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Meinen Eltern

Every accomplishment starts with the decision to try.

(anonym)

Kurzfassung

Bewertung unterschiedlicher Gaszusammensetzungen für die Geflügelfleischindustrie auf Grundlage eines Gesamtqualitätsindex

Das Ziel der vorliegenden Arbeit war die Entwicklung eines Gesamtqualitätsindex (OQI) für unter Schutzgas verpacktes (MAP) Geflügelfleisch, um den Einfluss verschiedener Umweltfaktoren auf die Haltbarkeit und den Qualitätsverlust standardisiert zu bewerten. Zunächst wurde der Einfluss der Temperatur und unterschiedlicher Sauerstoffkonzentrationen auf die Entwicklung spezifischer Verderbskeime (*Brochothrix thermosphacta*, *Pseudomonas* spp., Enterobacteriaceae, *Lactobacillus* spp.) und sensorische Parameter untersucht. Weiterhin wurde die Entwicklung der Qualitätsparameter unter Hochsauerstoff und -stickstoff angereicherten Atmosphären verglichen. Insbesondere galt es dabei den Einfluss der Gasgemische auf das Wachstum von *Listeria monocytogenes* zu untersuchen. Des Weiteren wurde der Einfluss des Edelgases Argon getestet. Die Entwicklung der unterschiedlichen Parameter wurde mit dem modifizierten Gompertz Model modelliert. Basierend auf den Ergebnissen wurde ein Gesamtqualitätsindex entwickelt, der mikrobiologische und sensorische Parameter miteinander kombiniert.

Aus den jeweiligen Lagertests ging hervor, dass die Keimflora signifikant von den jeweiligen Umweltbedingungen beeinflusst wurde. Somit konnte kein spezifischer Verderbsorganismus (SSO) identifiziert werden, der allein einen standardisierten Vergleich der unterschiedlichen Einflussfaktoren ermöglichte. Sowohl die Temperatur als auch die Variation der Sauerstoffkonzentration zeigte einen starken Einfluss auf die Entwicklung der Verderbsflora und der sensorischen Parameter. 55-60% Sauerstoff in Kombination mit niedrigen Lagertemperaturen reduzierten die Wachstumsgeschwindigkeit der mikrobiologischen und sensorischen Parameter. Der Vergleich zwischen Sauerstoff und Stickstoff zeigte, dass die Sauerstoffatmosphäre am effektivsten das Wachstum von *L. monocytogenes* unterdrückte. Argon wies keinen zusätzlichen Effekt auf den Qualitätsverlust auf. Allerdings zeigte das mit 15% Ar angereichte Gasgemisch bei einigen Proben eine positive Wirkung auf die Fleischfarbe.

Aus dem entwickelte OQI ging hervor, dass niedrige Lagertemperaturen (<4°C) und 60% O_2/25% CO_2 mit 15% Ar oder 15% N_2 den Qualitätsverlust maßgeblich reduzierten. Der QQI kann bei qualitätsrelevanten Entscheidungsprozessen der Industrie ein wichtiges Hilfsmittel darstellen, um den Einfluss unterschiedlicher Faktoren auf den Qualitätsverlust von Geflügelfilet standardisiert zu vergleichen und zu bewerten. Langfristig können dadurch die Qualität und Sicherheit von frischem Geflügelfleisch verbessert und gleichzeitig die Menge an Ausschüssen reduziert werden.

Abstract

Assessment of different packaging atmospheres for the poultry meat industry based on an overall quality index

The objective of this thesis was the development of an Overall Quality Index (OQI) for modified atmosphere packaged (MAP) poultry fillets to compare and to assess the influence of different environmental factors on the quality loss. In particular, the influence of different temperature conditions and different oxygen combinations on the composition of the specific spoilage flora (*Brochothrix thermosphacta*, *Pseudomonas* spp., Enterobacteriaceae, *Lactobacillus* spp.) and sensory parameters was investigated. Furthermore, a comparison was made between oxygen enriched and nitrogen enriched atmospheres, which are the atmospheres typicially used by the industry for fresh poultry fillet. A special focus was laid on the growth of *Listeria monocytogenes* and the interaction with specific spoilage organisms. The influence on quality loss by the noble gas argon as an alternative to nitrogen was also tested. The development of the microbiological and sensory parameters was modeled by using the Gompertz function. Based on the obtained results, an Overall Quality Index (OQI) was developed, allowing a comparison and assessment of the different influence factors on the quality loss.

The storage trials showed that the quality loss is strongly influenced by changing environmental conditions, and no specific spoilage organisms (SSO) could be identified to compare and assess the effect of different environmental factors. Generally, the results showed that the temperature conditions and the oxygen content variation had a strong influence on the composition of the microflora and on the quality loss. 55-60% oxygen combined with low temperature decreased microbial growth and the sensory quality loss. The comparison between the oxygen free and the oxygen enriched atmosphere on the growth of *L. monocytogenes* showed that the oxygen enriched atmosphere was most effective at suppressing the growth. Argon, as a novel packaging gas, had no additional effect on the growth of typical spoilage microorganisms. However, using 15% Ar seems to have a beneficial effect on meat color.

The developed OQI showed that low storage temperature (<4°C) and 60% O_2/25% CO_2, with 15% N_2 or 15% Ar, had the main beneficial effect on the quality loss. The developed index can be used to support the decision making process of meat companies for a reliable and standardized comparison between the influence of different environmental parameters on the quality loss of fresh poultry fillet. Thus, the application of the OQI will support the process of delivering high quality products and will reduce food waste at the same time.

Contents

1. General Introduction

1.1 Modified atmosphere packaging (MAP) for meat and poultry

Modified atmosphere packaging is a preservation technique, where the air surrounding the product is replaced by a selected mixture of gases (Bilska 2011). This preservation technique was introduced to the market in 1979 by Marks and Spencer (Church 1994) and is commonly established. Approximately 50% of meat products were purchased by private households in self-service (AMI 2013). During the last years, the trend of self-service merchandising of fresh meat became more and more important. Furthermore, sensory attributes, especially the color of the meat, are of great relevance to the consumer's choice at the point of sale (Bell 2001). Additionally, the retailer and the consumer ask for long shelf life times and high product quality as well (Balev et al. 2011). Consequently, gas producers and the meat industry have an increasing interest in creating novel gas mixtures to improve the sensory attributes of the meat and to achieve longer shelf life times for their products (Day 2007). The gases commonly used for fresh meat are oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2), which were added to the package headspace in different proportions and combinations, depending on the product (Phillips 1996; Floros & Matsos 2005). Alternative gases like Argon are also permitted in the European Union (EU, 1995, directive 92/02/CE). The mentioned gases are classified as additives (E-numbers) on the package. According to (EC) No. 1333/2008, the food packaged under modified atmosphere conditions has to be marked with "Packaged in a Protective Atmosphere". The choice of the used gas is influenced by the microflora of the product (Church 1994; Day 2007). Each gas has different functions and effects on microbiological growth and physical properties:

Oxygen (O_2)

Oxygen is chemically characterized as a colourless, odourless, tasteless, and highly reactive gas (Greenwood & Earnshaw 1998). As a packaging gas, O_2 has different effects on meat:

The concentration of O_2 in MAP has a direct influence on the chemical form of the meat muscle pigment myoglobin and therefore on the stability of the meat colour (Figure 1.1). High levels of oxygen maintain myoglobin in its oxygenated form (oxymyoglobin), which results in a bright red fresh meat colour. However low concentrations of O_2 leads to the formation of the brown oxidized form metmyoglobin and anaerobic conditions favors the formation of the reduced purple deoxymyoglobin (Church 1994, Phillips 1996, Martínez et al.

2005, Sørheim et al. 2009). To insure a stable and bright red colour, fresh red meat (e.g. beef, pork, lamb) is conventionally packaged under a high oxygen atmosphere containing 80% O_2 and 20% CO_2 (Borch et al. 1996).

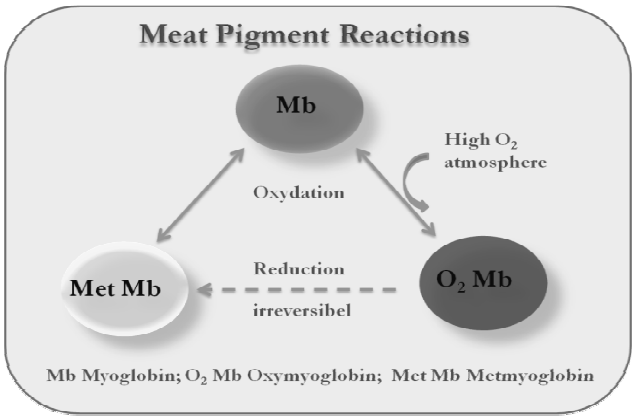

Figure 1.1 Pigment reactions of fresh meat (modified according to Fox 1966).

Besides the stabilizing effect on the muscle pigment myoglobin, Oxygen also affects the growth of specific microorganisms, depending on the oxygen demand of the bacteria (Church 1994). Generally, oxygen favors the growth of aerobic bacteria and reduces the growth of strictly anaerobic species. Therefore, the composition of the microflora changes depending on the oxygen concentration inside the package (Phillips 1996).
Oxygen, depending on concentration and partial pressure, can also be toxic to microorganisms. The toxicity is explained by several theories:

1. Enzyme inactivation
2. Increased formation of intracellular hydrogen peroxide
3. Membrane lipid oxidation
4. Formation of superoxide radicals (Halliwell 1984, Halliwell & Gutteridge 1984).

Because the oxygen resistence of bacterial cells is correlated to the activity of their superoxide dismutase, the formation of superoxide radicals is of great relevance (Gregory & Fridovic 1973). These are enzymes that catalyse the destruction of superoxide radicals by dismutation as follows (Lavelle et al. 1973):

$$2H^+ + 2O_2^{\bullet -} \rightarrow O_2 + H_2O_2$$

Oxidative stress situations for microorganisms take place, when the level of reactive oxygen species in an organism is higher than the scavenging capacity of the cell (Amanatidou 2001). Concentrations up to 60% slow down the growth of particular 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). Another effect is that high oxygen concentrations enhance the risk of lipid and protein oxidation reactions and the formation of cholesterol oxides. These products lead to discolorations, off-flavors and the production of potentially toxic compounds (Kim et al. 2010, Zakrys-Waliwander et al. 2012).

Carbon Dioxide (CO_2)

Carbon dioxide is the most important part of a gas mixture because of its antimicrobial properties against a wide range of microorganisms (Gill & Tan 1980, Faber 1991). Generally, CO_2 leads to an extension of the lag phase and slows down the exponential growth (Stanbridge & Davis 1998), but high concentrations up to 35-40% can cause meat discoloration (Phillips 1996, Arvanitoyannis & Statakos 2012) and are not recommended because of a potential pack collapse and an increased drip loss (Mullan & McDowell 2003). CO_2-concentrations above 20% lead especially to an inhibition of molds and gram-negative bacteria (e.g. *Pseudomonas* spp.), whereas yeasts and lactic acid bacteria (*Lactobacillus* spp.) were relatively unaffected by CO_2 (Gill & Tan 1980, Fierheller 1991). The mode of action of CO_2 on the microbiological growth is not yet clear. There are several hypothesis described in the scientific literature:

One is based on the solubility of CO_2 in water and fat. CO_2 gets partly dissolved in the water – and fat phase of the product under formation of carbonic acid with a direct ionization, which results in a decrease of the meat surface pH (Devlieghere et al. 1998). The solubility is dependent on the temperature, the pH-value, the product gas ratio and the permeability of the packaging material (Devlieghere et al. 2000). But the solubility of CO_2 is mostly influenced by the storage temperature; CO_2-solubility decreases with increasing temperature (Gill 1988, Walsh & Kerry 2000). However, Phillips (1996) pointed out, that this small pH-decrease is not the only reason for the bacteriostatic effect of CO_2. Further theories are based on the inhibition of microorganisms due to a decrease of intracellular pH, the inhibition of enzyme syntheses and/or enzymatically catalyzed reactions, interactions with cell membranes and influence of the nutrient uptake (Farber 1991). In conclusion, the effect of carbon dioxide is not induced by a single mechanism, but rather a result of a complex process between several physiological reactions (Dixon & Kell 1989).

Nitrogen (N_2)

Nitrogen (N_2) is an inert gas. Because of its inert properties, there is no direct effect on microbiological growth reported. It is used as a filling gas in MA-packages to prevent a package collapse because of its low solubility in water and fat (Stanbridge & Davis 1998, Dangel 2006, Bilska 2011). Especially meat packs with high CO_2 concentrations are very sensitive to package collapsing because of the solubility of carbon dioxide in the water and fat phase of the meat tissue. The flushing of the packs with N_2 before introducing the protection gas has the effect to remove the remaining oxygen, which results in prevention of rancidity and aerobic bacterial growth (Phillips 1996, Arvanitoyannis & Statakos 2012).

Argon (Ar)

Argon is also chemically inert like nitrogen and belongs to the group of noble gases. Argon is supposed to have an effect on the activity of enzymes, on the growth of microorganism and on deteriorative chemical reactions in food. This is possibly based on the similar atomic size to oxygen and its higher density and solubility in fat and water than oxygen and nitrogen. Therefore, argon is able to displace oxygen more effectively than nitrogen (Spencer 2005, Day 2008). The scientific literature reports other potential beneficial effects of argon on the shelf life and sensory attributes for a wide range of food and food products. The effects are summarized in Table 1.

Table 1.1 Reported effects of argon treatments on different food and food products

Product	Effect	Reference
Asparagus spears	• inhibition of enzymatic reaction • restricted intracellular water activity • positive effect on chlorophyll preservation and weight loss • longer shelf life	Zhang et al. (2008)
Cut apples	• improvement in quality after high argon treatment	Wu et al. (2012)
In vitro study	• significant reduction of tyrosinase and malic dehydrogenase activity (responsible for browning reaction of fresh cut fruits/vegetables)	Zhang et al. (2001)
Kiwifruit slices	• better firmness • lower CO_2-production	Rocculi et al. (2005)
In vitro study	• inhibitory effect of low-oxygen atmospheres with argon on apple and mushroom-polyphenoloxidase	O`Beirne et al. (2011)
Fresh cut lettuce/broccoli	• no effect on phenolics of fresh cut-lettuce • no effect on chlorophyll preservation in broccoli	Jamie & Saltveit (2002)
Turkey meat	• inhibitory effect on total anaerobic counts, total psychotropic counts and on *Brochothrix thermosphacta* • no effect on lipid oxidation	Fraqueza & Barreto (2009)
Pork sausages	• no effect on microbial growth and biogenic amines • sensory evaluation achieved the most effective scores using argon	Ruiz-Capillas & Jiménez-Colmenero (2010)
In vitro study	• inhibition of *Carnobacterium divergens* • favor the growth of agmatine producing *Enterobacteriaceae* (both isolated from pork sausages)	Curiel et al. (2011)
Dry-cured Iberian ham	• no significant influence on shelf life	Parra et al. (2010)
Poultry meat	• increase in the microbiological growth • unpleasant odor	Tománková et al. (2012)

1.2 Recommended gas mixtures for poultry meat

Poultry meat is relatively sensitive to microbial spoilage and also supports the growth of pathogenic bacteria due to its physical and chemical properties (Yavas & Bilgin 2010). Guaranteeing the quality as well as the safety aspects of the food by optimizating mixture of used gases is challenging for the food industry (Moller et al. 2000). Therefore, the gaseous atmosphere is often not adjusted optimally for the composition and the specific microflora of each kind of meat. Only general and inconsistent recommendations of gas producers exist without adaptation to the specific product. The result is that some poultry producers use high oxygen atmospheres, whereas others are using mixtures with less oxygen (Thoden van Velzen & Linnemann 2008), as emphasised in Table 1.2.

Table 1.2 Recommended gas compositions of different gas producers for fresh MA-packaged poultry meat in Germany (packages for self service).

Gas producer	% CO_2	% N_2	% O_2
Linde AG	50-80	20-50	
Messer Industriegase GmbH	25-100	75-0	0
Air Products GmbH	30	70	
Air Liquide Deutschland GmbH	30	0	70
Praxair	30	70	
Westfalen AG[1]	30		70
	30	30	40

*Date: 10.12.13;
1 = recommended for fresh meat in general

1.3 Spoilage process of meat and poultry under MAP conditions

Food spoilage can be defined as a process, which leads to a deteriorative change of the product and makes it unacceptable for human consumption (Hayes 1985). The most important factor causing spoilage and shelf life reduction is microbiological growth. Therefore the control of microbial activity is the prerequisite to preventing food spoilage during distribution and storage (Genigeorgis 1985). Responsible for the rate of spoilage are several intrinsic (e.g. pH, a_w-value), process (e.g. initial bacterial count) and extrinsic factors (e.g. temperature, humidity, gas atmosphere) (Mossel 1971). After the processing step, the packaging of a product plays an important role in slowing down the spoilage process. Generally, the packaging of a food or food product has different functions:

- protection against the environment,
- preservation of color,
- preservation of flavor and odor,
- protection against nutrient and texture loss,
- shelf life extension (Dallyn & Shorten 1988, Zhou et al. 2010).

The effect on the extension of shelf life of packaged fresh meat is also influenced by the product itself, the gas atmosphere, the packaging material and headspace, the packaging equipment, and the storage temperature during the supply chain (Han 2005, Zhou et al. 2010). Low temperatures for example combined with MAP-treatment leads to an extended shelf life and an improved storage stability of the food product (Leister 1995). Furthermore, the packaging has a main influence on the development of the microflora of poultry meat, whereby the surface diversity consists of mesophilic and psychrotrophic bacteria as well as pathogenic species.

Under aerobic conditions, ***Pseudomonas* spp.** dominates the spoilage flora of fresh aerobic stored chilled meat (Nychas & Drosinos 2000, Kreyenschmidt 2003, Gospavic et al. 2008; Bruckner 2010). Because of its psychrotrophic properties, *Pseudomonas* spp. is responsible for spoilage at -1 – 25°C (Hood & Mead 1993). Regarding the oxygen requirements, *Pseudomonas* spp. is an aerobic bacterium, but is also able to grow under reduced oxygen concentrations. As stated by Clark & Burki (1972), *Pseudomonas* spp. shows growth stability even under an oxygen content of less than 1%. Additionally, the bacteria show sensitivity to CO_2 in a gas mixture.

Under reduced oxygen concentration and because of the improved resistance against CO_2, ***Brochothrix thermosphacta*** is often associated as the main spoilage microorganism in MAP meat (Branscheid et al. 2007, Kreyenschmidt & Ibald 2012). As a facultative anaerobic microorganism, *Brochothrix thermosphacta* is able to grow under oxygen atmospheres as well as under reduced oxygen concentrations, but they prefer to grow in the presence of oxygen (Mullan & McDowell 2003). Also its psychrotrophic character contributes to the dominance of *B. thermosphacta* under MAP conditions on a wide range of meat and meat products (Pennacchio et al. 2009). Based on these characteristics, meat is an ecological niche for these bacteria, which cause spoilage through the production of spoilage metabolites (Labadie 1999, Pin et al. 2002).

Another facultative anaerobic group of bacteria associated with spoilage under MAP-conditions are **Enterobacteriaceae**. This group of bacteria includes mesophilic and psychrotrophic species (Weber 2008) and is more prevalent on pork and lamb (Dainty & Mackey 1992). The main species associated with meat spoilage are *Serratia*, *Pantoea, Klebsiella*, *Proteus* and *Hafnia* (Borch et al. 1996, Weber 2008).

Lactic acid bacteria (LAB) are strictly anaerobic microorganisms which are highly tolerant towards CO_2-enriched atmospheres. In a vacuum and under modified atmosphere conditions with low oxygen concentrations, Lactic acid bacteria are able to achieve high bacteria counts on the fresh meat surface (Borch et al. 1996, Shaw & Harding 2008). On refrigerated poultry meat, the bacteria play a minor role as part of the spoilage flora (Herbert et al. 2013, Rossaint et al. 2014) due to their mesoplilic properties (Huis in`t Veld, 1996; Jay et al., 2005).Table 1.3 gives an overview about the different tested gas mixtures for poultry meat and the main microorganisms occurring on the meat surface.

Table 1.3. Microflora on poultry meat under different gas atmospheres.

Meat type	Packaging	Temperature	Microflora	Reference
Chicken breast fillet	60% CO_2/39,6% N_2/0.4% CO (variation of packaging material)	4°C and 8°C	***Pseudomonas* spp.**, **Enterobacteriaceae**, *B. thermosphacta*, **LAB**	Pettersen et al. 2004
Chicken breast fillet	15% N_2/60% O_2/25% CO_2 15% Ar/60% O_2/25% CO_2 25% N_2/ 45% O_2 / 30% CO_2 25% Ar/ 45% O_2 / 30% CO_2 82% N_2/18% CO_2 82% Ar/18% CO_2	4°C	***Pseudomonas* spp.**, Enterobacteriaceae, ***B. thermosphacta***, LAB	Herbert et al. 2013
Chicken breast fillet	aerobic	4°C	***Pseudomonas* spp**.	Rossaint et al. 2014
	70% O_2/30% CO_2	4°C	***Pseudomonas* spp.**, Enterobacteriaceae, ***B. thermosphacta***, LAB	
	70% N_2/30% CO_2	4°C	***Pseudomonas* spp.**, Enterobacteriaceae, ***B. thermosphacta***, LAB	
Chicken breast meat	30% CO_2/70% N_2	4°C	***Pseudomonas* spp.**, Enterobacteriaceae, ***B. thermosphacta*, LAB**	Chouliara et al. 2007
	30% N_2/70% CO_2	4°C	***Pseudomonas* spp.**, Enterobacteriaceae, ***B. thermosphacta***, **LAB**	
Chicken breast with skin	30% CO_2/70% N_2	4°C	*Pseudomonas* spp., **Enterobacteriaceae, *B. thermosphacta***, **LAB**	Jiménez et al. 1997
	70% CO_2/30% N_2	4°C	*Pseudomonas* spp., **Enterobacteriaceae, *B. thermosphacta***, **LAB**	
Chicken breast meat	30% CO_2/70% N_2	4°C	*Pseudomonas* spp., **Enterobacteriaceae,** *B. thermosphacta*, **LAB**	Balamatsia et al. 2006

in bold = dominant species

Currently, a wide range of possible gas mixtures for fresh poultry meat exist on the market. The combinations of the commonly used packaging gases (O_2, CO_2, N_2) varies according to the recommendations given by the gas companies. In the scientific literature, contradictory results and recommendations about the optimal gas mixture for fresh poultry fillets are also published. The results are frequently not comparable due to different testing conditions and parameters.

Fluctuating temperature conditions, which frequently occur during storage and distribution, have an effect on the composition of the spoilage flora especially on MAP poultry meat. As a result, a reliable assessment of the quality loss of poultry fillets under modified atmosphere conditions by identifying a specific spoilage organism (SSO) is challenging.

Contradictory results are also published about the effect of different oxygen concentrations on the spoilage process of MAP poultry fillets. Detailed information about the behavior of typical spoilage microorganisms in combination with sensory deteriorative changes is important for the poultry meat industry due to the fact that the application of high oxygen atmospheres is still common especially in the German market.

In this context, safety aspects are also a prerequisite for the assessment of different gas mixtures regarding their contribution to producing a safe product which is suitable for human consumption.

Additionally, gas producers are searching for novel gas mixtures which deliver beneficial effects regarding the extension of shelf life and/or the preservation of sensory attributes. Tests on the effects of alternative gases on the quality and shelf life of poultry fillets are still rare.

But complex changes occurring during spoilage by varying different environmental influence factors which are reflected in a single index which combines microbiological and sensory changes is not available till now yet.

1.4 Research questions and outline of the thesis

The main objective of this thesis is the assessment and comparison of different environmental influence factors on the quality loss of modified atmosphere packaged poultry fillets. For this purpose, the following research questions are proposed:

- How are different temperature conditions and different oxygen combinations influencing the composition of the specific spoilage flora, and can a specific spoilage organism be identified to determine the quality loss of poultry fillets?
- How is the growth of specific spoilage bacteria and pathogens influenced by oxygen enriched atmospheres in comparison to nitrogen enriched atmospheres?
- How is the growth of specific spoilage bacteria and the development of sensory parameter influenced by using argon as an alternative to nitrogen?
- Is it possible to develop an overall quality index based on microbiological and sensory parameter to assess the influence of different environmental influence factors on the quality loss of MAP poultry?

In the first part of the thesis (*chapter 2*), the spoilage of fresh skinless poultry breast meat (70% O_2/30% CO_2) under different constant temperature conditions (2, 4, 10, 15°C) is characterized and the development and the composition of typical spoilage microorganisms and sensory parameter are investigated.

In *chapter 3*, several storage trials are conducted under different gas atmospheres to figure out the influence of the different oxygen concentrations on the development of the spoilage flora and sensory parameters and the overall shelf life of poultry fillets. Especially the influence of different oxygen concentrations on the growth rate and lag-phase of the spoilage microorganisms were analyzed and compared.

In *chapter 4* of the thesis, the growth of *Listeria monocytogenes* in presence of the typical spoilage microorganisms is investigated. The growth was compared by using two different gas combinations commonly used in Germany 70% O_2/30% CO_2) and the EU (70% O_2/30% CO_2).

In the next part (*chapter 5*), the quality loss of poultry fillets under different argon concentrations and nitrogen containing atmospheres is analyzed and compared. For this purpose, poultry fillet is stored under argon and nitrogen atmospheres and the growth of typical spoilage organisms and sensory parameter is compared to gather information about similarities and differences.

The last chapter (*chapter 6*), an overall quality index based on the combination of microbiological and sensory parameter is developed. The quality index is calculated for all storage trials which are described in chapter 2-5. Based on the results the influence of all tested environmental parameter is compared to release an assessment about the appropriate gas mixtures for MAP poultry fillets. The index can support poultry processing companies to assess the effect of different environmental parameter for poultry meat in a more standardized way.

1.5 References

Amanatidou, A. 2001. High oxygen as an additional factor in food preservation. Ph.D. Thesis, Wageningen University, The Netherlands.

AMI. 2013. Agrarmarkt Informations-Gesellschaft mbH. www.ami-informiert.de/ami/english.html. Stand: 25.03.2014.

Arvanitoyannis, I. S., A. Ch. Stratakos. 2012. Application of modified atmosphere packaging ans active/smart technologies to red meat and poultry: A review. *Food Bioprocess Technology,* 5:1423-1446.

Balamatsia, C. C., E. K. Paloelogos, M. G. Kontominas, I. N. Savvaidis. 2006. Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4°C: possible role of biogenic amines as spoilage indicators. *Antonie van Leeuwenhoek*, 89:9-17.

Balev, D. K., A. S. Staykov, G. Y. Ivanov, St. G. Drageov, E. H. Filizov. 2011. Colour stability improvement of chilled beef by natural antioxidant treatment and modified atmosphere packaging. *American Journal of Food Technology,* 6:117-128.

Bell, R. G. 2001. Meat Packaging: Protection, Preservation and Presentation Pages 463-490 in Meat Science and Applications. 1st ed., Hui, Y. H. (eds.), Marcel Dekker. Inc., New York, Basel.

Bilska, A. 2011. Packaging systems for animal origin food. *LogForum* 7(4):35-44.

Borch, E., M. L. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and meat products. *International Journal of Food Microbiology,* 33(1):103-120.

Branscheid, W., K. O. Honikel, G. von Lengerken, K. Troeger. 2007. Qualität von Fleisch und Fleischwaren. 2nd Edit, Deutscher Fachverlag GmbH, Frankfurt am Main.

Church, N. 1994. Review - Developments in modified-atmosphere packaging and related technologies. *Trends Food Science & Technology,* 5:345-352.

Chouliara, E., A. Karatapanis, I. N. Savvaidis, M. G. Kontominas. 2007. Combined effect of oregano essential oil on modified atmosphere packaging and shelf-life extension of fresh chicken breast meat, stored at 4°C. *Food Microbiology*, 24:607-617.

Clark, D. S., and T. Burki. 1972. Oxygen requirements of strains of *Pseudomonads* and *Achromobacter*. *Canadian Journal of Microbiol*ogy, 18:321-326.

Curiel, J. A., C. Ruiz-Capillas, B. de las Rivas, A. V. Carrascosa, F. Jiménez-Colmenero, and R. Munoz. 2011. Production of biogenic amines by lactic acid bacteria and erterobacteria

isolated from fresh pork sausages packaged in different atmospheres kept under refrigeration. *Meat Science,* 88:368-373.

Dainty, R. H., and B. M. Mackey. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage process. *Journal of Applied Bacteriology*, 73(21):103S-114S.

Dallyn, H., D. Shorten. 1988. Hygiene aspects of packaging in the food industry. *International Biodeterioration,* 24:387-392.

Dangel, S. 2006. MAP-Verfahren bei Frischgeflügel. *Fleischmagazin*, 7-8:36-38.

Day, B. P. F. 2007. Modified atmosphere packaging (MAP) – A global perspective on new developments. 40th AIFST Convention, Melbourne, 25 June 2007.

Day, B. P. F. 2008. Modified Atmosphere Packaging (MAP). In: Food Biodeterioration and Preservation, Tucker, G. S. (ed.). Blackwell Publishing Ltd.,Ames, pp 165-192.

Devlieghere, F., J. Debevere, and J. van Impe. 1998. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. *International Journal of Food Microbiology,* 43:105-113.

Dixon, N. M., D. B. Kell. 1989. The inhibition by CO_2 of the growth ad metabolism of micro-organisms*. Journal of Applied Bacteriology,* 67:109-136.

EU 1995. Directive No. 95/02/CE. Commission Decision 20/02/1995 on food additives other than colours and sweeteners. Off. J. L61:0001-0040.

Farber, J. M. 1991. Microbiological aspects of modified-atmosphere packaging technology – A review. *Journal of Food Protection,* 54:58-70.

Fierheller, M. G. 1991. Modified atmosphere packaging of miscellaneous products. Pages 246-257 in Modified Atmosphere Packaging of Foods. Ooraikul B. M. E. Stiles (eds,), Ellis Horwood Ltd, Chichester, UK.

Floros, J. D., K. I. Matsos (2005). Introduction to modified atmosphere packaging. In: Han et al. Innovations in food packaging. Elsevier Academic Press. pp 151-171.

Fox, J. B. 1966. The Chemistry of Meat Pigments. *J. Agric. Food Chem.* 14(3):207-210.

Fraqueza, M. J., A. S. Barreto. 2009. The effect on turkey meat shelf life of modified-atmosphere packaging with an argon mixture. *Poultry Science,* 88:1901-1998.

Genigeorgis, C. A. 1985. Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. *International Journal of Food Microbiology,* 1:237-251.

Gill, C. O.; K. H. Tan. 1980. Effect of carbon dioxide on growth of meat spoilage bacteria. *Applied Environmental Microbiology,* 39(2):317-319.

Gill, C. O. 1988. The solubility of Carbon Dioxide in Meat. *Meat Science,* 22:65-71.

Gospavic, R., J. Kreyenschmidt, S. Bruckner, V. Popov, N. Haque. 2008. Mathematical modelling for predicting the growth of *Pseudomonas* spp. in poultry under variable temperature conditions. *International Journal of Food Microbiology,* 127:290-297.

Gregory, E. M., I. Fridovich. 1973. Induction of superoxide dismutase by molecular oxygen. *Journal of Bacteriology,* 114:543-548.

Halliwell, B. 1984. Superoxide dismutase and the superoxide theory of oxygen toxicity. A critical appraisal. In Copper Proteins and Copper Enzymes, Lonti, R. (ed.), CRC Press, Boca Raton, pp 63-102.

Halliwell, B. ,J. M. C. Gutteridge. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal,* 219:1-14.

Han, J.H. (2005). Innovations in food packaging. Food Science and Technology, International Series, Elsevier Ltd.

Hayes, P. R. 1985. Food Microbiology and Hygiene. 1st Edit., Elsevier Academic Press, London, pp 80-139.

Hood, D. E., G. C. Mead. 1993. Modified atmosphere storage of fresh meat and poultry, In: Parry, R. T. (edit.), Principles and Applications of Modified Atmosphere Packaging of Food, Blackie Academic and Professional, London, pp 269-298.

Huis in`t Veld, J. H. J. 1996. Microbial and biochemical spoilage of foods: an overview. *Food Microbiology*, 33:1-18.

Jacxsens, L., F. Devlieghere, C. Van der Steen, and J. Debevere. 2001. Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71:197-210.

Jamie, P., M. E. Saltveit. 2002. Postharvest changes in broccoli and lettuce during storage in argon, helium, and nitrogen atmospheres containing 2% oxygen. *Postharvest Biology and Technology,* 26, 113-116.

Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Modern Food Microbiology. 7th Edition, Springer, New York, London, Berlin.

Jiménez, S. M., M. S. Salsi, M. C. Tiburzi, R. C. Rafaghelli, M. A. Tessi, V. R. Coutaz. 1997. Spoilage microflora in fresh chicken breast stored at 4°C: influence of packaging methods. *Journal of Applied Microbiology*, 83:613-618.

Kim, Y. H., E. Huff-Lonergan, J. G. Sebranek, S. M. Lonergen. 2010. High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerization. *Meat Science,* 85:759-767.

Kreyenschmidt, J. (2003). Modellierung des Frischeverlustes von Fleisch sowie des Entfärbeprozesses von Temperatur-Zeit- Integratoren zur Festlegung von Anforderungsprofilen für die produktbegleitende Temperaturüberwachung. PhD thesis, Rheinische Friedrich-Wilhelms-Universität Bonn. Bergen/Dumme, Germany: AgriMedia GmbH.

Kreyenschmidt, J, R. Ibald. 2012. Modeling shelf life using microbial indicators. In: Nicoli, M. C. (edit.). Shelf life Assessment of Food. CRC Press. pp 127-168.

Labadie, J. (1999). Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science*, 52, 299-305.

Lavelle, F., A. M. Michelson, L. Dimitrijevic. 1973. Biological protection by superoxide dismutase. *Biochemicial and Biological Research Communication*, 55(2):350-357.

Leister, L. (1995). Principles and applications of hurdle technology. In: G. W. Gould (ed.) New Methods of Food Preservation. Blackie, Glasgow, UK, pp 1-21.

Martínez, L., D. Djenane, I. Cilla, J. A. Beltrán, P. Roncalés. 2005. Effect of different concentrations of carbon dioxide and low concentration of carbon monoxide on the shelf-life of fresh pork sausages packaged in modified atmosphere. *Meat Science,* 71:563-570.

Moller, J. K. S., J. S. Jensen, M. B. Oslen, L. H. Skibsted, G. Bertelsen. 2000. Effect of residual oxygen on colour stability during chill storage of sliced, pasteurized ham packaged in modified atmosphere – What to do about it. *Meat Science,* 54:399-405.

Mossel, D. A. A. 1971. Physiological and metabolic attributes of microbial groups associated with foods. *Journal of Applied Bacteriology*, 34(1):95-118.

Mullan, M., D. McDowell. 2003. Modified atmosphere packaging. In: R. Coles, D. McDowell, M. J. Kirwan (Eds.): Food Packaging Techology, Blackwell Publishing, London, pp. 303-339.

Nychas G. J. E., and E.H. Drosinos (2000). Meat and Poultry – Spoilage of Meat, Pages 1253-1284 in Encyclopedia of Food Microbiology, 2nd Edition. R. K Robinson, C.A. Batt, and P.D. Patel, ed. Academic Press, London, UK.

O`Beirne, D., E. Murphy, and D. N. Eidhin. 2011. Effects of argon enriched low-oxygen atmospheres and of high-oxygen atmospheres on the kinetics of polyphenoloxidase (PPO). *Journal of Food Science,* 76(1):73-77.

Pennacchia, C., Ercolini, D. & Villani, F. (2009). Development of a Real-Time PCR assay for the specific detection of Brochothrix thermosphacta in fresh and spoiled raw meat. *International Journal of Food Microbiology*, 134, 230-236.

Pettersen, M. K., H. Nissen, T. Eie, A. Nilsson. 2004. Effect of packaging materials and storage conditions on bacterial growth, off-odour, pH and colour if chicken breast fillets. *Packaging Technology and Science*, 17:165-174.

Parra, V., J. Viguera, J. Sanchez, J. Peinado, F. Esparrago, J. I. Gutierrez, and A. I. Andrés. 2010. Modified atmosphere packaging and vacuum packaging for long period chilled storage of dry-cured Iberian ham. *Meat Science,* 84:760-768.

Phillips, C. A. (1996). Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology,* 31:463–479.

Pin, C., Garcia de Fernando, G. and Ordonez, J. A. (2002). Effect of modified atmosphere on the metabolism of glucose by *Brochothrix thermosphacta*. *Applied Environmental Microbiology*, 68, 4441-4447.

Rocculi, P., S. Romani, and M. D. Rosa. 2005. Effect of MAP with argon and nitrous oxide on quality maintenance of minimally processed kiwifruit. *Postharvest Biology and Technology,* 35:319-328.

Rossaint, S., Klausmann, S., Herbert, U. & Kreyenschmidt, J. (2014). Effect of package perforation on the spoilage process of poultry stored under different modified atmospheres. *Food Packaging and Shelf life*, 1:68-76.

Ruiz-Capillas, C., and F. Jiménez-Colmenero. 2010. Effect of an argon-containing packaging atmosphere on the quality of fresh pork sausages during refrigerated storage. *Food Control,* 21:1331-1337.

Shaw, B. G., and C. D. Harding. 2008. A numerical taxonomic study of lactic acid bacteria from vacuum packed beef, pork, lamb and bacon. *Journal of Applied Bacteriology*, 56(1):25-40.

Sørheim., O., F. Westad, H. Larsen, O. Alvseike. 2009. Colour of ground beef as influenced by raw materials, addition of sodium chloride and low oxygen packaging. *Meat Science,* 81:467-473.

Spencer, K. C. 2005. Modified atmosphere packaging of ready-to-eat foods, Pages 185-203 in Innovations in food packaging, Han, J. H., ed. Food Science and Technology, International Series.

Stanbridge, L. H., A. R. Davies. 1998. The microbiology of chill-stored meat. In: A. Davies, R. Board (Eds.). The microbiology of meat and poultry. Blackie Academic & Professional, London, UK, pp 174-219.

Thoden van Velzen, E. U. & A. R. Linnemann. 2008. Modified atmosphere packaging of fresh meats – Sudden partial adaption caused an increase in sustainability of dutch supply chains of fresh meat. *Packaging Technology and Science,* 21:37-46.

Tomankova, J., G. Borilova, I. Steinhauserova, and L. G. Las. 2012. Volatile Organic Compounds as Biomarkers of the Freshness of Poultry Meat Packaged in a Modified Atmosphere. *Czech Journal of Food Science,* 30(5):395-403.

Walsh, H. M., J. P. Kerry. 2000. Meat Packaging. In: J. Kerry, J. Kerry, D. Ledward (Eds.). Meat processing – Improving quality. CRC Press, Woodhead Publishing Limited, Cambridge, UK, pp 417-452.

Weber, H. 2008. Mikrobiologie der Lebensmittel, Fleisch-Fisch-Feinkost. 2nd Edition, Behrs Verlag, Hamburg.

Wu, Z. S., M. Zhang, and S. Wang. 2012. Effects of high pressure argon treatments on the quality of fresh-cut apples at cold storage. *Food Control,* 23(1):120-127.

Yavas, E. and B. Bilgin. 2010. Effect of calcium lactate, sodium diacetate and sodium chloride mixture on the microbial, chemical and sensory properties of chicken nuggets stored in refrigerated and under modified atmospheres. *International Journal of Poultry Science,* 9:66-71.

Zakrys-Waliwander, P. I., M. G. O`Sullivan, E. E. O`Neil, J. P. Kerry. 2012. The effect of high oxygen modified atmosphere packaging on protein oxidation of bovine M. longissimus dorsi muscle during chilled storage. *Food Chemistry,* 131:527-532.

Zhou, G. H., X. L. Xu, Y. Liu (2010). Preservation technologies for fresh meat – A review. *Meat Science,* 86:119-128.

Zhang, D., P. C. Quantick, J. M. Grigor, R. Wiktorowicz, and J. Irven. 2001. A comparative study of effects of nitrogen and argon on tyrosinase and malic dehydrogenase activities. *Food Chemistry,* 72:45-49.

Zhang, M., Z. G. Zhan, s. J. Wang, and J. M. Tang. 2008. Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases. *LWT-Food Science & Technology,* 14:686-691.

2. Definition of predictor variables for MAP poultry filets stored under different temperature conditions

2.1 Abstract

Storage tests under different temperatures (2, 4, 10, 15°C) were conducted to identify the best predictor variable, which is most effective to explain the loss of the shelf life and quality of MAP poultry and constitutes the basis for the prediction of the remaining shelf life. The Samples were packed in 70% O_2 and 30% CO_2, which is the common used gas atmosphere for poultry fillets in Germany. Typical spoilage microorganisms (*Pseudomonas* spp., *Brochothrix thermosphacta*, Enterobacteriaceae, *Lactobacillus* spp.) and total viable count (TVC) were investigated frequently. Additionally, samples were analyzed to sensory changes, pH and gas concentration. The data extraction and selections by stepwise regression and principle component analyses was carried out to identify a variable which has the main influence on shelf life and freshness loss. The results accentuate that the spoilage is caused by a wide range of microorganisms. No specific microorganism could be identified as the dominant originator for the deteriorative changes. Solely TVC showed significant correlations between the development of the sensory decay and the development of the TVC for each single storage temperature.

Submitted in *Poultry Science*, see list of publications (Herbert et al. 2014).

2.2 Introduction

Raw poultry meat is sensitive for microbial spoilage due to its physical and chemical properties. Therefore, packaging under modified atmosphere conditions is widely established to improve the quality attributes and shelf life (Rokka et al., 2004). The main gases used for packaging are oxygen, carbon dioxide and nitrogen, combined in different mixtures (Philips, 1996; Mullan & McDowell, 2003). Changes of the packaging conditions due to oxygen requirements of the bacteria have a selective effect on the microbial population (Farber, 1991; Labadie, 1999). Next to packaging, temperature is the most important influence factor on shelf life and quality of fresh meat and meat products during processing, distribution and storage (Cox et al., 1998; McDonald & Sun, 1999; Labadie, 1999; Bruckner et al., 2012a, b). Thereby, MAP-treatment combined with the storage at chill temperatures leads to an extended shelf life and an improved storage stability of the food product (Leister, 1995). Thus, increasing temperature conditions results in an increase of microbial growth with a decrease in the lag phase and the generation time (Herbert & Sutherland, 2000). For example, an increase of the storage temperature from 2°C to 4°C leads to a decrease in shelf life of fresh aerobic packed poultry by nearly 22% (Bruckner et al., 2012c). The spoilage of poultry under aerobic conditions is mainly caused by different species of psychrotropic *Pseudomonas spp.* (Barnes, 1976; Gill & Newton, 1977; Pooni & Meat, 1984; Gram et al., 2002; Koutsoumanis et al., 2006; Raab et al., 2008). The detailed knowledge about the growth behaviour of these so called specific spoilage organism (SSO) are the basis for the development of predictive shelf life models (Gram et al., 2002). Under modified atmosphere conditions, *B. thermosphacta* and *Lactobacillus* spp. are often described as the main spoilage microorganisms on meat stored at cold temperatures (Dainty & Mackey, 1992; Davies, 1995). Actually, inconsistent information regarding the identification of a main spoilage microorganism of MAP poultry is described in the scientific literature. Most studies are only focused on CO_2-N_2 gas mixtures for the packaging of MAP poultry, but the German poultry industry packed the meat under high oxygen (50-70% O_2) atmospheres (Rossaint et al., 2013, unpublished). But the spoilage process is mainly caused by a wide spectrum of microorganisms like *B. thermosphacta*, *Pseudomonas* spp., *Enterobaceriaceae* and *Lactobacillus* spp. (Borch et al., 1996; Saucier et al., 2000; Walsh & Kerry, 2000) and the growth is mainly influenced by the initial bacterial load, the gas mixture, the product-gas ratio and the storage temperature (Sivertsvik et al., 2002). Jiménez et al. (1997) demonstrated a good growth of *Lactobacillus* spp., Enterobacteriaceae and *B. thermosphacta* on fresh chicken breast stored at 4°C in MAP under both used atmospheres (30% CO_2/70% N_2 and 30% N_2/70% CO_2). A study by Smolander et al. (2004) and Rajamäki et

al. (2006) pointed out that varying storage temperatures and under 80% CO_2 / 20% N_2 affected most the growth of Enterobacteriaceae on MAP poultry meat. In contrast, Balamatsia et al. (2007) identified *B. thermosphacta* and Lactic acid bacteria as the main spoilage microorganisms on aerobic and MAP (30% CO_2 / 70% N_2) chicken fillets. Therefore, the reliable determination of remaining shelf life based on the definition of SSOs under changing extrinsic influence factors is challenging for the development of a predictive shelf life model. Consequently, the present study analyzed the spoilage process of poultry, packed under the in Germany used gas atmosphere with high oxygen at varying temperature conditions to identify the best predictor variable, which is most effective in predicting the deteriorative changes of MAP poultry during storage.

2.3 Materials & Methods

Preparation of meat samples

Unsexed 42-days-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 hours after slaughtering. In the laboratory the double breast fillets were divided into single fillets using a sterile scalpel.

Packaging and storage of meat samples

For modified atmosphere packaging, the poultry 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 2:1. The meat samples were packaged under an atmosphere containing 30% CO_2/70% O_2. Thereafter, the trays were heat-sealed with a polypropylene foil (Suedpack Verpackungen GmbH & Co. KG, Ochsenhausen, Germany; water vapour permeability < 3.5 g/m^2d 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 four-component gas blender machine (KM 60-4 MEM SO, Witt Gasetechnik, Witten, Germany). The packaged meat samples were stored at 2, 4, 10 and 15°C in low-temperature high precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). The storage

temperatures were monitored by data logger (ESCORT JUNIOR Internal Temperature Data Logger, Escort, Auckland, New Zealand) every 5 minutes. The microbiological, sensory and chemical analyses were conducted at appropriate time intervals. Each measurement was repeated three times.

Microbiological analyses

For microbiological analyses, the meat surfaces were removed aseptically by using a sterile scalpel. The product sample had an admeasurement of 4 x 7 x 0.5 cm to achieve a total weight of nearly 25 g, which were transferred to a filtered sterile stomacher bag and filled up with 225 ml saline peptone diluent (0.85 % NaCl with 0.1% peptone Saline-Tablets, Oxoid BR0053G, Cambridge, United Kingdom). 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 (TVC), 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 (PCA, Merck, Darmstadt, Germany) and plates were incubated at 30°C for 72 hours. Pseudomonas spp. were detected by spread plate technique on Pseudomonas Agar with Cetrimide-Fucidin-Cephalosporin selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 hours. 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 hours. Enterobacteriaceae were identified by overlay treatment on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany) by incubation of the agar plates at 30°C for 48 hours. Lactobacilli spp. were detected by pour plate technique on de Man, Rogosa, Sharpe Agar (MRS, Oxoid, Cambridge, United Kingdom). Plates were incubated aerobically at 37°C for 72 hours. Counts of colony forming units were expressed as $\log_{10}$cfu/g for each medium and sample.

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.

Gas analysis

Concentrations of oxygen and carbon dioxide inside the trays were monitored over the storage period, using a hand-held gas analyser (Oxybaby V O_2/CO_2, Witt Gasetechnik, Witten, Germany). Before starting the gas measurement inside the trays, the composition of air was analysed to control the accuracy of the gas analyser. 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 seconds and the oxygen concentration was detected by an electrochemical sensor; carbon dioxide concentration was detected by IR-absorption. Control packages containing no meat samples were stored as reference and the gas composition was also monitored over the entire storage period.

Sensory evaluation

Sensory analyses were carried out by trained sensory panellists, which were recruited from the Institute of Animal Science (University of Bonn) and experienced in poultry evaluation. During the trials, each sample was evaluated directly after opening the tray, using a developed sensory scheme according to the Quality Index Method (QIM) for fish evaluation (Bremner, 1985). A picture of fresh chicken breast fillets was used as reference during the sensory evaluations.

Attributes were defined as general appearance (G), colour (C), odour (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 quality index (QI) was calculated by the following equation (Kreyenschmidt, 2003):

$$QI = \frac{2G + 2C + 1T + 1D + 2O}{8} \qquad (1.1)$$

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

Primary Modelling

The Gompertz equation was used to model the growth of the total viable count, Enterobacteriaceae, Pseudomonas spp., Brochothrix 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., U.S.A.).

Statistical analysis

Man-Whitney-U-test was used to make comparisons between the measured counts of colony forming units and pH-values with a level of significance of 0.05. Further on, a stepwise regression was carried out of the original data set to reduce the number of data and find the best predictor variable for shelf life assessment, which is most effective in predicting the dependent variable. Additionally, a Principle Component Analyses (PCA) was used to extract the variables (components) with the highest explanatory power for the data set. Before performing the PCA, a z-transformation was conducted to standardise data measured in different scales. SPSS statistics 20 for Windows was used.

2.4 Results & Discussion

Development of spoilage microflora under different temperature conditions on MAP poultry fillets

Figure 2.1 shows the development of TVC under different constant temperature conditions (2-15°C) on poultry breast fillets packed under MAP. The graph shows that increasing storage temperatures lead to a faster microbiological growth on fresh meat as also described by several authors (Baranyi et al. 1995, Kreyenschmidt 2003, Raab et al. 2008, Bruckner et al. 2012a).

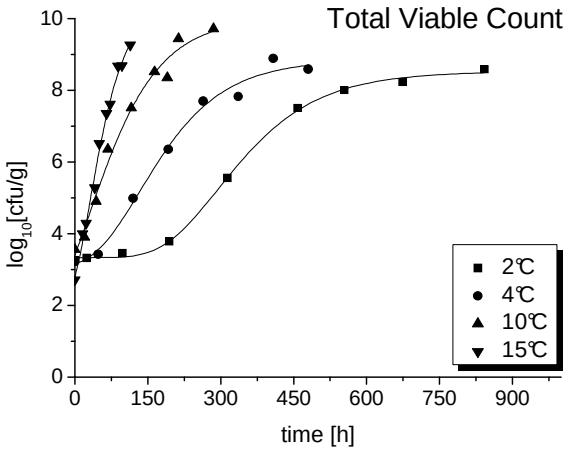

Figure 2.1 Development of TVC on MAP poultry fillets under different temperature conditions (n=3).

The changes in the different microbial groups during MAP storage of poultry fillets under different storage temperatures (2-15°C) are illustrated in Figure 2.2. In general, the growth curves demonstrate a faster growth for all investigated microorganisms with increasing temperatures. The results show that *B. thermosphacta* dominates the spoilage flora at lower temperature conditions (2-4°C), whereas *Pseudomonas* spp. shows an increased growth during storage at higher temperatures (10-15°C).

B. thermosphacta is often associated with spoilage under MAP conditions based on the improved resistance to CO_2 (Borch et al. 1996, Branscheid et al. 2007). During storage, *B. thermosphacta* becomes the predominant spoilage microorganism as also emphasized in Table 2.1 with the highest growth rates during storage at 2-4°C. The results show, that *B. thermosphacta* is relatively resistant against refrigeration temperatures because of its psychotropic properties (McClure et al. 1993). Also the used gas mixture in this study showed no effect in delaying the growth of B. thermosphacta, as stated also by Santé et al. (1994). Even though the microorganism is a facultative anaerobic competitor, it prefers to grow under oxygen atmospheres. Therefore the microorganisms are able to dominate the microflora, when oxygen is present (Gribble & Brightwell 2013).

Pseudomonas spp. are aerobic microorganisms and they grow preferably under oxygen conditions (Chouliara et al. 2008, Fraqueza & Barreto 2009, Herbert et al. 2013). Besides the fact that *Pseudomonas* spp. is a psychrotrophic bacteria, which prefers to grow under refrigeration temperatures, the results show that the growth of P*seudomonas* spp. is retarded at low storage temperatures between 2-4°C (Figure 2.2, Table 2.1). This is

presumable due to the fact that Pseudomonads are sensitive to the antimicrobial component CO_2 (Saucier et al. 2000, Fraqueza & Barreto 2009), which leads to an increase in the lag phase and the generation time (Kreyenschmidt & Ibald 2012). At higher temperatures, *Pseudomonas* spp. dominates the spoilage flora, accordingly to the lower solubility of CO_2 in water and fat and the reduced antimicrobial activity.

Enterobacteriaceae are facultative anaerobic microorganisms, which prefer to grow under oxygen conditions. The initial counts of Enterobacteriaceae are approximately 2 $\log_{10}$cfu/g under all storage conditions, as also shown by Smolander et al. (2004). During storage, the growth of Enterobacteriaceae is slowed down under refrigeration temperatures (2-4°C), whereas at higher temperatures (10-15°C), no delay in the growth can be observed (Table 2.1). This is due to the fact that these microorganisms prefer to grow under mesospheric temperatures and their growth is favoured in comparison to the other microorganisms when temperature increases.

Lactobacillus spp. are only dominant at the beginning of storage under refrigeration temperatures (2-4°C), as also observed by Santé et al. (1994) and Herbert et al. (2013). But their growth becomes not dominant over the entire storage period under all investigated temperatures and they play a minor role in the overall spoilage flora. This is based on the growth conditions of *Lactobacillus* spp., which belongs to a slow growing group of microorganism with preferred growth under anaerobic conditions. Despite the fact that *Lactobacillus* spp. are showing an enhanced tolerance to CO_2, the slow growth at 2-4°C is possibly related to cold temperatures, because Lactobacilli spp. are also mesophilic bacteria (Huis in`t Veld 1996, Jay et al. 2005). At storage temperatures between 10-15°C, the microorganisms growth is favored and possibly caused by the reduced solubility of CO_2 at higher temperatures (Gill 1988) and the mesophilic properties.

Table 2.1 Calculated growth parameters for typical spoilage organisms (Gompertz function), stored under different temperature conditions (70% O_2 / 30% CO_2).

	Growth Parameters							
	Lag Phase [h]				Growth rate [1/h]			
Temperature [°C]	**2**	**4**	**10**	**15**	**2**	**4**	**10**	**15**
TVC	181.25	38.25	9.81	4.56	0.017	0.022	0.040	0.080
***Pseudomonas* spp.**	274.90	27.17	11.50	19.47	0.009	0.016	0.038	0.096
B. thermosphacta	236.10	33.92	19.74	23.23	0.018	0.029	0.038	0.012
Enterobacteriaceae	250.65	47.37	54.79	9.60	0.009	0.021	0.042	0.079
***Lactobacillus* spp.**	345.76	203.68	94.33	33.95	0.009	0.007	0.056	0.082

Generally, temperature has the main influence on microbiological growth also under MAP conditions. The growth curves indicate some kind of synergistic effect between the improved solubility of CO_2 and refrigeration temperatures (2-4°C), while higher temperatures are reduce the solubility of CO_2 and favor the growth of each microorganism. These results are also in accordance to Devlieghere and coauthors (1998), which established a significant interaction term between temperature and dissolved CO_2 on the growth of *Lactobacilli sake*. Regarding the effect of temperature on the development of microorganism's growth curves, the results show that no specific spoilage microorganism could be observed as main spoilage originator under MAP conditions. Also Alfaro et al. (2013) showed for MAP fish products, that the spoilage microflora is changing by varying the storage temperature (0-20°C). Further on Coton and coauthors (2013) stated out that the packaging conditions have a further effect on the microbial ecosystem and lead to an increase in the bacterial diversity.

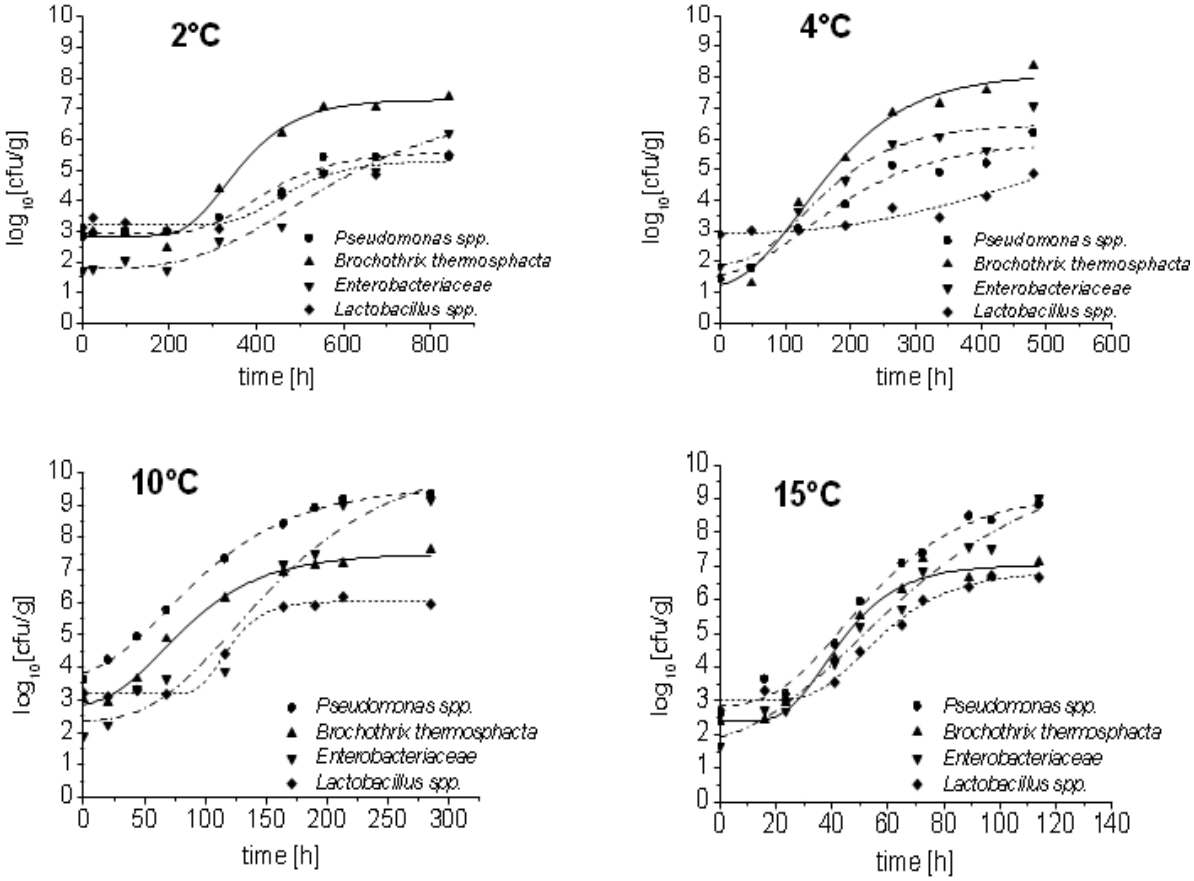

Figure 2.2 Comparison of spoilage microflora development during storage of MAP poultry fillets under various temperature conditions (n=3).

Development of the gas atmosphere

Figure 2.3 shows the development of the gas atmosphere (30% CO_2 / 70% O_2) under different constant temperature conditions with product inside the trays and without any sample as reference. In the beginning of storage, a small decrease of CO_2 could be detected in all test packages with product inside. 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 et al. (2013) and Parra et al. (2010) reported similar results for MA packed meat. But the solubility of the antimicrobial component CO_2 is, besides the muscle tissue pH and the proportion and composition of the fat, dependent from the storage temperature. The solubility of CO_2 in muscle tissue decrease with increasing temperature (Gill 1988), which is reflected in the faster increase of CO_2 at 10°C and 15°C (Figure 2.3). But the proportion which gets dissolved on the meat tissue cannot be quantified due to microbiological growth. During storage, microorganisms consume O_2 for their metabolism and produce CO_2. This effect occurs faster with increasing temperature conditions due to the accelerated microbial growth. During the

entire storage period, the O_2 concentration inside the trays shows a small decrease at lower temperature (2-4°C) and a rapid decrease with rising temperature condition (10-15°C). This is 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). Generally, changes in the gas atmosphere, especially at 10°C and 15°C, were initiated when TVC reaches 7 $\log_{10}$cfu/g, which also corresponds with the sensory end of shelf life. Temperature has also an effect on the gas and water vapor permeability of the packaging material, whereas the gas transmission rate increases with increasing temperatures (Kirwan & Stawbridge 2003, Mullan & McDowell, 2003). Comparing the development of the gas concentration in reference samples, no significant change could be observed. Therefore, the changes in the gas proportions are caused by the increased microbial growth due to temperature increase.

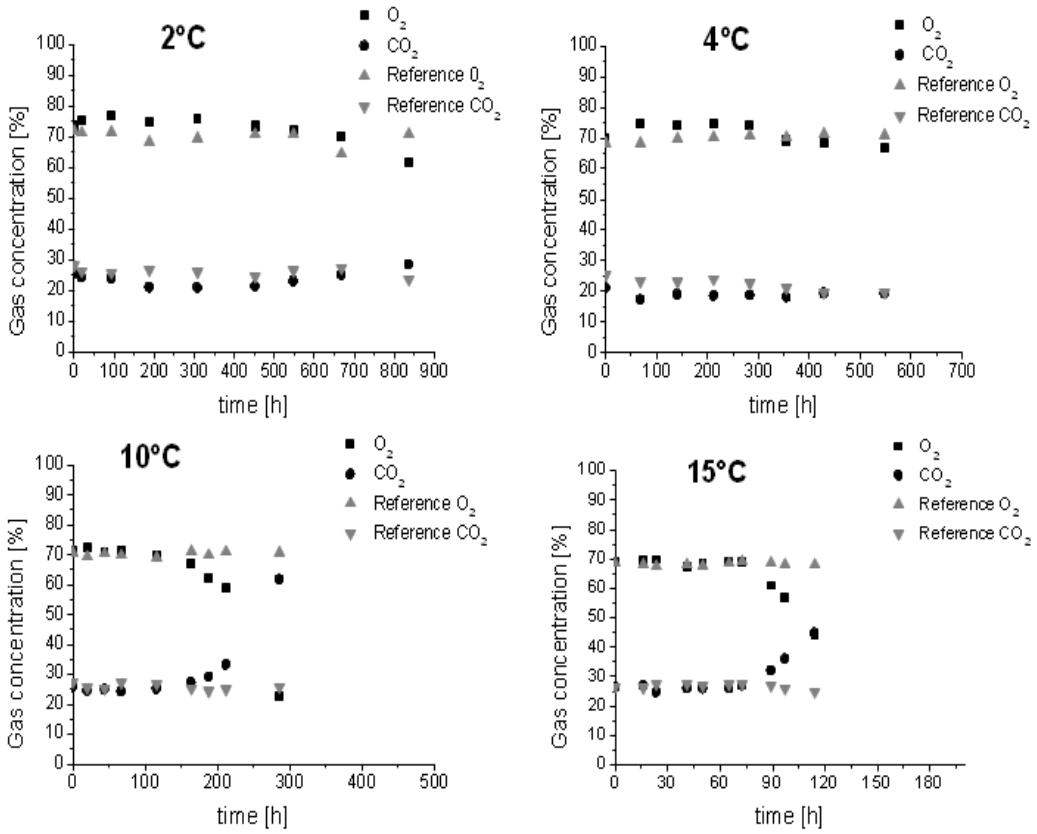

Figure 2.3 Development of the gas atmosphere under different temperature conditions during storage of MAP poultry fillet (n=3).

Development of meat pH

The initial broiler breast meat pH 24h post mortem varies between 5.7 and 6.2 (data not shown), which is in a normal range for poultry meat (ICMSF 1988, Lund and Eklund 2000, Herbert et al. 2013). As reported by Devlieghere et al. (1998) CO_2 gets partly dissolved in the water – and fat phase of the product under formation of carbonic acid with a direct ionization, which results in a decrease of the surface meat pH. This effect is mainly influenced by the storage temperature: the CO_2-solubility decreases with increasing temperature (Gill 1988, Walsh & Kerry 2000). In contrast, the results show, that the pH-value was not significantly influenced by any storage temperature ($p>0.05$) over the entire storage periods. This is due to the buffer effect of the meat proteins which limits significant variations in pH while storing the meat under MAP, as also stated out by Gilka et al. (1980) and Dixon & Kell (1989).

Development of sensory parameter and shelf life determination

The Quality Index (QI) increases for poultry, with increasing storage time for all temperatures. An quality index of 2.5 was taken as the lower limit of acceptability, corresponding to initial deteriorative changes regarding colour, odour, texture, general appearance and drip loss. So, an increase of the storage temperature from 2°C up to 4°C results in a shelf life reduction of approximately 45% (Table 2.2).

Table 2.2 Bacterial counts at the end of sensory shelf life during storage of poultry fillets under different temperature conditions (70% O_2 / 30% CO_2).

	TVC	***Pseudomonas* spp.**	***B. thermosphacta***	**Enterobacteriaceae**	***Lactobacillus* spp.**
End of shelf life (QI=2.5)			**log_{10}cfu/g**		
2°C (423 h)	7.0	4.3	6	3.9	3.2
4°C (228 h)	7.0	4.4	6.1	5.2	3.2
10°C (107 h)	7.3	7.0	5.9	4.4	3.7
15°C (75 h)	7.5	7.6	6.7	6.7	6.0

Generally, the decay of shelf life follows also an exponential function (Figure 2.4). Table 2 shows the bacterial counts of the different investigated bacteria at the end of sensory shelf

life. The results show that with increasing temperature the variation of the bacteria which are influencing the spoilage process is increasing. At 2 and 4°C B. thermosphacta dominates the flora at the end of the shelf life and at 10° and 15°C dominates Pseudomans spp., but at 15°C also the other bacteria have a main part on the microbial flora. Therefore, the definition of a specif spoilage organisms and common bacteria acceptance level for all temperature is challenging. Only the microbiological count of the TVC indicate, that TVC-counts are in the same range (7.0-7.5 $\log_{10}$cfu/g) under all investigated temperature conditions, which represents the upper microbiological acceptability limit of 7 $\log_{10}$cfu/g, which is in to the International Commission on Microbiological Specifications for Foods (ICMSF, 1978). Therefore, the microbiological spoilage regarding TVC 7 $\log_{10}$cfu/g is in compliance with the end of sensory shelf life defined at QI = 2.5.

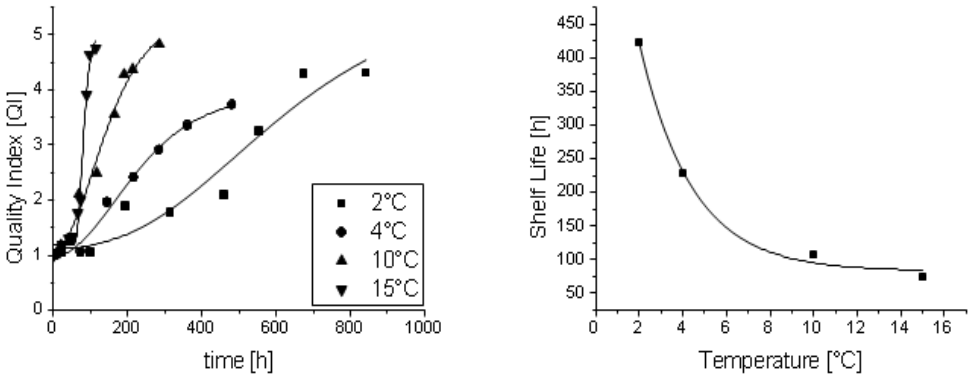

Figure 2.4 Development of the Quality Index.

Definition of predictor variable

As stated out in Table 2.2, the spoilage of MAP poultry under different temperature conditions is caused by a wide range of microorganisms and the bacterial counts at the end of sensory shelf life are varying. For the identification of predictor variables for a reliable shelf life prediction under MAP conditions, a stepwise regression and a principle component analyses were carried out. The results of the stepwise regression analyses shows a significant correlation between the sensory shelf life and the time when *Pseudomonas* spp. and TVC pass over into the stationary phase ($p<0.05$). Additionally, a Principle Component Analyses was conducted. Table 2.3 shows the component matrix, where three main components

could be identified: (1) the time when *Pseudomonas* spp. and *B. thermosphacta* reach the stationary phase, (2) the initial bacterial count of *B. thermoasphacta* and (3) the initial bacterial count of Enterobacteriaecae.

Table 2.3 Factor Loadings (Varimax Normalized).

	Component		
	1	**2**	**3**
***B. thermosphacta* (plateau)**	**-,994**		
***Pseudomonas* spp. (plateau)**	**-,994**		
TVC (plateau)	-,990		
QI	-,986		-,149
Enterobacteriaceae (plateau)	-,975	,219	
Enterobacteriaceae (k)	,935	,156	-,319
Temperature	,892	,437	-,118
TVC (k)	,836	,466	-,289
TVC (N_{max})	,785	,280	,552
***Pseudomonas* spp. (k)**	,755	,468	-,460
***Lactobacillus* spp. (k)**	,739	,654	,162
***B. thermosphacta* (k)**	,727	,455	-,513
Enterobacteriaceae (N_{max})	,699	,176	,693
***B. thermosphacta* (N_0)**	-,190	**,955**	,228
***B. thermosphacta* (N_{max})**	-,130	-,949	,289
***Lactobacillus* spp. (N_{max})**	,599	,797	
***Pseudomonas* spp. (N_{max})**	,512	,769	,323
***Lactobacillus* spp. (plateau)**	-,617	-,780	-,103
***Lactobacillus* spp. (N_0)**	-,377	,780	,500
Enterobacteriaceae (N_0)	,113		**,992**
***Pseudomonas* spp. (N_0)**	,166		,986
TVC (N_0)	-,476	,118	,872

Values in bold: Principle Components

Figure 2.5 shows the loading plot of the PCA. As well as the stepwise regression analyses, the PCA indicates that the time, when *Pseudomonas* spp. pass over into the stationary phase is an important variable on the shelf life. However, the PCA shows also that *Brochothrix thermoshacta* ($t_{plateau}$) has a main influence on the shelf life, because component one is mainly explained by $t_{plateau}$ of *Pseudomonas* spp. and $t_{plateau}$ of *Brochtothrix thermosphacta*. But also component two is influenced by *Brothothrix thermosphacta* (N_o). The results emphasize, that no common variable could be identified, which has the highest explanatory power for the data set regarding the identification of a specific spoilage microorganism.

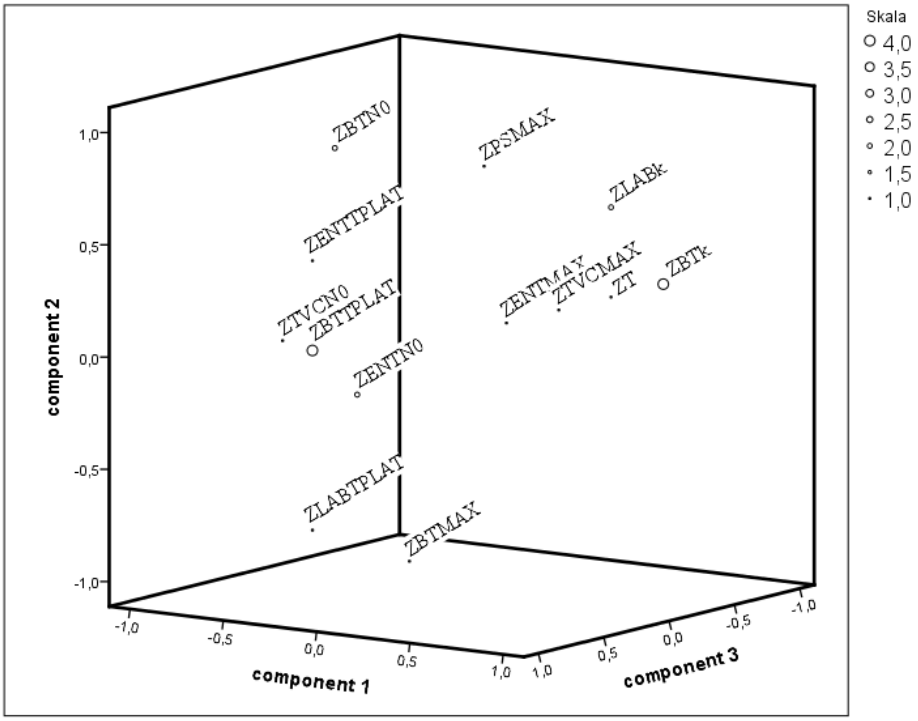

Figure 2.5 Loading plot of the PCA (Data z-tansformed).

2.5 Conclusion

Despite the fact that MAP results in a remarkable extension of the shelf life of meat and meat products in comparison to aerobic storage, the results emphasise also a strong influence of the storage temperature on the packaging under modified atmosphere conditions. The shelf life reduction while increasing the temperature from 2°C to 4°C is comparatively high (45%) due to storage under aerobic conditions (22%) (Bruckner 2010). No significant influence of the storage temperature on the development of the meat pH could be observed. The development of gas atmosphere was strongly influenced by the storage temperature due to an increase of CO_2 and a decrease of O_2. From the microbiological point of view, *B. thermosphacta* showed higher growth rates at 2 and 4°C, whereas the microflora changed under 10°C and 15°C with the highest growth rates for *Pseudomonas* spp. In contrast to aerobic storage where *Pseudomonas* spp. is the main spoilage microorganism, the spoilage microflora under MAP consists of a wide mixture of species and the contribution of each microorganism to the spoilage process strongly depends from the temperature. Therefore, the definition of an acceptance level based on SSO is not feasible. Also the results of the stepwise regression and PCA reflected that no single predictor variable could be identified as main spoilage organism. Therefore, the shelf life under high oxygen conditions is caused by several factors. In conclusion, TVC seems to be the best predictor variable for the prediction of remaining shelf life for MAP poultry based on the significant correlation between the development of the sensory decay and the development of the TVC and the number of TVC at the end of sensory shelf life for each single storage temperature. But in dependence of the composition and variation of the microflora, the TVC is not always meaningful. Therefore, further research is needed to gather a detailed knowledge of the growth and the interaction of SSOs under various packaging and temperature conditions and the initial composition of the flora. Especially a more detailed knowledge about the contribution of the different metabolites of each microorganism to the spoilage process as function of temperature conditions is necessary. Furthermore, for the development of a general shelf life model for MAP packed poultry the combination of different quality parameter in one single model is required. Also the development of shelf life models based on rapid technologies like Raman Spectroscopy or Hyperspectral Imaging can deliver an important contribution to a more flexible use of such models under practical conditions in different enterprises.

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2.6 References

Balamatsia, C. C., A. Patsias, M. G. Kotominas, and I. N. Savvaidis. 2007. Possible role of volatile amines as quality-indicating metabolites in modified atmosphere-packaged chicken fillets: correlation with microbiological and sensory attributes. Food Chem. 104:1622-1628.

Baranyi, J., T. P. Robinson, A. Kaloti, and B. M. Mackey .1995. Predicting growth of *Brochothrix thermosphacta* at chainging temperatures. Int. J. Food Microbiol. 27(1):61-75.

Barnes, E. M. 1976. Microbiological problems of poultry at refrigerator temperatures – A review. J. Sci. Food Agric. 27:777-782.

Betts, G. 1995. The microbiological consequences of MAP and vacuum packaging. Proceedings of the International Conference on Modified Atmosphere Packaging and Related Technologies, Campden & Chorleywood Research Association, Chipping Campden, UK.

Borch, E., M. L. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and meat products. Int. J. Food Microbiol. 33(1):103-120.

Branscheid, W., K. O. Honikel, G. von Lengerken, and K. Troeger. 2007. Qualität von Fleisch und Fleischwaren. 2nd Edit, Deutscher Fachverlag GmbH, Frankfurt am Main.

Bremner, A. 1985. A convenient easy-to-use system for estimating the quality of chilled seafood. Fish Processing Bulletin 7:59-70

Bruckner, S., A. Albrecht, B. Petersen, and J. Kreyenschmidt. 2012a. A predictive shelf life model for the improvement of quality management in pork and poultry chains. Food Cont. 29:451-460.

Bruckner, S., A. Albrecht, B. Petersen, and J. Kreyenschmidt. 2012b. Influence of cold chain interruptions on the shelf life of fresh pork and poultry. Int. J. Food Sci. Tech. 47(8):1639-1646.

Bruckner, S., A. Albrecht, B. Petersen, and J. Kreyenschmidt. 2012c. Characterization and comparison of spoilage process in fresh pork and poultry. J. Food Quality. 35:372-382.

Chouliara E., A. Karatapanis, I. N. Savvaidis, and M. G. Kontominas. 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. Food Microbiol. 24:607–617.

Coton, M., J. J. Joffraud, L. Mekhtiche, F. Leroi, and E. Coton. 2013. Biodiversity and dynamics of the bacterial community of packaged king scallop (*Pecten maximus*) meat during cold storage. Food Microbiol. 35:99-107.

Cox, N. A., S. M. Russell, and J. S. BAILEY. 1998. The microbiology of stored poultry, Pages 266-287 in The microbiology of meat and poultry, Vol. 1. A. Davies, & R. Board, ed. Blackie Academic & Professional, London, UK.

Dainty, R. H., and B. M. Mackey. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. J. Appl. Bacteriol. - Symposium Supplement, 73:103-144.

Davies, A. R. 1995. Advances in modified atmosphere packaging Pages 304-320 in New Methods of Food Preservation, Vol. 1. Gould, G. W., ed. Blackie Academic & Professional, London, UK.

Devlighere, F., J. Debevere, and J. van Impe. 1998. Effect of dissolved carbon dioxide and temperature on the growth of *Lactobacillus sake* in modified atmospheres. Int. J. Food Microbiol. 41:231-238.

Dixon, N. M., and D. B. Kell. 1989. The inhibition by CO_2 of the growth and metabolism of micro-organisms. J. Appl. Bacteriol. 67:109-136.

Farber, J. M. 1991. Microbiological aspects of modified-atmosphere packaging technology – A review. J. Food Protect. 54:58-70.

Fraqueza, M. J., and A. S. Barreto. 2009. The effect on turkey meat shelf life of modified-atmosphere packaging with an argon mixture. Poultry Sci. 88:1901-1998.

Gibson, A., N. Bratchell, and T. Roberts. 1987. The effect of sodium chloride and temperature on rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. 62, 479–490.

Gilka, J., I. Ingr, and J. Palasek. 1980. Beurteilung des Frischegrades von zu Halbfertigerzeugnissen bestimmtem Fleisch. Fleischwirtschaft. 60:118-122.

Gill, C. O., and K. G. Newton. 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. J. Appl. Bacteriol. 43(2):189-195.

Gill, C. O. 1988. The solubility of carbon dioxide in meat. Meat Sci. 22:65-71.

Gram, L., L. Ravn, M. Rasch, J. B. Bruhn, A. B. Christensen, and M. Givskov. 2002. Food spoilage – interactions between food spoilage bacteria. Int. J. Food Microbiol. 78:79-97.

Gribble, A. and G. Brightwell. 2013. Spoilage characteristics of *Brochtothrix thermosphacta* and *campestris* in chilled vacuum packaged lamb, and their detection and identification by real time PCR. Meat Sci. 94:361-368.

Herbert, R. A., and J. P. Sutherland. 2000. Chill storage, Pages 101-121 in The microbial safety and quality of food. B. M. Lund, T. C. Baird-Parker, and G. W. Gould, ed. Gaithersburg, MD:Aspen.

Herbert, U., S. Rossaint, M.-A. Khanna, and J. Kreyenschmidt. 2013. Comparison of argon-based and nitrogen-based modified atmosphere packaging (MAP) on bacterial growth and product quality of chicken breast fillets. Poultry Sci. 92(5):1348-56.

Huis in`t Veld, J. H. J. 1996. Microbial and biochemical spoilage of foods: an overview. Food Microbiol. 33:1-18.

ICMSF (International Commission Microbiological Specifications for Foods).1978. Page 434 in Microorganisms in Foods, 1. Their significancs and methods of enumeration. 2nd ed. University of Toronto Press, Toronto.

ICMSF (International Commission Microbiological Specifications for Foods). Part 1:Principles.1988. Pages 7-12 in HACCP in microbiological safety and quality. Great Britain. Silliker, J.H., A. C. Baired-Parker, F. L.Bryan, J. H. B. Christian, T. A. Roberts, and R. B. Tompkin, ed. Blackwell Scientific Publications, London, UK.

Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Modern Food Microbiology. 7th Edition, Springer, New York, London, Berlin.

Kirwan, M. J., and J. W. Stawbridge. 2003. Plastics in food packaging, Pages 174-240 in Food Packaging Techology. Coles, R., D. McDowell, and M. J. Kirwan, ed. Blackwell Publishing, London, UK.

Koutsoumanis, K., A. Stamatiou, P. Skandamis, and G.-J. E. Nychas. 2006. Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat, and validation of the model under dynamic temperature conditions. Appl. Environ. Microb. 72(1):124-134.

Kreyenschmidt, J. 2003. Modellierung des Frischeverlustes von Fleisch sowie des Entfärbeprozesses von Temperatur-Zeit- Integratoren zur Festlegung von Anforderungsprofilen für die produktbegleitende Temperaturüberwachung. PhD thesis, (Rheinische Friedrich-Wilhelms-Universität Bonn. Bergen/Dumme, Germany: AgriMedia GmbH).

Kreyenschmidt, J., and R. Ibald. 2012. Modeling shelf life using microbial indicators, Pages 127-168 in Shelf life Assessment of Food, 1st Edition. Nicoli, M. C., ed. CRC Press.

Labadie, J. 1999. Consequences of packaging on bacterial growth. Meat is an ecological niche. Meat Sci. 52:299-305.

Leister, L. 1995. Principles and applications of hurdle technology, Pages 1-21 in New Methods of Food Preservation, Gould, G. W., ed. Blackie, Glasgow, UK.

Limbo, S., L. Torri, N. Sinelli, L. Franzetti, and E. Casiraghi. 2010. Evaluation and predictive modeling of shelf life of minced beef stored in high-oxygen modified atmosphere packaging at different temperatures. Meat Sci. 84:129-136.

Lund, B. M., and T. Eklund. 2000. Control of pH and use of Organic Acids, Pages 175-199 in The microbial safety and quality of food. B. M. Lund, T. C. Baird-Parker, and G. W. Gould, ed. Gaithersburg, MD:Aspen.

McClure, P.J., J. Baranyi, E. Boogard, T. M. Kelly, and T. A. Roberts. 1993. A predictive model for the combined effect of pH, sodium chloride and storage temperature on the growth of *Brochothrix thermosphacta*. Int. J. Food Microbiol. 19:161-178.

McDonald, K., and D.-W. Sun. 1999. Predictive food microbiology for the meat industry: a review. Int. J. Food Microbiol. 52(1-2):1-27.

Mullan, M., D. McDowell. 2003. Modified atmosphere packaging. In: R. Coles, D. McDowell, M. J. Kirwan (Eds.): Food Packaging Techology, Blackwell Publishing, London, pp. 303-339.

Parra, V., J. Viguera, J. Sanchez, J. Peinado, F. Esparrago, J. I. Gutierrez, and A. I. Andrés. 2010. Modified atmosphere packaging and vacuum packaging for long period chilled storage of dry-cured Iberian ham. Meat Sci. 84:760-768.

Phillips, C. A. 1996. Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce. Int. J. Food Sci. Tech. 31:463–479.

Pooni, G. S., and G. C. Mead.1984. Prospective use of temperature function integration for predicting the shelf-life of non-frozen poultry-meat products. Food Microbiol. 1:67-78.

Raab, V., S. Bruckner, E. Beierle, Y. Kampmann, B. Petersen, and J. Kreyenschmidt. 2008. Generic model for the prediction of remaining shelf life in support of cold chain management in pork and poultry supply chains. J. Chain Network Sci. 8(1):59-73.

Rajamäki, T., H.-L. Alakomi, T. RITVANEN, E. SKYTTÄ, M. SMOLANDER, and R. Ahvenainen. 2006. Application of an electronic nose for quality assessment of modified atmosphere packed poultry meat. Food Control. 17:5-13.

Rokka, M., S. Eerola, M. Smolander, H.-L. Alakomi, and R. Ahvenainen. 2004. Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions. B. biogenic amines as quality-indicating metabolites. Food Control. 15:601-607.

Santé, V., M. Renerre, and A. Lacourt. 1994. Effect of modified atmosphere packaging on colour stability and on microbiology of turkey breast meat. J. Food Quality. 17:177.195.

Saucier, L., C. Gendron, and C. Gariépy. 2000. Shelf life of ground poultry meat stored under modified atmosphere. Poultry Sci. 79:1851-1856.

Sivertsvik, M., W. K. Jeksrud, and T. Rosnes. 2002. A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. Int. J. Food Sci. Tech. 37:107-127.

Smolander, M., H.-L. Alakomi, T. Ritvanen, J. Vainionpää, and R. Ahvenainen. 2004. Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions. A. Time-Temperature indicators as quality-indication tools. Food Cont. 15:217-229.

Walsh, H. M., and J. P. Kerry. 2000. Meat Packaging, Pages 417-452 in Meat processing – Improving quality, Kerry, J., J. Kerry, and D. Ledward, ed. CRC Press, Woodhead Publishing Limited, Cambridge, UK.

3. Effect of different oxygen concentrations on shelf life and quality parameter of modified atmosphere packaged (MAP) poultry breast filets

3.1 Abstract

The objective of the study was to investigate the effect of varying oxygen concentrations on the development of the spoilage microbiota and on sensory deteriorations to select the most appropriate gas combination for fresh poultry breast fillets stored at 4°C. Gas combinations included: (MAP 1): 45% O_2 / 25% N_2 / 30% CO_2; (MAP 2): 60% O_2 / 15% N_2 / 25% CO_2; (MAP 3): 70% O_2 / 30% CO_2; (MAP 4): 90% O_2 / 10% N_2 and aerobic samples. For microbiological investigation, *Pseudomonas* spp., *Brochothrix thermosphacta*, Enterobacteriaceae, *Lactobacillus* spp. and total viable count (TVC) were determined and modelled (Gompertz function). Moreover, sensory changes, pH-values and gas concentrations were monitored. Microbiological growth slowed down on poultry breast due to the combination of CO_2 in the mixture with O_2 between 45-70%. Sensory evaluation indicated beneficial effects on meat color using 60-90% O_2. 45% O_2 decreases the color stability. Concentrations between 55-60% O_2 were selected as the best combinations for fresh poultry breast fillets.

Submitted in the *Journal of Food Science & Technology*, see list of publications (Herbert et al. 2014).

3.2 Introduction

Fresh meat and meat products are sensitive to storage and distribution, presenting deteriorative changes (Pérez-Alvarez & Fernández-Lopez, 2007). With the increasing trend of self-service merchandising of fresh meat, the color of the meat is of great relevance for the consumer's choice at the point of sale (Bell, 2001). Additionally, the retailer and the consumer ask for long shelf lives and high quality products as well (Balev *et al.*, 2011). Therefore, packaging under modified atmosphere conditions is widely established to preserve the quality attributes and to prolong the shelf life of a product, but the optimization of the gas atmosphere is still critical (Narasimha & Sachindra, 2002). Fresh red meat is usually packed under 70-80% oxygen and 20-30% carbon dioxide. The high oxygen content is necessary to preserve the bright red color by keeping the muscle pigment myoglobin in its oxygenated form (Church, 1994; Faustman & Cassens, 1990). In contrast, poultry breast is a less used muscle with a reduced quantity of myoglobin and is categorized to white meat (McKee, 2007). For example, the concentration of myoglobin of 8 weeks old poultry is 0.01 mg/g, whereas 12 days old beef contains 0.70 mg/g (Miller, 1994). Because of the less myoglobin content, controversial recommendations by gas producers were given for the use of oxygen or nitrogen as a packaging gas for poultry breast fillets. Also in the scientific literature are contradictory results published. The consequence is that some companies use high oxygen atmospheres whereas others are using mixtures with less oxygen (Thoden van Velzen & Linnemann, 2008). But regarding the microbial growth and shelf life of poultry breast fillets using oxygen or nitrogen, no difference could be observed (Rossaint *et al.*, 2014, *data unpublished*).

Besides the stabilizing effect on the muscle pigment myoglobin, oxygen also affects the growth of specific microorganisms, which depends on the oxygen demand of the bacteria (Church, 1994). Generally, oxygen favors the growth of aerobic bacteria and reduces the growth of strictly anaerobic species. Therefore, the composition of the microbiota changes depending from the headspace gas atmosphere inside the package (Phillips, 1996). However, most investigations are focused on the development of sensory attributes and lipid oxidation for fresh beef and pork meat. Zakrys *et al.* (2008) investigated the effect of different oxygen concentrations (0, 10, 20, 50 and 80%) on the quality of beef. Regarding quality parameters, 50% and 80% O_2-packed meat showed a reduced tenderness but was more acceptable than lower O_2-concetrations. Resconi *et al.* (2012) investigated the effect of different high oxygen MAP (50/60/80%) in comparison to vacuum packaging on quality and sensory parameter for beef steaks. The authors showed that beef steaks had the lowest color stability under 50% O_2. Even if high rancidity levels were identified at 50 and 60% O_2, sensory characteristics

between 60% and 80% did not differ significantly. For pork meat, Zhang & Sundar (2005) showed that 45% oxygen with 20% CO_2 seems to be the best gas atmosphere regarding lipid oxidation, while 55% O_2 showed better color results. For modified atmosphere packed poultry meat, studies on the effect of varying oxygen concentrations on the development of meat spoilage microorganisms are rare, even if the German poultry industry uses high oxygen MAP (Rossaint *et al.*, 2014). Most studies are only focused on the use of N_2/CO_2 combinations (Jiménez *et al.*, 1997; Patsias *et al.*, 2006; Chouliara *et al.*, 2007).

Therefore, the aim of the study was to compare the effect of different oxygen concentrations in modified atmosphere packaged poultry breast fillets on the behaviour of typical spoilage microorganism and sensory attributes to figure out the optimal concentration of O_2 in a gas mixture.

3.3 Materials & Methods

Preparation of meat samples and packaging

42-days-old-unsexed 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 hours after slaughtering. In the laboratory, the double-breast fillets were divided into single fillets using a sterile scalpel. Samples were obtained for each trial from the same batch.

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 2:1. The meat samples were packaged under aerobic conditions and under four different modified atmospheres: (MAP 1): 45% O_2, 25% N_2, 30% CO_2; (MAP 2): 60% O_2, 15% N_2, 25% CO_2; (MAP 3): 70% O_2, 30% CO_2; (MAP 4): 90% O_2, 10% N_2. Thereafter, the trays were heat-sealed with a polypropylene foil (Suedpack Verpackungen GmbH & Co. KG, Ochsenhausen, Germany; water vapour permeability < 3.5 g/m^2d 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 four-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 used gas mixture in low-temperature high precision incubators (Sanyo

model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). Storage temperature was monitored by data logger (ESCORT JUNIOR Internal Temperature Data Logger, Escort, New Zealand) every 5 minutes. The microbiological, sensory and chemical analyses were conducted at appropriate time intervals. Each measurement was repeated three times. Table 1 gives an overview about the storage and sampling conditions during the trials.

Microbiological analyses

After opening the packages, a representative amount (25g) of meat surface sample in size of 4 x 7 x 0.5 cm was aseptically taken using a sterile scalpel, which was transferred to a filtered sterile stomacher bag and filled with 225 ml saline peptone diluent (0.85 % NaCl with 0.1% peptone Saline-Tablets, Oxoid BR0053G, Cambridge, United Kingdom). Samples were homogenised with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. Ten-fold dilutions of the homogenate were prepared in saline peptone diluents. Total Viable Count (TVC), *Pseudomonas* spp., *B. thermosphacta*, Enterobacteroaceae and Lactic acid bacteria in rinsates were enumerated.

Total Viable Count was determined by pour plate technique on Plate Count Agar (PCA, Merck, Darmstadt, Germany) and plates were incubated at 30°C for 72 hours. Pseudomonas were counted by spread plate technique on Pseudomonas Agar with Cetrimide-Fucidin-Cephalosporin selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 hours. *B. thermosphacta* was detected by drop plate technique (10 drops à 10µl per dilution step) and counted on Streptomycin Inosit Toluylene Red Agar (SIN-Agar) according to Hechelmann (1981). Petri dishes were incubated at 25°C for 48 hours. *Enterobacteriaceae* were identified by overlay treatment on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany). Therefore, 1.0 ml of the sample was inoculated in approximately 10 ml VRBD-Agar. After setting, approximately 10 ml overlay of liquefied VRBD-medium were added and plates were incubated at 30°C for 48 hours. Lactic acid bacteria were counted by pour plate technique on de Man, Rogosa, Sharpe Agar (MRS, Oxoid, Cambridge, United Kingdom). Plates were incubated aerobically at 37°C for 72 hours. Counts of colony forming units were expressed as $\log_{10}$cfu/g of sample. Table 3.1 gives an overview about the packaging atmospheres, the storage- and sampling conditions during the trials.

Table 3.1 Packaging and sampling conditions during the trials.

Packaging	Storage conditions	Sampling intervals (h)*	Number of packages
Aerobic	20.9% O_2	0, 48, 96, 120, 168, 240	18
(MAP 1)	45% O_2 / 25% N_2 / 30% CO_2	0, 21, 70, 141, 214, 309, 381, 453	24
(MAP 2)	60% O_2 / 15% N_2 / 25% CO_2	0, 72, 144, 216, 288, 360, 432, 552, 576	24
(MAP 3)	70% O_2 / 30% CO_2	0, 73, 144, 214, 288, 363, 483	21
(MAP 4)	90% O_2 / 10% N_2	0, 71, 145, 215, 287, 360, 430	21

*three replicates per sampling point

Sensory evaluation

Sensory analyses were carried out by trained sensory panelists. All assessors were recruited from the Institute of Animal Science (University of Bonn) and experienced in poultry evaluation. A picture of fresh chicken breast fillets was used as reference during the sensory evaluations.

During the trials, each sample was evaluated directly after opening the tray, using a developed sensory scheme according to the Quality Index Method (QIM) for fish evaluation (Bremner, 1985). Attributes were defined as general appearance (G), colour (C), odour (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 quality index (QI) was calculated by the following equation (Kreyenschmidt, 2003):

$$QI = \frac{2G + 2C + 1T + 1D + 2O}{8} \qquad (1.1)$$

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 hand-held gas analyser (Oxybaby V O_2/CO_2, Witt Gasetechnik, Witten, Germany). Before starting the gas measurement inside the trays, the composition of air was analysed to control the accuracy of the gas analyser. 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 seconds and the oxygen

concentration was detected by an electrochemical sensor; carbon dioxide concentration was detected by IR-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 Modelling

The Gompertz equation was used to model the growth of the total viable count, *Enterobacteriaceae*, *Pseudomonas* spp., *Brochothrix 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., U.S.A.)
The development of the specific growth rates of the investigated microorganisms as function of the used oxygen content during storage of fresh poultry fillets were fitted using a second order polynomial function.

Statistical analysis

Man-Whitney-U-test was used to make comparisons between sensory colour evaluation, pH-values and the measured counts of colony forming units with a level of significance of 0.05. SPSS statistics 20 for Windows was used.

3.4 Results & Discussion

Comparison of the spoilage process under various oxygen concentrations

Figure 3.1 shows the development of typical spoilage microorganism and total viable count on poultry packed under different oxygen enriched atmospheres at a constant temperature of 4°C.

Because of the presence of oxygen in all used gas mixtures, the growth of *Lactobacillus* spp. remains relatively constant over the entire storage periods. This is due to the fact that the growth is favored under anaerobic conditions and under high CO_2 atmospheres. Also the cold storage influences the growth because of its mesophilic properties (Jay *et al.*, 2005). The findings are also in accordance to Santé *et al.* (1994). Therefore, the bacteria play a minor role in the spoilage process and were not considered in the present study.

Under aerobic conditions (20.9% O_2), *Pseudomonas* spp. is dominating the spoilage flora. The microorganism is the main spoilage bacteria under these conditions because of its aerobic properties and psychrotropic character, which is also in accordance to several authors (Pooni & Meat, 1984; Arnaut-Rollier *et al.*, 1999; Gram *et al.*, 2002; Sivertsvik *et al.*, 2002; Koutsoumanis *et al.*, 2006; Bruckner *et al.*, 2012). Also the concentration of CO_2 in ambient air is diminished and leads to an additional selective effect in favor of the growth of Pseudomonads because of its high sensitivity to CO_2. At the beginning of storage the microorganisms show the highest initial counts with 2.6 $\log_{10}$cfu/g in comparison to the other investigated bacteria, which reflects a direct correlation between the initial number of *Pseudomonas* spp. and the shelf life at chill temperatures, as stated out by Barnes *et al.* (1979). During storage, *Pseudomonas* spp. continues to show the best growth which is reflected in the highest maximum growth rate with 0.045 1/h (Figure 3.2) and the highest maximum microbial counts with 9.6 $\log_{10}$cfu/g (Table 3.2). Moreover, *B. thermosphacta* and Enterobacteriaceae are also present as part of the spoilage flora of poultry under aerobic conditions (Corry 2006). During storage, *B. thermosphacta* could be identified as the second main spoilage competitor (Table 3.2), which is also shown by Gallo *et al.* (1988). Enterobacteriaceae are also present on aerobic stored poultry fillets. Because of its mesophilic properties, the bacterial flora was presumably suppressed by the psychrotrophic groups and plays a minor role in the spoilage process.

The 45% O_2 enriched atmosphere leads to a change in the mircroflora in favor of *B. thermosphacta* (Figure 3.1). Storing the samples under 45% O_2 enriched atmospheres, the

initial bacterial loads of *Pseudomonas* spp. and Enterobacteriaceae are in the same range and dominates the spoilage flora. *B. thermosphacta* shows the lowest initial concentration (Figure 3.1), but becomes dominant after approximately 180h of storage. The microorganism shows also a similar duration of the lag-phase compared to Enterobacteriaceae, but a higher maximum growth rate and therefore an increase growth during storage (Table 3.2). This is due to the fact that *B. thermosphacta* is a facultative anaerobic microorganism but prefers to growth under oxygen containing atmospheres. Therefore the microorganisms are able to dominate the microflora, when oxygen occurs (Gribble & Brightwell, 2013). *B. thermosphacta* is also often associated with spoilage under MAP conditions based on the improved resistance to CO_2 (Borch *et al.*, 1996; Branscheid *et al.*, 2007) and its psychrothrophic character (Pennacchio *et al.*, 2009).

With increasing oxygen concentrations to 60% and 70%, the dominance of *B. thermosphacta* shows a distinct behaviour with the highest maximum growth rate (Table 3.2) as illustrated in Figure 2. The growth of *Pseudomonas* spp. and Enterobacteriaceae is slowed down with increasing oxygen concentrations (60/70%) compared to the growth of B*. thermosphacta*. This effect could be explained by the growth behaviour of *B. thermosphacta* in presence of other meat spoilage bacteria. As shown in vitro by Russo *et al.* (2006), the growth of *B. thermosphacta* is strongly influenced by the presence of *Lactobacillus* spp., *Pseudomonas* spp. and Enterobacteriaceae. The interactions are a possible explanation for the dominance of the bacteria. Further on, *B. thermosphacta* shows the ability to switch between aerobic and anaerobic metabolism due to the oxygen and carbon dioxide environment and the meat matrix seems to be an ecological niche for *B. thermosphacta* as stated out by Labadie (1999) and Pin *et al.* (2002). Therefore, the bacterium has a kind of selective advantage, which supports the bacteria to be an important spoilage originator as part of the spoilage microflora, as shown in this study.

The 90% oxygen atmosphere was most effective in favour the growth of *Pseudomonas* spp. (Table 3.2). Similar results were reported by Viana *et al.* (2005) for the growth of Pseudomonads on refrigerated pork loins during storage under 100% O_2. The growth behaviour could be explained by the high affinity to oxygen and the absence of CO_2 as growth limiting factor for Pseudomonads. Also *B. thermosphacta* shows a good growth under 90% oxygen because of its psychrotrophic properties and preferred growth under oxygen atmospheres. Merely the growth of the mesophilic Enterobacteriaceae is slightly slowed down compared to the remaining bacteria because of the low storage temperature. Also Smolander *et al.* (2004) showed that temperature conditions have an effect on delaying

the growth of Enterobacteriaceae, but the microorganisms also show the ability to adapt quickly to changing environments.

Further on, all samples stored under 90% oxygen achieve the highest maximum bacterial counts next to aerobic packaging for all investigated microorganisms (Table 3.2), what is also in accordance to Santé *et al.* (1994). The affinity of the microorganisms to oxygen and the absence of the antimicrobial component CO_2 allow the outgrowth of the spoilage flora, as also reflected in Table 3.2 and Figure 1.

The development of the specific growth rates of the investigated microorganisms as function of the used oxygen content during storage of fresh poultry fillets is illustrated in Figure 3.2. For all investigated microorganisms, the growth rates decrease with increasing oxygen concentrations. The local minimum of the growth rate development for each microorganism in dependence of the used gas mixture is shown in Table 3.3. Therefore, a local minimum of the growth rates results in an appropriate oxygen concentration in a range between 55-60%. Merely the local minimum (μ_{max}) of *B. thermosphacta* is located at 46% O_2. Generally, the results emphasize that the storage of the samples under different gas mixtures causes a change of the environmental conditions and induces a selection pressure on specific microorganism in dependence from the used O_2 concentration (Molin 2000).

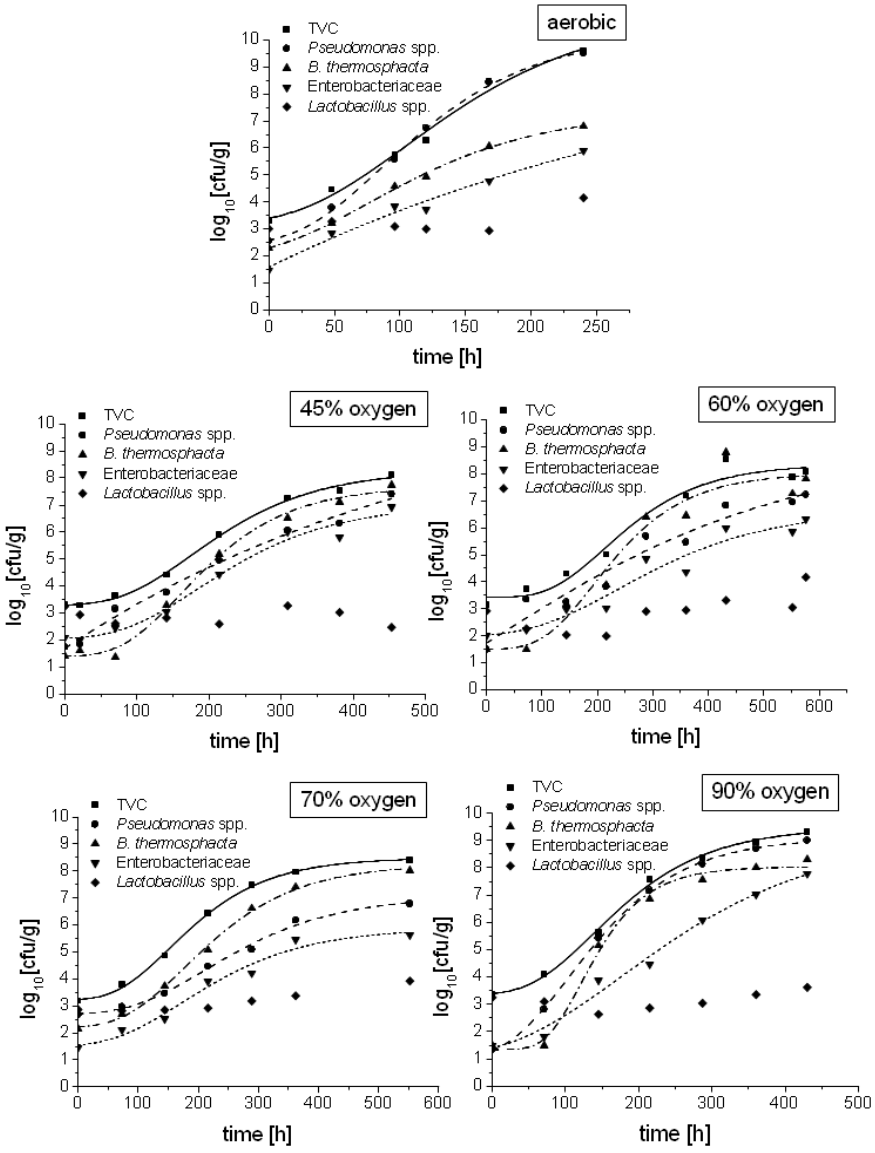

Figure 3.1 Growth of typical spoilage microorganism on poultry stored under different oxygen enriched atmospheres fitted with the Gompertz model, n = 3.

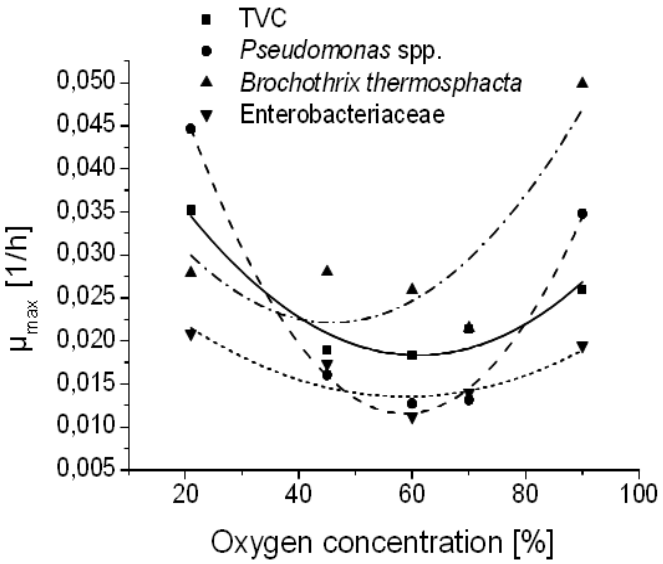

Figure 3.2 Development of the maximum growth rate, calculated with the Gompertz model.

Table 3.2 Development of growth parameter during storage of poultry under different oxygen enriched atmospheres calculated with the Gompertz function, n = 3.

O_2 (%)	TVC			*Pseudomonas* spp.			*B. thermosphacta*			Enterobacteriaceae		
	μ_{max} [1/h]	T_{lag} [h]	N_{max} log_{10}cfu/g	μ_{max} [1/h]	T_{lag} [h]	N_{max} log_{10}cfu/g	μ_{max} [1/h]	T_{lag} [h]	N_{max} log_{10}cfu/g	μ_{max} [1/h]	T_{lag} [h]	N_{max} log_{10}cfu/g
20.9	0.035	22	9.6	0.045	22	9.5	0.028	18	6.8	0.021	12	5.9
45	0.019	78	8.1	0.016	15	7.4	0.028	78	7.6	0.017	77	6.9
60	0.018	114	8.1	0.013	19	7.2	0.026	101	7.8	0.011	81	6.3
70	0.021	62	8.4	0.013	86	6.8	0.022	74	8.0	0.014	57	5.6
90	0.026	52	9.3	0.035	20	9.0	0.050	73	8.3	0.019	23	7.8

Table 3.3 Calculated local minima of μ_{max} for the investigated microorganisms and cumulated μ_{max} with the derived oxygen concentration.

	TVC	***Pseudomonas* spp.**	***B. thermosphacta***	**Enterobacteriaceae**
Local minimum of μ_{max} [1/h]	0.018	0.012	0.022	0.014
Local minimum of O_2 concentration [%]	61	58	46	59

Sensory analyses and shelf life determination

Figure 3.3 shows the development of the quality index of poultry breast fillets packed under different oxygen concentrations. A QI score of one is related to fresh meat and increases linearly when the meat deteriorates during storage. The shelf life time based on sensory evaluation increases in order: aerobic < 45% < 70% < 90% < 60% (Table 3.4). The storage under 45% O_2 shows the lowest sensory shelf life within the different used oxygen atmospheres. This is presumably related to the color evaluation, which shows the lowest stability compared to higher oxygen concentrations, as also reported for fresh pork meat by Zhang & Sundar (2005). The difference in sensory shelf life times of the samples stored under 90% oxygen (238h) and samples stored under 60/70% oxygen (247/212h) is mainly based on the evaluation of meat color as emphasized in Figure 3.3. The development of meat color under 90% O_2 is evaluated with a score of 1 constantly and shows a rapid increase after 150h of storage. The results are also in compliance with the study of Santé et al. (1994), during storage of turkey meat under 100% O_2. The atmosphere caused the best redness value compared to storage under 100% N_2, 100% CO_2 or 66% O_2 / 9% N_2 /25% CO_2. Also Blacha *et al.* (2014) showed in their study for turkey meat, that the storage under high oxygen (80% O_2 / 20% N_2) resulted in the highest sensory and redness scores but showed also the highest thiobarbituric acid-reactive substance values, associated with an increased fat oxidation. Further on, the storage of the samples under 70% oxygen enriched atmosphere shows a reduction in shelf life of 35h compared to 60% oxygen. The relatively small difference is, next to the differences in the oxygen concentrations, presumably related to variations in the initial bacterial load of *Pseudomonas* spp. and *B. thermosphacta* due to preliminary meat contamination with approximately 0.5 log level difference between the samples stored under 60% and 70%. Therefore, the surface contamination of the meat is directly related to the determination of shelf life (Borch *et al.* 1996.) Also interactions

between the spoilage bacteria and animal specific factors like age, sex, fat content, meat moisture or genetic factors have a further influence on the sensory shelf life development.

Table 3.4 Sensory shelf life times under different oxygen concentrations during storage at 4°C.

	Aerobic (20.9% O_2)	**45% O_2**	**60% O_2**	**70% O_2**	**90% O_2**
End of sensory shelf life [h] QI = 2.5	100	142	247	212	238

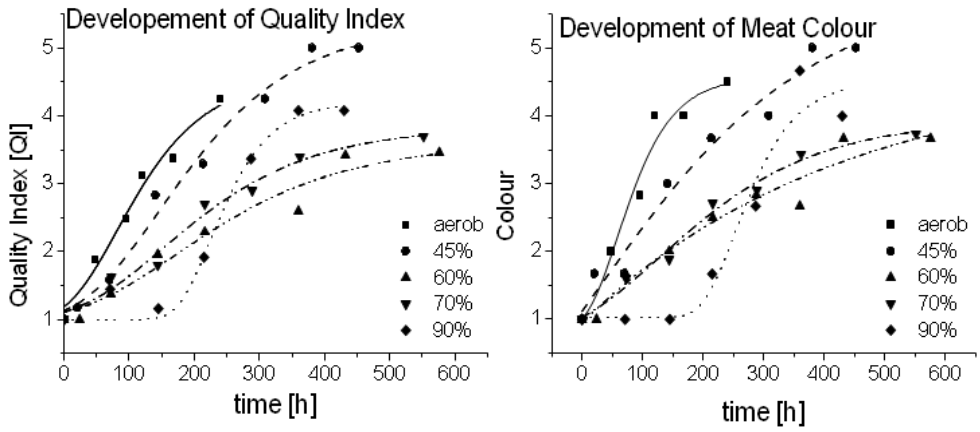

Figure 3.3 Development of quality index and poultry meat color under different oxygen concentrations, n = 3.

Development of meat pH and gas atmosphere

The initial poultry breast pH (24h after slaughtering) varies between 5.62 and 6.15 (data not shown) and is in a normal range for fresh poultry as also shown in the literature (Herbert *et al.*, 2013; Bruckner *et al.*, 2012; Lund & Eklund, 2000). The results show that the pH value is not significantly influenced by any used gas mixture ($p>0.05$). This is due to the buffer effect of the meat proteins which limits significant variations in pH while storing the meat under MAP, as also stated out by Gilka *et al.* (1980), Dixon & Kell (1989) and Herbert *et al.* (2013).

For the development of the gas concentration in packages containing CO_2/O_2 mixtures (MAP 1-MAP3), a small decrease of CO_2 could be detected in the beginning of storage. This is caused by the high solubility of carbon dioxide in the fat tissue and water on the meat surface (Betts, 1995; Gill, 1988). Parra et al. (2010) and Herbert et al. (2013) reported similar results for MA packed meat. For the packaging under 45-90% oxygen, a decrease of the O_2 concentration around 2-3% could be observed at the end of storage. The effect is related to the microbial O_2 consumption for their metabolism during growth and multiplying and accompanied by contemporaneous CO_2 production. Also the respiration of meat enzymes and gaseous exchanges between the gas composition inside the trays and the environment are influence factors for the gas change during storage (Mullan & McDowell, 2003).

3.5 Conclusion

In conclusion, the storage of poultry fillets under different oxygen concentration showed that oxygen has a stabilising effect on the color and increases the shelf life of poultry fillet with increasing O_2 concentrations. However, the quality is reduced by using low oxygen concentrations (aerobic) and high concentrations (90%). The 45% O_2 enriched atmsophere results in a reduced sensory shelf life compared to 60/70 and 90% O_2, which is mainly caused by the reduced color stability. 90% oxygen in the atmosphere leads to an improved color stability, but favours the growth of all bacteria. According to several authors, the lipid oxidation increases with increasing oxygen concentrations. But poultry meat relates to a meat with less fat content in comparison to pork or beef meat and seems to be comparatively lower. Therefore, fat oxidation seems to play a minor role in the deteriorative changes during storage. But further research is needed to investigate also the oxidation potential of poultry meat under varying oxygen concentrations. From the safety aspects, most pathogenic bacteria are facultative anaerobic competitors and can grow uncontrolled in packages without oxygen. Therefore, spoilage bacteria are no longer indicators for environmental changes and allow the outgrowth of pathogenic ones when oxygen is absent. Hence, the packaging of poultry under high oxygen atmospheres is a more optimal packaging mixture comparing with nitrogen packaging. Regarding the investigated atmospheres and the local minima of the different bacteria, 55-60% O_2 mixed with CO_2 can be concluded as the appropriate concentration for fresh poultry breast meat at 4°C.

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3.6 References

Arnaut-Rollier, I., L. De Zutter, J. Van Hoof. 1999. Identities of the *Pseudomonas* spp. in flora from chilled chicken. *International Journal of Food Microbiology*, 48:87-96.

Balev, D. K.., A. S. Staykov, G. Y. Ivanov, St. G. Drageov, E. H. Filizov. 2011. Colour stability improvement of chilled beef by natural antioxidant treatment and modified atmosphere packaging. *American Journal of Food Technology*, 6:117-128.

Barnes, E. M., G. C. Mead, C. S. Impey, B. W. Adams. (1979). Spoilage organisms of refrigerated poultry meat, Pages 101-116 in Cold tolerant microbes in spoilage and the environment. Society for Applied Bacteriology Series no. 13, Russell, A. D. & R. Fuller, ed., Academic Press, London, UK.

Blacha, I., C. Krischek, G. Klein. 2014. Influence of modified atmosphere packaging on meat quality parameters of turkey breast muscles. *Journal of Food Protection*, 77(1):127-32.

Betts, G. 1995. The microbiological consequences of MAP and vacuum packaging. Proceedings of the International Conference on Modified Atmosphere Packaging and Related Technologies, Campden & Chorleywood Research Association, Chipping Campden, UK.

Borch, E., M. L. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and meat products. *International Journal of Food Microbiology*, 33(1):103-120.

Branscheid, W., K. O. Honikel, G. von Lengerken, and K. Troeger. 2007. Qualität von Fleisch und Fleischwaren. 2nd Edit, Deutscher Fachverlag GmbH, Frankfurt am Main.

Bruckner, S., Albrecht, A., Petersen, B., & Kreyenschmidt, J. (2012). Characterization and Comparison of Spoilage Processes in Fresh Pork and Poultry. *Journal of Food Quality*, *35*(5):372-382.

Bell, R. G. 2001. Meat Packaging: Protection, Preservation and Presentation Pages 463-490 in Meat Science and Applications. 1st ed., Hui, Y. H. (eds.), Marcel Dekker. Inc., New York, Basel.

Bremner, A. 1985. A convenient easy-to-use system for estimating the quality of chilled seafood. *Fish Processing Bulletin*, 7:59-70.

Church, N. 1994. Review - Developments in modified-atmosphere packaging and related technologies. *Trends in Food Science and Technology,* 5:345-352.

Chouliara, E., A. Karatapanis, I. N. Savvaidis, M. G. Kontominas. 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. *Food Microbiology*, 24:607-617.

Corry, J. E. L. 2006. Spoilage organisms of red meat and poultry, Pages 101-122 in Microbiological analysis of red meat, poultry and eggs, 1st Edition. Mead G. C., ed. Woodhead Publishing Limited, CRC Academic Press, UK.

Dixon, N. M., and D. B. Kell. 1989. The inhibition by CO_2 of the growth and metabolism of micro-organisms. *Journal of Applied Bacteriology*, 67:109-136.

Gallo, L., R. E. Schmitt, W. Schmidt-Lorenz. 1988. Microbial spoilage of refrigerated fresh broilers. I. Bacterial flora and growth during storage. *Lebensmittel Wissenschaften und Technologie*, 21:216-223.

Gibson, A., N. Bratchell, and T. Roberts. 1987. The effect of sodium chloride and temperature on rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *Journal of Applied Bacteriology*, 62, 479–490.

Gilka, J., I. Ingr, and J. Palasek. 1980. Beurteilung des Frischegrades von zu Halbfertigerzeugnissen bestimmtem Fleisch. *Fleischwirtschaft*, 60:118-122.

Gill, C. O. 1988. The solubility of carbon dioxide in meat. *Meat Science*, 22:65-71.

Gram, L., L. Ravn, M. Rasch, J. B. Bruhn, A. B. Christensen, and M. Givskov. 2002. Food spoilage – interactions between food spoilage bacteria. *International Journal of Food Microbiology,* 78:79-97.

Gribble, A. and G. Brightwell. 2013. Spoilage characteristics of *Brochtothrix thermosphacta* and *campestris* in chilled vacuum packaged lamb, and their detection and identification by real time PCR. *Meat Science*, 94:361-368.

Han, J.H. 2005. Innovations in food packaging. Food Science and Technology, International Series, Elsevier Ltd.

Hechelmann, H. 1981. Vorkommen und Bedeutung von *Brochothrix thermosphacta* bei Kühllagerung von Fleisch und Fleischerzeugnissen. Mitteilungsblatt der Bundesanstalt für Fleischforschung, Kulmbach, 4435-4437.

Herbert, U., Rossaint, S., Khanna, M. A., & Kreyenschmidt, J. 2013. Comparison of argon-based and nitrogen-based modified atmosphere packaging on bacterial growth and product quality of chicken breast fillets. *Poultry Science*, 92(5):1348-1356.

Jay, J. M., M. J. Loessner, D. A. Golden. 2005. Modern Food Microbiology. 7th ed., Springer, New York, NY.

Jiménez, S. M., M. S. Salsi, M. C. Tuburzi, R. C. Rafaghelli, M. A. Tessi, V. R. Coutaz. 1997. Spoilage microflora in fresh chicken breast stored at 4°C: influence of packaging methods. *Journal of Applied Microbiology*, 83:613-618.

Koutsoumanis, K., A. Stamatiou, P. Skandamis, and G.-J. E. Nychas. 2006. Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat, and validation of the model under dynamic temperature conditions. *Applied Environmental Microbiology*, 72(1):124-134.

Kreyenschmidt, J. 2003. Modellierung des Frischeverlustes von Fleisch sowie des Entfärbeprozesses von Temperatur-Zeit- Integratoren zur Festlegung von Anforderungsprofilen für die produktbegleitende Temperaturüberwachung. PhD thesis, Rheinische Friedrich-Wilhelms-Universität Bonn. Bergen/Dumme, Germany: AgriMedia GmbH.

Labadie, J. 1999. Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science*, 52:299-305.

Leister, L. (1995). Principles and applications of hurdle technology. In: G. W. Gould (ed.) New Methods of Food Preservation. Blackie, Glasgow, UK, pp 1-21.

McKee, L. 2007. Microbiological and sensory properties of fresh and frozen poultry. In: Nollet, L. M. L. (Eds.), Handbook of Meat, Poultry and Seafood Quality., Blackwell Publishing Ltd, 1st Edition, pp 429-474.

Miller, R. K. 1994. Quality Characteristics. In: D. M. Kinsman, A. W. Kotuala, B. C. Breidenstein (Eds.). Muscle Foods. Chapman & Hall, New York, pp 296-332.

Molin, G. 2000. Modified Atmospheres. In: B. M. Lund, T. C. Baired-Parker, G. W. Gould (Eds.). The microbiological safety and quality of foods. Vol. 1, Aspen Publishers Inc., Gaithersburg, US, pp 214-234.

Mullan, M., D. McDowell. 2003. Modified atmosphere packaging. In: R. Coles, D. McDowell, M. J. Kirwan (Eds.): Food Packaging Techology, Blackwell Publishing, London, pp. 303-339.

Narasimha, R. D. and N. M. Sachindra. 2002. Modified atmosphere and vacuum packaging of meat and poultry products. *Food Reviews International*, 18(4):263-293.

Parra, V., J. Viguera, J. Sanchez, J. Peinado, F. Esparrago, J. I. Gutierrez, and A. I. Andrés. 2010. Modified atmosphere packaging and vacuum packaging for long period chilled storage of dry-cured Iberian ham. *Meat Science*, 84:760-768.

Patsias, A., I. Chouliara, A. Badeka, I. N. Savvaidis, M. G. Koutominas. 2006. Shelf life of precooked chicken product stored in air and under modified atmospheres: microbiological, chemical, sensory attributes. *Food Microbiology*, 23:423-429.

Pennacchia, C., D. Ercolini, F. Villani. 2009. Development of a Real-Time PCR assay for the specific detection of Brochothrix thermosphacta in fresh and spoiled raw meat. *International Journal of Food Microbiology*, 134:230-236.

Pérez-Alvarez, J. A. and J. Fernández-Lopez. 2007. Chemical and biochemical aspects of color in muscle foods. In: Nollet, L. M. L. (Eds.), Handbook of Meat, Poultry and Seafood Quality., Blackwell Publishing Ltd, 1st Edition, pp 25-45.

Phillips, C. A. (1996). Review: Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology*, 31, 463-479.

Pin, C., G. Garcia de Fernando, J. A. Ordonez. 2002. Effect of modified atmosphere on the metabolism of glucose by *Brochothrix thermosphacta*. *Applied Environmental Microbiology*, 68:4441-4447.

Pooni, G. S., and G. C. Mead.1984. Prospective use of temperature function integration for predicting the shelf-life of non-frozen poultry-meat products. *Food Microbiology,* 1:67-78.

Raab, V., S. Bruckner, E. Beierle, Y. Kampmann, B. Petersen, and J. Kreyenschmidt. 2008. Generic model for the prediction of remaining shelf life in support of cold chain management in pork and poultry supply chains. *Journal on Chain and Network Science*, 8(1):59-73.

Resconi, V. C., A. Escudero, J. A. Beltrán, J. L. Olleta, C. Sanudo, M. Del Mar Campo. 2012. color, lipid oxidation, sensory quality, and aroma compounds of beef steaks displayed under different levels of oxygen in a modified atmosphere package. *Journal of Food Science*, 71(1):10-18.

Russo, F. D. Ercolini, G. Mauriello, F. Villani. 2006. Behaviour of Brochothrix thermosphacta in presence of other meat spoilage microbial groups. *Food Microbiology*, 23:797-802.

Santé, V., M. Renerre, A. Lacourt. 1994. Effect of modified atmosphere packaging on color stability and on microbiology of turkey breast meat. *Journal of Food Quality*, 17:177:195.

Sivertsvik, M., W. K. Jeksrud, and T. Rosnes. 2002. A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Science and Technology*, 37:107-127.

Smolander, M., H.-L. Alakomi, T. Ritvanen, J. Vainionpää, and R. Ahvenainen. 2004. Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions. A. Time-Temperature indicators as quality-indication tools. *Food Control*, 15:217-229.

Thoden van Velzen, E. U., A. R. Linnemann. 2008. Modified atmosphere packaging of fresh meats – Sudden partial adaption caused an increase in sustainability of dutch supply chains of fresh meat. *Packaging Technology and Science*, 21:37-46.

Viana, E. S., L. A. M. Gomide, M. C. D. Vanetti. 2005. Effect of modified atmospheres on microbiological, color and sensory properties of refrigerated pork. *Meat Science*, 71:696-705.

Zakrys, P. I., S. A. Hogan, M. G. O`Sullivan, P. Allen, J. P. Kerry (2008). Effect of oxygen concentrations on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. *Meat Science*, 79:648-655.

Zhang, M., S. Sundar. 2005. Effect of oxygen concentration on the shelf life of fresh pork packed in a modified atmosphere. *Packaging Technology and Science*, 18:217-222.

Zhou, G. H., X. L. Xu, Y. Liu (2010). Preservation technologies for fresh meat – A review. *Meat Science*, 86:119-128.

4. Comparison of oxygen and nitrogen enriched atmospheres on the growth of *Listeria monocytogenes* inoculated on poultry breast fillets in presence of natural background flora

4.1 Abstract

In this study, the growth behaviour of *Listeria monocyogenes* in presence of typical spoilage microorganisms on poultry breast fillets (*Pseudomonas* spp., *Brochothrix thermosphacta*, Enterobacteriaceae, *Lactobacillus* spp., Total Viable Count) was investigated. The aim of the study was to compare the growth of *L. monocytogenes* under oxygen and nitrogen enriched atmospheres and to figure out possible interactions between *L. monocytogenes* and typical spoilage microorganisms on skinless poultry breast fillets. Therefore, the natural meat surface was inoculated with an initial concentration of *L. monocytogenes* between 180-280 cfu/g and packed under two different gas atmospheres, which were commonly used by poultry processing plants (70% O_2 / 30% CO_2 and 70% N_2 / 30% CO_2). Additionally, the gas atmosphere was monitored over the entire storage period and typical sensory attributes (color, odor, texture, drip loss and general appearance) were evaluated in parallel to each microbiological investigation point. Generally, the results show that the storage under 70% N_2/30% CO_2 favored the growth of *L. monocytogenes* in comparison to the 70% O_2/30% CO_2 atmosphere. Under nitrogen, *L. monocytogenes* reached a maximal bacterial count (N_{max}) of 5.7 (log_{10}cfu/g) after approximately 500h storage, but no specific microorganism could be identified responsible for the favoured growth. The growth of *L. monocytogenes* was suppressed in the oxygen enriched atmosphere, whereas *Brochothirx thermosphacta* dominated the spoilage flora. The results indicate, that the combination of high oxygen with carbon dioxide and possible interactions with the spoilage background flora cause a delay of the *L. monocytogenes* growth under the 70% O_2 / 30% CO_2 atmosphere.

4.2 Introduction

Nowadays, food producers are confronted with the challenge to produce high quality food with long shelf life times and products which are safe for human consumption as well (Bilska 2011). Especially fresh meat is susceptible to microbiological growth because of its nutritional profile. Thereby, the packaging under modified atmosphere conditions became one of the most popular preservation techniques for fresh and processed products. The commonly used gases for MAP application are carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2), which are added in different proportions according to the product properties (Phillips 1996, Floros & Matsos 2005). For poultry meat, inconsistent recommendations about the optimal gas mixture are given by the gas producers. The consequence is that some companies use high oxygen atmospheres whereas others are using mixtures with less oxygen (Thoden van Velzen & Linnemann 2008). However, Rossaint et al. (2014) showed that there is no difference between the two used gas atmospheres in on the shelf life determined by sensory parameter. Besides the effect of MAP gas mixtures on the sensory shelf life of poultry, producers also have to guarantee that pathogenic bacteria are not able to multiply on the surface. Especially poultry meat shows nearly the highest bacterial growth of spoilage and pathogenic bacteria than other foods (Synder 1998). Thereby, *Listeria monocytogenes* is one of the most important food-borne pathogen on fresh meat and the growth is of particular concern for the food industry. The reason is that *L. monocytogenes* is able to proliferate at refrigeration temperatures up to -0.4°C (Philips 1996). Especially poultry meat supports the growth of *Listeria monocytogenes* better than other kind of meat (Farber & Peterkin 1991).

The growth of *L. monocytogenes* is generally influenced by the spoilage background flora, whereas inconsistent results are published in the scientific literature. For *Pseudomonas* spp. for example, Marshall & Schmidt (1991) reported an increased growth of *L. monocytognes* by in milk after the inoculation with Pseudomonds. In contrast, nearly no effect in delaying the growth of *L. monocytogenes* by *Pseudomonas* spp. was found in Tryptose broth by Farrag & Marth (1989). Carlin et al. (1996) reported a slightly reduction of L. monocytogenes by different strains of fluorescent Pseudomonads in liquid endive leaf medium.

The contradictory results can be explained by the performance in liquid media, which simulates ideal growth conditions and did not take into account the complex food matrix. Several studies are published about interactions of the spoilage flora with *L. monocytogenes* in liquid media (Mattila-Sandholm & Skyttä 1991, Bennik et al. 1995, Francis & O`Beirne 1998, Malakar et al. 1999, Malakar et al. 2003, Mellefont et al. 2008). In contrast, structured

food provides the basis for a huge bacterial diversity and the growth of microorganisms is mostly slowed down due to additional stress for the bacteria (Van Impe et al. 2010).

Therefore, the objective of this work is to investigate the growth of *Listeria monocytogenes* inoculated on fresh poultry breast fillets in the presence of the natural background spoilage flora and compare the growth behavior using two commonly used gas mixtures for poultry breast meat (70% O_2 / 30% CO_2 vs. 70% N_2 / 30% CO_2).

4.3 Materials & Methods

Preparation of meat samples

As test samples, unsexed 42-days-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 hours after slaughtering.

Preparation of inocula and inoculation

For inoculation *Listeria monocytogenes* (ATCC 19111) was used. The strain was deep-freeze in cryogen pellets for preservation and purchased from the German Resource Centre for Biological Material (DSMZ). Before cultivation of the strain, the nutrient broth (Carl Roth, Karlsruhe, Germany) was pre-tempered for 24 hours at the optimal growth temperature according to the instructions of DSMZ for each microorganism. Afterwards, *Listeria monocytogenes* was grown separately in 10ml nutrient broth and sub-cultured after 24 hours and 48 hours. Afterwards the dilution of the broth to a concentration of 10^4 cfu/ml was conducted.
For inoculation of the samples, the double breast fillets were divided into single fillets using a sterile scalpel. Afterwards, a defined area of the meat surface (28cm^2) was marked using a rectangular metallic frame (4 x 7cm). The defined meat surface was inoculated with 0.1ml of the bacteria dilution to achieve an initial concentration between 180-280cfu/g. The inoculum was dispersed on the meat surface using a sterile drigalski applicator.

Packaging and storage of meat samples

For modified atmosphere packaging, the inoculated poultry 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 2:1. The inoculated meat samples were packaged under an atmosphere containing 70% O_2/30% CO_2 and 70% N_2/30% CO_2. The trays were heat-sealed with a polypropylene foil (Suedpack Verpackungen GmbH & Co. KG, Ochsenhausen, Germany; water vapour permeability < 3.5g/m^2d at 23°C / 85% RH; oxygen permeability </=1.5 cm^2/m^2d bar at 23°C / 35% RH) for 3s/175°C using a tray sealer packaging machine (Traysealer T200, Multivac Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany). Gas mixtures were prepared by a four-component gas blender machine (KM 60-4 MEM SO, Witt Gasetechnik, Witten, Germany). The packaged meat samples were stored at 4°C in low-temperature high precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). The storage temperatures were monitored by data logger (ESCORT JUNIOR Internal Temperature Data Logger, Escort, Auckland, New Zealand) every 5 minutes. The microbiological, sensory and chemical analyses were conducted at appropriate time intervals. Each measurement was repeated three times.

Microbiological analyses

For microbiological analyses, the inoculated meat surfaces were removed aseptically by using a sterile scalpel. The product sample had an admeasurement of 4 x 7 x 0.5cm to achieve a total weight of nearly 25g. Samples were transferred to a filtered sterile stomacher bag and filled up with 225ml saline peptone diluent (0.85 % NaCl with 0.1% peptone Saline-Tablets, Oxoid BR0053G, Cambridge, United Kingdom). Sample homogenization was conducted with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60s. A 10-fold dilution series were made of the homogenate using saline peptone diluent and investigated for total viable count (TVC), *Pseudomonas* ssp., *Brochothrix thermosphacta*, *Enterobacteroaceae*, *Lactobacillus* ssp. and *Listeria monocytogenes*. Total viable count was determined by pour plate technique on Plate Count Agar (PCA, Merck, Darmstadt, Germany) and plates were incubated at 30°C for 72 hours. *Pseudomonas* sp. was detected by spread plate technique on Pseudomonas Agar with Cetrimide-Fucidin-Cephaloridine selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 hours. *Brochothrix 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 hours. *Enterobacteriaceae* were identified by overlay-treatment on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany) by incubating the agar plates at 30°C for 48 hours and *Lactobacillus* sp. was determined by pour plate technique on de Man, Rogosa, Sharpe Agar (MRS, Oxoid, Cambridge, United Kingdom). Plates were incubated aerobically at 37°C for 72 hours. The determination of Listeria monocytogenes was performed on Agar Listeria according to Ottaviani and Agosti (ALOA, Bio Mérieux, Paris, France). Plates were incubated at 37°C for 24hours. During storage, the presence of *L. monocytogenes* was tested on samples without inoculation treatment. In all samples, *L. monocytogenes* was not detected. Counts of colony forming units were expressed as $\log_{10}$ cfu/g for each medium and sample.

Gas analysis

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

Sensory evaluation

Sensory analyses were carried out by trained sensory panellists, recruited from the Institute of Animal Science (University of Bonn) and all were experienced in poultry sensory evaluation. During the trials, each sample was evaluated directly after opening the tray, using a developed sensory scheme according to the Quality Index Method (QIM) for fish evaluation (Bremner, 1985). A picture of fresh chicken breast fillets was used as reference during the sensory evaluations.
Attributes were defined as general appearance (G), colour (C), odour (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 quality index (QI) was calculated by the following equation (Kreyenschmidt 2003):

$$QI = \frac{2G + 2C + 1T + 1D + 2O}{8} \qquad (1.1)$$

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

Primary Modelling

The Gompertz equation was used to model the growth of the total viable count, Enterobacteriaceae, *Pseudomonas* spp., *B. thermosphacta*, *Lactobacillus* spp. and *L. monocytogenes* 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 [h] at which maximum growth rate is obtained (reversal point), and t is time.

The time [h], when the bacterial count achieves the plateau ($t_{plateau}$) is calculated according to the following equation:

$$t_{plateau} = M + \frac{1}{B} \qquad (1.3)$$

With M: time [h] at which maximum growth rate is obtained (reversal point); and B: relative maximum growth rate at time M [h^{-1}].

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

4.4 Results & Discussion

Influence of the gas atmospheres on the growth of Listeria monocytogenes

Figure 4.1 compares the growth of *Listeria monocytogenes* during storage, inoculated on natural skinless chicken breast fillets under nitrogen and oxygen enriched atmospheres at 4°C. During the trials, *L. monocytogenes* shows a growth potential up to a maximal bacterial count (N_{max}) of 5.7 (log_{10}cfu/g) under the nitrogen containing atmosphere. Also Farber & Daley (1994) showed that *L. monocytogenes* is able to grow under 70% N_2 / 30% CO_2 on turkey roll slices. Jydegaard-Axelson et al. (2004) showed that the metabolite production and the growth of *L. monocytogenes* are more increased in N_2 then in CO_2 atmospheres. Several studies also emphasise that *L. monocytogenes* shows the ability to grow under reduced O_2-tensions and chilled temperatures on several foods and nutrient media (Brackett 1988, Doyle 1988, Wimpfheimer et al. 1990).

During storage under high oxygen atmosphere, the growth of *L. monocytogenes* remains relatively constant during the entire storage period. This agrees with the results of Nissin et al. (2000), who found no growth of *L. monocytogenes* in inoculated ground beef during storage at 4°C in 70% O_2 / 30% CO_2 mixture. High oxygen concentrations lead to the formation of oxygen radical species and causes cell damages and cell death. But according to Fisher et al. (2000), *L. monocytogenes* is able to produce the enzyme superoxide dismutase during exposure to high oxygen concentrations. These are enzymes that catalysis the destruction of superoxide radicals by dismutation (Lavelle et al. 1973). As stated out by Amanatidou et al. (1999), the growth of *L. monocytogenes* is strongly retarded by 90% O_2 mixed with 10% CO_2, whereas the use of 90% O_2 alone did not inhibit the growth. Therefore, only the combination between oxygen and carbon dioxide indicates an inhibitory effect on *L. monocytogenes*, as also shown in this study. Krämer & Baumgart (1992) showed that the microorganism is merely inhibited up to 50% CO_2 during storage of sausages boiled in broth. But the authors also mentioned that high CO_2 concentrations lead to unacceptable souring of the product. In contrast, Wimpfheimer at al. (1990) showed that *L. monocytogenes* is able to grow under reduced oxygen concentrations and is relatively unaffected by high CO_2-concentrations. In the study, the microorganism, inoculated on raw chicken stored at 4°C under 72.5% CO_2 / 22.5% N_2 / 5% O_2, reached bacterial counts of 10^8 cfu/g after 20 days. In contrast, the absence of oxygen resulted in growth stagnation.

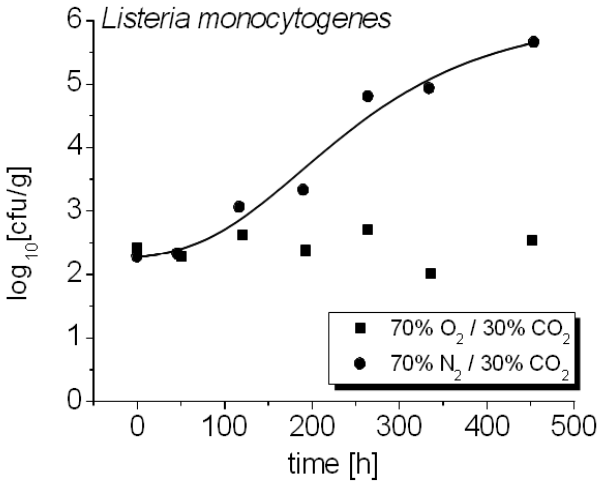

Figure 4.1 Development of *Listeria monocytogenes* on poultry fillets stored under two different gas atmospheres.

Influence of the natural spoilage background flora on the growth of *Listeria monocytogenes*

Figure 4.2 shows the development of the spoilage microflora in presence of inoculated *Listeria monocytogenes* during storage under nitrogen or oxygen enriched gas mixtures. The development of TVC is nearly the same for both investigated gas mixtures (Table 4.1). Regarding the development of the spoilage bacteria, the results show that the development of the flora is strongly influenced by the used gas atmosphere.

L. monocytogenes and Pseudomonas spp.

Comparing the growth of *Pseudomonas* spp., the microorganisms show a comparable growth under both atmospheres with the same duration of the lag phases (83h) and comparable maximum bacterial counts at the end of storage (N_2:7.8 $\log_{10}$cfu/g and O_2: 7.4 $\log_{10}$cfu/g). Even though the bacterium is an aerobic competitor, *Pseudomonas* spp. is able to grow up to oxygen concentrations of 1% O_2 (Clark & Burki 1972). During storage of the samples, a residual oxygen concentration of approximately 1% was detected. Similar results were reported by Fraqueza & Barreto (2009) during storage of turkey meat (100% N_2 and 50% Ar / 50% N_2) and Herbert et al. (2013) during storage of skinless poultry breast fillets (82% N_2 / 18% CO_2 and 82% Ar / 18% CO_2). Also Chouliara et al. (2008) showed a good

growth of *Pseudomonas* spp. under 70% N_2 / 30% CO_2. Even through *Pseudomonas* spp. shows an analogous growth under both atmosphere, the growth is slightly reduced under 70% O_2 compared to the 70% N_2 containing atmosphere, which is reflected in the calculated growth rate (N_2: 0.024h^{-1} and O_2: 0.016h^{-1}) This is presumably related to the formation of oxygen radical species which leads to an inhibition of growth. Also the results of Jacxsens et al. (2001) underline the results of the present study. The authors showed an increased sensitivity towards oxygen concentrations between 70-95% for *Pseudomonas fluorescens*, whereas the growth was favoured under 5% O_2. Also Amanatidou et al. (1999) reported a reduced growth of *Pseudomonas fluorescens* in vitro at high oxygen atmospheres.

Marshall et al. (1992) published that *Pseudomonas* spp. seems to support the growth of *L. monocytogenes*. For milk, it has been shown that the growth of *L. monocytogenes* is favoured in the presence of Pseudomonads (Marshall et al. 1988). Marshall & Schmidt (1991) concluded, that the hydrolyses of proteins due to Pseudomonads and the formation of free amino acids stimulates the proliferation of *L. monocytogenes*. Also Mattila-Sandholm & Skyttä (1991) reported an inhibitory effect of Pseudomonads on the growth of *L. monocytogenes* in a medium prepared from minced beef meat. Carlin et al. (1996) showed in a liquid endive leafs media that high numbers of *Pseudomonas* spp. reduced the growth of *L. monocytogenes*. But the authors also accentuated that the effect of a single isolates varied between the experiments. In contrast, Farrag & Marth (1989) found nearly no effect in delaying the growth of *L. monocytogenes* by *Pseudomonas* spp. in Tryptose broth.

The results of the present study lead to the conclusion, that *Pseudomonas* spp. seems to have nearly no influence in delaying or supporting the growth of Listeria on natural meat because of the slightly difference in growth during storage under both atmospheres. It has to be taken into account also, that the reported interactions between *Pseudomonas* spp. and Listeria observed in the studies carried out with isolated species of Pseudomonads. Such data are often not comparable to meat.

L. monocytogenes and Brochothrix thermosphacta

The growth of *B. thermosphacta* is slowed down under 70% N_2 / 30% CO_2 compared with the oxygen enriched atmosphere, achieving a maximum microbial count of 6.6 $\log_{10}$cfu/g in comparison to 8.5 $\log_{10}$cfu/g (Table 4.1). The microorganism is a psychrotrophic, facultative anaerobic competitor and prefers to grow under oxygen containing atmospheres (Gribble & Brightwell 2013). Therefore *B. thermosphacta* dominates the spoilage flora under the oxygen enriched atmosphere. The microorganism is often associated with spoilage of meat under MAP conditions (Labadie 1999, Kreyenschmidt & Ibald 2012, Gribble & Brightwell 2013). Further on, the microorganism is relatively unaffected by CO_2 (Branscheid et al. 2007), which

explains the favored growth during the trials. Regarding the growth of *L. monocytogenes* in presence of *B. thermosphacta* under O_2 enriched atmospheres, the growth of *L. monocytogenes* seems to be suppressed when *B. thermosphacta* dominates the flora. These results are also in accordance to Tsigarida et al. (2000). The authors demonstrated a growth inhibition of *L. monocytogenes* by *B. thermosphacta* either on naturally contaminated and sterile beef surface under MAP and vacuum conditions. Greer & Dilts (2006) reported for *Brochothrix campestris* to inhibit *L. monocytogenes* in All Purpose Tween broth or on discs of pork adipose tissue at 4°C by producing a bacteriocin. However, a bactericin production was not tested in the present study. Ludwig et al. (1984) demonstrated a strong relationship between *L. monocytogenes* and *B. thermosphacta* by 16S rRNA analyses. This close relationship is presumably a reason for the reduced growth of *L. monocytogenes* in the presence of *B. thermosphacta* during the storage tests. Due to the fact that meat is an ecological nice especially for *B. thermosphacta* (Labadie 1999), the microorganism is optimal adapted to the meat matrix and seems to have a selective advantage due to nutrient competition in comparison to *L. monocytogenes*.

L. monocytogenes and Enterobacteriaceae

Enterobacteriaceae play a subordinated role under both gas atmospheres. The microorganisms are mesophilic and their growth is reduced due to the refrigerated temperature of 4°C during storage (Smolander et al. 2004). Considering the growth characteristics under MAP conditions, the nitrogen containing atmosphere seems to retard the growth of Enterobacteriaceae at the beginning of storage, which is reflected in the prolongation of the lag phase compared to the O_2-CO_2 mixture (N_2: 134h / O_2: 35h). This is presumably related to the facultative anaerobic properties of the bacterium, which prefers oxygen to multiply. During storage, Enterobacteriaceae show a similar growth under both gas atmospheres (Figure 4.2), reflected in a similar maximal microbial count under both gas atmospheres (N_2: 6.7 $\log_{10}$cfu/g and O_2: 7.2 $\log_{10}$cfu/g). After 250h the bacterial counts of Enterobacteriaceae under both atmospheres achieve 5 $\log_{10}$cfu/g, where the shelf life under these conditions has been exceeded already (Rossaint et al. 2014). Therefore it can be concluded that the growth of Enterobacteriaceae has presumably no effect on the growth development of *L. monocytogenes*. Also Tsigarida et al. (2000) stated out, that Enterobacteriaceae played a minor role on vacuum and MAP packed beef meat fillets and were not considered as competitors for Listeria. In contrast, Francis & O`Beirne (2002) reported a significant reduction in *Listeria inoccua* (in lieu of *L. monocytogenes*) in liquid lettuce medium by *Enterobacter cloacae*, whereas *Enterobacter sakazakii* caused a slightly

growth reduction. Therefore, an inhibitory effect by Enterobacteriaceae on the growth of *L. monocytogenes* seems to be related to the composition of the species present on poultry.

L. monocytogenes and Lactobacillus spp.

Lactobacillus spp. shows a slightly favoured growth under the nitrogen enriched atmosphere, reflected in the growth rate (N_2: 0.027h^{-1} and O_2: 0.012h^{-1}) and the population density at the end of storage (N_2: 5.8 log_{10}cfu/g and O_2: 4.9 log_{10}cfu/g). But their growth becomes not dominant over the entire storage period in both atmospheres and the bacteria plays a minor role for the spoilage process of MAP poultry. Similar results were reported by Herbert et al. (2013) und Rossaint et al. (2014). Generally, *Lactobacillus* spp. belong to a slow growing group of microorganisms and they preferably to grow under anaerobic conditions and are highly tolerant towards high levels of CO_2 (>50%) (Huis in`t Veld 1996, Jay et al. 2005). In the present study, relatively low CO_2 (30%) concentrations occur and resulting additionally in a reduced growth of *Lactobacillus* spp. Regarding the effect of *Lactobacillus* spp. on the growth of *L. monocytogenes*, it is reported that the microorganisms are able to inhibit the growth of *L. monocytogenes* in various food products due to bacteriocin production, when Lactic acid bacteria reach high counts (Hugas 1998). Bredholt et al. (2001) showed that *Lactobacillus sakei* inhibited the Listeria growth in cooked, sliced vacuum packed meat. Nilsson et al. (1999) reported an inhibition of *L. monocytogenes* by *Lactobacillus* spp. in cold-smoked salmon. In the present study, *Lactobacillus* spp. seems to have no effect on the growth of *L. monocytogenes* due to the minor quantities of the bacteria under both atmospheres. Also Malakar et al. (2003) stated out that interactions between Lactobacillus spp. and *L. monocytogenes* only occur at very high of population densities of LAB (10^7cfu/ml). These results are also in accordance to Barakat & Harris (1999), who showed that the growth of *L. monocytogenes* was not inhibited in the presence of various naturally Lactobacillus species on cooked modified atmosphere packed poultry under 44% CO_2 / 56% N_2. Therefore, the inhibition of Listeria due to LAB seems to be strongly dependent from the prevailing Lactobacillus species, able to produce antilisterial compounds, as also stated out by Hwang & Sheen (2011).

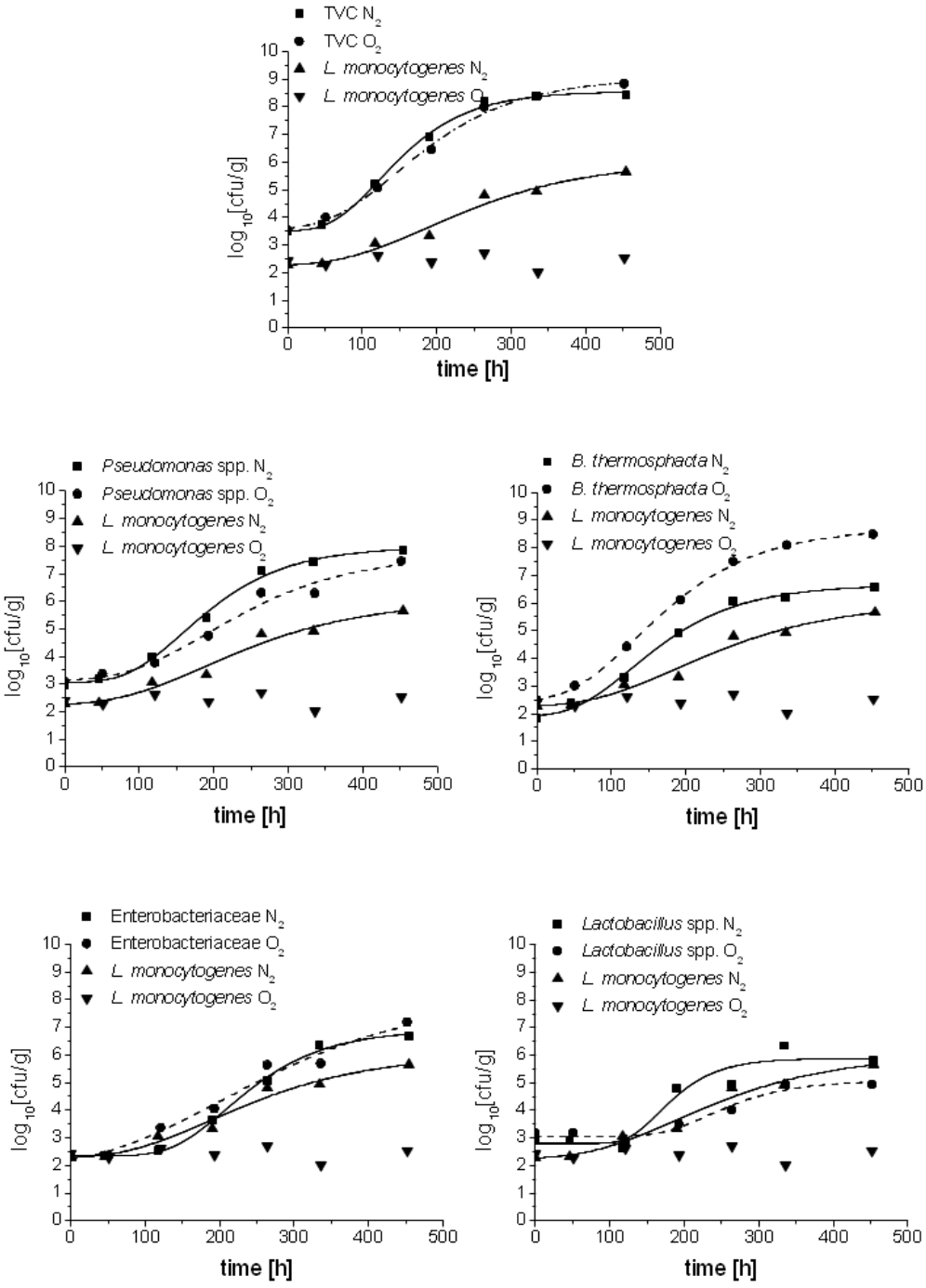

Figure 4.2 Development of natural spoilage flora and *L. monocytogenes* on poultry breast fillets stored under tow different atmospheres.

Table 4.1 Calculated growth parameters of the investigated microorganisms (Gompertz function).

Atmosphere	70% N_2/30% CO_2				70% O_2/30% CO_2			
Growth parameter	t_{lag} [h]	growth rate [1/h]	N_{max} [log_{10}cfu/g]	$t_{plateau}$	t_{lag} [h]	growth rate [1/h]	N_{max} [log_{10}cfu/g]	$t_{plateau}$
TVC	58	0.029	8.4	188	54	0.022	8.8	235
***Pseudomonas* spp.**	83	0.024	7.8	235	83	0.016	7.4	297
B. thermosphacta	48	0.022	6.6	207	43	0.023	8.5	223
Enterobacteriaceae	134	0.023	6.7	282	35	0.014	7.2	361
Lactic acid bacteria	124	0.027	5.8	209	169	0.012	4.9	294
Listeria monocytogenes	70	0.012	5.7	306	-	-	2.5	-

Development of the Quality Index

The Quality Index (QI) increases for poultry, with increasing storage time for both used gas atmospheres. A quality index of 2.5 was taken as the lower limit of acceptability, corresponding to initial deteriorative changes regarding colour, odour, texture, general appearance and drip loss (Figure 4.3). According to Rossaint et al. (2014), no significant difference in the development of sensory shelf life using oxygen or nitrogen enriched atmospheres could be conducted. In the present study, shelf life of poultry meat was prolonged by approximately 40h during storage under the O_2-CO_2 mixture compared to the N_2-CO_2 mixture. This was mainly related to slightly differences in color ond odor. Animal specific factors like age, sex, moisture content or processing can have an influence on meat color development (Totosaus et al., 2007).The changes in odor are presumably related to the differences in the bacterial diversity due to individual oxygen requirements. The bacterial load of *L. monocytogenes* at the end of shelf life under the N_2-CO_2 mixture reached a level of 3.1 log_{10}cfu/g. According to the EU (No) 2073/2005 the concentration of *L. monocytogenes* should be lower than 100 cfu/g food at the time of consumption. The growth development of *L. monocytogenes* under the nitrogen atmosphere can be critical for human consumption. The counts of *L. monocytogenes* under the oxygen containing atmosphere stayed relatively constant (approximately 2.3 log_{10}cfu/g) throughout the entire storage period. Therefore, the use of the high oxygen atmosphere can be recommended in comparison to the oxygen free

atmosphere. Due to prolongation of shelf life using MAP treatment by delaying the growth of spoilage bacteria, it is possible that pathogenic bacteria achieve high levels and produce toxins before the spoilage of the food occurs, as proposed by Farber et al. (2003). Also Bohm (2006) stated out that most pathogenic bacteria are facultative anaerobic bacteria and the absence of oxygen in the atmosphere enables an unheeded outgrows.

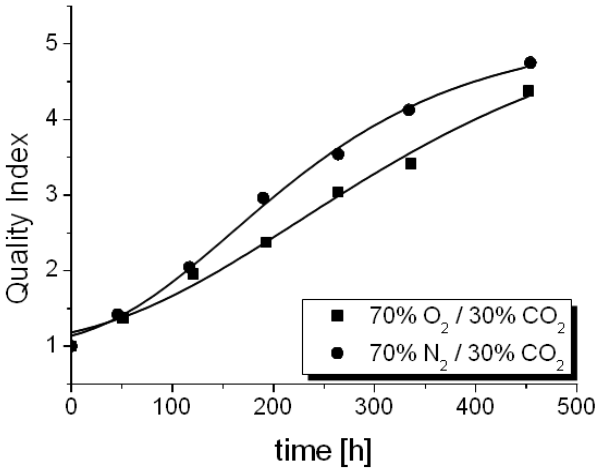

Figure 4.3 Development of the Quality Index.

Development of the gas atmosphere

Within the first 24 hours of storage, the CO_2 concentration shows a small decrease (approximately 2%) in all packages (data not shown). This is related to the high solubility of carbon dioxide in the fat tissue and water on the meat surface (Gill 1988, Betts 1995). Similar results for MA packed meat were reported by Parra et al. (2010) and Herbert et al. (2013). During storage, CO_2 concentrations increased slowly due to metabolic CO_2 production of bacteria and meat enzymes (Abdullah et al. 1994). For the packaging under 70% O_2 / 30% CO_2, an O_2 depletion is shown. The oxygen levels inside the trays show a continuous decrease during storage to a final concentration of to approximately 65% at the end of storage, which is caused by microbiological consumption of O_2, the respiration of meat enzymes and gas exchanges between the gas composition inside the trays and the environment (Mullan & McDowell 2003, Esmer et al. 2011).

4.5 Conclusion

Regarding the used gas mixture, it can be summarized that the use of high oxygen in combination with 30% CO_2 is effective in suppressing the growth of *L. monocytogenes.* Also the sensory evaluation resulted in a longer shelf life compared to the N_2-CO_2 mixture. The contribution of each microorganism to the spoilage process was affected by the MAP conditions. Under high oxygen atmosphere, the growth of *L. monocytogenes* was delayed during the entire storage period, which is presumably related to the dominance of *Brochothrix thermosphacta* and the mixture of O_2 with CO_2. Under high nitrogen atmosphere no specific microorganism could be identified responsible for the favoured growth of Listeria. Due to the fact that 70% O_2/30% CO_2 as well as 70% O_2/30% CO_2 gas mixtures were commonly used in Europe for the packaging of poultry meat, the results of the present study indicate that the use of high O_2 atmospheres enriched with CO_2 are safer then the nitrogen enriched atmospheres for the packaging of poultry fillets. But also pathogenic bacteria like *Staphylococcus aureus* and *Camphylobacter* spp. are important microorganisms occurring on poultry meat. Therefore, further research is needed consider also possible effects of the investigated gas atmospheres on the growth of other pathogenic bacteria. To gather precise information about interactions of pathogens with spoilage microorganisms, further investigations have to be performed on naturally meat surface to consider also the influence of different subspecies and their by-products on the growth behaviour of pathogenic microorganisms.

4.6 References

Abdullah, B., F. Gardini, A. Paparella, M. E. Guerzoni (1994). Growth modelling of the predominant microbial groups in relation to the modulation of atmosphere composition, storage temperature, and diameter of meat particle. *Meat Science*, 38:511-526.

Amanatidou, A., E. Smid, L. Gorris (1999). Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated micro-organisms. *Journal of Applied Microbiology*, 86:429-438.

Barakat, R. K., L. J. Harris (1999). Growth of *Listeria monocytogenes* and *Yersinia enterocolitica* on cooked modified-atmosphere-packaged poultry in the presence and absence of naturally occurring microbiota. *Applied Environmental Microbiology*, 65:342-345.

Bennik, M. H. J., E. J. Smid, F. M. Rombouts, L. G. M. Gorris. 1995. Growth of psychrotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. *Food Microbiology*, 12:509-519.

Betts, G (1995). The microbiological consequences of MAP and vacuum packaging. International Conference on Modified Atmosphere Packaging and Related Technologies. Campden & Chorleywood Research Association, Chipping Campden, UK.

Bilska, A. (2011). Packaging systems for animal origin food. *LogForum* 7(4):35-44.

Bohm, S. B. 2006. What is Reduced Oxygen Packaging and what are the Food Safety Concerns & Controls?. National Environmental Health Association Annual. Educational Conference and Expedition. June 2006, San Antonio, Texas.

Brackett, R. E. (1988). Presence and persistance of *Listeria monocytogenes* in food and water. Food Technology, 42:162-164.

Branscheid, W., K. O. Honikel, G. von Lengerken, K. Troeger (2007). Qualität von Fleisch und Fleischwaren. 2nd Edit, Deutscher Fachverlag GmbH, Frankfurt am Main.

Bredholt, S. T. Nesbakken, T. Holk (2001). Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats. *International Journal of Food Microbiology*, 66:191-196.

Bremner, A. (1985). A convenient easy-to-use system for estimating the quality of chilled seafood. *Fish Processing Bulletin*, **7**, 59-70.

Chouliara E., A. Karatapanis, I.N. Savvaidis, and M.G. Kontominas (2007). Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. *Food Microbiology*, 24:607–617.

Clark, D. S., and T. Burki (1972). Oxygen requirements of strains of *Pseudomonads* and *Achromobacter*. *Canadian Journal of Microbiology*, 18:321-326.

Doyle, M. P. (1988). Effect of environmental and processing conditions on *Listeria monocytogene*s. *Food Technology*, 42:169-171.

Esmer, O. K. R. Irkin, N. Degirmenicoglu, and A. Degirmenicoglu (2011). The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. *Meat Science*, 88:221-226.

EU (No) 2073/2005. Commission Regulation on microbiological criteria for foodstuff. 15/11/2005, L 338/1-L338/25.

Farber, J. M. & P. I. Peterkin (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiololgical Reviews,* 55(3):476-511.

Farber, J. M., and E. Daley (1994). Fate of *Listeria monocytogenes* on modified-atmosphere packaged turkey roll slices. *Journal of Food Protection*, 57:1098-1100.

Farber, J. M., L. J. Harris, M. E. Parish, L. R. Beuchat, T. V. Suslow, J. R. Gorney, E. H. Garrett, F. F. Busta (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, 2:142-160.

Fisher, C. W., D. Lee, B. A. Dodge, K. M. Hamman, J. B. Robbins, S. E. Martin (2000). Influence of catalase and superoxide dismutase on ozone inactivation of *Listeria monocytogenes*. *Applied & Environmental Microbiology*, 66:1405-1409.

Fraqueza, M. J., and A. S. Barreto (2009). The effect on turkey meat shelf life of modified-atmosphere packaging with an argon mixture. *Poultry Science,* 88:1901-1998.

Floros, J. D., K. I. Matsos (2005). Introduction to modified atmosphere packaging. In: Han et al. Innovations in food packaging. Elsevier Academic Press. pp 151-171.

Gibson, A., N. Bratchell, and T. Roberts. 1987. The effect of sodium chloride and temperature on rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *Journal of Applied Bacteriology*, 62, 479–490.

Gill, C. O. (1988). The solubility of carbon dioxide in meat. *Meat Science* 22:65-71.

Greer, G. G. & B. D. Dilts (2006). Control of meatborne *Listeria monocytogenes* and *Brochothrix thermosphacta* by a bacteriocinogenic *Brochothrix campestris* ATCC 43754. *Food Microbiology*, 23:785-790.

Gribble, A. & G. Brightwell. (2013). Spoilage characteristics of *Brochtothrix thermosphacta* and *campestris* in chilled vacuum packaged lamb, and their detection and identification by real time PCR. *Meat Science*, 94, 361-368.

Hechelmann, H. (1981). Vorkommen und Bedeutung von *Brochothrix thermosphacta* bei Kühllagerung von Fleisch und Fleischerzeugnissen. *Mitteilungsblatt der Bundesanstalt für Fleischforschung, Kulmbach*, 4435-4437.

Herbert, U., Rossaint, S., Khanna, M. A. & Kreyenschmidt, J. (2013). Comparison of argon-based and nitrogen-based modified atmosphere packaging on bacterial growth and product quality of chicken breast fillets. *Poultry Science*, 92(5), 1348-1356.

Hugas, M. (1998). Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products. *Meat Science*, 49:S139-S150.

Huis in`t Veld, J. H. J. 1996. Microbial and biochemical spoilage of foods: an overview. *Food Microbiology*, 33:1-18.

Hwang, C.-A., S. Sheen (2011). Growth characteristics of *Listeria monocytogenes* as affected by a native microflora in cooked ham under refrigerated and temperature abuse conditions. *Food Microbiology*, 28:350-355.

Jacxsens, L., F. Devlieghere, C. Van der Steen, and J. Debevere. 2001. Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71:197-210.

Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Modern Food Microbiology. 7th Edition, Springer, New York, London, Berlin.

Jydegaard-Axelson, A., P. E. Høiby, K. Halmstrøm, N. Russel, S. Knøchel (2004). CO_2 -and anaerobiosis-induced changes in physiology and gene expression of different *Listeria monocytogenes* strains. *Applied Environmental Microbiology*, 70:4111-4117.

Krämer, K., J. Baumgart (1992). Brühwurstaufschnitt. Hemmung von Listeria monocytogenes durch modifizierte Atmosphären. *Fleischwirtschaft*, 72:666-668.

Kreyenschmidt, J. (2003). Modellierung des Frischeverlustes von Fleisch sowie des Entfärbeprozesses von Temperatur-Zeit- Integratoren zur Festlegung von Anforderungsprofilen für die produktbegleitende Temperaturüberwachung. PhD thesis,

Rheinische Friedrich-Wilhelms-Universität Bonn. Bergen/Dumme, Germany: AgriMedia GmbH.

Kreyenschmidt, J, R. Ibald (2012). Modeling shelf life using microbial indicators. In: Nicoli, M. C. (edit.). Shelf life Assessment of Food. CRC Press. pp 127-168.

Labadie, J. 1999. Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science*, 52:299-305.

Lavelle, F., A. M. Michelson, L. Dimitrijevic. 1973. Biological protection by superoxide dismutase. *Biochemicial and Biological Research Communication*, 55(2):350-357.

Ludwig, W., K.-H. Schleifer, E. Stackebrandt (1984). 16S rRNA analysis of *Listeria monocytogenes* and *Brochothrix thermosphacta*. *FEMS Microbiology Letters*, 25:199-204.

Malakar, P. K.,D. E. Martens, M. H. Zwietering,C. Béal, K. van`t Riet. 1999. Modelling the interactions Lactobacillus carvatus and Enterobacter chloaceae. II. Mixed cultures and shelf life predictions. *International Journal of Food Microbiology*, 51:67-79.

Malakar, P. K., G. C. Barker, M. H. Zwietering, K. van`t Riet. 2003. Relevance to microbial interactions to predictive mocrobiology. *International Journal of Food Microbiology*, 84:263-272.

Marshall, D. L., R. H. Schmidt (1988). Growth of *Listeria monocytogenes* at 10°C in milk preincubated with selected pseudomonads. *Journal of Food Protection*, 51:277-282.

Marshall, D. L., R. H. Schmidt (1991). Physiological evaluation of stimulated growth of *Listeria monocytogenes* by *Pseudomonas* species in milk. *Canadian Journal of Microbiology*, 37(8):594-599.

Marshall, D. L., L. S. Andrews, J. H. Wells, A. J. Farr (1992). Influence of modified atmosphere packaging on the competitive growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken. *Food Microbiology*, 9:303-309.

Mattila-Sandholm, T., and E. Skyttä. 1991. The effect of spoilage flora on the growth of food pathogens in minced meat stored at chilled temperatures. *Lebensmittel-Wissenschaft und Technologie*, 24(2):116-120.

Mellefont, L. A., T. A. McMeekin, T. Ross. 2008. Effect of relative inoculum concentration on Listeria monocytogenes growth in co-culture. *International Journal of Food Microbiology*, 121:157-168.

Mullan, M., D. McDowell (2003). Modified atmosphere packaging. In: R. Coles, D. McDowell, M. J. Kirwan (Eds.): Food Packaging Techology, Blackwell Publishing, London, pp. 303-339.

Nilsson, L., L. Gram, H. H. Huss (1999). Growth control of *Listeria monocytogenes* on cold-smoked salmon using a competetive lactic acid bacteria flora. *Journal of Food Protection*, 62:336-342.

Nissin, H., O. Alvseike, S. Bredholt, A. Holck, T. Nesbakken (2000). Comparison between the growth of *Yersinia enterocolitica*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* spp. in ground beef packed by three commercially used packaging techniques. *International Journal of Food Microbiology*, 59:211-220.

Parra, V., J. Viguera, J. Sanchez, J. Peinado, F. Esparrago, J. I. Gutierrez, and A. I. Andrés (2010). Modified atmosphere packaging and vacuum packaging for long period chilled storage of dry-cured Iberian ham. *Meat Science*, 84:760-768.

Phillips, C. A. (1996). Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science & Technology,* 31:463–479.

Rossaint, S., Klausmann, S., Herbert, U. & Kreyenschmidt, J. (2014). Effect of package perforation on the spoilage process of poultry stored under different modified atmospheres. *Food Packaging and Shelf life*, 1(1):68-76.

Smolander, M., Alakomi, H.-L., Ritvanen, T., Vainionpää, J. & Ahvenainen, R. (2004). Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions. A. Time-Temperature indicators as quality-indication tools. *Food Control*, 15:217-229.

Snyder, O.P. (1998). Menu management and purchasing. In: Food Safety Through Quality Assurance Management (eds. Hospitality Institute of Technology and Management). Chapter 6, pp. 11-13. Saint Paul, Minnesota.

Thoden van Velzen, E. U. & A. R. Linnemann (2008). Modified atmosphere packaging of fresh meats – Sudden partial adaption caused an increase in sustainability of dutch supply chains of fresh meat. *Packaging Technology and Science,* 21:37-46.

Totosaus, A., M. L. Pérez-Chabela, and I. Guerrero. 2007. Color of fresh and frozen poultry, Pages 455-465 in Handbook of meat, poultry & seafood quality, 1st Edition. L. M. L. Nollet, ed. Blackwell Publishing, Oxford, UK.

Tsigarida, E., P. Skandamis, G.-J. E. Nychas (2000). Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with and without the presence of oregano essential oil at 5°C. *Journal of Applied Microbiology*,89:901-909.

Van Impe, J. F., A. M. Cappuyns, E. Van Derlinden (2010). Predictive modeling for microbial food safety and shelf life. oral Presentation, 3rd Food Spot Workshop, 20th May, Gent, BE.

Wimpfheimer, L., N. S. Altman, J. H. Hotchkiss (1990). Grwoth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. *International Journal of Food Microbiology*, 11:205-214.

5. Comparison of argon-based and nitrogen-based modified atmosphere packaging (MAP) on bacterial growth and product quality of chicken breast fillets

5.1 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 analysed and modelled by using the Gompertz function. Sensory analyses of the poultry samples were carried out by trained sensory panellists for colour, odour, 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 to nitrogen, except the *B. thermosphacta* stored under 82% argon. The sensory evaluation showed a significant effect of an argon-enriched atmosphere, particularly on colour 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.

Article has been published in *Poultry Science*, see list of publications (Herbert et al. 2013).

5.2 Introduction

Recently, there is an increasing interest by the food industry and gas producer for effective gas mixtures to further extend the shelf life of fresh and/or processed food products (Day, 1995; 2007). The three traditional gases for modified atmosphere packaging are oxygen, carbon dioxide and nitrogen (Farber, 1991; Rao & Sachindra, 2002; Floros & 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, odourless and tasteless (Greenwood & 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 gL^{-1}) and higher density (1.650 vs 1.153 kg/m^3) compared to nitrogen (Spencer, 1995; 2005). Regarding the inhibitory activity against bacterial growth, argon was suggested to have a better solubility in fat, resulting improved membrane permeability of CO_2, salts, acids to bacterial cells (Betts, 1995). Several studies were conducted to investigate the effect of argon on enzyme activities and sensory characteristics in the field of fruits and vegetables (Zhang et al. 2008, Wu et al. 2012, Zhang et al. 2001, Rocculi et al. 2005, O`Beirne et al. 2011, Jamie & Saltveit 2002). 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 *B. thermosphacta* with 1 log difference after the 25 days of storage, in comparison to nitrogen, but no effect on lipid oxidation (Fraqueza & 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 the microbiological growth and an unpleasant odour compared to the oxygen containing atmosphere. The storage of pork sausages under an argon-enriched atmosphere also shows 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 & Jiménez-Colmenero, 2010). Curiel et al. (2011) investigated in vitro the biogenic amine production of *lactic acid bacteria* and *Enterobacteriaceae* isolated from pork sausages packed in different atmospheres. The authors found an inhibition of *Carnobacterium divergens* under an argon atmosphere after 28 day storage, but the argon atmosphere also seemed to favour the growth of agmatine-producing *Enterobacteriaceae* in comparison to 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.

5.3 Materials & Methods

Preparation of meat samples and packaging

Unsexed 42-days-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 hours 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 six 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 & Co. KG, Ochsenhausen, Germany; water vapour permeability < 3.5 g/m^2d 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 four-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 used gas mixture in low-temperature high precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). Storage temperature was monitored by data logger (ESCORT JUNIOR Internal Temperature Data Logger, Escort, New

Zealand) every 5 minutes. The microbiological, sensory and chemical analyses were conducted at appropriate time intervals. Each measurement was repeated three times.

Microbiological analyses

Immediately after opening the packages, the amount (25g) of meat surface sample in size of 4 x 7 x 0.5 cm was aseptically taken using a sterile scalpel, which was transferred to a filtered sterile stomacher bag and filled with 225 ml saline peptone diluent (0.85 % NaCl with 0.1% peptone Saline-Tablets, Oxoid BR0053G, Cambridge, United Kingdom). 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 (TVC), *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 (PCA, Merck, Darmstadt, Germany) and plates were incubated at 30°C for 72 hours.

Presumptive *Pseudomonas* spp. were detected by spread plate technique on Pseudomonas Agar with Cetrimide-Fucidin-Cephalosporin selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 hours.

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 hours.

Presumptive *Enterobacteriaceae* were identified by overlay treatment on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany) by incubation of the agar plates at 30°C for 48 hours.

Presumptive *Lactobacilli* spp. were detected by pour plate technique on de Man, Rogosa, Sharpe Agar (MRS, Oxoid, Cambridge, United Kingdom). Plates were incubated aerobically at 37°C for 72 hours.
Counts of colony forming units 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 round about 1 hour before the investigation started. For the training, all participants had to describe and define typical sensory attributes (colour, odour, 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 Method (QIM) for fish evaluation (Bremner, 1985).
Attributes were defined as general appearance (G), colour (C), odour (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 quality index (QI) was calculated by the following equation (Kreyenschmidt, 2003):

$$QI = \frac{2G + 2C + 1T + 1D + 2O}{8} \qquad (1.1)$$

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 hand-held gas analyser (Oxybaby V O_2/CO_2, Witt Gasetechnik, Witten, Germany). Before starting the gas measurement inside the trays, the composition of air was analysed to control the accuracy of the gas analyser. 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 seconds and the oxygen concentration was detected by an electrochemical sensor; carbon dioxide concentration was detected by IR-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 Modelling

The Gompertz equation was used to model the growth of the total viable count, Enterobacteriaceae, *Pseudomonas* spp., *Brochothrix 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., U.S.A.).

Statistical analysis

Man-Whitney-U-test was used to make comparisons between sensory colour evaluation, pH-values and the measured counts of colony forming units with a level of significance of 0.05. SPSS statistics 20 for Windows was used.

5.4 Results & Discussion

Comparison of the spoilage process under various gas concentrations

Figure 5.1 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.

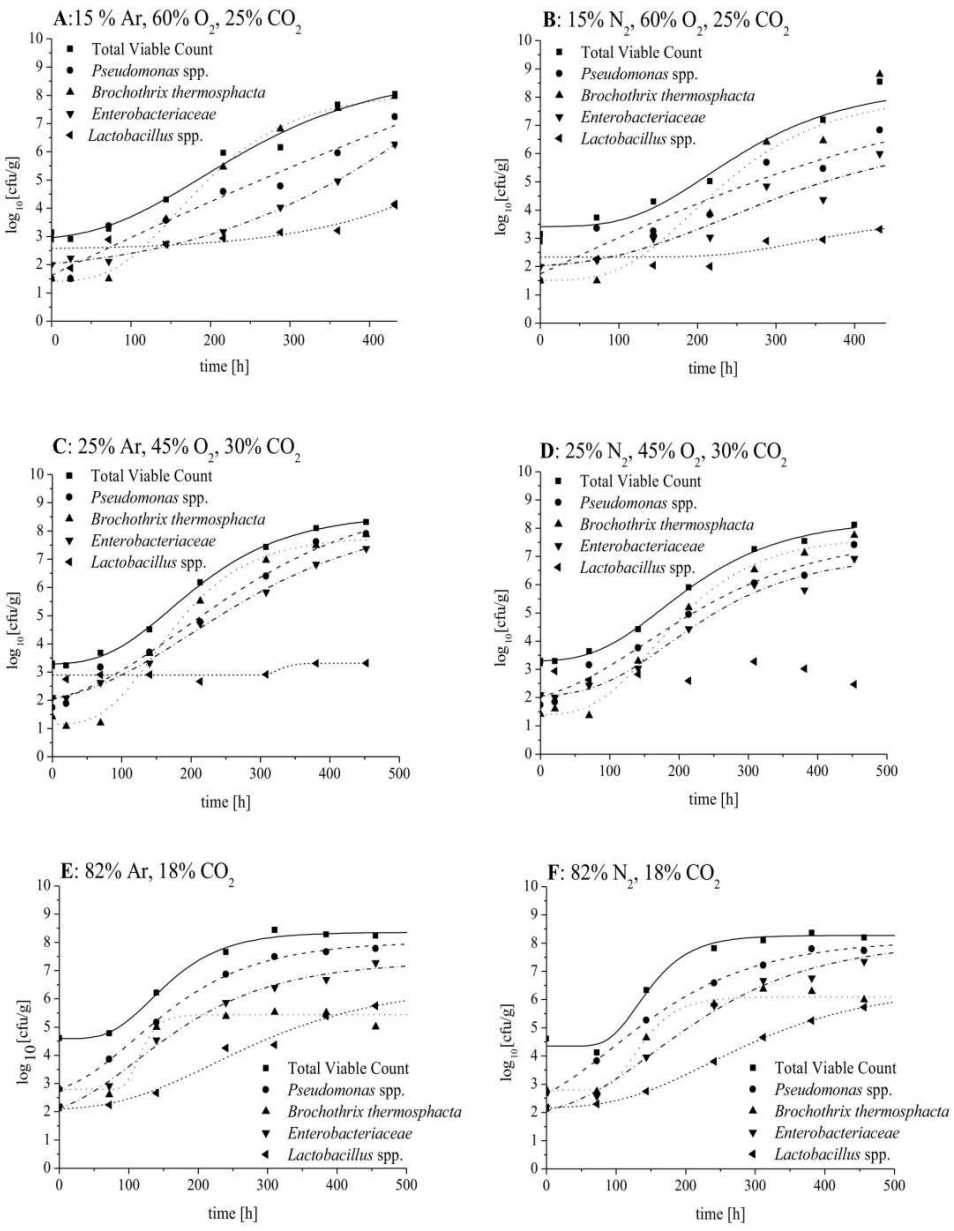

Figure 5.1 Growth of the spoilage microflora under different argon (left) and nitrogen (right) concentrations fitted with the Gompertz model, n=3.

Argon or nitrogen treatments of 15% show no significant effect on the growth of typical spoilage organisms (Table 5.2). The development of bacterial growth curves and the calculated growth rates (Table 5.1) are almost the same for both atmospheres.

Table 5.1 Development of growth parameter during storage of poultry under different atmospheres calculated with the Gompertz function.

Gas concentration	**15% Ar/60% O_2/30% CO_2**	**15% N_2/60% O2/30% CO_2**	**15% Ar/60 % O_2/30% CO_2**	**15% N_2/60% O_2/30% CO_2**
	μ_{max} [h^{-1}]		**Duration of lag-phase [h]**	
TVC	0.018	0.017	82.38	**84.37**
Brochothrix	0.031	0.026	78.04	**100.89**
Pseudomonas spp.	0.014	0.013	18.73	**34.37**
Enterobacteriaceae	0.014	0.011	130.51	**83.51**
Lactobacillus spp.	0.007	0.005	233.57	**232.52**
Gas concentration	**25%Ar/45% O_2/30% CO_2**	**25% N_2/45% O2/30% CO_2**	**25% Ar/45% O_2/30% CO_2**	**25% N_2/45% O2/30% CO_2**
	μ_{max} [h^{-1}]		**Duration of lag-phase [h]**	
TVC	0.021	0.019	76.30	**77.70**
Brochothrix	0.034	0.028	72.70	**78.14**
Pseudomonas spp.	0.018	0.016	29.67	**15.04**
Enterobacteriaceae	0.016	0.017	40.87	**77.54**
Lactobacillus spp.	0.012		309.70	
Gas concentration	**82% Ar/18% CO_2**	**82% N_2/18% CO_2**	**82% Ar/18% CO_2**	**82% N_2/18% CO_2**
	μ_{max} [h^{-1}]		**Duration of lag-phase [h]**	
TVC	0.024	0.035	98.09	**89.41**
Brochothrix	0.060	0.090	94.22	**86.13**
Pseudomonas spp.	0.021	0.020	13.90	**17.37**
Enterobacteriaceae	0.019	0.018	28.58	**27.67**
Lactobacillus spp.	**0.012**	**0.012**	**84.25**	**102.99**

Table 5.2 Comparison of argon and nitrogen atmospheres on the growth of spoilage bacteria (level of significance $p < 0.05$).

Ar / N_2 concentration	15% Ar/15% N_2	25% Ar/25% N_2	82% Ar/82% N_2
Microorganism	**Significance**		
TVC	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

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., Enterobacteriaecea and *B. thermosphacta* become dominant with *B. thermosphacta* being the predominant microorganism after approx. 210h (Figure 5.1). These results agree with other studies, where *B. thermosphacta* is also associated with spoilage under MAP-conditions (Nychas & Drosinos, 2000; Borch et al., 1996; Pin et al., 2002). The number of *Lactobacilli* spp. remains relatively constant throughout the entire storage period (Table 5.1) 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 favoured by anaerobic conditions or/and high amounts of CO_2 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 5.2). The comparison of growth revealed that growth of *Lactobacilli* spp. was constant during storage, while *B. thermosphacta* becomes 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 5.1). However, the maximum number of Enterobacteriaceae at the end of storage (25% Ar/N_2) is approximately 1 log level higher in comparison to the 15% Ar/N_2-atmosphere. This is presumably due to the fact that 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 shows a stable growth, even though these microorganisms are aerobic. Clark & 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 & 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 days of storage.

Ar/CO_2-mixture was the most effective in delaying the growth of *B. thermosphacta* (Table 5.1: Ar: 0.060 h^{-1} / N_2: 0.090 h^{-1}), but not significant (Table 5.2). Similar results were reported in a study by Fraquezza & Barreto (2009) for the growth of *B. thermosphacta* during the storage of turkey meat under a 50% Ar – 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. But 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 Quality Index (QI) and meat colour during storage under different argon and nitrogen treatments is illustrated in Figure 5.2. The Quality Index (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 Quality Index or on surface meat colour. Comparing the Quality Index under 15% argon with 15% nitrogen concentrations in the mixture, samples stored under nitrogen atmosphere achieve the Quality Index (QI = 2.5) approximately 100 hours earlier than samples stored under argon atmosphere. The differences in Quality Index development between 15% Ar/N_2-atmospheres were mainly based on the evaluation of meat colour. The results of the sensory colour evaluation showed a significant difference ($p < 0.05$) throughout the storage period after packaging under a 15% argon-containing atmosphere in comparison to 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 non-covalent van der Waals forces and build up a complex with myoglobin. This effect could be an explanation for the beneficial colour evaluation of samples stored under 15% argon. Parra et al. (2010) also found a positive effect on colour development of Iberian ham. Samples packed under 70% Ar/30% CO_2 showed higher a-values after 60 days 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 colour of poultry is additionally influenced by several factors such as age, sex, meat moisture content, pre-slaughter 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 colour 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.

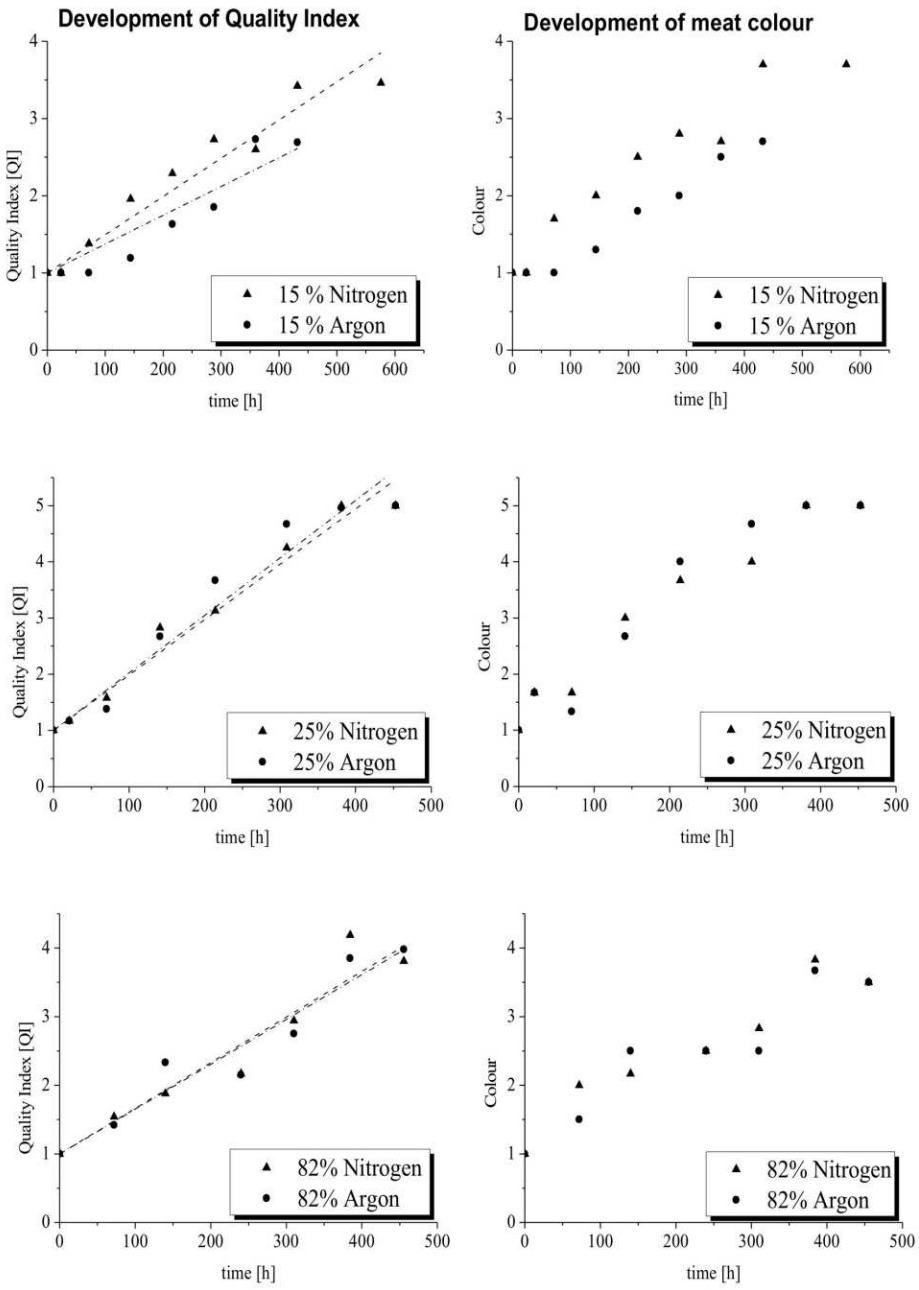

Figure 5.2 Development of quality index and poultry meat color under different argon and nitrogen concentrations, n=3**.**

Development of gas composition

During the first 24 hours after MAP, CO_2 concentration shows a small decline in all packages (data not shown). This is due to the high solubility of CO_2 to lipid and water on meat surface (Betts, 1995; Parra et al., 2010). The maximum CO_2-decline (5%) was in 82% argon-containing packages while 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 like 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 & McDowell, 2003; Yam et al., 2005; Esmer et al., 2011).

Development of pH value

Broiler breast pH at 24h post mortem varied between 5.7 and 6.2 (data not shown), which is also described by Lund & 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; Martinez 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 & Bertelsen (2005) pointed out that CO_2, in case of 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.

5.5 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-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 to the nitrogen atmosphere. Storing the samples under a 15% argon-enriched atmosphere stabilized the light pink colour of parts of the poultry fillets samples. In this context, it has to be considered that the colour 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 discolouration, which leads to huge economic loss. However, it has to be taken into account that animal specific factors, which are not yet known, have an additional influence on colour particularly after slaughtering 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 on colour, 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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5.6 References

Amanatidou, A. 2001. High oxygen as an additional factor in food preservation. Ph.D. Thesis, Wageningen University, The Netherlands.

Betts, G. 1995. The microbiological consequences of MAP and vacuum packaging. International Conference on Modified Atmosphere Packaging and Related Technologies. Campden & Chorleywood Research Association, Chipping Campden, UK.

Bremner, A. 1985. A convenient easy-to-use system for estimating the quality of chilled seafood. *Fish Processing Bulletin*, 7:59-70

Borch, E., M. L. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and meat products. *International Journal of Food Microbiology*, 33(1):103-120.

Clark, D. S., and T. Burki. 1972. Oxygen requirements of strains of *Pseudomonads* and *Achromobacter*. *Canadian Journal of Microbiology*, 18:321-326.

Curiel, J. A., C. Ruiz-Capillas, B. de las Rivas, A. V. Carrascosa, F. Jiménez-Colmenero, and R. Munoz. 2011. Production of biogenic amines by lactic acid bacteria and erterobacteria isolated from fresh pork sausages packaged in different atmospheres kept under refrigeration. *Meat Science*, 88:368-373.

Daniels, J. A., R. Krishnamurth, and S. S. H. Rizvi. 1985. A review of the effect of CO2 on microbial growth and food quality. *Journal of Food Protection*, 48:532-537.

Day, B. P. F. 1995. Commercial and high oxygen MAP of fresh prepared produce. International Conference on Modified Atmosphere Packaging and Related Technologies. Campden & Chorleywood Research Association, Chipping Campden, UK.

Day, B. P. F. 2007. Modified atmosphere packaging (MAP) – A global perspective on new developments. 40th AIFST Convention, Melbourne, 25 June 2007.

Devlieghere, F., J. Debevere, and J. van Impe. 1998. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. *International Journal of Food Microbiology*, 43:105-113.

Esmer, O. K. R. Irkin, N. Degirmenicoglu, and A. Degirmenicoglu. 2011. The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. *Meat Science*, 88:221-226.

EU 1995. Directive No. 95/02/CE. Commission Decision 20/02/1995 on food additives other than colours and sweeteners. Off. J. L61:0001-0040.

Farber, J. M. 1991. Microbiological aspects of modified-atmosphere packaging technology – A review. *Journal of Food Protection*, 54:58-70.

Faustmann, C. 1990. The biochemical basis for discoloration in fresh meat: a review. *Journal of Muscle Foods*, 1:217-243.

Floros, J. D., K. I. Matsos. 2005. Introduction to modified atmosphere packaging, Pages 151-171 in Innovations in food packaging, Han, J. H., ed. Food Science and Technology, International Series.

Fraqueza, M. J., A. S. Barreto. 2009. The effect on turkey meat shelf life of modified-atmosphere packaging with an argon mixture. *Poultry Science*, 88:1901-1998.

Gibson, A., N. Bratchell, and T. Roberts. 1987. The effect of sodium chloride and temperature on rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *Journal of Applied Bacteriology*, 62, 479–490.

Giménez, B., P. Roncalés, and J. A. Beltrán. 2002. Modified atmosphere packaging of filleted rainbow trout. *Journal of Science and Food Agriculture*, 82:1154-1159.

Greenwood, N. N., and A. Earnshaw. 1998. Chemistry of the Elements. 2nd Edition, p 891.

Hechelmann, H. 1981. Vorkommen und Bedeutung von *Brochothrix thermosphacta* bei Kühllagerung von Fleisch und Fleischerzeugnissen. Mitteilungsblatt der Bundesanstalt für Fleischforschung, Kulmbach, 4435-4437.

Jacxsens, L., F. Devlieghere, C. Van der Steen, and J. Debevere. 2001. Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71:197-210.

Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Modern Food Microbiology. 7th Edition, Springer, New York, London, Berlin.

Jakobsen, M., and G. Bertelsen. 2005. Solubility of carbon dioxide in fat and muscle tissue. *Journal of Muscle Foods*, 17:9-19.

Jamie, P., and M. E. Saltveit. 2002. Postharvest changes in broccoli and lettuce during storage in argon, helium, and nitrogen atmospheres containing 2% oxygen. *Postharvest Biology and Technology*, 26, 113-116.

Kreyenschmidt, J. 2003. Modellierung des Frischeverlustes von Fleisch sowie des Entfärbeprozesses von Temperatur-Zeit- Integratoren zur Festlegung von Anforderungsprofilen für die produktbegleitende Temperaturüberwachung. PhD thesis,

Rheinische Friedrich-Wilhelms-Universität Bonn. Bergen/Dumme, Germany: AgriMedia GmbH.

Lund, B. M., and T. Eklund. 2000. Control of pH and use of Organic Acids. In The Microbiological Safety and Quality of Food, ed. B. M. Lund, T. C. Baird-Parker, and G. W. Gould, 175-199. Maryland: Aspen Publishers, Inc. Gaithersburg.

Martinez, L., D. Djenane, I. Cilla, J. A. Beltrán, and P. Roncalés. 2005. Effect of different concentrations of carbon dioxide and low concentrations of carbon monoxide on the shelf-life of fresh pork sausages packaged in modified atmosphere. *Meat Science*, 71:563-570.

Mullan, M., D. McDowell. 2003. Modified atmosphere packaging. In: R. Coles, D. McDowell, M. J. Kirwan (Eds.): Food Packaging Techology, Blackwell Publishing, London, pp. 303-339.

Nychas G. E., and E.H. Drosinos. 2000. Meat and Poultry – Spoilage of Meat, Pages 1253-1284 in Encyclopedia of Food Microbiology, 2nd Edition. R. K Robinson, C.A. Batt, and P.D. Patel, ed. Academic Press, London, UK.

O`Grady, M. N., F. J. Monahan, R. M. Burke, and P. Allen. 2000. The effect of oxygen level and exogenous α-tocopherol on the oxidative stability of minced beef in modified atmosphere packs. *Meat Science*, 55:39-45.

O`Beirne, D., E. Murphy, and D. N. Eidhin. 2011. Effects of argon enriched low-oxygen atmospheres and of high-oxygen atmospheres on the kinetics of polyphenoloxidase (PPO). *Journal of Food Science*, 76(1):73-77.

Parra, V., J. Viguera, J. Sanchez, J. Peinado, F. Esparrago, J. I. Gutierrez, and A. I. Andrés. 2010. Modified atmosphere packaging and vacuum packaging for long period chilled storage of dry-cured Iberian ham. *Meat Science*, 84:760-768.

Pin, C., G. D. Garcia de Fernanado, and J. A. Ordonez. 2002. Effect of modified atmosphere composition on the metabolism of glucose by *Brochothrix thermosphacta*. *Applied Environmental Microbiology*, 68:4441-4447.

Prangé, T., M. Schiltz, L. Pernot, N. Colloc`h, S. Longhi, W. Bourguet, and R. Fourme. 1998. Exploring hydrophobic sites in proteins with xenon and krypton. *Proteins*, 30:61-73.

Rao, D. N., and N. M. Sachindra. 2002. Modified atmosphere and vacuum packaging of meat and poultry products. *Food Reviews International*, 18 (4):263-293.

Rocculi, P., S. Romani, and M. D. Rosa. 2005. Effect of MAP with argon and nitrous oxide on quality maintenance of minimally processed kiwifruit. *Postharvest Biology and Technology,* 35:319-328.

Rotabakk, B. T., S. Birkeland, W. K. Jeksrud, and M. Sivertsvik. 2006. Effect of modified atmosphere packaging and soluble gas stabilization on the shelf life of skinless chicken breast fillets. *Journal of Food Science*, 71(2):124-131.

Ruiz-Capillas, C., and F. Jiménez-Colmenero. 2010. Effect of an argon-containing packaging atmosphere on the quality of fresh pork sausages during refrigerated storage. *Food Control*, 21:1331-1337.

Spencer, K. C. 1995. The use of argon and other noble gases for the MAP of foods. International Conference on Modified Atmosphere Packaging and Related Technologies. Campden & Chorleywood Research Association, Chipping Campden, UK.

Spencer. K. C. 2002. Method of preservation foods using noble gases. US patent number 6,342,261 B1. American Air Liquide.

Spencer, K. C. 2005. Modified atmosphere packaging of ready-to-eat foods, Pages 185-203 in Innovations in food packaging, Han, J. H., ed. Food Science and Technology, International Series.

Tomankova, J., G. Borilova, I. Steinhauserova, and L. G. Las (2012). Volatile Organic Compounds as Biomarkers of the Freshness of Poultry Meat Packaged in a Modified Atmosphere. *Czech Journal of Food Science*, 30(5):395-403.

Totosaus, A., M. L. Pérez-Chabela, and I. Guerrero. 2007. Color of fresh and frozen poultry, Pages 455-465 in Handbook of meat, poultry & seafood quality, 1st Edition. L. M. L. Nollet, ed. Blackwell Publishing, Oxford, UK.

Wu, Z. S., M. Zhang, and S. Wang. 2012. Effects of high pressure argon treatments on the quality of fresh-cut apples at cold storage. *Food Control*, 23(1):120-127.

Yam, K. L., P. T. Takhistov, and J. Miltz. 2005. Intelligent packaging: Concepts and Applications. *Journal of Food Science*, 70(1):R1-R10.

Zhang, D., P. C. Quantick, J. M. Grigor, R. Wiktorowicz, and J. Irven. 2001. A comparative study of effects of nitrogen and argon on tyrosinase and malic dehydrogenase activities. *Food Chemistry*, 72:45-49.

Zhang, M., Z. G. Zhan, s. J. Wang, and J. M. Tang. 2008. Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases. *LWT-Food Science and Technology*, 14:686.

6. Development of an overall quality index for modified atmosphere packaged (MAP) poultry fillets based on sensory and microbiological parameter

6.1 Abstract

The objective of the present study was to determine and to compare the freshness loss of poultry fillets stored under different conditions to develop an Overall Quality Index (OQI) for MAP poultry. The different storage conditions are described in detail in the previous chapters: (A) Different storage temperatures (chapter 2), (B) Different oxygen concentrations (chapter 3), (C) High oxygen and oxygen free atmospheres inoculated with *Listeria monocytogenes* (chapter 4), (D): Different argon concentrations (chapter 5). Quality changes of fresh poultry fillets were monitored by microbiological and sensory evaluation. For the microbiological evaluation, typical spoilage microorganisms (*Pseudomonas* spp., *Brochrothrix thermosphacta*, Enterobacteriaceae, Lactic acid bacteria) and Total viable count were investigated. The sensory evaluation was performed according to deteriorative changes of the attributes color, odor, texture, drip loss and general appearance. Afterwards, the microbiological and sensory data were fitted by using the Gompertz function. Based on selected fit parameter an Overall Quality Index was developed. The results of the study highlighted that the developed Overall Quality Index can be used to assess and compare the influence of different parameters on the spoilage rate of poultry. This allows a more standardized comparison of the effect of different environmental parameter on the freshness loss. Low storage temperature (<4°C) and the gas atmospheres containing 60% O_2/25% CO_2/15% Ar or 15% N_2 showed the highest retardation of the quality loss compared to the commonly used gas mixture for fresh poultry fillets in Germany (70% O_2/30% CO_2 at 4°C).

6.2 Introduction

Nowadays, the consumer preference in meat consumption changed regarding the increased consumption of white meat like chicken and turkey due to the healthy nutritional profile, which resulted in a significantly increase of the poultry meat production during the last decades (Balamatsia et al. 2006). At the same time, the trend of self-service merchandising of fresh meat became important to cover the increased demand for fresh poultry meat and to ensure a good quality and a prolonged shelf life simultaneously. Therefore, the packaging of fresh poultry meat under modified atmospheres is steadily increasing as a preservation technique for the poultry meat industry.

In food supply chains, the quality and shelf life of food products can vary due to the exposure to dynamic influence factors, leading to unexpected microbiological growth and deteriorative sensory changes. The main extrinsic influence factors to extend the shelf life and to maintain the quality status of fresh meat is the variation of the packaging atmosphere and the continuous storage under chilled temperatures (Church & Parsons 1995). The commonly used packaging gases are CO_2, O_2, and N_2, which have different influences on the quality loss and shelf life. The main effect of CO_2 is the growth inhibition of microorganisms. The optimal concentration of CO_2 is in the range of 20-30% (Stiles 1991, Sivertsvik et al. 2002). According to Jakobsen & Bertelsen (2002), atmospheres with higher CO_2 concentrations can act as pro-oxidant and can lead to an unpleasant color and odor of the meat. Therefore, changes in the color are not only related to spoiled meat (USDA 2011). O_2 is responsible to maintain the red color of the meat (Farber 1991, Phillips 1996) and to inhibit particular microorganisms due to their oxygen requirements (absence of oxygen) or oxygen radical formation, when high concentrations (>60%) occur (Amanatidou et al. 1999, Imlay 2003). Consequently, both gases have an effect on the microbial growth as well as on sensory changes. The effect is dependent on the gas mixture and the meat product itself, as shown in Chapter 3 and 4. Besides the two mentioned gases, also the inert gas nitrogen is used in gas packs. Because of the inert character, nitrogen has no direct effect on the meat quality or spoilage process, but it is necessary to avoid a pseudo-vacuum (Floros & Matsos, 2005). In this context, also the application of argon as an alternative to nitrogen is discussed because of several potential beneficial effects (Spencer 2005). Therefore, also alternative gases can have an effect on the shelf life and sensory attributes, where the focus was drawn in Chapter 5. But also variations of the storage temperature occur in real meat supply chains (Raab et al. 2008). In MAP systems, temperature interruptions may cause a decrease of the gas functions and result in an increased growth of the typical spoilage microflora and on the

sensory appearance, as shown in Chapter 2. The effect of CO_2 for example is reduced, when high temperatures occur due to the decreased solubility (Gill 1988).
Consequently, it is evident that the extrinsic influence factors gas atmosphere and temperature are strongly influencing each other. This means, several studies are available focusing on one influence factor like temperature or the variation of the gas atmosphere. However, studies combining different environmental influence factors on product shelf life and quality loss are still missing. This makes a reliable and precise assessment and comparison between different gas mixtures for poultry exceptionally challenging. Therefore, the objective of the present study is to evaluate different gas mixtures and the influence of the storage temperature on MAP poultry meat on the basis of the results obtained in Chapter 2-5, to develop an Overall Quality Index by combining both, microbiological and sensory parameter. An Overall Quality Index built up the basis for a reliable assessment of different gas mixtures for the poultry meat industry.

6.3 Materials & Methods

Preparation of meat samples and packaging conditions

42-days-old-unsexed broiler chickens (Ross 308/708) were used as test samples. The chickens were slaughtered and air-chilled in a poultry processing plant in Germany. Afterwards, 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 hours after slaughtering. In the laboratory, the double-breast fillets were divided into single fillets using a sterile scalpel.

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 2:1. The different packaging scenarios are shown in Table 6.1. Thereafter, the trays were heat-sealed with a polypropylene foil (Suedpack Verpackungen GmbH & Co. KG, Ochsenhausen, Germany; water vapour permeability < 3.5 g/m^2d 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 four-component gas blender machine (KM 60-4 MEM SO, Witt Gasetechnik, Witten, Germany). The packaged meat samples were stored at 4°C in low-temperature high precision incubators (Sanyo model MIR 153, Sanyo Electric Co.,

Ora-Gun, Gumma, Japan). Storage temperature was monitored by data logger (ESCORT JUNIOR Internal Temperature Data Logger, Escort, New Zealand) every 5 minutes. The microbiological, sensory and chemical analyses were conducted at appropriate time intervals. Each measurement was repeated three times.

Table 6.1 Overview about the tested extrinsic influence factors on MAP poultry.

Scenarios	Research trials	Gas concentrations				Storage temperatures
		O_2	CO_2	N_2	Ar	
A	Influence of storage temperature	70%	30%			**2, 4, 10, 15°C**
B	Influence of oxygen concentration	45% 60% 70% 90%	30% 25% 30%	25% 15% 10%		**4°C**
C	High oxygen and oxygen free atmospheres inoculated with *Listeria monocytogenes*	70%	30% 30%	70%		**4°C**
D	Different argon concentrations	60% 60% 45% 45%	25% 25% 30% 30% 18% 18%	15% 25% 82%	15% 25% 82%	**4°C**

Microbiological analyses

After opening the packages, a representative amount (25g) of meat surface sample in the size of 4 x 7 x 0.5cm was aseptically taken using a sterile scalpel. The sample was transferred to a filtered sterile stomacher bag and filled with 225ml saline peptone diluent (0.85% NaCl with 0.1% peptone Saline-Tablets, Oxoid BR0053G, Cambridge, United Kingdom). Test pieces were homogenised with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60s. Ten-fold dilutions of the homogenate were prepared in saline peptone diluents and

Total Viable Count (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, Enterobacteroaceae and *Lactobacillus* spp. were enumerated.

Total Viable Count was determined by pour plate technique on Plate Count Agar (PCA, Merck, Darmstadt, Germany) and plates were incubated at 30°C for 72 hours. *Pseudomonas* spp. were detected by spread plate technique on Pseudomonas Agar with Cetrimide-Fucidin-Cephalosporin selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 hours. *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 hours. Enterobacteriaceae were identified by overlay treatment on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany) by incubation of the agar plates at 30°C for 48 hours. *Lactobacillus* spp. were detected by pour plate technique on de Man, Rogosa, Sharpe Agar (MRS, Oxoid, Cambridge, United Kingdom). Plates were incubated aerobically at 37°C for 72 hours. The determination of *Listeria monocytogenes* (ATCC 19111) was performed on Listeria Agar (ALOA, Bio Mérieux, Paris, France). Plates were incubated at 37°C for 24h. During storage, the presence of *L. monocytogenes* was tested on samples without inoculation treatment. The pathogenic bacterium was not detected. Counts of colony forming units were expressed as $\log_{10}$cfu/g for each medium and sample.

Sensory evaluation

Sensory analyses were carried out by trained sensory panellists. All assessors were recruited from the Institute of Animal Science (University of Bonn) and experienced in poultry evaluation. A picture of fresh chicken breast fillets was used as reference during the sensory evaluations.

During the trials, each sample was evaluated directly after opening the tray, using a developed sensory scheme according to the Quality Index Method (QIM) for fish evaluation (Bremner, 1985). Attributes were defined as general appearance (G), colour (C), odour (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 quality index (QI) was calculated by the following equation (Kreyenschmidt, 2003):

$$QI = \frac{2G + 2C + 1T + 1D + 2O}{8} \qquad (1.1)$$

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

Selection of relevant parameter for the development of the Overall Quality Index

Quality has an objective and a subjective dimension (Grunert 2005). For the development of an Overall Quality Index, sensory attributes (subjective) and microbiological as well as safety attributes (objective) were selected, as listed in Figure 6.1. The selection of the sensory attributes is based on the sensory parameters, evaluated during the storage trials under various gas mixtures.

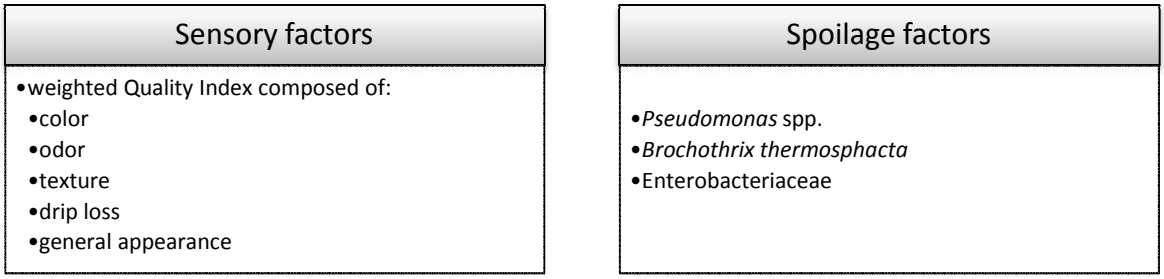

Figure 6.1 Selection of relevant parameters for the development of an Overall Quality Index.

As relevant microorganisms for the calculation of an Overall Quality Index, *Pseudomonas* spp., *B. thermosphacta* and Enterobacteriaceae were chosen according to the results of Chapter 2-4. Lactic acid bacteria played a minor role during spoilage and were not taken into account. *L. monocytogenes* was chosen exemplarily as pathogenic bacteria to simulate the decision when microbiological hazards occur.

The Gompertz equation was used to model the development of the sensory Quality Index and the growth of the Enterobacteriaceae, *Pseudomonas* spp., *B. thermosphacta* and 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 growth data were fitted using the statistical software program Origin 8.0G (OriginLab Corporation, Northampton, Ma., U.S.A.).

The development of an Overall Quality Index is based on the parameter M, obtained from the Gompertz equation. The parameter M is the time point, at which the maximum growth rate is obtained (reversal point). According to the calculated time, the parameter allows a standardized comparison of the growth rate of each microorganism and the sensory decay and therefore a comparison between the influence of different environmental influence factors like temperature and gas mixtures.
The obtained parameter M (reversal point) of the relevant microorganisms responsible for the spoilage process was averaged ($M_{(Microflora)}$) according to the following equation:

$$M_{(Microflora)} = \frac{M(P) + M(B) + M(E)}{3} \qquad (1.3)$$

With: M (P) = time at which the maximum growth rate is obtained for *Pseudomonas* spp. [h], M (B): time at which the maximum growth rate is obtained for *B. thermosphacta*, M (E): time at which the maximum growth rate is obtained for Enterobacteriaceae.

A mean value was calculated of $M_{(Microflora)}$ and $M_{(Sensory)}$ and resulted in a combined Overall Quality Index (M) to compare the gas mixtures in each scenario with each other.

$$OQI(M) = \frac{M_{(Microflora)} + M_{(sensory)}}{2} \qquad (1.4)$$

The Overall Quality Index (M) was determined for each trial and compared to gather information about the rate of the quality loss under different atmospheres and temperatures. The commonly used gas mixture used by the German poultry industry with 30% CO_2/70% O_2 was defined as the reference gas mixture with a calculated Overall Quality Index (M) of 222h. The assessment of the tested gas mixture and storage conditions was made according to the following assessment scheme:

Table 6.2 Assessment scheme for the freshness loss of MAP poultry fillets based on the Overall Quality Index (M).

OQI_M (h)	**Description**
222	Reference gas mixture
< 222	Accelerated growth
> 222	Retarded growth

6.4 Results

Figure 6.2 shows the development of $M_{(Microflora)}$, the development of $M_{(sensory)}$ and the development of the calculated OQI(M) as a function of the storage temperature. The time at which the maximum growth rate is obtained (M, reversal point) increases with increasing temperatures. The comparison of the fits shows a good exponential description of the temperature dependency, which enables the parameter M as an indicator variable for the development of an Overall Quality Index (M).

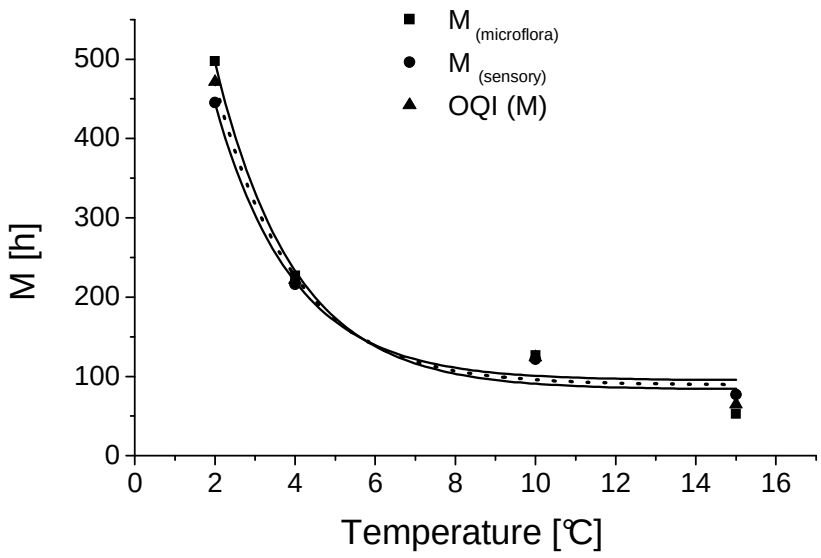

Figure 6.2 Development of $M_{(Microflora)}$, $M_{(Sensory)}$ and the calculated OQI (M) as a function of the used storage temperature.

Figure 6.3 shows the development of $M_{(Microflora)}$, the development of $M_{(sensory)}$ and the development of the calculated OQI (M) as a function of the oxygen concentration inside the package. The comparison of the fits shows a good polynomial description of the data as a function of the oxygen concentration. The correspondent fits also enable the parameter M as an indicator variable for the development of the Overall Quality Index (M) as a function of the used gas O_2/CO_2 mixture.

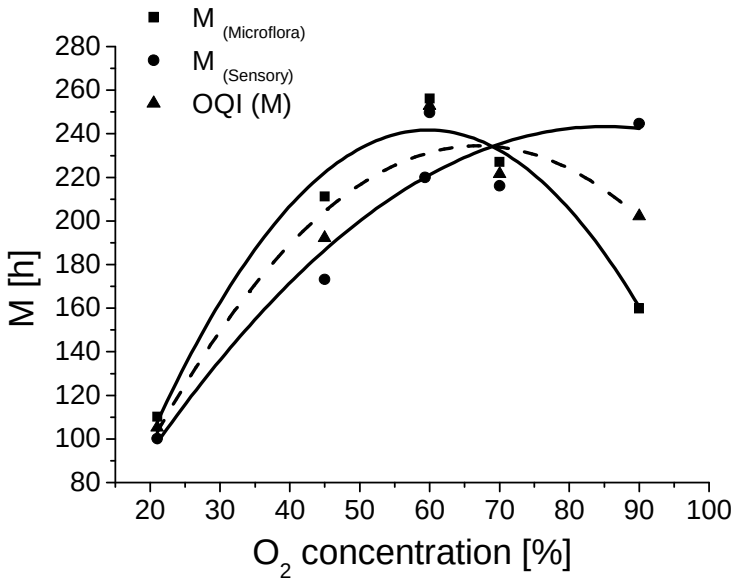

Figure 6.3 Development of $M_{(Microflora)}$, $M_{(Sensory)}$ and the calculated OQI (M) as a function of the used oxygen concentration.

Assessment of different gas mixtures and storage conditions based on OQI (M)

Table 6.3 gives an overview about the calculated Overall Quality Index (M) for each trial. The commonly used gas mixture for poultry (30% CO_2/70% O_2 at 4°C) is taken as reference.
Within scenario A, the Overall Quality Index (M) enables the standardized comparison of the growth acceleration as a function of temperature during storage of MAP poultry (70% O_2/30% CO_2). The storage of the samples at 2°C leads to a decrease of the bacterial growth and the time, where the microbial and sensory reversal point is reached at approximately 250h. With increasing temperatures, also the OQI (M) increases and therefore the spoilage process is accelerated.

Further on, the Overall Quality Index (M) allows also the standardized comparison of the growth acceleration as a function of changing oxygen concentrations. Comparing the different oxygen concentrations with each other, aerobic packaging and the use of 45% O_2 or 90% O_2 leads to an increase in microbial growth compared to the reference gas mixture, which is reflected in a lower OQI(M). The effect on the quality loss under 45% (OQI(M) = 192h) and 90% (OQI(M) = 222h) oxygen is comparable. Therefore, the Overall Quality Index (M) considers also over- and underestimations of the quality loss by combining microbiological changes and sensory deteriorations. As shown in Chapter 3, the oxygen concentration has a strong influence on the sensory evaluation. For example, 90% O_2 resulted in a prolonged sensory shelf life, whereas the microbiological spoilage process was

comparable to aerobic storage. These findings emphasise, that the color of the meat can lead to an overestimation of the real shelf life of the product. In contrast, 45% O_2 resulted in the lowest sensory shelf life, whereas the microbiological growth was more delayed compared to 90% O_2, which resulted in an underestimated of the real shelf life. Comparing the OQI(M) of the used atmospheres with the reference mixture, merely the implementation of 60% O_2 in a gas mixture leads to a retarded quality loss. The calculated OQI(M) is retarded by approximately 31h.

Comparing the calculated OQI(M) of the used high oxygen and oxygen free atmospheres with each other, the calculated OQI(M) is in a similar range with 18h difference between both gas mixtures (N_2: 204h and O_2: 222h). Therefore, nitrogen or oxygen has the same effect on the development of microbiological and sensory parameters. But it has to be taken into account that the oxygen free atmosphere favoured the growth of *Listeria monocytogenes*, as shown in Chapter 4.

The comparison between argon and nitrogen in a gas mixture was tested in scenario D. The calculated Overall Quality Index (M) reflects nearly no differences between the used gas mixtures. Therefore, the results emphasise that argon has no additional beneficial effect in comparison to nitrogen as also shown in chapter 5 (Table 6.3). The beneficial effect on meat colour in parts of the samples by using 15% Ar is realtivized by the OQI(M) and allows an objective comparison of the used mixtures. The comparison of the used atmospheres with the reference mixture by using the OQI(M) shows, that 15% Ar or 15% N_2 as well as 82% Ar or 82% N_2 in a gas mixture lead to a decrease of the quality loss. The use of 15% Ar/15%N_2 results in a prolongation of 31h; the OQI(M) of 82% Ar or 82% N_2 in a mixture retards the quality loss of 10h and 21h, respectively. In contrast, 25% Ar or 25% N_2 in a gas mixture lead to an accelerated quality loss between 11h and 22h, respectively (Table 6.3).

Table 6.3 Calculated Overall Quality Indices (M) of microbiological and sensory evaluation of MAP poultry fillets (Gompertz function).

Scenario	Temp. (°C)	Gas atmosphere	Sensory evaluation$_M$ (h)	Microbiological evaluation$_M$ (h)	Overall Quality Index$_M$ (h)
A	2	70% O_2 / 30% CO_2	445	498	472
	4		216	227	222
	10		121	127	124
	15		77	53	65
B	4	aerob	100	110	105
	4	45% O_2/25% N_2/30% CO_2	173	211	192
	4	60% O_2/15% N_2/25% CO_2	250	256	253
	4	70% O_2/30% CO_2	216	227	222
	4	90% O_2/10% N_2	245	160	203
C	4	70% N_2/30% CO_2*	186	221	204
	4	70% O_2/30% CO_2	216	227	222
D	4	15% Ar/60% O_2/25% CO_2	277	229	253
	4	15% N_2/ 60% O_2/25% CO_2	250	256	253
	4	25% Ar/45% O_2/30% CO_2	167	200	184
	4	25% N_2/45% O_2/30% CO_2	173	211	192
	4	82% Ar/18% CO_2	229	234	232
	4	82% N_2/18% CO_2	242	243	243

*the gas mixture supports the growth of *Listeria monocytogenes*

Figure 6.4 gives an overview about the calculated OQI_M[h] for each tested scenario. For the assessment of the different used gas mixtures, the commonly used gas atmosphere for poultry fillet was used as a reference mixture (30% CO_2/ 70% O_2) during storage at 4°C. The grey line represents the OQI(M) of the reference gas mixture (222h). Thereafter, the OQI(M) above the line can be assessed as appropriate mixtures compared to the reference.

Scenarios with an OQI(M) lower then 222h can be assessed as mixtures causing an acceleration of the quality loss and can be rejected compared to the reference mixture. Figure 6.4 points out that decreasing storage temperatures have the main effect in delaying the quality loss of poultry meat, compared to the variation of the gas mixture. Regarding the different gas mixtures, the use of low oxygen concentrations (21% O_2, aerobic storage) has a similar effect on the quality loss then increasing the storage temperature. The OQI(M) for aerobic storage (105h) is in the range between 10°C (123h) and 15°C (65h).

The variation of the gas mixture 60% O_2 with 25% CO_2 and 15% N_2 shows the best effect on the decrease of the quality loss compared to the reference mixture. This mixture is also comparable to the 15% Ar containing atmosphere and can be assessed as equal. Also the use of 82% Ar or 82% N_2 resulted in a enhanced OQI(M) compared to the reference. Merely the use of 45% O_2 in a mixture with 30% CO_2 and 25% N_2 or 15% Ar and the use of 90% O_2/10% N_2 show a lower OQI(M) compared with the reference mixture and can be rejected as an appropriate mixture for MAP poultry fillets.

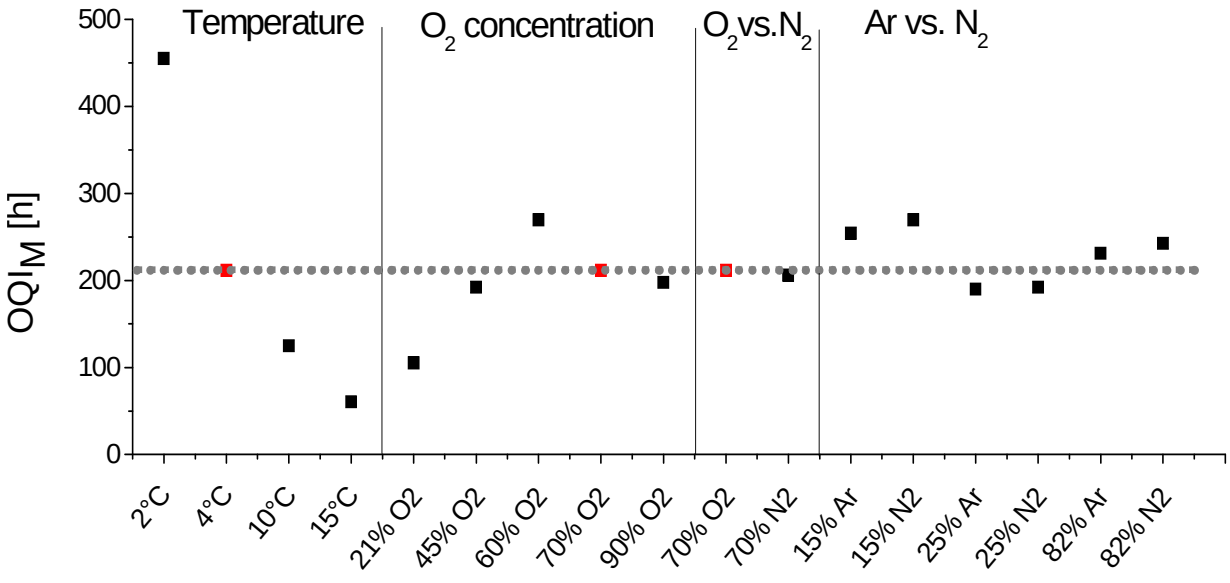

Figure 6.4 Illustration of the calculated OQI(M) of the tested gas mixtures and storage temperatures for MAP poultry meat.

6.5 Conclusion

In this study a new Overall Quality Index was developed for MAP poultry fillets, which combines microbial and sensory parameters with each other. Based on the results of chapter 2-4, the influence of all tested environmental parameters was compared and the index allows a reliable assessment of the tested scenarios in comparison to the commonly used mixture for poultry fillets in Germany (30% CO_2/70% O_2 at 4°C). For the development of an Overall Quality Index, the parameter M (time, at which the maximum growth rate is obtained) of the Gompertz function (sensory and microbial curves) was chosen as a suitable parameters to compare the influence of the tested environmental influence factors with each other.

It became evident that the increase of temperature and the decrease of oxygen under 55% has the main effect on the quality preservation whereas 60% O_2/25% CO_2/15% Ar or 15% N_2 showed the highest retardation of the quality loss. Therefore, the use of the mentioned gas mixtures can be assessed as the appropriate gas mixture for MAP poultry fillets within all tested scenarios. A combination of the mixture with low storage temperatures <4°C may lead to an additional beneficial effect on the quality loss of MAP poultry.

In conclusion, the Overall Quality Index can support the decision making process of companies about the implementation of gas mixtures. Further on, the Index can be applicable for a reliable and objective assessment of the influence of different environmental influence factors like temperature and gas atmospheres also for other kind of perishable products, e.g. pork, beef and fish.

6.6 References

Amanatidou, A., E. Smid, L. Gorris (1999). Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated micro-organisms. *Journal of Applied Microbiology*, 86:429-438.

Balamatsia, C. C., E. K. Paloelogos, M. G. Kontominas, I. N. Savvaidis. 2006. Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4°C: possible role of biogenic amines as spoilage indicators. *Antonie van Leeuwenhoek*, 89:9-17.

Bremner, A. 1985. A convenient easy-to-use system for estimating the quality of chilled seafood. *Fish Processing Bulletin*, 7:59-70.

Church, I. J., and A. L. Parsons. 1995. Modified atmosphere packaging technology: A review. *Journal of the Science of Food and Agriculture*, 67:143-152.

Farber, J. M. (1991). Microbial Aspects of modified-atmosphere packaging technology – A review. *Journal of Food Protection*, 54(1):58-70.

Floros, J. D., K. I. Matsos. 2005. Introduction to modified atmosphere packaging, Pages 151-171 in Innovations in food packaging, Han, J. H., ed. Food Science and Technology, International Series.

Galgano, F., F. Favati, M. Bonadio, V. Lorusso, P. Romano. 2009. Role of biogenic amines as index of freshness in beef meat packed with different biopolymeric materials. *Food Research International*, 42:1147-1152.

Genigeorgis, C. A. 1985. Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. *International Journal of Food Microbiology,* 1:237-251.

Gibson, A., N. Bratchell, and T. Roberts. 1987. The effect of sodium chloride and temperature on rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *Journal of Applied Bacteriology*, 62, 479–490.

Gill, C. O. 1988. The solubility of carbon dioxide in meat. Meat Sci. 22:65-71.

Hechelmann, H. 1981. Vorkommen und Bedeutung von *Brochothrix thermosphacta* bei Kühllagerung von Fleisch und Fleischerzeugnissen. Mitteilungsblatt der Bundesanstalt für Fleischforschung, Kulmbach, 4435-4437.

Imlay, J. A. 2003. Pathways of oxidative damage. *Annual Review of Microbiology*, 57:395-418.

Jakobsen, M., and G. Bertelsen. 2002. The use of CO_2 in packaging of fresh red meat and its effect on chemical quality changes in the meat: a review. *Journal of Muscle Foods*, 13:143-168.

Kreyenschmidt, J. 2003. Modellierung des Frischeverlustes von Fleisch sowie des Entfärbeprozesses von Temperatur-Zeit- Integratoren zur Festlegung von Anforderungsprofilen für die produktbegleitende Temperaturüberwachung. PhD thesis, Rheinische Friedrich-Wilhelms-Universität Bonn. Bergen/Dumme, Germany: AgriMedia GmbH.

Phillips, C. A. 1996. Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science & Technology,* 31:463–479.

Sivertsvik, M., W. K. Jeksrud, and T. Rosnes. 2002. A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. Int. J. Food Sci. Tech. 37:107-127.

Stiles, M. E. 1991. Scientific principles of controlled/modified atmosphere packaging. In: Ooraikul, M. & M. E. Stiles (Edit.), *Modified Atmosphere Packaging of Food*. Pp 18-25, Ellis Horwood Ltd,.Chichester, UK.

Spencer, K. C. 2005. Modified atmosphere packaging of ready-to-eat foods, Pages 185-203 in Innovations in food packaging, Han, J. H., ed. Food Science and Technology, International Series.

USDA (United States Department of Agriculture). 2011. Food Safety and Inspection Service. Food Safety Information – The colour of meat and poultry, http://www.fsis.usda.gov/wps/portal/fsis/home.

7. General conclusion

Raw poultry meat is susceptible to microbial spoilage due to its nutritional, physical and chemical properties. Therefore, packaging under modified atmosphere conditions is widely established in order to preserve the quality status and to prolong the shelf life of fresh poultry. Additionally, discussion about reducing the amount of food waste along the meat supply chain creates additional pressure on the meat companies. In this context, the meat industry, in cooperation with gas producers is searching for new gas mixtures to achieve additional beneficial effects on the quality loss of the product. Therefore, the application of alternative gases like argon or xenon, or the variation of the gas atmosphere is discussed more and more. Besides packaging, the temperature conditions in the chains are the most important extrinsic influence factor. Until now, the real influence of different environmental factors on the quality loss of fresh poultry is intensively discussed.

Therefore, the main objective of this thesis was the comparison and assessment of different environmental influence factors on the shelf life and quality loss of modified atmosphere packaged poultry fillets. For this purpose, the following research questions were proposed:

- How are different temperature conditions and different oxygen combinations influencing the composition of the specific spoilage flora, and can a specific spoilage organism be identified to determine the quality loss of poultry fillets?
- How is the growth of specific spoilage bacteria and pathogens influenced by oxygen enriched atmospheres in comparison to nitrogen enriched atmospheres?
- How is the growth of specific spoilage bacteria and the development of sensory parameters influenced by using argon as an alternative to nitrogen?
- Is it possible to develop an overall quality index based on microbiological and sensory parameters to assess the influence of different environmental influence factors on the quality loss of MAP poultry?

The first research question was focused on determining the influence of different temperature conditions on the composition of the spoilage flora and the quality loss of MAP poultry. Samples were packed under the commonly used gas atmosphere for fresh poultry fillets in Germany (70% O_2 and 30% CO_2) and stored under four constant temperature conditions (2, 4, 10, 15°C). Typical spoilage microorganisms were investigated (*Pseudomonas* spp., *Brochothirx thermosphacta*, Enterobacteriaceae, *Lactobacillus* spp.) and the growth was modeled using the Gompertz function. Parallel to the microbiological investigations, each sample was investigated for sensory deteriorative changes, concentration of the

headspace gas atmosphere and the development of the pH-value. On the basis of the microbiological and sensory data, a stepwise regression and a principle component analyses (PCA) were carried out to identify the variables with the highest explanatory power for the data set.

The results showed that the storage temperature has a significant influence on the composition of the spoilage flora and on the length of sensory shelf life under MAP conditions. The storage of poultry meat under low temperature conditions (2°C and 4°C) favored the growth of *Brochothrix thermosphacta*, whereas higher temperatures (10°C and 15°C) supported the growth of *Pseudomonas* spp. Enterobacteriaceae also comprised a substantial part of the spoilage flora and contributed to the loss of quality and shelf life. *Lactobacillus* spp. played merely a minor role in the spoilage process. The results of the stepwise regression and PCA also supported that no single predictor variable could be identified as the main spoilage originator and that the spoilage was induced by the mix of the different spoilage microorganisms.

The identification of one single specific spoilage organism (SSP) for the definition of a common acceptance level for MAP poultry stored under different temperature conditions was not feasible. Significant correlations between TVC and the development of the sensory decay and the number of TVC at the end of sensory shelf life for each single storage temperature were observed. But it has to be taken into account that *Pseudomonas* spp. and *Brochothrix thermosphacta* also play an important part of the spoilage process, which has been shown by the principle component analyses.

In addition to temperature, also changes in the gas atmosphere, which also have a significant influence on the composition of the microflora and the length of shelf life, were also analysed in this thesis. Therefore, the effect of varying oxygen concentrations on the development of microbiological and sensory parameters during storage was investigated for the most appropriate oxygen concentration for MAP poultry fillets. Storage tests were conducted by changing the oxygen concentrations to (MAP 1): 45% O_2 / 25% N_2 / 30% CO_2; (MAP 2): 60% O_2 / 10% N_2 / 20% CO_2; (MAP 3): 70% O_2 / 30% CO_2; (MAP 4): 90% O_2 / 10% N_2 and aerobic storage. Samples were stored at a constant temperature of 4°C and were investigated for the typical spoilage microorganisms for MAP poultry, which were identified in the first study. The effect on sensory parameters was investigated also. Microbiological and sensory data were fitted with the Gompertz function.

The oxygen concentration had a strong influence on the development of typical sensory parameters of the poultry fillets during storage. The results revealed that increasing oxygen

concentrations had a stabilizing effect on the surface meat color and thus an effect on the sensory shelf life. The 45% O_2 atmosphere resulted in reduced color stability in comparison to the higher O_2 concentrations, whereas 90% oxygen stabilized the color but favored the growth of all investigated microorganisms.

The results of the microbiological investigations revealed that the microbiological growth was also strongly influenced by the different oxygen concentrations. For example, under aerobic (21% O_2) conditions, *Pseudomonas* spp. was the dominant species, whereas with increasing (45-70%) oxygen inside the packaging atmosphere, *B. thermosphacta* became the predominant bacteria. It can generally be concluded that aerobic storage and the storage under 90% oxygen favored the growth of all bacteria, which was emphasized by the calculated maximum growth rates of the bacteria using the Gompertz function. Comparing the calculated local minima of the growth curves of different bacteria with each other, 55-60% oxygen in a gas mixture can be assumed to be the optimal concentration to prolong the shelf life and delay the freshness loss.

Regarding the increased risk of lipid oxidation due to the recommended oxygen concentration (55-60%) of this study, poultry fillets are presumably not susceptible to deteriorative sensory changes because poultry refers to white meat with a low fat content. However, the investigation and the comparison of different oxygen atmospheres on the proportion of the lipid oxidation should be continued in further studies.

The stabilizing effect of oxygen on the oxymyoglobin formation of the meat further emphacized the applicability of high oxygen atmospheres in the range of 55-60% for poultry as an alternative to oxygen free atmospheres, even through the myoglobin content of poultry breast fillet is much less compared to beef or pork. In this context, it has to be taken into account that different animal breeds can have an influence on the initial color development and therefore the results should be evaluated in further studies considering different animal specific factors.

The third research question was focused on the comparison between oxygen and nitrogen enriched atmospheres to investigate the influence on the growth of specific spoilage bacteria, pathogens and sensory parameters. Therefore, the focus was laid on the development of the pathogenic bacteria *Listeria monocytogenes* in the presence of typical spoilage microorganisms, whereby possible interactions with the natural spoilage bacteria were also considered. For that purpose, the poultry meat surface was artificially contaminated with *Listeria monocytogenes* and stored under 70% O_2 / 30% CO_2 and 70% N_2 / 30% CO_2 at 4°C.

Regarding the comparison between oxygen and nitrogen in the packaging atmosphere for poultry, the oxygen enriched mixture in combination with CO_2 suppressed the proliferation of *Listeria monocytogenes*. In contrast, the nitrogen enriched atmosphere in combination with CO_2 favored the pathogenic growth. Presumably, the suppression was not solely related to the gas mixture, because *L. monocytogenes* is relatively unaffected by O_2 and has the enzyme superoxide dismutase to protect themselves at high oxygen concentrations. The predominance of *B. thermosphacta* under the high oxygen atmosphere seems to have an effect on the growth inhibition of the pathogenic bacteria.

These results lead to the conclusion that interactions between different microorganisms with pathogenic bacteria on natural products, have to be taken into account as an important influence factor on the shelf life and quality deteriorations of the meat. Most of the analytical studies were carried out in liquid laboratory media and do not consider the influence of the food structure, which can lead to fail safe interpretations of the results. Also further research is needed to obtain growth data under oxygen and nitrogen atmospheres for other pathogenic bacteria like *Staphylococcus aureus* and *Camphylobacter* spp.

Besides different oxygen mixtures, the application of alternative gases is also in discussion to influence the growth of the spoilage flora, which leads to the fourth research question. Besides the commonly used packaging gases (O_2, CO_2, N_2), argon is also permitted in the EU as an alternative to nitrogen for food packaging. In this context, the study was focused on testing different argon enriched atmospheres in comparison to nitrogen enrichments in the equivalent amount. Six different atmospheres were used: (MAP 1) 15% Ar, 60% O_2, 30% CO_2; (MAP 2) 15% N_2, 60% O_2, 30% CO_2; (MAP 3) 25% Ar, 45% O_2, 30% CO_2; (MAP 4) 25% N_2, 45% O_2, 30% CO_2; (MAP 5) 82% Ar; 18% CO_2; (MAP 6) 82% N_2, 18% CO_2) and stored at 4°C. The samples were investigated for the typical spoilage microorganisms and for sensory changes.

The comparison between argon and nitrogen showed that argon had no beneficial effect on the growth of the typical spoilage microorganisms compared to the use of nitrogen. Merely the growth *B. thermosphacta* was reduced by approximately 1-log level at the end of storage using 82% argon and could be neglected. The results indicate that the application of the novel gas argon had no beneficial effect on the quality loss of poultry fillets. Merely the meat color was positively influenced using 15% argon, but the effect was shown only in some of the samples. Animal specific factors can also lead to the stabilising effect, which has to be clarified in further studies.

The final research question aimed at the development of an Overall Quality Index (OQI) for modified atmosphere packaged poultry. Generally, the results obtained in Chapter 2-5

showed, that changing temperature conditions as well as variations of the gas atmosphere have a strong influence on the microbial growth, on the composition of the spoilage flora, and on the sensory decay during storage. The results of the thesis show, that no single parameter could be identified to compare the influence of different environmental influence factors on MAP poultry with each other. No specific spoilage organism for MAP poultry could be identified when environmental influence factors were variable. Hence, a single index which combines microbiological and sensory changes on the basis of complex changes occurring during spoilage has not been available till now. For the development of an Overall Quality Index, the growth of typical spoilage microorganisms (*B. thermosphacta*, *Pseudomonas* spp., Enterobacteriaceae) and sensory parameters was taken into account. The integration of the typical spoilage microorganisms into an index is considered more reliable than using the total viable count to determine the spoilage process and quality loss of MAP poultry fillets. The OQI was developed by using the parameter M of the Gompertz function, which represents the time at which the maximum growth rate is obtained (reversal point). For a reliable and standardized comparison of the scenarios, the commonly used gas mixture for poultry fillets (30% CO_2/70% O_2 at 4°C) was used as reference. Based on this parameter, it was possible to develop a single index which combines microbiological and sensory parameters with each other. As a result, the OQI enables a standardized comparison and assessment of different environmental influence factors on the shelf life and quality loss of modified atmosphere packaged poultry fillets.

The lowest OQI(M) was calculated for the storage of MAP poultry (70% O_2/30% CO_2) at 2°C, which underlines the importance of a stable cold chain in meat supply chains for modified atmosphere packaged poultry also. Comparing the different gas mixtures at a storage temperature of 4°C with each other, the results of the OQI(M) showed that the storage under 60% O_2/25%CO_2/15%N_2 as well as under 60% O_2/25% CO_2/15% Ar showed the highest retardation of the quality loss with an OQI(M) of 253h compared to OQI(M) of 222h of the reference mixture. In conclusion, the use of the mentioned gas mixtures can be assessed as the appropriate gas mixture for MAP poultry fillets within all tested scenarios. Combining the mixtures with reduced storage temperatures <4°C may lead to an additional beneficial effect on the quality loss of MAP poultry.

The overall result of the thesis was the development of a single index, which allows a standardized comparison of the influence of different environmental factors on the quality loss of MAP poultry based on sensory and microbiological parameters. In future, the application of new sensor technologies like Raman Spectroskopie or Hyperspectral Imaging is a promising approach. These sensor technologies will presumably replace the traditional

microbiology within the next decades and will deliver additional information about the quality loss and shelf life of meat dependent on different environmental factors. The data of the present thesis can be used as reference data, which may form the basis for the development of an Overall Quality Index based on real time measurements by new sensor technologies. Furthermore, the integration of the developed assessment scheme in user-friendly software is conceivable, which also allows an improvement of the quality management system in poultry supply chains.

Generally, the developed Index can be used to support the decision making process of meat companies for a reliable and objective comparison between the influence of different environmental parameters on the spoilage process of fresh poultry and thus on the quality loss. The procedure of the development of an Overall Quality Index (M) can also be adapted to different kind of products like pork, beef and fish. Therefore, it is first important to define the relevant microorganisms responsible for the spoilage process and second to define characteristic sensory attributes during storage. The application of the OQI will support the delivering process of high quality products and at the same time the amount of food waste will be reduce.

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List, U., B. Petersen, J. Kreyenschmidt (2010). Effect of argon-enriched atmospheres on the spoilage process of modified atmosphere packaged (MAP) poultry. Oral Presentation. 4th International Cold-Chain-Management Workshop, 27-28th Sept, Bonn.

List, U., D. Mack, A. Nawrath, H. Ostrowski, A. Marquardt, J. Kreyenschmidt (2011). Comparison of argon and nitrogen containing atmospheres on quality parameters and shelf life of fresh meat. Oral Presentation. 1st International Conference on Food and Environment, 21-23th June, New Forest, UK.

List, U., S. Rossaint, J. Kreyenschmidt (2011). Effect of Argon on quality loss of fresh poultry. Poster Presentation. 57th International Congress of Meat Science and Technology, 7-12th August, Gent, Belgium.

List, U., Rossaint, S. & J. Kreyenschmidt (2011): Effect of argon on the quality loss of fresh poultry. In: Proceedings "International Congress of Meat Science and Technology" (ICoMST), 7-12th August, Gent, p 266.

Herbert, U., L. Heres, J. Kreyenschmidt (2012). Vergleich der Qualität von SB-Hackfleisch unterschiedlicher Hersteller. *Fleischwirtschaft*, 4:95-100.

Herbert, U. (2012). Cold-Chain Management and important influence factors on meat spoilage. Training activity IQ-Freshlabel Project (FP 7), 19.09.2012, Romanian Meat Association, Bucharest.

Herbert, U. & J. Kreyenschmidt (2012). Optimising shelf life by using innovative packaging solutions. Vortrag im Rahmen der 2nd International Summer School, 25.-28.09.2012, Kulmbach.

Herbert, U., M. Mack, S. Rossaint, Judith Kreyenschmidt (2012). Transport, Cool Chain and Packaging. Vortrag im Rahmen des ISTA European Symposium 2012, 6-7.11.2012, Dortmund.

Herbert, U., S. Rossaint, M.-A. Khanna, J. Kreyenschmidt (2013). Comparison of argon-based and nitrogen-based modified atmosphere packaging (MAP) on bacterial growth and product quality of chicken breast fillets. *Poultry Science*, 92(5):1348-56. doi: 10.3382/ps.2012-02590.

Braun, C., **U. Herbert**, M. Mack, S. Rossaint, J. Kreyenschmidt (2013). Cold Chain Optimisation and Predictive Tools and Technologies. Oral Presentation. CSS Symposium. 6.-8.02.2013. Brussels.

Herbert, U., M. Halim, J. Wahl, H. Ostrowski, J. Kreyenschmidt (2013). Assessment of different packaging strategies on the quality and shelf life of poultry meat. Oral Presentation. 5th International Cold-Chain-Management Workshop, 10-11th June, Bonn.

Jaafreh S., J. Kreyenschmidt, **U. Herbert**, P. Kaul (2013). Detection of Poultry Meat Spoilage using Raman and ATR-IR Spectrometers. Poster Presentation. 5th International Cold-Chain-Management Workshop, 10-11th June, Bonn.

Kreyenschmidt, J., A. Albrecht, C. Braun, **U. Herbert**, M. Mack, S. Rossaint, G. Ritter, P. Teitscheid, Y. Ilg (2013). Food Waste in Fleisch verarbeitenden Ketten. *Fleischwirtschaft*, 10:57-63.

Herbert, U. A. Albrecht, J. Kreyenschmidt (2014). Definition of predictor variables for MAP poultry filets stored under different temperature conditions. *Poultry Science,* submitted 2.

Rossaint, S., S. Klausmann, **U. Herbert**, J. Kreyenschmidt (2014). Effect of package perforation on the spoilage process of poultry stored under different modified atmospheres. *Food Packaging and Shelf Life.* 1:68-76.

Kreyenschmidt, J., **U. Herbert**, S. Rossaint, Y. Ilg (2014). Cold Chain Management: New packaging concepts to reduce food waste. In: B. Petersen, M. Nüssel, M. Hamer (Eds.), Quality and risk management in agri-food chains. pp. 161-167.

Herbert, U., U. Schmalz, J. Kreyenschmidt (2014). Effect of different oxygen concentrations on shelf life and quality parameter of fresh poultry breast fillet. *International Journal of Food Science & Technology*, submitted.

Curriculum Vitae

Ulrike Herbert (geb. List) was born on 30th of November 1981 in Magdeburg, Germany. She studied Nutritional Science at the Rheinische-Friedrich Wilhelms University in Bonn, Germany. During her studies, she was working as a student assistant at the department of Preventive Health Management of the Institute of Animal Sciences (University of Bonn). In the year 2008, she finished her diploma thesis ("Generisches Prognosemodell zur Bestimmung der Haltbarkeit von Geflügel- und Schweinefleisch als Unterstützungswerkzeug im Rahmen der präventiven Qualitätssicherung"). Afterwards she started working at Erlenbacher Backwaren GmbH/Nestlé, Groß-Gerau, Germany in the department of Six Sigma. During that time, she received a postgraduate scholarship from the University of Bonn and started as a PhdD student in the department of Preventive Health Management of the University of Bonn. Within her studies, she started working as a junior researcher in the year 2010/2011 for VION FOOD Group in the area of Quality and Risk assessment for meat and meat products and came back afterwards to the University of Bonn to fianlise the phd thesis.

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