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Effect of VP, MAP and combined packaging systems on the physicochemical properties and microbiological status of veal from unweaned calves

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ABSTRACT

The packaging system is one of the factors influencing the preservation of the nutritional value, microbiological safety, and sensory attributes of meat. The study investigated changes in physicochemical and microbiological properties taking place during 15-day refrigerated storage of two calf muscles, the *longissimus lumborum* (LL) and *semitendinosus* (ST), packaged in three systems, respectively, vacuum packing (VP), modified atmosphere packaging (MAP, 80% O_2 + 20% CO_2), and a combined system (VP + MAP, 8 d in VP followed by 7 d in MAP). LL and ST stored in VP had significantly lower levels of lipid oxidation, higher α-tocopherol content, and higher instrumentally measured tenderness in comparison with the samples stored in MAP. On the other hand, the MAP samples had lower purge loss at 5 and 15 days, a higher proportion of oxymyoglobin up to 10 days of storage, and a better microbiological status. Calf muscle samples stored in the VP + MAP system had intermediate values for TBARS and α-tocopherol content and at the same time were the most tender and had the lowest counts of *Pseudomonas* and *Enterobacteriaceae* bacteria at 15 days. All packaging systems ensured relatively good quality of veal characteristics up to the last day of storage. However, for MAP at 15 days of storage, unfavourable changes in colour (a high level of metmyoglobin and a decrease in oxymyoglobin, redness and R630/580 ratio) and in the lipid fraction (a high TBARS value and a significant decrease in α-tocopherol content) were observed.

1. Introduction

The popularity of beef and veal varies depending on geographic and cultural determinants. In European Union countries, beef accounts for about 17% of meat consumption, which corresponds to 10.45 kg per capita. Higher interest in beef is observed in Argentina (48% of total meat consumption), Brazil (35%) and the United States (30%) (Hocquette et al., 2018; OECD, 2024). Veal is consumed in smaller amounts than other types of meat, varying widely among countries. Nearly 90% of global consumption occurs within the EU, mainly in Spain, France and Italy (exceeding 2 kg per person), with an average of 1.85 kg for the EU. The EU is the world's leading producer of veal, accounting for up to 90% of total production (Gira Consultancy and Research, 2020), most of which takes place in the Netherlands (36%),

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France (28%), Italy (13%), Belgium (9%) and Germany (7%) (www.fefac.eu, 2024).

Despite the small global scale of production, veal is recognized as a relatively high-quality and nutritious meat. It is a good source of protein and health-promoting peptides, B vitamins (mainly B_{12}, B_2, PP and B_6), and minerals, such as zinc, iron, copper, and selenium (Cabrera & Saadoun, 2014; Gálvez et al., 2019). Veal is regarded as a lean meat, due to low fat content, which results in a low caloric value. Moreover, meat obtained from grazing suckling calves has a favourable fatty acid profile, including a relatively high proportion of polyunsaturated fatty acids (PUFA), the presence of vaccenic acid and conjugated linoleic acid isomers (CLAs), and good PUFA/SFA and n6/n3 ratios, as well as relatively low cholesterol content (Domaradzki, Stanek, Litwińczuk, Skałecki, & Florek, 2017).

The shelf-life of food can be extended by using suitable packaging methods and storage conditions. Packaging systems influence the shelf-life as well as the nutritional value and sensory properties of meat, such as colour and tenderness. One of the most popular packaging solutions in the meat industry is vacuum packing (VP), which reduces oxidation processes and thereby helps to preserve the desired sensory characteristics of meat. This system, however, directly affects the proportions of forms of myoglobin and thus the colour of meat. The colour of vacuum-packed beef quickly changes from the bright red of oxymyoglobin (OMb) to the purple form of deoxymyoglobin (DMb), which consumers find less appealing (Hur, Jin, Park, Jung, & Lyu, 2013; Wagoner et al., 2022). Moreover, with longer storage, exudate held in VP folds is also undesirable from the consumer's point of view and may be more susceptible to bacterial growth (Li, Lindahl, Zamaratskaia, & Lundström, 2012).

An alternative is packaging in a modified atmosphere (MAP) with high oxygen content (>70%), which makes it possible to preserve the light red colour valued by consumers (Ripoll, Albertí, Casasús, & Blanco, 2013). The high oxygen concentration leads to oxygenation of myoglobin to oxymyoglobin, which is favourable in terms of sensory attributes (Kameník et al., 2014), but it is also conducive to oxidation (e.g. of lipids and proteins) and causes undesirable flavours and a decrease in the content of vitamins such as α-tocopherol, thereby reducing the nutritional value of the meat (De Palo, Maggiolino, Tateo, & Centoducati, 2014; Łopacka, Półtorak, & Wierzbicka, 2016). In addition, the presence of oxygen in a certain range of concentrations promotes the development of aerobic microbes. MAP has been shown to increase microbiological shelf-life owing to the presence of a modified atmosphere of carbon dioxide, which at a concentration of 20–30% inhibits some bacterial growth (Lorenzo & Gómez, 2012). The packaging systems mentioned above undoubtedly have both advantages and disadvantages. Therefore it is necessary to seek alternative solutions which could be a compromise between satisfactory stability and shelf-life of raw meat and high nutritional value and sensory appeal.

There are many studies on the impact of packaging systems on the quality of meat, but those regarding veal are very limited (De Palo et al., 2014; Lušnic Polak, Kuhar, Zahija, Demšar, & Polak, 2023) and studies concerning medium- and long-term storage of veal are scarce (Lušnic Polak et al., 2023). Veal is fundamentally different from beef from both a physical and chemical perspective, due to its low fat and myoglobin content, slightly different fatty acid profile, higher tenderness and a good smooth flavour. Therefore, a direct translation of research results from beef to veal is not always appropriate (De Palo et al., 2014).

The study aimed to determine the effect of vacuum packing (VP), modified atmosphere packaging (MAP), and a combination of these two systems (VP + MAP) on the physicochemical properties and microbiological quality of veal stored in refrigerated conditions for 15 days.

2. Materials and methods

2.1. Animals, sample collection and preparation

The research material comprised samples of two muscles, the *longissimus lumborum* (LL; sampled between the 13th thoracic vertebrae and first and fifth lumbar vertebrae) and the *semitendinosus* (ST, whole muscle), taken from 8 left half-carcasses of unweaned Limousin calves. The animals were 7 to 8 months of age (7.4 ± 0.6 months), with an average body weight of 302 kg ± 29.6 kg and carcass weight of 184.5 kg ± 21.7 kg. They were reared with their mothers on pasture and kept suckling and grazing until slaughter. Details of animal management, feeding, and slaughter procedures (without electrical stimulation) were previously described by Kaliniak-Dziura et al. (2022). The calves were slaughtered on two dates, two weeks apart (four calves per slaughter date, Fig. S1). For the purposes of this study carcasses of the calves were suspended from the Achilles tendon and chilled under commercial conditions at 2 °C for 72 h.

During dissection, the LL and ST muscles from each half-carcass ($n = 8$), were divided into eight parts of similar weight (approx. 400 g each, in total $n = 64$ parts from LL, and $n = 64$ parts from ST), which were then randomly assigned to three packaging systems and packaged individually. Hence, twenty-four pieces per each muscle were allocated for vacuum packing (VP), twenty-four pieces per each muscle for packaging in a modified atmosphere with a high oxygen concentration (MAP – 80% O_2 + 20% CO_2), and eight pieces from each muscle for the combined VP + MAP system. Irrespective of the packaging system, the pieces were stored in a refrigerated cabinet (EVERLASTING s.r.l., Suzzara, Italy) at 4 ± 1 °C until physicochemical and microbiological analysis. The muscle samples from the first two systems (VP and MAP) were analysed 5, 10 and 15 days after packaging, i.e. at 8, 13 and 18 days post mortem (on each storage day $n = 8$ per each muscle, per each system), while the samples from the combined system were kept in VP for 8 days, then repackaged in MAP for another 7 days, and analysed after 15 days of storage (VP 8 d + MAP 7 d; $n = 8$ per each muscle). To determine the initial values of the parameters, the remaining not packed pieces of each muscle (in total $n = 8$ from LL and $n = 8$ from ST) were used as a control group, and its storage time was designated as day 0. The experiment design and timeline of analysis are presented in Fig. S1.

Muscle samples in the VP system were packed using a Multivac C200 tabletop chamber machine (MULTIVAC Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany) in 20/70 μm PA/PE side seal bags with oxygen permeability of <56 cm^3/m^2/24 h/bar at 50% RH and water vapour permeability of <3 g/m^2/24 h/bar at 85% RH (MULTIVAC, Bucharest, Romania). Samples in the MAP system were placed in PET/PE trays with a modified atmosphere (80% O_2 + 20% CO_2) (Messer Group GmbH, Germany), keeping a 2:1 gas to meat headspace, using the Multivac Traysealer T250 (MULTIVAC Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany). The cover film was a 90 μm thick PA/EVOH/PE laminate with oxygen permeability of <5 cm^3/m^2/24 h/bar at 23 °C and 50% RH and water vapour permeability of <7 g/m^2/24 h at 38 °C and 90% RH.

2.2. pH value and water activity

The pH was measured in triplicate in each sample using a portable pH-meter with automatic buffer detection and automatic temperature compensation (CP-401, Elmetron, Zabrze, Poland). A penetrating glass electrode (ERH-12-6, Hydromet, Gliwice, Poland) calibrated at the 2 points pH 4.00 and pH 7.00 with high-accuracy pH buffer solutions (± 0.02 at 20 °C, Elmetron, Zabrze, Poland) was used.

2.3. Water-holding capacity (WHC)

Purge loss (PL) was expressed as the percentage difference between the initial weight of muscle sample on the sampling day (0 d) and the weight of the sample after storage on a given day of measurement, following gentle blotting with tissue paper before weighing. Cooking loss (CL) was calculated as the percentage difference between the initial weight of the meat sample and that of the sample after heat treatment in a water bath (HENDI sous-vide system GN 1/1, Rhenen, The

Netherlands) at 70 °C to an internal temperature of 65 °C, followed by cooling with tap water and overnight storage at 4 °C. Samples from each slaughter date were cooked within a single batch per each muscle. Expressible water content (mg) was determined by the filter paper press method (Grau & Hamm, 1953). The amount of expressed water is inversely proportional to the water-holding capacity (WHC) of the meat, i.e. a higher amount of expressible water indicates a lower WHC.

2.4. Colour measurements

Meat colour was measured using a Konica Minolta CM-600d portable spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) with a pulsed xenon lamp and 8 mm aperture size, including a specular component, illuminant D65, standard observer at 10°, and zero and white calibrations. The results of the measurements were given in the CIE $L^*a^*b^*$ colour space of the Commission Internationale de L'Eclairage (Commission International de l'Eclairage, 2004), including the following spectral values: L^* (lightness), a^* (redness), and b^* (yellowness). The reflectance between 400 and 700 nm at 10 nm intervals was also recorded and used to calculate the R630/580 reflectance ratio and the percentages of myoglobin isoforms (metmyoglobin (%MMb), deoxymyoglobin (%DMb), and oxymyoglobin (%OMb), according to the 'calculating myoglobin forms via selected wavelengths' method described by AMSA (2012). Reflectance values at 473, 525, 572 and 730 nm were not given by the instrument but were calculated by linear interpolation. The colour values of the surface of raw meat samples were recorded after 30 min of exposure to atmospheric oxygen under PVC wrapping at 4 °C to allow blooming. At least five readings at different points of the sample were taken each time.

2.5. Shear force measurement

A minimum of six strips (10 × 10 mm, about 20 mm long) were cut from the meat samples parallel to the longitudinal orientation of the muscle fibres, following heat treatment (described above: 'Water holding capacity' section CL) and sheared at room temperature (20–22 °C) perpendicular to the fibres using the Zwick/Roell ProLine BDO125 FB0.5TS (Zwick GmbH and Co, Ulm, Germany) with a V-shaped shear blade and crosshead equipped with a 500 N load cell, at a speed set at 100 mm/min. Shear force (SF, N) was evaluated using device-specific testXpert II software.

2.6. TBARS value and α-tocopherol content

Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS) according to Witte, Krause, and Bailey (1970). The absorbance was measured at 530 nm using a Varian Cary 300 Bio spectrophotometer (Varian Australia PTY, Ltd., Mulgrave, Australia) and expressed in mg of malondialdehyde (MDA) per kg of sample. The content of α-tocopherol was determined by an accredited method (No AB 512; Reference document SOP M.001, 7th edition of 27.01.2020) based on the procedure of Eitenmiller and Landen (2007), using a high-performance liquid chromatograph with a fluorescence detector. The α-tocopherol content was expressed as μg per g of sample.

2.7. Microbial profile

Samples for microbiological analysis were taken at 0 d, 5 d and 15 d and prepared according to PN ISO 6887-1, 2005 and PN ISO 6887-2, 2005. The analysis included the total viable count (TVC), total viable psychrotrophic (TVPC) count, total yeast and mould count (TYMC), lactic acid bacteria count (LABC), total staphylococcal count (TSC), *Enterobacteriaceae* count (EBC), *Pseudomonas* spp. count (PC) and *Campylobacter* spp. count (CC). Tests for the presence of *Listeria monocytogenes* were also performed. TVC and TVPC were assessed by surface plating on plate count agar (BTL, Łódź, Poland), incubated at 30 °C for 3 days and at 6.5 °C for 10 days, respectively, according to PN-ISO 4833-2, 2013–12 and PN-ISO-17410, 2004. Yeasts and moulds were isolated on Sabouraud agar (BTL, Łódź, Poland) at 25 °C for 5 days according to PN-ISO 21527-2, 2009. LABC was determined by anaerobic growth (AnaeroPack™, Atmospheric Generation Systems, BioMérieux, France) on MRS agar (BTL, Łódź, Poland) on plates incubated at 30 °C for 3 days according to PN-ISO 15214, 2002. TSC was determined on Baird Parker agar (BTL, Łódź, Poland) following incubation at 37 °C for 24–48 h according to PN-ISO 6888-1, 2001. *Enterobacteriaceae* (EBC) were determined on Violet Red Bile Glucose agar – VRBG (BTL, Łódź, Poland), on plates incubated at 37 °C for 24 h according to PN-ISO 21528-2, 2005. PC was determined on CFC agar (BTL, Łódź, Poland) incubated at 25 °C for 3 days according to PN-EN ISO 13720, 2010. CC was determined according to PN-EN ISO 10272-2, 2017–10 (Biocorp, Warsaw, Poland) with incubation at 42 °C for 48 h under anaerobic conditions (AnaeroPack™, Atmospheric Generation Systems, BioMérieux, France). *Listeria* spp. was determined according to PN-EN ISO 11290-1, 2017. The isolated colonies were identified using the API®Listeria test (BioMérieux, France). All bacteria populations were determined as a log of colony-forming units (log CFU/g).

2.8. Statistical analysis

The Statistica 13 software (TIBCO Software Inc., Palo Alto, CA, USA) was applied to build the statistical model. The main analysis was preceded by a normality and homogeneity of variance check. Due to the incomplete arrangement of main factors (storage time, type of packaging), analysis was performed using the general linear model (GLM) nested ANOVA design. Individual records of variables were processed in terms of packaging type nested in storage time as a fixed effect and slaughter day as a random effect within individual muscle type (LL and ST).

In addition, the full data concerning packaging systems (VP and MAP) and storage time (5, 10 and 15 days) as main effects including their interaction were analysed by a two-way complete ANOVA model. Finally, to check whether the simple two-way 'packaging system x storage time' interactions differ between the levels of muscle (i.e. vary for LL and ST) a three-way ANOVA was performed to identify the a three-way interaction significance. The means of variables among different groups were compared using the Tukey's (HSD) test, and the level of significance was set at $P < 0.05$.

3. Results and discussion

3.1. pH value, water-holding capacity and shear force

The pH value in *longissimus lumborum* (LL) samples increased during storage in both packaging systems (VP vs. MAP, $P > 0.05$; Table 1), attaining a higher value than in the control (0 d) at 15 days ($P < 0.05$; Table 2). The *semitendinosus* (ST) also showed an increasing tendency ($P > 0.05$). No significant differences in either LL or ST were confirmed in the case of samples stored for 15 d in the combined system (VP 7 d + MAP 8 d). Our results are consistent with previous observations by other authors. Baldi et al. (2015) observed during 16-day storage of calf muscles in VP a significant increase in pH only in the initial period in *longissimus dorsi* (LD), while in *biceps femoris* (BF) only a negligible growth trend occurred. D'agata et al. (2010), in both the VP and the MAP system, noticed only a slight increase in pH ($P > 0.05$) in beef during 21-day storage, and at the same time emphasized the stability of the pH of the raw meat. Similar results were also reported by Cullere et al. (2018) for rabbit meat during 10 d of storage in VP and MAP.

In the initial post-slaughter period, the pH value of beef declined due to the glycolysis and accumulation of lactic and phosphoric acids (Huang et al., 2022). The ultimate pH value observed in our study was typical for beef in this postmortem period (72 h) and allowed to exclude the quality defects (Ijaz et al., 2020). The gradual increase in pH value

Table 1
Summary results of the two-way complete ANOVA model and a three-way interaction term for the physicochemical properties, colour parameters and microbial counts of muscles.

Parameter	Packaging system		Storage time		Packaging system x Storage time		Packaging system x Storage time x Muscle type
	LL	ST	LL	ST	LL	ST	
				Physicochemical properties			
pH	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.
Purge loss	n.s.	n.s.	<0.05	n.s.	n.s.	<0.05	n.s.
Cooking loss	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05
Expressible water	n.s.	n.s.	<0.05	<0.05	n.s.	n.s.	n.s.
SF	<0.05	<0.05	<0.05	<0.05	n.s.	n.s.	<0.05
TBARS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
α-tocopherol	<0.05	<0.05	n.s.	<0.05	n.s.	<0.05	n.s.
				Colour parameters			
L^*	<0.05	n.s.	<0.05	n.s.	<0.05	n.s.	<0.05
a^*	n.s.	n.s.	<0.05	n.s.	n.s.	<0.05	<0.05
b^*	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.
C^*	<0.05	<0.05	<0.05	n.s.	n.s.	n.s.	n.s.
h^o	n.s.	<0.05	n.s.	<0.05	<0.05	<0.05	<0.05
%DMb	<0.05	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.
%OMb	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	n.s.
%MMb	<0.05	n.s.	n.s.	n.s.	<0.05	<0.05	n.s.
R630/580	<0.05	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.
				Microbiological analysis			
TVC	<0.05	n.s.	<0.05	<0.05	n.s.	n.s.	n.s.
TVPC	<0.05	<0.05	<0.05	<0.05	n.s.	<0.05	n.s.
EBC	n.s.	<0.05	<0.05	<0.05	n.s.	n.s.	n.s.
PC	n.s.	n.s.	<0.05	<0.05	n.s.	n.s.	n.s.
TSC	n.s.	n.s.	<0.05	n.s.	n.s.	n.s.	n.s.
CC	n.s.	<0.05	n.s.	n.s.	n.s.	<0.05	n.s.
LABC	<0.05	<0.05	<0.05	<0.05	n.s.	n.s.	n.s.
TYMC	<0.05	n.s.	<0.05	<0.05	n.s.	n.s.	n.s.

LL – m. *longissimus lumborum*, ST – m. *semitendinosus*, n.s. – non-significant.

Table 2
pH, water-holding capacity and shear force (SF) of calf muscles stored in different packaging systems.

Packaging system	Control	VP			MAP			VP + MAP	SE	Sig.
Storage time	0	5	10	15	5	10	15	15		
m. *longissimus lumborum* – LL										
pH	5.56a	5.59ab	5.61ab	5.63b	5.56ab	5.57ab	5.63b	5.61ab	0.01	<0.05
Purge loss (%)	0.8a	2.3abc	3.4bcd	4.8d	1.6ab	3.3bcd	3.8cd	6.5e	0.3	<0.05
Cooking loss (%)	23.9	24.1	23.2	22.6	21.6	24.0	23.4	21.8	0.3	n.s.
Expressible water (mg)	47.7a	38.7abc	43.3ab	28.7c	40.7ab	45.4a	32.0bc	48.6a	1.1	<0.05
SF (N)	67.9a	35.7cd	33.4d	31.2d	47.6b	45.8bc	35.7d	28.7d	1.0	<0.05
m. *semitendinosus* – ST										
pH	5.60	5.65	5.69	5.72	5.63	5.69	5.65	5.60	0.01	n.s.
Purge loss (%)	1.5a	3.1ab	2.4ab	4.6b	1.7a	3.7ab	3.0ab	2.2a	0.2	<0.05
Cooking loss (%)	24.8	25.9	25.1	23.2	28.9	23.8	24.9	24.4	0.4	n.s.
Expressible water (mg)	58.5a	49.7ab	36.0c	34.7c	43.7bc	42.9bc	41.3bc	47.6abc	1.3	<0.05
SF (N)	60.7a	41.0bcd	41.2bc	35.4de	44.7b	41.3bc	39.3cd	34.6e	0.6	<0.05

a, b, c, d, e – Means with different letters differ statistically significantly at $P < 0.05$, n.s. – non-significant.

during storage was probably caused by oxidation, proteolytic activity of endogenous enzymes and to some extent microbial action. With the extension of the storage, lactic acid gradually degrades and alkaline protein decomposition products accumulate in meat, causing an increase in pH (Florek, Litwińczuk, Skałecki, & Ryszkowska-Siwko, 2007; Huang et al., 2022; Ijaz et al., 2020; Vaskoska, Ha, Naqvi, White, & Warner, 2020). It is hypothesized that enzymes, especially those involved in the meat tenderization might be oxidized during storage in MAP (Lagerstedt, Lundström, & Lindahl, 2011).Therefore, the higher rate of protein degradation may be the explanation of slightly higher pH values in VP, than in MAP ($P > 0.05$). This phenomenon may also be related to the presence of CO_2 in the MAP system, as this molecule can dissolve in muscle tissue and forms weak carbonic acid (Ząbek, Miciński, Milewski, & Sobczak, 2021).

Many traits of beef are affected by the pH value, which is used as an indicator of meat quality. For example, ultimate pH plays a role in myofibrillar protein degradation, facilitating meat tenderization (Wu, Farouk, Clerens, & Rosenvold, 2014). Another trait, that may be impacted by pH, is water-holding capacity, as pH reaching values close to the isoelectric point of myofibrillar proteins (5.3–5.5) may decrease the water-holding capacity of myosin, which results in increased losses (de Oliveira Ferreira, Rosset, Lima, Campelo, & de Macedo, 2019). Nevertheless, our results did not show a clear relation between pH value and such traits as shear force or purge and cooking losses. There was no significant effect of the packaging system on pH and the storage time affected significantly this parameter only in LL.

Purge loss (PL) in LL increased with storage time in all packaging systems, with significantly the highest value noted at 15 d in the combined system (6.5%). The LL samples from the combined system also had the highest expressible water content at 15 d of storage ($P < 0.05$; Table 2). Lindahl, Lagerstedt, Ertbjerg, Sampels, and Lundström (2010) reported significantly higher PL after 10 d of ageing of the LD muscle in a combined system (VP 5 d + MAP 5 d) than in MAP. Similarly, in a comparison of various packaging systems for the LD muscle, Lagerstedt, Lundström et al. (2011) recorded significantly higher thawing loss from steaks stored for 15 d in a combined system (VP 5 d + MAP 10 d) than in vacuum-packed or MAP samples, which in combination with high cooking loss contributed to a higher total loss. In the authors' opinion, this was most likely linked to the negative effect of the additional procedure associated with transferring the samples from VP to MAP. For economic reasons, all types of water losses from raw meat are highly undesirable for the meat industry (Lindahl et al., 2010).

For the other muscle analysed, ST, the reverse pattern was observed to LL, as the lowest PL at 15 d was recorded for the samples packed in the combined system – >2.3 p.p. (about 52%) lower than in VP ($P < 0.05$) and 0.8 p.p. (27%) lower than in MAP ($P > 0.05$). In both muscles, purge loss increased with storage time (0 d vs. 15 d; $P < 0.05$ in LL), with generally greater loss in VP than in MAP ($P > 0.05$). Additionally, in the case of ST samples, the significant interaction of the packaging system and storage time was confirmed (Table 1). The opposite was observed for expressible water content, which decreased with storage (0 d vs. 15 d; $P < 0.05$ for both LL and ST storage under VP and MAP; Table 2) and was lower for the samples stored in VP at 10 and 15 days ($P > 0.05$). The higher PL in VP as well as the progressive degradation of cytoskeletal proteins as the meat aged most likely resulted in lower availability of free water and thus a lower level of expressible water.

Łopacka, Żontała, Pietras, Półtorak, and Wierzbicka (2015) observed an increase in purge loss during storage of steaks from the *infraspinatus* and *supraspinatus* muscles, in both MAP and a combined system (7 d in VAC + MAP up to 12 d), but these differences were not significant. Similarly, Kameník et al. (2014), despite the increase in purge loss from the LL muscle during 35-day storage, did not confirm a significant effect of either the storage time or the packaging system (VP vs. MAP vs. VSP – vacuum skin packaging); however, the authors stress that the lowest PL was noted in the steaks stored in the VSP system. An earlier study by Lagerstedt, Ahnström, and Lundström (2011) confirmed that PL significantly increased with storage time, with higher losses noted for steaks packed in VP than in MAP (on average by 1% in 14 d and 0.6% in 21 d storage). Purge losses are generally lower in MAP packaging compared to VP, which may be linked to the greater physical pressure of vacuum packaging on the surface of the meat (Sebranek & Houser, 2006). The greater purge loss in VP makes the product less appealing and also increases the susceptibility of meat to the development of microbes.

As in the present study, many authors (Domaradzki, Litwińczuk, Florek, & Żółkiewski, 2017; Farouk, Mustafa, Wu, & Krsinic, 2012; Horcada-Ibáñez et al., 2016; Modzelewska-Kapituła, Kwiatkowska, Jankowska, & Dąbrowska, 2015) have previously shown a significant improvement in the water-holding capacity (assessed by the Grau-Hamm method) of beef stored for 15 d. According to Farouk et al. (2012), the increase in the water-holding capacity of meat during ageing is caused by structural changes, i.e. progressive disintegration of muscle proteins, which results in the appearance of proteins of lower molecular weight and greater extractability and in the disintegration of the spaces ('channels') through which water (the source of the loss) could initially be removed to outside the muscle fibres.

Additional losses in the weight of raw meat are caused by the loss of meat juices during heat treatment. In the present study, the packaging system was not shown to significantly affect cooking loss (CL), which has remained stable throughout the storage period (Table 2), and averaged 23% in LL and 25% in ST. The significant three-way interaction term (packaging system x storage time x muscle type) was confirmed (Table 1) as at 5 d LL and ST displayed quite opposite pattern (Table 2). Hence, CL was significantly lower in LL stored in MAP (21.6%) than in ST samples packed as well in MAP (28.9%) as in VAC (25.9%).

These results are consistent with previous observations by other authors. Baldi et al. (2015) found, that CL in LD and BF calf muscles was not affected by storage time, ranging from 25% to 30%. Moczkowska, Półtorak, Montowska, Pospiech, and Wierzbicka (2017) showed no significant effect of the packaging system (VSP vs. MAP vs. VSP + MAP) on CL, which averaged 24% in steaks from the LL muscle and 26% in steaks from the BF. Lagerstedt, Lundström et al. (2011) also found no significant differences in CL between steaks stored in VP (for 5 and 15 d), in MAP (for 5 d), or in a combined system (VP 5 d + MAP 10 d), and the average value of this parameter was about 25%. In another study, Lagerstedt, Ahnström et al. (2011) reported higher CL in samples stored in MAP for 21 d in comparison with VP (28.6% vs. 26.3%; $P < 0.05$). Łopacka et al. (2016), on the other hand, did not confirm a significant effect of the steak packaging system (VSP vs. MAP vs. VSP + MAP – a combination of modified atmosphere and skin packaging with oxygen semi-permeable inner skin film) on cooking loss, but it significantly increased with storage time for the samples in MAP and VSP + MAP.

Packaging system and storage time significantly affected the shear force (SF) of the muscles. Generally, throughout the storage period the LL and ST samples stored in VP had a lower SF, i.e. instrumentally measured tenderness, than MAP, and these differences were particularly noticeable in the LL at 5 and 10 d ($P < 0.05$; Table 2). Similar patterns were observed by Moczkowska, Półtorak, Montowska et al. (2017) for LL steaks stored for 14 d (29.6 N in VSP vs. 38.9 N in MAP; $P < 0.05$) and by Lagerstedt, Ahnström et al. (2011) for steaks ageing for 14 d (37.5 N in VP vs. 49.5 N in MAP; $P > 0.05$) and 21 d (34.4 N in VP vs. 52.9 N in MAP; $P < 0.05$). Irrespective of the packaging system of calf muscles, a significant decrease in SF over storage time was observed; after 5 d the SF values were already at a level (< 45.0 N) which would allow beef samples to be classified as moderately tender (Boleman et al., 1997).

It should be noted that literature reports on the relationship between the oxygen concentration in the packaging atmosphere and the tenderness of beef are not conclusive. Łopacka et al. (2016) did not confirm a significant effect of the packaging system on the SF of steaks, which after 12 d was 25.0 N in VSP and 26.3 N in MAP. Similarly, results obtained by Lindahl et al. (2010) indicate that ageing in MAP did not negatively affect shear force compared with ageing in a vacuum. On the other hand, some researchers indicate that storage in MAP containing >70% oxygen can negatively affect beef tenderness (Lagerstedt, Ahnström et al. (2011)). This is linked to increased oxidation of enzymes responsible for the tenderization of meat, which significantly slows down this process (Lagerstedt, Lundström et al. (2011); Lund, Hviid, & Skibsted, 2007). Similarly, Zakrys, Hogan, O'Sullivan, Allen, and Kerry (2008) reported that meat packaging atmospheres with a high (≥ 50%) oxygen content were correlated positively with SF ($P > 0.05$) and negatively with sensory assessment of tenderness ($P > 0.05$), which suggests that the beef samples became less tender as the oxygen level increased. Meat stored under high oxygen MAP is more prone to oxidative changes occurring in the structure of proteins which are followed by the reduced water holding capacity (Zakrys-Waliwander, O'Sullivan, O'Neill, & Kerry, 2012). Myosin due to oxidation and presence of free-radicals form disulphide bonds promoting cross-linking of proteins. Formulation of cross-linked proteins has negative effect on the meat tenderness as intermolecular cross-links make proteins less susceptible to enzymatic proteolysis (Zakrys-Waliwander, O'Sullivan, Allen, O'Neill, & Kerry, 2010). Therefore meat is perceived as less tender, tougher and less juicy in sensory evaluation.

Muscle type in interaction with the storage time and packaging system significantly affected the SF (Table 1). Relatively high shear force values in the initial storage period (up to 10 d) showed samples (particularly LL) packed in MAP system. These differences, apart from the reasons mentioned above, may result from different rates of muscle ageing due to distinct glycolytic and oxidative properties as well as muscle fibre characteristics (Moczkowska, Półtorak, & Wierzbicka,

2017; Nair, Canto, Rentfrow, & Suman, 2019).

Among the packaging systems tested, the lowest SF was recorded at 15 d for the samples stored in the VP + MAP system, with significant differences in comparison to MAP confirmed in the ST. In contrast to our results, Lagerstedt, Lundström et al. (2011) demonstrated significantly lower tenderness of beef steaks stored in a combined system (VP 5 d + MAP 10 d) than for samples stored for the same period in VP (55 N and 42 N, respectively; $P < 0.05$). Moczkowska, Półtorak, Montowska et al. (2017) also obtained significantly lower SF for steaks stored in VSP for 28 d compared to samples stored in a combined system (VSP 14 d + MAP 14 d). The slightly different results may be due to the type of meat, which in our study was veal, considered to be more tender than other meats. Although post mortem storage improves the tenderness of veal similarly to beef, veal is much more tender in the initial period (Domaradzki, Litwińczuk, et al., 2017).

3.2. Colour parameters

The significant effects of a three-way interaction (storage time x packaging system x muscle type) as well as a two-way interaction (storage time x packaging system) in LL on lightness (L^*) were confirmed (Table 1). In LL samples, the L* value at 5 d was lower in VP than in MAP ($P < 0.05$), remained at a similar level at 10 d, and then at 15 d again in the VP system reached values lower than in MAP and VP + MAP ($P < 0.05$; Fig. 1A). In the ST muscle, L^* values remained stable throughout the storage period in the VP and MAP systems, although at 15 d the L^* parameter was significantly higher in the combined system than in the samples stored in MAP (Fig. 1F).

Most studies indicate lower lightness values for beef, veal and foal meat stored in vacuum conditions compared to MAP (Insausti et al., 1999; Łopacka et al., 2016; Ripoll et al., 2013) or a combined VSP + MAP system (Lagerstedt, Lundström et al., 2011; Łopacka et al., 2016), and this effect is more evident with increasing storage time. Over the course of post mortem ageing and in an atmosphere with a high oxygen concentration, conformational changes in proteins may take place, causing greater dispersion of light and thus an increase in the lightness/paleness of meat (Łopacka et al., 2016; Lorenzo & Gómez, 2012; Ripoll et al., 2013). In contrast, in VP the L^* value may be kept constant due to a considerable delay in changes in the meat structure, thereby avoiding scattering (Ripoll et al., 2013). Generally, ST samples were lighter than LL, with differences more apparent in VP system. Additionally, in the case of the LL muscle, an increasing tendency of the L^* value in the MAP system was observed with the storage time, while in VP fluctuated. Hence, a significant three-way interaction effect was observed (Table 1). Our results are in agreement with observation of McKeena et al. (2005) who noted that muscles from round (*m. semitendinosus* and m. *tensor fasciae latae)* had higher L^* values, than from loin (m. *longissimus lumborum,* m. *longissimus thoracis*) or from shoulder *(*m. *triceps brachi)*. The differences in colour parameters between muscles are probably the result of the metabolic functions and anatomical locations (LL - more static muscle, while ST is a locomotive muscle) which is also reflected in naturally occurring differences in proportions of muscle fibres and myoglobin levels (Jayasooriya, Torley, D'arcy, & Bhandari, 2007; McKenna et al., 2005).

Red colour is a critical feature for the acceptability of red meats (Holman, van de Ven, Mao, Coombs, & Hopkins, 2017; Isdell, Allen, Doherty, & Butler, 2003; Ripoll et al., 2013). Similarly to L^*, also redness (a^*) was significantly affected by a three-way interaction term (packaging system x storage time x muscle type), and a two-way interaction (time of storage x packaging) in ST muscle (Table 1). In the LL samples, both vacuum-packed and MAP, after an initial (from 0 to 5 d) significant increase in redness, on subsequent days it decreased slightly ($P > 0.05$) to a level similar to that noted in the combined system ($P > 0.05$; Fig. 1B). In the ST muscle in both packaging systems, the a^* value increased in initial period (up to 10 d), after which at 15 d in MAP it fell to a value below that recorded on day 0 ($P > 0.05$), and was also lower than in combined ($P > 0.05$) and VP system ($P < 0.05$) (Fig. 1G).

Changes in yellowness (b^*) in the muscles, were similar to those obtained for redness, i.e. following an initial increase at 5 d (VP vs. MAP in LL, $P < 0.05$), minor decreases were observed on subsequent days (Fig. 1C and H). Although there is a lack of agreement in the literature regarding the effect of the packaging system and ageing time on a^* and b^* values, changes in the redness of the *longissimus thoracis* muscle of calves similar to those noted in our study were reported by Ripoll et al. (2013), who also observed that it increased between 0 and 5 d of storage without differences between the packaging systems, followed by a significant decrease from 5 to 13 d in MAP and a further significant increase in in VP. Similarly, Insausti et al. (2001) and Yang et al. (2016) observed an increase in the a^* and b^* parameters in the initial period of storage in beef packaged in MAP, followed by a gradual decrease.

Generally, the changes in chroma (C*) over the storage period were similar to the ones observed in the case of yellowness (Fig. 1D and I.). Both in LL and ST, after an initial increase (0 d vs. 5d; $P < 0.05$ in LL), a slight decline at 10 d was observed, after which the value remained stable up to 15 d (10 d vs. 15 d; $P > 0.05$). An increase in chroma during the initial period of storage (from 0 to 5 d) was also confirmed by Ripoll et al. (2013), but in the following days remained stable only in VP samples and declined in MAP-stored steaks (VP vs. MAP at 13 d; $P < 0.05$). In our study this parameter in both muscles was significantly affected by the packaging system, attaining higher values in MAP (Table 1). Łopacka et al. (2016) observed a similar trend and confirmed significantly higher values of chroma in beef packed in MAP on each storage time.

The three-way interaction (storage time x packaging system x muscle type) as well as a two-way interaction (storage time x packaging system) in both muscles, had a significant effect on hue angle (h°). However, LL and ST displayed different patterns throughout the storage period. In the LL samples, both vacuum-packed and MAP, the hue angle value decreased significantly in the initial storage period (from 0 to 5 d). During further storage was higher in VP samples (VP vs. MAP at 10 d; $P > 0.05$), after which at 15 d it fell to values significantly lower than in the combined system (Fig. 1E). Contrary to LL, the h° value in the ST muscle remained stable in the initial period of storage (from 0 to 5 d), after which at 10 d it decreased both in VP and MAP to a value below that recorded on day 0 ($P > 0.05$). At 15 d, higher hue angle were observed in VP and combined system and the significant differences were confirmed compared to MAP (Fig. 1J).

Although, chroma is the measure of colour stability and its decrease during storage is the effect of colour deterioration (Strydom & Hope-Jones, 2014). Young, Priolo, Simmons, and West (1999) proposed hue angle (h°) as a better measure of browning. Our study did not confirm this statement. It was clear that in the case of ST, a significantly higher hue angle in VP at 15 d was accompanied by the higher a* ($P < 0.05$), L* and %OMb as well as lower %MMb. These results are in agreement with the findings obtained by Wyrwisz et al. (2016) who also recorded an increase not only in hue angle but also in lightness, redness and %OMb during vacuum-ageing of *semimembranosus* muscle.

The packaging system is only one of the factors affecting the colour of the muscle surface, which depends mainly on the structure of the meat and the concentration and redox state of muscle pigments (mainly myoglobin), which in turn are conditioned by many other factors, both ante-mortem (e.g. breed, sex, diet, housing and age) and post mortem (e.g. bleeding and cold chain management) (Franco et al., 2012; Mancini & Hunt, 2005). Irrespective of the packaging system, in the initial period between 0 d and 5 d (i.e. 3 d and 8 d post mortem), a decrease in %DMb ($P < 0.05$) was observed in both muscles, as well as a increase in %OMb ($P < 0.05$) and %MMb ($P < 0.05$ only in LL; Fig. 2A-G). In the initial post mortem period, meat generally shows a lower blooming ability and lower colour stability, which is directly linked to the high mitochondrial oxygen consumption rate (OCR). OCR declines with storage time, and the protein structure becomes more degraded, enabling deeper oxygen penetration and oxygenation of myoglobin, which leads to blooming

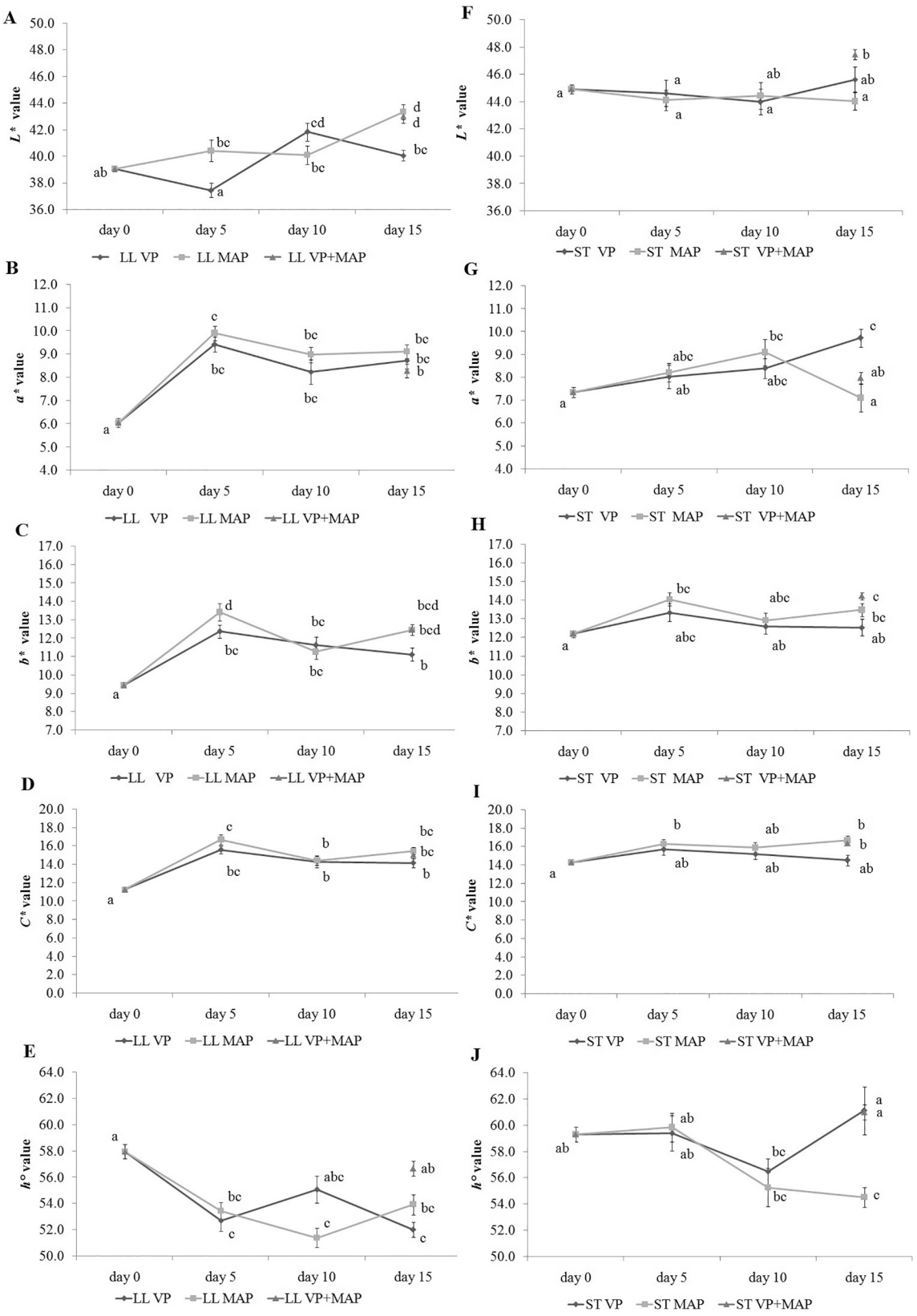

Fig. 1. Results of instrumental measurement of the colour attributes (L*, a*, b*, C*, h°) of calf muscles (LL – 1A-1E; ST – 1F-1J) stored in different packaging systems (mean ± SE); a, b, c, d - means with different letters are significantly different ($P < 0.05$).

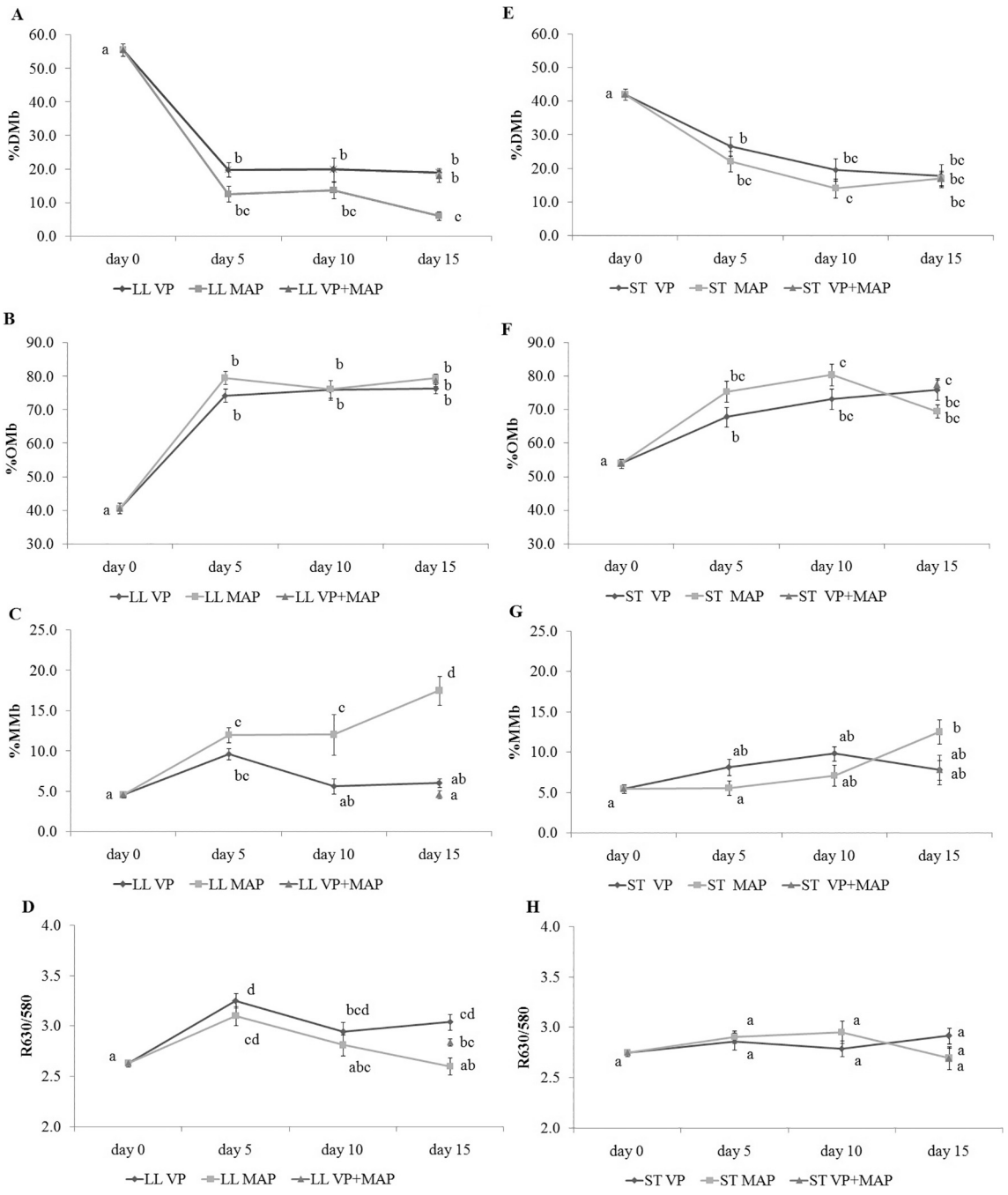

Fig. 2. Percentage of myoglobin forms (%DMb, %OMb, %MMb) and R630/580 ratio of calf muscles (LL – 2A-2D; ST – 2E-2H) stored in different packaging systems (mean ± SE); a, b, c, d - means with different letters are significantly different ($P < 0.05$).

(Lindahl, 2011). Beriain, Goñi, Indurain, Sarriés, and Insausti (2009) state that beef aged 7 d in VP probably had the most favourable oxygen partial pressure for myoglobin oxygenation.

On successive days of storage (5 vs. 10 vs. 15 d) no significant changes in individual myoglobin fractions were shown in the VP samples. Both muscles stored in MAP displayed lower %DMb than samples in VP, albeit the significant difference was confirmed only in case of LL (19.55% for VP vs. 10.97% for MAP; $P < 0.05$). Difference between packaging systems was especially evident at 15 d as %DMb for LL in MAP was significantly higher than in VP. In turn, storage time significantly affected the parameter in ST samples (Table 1), leading to the clear decline in %DMb between 5 d and 10 d (25.08% and 16.73%, respectively, $P < 0.05$). Although, %OMb in LL muscle gradually increased during storage period and its percentages was higher in MAP, neither packaging system nor day of storage significantly affected this parameter. In turn, in ST samples significant effect of two-way interaction (packaging system x storage time) on %OMb was confirmed. The samples stored in both VP and MAP systems displayed increasing tendency up to 10 d, which was especially fast-paced in MAP (5 d in VP vs. 10 d in MAP; $P < 0.05$). However, during the final storage period %OMb in MAP dropped to values below 70% (5 d vs. 15 d; $P > 0.05$), whereas in the samples stored in VP the increasing tendency was maintained and the parameter approached values over 75% (5 d vs. 15 d; $P < 0.05$).

The significant effect of two-way interaction (storage time x packaging system) on %MMb was confirmed in both muscles (Table 1). In case of LL stored in MAP, the level of %MMb kept stable up to 10 d and increased notably at 15 d, reaching the values significantly higher than VP samples both at 10 d and 15 d. Slightly different tendencies were

observed in ST samples. Following initial (within 10 d of storage) minor increase in the proportion of %MMb in both systems (VP vs. MAP; $P > 0.05$), a further upward trend was maintained only in MAP (5 d vs. 15 d; $P < 0.05$), while at 15 d for the samples stored in VP it decreased (5 d vs. 10 d vs. 15 d; $P > 0.05$). In the combined system, the proportions of individual myoglobin fractions in both the LL and ST muscles at 15 d of storage were similar to the values in VP (Fig. 2A-G).

Many studies have shown that the colour of meat packaged in MAP is more appealing than that of VP meat (higher L^*, a^* and %OMb), but only for a limited time (Li et al., 2012; Lindahl, 2011; Ripoll et al., 2013). During storage in an atmosphere with a high oxygen concentration, there is an increase in the proportion of the least desired form of myoglobin, i.e. metmyoglobin (MMb), which gives meat a brown colour, having a significant negative effect on its appearance. A brown colour on the surface of meat can be seen when the share of MMb reaches 40% (Van den Oord & Wesdorp, 1971), but at a level of just 20% MMb about half of consumers find the meat unacceptable (Hood & Riordan, 1973), and at 50% it is rejected by all customers (Van den Oord & Wesdorp, 1971).

Unfavourable changes in meat colour are indicated by the R630/580 ratio, due to its relationship with the proportion of metmyoglobin on the meat surface. Lower values for the ratio indicate an increase in brownness and a decrease in redness (Reyes et al., 2022; Wagoner et al., 2022). In both muscles, R630/580 increased up to 5 d of storage ($P < 0.05$ in the LL; Fig. 2D and H) and remained at a similar level up to 10 d ($P > 0.05$ in LL and ST). At 15 d higher values for the ratio were observed for the samples stored in VP, and in the LL these differences were confirmed statistically in comparison to MAP. LL samples in the combined system took on intermediate values between VP and MAP ($P > 0.05$), while in the ST they were similar to the MAP samples ($P > 0.05$).

In the case of beef, the R630/R580 ratio decreases from about 3.9 ± 0.1 when no MMb is present (0% MMb) to 1.01 ± 0.02 in a completely oxidized sample (100% MMb) (Hernández Salueña, Sáenz Gamasa, Diñeiro Rubial, & Alberdi Odriozola, 2019). Ripoll, Joy, and Muñoz (2011) report that at values below 2.5 consumers tended to perceive meat to be more brown than red. In our study, at 15 d the values of the ratio for samples stored in MAP (2.60 in LL and 2.70 in ST), and in the case of ST also in VP + MAP (2.69), approached the threshold level indicated in the literature.

Longer storage of packaged meat, i.e. >15 d for beef (Lindahl, 2011) and >7 d for veal and foal meat (Lorenzo & Gómez, 2012; Ripoll et al., 2013), should take place in vacuum conditions (VP). Insausti et al. (1999) report that the colour of beef in MAP was acceptable only up to 10 d, whereas in VP or a combined system (VP 10 d + MAP 5 d), it remained satisfactory up to the end of the storage period, i.e. 15 d. In our study, although at 15 d of storage there were certain signs of unfavourable changes in the colour of samples packaged in MAP (a decrease in a^*, %OMb and R630/R580 and an increase in %MMb in the ST, and a decrease in R630/R580 and an increase in %MMb in the LL), they were not sufficiently great and perceptible in sensory assessment to disqualify the meat.

Many studies indicate that the conversion of oxymyoglobin to metmyoglobin resulting from oxidation is associated with a decrease in the a^* value (Insausti et al., 2001, Łopacka et al., 2016, Łopacka, Półtorak, & Wierzbicka, 2017). A strong positive relationship has also been shown between metmyoglobin percentages and the TBARS value (Łopacka et al., 2016, Łopacka et al., 2017), which was also reflected in the present study. In addition, the higher level of %MMb and TBARS in MAP muscles could explain the lower content of α-tocopherol compared with VP, since α-tocopherol in the antioxidant system delays lipid oxidation during storage (Clausen, Jakobsen, Ertbjerg, & Madsen, 2009) and has a role in the regeneration of MetMb-reductase (Lagerstedt, Lundström et al., 2011).

3.3. TBARS value

The three-way interaction (time of storage x packaging system x muscle type) and a two-way interaction (time of storage x packaging) in both muscles had a significant effect on lipid oxidation (Table 1). At 5 d of storage, the TBARS value of samples in MAP was higher ($P > 0.05$) than in VP, and on subsequent days it reached significantly higher values, i.e. about 3.5-fold at 10 d (LL and ST) and 8.5-fold for LL and 6-fold for ST at 15 d (Fig. 3A and B). Samples of both muscles packaged in VP were stable in terms of lipid oxidation throughout the storage period. The TBARS value ranged from 0.178 to 0.215 mg MDA/kg in the LL ($P > 0.05$) and from 0.195 to 0.240 mg MDA/kg in the ST ($P > 0.05$). In both muscles stored in MAP, the degree of lipid oxidation significantly increased with storage time (5 d vs. 10 d vs. 15 d). These results are in agreement with the observations of other authors, who point out the high concentration of oxygen in MAP, which intensifies oxidation processes during meat storage, as a key factor leading to the conversion of alkyl radicals to superoxide radicals at the propagation stage (Formanek et al., 2001; Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010; Lorenzo & Gómez, 2012; Resconi et al., 2012). Formanek, Kerry, Buckley, Morrissey, and Farkas (1998) investigated different oxygen levels in MAP and reported higher TBARS values in minced beef at increased oxygen levels in MAP. They proved that during 8 days of storage, TBARS values were higher for all MAP combinations than VP packaged meat. Similar results were observed by Franco et al. (2012) who confirmed significantly lower malonaldehyde concentrations for beef steaks stored up to 21 days in VP than in the MAP samples. Orkusz, Haraf, Okruszek, and Wereńska-Sudnik (2017) assessed the effect of the packaging system on the degree of lipid oxidation in goose meat and showed that at 7 d of storage the MDA content was more than twice as high in the samples packaged in MAP than in VAC (1.08 mg MDA/kg meat vs. 0.44 mg MDA/kg meat) and increased with each day of storage, tripling by day

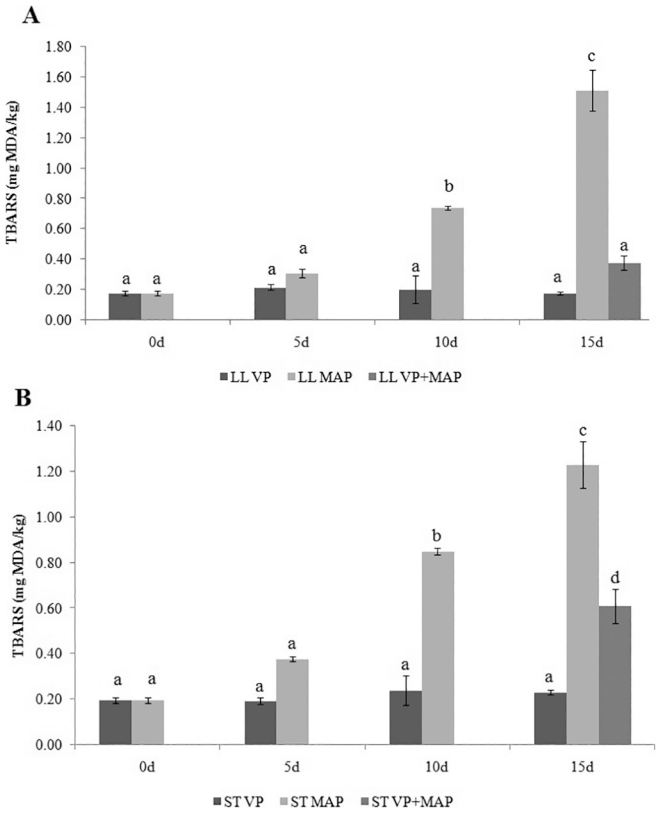

Fig. 3. TBARS values (mg MDA/kg) of calf muscles (LL - 3A; ST – 3B) stored in different packaging systems (mean ± SE); a, b, c, d - means with different letters are significantly different ($P < 0.05$).

11 (1.55 mg MDA/kg meat vs. 0.51 mg MDA/kg meat, respectively).

Similarly, Berruga, Vergara, and Gallego (2005) showed that during 28 d of storage of lamb, a mixture of protective gases consisting of 80% CO_2 and 20% O_2 resulted in a significantly higher degree of lipid oxidation than vacuum packaging or protective atmospheres consisting of various proportions of CO_2 and N_2. At 15 d, samples stored in the combined system had intermediate TBARS values between those obtained in VP and MAP; in the LL they were significantly lower than in MAP and did not differ significantly from VP. In the ST, significant differences were confirmed in comparison to both packaging systems (Fig. 3A and B). Łopacka et al. (2016) reported similar relationships for TBARS values of steaks stored for 8 and 12 days in three different systems (VSP < VSP-MAP<MAP).

3.4. α-Tocopherol content

Oxidation processes taking place during storage lead not only to the generation of harmful oxidation products, but also to the degradation of bioactive substances such as vitamins. Packaging system significantly affected the content of α-tocopherol in both muscles. Additionally, in case of ST the two-way interaction (packaging system and storage time) was confirmed ($P < 0.05$; Table 1). In the samples stored in MAP, it decreased successively from 0 d, reaching a significantly lower value (by about 25%) at 10 d in the ST and in both muscles (by >30%) at 15 d. A decrease in the α-tocopherol level was also observed in the samples stored in the VP + MAP system (compared to 0 d), but these changes were not significant in either the LL or ST (Fig. 4 A and B). Generally, content of the α-tocopherol was similar in both muscles and did not change significantly during storage in vacuum conditions.

An atmosphere with higher oxygen content may be favourable to the generation of reactive oxygen species, which can react with α-tocopherol, thus decreasing vitamin E (Franco et al., 2012). Clausen et al. (2009), reported that storage of beef in an atmosphere with a high (50%) oxygen concentration leads to a decrease in α-tocopherol content from 2.2 μg/g of meat at 0 d to 1.5 μg/g of meat after 6 d and to 1.2 μg/g of meat after 20 d of storage, whereas in the VSP system it remains at a high, stable level (~1.9 μg/g meat) even after 20 days. Similarly, Lagerstedt, Lundström et al. (2011) found that the content of α-tocopherol did not change with ageing time of LD steaks in a vacuum, whereas it decreased rapidly during exposure to high-oxygen MAP. Franco et al. (2012), also observed the higher content of α-tocopherol in beef steaks under vacuum storage rather than MAP ($P < 0.05$). Formanek et al. (1998) observed a progressive decline in α-tocopherol content in minced beef as oxygen concentrations increased.

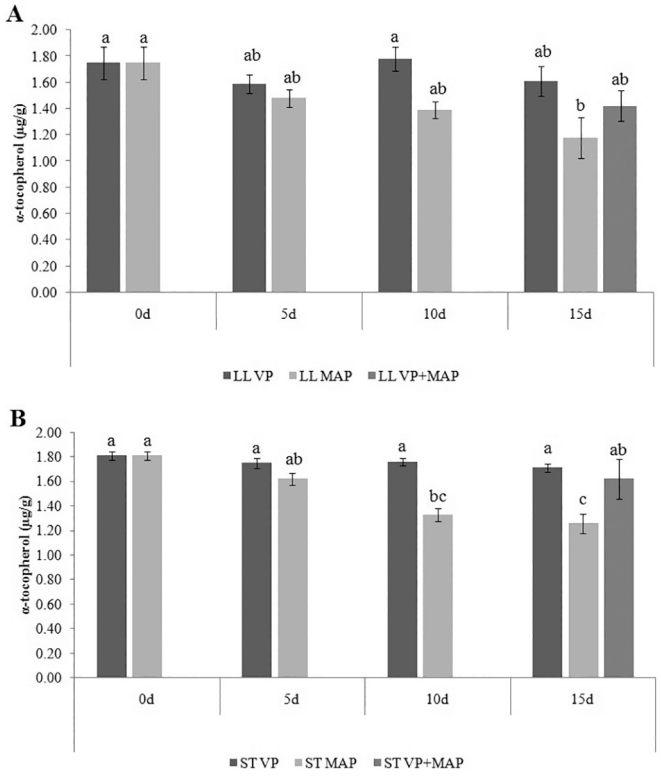

Fig. 4. α-Tocopherol content (μg/g meat) in calf muscles (LL - 4A; ST – 4B) stored in different packaging systems (mean ± SE); a, b, c - means with different letters are significantly different (P < 0.05).

3.5. Microbial analysis

The present study showed that the refrigerated storage time of veal significantly influenced all analysed groups of microbes. For both muscles analysed, irrespective of the packaging system, between 0 d and 5 d there was a significant increase in the total viable count (TVC) (except for ST in MAP), total viable psychrotrophic count (TVPC), and counts of bacteria of the genera *Pseudomonas* spp. (PC) and *Lactobacillus* spp. (LABC). A further increase in these groups of bacteria, often also significant, was observed up to the final day (15 d) of storage (Tables 1 and 3). Similar relationships were reported by Lorenzo and Gómez (2012), who after both 4-day and 7-day storage of foal meat in VP and MAP (80% O_2 + 20% CO_2) obtained similar TVC, TVPC, PC, and *Enterobacteriaceae* and *Lactobacillus* spp. counts (EBC and LABC) in both packaging systems. Generally, samples stored in MAP showed a lower counts of analysed bacterial groups compared to VP, which was reflected especially in the LL muscle ($P < 0.05$ for TVC, TVPC and LABC; Table 1). However, comparing the numbers of microbes between both systems on individual days of storage (5 d vs. 15 d), significant differences were confirmed in only a few cases, i.e. at 15 d in the ST for TVPC, LABC and *Campylobacter* spp. (CC) (Table 3). Lorenzo and Gómez (2012) also reported that MAP was the most effective treatment for the inhibition of TVC, TVPC, LABC, and EBC in foal meat. Kameník et al. (2014) reported a lower TVC in MAP after 7 and 14 days of storage of steaks in comparison to VP. A higher shelf-life of minced water buffalo meat stored in MAP is confirmed by Jaberi, Kaban, and Kaya (2019). The presence of CO_2 may be responsible for the lower bacterial counts in the MAP system, as the inclusion of 20–30% CO_2 prolongs shelf life by inhibiting bacterial growth (McMillin, 2008).

One of the tasks of packaging and food storage systems is to limit unfavourable microbiological changes affecting the shelf-life and safety of products. The atmosphere and conditions formed inside the packaging largely affect the development of individual groups of microorganisms (D'agata et al., 2010). In the case of LAB, many studies confirm that their levels are higher in meat stored in vacuum conditions than in MAP (Berruga et al., 2005; Jaberi et al., 2019), due to their ability to grow in anaerobic conditions. Microbial spoilage of food occurs when total aerobic counts reach 10^7 CFU/g (ICMSF, 1984), and sensory spoilage can occur when LAB concentrations reach approximately 10^7 CFU/g (Mejlholm & Dalgaard, 2013). In the present study, values below these limits were obtained throughout the storage period in all packaging systems.

Irrespective of the packaging system, in both muscles a significant increase in EBC was observed, and at 15 d of storage the level of these bacteria was lowest in the combined system (3.44 log CFU/g in LL and 3.51 log CFU/g in ST; $P > 0.05$). Bacteria of the family *Enterobacteriaceae* lead to degradation of amino acids and the formation of sulfur compounds, amines and volatile substances responsible for the specific odour of spoilt meat (Djordjević et al., 2019). The EBC level noted by Wang et al. (2016) at 14 days of storage of lamb was similar to that obtained in the present study (~4.66 log CFU/g), with no significant differences between VP and MAP with either a low (20% CO_2 + 80% N_2) or intermediate concentration of carbon dioxide (60% CO_2 + 40% N_2).

The meat spoilage process can additionally be intensified by the

Table 3
Results of microbiological analysis (log CFU/g) of calf muscles stored in different packaging systems.

Packaging system	Control	VP		MAP		VP + MAP	SE	Sig.
Storage time	0	5	15	5	15	15		
m. *longissimus lumborum* – LL								
TVC	2.87a	4.77bc	6.65d	4.52b	5.64bcd	5.86cd	0.19	<0.05
TVPC	2.26a	4.72bc	6.72d	4.32b	5.82cd	6.25d	0.22	<0.05
EBC	1.36a	2.44ab	4.33cd	2.83abc	4.77d	3.44bcd	0.23	<0.05
PC	3.33a	4.52b	6.52d	4.72bc	6.69d	5.53c	0.18	<0.05
TSC	3.11bc	2.89ab	1.99a	2.99abc	2.47ab	4.02c	0.13	<0.05
CC	2.71a	2.90ab	2.98ab	2.79ab	2.61a	4.02b	0.14	<0.05
LABC	1.22a	4.32bc	6.23d	3.42b	5.53cd	4.42bc	0.24	<0.05
TYMC	0.9a	1.95ab	3.24c	2.13bc	4.12cd	4.39d	0.19	<0.05
m. *semitendinosus* – ST								
TVC	2.93a	3.95b	5.67c	3.82ab	5.49c	6.28c	0.18	<0.05
TVPC	1.89a	3.78b	6.37c	3.91b	4.96b	6.45c	0.24	<0.05
EBC	0.63a	2.37b	5.01c	1.72ab	3.54bc	3.51bc	0.23	<0.05
PC	2.36a	4.63b	6.59c	4.48b	6.25c	6.12c	0.21	<0.05
TSC	2.64a	2.09a	1.69a	2.35a	2.30a	4.47b	0.16	<0.05
CC	2.88bc	2.99bc	3.19bc	2.70b	1.62a	3.80c	0.13	<0.05
LABC	1.31a	3.70bc	5.58d	3.31b	4.34bc	4.56cd	0.20	<0.05
TYMC	1.11a	2.04ab	3.30bc	1.78a	2.28ab	3.85c	0.17	<0.05

a, b, c, d – Means with different letters differ statistically significantly at $P < 0.05$.
TVC – total viable count, TVPC – total viable psychrotrophic count, EBC - *Enterobacteriaceae* count, PC - *Pseudomonas* spp. count, TSC - total staphylococcal count, CC – *Campylobacter* spp. count, LABC - lactic acid bacteria count, TYMC - total yeast and mould count.

development of bacteria of the genus *Pseudomonas*. In the present study, at 15 d of storage the lowest number of bacteria from this group was once again shown in the meat samples in the combined system, and in the case of LL the differences in comparison with MAP and VP were confirmed ($P < 0.05$).

In both VP and MAP conditions, the total staphylococcal count (TSC) showed a decreasing tendency during refrigerated storage of the muscles ($P < 0.05$ in LL; Table 1), reaching a significantly lower counts at the end of storage for LL packaged in VP (0 d vs. 15 d; $P < 0.05$). Yu et al. (2020) assessed the microbiological quality of beef packaged in VSP and showed a significant decrease in *Staphylococcus aureus* from 4.30 log CFU/g at 0 d to 3.44 log CFU/g after 7 d storage and a further, but non-significant decrease up to 21 d of storage. Among the packaging systems evaluated in the study, the highest TSC was shown in the samples stored in the combined system ($P < 0.05$), on average about 2 log CFU/g higher than in VP and MAP (Table 3). Staphylococci make up a significant portion of the microbiome of human skin (Skowron et al., 2021), and therefore inadequate hygiene during technological processes may result in secondary contamination of raw meat. The high TSC value in the VP + MAP system may be due to secondary contamination, which may have taken place during the additional operations associated with repackaging of the meat.

Throughout the storage period the *Campylobacter* spp. counts in LL samples in both system packaging remained at a comparable level, while in ST stored in MAP showed a decreasing tendency, being significantly lower at 15 d compared to the control samples (0 d) and those packed in the VP system. *Campylobacter* spp. *bacteria* are microaerobic and grow optimally at about 5% O_2. Storage under oxygen has proven effective in reducing *CC (*Meredith et al., 2014). Beterams et al. (2023) showed a marked decrease in CC on poultry meat during 13-day storage in MAP. In the present study, the highest CC was shown at 15 d in the combined system, and the differences in comparison with MAP were confirmed for both LL and ST muscles ($P < 0.05$).

The number of yeasts and moulds in the LL and ST samples significantly increased during storage in both packaging systems, in LL reaching values higher in MAP than in VP ($P < 0.05$; Tables 1 and 3). The highest TYMC was obtained for samples stored in the combined system, which in the case of LL was higher than in VP ($P < 0.05$), and in the ST in comparison to MAP ($P < 0.05$). Literature data on the effect of the meat packaging method and storage time on the growth of yeast and moulds are not conclusive, but some studies indicate a lower mould count in vacuum-packed samples. Lorenzo and Gómez (2012) reported a decreasing tendency for mould and yeast counts during 14-day storage of foal meat, which was most pronounced for the samples packaged in MAP with a high concentration of carbon dioxide (MAP 30% O_2 + 70% CO_2). Jaberi et al. (2019), on the other hand, reported that vacuum packaging showed an inhibitory effect on the mould and yeast count during chilled storage in comparison to MAP with a high oxygen concentration (MAP 80% O_2 + 20% CO_2). It is worth emphasizing that in the veal meat assessed in the present study, pathogenic bacteria such as *Listeria monocytogenes* were not found in any of the samples tested.

4. Conclusions

The packaging system significantly influenced the physicochemical and microbiological parameters of veal during 15-day storage. Both the *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles packaged in a vacuum (VP) had significantly lower lipid oxidation levels, lower expressible water content, better α-tocopherol retention, higher instrumentally measured tenderness, and a lower proportion of metmyoglobin than the meat stored in a modified atmosphere (MAP), especially at the end of the storage period. The samples packaged in MAP, on the other hand, had lower purge losses, a higher proportion of oxymyoglobin with a higher share of red colour at 5 and 10 days of storage and better microbiological quality throughout the storage period (lower numbers of mesophilic and psychrophilic bacteria, *Enterobacteriaceae*, *Campylobacter* and *Lactobacillus*). Muscle type in interaction with packaging system and storage time significantly affected parameters, such as shear force, cooking loss (specific differences were mainly observed in the initial period), lightness and redness (specific differences mostly occurred in the final period). However, no effect of this interaction was observed on the microbial counts.

The combined system proposed in the study (VP 8 d + MAP 7 d) is an alternative worth considering for packaging of veal, as it reduces the occurrence of unfavourable qualitative changes typical of both systems used separately. The meat stored in the combined system had the highest tenderness (lowest SF), the lowest counts of *Pseudomonas* and *Enterobacteriaceae*, and favourable colour parameters (a low %MMb and high %OMb at 15 d of storage). In addition, the samples stored in this system had intermediate (between VP and MAP) values for lipid oxidation

(TBARS) and α-tocopherol content. A critical point in the combined packaging system seems to be the repackaging, which can lead to secondary microbiological contamination and in combination with the change from anaerobic to aerobic conditions may foster intensive growth of some groups of microbes, such as yeasts and moulds or *Staphylococcus* and *Campylobacter* bacteria, whose numbers in this system were higher than in MAP and VAC.

In general, all the packaging systems ensured the preservation of good quality characteristics of veal up to the last day of storage. However, in the case of the MAP system at 15 days of storage unfavourable signs associated with changes in the lipid fraction (high TBARS value and a significant decrease in α-tocopherol content) and with colour (a high level of metmyoglobin and a decrease in oxymyoglobin and in redness) were observed. To confirm the usefulness of the combined system and to give stronger confirmation of the results obtained for VP and MAP additional studies are necessary, involving a higher number of replicates and broadened representation of the calf population. Additionally, nutritional value assessment (including vitamins, antioxidants, fatty acid, amino acid profiles) are vital to provide more complex insight into the advantages and disadvantages of the tested packaging systems.

Ethical approval

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Declaration of competing interest

There is no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2024.109590.

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