Effects of modified atmosphere packaging on an extended-spectrum beta-lactamase–producing *Escherichia coli*, the microflora, and shelf life of chicken meat

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ABSTRACT The effects of modified atmosphere packaging on an extended-spectrum beta-lactamase–producing *Escherichia coli* strain and product quality characteristics of skinless chicken meat were determined. The samples were packed separately in air, 100% N_2, and prefabricated gas mixtures with 75% O_2 + 25% CO_2 and 70% CO_2 + 15% O_2 + 15% N_2 and incubated at 3°C for 7 d. To investigate the influence of the headspace ratio, samples were packed in identical trays to 600 g and 120 g. After 0, 2, 5, and 7 d of incubation, the samples were analyzed microbiologically and photometrically, and pH was measured. The results show that the development of the microorganisms depends on the atmosphere, with the 70% CO_2 + 15% O_2 + 15% N_2 atmosphere having the highest development-inhibiting effect. This effect is increased with increased headspace. No significant effects on the pH and color of the samples were observed.

Key words: ESBL-producing *E. coli*, chicken meat, shelf-life, modified atmosphere packaging

INTRODUCTION

In 2017, more than 11.2 billion tons of chicken meat (carcass weight) were consumed in the European Union (Statista, 2018). The typical microflora on chicken carcasses consists of shelf life-decreasing microorganisms, pathogenic microorganisms or facultative pathogens such as *Campylobacter* spp., *Salmonella* spp., *Arcobacter* spp., *Helicobacter* spp., *Pseudomonas* spp., *Escherichia coli* (*E. coli*), and other bacteria (Mead, 2004; Nieminen et al., 2012; Bhaisare et al., 2014; Rouger et al., 2017). In particular, *Salmonella* spp. and *E. coli* are able to carry antibiotic resistance genes such as the extended-spectrum beta-lactamase (**ESBL**) gene (EFSA Panels on Biological Hazards [BIOHAZ], 2012). As a result, these microorganisms can deactivate beta-lactam antibiotics such as cephalosporin, penicillin, and monobactam, which are commonly used in human medicine (Bush et al., 1995; Sturenburg and Mack, 2003; Paterson and Bonomo, 2005; Dhanji et al., 2010). This antibiotic resistance is mainly encoded on plasmids, which facilitates horizontal interspecies gene transfer to other microorganisms to confer antibiotic resistance (Pfeifer et al., 2010).

Zarfel et al. (2014) observed ESBL-producing *E. coli* with a prevalence of 42% in Austrian chicken meat (Zarfel et al., 2014). In Germany, out of 418 retail chicken meat samples, 137 (33%) were contaminated with ESBL-producing *E. coli* in 2016 (BVL, 2017). A Dutch study compared the prevalence of ESBL-producing bacteria in organic and conventional retail chicken meat. These results showed a higher prevalence in conventionally produced chicken meat samples (100%) than in organically (84%) produced ones (Stuart et al., 2012).

Different nonthermal methods are known to reduce the development or number of microorganisms on food, for example, active packaging and the use of nonthermal plasma (Conrads and Schmidt, 2000; Panea et al., 2014; Dohlen et al., 2016; Lee et al., 2017; Yildirim et al., 2017; Souza et al., 2018; Moutiq et al., 2020).

Furthermore, modified atmosphere packaging (**MAP**) has become a routinely used nonthermal technology for food preservation because of its potential for increasing the shelf-life of many kinds of food products as compared to air packaging (Daniels et al., 1985; Farber, 1991; Lambert et al., 1991). Typically oxygen (O_2), nitrogen (N_2), and carbon dioxide (CO_2) gases are used in MAP (Air Products, 2015; Dansensor, 2018).

Oxygen is responsible for several undesirable reactions in food, including oxidation of fats and oils. Color

changes and spoilage due to aerobic microbial development are also induced by oxygen. Oxygen generally promotes the development of aerobic microbes but inhibits the development of anaerobic microorganisms (He and Hoseney, 1990; Jayasingh et al., 2002; Seydim et al., 2006). Owing to the negative effects of oxygen on the preservation of food quality, it is generally avoided in the MAP of many products (Paul and Pandey, 2014). But in the case of meat, especially red meat, a high oxygen concentration is used to preserve the red color (Jayasingh et al., 2002; Dansensor, 2018).

Nitrogen is an inert and tasteless gas, without any antimicrobial activity in food products, similar to the noble gas argon (Herbert et al., 2013). It is not very soluble in water and is primarily used to displace oxygen and prevent package collapse (Floros and Matsos, 2005).

Carbon dioxide is soluble in water and lipids, and with decreasing temperatures, its solubility increases. This characteristic can result in package collapse (Gill and Penney, 1988). CO_2 slows down the respiration of many products and reduces the growth of different microorganisms on poultry meat by inhibiting their metabolism and, therefore, cell division (Lee et al., 1991). When dissolved in the cellular fluids, CO_2 freely permeates the bacterial membrane. Once inside the cells, it can form bicarbonate ions (HCO^3) and liberate protons. To maintain the internal pH, protons are actively transported out of the cells, resulting in the dissipation of energy (Dixon and Kell, 1989; Ma et al., 2017). Because of complex effects of carbon dioxide on bacterial development, different inhibitory mechanisms have been described, including the alteration of cell membrane function, decreases in the rate—or complete inhibition—of enzymatic reactions, changes in the physicochemical characteristics of proteins, and intracellular changes of the pH (Parkin et al., 1982; Daniels et al., 1985; Dixon and Kell 1989; Farber 1991).

The optimal concentration of each gas depends on the matrix, the target microorganism, and the desired effect. To increase the positive and reduce the negative effects to the matrix and the microbiome, the optimal gas concentration must be determined for each product (Floros and Matsos, 2005).

To test the effect of MAP on an ESBL-producing *E. coli*, the present study investigated the effect of 3 different modified atmospheres (**MA**) compared with atmospheric air. The first MA consisted of 75% O_2 and 25% CO_2. These concentrations are similar to typical MA conditions in poultry packaging, with 70% O_2 and 30% CO_2 or 80% O_2 and 20% CO_2 being typical (Air Products, 2015; Dansensor, 2018). The second MA consisted of 100% N_2. The inert properties of N_2 had the effect of simulating a skin package without changes in the study design. In addition, dissolved gases from inside of the samples were diluted and less available for the microorganisms. The third MA consisted of 70% CO_2, 15% O_2, and 15% N_2. This MA was described as being able to reduce the number of *E. coli* on chicken drumsticks by 2 log cfu g^{-1} after 7 d of incubation at 3°C (Al-Nehlawi et al., 2013).

MATERIALS AND METHODS

Strain Used

An ESBL (TEM-52)-producing strain of *E. coli* 10,714 assigned to phylogroup B1, isolated from a chicken meat sample, was used in this study. The strain was stored at −20°C in Luria-Bertani Miller broth (Merck Millipore, Darmstadt, Germany) containing 25% (vol/vol) glycerol and cultured at 37°C on tryptone soya agar (Oxoid, Wesel, Germany) for 24 h. Colony material was packaged in Luria-Bertani to a concentration of 7–8 log cfu mL^{-1}. The concentration was verified by inoculating 50 μL of appropriate dilutions in maximum recovery diluent (**MRD**) (Merck) by the drop-plating method on Tryptone Bile X-glucuronide agar (TBX-Agar) (Oxoid). Incubation conditions were 37°C for 24 h. The suspension was stored at 4°C for 24 h until use in the experiments.

Chicken Meat Weight/Volume Ratio

For this study, 2 different types of meat were obtained from a local slaughterhouse directly after slaughter: fresh chicken breast filets and inner breast filets. These products are expected to show similar slaughtering- and cutting-caused surface contaminations in terms of type and amount of contamination per gram. Samples were immediately chilled and maintained as such during transport to the laboratory. The chicken breast filet samples were packaged according to common industrial specifications. Approximately 600 g chicken breast filet (2-3 pieces per package) were sealed under gas in trays with a final volume of approximately 860 mL. The samples were weighed by the chicken slaughter company. These samples were designated as "regular headspace." To examine the influence of the headspace ratio, the amount of chicken meat was reduced to approximately 120 g per package, by packaging 3 pieces of inner breast filet. The volume of the package was maintained at 860 mL. These samples were designated as "increased headspace."

Sample Preparation and Packaging

Chicken breast filets from each 600-g package were cut into 6 pieces (each approximately 100 g) and transferred into a new tray. Three of these pieces were inoculated with *E. coli* 10,714. One of the 3 uninoculated pieces was used for the color and the pH measurement on the subsequent sampling days. On the remaining 2 pieces, the development of the microbiological contamination was investigated.

For tests with increased headspace, 2 inner filets were halved. Two of these pieces (each approximately 20 g) were inoculated with *E. coli* 10,714. The third inner filet was used for the color and the pH measurements.

The experimental meat samples were inoculated to a concentration of 4–5 log cfu g^{-1}. Directly after inoculation, the samples were packed under the specific gas

mixtures. The gas mixtures used were atmospheric air (air), an oxygen-dominated gas mixture with 75% O_2 + 25% CO_2 (O_2), a carbon dioxide–dominated gas mixture with 70% CO_2 + 15% O_2 + 15% N_2 (CO_2), and 100% nitrogen (N_2). The packaged samples were stored at 3 ± 1°C.

Sampling

Samples were analyzed on day 0, 2, 5, and 7 after packaging. On day 0, the initial bacterial count was examined on 2 samples before packaging. At each following sampling day, 2 trays held under the same incubation conditions were examined. One piece of meat from each package was examined for color and pH measurement. For examination of *E. coli* 10,714 and bacterial contamination, 2 pieces for each were sampled out of each tray. Tests were repeated 3 times independently for each gas and headspace ratio.

Microbiological Analysis For the microbiological assessment, samples were analyzed after 0, 2, 5, and 7 d of incubation. Two samples from each tray were analyzed separately for enumeration of total viable aerobic bacteria (**TAB**), *Pseudomonas* spp., *Enterobacteriaceae*, and *E. coli* 10,714. On day 0, the initial number of *E. coli* was analyzed on uninoculated meat samples. Only batches of meat with less than 2 log cfu *E coli* g^{-1} were used for this study. Directly after opening the packages, samples were placed in stomacher bags (Interscience, Saint Nom, France). The higher weight pieces of chicken breast filets (approximately 100 g) were homogenized in 100-g MRD. The lower weight pieces of inner breast filets (approximately 20 g) were homogenized in 20-g MRD. All samples were homogenized for 4 min in a bag mixer (Interscience). Thereafter, a decadal dilution series in MRD + 0.75 g Agar l^{-1} was spread on the following culture media by the drop-plating method (50 μL).

The amount of TAB was determined on tryptone soya agar by incubation at 30.0 ± 1.0°C for 72 ± 2 h with a precounting after 48 h. The *E. coli* 10,714 cell count was determined on TBX-Agar incubated at 37.0 ± 1.0°C for 24 ± 2 h. *Enterobacteriaceae* were determined on Violet Red Bile Glucose agar (Merck), incubated at 37.0 ± 1.0°C for 24 ± 2 h. The number of *Pseudomonas* spp. was determined on glutamate starch phenol red agar (GSP-Agar Pseudomonas Aeromonas selective agar) (Merck), incubated at 28.0 ± 1.0°C for 48 ± 2 h. The initial amount of *E. coli* on day 0 was determined by the spread-plate method using 0.5 mL of homogenate on TBX-Agar, incubated at 37.0 ± 1.0°C for 24 ± 2 h.

The calculations of the results were performed with the respective exact sample weights.

We refer to the growth or cell multiplication of these organisms under the conditions tested throughout the text as "development."

Color Assessment The superficial color on one meat sample out of each package was measured using a colorimeter (Chroma Meter CR-210; Konica Minolta Sensing, Japan). To get representative measurements of the sample, 3 measurements were taken, for each replication,

each time on the surface that was directly in contact with the gas mixture. The assessed parameters were lightness (L*), the balance between green and red (a*), and the balance between blue and yellow (b*).

pH Measurement The pH was measured according to ISO 2917:1999 (ISO, 2917, 1999). After conditioning the meat samples at room temperature (approximately 25 ± 2°C), measurements were performed using a Knick pH meter (Knick laboratory-ph-meter-766; Berlin, Germany), with an insertion electrode (InLab Solids; Mettler Toledo, Mississauga, Canada). The measurement of the pH in high-weight samples was performed in one piece of chicken breast filet at 3 different positions. For the measurement of the pH in low-weight samples, a 40-g piece of inner breast filet was placed into a cup and covered with deionized water.

Statistical Analyses

The described experiment was repeated 3 times separately with each gas and headspace ratio. All results of microbiological investigations beneath the detection limit were set to the respective detection limits of approximately 1.6 log cfu mL^{-1} for the statistical analyses. Results were summarized as the mean count. The statistical analyses of the influence of the present atmosphere and the headspace ratio were performed with the nonparametric Mann-Whitney U test for independent samples using SPSS (IBM SPSS Statistics for Windows, version 21) (Sachs, 2003). Results were considered significant at $P \leq 0.008$ for the influence of the atmosphere by comparing all atmospheres to each other. Multiple testing was taken into account using a corresponding Bonferroni-adjusted significance level of $\alpha < 0.008$ and at $P \leq 0.05$ for the influence of the headspace ratio. A significance level of $\alpha < 0.05$ was applied when analyzing the influence of headspace ratio on the development of microorganisms.

RESULTS

Microbiological Examination

Changes in microbial populations are shown in Figures 1A and 1B. Microbial counts were expressed as differences in the mean on the respective day to the mean on day 0 in Δlog cfu g^{-1}. Significant differences in the development of microorganisms were induced by gas conditions ($P < 0.001$) and the headspace ratio ($P < 0.001$).

Influence of the Atmosphere

Regular Headspace All measurements of bacterial development showed continuously increasing numbers of TAB, *Pseudomonas* spp., and *Enterobacteriaceae* during incubation of the samples at 3°C (Figure 1A). The development of these microorganisms was significantly increased ($P < 0.001$) after 7 d of incubation in the presence of air compared with incubation in the presence of the other gases (Table 1). No significant influence of the other gases on the development of TAB was observed. CO_2 was able to hinder the development of *Pseudomonas* spp.
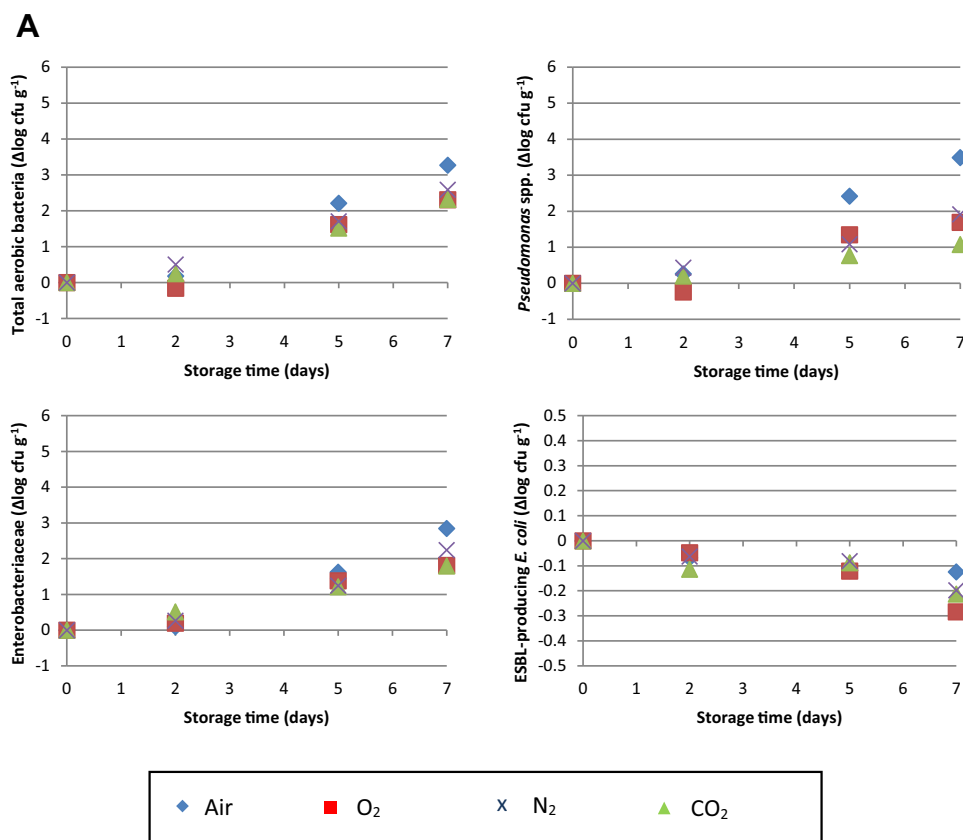
Figure 1. Development of microorganisms. Effect of the atmospheres (air, O_2, CO_2, and N_2) on the development of total viable aerobic bacteria (TAB), *Pseudomonas* spp., *Enterobacteriaceae*, and *E. coli* 10,714 in samples of meat packed to 600 g (A) and 120 g (B) per tray, stored for 7 d at $3 \pm 1°C$. Pictured are the differences in the mean of the respective day from the mean of day 0 in Δlog cfu g^{-1}. D 0-> n = 6; D 2, 5, and 7 -> n = 12.

significantly in comparison to the development under the O_2 atmosphere ($P < 0.008$) and in the presence of N_2 ($P < 0.008$). The development of *Enterobacteriaceae* was also significantly decreased ($P < 0.001$) in the presence of CO_2 as compared to N_2. After 7 d of incubation, the count for *E. coli* 10,714 had decreased under all atmospheres. The greatest decrease of *E. coli* 10,714 was determined in the presence of O_2, although no significant difference induced by the atmospheres was observed.

Increased Headspace Already after 5 d of incubation, the development of TAB, *Pseudomonas* spp., and *Enterobacteriaceae* had increased significantly ($P < 0.001$) in the presence of air as compared to the other gases (Figure 1B, Table 1). Comparing the influence of N_2 to the influences of O_2 and CO_2, the development of TAB was significantly higher ($P < 0.008$) at day 5 and later. While the count for TAB increased continuously in the presence of CO_2, the development of *Pseudomonas* spp. and *Enterobacteriaceae* was inhibited under the same conditions. CO_2 significantly reduced the development of these 3 bacterial groups also already after 5 d of incubation ($P < 0.008$). Similar to the investigations with regular headspace, the count of *E. coli* 10,714 had decreased by day 7. No significant impact by the atmosphere on *E. coli* 10,714 was determined.

Influence of the Headspace Ratio The influences of the headspace ratio to the development of the investigated microorganisms are shown in Table 1. The development of TAB was not influenced by the headspace

ratio in the presence of an O_2 atmosphere. However, the increased headspace significantly facilitated development of TAB under the air and N_2 atmospheres ($P < 0.05$). Furthermore, a significant reduction of the rate of development of TAB was observed in the presence of CO_2 in samples with increased headspace.

While the increased headspace supported the development of *Pseudomonas* spp. in the presence of air significantly ($P < 0.008$) already after 5 d of incubation, O_2 and CO_2 significantly reduced the rate of development ($P < 0.05$) already after 5 d of incubation.

The observations of *Enterobacteriaceae* as a function of the headspace ratio showed a significant ($P < 0.01$) inhibition of their development in the presence of all tested MAs after 5 d of incubation.

No significant influence of the headspace ratio on *E. coli* 10,714 was observed after 7 d of incubation in the presence of all tested MAs. In the presence of air, significant differences ($P < 0.05$) in the development were observed after 2 and 5 d of incubation. While the counts of *E. coli* 10,714 were reduced after 2 and 5 d of incubation with regular headspace, an increase in counts was observed with increased headspace.

pH Assessment

The means of the pH values of all samples under different atmospheric conditions, weight, and incubation times (0, 2, 5, and 7 d) are shown in
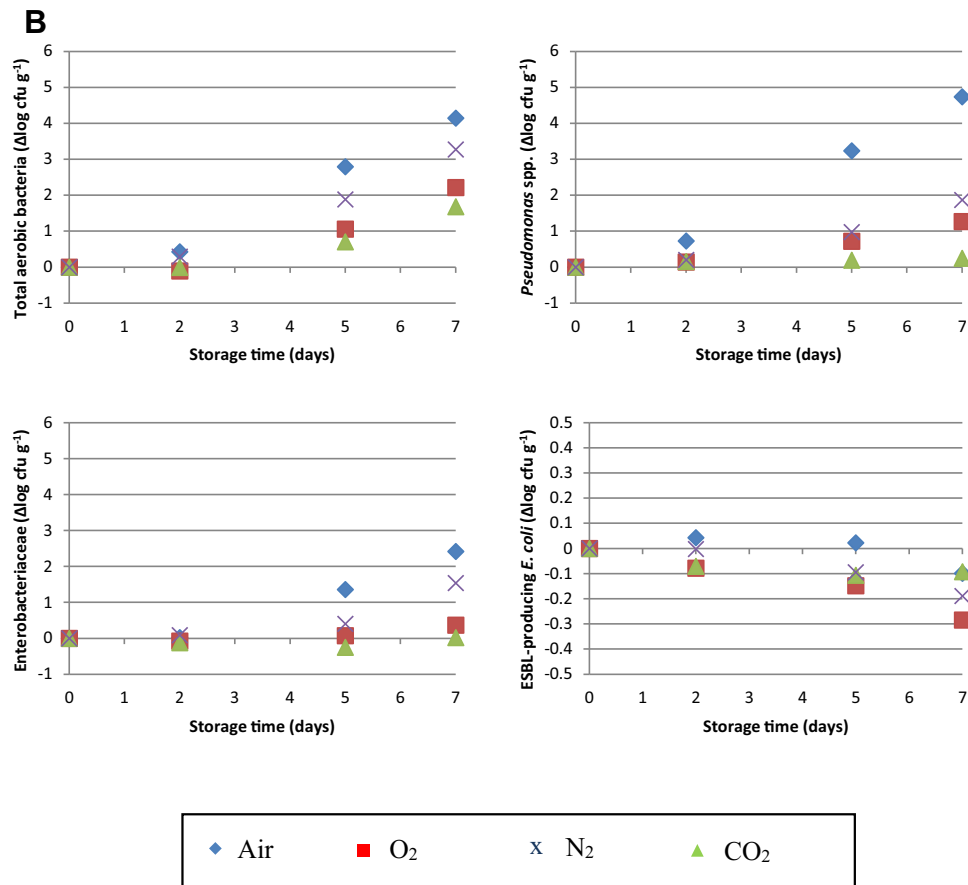
Figure 1. Continued

(Figures 2A and 2B) and Table 2. The initial pH of samples with regular headspace ratio varied between 5.70 in samples packed under O_2 and 5.93 in the samples packed under N_2. After 7 d of incubation, the pH varied between 5.66 in O_2 samples and 5.91 in the N_2 samples. Samples stored in the presence of O_2 showed a significantly reduced ($P < 0.001$) pH in comparison to the influence of the other gases. In comparison to CO_2, the samples stored in N_2 showed an increase ($P < 0.008$) in pH (Table 2).

The initial pH of the samples with increased headspace varied between 5.94 in samples packed under air and O_2 and 6.06 in samples packed under N_2. After 7 d of incubation, the pH varied between 5.87 in CO_2 samples and 5.91 in the N_2 samples. The increased headspace did not result in a significant change in the development of the pH of the meat samples.

Surface Color Measurements

The mean results of the color measurements (Lightness [L*], redness [a*], and yellowness [b*]) are shown in Figure 3. The values of L*, a*, and b* were compared in each possible combination for the respective incubation atmospheres. The significance values of the comparisons between the influences of the individual gases are shown in Table 3.

The initial values of L* of the samples stored with regular headspace ratio varied between 64.50, measured on samples packed in N_2, and 67.90, measured on samples packed in air. After 7 d of incubation, the L* values varied between 64.60 (N_2) and 69.09 (air). The rank order of the respective gases did not change. After 7 d of incubation, the L* values of the meat stored in the presence of N_2 differed significantly ($P < 0.008$) from those of the samples incubated in the presence of the other atmospheres. The a* values were also influenced by the incubation under N_2. There was an increase ($P < 0.001$) in this parameter as compared to the results of the samples incubated in the presence of the other gases after 5 d of incubation. After 7 d of incubation, the b* values varied significantly between almost all individual gases. Except for the comparison of the samples packed under O_2 to the samples packed under CO_2, a significant difference in this parameter was also determined on day 0. After 7 d of incubation, only the comparison of the samples packed under air to the samples packed under O_2 showed no significant differences in the b* values.

The L* values of the samples with increased headspace showed significant differences only between the samples packed under CO_2 and the samples packed under N_2. The a* values differed partially significant since d 0. Only the differences of the values between CO_2 and N_2 have developed to significant differences ($P < 0.001$) during the incubation. After 7 d of incubation, only

Table 1. Influence of the respective atmosphere and the headspace ratio on the development of microorganisms.

Weight per package	Microbiological parameter	Atmosphere	Days of incubation			
			0	2	5	7
600 g	TAB	Air	4.40	4.58	6.61[2]	7.68[a+,b+,c+2]
		O_2	4.68	4.52	6.30[1]	6.99[a+]
		CO_2	4.43	4.68	5.95[1]	6.74[b+1]
		N_2	4.33	4.84	6.04	6.92[c+2]
	Pseudomonas spp.	Air	3.77	4.03[a,1]	6.18[a+,b+,c+1]	7.26[a+,b+,c+2]
		O_2	4.03	3.79[a,b,1]	5.37[a+1]	5.72[a+,d,1]
		CO_2	3.75	3.95	4.52[b+1]	4.83[b+,d,e+1]
		N_2	3.70	4.13[b]	4.79[c+]	5.62[c+,e+]
	Enterobacteriaceae	Air	2.12	2.22	3.45[a,1]	4.97[a+,b+,c+1]
		O_2	2.24	2.44	3.38[2]	4.05[a+2]
		CO_2	2.14	2.65[1]	3.28[a,2]	3.93[b+,d,2]
		N_2	2.13	2.40	3.26[1]	4.37[c+,d,2]
	E. coli 10,714	Air	7.66	7.59[1]	7.54[1]	7.53
		O_2	7.60	7.55	7.48	7.31
		CO_2	7.71	7.60	7.63	7.50
		N_2	7.90	7.83	7.81	7.70
120 g	TAB	Air	3.01	3.43[a,b]	5.79[a+,b+,c+1]	7.15[a+,b+,c+2]
		O_2	3.16	3.05[a]	4.21[a+,d,1]	5.37[a+,d+]
		CO_2	3.13	3.12[b]	3.83[b+,e+1]	4.80[b+,e+1]
		N_2	3.14	3.43	5.03[c+,d,e+]	6.41[c+,d+,e+2]
	Pseudomonas spp.	Air	2.12	2.85[a,b,c,1]	5.36[a+,b+,c+1]	6.86[a+,b+,c+2]
		O_2	2.12	2.27[a,1]	2.84[a+1]	3.39[a+,d+1]
		CO_2	1.86	2.02[b]	2.05[b+,d,1]	2.10[b+,d+,e+1]
		N_2	2.13	2.33[c]	3.11[c+,d]	3.99[c+,e+]
	Enterobacteriaceae	Air	1.75	1.77	2.83[a+,b+,c+1]	4.16[a+,b+,c+1]
		O_2	1.75	1.67	1.82[a+2]	2.12[a+,d+2]
		CO_2	1.74	1.62[1]	1.61[b+,d+2]	1.76[b+,e+2]
		N_2	1.71	1.80	2.03[c+,d+1]	3.25[c+,d+,e+2]
	E. coli 10,714	Air	7.70	7.74[1]	7.72[1]	7.60
		O_2	7.70	7.62	7.55	7.42
		CO_2	7.73	7.66	7.62	7.64
		N_2	7.77	7.77	7.67	7.58

[a-e]Means of microbial population (log cfu g^{-1}). Means with same superscript letter (in the respective bacterial group and weight) suggest significant differences ($P < 0.008$) in the development of the microorganisms. Means with same superscript letter and "+" (in the respective bacterial group and weight) suggest significant differences ($P < 0.001$) in the development of the microorganisms. The statistical analyses were performed with the Mann-Whitney U test by comparing the development of the respective parameter in the presence of the respective atmosphere.

Abbreviation: TAB, total viable aerobic bacteria.

[1]Differences in the mean are significantly ($P < 0.05$) influenced by the headspace ratio.

[2]Differences in the mean are significantly ($P < 0.001$) influenced by the headspace ratio.

samples stored in O_2 and samples stored in CO_2 did not differ significantly from each other ($P < 0.008$).

DISCUSSION

In the present study, the effect of modified packaging atmosphere with different gas mixtures and headspace ratios on an inoculated ESBL-producing *E. coli* strain and the typical microflora on skinless chicken meat was examined. The aim was to identify a gas-headspace combination generating a safer product with an increased shelf life.

In comparison to the development in air, the O_2 atmosphere, similar to common MA used in praxis, slowed the development of TAB and *Pseudomonas* spp. significantly ($P < 0.001$) (Air Products, 2015; Dansensor, 2018). Similar results concerning the development of TAB and *Pseudomonas* spp. were reported by Bingol and Ergun (2011). Their investigations have shown that an MA consisting of 80% O_2 + 20% CO_2 reduces the development of TAB and *Pseudomonas* spp. in comparison to air-packed samples. However, the results of Bingol and Ergun (2011) did not show an increased development of TAB and *Pseudomonas* spp. in the presence of air in

samples packed with increased headspace compared to samples packed with regular headspace, as investigated in the present study. This difference could be linked to the different sampling method we used in comparison to that used in the Bingol and Ergun study. While Bingol and Ergun (2011) have used similar meat samples to examine the influence of the different headspace ratios, whole chicken breast filets and inner filets were used in the present study to achieve the different headspace ratios. These meat parts had different initial microbial loads. CO_2 and N_2 were also able to reduce the development ($P < 0.001$) of TAB and *Pseudomonas* spp. in comparison to air. The most inhibiting effect on *Pseudomonas* spp. in both headspace ratios was observed for CO_2. Compared to the influence of the other atmospheres, this MA significantly reduced the development of *Pseudomonas* spp. ($P < 0.008$). Inhibiting effects on the development of *Pseudomonas* spp. were increased significantly ($P < 0.05$) by an increased headspace in samples packed under O_2 and CO_2. The development of TAB was also decreased ($P < 0.05$) by increased headspace in the presence of CO_2. Inhibiting effects of high-CO_2 atmospheres to TAB and *Pseudomonas* spp. on chicken breast meat, fresh beef, minced beef, and broiler meat have also been
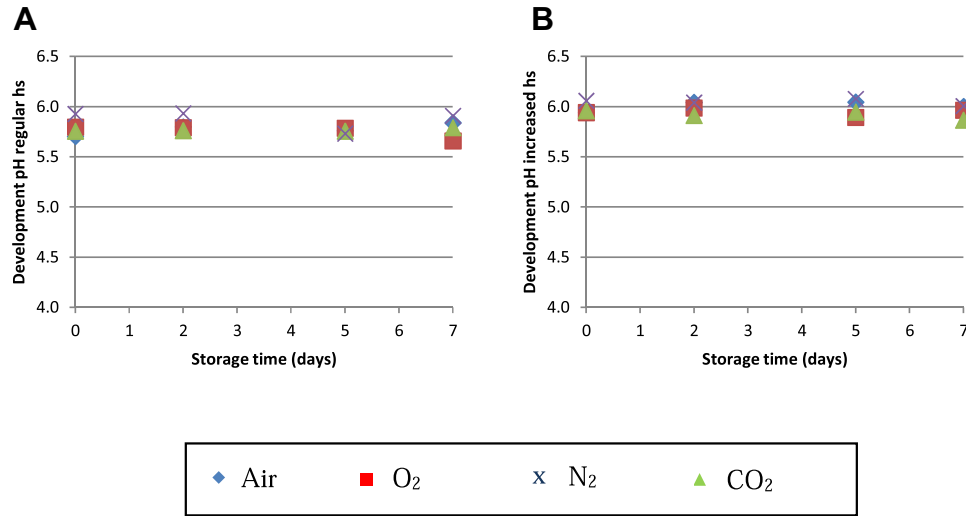
Figure 2. Development of pH. Trend of the pH in samples of chicken meat packaged under different atmospheres with regular headspace (hs) (A) and increased headspace (B), stored for 7 d at $3 \pm 1°C$. D 0- > n = 3; D 2, 5 and 7- > n = 6.

observed in other studies (Skandamis and Nychas, 2002; Chouliara et al., 2007; Esmer et al., 2011; Zhang et al., 2015).

The initial counts for *Enterobacteriaceae* per gram meat were nearly the same in the 600-g and 120-g packages (Table 1), and the differences in development seem to be affected by both the atmospheric conditions and the headspace ratio: In the 600-g packages, the development decreased in the modified atmospheres compared to in air ($P < 0.001$). Comparing the results of incubation with regular to those with increased headspace, the increased headspace was able to reduce the development of *Enterobacteriaceae* ($P < 0.01$) in the presence of all investigated atmospheres. The development of the *Enterobacteriaceae* was completely inhibited in the presence of CO_2 in samples packed with increased headspace. The decelerated development of the *Enterobacteriaceae* in the presence of the 80% O_2 + 20% CO_2 mixture compared to the development in air as well as the decelerating effect of the increased headspace were well described by Bingol and Ergun (2011). Chouliara et al. (2007) described a slower development of *Enterobacteriaceae* on chicken breast meat packed under a high-CO_2 atmosphere compared to the development in air. The

influence of a high-CO_2 atmosphere on the development of *Enterobacteriaceae* on minced beef was described by Esmer et al. (2011) as completely inhibiting during the first 7 d of incubation, with similar findings in a highly concentrated O_2 atmosphere. Also Skandamis and Nychas (2002) have reported that the development of *Enterobacteriaceae* on fresh beef was inhibited by a high-CO_2 atmosphere.

As expected no increase in the *E. coli* 10,714 number induced by the atmosphere nor the headspace ratio was detected in the investigations. A temperature of 3°C was completely inhibiting, and slight decreases in the cell numbers were detected under all testing conditions. The main influencing atmosphere was O_2. The cell number was decreased by approximately 0.3 log cfu g^{-1} after 7 d of incubation in both investigated headspace ratios. These results are similar to those found by Jones et al. (2004). Their investigations have shown a decreased count for *E. coli* caused just by storing at 2°C. Heinrich et al. (2016) also determined a decreased count for *E. coli* inoculated on ham, with values similar to those reported in the present study. But the results of the present study differ from the results of Bingol and Ergun (2011) and Al-Nehlawi et al. (2013). While

Table 2. Influence of the atmosphere on the development of pH.

Weight per package	Modified atmosphere combination	Days of incubation			
		0	2	5	7
600 g	Air	5.7	5.8	5.8	5.8[a]
	O_2	5.8	5.8[a]	5.8	5.7[a-c]
	CO_2	5.8	5.8	5.8	5.8[b,d]
	N_2	5.9	5.9[a]	5.7	5.9[c,d]
120 g	Air	5.9	6.0	6.0	6.0
	O_2	5.9	6.0	5.9	6.0
	CO_2	6.0	5.9	5.9	5.9
	N_2	6.1	6.0	6.1	6.0

[a-d]Means of the pH. The statistical analyses were performed with the Mann-Whitney U test by comparing the respective pH values in the presence of the respective atmosphere. Means with the same superscript letter (in the respective weight) suggest significant differences ($P < 0.008$) of the pH.
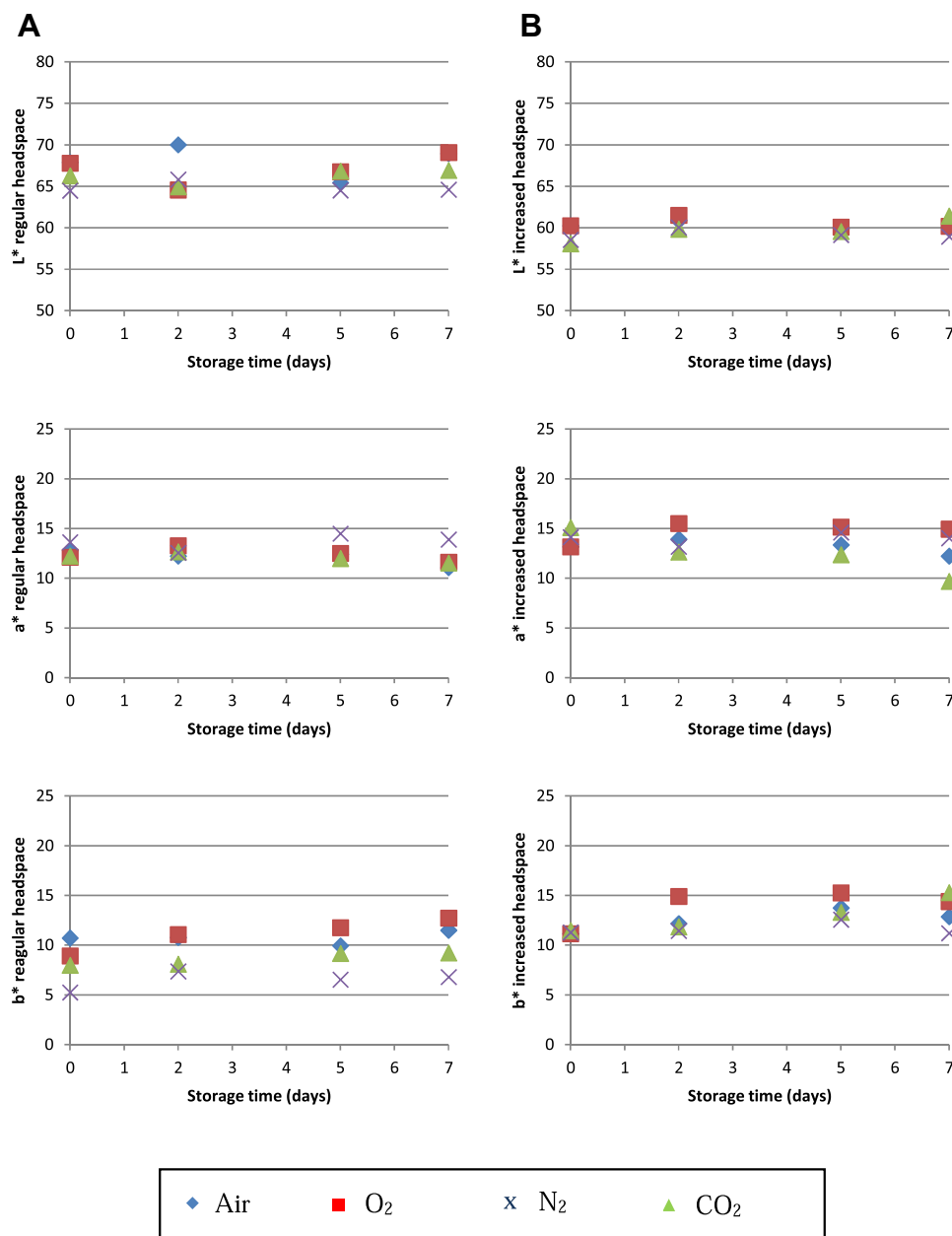
Figure 3. Development of color. Effect of the atmospheres (air, O_2, CO_2, and N_2) on the color parameters L*, a*, and b* in samples of meat packed with regular (A) and increased (B) headspace, stored for 7 d at $3 \pm 1°C$. D 0- > n = 3; D 2, 5, and 7- > n = 6.

Bingol and Ergun (2011) reported increasing *E. coli* counts during storage in all tested atmospheres, Al-Nehlawi et al. (2013) observed lower *E. coli* counts under the same CO_2 atmosphere used in the present study. The reasons for this may be the different set ups compared to the present study. In the present study, the effect of the atmospheres on a known and inoculated *E. coli* strain was investigated. The initial counts of *E. coli* on all samples were known and similar at day 0. Differences from day 0 were therefore not due to different initial cell counts. Furthermore, the incubation temperature in the present study (3°C) was lower than that in the investigations of Bingol and Ergun (2011) (4°C).

Significant differences ($P < 0.008$) were observed in the effect of the atmosphere on the pH in samples packed with regular headspace. The values varied from 5.57 to 5.89 in the mean of the single samples at day 0 and from 5.57 to 5.92 after 7 d of incubation. No significant differences were observed in samples packed with an increased headspace. The pH varied from 5.87 to 6.04 at day 0 and from 5.68 to 6.32 after 7 d of incubation. Therefore, the significance of the regular headspace sample simply resulted from the low standard derivation, not by large differences caused by the atmospheres. The differences between the means of the pH measurements per atmosphere and headspace ratio were less than 0.16 from day 0 to day 7 (regular headspace, O_2) in all pH investigations. Similar observations were also reported by Mbaga et al. (2014). Al-Nehlawi et al. (2013) reported that the incubation of chicken drumsticks under a CO_2 atmosphere decreases the pH from approximately 6.5 to 6.2. Bingol and Ergun (2011)

Table 3. Influence of the atmosphere on the development of color.

Weight per package	Attribute	Modified atmosphere combination	Days of incubation			
			0	2	5	7
600 g	L	Air	67.90	70.03	65.47	68.98[a+]
		O_2	67.79	64.57	66.79	69.09[b+]
		CO_2	66.28	64.98	66.85	66.92[c]
		N_2	64.50	65.86	64.53	64.60[a+,b+,c]
	a	Air	12.81	12.23	12.43[a+]	11.07[a+]
		O_2	12.12	13.28	12.50[b]	11.62[b+]
		CO_2	12.23	12.66	12.00[c+]	11.55[c+]
		N_2	13.63	12.55	14.49[a+,b,c+]	13.90[a+,b+,c+]
	b	Air	10.72[a,b+]	10.79[a,b+]	9.96[a+,b+]	11.51[a,b+]
		O_2	8.93[c+]	11.09[c,d+]	11.79[a+,c+,d+]	12.73[c+,d+]
		CO_2	7.99[a,,d+]	8.11[a,c]	9.17[c+,e]	9.24[a,c+,e]
		N_2	5.27[b+,c+,d+]	7.38[b+,d+]	6.56[b+,d+,e]	6.80[b+,d+,e]
120 g	L	Air	60.26	60.87	59.88	60.07
		O_2	60.26	61.51	60.10	60.20
		CO_2	58.10	59.86	59.57	61.44[a+]
		N_2	58.56	60.06	59.15	58.96[a+]
	a	Air	13.17[a,b]	13.92	13.37	12.24
		O_2	13.17[c,d]	15.51[a+,b+]	15.16[a+]	14.95[a+]
		CO_2	15.11[a,c]	12.62[a+]	12.38[a+,b+]	9.68[a+,b+]
		N_2	14.14[b,d]	13.17[b+]	14.61[b+]	14.03[b+]
	b	Air	11.17	12.18[a]	13.75	12.89[a,b+,c]
		O_2	11.17	14.92[a,b+,c+]	15.25[a,b+]	14.40[a,d]
		CO_2	11.47	11.87[b+]	13.30[a]	15.30[b+,e+]
		N_2	11.30	11.46[c+]	12.58[b+]	11.23[c,d,e+]

[a-d]Means of the color parameters L*, a*, and b*. Means with the same superscript letter (in the respective color parameters group and weight) suggest significant differences ($P < 0.008$) of the color parameters. Means with the same superscript letter and "+" (in the respective bacterial group and weight) suggest significant differences ($P < 0.001$) in the development of the microorganisms. The statistical analyses were performed with the Mann-Whitney U test by comparing the respective color parameter in the presence of the respective atmosphere.

reported a decrease in pH under all investigated atmospheres with both tested headspace ratios, but the increased headspace reduced the differences in pH induced by the atmosphere. The investigations of Lorenzo et al. (2014) have shown that a high-O_2 atmosphere does not influence the pH of pork patties. The results of the present study suggest that the final pH was reached before the chicken meat was packed and investigated in this study. In addition, no further influence of the atmosphere on the meat samples was investigated. The data of the statistical analysis of the comparison between the headspace ratio and the pH are not shown because the initial pH at day 0 differs significantly.

After 7 d of incubation with regular headspace, the L* and a* values differed significantly ($P < 0.008$) only in N_2 compared to the results of the samples packed in presence of the other atmospheres. These N_2 samples were darker and more reddish. Excepting the gas combinations being significantly different since day 0 in b* value, only samples packed in O_2 compared to samples packed in CO_2 developed a significant difference ($P < 0.001$) during incubation, and samples packed in O_2 and with regular headspace were more yellowish. Comparing the L* values of the samples packed with increased headspace, a significant difference ($P < 0.001$) was detected only between CO_2 samples and N_2 samples, with samples packed in CO_2 being brighter. Significant differences in the b* value developed in samples packed in air compared to samples packed in O_2. Samples packed in O_2 became more reddish. Also, samples packed in CO_2 differed from samples packed in N_2, with CO_2 samples

becoming more greenish. With increased headspace, except for the samples packed in O_2 compared to samples packed in CO_2, all comparisons of the influence of the atmosphere on the b* value showed significant differences. With regular headspace, the b* values only of air-packed and O_2-packed meat did not differ significantly. Comparisons of the effect of the headspace would not be purposeful because of significant differences of L*, a*, and b* since the first measurements at day 0 (Table 3). Chouliara et al. (2007) have also found nearly constant L*, a*, and b* values on chicken meat over an incubation period of 12 d.

Seydim et al. (2006) have reported effects on the L* value of ostrich meat in air and in an 80% O_2 + 20% CO_2 atmosphere similar to those of the respective atmospheres in the present study. They described an almost constant L* value over an incubation period of 6 d in the presence of both gases. Bingol and Ergun (2011) also described nearly constant values of L* of ostrich meat packed with 2 different headspace ratios after 7 d of incubation. But in both studies, the a* value decreased over the incubation time of 6 and 7 d, respectively. The b* value also decreased slightly in the presence of air and the 80% O_2 + 20% CO_2 atmosphere in both studies. Slight decreases in the L* and a* values and an increased b* value were reported by Lorenzo et al. (2014) on pork patties packed under a high-O_2 atmosphere. Except for the slightly darker initial color, the a* and b* values were similar to the values measured in the present study. Even when some differences of the influence of the atmosphere on the color parameters L*, a*, and b* were

significant, no unappetizing colors were detected in the present study. The meat was still pink in different nuances and did not become pale or dark.

CONCLUSION

The use of MAP has increased the shelf life of chicken meat. The atmosphere with the highest potential for reducing the development of the corresponding microorganisms is composed of 70% CO_2 + 15% O_2 + 15% N_2. Samples packed with increased headspace have shown an increased shelf life depending on the composition of MA, but no significant decrease of the number of *E. coli* 10,714 has been observed. However, before using the most efficient MA in praxis, it is necessary to investigate the influence on the flavor and the odor of chicken meat during incubation.

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DISCLOSURES

The authors declare that there are no conflicts of interest.

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