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Korrespondenzadresse:
mstasiewicz76@o2.pl

Department of Animal Nutrition and Feed Science; University of Warmia and Mazury in Olsztyn, Poland

Lipid oxidation in meat products packaged stored under vacuum and modified atmosphere with different concentrations of carbon dioxide and nitrogen

Lipidoxidation in Vakuum bzw. unter Gasatmosphäre mit unterschiedlichen Gehalten an Kohlenstoffdioxid und Stickstoff verpackten und gelagerten Fleischerzeugnissen

Małgorzata Stasiewicz, Krzysztof Lipinski, Marek Cierach, Cezary Purwin

Summary

The objective of this study was to determine the effect of storage time and modified atmosphere on lipid oxidation in pre-cooked sausages with varying degrees of coarseness. The sausages, produced in accordance with standard procedures and technologies, were stuffed into artificial protein casings that provide optimal steam, meat juice and smoke permeability, and 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). Sausage samples were stored at around 4 °C (±1 °C) for 15 days. Determinations were made at three-day intervals (day 0, 3, 6, 9, 12, 15) during storage. 8 replicates of each experiment were made (n = 8). For each of 8 experiments 3 parallel measurements were carried out.

The presence of carbon dioxide in modified atmosphere slowed down the rate of lipid oxidation in meat products. The atmosphere with the highest carbon dioxide concentrations was most suitable for storing pre-cooked sausages. Coarsely-ground sausages were characterized by the lowest oxidative stability throughout storage.

Keywords: meat products, storage, modified atmosphere, lipid oxidation

Zusammenfassung

Ziel dieser Studie war es, den Effekt von Lagerzeit und modifizierter Atmosphäre auf die Lipidoxidation zu untersuchen. Als Untersuchungsmaterial dienten Brühwürste mit unterschiedlichem Zerkleinerungsgrad. Die Würste wurden nach Standardrezepten und -technologien hergestellt. Nach Beendigung des Produktionsprozesses wurden sie unter modifizierter Atmosphäre verpackt. Als Verpackung wurden wasserdampf-, fleischsaft-, rauch- und luftdurchlässige Eiweiß-Kunststoffhüllen verwendet. Es wurden 4 Varianten angewandt Vakuum (P); 20 % CO_2, 80 % N_2 (A1); 50 % CO_2, 50 % N_2 (A2); 80 % CO_2, 20 % N_2 (A3). Die Produkte wurden bei einer Temperatur von ca. 4 °C (±1 °C) 15 Tage lang gelagert. Die Bestimmungen wurden nach der Herstellung sowie nach 3, 6, 9, 12, 15 Tagen der Lagerung vorgenommen. Es wurden 8 Wiederholungen der Untersuchung durchgeführt (n = 8). Für jede der 8 Untersuchungen wurden gleichzeitig 3 Messungen durchgeführt.

Das Vorhandensein von Kohlenstoffdioxid in modifizierter Atmosphäre trug zur wesentlichen Verlangsamung der Lipidoxidation in den Fleischprodukten bei. Die Atmosphäre mit dem größten Gehalt an Kohlenstoffdioxid hat sich als die günstigste Lagerungsmethode für die Fleischprodukte erwiesen. Die grob zerkleinerten Würste zeigten die geringste Oxidationsstabilität während der gesamten Lagerungsdauer der Brühwürste.

Schlüsselwörter: Fleischprodukte, Lagerung, modifizierte Atmosphäre, Lipidoxidation

Introduction

Lipid oxidation changes in meat have a significant effect on the end product's palatability, flavor, aroma, nutritional value and safety. The rate of lipid oxidation is determined by several factors that initiate the oxidation process, such as oxygen, temperature and light. The higher the oxygen content of the packaging, the higher the TBA values are during storage (Liu et al., 2007; Martínez et al., 2007; Nowak and Piotrowska, 2012).

The presence of substances with antioxidant properties delays lipid oxidation in meat products. Those substances are inclusive of antioxidants that occur naturally in fat (vitamin E), synthetic antioxidants, herbal spices and curing smoke (Martínez et al., 2007; Valencia et al., 2008; Fredriksen et al., 2009; Liu et al., 2009; Ponnampalam et al., 2012). The rate of lipid oxidation in meat products is largely dependent on the properties of fat, in particular on the degree of unsaturation (Rubio et al., 2008), and it is proportional to the concentrations of unsaturated fatty acids in meat (Martínez et al., 2007; Rubio et al., 2008). Due to its high iron content, the type of lean meat used in the production process has a significant effect on the rate of lipid oxidation. Iron initiates lipid oxidation, and it is capable of decomposing H_2O_2 and ROOH to form free radicals (Carlsen et al., 2005; Rubio et al., 2008; Ponnampalam et al., 2012). The oxidative stability of meat products is also affected by technological processes such as grinding, cutting and mixing, which cause damage to cell membranes, as well as air and oxygen forcing into tissues. An increase in the surface area of meat exposed to light and oxygen accelerates lipid oxidation (Martínez et al., 2006a, Rubio et al., 2008) since heme iron present in meat is photosensitive.

Modified atmosphere packaging has evolved from the vacuum packaging technique that has been in use for many years. In this process, the internal atmosphere surrounding a product is modified with a selection of gases. The use of protective gas supports the microbiological and chemical stability of meat products, thus enhancing the quality of the end product (Gajewska-Szczerbal, 2005).

The objective of this study was to determine the effect of storage time and modified atmosphere on lipid oxidation in pre-cooked sausages with varying degrees of coarseness.

Materials and Methods

Materials

The experimental materials comprised pre-cooked sausages with varying degrees of coarseness and various processing parameters to differentiate the rate of gas diffusion from the outside to the inside of the product. The sausages were produced from class I (lean, non-tendinous), class IIA (medium fatty, non-tendinous), class III (lean or medium fatty, tendinous) pork, and class I (lean, non-tendinous) and class II (lean, tendinous) beef. Meat was diced into 50 mm/50 mm cubes, dry cured for 24 hours at 4 °C±1 °C with the addition of 2 % curing salt with the following composition: 99.4 % NaCl, 0.6 % $NaNO_2$.

Experimental sausages were stuffed into artificial protein casings (Fabios, Kielce, Poland) that provide optimal steam, meat juice and smoke permeability.

Technological process – coarsely-ground sausages:
Class I pork was diced into 40 mm/40 mm cubes, 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 °C and spices to produce stuffing with a final temperature of 15 °C. The ingredients were then mixed manually with class I pork and class I beef until fully blended. Artificial casings were filled with the stuffing using a manual stuffer to produce links with a diameter of 85 mm. Coarsely-ground 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 °C.

Technological process – semi-coarsely ground sausages:
Class I pork was ground into pieces of approximately 20 mm in a grinder, class IIA pork was ground in a grinder, mesh size 10 mm, class II beef was ground in a grinder, mesh size 3 mm. In the production process, class II beef was chopped with the addition of water with a temperature of 4 °C and spices to produce stuffing with a final temperature of 15 °C. The ingredients were then mixed manually with class I and IIA pork until fully blended. Artificial casings were filled with the stuffing using a manual stuffer to produce links with a diameter of 30 mm. Semi-coarsely ground sausages were cooked in a water bath at 100 °C for 5 minutes, after which water bath temperature was reduced to 72 °C and sausages were cooked for a further 20 minutes until the inside of the link reached a temperature of 72 °C.

Technological process – finely-ground sausages:
Meat was ground in a grinder, mesh size 3 mm. In the production process, meat was chopped with the addition of water with a temperature of around 4 °C, skinned jowl and spices were added, and the mixture was chopped until fully blended to produce stuffing with a final temperature of

Composition of experimental sausages

Main ingredients	Coarsely-ground sausages [%]	Semi-coarsely ground sausages [%]	Finely-ground sausages [%]
class I pork – lean, non-tendinous	60	20	–
class IIA pork – medium fatty, non-tendinous	–	70	15
class III pork – lean or medium fatty, tendinous	15	–	20
class I beef – lean, non-tendinous	15	–	30
class II beef – lean, tendinous	5	10	–
cutting fat	5	–	–
skinned jowl	–	–	30
skin	–	–	5
Additives	**[%]**	**[%]**	**[%]**
water	9	9	32
natural pepper	0.10	0.10	0.08
coriander	0.01	0.07	–
nutmeg	0.01	–	0.04
sugar	0.20	–	0.20
marjoram	–	0.10	–

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15 °C. Artificial casings were filled with the stuffing using a manual stuffer to produce links with a diameter of 18 mm. Finely-ground sausages were hanged at 25 °C ± 5 °C for around 45 minutes, hot smoked at 50 °C ± 10 °C for around 25 minutes, and then scalded at 75 °C for 15 min. After the production process, experimental sausages were cooled under cold water until the inside of the bar reached a temperature of 20 °C.

Packaging and storage of samples

After 24 hours of cooling at around 4 °C ± 1 °C, the products were packaged in a modified atmosphere with the following composition:

P: vacuum; **A2:** 50 % CO_2, 50 % N_2;
A1: 20 % CO_2, 80 % N_2; **A3:** 80 % CO_2, 20 % N_2.

Modified atmospheres composed of carbon dioxide and nitrogen at different proportions were used in the present experiment since mixtures of those two gases are most commonly applied in the meat industry for processed meat packaging.

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). Approximately 0.5 kg of sausage was placed in each bag. All sausages were packaged individually with the use of the Multivac A300 device (Wolfertschwenden, Germany), and were stored at around 4 °C (±1 °C) for 15 days. 8 replicates of each experiment were made (n = 8). For each of 8 experiments 3 parallel measurements were carried out. 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

Lipid oxidation changes in sausages stored under modified atmosphere were determined by the modified Salih method, and the results were expressed as TBARS, in mg of malondialdehyde per kg of sample (Pikul, 1993). The method involves colorimetric determination of compounds that are formed during reactions between 2-thiobarbituric acid (TBA) and lipid oxidation products, after heating in an acidic environment. 10 g samples of ground sausages were homogenized in a stomacher (Model 400, Seward Medical, London, UK) for 2 minutes at 4000 rpm, with the addition of 34.25 cm^3 of 4 % chloric acid (VII) at 4 °C and 0.75 cm^3 of an alcoholic solution of butylated hydroxytoluene (BHT). The homogenate was filtered through Whatman No. 1 filter paper into an Erlenmeyer flask. The filtrate was made up to 50 cm^3, the sediment was rinsed with 4 % of chloric acid (VII), 5 cm^3 samples of the resulting solution were transferred to 20 cm^3 tubes, and 5 cm^3 of 0.02 mol aqueous solution of TBA was added as a reagent. The tubes were cooked in a water bath at 100 °C for 1 hour, and then they were cooled under cold water for 10 minutes. Absorbance was measured at 532 nm wavelength against a control sample containing 5 cm^3 of 4 % chloric acid (VII) and 5 cm^3 of TBA as a reagent. The TBARS value (mg malondialdehyde per kg of sausage) was calculated as:

$$L_{TBARS} = B \cdot K$$

where:
L_{TBARS} – TBARS value [mg/kg of sample],
B – absorbance of the analyzed sample,
K – conversion factor K of 5.5.
The average percent recovery of the standard is 94.

Statistical analysis

The effect of atmosphere type, storage time and the atmosphere x time interaction on changes in the qualitative attributes of the studied products was determined by two-way ANOVA (Fisher's F test). Mean values were compared by the Newman-Keuls test at $\alpha = 0.05$. Calculations were performed using STATISTICA 6.0 PL software (Stanisz, 1998).

Results and Discussion

The initial malondialdehyde content of coarsely-ground, semi-coarsely ground and finely-ground sausages was 0.64 mg/kg, 0.58 mg/kg and 0.66 mg/kg, respectively. A significant upward trend was observed concerning malondialdehyde levels depending on storage time for coarsely-ground sausages stored in P, A1, A2, A3 pack, for semi-coarsely-ground sausages stored in P pack and for finely-ground sausages stored in stored in protective atmosphere bags P, A1, A2, A3. Throughout storage, for coarsely-ground sausages significant differences were observed between atmosphere types on days 6, 12 and 15, for semi-coarsely-ground sausages no significant differences were observed between packaging methods and for finely-ground sausages significant differences were observed between pack type on days 9, 12 and 15. On day 15, the highest TBARS values were reported in vacuum-packaged products. Malondialdehyde content increased by 0.21 mg/kg in coarsely-ground sausages, by 0.05 mg/kg in semi-coarsely ground sausages and by 0.06 mg/kg finely-ground sausages, compared with day 0 (Tab. 1).

During the storage of vacuum-packaged products, the lowest oxidative stability was noted in coarsely-ground sausages (links with a diameter of 85 mm), followed by finely-ground sausages (links with a diameter of 18 mm) and semi-coarsely ground sausages (links with a diameter of 30 mm). The observed changes in TBARS values most likely resulted from a higher residual oxygen content of the packaging with coarsely-ground sausages, and higher oxygen permeability in sausages with a larger diameter. Our results support the findings of Zanardi et al. [2002] who observed lower oxidative stability in vacuum-packaged fermented sausages, compared with sausages stored in a gas atmosphere of 100 % nitrogen. After 60 days of storage, the TBARS values of vacuum-packaged sliced fermented sausages increased by 0.72 mg/kg. In the protective atmosphere (100 % N_2), TBARS values remained at a stable level of 0.33 mg/kg throughout storage. According to Zanardi et al. (2002), both vacuum and modified atmosphere packaging can extend the shelf life of sliced sausages by elimination of oxygen from the package, but in both cases low residual levels of oxygen cannot be avoided. Similar observations were made by Rubio et al. (2008) who analyzed lipid oxidation in sliced salchichón manufactured from raw meat with different fatty acid composition, packaged by two methods (vacuum and 20 % CO_2/80 % N_2) and stored for a long period of time (210 days) at 60 °C.

Sausages packaged in a modified atmosphere of 20 % CO_2/80 % N_2 had lower TBARS values ($P < 0.05$) than vacuum-packaged sausages. During 210 days of chilled storage, both packaging methods (vacuum and 20 % CO_2/80 % N_2) had a minor effect on the color and lipid oxidation stability of salchichón. In a study by Wang et al. (1995), modified atmosphere had a similar effect on

changes in the TBARS values of fermented sausages. The sausages were packaged under vacuum and in a modified atmosphere (75 % N_2/25 % CO_2), and were stored at 4 °C and 15 °C for 5 months. TBARS values were significantly higher in sausages stored at 15 °C than in those stored at 4 °C. After 5 months of storage, the TBARS values of sausages increased significantly ($P < 0.05$) in both types of packages, but the noted increase was higher in vacuum-packaged sausages than in those packaged in a modified atmosphere (75 % N_2/25 % CO_2). After 5 months, the TBARS values of vacuum-packaged sausages stored at 15 °C and 4 °C increased by 2.1 mg/kg and 2.05 mg/kg, respectively, whereas the TBARS values of MA-packaged sausages stored at 15 °C and 4 °C increased by 1.05 mg/kg and 0.85 mg/kg, respectively.

Our studies demonstrate significant changes in TBARS content for 3 types of vacuum-stored sausages. The differences between our results and some published studies could be due to the different type, composition, size (whole links or slices) and degree of coarseness/fineness of the analyzed sausages, different type of casings used, different processing parameters and different pressure exerted on the product during packaging. The above factors determine how much residual oxygen is left in the package, and affect the rate of gas diffusion from the modified atmosphere to the inside of the product and the rate of lipid oxidation (Lin and Lin 2002; Cilla et al., 2006; Martínez et al., 2006a, Parra et al., 2010).

On the last day of storage TBARS values were considerably lower for sausages stored in A3 atmosphere pack, i.e. the one of the highest carbon dioxide content. The increase for coarsely- and finely-ground sausages was approx. 0.04 mg/kg on the 15th day of storage compared to the values on the day of packaging. For semi-coarsely-ground sausages malondialdehyde levels increased by approx. 0.03 mg/kg on the last day of storage in A1, A2, A3 pack compared to day 0. Lower malondialdehyde levels in sausages stored in A3 atmosphere pack were probably due to the presence of carbon dioxide that shows antioxidative effects by lowering reduction and oxidation potential of a product [Polinski 2004].

Cachaldora et al. (2013) studied *"morcillas"*, traditional cooked blood sausages, pakkaged 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 found that storage time had a significant effect on TBARS values. The initial TBARS values were low, at 0.35 mg malonaldedyde/kg of sample, and they increased significantly ($P < 0.05$) with time. After 8 weeks of storage in different atmospheres, TBARS values were below 1 mg malondialdehyde/kg of sample, which suggests that vacuum and modified atmospheres suppressed lipid oxidation in *"morcillas"*. Samples packaged with high CO_2 concentrations (40:60/N_2:CO_2) had the lowest TBARS values at the end of storage (an increase by 0.05 units after 8 weeks), which is consistent with our findings. In the other types of atmosphere, TBARS values increased during 8 weeks of storage, as follows: atmosphere 2 (60:40/N_2:CO_2) – by 0.25 units, vacuum – by 0.17 units, atmosphere 1 (15:35:50/O_2:N_2:CO_2) – by 0.15 units. Carbon dioxide suppressed lipid oxidation also in our study (Tab. 1), which supports previous research (Cachaldora et al., 2013; Bingol and Ergun, 2011; Gokoglu et al., 2010; Martínez et al., 2006a). In a study by Cachaldora et al. (2013), the final TBARS values of *"morcillas"* (below 1 mg malondialdehyde/kg of sample) were lower than those obtained in different types of sausages packaged under vacuum and modified atmospheres (Martínez et al., 2006a; Rubio et al., 2008; Zanardi et al., 2002), which points to delayed or slow development of rancidity due to oxidation in *"morcillas"*.

In the current experiment, modified atmosphere caused a lower increase in TBARS formation in sausages than vacuum packaging, which corroborates the findings of other authors (Wang et al., 1995; Krala and Kulagowska, 2005; Rubio et al., 2008). The malondialdehyde content of sausages was most likely affected by the composition and grinding degree of raw material, and by the levels of fat inclusion. Semi-coarsely ground sausages, which had the lowest malondialdehyde content throughout storage, contained no added fat. Fat was added at 5 % and 30 % to coarsely-ground and finely-ground sausages, respectively. Spices added to meat products can also exert antioxidant effects (Pyrcz and Kowalski, 2005; Martinez et al. 2006b).

Two-way ANOVA revealed that changes in the TBARS values of coarsely-ground and finely-ground sausages were significantly affected by the type of atmosphere and storage time, and the interaction between the two factors. Storage time had a significant effect on the malondialdehyde content of semi-coarsely ground sausages (Tab. 2).

Throughout storage, sausages packaged under vacuum and in gas atmosphere composed of carbon dioxide and nitrogen had TBARS values lower than 1 mg malondialdehyde per kg

TABLE 1: *Changes in the malondialdehyde content (mg/kg) of coarsely-ground, semi-coarsely ground and finely-ground sausages during storage (4 °C).*

Packaging method	Storage (4 °C) period (days) 0	3	6	9	12	15
Coarsely-ground sausages						
P	0.64^{c}	0.66^{ab}	0.70^{1ab}	0.70^{ab}	0.78^{1bc}	0.85^{1a}
A1	0.64^{b}	0.66^{ab}	0.66^{12ab}	0.68^{a}	0.70^{2a}	0.71^{2a}
A2	0.64^{c}	0.65^{ab}	0.66^{12ab}	0.67^{ab}	0.68^{2bc}	0.70^{2a}
A3	0.64^{b}	0.64^{b}	0.65^{2ab}	0.66^{ab}	0.66^{2ab}	0.68^{2a}
Semi-coarsely ground sausages						
P	0.58^{b}	0.59^{b}	0.60^{b}	0.60^{b}	0.62^{a}	0.63^{a}
A1	0.58	0.59	0.60	0.60	0.61	0.61
A2	0.58	0.59	0.60	0.60	0.61	0.61
A3	0.58	0.59	0.59	0.60	0.60	0.61
Finely-ground sausages						
P	0.66^{f}	0.68^{e}	0.69^{d}	0.69^{1c}	0.70^{1b}	0.72^{1a}
A1	0.66^{e}	0.67^{d}	0.68^{c}	0.69^{12bc}	0.70^{1b}	0.71^{2a}
A2	0.66^{f}	0.67^{e}	0.68^{d}	0.69^{12c}	0.70^{1b}	0.71^{2a}
A3	0.66^{e}	0.67^{d}	0.68^{cd}	0.68^{2cb}	0.69^{2b}	0.70^{2a}

$^{a, b}$: mean values in lines denoted with different letters are statistically significantly different ($\alpha = 0.05$); $^{1, 2}$: mean values in columns denoted with different letters are statistically significantly different ($\alpha = 0.05$); 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.

TABLE 2: *Effect of modified atmosphere and storage time on changes in the malondialdehyde content of sausages.*

Variable	Coarsely-ground sausages		Semi-coarsely ground sausages		Finely-ground sausages	
	F	P	F	P	F	p
Atmosphere	15.96	<0.001*	0.99	0.400	19.66	<0.001*
Time	15.69	<0.001*	0.62	<0.001*	279.34	<0.001*
Interaction	2.71	0.001*	0.27	0.997	2.00	0.002*

*: significant at $\alpha = 0.05$; F: Fisher's F test; p: probability at $p < 0.05$.

of sample, which indicates that they were fit for human consumption after 15 days of storage. This finding is in agreement with previous research (Martínez et al., 2006a, 2006b).

Conclusions

The observed minor changes in the TBARS values of the analyzed sausages indicate that the storage of meat products under modified atmosphere slows down the rate of lipid oxidation, which is highly desirable. Optimal results were obtained when meat products were stored in an atmosphere with the highest concentrations of carbon dioxide that exerts antioxidant effects and delays lipid oxidation in meat.

The malondialdehyde content of meat products is affected by the composition and grinding degree of raw material. Throughout storage, coarsely-ground sausages were characterized by the lowest oxidative stability due to their composition and larger diameter, which improved the steam and meat juice permeability of casings.

Conflict of interest

The authors declare that no conflicts of interest exist.

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Address of corresponding author:
Małgorzata Stasiewicz, PhD, Eng
Department of Animal Nutrition and Feed Science
University of Warmia and Mazury in Olsztyn
Oczapowskiego 5
10-718 Olsztyn-Kortowo
Poland
mstasiewicz76@o2.pl