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DOI : 10.2478/aucft-2023-0015

THE FATTY ACID PROFILE, AND THE CONTENT OF VITAMIN A, VITAMIN E AND CHOLESTEROL IN BEEF (*M. LONGISSIMUS LUMBORUM*) STORED UNDER DIFFERENT MODIFIED ATMOSPHERES

– Research paper –

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Abstract: The aim of this study was to analyze the fatty acid profile of intramuscular fat and the content of vitamin A, vitamin E and cholesterol in samples of the *longissimus lumborum* (LL) beef muscles and stored for 7, 14 and 21 days under modified atmospheres (MA): vacuum, 80% O_2 + 20% CO_2, 60% O_2 + 30% CO_2 + 10% N_2, 40% CO_2 + 60% N_2, 30% CO_2 + 70% Ar). The experimental factors, i.e., storage time (ST) and MA composition, had no significant effect on the proportions of individual saturated fatty acids (SFAs), but they significantly modified the proportions of individual unsaturated fatty acids (UFAs) and the fatty acid profile of intramuscular fat (IMF). The study demonstrated that in order to minimize the loss of total UFAs, samples of the LL muscle should be cold-stored in MA 40% CO_2 + 60% N_2 for up to 14 days; MA 30% CO_2 + 70% Ar also effectively prevented the loss of total PUFAs and eicosapentaenoic acid (EPA). The losses of vitamins A and E were lowest when beef was packaged under oxygen-free MA, in particular MA 30% CO_2 + 70% Ar. Prolonged storage (21 days) did not contribute to significant vitamin losses, but it induced a decrease in cholesterol concentration, which was lower in vacuum-packaged samples than in those packaged in MA containing 60% O_2 and MA composed of 40% CO_2 + 60% N_2.

Keywords: beef quality, fatty acids, vitamins, cholesterol, modified atmosphere, storage period.

INTRODUCTION

Meat are a good source of bioactive compounds with positive effect on human health such as vitamins or fatty acids. (Pogorzelska-Nowicka et al., 2018). Meat belongs to the group of food products with limited durability, and it is to natural, continuous, and usually physical, chemical, biochemical, and microbiological changes. Meat may undergo different qualitative changes depending on the initial quality of raw material and the applied preservation technology (Wood, 2017). A particularly important process in the preservation and storage of beef is cold storage, which is important for this type of meat due to the requirement of a long aging process. (Mancini and Ramanathan, 2014; Dashdorj et al., 2016; Bhat et al., 2018). Therefore, obtaining the desired quality of a specific type of meat raw material requires the use of appropriate technological parameters of refrigerated storage. The quality attributes of fresh packaged meat are considerably affected by storage time (ST) and temperature, type of meat, composition of gas mixture, and type of packaging (Yang et al., 2022; Nethra et. al., 2023).

The proportions of individual fatty acids in intramuscular fat (IMF) may vary depending on animal species (Hwang and Joo, 2017), type of feed (Horcada et al., 2018), carcass fat content (Jacyno et al., 2015), the animal's age (Park et al., 2018), breed and genotype (Brugiapaglia et al., 2014; Ekine-Dzivenu et al., 2014). As demonstrated by some studies, an important role is also played by the duration and conditions of storage (Orkusz et al., 2015; Moczkowska et al., 2017; Rosa, 2019). Changes in the fatty acid profile of IMF may be indicative of lipolytic and oxidative changes in stored meat (Scollan et al., 2014). This applies in particular to phospholipids which contain high concentrations of polyunsaturated fatty acids (PUFAs), which increases their susceptibility to oxidation. The duration and conditions of storage exert a significant impact on the rate and effects of undesirable changes observed in fats, which can be

Received: 01.06.2023
Accepted in revised form: 30.11.2023

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inhibited or slowed down when storage parameters are appropriately set.

Similarly, to other fat-soluble vitamins, vitamins A and E are susceptible to oxidation, which is accelerated in the presence of oxidized fats. Therefore, meat storage may lead to the loss of these vitamins (Horbańczuk and Wierzbicka, 2016). Vitamins are essential for maintaining health and normal bodily functions. Vitamin E has antioxidant effects post mortem as well as in living organisms (Bellés et al., 2019). Meat contains small amounts of vitamins, thus their losses during refrigerated storage should be minimized. Appropriate parameters of the storage process are the key to retaining the vitamin content of meat.

Optimal ST and storage conditions are also important due to the presence of cholesterol in meat because similarly to fatty acids, cholesterol undergoes free radical-mediated oxidation (Cardenia et al., 2013; Chmiel et al., 2019). Cholesterol oxidation occurs during meat storage, and leads to the formation of oxysterols. The concentration of oxysterols in meat may increase during prolonged storage under improper conditions (Oliveira de et al., 2018). However, changes in cholesterol content during storage under different conditions have not been described in the literature. It should be stressed that cholesterol plays many important roles in the body. It participates in the synthesis of hormones, bile acids, and provitamin-D3. Phospholipids and cholesterol are major components of animal cell membranes, and contribute to maintaining the membrane structure. Cholesterol is a key molecule for the function of synapses; it protects and insulates nerves in the myelin sheath, thus supporting the conduction of nerve impulses (Shabanzadeh et al., 2021).

Technological processes carried out during meat storage may cause nutrient losses and decrease the nutritional value of the product. Therefore, the parameters of these processes should be appropriately adjusted to preserve meat nutrients (Horbańczuk et al., 2019). Changes in the quality of meat stored under different modified atmospheres (MA) have been investigated, but a few studies have compared the effects of several different MA combinations on changes in the fatty acid content of meat, whereas changes in the levels of vitamin A, vitamin E and cholesterol in MA-packaged meat have not been researched to date. Therefore, the aim of this study was to analyze changes in the content of fatty acids, vitamin A, vitamin E and cholesterol in samples of the *longissimus lumborum* (LL) muscles from Holstein-Friesian Black-and-White (HF BW) bulls and stored for 7, 14 and 21 days under MA conditions (vacuum, 80% O_2 + 20% CO_2, 60% O_2 + 30% CO_2 + 10% N_2, 40% CO_2 + 60% N_2, 30% CO_2 + 70% Ar). The research hypothesis postulates that ST and MA composition significantly affect the fatty acid profile of IMF and the content of vitamin A, vitamin E and cholesterol in beef (LL muscles).

MATERIALS AND METHODS

Materials

The experimental materials comprised lumbar segments (*longissimus lumborum* – LL) of the right and left *longissimus dorsi* (LD) muscles. The muscles were purchased in the abattoir where 10 HF BW bulls (at 19 months of age), raised on the same farm. Samples weighing approximately 400 g were collected and assigned to six groups (Figure 1): not packaged samples - evaluated immediately after acquisition; samples A - vacuum-packaged; samples B - packaged in MA 80% O_2 + 20% CO_2; samples C - packaged in MA 60% O_2 + 30% CO_2 + 10% N_2; samples D - packaged in MA 40% CO_2 + 60% N_2; samples E - packaged in MA 30% CO_2 + 70% Ar. Packaged meat samples A, B, C, D and E were stored at a temperature of 2 °C for 7, 14 and 21 days, and analyzed after the specified time periods, and were packaged in bags of ethylene-vinyl alcohol (EVOH) copolymer with enhanced gas barrier performance (permeability: $O_2 \leq 1.5$ cm^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; $H_2O \leq 2.5$ g/m^2/24 h/bar/23 °C), using the PP15 (MGO) Tepro Vacu Tronic 2000 machine (Tepro S.A., Poland).

Methods

Fat extraction and fatty acid profile

Intramuscular fat (IMF) was extracted from muscles LL by Soxhlet extraction (with diethyl ether as the solvent, in the Soxtec™ Avanti 2050 Auto Fat Extraction System,FOSS Analytical, Hilleroed, Denmark) (AOAC, 2007). Fatty acid methyl esters were prepared by the modified method of Peisker (Żegarska et al., 1991) with the use of a chloroform:methanol:sulfuric acid mixture (100:100:1 v/v). The fatty acid profile of IMF was determined in accordance with the methodology given by Śmiecińska et al. (2022).

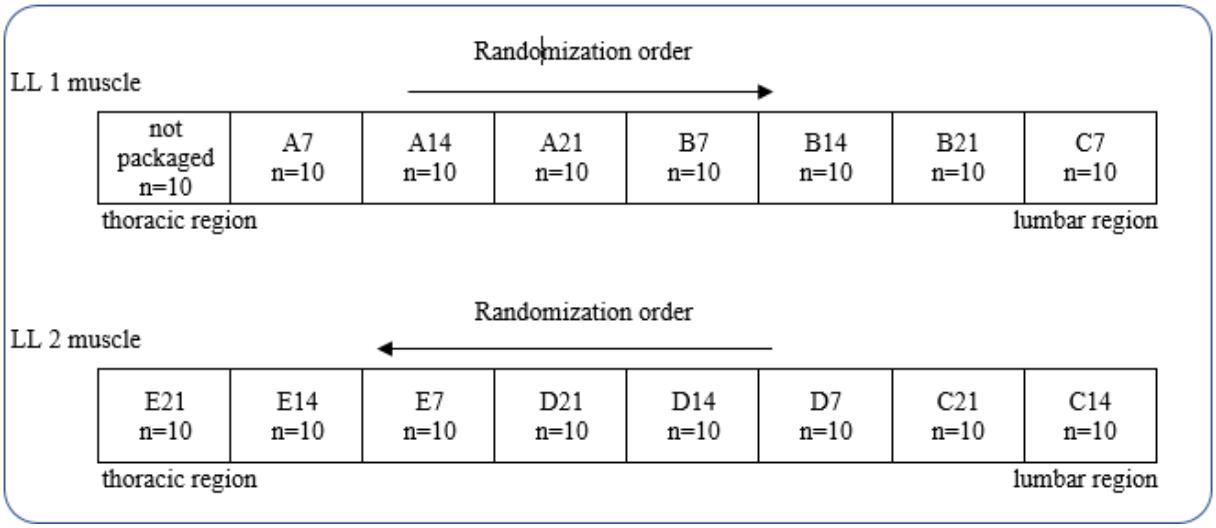

Figure 1. Division of *musculus longissimus lumborum* (right - LL 1 and left - LL 2) into samples, including the packaging method (A, B, C, D, E) and storage time (7, 14 and 21 days).

The results were expressed as percentages of saturated and unsaturated fatty acids in the total fatty acid pool in intramuscular fat (IMF) and specified: saturated fatty acids (SFAs), unsaturated fatty acids (UFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), and calculated: UFA/SFA, MUFA/SFA, PUFA/SFA and PUFA/MUFA ratios.

Vitamin A and vitamin E content

The content of vitamin A (retinol) and vitamin E (α-tocopherol) in beef was determined according to the description of Högberg et al. (2002) and Fredriksson Eriksson and Pickova (2007). Meat samples (2 g) were homogenized (Ultra-Turrax® T25 homogenizer, IKA® Poland Ltd.) and saponified in the presence of 50% KOH (w/v), in Falcon test tubes with snap caps, in a shaking water bath (200 rpm, 80 °C, 15 min), following the addition of 20% aquatic solution of ascorbic acid (1.2 cm^3). Saponified and cooled samples were combined with 5 cm^3 of 35% ethanol and 5 cm^3 of 10% NaCl (w/v). Each time, the samples were shaken for 1 min (IKA MS3 basic vortex mixer, IKA® Poland Ltd.) and left for 5 min until phase separation. Next, 4 cm^3 of n-hexane was added to samples, shaken again and centrifuged at 1500 x g for 15 min (MPW-350R centrifuge, MPW Med. Instruments, Poland).

Three cm^3 of the extract was collected from the supernatant, andextraction was repeated (4 cm^3 of n-hexane, 2 min shaking, 1500 x g centrifugation for 15 min). The extracts were evaporated to dryness in a stream of nitrogen. The samples were dissolved in 1 cm^3 of anhydrous ethanol, filtered, and analyzed in the reverse-phase high performance liquid chromatography system (SHIMADZU, "SHIM-POL A.M. Borzymowski", Poland). A Nucleosil C_{18} column was used, mobile phase was a methanol:water mixture (95:5). A fluorescence detector was used (E_x - 295 nm, E_m - 330 nm). The content of vitamins was calculated based on the peak areas of external standards (Sigma-Aldrich® Brand: (±)-α-Tocopherol).

Cholesterol content

The cholesterol content of beef was determined using the method proposed by Rhee et al. (1982). Meat samples (5 g) were extracted with a chloroform:methanol mixture (2:1). The extracts were evaporated in a water bath, in a stream of nitrogen, at a temperature of 55-60 °C. The fat that remained after evaporation was saponified with 15% solution of KOH (w/v) in ethanol (96%), in a water bath (90 °C, 15 min, reflux condenser). The samples were cooled to room temperature, and the non-saponified fraction was extracted twice with 5 cm^3 of hexane. The samples were centrifuged (3000 x g, 15 min) to separate the saponified and non-saponified fractions. The hexane supernatants were combined, evaporated to dryness (as described above), and dissolved in 5 cm^3 of ethanol (96%). Cholesterol concentration was determined by colorimetry, based on an enzymatic reaction involving cholesterol esterase and oxidase (horseradish peroxidase). Measurements were performed at a wavelength of 520 nm (the spectrophotometer Specord® 40, Analytik Jena AG, Jena, Germany), The Pointe Scientific cholesterol test kit was used.

Statistical analysis

The results were processed using the Statistica program, ver. 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). The normality of data distribution was checked by the Shapiro-Wilk test. The effects of experimental factors – storage time (ST – 0, 7, 14

and 21 days) and modified atmospheres composition (MA) on the analyzed parameters of the LL muscles, and their interactions, were determined by two-way ANOVA. Since interactions between the experimental factors were not found (P>0.05), the significance of differences between group means was estimated by Duncans's test. The significance of differences was determined at P≤0.05 andvariability was expressed as the standard error of the mean (SEM)

RESULTS AND DISCUSSION

Fatty acid profile

The composition of MA had a significant effect on the proportions of only two SFAs identified in IMF, arachidic (C20:0) (P=0.041) and behenic (C22:0) (P=0.035) fatty acids (Table 1). Prolonged storage (21 days) increased the content of palmitic (C16:0) (P=0.025), stearic (C18:0) (P=0.015) and arachidic (C20:0) (P=0.034) fatty acids, and decreased behenic acid content (C22:0) (P=0.025).

The experimental factors (MA and ST) had a greater influence on the proportion of UFAs in IMF extracted from the LL muscle of HF BW bulls (Table 2). Storage in MA containing 80% O_2 decreased the concentrations of linoleic (C18:2) (P=0.021), eicosadienoic (C20:2) (P=0.015), arachidic (C20:4) (P<0.001) and eicosapentaenoic (C20:5 - EPA) (P=0.025) fatty acids, and increased the concentrations of margaroleic (C17:1) (P=0.024) and conjugated linoleic (C18:2 c9, t11 – CLA) (P<0.001) fatty acids. A decrease in the levels of most UFAs was noted only after 21 days of storage. Prolonged storage increased the content of C20:2 (P=0.011) and EPA (P<0.001).

The proportion of total SFAs was lower (P=0.025), and the proportion of total UFAs was higher (P=0.038) in samples stored in MA 40% CO_2 + 60% N_2 (D), compared with the remaining MAs (A, B, C, E) (Table 3). Storage in MA containing Ar (E) decreased the proportions of total UFAs (P=0.038) and MUFAs (P=0.026) in IMF, and increased the proportion of total PUFAs (P=0.038).

Samples stored for 21 days were characterized by a higher proportion of total SFAs (P<0.001), and lower total concentrations of UFAs, MUFAs and PUFAs, and their ratios, than samples stored for 0, 7 and 14 days. In many countries, consumers avoid fat contained in meat due to health concerns. However, fatty acids in IMF are central to the nutritional value and health-promoting properties of meat Wood et al. 2008.

Mahecha et al. (2009) found that 14-day storage of vacuum-packaged *longissimus* muscles of German Simmental bulls had no significant effect on the concentrations of individual fatty acids or total SFAs, MUFAs and PUFAs in IMF (P>0.05). However, the proportion of total SFAs tended to increase and the proportion of total PUFAs tended to decrease in samples stored for 14 days. A similar, statistically significant (P≤0.05) relationship was also observed in the present study.

Chen et al. (2007) evaluated the influence of ST on the fatty acid content of the *semitendinosus* muscle in Chinese yellow cattle and noted differences (P≤0.05) in selected fatty acids and total MUFAs, whereas total PUFAs, SFAs and CLA remained unchanged in vacuum-packaged samples stored for 10 days at a temp. of 7 °C. Similar observations were made in the current study where only ST longer than 14 days induced significant differences in the concentrations of SFAs, UFAs, MUFAs, PUFAs and CLA in IMF extracted from the LL muscle of HF BW bulls (P≤0.05). In a study by Insausti et al. (2004), samples of the LD muscle collected from cattle of five Spanish breeds were packaged in MA 60% O_2 + 30% CO_2 + 10% N_2 and aged for 0, 5, 10 and 15 days.

The content of fatty acids in IMF was affected by ST in only one breed (Asturiana de los Valles) where the content of C18:3 increased (P≤0.05) during the first 5 days of storage, and decreased between days 5 and 15 (P≤0.05). In the present study, ST had no influence on the content of C18:3 in IMF (P>0.05). The cited authors concluded that the fact that ST had no significant (P>0.05) effect on PUFA content was surprising because beef samples were stored in oxygen-enriched MA.

The duration and conditions of storage may induce considerable changes in the content of PUFAs in IMF due to their susceptibility to oxidation (Domínguez et al., 2019). Such changes were noted in the current study where the proportion of PUFAs in IMF extracted from the LL muscle of HF BW bulls was lower after 21 than 14 days of storage (P≤0.05). The content of PUFAs was also lower in the LL muscle stored in MA containing 60% and 80% O_2 than in samples stored in oxygen-free MA (P≤0.05).

The oxidation susceptibility of IMF extracted from the LL muscle of HF BW bulls during cold storage in MA containing 80% and 60% O_2 was also described by Śmiecińska and Daszkiewicz (2021). In the cited study, low oxidative changes in lipids, which resulted in low TBARS values, were

observed throughout storage in vacuum and MA:40% CO_2 + 60% N_2 and 30% CO_2 + 70% Ar.
The results of studies investigating the IMF content in beef and the fatty acid content of IMF may vary because it can be formed by including animal species, breed and age, muscle type and diet (Alberti et al., 2014; Ekine-Dzivenu et al., 2014; Wiśniewski et al., 2021). The results of this study and the findings of other authors indicate that the fatty acid profile of beef may also be affected by MA composition and ST.

Content of vitamin A and vitamin E

An analysis of the content of vitamins in the LL muscle of HF BW bulls revealed that it decreased considerably during storage in MA containing O_2 ($P<0.001$) (Table 4). Storage in MA containing Ar (E) increased vitamin A content, relative to the remaining MAs (A, B, C, D), whereas storage in MA 40% CO_2 + 60% N_2 (D) increased vitamin E content, compared with MA containing 80% O_2 (B). In turn, ST induced significant changes only in vitamin A content ($P<0.001$). Beef samples stored for 14 and 21 days had higher retinol content than those evaluated before storage and after 7 days of storage.No results of studies on changes in vitamin A content during meat storage in MA have been found in the available literature, so it is difficult to compare the results with the results of other researchers. Conte et al. (2019) analyzed changes in the vitamin A content of minced beef (*Longissiumus thoracis*) wrapped in oxygen-permeable film and stored for 6 days at a temperature of 4 °C. The cited authors found that retinol concentration was significantly ($P\leq0.01$) higher before storage than after 2 and 6 days of storage, most likely due to oxygen access to the samples. In the present study, the effect of storage conditions on the vitamin A content of the LL muscle was similar to that described by the cited authors, but the effect exerted by ST was different because vitamin A content did not decrease after 7 days of storage. It should be noted that in the current study, samples stored in MA 30% CO_2 + 70% Ar had the highest vitamin A content. The few studies that have investigated Ar as a component of MA gas mixtures, mostly in poultry (Fraqueza and Barreto, 2009; Tománková et al., 2012; Pérez-Rodríguez et al., 2014) and beef (Śmiecińska and Daszkiewicz, 2021), suggest that Ar may have a beneficial influence on meat quality. However, further research is needed to confirm this hypothesis, especially that the cited studies have focused on the microbiological quality and color of meat as well as lipid oxidation during storage rather than the vitamin content of meat. Nevertheless, their results are interesting because they revealed that MA packaging with Ar effectively inhibited lipid oxidation.
The effect of ST and MA composition on the vitamin E content of meat has been more thoroughly researched. Li et al. (2012) reported that α-tocopherol content was lower ($P>0.05$), in beef LL muscles that were aged in vacuum for 14 days, than repackaged and stored for 5 days in MA 80% O_2 + 20% CO_2, compared with vacuum-packaged samples. Franco et al. (2012) demonstrated that the vitamin E content of beef decreased during 21-day storage ($P<0.05$), which was not observed in the present study. The cited authors also found that vitamin E loss was significantly ($P<0.05$) lower in vacuum-packaged steaks of *Longissimus thoracis* than in samples packaged in MA 80% O_2 + 20% CO_2. According to Clausen et al. (2009) and Yang et. al. (2016), the vitamin E content of meat is affected by MA packaging system, and systems including high O_2 MAP may lead to its decrease during storage, which is consistent with the present findings. Fredriksson Eriksson and Pickova (2007) noted a minor decrease ($P>0.05$) in tocopherol levels in vacuum-packaged LD muscles of steers during 14 days of refrigerated storage. In the present study, ST had no significant effect on the vitamin E content of beef, either. Lagerstedt et al. (2011) also found that α-tocopherol levels in vacuum-packaged LD muscles of bulls remained unchanged during 15 days of storage ($P>0.05$), whereas high-oxygen MAP (80% O_2) decreased the vitamin E content of the analyzed samples already after 5 and 10 days of storage. According to Formanek et al. (1998), high-oxygen MA contributes to the formation of reactive oxygen species that can react with α-tocopherol, thus decreasing its concentration in meat. It appears that changes in vitamin A content may follow the same pattern.

Cholesterol content

Cholesterol levels in beef have been determined in different muscles of cattle of different breeds, genders and ages, fed different diets (Muchenje et al., 2009; Zdanowska-Sąsiadek et al., 2016). However, the information on changes in the cholesterol content of meat stored under different MAs is scant. In the present study, cholesterol concentration was higher in the LL muscle stored in MA:60% O_2 + 30% CO_2 + 10% N_2 (C) and 40% CO_2 + 60% N_2 (D), compared with vacuum-packaged samples (A) ($P<0.001$) (Table 4). Insausti et al. (2004) analyzed the cholesterol content of the LD muscle stored in 60% O_2 + 30% CO_2 + 10% N_2 for 0, 5, 10 and 15 days and found that it increased significantly ($P\leq0.05$) in beef from three out of five Spanish cattle breeds. However, the increase in

cholesterol content noted by the cited authors was not confirmed by other researchers. In the current study, samples analyzed before storage and after 7 days of storage were characterized by higher cholesterol levels than those stored for 14 and 21 days ($P<0.001$). Similar to the present experiment, a study by Śmiecińska (2016) investigated the effect of ST and MA composition on the cholesterol content of the LL muscle of young bulls, commercial crosses of HF BW and Belgian Blue (BB) breeds. In the cited study, cholesterol content was highest in beef samples stored in MA 30% CO_2 + 70% Ar for 14 days ($P\leq0.01$), and lowest in samples stored in vacuum packages for 21 days ($P\leq0.01$), which is consistent with the present findings. Conte et al. (2019) demonstrated that the cholesterol content of minced beef (*Longissiumus thoracis*) wrapped in oxygen-permeable film decreased during 6 days of storage at a temperature of 4 °C ($P\leq0.05$). Studies involving other types of meat also revealed that cholesterol levels may decrease during storage. Mello et al. (2016) found that refrigerated storage (2 °C) induced a decrease in the cholesterol content of chicken breast muscles already after 3 days ($P<0.05$).

The content of cholesterol oxidation products in meat has been more extensively researched. Similar to fatty acid oxidation, cholesterol oxidation in meat can also be prevented with the use of antioxidants, in particular natural antioxidants such as vitamin E, under optimal storage conditions (Ferioli et al., 2008). Lipid oxidation in meat may be affected by factors such as temperature, packaging method and ST (Baggio et al., 2005). Ferioli et al. (2008) noted a continuous increase in cholesterol oxidation in beef stored in MA with oxygen. Cholesterol oxidation derivatives exert mutagenic and cancerogenic effects, disrupt membrane functions, inhibit cholesterol biosynthesis, and DNA synthesis (Sottero et al., 2009). The formation of these undesirable compounds can be effectively inhibited by maintaining a minimum temperature to ensure the safety of meat products, the use of vacuum or MA packages, low-temperature storage away from light, addition of antioxidants to processed meat, and fortification of meat products with antioxidants (Cardenia et al., 2013)

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CONCLUSIONS

The experimental factors, i.e., ST and MA composition, had no effect on the proportions of individual SFAs, but they significantly modified the proportions of individual UFAs and the fatty acid profile of IMF in the LL muscle. On the basis of the results of the conducted study, it is difficult to clearly indicate the specific method and time of storage that would cause the smallest changes and/or losses of the analyzed ingredients. Perhaps more research is needed in this area. However, the study demonstrated that in order to minimize the loss of total UFAs (including MUFAs and PUFAs), samples of the LL muscle should be cold-stored in MA composed of 40% CO_2 + 60% N_2 for up to 14 days; MA composed of 30% CO_2 + 70% Ar also effectively prevented the loss of total PUFAs and EPA. The losses of vitamins A and E were lowest when beef was packaged under oxygen-free MA, in particular MA composed of 30% CO_2 + 70% Ar. Prolonged storage (21 days) did not contribute to significant vitamin losses, but it induced a decrease in cholesterol concentration, which was lower in vacuum-packaged samples than in those packaged in MA containing 60% O_2 and MA composed of 40% CO_2 + 60% N_2.

ACKNOWLEDGEMENTS

Project financially supported by Minister of Education and Science in the range of the program entitled "Regional Initiative of Excellence" for the years 2019–2023, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.

COMPLIANCE WITH ETHICS REQUIREMENTS

In the conducted studies, no experimental procedures were carried out on live animals.
The research material was purchased beef. Thus, ethical approval was not required.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

Table 1. Proportions of saturated fatty acids (SFAs) in IMF (% of total fatty acids) extracted from the *longissimus lumborum* muscle of HF BWbulls after MA storage (means±SEM)

Item	Modified atmosphere (MA)*					Storage time (ST) (days)				SEM	p-Value	
	A n=30	B n=30	C n=30	D n=30	E n=30	0 n=50	7 n=50	14 n=50	21 n=50		MA	ST
Crude fat (%)	2.97	3.41	3.46	3.01	2.99	2.75	2.83	3.38	2.99	0.05	0.117	0.798
$C_{10:0}$ (capric)	0.056	0.055	0.054	0.055	0.056	0.056	0.055	0.054	0.056	0.002	0.286	0.371
$C_{12:0}$ (lauric)	0.068	0.062	0.062	0.065	0.066	0.066	0.062	0.065	0.067	0.002	0.872	0.984
$C_{14:0}$ (myristic)	2.953	2.970	2.943	2.922	2.968	2.965	2.975	2.935	3.028	0.07	0.356	0.558
$C_{15:0}$ (pentadecanoic)	0.403	0.399	0.396	0.387	0.383	0.395	0.391	0.402	0.393	0.03	0.654	0.556
$C_{16:0}$ (palmitic)	30.212	30.285	30.161	29.885	30.403	30.102 ^{b}	30.129 ^{b}	29.839 ^{b}	30.796 ^{a}	0.35	0.855	0.025
$C_{17:0}$ (margaric)	1.018	1.032	1.045	0.999	1.010	1.066	1.006	1.041	1.023	0.04	0.722	0.359
$C_{18:0}$ (stearic)	16.591	16.552	16.748	16.249	16.970	16.669 ^{b}	16.514 ^{b}	16.415 ^{b}	17.144 ^{a}	0.31	0.598	0.015
$C_{20:0}$ (arachidic)	0.139 ^{a}	0.132	0.133	0.128 ^{b}	0.132	0.139 ^{a}	0.131 ^{b}	0.131 ^{b}	0.137 ^{a}	0.004	0.041	0.034
$C_{22:0}$ (behenic)	0.083 ^{b}	0.078 ^{b}	0.078 ^{b}	0.099 ^{a}	0.102 ^{a}	0.075 ^{b}	0.089 ^{a}	0.086	0.076 ^{b}	0.002	0.035	0.025

* Modified atmosphere: A – vacuum; B – 80% O_2 + 20% CO_2; C – 60% O_2 + 30% CO_2 + 10% N_2; D – 40% CO_2 + 60% N_2; E – 30% CO_2 + 70% Ar. Values within a row followed by different superscript letters, within experimental factors, are significantly different a, b – P≤0.05

Table 2. Proportions of unsaturated fatty acids (USFAs) in IMF (% of total fatty acids) extracted from the *longissimus lumborum* muscle of HF BW bulls after MA storage (means± SEM)

Fatty acid	Modified atmosphere (MA)*					Storage time (ST) (days)				SEM	p-Value	
	A n=30	B n=30	C n=30	D n=30	E n=30	0 n=50	7 n=50	14 n=50	21 n=50		MA	ST
$C_{14:1}$ (myristoleic)	0.721	0.741	0.712	0.759	0.708	0.735	0.717	0.742	0.708	0.03	0.075	0.089
$C_{16:1}$ (palmitoleic)	4.096	4.121	4.026	4.218	4.005	4.052	4.046	4.184 ^{a}	3.944 ^{b}	0.185	0.095	0.025
$C_{17:1}$ (margaroleic)	0.982	1.006 ^{a}	0.988	1.011 ^{a}	0.964 ^{b}	0.984 ^{b}	0.966 ^{b}	1.042 ^{a}	0.944 ^{b}	0.01	0.024	0.036
$C_{18:1}$ (oleic)	39.820	39.830	39.901	40.262 ^{a}	39.237 ^{b}	40.155 ^{a}	40.135 ^{a}	40.166 ^{a}	38.936 ^{b}	0.35	<0.001	<0.001
$C_{20:1}$ (gadoleic)	0.080	0.081	0.081	0.081	0.078	0.079	0.082 ^{a}	0.080	0.079 ^{b}	0.01	0.085	0.041
$C_{18:2}$ n-6 (linoleic)	1.998	1.926 ^{b}	1.944	2.114	2.142 ^{a}	2.081 ^{a}	2.033 ^{a}	2.049 ^{a}	1.911 ^{b}	0.08	0.021	0.035
$C_{18:2}$ c9, t11 (CLA) (conjugated linoleic)	0.202	0.218 ^{a}	0.208	0.202	0.200 ^{b}	0.203 ^{b}	0.200 ^{b}	0.215 ^{a}	0.202 ^{b}	0.06	<0.001	<0.001
$C_{18:3}$ n-3 (linolenic)	0.372	0.368	0.365	0.379	0.391	0.375	0.379	0.378	0.364	0.02	0.095	0.073
$C_{20:2}$ n-6 (eicosadienoic)	0.028 ^{b}	0.028 ^{b}	0.030	0.029 ^{b}	0.032 ^{a}	0.032 ^{a}	0.031 ^{a}	0.027 ^{b}	0.030 ^{a}	0.001	0.015	0.011
$C_{20:4}$ n-6(arachidonic)	0.101 ^{a}	0.063 ^{b}	0.064 ^{b}	0.072 ^{b}	0.073 ^{b}	0.085 ^{a}	0.081 ^{a}	0.069 ^{b}	0.076	0.03	<0.001	0.038
$C_{20:5}$ n-3 (EPA) (eicosapentaenoic)	0.077 ^{a}	0.053 ^{b}	0.061	0.083 ^{a}	0.079 ^{a}	0.045 ^{b}	0.044 ^{b}	0.077 ^{a}	0.084 ^{a}	0.005	0.025	<0.001

* Explanations the same as under Table 1

Table 3. Fatty acid profile of IMF (% of total fatty acids) extracted from the *longissimus lumborum* muscle of HF BW bulls after MA storage (means ± SEM)

Item	Modified atmosphere (MA)*					Storage time (ST) (days)				SEM	p-value	
	A n=30	B n=30	C n=30	D n=30	E n=30	0 n=50	7 n=50	14 n=50	21 n=50		MA	ST
Saturated fatty acids (Total SFAs)	51.523 [a]	51.665 [a]	51.620 [a]	50.790 [b]	52.197 [a]	51.245 [b]	51.288 [b]	50.970 [b]	52.723 [a]	0.75	0.025	<0.001
Unsaturated fatty acids (Total UFAs)	48.477 [b]	48.435 [b]	48.380 [b]	49.210 [a]	47.910 [b]	48.755 [a]	48.712 [a]	49.030 [a]	47.277 [b]	0.46	0.038	<0.001
Monounsaturated fatty acids (Total MUFAs)	45.698 [a]	45.779 [a]	45.708 [a]	46.331 [a]	44.991 [b]	45.960 [a]	45.945 [a]	46.215 [a]	44.611 [b]	0.52	0.026	<0.001
Polyunsaturated fatty acids (Total PUFAs)	2.779	2.656 [b]	2.672 [b]	2.879 [a]	2.918 [a]	2.695	2.767	2.815 [a]	2.666 [b]	0.62	0.038	0.041
UFA / SFA ratio	0.942	0.940	0.938	0.970 [a]	0.923 [b]	0.944 [a]	0.951 [a]	0.963 [a]	0.847 [b]	0.03	0.014	<0.001
MUFA / SFA ratio	0.888	0.888	0.887	0.913 [a]	0.867 [b]	0.896 [a]	0.897 [a]	0.907 [a]	0.851 [b]	0.02	<0.001	<0.001
PUFA / SFA ratio	0.054	0.052	0.052	0.057	0.056	0.058 [a]	0.054 [a]	0.055 [a]	0.051 [b]	0.003	0.065	0.011
PUFA / MUFA ratio	0.061	0.058 [b]	0.058 [b]	0.062	0.065 [a]	0.060	0.060	0.061	0.060	0.004	0.013	0.859

* Explanations the same as under Table 1

Table 4. Vitamin (µg/g) and cholesterol (mg/100g tissue) content of the *longissimus lumborum* muscle of HF BW bulls after MA storage (means ± SEM)

Item	Modified atmosphere (MA)*					Storage time (ST) (days)				SEM	p-value	
	A n=30	B n=30	C n=30	D n=30	E n=30	0 n=50	7 n=50	14 n=50	21 n=50		MA	ST
Vitamin A	0.10 [b]	0.09 [b]	0.10 [b]	0.09 [b]	0.12 [a]	0.08 [b]	0.08 [b]	0.11 [a]	0.11 [a]	0.03	<0.001	<0.001
Vitamin E	1.36	1.13 [b]	1.26	1.55 [a]	1.32	1.12	1.37	1.27	1.34	0.11	<0.001	0.758
Cholesterol	55.34 [b]	58.97	62.49 [a]	65.63 [a]	60.10	65.28 [a]	65.22 [a]	59.22 [b]	57.08 [b]	0.61	<0.001	<0.001

* Explanations the same as under Table 1

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