Scientific Papers. Series D. Animal Science. Vol. LXIX, No. 1, 2026
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECT OF KOMBUCHA-ENRICHED BRINE ON THE TENDERIZATION OF INJECTED BEEF: IMPLICATIONS FOR TEXTURE PROFILE, PHYSICOCHEMICAL PROPERTIES, AND SENSORY ACCEPTABILITY

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Abstract

This study evaluated the potential of kombucha – a fermented beverage produced through the symbiotic activity of acetic acid bacteria and yeasts – incorporated at three concentration levels (5%, 10%, and 15%) into the injection brine, as a natural source of organic acids with potential tenderizing and quality-modifying effects for smoked beef loin. Four batches were produced and assessed: one control (CB0%, with standard brine) and three formulations (KB5%, KB10%, KB15%), all injected at a rate of 10% (w/w). The products were evaluated for chemical composition, color parameters, texture profile, pH, water activity, and sensory acceptability. The addition of kombucha to the injection brine was associated with an improvement in meat tenderness, reflected by significantly lower Warner-Bratzler shear force and TPA hardness values, decreasing from 116.81 N and 57.60 N in the control sample to 71.66 N and 37.78 N in the sample containing 15% kombucha ($p < 0.05$). Sensory evaluation indicated good overall acceptability for all experimental formulations. Among the tested variants, the KB5% sample showed the highest scores for smoked aroma (7.86 ± 0.95), flavour (7.33 ± 0.66) and overall acceptability (7.58 ± 0.32), suggesting it as the most favorable formulation.

Key words*: injection brine, kombucha, meat tenderization, smoked beef, texture profile analysis.*

INTRODUCTION

The growing consumer demand for more natural and healthier meat products is posing new challenges for manufacturers in terms of the use of plant-based ingredients to improve the nutritional quality of products (Anchidin et al., 2024a; Manoliu et al., 2024). The meat industry, in particular, has been affected by this new movement owing to negative attitudes consumers associate with consuming heavily processed meat products (Inguglia et al., 2023). In this sense, nutritional enrichment with natural bioactive plant compounds including antioxidants, dietary fibers, probiotics, as well as the reduction of potentially harmful components such as salt, nitrate/nitrite, and artificial additives, are among the most commonly applied current strategies to improve the quality and safety of meat products (Ursachi et al., 2020; Flocea et al., 2024; Manoliu et al., 2025a).

At the same time, meat, and particularly beef, remains a nutritionally valuable food matrix, providing high-quality proteins with a complete amino acid profile, alongside essential lipids and micronutrients contributing to human health (Mădescu et al., 2025). Moreover, bovine resources can be further valorised through the superior utilisation of bones, which are used to obtain collagen-rich preparations such as bone broths, contributing additional nutritional and functional benefits (Anchidin et al., 2024b).

The use of fermented ingredients of natural origin, such as starter cultures (Baytukenova et al., 2025), plant extracts (Anchidin et al, 2025a; Ciobotaru et al., 2025a), vegetables (Bento de Carvalho et al., 2024; Boișteanu et al., 2023), or acid whey/vinegar (Sionek et al., 2024), as functional components in meat processing has gained considerable scientific interest, offering both technological advantages and improved consumer acceptability aligned with clean-label principles (Inguglia et al., 2023; Coșarcă et al., 2025).

The functional potential of plant-based bioingredients in food systems has been increasingly explored in recent years, with

numerous studies focusing on their incorporation into diverse food matrices. These investigations demonstrate significant antioxidant, antimicrobial, and nutritional enrichment effects, as well as improvements in sensory quality, across a wide range of products such as fish-based products (Flocea et al., 2025), beef snacks (Anchidin et al., 2025), chicken roulades (Boișteanu et al., 2025), or pork tenderloin (Anchidin et al., 2023).

Meat tenderness is considered one of the most decisive quality attributes influencing consumer acceptance of beef and processed beef products (Joo et al., 2023). The fundamental mechanism by which post-mortem aging and technological treatments improve the tenderness of meat involves the activation of endogenous proteolytic systems, particularly the calpain system, which causes the degradation of key myofibrillar and cytoskeletal proteins, disrupting the overall structural integrity of muscle cells (Inguglia et al., 2023; Joo et al., 2023). The optimal conditions for calpain activity, measured at the temperature of 25°C, have been estimated to occur around pH 7.5, with residual activity still detectable at pH 5, and tenderization in beef is known to occur at approximately pH 6.3 post-mortem, as μ-calpain is activated at low calcium concentrations (Kaur et al., 2021). Beyond the calpain system, lysosomal cathepsins – particularly cathepsins B, D, H, and L – are active under acidic conditions and contribute to tenderization during prolonged cold storage or under conditions of controlled pH reduction (Sentandreu et al., 2002; Huff-Lonergan & Lonergan, 2005).

In addition to technological and nutritional considerations, ensuring the safety of meat products remains essential, particularly in the context of processing operations that may introduce contaminants through interactions with equipment or food contact materials (Matei et al., 2024). In the context of increasingly complex food supply chains, systems such as the Rapid Alert System for Food and Feed (RASFF) highlight the persistent occurrence of contamination events and the need for continuous monitoring and risk management strategies (Postolache et al., 2020). These risks encompass a wide range of hazards, including microbiological contamination and chemical contaminants such as heavy metals (Ciobanu et al., 2020), which may accumulate in animal tissues and pose potential risks to human health, particularly due to their persistence and bioaccumulation along the food chain (Boișteanu et al., 2024), while contamination may also originate from upstream sources such as animal feed, where pollutants including mineral oil hydrocarbons have been identified (Matei et al., 2023).

Marination is a well-established meat processing technique that improves the natural rate of proteolysis by lowering the pH of the meat after slaughter, and stimulating enzymatic and proteolytic activities during muscle maturation. In some applications, the use of different acids helps break down the connective tissue, contributing to tenderness, while also exerting antimicrobial effects that extend shelf life (Rahman et al., 2023). A variety of ingredients have been incorporated into marinades over time, shifting the primary focus from preservation or pickling toward tenderization and flavor enhancement. Among these, biological marinades, including fermented dairy products such as kefir, yogurt, and buttermilk (Latoch, 2020; Rahman et al., 2023), fruit vinegars (Hafez et al., 2024), or juices (Zhang et al., 2024) – have demonstrated promising effects on the textural and sensory properties of meat. In marinated chilled chicken meat treated with papaya juice, an increase in protein solubility and a decrease in shear force was observed with the increase of papaya juice concentration (Zhang et al., 2024).

Injection marination, as opposed to surface marination, represents a particularly effective method for the uniform delivery of functional ingredients into the muscle matrix, enabling deeper penetration of bioactive compounds while improving water-holding capacity and overall technological yield (Sikes et al., 2009; Pietrasik & Shand, 2005; Kim et al., 2020). Brine injection has been reported to positively affect water retention and thereby the tenderness of meat, as salt and other dissolved compounds penetrate the protein-ion interactions within meat fibers, causing swelling of the matrix and solubilization of myofibrillar proteins (Rahman et al., 2023). The combination of injection with naturally acidic functional liquids thus provides a dual technological advantage: mechanical

distribution of the marinade throughout the muscle structure, and biochemical stimulation of proteolytic pathways (Chen et al., 2024; Ciobotaru et al., 2025b).

Kombucha is a fermented beverage produced by the fermentation of sweetened tea with a symbiotic culture of bacteria and yeasts (SCOBY), typically dominated by acetic acid bacteria of the genus *Komagataeibacter* and osmophilic yeast genera such as *Zygosaccharomyces* and *Saccharomyces* (Jayabalan et al., 2014). Through fermentation, this microbial consortium converts sucrose into a variety of metabolites, including ethanol, carbon dioxide, vitamins, polyphenolic derivatives, and organic acids such as acetic acid, gluconic acid, and glucuronic acid – bioactive compounds associated with antioxidant, antimicrobial, and anti-inflammatory effects (Tandhanskul et al., 2025). Several species of bacteria and yeasts involved in the fermentation process generate beneficial compounds, including polyphenols, organic acids, amino acids, vitamins, minerals, organic nitrogens, and hydrolytic enzymes, which confer significant antioxidant, anti-inflammatory, anticancer, and antimicrobial properties (Kitwetcharoen et al., 2023). The resulting low pH of kombucha (typically 2.5-3.5), combined with its content of organic acids and hydrolytic enzymes, positions it as a candidate for use as a natural tenderizing agent in meat processing applications, analogous to other acidic fermented substrates previously studied in this context (Rahman et al., 2023).

Despite the growing body of literature on the functional properties of kombucha as a beverage or as feed supplement (Adriani et al., 2007; Adriani et al., 2012; Ahmed et al., 2024), there are currently few studies regarding the application of kombucha in comminuted meat products (Bordea et al., 2017a; Bordea et al., 2017b), with no research regarding its use in meat marination or as a functional component of injection brine formulations.

This study aimed to investigate the effect of incorporating kombucha at different concentrations into the injection brine on the physicochemical properties, textural characteristics, color attributes, and sensory acceptability of smoked beef, with particular emphasis on tenderization as a key quality outcome.

MATERIALS AND METHODS

The study was conducted in two stages: the production of the experimental formulations and the qualitative analysis of the finished products, both carried out in the meat processing workshop and the specialized laboratories of the "Ion Ionescu de la Brad" Iași University of Life Sciences.

The beef loin and kombucha beverage were purchased from a local supplier in Iași County, kombucha having an initial pH of 4.1 Four experimental batches were produced, consisting of one control batch (CB 0%) injected with a standard brine solution, and three experimental batches (KB 5%, KB 10%, KB 15%) injected with brine solutions containing 5%, 10%, and 15% kombucha, respectively. Prior to incorporation into the brine, the kombucha was homogenized and used as a direct liquid replacement for a portion of the water in the brine solution, while the salt concentration in the brine was constant at 10% across all experimental batches. All batches were injected at a rate of 10% (w/w) relative to the meat weight, using an automatic injector (Inwestpol A-10), following the protocol described by Ciobotaru et al. (2025a). The brine uptake corresponding to the target injection level of 10% was verified gravimetrically by weighing the meat before and after injection. The brine composition for each formulation is presented in Table 1.

After production, the finished products were cooled and stored at refrigeration temperature (0-3°C) until the subsequent analysis stages.

All analyses were conducted in the Meat and Meat Products Technology Laboratory and the Sensory Analysis Laboratory of "Ion Ionescu de la Brad" Iași University of Life Sciences.

The chemical composition was determined using standard analytical methods: moisture by drying at 105°C to constant weight (ISO 1442:2023), ash by calcination at 550°C (ISO 936:1998), total fat by Soxhlet extraction (ISO 1443:1973) and total protein by the Kjeldahl method, with a conversion factor of 6.25 (ISO 937:2023).

The instrumental color parameters (L*, a*, b*) were assessed in the CIELAB color space on both the external surface and the cross-section of each sample, using the Chroma Meter CR-410 (Konica Minolta Inc., Japan). The instrument was calibrated using a standard white calibration plate under a D65 illuminant and a 2° standard observer. Based on the measured values for CIELAB color coordinates (L* – lightness, a* – green-red, b* – blue-yellow), the derived color parameters, hue angle (h*), Chroma (C*) and color difference (ΔE) were calculated according to the formulas (1), (2), and (3) described by Ciobanu et al. (2024).

Table 1. Formulations and heat treatment of experimental batches for beef loin injected with different concentrations of kombucha

Experimental batch	Injection level	Brine composition			Heat treatment		
		Water (%)	Kombucha (%)	Salt (%)	Air drying (°C/min)	Smoking (°C/min)	Hot air cooking (°C/min)
CB 0%	10%	90	0	10	65/30	72/30	86/50
KB 5%	10%	85	5		65/30	72/30	86/50
KB 10%	10%	80	10		65/30	72/30	86/50
KB 15%	10%	75	15		65/30	72/30	86/50

CB 0% – Control beef loin batch; KB 5%, KB 10%, KB 15% – Beef loin injected with brine containing 5%, 10% and 15% kombucha.

The pH value of the products was determined using a direct insertion pH meter (HI98163, Hanna Instruments Romania). The water activity (a_w) of the samples was determined using the RH2 humimeter instrument (Schaller Messtechnik, Austria).

$$h^* = tan^{-1} \times \frac{b^*}{a^*} \quad (1)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

$$\Delta E = \sqrt{(L_0 - L_1)^2 + (a_0^* - a_1^*)^2 + (b_0^* - b_1^*)^2} \quad (3)$$

The textural parameters were determined using the TA1Plus texture analyzer (Lloyd Instruments, AMETEK, UK), equipped with a 500 N load cell. Two types of measurements were performed, as described by Manoliu et al. (2025b), with minor modifications regarding sample preparation: the Warner-Bratzler shear test, in which samples were prepared as strips (18 × 10 mm) aligned parallel to the muscle fibers, and shear force (N) and shear work (mJ) were recorded; and texture profile analysis (TPA), in which cylindrical samples of approximately 15 mm in height were subjected to double compression, and the following parameters were determined: hardness (N), cohesiveness, elasticity and gumminess (N).

The sensory analysis, an essential tool for ensuring that the products meet the required quality standards, helping to identify any unfavorable sensory characteristics (Ciobanu et al., 2023), was conducted under controlled temperature and lighting conditions in the Sensory Analysis Laboratory of University of Life Sciences, following procedures previously applied for similar meat products evaluated in the laboratory (Manoliu et al., 2023). A panel of 21 semi-trained evaluators, familiar with meat products, participated in a hedonic session, evaluating the following sensory attributes: appearance, section color, odour (smoked aroma), flavour, tenderness, juiciness, overall texture and overall acceptability. Each attribute was rated on a nine-point hedonic scale (1 – dislike extremely; 9 – like extremely). Samples were presented in uniform portions, coded with randomly assigned three-digit codes, and served in randomized order at room temperature.

All results for physicochemical evaluations were expressed as mean values (± standard deviation) from five analytical determinations performed on subsamples obtained from each experimental batch. Differences between the experimental batches and the control were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test at a 5% significance level ($p < 0.05$), using IBM SPSS Statistics v. 21.

RESULTS AND DISCUSSIONS

The results for the physico-chemical analyses obtained for the beef loin injected with brine and smomed are shown in Table 2.

Moisture content increased significantly ($p < 0.0001$) with increasing kombucha concentration in the injection brine, ranging from 62.30 ± 0.69% (CB 0%) to 65.55 ± 0.27% (KB 15%), with corresponding decreases in dry matter from 37.70 ± 0.69% to 34.45 ± 0.27%. These values are consistent with the typical proximate composition of thermally processed whole-muscle beef products reported in the literature, ground beef samples analyzed showed moisture contents ranging from 59.6 to 72.9%, confirming the broad natural variability of this parameter across beef products (Isaksson et al., 1996).

The progressive increase in moisture with increasing kombucha concentration may be associated with the biochemical effects of the organic acids naturally present in kombucha beverage - primarily acetic, lactic, and gluconic acids (Jayabalan et al. 2014) - which can contribute to the local pH of the injected muscle tissue, shift the net charge of myofibrillar proteins away from their isoelectric point (~5.1), and thereby increase electrostatic repulsion between myofilaments and water-holding capacity of the protein matrix, , although this interpretation remains indicative due to the limited characterization of the kombucha used. However, a similar mechanism has been reported for acidic brine injection in beef, where pH reduction following organic acid treatment was associated with myofibrillar swelling and improved moisture retention within the muscle structure (Berge et al., 2001; Burke & Monahan, 2003).

Fat content decreased significantly ($p < 0.0001$) from 16.31 ± 1.06% (CB 0%) to 11.86 ± 0.42% (KB 15%). This decline is primarily attributable to a dilution effect: as moisture content increases through brine injection, the lipid fraction expressed as a percentage of total product mass decreases proportionally, even without actual lipolytic degradation of the lipid constituents. A similar inverse relationship between moisture and fat content has been consistently reported in the literature (Renou, 1995). One-point increase in intramuscular fat resulted in linear decreases in moisture and crude protein at an approximate rate of 0.7 and 0.3 percentage points, respectively, confirming the compositional interdependence between moisture, fat, and protein in muscle tissue (Matsumoto et al., 2024). An additional contribution to fat reduction at higher kombucha concentrations may arise from the organic acid content of kombucha (Cao & Qiu, 2024), which could promote limited lipolytic activity at the injection site; however, this mechanism requires further investigation to be confirmed in this specific matrix.

Table 2. Physico-chemical data of control and kombucha brine injected beef loin

Chemical compound	Sample				p-value
	CB 0%	KB 5%	KB 10%	KB 15%	
Moisture (%)	62.30 ± 0.69 ^{c}	62.97 ± 0.24 ^{bc}	63.20 ± 0.23 ^{b}	65.55 ± 0.27 ^{a}	<0.0001(***)
Dry Matter (%)	37.70 ± 0.69 ^{a}	37.03 ± 0.24 ^{ab}	36.80 ± 0.23 ^{b}	34.45 ± 0.27 ^{c}	<0.0001(***)
Fat (%)	16.31 ± 1.06 ^{a}	15.28 ± 0.46 ^{ab}	14.74 ± 0.33 ^{b}	11.86 ± 0.42 ^{c}	<0.0001(***)
Protein (%)	20.28 ± 0.37 ^{b}	20.53 ± 0.35 ^{b}	20.66 ± 0.21 ^{ab}	21.12 ± 0.22 ^{a}	0.003(**)
Ash (%)	1.09 ± 0.07 ^{b}	1.22 ± 0.05 ^{b}	1.40 ± 0.09 ^{a}	1.47 ± 0.09 ^{a}	<0.0001(***)
a_w	0.908 ± 0.00 ^{c}	0.935 ± 0.00 ^{ab}	0.937 ± 0.00 ^{a}	0.933 ± 0.00 ^{b}	<0.0001(***)
pH	6.32 ± 0.06 ^{a}	6.22 ± 0.02 ^{ab}	6.06 ± 0.06 ^{b}	6.34 ± 0.24 ^{a}	0.015(*)

Values are given as means ± standard deviation. Means with different superscripts in a row orientation indicate significant differences ($p < 0.05$) between samples determined by the Tukey test. Signification codes for the p-value: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns.

Crude protein content showed a moderate but statistically significant increase ($p = 0.003$) from 20.28 ± 0.37% (CB 0%) to 21.12 ± 0.22% (KB 15%), with significant differences identified between the control and the two highest kombucha concentrations. The observed protein values (20.28-21.12%) fall within the range of 18.1-20.7% reported for raw ground beef and above it for thermally processed products, reflecting the concentrating effect of the smoking and cooking process applied in the present study (Isaksson et al., 1996).

Ash content increased significantly ($p < 0.0001$) from 1.09 ± 0.07% (CB 0%) to 1.47 ± 0.09%

(KB 15%), consistent with the incorporation of mineral components from the injection brine. Bolognesi et al. (2020) reported that brine solutions containing 2.5% and 5.0% NaCl increased the mineral residue content from 1.04% (raw meat) to 3.1% at the higher salt concentration, demonstrating the direct and proportional relationship between NaCl concentration in the brine and ash content in the finished product. Moreover, different studies showed that the chemical composition of kombucha includes minerals, such as potassium, magnesium, calcium, copper, iron, zinc and manganese (Bauer-Petrovska & Petrushevska-Tozi, 2000; Bishop et al., 2022), which may contribute to the progressive increase in ash content from 1.09% to 1.47%.

In terms of pH, Neffe-Skocińska et al. (2017) state that the organic acids naturally present in kombucha beverage (acetic, lactic, and gluconic acids) are the main bioactive products of kombucha fermentation and are responsible for the characteristic acidic pH of the beverage. In the present study, pH values decreased from 6.32 ± 0.06 (CB 0%) to 6.06 ± 0.06 (KB 10%), followed by an increase at KB 15% (6.34 ± 0.24), as shown in Table 2. The initial pH decrease at intermediate concentrations may be associated with the presence of organic acids (Neffe-Skocińska et al., 2017). Moreover, Shi et al. (2021) show that changes in pH relative to the isoelectric point of myofibrillar proteins may influence their structural organization and water-holding capacity. When the pH departs from the isoelectric point of myosin (pI ≈ 5.4), the net charge of the proteins increases, which may lead to electrostatic repulsion between myofilaments and expansion of the interfibrillar space (Huff-Lonergan & Lonergan, 2005).

These structural changes may be associated with the increase in water activity (aw) observed in the kombucha-treated samples (0.908 for CB 0% vs. 0.935-0.937 for KB 5-10%), as a greater amount of water may be retained within the protein matrix.

In contrast to the trends observed at lower kombucha concentrations, the KB 15% sample showed an increase in pH accompanied by a slight decrease in water activity. This behavior indicates a non-linear response, suggesting the influence of additional factors beyond the effects typically attributed to organic acids reported in kombucha in the literature. This divergence may be associated with interactions between the brine constituents and the muscle matrix at higher incorporation levels, as well as with structural or compositional variability within the sample.

Table 3 shows the mean results registered for the colorimetric parameters (L*, a*, b*, C*, h*, and ΔE) for the injected smoked beef loin samples, measured both on the external surface and at the cross-section. These parameters are key indicators of product visual quality, serving as determinants of consumer acceptance before any tasting occurs.

In terms of surface color, lightness (L*) values on the product surface did not differ significantly between CB 0%, KB 5%, and KB 10% (27.73, 28.82, and 28.80, respectively), while KB 15% recorded a significantly higher value of 34.06 ± 0.98 ($p = 0.000$). This increase in surface lightness at the highest kombucha concentration can be attributed to the higher moisture content (65.55%) that alters the reflection of light at the surface by increasing the light reflectance at the product surface through modification of the surface microstructure during smoking and thermal treatment (Karamucki et al., 2013).

Redness (a*) showed a slight but significant decrease with increasing kombucha concentration ($p = 0.020$), from 9.45 ± 1.02 (CB 0%) to 7.57 ± 0.76 (KB 10%), with a partial recovery at KB 15% (8.93 ± 0.82). This reduction in surface redness is consistent with the dilution of myoglobin-derived pigments caused by the higher moisture content retained in the injected samples. Redness (a*) depends primarily on the forms of myoglobin present in the tissue and on the chromatic absorbance at 525 nm, and is directly influenced by the relative proportions of oxymyoglobin, metmyoglobin, and deoxymyoglobin in the muscle matrix (Karamucki et al., 2013). The introduction of organic acids from kombucha may additionally promote the formation of metmyoglobin at the product surface, further reducing the perceived redness.

The blue-yellow (b*) values on the surface showed significant differences among samples ($p = 0.008$), with KB 15% recording the highest value (9.68 ± 1.32) compared to KB 10% (5.56 ± 0.67), which was the lowest. Yellowness (b*)

increases with a decrease in the relative amount of deoxymyoglobin and an increase in oxymyoglobin and metmyoglobin, indicating that the oxidative state of myoglobin directly drives the yellow tone of the product (Karamucki et al., 2013). The elevated b* at KB 15% is consistent with the greater oxidative challenge posed by the higher concentration of organic acids introduced through kombucha, which may accelerate lipid oxidation secondary products contributing to yellow hue development.

Table 3. Colorimetric parameters of injected smoked beef (surface and section measurements)

Sample	L* (D65)	a* (D65)	b* (D65)	C*	h*	ΔE
			Surface color			
CB 0%	27.73 ± 0.60 ^{b}	9.45 ± 1.02 ^{a}	6.35 ± 1.06 ^{b}	11.39 ± 1.42 ^{ab}	33.74 ± 1.95 ^{b}	–
KB 5%	28.82 ± 3.35 ^{b}	8.77 ± 0.78 ^{ab}	7.23 ± 2.82 ^{ab}	11.48 ± 2.26 ^{ab}	38.12 ± 9.38 ^{ab}	4.55 ± 3.09 ^{ab}
KB 10%	28.80 ± 0.51 ^{b}	7.57 ± 0.76 ^{b}	5.56 ± 0.67 ^{b}	9.39 ± 1.01 ^{b}	36.25 ± 0.66 ^{b}	2.61 ± 0.93 ^{b}
KB 15%	34.06 ± 0.98 ^{a}	8.93 ± 0.82 ^{ab}	9.68 ± 1.32 ^{a}	13.19 ± 1.23 ^{a}	47.15 ± 4.05 ^{a}	7.39 ± 2.22 ^{a}
p-value	0.000 (***)	0.020(*)	0.008 (**)	0.012(*)	0.005(**)	0.000 (***)
			Section color			
CB 0%	49.33 ± 0.71 ^{d}	14.22 ± 0.49 ^{ab}	10.99 ± 0.20 ^{b}	17.97 ± 0.48 ^{ab}	37.71 ± 0.74 ^{bc}	–
KB 5%	54.19 ± 1.21 ^{b}	11.78 ± 0.35 ^{c}	11.62 ± 0.33 ^{a}	16.55 ± 0.34 ^{c}	44.61 ± 1.20 ^{a}	5.52 ± 1.83 ^{b}
KB 10%	59.22 ± 1.21 ^{a}	13.24 ± 0.41 ^{bc}	11.38 ± 0.15 ^{ab}	17.47 ± 0.27 ^{bc}	40.69 ± 1.16 ^{b}	9.97 ± 1.89 ^{a}
KB 15%	52.39 ± 0.49 ^{c}	15.17 ± 1.58 ^{a}	11.05 ± 0.21 ^{b}	18.80 ± 1.13 ^{a}	36.23 ± 3.51 ^{c}	3.56 ± 1.10 ^{b}
p-value	<0.0001(***)	0.000 (***)	0.002(**)	0.000 (***)	<0.0001(***)	0.019 (*)

L* = 0 (black); L* = 100 (white); –a * = green color; +a* = red color; –b* = blue color; +b* = yellow color; h* (hue angle, describes the dominant color tone), values closer to zero = more pure red, while increasing values indicate a shift toward yellow hues. Chroma (C*, color saturation or vividness) = larger values indicating more intense and saturated color. Results are given as means ± standard deviation from five repeated determinations. Means with different superscripts in a column orientation indicate significant differences ($p < 0.05$) between samples determined by the Tukey test. Signification codes for the p value: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns.

The significant differences in surface colorimetric parameters observed among the experimental batches are consistent with findings from studies conducted on smoked meat products, which confirm that both the intensity of the thermal treatment and the type of smoking method applied are key determinants of the colorimetric profile of thermally processed products (Gucianu et al., 2023; Ciobotaru et al., 2024a).

Color saturation (Chroma, C*) showed significant differences ($p = 0.012$), with KB 15% recording the highest value (13.19 ± 1.23) and KB 10% the lowest (9.39 ± 1.01). The hue angle (h*) increased significantly with kombucha concentration ($p = 0.005$), from 33.74° ± 1.95 (CB 0%) to 47.15° ± 4.05 (KB 15%), indicating a progressive shift of the dominant surface color away from red tones toward yellow-orange hues. This trend is directly interpretable in the context of the simultaneous decrease in a* and increase in b* described above, and is consistent with the increased moisture content and modified myoglobin redox state of the KB15% sample.

At the cross-section level, all four colorimetric parameters (L*, a*, b*, and C*) showed highly significant differences among samples ($p < 0.0001$ to $p = 0.002$), reflecting a more systematic influence of kombucha on the internal protein matrix than on the smoked surface, where Maillard reaction products and smoke deposits partially mask the treatment effects (Gucianu et al., 2024).

Lightness (L*) increased progressively from 49.33 ± 0.71 (CB 0%) to a maximum of 59.22 ± 1.21 (KB 10%), then decreased to 52.39 ± 0.49 (KB 15%), consistent with the non-linear pH pattern observed in Table 2, where KB 10% recorded the lowest pH (6.06 ± 0.06) and KB 15% showed a partial recovery (6.34 ± 0.24). This relationship is explained by a well-established structural mechanism: as muscle pH increases, the water-holding capacity of proteins increases and the higher intracellular water content causes swelling of fibers and shrinkage of the interfibrillar space, which decreases light scattering and increases light absorption by myoglobin, making the muscle appear darker (Wu et al., 2020).

Redness (a*) at the cross-section showed a distinct pattern from the surface, with KB 15% recording the highest value (15.17 ± 1.58), suggesting greater myoglobin preservation in the internal layers away from oxidative surface effects. Heat-induced denaturation of myoglobin in cooked meats is influenced by multiple endogenous factors including pH,

muscle source, and redox state, as well as exogenous factors such as ingredients incorporated during processing (Suman et al., 2016).

Hue angle (h*) showed significant differences ($p < 0.0001$), with CB 0% (37.71) and KB 15% (36.23) presenting lower values indicating a more red-dominant internal color, while KB 5% (44.61) and KB 10% (40.69) registered higher angles. Hue angle values closer to zero represent more pure red, while increasing values indicate a progressive shift toward yellow hues; chroma quantifies color saturation, with larger values indicating greater color intensity at a given hue (Claus, 2006).

The total color difference (ΔE) quantifies the overall chromatic distance between each kombucha-treated formulation and the control batch. Regarding the color difference at the external surface, ΔE values ranged from 2.61 ± 0.93 (KB 10%) to 7.39 ± 2.22 (KB 15%) on the surface. Altmann et al. (2022) state that a total color difference (ΔE) of 1 is discriminable by consumers under normal observation conditions, indicating that even modest ΔE values in the range observed for KB 10% represent perceptible color changes relative to the control. The ΔE of KB 15% (7.39) substantially exceeds this threshold, confirming that the color modification induced by 15% kombucha incorporation is clearly perceptible to consumers on the product surface.

At the section surface, the ΔE values ranged from 3.56 ± 1.10 (KB 15%) to 9.97 ± 1.89 (KB 10%), confirming that the color differences induced by kombucha incorporation are perceptible to consumers at the internal level and are most pronounced at the 10% incorporation level, consistent with the most pronounced pH reduction observed at this concentration.

In terms of textural attributes, this study evaluated the effect of kombucha beverage incorporation at varying concentrations (5%, 10%, and 15%) in the injection brine by Warner-Bratzler shear force and texture profile analysis (TPA) parameters including hardness, cohesiveness, gumminess, and elasticity. The data in Table 4 shows a gradual and significant reduction ($p < 0.0001$) of shear force with increasing kombucha concentration – from 116.81 ± 3.93 N in the control sample to 71.66 ± 2.15 N in the KB 15% formulation. The work of shear followed the same pattern, decreasing significantly from 1349.20 ± 9.61 mJ (CB 0%) to 948.57 ± 11.68 mJ (KB 15%), with all batches differing significantly from each other.

These results demonstrate that kombucha incorporation contributes to a progressive improvement in tenderness of the injected smoked beef loin, consistent with the tenderizing mechanisms of organic acids described in the literature. The tenderizing effects of organic acid injection in beef have been attributed to two concurrent mechanisms: (1) direct weakening of the perimysial connective tissue; and (2) acceleration of post-mortem proteolysis by cathepsins, whose activity is optimal at low pH values (Berge et al., 2001; Shi et al., 2021). In the present study, the organic acids naturally present in kombucha - primarily acetic, lactic, and gluconic acids - delivered via injection into the muscle matrix, reduced the local pH and thereby activated these mechanisms in a dose-dependent manner.

Hardness results followed a similar pattern to shear force, decreasing significantly ($p < 0.0001$) from 57.60 ± 5.03 N (CB 0%) to 37.78 ± 3.53 N (KB 15%). This may reflect the water-holding and myofibrillar swelling behavior of the organic acids within the protein matrix, leading to increased matrix density reduction and a softer internal consistency. Moisture enhancement with salt and phosphate solutions has been demonstrated to significantly reduce Warner-Bratzler shear force and to increase sensory scores for juiciness and tenderness in beef, confirming the structural softening effect of brine injection on the meat protein matrix (Pietrasik & Shand, 2011). In the present study, the additional biochemical contribution of kombucha's organic acids amplifies this effect beyond what would be expected from the NaCl component of the brine alone.

Table 4. Texture profile of beef loin injected with different concentrations of kombucha in brine

Analyzed parameter	CB 0%	KB 5%	KB 10%	KB 15%	p-value
Shear force (N)	116.81 ± 3.93 [a]	106.80 ± 5.65 [b]	74.39 ± 4.02 [c]	71.66 ± 2.15 [c]	<0.0001(***)
Work of shear (mJ)	1349.20 ± 9.61 [a]	1248.13 ± 8.89 [b]	980.52 ± 12.56 [c]	948.57 ± 11.68 [d]	<0.0001(***)
Hardness (N)	57.60 ± 5.03 [a]	45.63 ± 2.96 [b]	44.47 ± 4.61 [bc]	37.78 ± 3.53 [c]	<0.0001(***)
Cohesiveness (N)	0.03 ± 0.01 [b]	0.06 ± 0.01 [a]	0.06 ± 0.01 [a]	0.05 ± 0.02 [ab]	0.002(**)
Gumminess (N)	1.75 ± 0.19 [c]	2.87 ± 0.29 [a]	2.50 ± 0.38 [ab]	1.83 ± 0.65 [bc]	0.001(**)
Elasticity	1.01 ± 0.02 [a]	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.01	0.871(ns)

Values are given as means ± standard deviation from five repeted determinations. Means with different superscripts in a row orientation indicate significant differences ($p < 0.05$) between samples determined by the Tukey test. Signification codes for the p value: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns.

In terms of cohesiveness, the samples showed significant differences ($p = 0.002$), with KB 5% and KB 10% recording the highest values (0.06 ± 0.01), significantly greater than CB 0% (0.03 ± 0.01), while KB 15% returned to intermediate values (0.05 ± 0.02). This pattern suggests that at low and intermediate kombucha concentrations, the organic acids enhance the integrity of the internal protein network by promoting myofibrillar swelling and water retention within the gel matrix, creating a more cohesive structure. At the highest concentration (KB 15%), the more extensive proteolysis and connective tissue weakening may partially disrupt this internal cohesion, reducing the cohesiveness value relative to KB 5% and KB 10%.

Gumminess decreased significantly ($p = 0.001$) from 2.87 ± 0.29 N (KB 5%) to 1.83 ± 0.65 N (KB 15%), with the control recording the lowest value (1.75 ± 0.19 N). The superior value at KB 5% is consistent with the increase in cohesiveness observed at this concentration, as gumminess is is a parameter derived from the results obtained for hardness and cohesiveness. The decrease at KB 15% reflects the dominant effect of hardness reduction at the highest kombucha concentration, suggesting that the viscoelastic matrix was partially disrupted at this level, facilitating easier breakdown during mastication.

Elasticity did not differ significantly among samples ($p = 0.871$), with all batches recording values of 1.00 ± 0.00 to 1.01 ± 0.02. This result indicates that kombucha incorporation, regardless of concentration, did not alter the elastic recovery properties of the protein matrix under the compression conditions applied, suggesting that the structural modifications induced by organic acid injection affect primarily the resistance to shear and the firmness of the matrix, rather than its capacity for elastic recovery after deformation.

The sensory evaluation of the four experimental batches, performed by a panel of 21 semi-trained assessors on a 9-point hedonic scale, revealed that all samples scored above 6.0 for all evaluated attributes, indicating an acceptable sensory quality across all kombucha concentrations tested (Table 5).

For the attributes appearance, odour, and flavour, the sensory perception did not differ significantly among samples ($p = 0.614$, $p = 0.176$, and $p = 0.691$, respectively), confirming that kombucha incorporation into the injection brine did not alter the visual exterior or the characteristic smoked aroma and flavor profile of the product. The organic acids naturally present in kombucha, such as acetic, lactic, and gluconic acids, did not introduce perceptible off-notes at the concentrations used in the finished product after thermal treatment, a finding consistent with results reported by Braïek & Smaoui (2021), who demonstrated that lactic acid and sodium lactate incorporation into meat products improved sensory attributes including odour, flavour, and texture, with the highest overall acceptability score recorded at 1% lactic acid, without introduction of undesirable acidic off-notes perceptible to consumers.

Significant differences among samples were observed for section color, tenderness, juiciness, and overall texture ($p = 0.003$, $p = 0.016$, $p = 0.007$, and $p = 0.004$, respectively), with scores generally improving with increasing kombucha concentration. KB15% received the highest scores for tenderness (7.71 ± 0.81), juiciness (8.19 ± 0.88), and overall texture (7.86 ± 0.62).

Table 5. Sensory evaluation for control and kombucha brine injected smoked beef

Sensory attribute	Sample				p-value
	CB0%	KB 5%	KB 10%	KB 15%	
Appearance	7.33 ± 1.17 [a]	7.52 ± 0.89 [a]	7.57 ± 0.89 [a]	7.24 ± 0.77 [a]	0.614(ns)
Section color	7.05 ± 0.76 [ab]	7.29 ± 1.27 [a]	7.67 ± 0.94 [a]	6.38 ± 1.36 [b]	0.003(**)
Odour (smoked aroma)	7.33 ± 0.68 [a]	7.86 ± 0.95 [a]	7.48 ± 1.23 [a]	7.29 ± 0.55 [a]	0.176(ns)
Flavour	7.14 ± 0.88 [a]	7.33 ± 0.66 [a]	7.24 ± 0.89 [a]	7.05 ± 0.83 [a]	0.691(ns)
Tenderness	6.95 ± 0.86 [b]	7.57 ± 0.89 [ab]	7.05 ± 1.00 [ab]	7.71 ± 0.81 [a]	0.016(*)
Juiciness	7.19 ± 0.85 [b]	7.90 ± 1.12 [ab]	7.62 ± 0.89 [ab]	8.19 ± 0.88 [a]	0.007(**)
Overall texture	7.10 ± 0.67 [b]	7.57 ± 0.68 [ab]	7.40 ± 0.60 [ab]	7.86 ± 0.62 [a]	0.004(**)
Overall acceptability	7.16 ± 0.40 [b]	7.58 ± 0.32 [a]	7.43 ± 0.39 [ab]	7.39 ± 0.33 [ab]	0.006(**)

Values are given as means ± standard deviation (n=21). Means with different superscripts in a row orientation indicate significant differences ($p < 0.05$) between samples determined by the Tukey test. Signification codes for the p value: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < ns.

These results are consistent with the established tenderizing and moisture-retaining effects of organic acid injection reported in the literature. Berge et al. (2001) demonstrated that lactic acid injection significantly improved the textural traits of beef, including shear force values and sensory scores, through a combination of myofibrillar swelling, lysosomal enzyme release, and collagen weakening. Similarly, marination of beef with acid whey, a matrix rich in lactic acid, increased tenderness and juiciness ratings without decreasing hedonic liking scores for overall flavor or aftertaste (Kim, 2018).

Overall acceptability differed significantly among samples ($p = 0.006$), with KB 5% recording the highest score (7.58 ± 0.32) and CB 0% the lowest (7.16 ± 0.40), suggesting that, as overall perception is shaped by the combined contribution of all sensory attributes, moderate kombucha concentrations achieve an optimal sensory balance from the consumer's perspective. The slight decrease in section color score at KB 15% (6.38 ± 1.36), combined with greater inter-assessor variability for this attribute, may have partially offset the textural advantages of this batch in the overall hedonic evaluation. These findings indicate that KB 5% represents the optimal kombucha concentration for brine injection in smoked beef loin, achieving the most favorable balance between physicochemical improvement and overall consumer acceptability This observation aligns with the broader principle that functional additives incorporated into meat products at moderate concentrations tend to optimize sensory acceptance, whereas excessive levels risk compromising texture and overall sensory perception (Ciobotaru et al., 2024b).

The PCA biplot presented in Figure 1 covers 91.68% of the total variability of the dataset, with F1 accounting for 46.25% and F2 for 45.42%, indicating that the two principal components together provide a highly representative projection of the relationships among the analyzed parameters and experimental batches.

The control batch (CB 0%) is positioned in the lower-left quadrant of the biplot, strongly correlated with the parameters associated with textural resistance, shear force, work of shear, hardness, and dry matter and fat content, reflecting the firmer structure of smoked beef loin. These results confirm that the control sample presents the highest mechanical resistance among the analyzed batches, as showed by the instrumental texture data.

KB 5% is located in the upper-left area of the biplot and is associated primarily with gumminess, hue angle (h*), and b* values, suggesting that this batch is characterized by a moderate tenderizing effect combined with a slight shift toward yellow hues at the product surface.

Batch KB 10% is positioned in the upper-central area and correlates positively with cohesiveness, lightness, and water activity, reflecting the combined effect of pH reduction at this concentration on myofibrillar structure, promoting greater light scattering at the cross-section (higher L*) and improved internal cohesion, as well as the increased moisture retention.

Sample KB 15% occupies the lower-right quadrant and is strongly associated with moisture, protein, ash, chroma (C*), and redness (a*), as well as with a lower pH. This positioning confirms that the highest kombucha concentration produces the most pronounced

modification of the internal protein matrix and mineral profile, driving the highest redness and color saturation at the cross-section while simultaneously achieving the greatest moisture and protein retention. The pH vector points downward in the same general direction as KB 15%, consistent with the non-linear pH trend, where KB 15% records a partial pH recovery relative to KB 10%.

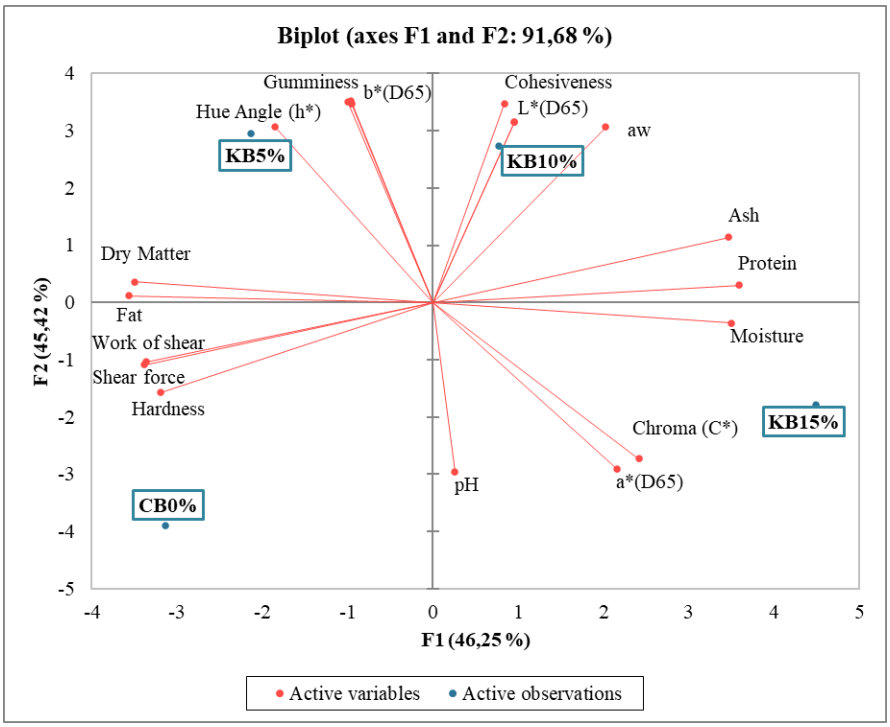

Figure 1. Principal component analysis (PCA) biplot illustrating the relationships between physicochemical, colorimetric, and textural parameters of control and kombucha brine-injected smoked beef loin (CB 0%, KB 5%, KB 10%, KB 15%).

CONCLUSIONS

The incorporation of kombucha beverage into the injection brine at concentrations of 5%, 10%, and 15%, from a textural perspective, resulted in a dose-dependent reduction in meat toughness, reflected by decreases in Warner–Bratzler shear force and hardness values.

In addition, kombucha incorporation was associated with significant modifications in the physicochemical profile of smoked beef loin, including progressive increases in moisture content, protein, and ash, alongside a corresponding decrease in fat content. Colorimetric analysis revealed perceptible color differences ($\Delta E > 1$) in all kombucha-treated samples relative to the control, both at the surface and cross-section, with the most pronounced internal lightness recorded at KB 10% and the highest surface redness and chroma at KB 15%, effects attributable to the pH-driven structural modifications of the myofibrillar matrix.

In terms of sensory evaluation, all four experimental batches maintained acceptable hedonic scores above 6.0 for all evaluated attributes. Although improvements were observed in tenderness, juiciness, overall texture, and section color, with KB 15% achieving the highest scores for textural attributes, the KB 5% sample showed a more balanced sensory profile in terms of appearance, odour, and flavour, resulting in the highest overall acceptability.

The mechanisms involved remain hypothesized based on literature data rather than directly

demonstrated in the present study; therefore, further research should aim to characterize the chemical composition of the kombucha used and to further explore its effects on product quality and stability.

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