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INVESTIGATION OF TREATMENT PARAMETERS INFLUENCING THE QUALITY ATTRIBUTES OF WILD RED DEER (*CERVUS ELAPHUS*) MEAT

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The PhD School

Name: Doctoral School of Food Sciences

Discipline: Food Sciences

Head: **Livia Simonné Sarkadi**

Professor, DSc
Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Department of Nutrition

Supervisors: **Adrienn Varga-Tóth**

Researcher, PhD
Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Department of Livestock Products and Food Preservation Technology

Attila Lőrincz

Honorary Professor, PhD
Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Department of Livestock Products and Food Preservation Technology

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Approval of the Supervisor
Adrienn Varga-Tóth, PhD

.....

Approval of the Head of Doctoral School
Livia Simonné Sarkadi, DSc

.....

Approval of the Supervisor
Attila Lőrincz, PhD

1. INTRODUCTION AND OBJECTIVES

Wild red deer (*Cervus elaphus*) meat has gained increasing attention in recent years due to its high nutritional value, unique sensory attributes, and alignment with consumer preferences for natural and sustainable food sources (Demartini et al., 2018). Unlike conventionally farmed meats such as beef, pork, and poultry, wild red deer meat is characterized by higher protein content, lower fat levels, and a favorable omega-3 to omega-6 fatty acid ratio, making it a desirable alternative for health-conscious consumers (Milczarek et al., 2021).

Despite these advantages, large-scale commercialization is limited. Wild game harvesting occurs under uncontrolled conditions, resulting in variable quality, higher microbial loads, and greater spoilage risk (Kunová et al., 2022). Its low intramuscular fat and dense muscle fibers make it more prone to toughness and oxidation (Soriano et al., 2006).

To address these issues, the meat industry and scientific research have explored innovative processing technologies that can enhance microbial stability, textural properties, and shelf life while maintaining the natural quality of wild red deer meat. Among these emerging technologies, organic acid treatment, high hydrostatic pressure (HHP), and sous-vide treatment have shown promising potential in improving food safety and quality retention in meat products (Majzinger et al., 2025). Organic acid treatment, particularly with lactic acid (LA) and ascorbic acid (AA), has been widely studied for its ability to reduce microbial contamination, delay oxidative deterioration, and maintain color stability in fresh meat (Rodríguez-Melcón et al., 2017; Manzoor et al., 2020). However, no studies had investigated the effectiveness of a combined LA and AA treatment for wild red deer meat.

HHP processing is a non-thermal preservation method that applies high pressure (100–600 MPa) to inactivate microorganisms, modify protein structures, and

enhance meat texture. Studies on beef, pork, and poultry have demonstrated that HHP can improve meat tenderness, reduce microbial growth, and extend shelf life (Csehi et al., 2016; Han et al., 2021). However, its effects on wild game meat, particularly wild red deer meat, remain largely unexplored.

Sous-vide treatment, a low-temperature, long-time cooking technique, is another promising method for enhancing the tenderness and sensory properties of meat while preserving its nutritional quality (Misu et al., 2024). This technique has been successfully applied to beef and pork to improve juiciness, texture, and oxidative stability. However, limited data exist on the effectiveness in wild red deer meat, especially regarding how different cooking temperatures affect meat quality parameters such as water holding capacity, protein denaturation, and sensory characteristics. Moreover, an important but often overlooked factor in sous-vide processing is the age of the animal. Meat from older animals typically contains higher levels of connective tissue, which may influence the effectiveness of sous-vide cooking in tenderizing wild red deer meat. There is no research on how age impacts the sous-vide treatment of wild red deer meat presents a critical gap in knowledge.

This study aims to evaluate and compare the effects of organic acid treatment, HHP, and sous-vide cooking on the quality attributes of wild red deer meat.

The primary goal of this research is to evaluate the effects of **organic acid treatment, HHP, and sous-vide treatment** on the quality attributes of wild red deer meat. Additionally, the research examines the influence of **animal age on meat quality**, particularly in the sous-vide experiment, to provide insights into how aging affects tenderness, protein structure, and overall quality. The findings aim to support optimal processing strategies for enhancing the commercial viability of wild game meat.

To achieve the general research goal, the study is structured around the following specific objectives:

1. **Effect of organic acids treatment (Experiment 1):** Assess a 2% lactic acid + 2% ascorbic acid spray on drip loss, water-holding capacity (WHC), dry matter, water activity (a_w), pH, instrumental color, texture, SDS-PAGE protein profile, and microbiological evaluation during vacuum storage at 4 ± 1 °C (days 1, 7, 14, 21).
2. **Effect of HHP treatment (Experiment 2):** Characterize meat responses to 150–600 MPa for 5 min at 22 °C on drip loss, WHC, dry matter, pH, instrumental color, texture, SDS-PAGE, microbiological evaluation during vacuum storage at 4 ± 1 °C (days 1 and 14).
3. **Effects of Sous-Vide Treatment and Influence of Animal Age (Experiment 3):** Evaluate sous-vide at 60, 65, and 70 °C for 3 h in samples from nine known-age wild red deer plus one unknown-age sample (for age inference) measuring drip loss, WHC, pH, color, texture, SDS-PAGE, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and microbiology.

2. MATERIALS AND METHODS

2.1 Preparation of Samples

2.1.1 Organic Acid Treatment **(Experiment 1)**

Wild red deer (*Cervus elaphus*) meat used in this study was sourced from the local processing plant "VADEX" Mezőföldi Ltd. in Hungary. The wild red deer, which were hunted in Western Hungary, included both stags (males) and hinds (females) and were aged between 4 and 6 years. Fresh meat samples were packed in low-density polyethylene pouches, transported to the laboratory under chilled conditions, and stored at 4 ± 1 °C for one day. *Semimembranosus* muscles were dissected from 12 individual deer. Each muscle was cut into four steaks of similar

sizes (approximately 14 × 7 × 2 cm), with an average weight of 250 ± 10 g. Steaks in the control group were vacuum-packed and kept in a refrigerated cabinet at 4 ± 1 °C. For the treatment group, individual meat steaks were placed inside vacuum bags, and a water-based mixture containing 2% lactic acid and 2% ascorbic acid was sprayed inside each bag using a manual sprayer. The applied solution concentration was 1% in relation to the initial meat weight. After treatment, all samples were vacuum-packed. The assessment of quality parameters occurred on days 1, 7, 14, and 21 during the storage period.

2.1.2 High Hydrostatic Pressure (HHP) Treatment **(Experiment 2)**

Fresh wild red deer (*Cervus elaphus*) meat samples used in this part were sourced from the local processing plant "VADEX" Mezőföldi Ltd. The wild red deer, hunted in Western Hungary, included both stags and hinds aged between 4 and 6 years. The meat was transported to the laboratory in low-density polyethylene pouches under chilled conditions (4 ± 1 °C) and stored for one day. *Semimembranosus* muscles were dissected from 22 individual deer. Each muscle was cut into three steaks of similar size (approximately 14 × 7 × 3 cm), with an average weight of 300 ± 10 g. All samples were vacuum-packed and packed samples were subjected to HHP using the Resato FPU-100-2000 HHP equipment (Resato Int. B.V., Roden, Netherlands). The samples were pressurized at 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 MPa for 5 minutes at 22 °C. Following treatment, all samples were stored at 4 ± 1 °C for 14 days. Quality parameters were evaluated on days 1, and 14 of storage.

2.1.3 Sous-Vide Treatment **(Experiment 3)**

Meat samples for this experiment were obtained from local hunters in Western Hungary. The *semimembranosus* muscle was dissected from nine different red deer (*Cervus elaphus*) (7, 8, 9, 12, 18, 32, 36, 37, 48 months) and one sample of wild red deer with unknown age was sourced from a local processing plant,

“VADKONYHA” Fiwi-Hűt Ltd. As part of this study, we will attempt to predict the age of this sample. Each sample was cut into four equal-sized steaks of approximately 14 × 7 × 2 cm and 250 ± 10 g, then vacuum-packed. The vacuum-packed samples were subjected to sous-vide treatment using Maxima sous-vide stick (Maxima, Model 09500500, Mijdrecht, Netherlands). Each sample underwent cooking for 3 hours at three different temperatures: 60 ^{O}C, 65 ^{O}C, and 70 ^{O}C. Sample codes denote age in months; “U” indicates unknown.

2.2 Quality Evaluation

- **Drip Loss Measurement**

Drip loss was determined by weight difference before and after treatment in vacuum-packing. The weight of each meat sample was measured before treatment. After the designated storage period, samples were taken from the vacuum bags, blotted dry and immediately weighed.

- **Cooking Loss Measurement**

Cooking loss indicates the amount of water and soluble matter lost from meat during cooking. After cooking, samples were cooled in an ice slurry, stored at 4 ±1 °C until equilibrated, removed from the bag, blotted dry, and weighed.

- **Water Holding Capacity Determination (WHC)**

The WHC of the red deer meat samples was determined using a modified filter paper press method described by Honikel and Hamm (1994). Briefly, a 0.2-0.4 g meat sample was measured in analytical scale and placed in filter paper between two glass plates, and a 500 g metal weight was applied for 5 min. Three replicates were analyzed from each sample. After pressing, the meat juice area was cut from the filter paper and measured.

- **Determination of Dry Matter Content**

The dry matter content of wild red deer meat was determined according to the Hungarian Standard MSZ ISO 1442:2000. The weight of petri dishes was measured first. Subsequently, 1-2 g of the sample was measured using an analytical scale and placed into the pre-weighed petri dishes. Three replicates were analyzed from each sample. The samples were then transferred to a drying cabinet, and their weights were re-measured after 8-9 hours.

- **Water Activity Measurement**

Water activity (a_w) was performed using a LabMaster-aw neo water activity meter (Novasina AG, Lachen, Switzerland) at a controlled room temperature of 20 ± 1.5 ^{O}C. Three replicates were analyzed from each sample.

- **pH Determination**

The pH values of wild red deer meat samples were measured using one hand digital pH meter (Testo, Model 206-pH2, Alton, UK). The pH was directly determined from the muscle tissues of the chilled samples at room temperature. Each sample was measured three times.

- **Instrumental Color Measurement**

The surface color of the vacuum-packed wild red deer samples was determined using a Chroma Meter CR-400 (Konica Minolta, Tokyo, Japan) with a 4 mm diameter aperture, an illuminant D65, and a 10^{o} standard observer. For each sample, 10 replicate measurements were taken across the entire surface of the meat while still in vacuum packaging. The measurement method followed the same approach as used by other researchers (Park et al., 2010).

- **Instrumental Texture Measurement**

Texture was measured on a TA.XT Plus (Stable Micro Systems, UK). For Warner–Bratzler shear force (WBSF), slabs (15 × 15 × 50 mm) cut perpendicular to fibres

were sheared with a flat-ended blade at 2 mm/s; peak force (N) was recorded as WBSF. Ten replicates per sample were tested and processed in Texture Exponent 32. For texture profile analysis, cylinders with a diameter and height of 12 mm were compressed twice with a 35 mm probe to 70% height at 3.0 mm/s (pre-test), 1.0 mm/s (test), and 3.0 mm/s (post-test), with a 2 s pause; force was sampled every 0.01 s to derive TPA parameters.

- **Analysis of Sarcoplasmic and Myofibrillar Proteins by Sodium Dodecyl-Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

Sarcoplasmic and myofibrillar proteins from wild red deer were extracted per a modified Csehi et al. (2016) method based on Kretzschmar (1995). Minced meat (5 g) was homogenized in 10 mL 0.05 M NaCl (13,500 rpm, 3.5 min, on ice) and centrifuged (6,000 rpm, 20 min, 4 °C); the supernatant (sarcoplasmic) was collected and stored at −24 °C. The pellet was washed twice with 0.05 M NaCl, resuspended in 10 mL 0.7 M NaCl, homogenized (1 min), centrifuged as above, and the supernatant (myofibrillar) stored at −24 °C. SDS-PAGE followed Laemmli (1970) with minor modifications: extracts were mixed with 2× Laemmli buffer containing 10% 2-mercaptoethanol, diluted 1:20 (sarcoplasmic) or 1:2 (myofibrillar), heated at 95 °C for 2 min, and 4 µL loaded per lane. Gels were run at 200 V for 45 min with a 250-10 kDa marker, imaged, and analyzed in ImageLab 6.1 software (Bio-Rad, USA).

- **Protein Analysis Using Differential Scanning Calorimetry (DSC)**

Protein thermal properties were analyzed using a Micro DSC III microcalorimeter (Setaram Inc., Caluire, France). Distilled water was used as a reference for all measurements. Measurements were conducted within a temperature range of 20^{o}C to 90^{o}C. The heating rate from 20^{o}C to 90^{o}C was set at 0.8^{o}C/min, while the cooling rate from 90^{o}C to 20^{o}C was 1^{o}C/min. Sample weights were standardized at 200 $\pm$ 5 mg, and three replicates were analyzed for each sample to ensure data

accuracy. Heat flow curves obtained from the measurements were evaluated using the Calisto Processing thermal analysis software (Version 1.08, Setaram Inc., Caluire, France).

- **Scanning Electron Microscope Imaging**

Wild red deer meat samples were cut into very thin slices using a sterile razor blade to ensure adequate fixative penetration and minimize structural distortion. The samples were fixed in 2.5% glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 24 hours at 4 ºC. Rinsed in phosphate-buffered saline and dehydrated in ethanol for 24 hours. The final step involved critical point drying using carbon dioxide (Lach-Ner, Neratovice, Czech Republic) to remove residual moisture. Electron micrographs were captured using a Prisma E SEM (Thermo Fisher Scientific Inc., Waltham, MA, USA).

- **Microbiological Evaluation**

The treated and non-treated wild red deer meat samples were separately vacuum-packed from other measurement samples. The storage temperature before measurement was 4 ± 1 ^{o}C. For microbial analysis, 10 g of each meat sample was homogenized with 90 mL of 0.1% sterile peptone water. To prepare for the Aerobic Plate Count (APC), serial dilutions were conducted by mixing one milliliter of the homogenate with nine milliliters of 0.1% peptone water. The APC was determined using the pour plate method with nutrient agar on duplicate plates. These plates were incubated at 30 ^{o}C for 48 h. After incubation, colonies were counted to determine the total number of colony-forming units per gram of meat (CFU/g) (Roberts, 1996).

2.3 Statistical Analysis

All experimental data obtained from Experiment 1, 2, and 3 were statistically analyzed using IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA). Each experiment was conducted with at least three replicates. However, some

experiments had up to ten replicates. Results are expressed as mean values ± standard deviation (mean ± SD). Assumptions of normality and homogeneity of variance were checked using Shapiro‑Wilk and Levene's tests, respectively. To assess the significance of differences among treatments and during storage periods, a two-way analysis of variance (ANOVA) was conducted. When significant differences were detected ($p < 0.05$), Tukey's Honest Significant Difference (HSD) post-hoc tests were used to determine specific differences between groups. Graphical representations and data visualizations were generated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

3. RESULTS AND DISCUSSION

3.1 Organic Acid Treatment (Experiment 1)

Across 21 days at 4 ± 1 °C, spraying 2% lactic acid + 2% ascorbic acid produced clear early benefits for moisture retention. Drip loss did not differ on Day 1 but was significantly lower in treated meat by Day 7 (8.07 ± 1.26% vs 11.07 ± 1.10%, $p < 0.05$), after which differences narrowed and were no longer significant by Days 14–21. Water-holding capacity (WHC) followed similar trajectories in both groups and declined over time; the only notable divergence was on Day 21, when WHC was lower in treated samples (0.00069 ± 0.00011 g/mm²) than controls (0.00089 ± 0.00011 g/mm², $p < 0.05$). Consistent with reduced drip loss earlier in storage, dry matter was lower in treated meat at mid-to-late storage (Day 14: 24.96 ± 0.22% vs 26.70 ± 0.25%, $p < 0.001$; also lower at Day 21, $p < 0.01$). Water activity (a_w) was reduced by treatment at Day 1 ($p < 0.001$) and remained lower at Days 14 and 21 ($p < 0.05$), indicating less free water and potential suppression of microbial growth.

The pH response was modest but directionally consistent with acidification: treated samples were 0.08 units lower on Day 1 ($p < 0.05$). Over storage, treated meat showed an initial decrease followed by a slight rise toward Day 21, while

non-treated samples declined more steadily patterns attributable to the immediate effect of the acids and subsequent microbial activity (Aktaş et al., 2003).

Instrumental color was largely preserved. Lightness (L^*) was similar between groups except for a transient difference mid-storage, and redness (a^*) increased in both groups from 9.2–9.4 on Day 1 to 11.0–11.8 by Day 21, consistent with metmyoglobin reduction in vacuum. Yellowness (b^*) and chroma rose slightly over time. The overall color difference between groups became perceptible later in storage (ΔE 3.4 at Day 14; 2.8 at Day 21), but treatment did not materially impair color stability.

Texture softened with aging irrespective of treatment. Hardness, cohesiveness, and chewiness declined from Day 1 to Day 21 (chewiness decreased from 6.69 to 2.42 mJ in treated, and from 8.36 to 3.25 mJ in controls), with only sporadic treatment-day interactions (notably around Day 14). Springiness was stable in treated samples (0.80–0.81 mm), while controls were slightly higher early and late in storage.

Protein profiling supported a protective effect of the acid mix. Non-treated meat showed pronounced loss of sarcoplasmic enzymes (β-enolase, GAPDH, TPI) and degradation of myofibrillar proteins (α-actinin, troponin-T, desmin, actin) after Day 14, whereas treated samples retained clearer bands and preserved myoglobin (~17 kDa) longer. This suggests attenuated proteolysis/oxidation, plausibly via the antimicrobial action of lactic acid and the antioxidative role of ascorbic acid.

Aerobic counts increased during storage from 3.3 log CFU/g at Day 1 but remained below 7 log CFU/g at Day 21 in all cases; treated samples were consistently lower and significantly reduced by Day 21, indicating a modest shelf-life advantage. Overall, the 2% LA + 2% AA spray offers measurable early-to-mid storage quality benefits, lower drip loss, lower dry matter and a_w, preserved proteins, and reduced microbial loads while maintaining acceptable color and typical aging-related tenderization. Effects taper by Day 21, suggesting value as

part of a multi-hurdle preservation strategy rather than a standalone long-term solution.

3.2 HHP Treatment (Experiment 2)

High hydrostatic pressure (150–600 MPa) applied to vacuum-packed wild red deer meat and stored at 4 ± 1 °C produced pressure and time-dependent changes in moisture, pH, color, texture, proteins, and microbiology. Drip loss increased with pressure, most markedly ≥500 MPa (Day 1: 0 MPa = 1.56% vs 600 MPa = 5.84%; Day 14: 0 MPa = 6.19% vs 600 MPa = 18.67%; $p < 0.05$), consistent with myofibrillar denaturation and reduced water binding. WHC varied non-linearly: higher values were observed at moderate–higher pressures on Day 1 (450–500 MPa) and around 350 MPa during storage, but WHC generally declined from Day 1 to Day 14 ($p < 0.05$). Dry matter showed pressure-specific shifts (elevated at some low and high pressures on Day 1 and at 550–600 MPa by Day 14), reflecting concomitant moisture loss.

pH rose with pressure on Day 1 (control 5.60 to 5.94 at 600 MPa) and diverged over storage: it decreased at ≤250 MPa, was largely stable at 300–350 MPa, and increased at 400–550 MPa, remaining high at 600 MPa patterns consistent with pressure-induced protein/ion rearrangements and differing microbial/enzymatic activity during storage. Color responses were characteristic of pressure-induced pigment/protein changes: lightness (L^*) increased from ≥350 MPa and remained elevated at Day 14; redness (a^*) was variable initially but declined during storage, especially ≥550–600 MPa; yellowness (b^*), hue angle, and (at high pressure over time) chroma shifts indicated paler, less vivid surfaces at higher pressures.

Texture reflected competing denaturation and proteolysis. On Day 1, WB shear force and TPA hardness rose with pressure (notably ≥450 MPa), indicating firmer gels/cross-linking; by Day 14, moderate pressures (250–300 MPa) showed reduced shear force (tenderization), whereas high pressures (≥500–600 MPa)

maintained higher hardness, chewiness, and cohesiveness. SDS-PAGE supported these outcomes: sarcoplasmic proteins diminished with increasing pressure (marked at 550–600 MPa and, non-linearly, at 250 MPa), with greater losses by Day 14 (reduced aldolase). Myofibrillar proteins above 250 MPa showed decreased band intensity and evidence of aggregation (MLC-1) with MLC-2 persistence and a 37–50 kDa fragment consistent with proteolysis mechanisms that jointly influence WHC and texture.

Microbiologically, pressure produced dose-dependent inactivation on Day 1 (≥500 MPa ≤ 1 log CFU/g). After 14 days all samples increased, but counts remained markedly lower with higher pressures (2.8 log CFU/g at 600 MPa vs 7.6 log CFU/g control), indicating a clear shelf-life advantage. Overall, HHP offers strong microbial control; a moderate window (300–450 MPa) best balances safety with product quality (color, WHC, tenderness), while very high pressures (≥500–600 MPa) maximize decontamination at the cost of paler color, higher drip loss, and firmer texture.

3.3 Sous-Vide Treatment (Experiment 3)

Sous-vide (60, 65, 70 ºC; 3 h) produced temperature- and age-dependent shifts in moisture, pH, color, texture, proteins, microstructure, and microbiology. Cooking loss rose with temperature (lowest at 60 ^{O}C, highest at 70 ºC) and was greater in older deer (36‑48 months), consistent with enhanced collagen cross-linking; younger meat (7‑12 months) retained more moisture (Tornberg, 2005). WHC declined monotonically with temperature, with higher values in younger vs older animals; patterns for the unknown sample most closely matched mid-aged deer (32‑36 months). pH increased after cooking (raw 5.52‑6.09; sous-vide 5.81‑6.39) and tended to rise with temperature across ages; raw pH varied by age, peaking around 12 months and declining thereafter, and the unknown sample's pH aligned with mid-aged animals (Maggiolino et al., 2019; Soriano et al., 2020).

Color responses reflected myoglobin/protein alterations: raw meat showed low lightness ($L^* < 40$) typical of wild deer, while sous-vide slightly increased L^* and reduced redness (a^*), especially at 65–70 ºC, with a concomitant rise in yellowness (b^*). Total color difference (ΔE) relative to raw grew with temperature and was generally higher in older deer, indicating more perceptible shifts; the unknown sample showed intermediate ΔE consistent with mid-age classification.

Texture changed in line with moisture loss and protein gelation. Shear force, hardness and chewiness increased with temperature, most at 70 ºC; younger deer remained more tender than older animals. Cohesiveness generally rose post-cooking, and springiness responses were sample-dependent. Protein analyses supported these outcomes: SDS-PAGE revealed progressive loss/aggregation of sarcoplasmic and myofibrillar proteins with temperature (myoglobin bands persisted but diminished at 70 ºC), with more pronounced reductions in older muscle. DSC of raw meat displayed three endotherms (50–55, 60–65, 70–75 ºC) attributable to myosin/head–tail domains, connective tissue and actin; these peaks flattened after sous-vide at 65–70 ^{O}C, especially in older samples, indicating extensive denaturation. SEM at 65 ºC showed fiber flattening, fragmentation and increased porosity, accentuated with age, consistent with observed toughness and water loss.

Microbiologically, aerobic plate counts in raw meat were 4.3–4.7 log CFU/g and fell below detection (<1.00 log CFU/g) after all sous-vide treatments, confirming robust lethality for vegetative cells (spore control still requires chilled storage). Overall, 60–65 ºC offered a better balance between safety and quality (lower cooking loss, higher WHC, less discoloration and toughness), whereas 70 ºC maximized microbial reduction at the cost of paler color, higher exudation and

firmer texture. The unknown sample most consistently aligned with mid-aged deer across moisture, pH, color and texture metrics.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusion of Experiment 1 (Organic Acid Treatment)

Applying a 2% lactic acid + 2% ascorbic acid spray to vacuum-packed wild red deer meat (4 ± 1 ºC, 21 days) improved microbiological stability and enhanced tenderness without compromising color or overall pH stability. pH dropped slightly immediately after treatment but remained comparable to controls during storage. Texture measures (hardness, cohesiveness, chewiness) declined more in treated samples, indicating greater tenderization. SDS-PAGE showed slower degradation of key sarcoplasmic and myofibrillar proteins including myoglobin treated meat, consistent with reduced oxidation and better-quality preservation. Overall, the acid mix offers a practical, short-term shelf-life and quality benefit under vacuum storage. Future work should include sensory validation and a techno-economic assessment for commercial use.

4.2 Conclusion of Experiment 2 (High Hydrostatic Pressure Treatment)

HHP markedly altered quality attributes of wild red deer meat. Pressures ≥300 MPa stabilized pH over 14 days consistent with suppressed microbial/enzymatic activity and reduced aerobic counts in a pressure-dependent manner; ≥500 MPa achieved <1 log CFU/g on Day 1 and maintained low levels during storage. Color shifted toward higher lightness/yellowness and lower redness/chroma, reflecting pigment/protein changes. Texture diverged by regime: moderate pressures (150–300 MPa) improved tenderness by balancing denaturation with proteolysis, whereas higher pressures (450–600 MPa) increased firmness via aggregation. SDS-PAGE corroborated pressure-induced denaturation/aggregation of sarcoplasmic and myofibrillar proteins, most pronounced ≥500 MPa. Overall, 300 MPa offers the best quality–safety balance (tenderness, acceptable color,

extended shelf life) while avoiding excessive gelation; 500 MPa is recommended when maximum decontamination is the priority. Future work should test shorter holds (3–4 min), include sensory evaluation, explore hurdle combinations (e.g., organic acids or sous-vide), and assess nutritional impacts.

4.3 Conclusion of Experiment 3 (Sous-Vide Treatment and Animal Age Influence)

Sous-vide at 60, 65, and 70 °C (3 h) markedly affected wild red deer meat quality in a temperature- and age-dependent manner. All treatments reduced aerobic plate counts below detection, confirming strong lethality for vegetative cells. Heat effects were evident across scales: SDS-PAGE and DSC showed progressive denaturation/aggregation of sarcoplasmic and myofibrillar proteins (most at 65–70 °C), and SEM revealed fiber flattening/fragmentation and greater porosity, accentuated in older animals. Functionally, cooking loss increased with temperature and age, while WHC declined with temperature; age modulated this response, with some older samples retaining relatively higher WHC at elevated temperatures. Texture (shear force, hardness, chewiness) rose with temperature, and older deer remained consistently tougher than younger ones. Overall, 65 °C provided the best balance between microbial safety, limited colour/juiciness losses, and acceptable texture particularly for younger animals, whereas 70 °C maximized lethality at the cost of higher exudation and firmness. The unknown sample's moisture, pH, color, and texture most closely matched mid-aged deer (36 months). Future work should vary time temperature combinations (including shorter or stepwise treatments), expand sample size for greater statistical power, and test flavor-oriented hurdles (e.g., herb marinades) to further optimize quality.

4.4 General Conclusion

This work shows that targeted processing can improve quality, safety, and shelf life of wild red deer meat; the optimal choice depends on desired product traits and animal age.

- Organic acids (2% lactic + 2% ascorbic): Best for short-term (≤21 days) refrigerated, vacuum-packed meat. Simple to implement, lowers microbial load, preserves proteins, and has minimal impact on color/texture well suited to small or artisanal processors.
- High hydrostatic pressure (HHP): ~300 MPa offers the best quality–safety balance (tenderness, color, protein stability) for moderate shelf-life extension. 500 MPa maximizes microbial inactivation but can yield firmer texture and paler color, fitting applications prioritizing safety above texture.
- Sous-vide: 65 °C for 3 h is ideal for ready-to-eat products, especially from younger animals (≤12 months) combining safety with favorable tenderness and structure; parameters can be age-adjusted for consistent quality.

Producers can deploy these individually or as hurdle combinations (e.g., acid + HHP or sous-vide) to tailor outcomes. Future work should refine combined/stepwise processes for specific market targets.

5. NEW SCIENTIFIC RESULTS

1. This study provides the first comprehensive evaluation of the effects of a **2% lactic acid and 2% ascorbic acid mixture**, applied via surface spraying, on wild red deer (*Cervus elaphus*) meat harvested in Western Hungary, examining its impact on physicochemical properties and protein integrity during 21 days of vacuum storage at 4 ± 1 °C. The treatment improved moisture retention (Day 7 drip loss: 8.07 ± 1.26% vs 11.07 ± 1.10%, $p < 0.05$), improved tenderness (Day 14 hardness: 10.22 vs 16.17 N, $p < 0.05$) and increased protein stability (SDS-PAGE indicated slower degradation) without compromising pH or color attributes, as no significant differences ($p > 0.05$) were observed in redness (a^*), and only on Day 14 significant differences ($p < 0.05$) were found for lightness (L^*), yellowness (b^*).
2. I found based on my data the combination of **2% lactic acid and 2% ascorbic** acid effectively reduced microbial growth in vacuum-packed wild red deer (*Cervus elaphus*) meat during 21 days of refrigerated storage at 4 ± 1 ºC. By Day 21, treated samples exhibited a mean aerobic plate count of 4.55 log CFU/g, compared to 5.60 log CFU/g in non-treated sample indicating a statistically significant reduction ($p < 0.05$). These results demonstrate the treatment's efficacy in improving microbial stability and extending the microbiological shelf life of wild red deer meat under chilled storage conditions.

Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., Barkó, A., Csurka, T., Boros, A., Hitka, G., & Varga-Tóth, A. (2024). Effects of Lactic Acid and Ascorbic Acid Mixture on Quality Properties of Wild Red Deer (*Cervus elaphus*) Meat. *Applied Sciences*, *14*(19), 8915. DOI: https://doi.org/10.3390/app14198915

3. This study is the first to apply a comprehensive **high hydrostatic pressure (HHP) treatment** (150–600 MPa for 5 minutes at 22 °C) to wild red deer (*Cervus elaphus*) meat harvested in Western Hungary, assessing its effects on texture, color, and protein profiles during 14 days of vacuum-packaged refrigerated storage (4 ± 1 °C). Pressures ≥300 MPa significantly suppressed microbial growth, with 500–600 MPa reducing aerobic plate counts to <1 log CFU/g on Day 1 and maintaining lower counts (e.g., 2.83 log CFU/g at 600 MPa) on Day 14 ($p < 0.05$), confirming enhanced microbial stability and extended shelf life.
4. Texture profile analysis showed that moderate pressures (150–300 MPa for 5 minutes at 22 °C) applied to wild red deer (*Cervus elaphus*) meat resulted in reduced hardness and shear force indicating improved tenderness, whereas higher pressures (≥450 MPa) significantly increased hardness, chewiness, and cohesiveness due to protein aggregation and gelation, as confirmed by SDS-

PAGE analysis showing sarcoplasmic and myofibrillar protein denaturation above 500 MPa.

Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., Barkó, A., Hidas, K. I., & Varga-Tóth, A. (2025). Effect of High Hydrostatic Pressure on the Quality Parameters of Wild Red Deer (*Cervus elaphus*) Meat. *Applied Sciences*, *15*(8), 4336. DOI: https://doi.org/10.3390/app15084336

5. This study is the first to systematically evaluate the effect of **sous-vide treatment** (60 °C, 65 °C, and 70 °C for 3 hours) on wild red deer (*Cervus elaphus*) meat samples across a broad age range (7, 8, 9, 12, 18, 32, 36, 37, and 48 months), all hunted in Western Hungary, revealing a significant temperature- and age-dependent effect on physicochemical and structural meat quality parameters. Sous-vide also revealed age-related differences in protein thermal stability and degradation: SDS-PAGE analysis of sarcoplasmic and myofibrillar proteins showed that younger samples (7 months) retained greater band intensity for proteins such as myoglobin and actin across sous-vide treatments, whereas older samples (18 and 48 months) exhibited more pronounced protein degradation, especially at 70 °C. These findings were corroborated by DSC thermograms, where the 7-month-old samples displayed distinct endothermic peaks indicative of partial myosin and actin denaturation while in older samples, even 60 °C treatment led to flattened curves with no detectable transitions, suggesting complete denaturation. This combined molecular and thermal evidence confirms that muscle protein stability during sous-vide cooking diminishes with increasing animal age.
6. **Sous-vide treatment** of wild red deer (*Cervus elaphus*) meat at 60 °C, 65 °C, and 70 °C for 3 hours reduced aerobic plate counts to below detectable limits (<1 log CFU/g) across all age groups (7–48 months). This demonstrates effective microbial stabilization without the use of chemical additives, supporting the technique's potential as a clean-label preservation strategy for vacuum-packaged wild game meat.

6. LIST OF PUBLICATION

6.1 Publications in Q-ranked Journals

First Author Publications

1. Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., Barkó, A., Hidas, K. I., & Varga-Tóth, A. (2025). Effect of High Hydrostatic Pressure on the Quality Parameters of Wild Red Deer (Cervus elaphus) Meat. *Applied Sciences*, *15*(8), 4336. DOI: https://doi.org/10.3390/app15084336
2. Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., Barkó, A., Csurka, T., Boros, A., Hitka, G., & Varga-Tóth, A. (2024). Effects of Lactic Acid and Ascorbic Acid Mixture on Quality Properties of Wild Red Deer (Cervus elaphus) Meat. *Applied Sciences*, *14*(19), 8915. DOI: https://doi.org/10.3390/app14198915
3. Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., Solymosi, A., Wieszt, B., Jáni, K., & Tóth, A. (2024). Influence of lactic acid and ascorbic acid mixture on the quality of wild boar meat stored under vacuum packaging at chilled storage. *Journal of Hygienic Engineering and Design,* Vol. 46, pp. 45-50.
4. Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., Solymosi, A., Wieszt, B., Jáni, K., & Tóth, A. (2023). Comparison of the effect of organic acid mixture on quality parameters of red deer meat and beef. *Progress in Agricultural Engineering Sciences*, *19*(S1), 27-33. DOI: https://doi.org/10.1556/446.2023.00079
5. Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., Surányi, J., & Tóth, A. (2023). Improvement of shelf-life of beef using lactic acid, ascorbic acid mixture and potassium sorbate. *Journal of Hygienic Engineering and Design,* Vol. 42, pp. 45-50.

Co-authored Publications

1. Elayan, M., Németh, C., Enkhbold, M., Friedrich, L., Dalmadi, I., & Varga-Tóth, A. (2025). Effects of Supplementation of Different Proteins on the Rheological Properties of Liquid Whole Eggs. *Applied Sciences*, *15*(3), 1660. DOI: https://doi.org/10.3390/app15031660
2. Varga-Tóth, A., Németh, C., Dalmadi, I., Csurka, T., Csorba, R., Elayan, M., Enkhbold, M., Hidas, K., & Friedrich, L. F. (2023). Investigation of the effects of bovine collagen peptides and mixed berries on rheological properties and biological activity of egg white-based beverage via central composite design. *Frontiers in Nutrition*, *9*, 1011553. DOI: https://doi.org/10.3389/fnut.2022.1011553
3. Elayan, M., Németh, C., Enkhbold, M., & Varga-Tóth, A. (2023). The effect of adding different oils on liquid egg products chemical and physical properties. *Journal of Hygienic Engineering and Design,* Vol. 45.
4. Elayan, M., Németh, C., Enkhbold, M., & Varga-Tóth, A. (2023). The effect of adding egg white powder on liquid egg products properties. *Journal of Hygienic Engineering and Design,* Vol. 43, pp. 141-146.

6.2 Conference Full Paper Publications

1. Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., & Varga-Tóth, A. (2024). Effects of high hydrostatic pressure on the textural properties of cooked wild red deer meat. *Review on Agriculture and Rural Development*, *13*(1-2), 3-9. DOI: https://doi.org/10.14232/rard.2024.1-2.3-9
2. Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L., Solymosi, A., Wieszt, B., Jáni, K., & Tóth, A. (2023). The impact of lactic acid and ascorbic acid mixture on quality parameters of wild boar meat. *Review on Agriculture and Rural Development*, *12*(1-2), 15-21. DOI: https://doi.org/10.14232/rard.2023.1-2.15-21

3. Enkhbold, M., Lőrincz, A., Elayan, M., Friedrich, L. F., & Tóth, A. (2023). Effects of high hydrostatic pressure on quality properties of wild red deer meat. Acta Agronomica Óváriensis, Vol 64(Ksz 1), pp. 181-187.
4. Enkhbold, M., Tóth, A., Elayan, M., Friedrich, L., Surányi, J., & Lorincz, A. (2021). Possibilities for game meat processing in Hungary. *Acta Agronomica Óváriensis* Vol. 62, pp. 40-45.

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