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THE IMPACT OF AGING ON THE QUALITY OF MEAT FROM RED DEER (*CERVUS ELAPHUS* L.) HINDS FOLLOWING FROZEN STORAGE

– Research paper –

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Abstract: The impact of aging on the quality of meat (*longissimus thoracis et lumborum* muscle) from red deer hinds, stored in a frozen state, was analyzed in this study. The muscles were cut out from 10 half-carcasses of hunter-harvested hinds aged 4-6 years. Each muscle was divided into portions 1 and 2. Portion 1 was further subdivided into four samples (0, 3F, 6F, and 9F). Samples 0 were subjected to analyses, and the remaining samples were vacuum-packaged, frozen, and stored at a temperature of -26ºC for three, six, and nine months. Portion 2 of the muscle was vacuum-packaged and stored at a temperature of 2ºC for 10 days. Next the muscle was unpackaged and divided into four samples (10A, 3AF, 6AF, and 9AF). Samples 10A were subjected to analyses, and the remaining samples were vacuum-packaged, frozen, and stored at a temperature of -26ºC for three, six, and nine months. Aging prior to freezing resulted in a significantly higher content of water-soluble nitrogen compounds and non-protein nitrogen compounds in muscle samples, their higher water-holding capacity, lighter color, and higher tenderness. The color of these samples was also characterized by a lower contribution of redness (a*) and a lower chroma (C*) value, compared with unaged and frozen samples, and by lower taste desirability after nine months of storage. Due to the specific characteristics of the experimental materials, the results of this study should be validated in terms of repeatability, including under different freezing-thawing conditions.

Keywords: game meat, aging, freezing, meat quality

INTRODUCTION

Appropriate aging processes are crucial for developing the full technological properties of meat and improving its eating quality (Rehman et al., 2024). Meat aging is driven by biochemical reactions that occur under controlled conditions (temperature, humidity), enhancing the sensory attributes (tenderness, taste, juiciness) of meat as well as sensory experience and nutrient digestibility (Dashdorj et al., 2016). Currently, two primary meat aging methods are used in practice: dry aging and wet aging (Kim et al., 2022).

Dry meat aging is a traditional method that has been applied for centuries (Ortez et al., 2022). It involves storing (for 21-35 days) whole carcasses (typically beef carcasses) or carcass cuts in the air, under specific conditions, i.e. relative humidity of 75-85%, temperature of 1-4°C, and airflow of 0.5-2.5 m/s (EFSA BIOHAZ Panel et al., 2023). However, dry aging leads to losses (weight loss due to water evaporation, microbial growth) that decrease production profitability by increasing the overall cost. Therefore, the practical applicability of this method is limited (Kim et al., 2020). However, it continues to be used by small local producers that supply meat to high-end hotels, restaurants, and delicatessens (Ortez et al., 2022) as consumers appreciate the unique flavor profile of dry-aged meat (Pogorzelski et al., 2021). In the modified dry aging method, meat is wrapped in high moisture permeability foil (Setyabrata et al., 2022) to increase the efficiency of the aging process and reduce losses resulting from the growth of spoilage microorganisms.

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Wet aging is the most popular aging method in the meat industry (Sirtori et al., 2022). In this method, beef, pork, and lamb cuts are vacuum-packaged in barrier bags and stored under refrigerated conditions (beef: 0-4°C for 14-49 days, pork: 0-4°C for 4-6 days, lamb: -1-5°C for 7-77 days) (EFSA BIOHAZ Panel et al., 2023). Unlike dry aging, wet aging does not require controlled humidity and airflow because meat is packaged prior to the process (Hulánková et al., 2018). The major disadvantage of wet aging is the development of undesirable flavor changes in meat due to the proliferation of lactic acid bacteria and the accumulation of their metabolites (Li et al., 2021). In addition, the presence of juice drip in the package can negatively impact consumer perceptions due to concerns about microbial growth.

Freezing is a standard method for preserving meat and extending its shelf-life (Zhang and Ertbjerg, 2018) and meat can be aged both before and after frozen storage (da Silva Bernardo et al., 2020). However, it should be stressed that freezing does not inhibit biophysicochemical processes that occur naturally in meat and may alter its quality by affecting its water-holding capacity, color, texture, and sensory attributes (Stafford et al., 2024). Since both meat aging and freezing are commonly applied in the meat industry, the optimal sequence of these treatments should be established to maximize their benefits. The results of some studies (Kim et al., 2011, 2015; Li et al., 2019) suggest that meat aging prior to freezing may alleviate the negative effects of the latter treatment, particularly by improving the water-holding capacity of meat.

Research on the effects of wet aging and freezing on the quality attributes of livestock meat is extensive. However, the impact of these processes on game meat, including that from wild cervid populations, remains insufficiently investigated. Game meat is harvested by hunters on a seasonal basis (outside the closed season), and it is subsequently frozen following aging within a wide range of temperatures (4-10°C) and durations (1-4 days), using various methods (skinned or unskinned carcasses) (Soriano et al., 2016). In view of the above, the research hypothesis postulates that the sequence of aging and freezing processes affects the quality of game meat. The aim of the present study, undertaken to validate the above hypothesis, was to analyze the influence of 10-day wet aging on the quality of meat (*longissimus thoracis et lumborum*, *LTL* muscle) from red deer (*Cervus elaphus* L.) hinds after long-term (3, 6, and 9 months) frozen storage.

MATERIALS AND METHODS

Materials

Pursuant to the provisions of Polish regulations and Directive 2010/63/EU (OJEU, 2010), the experiment did not require the approval of the Local Ethics Committee for Animal Experimentation. The experimental materials comprised the right *LTL* muscles of 10 red deer (*Cervus elaphus* L.) hinds aged 4-6 years. The hinds were hunter-harvested in the forests of north-eastern Poland during one hunting season. The *LTL* muscles were cut out during carcass dressing in a meat processing plant, approximately 48-54 h post mortem. The animals' age was estimated based on the wear of mandibular premolars and molars (Morow, 1993). All analyzed carcasses were characterized by the absence of bullet damage to the *LTL* muscle, the absence of digesta contamination, adequate chilling (temperature of <7°C in the geometric center of the leg), and the pH of the *LTL* muscle (measured behind the last rib) in the range of 5.4-5.8. The *LTL* muscles were placed in polyethylene bags and transported to the laboratory in isothermal containers with ice packs.

In the laboratory, each *LTL* muscle was divided into portions 1 and 2 of similar weight. Portion 1 was further subdivided into four samples: 0, 3F, 6F, and 9F. Samples 0 were subjected to laboratory analyses immediately, whereas the remaining samples were vacuum-packaged, frozen at a temperature of -26°C, and stored at that temperature for three (samples 3F), six (samples 6F), and nine (samples 9F) months. Portion 2 of the muscle was weighed, vacuum-packaged, and stored at a temperature of 2°C for 10 days. At the end of the 10-day period, the muscle was unpackaged, dried, and divided into four samples: 10A, 3AF, 6AF, and 9AF. Samples 10A were subjected to laboratory analyses immediately, whereas the remaining samples were vacuum-packaged, frozen at a temperature of -26°C, and stored at that temperature for three (samples 3AF), six (samples 6AF), and nine (samples 9AF) months.

Muscle samples were vacuum-packaged in barrier bags made of ethylene-vinyl alcohol (EVOH) copolymer (gas permeability: O_2=1cm^3/m^2/24 h/bar/23°C, N_2<0.1 cm^3/m^2/24 h/bar/23°C, CO_2=1.6 cm^3/m^2/24 h/bar/23°C, H_2O=3 g/m^2/24 h/23°C) using the PP-5MG (015) vacuum packaging machine (TEPRO S.A., Koszalin, Poland) The samples were stored in a cooling chamber (without

forced air-flow) with freon as a refrigerant, and they were frozen and stored in a freezing chamber with freon as a refrigerant and an electronic temperature control system.

Methods

The rate of proteolysis in the *LTL* muscle was determined (Kjeldahl method; AOAC, 2005; Kjeltec™ 2200 Auto Distillation Unit, FOSS Analytical, Hillerod, Denmark) based on the content of total nitrogen compounds and non-protein nitrogen compounds (proteins were precipitated with trichloroacetic acid) in an aqueous meat extract prepared according to the method of Herring et al. (1971). The content of protein nitrogen compounds in the aqueous meat extract was calculated as the difference between the content of total nitrogen compounds and non-protein nitrogen compounds.

The water-holding capacity of meat was assessed based on natural drip loss (Honikel, 1998), forced drip loss – determined by the Grau and Hamm method (Van Oeckel et al., 1999), cooking loss (Honikel, 1998), and the ability to retain added water – determined by the modified centrifugal method described by Dzierżyńską-Cybulko (1963). The modifications of the original method involved adding redistilled water with a temperature of 4°C to meat and storing the homogenized sample at a temperature of 4°C (Daszkiewicz et al., 2009). Meat was thawed (2°C, 18 h) after the respective storage periods, and a reduction in meat weight during thawing (thaw loss) was determined as the difference between initial sample weight before freezing and sample weight after thawing.

The pH of the *LTL* muscle was measured behind the last rib prior to carcass cutting to eliminate PSE and DFD meat, using a Double Pore combination electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland) and a 340i pH-meter with a TFK 150/E temperature sensor (WTW Wissenschaftlich-Technische Werkstatten GmbH, Weilheim, Germany). The pH values of unstored meat and meat stored under refrigerated and frozen conditions for the assumed periods of time were determined in homogenates of meat and redistilled water (1:1, m/v), using a Polilyte Lab combination electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland) and an inoLab Level 2 pH-meter with a TFK 325 temperature sensor (WTW Wissenschaftlich-Technische Werkstatten GmbH, Weilheim, Germany).

The rate of lipid oxidation in meat was determined based on thiobarbituric-acid-reactive substances (TBARS) values (Pikul et al., 1989). Absorbance was measured with the Specord 40 spectrophotometer (Analytik Jena AG, Germany), and TBARS values were expressed in mg malondialdehyde (MDA) per kg of meat.

Meat color was assessed based on lightness (L*), redness (a*), yellowness (b*), and chroma (C*) in the CIELAB color space (CIE, 1978). The values of parameters L*, a*, and b* were measured three times at different points over the muscle cross-section area, by the reflectance method, using the MiniScan XE Plus spectrocolorimeter (Hunter Associates Laboratory, Reston, Virginia, USA) with standard illuminant D65, a 10° standard observer angle, and a 2.54-cm-diameter aperture. The value of C* was calculated using the following formula 1:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad [1]$$

The sensory properties of meat were evaluated after thermal processing, as described by Baryłko-Pikielna et al. (1964). Following the removal of fat and epimysium (with the perimysium intact), meat was divided into portions. Meat samples were cooked in 0.6% NaCl solution at a temperature of 96°C (±2°C) for 1.5 h. A sensory analysis was conducted by five trained panelists. The sensory attributes of meat, including aroma, juiciness, tenderness, and taste, were assessed on a five-point scale (5 points – most desirable, 1 point – least desirable) (Daszkiewicz et al., 2012).

Statistical analysis

The results were processed statistically using STATISTICA ver. 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). One-way analysis of variance (ANOVA) was performed to compare the quality of the *LTL* muscle of red deer hinds, which was stored in a frozen state for three, six, and nine months, either with or without a 10-day aging period prior to storage. The significance ($p \leq 0.05$) of differences between group means was determined by Duncan's test

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RESULTS AND DISCUSSION

Post-mortem proteolysis, i.e. the breakdown of muscle proteins by endogenous proteases such as calpains and cathepsins, is a key process during meat aging (Han et al., 2024b). The proteolysis of muscle proteins induces changes in the technological properties and sensory attributes of meat, including color, water-holding capacity, tenderness, and taste (Joo et al., 2023). This process

increases the proportion of soluble nitrogen compounds in the total nitrogen content of meat (Bruas-Reignier and Brun-Bellut, 1996; Došler et al., 2007; Feidt et al., 2008; Bulgaru et al., 2022).

The analyses conducted in the present study revealed a higher ($p \leq 0.05$) proportion of nitrogen of water-soluble compounds (after 6 and 9 months of frozen storage) and nitrogen of water-soluble non-protein compounds (after 3, 6, and 9 months of frozen storage), and a lower ($p \leq 0.05$) proportion of nitrogen of water-soluble protein compounds (after 3 and 6 months of frozen storage) in the total nitrogen content of aged/frozen meat samples (Table 1). The above changes resulted from proteolysis that occurred in meat during refrigerated aging, freezing, and thawing. According to Stafford et al. (2024), enhanced proteolysis in frozen-thawed meat can be caused by the release of proteases from muscle cells and calcium ions from the sarcoplasmic reticulum (Ca^{2+} ions activate calpains) damaged by ice crystals that are formed during freezing. The available literature on postmortem proteolysis in wild game meat during its frozen storage is scarce. Therefore, the results of this study cannot be directly compared with previous research findings. Dzierżyńska-Cybulko and Fruziński (1997) reported that freezing increased the content of non-protein nitrogen in meat from wild boars, roe deer, and fallow deer, but specific storage conditions (temperature and time) were not detailed in their study.

Meat thawing is associated with drip loss (known as thaw loss), leading to weight loss. In the current study, no significant ($p > 0.05$) differences were observed in weight loss between *LTL* muscles that were aged prior to freezing and those that were not (Table 2). However, weight loss tended to be lower in muscle samples that were aged and then stored in the freezer for three and nine months.

Table 1. Content of water-soluble nitrogen compounds in the *longissimus thoracis et lumborum* muscle of red deer hinds (arithmetic mean ± SD)

Parameter	Aging period (days)	Frozen storage period (months)			
		0	3	6	9
Total nitrogen of water-soluble compounds/ total nitrogen in meat ratio (%)	0	28.37 ± 2.27	28.06 ± 1.75	28.06^{y} ± 1.26	27.82^{y} ± 1.88
	10	29.69^{a} ± 1.88	27.54^{b} ± 3.49	29.74^{ax} ± 1.38	30.26^{ax} ± 1.50
Nitrogen of water-soluble protein compounds/ total nitrogen in meat ratio (%)	0	13.27 ± 1.75	13.22^{x} ± 2.66	13.65^{x} ± 1.82	13.10 ± 1.26
	10	13.34^{a} ± 0.95	10.49^{by} ± 2.11	12.25^{ay} ± 0.95	13.32^{a} ± 1.06
Nitrogen of water-soluble non-protein compounds/ total nitrogen in meat ratio (%)	0	15.10 ± 0.68	14.84^{y} ± 1.62	14.41^{y} ± 1.14	14.72^{y} ± 1.35
	10	16.35 ± 1.80	17.05^{x} ± 2.01	17.49^{x} ± 1.07	16.94^{x} ± 1.06

[a,b] Means with different superscripts within a row are different at $p \leq 0.05$.

[x,y] Means with different superscripts within a column are different at $p \leq 0.05$.

Table 2. Weight loss and water-holding capacity of the *longissimus thoracis et lumborum* muscle of red deer hinds (arithmetic mean ± SD)

Parameter	Aging period (days)	Frozen storage period (months)			
		0	3	6	9
Weight (thaw) loss (%)	0	-	4.38 ± 1.27	3.75 ± 0.90	3.55 ± 0.83
	10	-	3.49^{ab} ± 0.62	3.96^{a} ± 0.85	3.28^{b} ± 0.57
Natural drip loss (%)	0	2.01^{c} ± 0.75	5.15^{bx} ± 1.77	7.08^{ax} ± 1.71	5.52^{bx} ± 1.32
	10	1.76^{b} ± 1.31	3.24^{ay} ± 0.60	4.09^{ay} ± 1.91	4.08^{ay} ± 1.01
Cooking loss (%)	0	33.19 ± 1.97	32.68 ± 2.01	33.52 ± 1.67	33.78 ± 1.10
	10	34.46 ± 1.75	34.10 ± 1.85	33.52 ± 1.91	33.98 ± 1.20
Water-holding capacity Grau and Hamm method (cm^2)	0	5.95^{x} ± 1.03	6.26^{x} ± 1.21	6.08^{x} ± 0.93	6.63^{x} ± 1.34
	10	4.26^{y} ± 1.13	4.64^{y} ± 1.49	4.18^{y} ± 1.02	3.94^{y} ± 1.08
Ability to retain added water (%)	0	18.97^{ay} ± 6.08	10.98^{b} ± 3.44	5.43^{cy} ± 5.02	2.91^{cy} ± 3.75
	10	24.31^{ax} ± 3.09	14.35^{b} ± 4.15	13.80^{bx} ± 3.07	10.96^{bx} ± 4.52

[a,b,c] Means with different superscripts within a row are different at $p \leq 0.05$.

[x,y] Means with different superscripts within a column are different at $p \leq 0.05$.

An analysis of natural and forced drip loss (Grau and Hamm method) and the amount of added water retained by meat revealed greater differences in the water-holding capacity of meat between the compared groups (Table 2). The values of all parameters were more favorable in *LTL* muscles that were aged prior to freezing. These samples were characterized by lower ($p \leq 0.05$) natural and forced drip loss, and retained more ($p \leq 0.05$) added water. In turn, no significant ($p > 0.05$) differences in cooking loss were found between *LTL* muscles that were aged prior to freezing and those that were not (Table 2).

The higher water-holding capacity of thawed meat, which was first aged and then frozen, was also reported by other authors (Farouk et al., 2009; Kim et al. 2011, 2013, 2015; Rehman et al., 2024). This phenomenon was attributed to the autolysis of cytoskeletal proteins (desmin, talin, vinculin) during meat aging (Kristensen and Purslow, 2001; Huff-Lonergan and Lonergan, 2005). Their degradation weakens the connections between myofibrils and the sarcolemma as well as the connections between myofibrils, which are involved in longitudinal and lateral contractions of myofibrils and the entire muscle fiber post mortem, which causes water to flow out of the cells and contributes to drip loss. In addition, the proteolysis of the muscle cell cytoskeleton eliminates pressure within the sarcolemma, which facilitates the inflow of water to the muscle cell from the extracellular space. In both cases, these processes improve the water-holding capacity of meat subjected to aging. During aging, changes in meat structure, induced by enzymatic degradation of muscle proteins, destroy the channels that facilitate water migration out of muscle cells during freezing and thawing (Setyabrata and Kim, 2019). As a result, water is retained in meat ("sponge effect") (Farouk et al., 2012).

Meat pH is a key quality indicator because its value is related to many other important attributes of meat, such as color, water-holding capacity, tenderness, and microbial stability (Kim et al., 2024). In this study, differences in average pH values between *LTL* muscles that were aged prior to freezing and those that were not were very small and not significant (Table 3). Changes in the pH of frozen meat are driven by various complex processes; therefore, they can vary depending on their underlying mechanisms. Farouk et al. (2009) found no significant differences in the average pH value between samples of venison (the *longissimus dorsi* (*LD*) muscle of red deer stags) and beef (the *semimembranosus (SM)* muscle of young bulls) that were frozen before and after aging. In turn, Kim et al. (2011) demonstrated that aged/frozen samples of the ovine *LD* muscle had a significantly ($p \leq 0.05$) higher pH than frozen-only samples. Different results were reported by Kim et al. (2015) who observed a decrease in the pH of beef *longissimus lumborum* muscles frozen after three weeks of aging, and an increase in the pH of samples frozen after four weeks of aging, relative to frozen-only loins. Despite their statistical significance, the differences between group means were small (below 0.1 units) and had no impact on other attributes of meat.

Table 3. Values of pH, TBARS, and color parameters of the *longissimus thoracis et lumborum* muscle of red deer hinds (arithmetic mean ± SD)

Parameter	Aging period (days)	Frozen storage period (months)			
		0	3	6	9
pH	0	$5.50^{b} \pm 0.06$	$5.56^{a} \pm 0.06$	$5.54^{ab} \pm 0.06$	$5.56^{a} \pm 0.06$
	10	5.54 ± 0.03	5.55 ± 0.05	5.54 ± 0.06	5.56 ± 0.03
TBARS (mg malondialdehyde / kg meat	0	$0.85^{a} \pm 0.16$	$0.67^{b} \pm 0.18$	$0.44^{c} \pm 0.09$	$0.72^{b} \pm 0.08$
	10	$0.75^{a} \pm 0.13$	$0.78^{a} \pm 0.45$	$0.48^{b} \pm 0.07$	$0.77^{a} \pm 0.09$
L*	0	$30.82^{ay} \pm 1.60$	$28.34^{b} \pm 1.23$	$27.74^{by} \pm 1.21$	$27.46^{b} \pm 3.33$
	10	$32.51^{ax} \pm 1.42$	$29.11^{b} \pm 2.67$	$29.79^{bx} \pm 1.38$	$29.92^{b} \pm 3.47$
a*	0	$16.34^{ay} \pm 1.14$	$15.27^{abx} \pm 1.53$	$14.36^{b} \pm 1.94$	$15.13^{abx} \pm 2.14$
	10	$19.20^{ax} \pm 1.32$	$12.06^{by} \pm 1.74$	$10.60^{b} \pm 1.85$	$10.51^{by} \pm 1.83$
b*	0	$10.45^{y} \pm 1.29$	9.78 ± 0.98	$9.72^{x} \pm 1.30$	10.52 ± 1.62
	10	$12.54^{ax} \pm 1.57$	$10.20^{b} \pm 1.02$	$9.56^{by} \pm 0.99$	$9.60^{b} \pm 1.67$
C*	0	$19.42^{ay} \pm 1.45$	$18.14^{abx} \pm 1.73$	$17.36^{bx} \pm 2.18$	$18.44^{abx} \pm 2.62$
	10	$22.95^{ax} \pm 1.84$	$15.82^{by} \pm 1.82$	$14.30^{by} \pm 1.92$	$14.27^{by} \pm 2.25$

[a,b] Means with different superscripts within a row are different at $p \leq 0.05$.

[x,y] Means with different superscripts within a column are different at $p \leq 0.05$.

In general, the enzymatic proteolysis of muscle proteins by exogenous proteases during aging and frozen storage can contribute to changes in meat pH (Kim et al., 2024). This process leads to the cleavage of peptide bonds in proteins and the release of amino acids that may lower meat pH (Wereńska and Okruszek, 2023). However, the observed pH-decreasing effect is affected by the buffering capacity of muscle fibers in different muscle types. According to Puolanne and Kivikari (2000), white muscle fibers (with higher histidine content) are characterized by greater buffering capacity than red muscle fibers. Leygonie et al. (2012) demonstrated that the pH of thawed meat is generally lower than that of fresh meat before freezing due to denaturation of buffer proteins exposed to subzero temperature and the subsequent release of hydrogen ions, leading to a drop in pH. An increase in the concentrations of hydrogen ions and other acidic compounds in thawed meat may also be caused by thaw loss (Leygonie et al., 2012). Regardless of the described biochemical factors affecting the pH of frozen/thawed meat, pH values may vary depending on the applied freezing and thawing methods (Kim et al., 2015; Lu et al., 2022).

Undesirable biochemical processes that occur in food products during storage include oxidation, in particular lipid oxidation (Leygonie et al., 2012). Therefore, standard analytical procedures include monitoring of the content of oxidation products in foods. In practice, TBARS values are often determined as an indicator of autoxidation of polyunsaturated fatty acids (Abeyrathne et al., 2021). In the current study, no significant ($p > 0.05$) differences in average TBARS values were found between *LTL* muscles that were aged prior to freezing and those that were not (Table 3). Similarly, Kim et al. (2018) and Setyabrata and Kim (2019) observed no significant differences in average TBARS values in frozen-only and aged/frozen pork and beef loins, respectively, analyzed immediately after thawing. In contrast, Kim et al. (2011) found that aged/frozen lamb loins had significantly higher TBARS values than frozen-only samples. In the experiments conducted by Kim et al. (2011) and Kim et al. (2018), analyses of meat samples performed on successive days after thawing revealed considerably higher TBARS values in aged/frozen loins.

The direct causes of oxidative changes in refrigerated and frozen meat are identical and include the effects exerted by released oxidative enzymes and progressing degradation of antioxidant substances. As regards enzyme release, the integrity of muscle cell structure, which is disrupted during both aging and freezing of meat, is an important consideration.

During meat aging, the structure of muscle fibers is weakened due to the activity of endogenous proteolytic enzymes (Setyabrata and Kim, 2019). In turn, during freezing, muscle cell structures are damaged by ice crystals, leading to the release of prooxidants and mitochondrial enzymes (Dang et al., 2021). In view of the above, the potential for oxidative changes is greater in aged and then frozen meat due to the co-occurrence of contributing processes (enzymatic and physical), observed in muscle tissue during aging and subsequent freezing. Similar observations were made by Kim et al. (2011, 2018) who found that the rate and extent of lipid oxidation were greater in aged/frozen/thawed meat than in frozen/thawed meat that was subsequently stored under high-oxygen conditions. However, further research involving various combinations of meat aging, freezing, frozen storage, and thawing (under different conditions including packaging type, temperature, and time) is needed to validate this hypothesis.

Maintaining consumer-acceptable color is one of the key priorities in the processing and distribution of meat and meat products (Ruedt et al., 2023; Han et al., 2024a). In the present study, an analysis of meat color revealed that the *LTL* muscles of red deer hinds that were aged prior to freezing were characterized by a lighter color (L*), a lower contribution of redness (a*), and a lower chroma (C*) value (Table 3). In turn, b* values varied during frozen storage. Significant differences between group means were observed for C* values after all storage periods, for L* and b* values after six months, and for a* values after three and nine months.

The present results corroborate the findings of other authors (Kim et al., 2011, 2015, 2018) who observed lower L* values in loins frozen without prior aging. However, the values of C*, a* (Kim et al., 2015), and b* (Kim et al., 2018) were lower in frozen-only samples in the cited studies, but not in the current research. Farouk et al. (2009), who analyzed venison and beef *LD* muscles, also found that aging prior to freezing improved the color of thawed meat (higher values of C* and a*). Only Kim et al. (2011) noted higher b* values in frozen-only loins. According to Kim et al. (2015), the reasons for a desirable color of aged/frozen meat have not been fully elucidated. The lower L* value of frozen-only samples could result from greater thaw loss, accompanied by an increased concentration of water-soluble substances within the muscle tissue, including in the outer layer, leading to increased light absorption (Aroeira et al.,

2017). Myoglobin undergoes denaturation during freezing, frozen storage, and thawing, which increases its susceptibility to autoxidation (Leygonie et al., 2012). In turn, oxidation processes in frozen meat are accelerated by an increased concentration of solutes (heme pigments, metal ions) in unfrozen water (Lee et al., 2024). These solutes, along with mitochondrial and lysosomal enzymes, are released during meat freezing and thawing, owing to the destruction of muscle cell structure (Jeong et al., 2025). Kim et al. (2011) suggested that the differences in color between frozen-only and aged/frozen lamb meat could be due to the higher redox stability of myoglobin and lower respiratory activity of mitochondrial enzymes in aged loins. Lower oxygen consumption by cellular respiration promotes myoglobin oxygenation, exerting a beneficial influence on the color of meat surface. Regardless of these data, it should be stressed that meat susceptibility to discoloration varies depending on meat type. In a study by Farouk et al. (2009), the color of venison deteriorated more quickly during storage than the color of beef. It appears that the specific characteristics of game meat (low pH, high concentrations of heme iron and unsaturated phospholipids) (Williams et al., 1983; Ishida et al., 2001; Okabe et al., 2002), which promote autoxidation, could be responsible for the fact that aging had no positive effect on the color of frozen meat from red deer hinds in this study.

Previous research indicates that refrigerated aging (and enzymatic proteolysis of muscle proteins) has a beneficial influence on the sensory properties of meat, primarily its tenderness and taste (Joo et al., 2023). However, freezing may exert varied effects on the above quality attributes of meat. In general, changes in the sensory properties of frozen/thawed meat are linked to its altered structure caused by crystals of frozen water. A slower rate of freezing leads to the formation of larger ice crystals in the intercellular space, a process that utilizes intracellular water within muscle cells. Larger ice crystals damage and deform muscle cells, leading to water outflow from the cells (Dang et al., 2021). A likely cause of water migration from muscle cells into the intercellular space is also the equalization of osmotic pressure, which increases around growing ice crystals due to the high concentration of solutes in the remaining unfrozen water. All of the above processes contribute to greater thaw loss, which in turn may reduce the juiciness of meat subjected to thermal treatment (Lagerstedt et al., 2008). The described physical phenomena that occur in frozen/thawed meat with the involvement of water (regardless of the aging process) may explain the decrease in juiciness over prolonged frozen storage and the absence of significant differences in juiciness scores between *LTL* muscle samples that were aged prior to freezing and those that were not, observed in the present study (Table 4).

Table 4. Sensory attributes of the *longissimus thoracis et lumborum* muscle of red deer hinds (arithmetic mean ± SD)

Parameter	Aging period (days)	Frozen storage period (months)			
		0	3	6	9
Aroma - intensity (points)	0	4.55 ± 0.44	4.65 ± 0.34	4.50 ± 0.47	4.20 ± 0.82
	10	4.65^{a} ± 0.41	4.65^{a} ± 0.41	4.75^{a} ± 0.42	3.95^{b} ± 0.80
Aroma - desirability (points)	0	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
	10	5.00 ± 0.00	4.95 ± 0.16	5.00 ± 0.00	4.85 ± 0.34
Taste - intensity (points)	0	4.80^{a} ± 0.42	4.65^{ab} ± 0.24	4.40^{bc} ± 0.46	4.05^{c} ± 0.44
	10	4.70^{a} ± 0.35	4.70^{a} ± 0.26	4.70^{a} ± 0.48	4.30^{b} ± 0.48
Taste - desirability (points)	0	4.90 ± 0.32	4.90 ± 0.21	5.00 ± 0.00	4.85^{x} ± 0.34
	10	4.85^{a} ± 0.24	4.75^{a} ± 0.35	4.90^{a} ± 0.32	4.30^{by} ± 0.42
Tenderness (points)	0	4.45 ± 0.64	4.65^{y} ± 0.41	4.70^{y} ± 0.42	4.70 ± 0.54
	10	4.85^{ab} ± 0.24	4.95^{ax} ± 0.16	5.00^{ax} ± 0.00	4.70^{b} ± 0.42
Juiciness (points)	0	4.60^{ax} ± 0.39	4.60^{a} ± 0.46	4.45^{a} ± 0.37	3.70^{b} ± 0.42
	10	4.15^{by} ± 0.24	4.70^{a}± 0.48	4.10^{b} ± 0.46	3.90^{b} ± 0.61

^{a,b,c} Means with different superscripts within a row are different at $p \leq 0.05$.

^{x,y} Means with different superscripts within a column are different at $p \leq 0.05$.

Increased concentrations of solutes (including proteins and lipids) in unfrozen water that remains in the intercellular space and the release of compounds such as enzymes and prooxidants from muscle cells damaged by ice crystals contribute to autoxidation. The accumulation of autoxidation products may deteriorate the taste of frozen/thawed meat over time (Dang et al., 2021). This process was probably responsible for the lower taste scores of meat after the longest period (9 months) of frozen storage in the current study (Table 4).

In the present experiment, meat samples frozen after aging and stored for three and six months were characterized by higher tenderness than unaged samples (Table 4), which indicates that tenderness improved during refrigerated aging. In turn, the increased tenderness of meat from both groups after three and six months of frozen storage could be due to mechanical damage to myofibril structure by ice crystals and the activity of proteolytic enzymes (Stafford et al., 2024; Jeong et al., 2025). However, it should be noted that changes in muscle proteins due to freezing do not always enhance meat tenderness. According to Jeong et al. (2025), their effect is determined by freezing conditions. Zhang and Ertbjerg (2018) suggested that the potential decrease in shear force (reflecting meat tenderization), related to calpain activity, could be counterbalanced by the opposite effect of moisture loss during thawing.

CONCLUSIONS

An analysis of the proportions of nitrogen fractions in the total nitrogen content of meat revealed greater potential for proteolytic changes in frozen-thawed meat that was subjected to refrigerated aging for 10 days before freezing. The benefits of aging prior to freezing included improved tenderness of meat thawed after three and six months of storage, and its higher water-holding capacity after each frozen storage period (3, 6, and 9 months). Aging prior to freezing negatively affected the color parameters of thawed meat and its taste after the longest storage period (9 months). Due to the specific characteristics of the experimental materials (carcasses of wild animals living in different natural habitats) and the unique attributes of the analyzed game meat, the results of this study should be validated in terms of repeatability, including under different freezing-thawing conditions.

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