Comparison of argon-based and nitrogen-based modified atmosphere packaging on bacterial growth and product quality of chicken breast fillets

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ABSTRACT Poultry fillets were packaged under 6 different gas atmospheres (A: 15% Ar, 60% O_2, 25% CO_2; B: 15% N_2, 60% O_2, 25% CO_2; C: 25% Ar, 45% O_2, 30% CO_2; D: 25% N_2, 45% O_2, 30% CO_2; E: 82% Ar; 18% CO_2; F: 82% N_2, 18% CO_2) and stored at 4°C. During storage, the growth of typical spoilage organisms (*Brochothrix thermosphacta*, *Pseudomonas* spp., *Enterobacteriaceae*, and *Lactobacilli* spp.) and total viable count were analyzed and modeled using the Gompertz function. Sensory analyses of the poultry samples were carried out by trained sensory panelists for color, odor, texture, drip loss, and general appearance. No significant difference in microbiological growth parameters was observed for fresh poultry stored under an argon-enriched atmosphere in comparison with nitrogen, except the *B. thermosphacta* stored under 82% argon. The sensory evaluation showed a significant effect of an argon-enriched atmosphere, particularly on color of meat stored under 15% argon ($P < 0.05$). In contrast, 25 and 82% argon concentrations in place of nitrogen showed no beneficial effect on sensory parameters.

Key words: argon, modified atmosphere packaging, chicken breast fillet, spoilage flora, shelf life

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INTRODUCTION

Recently, the food industry and gas producers have increased interest in effective gas mixtures to further extend the shelf life of fresh or processed food products (Day, 1995, 2007). The 3 traditional gases for modified atmosphere packaging (**MAP**) are oxygen, carbon dioxide, and nitrogen (Farber, 1991; Rao and Sachindra, 2002; Floros and Matsos, 2005). Argon, as an alternative to nitrogen (Day, 2007), has recently been allowed to be used for MAP in the European Union (**EU**, 1995, directive 92/02/CE) with the properties of being inert, odorless, and tasteless (Greenwood and Earnshaw, 1998). Although inert, argon is suggested to have biochemical activities such as interference with oxygen receptor sites of enzymes and protein conformation change. Furthermore, argon displaces oxygen more effectively than nitrogen. This is possibly based on its similar atomic size to molecular oxygen and its improved water solubility (0.034 vs. 0.016 $g \cdot L^{-1}$) and higher density (1.650 vs. 1.153 kg/m^3) compared with nitrogen (Spencer, 1995, 2005). Regarding inhibitory activity against bacterial growth, argon was suggested to have a better solubility in fat, resulting in improved membrane permeability of CO_2, salts, and acids to bacterial cells (Betts, 1995). Several studies were conducted to investigate the effect of argon on enzyme activities and sensory characteristics in fruits and vegetables (Zhang et al., 2001; Jamie and Saltveit, 2002; Rocculi et al., 2005; Zhang et al., 2008; O'Beirne et al., 2011; Wu et al., 2012). Also, controversial results were reported for meat and meat products. A study of packed turkey meat in an argon-CO_2-mixture reports an inhibitory effect on total anaerobic counts, total psychotropic counts, and *Brochothrix thermosphacta* with a 1 log difference after 25 d of storage, in comparison with nitrogen, but no effect on lipid oxidation (Fraqueza and Barreto, 2009). Tománková et al. (2012) compared the effect of 70% O_2/30% CO_2 and 70% Ar/30% CO_2 for the packaging of poultry meat. The authors showed that argon leads to an increase in microbiological growth and an unpleasant odor compared with the oxygen containing atmosphere. The storage of pork sausages under an argon-enriched atmosphere also had no effect on microbiological growth and biogenic amines, whereas sensory evaluation achieved the most effective scores using an argon atmosphere, in contrast to nitrogen or vacuum packaging (Ruiz-Capillas and Jiménez-Colmenero, 2010). Curiel et al. (2011) investigated in vitro the biogenic amine production of lactic acid bacteria and *Enterobacteriaceae* isolated from pork

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sausages packed in different atmospheres. The authors found an inhibition of *Carnobacterium divergens* under an argon atmosphere after 28 d of storage, but the argon atmosphere also seemed to favor the growth of agmatine-producing *Enterobacteriaceae* in comparison with nitrogen. Parra et al. (2010) reported no significant differences in dry-cured Iberian ham quality while storing the samples under argon or nitrogen atmospheres.

Recently, there has been a lack of information about the effect of argon in MAP application on the quality and shelf-life of fresh meat. Therefore, the aim of the study was to investigate and compare the development of typical spoilage microorganisms, sensory parameters, gas composition, and pH during storage of fresh poultry fillets under different argon- and nitrogen-containing atmospheres.

MATERIALS AND METHODS

Preparation of Meat Samples and Packaging

Unsexed 42-d-old-broiler chickens (Ross 308/708) were slaughtered and air-chilled in a poultry processing plant in Germany. The skinless double-breast chicken fillets were transported from the poultry slaughter plant to a wholesaler and forwarded to the laboratory under temperature-controlled conditions in isolated boxes with cooling packs. The first investigation started within 24 h after slaughtering. In the laboratory, the double-breast fillets were divided into single fillets using a sterile scalpel. Half of each double-breast fillet was packaged in an atmosphere containing argon; the other half was packed with an equivalent nitrogen concentration.

The chicken breast fillets were placed in polypropylene trays (R. Fearch Plast A/S, Holstebro, Denmark). Tray volume was 680 mL and approximately 230-g meat samples were packaged to achieve a package headspace to meat ratio of nearly 3:1. The meat samples were packaged under 6 different modified atmospheres (**A**: 15% Ar, 60% O_2, 25% CO_2; **B**: 15% N_2, 60% O_2, 25% CO_2; **C**: 25% Ar, 45% O_2, 30% CO_2; **D**: 25% N_2, 45% O_2, 30% CO_2; **E**: 82% Ar; 18% CO_2; **F**: 82% N_2, 18% CO_2). Thereafter, the trays were heat-sealed with a polypropylene foil (Suedpack Verpackungen GmbH and Co. KG, Ochsenhausen, Germany; water vapor permeability <3.5 g/m^2 per d at 23°C/85% RH; oxygen permeability </=1.5 cm^2/m^2d bar at 23°C/35% RH) for 3 s/175°C using a tray sealer packaging machine (Traysealer T200, Multivac Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany). Gas mixtures were prepared by a 4-component gas blender machine (KM 60–4 MEM SO, Witt Gasetechnik, Witten, Germany). The packaged meat samples were stored at 4°C between 450 and 570 h according to the gas mixture used in low-temperature, high-precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). Storage temperature was monitored by the data logger (Escort Junior Internal Temperature Data Logger, Escort Data Loggers Inc., Auckland, New Zealand) every 5 min. The microbiological, sensory, and chemical analyses were conducted at appropriate time intervals. Each measurement was repeated 3 times.

Microbiological Analyses

Immediately after opening the packages, the amount (25 g) of meat surface sample 4 × 7 × 0.5 cm in size was aseptically taken using a sterile scalpel, which was transferred to a filtered sterile stomacher bag and filled with 225 mL of saline peptone diluent (0.85% NaCl with 0.1% peptone saline tablets, Oxoid BR0053G, Cambridge, UK). Samples were blended with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. Ten-fold dilutions of the sample rinsates were prepared in saline peptone diluents. Total viable count, *Pseudomonas* spp., *B. thermosphacta*, *Enterobacteroaceae*, and *Lactobacilli* spp. in rinsates were enumerated.

Total viable count was determined by pour plate technique on plate count agar (Merck, Darmstadt, Germany), and plates were incubated at 30°C for 72 h. Presumptive *Pseudomonas* spp. were detected by spread plate technique on *Pseudomonas* agar with cetrimide-fucidin-cephalosporin selective supplement (Oxoid, Cambridge, UK). Plates were incubated at 25°C for 48 h.

Presumptive *B. thermosphacta* was detected by drop plate technique and counted on streptomycin inosit toluylene red agar (SIN agar) according to Hechelmann (1981). Petri dishes were incubated at 25°C for 48 h.

Presumptive *Enterobacteriaceae* were identified by overlay treatment on violet red bile dextrose agar (Merck) by incubation of the agar plates at 30°C for 48 h. Presumptive *Lactobacilli* spp. were detected by pour plate technique on de Man, Rogosa, Sharpe Agar (Oxoid, Cambridge, UK). Plates were incubated aerobically at 37°C for 72 h. Counts of cfu were expressed as $\log_{10}$ cfu/g for each medium and sample.

Sensory Evaluation

Sensory analyses were carried out by 6 trained sensory panelists. All assessors were recruited from the Institute of Animal Science (University of Bonn) and experienced in poultry evaluation. For the trials, panelists were intensively trained one time for about 1 h before the investigation started. For the training, all participants had to describe and define typical sensory attributes (color, odor, texture, drip loss) at different stages of spoilage during storage of poultry fillets. A picture of fresh chicken breast fillets was used as reference.

During the trials with different argon or nitrogen mixtures inside the package, each sample was evaluated directly after opening the tray, using a developed sensory scheme according to the quality index (**QI**) method for fish evaluation (Bremner, 1985).

Attributes were defined as general appearance (G), color (C), odor (O), texture (T), and drip loss (D). Changes of the attributes were expressed in a 5-point scoring system. The lower the score, the better the quality and freshness of the product. A weighted QI was calculated by the following equation (Kreyenschmidt, 2003):

$$\mathrm{QI} = \frac{2G + 2C + 1T + 1D + 2O}{8}. \qquad [1.1]$$

The end of sensory shelf life was defined as a QI of 2.5.

Gas Analysis

Concentrations of oxygen and carbon dioxide inside the trays were monitored over the storage period, using a handheld gas analyzer (Oxybaby V O_2/CO_2, Witt Gasetechnik, Witten, Germany). Before starting the gas measurement inside the trays, the composition of air was analyzed to control the accuracy of the gas analyzer. Headspace in packages was sampled, using a syringe needle to withdraw 10 mL of headspace gas through a self-adhesive sealing pad in the package. Gas volume was absorbed in 15 s and the oxygen concentration was detected by an electrochemical sensor; carbon dioxide concentration was detected by infrared-absorption. Control packages containing no meat samples were stored as reference, and the gas composition was also monitored over the entire storage period.

pH Measurement

The pH of the meat samples was measured over the entire storage period, using a portable pH-meter (Escort Junior EJ-2E-D-16L, Escort, Auckland, New Zealand). Three measurements were performed for each meat sample by placing the electrode onto the meat surface, and an average pH value was calculated.

Primary Modeling

The Gompertz equation was used to model the growth of the total viable count, *Enterobacteriaceae*, *Pseudomonas* spp., *B. thermosphacta*, and *Lactobacillus* spp. as a function of time (Gibson et al., 1987).

$$N(t) = A + C \cdot e^{-e^{-B\cdot(t-M)}}, \qquad [1.2]$$

with $N(t)$: microbial count [$\log_{10}$ cfu/g] at any time; A: lower asymptotic line of the growth curve (initial bacterial count); C: difference between upper asymptotic line of the growth curve ($N_{\max}$ = maximum population level) and the lower asymptotic line; B: relative maximum growth rate at time M (h^{-1}); M: time at which maximum growth rate is obtained (reversal point); and t is time.

The microbiological growth data were fitted using the statistical software program Origin 8.0G (OriginLab Corporation, Northampton, MA).

Statistical Analysis

The Mann-Whitney-U test was used to make comparisons between sensory color evaluation, pH values, and the measured counts of cfu with a level of significance of 0.05. The SPSS Statistics 2.0 for Windows (SPSS Inc., Chicago, IL) software was used.

RESULTS AND DISCUSSION

Comparison of the Spoilage Process Under Various Gas Concentrations

Figure 1 shows the development of total viable count and specific spoilage microorganisms on chicken breast fillets, packaged under different argon- and nitrogen-containing atmospheres at a constant temperature of 4°C. Argon or nitrogen treatments of 15% show no significant effect on the growth of typical spoilage organisms (Table 1). The development of bacterial growth curves and the calculated growth rates (Table 2) are almost the same for both atmospheres.

Storing the samples under low argon or nitrogen atmospheres (15%), the microbiological spoilage flora is dominated by *Lactobacilli* spp. at the beginning of storage. During storage, counts of *Pseudomonas* spp., *Enterobacteriaeceae*, and *B. thermosphacta* become dominant with *B. thermosphacta* being the predominant microorganism after approximately 210 h (Figure 1). These results agree with other studies, where *B. thermosphacta* is also associated with spoilage under MAP conditions (Borch et al., 1996; Nychas and Drosinos, 2000; Pin et al., 2002). The number of *Lactobacilli* spp. remains relatively constant throughout the entire storage period (Table 2) and plays a minor role in the spoilage flora. This is due to the fact that *Lactobacilli* spp. belong to a slow-growing group of bacteria and their growth is favored by anaerobic conditions or high amounts of CO_2 (or both) in a gas mixture. *Lactobacilli* spp. are also mesophilic bacteria, and their slow growth is probably related to the cold storage temperature (Jay et al., 2005).

The storage under 25% argon- or nitrogen-enriched atmosphere also shows no significant differences in microbiological growth between both gas mixtures (Table 1). The comparison of growth revealed that growth of *Lactobacilli* spp. was constant during storage, whereas *B. thermosphacta* became dominant in both gas mixtures used. The growth of *Enterobacteriaceae* and *Pseudomonas* spp. also shows the same trend comparing argon- and nitrogen-containing atmospheres (Table 2). However, the maximum number of *Enterobacteriaceae* at the end of storage (25% Ar/N_2) is approximately 1 log level higher in comparison with the 15% Ar/N_2

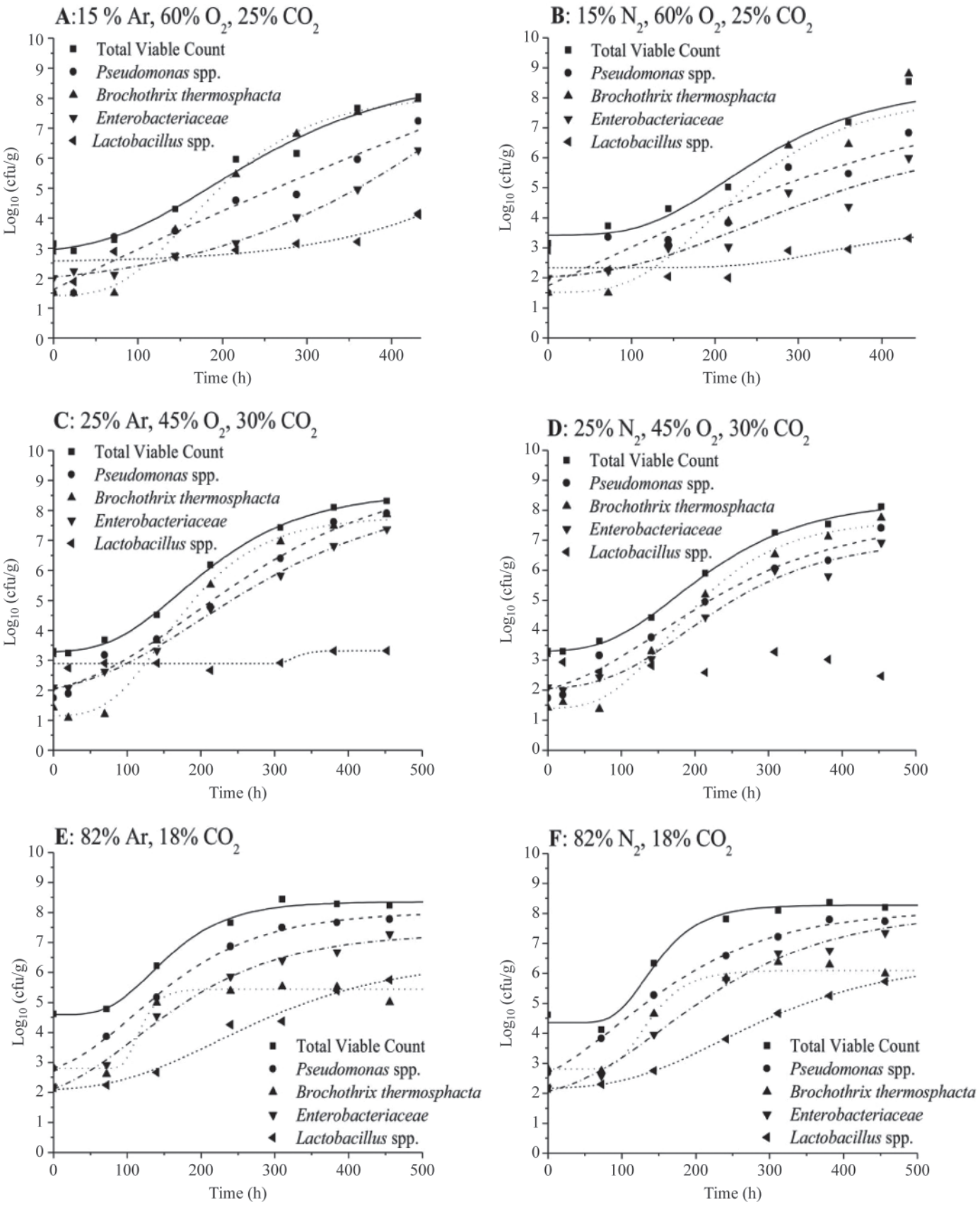

Figure 1. Growth of the spoilage microflora under different argon (left) and nitrogen (right) concentrations fitted with the Gompertz model, n = 3.

Table 1. Comparison of argon and nitrogen atmospheres on the growth of spoilage bacteria ($P < 0.05$)

	Ar/N_2 concentration (P-value)		
Microorganism	15% Ar/15% N_2	25% Ar/25% N_2	82% Ar/82% N_2
Total viable count	0.10	0.60	0.98
Brochothrix thermosphacta	0.45	0.54	0.28
Pseudomonas spp.	0.46	0.93	0.57
Enterobacteriaceae	0.46	0.48	0.96
Lactobacillus spp.	0.13	0.30	0.93

atmosphere. This is presumably because *Enterobacteriaceae* are facultative anaerobic bacteria, which grow preferably under oxygen conditions. However, concentrations up to 60%, as used in the first trials, slow down the growth of microorganisms and yeasts because of the formation of oxygen radical species, which leads to an inhibition of aerobic and anaerobic microbial growth (Amanatidou, 2001; Jacxsens et al., 2001).

The storage of the poultry samples under 82% argon- or 82% nitrogen-enriched atmospheres shows different effects on the spoilage of particular microflora. A high concentration of argon or nitrogen (82%) and the absence of oxygen lead to an increase of the growth of *Lactobacilli* spp. *Pseudomonas* spp. also show a stable growth, even though these microorganisms are aerobic. Clark and Burki (1972) also showed the growth stability of *Pseudomonas* spp. at oxygen concentrations of less than 1%. In these trials, a residual oxygen concentration of approximately 2% was monitored inside the trays. Similar results were shown by Fraqueza and Barreto (2009). The storage of turkey meat under 100% N_2 and 50% Ar/50% N_2 also resulted in good growth of *Pseudomonas* spp. of up to 7 $\log_{10}$ cfu/g after 15 d of storage.

The Ar/CO_2-mixture was the most effective in delaying the growth of *B. thermosphacta* (Table 2: Ar: 0.060 h^{-1}/N_2: 0.090 h^{-1}), but not significant (Table 1). Similar results were reported in a study by Fraqueza and Barreto (2009) for the growth of *B. thermosphacta* during the storage of turkey meat under a 50% Ar to 50% CO_2 atmosphere. This effect was explained by the biological activity of argon (Betts, 1995). It seems that argon works synergistically and supports the penetration of CO_2 into some microorganism species, while becoming dissolved into the lipid membrane, leading to a delay of microbial growth. However, no effect on the residual spoilage flora was observed using argon or nitrogen.

Comparison of Sensory Evaluation Under Various Argon and Nitrogen Concentrations

The development of the QI and meat color during storage under different argon and nitrogen treatments is illustrated in Figure 2. The QI increases linearly for poultry, with increasing storage time for all gas mixtures used. Storage of chicken breast fillets under 25% or 82% Ar/N_2-enrichment has no beneficial effect on the QI or on surface meat color. Comparing the QI under 15% argon with 15% nitrogen concentrations in the mixture, samples stored under nitrogen atmosphere achieve the QI (QI = 2.5) approximately 100 h earlier than samples stored under argon atmosphere. The differences in QI development between 15% Ar/N_2-atmospheres were mainly based on the evaluation of meat color. The results of the sensory color evaluation showed a significant difference ($P < 0.05$) throughout the storage period after packaging under a 15% argon-containing atmosphere in comparison with the nitrogen packages. However, the effect differed between samples. Spencer (1995, 2002) reported that argon is supposed to show a biological activity due to its physical and chemical properties. However, Prangé et al. (1998) demonstrated that noble gases may interact with proteins as a result of noncovalent van der Waals forces and build up a complex with myoglobin. This effect could be an explanation for the beneficial color evaluation of samples stored under 15% argon. Parra et al. (2010) also found a positive effect on color development of Iberian ham. Samples packed under 70% Ar/30% CO_2 showed higher a-values after 60 d than samples packed in nitrogen-containing atmospheres or under vacuum. Ruiz-Capillas and Jiménez-Colmenero (2010) also reported that argon in a gas mixture leads to a positive effect on the sensory evaluation of pork sausages. However, this took into account that, besides packaging conditions, the surface meat color of poultry is additionally influenced by several factors such as age, sex, meat moisture content, preslaughter conditions, and processing variables (Faustmann, 1990; Totosaus et al., 2007). Therefore, process- and animal-specific factors seem to have an additional effect on color development because the meat color stability could only be observed for parts of the samples. O'Beirne et al. (2011) pointed out that potential benefits of argon-containing atmospheres seem to be relatively small and need critical enzyme, substrate, and gas levels to be successful.

Development of Gas Composition

During the first 24 h after MAP, CO_2 concentration shows a small decline in all packages (data not shown).

Table 2. Development of growth parameter during storage of poultry under different atmospheres calculated with the Gompertz function[1]

	μ_{max} (h^{-1})				Duration of lag phase (h)				μ_{max} (h^{-1})				Duration of lag phase (h)			
Microorganism	15% Ar/ 60% O_2/ 25% CO_2	15% N_2/ 60% O_2/ 25% CO_2	15% Ar/ 60% O_2/ 25% CO_2	15% N_2/ 60% O_2/ 25% CO_2	25% Ar/ 45% O_2/ 30% CO_2	25% N_2/ 45% O_2/ 30% CO_2	25% Ar/ 45% O_2/ 30% CO_2	25% N_2/ 45% O_2/ 30% CO_2	82% Ar/ 18% CO_2	82% N_2/ 18% CO_2	82% Ar/ 18% CO_2	82% N_2/ 18% CO_2	82% Ar/ 18% CO_2	82% N_2/ 18% CO_2		
Total viable count	0.018	0.017	82.38	84.37	0.021	0.019	76.30	77.70	0.024	0.035	98.09	89.41				
Brochothrix thermosphacta	0.031	0.026	78.04	100.89	0.034	0.028	72.70	78.14	0.060	0.090	94.22	86.13				
Pseudomonas spp.	0.014	0.013	18.73	34.37	0.018	0.016	29.67	15.04	0.021	0.020	13.90	17.37				
Enterobacteriaceae	0.014	0.011	130.51	83.51	0.016	0.017	40.87	77.54	0.019	0.018	28.58	27.67				
Lactobacillus spp.	0.007	0.005	233.57	232.52	0.012		309.70		0.012	0.012	84.25	102.99				

[1]μmax = maximum growth rate.

This is due to the high solubility of CO_2 to lipid and water on the meat surface (Betts, 1995; Parra et al., 2010). The maximum CO_2 decline (5%) was in 82% argon-containing packages, whereas the minimum decline (2%) was seen in nitrogen-containing packages. The results indicate that the decline is possibly caused by the higher solubility of argon and therefore the synergistic effect with other gases such as CO_2, as proposed by Betts (1995). Furthermore, relative changes in the gaseous atmosphere were small and showed the most changes within packs containing low oxygen levels.

These findings are in accordance with O'Grady et al. (2000). The oxygen levels inside the trays were very small but decreased continuous during storage, potentially due to microbiological consumption, meat enzyme respiration, and gaseous exchanges between the trays and the environment (Mullan and McDowell, 2003; Yam et al., 2005; Esmer et al., 2011).

Development of pH Value

Broiler breast pH at 24 h postmortem varied between 5.7 and 6.2 (data not shown), which is also described by Lund and Eklund (2000). During storage, the pH value was not significantly influenced by any gas mixture used ($P > 0.05$). No differences could be observed using argon or an equivalent amount of nitrogen in the atmosphere. In contrast, several authors reported a decline of meat pH under CO_2-containing atmospheres (Giménez et al., 2002; Martínez et al., 2005; Rotabakk et al., 2006). This effect is explained by its high solubility in muscle and fat, which leads to the formation of carbonic acid (Daniels et al., 1985). However, Jakobsen and Bertelsen (2005) pointed out that CO_2, to 98%, becomes dissolved in water as carbonic acid and only a small amount dissociates into bicarbonate and hydrogen ions. Additionally, Devlieghere et al. (1998) reported that the initial pH of a product has a strong effect on the CO_2 solubility. The buffering effect of meat proteins also contribute to no significant variations in pH while storing the meat under MAP conditions.

Conclusion

The comparison between nitrogen and argon in a gas mixture showed no significant differences in the development of typical spoilage microorganisms using atmospheres A to D (15% Ar or N_2/25% Ar or N_2). The gas mixture containing 82% Ar/18% N_2 was the most effective in delaying the growth of *B. thermosphacta*, in comparison with the nitrogen atmosphere. Storing the samples under a 15% argon-enriched atmosphere stabilized the light pink color of parts of the poultry fillets samples. In this context, it has to be considered that the color of a product is the first visual impression that mainly influences the consumer choice at the point of sale, with meat being discounted in price or wasted due to surface discoloration, which leads to a huge economic loss. However, it has to be taken into account

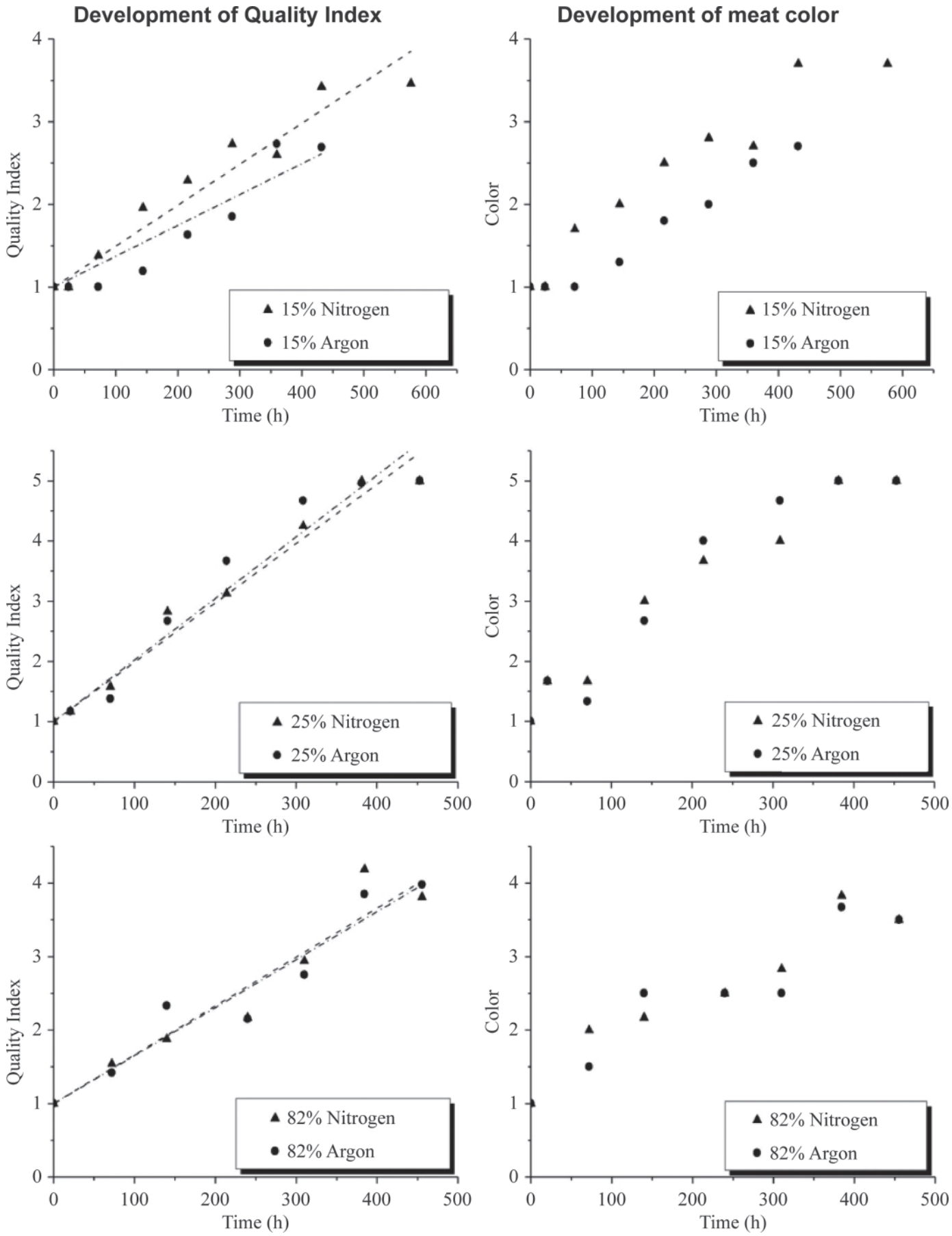

Figure 2. Development of quality index and poultry meat color under different argon and nitrogen concentrations, n = 3.

that animal-specific factors, which are not yet known, have an additional influence on color particularly after slaughter and might reduce the beneficial effect of argon. Therefore, further research is still needed to clarify the influence of the process- and species-specific factors influencing color, particularly after argon-based MAP. In conclusion, the results indicate that the creation of novel argon-containing gas mixtures is a very sensitive process. The potential benefits are probably marginal to compensate for the increasing argon cost as a noble gas and line adjustment for the argon-pack implementation.

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