Journal of Food Safety and Food Quality 63, Heft 2 (2012), Seiten 25–56

Arch Lebensmittelhyg 63,
42–46 (2012)
DOI 10.2376/0003-925X-63-42

ISSN 0003-925X

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Changes in the pH and microbiological parameters of coarsely-ground cooked sausages stored under different modified atmosphere conditions

Veränderungen ausgewählter Qualitätsmerkmale bei unter Schutzatmosphäre gelagerten grobzerkleinerten Brühwürsten

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Summary

Modified atmosphere packaging (MAP) is a modern preservation method used for prolonging the shelf-life of fresh and minimally processed foods. Modified atmosphere packaging 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. Since modified atmosphere packaging has a steadily growing number of applications, the objective of this study was to determine the effect of meat product storage under modified atmosphere of different composition (vacuum, CO_2 and N_2) on changes in selected quality attributes, and to indicate the most beneficial storage method. The experimental materials comprised coarsely-ground cooked sausages. The sausages were produced in accordance with standard procedures and technologies, and were packaged under 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 samples were stored at around 4 °C (±1 °C) for 15 days. The pH values were determined and a microbiological analysis was performed immediately after the production process (day 0) and on day 6 and 15 of the storage period.

Modified atmosphere packaging of processed meat products is a beneficial method of storage, as it does not cause significant changes in pH. The microbiological quality of the examined products was compliant with the relevant standards over the entire storage period in all types of the applied atmosphere. The results of the study indicate that modified atmosphere packaging combined with improvements in the chill chain contribute to prolonging the storage life of meat products.

Keywords: processed meat products, storage, modified atmosphere, pH, microbiological quality

Zusammenfassung

Das Verpacken unter modifizierter Gasatmosphäre ist eine Erweiterung des seit langem angewandten Vakuum-Verpackungssystems. Es beruht auf der Veränderung der Zusammensetzung der das Produkt umgebenden Atmosphäre mit entsprechend ausgewählter Gasmischung. In Bezug auf die Tatsache, da die Verpackung von Fleischwaren unter modifizierter Atmosphäre immer weiter verbreitet ist, hatte die durchgeführte Untersuchung zum Ziel, Veränderungen der Qualitätsmerkmale von Fleischerzeugnissen zu bestimmen, die unter modifizierter Atmosphäre unterschiedlicher Zusammensetzung gelagert werden.

Als Untersuchungsmaterial dienten grob zerkleinerte Brühwürste. Die Würste wurden nach den Standardrezepturen und -technologien hergestellt. Nach Ende des Herstellungsprozesses wurden sie in Behälter unter modifizierter Atmosphäre verpackt.

Es wurden 4 Varianten untersucht: Vakuum (P) bzw. 20 % CO_2, 80 % N_2 (A1) bzw. 50 % CO_2, 50 % N_2 (A2) oder 80 % CO_2, 20 % N_2 (A3). Die Verarbeitungsprodukte wurden bei ca. 4 °C (±1 °C) 15 Tage lang gelagert. Während der Lagerung hat man den pH-Wert überprüft sowie eine mikrobiologische Bewertung der Fleischverarbeitungsprodukte an Tag 0, 6 und 15 der Lagerungsdauer durchgeführt.

Das Verpacken von Fleischprodukten unter modifizierter Atmosphäre hat keine signifikanten Veränderungen des pH-Wertes verursacht. Die mikrobiologische Qualität der getesteten Erzeugnisse erfüllte die Normanforderungen für Brühwürste während der gesamten Lagerzeit in allen Arten von Verpackungen. Die

durchgeführten Untersuchungen ergaben, dass die Verpackung von Fleischprodukten unter modifizierter Atmosphäre mit der gleichzeitigen Einhaltung der Kühlkette eine effektive Methode der Haltbarkeitsverlängerung ist.

Schlüsselwörter: Fleischprodukte, Lagerung, modifizierte Atmosphäre, pH-Wert, mikrobiologische Qualität

Introduction

Quality attributes determine the processing suitability and consumer perception of meat products. Cooked sausages are particularly susceptible to microbial growth due to their specific chemical composition – they have a high moisture content (Liserre et. al., 2002).

Microbial growth during the storage of meat products leads to an increase in acidity and protein breakdown, thus deteriorating their sensory quality and nutritional value. The initial composition of microflora in meat is affected by product type and packaging method (Liserre et al., 2002). A number of packaging methods are used to reduce or eliminate the causative agents of meat spoilage, including microbial growth, drying, oxidation of hem pigments and lipids. Modified atmosphere packaging provides a viable alternative to traditional and vacuum packaging (which is a form of modified atmosphere packaging). The use of this technique has a beneficial effect on the microbial and chemical stability of meat, and on the quality characteristics of the end product (Paleari et al., 2004). The combined effect of low storage temperature and modified atmosphere packaging on the microbial stability of meat is greater than the effect of either method applied alone (Hou et al., 1998; Krala, 1999). In modified atmosphere packaging, carbon dioxide levels above 5 % can slow down the growth of bacteria. Gram-negative bacteria are more susceptible to carbon dioxide than Gram-positive bacteria (Devlieghere and Debevere, 2000). Effective control of microbial growth in meat products stored in a modified atmosphere is determined by the initial composition of microflora, the quality of additives used, sanitary conditions during the production process, the composition of modified atmosphere and storage temperature.

The objective of this study was to determine the effect of storage time and modified atmosphere compositions on changes in the pH and microbiological quality of cooked sausages.

Materials and Methods

Sausage processing

The experimental materials comprised coarsely-ground cooked 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 pork of class I and III and raw beef of class I and II was diced into 50 mm/50 mm cubes, dry cured for 24 hours at a temperature of 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 a homogeneous mixture was obtained. 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

The sausages were cooled to 20 ˚C under cold water, and then they were further cooled in the air to a temperature below 4 ˚C (±1 ºC). After 24 hours of cooling at around 4 ºC (±1 ºC), the products were packaged under modified atmosphere with the following compositions: vacuum (V) or 20 % CO_2, 80 % N_2 (A1) or 50 % CO_2, 50 % N_2 (A2) or 80 % CO_2, 20 % N_2 (A3). 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). Approximately 0.5 kg sausage was placed in each bag. The products were packaged with the use of the Multivac A300 (Wolfertschwenden, Germany) device and were stored at around 4 ºC (±1 °C) for 15 days. The experiment was performed in six series. Three samples were collected and analyzed each time. Measurements were performed immediately after the production process (day 0) and on day 6 and 15 of the storage period.

Analytical methods

- **pH** was measured using a 340/ION-SET pH-meter with a SenTix 21 combination electrode (PN – ISO 2917: 2001; PN – ISO 2917: 2001/ Ap 1: 2002).
- **Microbiological analysis**

A microbiological analysis was performed by standard methods, using Merck culture media (Merck, Darmstadt, Germany). 10 g specimens were taken from each samples in triplicate. 10 g analytical samples were mixed with 90 m^3 saline solution and homogenized in a stomacher (Model 400, Seward Medical, London, UK) for 2 minutes. Tenfold serial dilutions (10^{-1} to 10^{-5}) were prepared. The dilutions were plated and incubated (PN-EN ISO 6887-2: 2005). The

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counts of the following microbial groups were determined in the samples:

- mesophilic aerobic bacteria – nutrient agar, incubation temperature – 30 °C, incubation time – 72 hours (PN-ISO 15214: 2002),
- psychrotrophic bacteria – nutrient agar, incubation temperature – 6.5 °C, incubation time – 7 days (PN-ISO 17410: 2004),
- coliforms – Chromocult Coliform Agar, incubation temperature – 30 °C, incubation time – 48 hours (PN-ISO 4831: 2007),
- *Escherichia coli* – Chromocult Coliform Agar, incubation temperature – 30 °C, incubation time – 48 hours (PN-ISO 7251: 2006),
- acidifying bacteria – China Blue Lactose Agar (Demeter), incubation temperature – 30 °C, incubation time – 48 hours (PN-ISO 15214: 2002),
- spore-forming anaerobic bacteria of the genus *Clostridium* – Meat Lever, incubation temperature – 37 °C, incubation time – 48 hours; before plating, pasteurization was carried out at 80 °C for 15 minutes to destroy vegetative cells (PN-EN ISO 7937: 2005),
- fungi – YGC Agar, incubation temperature – 25 °C, incubation time – 96 hours (PN-ISO 7954: 1999),
- *Salmonella* spp.– MSRV, incubation temperature – 37 °C, incubation time – 24 hours (PN-EN ISO 6579: 2003; PN-EN ISO 6579: 2003/ A1: 2007),
- *Staphylococcus aureus* – Baird-Parkera, incubation temperature – 37 °C, incubation time – 24 hours (PN-EN ISO 6888-1: 2001; PN-EN ISO 6888-1: 2001/A1: 2004).

Bacterial counts were expressed as arithmetic means in colony-forming units per g of the product (cfu/g).

Statistical analysis

The results were processed statistically. Arithmetic means ($\bar{x}$) and the standard error of the mean (SEM) were calculated. Mean values were compared by the Newman-Keuls test at a significance level of $\alpha = 0.05$. Calculations were performed with the use of STATISTICA 6.0 PL software (Stanisz, 1998).

Results and Discussion

Changes in pH

Table 1 presents the average pH values of experimental sausages stored for 15 days under different modified atmospheres. The initial pH of coarsely-ground sausages chilled to 4 °C was 6.39. Inconsiderable changes in pH levels were observed over storage. Storage time and modified atmosphere had no significant effect on changes in the pH of sausages. After 15 days of modified atmosphere storage, the pH of sausages was similar to the initial values (Tab. 1).

According to published data, carbon dioxide used in modified atmosphere packaging dissolves and forms carbonic acid, thus lowering the pH of meat products (Cilla et al., 2006; Gajewska-Szczerbal, 2005; Juncher et al., 2003; Martinez et al., 2005). As demonstrated by Parra et al. (2010), the pH of sausages stored under different modified atmospheres (vacuum or 20 % CO_2, 80 % N_2 or 40 % CO_2, 60 % N_2 or 30 % CO_2, 70 % N_2 or 30 % CO_2, 70 % argon) at around 4 °C for 120 days increased insignificantly. Also

TABLE 1: *Changes in the pH* of coarsely-ground cooked sausages during storage at 4 °C for 15 days (n = 6).*

Packaging method	Storage period (days) 0	6	15
P	6.39	6.35	6.33
A1	6.39	6.32	6.35
A2	6.39	6.32	6.33
A3	6.39	6.33	6.34
SEM	0.02	0.02	0.02

*: mean values in the Table are not significantly different (a = 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, SEM: standard error of the mean.

Mingardi and Desenzani (1993) observed minor changes in the pH of meat products 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. The results of the present study indicate that packaging method has little effect on the pH of meat products stored in a modified atmosphere.

Microbiological quality

Before packaging, the counts of aerobic mesophilic bacteria in coarsely-ground sausages were 9.10 x 10^2 cfu/g. After six days of storage at 4°C, their counts increased by one order of magnitude in all types of package. After 15 days of storage, the counts of aerobic mesophilic bacteria were similar in all sausages, regardless of the type of modified atmosphere packaging (Tab. 2).

Prior to packaging, the counts of psychrotropic bacteria in experimental sausages were below 10 cfu/g, and they tended to increase over storage. On the last day of storage, the counts of psychrotropic bacteria were one to three orders of magnitude higher compared with the initial values. There were no statistically significant differences ($\alpha = 0.05$) between storage days in this respect (Tab. 2).

The initial counts of coliforms in coarsely-ground sausages were below 10 cfu/g, and they remained at this level for the first six days of storage. After 15 days of storage, coliform numbers increased in sausages packaged under modified atmosphere A2 (50 % CO_2, 50 % N_2), and they remained unchanged (relative to the initial values) in the other packages (Tab. 2).

The storage of coarsely-ground sausages under different modified atmospheres inhibited the growth of coliforms. The current results are consistent with the findings of other authors. In a study by Parra et al. (2010), total coliform bacteria counts did not increase in meat products stored for 120 days at around 4 °C under different modified atmospheres (vacuum or 20 % CO_2, 80 % N_2 or 40 % CO_2, 60 % N_2 or 30 % CO_2, 70 % N_2 or 30 % CO_2, 70 % argon). Similar observations were made by Pikul et al. (1997).

In the present study, the counts of *Escherichia coli* in experimental sausages remained below 10 cfu/g throughout storage under different modified atmospheres at 4 °C (Tab. 2a).

Our results corroborate those reported by other authors. According to Tyszkiewicz (1992), carbon dioxide used in modified atmosphere packaging suppresses the growth of *Escherichia coli.*

Before packaging, the counts of acidifying bacteria in coarsely-ground sausages were 45 cfu/g, and they increased during storage by two to three orders of magnitude. No

TABLE 2: *Microbiological quality* of coarsely-ground cooked sausages during storage at 4 ºC for 15 days (n = 6).*

Microbial groups	Packaging method	Storage period (days) 0	6	15
Mesophilic aerobic bacteria	P	9.10 x 10^2	4.2 x 10^3	2.5 x 10^3
	A1	9.10 x 10^2	4.17 x 10^3	3.45 x 10^3
	A2	9.10 x 10^2	7.69 x 10^3	3.64 x 10^3
	A3	9.10 x 10^2	4.99 x 10^3	6.10 x 10^3
	SEM	13.02	300.32	277.19
Psychrotrophic bacteria	P	<10	6.0 x 10^3	3.0 x 10^3
	A1	<10	<10	7.8 x 10^2
	A2	<10	1.2 x 10^2	2.0 x 10^3
	A3	<10	8.0 x 10^3	1.7 x 10^3
	SEM	–	733.61	165.67
Coliform bacteria	P	<10	<10	<10
	A1	<10	<10	<10
	A2	<10	<10	18
	A3	<10	<10	<10
	SEM	–	–	7.58

*: 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; SEM: standard error of the mean.

TABLE 2A: *Microbiological quality* of coarsely-ground cooked sausages during storage at 4 ºC for 15 days (n = 6).*

Microbial groups	Packaging method	Storage period (days) 0	6	15
Escherichia coli	P	<10	<10	<10
	A1	<10	<10	<10
	A2	<10	<10	<10
	A3	<10	<10	<10
	SEM	–	–	–
Acidifying bacteria	P	45	2.8 x 10^2	6.2 x 10^3
	A1	45	1.3 x 10^2	1.3 x 10^2
	A2	45	2.4 x 10^2	5.1 x 10^3
	A3	45	1.7 x 10^2	2.0 x 10^3
	SEM	0.17	12.21	357.26
Anaerobic *Clostridium* bacteria	P	12	ND	ND
	A1	12	ND	ND
	A2	12	ND	ND
	A3	12	ND	ND
	SEM	0.24	–	–
Fungi	P	<12	2.3 x 10^2	<10
	A1	<12	30	<10
	A2	<12	3.7 x 10^2	<10
	A3	<12	50	<10
	SEM	–	29.05	–

*: 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; SEM: standard error of the mean; ND: not detected.

statistically significant differences (α = 0.05) were found between storage days (Tab. 2a).

Pexara et al. (2002) investigated changes in the levels of acidifying bacteria in sausages stored under different modified atmospheres (vacuum or 60 % CO_2, 20 % O_2, 20 % N_2 or 0.4 % CO, 80 % CO_2, 19.6 % N_2 or 1 % CO, 80 % CO_2, 19 % N_2 or 0.5 % CO, 24 % O_2, 50 % CO_2, 25.5 % N_2 or 100 % N_2) for two weeks, at 4 °C or 10 °C, and found that the counts of acidifying bacteria increased to a similar degree in all types of packages.

Spore-forming anaerobic bacteria of the genus *Clostridium* were not found in the analyzed samples of sausages throughout storage, irrespective of the packaging method (Tab. 2a).

The initial fungal counts in coarsely-ground sausages were below 12 cfu/g. After six days of storage, they were found to increase by two orders of magnitude in sausages packaged under vacuum and modified atmosphere A2. After 15 days of storage, fungal counts were below 10 cfu/g. The increase in fungal counts in sausages packaged under vacuum and modified atmosphere A2, followed by a decrease on the last day of storage, could result from the composition of modified atmosphere (50 % CO_2, 50 % N_2) which inhibited the growth of fungi (Tab. 2a).

Pathogenic bacteria of the genus *Salmonella* and the species *Staphylococcus aureus* coag (+) were not identified in any of the analyzed samples. According to literature data, the growth of *Staphyloccocus aureus* is inhibited in a modified atmosphere containing 50 % or 100 % CO_2 at 10 °C, while the growth of *Salmonella* sp. is suppressed in a modified atmosphere composed of 60 % CO_2 and 40 % O_2 and in vacuum packages (Tyszkiewicz, 1992). Total bacterial counts in experimental sausages remained in the acceptable range. The counts of aerobic mesophilic bacteria were lower than 10^5 cfu/g (the maximum permissible level is 5 x 10^5 cfu/g, Journal of Laws 2001).

The counts of coliforms and spore-forming anaerobic bacteria were below 10 cfu/g in cooked sausages packaged under different modified atmospheres (Journal of Laws, 2001). Throughout storage, the microbiological quality of sausages was satisfactory and compliant with the relevant standards, due to the high quality of raw materials and additives used in the production process, and the assurance of optimum sanitary and storage conditions. The microbiological quality of sausages was high before packaging. Modified atmosphere storage did not elevate total bacterial counts to harmful levels that could pose a threat to human health – quite the opposite, it inhibited bacterial growth, thus prolonging the shelf-life of the studied products. The microbiological quality of modified atmosphere-packaged sausages remained unchanged during a 15-day storage period. The composition of the protective atmosphere was highly desirable. Carbon dioxide inhibits the growth of bacteria and molds, whereas nitrogen removes residual oxygen from the package, thus preventing the growth of aerobic bacteria and molds as well as lipid oxidation (Gajewska-Szczerbal, 2005).

The results of our study show that modified atmosphere packaging contributes to extending the shelf-life of meat products, and it does not lead to a significant increase in microbial counts.

Conclusions

The microbiological quality of cooked sausages packaged under differed modified atmospheres was comparable over the entire cold storage period, and compliant with the relevant standards. Modified atmosphere packaging combined with improvements in the chill chain contribute to prolonging the storage life of meat products.

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