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Changes in the physicochemical properties of scalded sausages stored under vacuum and modified atmosphere conditions

Änderungen der physikalisch-chemischen Eigenschaften von Brühwürsten, die im Vakuum und unter modifizierter Atmosphäre aufbewahrt werden

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Summary

The storage methods currently used by the meat industry are aimed at extending the shelf-life and maintaining the desired quality characteristics of meat products. Modern food processing technologies include modified atmosphere, vacuum, active and intelligent packaging systems.
The aim of this study was to determine changes in the physicochemical properties of scalded coarsely-ground sausages stored under vacuum and in a modified atmosphere composed of carbon dioxide and nitrogen in different proportions.

The experimental materials comprised scalded coarsely-ground ham sausages. Sausages were stuffed into artificial protein casings which provide optimal steam, meat juice and smoke permeability. The sausages were packaged in a modified atmosphere with the following composition: vacuum (P); 20 % CO_2, 80 % N_2 (A1); 50 % CO_2, 50 % N_2 (A2); 80 % CO_2, 20 % N_2 (A3). The Multivac A 300 packaging machine was used. The samples were stored at around 4∞C for 15 days. The experiment was performed in eight replications. Three samples were collected and analyzed each time. Determinations were made at three-day intervals (day 0, 3, 6, 9, 12, 15). Changes in the pH, drip loss and color of meat products were monitored during packaging. Color parameters were determined by the reflectance method, using a Spectro-color meter. The color attributes L*, a*, b* were determined in the CIE system, and the color stability coefficient DE* and color saturation C* were calculated.

Modified atmosphere packaging (MAP) of processed meat products is a beneficial storage method as it does not cause significant changes in pH values.

Drip loss was higher in vacuum-packaged products than in products stored under gas atmospheres. Greater drip loss resulted from high negative pressure in the package.

Insignificant changes in the color lightness (L*), redness (a*) and yellowness (b*) of meat products were noted over storage in all types of modified atmosphere.

Keywords: scalded sausages, storage, modified atmosphere, pH, drip loss, color parameters

Zusammenfassung

Gegenwärtig wird in der Fleischindustrie angestrebt, solche Methoden zur Aufbewahrung von Fleischprodukten anzuwenden, die zur Verlängerung der Haltbarkeit mit gleichzeitiger Erhaltung gewünschter Qualitätsmerkmale führen. In diese Richtung entwickeln sich verschiedene Verfahren zur Aufbewahrung von Fleischprodukten, u. a. Aufbewahrung im Vakuum, in Gasatmosphäre, in aktiven und intelligenten Verpackungen.

Ziel der durchgeführten Untersuchungen war es, Änderungen der physikalisch-chemischen Eigenschaften von Brühwürsten, die im Vakuum und unter modifizierter Atmosphäre aufbewahrt werden, zu bestimmen.

Das Untersuchungsmaterial waren grob zerkleinerte Brühwürste (Schinkenwurst). Zum Füllen der Würste wurden wasserdampf-, fleischsaft-, rauch- und luftdurchlässige Eiweiß-Kunststoffdärme verwendet. Die Würste wurden unter modifizierter Atmosphäre mit folgender Zusammensetzung verpackt: Vakuum (P); 20 % CO_2, 80 % N_2 (A1); 50 % CO_2, 50 % N_2 (A2); 80 % CO_2, 20 % N_2 (A3). Zur Verpackung wurde die Verpackungsmaschine Multivac A300 eingesetzt. Die verpackten Produkte wurden bei einer Temperatur von ca. 4 °C±1 °C 15 Tage lang aufbewahrt. Es wurden 8 Untersuchungsserien durchgeführt. Es wurden jeweils 3 Proben entnommen und analysiert. Die Bestimmungen wurden in 3-tägigen Abständen (0., 3., 6., 9., 12., 15. Tag) vorgenommen. Während der Aufbewahrung wurden Änderungen der pH-Werte, der Tropfsaftverlust sowie die Farbe der Fleischprodukte untersucht. Die Bestimmungen der Farbparameter wurden im Reflexionsverfahren mit dem Farbmessgerät Spectro Color vorgenommen. Es wurden die Farbparameter L*, a*, b* im CIE-System bestimmt sowie der Farbechtheitsfaktor DE* und der Farbsättigungsfaktor C* ermittelt.

Die Verpackung der Fleischprodukte unter modifizierter Atmosphäre verursachte keine wesentlichen pH-Wert-Änderungen.

Bei den in Vakuumverpackungen verpackten Produkten lag der Tropfsaftverlust höher als in den mit der Gasatmosphäre gefüllten Verpackungen. Der Tropfsaftverlust resultierte aus dem hohen Unterdruck auf das Produkt.

Die Aufbewahrung der Fleischprodukte unter modifizierter Atmosphäre verursachte hingegen unerhebliche Änderungen der Farbparameter der Helligkeit, der roten Farbe und der gelben Farbe während der gesamten Aufbewahrungszeit unter allen Atmosphärenarten.

Schlüsselwörter: Fleischprodukte, Aufbewahrung, modifizierte Atmosphäre, pH-Wert, Tropfsaftverlust, Farbparameter

Introduction

Meat products undergo a variety of biochemical, microbiological, chemical and physical changes affected by temperature, oxygen partial pressure, pH, relative air humidity and light. Water activity (a_w) and pH values can be used to predict the shelf-life of meat products in a given storage temperature range (Pikul, 2002). The quality, nutritional value and storage life of most food products is largely determined by water content. The water contained in meat products can be classified as free or bound. Free water contains dissolved organic and mineral compounds, it fills up the empty spaces and is not held by capillary forcers. Bound water can be subdivided into four categories: hygroscopic, capillary, crystallized and constitutional (Krełowska-Kułas, 1993).

Color is one of the most important quality attributes of meat and meat products. It is a visual sensation that depends on the presence of pigments, the tissue composition and texture of meat (Brewer and Novakofski, 1999; Brewer et al., 2002; Conforth, 1994; De Santos et al., 2007; Kłossowska and Tyszkiewicz, 2000; Lindahl et al., 2001; Nagapo et al., 2004; Summo et al., 2010). In the production process, meat color stability is most affected by curing. During the curing of meat, nitrogen oxide binds to myoglobin to form the red pigment nitrosyl myoglobin, which upon heating is converted into a bright pink pigment, nitrosyl hemochrome (Mroczek and Słowinski, 1997).

Color stability is determined by the composition of meat products. The red color of meat is stabilized with the use of various functional food additives that maintain redox balance (Dransfield, 2008; Jankiewicz, 2004; Keeton, 1994; Summo et al., 2010). Oxygen penetration into the meat tissue compromises color stability. Under the influence of light and air, the color of meat becomes grayish. The desirable color of meat can be stabilized by modified atmosphere packaging (MAP). High-oxygen (ca. 80 %) MAP delays adverse color changes by increasing the faction of oxygen-resistant oxymyoglobin in the pigment at the tissue surface. Moreover, the increased oxymyoglobin concentration intensifies the desirable red color of meat (Behrends et al., 2003; Buys, 2004; Czerniawski, 1999; Jankiewicz, 2004; John et al., 2005; Kłossowska and Olkiewicz, 2000; Loewenadler, 1994). High oxygen concentrations may contribute to lipid oxidation in meat and meat products, leading to changes in meat color, an increase in aerobic bacterial counts, deterioration in palatability and texture softening, in particular in finely-ground meat products (De Santos et al., 2007, Grobbel et al., 2008; Kim et al., 2010; Lund et al., 2007; Zarys-Wliwander 2010).

Although MAP has a steadily growing number of applications, there is a scarcity of information regarding changes occurring in meat products stored in modified atmosphere. MAP has evolved from the vacuum packaging technique which has been in use for many years. In this process, the internal atmosphere surrounding a product is modified with a selection of gases. This solution enhances the quality of the end product (Andres et al., 2002; Czerniawski and Sarzynski, 1996; Jayas and Jeyamkondan, 2002; Ruiz et al., 2002; Summo et al., 2010). Different gas compositions are used for MAP. Meat products are usually pakkaged under MAP composed of oxygen, carbon dioxide and nitrogen (Kerry et al., 2006; O'Sullivan et al., 2011).

Carbon dioxide (CO_2) has bacteriostatic properties, it inhibits the growth of Gram-negative bacteria, yeasts, molds and fungi. It is most effective when applied at a concentration higher than 20 %. CO_2 used in MAP dissolves and forms carbonic acid, thus lowering the pH of meat products. CO_2 absorption capacity is related to biological factors, i. e., pH, water and fat content. CO_2 is highly soluble in both muscle and fat tissue. CO_2 solubility in muscle tissue decreases with a decrease in pH and an increase in temperature, and it is largely determined by fat composition, packaging type and storage temperature (Cilla et al., 2006; Jakobsen and Bertelsen, 2004; Juncher et al., 2003; Kerry et al., 2006; Martinez et al., 2005; O'Sullivan et al., 2011; Parra et al., 2010). The presence of CO_2 in modified atmosphere packaging has been found to promote oxidation by lowering pH (Martinez et al., 2005).

Nitrogen (N_2) is an inert gas which slows down the growth of aerobic microorganisms and prevents pack deformation, since it is sparingly soluble in the water phase and fat phase of meat products. Nitrogen is an inactive component of a protective atmosphere, and it is used as a filler gas (McMillian 2008; O'Sullivan et al., 2011; Zhou et al., 2010).

Oxygen (O_2) is an undesirable component of a protective atmosphere in the majority of cases, but sometimes it is used as a component of a gas mixture. Oxygen delays the progress of metmyoglobin formation in raw meat, thus keeping its natural red color. Oxygen prevents the growth of anaerobic microbes in meat and meat products (McMillian 2008; O'Sullivan et al., 2011; Pikul, 2001; Zhou et al., 2010).

The physicochemical properties of meat products are key factors affecting the shelf-life and consumer acceptance of meat and meat products (Cava et al., 2000; Parra et al., 2010). Thus, the mixture of gases in the package has to assure adequate microbial quality and safety of foods (Møller et al., 2000).

The aim of this study was to determine the effect of storage time and the composition of modified atmosphere on changes in selected physicochemical properties of scalded coarsely-ground sausages stored under vacuum and in a modified atmosphere composed of carbon dioxide and nitrogen in different proportions.

Materials and Methods

Sausage manufacture

The experimental materials comprised scalded coarsely-ground sausages produced from:

- lean, non-stringy pork with a fat content of up to 15 % (class I) – 60 %,
- lean, non-stringy beef with a fat content of up to 15 % (class I) – 15 %,
- pork with a fat content of up to 35 % (class III) – 15 %,
- beef with a fat content of up to 25 % (class II) – 5 %,
- cutting pork fat – 5 %,
- water – 9 %,
- spices: natural pepper – 0.10 %, coriander – 0.01 %, nutmeg – 0.01 %, sugar – 0.20 %.

Raw meat was diced into 50 mm/50 mm cubes, dry cured for 24 hours at 4 ˚C±1 with the addition of 2 % curing salt mixture with the following composition: 99.4 % NaCl and 0.6 % $NaNO_2$. Class I pork was diced into 40 mm/40 mm cubes, class I beef was ground in a grinder, mesh size 20 mm, and the remaining raw materials were ground in a grinder, mesh size 3 mm. In the production process, class III pork, class II beef and cutting fat were chopped with the addition of water with a temperature of around 4 ºC and spices to produce stuffing with a final temperature of 10 ºC. The ingredients were then mixed with class I pork and class I beef until fully blended. Artificial protein casings which provided optimal steam, meat juice and smoke permeability were filled with the stuffing using a piston stuffer to produce bars with a diameter of 85 mm and a length of 300 mm.

The sausages were hanged at 25 ˚C ± 5 ˚C for around 45 minutes, hot smoked at 50 ˚C ± 10 ˚C for around 75 minutes, and scalded at 75 ˚C until the inside of the bar reached a temperature of 72 ˚C±1.

Packaging conditions

At the next stage of the production process, the sausages were cooled under cold water until the inside of the bar reached a temperature of 20 ºC. After 24 hours of cooling at around 4 ºC±1 ºC, the products were packaged under modified atmosphere with the following composition: vacuum (P) or 20 % CO_2, 80 % N_2 (A1) or 50 % CO_2, 50 % N_2 (A2) or 80 % CO_2, 20 % N_2 (A3).

Modified atmospheres composed of carbon dioxide and nitrogen in different proportions were used in the experiment because meat and meat products are most commonly packaged in gas mixtures composed of carbon dioxide and nitrogen. The sausages were packaged in polyamide and polyethylene bags with the following permeation rates: oxygen – 35 cm^3/ (m^2 x 24 h x Pa), nitrogen – 6 cm^3 / (m^2 x 24 h x Pa), carbon dioxide – 158 cm^3 / (m^2 x 24 h x Pa), water steam – 15 g / (m^2 x 24 h). The products were packaged with the use of the Multivac A300 device (Wolfertschwenden, Germany), and were stored at around 4 ºC (±1 °C) for 15 days. Tests were performed in eight replications. Three samples were collected and analyzed each time. Sausage samples were collected at three-day intervals: on the day of packaging (day 0) and after 3, 6, 9, 12 and 15 days of storage.

Analytical methods

pH

pH values were measured using a pH-meter, type 340/ ION-SET, equipped with a combination electrode SenTix 21 (PN – ISO 2917: 2001; PN-ISO 2917:2001/Ap 1:2002).

Drip loss

The drip formed inside the package (M) was determined as described by Pikul (1993), using the formula:

$$M = M1 - M2 - M3$$

M: drip inside the package (g),
M_1: weight of the product including the package (g),
M_2: weight of a dried product excluding the package (g),
M_3: weight of a dried package (g).

Measurement of color parameters

The color parameters of sausages were analyzed by measuring light reflectance with a Spectro-color meter, according to the CIE (Commission Internationale de l'Eclairage – International Commission on Illumination) system (Clydesdale, 1978). The following color parameters were determined:
L* – lightness,
a* – redness,
b* – yellowness.

Saturation C* was calculated using the formula:

$$C^* = (a^{*2} + b^{*2})^{0.5}$$

Total difference in the color of the sample ∅E* was calculated as follows:

$$\varnothing E^* = (\varnothing L^{*2} + \varnothing a^{*2} + \varnothing b^{*2})^{0.5}$$

where:
∅L* – difference in lightness calculated by the formula:

$$\varnothing L^* = L^*_{1,2\ldots5} - L^*_0$$

L^*_0 – lightness on the day of packaging (day 0),
$L^*_{1,2\ldots5}$ – lightness on day 3, 6, 9, 12, 15 of storage,

∅a* – difference in redness calculated by the formula:

$$\varnothing a^* = a^*1, 2\ldots5 - a^*0$$

a^*_0 – redness on the day of packaging (day 0),
$a^*_{1,2\ldots5}$ – redness on day 3, 6, 9, 12, 15 of storage,

∅b* – difference in yellowness calculated by the formula:

$$\varnothing b^* = b^*_{1,2\ldots5} - b^*_0$$

b^*_0 – yellowness on the day of packaging (day 0),
$b^*_{1,2\ldots5}$ – yellowness on day 3, 6, 9, 12, 15 of storage.

Statistical analysis

The results were processed statistically. Arithmetic means ($\bar{x}$) and the standard error of the mean (SEM) were calculated. The effects of atmosphere type, storage time and the atmosphere x time interaction on changes in the quality attributes of the studied products were determined by two-way ANOVA (Fisher F-test). Mean values were compared by the Newman-Keuls test at a significance level of α=0.05. All calculations were performed with the use of STATISTICA 6.0 PL software (Stanisz, 1998).

Results and Discussion

Changes in pH

At the completion of the production process (sausages cooled to 4 ˚C), the pH of sausages was 6.37. pH levels varied insignificantly during storage. Modified atmosphere had no significant effect on changes in the pH of sausages. After 15 days of storage under different gas compositions, the pH of sausages was similar to the initial values (Tab. 1).

ANOVA showed that storage time, atmosphere type and the interaction between these factors had no significant effect on changes in the pH of sausages (Tab. 1).

Similar observations were made by Cachaldora et al (2013) who studied *"morcillas"*, traditional cooked blood sausages packaged under vacuum and in modified atmosphere using three different gas mixtures: 15:35:50/O_2:N_2:CO_2 (atmosphere 1), 60:40/N_2:CO_2 (atmosphere 2) and 40:60/N_2:CO_2 (atmosphere 3), and stored for 2, 4, 6 and 8 weeks at 4 °C. The cited authors observed no significant differences (P>0.05) in pH values of sausages due to the packaging conditions – pH values increased insignificantly, by ca. 0.1 after 8 weeks of storage in all packaging types.

In the present study, the mean initial pH of sausages was 4.72±0.02, and it slightly increased during storage, reaching 4.75 to 4.84 after 8 weeks. This trend agrees with the results reported by several authors for other fermented sausages (Martínez et al., 2005; Tremonte et al., 2005).

Martinez et al. (2005) analyzed fresh pork sausages pakkaged in different modified atmospheres and stored for 20 days at 2±1 °C. The cited authors found that the pH of sausages packaged under modified atmospheres containing 60 % CO_2 – 40 % N_2 and 60 % CO_2 – 40 % O_2 decreased by ca. 0.2, while the pH of sausages stored in an atmosphere with a lower concentration of CO_2 (20 % CO_2 – 80 N_2%) dropped by only 0.1. The pH of sausages packaged under modified atmospheres containing 40 % O_2 – 20 % CO_2 – 40 % N_2 and 80 % O_2 – 20 % CO_2 increased by ca. 0.5 and 0.4, respectively. Minor changes in pH values indicate that the applied modified atmosphere packaging was suitable for preservation of fresh pork sausages.

Parra et al (2010) studied the pH of dry-cured Iberian ham slices stored under vacuum and under four different modified atmospheres (60/40 = 60 %N_2 + 40 %CO_2, 70/30 = 70 %N_2 + 30 %CO_2, 80/20 = 80 %N_2 + 20 %CO_2, argon = 70 % argon + 30 % CO_2) at 4 ± 1 °C during 120 days, and found that the pH of samples was not significantly (P > 0.05) affected by the type of gas mixture used in the study.

According to published data, carbon dioxide used in MAP dissolves and forms carbonic acid, thus lowering the pH of meat products by ca. 0.1 after 20 days of storage (Bruce et al., 1996; Cilla et al., 2006; Dixon and Kell, 1989, Gajewska-Szczerbal, 2005; Juncher et al., 2003; Martinez et al., 2005; Parra, 2010). The solubility of CO_2 used for preservation of meat and meat products is determined by the pressure exerted on the product during packaging, the size of meat portions, storage temperature and fat composition. All of those factors influence also the pH of meat products, which has been found to increase or decrease insignificantly over storage (Cilla et al. 2006; Jakobsen and Bertelsen, 2004). On the contrary, several authors observed that packaging with carbon dioxide produced a lower pH in ham (Cilla et al., 2006) and other meat products (Juncher et al., 2003; Martínez et al., 2005). This effect has been related to the absorption of CO_2 by meat, which results in the formation of carbonic acid (Bruce et al., 1996; Dixon and Kell, 1989).

Mingardi and Desenzani (1993) demonstrated that the pH of sausages stored in two types of modified atmosphere (30 % O_2, 70 % N_2 or 25 % O_2, 65 % N_2, 10 % CO_2) at a temperature of 4 to 6 °C for 13 days increased insignificantly. Manu-Tawiah et al. (1993) observed no significant changes in the pH of meat products stored in air and in modified atmosphere with various gas proportions (vacuum, 20 % CO_2, 80 % N_2 or 40 % CO_2, 60 % N_2 or 40 % CO_2, 10 % O_2, 50 % N_2). Research results show that the pH of meat products packaged under different gas atmospheres changes insignificantly over storage (Kim et al., 2010; Manu–Tawiah et al., 1993; Mingardi and Desenzani, 1993; Parra et al., 2010; Rubio et al., 2008; Schirmer and Langsrud, 2010).

Changes in drip loss

In all types of packages, the most profound changes in drip loss were observed on the first few days of storage, which corroborates the findings of other authors who demonstrated that in the initial stage of storage, drip loss is formed due to mechanical pressure exerted on the product during packaging (Daszkiewicz et al. 2008; Sekar et al. 2006). Similar observation were made by Krala (1998), Liaros et al. (2009) and Pikul (2001).

Throughout storage, the drip loss from sausages packaged in gas atmospheres was considerably lower and it remained at a stable level. On the last day of storage, drip loss was approximately five-fold greater in vacuum-packaged products than in sausages packaged under gas atmospheres (Tab. 2).

A statistical analysis showed that the type of the applied atmosphere and storage time had a significant effect on the drip loss from sausages. There was also a significant interaction between the analyzed factors (Tab. 2). According to reference data, storage time, atmosphere type and the interaction between them significantly affect drip loss, but storage time is the most important consideration (O'-Sullivan et. al., 2011; Torrieri et al., 2006).

Czerniawski (1998) found that the packaging of meat products in a modified atmosphere with high concentrations of carbon dioxide

TABLE 1: *Changes in the pH* of experimental sausages during storage. Results of ANOVA (F).*

Packaging method	Storage (4 °C) period (days) 0	3	6	9	12	15
P	6.37±0.02	6.33±0.02	6.36±0.02	6.37±0.02	6.37±0.02	6.35±0.01
A1	6.37±0.02	6.33±0.02	6.33±0.02	6.37±0.01	6.37±0.02	6.36±0.02
A2	6.37±0.02	6.36±0.02	6.34±0.03	6.35±0.02	6.37±0.02	6.34±0.01
A3	6.37±0.02	6.35±0.02	6.34±0.02	6.35±0.01	6.36±0.02	6.35±0.01
Results of ANOVA (F)						
Atmosphere	F=0.05	p=0.987				
Time	F=1.66	p=0.146				
Interaction	F=0.39	P=0.981				

* – mean values in the Table are not significantly different (α=0.05), P – vacuum, A1 – atmosphere containing 20 % CO_2, 80 % N_2, A2 – atmosphere containing 50 % CO_2, 50 % N_2, A3 – atmosphere containing 80 % CO_2, 20 % N_2, $\bar{x}$ – arithmetic mean, SEM – standard error of the mean (n=8), F – Fisher F-test, p – probability at significance level of α=0.05.

TABLE 2: *Changes in the drip formed inside the package during the storage of coarsely-ground ham sausages (%). Results of ANOVA (F).*

Packaging method	Storage (4 °C) period (days) 3	6	9	12	15
P	1.17±0.05[Ba]	1.25±0.04[Ba]	1.26±0.02[Ba]	1.36±0.02[Bb]	1.58±0.01[Bc]
A1	0.24±0.02[Ac]	0.28±0.01[Aa]	0.31±0.01[Aab]	0.33±0.01[Ab]	0.36±0.01[Ad]
A2	0.22±0.01[Aa]	0.25±0.01[Aa]	0.28±0.01[Ac]	0.31±0.01[Ab]	0.34±0.02[Ab]
A3	0.23±0.01[Ab]	0.26±0.01[Aa]	0.28±0.01[Aa]	0.32±0.01[Ac]	0.35±0.01[Ad]
Results of ANOVA (F)					
Atmosphere	F=4217.30	p<0.001			
Time	F=66.81	p<0.001			
Interaction	F=11.05	p<0.001			

A, B – mean values in columns denoted with different letters are significantly different (α=0.05), a, b – mean values in lines denoted with different letters are significantly different (α =0.05), P – vacuum, A1 – atmosphere containing 20 % CO_2, 80 % N_2, A2 – atmosphere containing 50 % CO_2, 50 % N_2, A3 – atmosphere containing 80 % CO_2, 20 % N_2, $\bar{x}$ – arithmetic mean, SEM – standard error of the mean (n=8), F – Fisher F-test, p – probability at significance level of α=0.05.

may increase drip loss in the package. The greater drip loss from vacuum-packaged sausages resulted from the effect of negative pressure on the products. The changes in weight loss, observed in our study, are consistent with previous research (Krala, 1998; Pikul, 2001). In our study, drip loss increased in all samples during storage, irrespective of the packaging method, which is consistent with the findings of other authors (Daszkiewicz et al. 2008; Liaros et. al 2009; O'Sullivan et. al 2011; Pikul 2001).

Changes in color parameters

The average value of color lightness (L*) of coarsely-ground sausages before packaging was 58.58, and it varied insignificantly during storage. On the last day of storage, an increase was noted in the lightness of experimental sausages packaged under various atmospheric conditions. The highest value of L* was observed in vacuum-packaged products, while the lowest – in sausages packaged in modified atmosphere A3, with the highest carbon dioxide content (Tab. 3). As shown by two-way ANOVA, changes in the lightness of coarsely-ground sausages were significantly affected by the type of the applied atmosphere and storage time (Tab. 3).

Similar results were obtained by Liaros et al. (2009) who found that low-fat fermented sausages packaged under vacuum were characterized by the highest color lightness L*. Other authors (Cilla et al., 2006; De Santos et al., 2007; Parra et al., 2010; Parra et al., 2012; Sørheim et al., 2004) demonstrated that high CO_2 levels (above 60 %) in the package may lead to meat discoloration resulting from myoglobin oxidation. In our study, changes in lightness were not affected by CO_2 levels in the modified atmosphere. According to Esmer et al. (2011) and Soldatou et al. (2009), CO_2 and O_2 concentrations in the modified atmosphere have no significant effect on changes in color lightness in stored ground beef.

Also Cachaldora et al. (2013) observed no significant differences (P>0.05) in L* values of *"morcillas"* packaged under vacuum and in different modified atmospheres: 15:35:50/O_2:N_2:CO_2 (atmosphere 1), 60:40/N_2:CO_2 (atmosphere 2) and 40:60/N_2:CO_2 (atmosphere 3), and stored for 2, 4, 6 and 8 weeks at 4 °C. After 8 weeks of storage, L* decreased by ca. 0.78 units in products stored under vacuum and by ca. 0.02 units in an atmosphere consisting of 40:60/N_2:CO_2, while an increase of 0.09 was observed in an atmosphere composed of 60:40/N_2:CO_2. Summo et al., (2010) reported that according to MacDougall (1982), a difference in visual lightness (L) of five units or more is perceivable in meat and meat products. Such a difference was not noted in our study.

Our results regarding changes in L* values of scalded sausages corroborate the findings of other authors who noted no significant differences in the color lightness of different types of sausages under different packaging conditions (Cachaldora et al., 2013, Rubio et al., 2008; Ruiz-Capillas and Jiménez-Colmenero, 2010).

The average redness of experimental sausages at the beginning of storage was 8.50. After 3 days of storage under various gas compositions the values of a* decreased insignificantly (Tab. 4).

TABLE 3: *Changes in the lightness (L*) of experimental sausages during storage. Results of ANOVA (F).*

Packaging method	Storage (4 °C) period (days) 0	3	6	9	12	15
P	58.58±1.16	59.24±1.31	58.33±0.57[B]	58.64±1.92	61.77±0.39[B]	62.66±0.55[B]
A1	58.58±1.16[a]	58.12±1.45[a]	53.78±0.84[Ab]	58.89±0.67[a]	58.58±0.96[Aa]	58.94±0.19[Aa]
A2	58.58±1.16	59.69±0.60	56.66±0.37[B]	58.16±2.02	58.14±0.29[A]	60.72±1.05[A]
A3	58.58±1.16[a]	59.00±1.33[a]	54.36±0.29[Ab]	58.95±0.70[a]	57.38±0.81[Aa]	58.71±0.18[Aa]
Results of ANOVA (F)						
Atmosphere	F=5.49	p=0.002				
Time	F=8.59	p<0.001				
Interaction	F=1.27	p=0.245				

A, B – mean values in columns denoted with different letters are significantly different (α=0.05), a, b – mean values in lines denoted with different letters are significantly different (α =0.05), P – vacuum, A1 – atmosphere containing 20 % CO_2, 80 % N_2, A2 – atmosphere containing 50 % CO_2, 50 % N_2, A3 – atmosphere containing 80 % CO_2, 20 % N_2, $\bar{x}$ – arithmetic mean, SEM – standard error of the mean (n=8), F – Fisher F-test, p – probability at significance level of α=0.05.

TABLE 4: *Changes in the redness (a*) of experimental sausages during storage. Results of ANOVA (F).*

Packaging method	Storage (4 °C) period (days) 0	3	6	9	12	15
P	8.50±0.18	8.48±0.49	7.62±0.21	7.74±0.53	7.76±0.36	8.85±0.12
A1	8.50±0.18[b]	7.79±0.20[a]	7.27±0.11[a]	7.69±0.16[a]	7.62±0.19[a]	8.59±0.17[b]
A2	8.50±0.18	8.47±0.07	8.04±0.23	7.98±0.40	7.77±0.38	8.63±0.16
A3	8.50±0.18	7.53±0.59	7.45±0.75	7.41±0.16	8.28±0.15	8.97±0.15
Results of ANOVA (F)						
Atmosphere	F=1.25	p=0.299				
Time	F=8.65	p<0.001				
Interaction	F=0.80	p=0.677				

a, b – mean values in columns denoted with different letters are significantly different (α=0.05), a, b – mean values in lines denoted with different letters are significantly different (α =0.05), P – vacuum, A1 – atmosphere containing 20 % CO_2, 80 % N_2, A2 – atmosphere containing 50 % CO_2, 50 % N_2, A3 – atmosphere containing 80 % CO_2, 20 % N_2, $\bar{x}$ – arithmetic mean, SEM – standard error of the mean (n=8), F – Fisher F-test, p – probability at significance level of α=0.05.

Small fluctuations in redness were observed throughout storage, most probably due to nitrosyl myoglobin oxidation resulting from the presence of residual oxygen in the package and the penetration of gases into the sausages (Gøtterup et. al. 2008; Liaros et. al. 2009, Parra et al. 2012). On day 15, the values of this color parameter increased in all samples. The highest value of a* was noted in sausages packaged in modified atmosphere A3 (change by ca. 0.47 units), with the highest carbon dioxide content, while the lowest – in products packaged in modified atmosphere A1 (change by ca. 0.09 units), with the lowest carbon dioxide content (Tab. 4).

Similar observations were made by Cachaldora et al. (2013) who reported the highest increase (by ca. 1.85 units) in a* values of cooked blood sausages packaged in a modified atmosphere with the highest carbon dioxide content (40:60/N_2:CO_2) and stored for 8 weeks at 4 °C, and the lowest increase (by ca. 0.5 units) in a* values of sausages packaged in a modified atmosphere containing oxygen. In vacuum-packaged samples, a* increased by ca. 1.37 units. The above results indicate that high oxygen concentrations enhance the bright-red color of fresh meat, but low concentrations accelerate the oxidation of myoglobin to metmyoglobin which turns the color brown. In fermented sausages to which nitrites are added (as in the present study), the red color turns brown in the presence of oxygen and light.

The evolution of a* values found in this study is similar to that described by other authors (Cachaldora et al., 2013, Martínez et al., 2006; Ruiz-Capillas and Jiménez-Colmenero, 2010; Tremonte et al., 2005). who observed similar changes in a* during the storage of meat products packaged under MAP. A minor increase in redness a* resulted from the reaction of nitric oxide, produced from nitrites, with myoglobin, producing nitric oxide myoglobin (nitrosyl myoglobin) (Gøtterup et al., 2008, Liaros et al., 2009; Rubio et al., 2008). According to reference data, the desired red color of meat and meat products can be obtained in MAP with low CO levels, which prevents discoloration even at high CO_2 concentrations in the package (De Santos et al., 2007; Esmer et al., 2011; Jayasingh et al., 2001; Sørheim et al., 1999).

The F-test showed that changes in the redness of coarsely-ground sausages were significantly affected by storage time (Tab. 4). In a study by Esmer et al. (2011), gas composition and storage time had a considerable effect on changes in the red color of ground beef, and high CO_2 concentrations led to a high decrease in redness.

The average value of yellowness of coarsely-ground sausages before packaging was 12.36. Modified atmosphere had no significant effect on changes in this color parameter throughout storage. Similar observations were made by Summo et al. (2010). On the last day of storage, the values of yellowness increased in all samples, irrespective of the packaging method. The highest value of b* was observed in sausages packaged in modified atmosphere A3 (increase by ca. 2.11 units), with the highest carbon dioxide content, while the lowest – in vacuum-packaged products (increase by ca. 1.65 units). Throughout storage, the range of changes in parameter b* was similar in the analyzed types of packages (Tab. 5).

Cachaldora et al. (2013) found that vacuum-packaged cooked blood sausages were characterized by the lowest changes in yellowness after 8 weeks of storage (increase by ca. 2.38 units). Differences in b* between samples packaged under modified atmospheres composed of nitrogen and carbon dioxide were small, and the highest increase (by ca. 2.62 units) was noted after 8 weeks of storage in the atmosphere with the highest CO_2 content. Our results presented in Table 5 are highly similar.

Similarly as in our study, in the experiment conducted by Cachaldora et al. (2013) storage time and packaging conditions did not cause significant changes (P>0.05) in b* values of sausages stored for 8 weeks. According to Cachaldora et al. (2013), the above could be due to the addition of nitrite which binds to myoglobin to form nitrosyl myoglobin. However, in samples packaged under 15:35:50/O_2:N_2:CO_2, b* values increased by ca. 3.84 units after 8 weeks of storage. Also other authors (Rubio et al., 2008, Ruiz-Capillas and Jiménez-Colmenero 2010) reported insignificant changes in b* values of other fermented sausages stored under different packaging conditions.

The influence of the composition of modified atmosphere on the majority of quality attributes of sausages was noted after 3 days of storage. Most probably, this resulted from the combined effect of negative pressure and the initial composition of gas mixtures. Subsequent small changes in the values of the analyzed color parameters were due to different rates of gas diffusion from the outside to the inside of the product.

The present findings are consistent with the results of previous studies. Pexara et al. (2002) demonstrated that sausages packaged in an atmosphere with a high CO_2 concentration (60 % CO_2, 20 % O_2, 20 % N_2) were characterized by faster changes in color during storage. In MA-packaged meat products, high carbon dioxide levels may speed up changes in color parameters. In a study by Martínez et al. (2005), high concentrations of carbon dioxide (60%) promoted myoglobin oxidation in fresh pork sausages packaged in modified atmosphere, which caused changes in the products' color. Lin and Lin (2002) studied the color of reduced-fat Chinese-style sausages packaged under modified atmosphere conditions (vacuum, 100 % CO_2 or 80 % CO_2, 20 % N_2 or 20 % CO_2, 80 % N_2) and stored for 10 weeks at 4 °C. The authors demonstrated that the lightness, redness and yellowness of the products

TABLE 5: *Changes in the yellowness (b*)* of experimental sausages during storage. Results of ANOVA (F).*

Packaging method	Storage (4 °C) period (days) 0	3	6	9	12	15
P	12.36±0.71	12.96±0.26	13.25±0.44	13.46±0.50	13.66±0.15	14.01±0.07
A1	12.36±0.71	12.59±0.52	12.25±0.62	14.14±0.38	13.16±0.53	14.19±0.54
A2	12.36±0.71	12.21±0.42	13.74±0.99	13.51±0.69	13.36±0.34	14.30±0.37
A3	12.36±0.71	12.84±0.35	14.35±0.90	14.30±0.39	13.93±0.34	14.47±0.20
Results of ANOVA (F)						
Atmosphere	F=1.36	p=0.262				
Time	F=6.92	p=0.001				
Interaction	F=0.57	P=0.887				

* – mean values in the Table are not significantly different (α=0.05), P – vacuum, A1 – atmosphere containing 20 % CO_2, 80 % N_2, A2 – atmosphere containing 50 % CO_2, 50 % N_2, A3 – atmosphere containing 80 % CO_2, 20 % N_2, $\bar{x}$ – arithmetic mean, SEM – standard error of the mean (n=8), F – Fisher F-test, p – probability at significance level of α=0.05.

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changed insignificantly over storage. Summo et al. (2010) reported that according to MacDougall (1982), a difference in visual lightness (L*) of five units or more is perceivable in meat and meat products. Such a difference was not noted in our study.

The color of meat products remains more stable during storage in gas atmospheres than in vacuum packaging (Ho et al., 2003; Narasimha and Sachindra, 2002). In order to account for changes in the color of sausages stored in various types of modified atmosphere, color saturation (C*) and the total difference in the color of samples (∅E*) were calculated (Tab. 6).

The color saturation (C*) of coarsely-ground sausages before packaging was 15.01. Modified atmosphere had no significant effect on this parameter throughout storage. On day 15, the color saturation of sausages increased by 1.56 (vacuum packaging) to 2.02 units (packaging in modified atmosphere A3), compared with the initial value. The increase in color saturation resulted from the increase in redness and yellowness, noted on the last days of storage (Tab. 6). There were minor differences in color saturation between the analyzed types of packages, which corroborates the findings of De Santos et al. (2007).

Two-way ANOVA revealed that changes in the color saturation of experimental sausages was significantly influenced by storage time (Tab. 6). Cachaldora et al (2013) demonstrated that the chroma value (C*) of *"morcillas"* remained stable during 8-week storage under vacuum and different modified atmospheres. Significant (P>0.05) differences were noted only after 2 weeks of storage, and the highest C* value was observed in samples packaged in an atmosphere with the highest carbon dioxide content (40:60/N_2:CO_2). After 8 weeks, C* increased by ca. 2,71 units in vacuum-packaged samples, and by ca. 3.2 units in samples packaged under 40:60/N_2:CO_2.

During storage, the color saturation of coarsely-ground sausages was in the range of 2.05–5.73. The total difference in the color of samples varied insignificantly throughout storage. On day 15, the lowest value of the above parameter was observed in sausages packaged in modified atmosphere A3, with the highest carbon dioxide content, while the highest – in vacuum-packaged products (Tab. 7).

The values of ∅E* changed similarly as color redness, which is consistent with the findings of other authors (Bingol et al., 2011; Fernandez-Lopez et al., 2008). Kennedy et al. (2004) demonstrated that a modified atmosphere composed of 80 % O_2, 20 % CO_2 and 0N_2 was most desirable with regard to maintaining the red color of lamb and hogget. Insausti et al. (1999) found that beef packaged under 60 % O_2, 30 % CO_2 and 10 % N_2 was characterized by higher lightness and total difference in the color, lower redness and color saturation.

Total color difference (∅E) indicates the distance between locations in the CIE L* a* b* color space. Higher DE values point to a greater difference between the analyzed color and the reference. DE values higher than 3 indicate a change in color visible to the naked eye, ∅E values between 2 and 3 indicate a color difference that is not very apparent, and ∅E values lower than 1 indicate a change in color not visible to the naked eye (Mastromatteo et al., 2011). In our study, differences in sausage color perceptible to the naked eye were noted after 15 days of storage, irrespective of the packaging method. The F-test indicated that the total difference in the color of samples was significantly affected by storage time, whereas the type of the applied atmosphere and the interaction between these factors had no significant effect on this parameter (Tab. 7). Our findings validated reference data, and confirmed that the type of modified atmosphere and storage time exert an insignificant effect on changes in the color parameters of meat products during storage (Rubio et al., 2008). The small changes in the color of sausages noted in the study could result from the diffusion of gases into the products and from transformations in the heme pigments of meat.

TABLE 6: *Changes in the color saturation (C*)* of experimental sausages during storage. Results of ANOVA (F).*

Packaging method	Storage (4 °C) period (days) 0	3	6	9	12	15
P	15.01±0.68	15.51±0.36	15.30±0.31	15.54±0.64	15.72±0.25	16.57±0.09
A1	15.01±0.68	14.82±0.36	14.25±0.56	16.10±0.27	15.22±0.50	16.59±0.51
A2	15.01±0.68	14.86±0.36	15.95±0.85	15.70±0.75	15.47±0.31	16.71±0.38
A3	15.01±0.68	14.90±0.55	16.22±0.93	16.11±0.40	16.21±0.37	17.03±0.25
Results of ANOVA (F)						
Atmosphere	F=1.20	p=0.310				
Time	F=5.81	p=0.001				
Interaction	F=0.60	P=0.867				

* – mean values in the Table are not significantly different (α=0.05), P – vacuum, A1 – atmosphere containing 20 % CO_2, 80 % N_2, A2 – atmosphere containing 50 % CO_2, 50 % N_2, A3 – atmosphere containing 80 % CO_2, 20 % N_2, $\bar{x}$ – arithmetic mean, SEM – standard error of the mean (n=8), F – Fisher F-test, p – probability at significance level of α=0.05.

TABLE 7: *Total difference (∅E*) in the color of experimental sausages during storage. Results of ANOVA (F).*

Packaging method	Storage (4 °C) period (days) 0	3	6	9	12	15
P	0.00±0.00[b]	2.39±0.37[a]	3.47±0.49[ab]	2.05±0.47[a]	4.08±0.57[ab]	4.95±0.83[b]
A1	0.00±0.00[b]	4.33±0.81[a]	5.73±1.46[a]	2.69±0.54[ab]	2.58±0.60[ab]	3.19±0.46[a]
A2	0.00±0.00[b]	2.38±0.53[a]	3.81±0.92[a]	2.43±0.41[a]	3.15±0.45[a]	4.52±1.02[a]
A3	0.00±0.00[b]	3.86±0.96[a]	5.13±1.20[a]	3.28±0.66[a]	3.45±0.88[a]	3.17±0.71[a]
Results of ANOVA (F)						
Atmosphere	F=0.51	p=0.675				
Time	F=20.25	p<0.001				
Interaction	F=1.38	p=0.183				

a, b – mean values in columns denoted with different letters are significantly different (α=0.05), a, b – mean values in lines denoted with different letters are significantly different (α =0.05), P – vacuum, A1 – atmosphere containing 20 % CO_2, 80 % N_2, A2 – atmosphere containing 50 % CO_2, 50 % N_2, A3 – atmosphere containing 80 % CO_2, 20 % N_2, $\bar{x}$ – arithmetic mean, SEM – standard error of the mean (n=8), F – Fisher F-test, p – probability at significance level of α=0.05.

Conclusions

The analyzed packaging methods (vacuum, gas mixtures) lead to insignificant changes in the pH of scalded sausages, which testifies to the physical, biochemical and microbiological stability of the stuffing used in the experiment.

Greater drip loss observed in vacuum-packaged sausages, compared with samples stored under gas atmospheres, results from higher negative pressure exerted on the product during packaging.

Sausages packaged in a modified atmosphere with the highest carbon dioxide content (A3) are characterized by the lowest color lightness and by the highest redness and yellowness values, most probably due to the effects exerted by carbon dioxide on meat components.

Changes in selected physicochemical properties of scalded sausages packaged under gas atmospheres are smaller, compared with vacuum-packaged products, and they remain at a similar level irrespective of the composition of gas mixtures.

Packaging in a protective atmosphere (vacuum, gas mixtures), combined with improvements in the chill chain, is an effective preservation technique for scalded sausages.

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