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ANALYSIS OF THE PHYSICO-CHEMICAL CHARACTERISTICS AND CONTENT OF SELECTED BIOACTIVE COMPONENTS IN LAMB MEAT, DEPENDING ON THE TYPE OF MUSCLE AND VACUUM AGING TIME*

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Abstract

The impact of processes related to meat aging on physico-chemical characteristics and biologically active compound content remain in the minority. Thus, the aim of the study was to analyze the physico-chemical traits (pH, meat color, expressed juice, moisture content, protein, fat, total collagen, and fatty acids) and carnosine, taurine, and L-carnitine content for fresh meat and vacuum-packaged meat aged for 7 and 14 days at temperature of 2°C, from the *longissimus lumborum* (LL) and *gluteus medius* (GM) muscles. This was carried out using 66 Polish Merino heavy lambs. In both muscles, the L* parameter decreased in aged meat, however, the differences with respect to fresh meat were not statistically confirmed. In the *gluteus medius* muscle, a higher (P<0.05) redness value (a*) and lower yellowness (b*) were recorded in meat after aging. A lower (P<0.05) expressed juice value was found in both LL and GM after 7 days of aging. An increase (P<0.05) in protein and fat content in both muscles, and collagen content in LL was observed in aging meat. There was no difference in fatty acid groups or C18:2 c9, t11 content between the GM and LL muscles, or fresh and aging meat. A higher TBARS (thiobarbituric acid reactive substances) content (P<0.05) was recorded in meat after aging for both GM and LL. The meat aging process increased (P<0.05) the carnitine and taurine content, while the carnosine content decreased. Apart from carnosine, the aging process did not significantly reduce the content of bioactive ingredients, which are important in the human diet. This process also did not negatively affect the physico-chemical characteristics determining consumers' evaluation of the meat.

Key words: lambs, muscle type, aging time, CLA, carnosine, L-carnitine, taurine

Meat is one of the basic components of the human diet. As an important source of wholesome protein and other compounds, including those that are biologically active, it guarantees the proper development of the body and prevents the occurrence of many lifestyle diseases (Szmid, 2010; Decker and Park, 2010). The consumer's acceptance of meat depends on its perceived quality based on physico-chemical features such as color, juiciness, and the amount of intramuscular fat. The health values determined by the presence of the bioactive compounds in meat, the properties of which have already been recognized, are also significant. These compounds which in the human body are not always synthesized in sufficient quantities, and which are characterized by properties that inhibit oxidative stress, inflammation, as well as cancer processes – include, among others, carnosine, taurine, L-carnitine, omega-3 polyunsaturated fatty acids, and conjugated isomers of linoleic acid (CLA). They are mainly found in red meat, especially from ruminants, so a diet that includes this type of meat can be an excellent source of bioactive compound supplementation (Wu, 2020; Hipkiss and Gaunitz, 2014; Ito et al., 2014; Shimada et al., 2015; Seidel et al., 2019).

Many studies have indicated that the physico-chemical characteristics of meat, as well as the level of bioactive compounds contained in it, depend on the animal species, breed, body weight at slaughter, feeding system, and the type of muscle and its aging time (Peraza-Mercado et al., 2010; Radzik-Rant et al., 2020; Martinez-Cerezo et al., 2005; Ablikim et al., 2016; Dou et al., 2023; Martin et al., 2022; Yang et al., 2022).

The basic components of mammalian skeletal muscles are muscle fibers, which have various morphological, metabolic, and functional characteristics. Each fiber type is present in all muscles, but in different proportions. The development of the fibers affects not only the overall muscle mass in the carcasses of slaughtered animals, but also the quality features of the meat (Dou et al., 2023; Joo et al., 2013, 2017). Better rates of biochemical change and post-slaughter meat aging are influenced by a greater

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share of white fibers in the muscle, which is associated with a faster decrease in pH and the meat having a greater susceptibility to protein degradation and the greater share of which in the *longissimus dorsi* muscle of ruminants was indicated by Sazili et al. (2005) and Joo et al. (2017). A higher proportion of white fibers may also contribute to improved meat tenderness (Warner, 2016). It has also been established that carnosine, L-carnitine and n-3 fatty acids are present in greater amounts in white muscles than in red ones (Hill et al., 2007; Sale et al., 2010; Shimada et al., 2004; Janovska et al., 2010).

The quality of meat meant for consumption is associated with the post-slaughter aging process, during which a number of physiological, metabolic, and biochemical changes occur in the meat. These changes, as mentioned above, mainly concern a decrease in pH, the degradation and oxidation of myofibrillar proteins, the chemical state of myoglobin, the production of heat shock proteins, and apoptosis, all of which ultimately affect the meat's tenderness, color, and water holding capacity (WHC) (Warner, 2016; Gramatina et al., 2017; Huang et al., 2020). Apart from the beneficial changes found in post-slaughter meat aging, the processes related to transforming myoglobin into metmyoglobin and the oxidation of lipid compounds can negatively affect the color of meat and its sensory characteristics (Hopkins et al., 2013; Ponnampalam et al., 2017; Bekhit et al., 2013). In addition, during meat aging, the bioactive compounds content, as a result of their participation in the processes of glycolysis, proteolysis, the Krebs cycle, or pro-oxidation, may change – and not always to the benefit of the consumer (Kim et al., 2016).

The length of time of meat aging, which allows obtaining the most favorable meat quality indicators from the consumer's point of view, varies depending on the animal species. According to Sosin-Bzducha and Puchała (2017), the aging time for beef should be 6 weeks, while according to Nowak (2005) appropriate time for pork is 6 to 10 days, and for poultry meat, 12 to 24 hours. The quality of lamb meat was tested in a range of studies for different aging periods (7, 30, and 60 days), mainly in terms of the physico-chemical characteristics that are available to be assessed by the consumer (Abdullah and Qudsieh, 2009; Ablikim et al., 2016; Ponnampalam et al., 2017). However, studies on the impact of processes related to meat aging on the content of biologically active compounds remain in the minority.

The aim of this study was to analyze the physico-chemical properties and the content of selected bioactive ingredients (carnosine, taurine and L-carnitine, CLA) in fresh heavy lamb meat, and in post-mortem aged meat on the seventh and fourteenth days, for various types of muscle.

Material and methods

The study was carried out on 66 Polish Merino ram lambs, which were fattened to a weight of 40 kg (±1.5 kg). The lambs came from a flock of 300 ewes with an average prolificacy of 140%. Ram lambs selected for the study came from single litters and were equal in age (all were born within a week). The lambs were reared in standardized conditions with their mothers for a period of 90 days, then weaned and fattened. The initial body weight of lambs was 23.5 kg (±1.5 kg), the fattening duration was 74 days with daily gains of 221.7 g/day. The fattened lambs were kept in identical environmental conditions under constant zootechnical and veterinary supervision, and were fed in accordance with the requirements for fattened animals (Osikowski et al., 1998). The rations consisted of grass hay, steamed potatoes, and a concentrate with the following composition: 23.5% rapeseed meal, 57.8% ground oat grain, 17.6% wheat bran, and 1% mineral mixture. The animals were fed twice a day. The amount of feed given to the lambs was calculated based on the demand and the previously determined chemical composition of the ratio components. The amount of feed was increased according to requirements after the lambs achieved a 5 kg body weight gain. The lambs were given grass hay separately, while potatoes and concentrate were mixed before each feeding. The animals were provided with constant access to water.

All lambs reached the target body weight in a similar time and were slaughtered within 8 days. Before slaughter, the animals were fasted for 12 hours and then transported to the slaughterhouse within 40 minutes. After 3 hours of lairage the lambs were slaughtered. Slaughter was carried out in accordance with Council Regulation (EC) No 1099/2009 of 24 September 2009 The carcasses, which were suspended from the Achilles tendon, were chilled at 4°C. The cooling period lasted 24 hours.

Sample preparation

After cooling, the carcasses were weighed. The average cold carcass weight was 17.9 kg, and the cold dressing percentage reached the level of 44.56%. The carcasses were then split longitudinally and the right carcass halves were cut into joints according to methodology recommended by the National Research Institute of Animal Production (Krupiński et al., 2009).

The *longissimus lumborum* (LL) and *gluteus medius* (GM) muscles were dissected from the loin and hind leg respectively, vacuum packed, and then randomly divided into three groups. Then, 22 LL and 22 GM samples were subjected to analysis as non-aged (fresh) meat. In turn, 22 LL and 22 GM muscles were aged for 7 days, and a further 22 LL and 22 GM for 14 days. The aging process took place at a constant temperature of 2°C, at a humidity of 68%.

In order to analyze the concentration of taurine, carnosine, and L-carnitine, samples taken from the central part of the muscle and weighing approximately 700 mg were fixed in liquid nitrogen in a specially prepared container, immediately transferred to Eppendorf tubes, and then stored at −80°C. The residue of the LL and GM muscle samples was used to determine the physico-chemical parameters of the meat.

Analysis of physical traits of the meat

The pH of the meat was measured 24 hours after slaughter (fresh meat) and after 7 and 14 days of aging, using an Elmetron CP-411 pH meter with a dagger electrode linked with temperature probe calibrated to pH 4.0, 7.0, and 9.0. After 30 minutes of blooming, the color of the LL and GM samples was measured at the meat's surface using a Minolta CR410 colorimeter (Konica-Minolta). Color coordinates were determined three times for each sample: lightness (L*), redness (a*), yellowness (b*), color saturation (C*), and hue angle (H*). Before each measurement session, the colorimeter was recalibrated using standards.

According to a method devised by Grau and Hamm (1953), three replicates of each meat sample (0.3 g) were placed on Whatman No. 1 filter paper and subjected to a pressure of 2 kg for 5 min to determine the expressed juice. The expressible juice outline area and the meat film were measured using a MultiScan 14.02 image analysis system and the results were calculated in units of square centimeters per gram of meat.

Chemical composition of the meat

By applying the spectrometric technique using near infrared transmission (NIR) (PNA-82109), the moisture, crude protein, intramuscular fat, and collagen content was determined. For this purpose, meat samples were homogenized in an Electrolux DITO K35 apparatus, and were then placed in the measuring cell with diameter of 140 mm of a FoodScan analyzer. This device uses the near infrared transmission method in the range of 850–1050 nm. The analyzer was calibrated based on the own calibration study for lamb meat. For each tested parameter, the analyzer scanned each sample 16 times, then the scans were averaged by the equipment software.

Fatty acid determination

Fat extraction from the fresh and aged meat samples was carried out according to a method determined by Folch et al. (1957). Fat saponification was performed in 0.5 M potassium hydroxide (KOH) in methanol, and esterification in 10% BF3 in methanol. The fatty acid methyl esters were extracted using hexane. Fatty acid composition analysis was performed by gas chromatography, using an Agilent Technologies GC 6890 N chromatograph equipped with a BP×70 capillary column (length 60 m, internal diameter 0.22 mm, film thickness 0.25 μm). The conditions for the analysis were helium gas (41 psi) and a FID (flame ionization detector) at 240°C. The temperature program included the following: 3 min at 130°C, an increase to 235°C by +2°C min^{-1}, and 4 min at 235°C. The fatty acids were identified using the BCR 163 reference material (a mixture of beef and pork fat). To standardize the measurements, methyl nonadecanoate (C19:0, Supelco, Bellefonte, USA) was used as the internal reference compound.

The standard cis-9, trans-11 octadecadienoic acid (Larodon AB, Sweden) was used to determine the isomer of linoleic acid (CLA).

Peroxidation of lipids

According to a method determined by Uchiyama and Mihara (1978), for the triplicate extracts from the tested meat samples, lipid peroxidation was assessed via substances reacting with thiobarbituric acid (TBARS). These extracts were obtained after homogenizing tissue lyophilisates (150 mg meat per 1 ml buffer) in a radioimmunoprecipitation assay (RIPA) buffer and centrifugation (1600 × g, 10 min). The malondialdehyde (MDA) precursor 1.2.3.3-tetraethoxypropane (TEP) was used as a standard to express TBARS as MDA equivalents. The absorbance was measured at 532 nm using a microplate reader (Infinite M200, Tecan, Männedorf, Switzerland).

L-carnitine, carnosine, and taurine analysis

ELISA immunoassay was used to determine the concentration of L-carnitine, carnosine, and taurine. For each meat sample previously stored at −80°C, triplicate 250 mg samples were homogenized in 25 mL PBS (phosphate-buffered saline). The resulting homogenates were then centrifuged for 5 min at 5000 × g on ice to obtain supernatants. These were then assayed using enzyme-linked immunosorbent assays kits according to the manufacturer's protocols (Cloud-Clone Corp., USA). Spectrophotometric readings of the results were made using a WHY101TX microplate reader (Novazym Poland Ltd.). The concentration of L-carnitine, carnosine, and taurine was calculated from the determined curves.

Statistical analysis

A statistical analysis of the data was performed using the SPSS 23.0 packet software (2016) based on a linear model that included the effects of aging time, type of muscle, and the interaction between aging time and type of muscle. All effects were tested against residual least squares means to determine the level of significance. Tukey's test was used for comparing mean values between groups when an F test for the main effect was significant.

Results

Physical traits of the meat

There were no statistical differences in the pH values between the LL and GM muscles in both fresh meat and aged meat. Among the types of studied muscle, those that were aged for 14 days had a higher pH ($P<0.05$) compared to fresh meat, but only for the GM muscle (Table 1).

The aging time and the type of muscle did have an effect on the color changes in the meat. A higher ($P<0.05$) redness value (a*) and higher chroma value (C*), but lower hue angle (H*) and lower ($P<0.05$) yellowness value (b*) were recorded in the meat after 7 days, and especially after 14 days of aging compared to fresh *gluteus medius* muscle meat. In the LL muscle, differences

(P<0.05) between fresh meat and meat aged for 7 days and 14 days were found for yellowness (b*) and hue angle (H*). As in the GM muscle, the values of these parameters were lower in aged meat (Table 1). In both muscles, the L* parameter decreased in aged meat but the differences with respect to fresh meat were not statistically confirmed.

A higher lightness (L*) in both fresh meat and meat aged for 7 days (P<0.05) was registered for GM muscle compared to LL muscle. On the other hand, fresh *longissimus lumborum* muscle had a higher (P<0.05) yellowness value (b*) and hue angle (H*). Analysis of the expressed juice showed no differences between the studied muscles. A lower value (P<0.05) of expressed juice was found in both LL and GM after 7 days of aging compared to fresh meat; in the GM muscle the value was even lower after 14 days of aging, but the differences between 7 and 14 days were not statistically confirmed. In the LL muscle, however, after 14 days of aging the expressed juice measurement was lower (P<0.05) compared to both fresh meat and meat aged for 7 days (Table 1).

Table 1. The mean values ±SD for meat quality properties for the various muscles and aging time

Item	Muscle	Aging time		
		fresh meat	meat aged 7d	meat aged 14d
pH	GM	5.53 a±0.17	5.60 ab±0.11	5.68 b±0.18
	LL	5.55±0.23	5.60±0.15	5.65±0.23
L*	GM	33.68 A±2.00	33.60 A±2.07	32.31±3.06
	LL	31.48 B±2.39	31.54 B±2.83	30.72±3.01
a*	GM	11.72 a±0.74	12.33 ab±0.86	12.70 b±0.99
	LL	11.70±1.18	12.30±0.87	12.26±1.28
b*	GM	2.82 Aa±1.46	1.65 b±0.43	1.97 b±0.79
	LL	3.86 Ba±0.90	1.87 b±1.07	1.80 b±1.02
C*	GM	12.12 a±0.94	12.45±0.87	12.87 b±1.06
	LL	12.33±1.33	12.49±0.90	12.44±1.19
H*	GM	13.21 Aa±6.29	7.61 b±1.92	8.69 b±3.04
	LL	18.10B a±3.12	8.61 b±4.80	8.44 b±5.41
Expressed juice (cm^2/g^{-1})	GM	24.07 a±4.47	18.32 b±5.32	15.40 b±3.00
	LL	24.27 a±2.69	19.01 b±2.97	15.54 c±2.22

GM – *M. gluteus medius*; LL – *M. longissimus lumborum*; a, b, c – the different letters in the same row represent significant differences between aging time within individual muscles (P<0.05); A, B – different letters in the same column represent significant differences between muscles within item and aging time (P<0.05).

Table 2. The mean values ±SD for meat chemical composition for the various muscles and aging time (%)

Item	Muscle	Aging time		
		fresh meat	meat aged 7d	meat aged 14d
Moisture	GM	75.68±0.81	75.56 A±1.23	75.06±1.04
	LL	75.41 a±0.66	74.26 Bb±1.69	74.79 ab±1.18
Fat	GM	3.15 a±0.79	4.20 Ab±1.86	3.46 ab±1.07
	LL	3.02±0.84	2.78 B±0.99	3.03±0.87
Protein	GM	20.61 a±0.46	20.94 ab±0.45	21.12 b±0.33
	LL	20.71 a±0.46	20.87 ab±0.68	21.10 b±0.45
Total collagen	GM	1.14±0.14	1.23 A±0.19	1.24 A±0.22
	LL	1.26 a±0.15	1.41 Bb±0.19	1.40 Bb±0.22

GM – *M. gluteus medius*; LL – *M. longissimus lumborum*; a, b, c – different letters in the same row represent significant differences between aging time within individual muscles (P<0.05); A, B – different letters in the same column represent significant differences between muscles within item and aging time (P<0.05).

Chemical composition of the meat and the peroxidation of lipids

An analysis of the basic chemical composition of fresh and aged meat for both tested muscle types is shown in Table 2. There were no differences in the water content between fresh meat and meat aged for 7 and 14 days for the GM muscle. The fat content of this muscle was higher (P<0.05) after 7 days of aging compared to fresh meat. After 7 days of aging, the LL muscle had a lower (P<0.05) water content, but there were no differences in fat content between the fresh and aged meat.

Table 3. The mean values ±SD for each group, and selected fatty acids for the various muscles and aging time (g/100 g total fatty acid)

Item	Muscle	Aging time		
		fresh meat	meat aged 7d	meat aged 14d
Σ SFA	GM	46.92±2.20	47.40±2.09	48.27±2.14
	LL	46.96±1.91	47.62±2.87	47.48±1.63
Σ MUFA	GM	46.01±2.03	45.84±1.80	44.95±1.51
	LL	46.70±1.33	46.43±1.46	46.41±1.89
Σ PUFA	GM	6.59±1.14	6.23±1.55	6.22±1.40
	LL	6.04±1.89	5.82±1.54	5.42±0.91
Σ n6	GM	4.53±0.99	4.06±1.40	4.14±1.30
	LL	3.98±1.31	3.77±1.25	3.49±0.72
Σ n3	GM	1.29±0.26	1.28±0.19	1.32±0.33
	LL	1.33±0.33	1.26±0.26	1.25±0.31
n6/n3	GM	3.55±0.60	3.15±0.89	3.20±0.93
	LL	2.99±0.61	2.97±0.62	2.86±0.63
C18:2c9t11	GM	0.63±0.17	0.58±0.18	0.57±0.13
	LL	0.54±0.09	0.59±0.14	0.48±0.05
TBARS (mmol g^{-1} meat)	GM	0.28 Aa±0.08	0.51 Ab±0.07	0.60 c±0.24
	LL	0.19 Ba±0.08	0.43 Bb±0.08	0.46 b±0.08

GM – *M. gluteus medius*; LL – *M. longissimus lumborum*; a, b, c – different letters in the same row represent significant differences between aging time within individual muscles (P<0.05); A, B – different letters in the same column represent significant differences between muscles within item and aging time (P<0.05).

Table 4. The mean values ±SD for L-carnitine, carnosine, and taurine concentrations for the various muscles and aging time (mg/100 g meat)

Item	Muscle	Aging time		
		fresh meat	meat aged 7d	meat aged 14d
L-carnitine	GM	169.32 a±5.88	192.01 b±6.40	224.70 Ac±9.91
	LL	165.16 a±4.10	191.14 b±7.57	204.15 Bc±9.30
Carnosine	GM	206.28 Aa±9.97	103.71 b±10.12	111.80 Ab±9.04
	LL	226.15 aB±13.42	101.99 b±9.10	96.26 Bb±13.13
Taurine	GM	75.10 Aab±8.87	70.92 Aa±9.69	80.71 Ab±8.38
	LL	61.63 abB±11.87	56.22 aB±5.75	67.01 Bb±11.16

GM – *M. gluteus medius*; LL – *M. longissimus lumborum*; a, b, c – different letters in the same row represent significant differences between aging time within individual muscles (P<0.05); A, B – different letters in the same column represent significant differences between muscles within item and aging time (P<0.05).

The protein content increased (P<0.05) after 14 days of aging compared to fresh meat in both GM and LL muscles. Collagen content increased with the aging of the meat, but a significant increase (P<0.05) in this component in the meat after 7 and 14 days of aging was recorded only in LL muscle. The *longissimus lumborum* muscle was also characterized by a higher (P<0.05) collagen content in the meat for both periods of aging compared to the GM muscle (Table 2).

An analysis of the fatty acid composition showed that neither aging nor muscle type had any effect on the proportion of selected fatty acid groups or the content of the main linoleic acid isomer, C18:2 c9,t11 (Table 3). Although a slight increase in the total saturated fatty acid (SFA) content and a decrease in the total monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were observed with the aging of the meat in both tested muscles, the differences in the content of these acid groups between fresh and aged meat were not statistically confirmed. There was also no difference in fatty acid groups between the GM and LL muscles.

However, aging time and muscle type did affect the degree of lipid peroxidation, expressed as TBARS (Table 3). A higher content of thiobarbituric acid reactive substances (P<0.05) was recorded in the meat after 7 and 14 days of aging for both GM and LL. A higher TBARS content (P<0.05) in fresh meat and meat aged for 7 days was recorded for the GM muscle compared to the *longissimus lumborum* muscle. The GM muscle that was aged for 14 days also had a higher thiobarbituric acid reactive content than LL, but the differences were not statistically confirmed (Table 3).

L-carnitine, carnosine, and taurine content

In the conducted study, the effect of aging and muscle type on taurine, carnosine, and L-carnitine content was

observed (Table 4). Among the tested muscles, the L-carnitine content was higher ($P<0.05$) in meat aged for either 7 or 14 days compared to fresh meat.

While the carnosine content was higher ($P<0.05$) in fresh meat, the content of this component decreased in aged meat. As for taurine, its amount was lower ($P<0.05$) in meat after 7 days of aging compared to meat aged for 14 days for both GM and LL. This bioactive amino acid content in meat after 7 days of aging was also lower than in fresh meat but the differences were not statistically significant (Table 4). The proportion of the aforementioned bioactive compounds also differed according to the type of muscle being tested. In the *longissimus lumborum*, the taurine content was lower ($P<0.05$) compared to the *gluteus medius* muscle for fresh meat as well as after 7 and 14 days of aging; while carnosine, conversely, was characterized by a higher content ($P<0.05$) in LL and a lower content in GM, but only for fresh meat. In meat aged for 14 days, the content of this dipeptide was lower for LL than for GM. Differences in L-carnitine content between the tested muscles were registered in meat aged for 14 days. In the *gluteus medius* muscle, the amount of L-carnitine was higher ($P<0.05$) than in LL (Table 4).

Discussion

Physical traits of the meat

The acceptable pH range for both mutton and lamb meat should be between 5.5 and 5.8 (Devine et al., 1993). In this study, this range of pH values was not exceeded by either fresh or aged meat for GM and LL muscles (Table 1). As in the present study, no differences in pH values between fresh meat and meat aged for 7 days were reported by Abdullah and Qudsieh (2009) in *semitendinosus*, *semimembranosus*, *biceps femoris*, or *longissimus* muscles in Awassi sheep meat. In turn, Gürbüz et al. (2022) observed significant increase in pH in sheep meat at 7th and 14th days of aging compared to the fresh meat. Conversely, a marked decrease in pH at 7 days post-slaughter in the *longissimus dorsi* and *semimembranosus* muscles in adult sheep was recorded by Yaner and Yetim (2001). Decreasing pH values in lamb meat for aging up to 35 days were recorded by Gramatina et al. (2019) in a study comparing the quality of beef, lamb, and pig meat. A significant reduction in the value of this parameter between zero and twenty-four hours after slaughter, was recorded by Yang et al. (2022) in the *longissimus thoracis* muscle of Sunit sheep, which the authors explained was due to the accumulation of lactic acid during the process of anaerobic glycolysis. In this study, the type of muscle had no effect on the pH value either for fresh meat or for meat aged 7 or 14 days. Similarly, no differences in the value of this parameter were recorded between muscles by Purchas and Zou (2008) when examining *longissimus* and *infraspinatus* muscles in fresh cattle meat. A significant decrease in pH is usually recorded within the first hours after slaughter, which may be greater in the LL muscle due to the greater proportion of white fibers in it; but in this study the first measurement was made after 24 h. The rate of pH decline is associated with muscle glycogen content, which can be affected by various environmental factors such diet composition, stress associated with pre-slaughter handling as well as with the type of muscle and muscle fiber composition (Komatsu et al., 2014). In the present study, the lack of differences in the pH may indicate a similar level of glycogen in both examined muscles and proper pre-slaughter handling of the animals.

Color is a key indicator of meat quality for consumers and is considered to be the main criterion for judging whether meat is fresh or not, which influences the decision to buy it (Mancini and Hunt, 2005). Two of the factors influencing changes in meat color may be the storage method and aging time (Yang et al., 2022). In the conducted research, the meat aging process did not affect the L* value, representing the lightness of color, which was favorable in terms of the consumers' perception of meat color. A higher saturation for redness and an increase in chroma values for the *gluteus medius* muscle with aging time that was obtained in the present study was confirmed by Yang et al. (2022), who analyzed changes in the quality parameters of lamb meat between zero and ninety-six hours aging. Likewise, Abdullah and Qudsieh (2009) reported a significant increase in the a* value and a slight decrease in the L* value for meat after 7 days of aging compared to fresh meat, in Awassi lambs.

The concentration of myoglobin in skeletal muscle, which is responsible for changes in meat color, can also be affected by the type of muscle fiber (Mancini and Hunt, 2005). Muscle with a higher proportion of oxidative, slow-contracting fibers has a higher myoglobin content than muscle with a predominance of glycolytic, fast-contracting fibers (Picard et al., 2002). According to Ithurralde et al. (2015), the type I fiber content in the *gluteus medius* muscle is higher than in the *longissimus lumborum*; whereas, Joo et al. (2017) determined a similar proportion of type I fibers in GM and LL, but also indicated a significantly higher proportion of oxidative-glycolytic, fast-contracting fibers (type IIA) in the *gluteus medius* muscle than in the LL muscle. The differences in L*, b*, and hue values between examined muscles in fresh meat and in L* values in meat aged for 7 days can be explained by the different proportion of muscle fibers in the studied muscle types (Table 1).

Water holding capacity, referred to as free water, is an important indicator for assessing meat quality, as it can directly affect the juiciness, tenderness, and color of the meat, which, as mentioned above, is important for consumers (Luca et al., 2016). Many factors can affect water loss, including the hydrolysis and oxidation of cytoskeletal proteins and cell membrane permeability. During aging, the contraction of the myofibrillar reticulum and myocytes, and the degradation of proteins leads to the relaxation of the sarcomere structure so that the muscle's water retention may increase (Lee et al., 2010; Huang et al., 2020).

The lower expressed juice value in aged meat, which indicates a better ability of the meat to hold its own water, that was obtained in the present study for both muscles was also found in an earlier study by the authors, comparing fresh meat and meat aged for 14 days (Rant et al., 2019). A lower value for this parameter in the *semitendinosus*, *semimembranosus*, *biceps femoris*, and *longissimus* muscles in Awassi sheep after 7 days of aging was also determined by Abdullah and Qudsieh (2009). In the current study, no differences in expressed juice were recorded between muscles (Table 1). However, different results were obtained by Kapase et al. (2021) when comparing the quality of different sheep muscle types, where the *longissimus thoracis et lumborum* muscle showed the higher value of water holding capacity compared to *gluteus medius* muscle.

Chemical composition of the meat

The aging time and type of muscle had an effect on the basic chemical composition of the meat (Table 2). In this study, it was found that meat aging had an effect on the water content in the LL muscle at 7 days after slaughter, and this was also reported by Ablikim et al. (2016) in meat from Chinese sheep breeds, but after 15 days of frozen storage. In contrast, Abdullah and Qudsieh (2009) found that aging time had no effect on the water content of various muscles of Awassi lambs; this was similar to the finding for the GM muscle in the present study. The 14-day aging period had a significant effect on the total protein content of both tested muscles, while a higher fat content after aging was recorded only in the GM muscle. This muscle also differed in terms of fat content from the LL muscle after 7 days of aging (Table 2). Abdullah and Qudsieh (2009) were not able to confirm differences in the protein content of various muscles according to aging, and, in contrast to the present study, also were not able to show any differences in fat content. It was also confirmed by Ablikim et al. (2016) that there were no differences regarding fat content between muscle types for fresh meat and meat aged for 14 days for *longissimus dorsi* and *gluteus medius* muscles; as well as by Esenbuga et al. (2009) who compared the *longissimus dorsi, semitendinosus*, and *triceps brachii* muscles in Awassi sheep. In turn, the previous research conducted by Rant et al. (2019) on LL and GM muscles from Polish Merino lambs showed also no differences in the protein and fat content between fresh meat and meat after 14 days of aging. However, significant differences concerned the fat content between LL and Gm muscles in both examined periods.

In this study, the 7- and 14-day aging period had the effect of increasing ($P<0.05$) the collagen content, but only in the *longissimus lumborum* muscle. The significant differences in the proportion of this component in aged meat also concerned the type of muscle. In a similar way to the present study, Ablikim et al. (2016) observed a higher collagen content in the *longissimus dorsi* muscle compared to the GM muscle in Chinese sheep. Differences in the content of this component depending on the type of muscle may be influenced by differences in the amount of intramuscular fat. According to Listrat et al. (2016), an increase in fat content may decrease collagen content – this was also observed in the present study. In the GM muscle, which had a higher fat content after the seventh day of aging compared to the LL muscle, the collagen content was lower (Table 2).

Fatty acid profile and the peroxidation of lipids

In the conducted study, no significant differences were noted in the content of fatty acid groups between fresh and aged meat and between the analyzed muscles. However, the TBARS value was higher in aged meat compared to fresh meat. The GM muscle was also characterized by a higher TBARS value compared to the LL muscle. (Table 3). The oxidative stability of meat can be significantly affected by even small changes in the concentration of fatty acids with double bonds in the chain (Yang et al., 2002). Thus the slight increase of SFA and reduction in the content of MUFA and PUFA during the aging of the meat in the present study may be due to the fact that unsaturated acids are more easily oxidized. A similar variation in fatty acid content during aging was obtained by Yang et al. (2022) in a study of the *longissimus thoracis* muscle in Sunit sheep subjected to different feeding regimes. An increase in the TBARS value in lamb meat aged for 7 and 14 days was also observed by Gürbüz et al. (2022). According to Choi et al. (2018), deep freezing at –60°C can keep lamb meat fresh without symptoms of lipid oxidation for even up to 5 months.

The higher degree of lipid oxidation (expressed by a higher TBARS value) found in the *gluteus medius* muscle compared to the LL muscle could be explained by the higher intramuscular fat content in GM. Greater lipid oxidation was also observed in cattle meat that had a higher fat content (Luciano et al., 2011; Torrecilhas et al., 2021). It is noteworthy that there was a lack of significant changes in C18:2c9,t11 isomer content under the influence of the processes occurring during meat aging. There were also no differences in the content of this component between the examined muscles (Table 3). Looking at the extremely important properties of this isomer for human health, such as the anti-atherosclerotic, antioxidant, and, above all, anti-cancer effects, the stability of the proportion of this compound in meat, regardless of the type of muscle or aging time, is beneficial for consumers (Szmid, 2010; Decker and Park, 2010).

Taurine, carnosine, and L-carnitine concentration

Other tested bioactive compounds such as L-carnitine, carnosine, and taurine, which are substrates in the chemical reactions occurring during the storage, aging, and thermal processing of meat, in addition to the health benefit effects, can be correlated with its taste and aroma. In addition to its antioxidant, metal ion chelating, and anti-glycation effects, carnosine is associated with the umami flavor (Kim et al., 2016). Processes related to

the aging time of the meat (glycolysis, proteolysis, the metabolism of fatty compounds), in which the above-mentioned bioactive compounds are involved, may lead to changes of their content. In the conducted studies, a decrease in carnosine and an increase in L-carnitine were recorded in association with meat aging (Table 4). This was not confirmed by Bischof et al. (2023) when examining non-aged beef and beef aged for 7 and 14 days. The carnosine content increased with the aging of the beef, while L-carnitine remained at similar levels in both fresh and aged meat. On the other hand, in a study by Kim et al. (2020) that compared metabolic changes in beef subjected to different aging treatments over a four-week period, the authors observed an initial increase in carnosine content in dry aging, followed by a decrease after 14 days of aging and a renewed increase and decrease on days 21 and 28 of the study, respectively. In the aforementioned studies, the taurine content decreased after 14 days of meat aging, followed by an increase after 28 days; while carnitine increased due to aging, but only for the first week, thereafter the levels of this component were stable (Kim et al., 2020). According to Roseiro et al. (2014), carnosine can reduce the formation of metmyoglobin, thereby improving the color stability of meat. In the present study, the decrease in its content in aged meat may explain the changes in meat color parameters (Table 1). From the consumer's point of view, the decrease in this bioactive compound, which scavenges oxidation products of sugars, lipids and proteins, is unfavorable. However, as the research of the above-mentioned authors shows, it is difficult to clearly determine the direction of changes in the carnosine content influenced by meat aging processes. In the present study, a decrease in taurine content occurred after 7 days of aging and increased after 14 days, while the L-carnitine content increased until the end of the experiment (Table 4). Carnitine, which is associated with the utilization of fatty acids and facilitates their removal from the mitochondria, can be synthesized from lysine and methionine. Thus, its increase during meat aging can be linked to the release of amino acids during processes associated with protein degradation (Jung et al., 2015). Proteolytic processes occurring during meat aging do not necessarily reduce the total protein content. The increase in this component obtained on the 14th day of meat aging in the presented study (Table 2), could be the result of water loss and not changes in the structure of proteins. Taurine is now recognized as playing a major role in human physiology and nutrition, however, humans' ability to synthesize this β-amino acid is very low compared to livestock. The higher content of taurine in meat after a longer aging time may be important information for people who require supplementation with this ingredient. The use of meat that has been aged for longer times in the diet can effectively prevent the occurrence of the cardiovascular, digestive, endocrine, immunological, muscular, and neurological disorders caused by taurine deficiency (Ito et al., 2014; Shimada et al., 2015; Seidel et al., 2019).

The differences in taurine, carnosine, and L-carnitine content by muscle type recorded in this study can also be seen in other authors' studies. The lower content of taurine in the *longissimus lumborum* muscle and the higher content in the GM muscle, and, conversely, the higher content of carnosine in LL and the lower content of this compound in GM in fresh meat obtained in the present study (Table 4) were confirmed in an earlier study by the authors, which they conducted on fresh meat from Polish Merino lambs (Radzik-Rant et al., 2020). However, in the presented study, in meat aged for 14 days, the carnosine content was higher in GM muscle compared to LL.

A higher carnosine and lower taurine content in the LL muscle compared to GM was also reported by Purchas et al. (2004) in lamb fresh meat. Glycolytic, fast-contracting muscle fibers (type IIB), which are more abundant in the *longissimus* muscle, have a significantly higher carnosine content compared to oxidative, slow-contracting fibers, due to their susceptibility to acidification and higher buffering capacity, as confirmed for beef by Ruiz et al. (2010) and for pork by Aristoy and Toldra (1998).

A significantly higher L-carnitine content in the *gluteus medius* muscle compared to LL was only registered in meat after 14 days of aging (Table 4). A higher L-carnitine content in GM in fresh meat was also determined by the authors in an earlier study on Polish Merino × Berrichon du Cher crossbred lambs (Radzik-Rant et al., 2020). Muscles with more oxidative or oxidative-glycolytic fibers may have a higher content of this component due to its association with oxygen metabolism. The higher L-carnitine content in GM may be explained by the fact that there is a high concentration of lipid compounds in red muscle, in the metabolism of which L-carnitine is involved.

Conclusions

The results of this study indicate that the aging process had a positive effect on the physical characteristics of the meat. Meat subjected to 7 days and 14 days of aging was characterized by no changes in color brightness and better parameters for expressed juice, which are very important in terms of consumer acceptance. When meat was aged for 7 days it contained significantly more fat, and when aged for 14 days it had significantly more protein and collagen. The aging time had an effect on the oxidative stability and the content of bioactive components. In meat aged to 7 days and 14 days, there was a higher L-carnitine and taurine content, while the carnosine content decreased under the influence of processes occurring during the aging.

The type of muscle had an effect on physico-chemical characteristics and the content of bioactive components. The GM muscle was characterized by better meat color brightness than LL. The fat content was higher in GM for aged meat, while LL had a higher share of collagen. The TBARS value indicated that the LL muscle had better oxidative stability.

The GM muscle was characterized by a higher L-carnitine and taurine content in meat aged up to 7 and 14 days, and higher taurine also in the fresh meat. The carnosine content in fresh meat was higher in LL, but in aged meat only for GM.

The obtained results regarding physico-chemical characteristics and most bioactive components indicate that for consumers the aged meat is recommended. The more desirable muscle seems to be the *gluteus medius*, which is part of the most valuable cut of the sheep carcass, which is the hind leg.

Ethical approval

The animal studies were conducted in accordance with the recommendations of the institutional committee for the use of animals (II Local Ethics Committee for Animal Experiments in Warsaw, consent form No. WAW2_20/2016).

Conflicts of interest

The authors declare that they have no conflicts of interests regarding the publication of this article.

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