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

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## LIST OF ABBREVIATIONS

*a** - In the CIE *Lab** color space: *a* represents the green (–) to red (+) axis.

AA – Ascorbic Acid

ANOVA – Analysis of variance

APC – Aerobic Plate Count

ATPase – Adenosine Triphosphatase

$a_w$ – Water Activity

*b** - In the CIE *Lab** color space: *b* represents the blue (–) to yellow (+) axis.

CFU- Colony-forming unit

CIE – Commission Internationale de l' Eclairage (International Commission on Illumination)

COMb – Carboxymyoglobin

DeoxyMb – Deoxymyoglobin

DSC – Differential Scanning Calorimetry

GRAS – Generally Recognized as Safe

HHP – High Hydrostatic Pressure

IBM SPSS – International Business Machines Corporation, Statistical Product and Service Solutions

*L** - In the CIE *Lab** color space: *L* represents lightness.

LA – Lactic Acid

LTLT – Low-Temperature, Long-Time

MetMb – Metmyoglobin

MPa – Megapascal (Unit of Pressure)

MUFA – Monounsaturated Fatty Acids

n-3 PUFA – n-3 Polyunsaturated Fatty Acids; the “n-3” (or omega-3) indicates the position of the first double bond from the methyl (omega) end of the fatty acid chain

PA/PE – Polyamide / Polyethylene

PDCAAS – Protein Digestibility-Corrected Amino Acid Score

SDS-PAGE – Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis

SE - Standard Error

SEM – Scanning Electron Microscopy

SFA – Saturated Fatty Acids

SV – Sous-Vide

TBARS – Thiobarbituric Acid Reactive Substances

TPA – Texture Profile Analysis

TVC – Total Viable Count

WBSF- Warner Bratzler Shear Force

WHC – Water Holding Capacity

$\Delta E$ – Total color difference between two colors in the CIE *Lab** color space

# 1. INTRODUCTION

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 (Polak et al., 2008; Milczarek et al., 2021). Additionally, wild deer meat is rich in iron, zinc, and vitamin B12, which are essential micronutrients contributing to its classification as a high-quality protein source (Bureš et al., 2015).

Despite its nutritional benefits, preserving the quality and extending the shelf life of wild red deer meat remains a significant challenge. Unlike farmed animals that undergo controlled slaughtering and processing conditions, wild game is harvested in uncontrolled environments, leading to variability in meat quality, higher microbial loads, and increased susceptibility to spoilage (Kunová et al., 2022). Moreover, due to its low intramuscular fat content and high muscle fiber density, wild deer meat is more prone to toughness and oxidation compared to conventional meats. These factors collectively limit the commercial potential of wild deer meat, particularly in large-scale meat processing and retail markets (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 (Gill and Badoni, 2004; 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 (Naveena et al., 2006; 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 (Sun and Holley, 2010; Szerman et al., 2011; Csehi et al., 2016; Sikes and Warner, 2016; Han et al., 2021). However, its effects on wild game meat, particularly wild red deer meat, remain largely unexplored. The variability in muscle structure and connective tissue composition between farmed and wild animals suggests that the impact of HHP on wild red deer meat may differ significantly from previously studied meat types.

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 (Przybylski et al., 2021; 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 of sous-vide cooking 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. The lack of research on how age impacts the sous-vide treatment of wild red deer meat presents a critical gap in knowledge.

While each of these processing techniques organic acid treatment, HHP, and sous-vide treatment has been extensively studied in farmed meats, there is no comprehensive study comparing their effects on wild red deer meat. A comparative analysis of these methods is essential to determine which approach best enhances wild red deer meat quality and shelf life while maintaining its natural attributes. Additionally, the combined effects of processing techniques and animal age on meat quality have not been thoroughly investigated. Without this knowledge, optimizing wild red deer meat processing for commercial applications remains a challenge.

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. Additionally, it seeks to examine the influence of animal age on processing outcomes, particularly in sous-vide treatment, to determine how aging affects tenderness, protein structure, and overall meat quality. By addressing these knowledge gaps, this research will provide valuable insights into optimizing wild red deer meat processing techniques, offering scientific guidance for meat producers and the food industry. The findings of this study have the potential to support the commercialization of high-quality wild red deer meat products, contributing to the sustainable utilization of game meat resources.

# 2. OBJECTIVES

## 2.1 General Research Goal

The primary goal of this research is to evaluate the effects of **organic acid treatment, high hydrostatic pressure (HHP), and sous-vide treatment** on the quality attributes of wild red deer (***Cervus elaphus***) 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.

## 2.2 Specific Research Goals

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

### 1. Effect of Organic Acid Treatment

- **Treatment:** This study applies a 2% lactic acid and 2% ascorbic acid mixture using a spray method to wild red deer meat.
- **Evaluation Parameters**: Measures Drip Loss, Water Holding Capacity (WHC), Dry Matter, Water Activity ($a_w$), pH, Instrumental Color, Instrumental Texture Analysis, Protein Profile Analysis Using SDS-PAGE, and Microbiological Evaluation.
- **Measurement Days**: Analysis is conducted on days 1, 7, 14, and 21 during vacuum-packaged storage at 4 ± 1 °C.

### 2. Effects of HHP Treatment

- **Treatment:** The study applies HHP at 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 MPa for 5 minutes at 22 °C on wild red deer meat.
- **Evaluation Parameters:** Measures Drip Loss, WHC, Dry Matter, pH, Instrumental Color Measurement, Instrumental Texture Analysis, Protein Profile Analysis Using SDS-PAGE, and Microbiological Evaluation.
- **Measurement Days:** Analysis is conducted on days 1, and 14 during vacuum-packaged storage at 4 ± 1 °C.

### 3. Effects of Sous-Vide Treatment and Influence of Animal Age

- **Sample Selection:** This study examines 9 different wild red deer samples from animals of varying ages, along with one unknown-aged wild red deer sample to predict the probable age based on meat quality attributes.
- **Treatment:** Sous-vide treatment is performed at 60 °C, 65 °C, and 70 °C for 3 hours.
- **Evaluation Parameters:** Measures Drip Loss, WHC, pH, Instrumental Color Measurement, Instrumental Texture Analysis, Protein Profile Analysis Using SDS-PAGE, Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM) and Microbiological Evaluation.

# 3. LITERATURE OVERVIEW

## 3.1 Introduction to Meat and Its Scientific Importance

### 3.1.1 Introduction

Meat has been a fundamental component of the human diet for over one million years, providing a dense and reliable source of energy, high-quality protein, essential fatty acids, and key micronutrients (Pereira and Vicente, 2013; Klurfeld, 2015). Its nutritional profile is unmatched, offering vital elements such as iron, zinc, selenium, and vitamin B12, which play critical roles in physiological processes including growth, tissue repair, enzymatic activity, immune function, and oxygen transport (Higgs, 2000; Klurfeld, 2015).

According to European legislation, the term meat encompasses edible parts removed from the carcass of domestic ungulates (such as bovine, porcine, ovine, and caprine species), domestic solipeds, poultry, lagomorphs, and both farmed and wild game, including small and large game species (European Commission, 2004). This broad definition highlights the diversity of meat sources available for human consumption. This broad definition highlights the diversity of animal species used for human consumption.

A fundamental classification distinguishes between fresh meat and processed meat. Fresh meat refers to raw muscle that has undergone no treatment beyond basic slaughtering and primary processing, whereas processed meat is subjected to preservation methods such as salting, smoking, curing, marination, or heat application (Linseisen et al., 2002).

Furthermore, meats are categorized based on their color and species of origin. Red meat typically includes beef, veal, pork, lamb, and goat, while white meat encompasses poultry such as chicken and turkey (Linseisen et al., 2002). Alongside these conventional categories, there is increasing interest in game meat, which includes wild species such as red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), and other hunted animals. Game meats are generally characterized by lower intramuscular fat content, a favorable fatty acid profile, and a distinctive flavor, making them a valuable alternative protein source for health-conscious and environmentally aware consumers (Demartini et al., 2018).

The inclusion of game meat in human diets not only diversifies protein sources but also aligns with growing consumer preferences for natural, sustainable, and minimally processed foods. Moreover, wild meats such as wild deer meat are often associated with higher nutritional value and unique

textural and sensory properties compared to conventional domesticated meats (Hoffman and Wiklund, 2006; Demartini et al., 2018).

### 3.1.2 Global Meat Consumption Trends

Meat consumption has significantly increased over the past several decades, largely driven by rising incomes, population growth, and urbanization. This trend is expected to continue, with projections indicating global meat consumption will nearly double between 2000 and 2050 (González et al., 2020; FAO, 2024). The most rapid growth is seen in poultry and pig meat, while ruminant meats like beef and goat are also increasing, albeit more gradually (Figure 1).

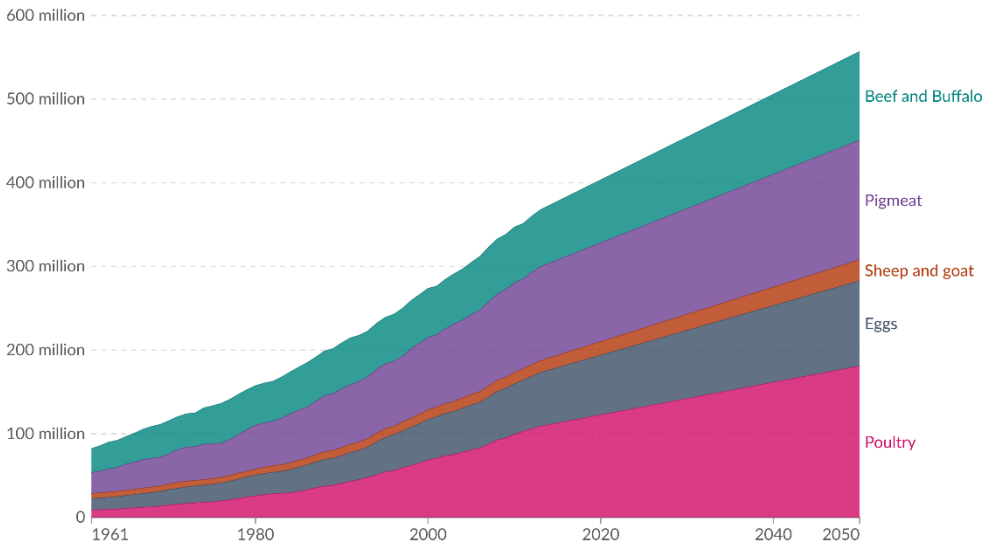


*Figure 1. Global meat and egg consumption, 1961 to 2050.*

*Global meat consumption by type, expressed in tonnes. Data from 1961–2022 are based on FAO estimates; projections from 2022–2050 are based on expected demographic and economic trends.*

*(Source: Food and Agriculture Organization of the United Nations (2024), via Our World in Data)*

Alongside global growth, meat consumption varies substantially by region. High-income countries such as the United States, Australia, and many in Western Europe typically consume more than 165 grams of meat per person per day, whereas much of Sub-Saharan Africa and South Asia remains below 40 grams per day (Figure 2) (FAO, 2024). These disparities reflect broader inequalities in food access and income, as well as cultural dietary practices.

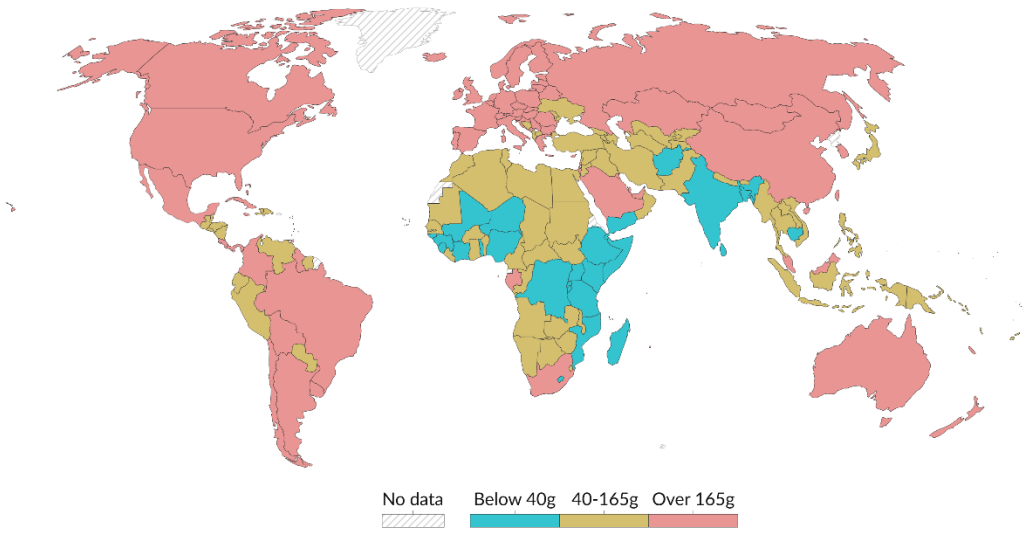


*Figure 2. Daily meat consumption per person, 2022.*

*Per capita meat supply by country compared to the EU's projected average of 165 g/day in 2030. Higher consumption is evident in North America, Europe, and parts of Latin America; lower levels dominate Africa and South Asia.*

*(Source: Food and Agriculture Organization of the United Nations (2024), via Our World in Data)*

In many Western countries, meat consumption has recently stabilized or even declined slightly, largely due to health concerns, environmental awareness, and ethical considerations. Simultaneously, consumer interest in more sustainable, lean, and natural alternatives such as wild game meat is increasing. Game meats like wild red deer meat offer lower environmental impacts and higher nutritional value, particularly in terms of lean protein and favorable fatty acid composition and may serve as a complementary solution in both high- and low-consumption contexts (Hoffman and Wiklund, 2006; Demartini et al., 2018).

### **3.1.3** Composition of Meat

Meat is a biologically complex and nutritionally dense tissue, primarily composed of approximately 75% water, 20% protein, 3% lipids, and about 2% soluble non-protein substances such as carbohydrates, vitamins, minerals, and other bioactive compounds. Its exact composition varies depending on factors such as species, age, sex, diet, and anatomical location of the muscle (Tornberg, 2005; Wyness et al., 2011).

#### *3.1.3.1 Protein and Amino Acids*

Proteins represent the most functionally significant component of meat, contributing directly to its nutritional value, structure, and texture. Meat contains complete proteins with all essential amino acids

in biologically available forms, making it a key dietary source for tissue repair and muscle maintenance. The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) for meat ranges from 0.92 up to 1.00, which is notably higher than most plant-based proteins (Pereira and Vicente, 2013).

Muscle proteins are broadly classified into three categories (Tornberg, 2005):

- **Myofibrillar proteins** (e.g., actin and myosin) are responsible for muscle contraction and meat structure, constitute approximately **50–55%** of total muscle protein.
- **Sarcoplasmic proteins** (e.g., enzymes and myoglobin) contribute to metabolic activity and meat color, constitute approximately **30–34%** of total muscle protein.
- **Connective tissue proteins** (e.g., collagen and elastin) influence the toughness and texture of meat, particularly in older animals or certain anatomical locations, constitute between **10-15%** of the total protein content.

The myofibrillar proteins are responsible for muscle contraction and significantly influence texture, especially postmortem, as they undergo conformational changes with temperature. Sarcoplasmic proteins contribute to muscle metabolism and meat color, while collagen provides tensile strength and determines toughness, particularly in older animals.

In addition to these main protein groups, meat also contains residual proteins, which, though present in smaller amounts, play essential roles (Boateng et al., 2020):

- Mitochondrial proteins (~3–5%), involved in energy production.
- Blood/plasma proteins (<1%), such as albumin and globulins, contributing to transport and immune functions.
- Membrane proteins (<1%), involved in cell signaling and ion exchange.
- Nuclear proteins (<0.5%), such as histones, associated with DNA regulation.

At the structural level, skeletal muscle is a complex, hierarchical tissue consisting of muscle bundles, fibers, and myofibrils organized into repeating units called sarcomeres. These sarcomeres contain contractile proteins, primarily actin and myosin, that govern both muscle function and postmortem meat texture. The connective tissue, cell membranes, and nuclei also play critical roles in meat quality attributes such as tenderness and juiciness (Lawrie and Ledward, 2014; Listrat et al., 2016) (Figure 3).

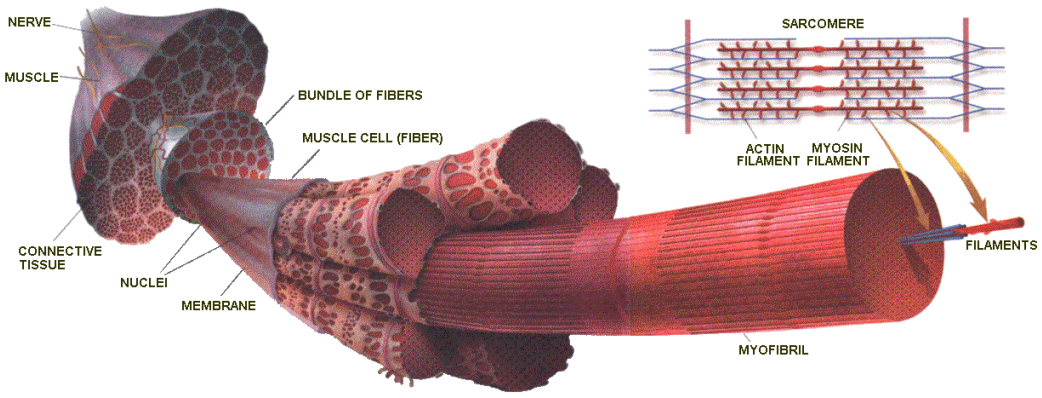


*Figure 3. Structure of Skeletal Muscle at the Microscopic Level.*

*The diagram illustrates the organization of skeletal muscle from the macroscopic muscle bundle down to the microscopic sarcomere, the functional unit responsible for muscle contraction and meat texture. (Image source: Listrat et al., 2016)*

### *3.1.3.2 Lipid and Fatty Acid Composition*

Though meat is often scrutinized for its fat content, the type and composition of fat vary significantly across species and cuts. Fat in meat exists as intramuscular (marbling), intermuscular, and subcutaneous fat. The fatty acid profile includes saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), with the latter including omega-3 and omega-6 fatty acids being essential for cardiovascular and neurological health. On average, meat lipids consist of approximately 30–40% SFA, 40–50% MUFA, and 5–10% PUFA, although these proportions vary with species and cut (Prates, 2024).

Compared to conventional livestock such as beef, pork, lamb, and poultry, wild red deer (*Cervus elaphus*) meat typically contains less total fat and saturated fatty acids, but higher levels of polyunsaturated fatty acids, especially long-chain n-3 PUFAs and conjugated linoleic acid. This gives deer meat a more favorable n-6 to n-3 ratio, a key indicator of fat quality in human diets (Woods and Fearon, 2009; Scollan et al., 2014). These nutritional advantages are complemented by a higher heme iron content and a leaner overall profile.

However, the higher PUFA content in game meat also increases its susceptibility to oxidative degradation, which can negatively impact shelf life, color, and flavor stability. Thus, understanding the lipid composition is essential for optimizing storage conditions, packaging techniques, and meat processing strategies.

#### *3.1.3.3 Vitamins and Minerals*

Meat is a dense source of micronutrients. Key vitamins include (Pereira and Vicente, 2013; Gille and Schmid, 2015):

- Vitamin B12, crucial for nerve function and red blood cell production,
- Niacin (B3), pyridoxine (B6), and riboflavin (B2), involved in energy metabolism,
- Vitamin A and folic acid, particularly abundant in organ meats like liver.

Mineral-wise, meat is especially rich in:

- Iron, mainly in the highly bioavailable heme form, helping to prevent anemia,
- Zinc, vital for immune function and enzymatic processes,
- Selenium, an antioxidant component of glutathione peroxidase.

These nutrients are present in amounts that significantly contribute to daily dietary requirements and are often more bioavailable than those in plant sources.

### **3.1.4** Meat Color and Consumer Perception

Another critical quality attribute of meat is color, which heavily influences consumer perceptions of freshness and acceptability at the point of purchase. Color is primarily determined by myoglobin, the oxygen-binding protein in muscle, and its oxidation state. Myoglobin exists in multiple forms deoxymyoglobin (purple red), oxymyoglobin (bright red), metmyoglobin (brown), and carboxymyoglobin (cherry red), each imparting a distinct hue to the meat surface depending on oxygen availability and redox reactions during storage and display (Mancini and Hunt, 2005).

Understanding these interconversion pathways is essential for meat science and processing, as discoloration is one of the leading causes of product rejection by consumers, even when safety and nutritional quality remain unaffected.

This diagram (Figure 4) illustrates the interconversion of myoglobin forms under different oxygenation and oxidation conditions, crucial for understanding meat discoloration during storage. As described by Mancini and Hunt (2005), the visible color of meat depends on the ligand bound at the sixth coordination site of the heme iron and its oxidation state:

- **Deoxymyoglobin** ($Fe^{2+}$) exists in the absence of ligands and gives meat a purplish-red appearance, typical of vacuum-packaged meat or freshly cut muscle.

- When exposed to oxygen, oxygenation occurs without a change in iron valence, forming **oxymyoglobin** ($Fe^{2+}$) and resulting in a bright cherry-red color that consumers associate with freshness.
- Upon further exposure and oxidative stress, oxymyoglobin or deoxymyoglobin oxidize to **metmyoglobin** ($Fe^{3+}$), producing a brown color. This discoloration intensifies as metmyoglobin accumulates on the surface and subsurface layers.
- Reduction reactions can revert metmyoglobin back to its ferrous forms (DeoxyMb or OxyMb), but this process depends on the muscle's enzymatic reducing capacity and availability of NADH, both of which decline postmortem.
- **Carboxymyoglobin** ($Fe^{2+}$) forms when carbon monoxide binds to deoxymyoglobin, producing a stable bright red color used in some modified atmosphere packaging. While CO binds strongly, its use in food packaging is regulated due to safety concerns, and the formation of COMb is more efficient from DeoxyMb than from OxyMb or MetMb.

These redox reactions are influenced by environmental factors such as oxygen partial pressure, temperature, pH, and the muscle's oxidative and reducing capacity, all of which affect meat's visual appeal and shelf life (Mancini and Hunt, 2005).

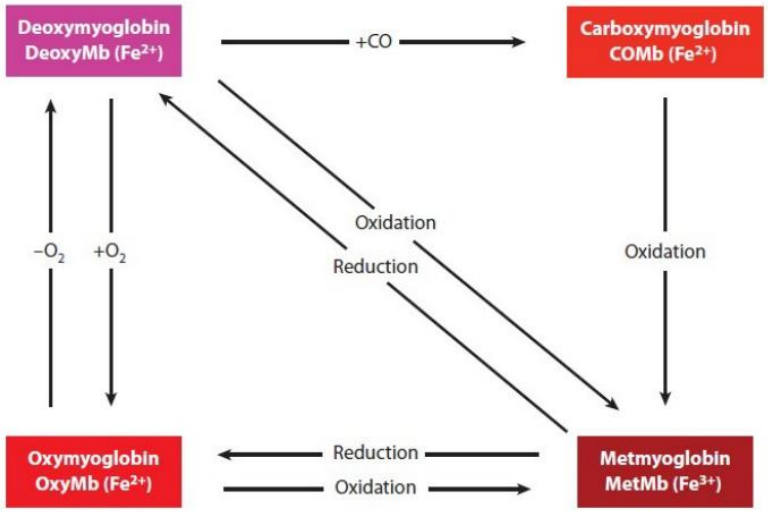


*Figure 4. Myoglobin Redox Chemistry and Meat Color Stability.*

*(Image source: Mancini and Hunt, 2005)*

### 3.2 Overview of Wild Red Deer Meat

The meat of wild animals has high nutritional value and specific sensory properties. Since ancient times, game meat has been an essential nutritional source for mankind. Due to their natural lifestyle,

wild animals have different eating habits and food quality than farm animals. As a result, game meat can be considered "organic" since it does not contain antibiotics or hormones (Vergara et al., 2003; Soriano et al., 2006). Consumers are becoming more concerned about the environment, and as a result, they are interested in organic products, as well as products made by natural production methods (Dransfield, 2003). Red deer (*Cervus elaphus*) is the one of most important species of game meat, popular in Europe, New Zealand, and Australia (Hoffman et al., 2006). Because of the low intramuscular fat and cholesterol content, as well as the high iron content (Polak et al., 2008), wild deer meat is an excellent dietary product for customers. Furthermore, this meat is high in unsaturated fatty acids, particularly long-chain n-3 polyunsaturated fatty acids, due to the natural diet and lifestyle of wild deer (Bureš et al., 2015). Wild deer primarily graze on grasses, shrubs, and other vegetation rich in omega-3 fatty acids, and unlike grain-fed domesticated animals, their natural diet and active, free-ranging lifestyle result in lower fat content and higher levels of unsaturated fats, particularly long-chain n-3 polyunsaturated fatty acids (Polak et al., 2008). Owing to its nutritional properties, deer meat consumption has been increasing each year. However, one of the challenges is its short shelf life, which is exacerbated by microbial growth (Milczarek et al., 2021). Unlike farm animals processed in controlled environments, deer are hunted in forests and fields. Factors like poor shot placement, delayed or insufficient cooling, and handling practices can compromise the microbiological safety of deer meat, further affecting its shelf life. Common bacterial species found on game meat include *Salmonella spp., E. coli, Listeria monocytogenes*, and *Campylobacter spp*., all of which can contribute to spoilage and food safety risks (Bureš et al., 2015). These issues not only affect consumer safety but also have economic implications for producers and retailers, leading to product losses and reduced marketability.

The production and sale of wild deer meat are governed by strict regulations in the European Union (EU), which ensure that wild game meat is safe for consumers. Regulation (EC) No. 853/2004 sets specific hygiene rules for the handling of wild game, requiring that stomachs and intestines be removed promptly after the kill and that a trained person conduct an inspection of the carcass to ensure it poses no health risks. The carcass must then be transported to an approved game-handling establishment for further processing (European Commission, 2004). These legal requirements, along with other food safety regulations (Regulation (EC) No. 178/2002, Regulation (EC) No. 852/2004), ensure the traceability and safety of wild red deer meat in the marketplace. The EU's regulations are aligned with similar standards worldwide, promoting consumer confidence in wild red deer meat as a safe and high-quality food product.

In Hungary, these EU regulations are complemented and enforced through national legislation, most notably Ministerial Decree No. 43/2011 (Hungarian Ministry of Rural Development, 2011), which sets detailed hygiene requirements for the treatment and marketing of killed wild game. This regulation mandates specific protocols for ante- and post-mortem inspection, evisceration immediately after shooting, and controlled transport to processing facilities. It also defines what constitutes small-scale direct sales by hunting units and outlines strict obligations for hunters when selling directly to final consumers.

Furthermore, all big game meat must be inspected and certified by a qualified game-meat inspector or veterinarian, and *trichinella* testing is mandatory for species such as wild boars. The regulation supports full traceability, requiring documentation of each animal's origin and health status. As noted by Komarek and Tóth (2019), Hungary's system reflects a strong alignment with EU law while also incorporating local oversight to maintain high-quality standards in game meat production and sales.

Various methods have been employed to inhibit microbial growth and extend meat shelf life, including hot water rinses (Castillo et al., 1998), high hydrostatic pressure, irradiation, steam pasteurization, bio preservatives (Aymerich et al., 2008), and organic acid spraying (Carpenter et al., 2011). These techniques are effective in prolonging meat shelf life by reducing or eliminating pathogenic bacteria and preventing oxidative rancidity (Naveena et al., 2006).

As global trends shift toward leaner, less processed meats, especially from wild species like red deer, understanding the scientific basis of meat composition, structure, and behavior under various treatments become increasingly important. This knowledge forms the foundation for developing novel, safe, and high-quality meat products suited to modern consumer preferences.

### 3.3 Processing Techniques to Improve Meat Quality

Modern consumers demand meat products that are not only safe and long-lasting but also retain their nutritional value, flavor, and visual appeal. Traditional preservation methods often compromise sensory and nutritional qualities due to high heat or chemical use. In response, the meat industry has increasingly adopted innovative processing technologies that can enhance safety and extend shelf life without degrading product quality. Techniques such as organic acid application, high hydrostatic pressure, and sous-vide cooking offer promising alternatives. These methods minimize thermal damage, inhibit spoilage and pathogenic microorganisms, and maintain desirable physicochemical and sensory attributes of meat products, aligning with current market expectations for minimally processed, high-quality foods (Aymerich et al., 2008).

Beyond the three methods investigated in this study, numerous other processing and preservation technologies are applied to meat, including steam pasteurization, hot water immersion, irradiation, pulsed electric fields, ultrasound, cold plasma, smoking, curing, and modified atmosphere packaging. These techniques differ in their primary aims (e.g., microbial inactivation, oxidative stability, texture modification), whether heat is applied, and the degree of structural change induced. As noted by Gómez et al. (2020), processing methods cause physicochemical and structural transformations in muscle proteins, such as denaturation, aggregation, and cross-linking, that alter water-holding capacity, tenderness, color stability, and juiciness. Moderate myofibrillar denaturation or collagen solubilization can improve tenderness, whereas excessive aggregation or cross-linking may lead to toughness and increased water loss. Non-thermal methods like cold plasma and irradiation achieve microbial safety with minimal structural alteration, while thermal processes and high-pressure treatments can produce more pronounced changes in protein conformation, influencing both sensory quality and shelf life (Beriain et al., 2018). Understanding these structural mechanisms is essential for interpreting quality outcomes and selecting the most appropriate processing technology for specific product goals.

### **3.3.1** Organic Acid treatment

Organic acids, particularly lactic acid (LA) and ascorbic acid (AA), have gained prominence as non-thermal interventions in meat processing for their dual functionality in microbial control and quality preservation.

In addition to LA and AA, other organic acids such as acetic acid, citric acid, propionic acid, and levulinic acid have also been widely studied for meat decontamination. These acids differ in antimicrobial strength, sensory impact, and effectiveness across species. For instance, acetic acid, a natural component of vinegar, has demonstrated broad-spectrum antimicrobial activity, while citric acid acts as a chelator and acidity regulator. Levulinic acid, often combined with other acids, shows promising results for reducing pathogens like *E. coli* and *Listeria monocytogenes* on meat surfaces (Carpenter et al., 2011). Propionic acid is also known for its antifungal properties and is sometimes used in combination with lactic acid in ready-to-eat products (Aymerich et al., 2008). In this study, we focused specifically on LA and AA due to their dual role in microbial inhibition and antioxidant action, particularly suited for wild red deer meat.

#### *3.3.1.1 Lactic Acid*

Lactic acid (LA) is commonly used as an organic antimicrobial agent for the decontamination of meat and meat products and is generally recognized as safe (GRAS). Its antimicrobial action is attributed to both bactericidal and bacteriostatic mechanisms, depending on application method, concentration, and meat type. Treatments using 2‑5% lactic acid sprays or dips have demonstrated significant reductions in *Salmonella Typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* on beef, buffalo, and rabbit meat surfaces (Friedrich et al., 2008; Manzoor et al., 2020).

Research has consistently shown that lactic acid reduces aerobic plate counts (APC) and spoilage organisms, thereby extending shelf life by several days under refrigerated storage. For instance, a 2% LA treatment of buffalo meat carcasses resulted in microbial reductions without negatively impacting sensory attributes such as flavor and odor, although higher concentrations (6%) could lead to surface discoloration (Manzoor et al., 2020).

However, some studies noted aesthetic and sensory drawbacks, particularly a decline in redness and consumer appeal due to pigment oxidation at higher acid concentrations. Therefore, concentration and application methods must be carefully optimized (Mancini and Hunt, 2005; Friedrich et al., 2008).

#### *3.3.1.2 Ascorbic Acid*

Ascorbic acid, commonly known as vitamin C, is valued in meat processing for its antioxidant properties and color stabilization effects. Unlike lactic acid, AA does not possess strong bactericidal properties alone, but it contributes significantly to the inhibition of lipid oxidation and metmyoglobin formation, which are primary causes of discoloration and rancidity in meat during storages (Lee et al., 1999; Naveena et al., 2006).

At concentrations of around 0.1%, ascorbic acid was shown to reduce thiobarbituric acid reactive substances (TBARS), indicating lower lipid peroxidation, and support red color retention, especially on exposed meat surfaces. However, its effectiveness is highly pH and concentration dependent, and it may act as a prooxidant in the presence of metal ions like iron and copper if not properly balanced (Lee et al., 1999).

#### *3.3.1.3 Combined Use of LA and AA*

The combined application of LA and AA via electrostatic spraying has emerged as a promising strategy to improve meat safety and extend shelf life during storage, particularly under mild-temperature conditions. In a recent study by (Yu et al., 2024), beef steaks treated with a mixture of

4% LA and 0.5% AA and stored at 10 $^{O}$C demonstrated superior microbiological stability, color retention, and sensory quality compared to individually treated or untreated controls

The treatment significantly reduced the total viable counts (TVC), *Enterobacteriaceae*, and *Brochothrix thermosphacta* throughout 24 days of storage, maintaining microbial levels below spoilage thresholds. This outcome is attributed to the antibacterial activity of LA and the antioxidant effect of AA, which together limited microbial growth and protein degradation markers such as total volatile basic nitrogen.

In terms of color stability, treated samples showed higher *L** (lightness) and *a** (redness) values, with improved surface brightness and reduced discoloration. These effects are linked to the reductive capacity of AA, which stabilizes myoglobin, and to better surface coverage achieved through electrostatic spraying.

Sensory evaluation supported the instrumental findings. The LA and AA treated beef was consistently rated higher for appearance freshness, odor, and overall acceptability especially during the later stages of storage. Volatile compound profiling further revealed that treated meat retained desirable aromatic profiles while minimizing spoilage associated volatiles.

In conclusion, combining LA and AA using electrostatic spraying effectively enhances both microbiological safety and sensory appeal of vacuum-packed beef under mild aging conditions. These results highlight its potential as a scalable intervention for improving the quality of premium or game meats such as wild red deer meat.

### **3.3.2** High Hydrostatic Pressure treatment

High Hydrostatic Pressure (HHP), also known as High Pressure Processing (HPP), is a non-thermal preservation method increasingly used to enhance the safety, shelf life, and textural quality of meat without significantly altering its nutritional or sensory characteristics. In this technique, vacuum-packed meat is subjected to pressures ranging from 100 to 600 MPa for a specified time (typically 1‑30 minutes), under controlled temperatures (4‑25 $^{O}$C). HHP effectively inactivates spoilage and pathogenic microorganisms while also inducing favorable changes in the muscle structure and water-binding capacity (Duma-Kocan et al., 2024).

### *3.3.2.1 Microbiological Effects*

High pressure disrupts microbial cells through multiple mechanisms including membrane damage, protein denaturation, impaired gene expression, and inhibited enzymatic function. Depending on the treatment intensity, these changes may lead to reversible sublethal injury or irreversible cell death. HHP significantly reduces microbial populations, including total viable counts (TVC), psychrotrophic bacteria, *Listeria monocytogenes*, and *Enterobacteriaceae*. For example, pork *semimembranosus* muscle treated at 200 MPa for 30 minutes at 20 $^{o}$C showed a sustained reduction in microbial counts over 10 days of storage, while untreated samples exceeded spoilage thresholds (Duma-Kocan et al., 2024). This microbial inactivation is achieved through mechanisms such as membrane disruption, enzyme inactivation, and loss of cell viability, all without applying heat or chemical preservatives (Garriga et al., 2004; Szerman et al., 2011; Wei et al., 2025).

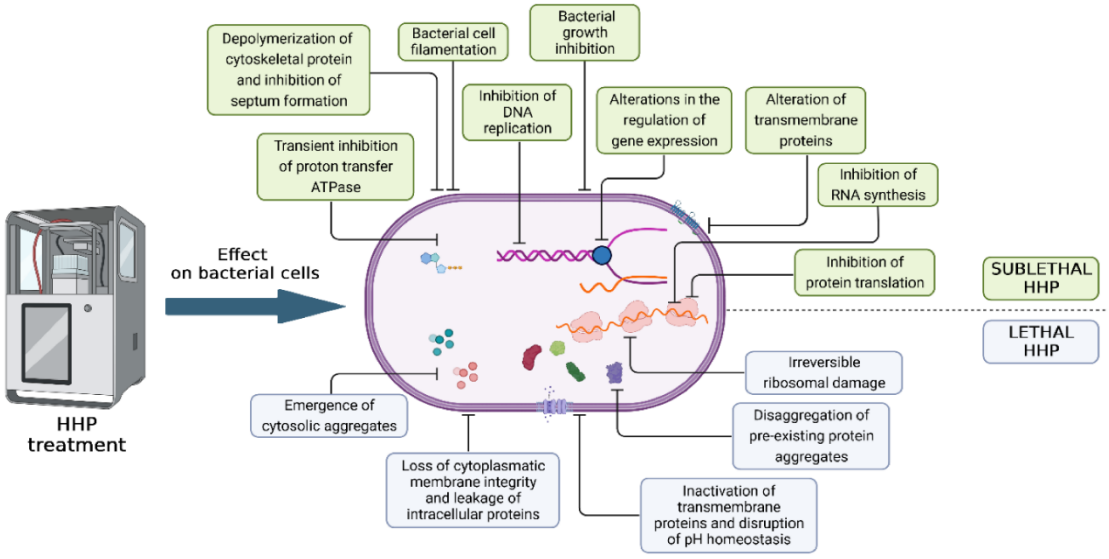


*Figure 5. Bacterial Inactivation by High Hydrostatic Pressure (HHP).*

*(Source: Shymialevich et al. 2024)*

### *3.3.2.2 Physicochemical and Structural Changes*

HHP alters the physicochemical properties of meat by modifying protein structure through the disruption of non-covalent bonds. These changes result in improved tenderness, reduced drip loss, and enhanced water holding capacity. Notably, muscle fiber shrinkage and partial collagen denaturation have been observed at pressures above 200 MPa, contributing to softer texture and better sensory appeal (Ros-Polski et al., 2015; Sikes and Warner, 2016; Şayin Sert and Coşkun, 2022). A slight pH increase in post-treatment also supports improved WHC during storage.

#### *3.3.2.3 Color and Sensory Attributes*

HHP has a measurable impact on meat color, particularly due to partial myoglobin denaturation. Generally, treatment increases *L** and *b**, while decreasing *a**, although the extent varies depending on pressure and storage time. These changes are often moderate and do not substantially affect consumer acceptance when managed with proper packaging (Şayin Sert and Coşkun, 2022; Reesman et al., 2023). Sensory evaluations confirm that HHP-treated meats retain acceptable flavor, odor, and tenderness scores (Szerman et al., 2011).

#### *3.3.2.4 Applications and Considerations*

HHP is particularly well-suited for vacuum-packed raw and cooked meat products, including wild game such as wild red deer meat. It enables sodium reduction strategies by maintaining texture and microbial safety without high salt content. However, pressures above 400 MPa can cause over-tenderization, excessive protein denaturation, and oxidative discoloration, especially in lean meats. Therefore, careful optimization of pressure level, holding time, and temperature is essential to maximize benefits while minimizing adverse effects (Yang et al., 2016; Jonas et al., 2017).

### **3.3.3** Sous-Vide technology

Sous-vide, meaning "under vacuum" in French, is a low-temperature long-time (LTLT) cooking technique in which food is vacuum-sealed in heat-stable pouches and cooked at precisely controlled temperatures for extended durations. LTLT typically involves heating meat at 50 °C to 65 °C for prolonged periods, ranging from hours to even days, allowing the product to reach its target temperature gradually and remain isothermally stable (Jeong et al., 2018). Originally developed in France, the method has transitioned from fine-dining kitchens to industrial food processing due to its efficiency in preserving nutritional quality, enhancing tenderness, and ensuring microbial safety of meat products (Thathsarani et al., 2022).

#### *3.3.3.1 Microbiological Effects*

The combination of a vacuum environment and pasteurization range temperatures (typically 55-65 ºC) in sous-vide treatment effectively reduces microbial loads and extends product shelf life. Studies have demonstrated that TVC, coliforms, and pathogens such as *Listeria monocytogenes* and *Salmonella spp*. remain stable or even decline during refrigerated storage following sous-vide cooking (Abel et al., 2020).

Shelf-life extensions of up to 30 days under chilled conditions have been reported, without significant increases in microbial growth or sensory degradation. However, precise control of time temperature parameters is critical. Inadequate thermal treatment may permit the survival of spores or psychrotrophic bacteria, posing food safety risks. Therefore, strict adherence to validated processing guidelines is essential to ensure microbiological safety throughout storage (Stanisławczyk et al., 2023).

#### *3.3.3.2 Physicochemical and Structural Changes*

Sous-vide significantly influences the structure and composition of muscle proteins and connective tissue. Long-time exposure to sub-boiling temperatures enables the gradual denaturation of collagen and softening of connective tissue without overcooking myofibrillar proteins. Compared to traditional cooking, sous-vide leads to less protein aggregation, reduced cooking loss, and improved water holding capacity, resulting in juicier meat (Roldán et al., 2013; Przybylski et al., 2021; Stanisławczyk et al., 2023). Moreover, the vacuum-sealed environment limits lipid oxidation and nutrient degradation, preserving the integrity of fatty acids, amino acids, and bioactive compounds (Stanisławczyk et al., 2023).

Instrumental color analysis also shows favorable results under sous-vide treatment. The method preserves desirable color attributes, particularly *a**, due to reduced myoglobin oxidation, while maintaining surface lightness and visual appeal (Roldán et al., 2013).

#### *3.3.3.3 Applications and Considerations*

Sous-vide technology has found increasing relevance in the commercial processing of meat products, including lean and collagen-rich meats such as wild game and older animals. Its controlled temperature environment and vacuum packaging make it particularly effective for species such as wild red deer (*Cervus elaphus*), which are characterized by low intramuscular fat content and dense muscle fibers. Sous-vide mitigates these challenges by enhancing tenderness and juiciness, while maintaining distinct flavor characteristics that are often diminished by conventional high-heat cooking methods (Lorenzo et al., 2019).

In addition to sensory benefits, sous-vide provides an effective microbial barrier when combined with refrigerated storage, making it suitable for ready-to-eat or cook‑chill products with extended shelf life (Thathsarani et al., 2022). The method is also gaining attention in the context of clean-label processing, as it does not rely on chemical preservatives to maintain product quality.

However, the adoption of sous-vide at an industrial scale involves several considerations. It requires specialized vacuum sealing and temperature control equipment, along with strict compliance to food safety standards to prevent pathogen survival (Shymialevich, Wójcicki and Sokołowska, 2024). Furthermore, because sous-vide cooking occurs below boiling point, the method is not suitable as a sterilization process and cannot replace traditional thermal processing for long-term ambient storage.

# 4. MATERIALS AND METHODS

## 4.1 Preparation of Samples

### 4.1.1 Organic Acid Treatment **(Experiment 1)**

#### *4.1.1.1 Preparation of Wild Red Deer Meat*

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 $^{o}$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. This resulting in a total of 48 steaks, which were randomly divided between two treatment groups: 24 steaks were treated with a 2% lactic acid and 2% ascorbic acid mixture, and 24 steaks were left non-treated as control samples. For packaging, two steaks were placed in each vacuum bag, resulting in 12 packs per group. Each treatment group was further divided into four storage time points (Day 1, 7, 14, and 21), with three packs assigned to each time point. Therefore, for each time point and treatment condition, three replicates were evaluated, and the mean of the three replicates was calculated for each quality parameter. The meat preparation and experimental setup were based on our previously published methodology (Enkhbold et al., 2024).

#### *4.1.1.2 Preparation of Spray Solution*

The lactic acid and ascorbic acid mixture solution was prepared by diluting lactic acid (Molar Chemicals Kft., Halásztelek, Hungary) and ascorbic acid (VitalTrend Kft., Budapest, Hungary) in distilled water to make a 2% + 2% (v/v) acid solution. The measured pH of the solution was 2.48 ± 0.04. A total of 500 mL of (v/v) lactic acid and ascorbic acid solution was applied through a manual sprayer to the meat samples. The temperature of the solution was maintained at 20 ± 1 $^{o}$C during application.

#### *4.1.1.3 Application of Treatment Solution*

Steaks in the control group were vacuum-packed and kept in a refrigerated cabinet at 4 ± 1 $^{o}$C. To reflect typical commercial handling, control samples were not sprayed with distilled water to avoid introducing moisture-related changes. 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, with 5 g of solution used for every 500 g of meat. After treatment, all samples were vacuum-packed using a C200 vacuum packer (Multivac Ltd., Geprüfte Scherhert, AGW, Wolfertschwenden, Germany) and stored at 4 ± 1 °C for 21 days. The vacuum packaging material used was PA/PE 90, with a moisture vapor permeability of 2.6 g/m$^2$ per day, oxygen permeability of 50 cm$^3$/m$^2$, carbon dioxide permeability of 150 cm$^3$/m$^2$, and nitrogen permeability of 10 cm$^3$/m$^2$. The assessment of quality parameters occurred on days 1, 7, 14, and 21 during the storage period.

### **4.1.2** High Hydrostatic Pressure (HHP) Treatment **(Experiment 2)**

#### *4.1.2.1 Preparation of Wild Red Deer Meat*

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. Resulting in a total of 66 steaks. The experiment included two sampling points (Day 1 and Day 14), with 33 vacuum-packed steaks per time point, randomly assigned to 1 control and 10 pressure treatments, each with 3 replicates. All samples were vacuum-packed in PA/PE 90 polyethylene bags using a C200 vacuum packer (Multivac Ltd., Geprüfte Scherhert, AGW, Wolfertschwenden, Germany).

#### *4.1.2.2 HHP Treatment*

Vacuum-packed samples were subjected to HHP using the Resato FPU-100-2000 HHP equipment (Resato Int. B.V., Roden, Netherlands) (Figure 6). The samples were pressurized at 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 MPa for 5 minutes at 22 °C. A total of 11 sample groups, including a non-treated control, were prepared.

Following treatment, all samples were stored at 4 ± 1 °C for 14 days. Quality parameters were evaluated on days 1, and 14 of storage. The meat preparation and experimental setup were based on our previously published methodology (Enkhbold et al., 2025).

*Figure 6. Resato FPU-100-2000 HHP equipment and loaded vacuum-packed meat samples into the HHP chamber. (Photo by the author)*

### **4.1.3** Sous-Vide Treatment **(Experiment 3)**

#### *4.1.3.1 Preparation of Meat Samples*

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*) of varying ages. Additionally, one sample of 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.

**Table 1**. Details of Deer Meat Samples Used in the Experiment

| Sample No. | Deer Type | Age | Gender |
|---|---|---|---|
| 7 | Red Deer | 7 months | Female |
| 8 | Red Deer | 8 months | Female |
| 9 | Red Deer | 9 months | Female |
| 12 | Red Deer | 12 months | Female |
| 18 | Red Deer | 18 months | Female |
| 32 | Red Deer | 32 months | Female |
| 36 | Red Deer | 36 months | Female |
| 37 | Red Deer | 37 months | Female |
| 48 | Red Deer | 48 months | Female |
| U | Red Deer | Unknown | Unknown |

The sample codes are structured as follows: The number represents the age of the deer in months, except for U, which has an unknown age.

Each sample was cut into four equal-sized steaks of approximately 14 × 7 × 2 cm and 250 ± 10 g, then vacuum-packed. One steak from each animal was assigned to each treatment group: raw control, 60 °C, 65 °C, and 70 °C sous-vide. This resulted in a total of 40 steaks (10 per group). To minimize biological variation, the control and all three sous-vide temperature treatments (60 °C, 65 °C, and 70 °C) were applied to samples taken from the same individual.

#### *4.1.3.2 Sous-Vide Treatment*

The vacuum-packed samples were subjected to sous-vide treatment using Maxima sous-vide stick (Maxima, Model 09500500, Mijdrecht, Netherlands) (Figure 7). Each sample underwent cooking for 3 hours at three different temperatures: 60 $^{O}$C, 65 $^{O}$C, and 70 $^{O}$C. Each sous-vide treatment group consisted of 10 vacuum packed samples, one from each of the 10 individual deer, allowing for direct within-animal comparison across treatments. After treatment, the samples were removed from the water baths and stored at 4 ± 1 $^{O}$C for further quality evaluation.

*Figure 7. Sous-vide equipment (Maxima) used for the experiment and treatment of vacuum-packed wild red deer meat samples during sous-vide processing (Photo by author)*

## 4.2 Quality Evaluation

### 4.2.1 Drip Loss Measurement

Drip loss reflects the amount of water lost from meat during storage and is a key indicator of water-holding capacity, which affects juiciness, appearance, and consumer acceptance (Huff-Lonergan and Lonergan, 2005). The method used was adapted from Honikel (1998).

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. Drip loss was calculated using the following Formula (1):

$$Drip\ loss\ (\%) = \frac{Initial\ weight - Drip\ weight}{Initial\ weight} \times 100 \qquad (1)$$

**4.2.2** Cooking Loss Measurement

Cooking loss indicates the amount of water and soluble matter lost from meat during cooking, primarily caused by protein denaturation, connective tissue shrinkage, and structural changes in the muscle (Hamm, 1977; Offer, 1984; Honikel, 1998). After cooking, samples were cooled in an ice slurry, stored at 4±1 °C until equilibrated, removed from the bag, blotted dry, and weighed. Cooking loss was calculated using the following Formula (2):

$$Cooking\ loss\ (\%) = \frac{Raw\ weight - Cooked\ weight}{Raw\ weight} \times 100 \qquad (2)$$

**4.2.3** Water Holding Capacity Determination

Water-holding capacity (WHC) is the ability of meat, or meat products, to retain all or part of its own or added water when subjected to external forces such as cutting, heating, pressing, or grinding. This property is largely determined by the structure and functional state of the myofibrillar proteins, the pH of the muscle, and post-mortem biochemical changes. WHC directly influences important quality traits such as juiciness, tenderness, appearance, and processing yield, and it can vary significantly with animal species, muscle type, and handling conditions (Huff-Lonergan and Lonergan, 2005).

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. WHC was calculated as the juice area per gram of meat using Formula (3):

$$WHC\left(\frac{mm^2}{g}\right) = \frac{Area\ of\ meat\ juice\ (mm^2)}{Sample\ weight\ (g)} \qquad (3)$$

### 4.2.4 Determination of Dry Matter Content

Dry matter is the portion of meat that remains after all water has been removed. 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. Dry matter content was calculated as:

$$Dry\ matter\ (\%) = \frac{Weight\ after\ drying}{Initial\ sample\ weight} \times 100 \qquad (4)$$

### 4.2.5 Water Activity Measurement

Water activity ($a_w$) was measured to assess the availability of free water in meat samples, an essential parameter affecting microbial stability, shelf life, and texture. The measurements were performed using a LabMaster-aw neo water activity meter (Novasina AG, Lachen, Switzerland) (Figure 8) at a controlled room temperature of 20 $\pm$ 1.5 $^{O}$C. Three replicates were analyzed from each sample.


*Figure 8. Water activity equipment (LabMaster-aw neo) used for the experiment (Image source: Internet 1)*

### 4.2.6 pH Determination

The pH values of deer meat samples were measured using a handheld 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. After each measurement, the pH meter was cleaned, and its calibration was verified using buffer solutions with pH values of 4.0 and 7.0, following the manufacturer's instructions.

### **4.2.7** 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. The instrument was routinely calibrated using a white tile and the same PE vacuum pack in which the samples were stored before each measurement session. 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). Color coordinates were recorded in the CIE *Lab** color space, where:

- *L** represents lightness from black (0) to white (100),
- *a** represents redness when positive and greenness when negative,
- *b** represents yellowness when positive and blueness when negative.

Additionally, chroma (*C**), indicating color vividness, was calculated using the following Formula (5):

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (5)$$

The hue angle (*h*), which describes the hue of the color (Savadkoohi *et al.*, 2014), was calculated as Formula (6):

$$h = \arctan\left(\frac{b^*}{a^*}\right) \quad (6)$$

The color difference ( $\Delta E$) was calculated using Formula (7):

$$E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (7)$$

To interpret the $\Delta E$ values, the observer criteria outlined by Mokrzycki and Tatol (2011) were applied, which categorize color differences as follows:

- When $0 < \Delta E < 1$, the observer does not notice the difference;
- When $1 < \Delta E < 2$, only an experienced observer may notice the difference;
- When $2 < \Delta E < 3.5$, an inexperienced observer also notices the difference;
- When $3.5 < \Delta E < 5$, a clear difference in color is noticed;
- When $\Delta E > 5$, the observer notices two different colors.

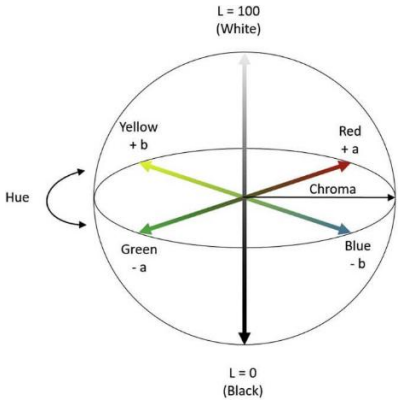


*Figure 9. Chroma Meter (CR-400, Konica Minolta) used for the experiment and CIELAB Color Space Diagram. (Image source: Internet 2 and Internet 3)*

### **4.2.8** Instrumental Texture Measurement

#### *4.2.8.1 Warner-Bratzler Shear Force*

The Warner–Bratzler shear force (WBSF) test is a widely used objective method for evaluating the tenderness of meat by measuring the maximum force required to cut through raw and cooked meat sample (Bratzler, 1958; Girard et al., 2012). Tenderness is influenced by both myofibrillar (muscle fibre) and connective tissue components, which contribute differently to the shear force deformation curve (Møller, 1980). The initial yield or first peak is generally attributed to the myofibrillar structure, while later peaks reflect the strength of connective tissue networks such as collagen fibres.

*Figure 10. TA.XT Plus Texture Analyzer (Stable Micro Systems) used for the measurement and the measurement process during the texture analysis of wild red deer samples (Photo by the author)*

For WBSF analysis, samples were cut on a slab shape in the size of 15 × 15 × 50 mm (width, thickness, length) and cut perpendicular to the orientation of fibers with a Warner‑Bratzler knife blade with a flat end at 2 mm/s speed using a TA.XT Plus texture analyzer (Stable Micro System, Surrey,

United Kingdom) (Figure 10). Texture Exponent 32 software for Windows (Stable Micro System) was used for processing the measured data. The obtained maximum peak force (N) was registered as a shear force value. Ten parallel measurements were performed for each sample. The measurements were performed at room temperature of 20 ± 1 $^{o}$C.

*4.2.8.2 Texture Profile Analysis (TPA)*

Texture Profile Analysis (TPA) is a method used to simulate the mechanical action of chewing and to quantify different texture characteristics of meat in a single test. The procedure involves two consecutive compressions of the sample, and the force–time data recorded from these compressions are used to calculate textural parameters.

All instrumental texture measurements were conducted using an SMS TA. XT Plus Texture Analyzer (Stable Micro System Ltd., Godalming, UK) (Figure 11a) with Texture Exponent 32 software. Wild red deer meat samples for TPA were shaped into cylinders with a diameter and height of 12 mm. Meat samples were positioned under a cylindrical probe with a diameter of 35 mm. The probe moved downward at a consistent speed of 3.0 mm/s during the pre-test phase, 1.0 mm/s during the test phase, and 3.0 mm/s during the post-test phase. The probe penetrated 70% of the sample height, retracted, paused for 2 s, and then performed a second compression. Resistance (N) was recorded every 0.01 s and plotted on a force-time plot (De Huidobro et al., 2005; Jonas et al., 2017).

a) b)

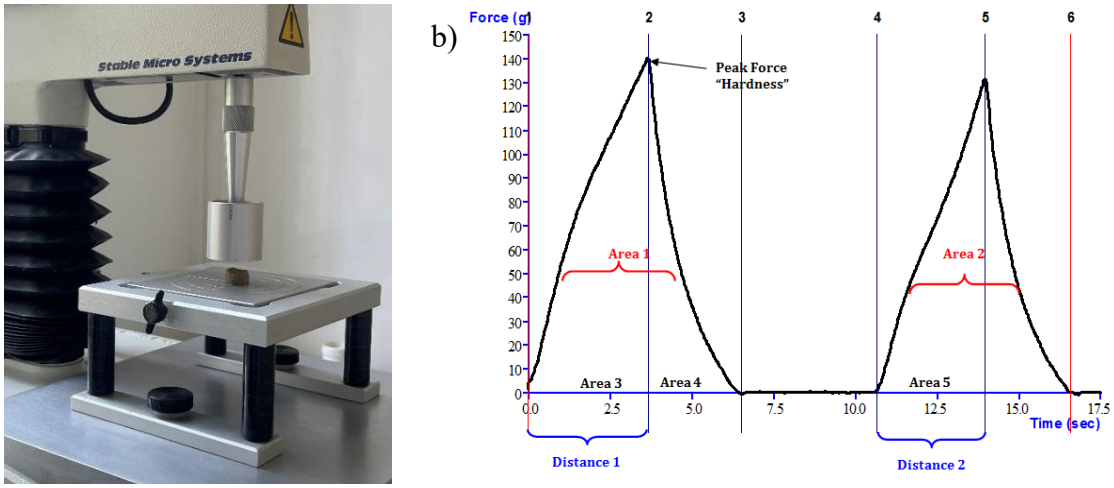


*Figure 11. Texture profile analysis (TPA) measurement: a) TA.XT Plus texture analyzer with a P/35 cylinder probe , (Photo by the author) b) Two-cycle compression graph (Image source: Internet 4)*

TPA parameters were derived from the data obtained from the two-cycle compression graph (Figure 11b). Hardness was determined as the peak force of the first compression (first bite) and expressed in Newtons. Cohesiveness was calculated as the ratio of the area under the second compression curve to

the area under the first compression curve. Springiness was defined as the sample's recovery distance after the first compression cycle or the time interval between the end of the first compression and the start of the second compression cycle, with results expressed in mm. Chewiness was calculated and expressed in mJ.

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

SDS-PAGE is a widely used analytical technique for separating proteins based on their molecular weight. It provides a visual profile of the protein composition in meat and allows comparison of sarcoplasmic and myofibrillar protein fractions under different treatments. The technique is particularly valuable for identifying postmortem protein degradation, heat-induced denaturation, and structural changes in muscle proteins during storage or processing (Jay, 2002).

#### *4.2.9.1 Isolation of Sarcoplasmic and Myofibrillar Proteins*

To analyze the protein composition of wild red deer (*Cervus elaphus*) meat, sarcoplasmic and myofibrillar proteins were isolated following a modified version of the method described by Csehi et al. (2016) based on Kretzschmar protocol (1995).

- **Sarcoplasmic Protein Extraction:** Minced deer meat samples (5 g) were homogenized with 10 mL of 0.05 M NaCl solution using an Ultra-Turrax T25 homogenizer (Ika Werke, Staufen, Germany) at 13,500 rpm for 3 minutes and 30 seconds, with intermittent pauses to prevent heating. Ice was placed under the tubes to maintain sample integrity. The homogenized suspension was centrifuged at 6,000 rpm (Beckman J2-21, Beckman Coulter, Brea, CA, USA) for 20 minutes at 4 °C. The supernatant, containing sarcoplasmic proteins, was filtered into test tubes and stored at −24 °C until further analysis.
- **Myofibrillar Protein Extraction:** The pellet remaining after sarcoplasmic protein extraction was washed twice with 10 mL of 0.05 M NaCl solution and centrifuged at 6,000 rpm for 20 minutes. The washed pellet was resuspended in 10 mL of 0.7 M NaCl solution and homogenized for 1 minute under the same conditions. Following another 20-minute centrifugation, the supernatant containing myofibrillar proteins was filtered and stored at −24 °C until analysis.

#### *4.2.9.2 SDS-PAGE Conditions*

SDS-PAGE was conducted using hand-cast polyacrylamide gels (acryla-mide/bis-acrylamide, 830 × 730 × 1.0 mm) in a vertical electrophoresis system (Mini-PROTEAN Tetra System, Bio-Rad, USA), following the method of Laemmli (1970), with modifications based on previous studies (Csehi et al.,

2016; Darnay et al., 2021). The molecular weight marker (Precision Plus Protein Standards All Blue, Bio-Rad, USA) covered a range of 250‑10 kDa.

Protein extracts were diluted with Laemmli sample buffer (2×) containing 10% 2-mercaptoethanol (Bio-Rad, USA). Sarcoplasmic proteins were diluted 20-fold, while myofibrillar proteins were diluted 2-fold before loading. The mixtures were heated at 95 °C for 2 minutes to denature proteins. A total of 4 μL of each diluted protein solution was loaded into the gel wells, and electrophoresis was performed at a constant voltage of 200 V for 45 minutes. Gel images were captured using a Bio-Rad Gel Doc XR system and analyzed with ImageLab 6.1 software (Bio-Rad, USA).

### **4.2.10** Protein Analysis Using Differential Scanning Calorimetry (DSC)

DSC is a thermoanalytical technique used to measure the heat flow associated with protein denaturation as a function of temperature. In meat science, DSC is commonly applied to determine the thermal stability and denaturation temperatures of muscle proteins, which reflect structural changes during processing and help predict functional properties such as texture and water-holding capacity (Hwang et al., 2019).

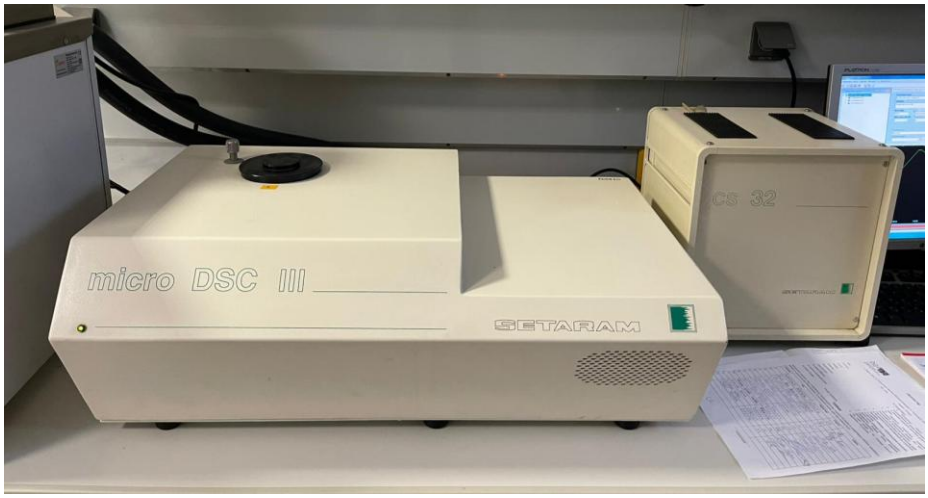


*Figure 12. Micro DSC III used for experiment (Photo by author)*

In this study, protein thermal properties were analyzed using a Micro DSC III microcalorimeter (Setaram Inc., Caluire, France) (Figure 12). Distilled water was used as a reference for all measurements. Measurements were conducted within a temperature range of 20°C to 90°C. The heating rate from 20°C to 90°C was set at 0.8°C/min, while the cooling rate from 90°C to 20°C was 1°C/min.

Sample weights were standardized at 200 ± 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).

#### **4.2.11** Scanning Electron Microscope Imaging

Scanning Electron Microscopy (SEM) is used to observe the surface microstructure of biological tissues at high magnification and resolution (Yin *et al.*, 2020). In meat science, SEM allows the examination of muscle fiber arrangement, connective tissue integrity, and changes due to storage, treatment, or cooking. It provides visual evidence of physical alterations that correlate with textural and structural quality.

Sample preparation for SEM was performed following standard protocols. Wild red deer meat samples were cut into very thin slices measuring approximately 0.5 mm × 1 mm × 3 mm (thickness × width × length) using a sterile razor blade to ensure adequate fixative penetration and minimize structural distortion.

- **Fixation:** Samples were immersed in 2.5% glutaraldehyde (Sigma-Aldrich, USA) for 24 hours at 4 °C to preserve ultrastructure by crosslinking proteins.
- **Rinse:** After fixation, samples were rinsed 3 times for 10 minutes each in phosphate-buffered saline (PBS, pH 7.2) to remove residual fixative and prepare tissues for dehydration.
- **Dehydration:** Samples were dehydrated in 99.8% ethanol, twice for 24 hours each. This step is critical, as SEM requires samples to be completely dry, residual water can cause structural collapse or imaging artifacts under vacuum.
- **Drying:** Freeze-drying was used to further preserve tissue morphology and eliminate remaining moisture while avoiding shrinkage, using a freeze dryer (Lach-Ner, Neratovice, Czech Republic).
- **Mounting:** Fully dried samples were mounted on aluminum SEM stubs using conductive adhesive tape.
- **Imaging:** Micrographs were obtained using a Prisma E SEM (Thermo Fisher Scientific, USA) at 20 °C, under high vacuum (130 Pa) and 100% relative humidity (Figure 13).

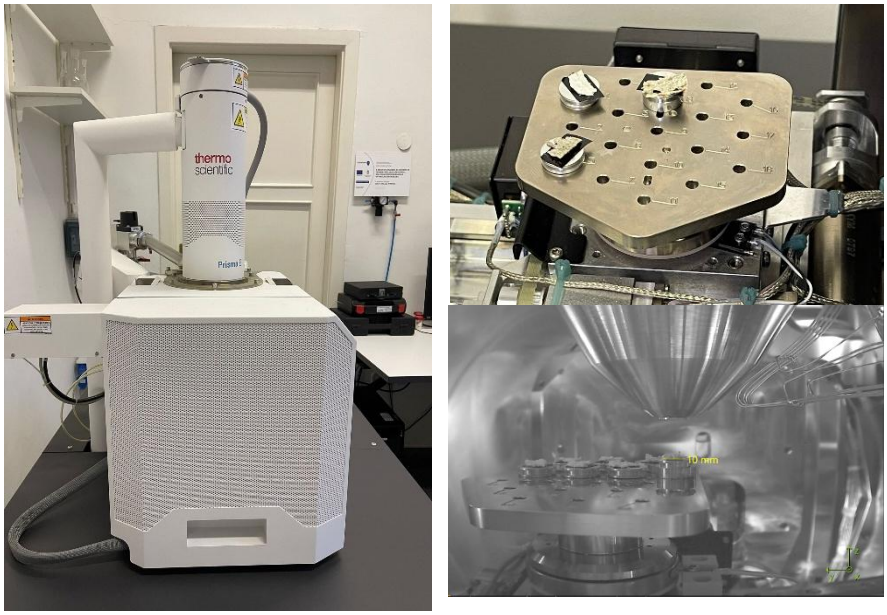


*Figure 13. Scanning Electron Microscope used for imaging and sample stage loaded with wild red deer meat samples (Photo by author)*

### **4.2.12** Microbiological Evaluation

Microbiological quality is a key indicator of meat safety and shelf life. The Aerobic Plate Count (APC) method was used to estimate the total viable count of mesophilic aerobic bacteria in wild red deer meat samples. APC are among the most widely used microbiological tests to assess the overall microbial load in fresh meat and meat products. APC results provide insight into the hygienic conditions of processing, handling, and storage, and are often used to estimate product freshness, shelf life, and potential safety concerns (ICMSF, 2006; Kim et al., 2018).

In the European Union, microbiological limits for foods are regulated under Commission Regulation (EC) No. 2073/2005, which defines food safety and process hygiene criteria. While specific APC limits may vary by product and country, APC values below $10^5$–$10^6$ CFU/g are generally considered acceptable for fresh meat, whereas levels above $10^7$ CFU/g are associated with spoilage and potential safety concerns.

In this study, 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 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).

### 4.3 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. For experiments involving multiple pressure levels or temperature variations, one-way ANOVA followed by Tukey's HSD test was applied.

Statistical significance was defined at $p < 0.05$ throughout the study. However, in certain cases, highly significant differences ($p < 0.001$) were also presented. Graphical representations and data visualizations were generated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

# 5. RESULTS AND DISCUSSION

## 5.1 Organic Acid Treatment (Experiment 1)

### 5.1.1 Moisture-Related Parameters (Drip Loss, WHC, Dry Matter, and Water Activity)

The impact of a 2% lactic acid and 2% ascorbic acid mixture on the moisture-related parameters of wild red deer meat was assessed over 21 days of refrigerated storage. The key parameters analyzed include drip loss, water holding capacity (WHC), dry matter content, and water activity ($a_w$). These results are presented collectively in Figure 14 (a-d) to highlight the correlations between these moisture-related properties.

**Drip Loss (Figure 14a):** On Day 1, no significant difference was observed between non-treated and treated samples (5.86%±0.51 and 5.25%±0.65, respectively). However, a statistically significant reduction ($p < 0.05$) in drip loss was observed on Day 7 for treated samples (8.07%±1.26) compared to non-treated samples (11.07%±1.1). This indicates that the organic acid treatment effectively reduced early-stage water loss. By Days 14 and 21, no significant differences were found, with drip loss levels stabilizing across both groups.

**Water Holding Capacity (Figure 14b):** No significant differences ($p > 0.05$) in WHC were observed across the storage period except Day 21. Both treated and non-treated samples exhibited similar trends, with WHC decreasing over time, particularly by Day 21, where non-treated samples maintained a significantly higher WHC (0.00089±0.00011 g/mm$^2$) compared to treated samples (0.00069±0.00011 g/mm$^2$).

**Dry Matter Content (Figure 14c):** A statistically significant difference ($p < 0.001$) was observed on Day 14, where treated samples exhibited lower dry matter content (24.96%±0.22) compared to non-treated samples (26.70%±0.25). This trend continued through Day 21 ($p < 0.01$), suggesting improved water retention in the treated meat. The significant reduction in dry matter percentage aligns with the observed lower drip loss and higher WHC in treated samples.

**Water Activity (Figure 14d):** On Day 1, treated samples exhibited significantly lower water activity values ($p < 0.001$) compared to non-treated samples, indicating reduced free water availability due to the treatment. Statistically significant differences persisted on Days 14 and 21 ($p < 0.05$), suggesting that the treatment effectively delayed the increase in water activity, which could inhibit microbial growth during storage.

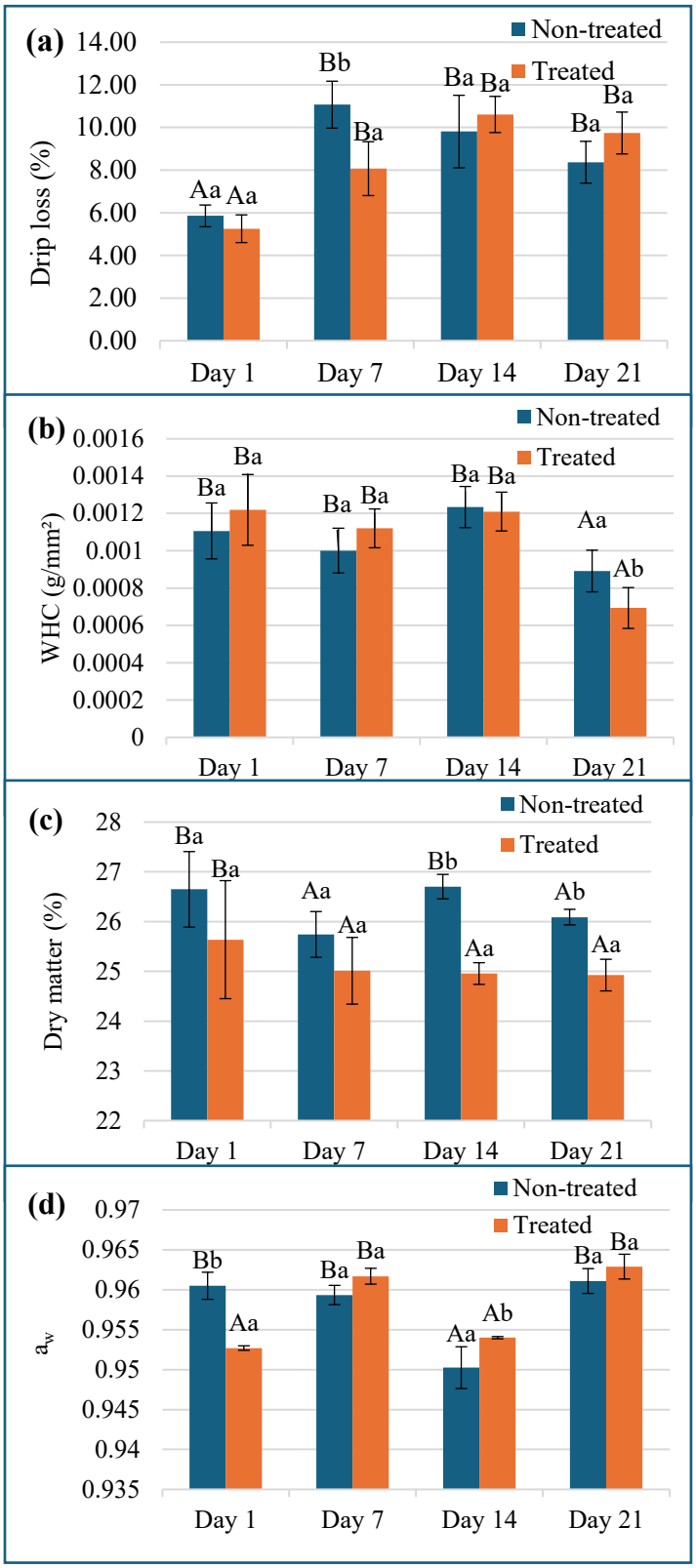


*Figure 14. Moisture-related parameters of treated and non-treated wild red deer meat during storage on Days 1, 7, 14, and 21: (a) Drip loss (%), (b) Water holding capacity (g/mm $^2$), (c) Dry matter (%), and (d) Water activity. Means with different letter (A, B) indicate significant differences ($p < 0.05$) between storage periods (Days 1, 7, 14, and 21), while means with different letter (a, b) indicate significant differences ($p < 0.05$) among non-treated and treated samples.*

Overall, good quality and stability in meat are typically characterized by low drip loss, high water-holding capacity, stable dry matter content, and low water activity, as these traits indicate better juiciness, texture, and microbial stability.

These findings indicate that the application of 2% lactic acid and 2% ascorbic acid mixture significantly enhanced the moisture retention properties of wild red deer meat, particularly during the initial two weeks of refrigerated storage. The treatment effectively reduced drip loss and dry matter content, while also maintaining a higher WHC and lowering $a_w$, thereby improving the overall quality and stability of the meat during early storage. However, the treatment effectiveness diminished over time, as differences between treated and non-treated samples became statistically non-significant by Day 21. This suggests that while the organic acid treatment is beneficial for short-term preservation, additional preservation methods may be necessary to maintain meat quality and prolong shelf life during extended storage periods. Similar results have been reported in previous studies (Cheng and Sun, 2008).

### **5.1.2** pH

The pH values of wild red deer meat at four time points (day 1, 7, 14, and 21) for both treated and non-treated groups are shown in Table 2. The pH did not significantly differ between treated and non-treated samples except on Day 1. The combination of LA and AA decreased the pH of meat samples by about 0.08 units by Day 1. This reduction aligns with prior studies indicating that the application of organic acids to beef can effectively reduce muscle pH post-treatment (Oreskovich et al., 1992; Seuss and Martin, 1993; Aktaş et al., 2003).

**Table 2.** Effects of 2% lactic acid and 2% ascorbic acid mixture on pH of wild red deer meat during 21 days at 4 ± 1 °C.

| **Characteristics** | | **Day 1** | **Day 7** | **Day 14** | **Day 21** | **SE** |
|---|---|---|---|---|---|---|
| pH | Treated | $5.60^{b}_{x}$ | $5.57^{b}_{x}$ | $5.33^{a}_{x}$ | $5.38^{a}_{x}$ | 0.06745 |
| | Non-treated | $5.68^{c}_{y}$ | $5.56^{bc}_{x}$ | $5.38^{a}_{x}$ | $5.31^{a}_{x}$ | 0.0843 |

Means in the same row within an attribute with no letters in common (a, b, c; superscript) indicate significant differences across storage periods ($p < 0.05$); means in the same column with no letters in common (x, y; subscript) indicate significant differences in treatment effects ($p < 0.05$). SE = Standard Error.

During storage, the pH of treated wild red deer meat samples displayed significant fluctuations. Initially, the pH decreased and then increased slightly toward the end of the storage period, with a few

exceptions. This initial decline can be attributed to the acid treatment, while the subsequent rise may be linked to microbial activity and metabolite accumulation during spoilage. Conversely, the non-treated samples exhibited a continuous decrease in pH throughout the storage period. This decline in pH may be due to the natural fermentation processes occurring as spoilage microorganisms metabolize the meat's nutrients. As bacteria proliferate, they can produce organic acids as metabolic byproducts, which contribute to a decrease in pH (Goddard et al., 1996). Additionally, as these microorganisms consume glucose and other energy sources, their activity can lead to a shift in the pH balance, resulting in a more acidic environment (Gill and Badoni, 2004). Jose et al. (1984) also observed that pH decreases at the onset of spoilage but subsequently increases as spoilage progresses.

### **5.1.3** Surface Color

Changes in CIE $L^*$, $a^*$, $b^*$, hue angle, and chroma values throughout the display of wild red deer meat samples are shown in Table 3. The $L^*$ values of treated and non-treated meat samples did not show a significant difference from each other except on day 14, when the non-treated sample was significantly higher than the treated one. Overall, $L^*$ values between 27.5 and 30.3 correspond to a moderately dark red appearance typical of wild red deer meat, with higher $L^*$ indicating a slightly lighter red tone. These results are comparable to the results of Naveena et al., (2006), who reported lactic acid (up to 5%) treated meat had no difference in $L^*$ value from control except on day one when 2% LA treated meat was lighter (higher $L^*$ value). However, ref. (Naveena et al., 2006) dipped Biceps femoris steaks in 2% lactic acid for 60 s, whereas Kotula and Thelappurate (1994) dipped Longissimus dorsi steaks in 0.6% and 1.2% lactic acid for 120 s, and Stivarius et al. (2002) tumbled beef trimmings in 5% lactic acid. All these studies showed that lactic-acid-treated meat had higher $L^*$ values than non-treated meat. These different results could be due to the fact that these researchers either dipped or tumbled steaks or trimmings into lactic acid solutions, whereas in this study and the one performed by Rodríguez-Melcón et al. (2017), lactic acid was only sprayed on the surface of the meat. Therefore, no difference in the $L^*$ value was measured.

As shown in Table 3, treated dear meat samples had not significantly different $a^*$ values compared to non-treated control samples. The $a^*$ values started around 9.2–9.4 on Day 1, which visually corresponds to a pale rose color, and significantly increased to 11.0–11.8 by Day 21, representing a bright cherry-red appearance. Metmyoglobin decrease and brown-to-red color reversion have also been recorded in vacuum-packed meats with increasing storage duration due to the action of endogenous muscle reductants in the absence of oxygen (Pierson et al., 1970). Although several

factors can impact meat color stability, metmyoglobin formation by free radicals is predominant (Renerre and Labas, 1987).

**Table 3.** Effects of 2% lactic acid and 2% ascorbic acid mixture on color parameters ($L^*$, $a^*$, $b^*$), hue angle, chroma, and total color difference ($\Delta E$) of wild red deer meat during 21 days at 4 ± 1 °C.

| Characteristics | | Day 1 | Day 7 | Day 14 | Day 21 | SE |
|---|---|---|---|---|---|---|
| $L^*$ | Treated | $30.2^{b}_{x}$ | $29.1^{ab}_{x}$ | $27.5^{a}_{x}$ | $28.6^{ab}_{x}$ | 0.56051 |
| | Non-treated | $30.3^{a}_{x}$ | $29.4^{a}_{x}$ | $30.0^{a}_{y}$ | $29.5^{a}_{x}$ | 0.21213 |
| $a^*$ | Treated | $9.4^{a}_{x}$ | $9.6^{a}_{x}$ | $11.1^{b}_{x}$ | $11^{b}_{x}$ | 0.44977 |
| | Non-treated | $9.2^{a}_{x}$ | $10^{a}_{x}$ | $11.3^{b}_{x}$ | $11.8^{b}_{x}$ | 0.59494 |
| $b^*$ | Treated | $2.0^{a}_{x}$ | $2.4^{b}_{x}$ | $2.1^{a}_{y}$ | $2.4^{b}_{x}$ | 0.10308 |
| | Non-treated | $2.2^{b}_{x}$ | $2.2^{b}_{x}$ | $1.8^{a}_{x}$ | $2.4^{b}_{x}$ | 0.12583 |
| Hue angle (°) | Treated | $0.21^{a}_{x}$ | $0.24^{b}_{x}$ | $0.18^{a}_{x}$ | $0.22^{b}_{x}$ | 0.0125 |
| | Non-treated | $0.23^{b}_{x}$ | $0.21^{b}_{x}$ | $0.16^{a}_{x}$ | $0.20^{ab}_{x}$ | 0.01472 |
| Chroma | Treated | $9.6^{a}_{x}$ | $9.9^{a}_{x}$ | $11.3^{b}_{x}$ | $11.1^{b}_{x}$ | 0.43842 |
| | Non-treated | $9.4^{a}_{x}$ | $10.3^{a}_{x}$ | $11.4^{b}_{x}$ | $12.0^{b}_{y}$ | 0.58768 |
| $\Delta E$ | Treated vs Non-treated | 0.25 | 1.26 | 3.37 | 2.76 | - |

Means in the same row within an attribute with no letters in common (a, b, c; superscript) indicate significant differences across storage periods ($p < 0.05$); means in the same column with no letters in common (x, y; subscript) indicate significant differences in treatment effects ($p < 0.05$). Comparisons were made on an individual trait level ($L^*$, $a^*$, $b^*$, hue angle, chroma). SE = Standard Error.

All the treated samples also had no significant difference in $b^*$ values compared to the non-treated ones except on Day 14. The $b^*$ values ranged from 1.8 to 2.4, indicating only very slight yellow-brown hues on the meat surface, with changes small enough to be visually subtle.

The chroma, which indicates the intensity of color, was observed to be not different in treated and non-treated samples. However, in the display period, chroma values increased in all samples. Sahoo and Anjaneyulu (1997) similarly found a significant increase in chroma values in ground buffalo meat preblended with 500 ppm of sodium ascorbate for 10-day refrigerated storage.

$\Delta E$ was calculated as the magnitude of the difference in color space between treated and non-treated wild red deer meat samples during the display period. According to the observer criteria defined by Mokrzycki and Tatol (2011), noticeable color differences were observed on Day 14 ( $\Delta E = 3.37$) and

Day 21 ( $\Delta E = 2.76$). These values exceed the threshold of 2.0, indicating that the differences in color between treated and non-treated samples became perceptible to inexperienced observers during the later stages of storage.

Our results indicate that the effect of LA and AA mixture on wild red deer meat color was not significantly different from non-treated samples, which is advantageous due to the fact that using acid might have a negative impact on meat color.

The significant interactions between treatment and display time for $L^*$, $a^*$, and $\Delta E$ values suggest that the combined effect of LA and AA treatment and display period on meat color is complex. Specifically, the significant difference in $L^*$ and $\Delta E$ values on day 14 highlights a critical time point where treatment effects are most pronounced.

These findings indicate that while the LA and AA treatments did not consistently alter meat color compared to non-treated samples, specific interactions over time did influence certain color metrics. This nuanced understanding underscores the importance of considering both treatment and display periods in assessing meat quality. By focusing on these interactions, we can better understand the optimal conditions for preserving meat color and quality during storage and display.

### **5.1.4** Texture Analysis

The results from TPA (hardness, cohesiveness, springiness, and chewiness) are presented in Table 4. The textural analysis of the treated and non-treated deer meat samples showed a significantly lower hardness on day 21 than on day 1, and the same tendency was observed in cohesiveness and chewiness. Hardness, cohesiveness, and chewiness decreased in the course of the storage period, which is in accordance with several studies (Marino et al., 2013; Bogdanowicz et al., 2018; Silva et al., 2019). These results could also be related to the changes in the patterns of myofibrillar degradation between 1 and 21 days, as mentioned by the authors: Buts et al. (1986) determined a positive correlation between the concentrations of troponin T and the hardness value.

Additionally, there is no significant difference in all texture analyses between treated and non-treated samples except on day 14. However, all treated sample values were slightly lower than non-treated ones. Multiple researchers have documented enhanced tenderness through a reduction in shear force values in meat following marination with organic acids (Wenham and Locker, 1976; Gault, 1985; Seuss and Martin, 1993). This impact could be due to some solubilization of the collagenous tissue. Stanton and Light (1990) and Eilers et al. (1994) found that lactic acid injection enhanced connective tissue degeneration.

**Table 4.** Hardness, cohesiveness, springiness, and chewiness of treated (2% lactic acid and 2% ascorbic acid mixture) and non-treated (control) wild red deer meat steaks at each measurement day at 4 ± 1 °C.

| Characteristics | | Day 1 | Day 7 | Day 14 | Day 21 | SE |
|---|---|---|---|---|---|---|
| Hardness (N) | Treated | $22.45^{b}_{x}$ | $20.43^{b}_{x}$ | $10.22^{a}_{x}$ | $10.64^{a}_{x}$ | 3.206094 |
| | Non-treated | $23.76^{b}_{x}$ | $22.61^{b}_{x}$ | $16.17^{a}_{y}$ | $12.52^{a}_{x}$ | 2.668772 |
| Cohesiveness (-) | Treated | $0.37^{b}_{x}$ | $0.32^{a}_{x}$ | $0.29^{a}_{x}$ | $0.29^{a}_{x}$ | 0.018875 |
| | Non-treated | $0.39^{b}_{x}$ | $0.34^{ab}_{x}$ | $0.31^{a}_{y}$ | $0.29^{a}_{x}$ | 0.021747 |
| Springiness (mm) | Treated | $0.80^{a}_{x}$ | $0.81^{a}_{x}$ | $0.80^{a}_{x}$ | $0.81^{a}_{x}$ | 0.002887 |
| | Non-treated | $0.89^{b}_{y}$ | $0.83^{a}_{x}$ | $0.89^{b}_{y}$ | $0.88^{b}_{y}$ | 0.014361 |
| Chewiness (mJ) | Treated | $6.69^{b}_{x}$ | $5.23^{b}_{x}$ | $2.42^{a}_{x}$ | $2.56^{a}_{x}$ | 0.889616 |
| | Non-treated | $8.36^{c}_{x}$ | $6.36^{b}_{x}$ | $4.46^{ab}_{y}$ | $3.25^{a}_{x}$ | 1.356622 |

Means in the same row within an attribute with no letters in common (a, b, c; superscript) indicate significant differences across storage periods ($p < 0.05$); means in the same column with no letters in common (x, y; subscript) indicate significant differences in treatment effects ($p < 0.05$). Comparisons were made on an individual texture parameter level. SE = Standard Error.

### 5.1.5 Protein Profile Analysis Using SDS-PAGE

The SDS-PAGE obtained by separating sarcoplasmic and myofibrillar proteins extracted from the non-treated and treated wild red deer meat at 1, 7, 14, and 21 days of storage is shown in Figure 15 (a, b) offers insight into the protective effects of the acid treatment on protein stability. The results demonstrate distinct differences in protein degradation between non-treated and treated samples, particularly regarding sarcoplasmic proteins involved in glycolysis and myofibrillar proteins critical for muscle structure.

Results showed that in non-treated samples, the storage period caused a notable reduction in the intensity of several sarcoplasmic protein bands, including phosphorylase b (~97 kDa), β-enolase (~47 kDa), aldolase (~40 kDa), glyceraldehyde 3-phosphate dehydrogenase, GaPDh (~37 kDa), lactate dehydrogenase, LDh (~36 kDa), phosphoglycerate mutase, PgaM (~28 kDa), myokinase, Mk (~23 kDa), and triosephosphate isomerase, TpI (~27 kDa). This decline in band intensity was particularly pronounced after 14 days of storage, which aligns with earlier studies indicating that protein degradation in aging meat is due to post-mortem enzymatic activity and the loss of protein solubility (Laville et al., 2009). The degradation of glycolytic enzymes such as GaPDh, beta-enolase, and TpI, which are integral to the second phase of glycolysis, is also linked to the meat tenderization process, as has been observed in beef (Joo et al., 1999; Marino et al., 2014).

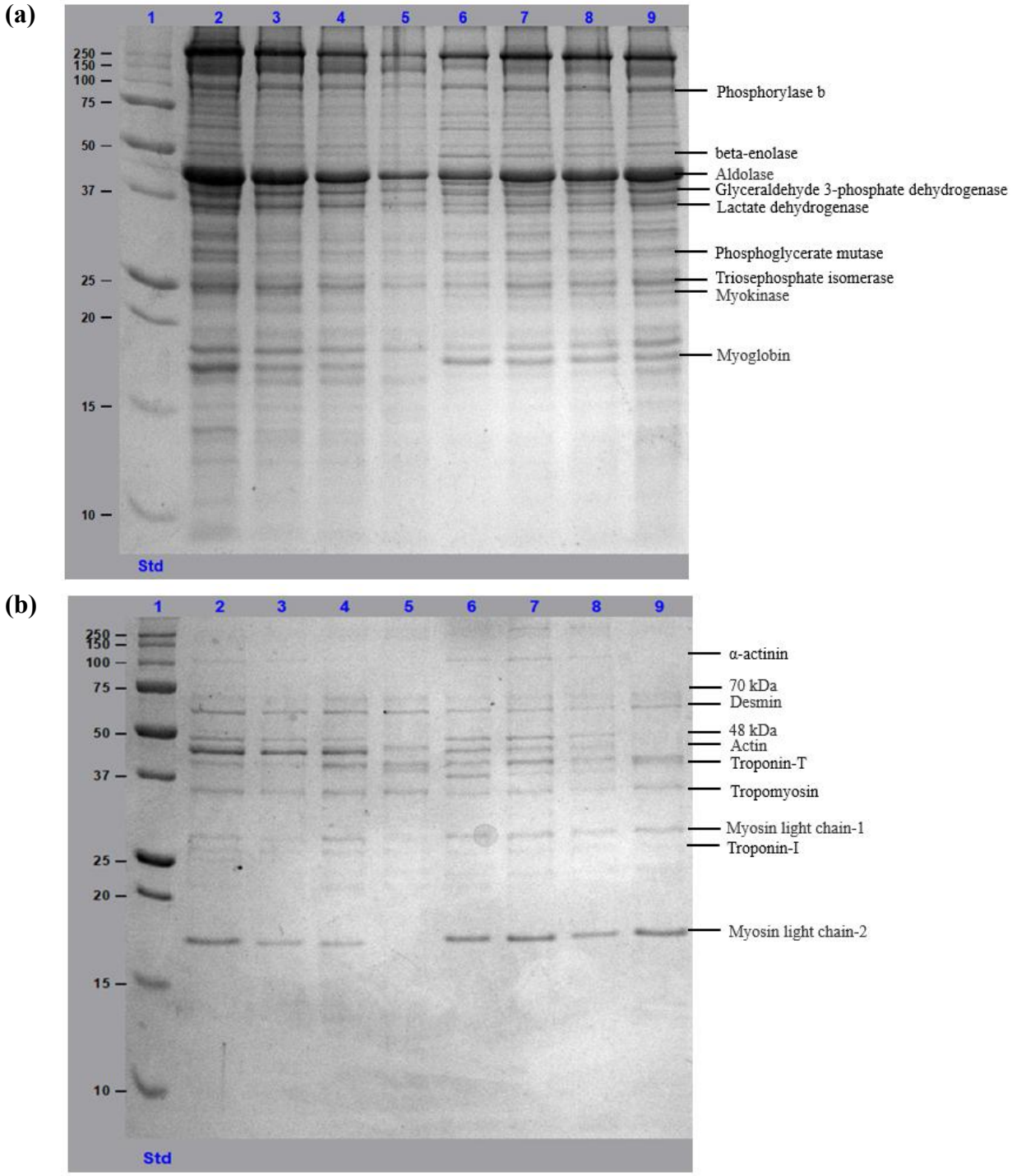


*Figure 15. SDS-PAGE patterns of (a) sarcoplasmic and (b) myofibrillar proteins from treated (2% lactic acid and 2% ascorbic acid mixture) and non-treated (control) wild red deer meat samples after 1, 7, 14, and 21 days in vacuum packaging at 4 ± 1° C. (Lane 1 molecular weight standard; Lanes from 2 to 5, non-treated samples of day 1, 7, 14, and 21; Lanes from 6 to 9, treated samples of day 1, 7, 14, and 21)*

In contrast, the sarcoplasmic proteins in the treated samples exhibited greater stability throughout the storage period. Specifically, the intensity of bands corresponding to GaPDh, beta-enolase, and TpI

was significantly higher in treated samples compared to non-treated samples, even after 21 days. This suggests that the acid treatment helped mitigate the degradation of these proteins, potentially through the antimicrobial and antioxidative properties of lactic acid and ascorbic acid, which can reduce proteolytic enzyme activity and oxidative stress. The preservation of these glycolytic enzymes could play a role in slowing down the tenderization process, contributing to prolonged meat quality during storage.

Another significant observation was the preservation of myoglobin (~17 kDa) in treated samples compared to non-treated ones. While myoglobin bands completely disappeared by day 21 in non-treated samples, they remained detectable, although at lower intensities, in treated samples. Since myoglobin is crucial for meat color, its preservation in treated samples is particularly important for maintaining a bright red color during storage, which is desirable for consumer acceptance (Mancini and Hunt, 2005).

The analysis of myofibrillar proteins further highlights the differences between non-treated and treated samples. In non-treated samples, aging led to the degradation of several key myofibrillar proteins, including α-actinin (~100 kDa), desmin (~70 kDa), actin (~42 kDa), troponin-T, TnT (~40 kDa), tropomyosin, Tm (~36 kDa), myosin light chain-1, MLC-1 (~25 kDa), troponin-I, TnI (~24 kDa), and myosin light chain-2, MLC-2 (~18 kDa). These proteins showed a marked decrease in band intensity during the storage period, with many of them disappearing by day 14 or 21. This degradation is consistent with previous studies showing the breakdown of structural proteins such as actin and myosin during aging, contributing to the tenderization process and the breakdown of muscle fiber integrity (Muroya et al., 2004; Rowe et al., 2004; Bowker et al., 2008).

However, the treated samples demonstrated better preservation of these myofibrillar proteins over the 21-day storage period. Proteins such as α-actinin, TnT, and MLC-1 exhibited significantly less degradation in treated samples compared to non-treated ones. In particular, TnT, which is known to degrade into multiple fragments during aging and plays a role in meat tenderization (Lonergan, Zhang and Lonergan, 2010), remained more intact in treated samples. The treated group showed fewer degradation products for TnT, suggesting that the acid treatment helped maintain the integrity of myofibrillar proteins, likely by limiting proteolytic activity and protein denaturation.

The preservation of these structural proteins, particularly TnT and α-actinin, in the treated samples is indicative of reduced enzymatic activity, which could lead to improved textural properties in the treated meat over time. The stabilization of myofibrillar proteins may result in less extensive tenderization compared to non-treated samples, potentially contributing to better meat firmness and

longer shelf life. The molecular weight bands at 70 kDa and 48 kDa, which increased in intensity after 14 days in non-treated samples, showed less pronounced changes in treated samples, further indicating that the acid mixture helped reduce protein degradation and maintain structural integrity.

The results of the SDS-PAGE analysis suggest that the combination of 2% lactic acid and 2% ascorbic acid effectively preserved both sarcoplasmic and myofibrillar proteins during aging, contributing to the overall stability of wild red deer meat during storage. The reduction in protein degradation in treated samples likely results from the antimicrobial properties of lactic acid and the antioxidative effects of ascorbic acid, which together help to mitigate both enzymatic proteolysis and oxidative stress. By preserving proteins involved in glycolysis and muscle structure, the acid treatment not only improved the chemical stability of the meat but also likely contributed to better color, texture, and tenderness over time.

In comparison, non-treated samples showed more rapid degradation of both sarcoplasmic and myofibrillar proteins, leading to greater tenderization but also a loss of protein integrity and potential declines in meat quality, such as reduced color stability and textural firmness. These findings highlight the importance of acid treatment in extending the shelf life and maintaining the quality of wild red deer meat during vacuum-packaged storage.

### **5.1.6** Microbiological Evaluation

Microbiological loads of treated and non-treated deer meat samples are shown in Figure 16. Initial aerobic plate count of non-treated and treated samples ranged between 3.39 and 3.25 log CFU/g, with counts increasing to a range of 5.60 and 4.55 log CFU/g after 21 days in vacuum packaging. Microbiological limit of shelf-life 7 log CFU/g was not reached in any of the samples.

The vacuum-packed wild red deer meat samples treated with 2% LA and 2% AA mixture showed significantly lower aerobic plate count as compared to vacuum-packed non-treated samples at day 21, which can lead to increased time on the shelf. However, in treated samples, slightly lower aerobic plate counts were recorded on days 1, 7, and 14 of the display.

The difference in the reductions in the microbial loads in different studies by LA and AA might be due to various factors such as dissimilarities in sampling site, sampling methods, acid concentration, temperature of the solution applied, method and type of application, handling, and initial surface load (Capita et al., 2002). Therefore, similar results cannot be achieved from the same decontaminant in different studies. In this study, only aerobic bacteria were counted, but Acuff et al. (1987) showed that lactic acid treatment decreased the number of both spoilage and pathogenic bacteria. Another research study, performed by Bosilevac et al. (2006), reported that spraying 2% lactic acid on pre-eviscerated

carcass decreased Enterobacteriaceae count by 1 log cfu/g, and Listeria by <1 log CFU/g. Shivas et al. (1984) showed that microbial populations were not reduced by treatment with ascorbic acid.

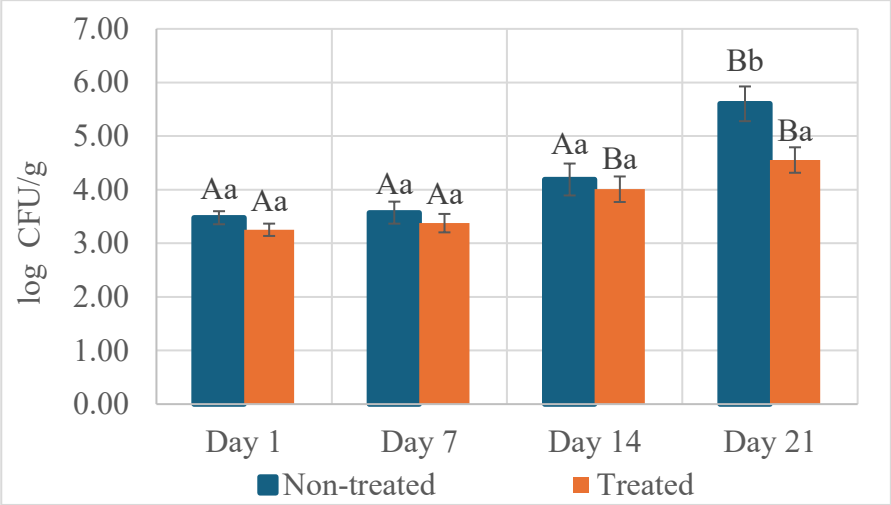


*Figure 16. Means ± SD for aerobic plate count (log CFU/g) of treated (2% lactic acid and 2% ascorbic acid mixture) and non-treated (control) vacuum-packed wild red deer meat samples during 21 days of retail display at 4 ± 1 °C. Means with different letter (A, B) indicate significant differences ($p < 0.05$) between storage periods (Days 1, 7, 14, and 21), while means with different letter (a, b) indicate significant differences ($p < 0.05$) among non-treated and treated samples.*

## 5.2 HHP Treatment (Experiment 2)

### 5.2.1 Moisture-Related Parameters (Drip Loss, WHC, and Dry Matter)

#### *5.2.1.1 Drip loss*

Drip loss increased with increasing pressure levels, particularly at ≥ 500 MPa, where a significant rise was observed ($p < 0.05$). On Day 1, the control sample (0 MPa) exhibited the lowest drip loss (1.56%), whereas the highest pressure treatment (600 MPa) resulted in a 5.84% loss. By Day 14, all samples exhibited increased drip loss, with the 600 MPa-treated sample reaching 18.67%, significantly higher than the control (6.19%).

The increase in drip loss at higher pressures can be attributed to structural modifications of muscle proteins, particularly myofibrillar proteins, leading to reduced water retention. Similar trends have been reported in other meat studies, where HHP-induced protein denaturation affects the water-binding ability of muscle tissue (Marcos and Mullen, 2014).

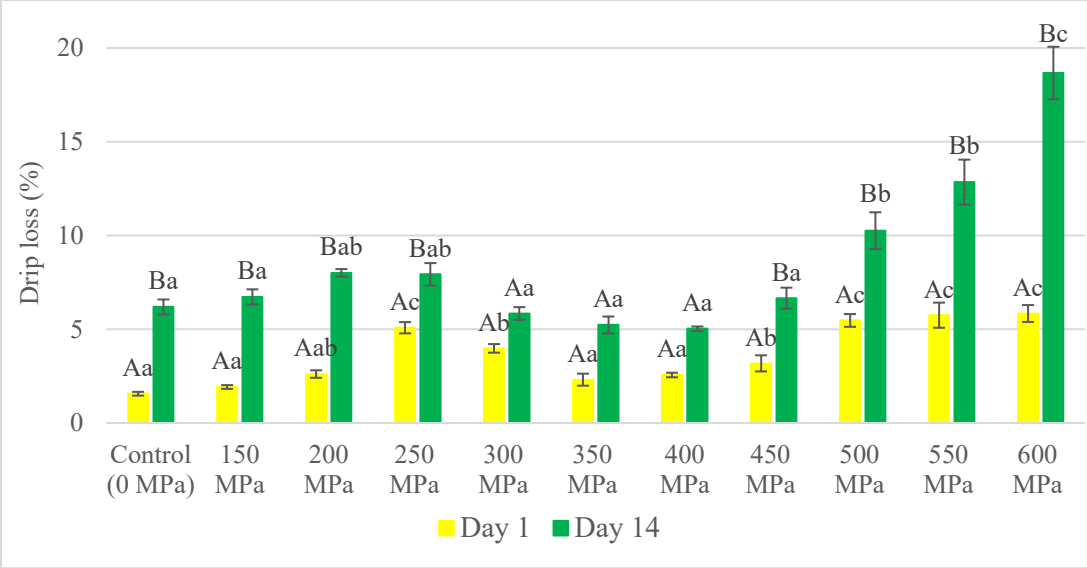


*Figure 17. Means ± SD for drip loss of vacuum-packed wild red deer meat samples subjected to HHP treatments (150‑600 MPa) and control (0 MPa) on Day 1 and Day 14 of storage at 4 ± 1 ° C. Means with different letter (A, B) indicate significant differences ($p < 0.05$) between storage periods (Day 1 vs. Day 14), while means with different letter (a, b, c) indicate significant differences ($p < 0.05$) among different pressure levels.*

*5.2.1.2 Water holding capacity*

Figure 18 illustrates the impact of HHP treatment on the water holding capacity (WHC) of wild red deer meat samples. On Day 1, 450 MPa and 500 MPa demonstrate the highest WHC, emphasizing the effectiveness of these pressures in preserving moisture. Conversely, 300 MPa exhibits the lowest WHC on the same day. Day 7 reveals 350 MPa with the highest WHC, while 150 MPa exhibits the lowest. By Day 14, 350 MPa maintains the highest WHC, contrasting with the control sample displaying the lowest.

Crucially, a temporal trend emerges as Day 1 results significantly surpass those of Day 14, indicating potential alterations in WHC during the storage period (Ma and Ledward, 2004). These findings underscore the importance of selecting optimal pressure conditions, such as 450 MPa, 500 MPa, and 350 MPa, to enhance the water retention capacity of wild red deer meat throughout processing, storage, and cooking.

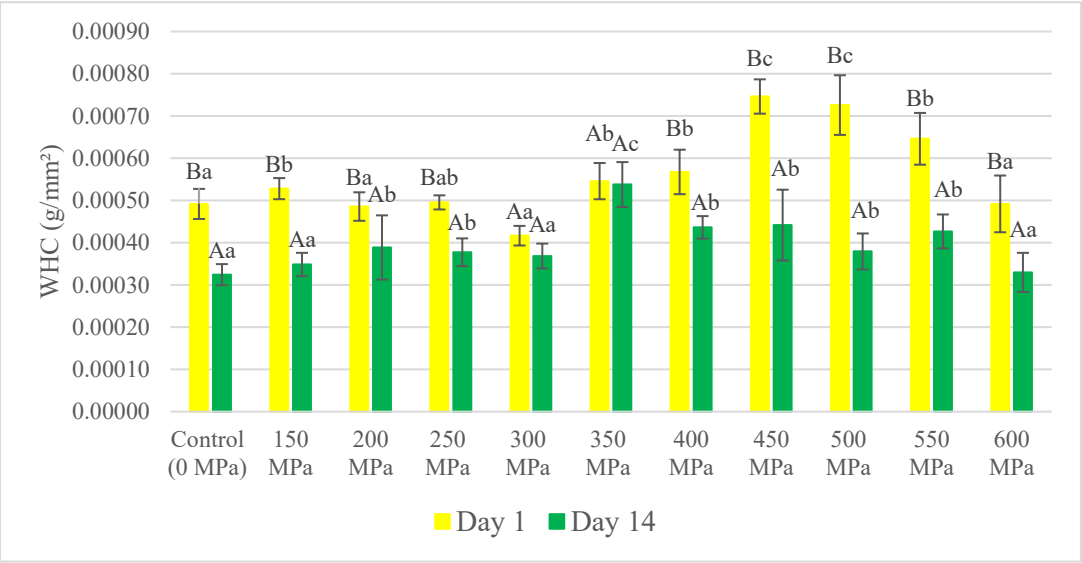


*Figure 18. Means ± SD for Water Holding Capacity (WHC) of vacuum-packed wild red deer meat samples subjected to HHP treatments (150 -600 MPa) and control (0 MPa) on Day 1 and Day 14 of storage at 4 ± 1 ° C. Means with different letter (A, B) indicate significant differences ($p < 0.05$) between storage periods (Day 1 vs. Day 14), while means with different letter (a, b, c) indicate significant differences ($p < 0.05$) among different pressure levels.*

*5.2.1.3 Dry matter*

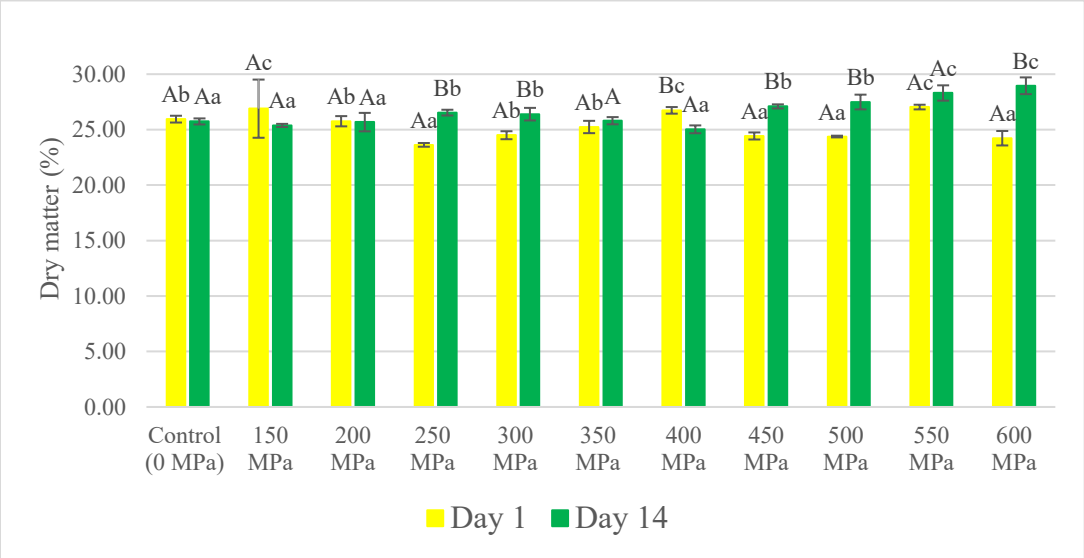


*Figure 19. Means ± SD for Dry matter content (%) of vacuum-packed wild red deer meat samples subjected to HHP treatments (150 -600 MPa) and control (0 MPa) on Day 1 and Day 14 of storage at 4 ± 1 ° C. Means with different letter (A, B) indicate significant differences ($p < 0.05$) between storage periods (Day 1 vs. Day 14), while means with different letter (a, b, c) indicate significant differences ($p < 0.05$) among different pressure levels.*

The dry matter analysis of wild red deer meat under HHP processing results were illustrated on Figure 19. Elevated dry matter content at 150, 400, and 550 MPa on Day 1 indicates positive effects on solid

component preservation, contrasting with the lowest content at 250 MPa. Notably, Day 14 shows a cumulative effect with increased content at 550 MPa and 600 MPa, but the lowest at 400 MPa suggests pressure-specific deviations. These findings underscore the dynamic relationship between HHP treatment and dry matter, emphasizing the need for tailored pressure selection in optimizing wild red deer meat composition.

### 5.2.2 pH

The pH values of wild red deer meat treated with different levels of high hydrostatic pressure (HHP) and stored for 14 days at 4 ± 1 °C are summarized in Table 5.

On Day 1, pH values ranged from 5.60 in the control group (0 MPa) to 5.94 at 600 MPa. Samples treated at 300, 350, 400, 450, and 550 MPa exhibited significantly higher pH values than the control ($p < 0.05$), while the 150, 200, and 250 MPa groups were not significantly different. The highest pH value at 600 MPa was significantly different from all other treatments ($p < 0.05$). These increases are consistent with previous findings under high-pressure conditions, often attributed to ion redistribution and structural changes in proteins (McArdle et al., 2011; Szerman et al., 2011).

**Table 5.** pH values of wild red deer meat treated with different levels of HHP during storage. (Mean ± SD).

| Pressure (MPa) | Day 1 | Day 14 |
|---|---|---|
| **Control** | $5.60\pm0.05^{b}_{x}$ | $5.13\pm0.03^{a}_{x}$ |
| **150** | $5.61\pm0.02^{b}_{x}$ | $5.30\pm0.04^{a}_{x}$ |
| **200** | $5.66\pm0.02^{a}_{x}$ | $5.59\pm0.05^{b}_{y}$ |
| **250** | $5.67\pm0.04^{b}_{x}$ | $5.55\pm0.04^{a}_{y}$ |
| **300** | $5.69\pm0.04^{a}_{y}$ | $5.68\pm0.03^{a}_{y}$ |
| **350** | $5.82\pm0.02^{a}_{y}$ | $5.80\pm0.03^{a}_{z}$ |
| **400** | $5.76\pm0.03^{a}_{y}$ | $5.93\pm0.02^{b}_{z}$ |
| **450** | $5.80\pm0.03^{a}_{y}$ | $5.92\pm0.02^{b}_{z}$ |
| **500** | $5.75\pm0.05^{a}_{y}$ | $5.88\pm0.02^{b}_{z}$ |
| **550** | $5.71\pm0.03^{a}_{y}$ | $5.85\pm0.01^{b}_{z}$ |
| **600** | $5.94\pm0.03^{a}_{z}$ | $5.90\pm0.01^{a}_{z}$ |

Different superscript letters (a, b) within a row represent statistically significant differences between storage times ($p < 0.05$), while different subscript letters (x, y, z) within a column indicate significant differences among treatment levels ($p < 0.05$).

By Day 14, a general decrease in pH was observed in most treatments. The control group exhibited a significant decline from 5.60 to 5.13 *(*$p < 0.05$), likely due to lactic acid and other metabolites accumulating during microbial activity, as reported in previous studies on vacuum-packaged meat (Enkhbold et al., 2024). Significant pH declines were measured at 150, 200, and 250 MPa between Day 1 and Day 14 ($p < 0.05$), likely due to continued microbial activity and post-mortem glycolysis leading to acid accumulation. In contrast, significant pH increases were observed at 400, 450, 500, and 550 MPa ($p < 0.05$), which may be attributed to pressure-induced denaturation of proteins and suppression of acid-generating microbial and enzymatic activity. pH remained stable at 300, 350, and 600 MPa ($p > 0.05$). The 600 MPa group retained a pH of 5.90 on Day 14, nearly identical to its initial value of 5.94. This stability could be explained by the suppression of microbial activity and enzymatic processes at higher pressure levels, as reported in similar studies on red meat (Şayin Sert and Coşkun, 2022).

### **5.2.3** Surface color

The surface color of wild red deer meat was evaluated using the CIE Lab system, where lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$) were measured on Days 1 and 14 of storage. Pressure treatments and storage duration significantly influenced the color parameters across all treatment levels (Table 6).

On Day 1, the $L^*$ values increased progressively with increasing pressure, particularly from 350 MPa onwards, with the highest $L^*$ values observed at 450 MPa (Table 6, Figure 20). The $a^*$ values showed variable trends: the control had higher $a^*$ values com-pared to samples treated at 150 MPa and pressures of 450 MPa or greater, while samples treated at intermediate pressures (250–400 MPa) showed elevated $a^*$ values. Yellowness also increased with pressure, with significant changes observed at 350 MPa and higher, peaking at 600 MPa. Hue angle and chroma values were significantly affected by pressure treatments. Hue angle increased sharply at pressures above 400 MPa, reflecting a shift in the color balance due to protein and pigment alterations. Chroma followed similar trends, with a noticeable increase from 250 MPa, peaking at 600 MPa. These changes suggest that higher pressures induced more pronounced color alterations, likely due to protein denaturation and pigment oxidation (Jung et al., 2003).


*Figure 20. Visual representation of color changes in wild red deer meat samples control (0 MPa) and treated at different HHP ranges (150‑600 MPa) on Day 1.*

After 14 days of storage, *L** values remained significantly elevated for samples treated at pressures of 300 MPa or greater (Table 6). However, a slight decrease in *L** was noted for lower-pressure treatments (150–250 MPa) and the control compared to Day 1, although this change was not statistically significant. Redness values significantly decreased over storage, particularly at higher pressures (350 MPa and above). The lowest *a** values were observed for 550 MPa and 600 MPa treatments, which may reflect oxidation of myoglobin and other pigments during extended storage, consistent with findings from Chmiel et al. (2025), who reported a progressive decline in redness in high-pressure-treated vacuum-packed beef. Similarly, Reesman et al. (2023) observed that while moderate pressure levels (300–450 MPa) improved the redness of dark, firm, and dry (DFD) beef, higher pressures (≥600 MPa) led to excessive pale coloration and further reductions in *a** values over time. The *b** values increased significantly in samples treated at pressures of 350 MPa or higher. This increase, combined with reductions in redness, contributed to shifts in hue angle, which was significantly higher in these samples. Chroma values, indicative of color intensity, were consistently lower for high-pressure treatments after 14 days, suggesting a loss of vividness due to protein aggregation and pigment degradation.

The results highlight the dual influence of HHP treatment and storage duration on the surface color of wild red deer meat. High-pressure treatments above 300 MPa caused significant protein denaturation, pigment oxidation, and aggregation, leading to in-creased lightness and yellowness but reduced redness and chroma values over time. These changes reflect the structural and chemical modifications induced by HHP on myoglobin and other meat proteins (Jung et al., 2003; McArdle et al., 2011; Şayin Sert and Coşkun, 2022).

The pronounced effects observed at higher pressures (450–600 MPa) could impact consumer perception, as discoloration and reduced redness may affect the visual appeal of meat products (Jung et al., 2003). The changes in hue angle and chroma also underscore the importance of optimizing pressure levels to balance preservation and appearance, particularly for fresh meat applications.

**Table 6**. Instrumental color measurement results of control and HHP-treated samples measured on Days 1 and 14 (Mean ± SD).

| Pressure (MPa) | Day 1 | | | | | Day 14 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *L** | *a** | *b** | Hue angle (°) | Chroma | *L** | *a** | *b** | Hue angle (°) | Chroma |
| **Control** | $38.9\pm1.39^{a}_{y}$ | $17.4\pm0.85^{b}_{x}$ | $3.0\pm0.57^{a}_{x}$ | $0.17\pm0.01^{a}_{x}$ | $17.64\pm0.57^{a}_{x}$ | $35.4\pm3.89^{a}_{x}$ | $16.8\pm2.04^{c}_{x}$ | $4.9\pm0.67^{a}_{y}$ | $0.28\pm0.01^{a}_{y}$ | $17.45\pm0.45^{c}_{x}$ |
| **150** | $40.9\pm1.58^{a}_{y}$ | $15.3\pm1.12^{a}_{x}$ | $2.4\pm0.48^{a}_{x}$ | $0.16\pm0.01^{a}_{x}$ | $15.47\pm0.34^{a}_{x}$ | $36.6\pm3.58^{a}_{x}$ | $14.9\pm2.47^{b}_{x}$ | $4.6\pm1.16^{a}_{y}$ | $0.30\pm0.02^{a}_{y}$ | $15.65\pm0.73^{b}_{x}$ |
| **200** | $41.8\pm0.83^{ab}_{y}$ | $15.9\pm1.19^{ab}_{x}$ | $2.6\pm0.77^{a}_{x}$ | $0.16\pm0.01^{a}_{x}$ | $16.08\pm0.43^{a}_{x}$ | $38.7\pm1.84^{a}_{x}$ | $17.4\pm1.58^{c}_{y}$ | $3.5\pm1.65^{a}_{y}$ | $0.20\pm0.01^{a}_{x}$ | $17.71\pm0.86^{c}_{y}$ |
| **250** | $42.7\pm2.22^{b}_{y}$ | $18.2\pm0.87^{c}_{y}$ | $3.8\pm0.61^{a}_{x}$ | $0.20\pm0.01^{a}_{x}$ | $18.58\pm0.59^{b}_{y}$ | $39.3\pm3.57^{ab}_{x}$ | $15.1\pm2.28^{b}_{x}$ | $5.8\pm1.48^{ab}_{y}$ | $0.37\pm0.02^{a}_{y}$ | $16.18\pm1.23^{b}_{x}$ |
| **300** | $43.9\pm0.99^{b}_{y}$ | $17.5\pm0.54^{b}_{x}$ | $3.6\pm0.62^{a}_{x}$ | $0.20\pm0.01^{a}_{x}$ | $17.84\pm0.78^{ab}_{x}$ | $41.5\pm1.49^{b}_{x}$ | $17.1\pm2.53^{c}_{x}$ | $4.2\pm1.72^{a}_{y}$ | $0.24\pm0.01^{a}_{x}$ | $17.65\pm0.96^{c}_{x}$ |
| **350** | $46.8\pm2.27^{bc}_{y}$ | $18.2\pm1.16^{c}_{y}$ | $5.1\pm1.27^{ab}_{x}$ | $0.27\pm0.01^{ab}_{x}$ | $18.88\pm0.92^{bc}_{y}$ | $45.7\pm2.8^{bc}_{x}$ | $15.5\pm1.74^{bc}_{x}$ | $7.2\pm1.27^{b}_{y}$ | $0.43\pm0.01^{b}_{y}$ | $17.06\pm0.83^{c}_{x}$ |
| **400** | $48.6\pm2.52^{c}_{y}$ | $19.1\pm1.48^{c}_{y}$ | $6.5\pm1.22^{b}_{x}$ | $0.33\pm0.02^{b}_{x}$ | $20.19\pm0.84^{c}_{y}$ | $46.9\pm3.17^{c}_{x}$ | $14.2\pm1.17^{b}_{x}$ | $8.6\pm0.89^{b}_{y}$ | $0.55\pm0.02^{b}_{y}$ | $16.57\pm1.14^{bc}_{x}$ |
| **450** | $51.3\pm3.12^{c}_{x}$ | $15.1\pm1.44^{a}_{y}$ | $10.8\pm0.90^{c}_{x}$ | $0.62\pm0.04^{c}_{x}$ | $18.61\pm1.12^{b}_{y}$ | $50.6\pm5.30^{c}_{x}$ | $11.9\pm0.99^{ab}_{x}$ | $10.9\pm0.80^{c}_{x}$ | $0.74\pm0.03^{c}_{y}$ | $16.20\pm0.36^{b}_{x}$ |
| **500** | $49.7\pm4.93^{c}_{x}$ | $14.8\pm2.11^{a}_{y}$ | $11.1\pm1.65^{c}_{x}$ | $0.64\pm0.01^{c}_{x}$ | $18.56\pm1.01^{b}_{y}$ | $50.5\pm5.94^{c}_{x}$ | $10.4\pm1.09^{a}_{x}$ | $11.6\pm0.78^{c}_{x}$ | $0.84\pm0.02^{c}_{y}$ | $15.54\pm0.88^{b}_{x}$ |
| **550** | $47.7\pm2.61^{bc}_{y}$ | $14.5\pm1.68^{a}_{y}$ | $10.3\pm0.77^{bc}_{x}$ | $0.62\pm0.02^{c}_{x}$ | $17.78\pm0.97^{ab}_{y}$ | $45.9\pm2.59^{bc}_{x}$ | $9.3\pm1.22^{a}_{x}$ | $10.1\pm0.57^{c}_{x}$ | $0.83\pm0.03^{c}_{y}$ | $13.78\pm0.62^{a}_{x}$ |
| **600** | $49.6\pm3.56^{c}_{x}$ | $16.6\pm1.96^{b}_{y}$ | $12.0\pm0.87^{c}_{x}$ | $0.63\pm0.01^{c}_{x}$ | $20.45\pm1.16^{c}_{y}$ | $49.8\pm3.51^{c}_{x}$ | $10.6\pm1.91^{a}_{x}$ | $11.7\pm1.36^{c}_{x}$ | $0.83\pm0.01^{c}_{y}$ | $15.78\pm1.46^{b}_{x}$ |

Superscript letters (a, b, c) within a column denote significant differences between pressure treatments ($p < 0.05$), while subscript letters (x, y) within a row reflect significant differences between Day 1 and Day 14 ($p < 0.05$). Comparisons were made separately for each color parameter (*L*, a*, b**, hue angle, and chroma).

### **5.2.4** Texture Analysis

The effects of HHP treatments on the texture properties of wild red deer meat samples were analyzed using Warner-Bratzler shear force (WB), texture profile analysis (TPA) hardness, cohesiveness, springiness, and chewiness. Results for samples measured on Day 1 and Day 14 are presented in Table 7.

Shear force, which reflects the resistance to cutting and is indicative of tenderness, showed significant differences across pressure treatments and storage days. On Day 1, the control samples exhibited a shear force of 40.32 ± 3.89 N, while increasing pressure levels generally led to higher shear forces, peaking at 87.39 ± 6.48 N for 550 MPa. Notably, treatments at 450 MPa and above resulted in significantly higher shear forces compared to the control, indicating increased firmness due to protein denaturation and cross-linking at high pressure levels (Macfarlane *et al.*, 1981). By Day 14, the shear force of control samples increased to 82.19 ± 4.98 N, which may reflect limited post-mortem proteolysis and higher connective tissue content typical of wild game meat. Unlike farm animals, where aging often improves tenderness, wild species such as wild red deer may exhibit reduced tenderization during storage (Hoffman and Wiklund, 2006). Interestingly, samples treated with 300

MPa and 450 MPa showed reductions in shear force on Day 14, suggesting that moderate pressures may promote proteolysis during storage, enhancing tenderness.

**Table 7**. Instrumental texture measurement results of control and HHP-treated samples measured on Days 1 and 14 (Mean ± SD).

| Pressure (MPa) | Day 1 | | | | | Day 14 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shear force WB (N) | Hardness TPA (N) | Cohesiveness TPA (-) | Springiness TPA (mm) | Chewiness TPA (mJ) | Shear force WB (N) | Hardness TPA (N) | Cohesiveness TPA (-) | Springiness TPA (mm) | Chewiness TPA (mJ) |
| **Control** | $40.32\pm3.89^{a}_{x}$ | $23.90\pm1.23^{c}_{x}$ | $0.24\pm0.01^{a}_{x}$ | $0.87\pm0.03^{c}_{x}$ | $4.80\pm0.12^{b}_{x}$ | $82.19\pm4.98^{b}_{y}$ | $21.93\pm1.99^{a}_{x}$ | $0.22\pm0.01^{a}_{x}$ | $0.88\pm0.05^{c}_{x}$ | $4.29\pm0.44^{a}_{x}$ |
| **150** | $56.94\pm3.11^{ab}_{x}$ | $9.34\pm1.64^{a}_{x}$ | $0.28\pm0.02^{b}_{x}$ | $0.87\pm0.04^{c}_{x}$ | $2.47\pm0.21^{a}_{x}$ | $80.87\pm5.11^{b}_{y}$ | $25.19\pm1.23^{b}_{y}$ | $0.25\pm0.02^{b}_{x}$ | $0.89\pm0.03^{c}_{x}$ | $5.50\pm0.34^{ab}_{y}$ |
| **200** | $59.09\pm2.45^{b}_{x}$ | $19.75\pm1.45^{bc}_{x}$ | $0.25\pm0.01^{a}_{x}$ | $0.79\pm0.02^{a}_{x}$ | $4.76\pm0.23^{b}_{x}$ | $86.08\pm4.21^{bc}_{y}$ | $26.05\pm2.02^{b}_{y}$ | $0.22\pm0.01^{ab}_{x}$ | $0.89\pm0.02^{c}_{y}$ | $5.13\pm0.56^{ab}_{x}$ |
| **250** | $58.03\pm4.36^{b}_{x}$ | $18.30\pm1.28^{b}_{x}$ | $0.25\pm0.01^{a}_{x}$ | $0.88\pm0.03^{c}_{x}$ | $4.91\pm0.29^{b}_{x}$ | $69.44\pm3.59^{ab}_{y}$ | $24.81\pm1.34^{a}_{y}$ | $0.24\pm0.01^{b}_{x}$ | $0.85\pm0.03^{b}_{x}$ | $4.99\pm0.23^{a}_{x}$ |
| **300** | $59.81\pm2.89^{b}_{x}$ | $10.38\pm1.56^{ab}_{x}$ | $0.23\pm0.01^{a}_{x}$ | $0.85\pm0.04^{bc}_{x}$ | $2.96\pm0.17^{a}_{x}$ | $56.84\pm4.36^{a}_{x}$ | $17.33\pm1.12^{a}_{y}$ | $0.20\pm0.01^{a}_{x}$ | $0.89\pm0.05^{c}_{x}$ | $3.12\pm0.29^{a}_{x}$ |
| **350** | $75.04\pm3.49^{c}_{x}$ | $4.30\pm0.34^{a}_{x}$ | $0.29\pm0.01^{b}_{x}$ | $0.86\pm0.04^{c}_{y}$ | $1.79\pm0.13^{a}_{x}$ | $80.52\pm6.15^{b}_{x}$ | $11.26\pm1.06^{a}_{y}$ | $0.26\pm0.02^{bc}_{x}$ | $0.73\pm0.07^{a}_{x}$ | $2.10\pm0.12^{a}_{x}$ |
| **400** | $52.42\pm3.56^{ab}_{x}$ | $6.54\pm0.27^{a}_{x}$ | $0.26\pm0.01^{a}_{x}$ | $0.79\pm0.05^{a}_{x}$ | $2.32\pm0.17^{a}_{x}$ | $75.17\pm3.98^{b}_{y}$ | $14.24\pm1.11^{a}_{y}$ | $0.27\pm0.01^{c}_{x}$ | $0.89\pm0.07^{c}_{y}$ | $3.41\pm0.21^{a}$ |
| **450** | $83.40\pm6.23^{c}_{y}$ | $23.45\pm1.69^{c}_{x}$ | $0.35\pm0.02^{c}_{y}$ | $0.77\pm0.02^{a}_{x}$ | $7.13\pm0.45^{c}_{y}$ | $57.79\pm4.12^{a}_{x}$ | $20.77\pm1.45^{a}_{x}$ | $0.28\pm0.02^{c}_{x}$ | $0.88\pm0.04^{c}_{y}$ | $5.05\pm0.25^{ab}_{x}$ |
| **500** | $64.55\pm3.12^{b}_{x}$ | $23.26\pm1.11^{c}_{x}$ | $0.29\pm0.01^{b}_{y}$ | $0.84\pm0.03^{b}_{x}$ | $7.88\pm056^{c}_{x}$ | $64.35\pm3.45^{a}_{x}$ | $41.81\pm3.67^{c}_{y}$ | $0.24\pm0.01^{b}_{x}$ | $0.89\pm0.04^{c}_{x}$ | $9.08\pm0.76^{bc}_{y}$ |
| **550** | $87.39\pm6.48^{c}_{x}$ | $24.94\pm1.98^{c}_{x}$ | $0.40\pm0.03^{c}_{y}$ | $0.86\pm0.04^{c}_{x}$ | $8.34\pm0.76^{c}_{y}$ | $100.64\pm8.88^{c}_{y}$ | $31.49\pm1.23^{b}_{y}$ | $0.24\pm0.01^{b}_{x}$ | $0.89\pm0.03^{c}_{x}$ | $6.84\pm0.13^{b}_{x}$ |
| **600** | $71.13\pm7.11^{c}_{y}$ | $27.38\pm1.99^{c}_{x}$ | $0.32\pm0.02^{b}_{x}$ | $0.87\pm0.05^{c}_{x}$ | $9.78\pm0.37^{c}_{x}$ | $64.63\pm4.58^{a}_{x}$ | $56.69\pm3.65^{c}_{y}$ | $0.28\pm0.01^{c}_{x}$ | $0.87\pm0.05^{c}_{x}$ | $13.81\pm0.99^{c}_{y}$ |

Superscript letters (a, b, c) within a column denote significant differences between pressure treatments ($p < 0.05$), while subscript letters (x, y) within a row reflect significant differences between Day 1 and Day 14 ($p < 0.05$). Comparisons were conducted separately for each texture attribute (shear force, hardness, cohesiveness, springiness, and chewiness).

Hardness, measured through TPA, revealed distinct trends with pressure and storage time. On Day 1, samples treated with higher pressures (450–600 MPa) exhibited significantly greater hardness compared to the control (23.90 ± 1.23 N), with the highest value observed for the 600 MPa treatment (27.38 ± 1.99 N). The hardness was determined to be higher in the pressurized samples during storage compared to the control samples ($p < 0.05$). This observation aligns with previous reports (Sun and Holley, 2010; Şayin Sert and Coşkun, 2022) that HHP can cause either softening or hardening of meat proteins, depending on factors such as temperature, pressure, and processing time. These changes are attributed to protein denaturation, aggregation, or gelation, which alter the structural properties of the meat matrix. By Day 14, the control samples showed a slight decrease in hardness (21.93 ± 1.99 N), while higher-pressure treatments (500–600 MPa) demonstrated further increases, indicating that the textural effects of high-pressure treatment persisted over time.

The texture analysis highlights the significant impact of high hydrostatic pressure on the structural and functional properties of wild red deer meat. At moderate pressures (150–300 MPa), the results suggest a balance between protein denaturation and proteolysis, leading to tenderness improvements over storage. In contrast, higher pressures (450–600 MPa) induced greater hardness, chewiness, and cohesiveness due to extensive protein gelation and matrix densification. These findings align with previous studies (Macfarlane et al., 1981; Sun and Holley, 2010; Şayin Sert and Coşkun, 2022) indicating that HHP alters meat texture through pressure-induced protein modifications, including partial denaturation, aggregation, and gel formation.

The storage period also influenced texture properties, as proteolytic activity likely contributed to tenderness in some pressure-treated samples, particularly at 250–300 MPa. However, at higher pressures, the structural rigidity imparted by HHP appeared to counteract proteolysis, resulting in firmer textures even after 14 days of storage.

### **5.2.5** Protein Profile Analysis Using SDS-PAGE

Figure 21 shows the SDS-PAGE profiles of sarcoplasmic (a) and myofibrillar (b) proteins extracted from vacuum-packed wild red deer meat treated with HHP (150–600 MPa) and a control (0 MPa) on Day 1 and Day 14 of storage at 4 ± 1 °C. The molecular weight standards in Lane 1 provided a reference for identifying key protein bands from 250 kDa to 10 kDa.

Proteins in meat, including sarcoplasmic and myofibrillar proteins, undergo significant changes during high-pressure treatment, primarily due to aggregation and denaturation (Marcos et al., 2010; Csehi et al., 2016). Sarcoplasmic proteins, which are water-soluble, include enzymes from the glycolytic pathway and myoglobin (~17 kDa), a key pigment responsible for meat color (Csehi et al., 2016). Myoglobin is one of the most critical sarcoplasmic proteins, making its analysis essential for understanding color changes in meat. On Day 1, sarcoplasmic protein profiles remained relatively consistent across treatments up to 500 MPa, with no major visual differences in band intensity observed between the control, 150 MPa, 450 MPa, and 500 MPa samples. Noticeable reductions in band intensity were more apparent at 550 and 600 MPa. High-pressure treatment induces conformational changes in myoglobin, such as denaturation of the globin component or oxidation of ferrous ions, which can result in meat discoloration (Marcos et al., 2010; Şayin Sert and Coşkun, 2022). As Bekhit et al. (2003) reported, this discoloration is closely linked to spontaneous structural changes in myoglobin.

Interestingly, the sample treated at 250 MPa (Lane 5) on Day 1 and Day 14 exhibited an unexpectedly strong reduction in band intensity similar to the 600 MPa treatment (Lane 12). This non-linear

behavior suggests that 250 MPa may represent a transitional pressure level where both pressure-induced tenderization and early stages of protein denaturation coexist. Although considered a moderate pressure, 250 MPa may still be sufficient to disrupt the solubility of heat- or pressure-sensitive sarcoplasmic proteins. This non-linear behavior highlights the complexity of protein responses under varying pressure conditions, where unfolding and solubility loss may not correlate directly with pressure intensity.

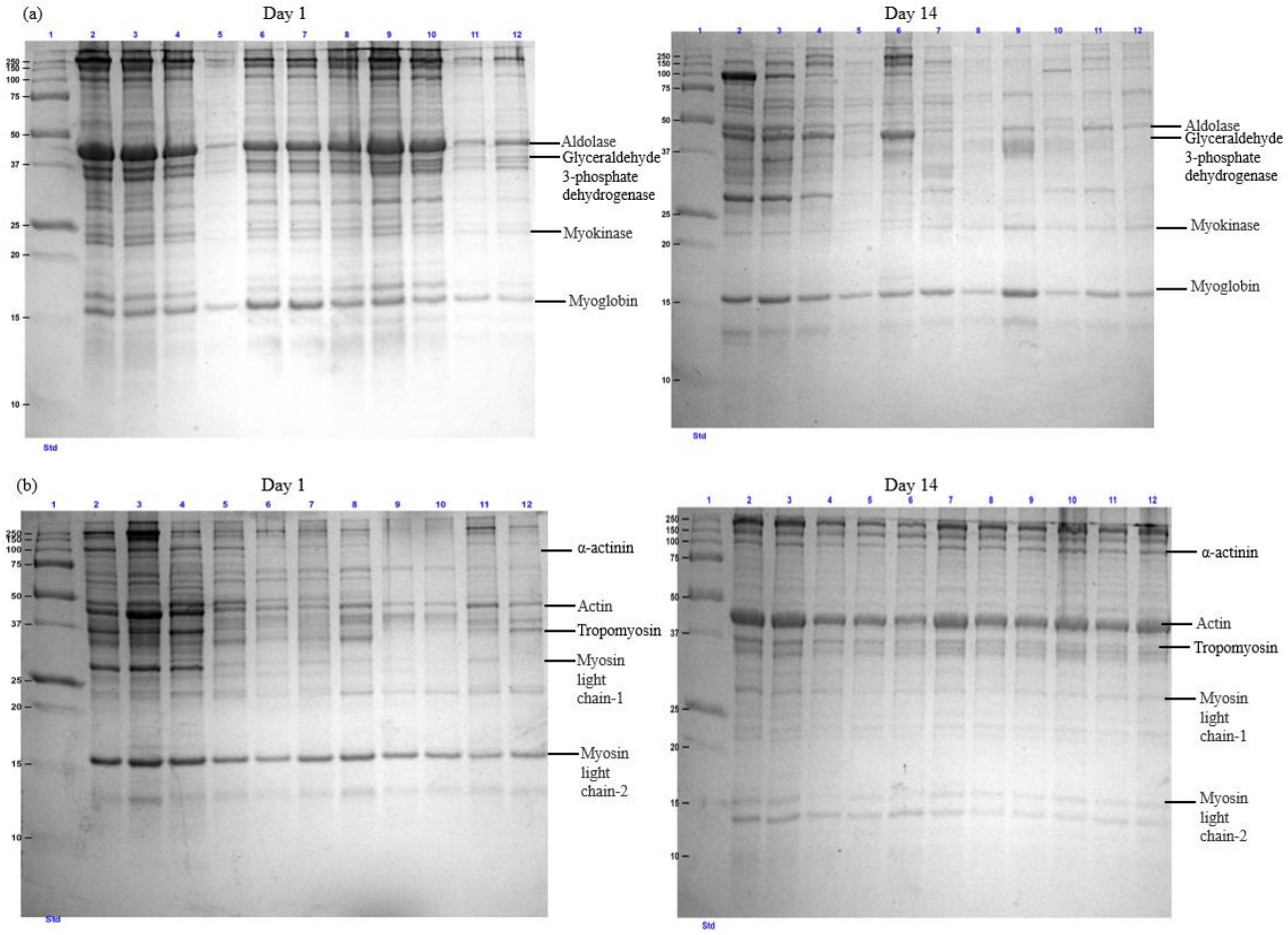


*Figure 21. SDS-PAGE patterns of (a) sarcoplasmic and (b) myofibrillar proteins of vacuum-packed wild red deer meat subjected to HHP treatments (150–600 MPa) and control (0 MPa) on Day 1 and Day 14 of storage at 4 ± 1 °C. (Lane 1: molecular weight standard; Lane 2: control (0 MPa); Lane 3: 150 MPa; Lane 4: 200 MPa; Lane 5: 250 MPa; Lane 6: 300 MPa; Lane 7: 350 MPa; Lane 8: 400 MPa; Lane 9: 450 MPa; Lane 10: 500 MPa; Lane 11: 550 MPa; Lane 12: 600 MPa)*

By Day 14, the degradation of sarcoplasmic proteins was more pronounced across all treatments. The control sample (Lane 2) still retained identifiable bands, but higher-pressure treatments (500-600 MPa; Lanes 10–12) exhibited marked reductions in band intensity, with the near disappearance of lower molecular weight proteins. Of note, aldolase (~40 kDa) showed a visible reduction in intensity in Day 14 HHP-treated samples. Aldolase, a glycolytic enzyme, is considered a quality biomarker associated with meat softness, colour stability, and physiological stress indicators (Marcos et al.,

2010). Its decreased abundance under HHP storage conditions could therefore be linked to post-mortem structural and metabolic changes.

The SDS-PAGE analysis of myofibrillar proteins in Figure 21 (b) also demonstrated significant changes due to HHP and storage duration. The majority of myofibrillar proteins consist of myosin, which is composed of two heavy chains and three light chains. Identified bands included α-actinin (~100 kDa), actin (~42 kDa), tropomyosin (~36 kDa), myosin light chain-1 (MLC-1, ~25 kDa), and myosin light chain-2 (MLC-2, ~18 kDa). As shown in Figure 21 (b), the MLC-1 protein primarily underwent aggregation and denaturation as a result of high-pressure treatment. In contrast, MLC-2 remained detectable even after exposure to 600 MPa. After 14 days of storage, the MLC-2 band showed decreased intensity, accompanied by the appearance of a stronger band in the ~37–50 kDa region, which may represent a degradation fragment or intermediate product derived from actin or myosin heavy chain proteolysis. Such protein changes can affect water-holding capacity and tenderness by altering the integrity of the myofibrillar network (Li et al., 2012).

At lower pressures (150 and 200 MPa), the intensity of myofibrillar proteins, as observed in the SDS-PAGE analysis, was comparable to that of the control. However, at pressures of 250 MPa or higher, a noticeable reduction in protein intensity was observed. This decline in intensity is attributed to protein aggregation caused by the high-pressure treatment.

### **5.2.6** Microbiological Evaluation

The impact of HHP treatments on the aerobic plate count (APC) of vacuum-packed wild red deer meat samples during storage is presented in Figure 22. The microbial counts on Day 1 and Day 14 demonstrate the effectiveness of HHP in reducing microbial loads and its influence on microbial growth during storage at 4 ± 1 °C.

On Day 1, a significant reduction in microbial counts was observed as the pressure increased, with the control (0 MPa) samples exhibiting the highest microbial load 4.67 log CFU/g. Samples treated at 150 MPa to 250 MPa showed moderate reductions, while pressures of 300 MPa and above resulted in a more pronounced decline in microbial counts, achieving levels below 1 log CFU/g at pressures of 500 MPa or higher. This dose-dependent microbial inactivation effect of HHP has been widely documented in the literature (Garriga et al., 2004; Martínez Bernié et al., 2021), attributed to the ability of high pressure to disrupt microbial cell membranes, denature cellular proteins, and inhibit enzyme activity. HHP is generally more effective against Gram-negative bacteria (e.g., *Pseudomonas spp*., *Enterobacteriaceae*) due to their thinner peptidoglycan layer, whereas Gram-positive bacteria (e.g.,

*Lactobacillus spp., Listeria monocytogenes*) exhibit greater resistance to pressure-induced inactivation due to their thicker cell walls (Wei et al., 2025). Bacteria typically present in chilled game meat include *Pseudomonas spp., Lactobacillus spp.,* and members of the *Enterobacteriaceae* family. Among these, *Pseudomonas spp*. is the primary contributor to meat spoilage, while facultative anaerobic bacteria, particularly *Lactobacillus spp*., tend to dominate in vacuum-packaged meat (Doulgeraki et al., 2012; Kunová et al., 2022).

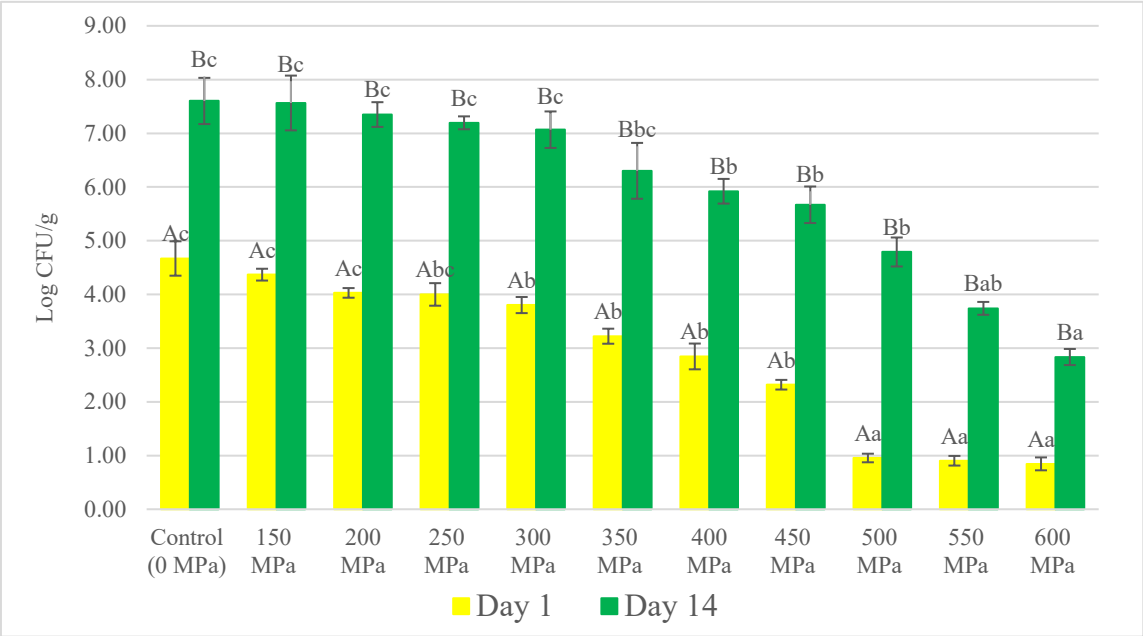


*Figure 22. Means ± SD for aerobic plate count (log CFU/g) of vacuum-packed wild red deer meat samples subjected to different high hydrostatic pressure (HHP) treatments (150–600 MPa) and control (0 MPa) on Day 1 and Day 14 of storage at 4 ± 1 °C. Means marked with different lowercase letters (a, b, c) indicate significant differences ($p < 0.05$) among different pressure levels, while means with different uppercase letters (A, B) indicate significant differences ($p < 0.05$) between storage periods (Day 1 vs. Day 14), based on Tukey's HSD test.*

By Day 14, microbial counts in all samples increased due to storage-related microbial growth, with the control samples reaching 7.6 log CFU/g. In contrast, HHP-treated samples demonstrated significantly lower microbial growth compared to the control, with higher pressures (500 MPa and above) maintaining microbial counts below 5 log CFU/g. Notably, microbial counts in samples treated at 600 MPa remained 2.83 log CFU/g, indicating the potential of high-pressure treatments to extend the shelf life of vacuum-packed meat products. Similar trends have been reported by McArdle et al. (2011), where pressures above 400 MPa were effective in suppressing microbial growth during chilled storage.

Additionally, microbial counts on Day 14 were consistently significantly higher than those on Day 1 for all pressure levels, reflecting microbial recovery or regrowth during storage. However, the extent of microbial growth was strongly dependent on the applied pressure, with higher pressures effectively limiting growth.

## 5.3 Sous-Vide Treatment (Experiment 3)

### 5.3.1 Moisture-Related Parameters (Cooking Loss and WHC)

#### *5.3.1.1 Cooking Loss*

Figure 23 presents the cooking loss (%) of wild red deer meat samples of different age subjected to sous-vide treatment at 60 °C, 65 °C, and 70 °C. The results indicate a significant increase in cooking loss with rising sous-vide temperature ($p < 0.05$) across all sample. At 60 °C, the lowest cooking loss values were observed, whereas at 70 °C, the highest levels of cooking loss were recorded, particularly for 36 and 37 months, where significant differences among treatments were noted ($p < 0.05$). These findings align with previous studies, which suggest that higher cooking temperatures lead to greater moisture loss due to increased protein denaturation and muscle shrinkage (Christensen et al., 2000; Tornberg, 2005).

The mechanism behind the observed trend can be attributed to heat-induced myofibrillar protein denaturation, which reduces the ability of muscle fibers to retain water. As temperature increases, collagen solubilization and connective tissue shrinkage further contribute to water expulsion from the muscle structure (Baldwin, 2012). Previous research has also confirmed that sous-vide treatment at temperatures above 65 °C leads to irreversible structural modifications in myofibrillar proteins, which correlates with increased water loss and reduced juiciness in cooked meat (Roldán et al., 2013).

These results highlight the trade-off between cooking temperature and moisture retention, indicating that while higher temperatures (70 °C) ensure food safety, they significantly increase cooking loss, which may affect meat texture and sensory quality. This suggests that moderate temperatures (60-65 °C) may be optimal for balancing moisture retention and tenderness in sous-vide-treated wild red deer meat.

Age-related differences were also observed: older animals (e.g., 36, 37, 48 months) tended to have higher cooking loss, especially at 70 °C, possibly due to greater collagen cross-linking and thermal shrinkage of more mature connective tissue. Conversely, younger samples (7 – 12 months) retained

more moisture, showing lower cooking loss at all temperatures. Based on its cooking loss and WHC patterns, the unknown sample exhibited characteristics most similar to mid-aged deer (approximately 32 months).

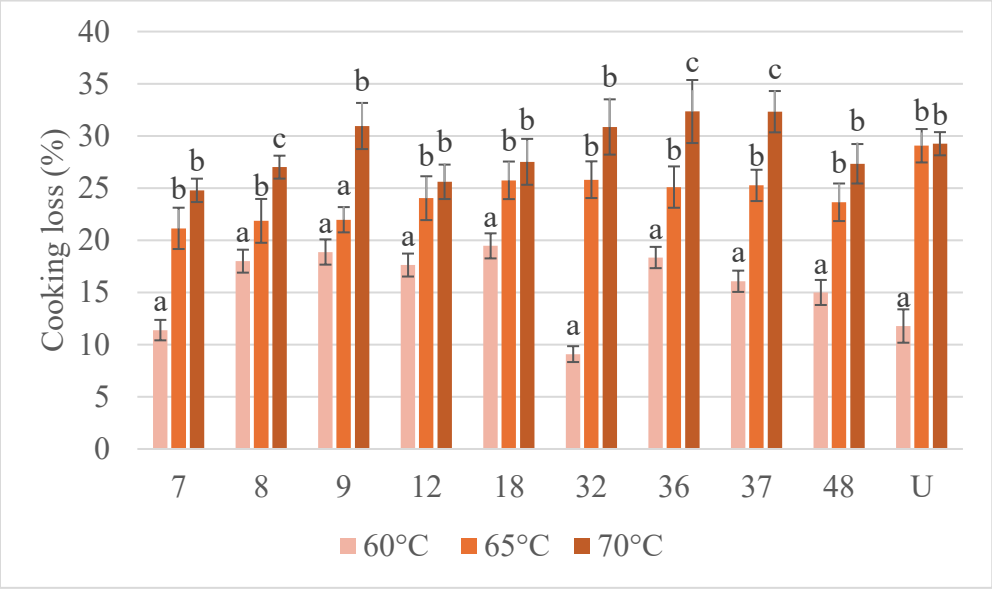


*Figure 23. Means ± SD for cooking loss of wild red deer meat samples subjected to sous-vide treatments at 60°C, 65°C, and 70°C. Means with different letters (a, b, c) indicate significant differences ($p < 0.05$) among sous-vide temperatures within each individual sample.*

#### *5.3.1.2 Water holding capacity (WHC)*

WHC showed a clear temperature-dependent decline across all samples (Figure 24). The highest WHC was consistently observed in control (raw) samples, while values decreased significantly ($p < 0.05$) with increasing sous-vide temperature.

The reduction in WHC at higher sous-vide temperatures is attributed to structural protein changes, specifically the denaturation of myosin and actin filaments, leading to increased muscle contraction and moisture loss (Christensen, Purslow and Larsen, 2000). At higher temperatures (>65 $^{o}$C), the sarcoplasmic proteins lose their ability to bind water, which corresponds with previous studies indicating that protein aggregation reduces WHC in cooked meat (Deng *et al.*, 2002; Tornberg, 2005).

Age also influenced WHC: younger samples (7-12 months) had generally higher WHC. In contrast, older animals exhibited lower WHC overall, likely due to lower protein solubility, greater connective tissue rigidity, and reduced intracellular water retention. The WHC values of the unknown sample closely resembled those of mid-aged deer (specifically 32–36 months), suggesting that it likely belongs to a similar age group.

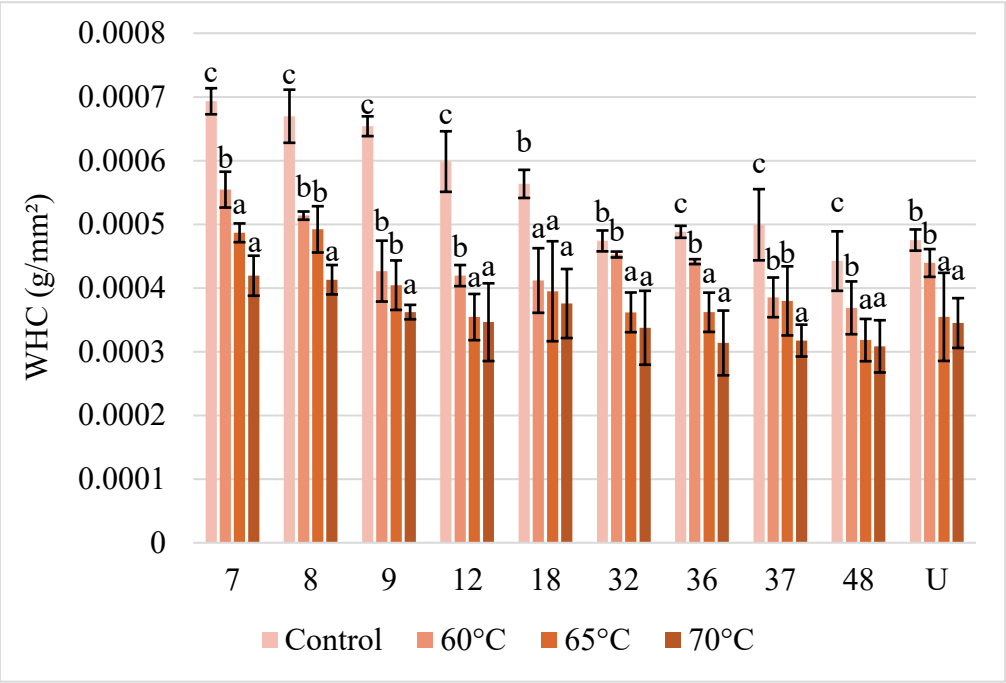


*Figure 24. Means ± SD for WHC of wild red deer meat samples subjected to sous-vide treatments at 60°C, 65°C, and 70°C. Means with different letters (a, b, c) indicate significant differences ($p < 0.05$) among sous-vide temperatures within each individual sample.*

### 5.3.2 pH

The pH values of wild deer meat samples subjected to different Sous-vide treatments (60 °C, 65 °C, and 70 °C) are summarized in Table 8. Sous-vide treatments significantly influenced the pH values across samples, resulting in increased pH compared to control meat samples.

With increasing sous-vide temperature, pH values increased. Control pH values ranged from 5.52 to 6.09, characteristic of normal postmortem meat conditions. However, upon sous-vide treatment, all samples exhibited significantly higher pH values, ranging from 5.81 to 6.39. These increases were consistent and statistically significant ($p < 0.05$) across all samples and treatment temperatures, reflecting pronounced protein denaturation and alterations in muscle fiber structure induced by heat treatment. Similar results can also be seen in the literature (Oz et al., 2017; Ayub and Ahmad, 2019).

In deer meat, the pH typically declines to 5.4–6.0 within 24–48 h after slaughter, with the optimum quality range being 5.5–5.7, as this favors desirable color, tenderness, juiciness, and processing characteristics. Values between 5.8 and 6.2 are considered intermediate, which may still be acceptable but can indicate changes in meat quality (Wiklund et al., 1995; Soriano et al., 2020).

**Table 8.** pH values of wild deer meat samples treated with different Sous-Vide temperatures (Mean ± SD).

| Sample age (months) | Control | 60 °C | 65 °C | 70 °C |
|---|---|---|---|---|
| 7 | $5.63\pm0.01^{a}_{x}$ | $5.95\pm0.03^{ab}_{y}$ | $6.07\pm0.10^{b}_{y}$ | $6.02\pm0.02^{b}_{y}$ |
| 8 | $5.79\pm0.19^{ab}_{x}$ | $5.87\pm0.02^{a}_{y}$ | $5.95\pm0.03^{a}_{y}$ | $6.11\pm0.05^{b}_{z}$ |
| 9 | $5.57\pm0.01^{a}_{x}$ | $5.95\pm0.08^{ab}_{y}$ | $6.02\pm0.02^{b}_{y}$ | $5.99\pm0.01^{a}_{y}$ |
| 12 | $6.09\pm0.03^{c}_{x}$ | $6.39\pm0.16^{c}_{z}$ | $6.24\pm0.08^{c}_{y}$ | $6.23\pm0.04^{c}_{y}$ |
| 18 | $5.64\pm0.03^{a}_{x}$ | $6.09\pm0.13^{b}_{y}$ | $6.26\pm0.06^{c}_{z}$ | $6.28\pm0.09^{c}_{z}$ |
| 32 | $5.83\pm0.01^{b}_{x}$ | $6.15\pm0.02^{b}_{y}$ | $6.23\pm0.01^{c}_{z}$ | $6.27\pm0.02^{c}_{z}$ |
| 36 | $5.84\pm0.14^{b}_{x}$ | $6.19\pm0.02^{b}_{y}$ | $6.28\pm0.01^{c}_{y}$ | $6.21\pm0.03^{c}_{y}$ |
| 37 | $5.61\pm0.11^{a}_{x}$ | $5.88\pm0.08^{a}_{y}$ | $5.9\pm0.01^{a}_{y}$ | $5.93\pm0.04^{a}_{y}$ |
| 48 | $5.52\pm0.01^{a}_{x}$ | $5.81\pm0.01^{a}_{y}$ | $5.96\pm0.02^{a}_{y}$ | $5.98\pm0.02^{a}_{y}$ |
| U | $5.87\pm0.01^{b}_{x}$ | $6.13\pm0.03^{b}_{y}$ | $6.16\pm0.05^{b}_{y}$ | $6.18\pm0.05^{bc}_{y}$ |

Means in the same column within an attribute with no letters in common (a, b, c; superscript) indicate significant differences across deer meat samples of different ages ($p < 0.05$); means in the same row with no letters in common (x, y, z; subscript) indicate significant differences in treatment effects ($p < 0.05$).

Comparison across age groups revealed noteworthy variations. The pH of raw deer meat samples ranged from 5.52 in the oldest red deer (48-month-old) to 6.09 in the 12-month-old red deer. Statistically significant differences were observed among samples ($p < 0.05$), as indicated by differing superscript letters. Notably, 12-month-old had the highest pH value, which was significantly greater than several other samples, including 7, 9, and 48-month-old. The unknown sample (U) had a pH of 5.87, statistically similar to the mid-aged group (32 and 36-month-old), suggesting a possible age estimation in that range.

Although pH trends in postmortem muscle can be influenced by multiple factors, the general pattern observed here shows a peak in pH at 12 months, followed by a gradual decline with increasing age. This is consistent with findings from (Maggiolino et al., 2019), who reported that pH at 72 h postmortem decreased significantly with age in Iberian wild red deer. The likely explanation is that younger animals, particularly around the weaning or early growth phase, may have more variable glycogen reserves and potentially higher stress sensitivity, leading to less complete postmortem glycolysis and higher ultimate pH (Wiklund et al., 1995; Maggiolino et al., 2019).

### 5.3.3 Surface Color

Meat color plays a critical role in consumer preference and acceptance (Bureš et al., 2015; Demartini et al., 2018). Wild animals have more physically active lifestyles compared to farmed animals, resulting in elevated levels of myoglobin in their muscles, which contributes to the darker coloration. Volpelli et al. (2003) reported that the dark red color of wild red deer meat, typically preferred by consumers, is defined by low lightness ($L^* < 40$) and high redness ($a^* > 21$) values. In the present study, while $L^*$ values were consistently below 40, indicating darker meat, the average $a^*$ values were lower than the threshold reported by Volpelli et al. (2003). Nonetheless, the results align with the findings of Hutchison et al. (2012), who observed similar color characteristics in deer meat.

**Table 9.** Color Parameters ($L^*$, $a^*$, $b^*$), hue angle, and chroma of deer meat samples (mean ± SD).

| Sample age (months) | *L** | *a** | *b** | Hue angle | Chroma |
|---|---|---|---|---|---|
| 7 | 35.1 ± 4.59$^{a}$ | 11.2 ± 2.54$^{a}$ | 4.0 ± 0.80$^{ab}$ | 0.34 ± 0.10$^{a}$ | 11.89 ± 2.41$^{a}$ |
| 8 | 37.2 ± 1.34$^{a}$ | 10.2 ± 1.00$^{a}$ | 2.6 ± 0.37$^{a}$ | 0.25 ± 0.04$^{a}$ | 10.53 ± 0.97$^{a}$ |
| 9 | 41.6 ± 4.59$^{b}$ | 14.6 ± 1.54$^{a}$ | 3.5 ± 0.50$^{a}$ | 0.24 ± 0.04$^{a}$ | 15.01 ± 1.50$^{b}$ |
| 12 | 35.8 ± 4.59$^{a}$ | 10.4 ± 1.30$^{a}$ | 4.6 ± 0.31$^{b}$ | 0.42 ± 0.05$^{b}$ | 11.37 ± 1.20$^{a}$ |
| 18 | 33.2 ± 1.20$^{a}$ | 9.9 ± 1.00$^{a}$ | 5.0 ± 0.17$^{b}$ | 0.47 ± 0.04$^{b}$ | 11.09 ± 0.90$^{a}$ |
| 32 | 35.3 ± 4.59$^{a}$ | 10.6 ± 2.54$^{a}$ | 3.3 ± 0.47$^{a}$ | 0.30 ± 0.08$^{a}$ | 11.06 ± 2.29$^{a}$ |
| 36 | 37.0 ± 2.34$^{a}$ | 9.3 ± 1.00$^{a}$ | 3.7 ± 0.22$^{a}$ | 0.38 ± 0.04$^{b}$ | 10.00 ± 0.95$^{a}$ |
| 37 | 41.8 ± 4.59$^{b}$ | 11.4 ± 2.54$^{a}$ | 4.9 ± 0.60$^{b}$ | 0.41 ± 0.09$^{b}$ | 12.45 ± 2.60$^{a}$ |
| 48 | 36.0 ± 3.67$^{a}$ | 12.0 ± 3.08$^{a}$ | 4.6 ± 0.40$^{b}$ | 0.37 ± 0.09$^{b}$ | 12.89 ± 3.12$^{b}$ |
| U | 37.0 ± 2.46$^{a}$ | 13.4 ± 1.00$^{a}$ | 2.8 ± 0.17$^{a}$ | 0.21 ± 0.02$^{a}$ | 13.69 ± 1.01$^{b}$ |

Means with different superscript letters (a, b, c) indicate significant differences across deer meat samples of different ages ($p < 0.05$).

Color parameters ($L^*$, $a^*$, $b^*$), as well as derived values such as hue angle and chroma of raw red deer meat with different ages, are presented in Table 9. The $L^*$ values did not follow a clear age-related trend. However, 9 and 37-month-old exhibited significantly higher $L^*$ values ($p < 0.05$) compared to the other samples, indicating lighter meat color in these individuals. In contrast, $a^*$ values, which represent redness, did not differ significantly among age groups, a finding consistent with the results reported by Maggiolino et al. (2019) in wild red deer. Yellowness ($b^*$) varied significantly between samples, with 12, 18, 37, and 48-month-old showing notably higher values than the others.

Hue angle was generally higher in samples 12, 18, 36, 37, and 48-month-old, suggesting a shift toward a yellower hue in older animals. Meanwhile, chroma values, which represent color vividness, were highest in 9, 48-month-old, and unknown, indicating more saturated color tones in these samples.

**Table 10**. Color difference (*ΔE*) between raw deer samples of varying ages and the 7-month-old reference sample.

| Sample No. | Δ*E* value | Interpretation |
|---|---|---|
| 7 | – | Reference sample |
| 8 | 2.74 | An inexperienced observer also notices the difference |
| 9 | 7.26 | The observer notices two different colors |
| 12 | 1.25 | Only an experienced observer may notice the difference |
| 18 | 2.51 | An inexperienced observer also notices the difference |
| 32 | 1.04 | Only an experienced observer may notice the difference |
| 36 | 4.29 | A clear difference in color is noticed |
| 37 | 6.70 | The observer notices two different colors |
| 48 | 1.28 | Only an experienced observer may notice the difference |
| U | 3.05 | An inexperienced observer also notices the difference |

Furthermore, the total color difference ( $\Delta E$) was used to quantify perceptible variations in meat color among deer samples, with 7-month-old serving as the reference due to its young age. The $\Delta E$ values ranged from 1.04 (32-month-old) to 7.26 (9-month-old), providing insight into the visual distinguishability of wild red deer meat from animals of different ages. Samples 12-month-old (1.25), 32-month-old (1.04), and 48-month-old (1.28) exhibited low $\Delta E$ values, indicating minimal perceptible color differences. These values suggest that only trained observers might notice any distinction when compared to 7-month-old. By contrast, unknown (3.05), 36-month-old (4.29), and 18-month-old (2.51) showed moderate differences, classified as clearly noticeable to inexperienced observers. The highest $\Delta E$ values were observed in 9-month-old (7.26) and 37-month-old (6.70), exceeding the perceptual threshold of $\Delta E > 5$, which indicates that these meats would appear distinctly different in color to consumers. Based on visual color metrics, the unknown sample demonstrated characteristics most comparable to 36 and 8-month-old, with a similar $L^*$ value and a hue angle close to that of 8 and 9-month-old. Its chroma profile more closely resembled that of 48-month-old, indicating a more vivid color appearance. Its moderate $\Delta E$ value relative to the youngest

deer (7-month-old) was also similar to 8-month-old, suggesting unknown is distinct from younger deer.

Surface color measurements ($L^*$, $a^*$, $b^*$) of sous-vide treated (60 °C, 65 °C, and 70 °C) samples of different age varies are detailed in Figures 25–27. Changes in meat color can be attributed to multiple factors, such as protein denaturation, oxidation, and the formation of various pigment compounds. Of these, the Maillard reaction plays a relatively minor role, as the low temperatures used in sous-vide cooking limit the formation of Maillard-derived color changes (Stanisławczyk et al., 2023). Sous-vide treatments generally increased the $L^*$ values in most samples, indicating a marginal increase in meat surface brightness. Statistical analysis revealed significant differences ($p < 0.05$) among treatments.

Redness values, as illustrated in Figure 26, decreased significantly at higher temperatures (65 °C and 70 °C), indicating the thermal denaturation of myoglobin. Younger samples maintained higher redness levels post-treatment compared to older samples, indicating potential variations in myoglobin stability linked to animal age. Yellowness values (Figure 27) significantly increased across sous-vide treatments for all samples, correlating with pigment degradation and browning (Stanisławczyk et al., 2023). Again, variations related to age were apparent, suggesting differential thermal stability of pigments across age groups.

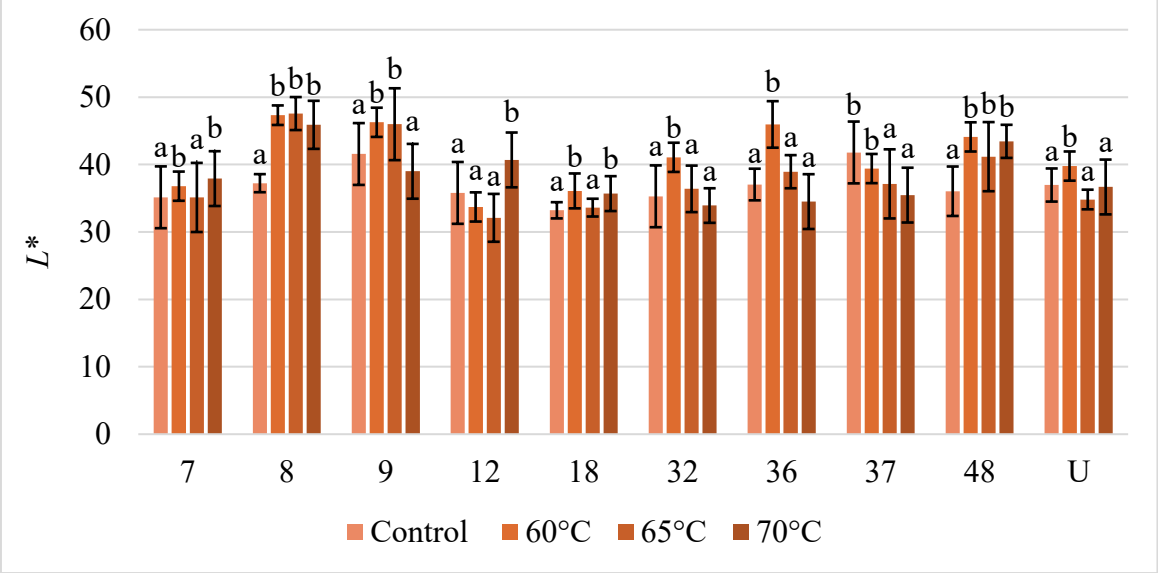


*Figure 25. Means ± SD for surface color lightness (L*) of wild red deer meat samples subjected to sous-vide treatments at 60°C, 65°C, and 70°C. Means with different letters (a, b, c) indicate significant differences (p < 0.05) among sous-vide temperatures within each individual sample.*

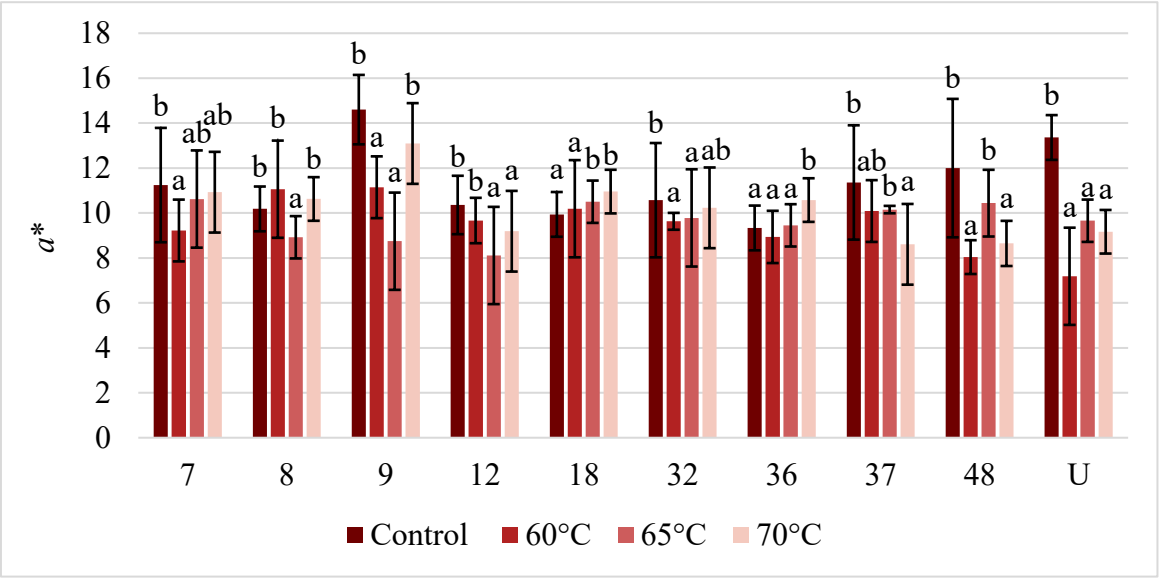


*Figure 26. Means ± SD for a* of wild red deer meat samples subjected to sous-vide treatments at 60°C, 65°C, and 70°C. Means with different letters (a, b, c) indicate significant differences ($p < 0.05$) among sous-vide temperatures within each individual sample.*

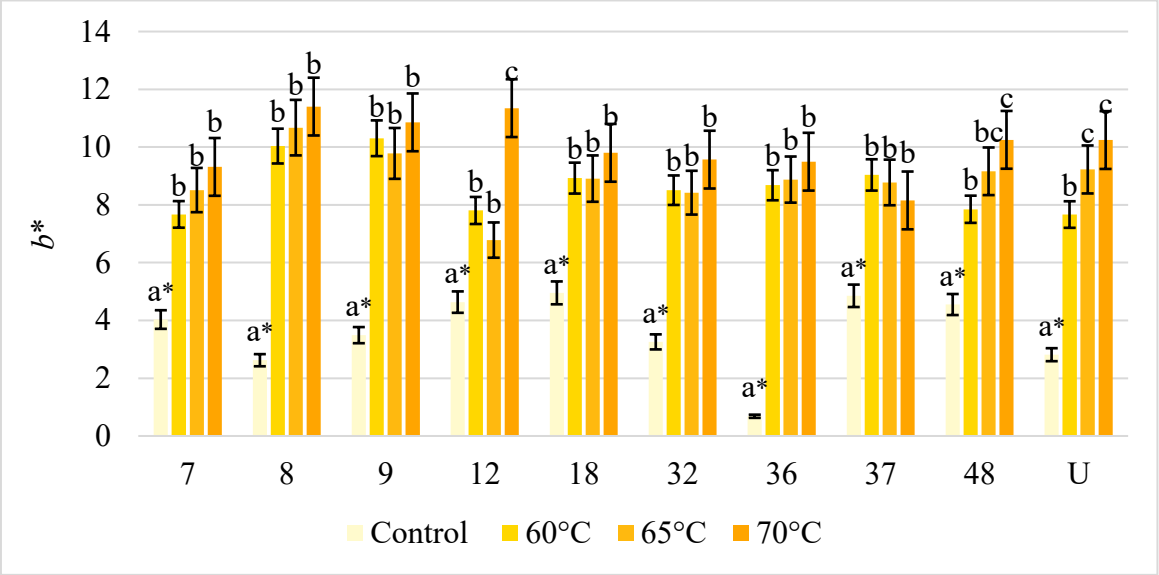


*Figure 27. Means ± SD for b* of wild deer meat samples subjected to sous-vide treatments at 60°C, 65°C, and 70°C. Means with different letters (a, b, c) indicate significant differences ($p < 0.05$) among sous-vide temperatures within each individual sample. * indicates control samples significantly lower ($p < 0.001$) compared to all treated samples.*

Visual appearance of wild red deer meat samples subjected to sous-vide treatments at temperatures of 60 °C, 65 °C, and 70 °C, compared to control samples, is presented in Figure 28. Noticeable visual changes are evident with increasing treatment temperatures and differences across meat samples of varying ages.

Surface color differences ($\Delta E$) of wild red deer meat samples subjected to sous-vide treatment at temperatures of 60 $^{O}$C, 65 $^{O}$C, and 70 $^{O}$C are summarized in Table 11. The $\Delta E$ values quantify the overall color differences, with most samples displaying significant increases with rising treatment temperatures. Older samples generally exhibited higher $\Delta E$ values, reinforcing visual observations of more substantial color shifts compared to younger samples. These findings highlight age-dependent differences in thermal susceptibility, important for tailoring sous-vide cooking conditions to optimize visual quality attributes and consumer appeal.

Overall, sous-vide treatments significantly influenced meat surface color, with age playing a substantial role in determining the extent and nature of color change. These findings provide crucial insights for selecting optimal sous-vide processing parameters based on meat age to enhance consumer acceptability.

**Table 11**. Color difference ($\Delta E$) between untreated and treated samples.

| Sample age (months) | 60°C | 65°C | 70°C |
|---|---|---|---|
| 7 | 4.48 | 4.52 | 5.97 |
| 8 | 12.55 | 13.16 | 12.33 |
| 9 | 8.97 | 9.66 | 7.94 |
| 12 | 3.86 | 4.83 | 8.39 |
| 18 | 4.91 | 4.01 | 5.54 |
| 32 | 7.87 | 5.34 | 6.45 |
| 36 | 11.98 | 8.41 | 9.25 |
| 37 | 4.98 | 6.21 | 7.65 |
| 48 | 9.57 | 7.07 | 9.92 |
| U | 8.34 | 7.71 | 8.54 |

Note: $\Delta E$ values represent the total color difference between each sous-vide treated sample and its corresponding untreated (raw) control. Higher values indicate greater visual deviation from the control sample color.

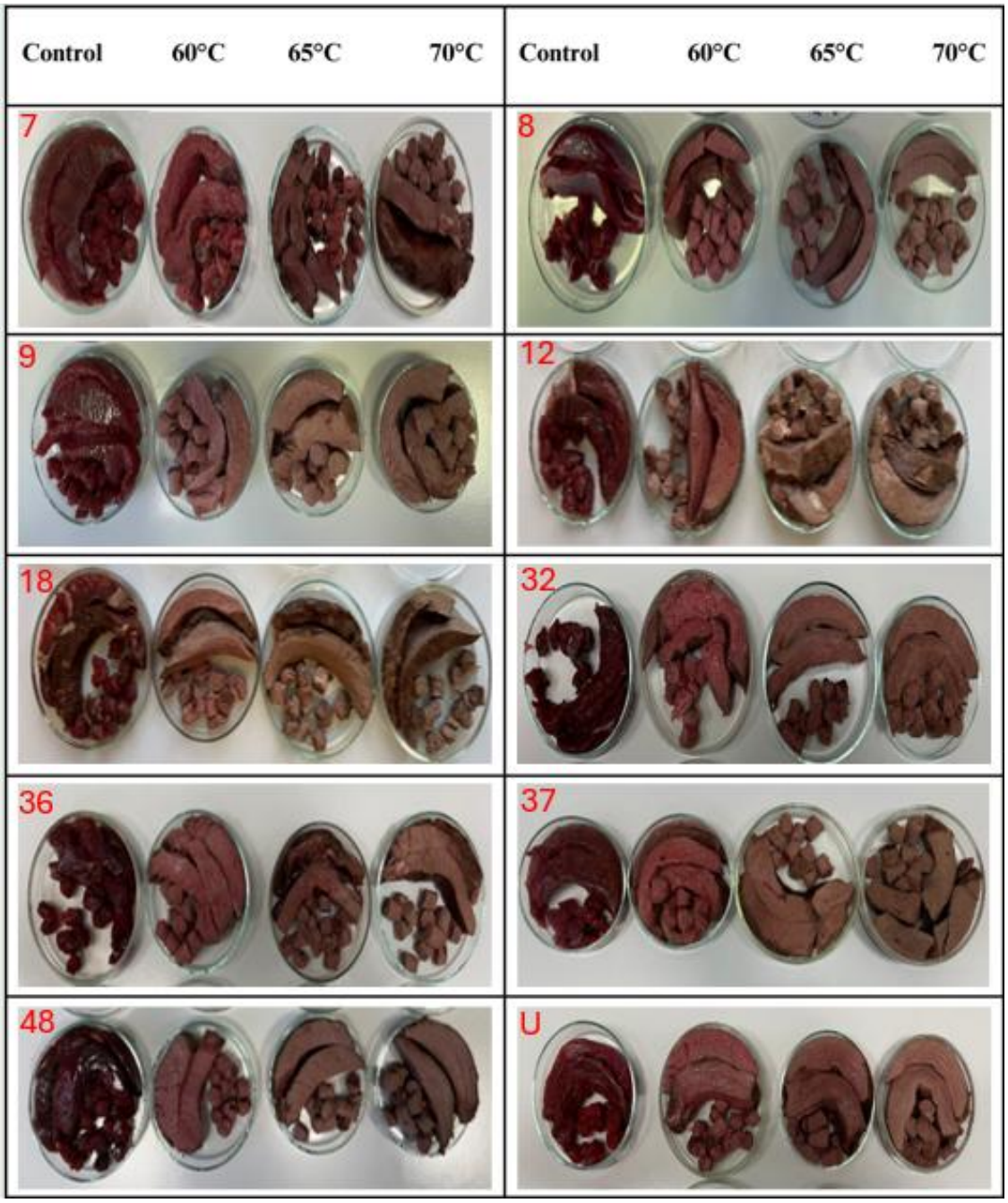


*Figure 28. Visual differences in wild red deer meat samples of varying ages subjected to sous-vide treatments at 60°C, 65°C, and 70°C compared to control (raw) samples.*

### **5.3.4** Texture analysis

Texture is one of the most critical quality attributes affecting consumer acceptance of meat, particularly in wild game, which is known for its unique muscular structure and low intramuscular fat content. In this study, the texture properties of wild red deer meat were evaluated through shear force

and TPA parameters, including hardness, cohesiveness, springiness, and chewiness (Table 12), in relation to animal age and sous-vide temperature (60 °C, 65 °C, and 70 °C for 3 hours).

**Table 12**. Instrumental texture measurement results of wild red deer meat samples subjected to sous-vide treatments at 60 °C, 65 °C, and 70 °C, compared to control samples (mean ± SD).

| Characteristics | Sample age (months) | Control | 60°C | 65°C | 70°C |
|---|---|---|---|---|---|
| Shear force (N) | 7 | $32.38\pm11.23^{a}_{x}$ | $65.93\pm2.45^{b}_{z}$ | $54.81\pm3.23^{a}_{y}$ | $69.11\pm8.56^{b}_{z}$ |
| | 8 | $39.99\pm9.66^{a}_{x}$ | $45.16\pm1.59^{a}_{y}$ | $46.18\pm3.98^{a}_{y}$ | $47.59\pm2.47^{a}_{y}$ |
| | 9 | $37.57\pm2.96^{a}_{x}$ | $38.42\pm2.55^{a}_{x}$ | $47.00\pm4.89^{a}_{y}$ | $56.21\pm4.25^{a}_{z}$ |
| | 12 | $39.83\pm6.76^{a}_{x}$ | $68.71\pm7.23^{b}_{y}$ | $83.79\pm9.47^{c}_{z}$ | $87.55\pm9.77^{c}_{y}$ |
| | 18 | $48.89\pm3.33^{b}_{x}$ | $61.12\pm5.21^{b}_{y}$ | $62.35\pm6.11^{b}_{y}$ | $79.22\pm7.29^{b}_{z}$ |
| | 32 | $42.43\pm3.65^{b}_{x}$ | $59.07\pm8.98^{b}_{y}$ | $61.26\pm8.67^{b}_{y}$ | $82.43\pm9.44^{c}_{z}$ |
| | 36 | $49.53\pm3.39^{b}_{x}$ | $52.59\pm4.42^{b}_{x}$ | $57.21\pm11.77^{a}_{y}$ | $61.93\pm5.73^{b}_{y}$ |
| | 37 | $56.70\pm11.02^{c}_{x}$ | $65.47\pm10.12^{b}_{y}$ | $64.80\pm5.16^{b}_{y}$ | $71.52\pm9.21^{b}_{z}$ |
| | 48 | $60.80\pm8.18^{c}_{x}$ | $63.17\pm5.21^{b}_{x}$ | $61.47\pm5.55^{b}_{x}$ | $77.10\pm8.05^{bc}_{y}$ |
| | U | $53.75\pm10.83^{c}_{x}$ | $61.99\pm11.62^{b}_{x}$ | $70.48\pm11.42^{b}_{y}$ | $78.35\pm15.34^{c}_{y}$ |
| Hardness (N) | 7 | $7.37\pm0.34^{a}_{x}$ | $55.85\pm4.56^{b}_{y}$ | $68.41\pm5.34^{b}_{z}$ | $87.54\pm7.23^{b}_{z}$ |
| | 8 | $6.32\pm0.45^{a}_{x}$ | $35.43\pm3.45^{a}_{y}$ | $40.16\pm3.12^{a}_{y}$ | $51.36\pm4.67^{a}_{z}$ |
| | 9 | $8.99\pm0.36^{b}_{x}$ | $52.29\pm4.12^{b}_{y}$ | $55.88\pm4.39^{ab}_{y}$ | $82.25\pm6.25^{b}_{z}$ |
| | 12 | $6.47\pm0.56^{a}_{x}$ | $43.83\pm3.67^{ab}_{y}$ | $85.21\pm7.87^{c}_{z}$ | $118.34\pm9.29^{c}_{zz}$ |
| | 18 | $12.20\pm0.82^{b}_{x}$ | $41.97\pm4.87^{a}_{y}$ | $56.08\pm4.45^{b}_{y}$ | $86.25\pm6.99^{b}_{z}$ |
| | 32 | $11.33\pm0.63^{b}_{x}$ | $53.21\pm4.12^{b}_{y}$ | $48.16\pm3.56^{a}_{y}$ | $83.85\pm7.24^{b}_{z}$ |
| | 36 | $6.44\pm0.12^{a}_{x}$ | $55.29\pm3.96^{b}_{y}$ | $66.10\pm5.97^{b}_{y}$ | $104.63\pm9.33^{c}_{z}$ |
| | 37 | $15.91\pm0.56^{c}_{x}$ | $58.93\pm4.12^{b}_{y}$ | $88.45\pm4.23^{c}_{z}$ | $113.19\pm9.11^{c}_{zz}$ |
| | 48 | $12.26\pm0.91^{b}_{x}$ | $39.90\pm3.86^{a}_{y}$ | $56.93\pm4.55^{b}_{z}$ | $83.12\pm7.21^{b}_{zz}$ |
| | U | $14.49\pm0.98^{c}_{x}$ | $69.07\pm5.32^{c}_{y}$ | $91.63\pm7.12^{c}_{z}$ | $68.50\pm5.12^{a}_{y}$ |
| Cohesiveness (-) | 7 | $0.23\pm0.01^{a}_{x}$ | $0.43\pm0.02^{bc}_{y}$ | $0.46\pm0.01^{b}_{y}$ | $0.50\pm0.04^{c}_{y}$ |
| | 8 | $0.26\pm0.01^{a}_{x}$ | $0.42\pm0.02^{b}_{y}$ | $0.40\pm0.02^{ab}_{y}$ | $0.44\pm0.03^{b}_{y}$ |
| | 9 | $0.35\pm0.01^{c}_{y}$ | $0.29\pm0.01^{a}_{x}$ | $0.38\pm0.03^{a}_{y}$ | $0.37\pm0.03^{a}_{y}$ |
| | 12 | $0.31\pm0.01^{bc}_{x}$ | $0.39\pm0.02^{b}_{y}$ | $0.46\pm0.03^{b}_{z}$ | $0.41\pm0.04^{b}_{y}$ |
| | 18 | $0.31\pm0.03^{bc}_{x}$ | $0.42\pm0.03^{b}_{yz}$ | $0.44\pm0.03^{b}_{z}$ | $0.50\pm0.02^{c}_{z}$ |

| | | | | | |
|---|---|---|---|---|---|
| | 32 | $0.25\pm0.02^{a}_{x}$ | $0.49\pm0.03^{c}_{y}$ | $0.55\pm0.04^{c}_{y}$ | $0.52\pm0.03^{c}_{y}$ |
| | 36 | $0.35\pm0.03^{c}_{x}$ | $0.34\pm0.02^{ab}_{x}$ | $0.45\pm0.04^{b}_{y}$ | $0.39\pm0.04^{a}_{x}$ |
| | 37 | $0.22\pm0.01^{a}_{x}$ | $0.47\pm0.04^{c}_{y}$ | $0.42\pm0.03^{b}_{y}$ | $0.40\pm0.03^{ab}_{y}$ |
| | 48 | $0.26\pm0.02^{a}_{x}$ | $0.38\pm0.03^{b}_{y}$ | $0.40\pm0.03^{ab}_{y}$ | $0.40\pm0.03^{ab}_{y}$ |
| | U | $0.27\pm0.01^{ab}_{x}$ | $0.37\pm0.02^{b}_{yz}$ | $0.36\pm0.02^{a}_{y}$ | $0.44\pm0.04^{b}_{z}$ |
| Springiness (mm) | 7 | $0.89\pm0.05^{b}_{y}$ | $0.80\pm0.04^{a}_{y}$ | $0.53\pm0.04^{a}_{x}$ | $0.78\pm0.06^{b}_{y}$ |
| | 8 | $0.90\pm0.09^{b}_{x}$ | $0.91\pm0.05^{b}_{x}$ | $0.90\pm0.08^{c}_{x}$ | $0.89\pm0.07^{c}_{x}$ |
| | 9 | $0.79\pm0.07^{a}_{x}$ | $0.89\pm0.08^{b}_{y}$ | $0.90\pm0.07^{c}_{y}$ | $0.85\pm0.08^{c}_{y}$ |
| | 12 | $0.87\pm0.08^{b}_{x}$ | $0.89\pm0.08^{b}_{x}$ | $0.86\pm0.08^{c}_{x}$ | $0.85\pm0.07^{c}_{x}$ |
| | 18 | $0.89\pm0.08^{b}_{y}$ | $0.82\pm0.09^{a}_{y}$ | $0.81\pm0.09^{b}_{y}$ | $0.71\pm0.08^{a}_{x}$ |
| | 32 | $0.88\pm0.07^{b}_{y}$ | $0.89\pm0.08^{b}_{y}$ | $0.89\pm0.08^{c}_{y}$ | $0.79\pm0.06^{b}_{x}$ |
| | 36 | $0.90\pm0.07^{b}_{z}$ | $0.88\pm0.07^{b}_{z}$ | $0.80\pm0.07^{b}_{y}$ | $0.79\pm0.06^{b}_{x}$ |
| | 37 | $0.90\pm0.07^{b}_{y}$ | $0.89\pm0.06^{b}_{y}$ | $0.79\pm0.07^{b}_{x}$ | $0.76\pm0.06^{a}_{x}$ |
| | 48 | $0.90\pm0.06^{b}_{y}$ | $0.90\pm0.07^{b}_{y}$ | $0.80\pm0.08^{b}_{x}$ | $0.79\pm0.07^{b}_{x}$ |
| | U | $0.89\pm0.05^{b}_{x}$ | $0.89\pm0.08^{b}_{x}$ | $0.87\pm0.09^{c}_{x}$ | $0.88\pm0.08^{c}_{x}$ |
| Chewiness (J) | 7 | $1.54\pm0.12^{a}_{x}$ | $19.18\pm1.21^{b}_{y}$ | $16.47\pm1.22^{a}_{y}$ | $34.08\pm2.93^{b}_{z}$ |
| | 8 | $1.46\pm0.13^{a}_{x}$ | $13.43\pm1.34^{a}_{y}$ | $14.67\pm1.04^{a}_{y}$ | $20.32\pm1.94^{a}_{z}$ |
| | 9 | $2.50\pm0.23^{b}_{x}$ | $13.39\pm1.12^{a}_{y}$ | $19.06\pm1.29^{a}_{y}$ | $25.70\pm2.34^{ab}_{z}$ |
| | 12 | $1.72\pm0.14^{a}_{x}$ | $15.27\pm1.98^{a}_{y}$ | $34.05\pm2.94^{c}_{z}$ | $41.51\pm3.88^{c}_{z}$ |
| | 18 | $3.33\pm0.32^{c}_{x}$ | $14.52\pm1.23^{a}_{y}$ | $19.91\pm1.20^{b}_{y}$ | $30.35\pm3.11^{b}_{z}$ |
| | 32 | $2.53\pm0.21^{b}_{x}$ | $23.30\pm2.45^{c}_{y}$ | $23.88\pm2.97^{b}_{y}$ | $34.26\pm2.87^{b}_{z}$ |
| | 36 | $1.78\pm0.22^{a}_{x}$ | $16.78\pm1.01^{b}_{y}$ | $25.92\pm2.22^{b}_{yz}$ | $32.77\pm2.19^{b}_{z}$ |
| | 37 | $3.12\pm0.23^{c}_{x}$ | $25.00\pm2.89^{c}_{y}$ | $29.39\pm1.83^{bc}_{y}$ | $34.53\pm3.29^{b}_{z}$ |
| | 48 | $2.86\pm0.02^{b}_{x}$ | $13.74\pm0.93^{a}_{y}$ | $18.34\pm1.96^{a}_{y}$ | $26.27\pm2.16^{ab}_{z}$ |
| | U | $3.46\pm0.03^{c}_{x}$ | $23.04\pm2.10^{c}_{y}$ | $28.74\pm2.45^{b}_{y}$ | $26.24\pm2.10^{ab}_{y}$ |

Means in the same column within an attribute with no letters in common (a, b, c; superscript) indicate significant differences across deer meat samples of different ages ($p < 0.05$); means in the same row with no letters in common (x, y, z; subscript) indicate significant differences in treatment effects ($p < 0.05$). Comparisons were made on an individual trait level (shear force, hardness, cohesiveness, springiness, and chewiness).

Shear force, an indicator of meat tenderness, was significantly influenced by both age and sous-vide temperature. Control (raw) samples exhibited the lowest shear force values, confirming their unaltered and intact muscle structure. Sous-vide treatment at 60 °C and 65 °C moderately increased shear force values, whereas treatment at 70 °C led to a pronounced increase. These results suggest that although sous-vide cooking is generally associated with tenderization, excessively high temperatures can lead

to protein hardening and increased toughness, particularly in wild game meat with dense muscle fibers and lower fat content.

Age had a compounding effect on shear force values. Young deer (7–9 months) displayed lower shear force post-treatment compared to older animals (32–48 months), likely due to less cross-linked collagen and more soluble connective tissue (Dominguez-Hernandez et al., 2018). The unknown-aged sample (U) demonstrated shear force behavior similar to older animals.

Hardness significantly increased ($p < 0.05$) with higher sous-vide temperatures across all samples. Control samples exhibited the lowest hardness values, while samples treated at 70 °C showed the highest values. 7, 12, 37, and 48 months demonstrated the most pronounced increase, with 12 months reaching 118.34 ± 9.29 N, indicating substantial protein denaturation and moisture loss at elevated temperatures. The significant differences ($p < 0.05$) between treatments suggest that sous-vide cooking strongly influences the mechanical resistance of the meat, with higher temperatures resulting in firmer textures. This increase in hardness at elevated temperatures is consistent with previous studies, which have reported that higher sous-vide temperatures can lead to increased meat toughness due to protein denaturation and moisture loss (Kurp et al, 2022).

Cohesiveness values varied among the samples, but a general trend of increased cohesiveness was observed in sous-vide-treated samples compared to controls. 7- and 32-month-old samples exhibited significantly higher cohesiveness at 70 °C ($p < 0.05$), while 8- and 37-month-old showed moderate increases. The increased cohesiveness at higher temperatures suggests improved protein gel formation, likely due to heat-induced cross-linking of muscle proteins.

Springiness, which indicates the meat's ability to return to its original shape after compression, showed mixed trends. Control samples exhibited relatively high springiness, but at 65 °C, a decline was observed in 7, 18, and 37-month-old, suggesting partial protein denaturation and loss of elasticity. However, at 70 °C, some samples (e.g., 8, 9-month-old) maintained their springiness, potentially due to different muscle fiber compositions and water-binding capacities.

Chewiness followed a similar pattern to hardness, increasing significantly with higher cooking temperatures. 7, 12, 37, and 48-month-old showed substantial increases at 70 °C, with 12-month-old reaching 41.51 ± 3.88 J, the highest value recorded. The observed increase in chewiness is consistent with the loss of water holding capacity and the hardening of myofibrillar proteins at elevated temperatures.

Overall, sous-vide cooking significantly altered the texture characteristics of wild red deer meat. Higher temperatures (65 °C and 70 °C) led to increased hardness and chewiness, while cohesiveness showed variable trends depending on muscle structure and age.

### **5.3.5** Protein Profile Analysis Using SDS-PAGE

Meat texture is a critical attribute influencing consumer acceptance, and it is strongly associated with protein-related structural changes (Larrea *et al.*, 2006). Sarcoplasmic proteins are known to influence key meat quality traits such as color, texture, and water holding capacity (Marcos et al., 2010). In the case of myofibrillar proteins, thermal denaturation during cooking leads to an increase in hydrophobic interactions, which in turn affects the meat's ability to retain moisture (Emel, 2023).

The SDS-PAGE pattern of **sarcoplasmic proteins** extracted from wild red deer samples treated at different sous-vide temperatures (60 °C, 65 °C, and 70 °C) is shown in Figure 29a. The analysis was conducted to evaluate heat-induced changes in protein solubility and stability under varying thermal conditions.

Lane 1 contains the molecular weight standard (10–250 kDa). Lanes 2–13 represent protein extracts from three wild red deer samples (7-, 18-, and 48-month-old), each including a raw control and three sous-vide treatments at increasing temperatures.

Across samples, band intensity and sharpness decreased progressively with temperature, particularly at 65 °C and 70 °C, suggesting partial protein denaturation or aggregation at higher sous-vide temperatures (Ayub and Ahmad, 2019). In comparison to control lanes (Lanes 2, 6, and 10), the treated samples exhibited:

- Reduced visibility of mid-range sarcoplasmic proteins (aldolase, Ald (~40 kDa), phosphorylase b, Phb (~97 kDa))
- Slight smearing or loss of bands at higher temperatures, especially in Lanes 5, 9, and 13 (70 °C)

The degradation or solubilization loss of these proteins under elevated heat conditions reflects temperature-dependent structural changes, consistent with literature describing the thermal sensitivity of glycolytic enzymes in sarcoplasmic fractions (Tornberg, 2005).

Interestingly, myoglobin (~17 kDa) bands remained visible across all treatments, though minor differences in band intensity were noted. This suggests partial resistance of Mb to denaturation at moderate sous-vide conditions, but potential structural alteration at 70 °C.

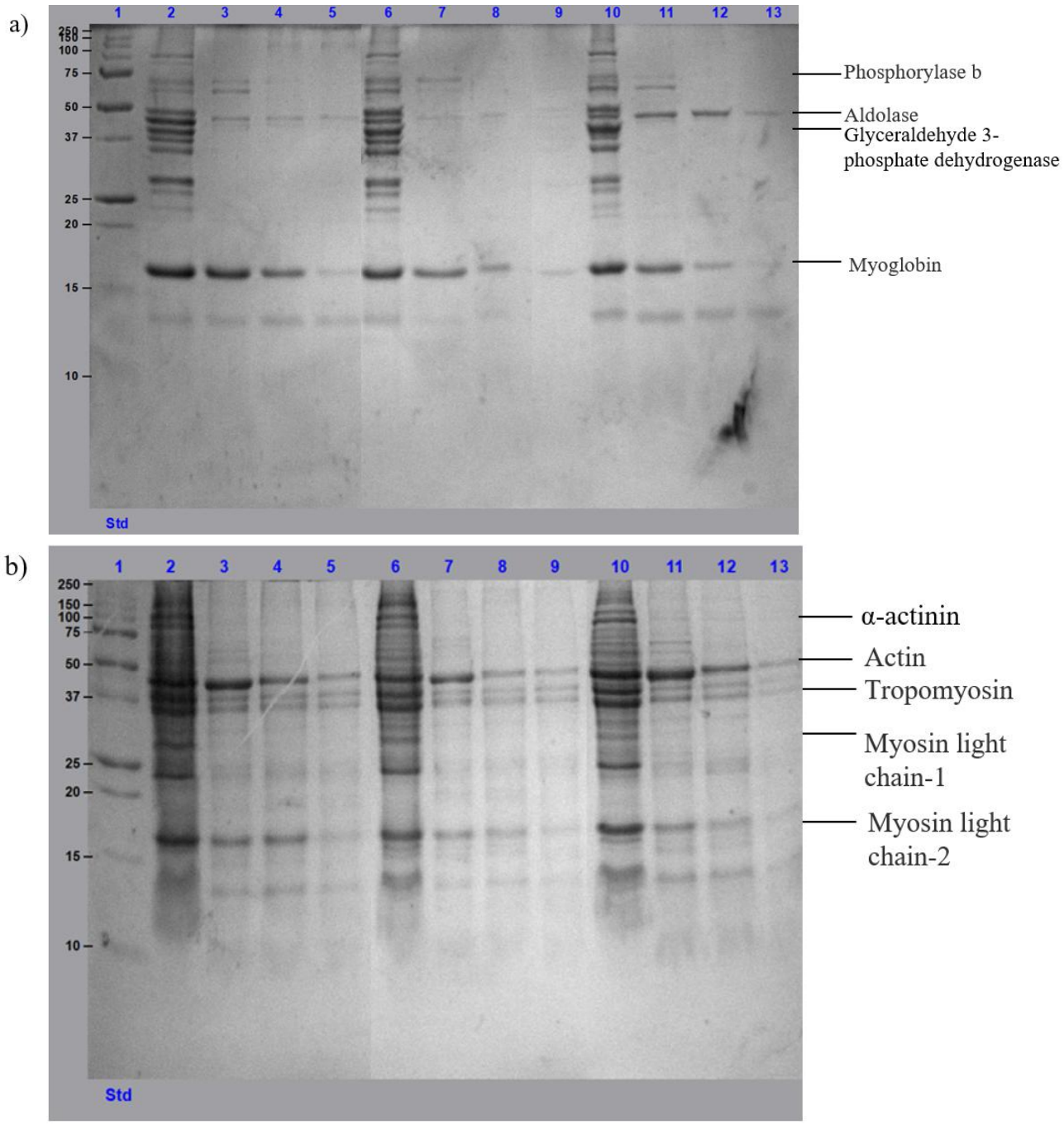


*Figure 29. SDS-PAGE patterns of (a) sarcoplasmic and (b) myofibrillar proteins of wild red deer meat subjected to sous-vide treatment.*

*(Lane 1: molecular weight standard; Lane 2: 7-month-old -control; Lane 3: 7-month-old -60°C; Lane 4:7-month-old -65°C; Lane 5:7-month-old -70°C; Lane 6:18-month-old -control; Lane 7:18-month-old -60°C; Lane 8:18-month-old -65°C; Lane 9:18-month-old -70°C; Lane 10:48-month-old -control; Lane 11:48-month-old -60°C; Lane 12:48-month-old -65°C; Lane 13:48-month-old -70°C)*

**Age-related differences were also observed in sarcoplasmic protein solubility.** In raw samples, the 7-month-old deer showed fewer and weaker sarcoplasmic protein bands compared to the 18- and 48-month-old deer, which displayed greater band intensity, likely due to increased postmortem protein solubilization. However, during sous-vide treatment, distinct age-related differences emerged among specific proteins. At 65 °C, the myoglobin band (~17 kDa) showed stronger intensity in the 7-month-old sample (Lane 4) compared to both the 18- and 48-month-old samples (Lanes 8 and 12). This may indicate greater Mb stability or retention in younger tissue under moderate heat, potentially due to less pre-existing oxidative damage or structural degradation. In contrast, aldolase exhibited its strongest band in the 48-month-old deer, while it was nearly undetectable in the 18-month-old sample. These findings indicate that protein response to heat varies not only with temperature but also with animal age, reflecting differences in muscle structure and protein stability.

The SDS-PAGE analysis of **myofibrillar proteins** extracted from sous-vide-treated wild red deer meat is presented in Figure 29b. Lane 1 corresponds to the molecular weight standard, while lanes 2 to 13 represent three individual wild red deer samples (7, 18, 48-month-old), each with a raw control and three sous-vide treatments (60 °C, 65 °C, and 70 °C).

In general, a progressive reduction in band intensity and clarity was observed with increasing temperature, especially at 70 °C (lanes 5, 9, and 13), indicating heat-induced denaturation or insolubilization (Tornberg, 2005).

**Myofibrillar protein degradation also varied with animal age.** The 7-month-old control sample (Lane 2) exhibited the strongest and most distinct protein bands. In contrast, the 18- and 48-month-old controls (Lanes 6 and 10) showed weaker, less defined bands, indicating partial degradation or reduced extractability with age.

Notably, after sous-vide treatment at 70 °C, the oldest sample (Lane 13) exhibited the most faded protein bands, with some nearly disappearing, highlighting severe protein breakdown or aggregation in aged muscle. These findings support the conclusion that younger muscle maintains greater structural protein stability, while older tissue is more prone to thermal denaturation and solubility loss under heat treatment.

### **5.3.6** Differential Scanning Calorimetry (DSC) Analysis

DSC thermograms for raw and sous-vide-treated wild red deer meat samples at three age groups (7, 18, and 48 months) are shown in Figures 30 (a-c). All measurements were performed from 20 °C to 90 °C at a heating rate of 0.8 °C/min to assess thermal transitions and protein denaturation patterns.

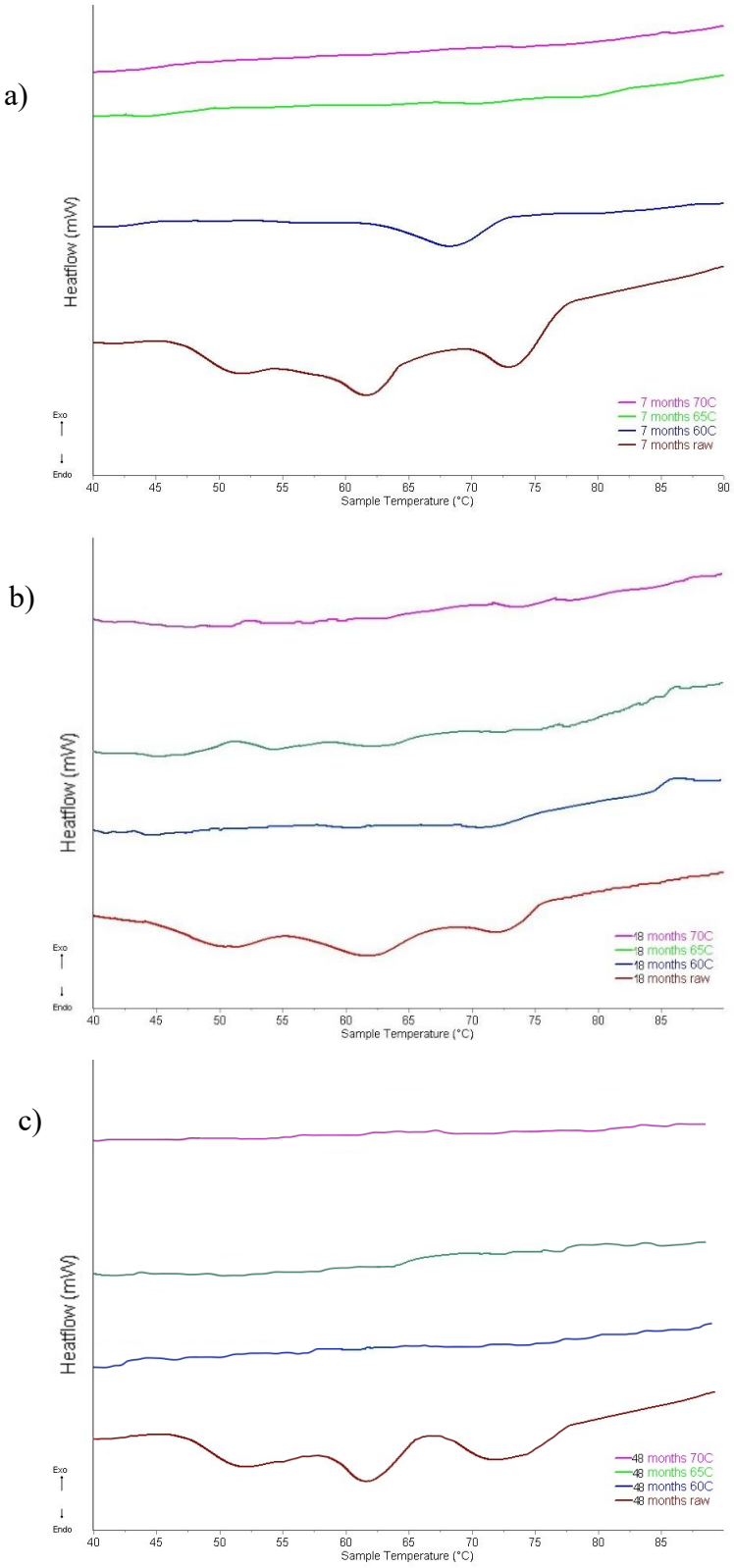


*Figure 30. Differential Scanning Calorimetry (DSC) thermograms of wild deer meat samples:*

*(a) 7, (b) 18, and (c) 48 months deer meat samples subjected to sous-vide treatments at 60°C, 65°C, and 70°C compared to control (raw) samples.*

Based on the DSC thermograms of wild red deer meat samples, three typical endothermic peaks were observed in the raw meat, corresponding to the denaturation of major muscle proteins.

The raw sample of the **7-month-old** wild red deer exhibited three distinct endothermic transitions: one around 50-55 °C, corresponding to myosin denaturation, and another broader peak near 60-65 °C. This thermal shift may reflect the interactions among sarcoplasmic proteins, myosin (tails), collagen, and actomyosin, as described by Xiong et al. (1987). The third peak (70-75 °C) is likely associated with actin and connective tissue proteins. With increasing sous-vide treatment temperature (60 °C, 65 °C, and 70 °C), the thermogram flattened progressively. The 65°C and 70 °C-treated sample showed no visible peaks, indicating that most thermally sensitive proteins had already denatured during cooking (Hwang et al., 2019). The 60 °C-treated sample retained a weak peak, suggesting partial preservation of structural proteins at this lower treatment temperature.

The DSC thermogram of the **18-month-old** wild red deer meat (Fig. 30b) showed three broad endothermic transitions in the raw sample: around 50–55 °C (myosin heads), 60–65 °C (myosin tails, sarcoplasmic proteins, and connective tissue), and 70–75 °C (actin denaturation). In sous-vide-treated samples, 60 °C, 65 °C, and 70 °C samples showed progressive flattening, suggesting nearly complete protein denaturation (Tornberg, 2005).

The DSC thermogram of the **48-month-old** sample (Fig. 30c) displayed three broad thermal transitions in the raw meat, observed near 50–55 °C, 60–65 °C, and 70–75 °C. In the treated samples the thermograms became almost completely flattened, indicating extensive or complete protein denaturation, highlighting the increased thermal sensitivity of proteins in older muscle.

These results confirm that aging decreases the clarity and intensity of thermal transitions, and that sous-vide processing at moderate to high temperatures leads to full protein denaturation in aged meat.

### **5.3.7** Scanning Electron Microscope

Scanning electron microscopy (SEM) images clearly illustrate microstructural alterations in three different age (7-, 18-, and 48-month-old) wild red deer (*Cervus elaphus*) meat samples subjected to sous-vide treatment at 65 $^{O}$C compared to control (raw) samples (Figure 31).

In the 7-month-old raw sample (Figure 31a), at 1000× magnification the muscle fibers appeared well-organized with a clear cylindrical morphology, indicating intact myofibrillar structure. However, after sous-vide treatment (Figure 31b), these cylindrical structures were no longer distinguishable, and the fibers appeared flattened and fragmented, suggesting thermal weakening and loss of structural integrity due to protein denaturation (Yin *et al.*, 2020).

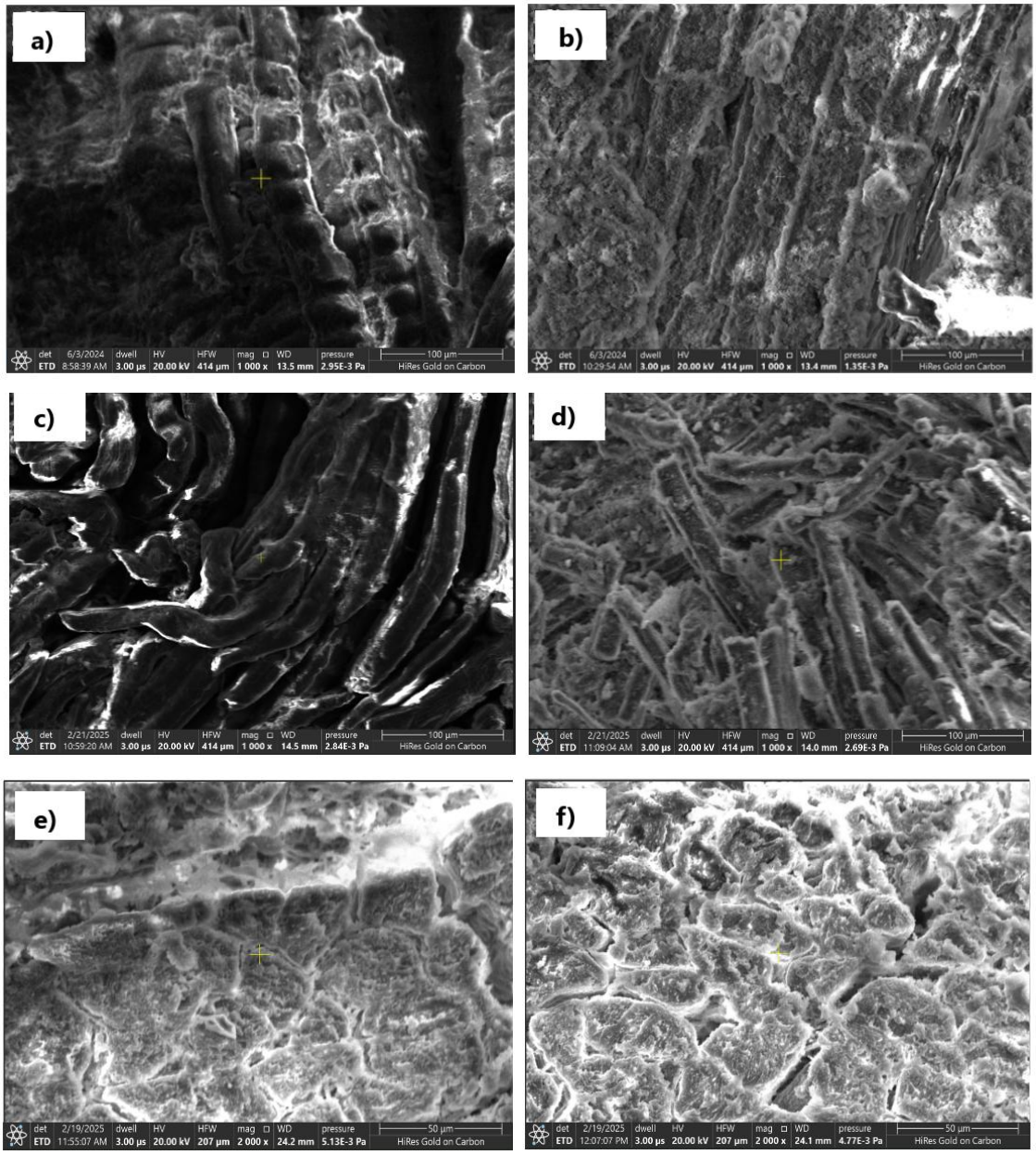


*Figure 31. Scanning Electron Microscopy (SEM) images showing the microstructural changes in wild deer meat samples:* ***(a)*** *7 month-old-control (raw) sample,* ***(b)*** *7 month-old - 65°C,* ***(c)*** *18month-old -control,* ***(d)*** *18 month-old -65°C (at 1000× magnification),* ***(e)*** *48 month-old -control, and* ***(f)*** *48 month-old -65°C (at 2000× magnification).*

For the 18-month-old sample, the raw muscle (Figure 31c) showed a more compact and aligned arrangement of muscle fibers than the younger group, reflecting age-related structural development. In the corresponding treated sample (Figure 31d), the muscle exhibited disruption of fiber alignment and apparent fragmentation, indicating breakdown of connective tissue and intracellular bonds following extended sous-vide heating.

In the 48-month-old group, SEM was conducted at 2000× magnification to observe finer structural details. The raw sample (Figure 31e) revealed a dense, tightly packed muscle fiber arrangement with visible 90° cuts, indicative of a more fibrous, matured tissue. After treatment (Figure 31f), the structure appeared more porous, with noticeable gaps and irregularities, likely due to extensive collagen degradation and myofibrillar collapse in response to prolonged thermal exposure.

These microstructural observations confirm that sous-vide treatment at 65 °C substantially alters muscle fiber integrity, resulting in considerable textural and structural changes in wild red deer meat. Such changes are directly correlated with the observed alterations in texture, juiciness, and sensory attributes, influencing consumer acceptance and quality perception of sous-vide processed meat products. These microstructural changes support earlier findings that sous-vide cooking promotes muscle fiber separation and connective tissue softening (Chotigavin et al., 2023; Shin et al., 2023).

### **5.3.8** Microbiological evaluation

Aerobic Plate Count (APC) results for wild red deer meat samples across ten individuals, including known and unknown-aged animals, are summarized in Table 13. In all control (raw) samples, APC values ranged from 4.25 ± 0.89 to 4.65 ± 0.98 log CFU/g, indicating a moderate microbial load typical for fresh wild game meat. These values are within the expected range for raw wild red deer meat, particularly when harvested and handled under field conditions (Atanassova et al., 2008). There is no statistically significant difference ($p > 0.05$) in the APC among the different age groups of raw wild red deer samples.

After sous-vide treatment at 60 °C, 65 °C, and 70 °C for 3 hours, microbial growth in all treated samples fell below the detection limit (<1.00 log CFU/g). This consistent reduction across all age groups highlights the effectiveness of sous-vide processing in significantly improving microbiological safety.

These results align with previous findings by Shin et al. (2023), who demonstrated that sous-vide cooking at 60 °C for 60 minutes eliminated detectable microbial populations in duck breast meat. Similarly, Kačániová et al. (2024) observed that sous-vide treatment of red deer meat at 60 °C and 65 °C for 5, 10, 15, and 20-minutes reduced APCs to undetectable levels. The reduction in bacterial load is primarily due to heat-induced protein denaturation and membrane disruption, coupled with oxygen limitation from vacuum packaging, which inhibits aerobic microbial activity.

**Table 13**. Aerobic plate count results of wild red deer meat samples before and after sous-vide treatment at different temperatures.

| Sample age (months) | Control | 60°C | 65°C | 70°C |
|---|---|---|---|---|
| 7 | 4.49±0.76 | <1.00 | <1.00 | <1.00 |
| 8 | 4.42±0.39 | <1.00 | <1.00 | <1.00 |
| 9 | 4.43±0.87 | <1.00 | <1.00 | <1.00 |
| 12 | 4.65±0.98 | <1.00 | <1.00 | <1.00 |
| 18 | 4.41±0.52 | <1.00 | <1.00 | <1.00 |
| 32 | 4.53±0.72 | <1.00 | <1.00 | <1.00 |
| 36 | 4.28±0.69 | <1.00 | <1.00 | <1.00 |
| 37 | 4.61±0.54 | <1.00 | <1.00 | <1.00 |
| 48 | 4.25±0.89 | <1.00 | <1.00 | <1.00 |
| U | 4.60±0.52 | <1.00 | <1.00 | <1.00 |

Values are expressed as mean ± standard deviation (log CFU/g). "<1.00" indicates counts below the detection limit.

It is important to note that while vegetative cells were effectively inactivated under the treatment conditions, bacterial spores are known to be more heat-resistant. As such, proper post-processing storage, particularly under refrigeration. Overall, the data confirm that sous-vide cooking is a highly effective method for microbiological stabilization of wild red deer meat.

# 6. CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusion of Experiment 1 (Organic Acid Treatment)

This study evaluated the effects of a 2% lactic acid (LA) and 2% ascorbic acid (AA) mixture on the quality of vacuum-packed wild red deer meat for 21 days at 4 ± 1 °C. The treatment significantly improved microbiological stability and meat tenderness without adversely affecting color or pH stability.

The pH of treated samples decreased initially due to the acidic treatment, but it remained stable compared to non-treated samples. The microbiological analysis revealed that the LA and AA mixture significantly reduced aerobic plate counts, suggesting potential for extending the shelf life of vacuum-packed wild red deer meat. Additionally, the treated wild red deer meat exhibited reduced hardness, cohesiveness, and chewiness during storage, which aligns with improved meat tenderness. While no consistent differences in color were observed between treated and non-treated samples, the treatment's non-significant impact on color is advantageous, as acids may otherwise negatively affect meat appearance.

Furthermore, the SDS-PAGE protein profile analysis showed that the LA and AA treatments helped preserve both sarcoplasmic and myofibrillar proteins, which are critical to maintaining meat quality during storage. Particularly, treated samples exhibited a slower degradation of key glycolytic enzymes and myoglobin, indicating that the acid treatment offers protective benefits against protein oxidation and degradation, contributing to prolonged quality preservation. Future research should focus on evaluating sensory attributes and the economic feasibility of implementing this treatment on a commercial scale.

## 6.2 Conclusion of Experiment 2 (High Hydrostatic Pressure Treatment)

This study highlights the significant impact of HHP on the quality attributes of wild red deer meat, including pH, surface color, microbial safety, texture, and protein stability.

HHP treatments, particularly at pressures ≥300 MPa, effectively stabilized pH during 14 days of storage, likely due to suppressed microbial and enzymatic activity. While these pressures enhanced microbial safety and extended shelf life by significantly reducing aerobic plate counts, pressures ≥500 MPa were most effective, achieving microbial counts below 1 log CFU/g on Day 1 and maintaining them at safe levels during storage. Treatments ≥300 MPa increased lightness and yellowness while reducing redness and chroma, reflecting protein denaturation and pigment oxidation. Textural analysis

revealed that moderate pressures (150–300 MPa) improved tenderness by balancing proteolysis and denaturation, whereas higher pressures (450–600 MPa) caused greater firmness due to protein aggregation and gelation. SDS-PAGE analysis confirmed significant denaturation and aggregation of sarcoplasmic and myofibrillar proteins at pressures ≥300 MPa, with more pronounced degradation at pressures ≥500 MPa.

Based on these findings, 300 MPa appears to be an optimal pressure level for improving tenderness, microbial safety, and shelf life while minimizing excessive protein aggregation. For maximum microbial reduction and extended shelf life, 500 MPa could be applied.

Future work should investigate the effects of shorter processing times (3–4 minutes) at selected pressure levels to assess time–efficiency, incorporate sensory evaluation to determine consumer acceptance, explore synergistic combinations with emerging preservation methods such as organic acid or sous-vide cooking, and assess impacts on nutritional quality.

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

This experiment aimed to evaluate the impact of sous-vide cooking at three different temperatures (60 °C, 65 °C, and 70 °C for 3 hours) on various quality parameters of wild red deer meat and assess the influence of animal age on these effects. The findings revealed that sous-vide treatment significantly influenced physicochemical, microbiological, and structural characteristics of wild red deer meat and that these effects varied with animal age.

Sous-vide treatment effectively reduced total mesophilic bacterial counts below detectable limits in all treated samples, highlighting its potential as a safe and effective cooking method for wild game meat. Thermal denaturation of sarcoplasmic and myofibrillar proteins was clearly observable in SDS-PAGE patterns, where band intensity diminished with increasing temperature, particularly in older animals. Scanning Electron Microscopy (SEM) confirmed age-dependent microstructural changes, showing more pronounced muscle fiber disruption in older deer following sous-vide treatment.

Differential Scanning Calorimetry (DSC) thermograms revealed typical protein denaturation peaks (myosin, sarcoplasmic proteins, actin) in raw meat, which diminished or disappeared post-treatment, especially at 65 °C and 70 °C. This confirmed temperature-induced structural modifications of key proteins.

Texture profile analysis showed that increasing cooking temperature generally elevated hardness, cohesiveness, chewiness, and shear force across all age groups. However, younger samples tended to

maintain more favorable textural properties, while older deer meat exhibited higher values, indicating increased toughness. Cooking loss tended to increase with both age and temperature, while WHC decreased at higher temperatures. Interestingly, age also played a role in water retention ability, with older animals demonstrating relatively higher WHC at elevated temperatures.

Overall, sous-vide treatment at 65 °C offered a promising balance between microbial safety, protein preservation, and acceptable texture, particularly for younger animals. Moreover, among the ten different wild red deer samples studied, the unknown-aged sample exhibited physicochemical and structural patterns similar to 36-month-old samples, suggesting a comparable age range. These insights offer valuable guidance for optimizing sous-vide protocols for wild game meat based on both temperature and animal age, promoting higher quality and safer meat products for niche markets.

Future studies should examine additional cooking durations, increase the number of deer samples to strengthen statistical power, and investigate the effects of herb-based marinades on quality attributes.

### 6.4 General Conclusion

The findings from this study provide valuable guidance for improving the quality, safety, and shelf life of wild red deer meat using organic acid treatment, high hydrostatic pressure (HHP), and sous-vide cooking. Each technique presents unique benefits that can be selectively applied depending on the intended product characteristics and processing goals.

- Organic acid treatment (2% lactic + 2% ascorbic acid) is recommended for short-term storage (up to 21 days) under refrigeration. It is easy to implement and effective in reducing microbial load and preserving protein integrity without altering meat color or texture, making it suitable for small-scale or artisanal processors handling vacuum-packed game meat.
- High hydrostatic pressure (HHP) at 300 MPa is optimal for balancing tenderness, microbial safety, and protein stability. It may be applied in industrial settings aiming for moderate shelf-life extension with minimal textural changes. For applications where maximum microbial inactivation is required, 500 MPa treatments are effective but may result in firmer texture due to protein aggregation.
- Sous-vide treatment at 65 °C for 3 hours is ideal for ready-to-eat game meat products, particularly from younger animals (≤12 months), offering favorable tenderness and structural preservation. This method ensures microbial safety and allows for targeted texture control based on age, supporting product standardization in niche meat markets.

Processors and producers can consider these methods not only to enhance product quality but also to improve food safety and develop differentiated products in the growing wild game meat market. Future work may explore combined or sequential use of these techniques to further optimize outcomes.

## 7. 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.

# 8. SUMMARY

The consumption of wild red deer (*Cervus elaphus*) meat is increasing due to its high nutritional value and alignment with consumer preferences for lean, sustainable protein sources. However, challenges related to its short shelf life, variable quality, and susceptibility to microbial spoilage limit its commercial potential. This dissertation aimed to investigate the effects of three processing methods organic acid mixture treatment, high hydrostatic pressure (HHP), and sous-vide treatment on the quality parameters of wild red deer meat. The study also examined the influence of animal age on meat quality in the context of sous-vide treatment.

A comprehensive experimental design was employed, incorporating chemical, physical, microbiological, and structural analyses. Organic acid treatment used a 2% lactic acid and 2% ascorbic acid mixture, applied via surface spraying to *semimembranosus* steaks and stored for up to 21 days. HHP was applied using 10 different pressure settings (150–600 MPa) for 5 minutes at 22 °C, followed by 14 days of storage. Sous-vide treatment was conducted at 60 °C, 65 °C, and 70 °C for 3 hours on 9 age-identified samples from 7–48 months old red deer and one unknown sample, to evaluate both temperature and age-related effects. All treatments were compared against controls, and results were statistically evaluated.

The organic acid treatment significantly reduced aerobic plate counts during 21 days of storage while preserving color and textural characteristics. Treated samples exhibited a lower microbial load (4.55 log CFU/g) compared to untreated samples (5.60 log CFU/g), with minimal negative effects on surface color or pH. Protein degradation was also delayed, as confirmed by SDS-PAGE analysis.

HHP treatment effectively reduced microbial load, with pressures ≥300 MPa maintaining pH stability and pressures ≥500 MPa reducing microbial counts to below 1 log CFU/g. However, pressures above 450 MPa led to increased hardness and reduced redness, attributed to protein aggregation and myoglobin oxidation. Moderate pressures (250–300 MPa) offered an optimal balance between microbial safety and textural quality.

Sous-vide cooking demonstrated excellent microbial safety across all temperatures, with aerobic plate counts reduced to undetectable levels. Texture, color, and protein stability were influenced both by temperature and animal age. Higher temperatures (70 °C) resulted in greater protein denaturation and tougher texture, particularly in older animals. Conversely, younger samples maintained better textural and structural integrity. Age estimation of an unknown sample was successfully conducted by comparing its quality parameters to known-age samples.

In conclusion, all three technologies improved certain aspects of meat quality, with each method having distinct strengths and limitations. Organic acid treatment was effective for short-term microbial control, HHP offered strong microbial reduction with variable effects on texture and color, and sous-vide ensured safety and acceptable texture, especially when tailored to animal age. These findings support the integration of optimized processing technologies for the commercialization of high-quality, safe, and consumer-acceptable wild red deer meat.

# 9. APPENDICES

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