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Influence of Oxygen and Carbon Dioxide Content in Modified Atmosphere Packaging on the Colour and Water-Holding Capacity of Pork Loin

Weronika Zduńczyk, Monika Modzelewska-Kapituła and Katarzyna Tkacz *

Department of Food Microbiology, Meat Technology and Chemistry, Faculty of Food Science, University of Warmia and Mazury in Olsztyn, Plac Cieszyński 1, 10-719 Olsztyn, Poland; weronika.zdunczyk@uwm.edu.pl (W.Z.); monika.modzelewska@uwm.edu.pl (M.M.-K.)
* Correspondence: ktkacz@uwm.edu.pl

Abstract: During storage, raw meat is exposed to many external factors, which cause visible changes on the surface of the meat and which affect its water-holding capacity. This study aimed to determine the effect of oxygen content in modified atmosphere packaging (MAP) used for storing fresh pork on the colour, pH, value and water-holding capacity during refrigerated storage. The study also analysed the dynamics of changes in colour using the colour difference (ΔE) coefficient and sensory quality. In the study, slices of pork loin were packed in MAP using the following gas compositions: 55% O_2/40% CO_2/5% N_2 and 75% O_2/20%, CO_2/5% N_2; they were then stored for 15 days at a temperature of 4 °C. The colour of pork stored in MAP was significantly affected by time, but not by the proportion of oxygen. During storage, the meat's lightness (L^*), yellowness (b^*), chroma, and hue angle increased, whereas its redness index (a^*/b^*) decreased. Significant differences in colour between freshly packed and stored samples were noted after days 7 and 9 in MAP containing 55% and 75% oxygen, respectively. The values of pH, free water, and purge and cooking loss were not affected by gas concentration but changed over time. Lowering the oxygen content from 75% to 55% in MAP opens the possibility of reducing the oxygen demand from the meat industry without compromising the quality of the meat.

Keywords: colour; meat; modified atmosphere; quality; storage

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1. Introduction

The global meat industry is based on red meat consumption, predominantly pork. According to OECD data, in highly developed countries, pork consumption in 2020–2023 was 22.8 kg/capita per year [1]. The production and distribution of pork meat is vitally important for the economy and agricultural sector of many countries, generating labour places and profits for producers and processors [2,3]. Pork is a rich source of high-quality protein. It also delivers many nutrients, such as B group vitamins (i.e., thiamine, niacin, B_{12} vitamin), iron, zinc, and selenium. Moreover, pork in used in many national and regional cuisines worldwide because of its taste and texture [4,5]. Many researchers indicate that visual attributes of meat, including colour, are of key importance for the consumer's perception of the quality of meat and have a direct impact on the decision to purchase it, while sensory attributes (tenderness, juiciness, and tastiness) determine the decision to purchase meat again [6–8]. The colour of raw meat and its changes occurring during storage are easily noticeable by consumers, which makes the colour a key indicator of the meat's quality and freshness [5,9,10]. Once the quality attributes of fresh meat do not meet consumers' expectations, the meat is withdrawn from retail, which leads to food waste through the loss of valuable and still safe sources of nutrients [5–8].

Pork colour is predominantly influenced by muscle biology, metabolism, and myoglobin chemistry, while important roles are played by metabolic, contractile, and stress

response pathways [10]. Meat quality is further affected by muscle fibre characteristics, notably fibre type and density. Specifically, meat lightness, a critical quality parameter, is associated with type I and IIB fibre proportion, which also inversely affects myoglobin content and perceived colour [11]. A significant role in shaping the colour of meat is played by myoglobin (Mb), which exists in three natural forms, depending on the degree of exposure to oxygen and the chemical state of iron. Under anaerobic conditions, myoglobin exists as deoxymyoglobin (DeoMb), giving the meat a characteristic purple–red colour. When meat is stored in the presence of oxygen, oxygen is bound to the myoglobin, forming oxymyoglobin (OxyMb), which makes the meat colour intense and bright red. During the storage process, myoglobin may be transformed into metmyoglobin (MetMb), which results in a change in the colour of the meat to an unattractive grey–brown or grey–beige. The proportion of different myoglobin forms determines the final meat colour, and it is a vital factor affecting the shelf life and aesthetics of meat products [3,5,12].

Modified atmosphere packaging (MAP) systems combined with a refrigeration process are used in the food industry to maintain the quality and extend the shelf life of meat and meat products. MAP uses combinations of various gasses, including oxygen, carbon dioxide, and nitrogen. The widespread use of MAP helps maintain product colour, especially through the use of high oxygen concentrations [13,14]. Oxygen in MAP is used not only to improve the stability of the ideal red colour of meat but also to inhibit the growth of anaerobic microorganisms. Atmospheres containing the appropriate concentration of CO_2 effectively extend the shelf life of meat products by limiting the development of aerobic microorganisms. However, using high CO_2 concentrations (>50%) can cause problems, such as flavour degradation and packaging collapse. Therefore, nitrogen, which is an inert gas, is also used in MAP. It is used as a balancing gas, preventing excessive oxidation, deterioration of taste, and collapse of packaging [4,13,15,16].

Packaging in a modified atmosphere with a high oxygen content (up to 80%) has become one of the most popular commercial methods of packaging chilled fresh meat to ensure a stable, attractive, "bloomed" colour of red meat. Despite the attractive presentation of meat in MAP, especially in the first week of exposure, this packaging method has certain limitations. High O_2 concentration promotes the development of undesirable quality defects, such as the growth of microbiota, increased storage leakage, lipid oxidation, discolouration, loss of nutrients, and changes in meat texture [3,5,13–15,17,18].

Therefore, various studies are conducted to mitigate adverse changes. Globally, researchers have explored variable proportions of gasses in packaging, looking for a universal mixture that can maintain the broadly understood quality of fresh meat at the highest level, but these researchers have encountered various obstacles [7,19–22]. The main problem indicated in studies was the accelerated process of protein oxidation, which is associated with increased weight loss during storage. Moreover, the gas mixtures used did not show any additional antimicrobial effect, and the results indicated that optimization of the oxygen concentration in the modified atmosphere (MA) is necessary when packaging various retail types of meat to significantly improve their oxidative stability [23–25]. The possibility of using MAP (30% O_2/70% N_2 and 10% O_2/90% N_2) combined with lowering the storage temperature to −3 °C was also investigated. However, the limitation of the method proposed was the need to maintain a lower cooling temperature than usually used in home refrigerators [26]. Also, the use of antimicrobial chitosan coating in combination with MAP (20% CO_2 and 80% O_2) did not provide the expected results due to the increased effect of protein peroxidation [27]. Kernberger-Fischer et al. [28] tested the possibility of using MAP with foil containing silver ions due to their commonly known antibacterial properties. However, their research results eliminated the use of a nanosilver coating to extend the shelf life of meat, due to the small impact of the addition of silver on myoglobin forms in the first days of storage and an ineffective attempt to limit the growth of dangerous microflora. Our previous research was an attempt to solve the problem of unfavourable changes in pork meat colour signalled by the meat industry by surface application of oregano essential oil [3]. The study showed that the surface application of the essential oil

allows for obtaining sensory-attractive products, but does not limit changes in the colour of meat during storage.

The colour of meat might be also associated with the husbandry system. Recent proteomic studies suggest that the type of chicken husbandry system (organic versus antibiotic-free) affected the proteome of chicken breast muscle after slaughter, which consequently modified meat quality [29]. Furthermore, an increased abundance of proteins associated with animal growth processes, such as α-actin isoforms (ACTA 1) and myosin isoforms (MYH 2), was observed in meat from the antibiotic-free group [29]. Wimmers et al. [30] indicated that the presence of MYH2 contributed to an increase in muscle mass in pigs. The presence of this fibre types affects many aspects of meat quality, including colour, WHC, tenderness, juiciness, and flavour [31]. The fact that the elimination of antibiotics from animal feeding might affect the quality of meat so deeply, and the lack of studies conducted on antibiotic-free pork loin in terms of colour changes during MAP storage, were drivers for designing our study. Moreover, despite some previous studies on the influence of MAP on meat quality, our experiment was designed to investigate the possibility of solving a particular industrial problem associated with unfavourable colour changes in antibiotic-free pork during storage. Therefore the study is innovative due to its individualised nature, but it also has a practical dimension. Through careful control of the experimental conditions, we have tried to develop practical solutions that can be applied by the meat industry to improve the product's colour. Bearing in mind that the meat industry is interested in improving strategies for meat storage in MAP, which consequently will improve consumer satisfaction and the competitiveness of the meat industry, this work aimed to investigate the influence of oxygen content in MAP on pork loin colour, pH value, and water-holding capacity during refrigerated storage.

2. Materials and Methods

2.1. Material and Sample Preparation

The study was carried out in the laboratory of the Goodvalley Polska meat plant in Przechlewo (Poland), which provided the research material. The research consisted of testing the influence of two gas compositions during storage (eight sampling times, including on day 0). The research material consisted of *longissimus thoracis et lumborum* (LTL) muscles, obtained from DanBred porkers (a crossbred of ♂Duroc × ♀(♂Landrace × ♀Yorkshire), which were raised without the use of antibiotics during fattening (RWA) [3]. A total of 12 LTL muscles were taken to the laboratory 24 h after slaughter. In each muscle, the pH was measured and, based on the results, 6 LTL muscles with pH values of 5.55 ± 0.05 were selected for further tests. Then, 17 samples weighing approximately 100 g were cut from each muscle (n = 102 samples in total obtained from 6 muscles). A total of 3 samples were selected from each muscle and tested at day 0: pH, colour, and proximate composition were measured (6 muscles × 3 repetitions resulted in n = 18 samples). The remaining samples (n = 84 samples) were mixed and randomly assigned to 2 experimental groups (n = 42 samples for each of the tested gas compositions in the package). Before packaging, the samples were divided into 7 groups (storage time), and each group contained 6 repetitions (3 trays of 2 slices). Samples packed in polypropylene trays with an absorbing pad were closed in a modified atmosphere using the following gas compositions: lower oxygen (LO) (55% O_2/40% CO_2/5% N_2) and higher oxygen (HO) (75% O_2/20% CO_2/5% N_2) using an automatic packaging machine Traysealer A6 SEALPAC (Sealpac, Oldenburg, Germany). The packed samples were stored in a refrigerator (Asber Ecp-G-1402 Glass, Asber, Palmiry, Poland) at 4 °C for 15 days. MA composition was monitored after packaging and during storage by Oxibaby 6.0 (Witt-Gasetechnik GmbH & Co., KG, Witten, Germany).

2.2. pH Measurements

Values of pH were determined on the 2nd, 5th, 7th, 9th, 12th, 14th, and 15th day of storage using a Testo 205 pH meter (electrode pH/NCT-sensor) (Testo, Titisee-Neustadt, Germany) with automatic temperature compensation. Before the measurements were

taken, the pH meter was calibrated using buffers 4.01 and 7.01. Measurements of the pH were conducted by inserting the pH meter's sensor directly into the centre of the meat sample [32]. The analyses were performed in triplicate for each sample and averaged.

2.3. Colour Measurements and Calculations

Colour measurements were conducted at the beginning of the experiment (day 0, before packaging of samples) and during storage (on the 2nd, 5th, 7th, 9th, 12th, 14th, and 15th day). On day 0, the colour of the samples was measured on the cross-sectional area of the pork loin after 20 min of blooming. The colour was measured using a Konica Chromameter CR-400 (Sensing Inc., Osaka, Japan), with a D65 illuminant, 10° observer, and aperture of 2.54 cm. Before the measurements, the device was calibrated using a white tile. The values of lightness (L^*), redness (a^*), and yellowness (b^*) were measured in three different locations on the surface of each pork slice. Hue (H°) and chroma (C^*) were calculated using Equations (1) and (2), as follows [33]:

$$H^\circ = \tan^{-1}\frac{b*}{a*} \quad (1)$$

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

To analyse differences in colour between LO and HO pork loin samples at each sampling time, the ΔE LO/HO coefficient was calculated. Also, the ΔE_0 LO and ΔE_0 HO coefficients were calculated to show the colour difference between samples before packaging (day 0) and during storage. All the calculations were performed according to the following Equation (3).

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (3)$$

where ΔE is colour change; ΔL^*, Δa^*, and Δb^* are changes in L^*, a^*, and b^*, respectively [34].

Colour changes were evaluated based on ΔE values and designated as trace if ΔE were below 0.5, slight if ΔE was in the range 0.5–1.5, noticeable if ΔE was in the range 1.5–3.0, appreciable if ΔE was in the range 3.0–6.0, much if ΔE was in the range 6.0–12.0, and very much if ΔE was above 12.0 [35].

The redness index (RI) was calculated according to Hunt and King [33], using the following Equation (4):

$$RI = \frac{a*}{b*} \quad (4)$$

2.4. Sensory Assessment of the Meat Colour

Sensory examination of pork loin packed in MAP was carried out by a five-person trained evaluation team. The evaluation was carried out on the 2nd, 5th, 7th, 9th, 12th, 14th, and 15th day of storage. During the evaluation procedure, the meat was visually assessed immediately after opening the package. Samples of stored meat were compared with the control sample, which consisted of meat samples taken directly from the packaging line (6 samples of freshly cut pork loin, randomly taken). The assessment was carried out using a descriptive scoring system, which allowed for quantifying the degree of change in meat colour (visible to consumers) over time in storage. The scoring system was defined as follows: 1 point—no differences in colour between fresh and stored samples or more vivid colour of stored samples; 2 points—less vivid colour of stored samples compared with fresh samples; 3 points—pale, slightly grey colour of stored samples; 4 points—light, grey colour of stored samples.

2.5. Nutritional Value

An automatic analyser, namely a FoodScan Lab Meat Analyser (Foss, Hilleroed, Denmark), was used to determine the content of moisture, fat, and protein in pork loins. Pork

loin samples (n = 6, from day 0) were minced through a 6 mm mesh, homogenized for 1 min. (K35, Electrolux Professionnel, Aubusson, France) and placed on a glass dish in a way that provided an even thickness of the meat and the absence of air holes.

2.6. Water-Holding Capacity

Water-holding capacity was determined based on measurements of free water content, purge loss, and cooking loss. Free water was determined according to the Grau–Hamm method [36] with modifications [37]. From each pork loin sample, 50 g was cut after colour and pH determination and minced twice through 3 mm mesh. Samples of 0.3 g were prepared and placed on a glass tile on Whatman 1 filter paper (Whatman Laboratory Division, Maidstone, UK), covered with a glass tile. A 2 kg weight was placed on top and kept there for 5 min to remove free water from the sample. Then, the filter paper with a pressed meat sample and water stain was photographed using a digital camera (Nikon D90, Nikon Corporation, Tokyo, Japan). The analysis was performed in triplicate for each meat sample. The images were analysed using NIS-Elements BR 2.20 image analysis software (Nikon Corporation, Tokyo, Japan), and free water content was calculated using the following Equation (5):

$$\text{Free water} = \frac{\text{FPabs} \cdot (\text{l} - \text{m})}{\text{c}} \, [\%] \quad (5)$$

where FPabs is the absorptiveness of the filter paper (cm^3); l is the area of liquid stain (cm^3); m is the area of the meat sample (cm^2); c is the meat sample weight (g).

To determine purge loss, starting from the second day of storage just after opening the packages, pork samples were weighed. Based on the weight of samples before and after storage, purge loss was calculated according to Equation (6) and expressed in % w/w.

To determine cooking loss, meat samples at each sampling time were ground through a 3 mm mesh and portions of 30 g (n = 2 from each muscle) were wrapped in gauze and cooked in a water bath at a temperature of 80 °C for 40 min. Before and after the thermal treatment, they were weighed, and the cooking loss was calculated according to the following Equation (6):

$$\text{Purge/cooking loss} = \frac{\text{m}_1 - \text{m}_2}{\text{m}_1} \cdot 100 \, [\%] \quad (6)$$

where m_1 is the weight of the sample before storage/cooking (g); m_2 is the weight of the sample after storage/cooking (g).

2.7. Statistical Analysis

The effects of MAP and storage time were evaluated using the variance components model. In the model, the fixed factors of atmosphere composition (2 levels) and storage time (8 levels) were included, as well as a random factor, namely repetition of the experiment (3 levels). The random factor did not affect pH, colour, and water-holding attributes. To compare mean values during storage, the following procedure was applied: (1) evaluation of the normal distribution of the data and variance homogeneity using the Shapiro–Wilk test and Levene's test, respectively. Depending on the results, there was an analysis of variance, and Tukey's reasonable significant difference (RIR) test was applied if the data showed a normal distribution and variance homogeneity; if not, the means were compared using a non-parametric Kruskal–Wallis test. The following attributes were compared using variance analysis: pH, a^*, C^*, free water, and purge and cooking losses, whereas L^*, b^*, H°, and RI were compared using the non-parametric test. All the calculations were conducted in the Statistica 13.3 software (Tibco Software Inc., Palo Alto, CA, USA).

3. Results

3.1. Proximate Composition of Pork Loin and the Composition of a Modified Atmosphere during Storage

The pork meat used in the research had the following chemical composition: water content 73.3% ± 0.27, protein 23.3% ± 0.06, and fat 0.85% ± 0.107. Table 1 shows the measurements of gas composition in the packages during storage. During storage, no differences ($p > 0.05$) in a modified atmosphere in packaging containing pork loin were noted, regardless of the composition used.

Table 1. Modified atmosphere composition in packages containing pork loins during cold storage (4 °C) (mean values ± SEM).

Attribute	Time (T)							*p*-Value
	2	5	7	9	12	14	15	T
LO								
O_2 (%)	53.6 ± 0.7	54.7 ± 0.9	54.8 ± 0.2	53.9 ± 0.5	54.8 ± 0.3	54.3 ± 0.2	54.9 ± 0.4	NS
CO_2 (%)	39.9 ± 0.1	38.9 ± 0.5	39.5 ± 0.8	39.3 ± 0.3	40.3 ± 0.5	38.5 ± 0.2	39.6 ± 0.4	NS
N_2 (%)	6.2 ± 0.9	6.5 ± 0.5	5.6 ± 0.6	6.7 ± 0.4	5.0 ± 0.5	7.2 ± 0.2	5.5 ± 0.7	NS
HO								
O_2 (%)	75.2 ± 0.3	76.4 ± 0.6	76.5 ± 0.4	76.7 ± 0.4	75.9 ± 0.3	76.1 ± 0.4	76.0 ± 0.3	NS
CO_2 (%)	19.8 ± 0.4	19.5 ± 0.3	19.0 ± 0.2	19.0 ± 0.3	19.2 ± 0.3	19.0 ± 0.1	19.1 ± 0.1	NS
N_2 (%)	5.0 ± 0.4	4.6 ± 0.9	4.5 ± 0.5	4.3 ± 0.3	5.6 ± 0.6	4.9 ± 0.5	4.9 ± 0.3	NS

LO—lower oxygen atmosphere (55% O_2/40% CO_2/5% N_2); HO—higher oxygen atmosphere (75% O_2/20% CO_2/5% N_2); T—storage time; NS—nonsignificant differences ($p \geq 0.05$); SEM—standard error of the mean.

3.2. Colour Changes

Time significantly affected the colour of meat stored in MAP, while the proportion of oxygen in the atmosphere did not affect the colour parameters (Table 2). The colour parameter *L** (lightness) increased during storage. The first significant change was visible on the second day of storage and persisted until the seventh day. The *L** value continuously increased during storage, which led to significant differences between days 7 and 15 of storage. Significant changes in the *a** parameter (increases in value) were found between days 0, 5, 7, 1, 14, and 15. However, the proportion of red colour remained at a similar level from the second day of storage until the end of the experiment.

Table 2. Changes in pork loin colour during cold storage (4 °C) under different modified atmospheres (mean values ± SEM).

Attributes	Modified Atmosphere (A)		Time (T)								*p*-Value		
	LO	HO	0	2	5	7	9	12	14	15	A	T	AxT
*L**	51.8 ± 0.3	51.7 ± 0.3	47.7^{d} ± 0.3	51.7^{c} ± 0.3	51.3^{c} ± 0.3	53.8^{bc} ± 0.4	54.7^{ab} ± 0.3	55.7^{ab} ± 0.3	56.2^{ab} ± 0.4	57.0^{a} ± 0.3	NS	<0.001	NS
*a**	4.12 ± 0.06	4.37 ± 0.06	3.87^{c} ± 0.06	4.2^{abc} ± 0.2	4.5^{ab} ± 0.2	4.6^{ab} ± 0.2	4.1^{bc} ± 0.2	4.5^{ab} ± 0.2	4.8^{a} ± 0.2	4.6^{ab} ± 0.2	NS	<0.05	NS
*b**	5.5 ± 0.2	5.6 ±0.2	3.24^{c} ± 0.06	5.7^{b} ± 0.2	6.0^{b} ± 0.2	6.8^{ab} ± 0.2	6.8^{ab} ± 0.2	7.7^{a} ± 0.2	7.9^{a} ± 0.2	8.1^{a} ± 0.2	NS	<0.001	NS
*C**	7.0 ± 0.2	7.2 ± 0.2	5.07^{f} ± 0.07	7.1^{e} ± 0.2	7.5^{de} ± 0.2	8.2^{cd} ± 0.2	7.9^{bc} ± 0.2	9.0^{ab} ± 0.2	9.3^{a} ± 0.2	9.4^{a} ± 0.2	NS	<0.001	NS
H°	51.3 ± 0.8	49.8 ± 0.8	39.9^{c} ± 0.5	53.7^{b} ± 0.6	53.6^{b} ± 0.7	55.9^{ab} ± 0.8	59.2^{a} ± 0.8	59.7^{a} ± 0.9	58.9^{ab} ± 0.8	60.8^{a} ± 0.5	NS	<0.001	NS
RI	0.85 ± 0.03	0.90 ± 0.03	1.23^{a} ± 0.03	0.74^{b} ± 0.02	0.74^{b} ± 0.02	0.68^{bc} ± 0.02	0.60^{c} ± 0.02	0.59^{c} ± 0.03	0.61^{bc} ± 0.02	0.56^{c} ± 0.02	NS	<0.001	NS

$^{a–f}$—mean values in rows with different superscripts differ significantly at $p < 0.05$; A—influence of the composition of the modified packaging atmosphere on the tested characteristics; T—influence of the storage time on the tested characteristics; AxT—influence of the composition of the modified packaging atmosphere and time on the tested characteristics; NS—nonsignificant differences ($p \geq 0.05$); LO—lower oxygen atmosphere (55% O_2/40% CO_2/5% N_2); HO—higher oxygen atmosphere (75% O_2/20% CO_2/5% N_2); SEM—standard error of the mean.

On day 2 of storage, the proportion of yellow colour (b^*) increased significantly compared to day 0, while from days 2 to 9, no significant differences were noted. On day 12, the value of the b^* parameter was significantly higher than that recorded from days 0 to 5, and did not differ significantly from the values on days 14 and 15. The consequences of the changes in a^* and b^* are significant differences in the colour change indexes C^* and H°. In general, the C^* value increased during the experiment, which indicates an increase in colour saturation. The H° value also increased, which indicates a change in the colour shade.

The redness index (RI) on day 0 reached the highest value and differed significantly from the remaining days of storage. Then, between the 2nd and 7th days of storage, the redness index remained at the same level. From days 9 to 15, the value of the index decreased significantly compared to from days 0 to 5. Therefore, the 7th day of storage may be considered as the limit after which changes in the colour affect meat appearance significantly.

3.3. The ΔE Colour Difference and Sensory Colour Evaluation

Table 3 shows the differences in the colour of pork meat that can be noticed by observers. The differences resulting from the oxygen content in the packaging ranged from trace to slight, which confirms the lack of influence of the oxygen content (55% vs. 75%) on the colour parameters. The colour of samples stored in modified atmospheres (LO and HO) was compared to fresh samples. In the case of the LO environment (with 55% oxygen), the differences in colour were appreciable from the 2nd to 5th day of storage, and were considered much observable from the 7th day until the end of the experiment. The application of HO (75% oxygen) in the packages maintained an appreciable colour difference until day 7, whereas from day 9 to the end of the experiment, differences described as "much" were visible between the colour of stored and fresh loin.

Table 3. The colour difference between pork loin samples packed in 55% (LO) and 75% (HO) oxygen in the modified atmosphere (ΔE LO/HO) and the relation to the initial pork loin colour (ΔE_0 LO and ΔE_0 HO for 55% oxygen and 75% oxygen, respectively).

Attribute	Storage Time (days)						
	2	**5**	**7**	**9**	**12**	**14**	**15**
ΔE LO/HO	0.81 Slight	0.53 Slight	0.36 Trace	0.57 Slight	1.34 Slight	0.60 Slight	1.42 Slight
ΔE_0 LO	4.21 Appreciable	3.45 Appreciable	6.15 Much	6.29 Much	8.45 Much	8.26 Much	8.59 Much
ΔE_0 HO	3.23 Appreciable	3.07 Appreciable	5.49 Appreciable	7.74 Much	6.95 Much	8.27 Much	9.56 Much

The results of the sensory evaluation of pork loin colour packed in MAP with different oxygen content (LO and HO) showed no significant differences between the analysed samples in the context of the atmospheric composition.

The results from the sensory evaluations are consistent with those from the instrumental colour measurements, which indicated that only storage time affected pork loin colour and that the gas composition in MAP had no effect. In the period up to day 5 of storage, no significant differences in the colour were observed, which was demonstrated by assigned scores of 1. However, on the following days of storage (7th and 9th), a decrease in colour intensity was observed, which resulted in a score of 2 (less vivid colour). On days 12 and 14, a pale, slightly grey colour was noted (3 points), whereas on day 15, a light, a grey colour was noted (scored for 4 points).

3.4. Changes in pH and Water-Holding Capacity

The composition of the atmosphere did not significantly ($p \geq 0.05$) affect pH and water-holding capacity during storage (Table 4), whereas storage time had a significant impact on these attributes. There were significant changes in pH during storage; however, it

should be highlighted that the range of pH changes was small and did not exceed 0.2 units. Changes (an increase) in the free water content were observed between days 0 and 2 of storage, but the values obtained on days 0 and 2 did not differ significantly from those obtained from days 5 to 15. Purge loss increased during meat storage in MAP, from 2.8% on day 2 to 7.4% on day 15 of storage (Table 4). This increase corresponded to an increase in cooking losses. Significantly higher cooking losses were recorded on days 12, 14, and 15 compared to from days 0 to 9.

Table 4. Changes in pH values, free water and losses in pork loin during storage under different modified atmospheres (mean values ± standard error of the mean).

Attributes	Modified Atmosphere (A)		Time (T)								*p*-Value		
	LO	HO	0	2	5	7	9	12	14	15	A	T	AxT
pH	5.48 ± 0.01	5.49 ± 0.01	5.58 ^{a} ± 0.01	5.45 ^{de} ± 0.01	5.44 ^{e} ± 0.01	5.45 ^{de} ± 0.01	5.48 ^{d} ± 0.01	5.49 ^{cd} ± 0.01	5.53 ^{c} ± 0.01	5.63 ^{b} ± 0.01	NS	<0.001	NS
Free water (%)	23.8 ± 0.4	24.4 ± 0.3	22.0 ^{b} ± 0.6	24.7 ^{a} ± 0.5	24.3 ^{ab} ± 0.5	23.7 ^{ab} ± 0.6	24.5 ^{ab} ± 0.7	23.3 ^{ab} ± 0.7	24.2 ^{ab} ± 0.6	23.5 ^{ab} ± 0.3	NS	<0.05	NS
Purge loss (%)	5.6 ± 0.4	5.2 ± 0.4	-	2.8 ^{d} ± 0.2	4.7 ^{c} ± 0.3	5.1 ^{bc} ± 0.2	5.7 ^{abc} ± 0.3	6.5 ^{ab} ± 0.7	6.9 ^{a} ± 0.8	7.4 ^{a} ± 0.6	NS	<0.05	NS
Cooking loss (%)	25.9 ± 0.4	26.2 ± 0.4	22.3 ^{d} ± 0.4	23.7 ^{cd} ± 0.3	24.3 ^{c} ± 0.3	23.2 ^{cd} ± 0.3	24.8 ^{c} ± 0.2	27.9 ^{b} ± 0.5	30.9 ^{a} ± 0.5	29.8 ^{a} ± 0.7	NS	<0.001	NS

$^{a–e}$—mean values in rows with different superscripts differ significantly at $p < 0.05$; A—influence of the composition of the modified packaging atmosphere on the tested characteristics; T—influence of the storage time on the tested characteristics; AxT—influence of the composition of the modified packaging atmosphere and time on the tested characteristics; NS—nonsignificant differences ($p \geq 0.05$).

4. Discussion

4.1. Colour Changes and Differences

Fresh red meat retailed in high-oxygen MAP should retain an acceptable red colour for up to 14 days of exposure, compared to less than 7 days for air-packed equivalents [38]. The presented research results, the aim of which was to investigate the possibility of reducing the oxygen content in the MAP packaging from 75% to 55%, clearly confirm that such a procedure does not have a negative impact on the quality of fresh meat. Considering that the meat industry may periodically struggle with the problem of lower oxygen availability (i.e., disturbances in the food sector caused by the COVID-19 pandemic [39,40]), our observations can help producers to make decisions in crisis situations. Statistical analyses of all colour parameters, i.e., measured (L^*, a^*, b^*) and calculated (C^*, H°, RI), did not reveal any significant statistical differences. Additionally, the analysis of the dynamics of colour changes during storage, taking into account the colour difference coefficient (ΔE), showed only slight differences between the colours of samples packed in different atmosphere compositions, which would not be noticeable by inexperienced observers. The lack of influence of oxygen concentration in packaging (LO and HO) is also consistent with the observations of Li et al. [18]. Reducing the oxygen content of the experimental MAP from 75% to 55% closely follows the observations of Bao and Ertbjerg [21], who showed that an increase in oxygen concentration from 20% to 80% had no significant effect on the a^* value in the case of pork steaks stored for 6 days. However, pork packed in a MAP, regardless of different oxygen concentrations, had higher a^* values compared to fresh meat. This is most likely because the presence of high levels of oxygen (50% or more) in the MAP saturates the surface of the meat with OxyMb, thereby improving colour fastness [38].

In the context of different gas compositions in MAP, Lukic et al. [41] indicated that during the first six days of storage, there were no significant differences in the colour of meat packed in the modified atmosphere. However, from day 7, significant differences were observed, with the worst colour rating being obtained for MAP3 (80% O_2, 20% CO_2), followed by MAP1 (75% O_2, 25% CO_2), and with the best ratings recorded for MAP2 (70% O_2, 30% CO_2). In the present study, similar differences in colour were noted from day 7 of storage, which is most likely not strictly due to the oxygen content, but is most likely

due to changes occurring as the meat ages. In addition, the recording of these significant differences may be due to a difference in the activity of enzymes present in the muscle, which are capable of regenerating the pigment and, thus, the pink colour [41]. Also, Viana et al. [23] indicated that the colour changes in meat stored in packages containing more than 25% CO_2 may be due to acidification of the meat surface and denaturation of proteins. They also explained that CO_2 as an agent alone does not affect the oxidation of myoglobin.

The observed significant increase in the a^* parameter, responsible for the red colour, is most likely due to the increased amount of oxygen in the modified packaging atmosphere (LO 55% O_2, HO 75% O_2), resulting in the oxygenation of myoglobin to oxymyoglobin [38]. This phenomenon was explained by Kropf and Mancini [42], who indicated that high oxygen content (above 50%) plays a key role in the interrelation of myoglobin and lipid oxidation processes, thus causing visible changes occurring on the meat surface. Myoglobin content and its redox state are responsible for a^* values, while only the redox state of the pigment affects b^* [43]. Also, Lee et al. [44] indicated that the formation of reactive secondary lipid oxidation products can affect the redox stability of Mb. This complex interplay of biochemical processes is a key element in understanding the colour changes of meat stored in modified atmosphere packaging. Also, Gagaoua et al. [10] drew attention to the increased redness value (a^*) in the case of MA-packaged meat compared to the fresh control sample. The a^* value is particularly important because it is closely related to the redness perceived by the consumer, where a higher a^* value indicates a brighter red colour [18].

A significant increase in the L^* parameter throughout the entire storage period in the present study coincides with the results of a study conducted by Li et al. [18], which revealed that pork packed in MAP is characterized by a lighter appearance than in the case of atmospheric packaging. The researchers attribute the observed increase in the L^* value in fresh pork to the process of protein denaturation [7], and, thus, the loss of the ability to bind water, which results in the migration of a large amount of water from the interior of the muscle fibres to the extracellular space, thus increasing the light reflectance coefficient [18]. The increased lightness of samples packed in a protective atmosphere suggests that changes in meat colour may be due to the effect of the type of packaging on moisture retention. This aspect is also confirmed by the results of other studies in which samples packed in a protective atmosphere were characterized by lower weight losses, which may be related to the reduction in surface drying in packaging with low water vapour permeability [20].

The results of the present study showed significantly higher values of the b^* parameter for samples packed in MAP compared to fresh samples. The observed increase in a^* and b^* values during storage in high oxygen concentrations results from fat oxidation processes and changes in protein structure [25,41]. In atmospheres containing a high oxygen content (80%), fat oxidation processes can occur more intensively, leading to the formation of larger amounts of free radicals, which can be promoters of protein oxidation processes. [21,24,45,46]. According to the research of Wang et al. [25] the increase in fat oxidation products, defined as the TBARS (thiobarbituric acid reactive substances) value, does not always correlate with changes in proteins related to their oxidation. Results obtained in the present study, indicating no effect of gas composition in MAP on the colour, pH, and water-holding capacity in pork loin, might lead to the assumption that reducing the oxygen content in packaging from 75% to 55% does not significantly reduce the oxidation processes of fats and proteins. The conclusions from the above analysis indicate that complex interactions between the composition of the atmosphere and the properties of pork meat have a key impact on its colour. These nuances are important both for producers who try to optimize MAP and for consumers whose meat colour preferences influence their purchasing decisions. Further research in this field is necessary to better understand the comprehensive factors influencing the colour changes in pork in MAP and to adapt packaging technologies to the expectations of the food market.

4.2. pH and Water-Holding Capacity

The pH values recorded at the beginning of the experiment (day 0) were typical for meat of normal quality, without PSE (pale, soft, exudative) and DFD (dark, firm, dry) defects [10]. The storage time, but not the gas concentration in the packaging, had a significant impact on the pH increase. During the first stage of cold storage, i.e., 0–5 days, pH values decreased as a result of glycolysis processes still taking place in the muscles and the accumulation of lactic acid and ATP decomposition products (mainly inorganic phosphate). With the extension of the refrigeration time, from the 5th to the 15th day, there was an increase in the pH value, which is related to the autolysis of proteins related to the action of microorganisms and the formation of trimethylamine and ammonia. This is a typical process for the pH during meat storage, which was reported in many studies [47–50].

The present study showed that different oxygen concentrations do not have a significant effect on the pH of pork muscles during storage, which stays in agreement with the results of Viana et al. [23] However, modified atmosphere composition might affect meat pH, as shown by Nieminen et al. [51], who reported that the meat stored under a high-oxygen MAP (80% O_2/20% CO_2) was less acidified than that stored with the same CO_2 concentration but without oxygen. It was associated with the growth of *Leuconostoc* sp., which produces acetoin and diacetyl as the end products and does not acidify the meat as much as, for example, homofermentative lactic acid bacteria (i.e., *Lactobacillus* sp.), which grow more intensively when the CO_2 concentration is over 80%. Therefore, the lack of differences between MAP used in the present study, containing 75% and 55% O_2, might be explained by a similar microbiota composition in HO and LO samples.

The water-holding capacity of meat (WHC) is another key aspect affecting its quality. WHC is defined as the ability of meat and meat products to bind water [52] during cutting, grinding, and pressing, as well as during transport, storage, processing, and cooking [36] and it is directly related to the pH value of fresh meat [53]. In the present study, the WHC changed during storage, which may result from processes related to changes in the structure of proteins degraded during meat storage [54]. However, there was no effect of increasing the oxygen concentration by 20% in packaging on free water, purge loss, and cooking loss. In contrast, other reports showed the impact of increasing the oxygen content on the WHC of meat samples during storage [25,46,55,56] as a result of increased dynamics of lipid and protein oxidation. However, the increase was noted when the differences in the oxygen concentrations were greater than those used in the present study, e.g., 20% vs. 50% vs. 80% O_2 [25] and 46% vs. 70% O_2 [55]. It might be assumed that the difference in the oxygen concentration used in the present study was too small to produce changes in WHC and pH caused by oxidation.

The oxygen concentration reduction from 75% to 55% in MAP investigated in the present study failed to fight against the colour deterioration of pork loin. Therefore, to obtain successful results, this intervention should be combined with some other treatment, such as active packaging or natural colourants with antioxidant potential. Souza et al. [57] developed PVA-active films containing $NaNO_2$ and used it for pork neck packaging, whereas Claus and Du [58] extended the colour stability of fresh beef by using FreshCase® (pouches made of nitrite-embedded film, NEF, Curwood Inc., Division of Bemis Company Inc., Neenah, WI, USA). Therefore, active packaging might effectively improve the colour stability of meat stored under MAP. Another possibility might be using betanin as a potential natural pigment and antioxidant [59]. However, a synergy between the reduced oxygen content in MAP with 55% oxygen and the addition of betanin as an additional antioxidant should be studied to understand whether the use of betanin can effectively minimise colour changes, especially after 7 days of storage. Undertaking such a study could contribute to the development of new packaging strategies based on the synergy of oxygen reduction and the use of natural antioxidants, which could be of practical importance to the meat industry in the context of maintaining meat quality over a longer storage period.

5. Conclusions

The colour, pH, and WHC of pork stored in MAP were significantly affected by time, but not by the proportion of oxygen. Between the 7th and 9th days of storage, the redness index (RI) decreased noticeably, and there were many (denoted as "much") differences in the colour between fresh and stored samples. Reducing the oxygen content in MAP from 75% to 55% does not limit changes occurring during storage. To maintain the colour of fresh meat throughout its shelf life, additional research should be undertaken to find ways to preserve the colour, e.g., the use of active packaging or shortening the storage period to 7–9 days of storage. Additionally, from the presented research results, it can be concluded that reducing the oxygen content in MAP to 55% will not cause unfavourable changes in meat quality that will influence consumer choices.

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