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Effect of package perforation on the spoilage process of poultry stored under different modified atmospheres

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ABSTRACT

The effect of a perforated package on the development of typical spoilage parameter and shelf life of poultry packed under high oxygen- (70% O_2; 30% CO_2) and high nitrogen- (70% N_2; 30% CO_2) containing atmospheres were studied. Perforations of 0.2 mm were made in the top foil and samples were stored at 4 °C for 20 days.

During storage the development of the total viable count and the growth of typical spoilage organisms (*Brochothrix thermosphacta*, *Pseudomonas* spp., *Enterobacteriaceae* and *Lactobacilli* spp.) were analyzed and modeled by using the Gompertz function. Sensory analysis of the samples was carried out to analyze color, odor, texture, drip loss and general appearance. Also the development of the gas atmosphere and the pH value was measured. The results showed that under both atmospheres the growths of all spoilage organisms and all sensory attributes were influenced by a perforation. Sensory shelf life was reduced under both atmospheres by 26% due to a perforation.

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1. Introduction

Modified atmosphere packaging (MAP) was introduced in the market in 1979 at the retailer Marks and Spencer (Church, 1994). This preservation technique is used for fresh meat to prolong the shelf life of this highly perishable product (Singh, Wani, Saengerlaub, & Langowski, 2011). In Europe different kinds of atmospheres are used for poultry: Several producers are using oxygen free atmosphere (70% N_2 and 30% CO_2) to pack fresh poultry. The residual oxygen content in such packages varies normally between 0.5% and 2% (Mills, 2005). Other producers (e.g. the German poultry industry) are using a high concentration of oxygen (>60%). The main reason for using high-oxygen packaging is to preserve the red color of meat, which is caused by the muscle pigments myoglobin and hemoglobin (Phillips, 1996; Totosaus, Pérez-Chabela, & Guerreo, 2007). Poultry breast muscles are referred to white meat, with a low quantity of myoglobin (McKee, 2007). Therefore the effect of a high oxygen concentration is controversially discussed (Löwenadler, 1994).

The gas atmosphere inside the package, after temperature, is one of the most important factors influencing the microbial growth respectively the composition of the spoilage flora and thus on the spoilage kinetic of the product. Changes in the gas atmosphere during storage caused e.g. by damaged packages decrease the positive effect of MAP and lead to an accelerated spoilage process. Tauschitz, Washüttl, Wepner, and Tacker (2003) analyzed the gas concentration inside the packages of different products (e.g. baked goods, cheese, snacks, meat) at the retailer. Only 48% of the modified packed products exhibit the optimal gas composition. At 4% of the tested packages the gas composition was similar to air (21% O_2). However, in this study of Tauschitz et al. (2003) only 14 of the 386 tested packages contained meat products. Reasons for perforation are improper

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sealing or mechanical damage during handling and transport. Particularly the trend to light-weight packaging components can contribute to packaging failure and product damage during the supply chain (Randell et al., 1995; Vergehese, Lewis, Lockrey, & Wiliams, 2013). Up to now, the control of the gas atmosphere inside the package is measured randomly. So far, one package out of 300–400 packages is tested during production. If a defect package is detected all of the 300–400 packages are scrapped or repackaged (Mills, 2005). Voidarou et al. (2011) investigated the quality of damaged MA packaged food products detected at the retailer stage. The samples were sorted out by the store manager due to the impression that the packaging was defective. The results of this study indicate that even a slight damage caused an increase in bacterial count.

Generally, the effect of damaged packages on the spoilage process depends on the product characteristic, the packaging material used, the initial atmosphere and the rate of change of the atmosphere. With increasing perforation size the oxygen concentration increases and the carbon dioxide concentration decreases. This leads to a faster spoilage process of the food. Recently, only a few studies are available which describe the influence of perforations on product quality. Randell et al. (1995) for example determined that the perforation affects the growths of yeast and molds and coliforms of modified atmosphere packed marinated chicken pieces. Eilamo, Ahvenainen, Hurme, Heiniö, and Mattila-Sandhoim (2005) determined that at 5 °C the development of the aerobic plate count in minced meat steaks increases with increasing perforation size. The maximum bacterial count increases 1–2 log levels in the packages with a perforation inside (33 d).

Ahvenainen, Eilamo, and Hurme (1997) studied the effect on perforation size on the sensory deterioration process of Pizza. The sensory spoilage of the product increased with increasing size of the leakage.

Most of the named studies are focusing on the effect of microholes of various sizes on a few typical spoilage parameter. But studies about the influence of leakages on the product specific spoilage organisms and sensory attributes under different gas atmospheres are rare.

Therefore the objective of the present study was to determine the influence of perforation on different sensory and microbiological spoilage parameter and the shelf life of MA packed poultry meat which is packed under high and low oxygen concentrations.

Poultry breast filets were packed under the two common used gas atmospheres 70% O_2; 30% CO_2 and 70% N_2; 30% CO_2 and one part was packed under air (reference). In half of the MA packages a perforation was made in the top foil (Ø = 0.2 mm). All samples were then stored at 4 °C for 20 days and typical spoilage parameters, the pH value and the change of the atmosphere with and without poultry inside were investigated after different time intervals.

2. Material and method

2.1. Poultry samples

Unisex 42-day-old broiler chickens (Ross 308/708) were slaughtered and air-chilled. Skinless chicken breast filets were used as test samples. The filets were obtained as double breast filets from a German slaughtering and processing plant. Filets were wrapped in polypropylene (PP) foil, packed in a card box and transported within 24 h after slaughtering to a German wholesaler and forwarded to a local retailer. Afterwards filets were transported to the laboratory under temperature-controlled conditions.

2.2. Packaging, storage conditions and experimental design

Before packaging the double breasts were divided into two single breast filets using a sterile scalpel. The bottom filet was removed, so that every filet weighed about 230 g to achieve a headspace to product ratio of nearly 3:1. Later one of the single breasts was stored in an intact package and one was stored in a perforated package. For each test day two samples per gas atmosphere were prepared and the storage test was repeated twice. Thus at one investigation point for each scenario were four samples tested.

The chicken filets were packed at the laboratory in polypropylene trays (171 mm × 127 mm × 50 mm; 680 ml, nominal foil thickness, my: 900, no absorber inlay, R. Fearch Plast A/s, Holstebro, Denmark) using a traysealer packaging machine (Traysealer T200 Multivac Sepp Haggenmüller GmbH & Co. KG Wolfertschwenden, Germany). As liddingfilm a low gas and water vapour permeable foil consisting of biaxial oriented polyester, polyethylene, EVOH and polypropylene. (Top Tray 50 LAF, SÜDPACK Verpackungen GmbH & Co. KG, Ochsenhausen, Germany) with an oxygen transmission rate of $\leq$1.5 cm^3/m^2 d bar 23 °C 35% r. F and a water vapor permeability of <3.5 g/m^2 d 23 °C 85% r. F was used.

The gas mixtures 70% N_2 and 30% CO_2, and 70% O_2 and 30% CO_2 were adjusted by a four-component gas blender machine (WITT-GASETECHNIK GmbH & Co KG, Witten, Germany). After packaging one perforation was made in each package by an acupuncture needle (Ø 0.2 mm) through the top foil of half of the samples. Reference samples were packed under normal atmosphere using the same trays and a low density polyethylene film as top foil (aerobic packaging). Further on, control blank samples without poultry were stored during the entire storage period to investigate the influence of the product on the headspace gas atmosphere. Table 1 shows the storage conditions of the different test scenarios.

Table 1 – Storage conditions of the different test scenarios.

Scenario	Gas concentration	Scenario description
A 1	70% N_2, 30% CO_2	Intact packages with product
A 2	70% N_2, 30% CO_2	Perforated packages with product
A 3	70% N_2, 30% CO_2	Intact packaging without product
A 4	70% N_2, 30% CO_2	Perforated packaging without product
B 1	70% O_2, 30% CO_2	Intact packages
B 2	70% O_2, 30% CO_2	Perforated packages
B 3	70% O_2, 30% CO_2	Intact packaging without product
B 4	70% O_2, 30% CO_2	Perforated packaging without product
C 1	Air	Aerobic packaging

For all storage experiments, packaged samples were stored at 4 °C under controlled temperature conditions in a low temperature high precision incubator (model Sanyo MIR-254, Sanyo Electric Co., Ora-Gun, Gumma, Japan). The air temperature was recorded every five minutes by data loggers (ESCORT JUNIOR Temperature Recorder, Escort, New Zealand).

Four samples of each packaging scenario (A and B) were analyzed at seven sample points during storage. Gas composition, pH value, sensory and microbial parameters were determined after 0, 3, 6, 9, 12, 15 and 20 days.

2.3. Gas analysis and measurement of pH value

The CO_2 and O_2 concentration were measured with a hand-held gas analyser (OXYBABY® O_2/CO_2 WITT-GASETECHNIK GmbH & Co KG, Witten, Germany). Headspace gas concentration was measured by penetrating a syringe needle onto the liddingfilm. The oxygen concentration was detected by an electrochemical sensor and the carbon dioxide concentration by IR-spectrophotometer.

Gas concentrations are given as volume percentages of the total packaging atmosphere. At every test point four packages were measured and the average was calculated.

The pH-value was measured on three different positions in each sample by inserting a portable pH-meter (Testo 171, Lenzkirchen, Germany) into the meat surface. From these three measurements, an average pH-value was calculated for each sample.

2.4. Microbiological analyses

For microbiological analyses, a representative surface sample of 25 g was separated under sterile conditions with a scalpel and transferred to a filtered stomacher bag and homogenized for 60 s in a Stomacher (Stomacher BagMixer® Interscience, Saint Nom, Frankreich) with 225 ml saline peptone diluent (0.85% NaCl with 0.1% peptone Saline-Tablets, Oxoid Br0053G, Cambridge, United Kingdom). Serial decimal dilutions of the homogenates (0.1%) were prepared using saline peptone diluent. The amount of 0.1 ml of these serial dilutions of poultry homogenates was used for pour plate technique and 0.01 ml for spread plate technique. total viable count (TVC) was performed on Plate Count Agar (PCA, Merck, Darmstadt, Germany) and plates were incubated at 30 °C for 72 h. *Pseudomonas* spp. was determined by spread plate technique on Pseudomonas Agar with Cetrimide-Fucidin-Cephaloridine selective supplement (CFC, Oxoid, Cambridge, United Kingdom). The incubation period was 48 h at 25 °C. *Brochothrix thermosphacta* was detected on Streptomycin Inosit Toluylene Red Agar (SIN-Agar) according to Hechelmann (1981) using the Drop-Plate technique (Sheep-Blood-Agar-Base, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25 °C for 48 h. *Enterobacteriaceae* were determined by Overlay technique after an incubation of 48 h at 30 °C on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany). De Man, Rogosa, Sharpe Agar (MRS, Oxoid, Cambridge, United Kingdom) was used to identify *Lactobacillus* spp. by pour plate technique. Plates were incubated aerobically at 37 °C for 72 h. All amounts of colony forming units were expressed as $\log_{10}$ cfu/g. For scenario A and B every amount of colony forming units is the average of four samples ($n = 4$), for scenario C every amount of colony forming units is the average of three samples ($n = 3$).

2.5. Sensory analysis

Sensory analysis of chicken breast filets was assessed by a trained sensory panel of five persons with experience in sensory assessment of meat. General appearance, odor, color, texture, drip loss, and cut (cut into single breast filets or double breast filets) were rated using a five-point scoring system (1–5) with 1 = ''highest quality'' and 5 = ''unacceptable quality''. A weighted sensory quality index (QI) was calculated. General appearance (*G*), color (*C*) and odor (*O*) were weighed twice and cut (*Z*) half in comparison to the texture (*T*) and drip loss (*D*). The end of shelf life was achieved at a QI of 2.5 or if a parameter was evaluated with five points.

$$QI = \frac{2 \times G + 2 \times C + T + 2 \times O + D + 0.5Z}{8.5} \quad (1.1)$$

2.6. Data analysis

The growth data of TVC, *Pseudomonas* spp., *B. thermosphacta*, *Enterobacteriaceae* and *Lactobacillus* spp. were analyzed by the statistical software Origin® 8.0G (OriginLab Corporation, Northampton, Ma., USA). The Gompertz model was used as the primary model to describe the growth of microorganisms with time (Gibson, Bratchell, & Roberts, 1987):

$$N(t) = A + C \cdot e^{-e^{-B \cdot (t-M)}} \quad (1.2)$$

with $N(t)$: microbial count [$\log_{10}$ cfu/g] at time *t*; *A*: lower asymptotic line of the growth curve (N_0 = initial bacterial count [$\log_{10}$ cfu/g]); *C*: difference between upper asymptotic line of the growth curve (N_{max} = maximum population density [$\log_{10}$ cfu/g]) and the lower asymptotic line (*A* [$\log_{10}$ cfu/g]); *B*: relative growth rate at time *M* [h^{-1}]; *M*: time at which maximum growth rate is obtained (reversal point); *t*: time [h].

The Mann–Whitney *U* test was used to compare between the measured counts of colony forming units with a level of significance of $p < 0.05$. The analyses were performed for each day separately. SPSS statistics 22 for Windows was used.

3. Results and discussion

3.1. Gas analysis

Fig. 1 shows the development of the gas atmosphere during storage in the intact and perforated product packages and the perforated blank control packages under the two different gas atmospheres. In the beginning of storage, a decrease of CO_2 could be measured in all product packages including the intact packages for both atmospheres (between 6% and 8% reduction). This is due to the high solubility of carbon dioxide in the fat tissue and water on the meat surface (Betts, 1995; Gill, 1988). Herbert, Rossaint, Khanna, and Kreyenschmidt (2013), Parra et al. (2010) and Dhananjayan, Han, Acton, and Dawson (2006) reported similar results for MA packed meat.

During the entire storage, the O_2 concentration inside the intact oxygen containing trays shows a small decrease. This is

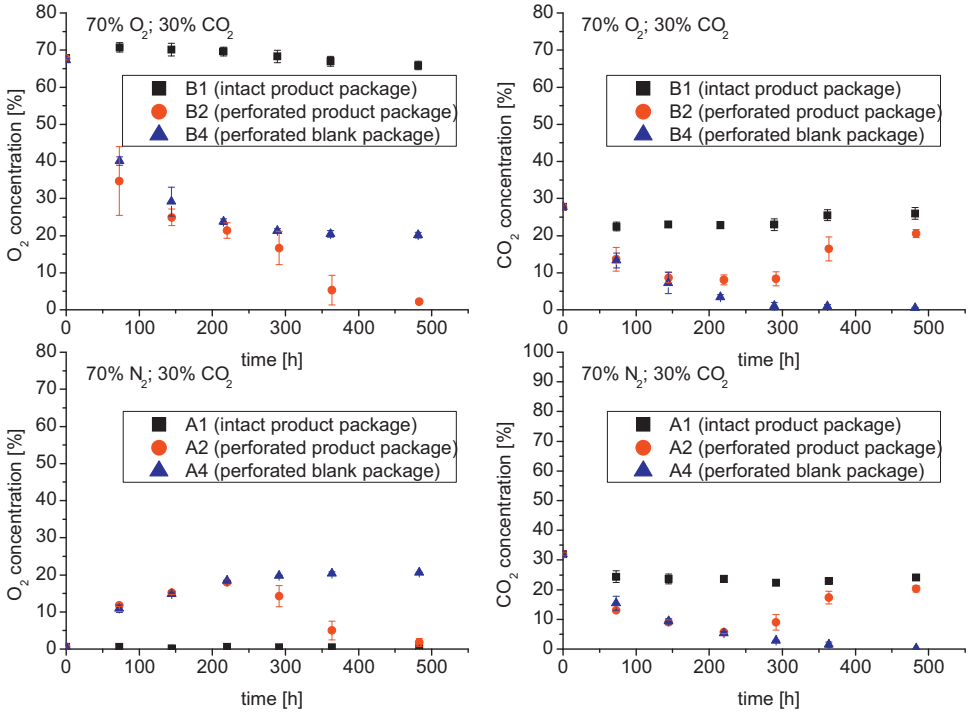

Fig. 1 – Influence of one perforation on the development of the gas atmosphere during storage with and without a product inside the package (above: oxygen enriched atmosphere, down: nitrogen atmosphere) (mean value ± SD of four analyses).

caused by microbiological consumption of O_2, the respiration of meat enzymes and gaseous exchanges between the gas composition inside the trays and the environment (Mullan & McDowell, 2003). The gas atmosphere in intact blank packages stays during storage nearly constant (data not shown).

In the perforated blank control packages under oxygen enriched atmosphere and under N_2 containing atmosphere the O_2 content adapted to ambient air (21% O_2) within 12 d. With poultry inside the perforated package, the increasing microbial growth causes the conversion of oxygen to carbon dioxide (Jakobsen & Bertelsen, 2002). In the high oxygen packages the O_2 content decreases under the 21% O_2 of air. At the end of the storage the O_2 content decreases to approx. 2%. At this point in time, the meat is already spoiled and the bacterial count is extremely high.

In the perforated product packages containing nitrogen the oxygen content increases to approx. 18% (9 d). The O_2 end concentration after 20 d is approximately 2%. The CO_2 content in product packages decreases from 30% to 5.7% in 9 days and increases again to approx. 20%. These results are in agreement with Randell et al. (1995), Eilamo et al. (2005), Ahvenainen et al. (1997) and O'Mahony, O'Riordan, Papkovskaia, Kerry, and Papkovsky (2006).

3.2. *Microbiological analysis*

The development of the TVC in intact packages, perforated packages and the reference test under aerobic storage conditions is shown in Fig. 2. The bacterial growth under intact oxygen and nitrogen atmosphere is nearly the same

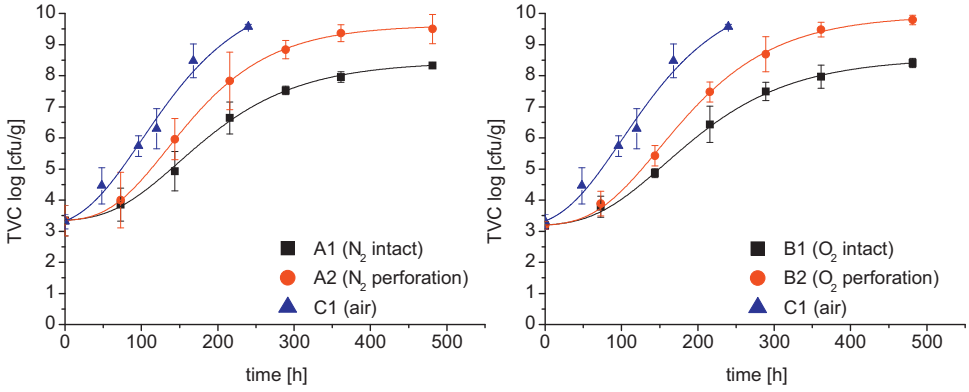

Fig. 2 – Comparison between the development of TVC under intact packaging, defect packaging and air (mean value ± SD for four analyses, except TVC air (three analyses)).

Table 2 – Development of growth parameters of TVC during storage of poultry under different package scenarios calculated with the Gompertz function.

	Growth parameters			
	N_0 [$\log_{10}$ cfu/g]	Duration lag-phase [h]	Maximum growth rate [1/h]	N_{max} [$\log_{10}$ cfu/g]
TVC O_2 intact	3.2 (±0.1)	64 (±7)	0.022 (±0.002)	8.4 (±0.2)
TVC N_2 intact	3.3 (±0.5)	62 (±10)	0.022 (±0.002)	8.3 (±0.0)
TVC O_2 perforation	3.2 (±0.1)	65 (±6)	0.030 (±0.002)	9.8 (±0.2)
TVC N_2 perforation	3.3 (±0.5)	58 (±5)	0.031 (±0.002)	9.5 (±0.5)
TVC air	3.3 (±0.2)	22 (±29)	0.036 (±0.014)	9.6 (±0.1)

Mean values ± SD for four analyses, except TVC air (three analyses).

(growth rate O_2: 0.022 h^{-1}, N_2: 0.022 h^{-1}). The growth is significantly influenced by the changes in gas atmospheres due to the perforation (O_2: 0.030 h^{-1}, N_2: 0.031 h^{-1}) (Table 2). The maximal bacterial count differs between intact and perforated packages about 1.4 log cfu/g (O_2) and 1.2 log cfu/g (N_2) at the end of storage. The bacterial count at the end of storage of aerobic packaging is in the same range as for the perforated packages, but the results show that the initial modified atmosphere has an influence on the lag phase of the TVC (Table 2). Gill and Tan (1980) reported that the application of CO_2 is especially effective if the gas is added to the product during the lag phase before bacterial growth occurs. The lag phase of TVC under air is shorter than the lag phase under perforated packages. The increasing bacterial growth in comparison to intact packages due to a perforation is in agreement with other studies (Ahvenainen et al., 1997; Eilamo et al., 2005; Randell et al., 1995).

Fig. 3 illustrates the development of the typical spoilage organisms under both tested modified atmospheres and air. The initial observed bacterial count was the same for the respective gas composition for the intact and perforated packages. The mean value for *Pseudomonas* spp. was 2.7 log cfu/g (O_2) and 3.0 log cfu/g (N_2), for *Enterobacteriaceae* 1.5 log cfu/g (O_2) and 2.5 log cfu/g (N_2), for *B. thermosphacta* 2.2 log cfu/g (O_2) and 2.2 log cfu/g (N_2) and for *Lactobacilli* spp. 2.9 log cfu/g (O_2) and 2.6 log cfu/g (N_2). Similar initial bacterial counts for fresh poultry were observed by Herbert et al. (2013). The results show that a perforation of 0.2 mm has a influence on the development of the typical spoilage flora of poultry packed under 70% oxygen or 70% nitrogen.

Generally the flora in the intact oxygen packages is dominated by *B. thermosphacta*, followed by *Pseudomonas* spp. Whereas in the perforated packages with time *Pseudomonas* spp. becomes the predominant spoilage organism. The spoilage flora in intact high nitrogen packages differs from the flora of high oxygen packages, the flora is dominated in intact and perforated packages by *Pseudomonas* spp. The main spoilage organisms of aerobically stored poultry are *Pseudomonas* spp. This result is in

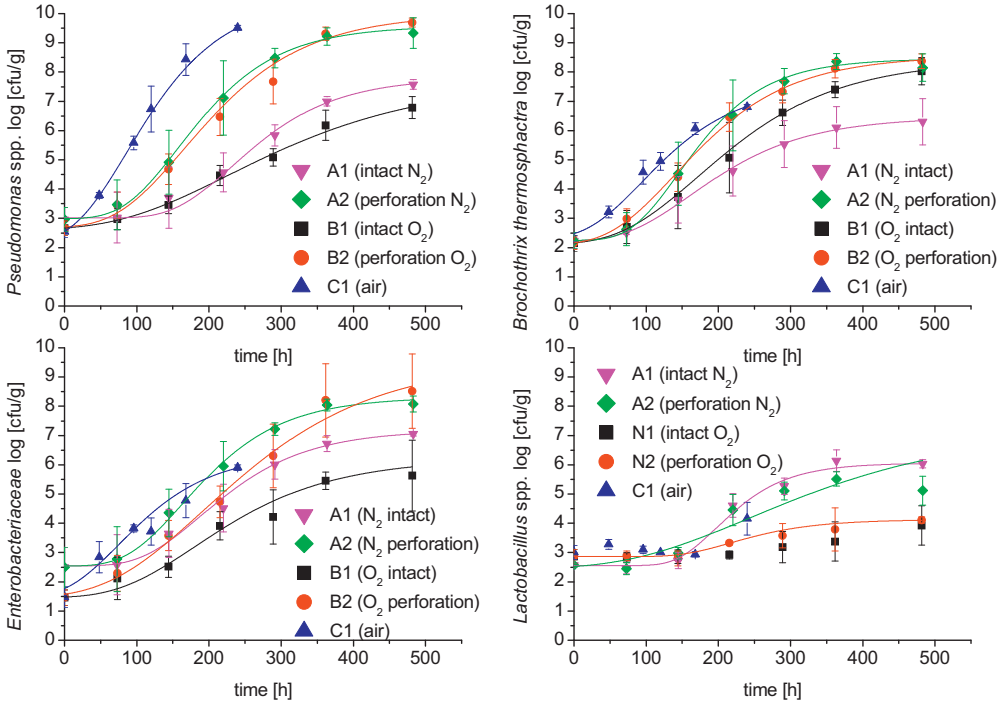

Fig. 3 – Development of spoilage organisms with and without a perforation (0.2 mm) under high oxygen and high nitrogen packaging (mean value ± SD of four analyses, except air (three analyses)).

agreement with Bruckner, Albrecht, Petersen, and Kreyenschmidt (2012) and Nychas, Skandamis, Tassou, and Koutsoumanis (2008).

Generally Fig. 3 illustrate, that the effect of a perforation on the growth of typical spoilage microorganism is different for both atmospheres. *Pseudomonas* spp. are strictly aerobic bacteria, but they are able to grow under near oxygen-free conditions (Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007; Herbert et al., 2013; Saucier, Gendron, & Gariépy, 2000). The influence of a perforation on the growth of *Pseudomonas* spp. is more visible under high oxygen atmosphere than under high nitrogen atmosphere. On the 3rd day of storage, the bacterial count of *Pseudomonas* spp. under oxygen atmosphere in the perforated packages is not significant (<0.05) higher (3.4 log cfu/g) in comparison to the intact packages (3.0 log cfu/g). During storage the difference between both curves increases steadily, so that at the end of storage the maximum number of *Pseudomonas* spp. shows a significant difference (<0.05) between perforated and intact packages of 2.9 log units (Table 3). This increase of the growth of *Pseudomonas* spp. is presumably due to the fact that the CO_2 content decreased in perforated packages and *Pseudomonas* spp. are gram negative bacteria, which are sensitive to CO_2 inhibition (Jay, Loessner, & Golden, 2005). The reduced growth of *Pseudomonas* spp. in intact packages are also caused by the high oxygen concentration, as described by Lee, Yam, and Piergiovanni (2008) and Mastromatteo, Lucera, Sinigaglia, and Corbo (2009). Due to the change of oxygen content in perforated package after 3 days from 70% to 35% this inhibitory effect of high oxygen concentrations is reduced. *Pseudomonas* spp. becomes, during storage under high nitrogen atmosphere, the main spoilage organism in intact and perforated packages. But the bacterial count of *Pseudomonas* spp. differs already on the 3rd day between perforated and intact packages by 0.4 log cfu/g. At the end of storage after 20 days counts of *Pseudomonas* spp. of 7.6 log cfu/g (intact) and 9.3 log cfu/g (perforated) were attained. Under aerobic conditions (air) the maximal bacterial count of 9.5 log cfu/g were already observed after 10 days.

Table 3 – Development of growth parameters of the main spoilage organisms during storage of poultry under different package scenarios calculated with the Gompertz function.

	Growth parameters		
	Duration lag-phase [h]	Maximum growth rate [1/h]	N_{max} [log_{10} cfu/g]
Pseudomonas spp. O_2 intact	74 (±36)	0.012 (±0.003)	6.8 (±0.4)
Pseudomonas spp. N_2 intact	151 (±14)	0.022 (±0.003)	7.6 (±0.2)
Pseudomonas spp. O_2 perforation	72 (±19)	0.029 (±0.005)	9.7 (±0.2)
Pseudomonas spp. N_2 perforation	81 (±10)	0.032 (±0.003)	9.3 (±0.5)
Pseudomonas spp. Air	11 (±9)	0.0 41 (±0.005)	9.5 (±0.1)
B. thermosphacta O_2 intact	63 (±9)	0.021 (±0.001)	8.0 (±0.2)
B. thermosphacta N_2 intact	70 (±9)	0.017 (±0.001)	6.3 (±0.8)
B. thermosphacta O_2 perforation	49 (±10)	0.026 (±0.003)	8.4 (±0.3)
B. thermosphacta N_2 perforation	78 (±16)	0.034 (±0.006)	8.2 (±0.5)
B. thermosphacta Air	17 (±7)	0.025 (±0.001)	6.8 (±0.1)
Enterobacteriaceae O_2 intact	74 (±43)	0.016 (±0.006)	5.6 (±1.2)
Enterobacteriaceae N_2 intact	106 (±17)	0.021 (±0.003)	7.1 (±0.1)
Enterobacteriaceae O_2 perforation	59 (±30)	0.022 (±0.005)	8.5 (±1.3)
Enterobacteriaceae N_2 perforation	86 (±22)	0.027 (±0.005)	8.1 (±0.3)
Enterobacteriaceae Air	3 (±25)	0.025 (±0.009)	5.9 (±0.0)

Mean values ± SD for four analyses, except TVC air (three analyses).

The comparison between intact oxygen and intact nitrogen packages shows that *Brochothrix thermospahcta* grows from day 7 onwards, faster under the high oxygen atmosphere (Table 3). The development of the growth of *B. thermosphacta* in perforated oxygen and nitrogen packages is nearly the same. This is due to the fact that *B. thermosphacta* grows better when oxygen is available for aerobic metabolism (Gill & Pearson, 1986). This preference for oxygen containing atmospheres is confirmed by the results of the perforated nitrogen packages. After 6 days, when the oxygen concentration increases to 15%, the bacterial count of *B. thermosphacta* differs not significant (<0.05) by 0.9 log cfu/g between intact and perforated packages. After 20 days the maximal bacterial count differs significant (<0.05) by 1.9 log cfu/g. Another explanation for the growth reduction in the intact nitrogen atmosphere is the interaction between the different microorganisms, particularly LAB (Russo, Ercolini, Mauriello, & Villani, 2006). The growth of *B. thermosphacta* under high oxygen atmosphere shows a small increase in the perforated packages after 3 days they differ about 0.3 log cfu/g. After 9 days the counts differ about 1.4 log cfu/g and at the end of storage about 0.3 log cfu/g.

The influence of a perforation on the development of counts of *Enterobacteriacea* at oxygen atmosphere was more pronounced. The significant difference (<0.05) of maximal bacterial counts for oxygen is 2.9 log levels after 20 days in comparison to 1 log cfu/g for nitrogen. This difference is presumably due to the fact that the maximal bacterial count of *Enterobacteriacea* is generally lower for the intact high oxygen packages than for the nitrogen packages. This result is in agreement with those of Gallas, Standarová, Steinhauserová, Steinhauser, and Vorlová (2010) who reported a higher value of coliform microorganism under high nitrogen atmosphere in comparison to a high oxygen atmosphere. Also Balamatsia, Paleologos, Kontominas, and Savvaidis (2006) reported similar maximal bacterial counts for *Enterobacteriaceae* under high nitrogen packaging.

Lactic acid bacteria are anaerobic aerotolerant organisms which are usually the dominant flora of meat stored anaerobically (Saucier et al., 2000). Generally they show a faster growth under nitrogen atmosphere but in comparison to the other microorganisms they play a minor role in the spoilage flora. The maximal bacterial count between intact oxygen and intact nitrogen atmosphere differs significant

(<0.05) by 2.1 log cfu/g. After 15 days of storage when the oxygen content increase in perforated nitrogen packages to 18% the growth is inhibited in comparison to the intact packages and after 20 days the count differs in nitrogen packages about 0.9 log cfu/g. The results show that the perforation has only a slight influence on the growth of *Lactobacilli* spp. under high oxygen packaging. These results are in agreement with Eilamo et al. (2005) where the lactic acid bacteria counts of minced meat steaks were not depending on the perforation size. After 20 days of storage under high oxygen atmosphere counts of 3.9 log cfu/g (intact) and 4.1 log cfu/g (leakage) and under air 4.2 log cfu/g were attained. The slow growth of *Lactobacilli* spp. through the entire storage period is in accordance with other studies (Gallas et al., 2010; Herbert et al., 2013) and related to the cold storage and the fact that their growth is favored by anaerobic conditions (Jay et al., 2005).

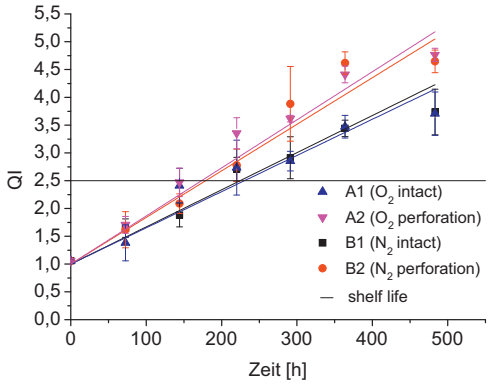

Fig. 4 – Development of sensory quality index (QI) with and without a perforation (0.2 mm) and oxygen and nitrogen enriched atmosphere (mean value ± SD of four analyses).

3.3. *Sensory analysis*

Fig. 4 shows the development of the sensory index with and without perforation. The QI increases linearly for both atmospheres with increasing storage time. Comparing the sensory criteria under oxygen with nitrogen, neither of the two gas atmospheres has a better effect on the scoring of the sensory parameters. Also the effect of a perforation on the sensory index is for both atmospheres similar. In both atmospheres, odor was the worst-rated sensory parameter. After 9 days, differences between intact and perforated packages were obtained in the evaluation of the odor. At this time the total viable counts in intact packages were lower than log 7 cfu/g whereas the TVC reached in perforated packages numbers higher than log 7 cfu/g. This is the level of spoilage organisms for off-odor formation which is reported in literature (Nychas et al., 2008). Randell et al. (1995) reported for marinated chicken that the odor was more affected by a perforation than the appearance of the products. The authors explained that by influence of the product marinade, which effectively covered many defects in appearance. Due to the limited ability of poultry to form oxymyoglobin as compared to pork or beef (Millar, Wilson, Moss & Ledward, 1994) no difference regarding the color between the oxygen or nitrogen containing atmosphere was noticed. Under nitrogen the evaluation of the drip loss is different between perforated an intact packages. Whereas under high oxygen packaging differs mainly the color evaluation between intact and perforated packages. But the calculated QI for the two gas atmospheres is nearly the same. The end of shelf life (calculated from time point zero of the laboratory investigations which means 24 h after slaughtering) is reached after approximately 10 days for intact packages. Normally the poultry producer gives a shelf life of 9 days. Due to a perforation (0.2 mm) the shelf life is reduced under both atmospheres by 26%.

Table 4 shows the bacterial count of spoilage organisms at the end of sensory shelf life for intact and perforated packages. The results indicate that the TVC is in the same range for all package scenarios (6.2–6.8 log cfu/g). *Pseudomonas* spp. varies between 4.6 log cfu/g and 5.8 log cfu/g and *B. thermosphacta* varies between 4.9 log cfu/g and 5.6 cfu/g (Table 4). Evidently no spoilage organism could be identified as consistently dominant at all scenarios at the end of sensory shelf life. Moreover Sone, Olsen, Dahl, and Heia (2011) determined changes in the physical properties of salmon by VIS/NIR which are not caused by microbiological spoilage. Thus not the composition of the spoilage flora or a single microorganism determines the shelf life but the sum of all spoilage organisms and sensory factors.

3.4. *Development of pH value*

The Initial broiler breast pH (24 h after slaughtering) are within the normal range for chicken breast filet and are consistent with generally observed pH values in the literature for fresh

Table 4 – Bacterial count of spoilage organisms at the end of sensory shelf life.

Microorganism	Package scenario			
	O_2 intact (10 d) [log cfu/g]	N_2 intact (10 d) [log cfu/g]	O_2 perforation (7 d) [log cfu/g]	N_2 perforation (7 d) [log cfu/g]
TVC	6.7 (±0.1)	6.8 (±0.2)	6.2 (±0.1)	6.7 (±0.1)
Pseudomonas spp.	4.6 (±0.2)	4.9 (±0.1)	5.4 (±0.4)	5.8 (±0.2)
Brochothrix thermosphacta	5.6 (±0.1)	4.9 (±0.1)	5.3 (±0.2)	5.3 (±0.4)
Enterobacteriaceae	3.9 (±0.4)	5.0 (±0.3)	4.0 (±0.5)	4.9 (±0.5)
Lactobacillus spp.	2.8 (±0.1)	3.6 (±0.3)	3.3 (±0.1)	4.8 (±0.2)

Mean values ± SD for four analyses.

poultry (Bruckner et al., 2012; Herbert et al., 2013; Lund & Eklund, 2000). The value varies between 5.8 and 6.4 (data not shown). In contrast to several authors who reported a decline of meat pH under CO_2-containing atmosphere (Martinez, Djenane, Cilla, Beltran, & Roncales, 2005; Rotabakk, Birkeland, Jeksrud, & Sivertsvik, 2006) is the pH value not significantly influenced by any of the different storage conditions. These results are presumably explained by the dependence on the CO_2 solubility of various factors, such as the pH of the product (Devlieghere, Debevere, & Van Impe, 1998). The non-significant change in pH during storage under MAP conditions is also due to the buffering capacity of the meat proteins (Jakobsen & Bartelsen, 2002).

4. Conclusion

In summary, the different storage tests with fresh poultry meat showed, independent from the two different gas atmospheres, an influence of a perforation (0.2 mm) on the development of spoilage. The growth of all spoilage organisms, expect *Lactobacilli* spp. under nitrogen, increases due to a perforation. The composition of the spoilage flora differed between the oxygen and nitrogen containing atmosphere. Thus the change of the gas atmosphere due to a perforation affected the growth of the specific spoilage organisms in different degrees. Under oxygen atmosphere, mainly the growth of *Pseudomonas spp*. and *Enterobacteriaceae* increases due to a perforation. Whereas under nitrogen atmosphere, mainly the growth of *B. thermosphacta* is influenced. But despite the change of gas atmosphere in perforated packages the bacterial growth is in comparison to air still inhibited. The result of sensory evaluation shows that not the composition of the spoilage flora determines the end of shelf life but the sum of all spoilage parameters. The sensory shelf life was reduced under both atmospheres by around 25% due to a perforation (0.2 mm). But a small perforation is not always visible to the eye, and the defect packages reach the end consumer. Therefore a continuous leakage detection along the entire supply chain is necessary. Thus the use of a leakage indicator would be helpful, to prevent that defect packages reach the end consumer.

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