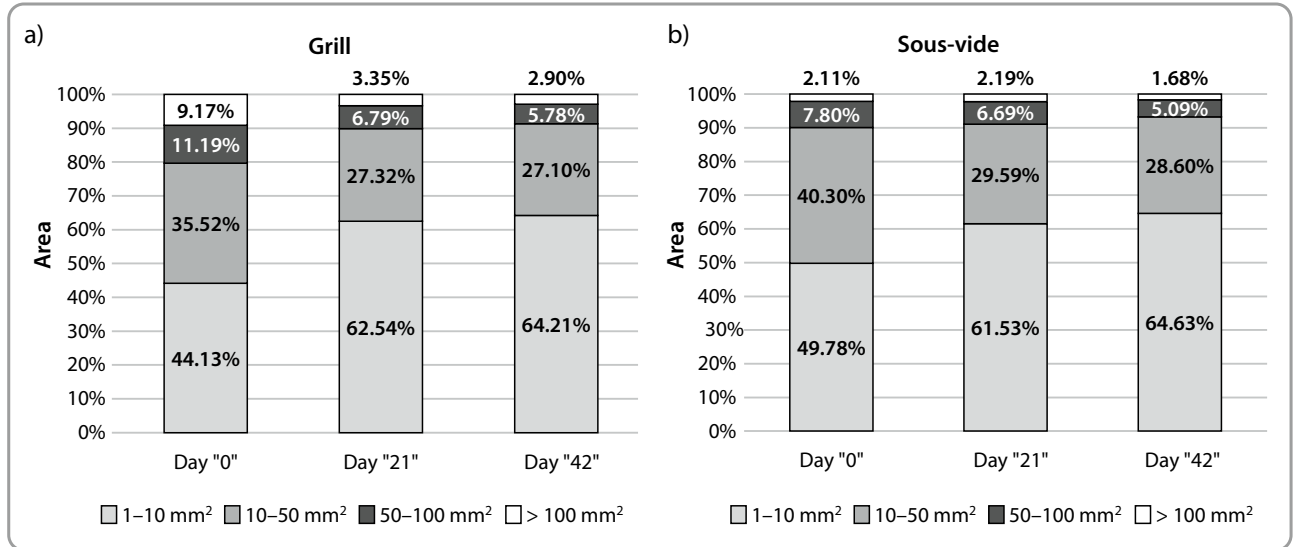


Figure 2. Percentage of area occupied by particles of size: 1–10 mm² (light grey), 10–50 mm² (dark grey), 50–100 mm² (black color), and >100 mm² (white color) depending on the aging time

grilled and sous-vide samples. The number of large particles (>100mm²) for grilled samples was greater than for sous-vide samples by 4-fold on day 0 and continued to remain on the lesser side during the whole period of dry ageing. We already know that a higher number of smaller particles in food samples may result in a smoother texture, while fewer, larger particles could create a coarser or chunkier sensation (Guo, 2021).

Eating rate, or how quickly one consumes food, plays a significant role in food oral processing, influencing both sensory perception and overall food intake (Wee et al., 2018). It affects the duration of oral exposure, the number of chews, and the bolus properties at the point of swallowing (Goh

et al., 2021). Chewing rate specifically measures how quickly someone chews their food and focuses solely on the mastication process, regardless of the amount of food consumed (Sánchez-Ayala et al., 2013). Surprisingly, our results did not reveal a statistically significant influence of either dry ageing or the cooking technique applied on any of the food oral processing parameters presented in Table 1. Chewing cycle duration, chewing and rate remained almost a constant throughout the study. Consumption time for one bite and the number of chews were altered by the duration of dry ageing and cooking method, but never to an extent where the differences could be perceived as statistically significant.

Table 1. Food oral processing parameters of beef subjected to grilling and sous-vide cooking methods.

Meat cooking method	Number of chews	Consumption time for one bite [s]	Number of swallows	Chewing cycle duration [s/chew]	Chewing rate [chew/s]	Eating rate [g/s]
Grilling						
Day 0	43.5 ± 12.4	31.2 ± 9.5	2.9 ± 1.3	0.7 ± 0.2	1.4 ± 0.3	0.4 ± 0.1
Day 21	39.0 ± 6.7	26.3 ± 4.7	2.4 ± 1.2	0.7 ± 0.1	1.5 ± 0.1	0.4 ± 0.1
Day 42	39.6 ± 11.7	26.9 ± 6.9	2.4 ± 1.0	0.7 ± 0.1	1.5 ± 0.1	0.4 ± 0.1
Sous-vide						
Day 0	40.9 ± 15.1	31.3 ± 20.5	2.9 ± 1.2	0.7 ± 0.2	1.4 ± 0.3	0.4 ± 0.1
Day 21	53.1 ± 13.5	37.9 ± 8.5	3.4 ± 0.9	0.7 ± 0.1	1.4 ± 0.2	0.3 ± 0.1
Day 42	41.4 ± 23.3	31.2 ± 20.2	2.8 ± 1.1	0.7 ± 0.1	1.4 ± 0.2	0.4 ± 0.2

Testing was performed in two replicates. Data are mean values ± standard deviation.

4. Conclusion

Three weeks of dry ageing resulted in partly improved oral processing of beef, but only in terms of the increased total number of particles and percentage of area occupied by particles of smaller size, which might have an indirect positive effect on meats' texture, digestion and nutrient absorption. In the same terms, sous-vide was not perceived as a favourable culinary method compared to grilling. Dry ageing for a prolonged period of time, i.e., an additional three weeks,

had no effect on beef oral processing. However, a limitation of this study was the food oral processing panel that was not tested for reliability, repeatability, or consistency. Also, further research is needed where the difference between dry ageing periods would be smaller, which could lead to more statistically robust and reliable results; instrumental textural analysis is also needed to confirm the sensory findings. The results of these studies could also be compared with the results of sensory evaluation and textural characteristics.

Uticaj perioda suvog zrenja i naknadnih kulinarskih metoda na parametre oralne obrade govedine

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INFORMACIJE O RADU

Ključne reči:

Suvo zrenje
Roštilj
Sous-vide
Veličina čestica
Brzina žvakanja
Brzina jedenja
Oralna obrada
Tekstura go

APSTRAKT

Ova studija istražuje uticaj trajanja suvog zrenja i naknadnih metoda termičke obrade na parametre oralne obrade govedine. Suvo zrenje je tradicionalna tehnika koja poboljšava senzorni kvalitet govedine u kontrolisanim uslovima usled dejstva enzima. Dva perioda suvog zrenja (21 i 42 dana) kombinovana su sa dve metode termičke obrade (roštilj i su-vid). Senzorni panel je ocenio uzorke govedine, procenjujući parametre kao što su veličina čestica, brzina žvakanja i brzina jedenja. Razgradnja strukture hrane tokom oralne obrade utiče na senzornu percepciju teksture, ukusa i arome, čime određuje ukupnu prihvatljivost i ukus mesa. Rezultati su pokazali da kraće trajanje suvog zrenja i metod termičke obrade značajno utiču samo na neke od parametara oralne obrade. Kraći period suvog zrenja (21 dan) generalno je doveo do povećanja ukupnog broja i udela čestica manjih veličina. U tom smislu, su-vid kuvanje je rezultiralo poboljšanim profilima oralne obrade u poređenju sa roštiljanjem. Duži period (42 dana) suvog zrenja nije imao uticaja na oralnu obradu govedine, bez obzira na primenjeni kulinarski metod. Ovi nalazi doprinose boljem razumevanju kako suvo starenje i metode termičke obrade utiču na senzorno iskustvo govedine, što potencijalno pomaže u razvoju proizvoda prilagođenih željama potrošača.

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Assessment of Mercury contamination in liver and muscle tissue of mallards (*Anas platyrhynchos*) as bioindicators at three locations in Serbia

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ABSTRACT

The aim of this study was to investigate the presence and distribution of mercury (Hg) in the liver and muscle tissue of Mallards (*Anas platyrhynchos*) collected from three locations in Serbia. A total of 55 samples were analyzed, including 23 from the Belegiš site, 12 from Pločica, and 20 from Vršacki Ritovi. Mercury concentrations were determined using the ICP-MS method. Mercury levels in the liver ranged from 0.009 to 0.239 mg kg⁻¹, while concentrations in muscle tissue ranged from 0.005 to 0.069 mg kg⁻¹. The mean values for liver tissue were 0.097 mg/kg (Belegiš), 0.108 mg/kg (Pločica), and 0.025 mg kg⁻¹ (Vršacki Ritovi), whereas for muscle tissue they were 0.030 mg kg⁻¹ (Belegiš and Pločica) and 0.008 mg kg⁻¹ (Vršacki Ritovi). Statistical analysis included descriptive statistics, Pearson correlation test, analysis of variance (ANOVA) with Tukey post hoc test, Levene's test for homogeneity of variances, Kruskal–Wallis test, and Mann–Whitney test. The findings provide insight into potential differences in mercury contamination levels between different ecosystems, as well as the relationship between different tissues in wild birds.

1. Introduction

Heavy metals have always been present as natural components of the environment, typically occurring at very low concentrations under normal conditions. However, both historically and in the present day, human activities have significantly contributed to their release into the environment. Particular concern is raised by the effects of certain metals, such as mercury, which can seriously threaten ecosystem stability. With the rise of public awareness regarding environmental issues, there is an increasing need for systematic monitoring, assessment, management, and remediation of pollution-related damage. Given the complexity of ecosystems, it is impossible to monitor all of their components, functions, and

properties; therefore, selected biological indicators are used for this purpose. Birds, as one of the organism groups for which exposure to and toxicity of heavy metals have been extensively studied, serve as useful bioindicators of overall ecological status (Zolfaghari *et al.* 2007).

Mercury is released into the environment through both natural processes (such as volcanic activity, oceans, and vegetation) and anthropogenic activities (Ribeiro & Germano, 2015). Anthropogenic sources of mercury emissions are divided into primary sources, in which geologically derived mercury is mobilized into the environment (e.g., mining and the combustion of fossil fuels), and secondary sources, which involve the intentional use of mercury in industry, consumer products, dentistry, and artisanal and small scale

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gold mining (ASGM). Among the primary sources, the largest contribution to pollution comes from sectors where mercury is released as an unintentional byproduct, particularly coal combustion, metal production, and cement manufacturing. Secondary sources also significantly contribute to mercury emissions, with ASGM predominantly occurring in impoverished regions of Africa, Asia, and South America remaining the largest global consumer of mercury (Pacyna *et al.*, 2010). In the environment, mercury can exist in three forms: elemental (Hg^0), inorganic (Hg^{2+}), and organic (methylmercury, MeHg). Through complex biogeochemical cycles, it enters the food chain and accumulates in the tissues of animals and fish. According to EFSA (European Food Safety Authority), the majority of mercury found in fish is in the form of methylmercury (80–100%), whereas in other food sources, it is predominantly present in its inorganic form (Ribeiro & Germano, 2015).

Over the past decades, the potential harmful effects of mercury (Hg) have been studied from both ecological and public health perspectives. Mercury is particularly dangerous because it can cause adverse effects in humans and other organisms, with these effects depending on various factors such as the chemical form of mercury, the amount, the route of exposure, and individual susceptibility. Humans can be exposed to mercury through different pathways, including food consumption (especially fish). The effects of mercury encompass a wide range of syndromes, including neurological, immunological, renal, cardiovascular, endocrine, and reproductive disorders. Exposure to high levels of mercury can cause tremors, emotional changes, insomnia, weakness, and sensory disturbances. Although toxic effects of mercury are present in many ecosystems, research on mercury contamination has primarily focused on freshwater fish and birds, as these

Table 1. Mercury Levels in Different Organs of Birds

Animal	Sample	Range (mg kg ⁻¹)	Mean Value (mg kg ⁻¹)	Technique	Source
Pheasant (Phasianidae)	Tail feathers	0.150–0.220	0.180	AAS	Zolfaghari <i>et al.</i> (2007)
Hawk	Tail feathers	0.950–1.500	1.250	AAS	Zolfaghari <i>et al.</i> (2007)
Mallard	Liver	0.010–0.689	0.154	CV-AAS	Żarski <i>et al.</i> (2017)
Mallard	Kidney	0.013–0.423	0.122	CV-AAS	Żarski <i>et al.</i> (2017)
Mallard	Muscle	0.009–0.925	0.110	CV-AAS	Żarski <i>et al.</i> (2017)
Mallard	Breast muscle	0.008–0.938	0.133	AAS	Kalisinska <i>et al.</i> (2013)
Mallard	Liver	0.016–0.966	0.248	AAS	Kalisinska <i>et al.</i> (2013)
Mallard	Kidney	0.010–1.499	0.270	AAS	Kalisinska <i>et al.</i> (2013)
Mallard	Breast feathers	0.037–3.475	0.634	AAS	Kalisinska <i>et al.</i> (2013)
Mallard	Liver	0.080–0.510	0.300	CV-AAS	Aazami <i>et al.</i> (2011)
Mallard	Kidney	0.070–0.450	0.260	CV-AAS	Aazami <i>et al.</i> (2011)
Mallard	Feathers	0.500–1.600	1.040	CV-AAS	Aazami <i>et al.</i> (2011)
Mallard	Muscle	0.030–0.160	0.110	CV-AAS	Aazami <i>et al.</i> (2011)
Pheasant	Leg skeletal muscle	0.003–0.019	0.009	AAS	Gasparik <i>et al.</i> (2010)
Mallard	Leg skeletal muscle	0.001–0.023	0.009	AAS	Gasparik <i>et al.</i> (2010)
Eurasian coot	Leg skeletal muscle	0.005–0.015	0.010	AAS	Gasparik <i>et al.</i> (2010)
Common pochard	Liver female	0.001–0.277	0.093	AAS	Florijancic <i>et al.</i> (2009)
	Liver male	0.001–0.016	0.006		
Mallard	Liver female	0.037–0.197	0.111	AAS	<i>Florijancic et al. (2009)</i>
	Liver male	0.072–0.262	0.122		

species represent a major protein source for many human populations (Lemaire et al., 2018; Kim et al., 2016).

The mallard (*Anas platyrhynchos*) is frequently used as a bioindicator of habitat pollution due to its wide distribution. This species is suitable for ecological studies because of its large population size, pronounced sexual dimorphism, long lifespan, and the ability to study long-term exposure to pollutants. Additionally, the mallard is a game species, and its meat is consumed by humans. Therefore, understanding the levels of toxic metals in its body is important for food safety (Żarski et al., 2017).

This study investigated mercury concentrations in liver and muscle tissue samples of wild mallards collected from three locations: near Belegiš on the Danube (Srem District), near Pločica on the Danube (South Banat), and from a fishpond near Vršački Ritovi during 2024. To better understand the extent of contamination, Table 1 presents results from scientific studies addressing similar topics, providing an overview of mercury concentrations in various tissues of wild birds.

The aim of this study was to examine and compare whether there is a statistically significant difference in mercury concentrations in the liver and muscle tissues of mallards collected from three different locations, each representing a distinct type of ecosystem. Two of the sites, Belegiš (Srem District) and Pločica (South Banat District), are located along the Danube River and are exposed to pollution due to intensive industrial activities in this section of the river. In contrast, the third site a fishpond near the Vršački Ritovi represents a relatively isolated and preserved ecosystem, without direct sources of anthropogenic contamination.

2. Materials and Methods

Mercury levels were measured in liver and muscle samples of mallards at three different locations: Belegiš (Srem District) and Pločica (South Banat District), along the course of the Danube River, and in a fishpond near the Vršački Ritovi during the 2024 calendar year. The total number of analyzed samples was 55, including both liver and muscle tissues of wild mallards. A total of 23 samples were collected from the Belegiš site (12 muscle tissue samples and 11 liver samples), 12 samples from the Pločica site (6 liver and 6 muscle samples), and 20 samples from the Vršački Ritovi site (10 liver and 10 muscle tissue samples).

The samples were stored at -18°C until the time of analysis. One day prior to laboratory processing, the frozen samples were gradually thawed at 4°C and subsequently homogenized. Approximately 0.3 g of tissue (± 0.001 g) was weighed for each sample and transferred into a Teflon vessel of a microwave digestion system. Nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and deionized water ($0.063\ \mu\text{S}/\text{cm}$) obtained from a water purification system (Purelab DV35, ELGA, Buckinghamshire, UK), were added to the sample in quantities of 5 mL each. The microwave digestion system (MARS 6, CEM Corporation, Matthews, NC, USA) was programmed as follows: 5 min from initial temperature to 180°C , hold at 180°C for another 10 min, cooling and venting for 20 min. Digested samples were quantitatively transferred into 100 mL polypropylene volumetric flasks and diluted with deionized water ($0.063\ \mu\text{S}$).

The determination of the ^{202}Hg isotope was performed using inductively coupled plasma mass spectrometry (ICP-MS) on an iCap Qc instrument (Thermo Scientific, Bremen, Germany), equipped with a collision cell and operated in kinetic energy discrimination (KED) mode. Quantitative analysis was based on a five-point calibration curve, including a zero point. A multielement internal standard (^6Li and ^{45}Sc at a concentration of $10\ \text{ng mL}^{-1}$; ^{71}Ga , ^{89}Y , and ^{209}Bi at a concentration of $2\ \text{ng mL}^{-1}$) was introduced via an additional peristaltic pump line to ensure measurement accuracy. Each sample was measured in duplicate, and the mean value was used, corrected for internal standard response factors. The quality of the analytical procedure was verified by the analysis of certified reference material (NIST 1577c - bovine liver, Gaithersburg, MD, USA), which was prepared in the same manner as the samples, using microwave digestion. Repeated measurements of the reference material yielded results within the range of certified values.

2.1.1 Statistical analysis

Statistical analysis was performed using Minitab® 17.1.0. Descriptive statistics and Pearson correlation were applied. ANOVA with Tukey post hoc test was used to assess significant differences in liver mercury concentrations between locations. For muscle tissue, the Kruskal–Wallis and Mann–Whitney U tests were employed to determine differences among sites.

3. Results

Tables 2 and 3 present the results of descriptive statistics for mercury concentrations in liver and muscle tissue samples of mallards from different locations. The tables include median values, ranges (min–max), arithmetic means, standard deviations, and the number of analyzed samples (n) for each sampling site.

Based on the presented results, mercury (Hg) concentrations in the liver of mallards from all three locations ranged from 0.009 to 0.239 mg kg⁻¹, with the highest recorded concentration found in an individual from the Belegiš site, measuring 0.239 mg kg⁻¹ (Table 2). According to the boxplot diagram (Figure 1), the highest

Table 2. Mercury Concentration in Liver Tissue of Mallards

Location	Number of Samples (n)	Median (mg kg ⁻¹)	Range (mg kg ⁻¹)	Mean ± standard deviation (mg kg ⁻¹)
Belegiš	11	0.100	0.023-0.239	0.097±0.056
Pločica	6	0.069	0.039-0.205	0.108±0.073
Vršački Ritovi	10	0.020	0.009-0.070	0.025±0.018

Table 3. Mercury Concentration in Muscle Tissue of Mallards

Location	Number of Samples (n)	Median (mg kg ⁻¹)	Range (mg kg ⁻¹)	Mean ± standard deviation (mg kg ⁻¹)
Belegiš	12	0.022	0.007-0.069	0.030±0.021
Pločica	6	0.027	0.013-0.054	0.030±0.018
Vršački Ritovi	10	0.007	0.005-0.022	0.008±0.005

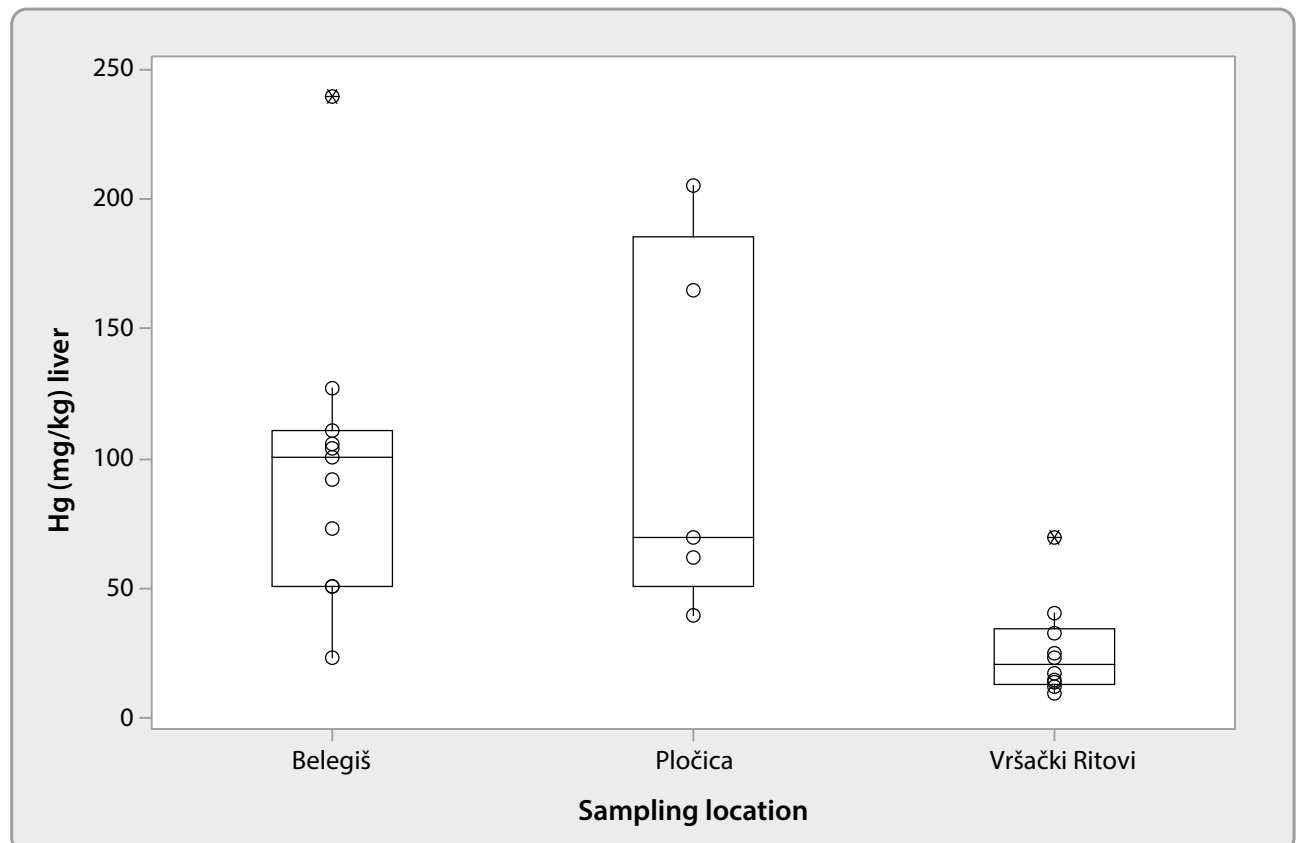


Figure 1. Mercury Levels (mg kg⁻¹) in Liver Samples of Mallards

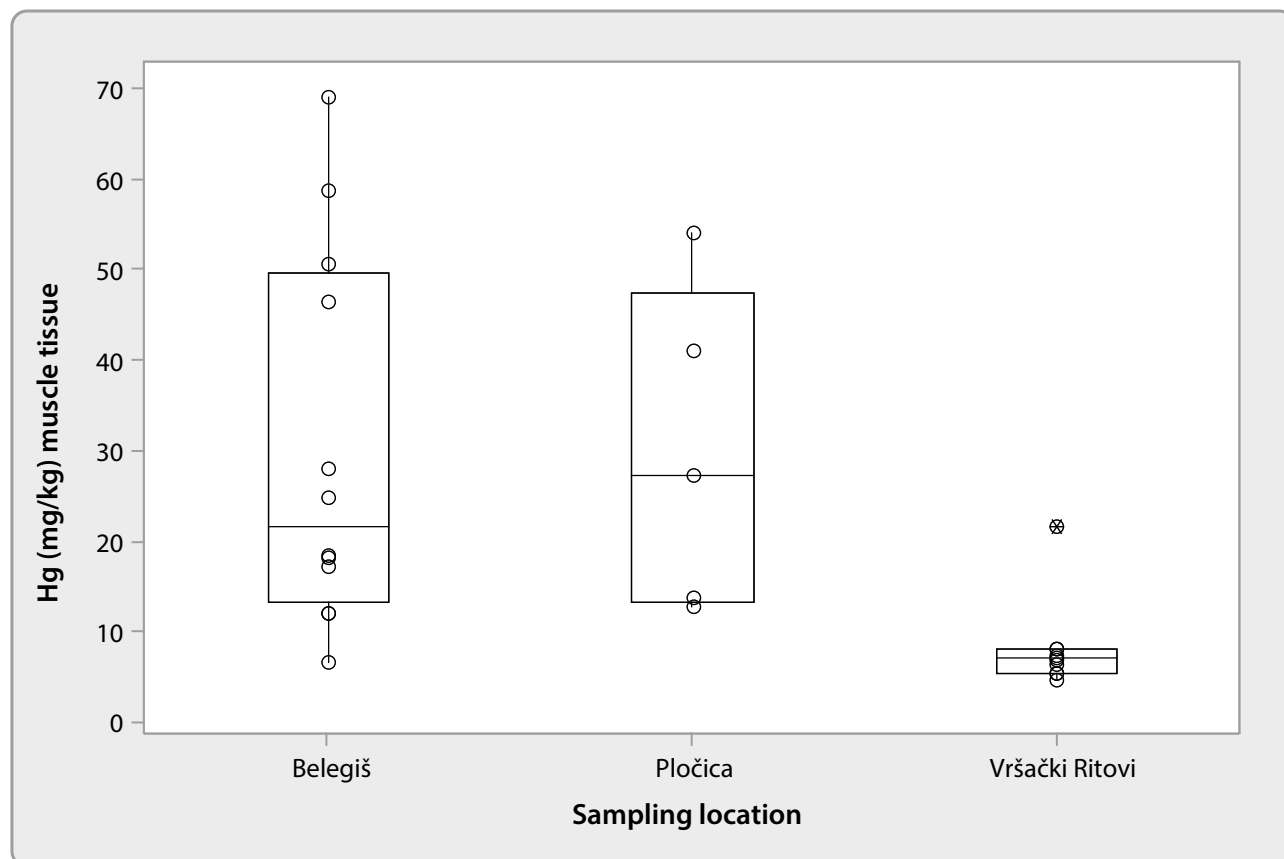


Figure 2. Mercury Levels (mg kg^{-1}) in Muscle Tissue Samples of Mallards

mean mercury concentration in liver samples was observed at the Pločica site, while the highest median value was recorded at Belegiš. Regarding mercury (Hg) concentrations in the muscle tissue of mallards, values at all three locations ranged from 0.005 to 0.069 mg kg^{-1} . The highest concentration was again recorded at the Belegiš site, measuring 0.069 mg kg^{-1} (Table 3 and Figure 2). The mean values for Belegiš and Pločica were identical at 0.030 mg kg^{-1} , while the median was slightly higher at Pločica.

Pearson correlation tests were performed at all three locations to examine the relationship between mercury concentrations in the liver and muscle tissues of mallards. At the Vršački Ritovi site, a very strong positive correlation coefficient ($r = 0.880$) was obtained, with a statistically significant p-value ($p = 0.001$), indicating a clear association between the two tissues. At Belegiš, a moderate positive correlation was found ($r = 0.539$), but the p-value ($p = 0.087$) suggests the relationship is not statistically significant. Similarly, at Pločica, a comparable correlation coefficient ($r = 0.508$) was observed, but with a high p-value ($p = 0.382$), indicating no statistically significant correlation.

Based on one-way analysis of variance (One-Way ANOVA), a statistically significant difference was found in mercury concentrations in the liver of mallards among the three studied locations ($P = 0.004$), indicating that location significantly affects mercury accumulation in liver tissue. A post hoc Tukey HSD test revealed no significant difference between Pločica and Belegiš, grouping them both into statistical group A, while Vršački Ritovi was significantly different from both (group B) with a notably lower average mercury concentration. Before analyzing differences in mercury concentrations in muscle tissue of mallards across the three studied locations, Levene's test was performed to check for homogeneity of variances. Since the test indicated unequal variances, the nonparametric Kruskal–Wallis test was applied. The results showed a statistically significant difference in mercury concentration in muscle tissue among the locations ($P = 0.001$). Following the Kruskal–Wallis test, a post hoc Mann–Whitney test revealed significant differences between Vršački Ritovi and Belegiš ($p = 0.0014$), as well as between Vršački Ritovi and Pločica ($p = 0.0059$). In contrast, no significant difference was found between Belegiš and Pločica ($p = 0.9580$).

4. Discussion

Differences in mercury concentrations between the examined locations were statistically confirmed using one-way analysis of variance (ANOVA) and Tukey post hoc test, with the most pronounced differences observed in liver tissue. The results indicate that habitat characteristics, as well as the presence of potential pollution sources along the course of the Danube River, significantly influence mercury accumulation in organisms. The Vršački Ritovi site clearly stands out as the least contaminated, which may be attributed to its isolation from direct sources of pollution and limited bird migration. In contrast, the Belegiš and Pločica sites, located along the open flow of the river, exhibited higher levels of contamination. Although it was expected, based on geographic position, that mercury concentrations would be higher at Pločica located downstream from Belegiš and directly following the urban and industrial zones of Belgrade and Pančevo the statistical analysis did not confirm this assumption, which is most likely explained by the limited number of samples collected at that site. A similar pattern of differences was observed in muscle tissue, where the Kruskal–Wallis and Mann–Whitney U tests revealed statistically significant differences between locations. The lowest mercury concentrations in this tissue were also recorded at the Vršački Ritovi site, clearly demonstrating the difference in mercury accumulation between distinct ecosystems, compared to the higher and more variable contamination levels observed at sites along the Danube River.

The results of this study were compared with data from two studies conducted in Poland. In the study by *Žarski et al.* (2017), carried out in the area of the Włocławek reservoir, the average mercury concentrations in the liver and muscle tissue of mallards were 0.154 mg kg⁻¹ and 0.110 mg kg⁻¹, respectively. Similarly, in the study by *Kalisinska et al.* (2013), conducted near the city of Szczecin, even higher concentrations were reported, with mean values of 0.248 mg kg⁻¹ in the liver and 0.133 mg kg⁻¹ in the pectoral muscles. In comparison, mercury concentrations at all study sites in Serbia were significantly lower. These differences are most likely the result of varying degrees of industrialization and exposure to anthropogenic pollution sources. The Polish sites, particularly near Szczecin, are heavily influenced by coal-fired power plants, chemical industries, and shipyards, whereas the Serbian locations especially Vršački Ritovi are situated

in considerably less contaminated ecosystems. The lower mercury burden observed in mallards from Serbia supports the conclusion that local ecological conditions and anthropogenic pressures play a crucial role in determining the level of heavy metal accumulation in bird tissues.

Additionally, the results obtained in this study were compared with those reported by *Gasparik et al.* (2010), in which the average mercury concentration in the skeletal muscles of mallards in Slovakia was 0.0086 mg kg⁻¹. This value is nearly identical to the concentration measured at the Vršački Ritovi site (0.008 mg kg⁻¹), suggesting that both locations share similar ecological characteristics, with limited industrial influence. In contrast, slightly higher mercury concentrations were recorded at the Belegiš and Pločica sites, which can be associated with greater anthropogenic impact, although these values remain substantially lower than those reported in studies from Poland. Furthermore, a comparison was made with the results of the study by *Florijančić et al.* (2009), conducted in eastern Croatia, where the average mercury concentration in mallard liver tissue was 0.116 mg kg⁻¹. In comparison, the values recorded in this study were slightly lower at Belegiš (0.097 mg kg⁻¹) and Pločica (0.108 mg kg⁻¹), and substantially lower at Vršački Ritovi (0.025 mg kg⁻¹). Given that the Croatian study site is geographically and ecologically closer to the Serbian localities than the Polish and Slovak sites, these findings further support the conclusion that the mercury concentrations observed in this study are consistent with regional environmental conditions. The differences in mercury levels between sites are likely attributable to varying degrees of exposure to local pollution sources, as well as differences in the feeding behavior of the birds.

5. Conclusion

The results of this study indicate that mallards can be considered reliable bioindicators of mercury contamination in aquatic ecosystems, due to their pronounced ability to accumulate this metal in both liver and muscle tissues. Statistical analyses revealed significant differences in mercury concentrations between the Vršački Ritovi ecosystem, which is isolated and subject to limited anthropogenic influence, and the Belegiš and Pločica sites, located along the open-flow section of the Danube River. However, no statistically significant difference was observed between Belegiš and Pločica,

which is likely attributable to the limited number of samples collected from the Pločica site. Based on the mean values of mercury concentrations in liver and muscle tissues, a higher degree of contamination

can be inferred in Pločica, consistent with its downstream position and greater exposure to potential urban and industrial pollution sources originating from Belgrade and Pančevo.

Procena kontaminacije živom u jetri i mišićnom tkivu divljih pataka (*Anas platyrhynchos*) sa tri lokaliteta u Srbiji

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INFORMACIJE O RADU

Ključne reči:

Živa

Divlja patka

Jetra

Mišić

Biomonitoring

APSTRAKT

Cilj ovog rada bio je da se ispita prisustvo i distribucija žive (Hg) u jetri i mišićnom tkivu divljih pataka (*Anas platyrhynchos*), prikupljenih na tri lokaliteta u Srbiji: Belegiš, Pločica i Vršački Ritovi. Ukupno je analizirano 55 uzorka, od čega 23 sa lokaliteta Belegiš, 12 sa lokaliteta Pločica i 20 sa lokaliteta Vršački Ritovi. Određivanje koncentracije žive vršeno je primenom ICP-MS metode. Koncentracije žive u jetri kretale su se u opsegu od 0,009 do 0,239 mg kg⁻¹, dok su u mišićnom tkivu bile u rasponu od 0,005 do 0,069 mg kg⁻¹. Srednje vrednosti za jetru iznosile su 0,097 mg kg⁻¹ (Belegiš), 0,108 mg kg⁻¹ (Pločica) i 0,025 mg kg⁻¹ (Vršački Ritovi), dok su za mišićno tkivo iznosile 0,030 mg kg⁻¹ (Belegiš i Pločica) i 0,008 mg kg⁻¹ (Vršački Ritovi). Statistička analiza obuhvatila je deskriptivnu statistiku, Pirsonov test korelacije, analizu varijanse (ANOVA) sa Tukey post hoc testom, test homogenosti varijansi (Levene), Kruskal-Wallis test i Mann-Whitney test. Dobijeni nalazi pružaju uvid u potencijalne razlike u nivou kontaminacije živom između dva različita ekosistema, kao i povezanost između različitih tkiva divljih pataka.

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The effect of marinating on the quality of deer meat

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ABSTRACT

Deer meat differs from common livestock meat by its distinctive odour and toughness. Hexanal, the primary odour-causing substance, can be reduced through casein adsorption or metal ion chelation. To improve texture, increasing water retention and utilising proteolytic enzymes may be effective. However, while these approaches show promise in isolated studies, their comprehensive efficacy and optimal application in deer meat processing, particularly in a practical culinary context, remain largely unverified and underexplored. Therefore, the aim of this study was to investigate the effects of marinating deer meat using lemon juice, honey, banana, and yoghurt, all of which have potential deodorising and tenderising properties. Deer meat was marinated for 12 h in each substance and then heated by baking. After cooking, the pH, moisture content, firmness, odour, and taste components of the meat were measured. The results showed that pH was higher in all groups of marinated deer meat after baking, water retention was highest in lemon- and honey-marinated meat, while lemon-, yoghurt-, and banana-marinated deer meat was less firm than the control meat. Yoghurt was the most effective food ingredient in reducing the deer meat's odour, while lemon and yoghurt induced higher inosinic acid and alanine levels than in the control. Overall, the findings indicate that lemon juice is the most effective food ingredient for improving deer meat quality.

1. Introduction

Deer meat has a distinctive odour (Kogiso and Kaneko, 2015) and is characterised by its toughness (Yoshimura *et al.*, 2011). Therefore, cooking methods that can improve both its aroma and tenderness are necessary. The primary odour-causing substance in deer meat is hexanal, which can be reduced by adsorption onto casein, a protein found in milk (Kogiso and Kaneko, 2015). Additionally, hexanal formation is promoted by copper and iron ions during lipid oxidation (Koyanagi, 2013). Adding iron chelators to pork, beef, and chicken meat has been shown to reduce hexanal production by more than 90% (Todokoro *et al.*, 2021). This suggests that food items containing chelating agents may help mitigate hexanal levels in deer meat. One such ingredient with chelating properties is citric acid, which is abundant in lemons.

Regarding firmness, immersing chicken meat in a honey concentration of 0–50% increased water retention proportionally, with a honey concentration of up to 40% leading to increased retention. In a sensory test, many respondents reported that the meat became softer as water retention increased (Shimizu and Nakamura, 2001). Moreover, soaking chicken meat in a solution containing the proteolytic enzyme ficin decreased its shear strength value (Güven *et al.*, 2021). Based on these findings, casein adsorption, citric acid chelation, improving water retention using honey, and adding proteolytic enzymes to enhance softness appear to be effective cooking methods for improving the odour and tenderness of deer meat.

Milk, yoghurt, cheese, and other dairy products contain casein, with yoghurt having a protein composition similar to that of milk (Naito *et al.*, 2019). Yoghurt is fermented with lactic acid bacteria

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(Tsuchihashi, 2022), which is expected to have a greater tenderising effect than unfermented cow's milk owing to its lactic acid content. Bananas also contain high levels of ficin, which may contribute to softening.

Amino acids and nucleotides influence flavour development in meat. After an animal's death, proteases in the muscle break down proteins, generating amino acids, such as glutamic acid. Simultaneously, inosinic acid is produced when ATP degrades to ADP, AMP, and IMP via ATP-degrading enzymes. In common livestock, meat is typically aged for a certain period after slaughter, which causes rapid degradation of nucleic acid-based taste components and an increase in amino acid-based taste components. Conversely, deer meat differs from other meats in that inosinic acid remains stable while amino acid accumulation is low (Yamazaki *et al.*, 2024), leading to lower concentrations of amino acid-based taste components. The glutamic acid content in deer meat is relatively low, whereas alanine and glutamine, which contribute to sweetness, are found in abundance (Fujiwara *et al.*, 2018).

However, despite these promising theoretical approaches and insights from other meat types, there are no comprehensive reports verifying the physical and chemical quality of deer meat using these specific, naturally derived ingredients in practical processing methods. While individual components such as citric acid or ficin have shown promise, their combined effects and overall efficacy in a real-world culinary context for deer meat remain largely unverified and underexplored. This highlights a significant gap in understanding how accessible ingredients can effectively address the distinctive challenges of deer meat quality.

Given this unexplored area, this study aimed to evaluate the effects of honey, lemon, yoghurt, and banana marinades on the pH, moisture content, firmness, odour, and taste of deer meat. Based on the results, the optimal seasoning conditions that improve the quality and palatability of deer meat were identified.

2. Materials and Methods

2.1. Ethical approval

Ethical approval for animal use was not required for this study as commercially sourced deer meat was purchased, and no animals were directly involved in the research.

2.2. Sample and marinade preparation

Frozen deer meat, sourced from Kyushu, Japan, was thawed overnight at 4 °C. It was then cut into small pieces (approximately 2 cm long × 2 cm wide × 1 cm high). Lemon juice, multifloral honey, yoghurt, and finely chopped un-peeled bananas were each mixed in a 1:1 ratio with water to prepare pickling solutions. These four pickling solutions defined the experimental groups, with each group comprising three replicates, containing one deer meat sample per replicate. The meat samples were marinated in their respective solutions for 12 h. Following marination, meat samples were baked using an induction cooker (IRIS OHY-AMA Inc., Miyagi, Japan) set at 1000 W. The meat was heated to 75 °C for 1 min and maintained at this temperature until its core temperature reached 75 °C.

2.3. Physical properties of deer meat after marination

2.3.1. pH

A pH meter (Sato Shoji Co., Ltd., Kanagawa, Japan) was used to measure the pH levels of the marinated deer meat both before and after heating. The samples were allowed to cool for 5 min before pH measurement.

2.3.2. Water holding capacity

After cooking, 0.5 g of accurately weighed deer meat was wrapped in filter paper (QL-C, particle retention capacity: 10 µm, diameter: 70 mm, SAN-SYO Co., Ltd., Tokyo, Japan). The wrapped samples were then centrifuged (2200 g, 30 min, 4 °C), and the samples were weighed once more.

2.3.3. Breaking strength

Deer meat was cooled for 5 min after heating, after which breaking stress was measured using a creep meter (YAMADEN Co., Ltd., Tokyo, Japan). The cylindrical plunger had a 3-mm diameter, and breaking strength was measured at a strain rate of 70% and a velocity of 1.00 mm/s.

2.3.4. Moisture content

After preheating to 105 °C, 1.0 g of chopped and cooked deer meat samples were accurately weighed into a weighing jar and then dried at 105 °C for 170 min. After cooling, the samples were weighed again, and the moisture content was calculated.

2.4. Chemical composition of deer meat after marination

2.4.1. Hexanal

The hexanal measurement method was modified based on the methods used by *Fujino et al.* (1991). Deer meat samples (2 g) were accurately weighed, and hexanal was extracted using 99% ethanol (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). The extract was centrifuged (20321 g, 5 min, 5 °C) and diluted to 5 mL.

Next, 500 µL of this solution were mixed with 500 µL 2,4-dinitrophenylhydrazine (DNPH) solution. The DNPH solution was prepared by dissolving 50 mg DNPH (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) in 40 mL ethanol (FUJIFILM Wako Pure Chemical Corporation), 1 mL hydrochloric acid (FUJIFILM Wako Pure Chemical Corporation), and 4 mL pure water.

The resulting mixture was heated in a heat block at 45 °C for 45 min, cooled in cold water, and filtered through a 0.22 µm membrane filter (Hangzhou Cobetter Filtration Equipment Co., Ltd., Hangzhou, China). The filtered solution was then analysed using high-performance liquid chromatography (HPLC, SHIMADZU CORPORATION, Kyoto, Japan).

The HPLC system utilised a Luster C18 column (150 × 4.6 mm, LCScience Co., Ltd., Nara, Japan) with a mobile phase of acetonitrile (FUJIFILM Wako Pure Chemical Corporation) and water (8:2). The flow rate was 1.0 mL/min, the column temperature was 40 °C, and detection was performed using a UV detector at a wavelength of 360 nm. The injection volume was 20 µL.

2.4.2. Inosinic acid

Inosinic acid was measured according to the methods used in *Seki* (2024). Accurately weighed deer meat (2.5 g) was placed in a 15 mL centrifuge tube and homogenised in 4 mL 10% perchloric acid (KANTO CHEMICAL Co., Inc., Tokyo, Japan) to extract amino acids and inosinic acid. After centrifugation (17075 g, 10 min, 5 °C), the supernatant was transferred to a new 15 mL centrifuge tube. This extraction and centrifugation procedure was repeated once more. The final volume was then adjusted to 10 mL.

Next, 1 mL of the extract was neutralised to pH 7 using 10 M potassium hydroxide solution (FUJIFILM Wako Pure Chemical Corporation)

and centrifuged (20321 g, 5 min, 5 °C). The supernatant was then collected and transferred to a new 15 mL centrifuge tube. The remaining precipitate was resuspended in 1 mL deionised water. This precipitation and supernatant collection procedure was repeated two times, and the combined supernatants were then diluted to 5 mL with water. The final solution was filtered through a 0.22 µm membrane filter and analysed using HPLC.

HPLC analysis was performed using a SHIMADZU CORPORATION system equipped with a COSMOSIL 5C18-PAQ column (150 × 4.6 mm, NACAL-AI TESQUE, INC., Kyoto, Japan). The mobile phase consisted of 20 mM NaH₂PO₄ adjusted to pH 2.8. A constant flow rate of 0.50 mL/min was maintained, and the column temperature was set at 40 °C. Detection was carried out using a UV detector at a wavelength of 260 nm, with an injection volume of 20 µL.

2.4.3. Alanine and glutamine

The measurement method for alanine and glutamine was modified based on the methods in *Seki* (2021). For this assay, 20 µL of the sample from the inosinic acid assay were combined with 70 µL ethanol (FUJIFILM Wako Pure Chemical Corporation), 20 µL triethylamine (FUJIFILM Wako Pure Chemical Corporation), and 20 µL phenyl isothiocyanate (KANTO CHEMICAL Co., Inc.). This combination was thoroughly mixed and then incubated at 25 °C for 30 min.

Following incubation, a 500 µL solution of 250 mM acetic acid buffer (pH 6.6) and acetonitrile (97:3) were added and mixed. The resulting solution was then filtered through a 0.22 µm membrane filter before HPLC analysis.

HPLC analysis was performed using a SHIMADZU CORPORATION system equipped with a COSMOSIL 5C18-MS-II column (150 × 4.6 mm, COSMOSIL; NACALAI TESQUE, Inc.). The mobile phase comprised two components: mobile phase A, which consisted of 250 mM acetic acid buffer (pH 6.6) with acetonitrile (97:3), and mobile phase B, which was a mixture of acetonitrile (FUJIFILM Wako Pure Chemical Corporation) and water (6:4).

A constant flow rate of 1.0 mL/min was maintained with the following gradient program: 0 min (B 5%) → 16 min (B 100%) → 20 min (B 5%) → 25 min (B 5%). The column temperature was set at 40 °C. Detection was performed using a UV detector at a wavelength of 254 nm, and the injection volume was 20 µL.

2.5. Statistical analysis

Statistical analysis was performed according to Fisher's three principles of experimental design. Comparisons between two groups were conducted using *t*-tests, while one-way analysis of variance (ANOVA) was applied for comparisons involving three or more groups. All analyses were performed using Microsoft Excel, with a significance level set at 5%.

3. Results

3.1. Physical properties of deer meat after marination

3.1.1. pH

Table 1 shows the pH, water holding capacity, breaking strength, and moisture content of deer meat marinated in different pickling solutions. The initial pH of the deer meat in the control (water-only marinade) was 6.11, significantly higher than that of deer meat marinated in honey (5.23), lemon (3.53), yoghurt (5.29), and banana (5.63) ($p < 0.001$ for all comparisons). Post heating, the pH levels were significantly higher in all deer meat groups than in corresponding groups prior to heating ($p < 0.001$). The pH of the deer meat in the control sample (water only) was 6.49, which remained significantly higher

than that of meat marinated in honey (5.74), lemon (3.96), yoghurt (5.72), and banana (6.05) ($p < 0.001$ for all comparisons).

3.1.2. Water holding capacity

In terms of water retention, lemon-marinated (85.6%) and honey-marinated (78.7%) deer meat showed significantly higher levels ($p = 0.0019$ and $p = 0.014$, respectively) than did the control deer meat (68.5%). Conversely, yoghurt-marinated (63.3%) and banana-marinated (66.5%) deer meat produced slightly lower water retention levels than did the control (deer meat marinated in water; 68.5%), although these differences were not statistically significant ($p = 0.15$ and $p = 0.39$, respectively).

3.1.3. Breaking strength

Regarding breaking strength, the control and honey-marinated deer meat exhibited similar values (13.6 N and 13.4 N, respectively, $p = 0.87$). In contrast, lemon-, yoghurt-, and banana-marinated deer meat showed significantly lower breaking strength than did the control ($p = 0.015$, $p = 0.018$, and $p = 0.032$, respectively). No significant difference in breaking strength was observed among the lemon, yoghurt, and banana treatments ($p = 0.88$; ANOVA).

Table 1. Physical properties of deer meat after marination

	pH		Water holding capacity (%)	Breaking strength (N)	Moisture (%)
	Before heating	After heating			
Control (water-only)	6.11±0.010	6.49±0.04	68.5±3.2	13.6±1.2	60.8±0.91
Honey	5.23±0.057 ^a	5.74±0.048 ^b	78.7±1.3 ^c	13.4±1.3	72.0±1.1 ^c
Lemon	3.53±0.06 ^a	3.96±0.05 ^b	85.6±2.6 ^c	9.57±1.2 ^d	66.3±1.2 ^c
Yoghurt	5.29±0.04 ^a	5.72±0.069 ^b	63.3±4.0	9.59±1.3 ^d	56.0±0.46 ^c
Banana	5.63±0.02 ^a	6.05±0.039 ^b	66.5±1.2	9.91±1.5 ^d	57.5±0.31 ^c
p	$p < 0.001^a$	$p < 0.001^b$	$p < 0.05^c$	$p < 0.05^d$	$p < 0.05^e$

Legend: Mean values for pH and breaking strength are reported for each marinade (n=9), based on three technical and three biological replicates. For water holding capacity and moisture content, mean values are given for each solution (n=3), derived from three technical and one biological replicate. In all cases ± denotes the standard deviation. Significance levels are as follows: $p < 0.001^a$: Significant difference compared to the control pH. $p < 0.001^b$: Significant difference compared to the control pH and the pH before heating. $p < 0.05^c$: Significant difference compared to the control water holding capacity. $p < 0.05^d$: Significant difference compared to the control breaking strength. $p < 0.05^e$: Significant difference compared to the control moisture content.

3.1.4. Moisture content

The moisture content of honey-marinated (72.0%) and lemon-marinated (66.3%) deer meat was significantly higher than that of the control (60.8%) ($p=0.00018$ and $p=0.0033$, respectively). In contrast, yoghurt-marinated (56.0%) and banana-marinated (57.5%) deer meat showed significantly lower moisture content than that did the control ($p=0.0038$ and $p=0.028$, respectively).

3.2. Chemical composition of deer meat after marination

3.2.1. Hexanal

Table 2 summarises the amounts of hexanal, alanine, glutamine, and inosinate in the marinated deer meat. The honey-marinated deer meat exhibited a significantly higher mean hexanal level ($1.74\text{ }\mu\text{g g}^{-1}$) than the control ($1.56\text{ }\mu\text{g g}^{-1}$) ($p=0.0027$). The banana-marinated deer meat showed an elevated hexanal content ($1.72\text{ }\mu\text{g g}^{-1}$) relative to the control ($p=0.17$). Conversely, the yoghurt-marinated deer meat had the lowest hexanal content ($0.859\text{ }\mu\text{g g}^{-1}$) ($p=0.017$) among the groups. The lemon-marinated meat had a slightly lower hexanal content ($1.54\text{ }\mu\text{g g}^{-1}$) than did the control, although this difference was not statistically significant ($p=0.46$).

3.2.2. Inosinic acid

Inosinic acid, a key taste compound, was significantly higher in lemon-marinated (1.31 mg g^{-1}) and honey-marinated (1.19 mg g^{-1}) deer meat ($p=0.0065$ and $p=0.026$, respectively) than in the control (0.966 mg g^{-1}). However, there was little change in the inosinic acid concentration of the yoghurt-marinated deer meat (0.957 mg g^{-1}) when compared with the control ($p=0.85$). Conversely, the banana-marinated deer meat exhibited the lowest inosinic acid concentration (0.725 mg g^{-1}) ($p=0.0179$) among the groups.

3.2.3. Alanine and glutamine

The alanine level in the yoghurt-marinated deer meat (0.918 mg g^{-1}) was significantly higher than that in the control (0.854 mg g^{-1}) ($p=0.0072$). The honey-marinated deer meat (0.884 mg g^{-1}) had a slightly higher alanine content than did the control ($p=0.15$). In contrast, lemon-marinated (0.742 mg g^{-1}) and banana-marinated (0.720 mg g^{-1}) deer meat contained significantly lower alanine levels than did the control ($p=0.0044$ and $p=0.0071$, respectively). Glutamine levels varied across treatments. The lemon-marinated deer meat (0.205 mg g^{-1}) exhibited slightly higher glutamine content than the control (0.187 mg g^{-1}) ($p=0.36$). Conversely, the glutamine content in the

Table 2. Chemical composition of deer meat after marination

	Hexanal ($\mu\text{g g}^{-1}$)	Inosinic acid (mg g^{-1})	Alanine (mg g^{-1})	Glutamine (mg g^{-1})
Control (water-only)	1.56±0.026	0.966±0.016	0.854±0.014	0.187±0.021
Honey	1.74±0.038 ^a	1.19±0.064 ^b	0.884±0.023	0.156±0.023
Lemon	1.54±0.040	1.31±0.046 ^b	0.742±0.021 ^c	0.205±0.020
Yoghurt	0.859±0.16 ^a	0.957±0.070	0.918±0.017 ^c	0.0871±0.0074 ^d
Banana	1.72±0.13	0.725±0.054 ^b	0.720±0.032 ^c	0.116±0.00054 ^d
P	$p<0.05^a$	$p<0.05^b$	$p<0.05^c$	$p<0.05^d$

Legend: Mean values for each solution are reported (n=3 technical replicates, n=1 biological replicate), with ± denoting the standard deviation. Significance levels are as follows: $p<0.05^a$: Significant difference compared to the control hexanal content. $p<0.05^b$: Significant difference compared to the control inosinic acid content. $p<0.05^c$: Significant difference compared to the control alanine content. $p<0.05^d$: Significant difference compared to the control glutamine content.

honey-marinated deer meat (0.156 mg g^{-1}) was slightly lower than that in the control ($p=0.16$). Yoghurt-marinated (0.0871 mg g^{-1}) and banana-marinated (0.0116 mg g^{-1}) deer meat contained significantly lower glutamine levels than did the control ($p=0.016$, $p=0.028$, respectively).

4. Discussion

This study examined the effects of different marinating solutions on the physicochemical properties of deer meat, specifically focusing on pH, water retention, texture, and biochemical composition.

4.1. Physical properties of deer meat after marination

4.1.1. pH

The pH of deer meat marinated in the different food ingredient solutions was highest in the control (water-only). The initial pH of the marinating solutions were 4.78 for honey, 2.44 for lemon, 5.57 for banana, and 4.22 for yoghurt. Therefore, the pH of the marinated deer meat was lower than that of the control, as it was influenced by the pH of the marinating solution. In addition, the pH of the deer meat immersed in each marinade was higher than that of the marinating solution. The pH of raw deer meat has been reported to be between 5.6 and 5.8 (Shimada et al., 2022), and in this study it was 6.11, which is close to this value. Since the pH of the meat itself was higher than that of the marinating solution, the pH of the marinating meat was higher than that of the marinating solution, particularly in the case of honey, lemon, and yogurt.

Furthermore, the pH was consistently higher after heating than before heating across all groups of marinated deer meat. In cheese, heating has been reported to decompose amino acids to produce ammonia (Buňka et al., 2004). Ammonia production increases pH, and in this study, an increase in pH was observed after heating in all experimental groups. This suggests that thermal denaturation contributed to the pH increase, regardless of the marinating solution. The pH increases due to heating were approximately 0.4 for the control, lemon-, banana-, and yoghurt-marinated meat, while honey induced a slightly higher pH increase of approximately 0.5. As the pH of acacia honey has been reported to increase by 0.1 after heat treatment at 90°C (Sulaiman and Sarbon, 2022), the use of honey in the marinade could have contributed intrinsically to the more pronounced rise in pH after heating compared to the other treatments.

4.1.2. Water holding capacity

The water retention properties of deer meat marinated in honey and lemon were higher than those of the control. Immersing pork meat in a solution of honey and water was also shown to improve its water-holding capacity, with increasing honey concentrations leading to nearly 75% water retention at 50% honey concentration (Shimizu and Nakamura, 2001). A similar trend was observed in the current study, where deer meat marinated in 50% honey retained 78.7% water. Hu et al. (2016) further demonstrated that adding glucose to chicken meat improved its water retention. Additionally, in kefir gel, the addition of 50% fructose increased water retention from approximately 45% to up to 100% (Zavala et al., 2015). This suggests that the sugar component of honey influenced the water retention capacity in the current study.

Similarly, improved water retention in chicken meat marinated in lemon juice has been reported (Unal et al., 2022), which aligns with the current findings. The lowest water retention in meat is typically observed at pH 5.5, near the isoelectric point of meat proteins (Abe et al., 2018). The pH of the lemon-treated meat was 3.53, sufficiently distant from the isoelectric point, which may have contributed to its enhanced water retention.

Conversely, both yoghurt- and banana-marinated deer meat exhibited slightly lower water retention levels than that of the control. Masoumi et al. (2022) showed that yoghurt lowers pH, thereby enhancing water retention in chicken. However, in the current study, the pH of deer meat marinated in yoghurt (5.29) and banana (5.63) remained close to the isoelectric point of meat, which likely played a role in the lower water retention of these two groups compared to the control.

4.1.3. Breaking strength

The breaking strength of deer meat marinated in honey was not significantly different to that of the control. In chicken meat, the addition of 10, 20, and 30% honey solution has been reported to increase water retention and tenderness, with a corresponding increase in honey concentration (Hashim et al., 1999). In contrast, more respondents answered that pork marinated in a 50% honey mixture was tougher than that in 30–40% honey (Shimizu and Nakamura, 2001), possibly because honey caused caramelisation, which toughened the meat surface after heating. Honey caramelisation also occurred in the current study.

Meat marinated in lemon, yoghurt, and banana exhibited lower breaking strength than that of the control. Similar results have been reported in beef, where the meat softened as water retention improved (Mega *et al.*, 1979). In the current study, the low pH measured after lemon marination could have improved water retention, thereby resulting in lower rupture strength. Lactic acid in marinades has been associated with the softening of pork, sheep, and rabbit meat (Simitzis *et al.*, 2021). As lactic acid is present in yoghurt, a similar effect was anticipated in this study.

Treatment of chicken meat with ficin, one of the proteolytic enzymes found in banana peels, has demonstrated a softening effect (Güven *et al.*, 2021). Our findings align with these results, suggesting that ficin contained in banana peels reduced the breaking strength of the deer meat.

4.1.4. Moisture content

Moisture content was higher in deer meat marinated in honey and lemon than that in the control. As fructose and glucose are major components of honey, and lemon shifts the pH away from the isoelectric point of deer meat, both these factors likely contributed to higher water retention and subsequent moisture holding. Higher water retention has been linked to higher moisture content in pork (Jankowiak *et al.*, 2021). Although lemon-marinated deer meat exhibited higher water retention than the honey-marinated in this study, the moisture content after heating was lower in lemon-marinated deer meat than that in honey-marinated meat (66.3 vs. 72.0%, respectively). In honey, water retention is primarily enhanced by sugar, whereas in lemon juice, it is predominantly influenced by pH-induced changes in the isoelectric point. Terazawa *et al.* (2014) compared the moisture content of ground beef marinated in unglazed soy sauce, mirin, and soy sauce, then cooked in teriyaki sauce, finding that the teriyaki group retained more moisture due to the caramelisation of sugar. In the current study, the caramelisation of sugar contained in the honey is thought to have contributed to the higher moisture content observed in the honey-marinated deer meat than in the lemon-marinated meat.

Conversely, the moisture content of deer meat marinated in yoghurt and banana was lower than that of the control. This reduction can be attributed to water loss due to heating, which decreased the water retention properties as the pH remained close to the meat's isoelectric point (approximately 5.5). Similarly, banana marination produced a lower moisture content

than did the control, a phenomenon also observed with yoghurt-marinated meat, both of which are attributable to the lower water content after heating.

4.2. Chemical composition of deer meat after marination

4.2.1. Hexanal

The hexanal content in deer meat marinated in honey and banana was higher than that in the control. Honey has been reported to contain hexanal as a volatile component, although its content varies depending on the nectar source (Zhu *et al.*, 2022). Similarly, as bananas have been reported to contain hexanal as a major volatile component (Mukherjee *et al.*, 2022), the higher hexanal levels observed in the meats marinated in honey and banana compared to the control can be attributed to the presence of hexanal in these marinating solutions.

Deer meat marinated in lemon showed no significant change in hexanal content compared to the control. Hexanal is produced by lipid oxidation, a process that involves metal ions (Koyanagi, 2013). Lipid oxidation can be inhibited by mitigating the action of metal ions via chelation (Todokoro *et al.*, 2021). As citric acid in lemons possesses chelating properties (Akatsuka *et al.*, 2015), it likely inhibits hexanal formation. However, chelation does not remove pre-existing hexanal, which explains why no change was observed in the lemon-marinated meat.

The amount of hexanal in deer meat marinated in yoghurt was lower than that in the control. Yoghurt contains casein, a protein that has been reported to adsorb hexanal in deer meat (Kogiso and Kanego, 2015). Thus, casein in yoghurt may have contributed to the removal of hexanal in this study.

4.2.2. Inosinic acid

The inosinic acid content in banana-marinated deer meat was lower than that in the control meat. Previous studies have reported a decrease in inosinic acid levels with increasing bacterial counts. For instance, when beef was stored at 5 °C for 8 days, the number of total viable bacteria increased from approximately 4 log CFU g⁻¹ to 8 log CFU g⁻¹, while the amount of inosinic acid decreased from 0.3 µmol g⁻¹ to 0.02 µmol g⁻¹ (Parris *et al.*, 1983). Similarly, when sterilised chicken meat was inoculated with *Staphylococcus* spp. and Enterobacteriaceae isolated from raw chicken meat and incubated at 35 °C for 48 h, the *Staphylococcus* count

increased. While the amount of inosinic acid in chicken without bacterial inoculation decreased slightly, inosinic acid levels were reported to disappear within 48 h in inoculated meat (Hayashi and Nakata, 2003). Given that Enterobacteriaceae have been detected in bananas (Issouffou *et al.*, 2018) and that *Staphylococcus* spp. is commonly found on human skin and other surfaces, these bacteria may have been present in the bananas. Moreover, bacterial growth has been observed in the epidermis and pulp of bananas after being cut, with an increase from 0 log₁₀ CFU g⁻¹ to 7 log₁₀ CFU g⁻¹ in the pulp within two days (Abe *et al.*, 2012). This evidence suggests that inosinic acid may have been degraded by the bacteria present in bananas.

Conversely, honey- and lemon-marinated deer meat contained higher levels of inosinic acid than that in the control meat. Inosinic acid-degrading enzymes degrade inosinic acid over time, and its levels can be maintained by suppressing enzyme activity. Seki and Kikuchi (2022) reported that honey inhibits inosinic acid-degrading enzymes in chub mackerel, suggesting that honey in the current study similarly inhibited these enzymes. In addition, inosinic acid-degrading enzymes in various fish species have different optimum pH values (Tomiooka and Endo, 1984). As honey and lemon create an acidic environment, enzyme activity in deer meat could have been suppressed, thereby preserving inosinic acid. This collectively suggests that honey and lemon may have supported the retention of inosinic acid in the marinated deer meat.

4.2.3. Alanine and glutamine

Honey contains gluconic acid, which has been reported to inhibit myosin degradation in squid (Kuwahara *et al.*, 2004). As myosin is a protein, inhibiting its degradation would prevent the production of amino acids. However, honey itself contains 3.43–27.0 mg/100 g alanine (Quintas *et al.*, 2021). Thus, while gluconic acid-induced proteolysis inhibition may have prevented the formation of amino acids, the alanine naturally present in honey may have offset this effect, resulting in the honey-marinated meat having a similar amount of alanine as the control.

The alanine content in deer meat marinated in lemon and banana was lower than that in the control. The addition of citric acid to squid inhibits the activity of metalloproteinase, an endogenous protease, and suppresses alanine production (Geng *et al.*, 2018). Similarly, the citric acid in lemons could have inhibited deer meat protease activity in this study, thereby inhibiting alanine production. Moreover, bananas

contain starch, and amino acids are involved in starch gelatinisation. Alanine is reported to bind to starch, albeit relatively weakly (Ito *et al.*, 2006), suggesting that heating could have facilitated the binding of alanine to starch, thereby resulting in less free alanine in the banana-marinated meat than in the control.

In contrast, the amount of alanine in deer meat marinated in yoghurt was higher than that in the control. In fermented sausages, the addition of lactic acid bacteria resulted in protein degradation and higher alanine content (Kato, 1991). Various proteolytic enzymes are present in meat, each requiring different conditions for their activation and inhibition. In this study, yoghurt likely promoted the activity of proteolytic enzymes that produce alanine, which may have caused the higher alanine content of yoghurt-marinated meat. The glutamine content in deer meat marinated with honey and banana was lower than that of the control. The addition of gluconic acid to squid inhibits degradation (Kuwahara *et al.*, 2004). Given the high gluconic acid levels in honey, this compound could have inhibited protein degradation in the current study. Similarly to alanine, the starch in bananas likely interacted with amino acids in deer meat during banana marination, which likely reduced glutamine content. Although the amount of glutamine in lemon-marinated deer meat was higher than that in the control, the difference was not significant, suggesting that lemon had no substantial effect on glutamine levels. However, deer meat marinated in yoghurt had significantly lower glutamine levels than that did the control. Yoghurt contains a type of lactic acid bacteria that produce high levels of gamma-aminobutyric acid (GABA). Ohmori *et al.* (2018) reported that when yoghurt containing these bacteria was mixed with sake, the L-glutamic acid in the sake was broken down by the lactic acid bacteria, leading to increased GABA levels. As glutamine serves as a precursor for glutamic acid, a similar phenomenon may have occurred in this study, resulting in the depletion of glutamine in the yoghurt-marinated deer meat.

4.3. Limitations

This study has some limitations that warrant discussion. A primary concern is the lack of precise information regarding sample storage conditions; specifically, details on the duration and temperature that deer meat samples were frozen at was not available from the supplier.

Additionally, specific data on the deer species, exact hunting location, or the hunting season could not be obtained. While these details would

significantly enrich the study's ecological context and reproducibility, they simply were not provided by the third-party supplier. Despite these gaps regarding the meat's origin, this study's conclusions remain solid given the available information.

Finally, many of the proposed mechanistic explanations in this study rely on findings from studies involving other types of meat, such as chicken, pork, beef, squid, and fish. While they offer plausible hypotheses, the specific physiological and biochemical responses of deer meat might differ. As these mechanisms could not be confirmed directly with individual deer meat samples, the direct applicability of these external findings to deer meat should be viewed with caution.

5. Conclusion

This study examined the effects of honey, lemon juice, yoghurt, and banana marination on deer meat quality. Regardless of the marinating solution

used, the pH increased with heat. Both honey and lemon treatments resulted in high water retention, while lemon-marinated deer meat exhibited the lowest breaking strength compared with the other groups. Marination in yoghurt resulted in the lowest odour intensity, whereas lemon marination yielded the highest inosinic acid levels. Glutamine and alanine levels were the highest in yoghurt-marinated meat. Overall, the results indicate that among the marinating solutions tested, lemon marination offers the most significant improvements to the quality of deer meat.

With the growing population, food shortages are a significant concern. Consequently, in addition to major livestock meats, the utilisation of meat from wild deer and boar is expected to increase in the future. This study investigated methods to enhance the palatability of deer meat. Future research will focus on assessing quality changes and conducting sensory evaluations of processed deer meat to further expand its culinary applications.

Uticaj rastvora marinade na kvalitet mesa divljači jelena

Hiroko Seki

INFORMACIJE O RADU

Ključne reči:

Meso jelenske divljači
Kvalitet mesa
Mariniranje limunom
Sposobnost zadržavanja vode
Jogurt
Smanjenje mirisa.

APSTRAKT

Meso divljači jelena se izdvaja od mesa domaćih životinja zbog svog karakterističnog mirisa i žilavosti. Heksanal, primarno supstanca koja uzrokuje neprijatan miris, može se smanjiti adsorpcijom kazeina ili helacijom metalnih jona. Poboljšanje teksture može se postići povećanjem sposobnosti zadržavanja vode i korišćenjem proteolitičkih enzima. U ovom radu istraživao je uticaj mariniranja na kvalitet mesa divljači jelena korišćenjem limuna, meda, banane i jogurta, koji imaju potencijalna svojstva dezodoracije i omekšavanja. Meso divljači jelena je marinirano 12 sati u svakom rastvoru marinade, a zatim zagrejano. Nakon kuvanja, mereni su pH, sadržaj vlage, čvrstoća, miris i komponente ukusa mesa. Rezultati su pokazali da se pH povećao u svim uzorcima nakon pečenja, sposobnost zadržavanja vode je bila najveća u mesu mariniranom limunom i medom, a čvrstoća se smanjila u uzorcima tretiranim limunom, jogurtom i bananom. Jogurt je bio najefikasniji u smanjenju mirisa, dok se upotrebom limuna i jogurta povećao sadržaj inozinske kiseline i alanina. Na kraju, ovi rezultati ukazuju da je limun najefikasniji sastojak hrane za poboljšanje kvaliteta mesa jelenske divljači.

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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 cfu g^{-1} (Table 2). A significantly lower ($P < 0.05$) TPC was observed in the BHT group (3.63 log cfu g^{-1}) compared to the control (4.16 log 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 cfu g^{-1}) and 50M (4.02 log cfu g^{-1}) groups relative to 30M (4.14 log 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 ⁻¹)	4.16±0.12 ^a	3.63±0.24 ^b	4.14±0.08 ^a	3.97±0.37 ^{ab}	4.02±0.28 ^a
TBA (mg Kg ⁻¹)	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 ^a	5.70±0.26 ^{ab}	5.40±0.12 ^b	5.46±0.19 ^b	5.43±0.33 ^b

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⁻¹ (Table 2). As expected, the BHT treatment exhibited the lowest TBA value (0.03 mg kg⁻¹), confirming its high oxidative stability. Among the treatments with senduduk leaf powder, the 50M group showed the lowest TBA value (0.04 mg kg⁻¹), followed by 30M and 40M (both at 0.05 mg kg⁻¹). 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 ^c	87.14±1.04 ^a	64.30±0.83 ^c	76.03±1.03 ^d	85.17±0.99 ^b
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 ^{bc}	2.40±0.96 ^{bc}	1.92±0.76 ^c	2.68 ± 0.85 ^b	3.52±1.00 ^a
Tenderness (mm g ⁻¹ s ⁻¹)	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 ± 1.00), indicating superior elasticity compared to all other treatments. Conversely, the lowest elasticity was observed in the 30M group (1.92 ± 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 ± 0.85), while the control (2.44 ± 1.12) and BHT (2.40 ± 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⁻¹ s⁻¹ (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 ^{ab}	5.50 ± 0.97 ^a	4.88 ± 1.12 ^b	5.06 ± 0.73 ^{ab}	5.53 ± 0.91 ^a
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 ^{ab}	6.24 ± 1.04 ^a	5.47 ± 1.02 ^b	5.14 ± 1.18 ^b	5.29 ± 0.99 ^b

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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The effect of using different bamboo on the characteristics of sui wu'u fermented meat from Bajawa, Nusa Tenggara Timur

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ABSTRACT

This research aimed to analyze the characteristics of beef sui wu'u prepared by storing the meat product in different types of bamboo. The research design used four treatments with five storage replications: P1: green betung bamboo (*Dendrocalamus asper*), P2: brown betung bamboo (*Dendrocalamus asper*), P3: talang bamboo (*Schizotachyum brachcladum*), P4: gombong bamboo (*Gigantocloa verticillata*). The research showed that the different types of bamboo storage had a significant effect ($P<0.05$) on the physical characteristics of beef sui wu'u in terms of yield, final pH, water holding capacity and hardness. Among the chemical characteristics, using different types of bamboo had a significant effect ($P<0.05$) on the water and protein content of beef sui wu'u. The organoleptic characteristics showed the different types of bamboo storage container had a significant effect ($P<0.05$) on colour, aroma, texture and overall acceptability, but for the colour dimensions, L^* , a^* , b^* , Cab , hab , Y , X , Z , x , y , Wi , Ti , and Tw , there were no significant effects ($P>0.05$). The microbiological characteristics of the sui wu'u for all types of bamboo used were within the standard limits fermented meat products. The use of different types of bamboo influences the characteristics of beef sui wu'u in terms of physical, chemical, colour dimensions, organoleptic characteristics and microbiology. The use of containers made from brown betung bamboo (*Dendrocalamus asper*) produces better beef sui wu'u than the other bamboo types. Nonetheless, the use of talang bamboo and gombong bamboo can also be recommended in the process of making sui wu'u, but the final beef product had different characteristics from the sui wu'u stored in brown betung bamboo, and was especially different from sui wu'u stored in talang bamboo.

1. Introduction

Traditional meat fermentation utilizes the activity of bacteria or enzymes that occur during the fermentation process, where bacterial or enzyme activity is obtained from the raw materials used in the meat fermentation process or from the type of container used during the process of fermentation. The fermented meat product traditional found in East Nusa Tenggara, Indonesia, is sui wu'u. The name sui wu'u, according to the local Flores

people, is derived from sui (meat) and wu'u (corn flour). Although sui is the general word for all types of meat, it can specifically refer to beef. A similar, related product is called hui wu'u when based on pork (hui in the local language). Therefore, the product's name depends on the meat used, but regardless, this product is fermented meat coated with salt and corn flour and then stored in bamboo containers, locally called tuku. According to (Rubak, Lalel, & Sanam, 2023), sui wu'u is a product produced by the people of Bajawa, East Nusa Tenggara, using

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preservation by traditional methods, i.e., by coating the meat with salt then storing it in bamboo (tuku) for six months. *Febrianti et al.* (2021) the type of bamboo used is betung (aka petung) bamboo (*Dendrocalamus asper*), which contains bioactive compounds, such as phenolics, flavonoids, lignans, tannins. Research conducted by (*Chee et al.*, 2020) showed that *D. asper* contains phenolics and flavonoids (total phenolic content measured via Folin-Ciocalteu), has antioxidant activity measurable using the DPPH method, as well as enzymatic activity, such as inhibiting α -amylase and α -glucosidase. In addition, *Lactococcus lactis* was isolated from sui wu'u (*Rubak, Lalel, Sanam, et al.*, 2023).

The characteristics of fermented meat products are their specific taste, texture and long shelf-life, all a result of both natural fermentation or from added cultured microorganisms (*Zhong et al.*, 2021). Various fermented meat products are traditionally produced across Asia, the principles of which are almost the same as sui wu'u (*Wang et al.*, 2022; *Zhong et al.*, 2021; 2022), including a local product from Hunan and Guizhou, China, with added rice flour and salt. Apart from that, the same principle applies to Cang-kuk fermented meat product and this processed product comes from Kuantan Singingi, Riau, Indonesia, and has added betung bamboo shoots, rice and salt (*Mirdhayati*, 2022). Furthermore *Endo et al.* (2014), a type of fermented processed product, Jinhua ham, is a fermented traditional product from Jinhua, China, a pork product coated with salt and stored for two months. Nham is a fermented pork product from Thailand, where the meat contains added rice, garlic, 2% salt (w/v), pepper, chilli, nitrate and is fermented for 3–4 days. Sai-krok-prieo and Mum are fermented sausages originating from Thailand that contain sugar, pepper, chilli, salt and are fermented for 2–3 days. Nem Chua is a processed fermented meat product from Vietnam where lean meat and skin are mixed with additives and chilies and fermented for 3–4 days. Urutan, a fermented sausages product from Bali, Indonesia contains lean meat mixed with chili, sugar, and salt. *Olatunde et al.* (2023), various fermented meat products are popular, namely Arjia from India, Yak Kargyong from Himalayan regions, Chartaysha, Kargyong, and Suka Ko Masu from India, Nem Chua from Vietnam, Sai-Krok-Prieo from Thailand and Tocin from the Philippines. According to *Bamidele et al.* (2023), many fermented meat products originate from Africa, namely Soudjouk/Sucuk, Boubnita, Pastirma, Afo-nnama, Beirta, Miriss, Dodery, Gueddid, and Khilii/Khlia.

Relatively few studies have been conducted on the characteristics of sui wu'u. *Naju et al.* (2022) showed that the use of different corn flours does not influence the organoleptic characteristics of the sui wu'u produced, and 0.5 kg of flour is optimal to preserve 250 g of landrace pork stored for one month using betung bamboo. *Febrianti et al.* (2021) found that using 0.5 kg of corn flour in 250 g of meat and 6% (w/w) salt, with storage for 1 month, did not affect the pH value, water holding capacity, cooking loss, or total lactic acid bacteria. Research into the physicochemical, microbiological and organoleptic characteristics after six months of storage by (*Rubak, Lalel, & Sanam*, 2023), using the ratio of meat (60%): corn flour (30%): salt (5%), showed the sui wu'u contained essential nutrients for the human body and was safe for consumption. According to *Di Gioia* (2015), the quality and manufacturing consistency of fermented processed products is greatly influenced by many factors, namely the raw materials used, which must be fresh and have low contamination, the consistent presence of fermentative microorganisms, application of strict sanitation, and proper control of time, temperature and humidity during fermentation. Furthermore *Bamidele et al.* (2023) stated that some fermented meat products can be classified as shelf-stable meat. Several variables that contribute to the stability of microbial populations in fermented meat products are low pH, high growth rate of lactic acid bacteria, low water activity, long drying time, and the added salt and seasonings. The previous research results were the basis for initial data on the formula used to make sui wu'u in the current study. This study aimed to analyze the characteristics of beef sui wu'u stored in different types of bamboo (tuku) in terms of physical, chemical, colour, organoleptic, and microbiological characteristics.

2. Materials and Methods

2.1 Sui wu'u preparation

Traditional sui wu'u preparation was previously described in detail (*Febrianti et al.*, 2021; *Naju et al.*, 2022).^{Briefly}, 250 g of beef was cut into dimensions of 5 cm × 6 cm × 0.5 cm. Dry corn crumble (500 g, i.e., 200% of the weight of beef) were coarsely ground into corn crumble. The cut beef was coated with 6% (w/w) salt, left to rest for 5 minutes, then completely coated with corn crumble (w/w). Corn crumble was layered into the bamboo (tuku). The first, followed by a layer of coated meat; thereafter, alternate layers of corn crumble and coated

beef were added, with corn crumble as the final layer. After that, covered with bamboo and stored for 30 days at room temperature (27–29 °C).

2.2 Research Design

The study was a completely randomized design with four treatments, each with five replications. Treatments were the four different types of bamboo, P1: brown betung bamboo (*Dendrocalamus asper*), P2: green betung bamboo (*Dendrocalamus asper*), P3: talang bamboo (*Schizotachyum brachcladum*), P4: gombong bamboo (*Gigantocloa verticallata*).

2.3 Physical Characteristics

Water holding capacity (WHC) was measured using the Hamm method (Soeparno, 1994); 0.3 g of sui wu'u was placed on Whatman 42 filter paper and then pressed using two glass plates (modified press equipment) with a weight of 35 kg for 5 minutes. The area of the flattened meat was drawn on the filter paper and then entered into the formula (equations 1, 2, 3). Cooking loss (CL) (Kong et al., 2023; Lu et al., 2022; Zhang et al., 2023) was measured by weighing 5 g of sui wu'u into a polythene plastic bag and heating in a water bath at 80 °C. Then, the cooked meat was cooled to 25 °C and held for 15 minutes before being dried and weighed and entered into the formula (equation 4) to yield the cooking loss. The pH was measured using a previously published method (Hiemori-Kondo et al., 2022). Sui wu'u was homogenized with twice its weight of distilled water, and the pH of the homogenized solution was measured using a pH meter (Ezdo PH5011, China). Hardness was measured (Zhang et al., 2023) using a texture analyzer (Brookfield CT3

4500, USA). Colours *L, *a, *b were measured using a colour meter (Color Meter TES 135A, Republic of China, Taiwan) with standards *L (brightness), *a (redness), and *b (yellowness).

$$\text{milimigrans H}_2\text{O} = \frac{\text{we area (cm}^2\text{)} - 8.0}{0.0948} = x \quad \text{Eq 1}$$

$$\text{wet area level} = \frac{X}{\text{sample weight (g)}} = x100\% \quad \text{Eq 2}$$

$$\text{wet holding capacity} = \frac{\% \text{ water content} - \% \text{ wet area level}}{\text{}} \quad \text{Eq 3}$$

$$\text{Cooking Loss (\%)} = \frac{W - W_o}{W} = x100\% \quad \text{Eq 4}$$

Information, w = weight of meat,
Wo = wight of meat after cookint

2.4 Chemical Characteristics

Contents of water, protein, fat, and carbohydrates in the sui wu'u were measured according to commonly used methods (AOAC, 2005).

2.5 Organoleptic Characteristics

Organoleptic quality of the sui wu'u was measured using 25 semi-trained panellists. Organoleptic testing used a hedonic quality test on a scale of 1–5 to look at colour, aroma, taste, texture and level of liking. The hedonic quality scale is shown in Table 1.

2.6 Microbiological Characteristics

Total plate count (TPC) and counts of *Escherichia coli*, and *Staphylococcus aureus* were conducted by standard methods (National Standardization Agency of Indonesia, 2008).

Table 1. Sui wu'u organoleptic quality hedonic scale

Parameter	Hedonic quality score				
	1	2	3	4	5
Colour	Pale red	Red	Slightly reddish brown	Quite brownish red	Brownish red
Aroma	Aroma off	Does not have the typical sui w'u aroma	Has a slight typical sui wu'u aroma	Has a quite typical sui wu'u aroma	Has a typical sui wu'u aroma
Taste	Tasty off	Not sour	Slightly sour	Quite sour	Sour
Texture	Not solid	Slightly not solid	Slightly solid	Quite solid	Solid
Acceptance	Do not like	Slightly do not like	Slightly like	Quite like	Like

2.7 Data analysis

Data on physical characteristics, i.e., yield, pH of meat, final pH, percentage of lactic acid, water holding capacity, cooking loss, colour characteristics of sui wu'u (L^* , a^* , b^* , Cab, hab values, Y, X, Z, x, y, Wi, Yi, Tw), as well as chemical characteristics (water, protein, fat and carbohydrate) were analyzed by one-way ANOVA, and if an effect was measured, Duncan's test was used. Organoleptic characteristics (colour, aroma, taste, texture and level of acceptance) were analyzed using Kruskal-Wallis, and if they were influential, with Mann-Whitney analysis. Microbiological characteristics (TPC, *E coli*, and *S. aureus* counts) were analyzed descriptively. Data were tabulated using Microsoft Excel and analysed using SPSS version 29 software. Data were presented as mean SD ($P < 0.05$).

3. Results and Discussion

3.1 Physical Characteristics

The measured physical characteristics of beef sui wu'u were yield, pH of fresh beef, final pH of the sui wu'u product, percentage of lactic acid, water holding capacity, cooking loss, and hardness. Different types of bamboo (tuku) influenced the physical characteristics of the beef sui wu'u produced after being stored for 30 days. The physical characteristics of beef sui wu'u produced using the different types of bamboo are shown in Table 2.

The sui wu'u yield value was in the range of 49.34 ± 9.19 (talang bamboo) to $65.49 \pm 5.04\%$ (green betung bamboo). The initial pH of the beef used was in the range 5.58 ± 0.086 to 5.61 ± 0.145 , which was

within the normal range. After storage for 30 days, the final pH of beef sui wu'u was in the range of 5.32 ± 0.346 (green betung bamboo) to 6.95 ± 0.934 (talang bamboo). The percentage of lactic acid in the sui wu'u was in the range $0.378 \pm 0.293\%$ (talang bamboo) to $0.678 \pm 0.146\%$ (green betung bamboo). The water holding capacity of sui wu'u was in the range $18.01 \pm 2.08\%$ (talang bamboo) to $28.79 \pm 1.79\%$ (brown betung bamboo). The cooking loss was in the range $23.31 \pm 5.98\%$ to $25.94 \pm 7.48\%$. The hardness was in the range of 119.30 ± 39.06 g (green betung bamboo) to 368.40 ± 262.77 g (talang bamboo) (Table 2). The statistical analysis showed that the physical characteristics of sui wu'u were influenced by the type of bamboo used. Namely, using different types of bamboo had significant effects ($P < 0.05$) on yield, pH, water holding capacity, and hardness, but no significant effects ($P > 0.05$) on lactic acid content or cooking loss.

Green betung bamboo produced a higher yield of sui wu'u compared to the use of other types of bamboo. This is likely because green betung bamboo has the characteristic texture of young bamboo and has a high-water content. The use of talang bamboo produced the lowest yield compared to the use of other types of bamboo. This is perhaps because talang bamboo is thinner than the other bamboos, so the better air circulation caused the sui wu'u in talang bamboo to be drier than that in the other bamboo types. Also, the water content reduces during storage, which logically is affected by the thin talang bamboo allowing more water reduction than the other bamboos used. The yield of sui wu'u produced was significantly affected ($P < 0.05$) by the characteristics of the bamboo used, as bamboo contains water, cellulose,

Table 2. Physical characteristics of beef sui wu'u prepared using different types of bamboo

Parameter	P1	P2	P3	P4	P-Value
Yield (%)	55.97 ± 3.79^a	65.50 ± 5.04^b	49.34 ± 9.19^a	56.67 ± 4.29^a	0.005
pH fresh beef	5.58 ± 0.086	5.61 ± 0.179	5.58 ± 0.126	5.61 ± 0.145	0.964
pH sui wu'u	5.46 ± 0.377^a	5.32 ± 0.346^a	6.95 ± 0.934^b	5.67 ± 0.467^a	0.002
Lactic acid percentage(%)	0.414 ± 0.117	0.678 ± 0.146	0.378 ± 0.293	0.564 ± 0.081	0.060
Water Holding Capacity (%)	28.79 ± 1.79^b	27.73 ± 2.25^b	18.01 ± 2.08^a	27.98 ± 0.678^b	0.000
Cooking Loss (%)	25.05 ± 6.10	25.94 ± 7.48	25.25 ± 5.92	23.31 ± 5.98	0.927
Hardness (g)	147.60 ± 28.82^a	119.30 ± 39.06^a	368.40 ± 262.77^b	229.80 ± 70.52^{ab}	0.049

P1: brown betung bamboo (*Dendrocalamus asper*), P2: green betung bamboo (*Dendrocalamus asper*) P3: talang bamboo (*Schizostachyum brachycladum*), P4: gombong bamboo (*Gigantocloa verticillata*).

^{a,b} Different superscript letters in a row indicate values are significantly different ($P < 0.05$)

lignin and hemicellulose. Previous research (Han et al., 2023) reported that the characteristics of structural bamboo segments, including their hierarchy, the vascular pattern bundle distribution, and fibre morphology have significant differences from multi-pore vascular bundles and irregular fibre morphology in the case of moso bamboo. Besides that, every type of bamboo has a specific structure, pore, porosity, chemical components and contents. According to Subekti et al. (2015), the chemical composition of bamboo is different for each species, so in betung bamboo, the chemical composition is 51.20% cellulose, 24.51% lignin and 0.32% nitrogen. Zhang et al. (2018); Zhang et al. (2019), bamboo is hygroscopic, which allows the processes of absorption and desorption of water from the environment when the relative humidity changes, thus effecting changes in bamboo's water content; the cellulose, hemicellulose, and lignin play roles in this.

The pH range of the meat used to produce sui wu'u was normal (Puolanne et al., 2001), as the normal post-rigor pH of beef ranges from 5.60 to 6.48. According to Wyrwisz et al. (2019), the pH of beef *longissimus lumborum* is pH 5.51 to 5.68. During 30 days of storage, the pH of the meat decreased, except for sui wu'u in talang bamboo and gombong bamboo—the pH of these products increased. This shows that the characteristics of the bamboo used influences the final pH of the sui wu'u produced. Differences could also be due to the high activity of lactic acid bacteria during the storage process in brown and green betung bamboo, causing the lower final pH of sui wu'u from these suku. According to Zhong et al. (2021), during the fermentation stage, the pH decrease from the initial pH of fresh beef to the final pH of sui wu'u provides the advantage of inhibiting the growth of several harmful bacteria and ensuring the quality of fermented meat. Furthermore Chelule et al. (2010), the decreasing pH from fermentation to below about pH 5 could be detrimental to the fermenting microorganisms, so usually the pH of fermented product remains at a pH somewhat below 5. According to Sun et al. (2016), the decrease in pH was greater in sausages inoculated with lactic acid bacteria. According to Puolanne et al. (2001), effect the combination of salt and pH is important for regulation of salt use and meat pH to reach a high enough level of water holding capacity. However, the pH of sui wu'u produced using containers made from talang bamboo and gombong bamboo had a higher pH compared to the initial pH of the fresh meat. According to Feiner (2006), pH

influences colour, shelf life, taste, microbiological stability, yield and texture of meat and meat products. The pH value of meat and meat product (raw fermented Salami) is around 4.6 and 6.4. Meat of pH >6.4 becomes damaged due to enzyme activity that produces large amounts of by-products from metabolism, unpleasant odours, and discolouration.

The lactic acid content was inversely correlated with the final pH of sui wu'u, where the higher the final pH, the lower the lactic acid content, and vice versa. According to Wang et al. (2022), fermentation reduces the pH drastically and the lactic acid content is negatively correlated, so the lactic acid content increases in line with decreasing pH. Sui wu'u is stored in talang bamboo had a lower percentage of lactic acid compared to other types of product. This is influenced by the lactic acid bacteria conversion of glycogen into lactic acid, as was indicated by the carbohydrate content of sui wu'u fermented in talang bamboo being numerically higher than in sui wu'u from other bamboo containers (Table 4). The low percentage of lactic acid could be influenced by the physical characteristics of talang bamboo, i.e., it is a thin bamboo, so outside air can likely more easily enter the internal bamboo spaces, thus giving the sui wu'u its characteristics.

The characteristics of the bamboo used influenced the sui wu'u water holding capacity, sui wu'u stored in talang bamboo had lower water holding capacity than product from the other types of bamboo. An increase in meat pH will increase the impression of juiciness and the water holding capacity and will decrease the cooking loss in sheep muscles linearly (Soeparno, 1994). Apple & Yancey (2013) reported positive and negative charges of muscle myofibril are in equal proportions at its isoelectric point, so the charged parts are attracted to each other, thereby reducing the amount of water that can be attracted to the myofibril. If the pH is below the isoelectric point, it causes an excess of positive charge, but if the pH is above the isoelectric point, there is an excess of negative charge, resulting in a greater attraction to water. According to Puolanne et al. (2001), salt is also involved in the ability to hold water, so water holding capacity is affected by the pH of the raw material. However, if meat is combined with NaCl, this combination is expected to keep the high pH so the water holding capacity remains high. However, this was in contrast to our results, where the high pH (pH=6.948) in sui wu'u stored in talang bamboo had a lower water holding capacity that other products with lower pH but higher water holding capacity. Other research Puolanne & Peltonen (2013) studied the combination of pH and salt content in dry sausages

during fermentation and drying, where at the beginning of fermentation, the ability to bind water was close to optimal. Then, simultaneously with a decrease in pH and an increase in ionic content, water holding capacity can end up above the optimal point so that it is possible to bind the particles cohesively, and this can have an impact on the resulting texture. The gel formed by the coagulation of dissolved proteins will stabilize the structural cohesiveness and low water-holding capacity during the fermentation process.

The use of different bamboos did not affect the cooking loss. Cooking loss is influenced by pH and water-holding capacity. According to *Torley et al.* (2000) cooking loss is influenced by a significant interaction between cooking temperature, and ionic strength, pH, and triphosphosphate. The high hardness value of sui wu'u was influenced by the characteristics of the bamboo used. Sui wu'u stored in talang bamboo had different characteristics compared to product from betung bamboo. Furthermore, the hardness of sui wu'u was also closely related to the water content. The sui wu'u produced using talang bamboo containers was drier compared to sui wu'u from other types of bamboo. Similar effects were also obtained by (*Wang et al.*, 2023), in that shear force was related to hardness and was influenced by the loss of water content as a result of the length of drying. Accordingly *Hu et al.* (2022);

Van Wezemaal et al. (2014), there is a relationship between water content and the level of hardness. Furthermore *Hu et al.* (2021) reported that hardness is influenced by water content and water activity. Apart from that, the level of hardness is influenced by the decrease in water content and pH of dry fermented sausages (*Bozkurt & Bayram*, 2006).

3.2 Colour Dimension Characteristics

The use of different bamboo types did not influence the colour characteristics of beef sui wu'u (Table 3). Treatment P3 (sui wu'u produced in Talang bamboo), with respect to L*, tended to be darker and with respect to a* tended to be redder than the other bamboo types, but differences were statistically insignificant ($P>0.05$). Physically, the results show that beef sui wu'u had a pale red colour unless using a talang bamboo container.

Colour characteristics of beef sui wu'u can be measured using the CIELAB method (*Warris*, 2000), this method develops through changing colours into imaginary primaries X, Y, and Z. The values X, Y, and Z are tristimulus that describe colours as points in space. The tristimulus value is used to calculate three coordinates L*, a*, and b*. L* is the component or value of lightness, while a* indicates red-greenish colour and b* indicates yellow-bluish colour. The

Table 3. Colour characteristics of beef sui wu'u prepared using different types of bamboo

Parameter	P1	P2	P3	P4	p-Value
L*	24.02±12.73	24.06±9.74	21.342±5.729	24.003±19.066	0.982
a*	19.59±16.18	29.76±43.47	33.755±28.945	24.512±42.805	0.923
b*	-37.56±30.36	-31.631±37.022	-36.786±35.995	-39.326±42.161	0.989
Cab*	62.80±41.25	53.388±49.488	59.710±30.280	66.454±37.665	0.962
hab	269.06±73.58	244.646±123.248	247.150±115.150	242.212±99.041	0.975
Y	7.22±9.15	4.834±2.499	3.635±1.397	8.841±14.244	0.774
x	0.254±0.107	0.304±0.090	0.283±0.108	0.251±0.130	0.847
y	0.186±0.092	0.224±0.121	0.190±0.134	0.190±0.159	0.963
X	8.13±8.33	6.716±1.601	5.769±1.196	10.172±12.154	0.803
Z	14.58±8.10	12.726±8.703	12.920±8.534	18.841±16.002	0.794
Wi	372.40±224.36	265.444±261.140	339.440±301.112	408.026±277.570	0.855
Yi	-272.064±224.18	61.122±197.306	-220.796±233.416	-188.060±190.574	0.102
Tw	-20.043±69.62	-46.418±63.925	-16.786±80.381	-14.446±79.160	0.893

P1: brown betung bamboo (*Dendrocalamus asper*), P2: green betung bamboo (*Dendrocalamus asper*) P3: talang bamboo (*Schizotachyum brachcladum*), P4: gombong bamboo (*Gigantocloa verticallata*).

L*, a*, b* values influence Wi, Yi, and Tw assessments. The low L* in sui wu'u was likely influenced by a browning reaction which is characterized by the formation of a dark colour. According to *Bozkurt & Bayram* (2006) the dark colour in fermented meat is formed as a result of the formation of nitrosomyoglobin, which plays a role the desired darker colour formation and is influenced by a decrease in water content. This indicates that fermented meat products will be a darker colour than the meat used to produce them. The positive a* value indicated similar red colours of the different sui wu'u products, although talang bamboo tended to produce a redder sui wu'u colour than the others (Figure 1). This was likely a result of salt reactions during fermentation. According to *Chelule et al.* (2010), stable haemoglobin S-nitroso, which is marked by a bright red colour, forms as a result of the reaction of nitrite with myoglobin during roasting, fermentation or cooking processes. Furthermore, according to (*Hu et al.*, 2021, 2022), the red colour is formed as a result of the formation of nitrosomyoglobin occurring due to the decrease in pH. The b* values measured reflected that some blueness was noticeable in our sui wu'u. This is different from other research (*Hu et al.*, 2022), where fermented sausage reflects a yellow colour. This is caused by a decrease in pH. The formation of yellow pigment is a reaction between lipid oxidation products and amines in the phospholipid group or amines in proteins.

3.3 Chemical Characteristics

The chemical characteristics of beef sui wu'u using different bamboo types are shown in Table 3. The statistical analysis showed that the use of different types of bamboo produced significant effects ($P<0.05$) on the water and protein content of the sui wu'u. However, bamboo type did not significantly affect ($P>0.05$) the carbohydrate or fat content of the sui wu'u. During fermentation, the chemical content is indicated to be changed by lactic acid bacteria, which can be naturally found in the bamboo used. Research conducted by (*Rubak, Lalel, Sanam, et al.*, 2023), discovered the lactic acid bacteria, *Lactococcus lactis* isolated from sui wu'u.

The water content of beef sui wu'u was in the range $41.47\pm8.14\%$ (talang bamboo) to $58.81\pm2.90\%$ (green betung bamboo). The protein content of beef sui wu'u was in the range of $26.99\pm2.50\%$ to $37.72\pm1.50\%$, seen in product from green betung bamboo and talang bamboo, respectively. The carbohydrate content of beef sui wu'u was in the range of $6.56 \pm 1.57\%$ to $11.65 \pm 7.04\%$, seen in product from gombong bamboo, and talang bamboo, respectively. The fat content of the sui wu'u was in the range of $1.13\pm 0.31\%$ (brown betung bamboo) to $1.99 \pm 0.812\%$ (green betung bamboo) (Table 4).

The characteristics of the bamboo used as a container in making sui wu'u influences the chemical content of the product, where the use of talang bamboo

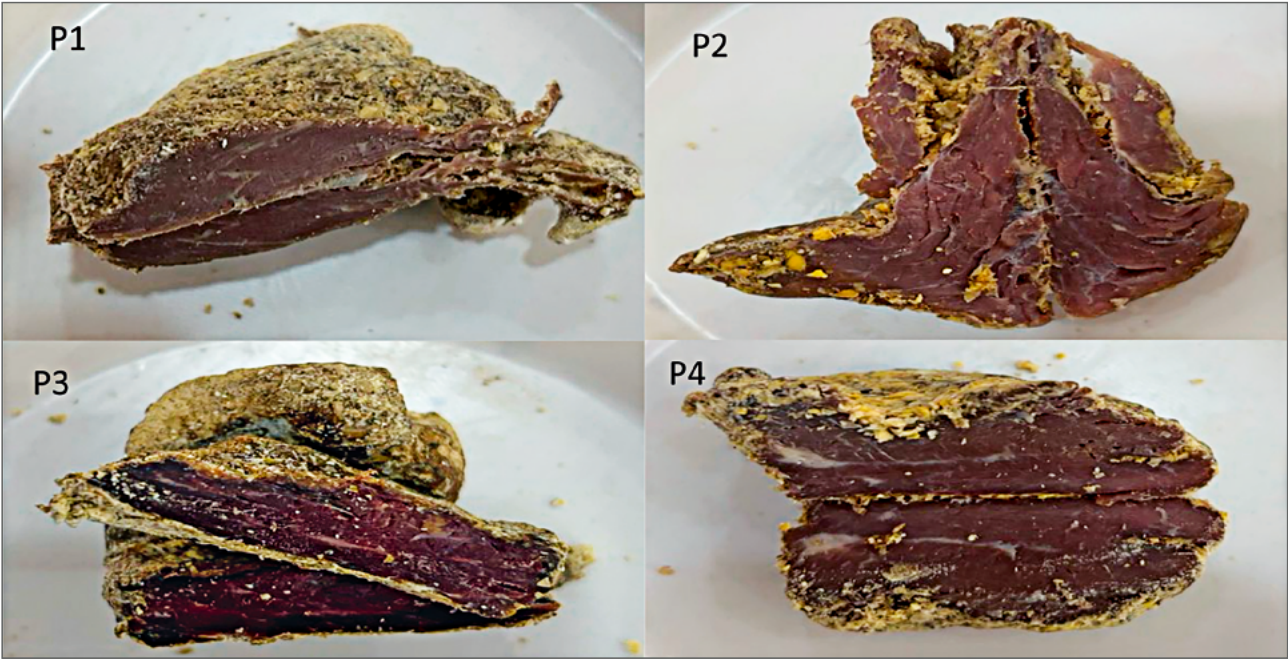


Figure 1. Beef sui wu'u prepared using different types of bamboo. P1: brown betung bamboo (*Dendrocalamus asper*), P2: green betung bamboo (*Dendrocalamus asper*) P3: talang bamboo (*Schizoztachyum brachcladum*), P4: gombong bamboo (*Gigantocloa verticallata*).

Table 4. Chemical characteristics of beef sui wu'u prepared using different types of bamboo

Parameter	P1	P2	P3	P4	P-Value
Water (%)	54.04±4.09 ^b	58.81±2.90 ^b	41.47±8.14 ^a	54.54±2.49 ^b	<0.001
Protein (%)	30.70±3.82 ^b	26.99±2.50 ^a	37.72±1.50 ^c	32.66±1.95 ^b	<0.001
Carbohydrate (%)	9.63±1.86	8.65±1.86	11.65±7.04	6.56±1.57	0.247
Fat (%)	1.13±0.314	1.99±0.81	1.84±0.594	1.57±0.498	0.143

P1: brown betung bamboo (*Dendrocalamus asper*), P2: green betung bamboo (*Dendrocalamus asper*) P3: talang bamboo (*Schizotachyum brachcladum*), P4: gombong bamboo (*Gigantocloa verticallata*).

^{a,b,c} Different superscript letters in a row indicate values are significantly different ($P < 0.05$)

results in lower water content and greater protein content. According to Subekti *et al.* (2015), each type of bamboo has different chemical components. Furthermore, according to (Han *et al.*, 2023), bamboo species have different morphologies and structures. The water and protein content of the sui wu'u produced in the current study was higher, but the fat content was lower compared to research by (Rubak, Lalel, & Sanam, 2023), where the water, protein and fat content of pork sui wu'u stored in betung bamboo for 6 months was 6.11±0.09, 24.29±0.21 and 59.68±0.07, respectively. This difference is influenced by the type of meat used and storage time. The decrease in water content is also influenced by the activity of microorganisms. According to Zhong *et al.* (2021), during fermentation there will be a decrease in water content, which can be beneficial for inhibiting pathogen microorganisms. The use of water by microorganisms and the addition of salt, the precipitation of water was facilitated, resulting in a decrease in water content during fermentation.

3.4 Organoleptic Characteristics

The change in chemical components in beef due to fermentation will affect the organoleptic characteristics of beef sui wu'u. The scores for the organoleptic characteristics of beef sui wu'u using different types

of bamboo are shown in Table 5. The statistical analysis showed that the use of different types of bamboo had significant effects ($P < 0.05$) on the colour, aroma, texture and overall acceptance of beef sui wu'u, but showed an insignificant effect ($P > 0.05$) on the taste of beef sui wu'u.

Sui wu'u colour scores ranged from 3.51±1.64 to 4.01±1.32, while aroma scores ranged from 2.50±1.26 to 3.31±1.30. The sui wu'u texture scores ranges from 2.57±1.34–4.03±1.11. The taste scores of sui wu'u ranged from 3.64±1.07 to 3.81±1.06, and overall acceptance scores of sui wu'u were from 3.23±0.899 to 3.60±0.916 (Table 5).

Sui wu'u stored in talang bamboo tended to have a lower aroma score compared to the products from the other bamboos, indicating the aroma produced differs in the talang bamboo product. The difference in the aroma of sui wu'u using talang bamboo (compared with the aroma of the other products) could have been caused by the higher pH as a result of the activity of by-products produced during fermentation. According to Feiner (2006), if the pH is around 6.4, meat can be damaged due to enzyme activity, producing large amounts of by-products from metabolism, unpleasant odours and discolouration. The beef sui wu'u produced in all the bamboo containers had quite a sour taste. This indicates that during storage in bamboo,

Table 5. Organoleptic characteristics of beef sui wu'u prepared using different types of bamboo

Parameter	–	P2	P3	P4	P-Value
Colour	3.51±1.64 ^a	3.64±1.30	3.63±1.42 ^a	4.01±1.32 ^b	0.035
Aroma	2.59±1.38 ^{ab}	3.31±1.30 ^c	2.50±1.26 ^a	2.86±1.44 ^b	0.000
Taste	3.81±1.06	3.71±1.01	3.64±1.07	3.65±1.02	0.487
Texture	2.57±1.36 ^a	3.06±1.44 ^b	4.03±1.11 ^c	3.99±1.18 ^c	0.000
Acceptance	3.60±0.916 ^b	3.35±0.927 ^a	3.23±0.899 ^a	3.38±0.905 ^{ab}	0.036

P1: brown betung bamboo (*Dendrocalamus asper*), P2: green betung bamboo (*Dendrocalamus asper*) P3: talang bamboo (*Schizotachyum brachcladum*), P4: gombong bamboo (*Gigantocloa verticallata*).

^{a,b,c} Different superscript letters in a row indicate values are significantly different ($P < 0.05$)

the expected fermentation processes occurred, characterised by a sour taste. The sui wu'u texture scoring showed that products made from talang and gombong bamboo containers were different from those produced using betung bamboo. This is closely related to the water content of sui wu'u, as the sui wu'u content of beef using containers made of talang bamboo had a lower water content compared to other types of bamboo (Table 4), and besides that, could also be influenced by the characteristics of the bamboo type. The overall acceptance assessed by the panellists showed they had a specific level of acceptance for each bamboo container used, and each sui wu'u produced from different types of bamboo had its own characteristics that were liked by the panellists. The degradation of protein and fat components by natural microorganisms found in bamboo and beef during the fermentation process, likely influences the characteristics of the aroma and taste of fermented sui wu'u. According to Zhong et al. (2021), microorganisms degrade both protein and fat during long fermentation processes to produce small molecules of peptides, amino acids fatty acids, etc., and the appropriate degradation process helps form a fermented meat taste.

The use of different types of bamboo influences the characteristics of beef sui wu'u in terms of colour, aroma, texture, and level of acceptance. The use of containers made from brown betung bamboo provided better organoleptic characteristics of beef sui wu'u compared with the other bamboo types studied.

3.5 Microbiological Characteristics

The microbiological characteristics of the beef sui wu'u produced using different types of bamboo are shown in Table 6. These results indicate that the sui wu'u produced was within the standard limits for *E. coli* and *S. aureus* contamination. The TPC of beef sui wu'u produced was in the range of

1.10x10⁹ CFU/g to 2.80x10⁹ CFU/g. Sui wu'u from gombong bamboo showed a higher TPC compared to the other treatments. Based on SNI 7388:2009 standards (National Standardization Agency of Indonesia, 2009), the TPC of processed whole or cut meat product should be below 1x10⁵ CFU/g. The higher TPC in our sui wu'u was likely not caused by the presence of pathogenic bacteria, but could be due to the presence of lactic acid bacteria which played a role in the fermentation process. The TPCs from our current research were higher than in research conducted by (Rubak, Lalel, & Sanam, 2023), i.e., the TPC of pork sui wu'u stored in betung bamboo for approx. 6 months was 4.4 × 10⁵ CFU/g. (Rubak, Lalel, Sanam, et al., 2023) isolated *Lactococcus lactis* from sui wu'u, and reported that the total lactic acid bacteria count in pork sui wu'u is around 3.7 × 10³ CFU/g.

According to (BPOM: Food and Drug Supervisory Agency) (Food and Drug Supervisory Agency, 2019), the maximum number of *E. coli* in meat and fermented processed meat products without heat treatment is around 10³ CFU/g. *E. coli* was not detected in the beef sui wu'u in our study, regardless of the type of bamboo used. *S. aureus* contamination in the sui wu'u was 1.00x10³ CFU/g for all types of bamboo used, which did not exceed the permitted level in SNI 7388:2009 standard (National Standardization Agency of Indonesia, 2009). According to (BPOM: Food and Drug Supervisory Agency) (Food and Drug Supervisory Agency, 2019), a maximum of 10⁴ CFU/g of *S. aureus* is permitted in meat, processed meat products and fermented meat without heat treatment.

Most fermented products can experience contamination by spoilage bacteria, expressed during storage. Several types of pathogenic bacteria are commonly found in fresh meat, such as *Campylobacter*, *E. coli* serotype O157:H7, and *Salmonella*, while *L. monocytogenes* and antibiotic-resistant bacteria, such as *Campylobacter* and *Salmonella* (Dixon

Table 6. Microbiological characteristics of beef sui wu'u prepared using different types of bamboo

Parameter	BPOM				
	PP BPOM No. 13/2019 Fermented processed meat product	P1	P2	P3	P4
TPC (CFU/g)	-	1.20×10 ⁹	1.50×10 ⁹	1.10×10 ⁹	2.80×10 ⁹
<i>Escherichia coli</i> (CFU/g)	10 ³ CFU/g	-	-	-	-
<i>Staphylococcus aureus</i> (CFU/g)	10 ⁴ CFU/g	1.00×10 ³	1.00×10 ³	1.00×10 ³	1.00×10 ³

P1: brown betung bamboo (*Dendrocalamus asper*), P2: green betung bamboo (*Dendrocalamus asper*) P3: talang bamboo (*Schizotachyum brachcladum*), P4: gombong bamboo (*Gigantocloa verticallata*). - : not found

et al., 2023) can also occur. According to *Ramos et al.* (2023), *S. aureus* and its toxins cannot be avoided with certainty in alkaline foods because its spores can survive. However, according to *Dong et al.* (2024): a decrease in pH not only affects organoleptic properties but can play a role in suppressing pathogens and spoilage microorganisms during fermentation and the final safety of the product. The sui wu'u produced, in terms of microbiological characteristics, was not influenced by the type of bamboo used, as can be seen from the microbiological profile.

Overall, beef sui wu'u produced in all four types of bamboo had acceptable levels of *E. coli* and *S. aureus* that were within the maximum permitted levels. Therefore, beef sui wu'u produced using the different bamboo types and stored for 30 days would be safe to consume with regard to these pathogens.

4. Conclusion

The use of different types of bamboo container influences the characteristics of beef sui wu'u in terms of physical, chemical, colour, organoleptic and microbiological aspects. The use of containers made from brown betung bamboo (*Dendrocalamus asper*) produces better beef sui wu'u. However, the individual use of talang and gombong bamboos can also be appropriate in the process of making sui wu'u, although compared with the sui wu'u stored in brown betung bamboo, the final products will have different characteristics in terms of physical, chemical and organoleptic aspects. Microbiological characteristics met the standards and showed the products were safe for consumption with respect to *S. aureus* and *E. coli* levels.

Uticaj korišćenja različitih vrsta bambusa na karakteristike fermentisanog mesa sui wu'u iz Bajave, Istočna Nusa Tenggara

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APSTRAKT

Ovo istraživanje imalo je za cilj analizu karakteristika govedeg sui wu'u, pripremljenog čuvanjem proizvoda od mesa u različitim vrstama bambusa. Eksperimentalni dizajn obuhvatio je četiri tretmana sa po pet ponavljanja skladištenja: P1 – zeleni betung bambus (*Dendrocalamus asper*), P2 – smeđi betung bambus (*Dendrocalamus asper*), P3 – talang bambus (*Schizostachyum brachycladum*) i P4 – gombong bambus (*Gigantochloa verticillata*). Rezultati istraživanja pokazali su da je vrsta bambusa imala značajan uticaj ($P < 0,05$) na fizičke karakteristike sui wu'u, uključujući randman, konačnu pH vrednost, sposobnost vezivanja vode i tvrdoću mesa. Kada je reč o hemijskim osobinama, korišćenje različitih bambusa značajno je uticalo ($P < 0,05$) na sadržaj vode i proteina. Organoleptičke analize pokazale su da je vrsta bambusove ambalaže značajno uticala ($P < 0,05$) na boju, aromu, teksturu i ukupnu prihvatljivost proizvoda. Međutim, kada su u pitanju dimenzije boje (L^* , a^* , b^* , C_{ab} , h_{ab} , Y , X , Z , x , y , W_i , T_i i T_w), nije uočen statistički značajan uticaj ($P > 0,05$). Mikrobiološke karakteristike sui wu'u kod svih korišćenih vrsta bambusa bile su u granicama standarda za fermentisane proizvode od mesa. Upotreba različitih vrsta bambusa utiče na fizičke, hemijske, organoleptičke i mikrobiološke karakteristike sui wu'u, dok razlike u dimenzijama boje nisu bile značajne. Najbolji kvalitet sui wu'u postignut je korišćenjem smeđeg betung bambusa (*Dendrocalamus asper*), ali se i talang i gombong bambus mogu preporučiti u procesu proizvodnje, iako gotov proizvod pokazuje različite karakteristike, naročito u poređenju sa sui wu'u skladištenim u talang bambusu.

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