

**Predictive shelf life model for the improvement of quality  
management in meat chains**

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*Meinen Eltern*

Essentially,  
all models are wrong,  
but some are useful.

George E.P. Box  
(British statistician, born 1919)

## Abstract

### **Predictive shelf life model for the improvement of quality management in meat chains**

The objective of this thesis was the development of a common predictive shelf life model for fresh pork and fresh poultry based on the growth of *Pseudomonas* sp. as specific spoilage organism (SSO). As an element of a decision support system the model should provide predictive information to improve quality management in meat chains. To define the relevant parameters of the shelf life model, the spoilage processes of both meat types were characterised and compared under constant and dynamic environmental conditions.

Altogether, 638 pork samples and 600 poultry samples were investigated in 42 time series. The growth of *Pseudomonas* sp. on fresh pork and fresh poultry was investigated at five different constant and nine dynamic temperature scenarios to quantify the influence of temperature on shelf life. Additionally, several intrinsic factors (pH-value,  $a_w$ -value, Warner-Bratzler shear force, D-glucose, L-lactic acid, fat and protein content) were analysed during storage at 4°C and correlated to the counts of *Pseudomonas* sp. The collected growth data have been the basis for the development of the common predictive shelf life model.

The results showed a good correlation between the counts of *Pseudomonas* sp. and the sensory characteristics under constant as well as dynamic temperature conditions. It was possible to determine a common spoilage level at 7.5 log<sub>10</sub> cfu/g for both meat types which defines the end of shelf life based on the growth of *Pseudomonas* sp. Temperature was identified as the most important influencing factor on the growth of *Pseudomonas* sp. and thus on the shelf life of both meat types. The investigated intrinsic factors had only minor or no influence and were therefore not considered in the predictive shelf life model. Investigation of the influence of dynamic temperature conditions on shelf life of fresh pork and poultry revealed similar spoilage patterns for both meat types under dynamic temperature conditions with remarkable shelf life reductions of up to two days (up to over 30 %) caused by short temperature abuses at the beginning of storage.

Based on the results, a common predictive shelf life model was developed by combining the Gompertz and the Arrhenius model. The predictive information from the model can be used in specific situations of decision making, for example by optimising the storage management from the FIFO concept (First In, First Out) to the LSFO concept (Least Shelf life, First Out). Furthermore, the predictive model can be combined with risk assessment tools which enables the development of a range of novel comprehensive quality and cold chain management systems.

## Kurzbeschreibung

### Vorhersagemodell für die Bestimmung der Haltbarkeit zur Verbesserung des Qualitätsmanagements in Fleisch erzeugenden Ketten

Ziel der vorliegenden Arbeit war die Entwicklung eines gemeinsamen Vorhersagemodells für die Bestimmung der Haltbarkeit von frischem Schweine- und Geflügelfleisch basierend auf dem Wachstum des Hauptverderbniserregers *Pseudomonas* sp. Als Teil eines Decision Support Systems stellt das Modell Informationen bereit, die zur Verbesserung des Qualitätsmanagements in Fleisch erzeugenden Ketten beitragen. Zur Ermittlung der Modellparameter erfolgte die Charakterisierung des Frischeverlustes beider Fleischsorten unter statischen und dynamischen Umweltbedingungen.

Für die Erstellung des Vorhersagemodells erfolgte die Untersuchung von insgesamt 638 Schweinekoteletts und 600 Geflügelbrustfilets in insgesamt 42 Zeitreihenmessungen. Das Wachstum des Hauptverderbniserregers *Pseudomonas* sp. wurde unter fünf konstanten sowie neun dynamischen Temperaturszenarien untersucht, um den Einfluss der Lagertemperatur auf die Haltbarkeit zu ermitteln. Die Analyse weiterer möglicher Einflussfaktoren auf das Wachstum von *Pseudomonas* sp. (pH-Wert,  $a_w$ -Wert, Warner-Bratzler-Scherkraft, D-Glukosegehalt, L-Milchsäuregehalt, Fettgehalt und Proteingehalt) erfolgte bei einer konstanten Lagertemperatur von 4°C. Die erhobenen mikrobiologischen Wachstumsdaten bildeten die Basis für die Entwicklung des Vorhersagemodells.

Die Eignung von *Pseudomonas* sp. als Frischeparameter für beide Fleischsorten wurde durch hohe Korrelationen mit den sensorischen Charakteristika unter konstanten und dynamischen Temperaturbedingungen bestätigt. Das Ende der Haltbarkeit wurde für beide Fleischsorten bei einer Keimzahl von  $7,5 \log_{10}$  KbE/g festgelegt. Als Haupteinflussfaktor auf den Frischeverlust bzw. das Wachstum von *Pseudomonas* sp. wurde die Lagertemperatur identifiziert, wohingegen die untersuchten intrinsischen Faktoren nur einen geringen bzw. keinen Einfluss auf das Wachstum von *Pseudomonas* sp. hatten. Daher fanden sie bei der Modellentwicklung keine weitere Berücksichtigung. Beide Fleischsorten zeigten ein vergleichbares Verderbsmuster unter dynamischen Temperaturbedingungen, wobei kurzzeitige Temperaturerhöhungen zu Beginn der Lagerung zu Haltbarkeitsverkürzungen von bis zu 2 Tagen (bis zu 30 %) führten.

Basierend auf den erhobenen Daten erfolgte die Entwicklung eines gemeinsamen prädiktiven Haltbarkeitsmodells durch Kombination zweier mathematischer Modelle (Gompertzmodell und Arrheniusmodell). Das entwickelte Modell ermöglicht die Vorhersage des Wachstums von *Pseudomonas* sp. und somit der Haltbarkeit von beiden Fleischsorten unter wechselnden Temperaturbedingungen. Die Haltbarkeitsvorhersagen des Modells können in spezifischen Entscheidungssituationen verwendet werden, um z.B. durch den schnelleren Vertrieb von Produkten mit kürzerer Haltbarkeitszeit das Lagermanagement zu optimieren. Des Weiteren ist eine Kombination mit Risikobewertungssystemen denkbar.

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**CHAPTER 1**

**GENERAL INTRODUCTION**

## 1.1 Meat spoilage

From the legislative perspective, meat is defined as all parts of warm-blooded animals, in fresh or processed form, which are suitable for human consumption in the Regulation (EC) 853/2004. Colloquially speaking the skeletal muscle with embedded fat and connective tissue is meant by the term meat (Belitz et al., 2009). Based on the colour, meat can be divided between red meat (e.g. pork, beef, lamb) and white meat (poultry). The difference in colour is caused by a different content of myoglobin in the muscle. “Red” muscles tend to have a higher proportion of narrow, myoglobin-rich fibres whereas “white” muscles have a greater proportion of broad, myoglobin-poor fibres (Lawrie et al., 1998; Belitz et al., 2009). However, the basic composition of both meat types is comparable. The main component is water (> 70 %), followed by protein (around 20 %), lipids (< 10 %) and ash (around 1 %). Carbohydrates are only present in very low concentrations of 0.05 – 2 % (Lambert et al., 1991; Krämer, 2002; Belitz et al., 2009). The average composition of several cuts of beef, pork and chicken is shown in Table 1.1 (Belitz et al., 2009).

**Table 1.1: Average composition of meat (%) (Belitz et al., 2009)**

Meat	Cut	Moisture	Protein	Fat	Ash
<b>Pork</b>	Boston butt ( <i>M. subscapularis</i> )	74.9	19.5	4.7	1.1
	Loin ( <i>M. psoas major</i> )	75.3	21.1	2.4	1.2
	Cutlets, chops <sup>a</sup>	54.5	15.2	29.4	0.8
	Ham	75.0	20.2	3.6	1.1
	Side cuts	60.3	17.8	21.1	0.85
<b>Beef</b>	Shank	76.4	21.8	0.7	1.2
	Sirloin steak <sup>a</sup>	74.6	22.0	2.2	1.2
<b>Chicken<sup>b</sup></b>	Hind leg (thigh + drum stick)	73.3	20.0	5.5	1.2
	Breast	74.4	23.3	1.2	1.1

<sup>a</sup> with adhering adipose tissue

<sup>b</sup> without skin

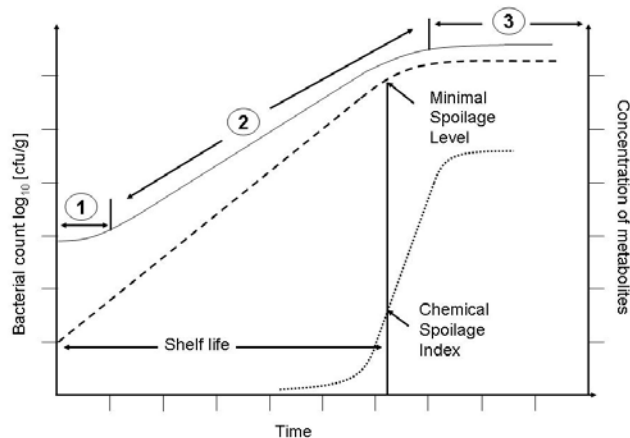
At the point of slaughter, the oxygen supply of the muscles breaks down as a result of the death of the animal. Thereby, the degradation of glycogen switches from the aerobic pathway to the anaerobic whereby glycogen is catabolised via pyruvate to lactic acid. The accumulation of lactic acid leads to a decrease of the pH to an ultimate pH of 5.4 – 5.8 in meat 24 h after slaughtering. (Gill & Newton, 1978; Lawrie, 1998; Krämer 2002).

When meat is considered as spoiled, it is no longer acceptable for human consumption - this is mainly attributed to sensory changes e.g. in colour, odour, flavour, aroma or texture (Mead, 2004; Singh & Anderson, 2004). These sensory changes during storage are mainly caused by microbiological growth, as the characteristics of fresh meat (high moisture content, moderate pH and readily available sources of energy, carbon and other nutrients) makes it ideal for microbiological growth (Gill, 1983; Huis in't Veld, 1996).

Usually, the muscles of healthy animals are essentially sterile at the point of slaughter (Gill, 1979; Krämer, 2002). However, during slaughtering and processing the meat surface is

contaminated with a variety of microorganisms (Krämer, 2002; Kleer, 2007) such as *Flavobacterium* sp., Enterobacteriaceae, *Pseudomonas* sp., *Aeromonas* sp., *Acitenobacter* sp., *Moraxella* sp. and *Staphylococcus* sp. (Barnes & Thornley, 1966; Blickstad & Molin, 1983; Gallo et al., 1988; Olsson et al., 2003). Generally, only one of these microorganisms is responsible for the spoilage of fresh meat during chill storage. This organism is called specific spoilage organism (SSO) (Gram & Huss, 1996; Gram et al., 2002). The growth and selection of the SSO is influenced by several factors, which are divided into intrinsic (properties of the food), extrinsic (storage environment), processing (treatments during processing) and implicit factors (microbial interactions) (Mossel, 1971). A more detailed explanation of these factors and their relevance for fresh meat is given in chapter 2.1.

From the initial microflora of fresh meat, less than 10 % is capable of growing at refrigeration temperature while the proportion of the SSO is even lower (Gill, 1986; Nychas et al., 1988; Borch et al., 1996). But during storage the SSO grows faster than the rest of the microflora producing metabolites responsible for e.g. off-odours or slime, which leads to the sensory rejection of the meat (Huis in't Veld, 1996, Koutsoumanis & Taoukis, 2005). At the point of spoilage, which is the point of sensory rejection, the cell concentration is termed minimal spoilage level. Shelf life is defined as the time from beginning of storage until the SSO reaches the minimal spoilage level (Dalgaard, 1993). Besides the determination of the minimal spoilage level, the concentration of the metabolites produced by the SSO can also be used for the estimation of shelf life. The metabolite which corresponds to spoilage caused by the growth of the SSO can be regarded as being a chemical spoilage index (CSI) (Dalgaard, 1993). The general pattern of microbial spoilage with different growth phases as well as the relations between changes in the total microflora, the SSO and the metabolites is shown in Figure 1.1.



**Figure 1.1: General pattern of microbial spoilage (modified after Dalgaard, 1993).**  
 SSO: specific spoilage organism, (—) total microflora; (- - -) SSO; (.....) metabolites.  
 Microbial growth phases: ① lag phase, ② exponential phase, ③ stationary phase

For fresh aerobically stored pork and poultry, *Pseudomonas* sp. have been identified as SSO (Gill & Newton, 1977; Pooni & Mead, 1984; Coates et al., 1995; Kreyenschmidt, 2003; Raab et al., 2008). *Ps. fragi*, *Ps. fluorescens* and *Ps. lundensis* are the main species which are detected during the aerobic spoilage of meat. Additionally, *Ps. putida* was found (Molin & Ternström, 1986; Nychas et al., 1988; Gennari & Dragotto, 1992; García-López et al., 1998; Krämer, 2002). Glucose is the first substrate utilised by *Pseudomonas* sp. during the spoilage of fresh meat. The dominance of these bacteria can partly be explained by its metabolism of glucose via the Entner-Doudoroff metabolic pathway (an alternative to glycolysis). In this pathway glucose is converted to the less commonly used 2-ketogluconate or gluconate which provides an extracellular energy source for *Pseudomonas* sp. and can not be metabolised by other bacteria (Farber & Idziak, 1982; Nychas et al., 1988; Dainty & Mackey, 1992; Montville & Matthews, 2007). After the depletion of glucose, the pseudomonads sequentially catabolise lactate, pyruvate, gluconate and in the end amino acids. The metabolism of nitrogenous compounds such as amino acids finally leads to the sensory changes (e.g. off-odours) which occur at the point of spoilage (Nychas et al., 2008).

## 1.2 Shelf life modelling

Generally, shelf life is understood as “the time period for the product to become unacceptable from sensory, nutritional or safety perspectives” (Fu & Labuza, 1993) which has been shown in Figure 1.1. Traditionally, the shelf life of a product has been mainly determined via challenge tests. In these tests, the effects of specific conditions on the growth and proliferation of the SSO were tested. To estimate the shelf life in real meat supply chains, challenge tests are mainly too expensive, labour intensive, time consuming and only valid for the product and conditions tested (Walker, 1994; Roberts, 1995;

McDonald & Sun, 1999; Wilson et al., 2002). But data generation with challenge tests are the basis for mathematical models which can predict the growth or decline of microorganisms. This field of research is called predictive microbiology or predictive food microbiology (McMeekin et al., 1993; Whiting, 1995; McDonald & Sun, 1999). According to Buchanan (1993) predictive models can be classified in several ways, e.g. based on the microbiological event studied (microbial growth or inactivation), the mathematical approach (probability-based or kinetics-based) and if they are mechanistic or empirical. Another generally accepted classification of predictive food models is the classification in primary, secondary and tertiary models proposed by Whiting and Buchanan (1993). Primary models describe the change of microbial numbers with time. The response can be measured directly by the microbial count, substrate levels or metabolic products and indirectly by absorbance, optical density or impedance (Whiting, 1995). The change of microbial count, especially the count of the SSO, can then be described by plotting the data with a primary model, for which particularly various sigmoidal functions are used. During the last years, several primary, secondary and tertiary models have been used to describe the growth of microorganisms as correlated to environmental factors. A selection of these models is listed in Table 1.2.

**Table 1.2: Selection of primary, secondary and tertiary models used for describing microbial growth (modified after McDonald & Sun, 1999)**

Classification	Model	Source
<b>Primary models</b>	Gompertz function	Gibson et al. (1987)
	Modified Gompertz	Zwietering et al. (1990)
	Logistic model	Jason (1983)
	Baranyi model	Baranyi et al. (1993), Baranyi & Roberts (1994)
	Three-phase linear model	Buchanan et al. (1997)
	Schnute model	Schnute (1981)
<b>Secondary models</b>	Belehradek model (square-root model)	Belehradek (1930)
	Ratkowsky model (square-root model)	Ratkowsky et al. (1982)
	Arrhenius model	Arrhenius (1889)
	Modified Arrhenius model	Davey (1989)
	(Davey or Schoolfield)	Schoolfield et al. (1981)
	Probability models	Hauschild (1982)
Polynomial or response surface models	Gibson et al. (1988)	
<b>Tertiary models</b>	USDA Pathogen Modelling Program	Buchanan (1991) <a href="http://pmp.arserrc.gov/">http://pmp.arserrc.gov/</a>
	Food Spoilage Predictor (Pseudomonas Predictor)	Neumeyer et al. (1997) Blackburn (2000)
	Seafood Spoilage and Safety Predictor (SSSP)	Dalgaard et al. (2008) <a href="http://sssp.dtuquaqua.dk/">http://sssp.dtuquaqua.dk/</a>
	Sym'Previs	Thuault & Couvert et al. (2008) <a href="http://www.symprevius.net/">http://www.symprevius.net/</a>
	ComBase	Baranyi & Tamplin (2004) <a href="http://www.combase.cc/">http://www.combase.cc/</a>
	Temperature History Evaluation for Raw Meats (THERM)	Ingham et al. (2007, 2009) <a href="http://www.meathaccp.wisc.edu/therm/">http://www.meathaccp.wisc.edu/therm/</a>
	Growth predictor & Perfringens Predictor	<a href="http://www.ifr.ac.uk/Safety/GrowthPredictor">http://www.ifr.ac.uk/Safety/GrowthPredictor</a>

The most widely used primary models are the Logistic model and the Gompertz model, which are comparable in applicability and accuracy. Both include four parameters to describe the sigmoidal growth curve. The difference is that the Logistic curve is symmetric



about the reversal point  $M$ , whereas the Gompertz is not (Gibson et al., 1987). Zwietering et al. (1990) statistically compared several sigmoidal functions to describe the growth of *Lactobacillus plantarum*, including the Logistic and the Gompertz function. They reparameterised the equations to include biologically relevant parameters e.g. lag time and specific growth rate. The modified Gompertz function was found to be statistically adequate to describe the microbiological growth and was easy to use. Buchanan (1993) also stated that the Gompertz function is easy to use with good curve-fitting software.

A secondary model is then used to describe the response of one or more parameters of the primary model to changes in environmental conditions (e.g. temperature, pH,  $a_w$ ) (Buchanan, 1993; Whiting & Buchanan, 1993; Whiting, 1995). As temperature is considered as the most important influence factor, secondary models mainly describe the temperature dependency of model parameters (Labuza & Fu, 1993; McDonald & Sun, 1999). Well-known secondary models are the Arrhenius model and the square root model as well as their modified forms (Table 1.2). The simple Arrhenius equation is often used in predictive microbiology but it is normally only accurate over a limited temperature range for microbial growth wherefore modified versions have been developed to achieve better fits with extreme temperature ranges (Buchanan, 1993; Fu & Labuza, 1993; McDonald & Sun, 1999). However, these modified forms have been reported as being complex and cumbersome in use (Buchanan, 1993) and successful applications of the simple Arrhenius model are available for many different meat types and meat products (Giannuzzi et al., 1998; Kreyenschmidt, 2003; Moore & Sheldon, 2003; Mataragas et al., 2006; Kreyenschmidt et al., 2010). Another often used secondary model is the Square root model which was first successfully applied by Ratkowsky et al. (1982) to describe satisfactorily the relationship between microbial growth rate and temperature with over 50 data sets.

The incorporation of primary and/or secondary models in “user-friendly” computer software to provide a complete prediction tool is called tertiary model (Buchanan, 1993; Whiting, 1995). They can be seen as an interface between the scientist and the end-user where the end-user can enter a set of product characteristics and receive a prediction of growth parameters (Betts & Walker, 2004). However, only a few predictive tertiary models are available for use in the industry. General predictive microbiology software with databases are for example ComBase (<http://www.combase.cc>) and the Seafood Spoilage and Safety Predictor (SSSP) (<http://sssp.dtuaqua.dk/>), which can be freely accessed worldwide via the internet. The use of tertiary models for the prediction of shelf life and remaining shelf life in the industry facilitates the making of better informed decisions by actors in the supply chains regarding further storage and the distribution of the product. This results in an improvement of quality management by the optimisation of the storage management from the FIFO concept (First In, First Out) to the LSFO concept (Least Shelf life, First Out) which reduces

product waste and thus economic losses (Giannakourou et al., 2001; Koutsoumanis et al., 2005).

For the successful development of a predictive shelf life model applicable for fresh meat, several points have to be considered. At first, a detailed knowledge of the spoilage process is required as it relates to various influencing factors (McMeekin et al., 1993; Blackburn, 2000). This includes the knowledge of the SSO of the product as well as the population level of the SSO at which spoilage occurs (minimal spoilage level) and the range of environmental conditions over which a particular SSO is responsible for spoilage Dalgaard (1995). To gather this information for the model development, storage tests are conducted at specified environmental conditions. These storage tests are often carried out in laboratory media like nutrient broth (e.g. Willocx et al., 1993; Baranyi et al., 1995; Greer et al., 1995). The problem is, that models based on microbiological growth data generated in broth often under- or overestimate microbial growth in real food. For example, Pin & Baranyi (1998) developed a microbial growth model based on growth data of a mixed microbial population in broth. In a later study, they showed that the overall error of this model was 53.5 % when comparing the predictions of the model with the observations in naturally spoiled food (Pin et al., 1999). Gill et al. (1997) also stated that their models derived from the cultivation of *Aeromonas hydrophila* and *Listeria monocytogenes* in commercial broths appear to be highly unreliable guides to the behaviours of those organisms on pork. Therefore, predictive models for the use in the meat industry should be developed using microbiological growth data obtained in naturally contaminated food products (Dalgaard, 1995; Koutsoumanis & Nychas, 2000).

Another important aspect is the validation of the model under dynamic environmental conditions since in meat chains major variations in temperature during storage and distribution are often observed (Raab & Kreyenschmidt, 2008; Koutsoumanis et al., 2010). As for the development of the model, the microbiological growth data used for validation under dynamic temperature conditions should be generated in storage tests with the naturally contaminated products (Dalgaard et al., 1997; McMeekin et al., 1997; Shimoni & Labuza, 2000; Membré & Lambert, 2008).

However, only a few models have been published which were, on the one hand developed in fresh meat or meat products instead of laboratory media, and on the other hand validated in these products under dynamic temperature conditions. These are e.g. the model of Koutsoumanis et al. (2006) for the growth of *Pseudomonas* sp. in ground meat, the model of Gospavic et al. (2008) for *Pseudomonas* sp. in poultry and the models of Mataragas et al. (2006) as well as Kreyenschmidt et al. (2010) for lactic acid bacteria in modified atmosphere-packed (MAP) cooked sliced ham. But all these models were only developed and validated

for just one type of meat or meat product. Predictive models that are applicable for different types of fresh meat (e.g. fresh pork and poultry) are unavailable.

### 1.3 Research objectives and outline of the thesis

The main objective of this thesis is the development of a common predictive shelf life model for fresh pork and poultry. In the context of quality management in meat chains the model should be a core element of a decision support system to provide predictive information. These objectives lead to the following research questions:

- How are the spoilage processes of fresh pork and poultry characterised and do they follow similar patterns?
- How is the growth of *Pseudomonas* sp. influenced by cold chain interruptions in fresh pork and poultry and what is the effect on shelf life?
- Is it possible to develop and validate a common predictive model for fresh pork and fresh poultry based on the growth of *Pseudomonas* sp. to estimate the shelf life and remaining shelf life under different temperature conditions?

In the first part of this thesis (*chapter 2*), the spoilage processes of fresh pork and fresh poultry are characterised and compared. For this purpose, intrinsic factors (pH-value,  $a_w$ -value, Warner-Bratzler shear force (WBSF), D-glucose, L-lactic acid, fat and protein) are analysed concerning their effect on *Pseudomonas* sp. growth for fresh pork and poultry during storage at 4°C. Additionally, the growth of *Pseudomonas* sp. is investigated at different constant storage temperatures and the minimal spoilage level is determined for fresh pork and fresh poultry to work out similarities and differences in the spoilage processes.

In *chapter 3* several storage trials are conducted with different dynamic temperature scenarios to figure out the influence of short cold chain interruptions on the spoilage processes and thus shelf life of fresh pork and poultry. Especially the influence of different amplitudes and durations of short temperature abuses, which can occur in the real cold chain of fresh meat, are analysed and compared for both meat types.

In the last chapter (*chapter 4*), model parameters for fresh pork and fresh poultry derived from storage experiments at constant storage temperatures in chapter 2 are presented. Based on this data, a predictive shelf life model is developed which is applicable for both meat types. The model is validated under dynamic temperature conditions using the growth data of chapter 3 and previous investigations. Furthermore, the improvement of quality management resulting from the implementation of the model in meat supply chains is discussed.

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**CHAPTER 2**

**CHARACTERISATION AND COMPARISON OF  
SPOILAGE PROCESSES IN FRESH PORK AND  
POULTRY**

## 2.1 Introduction

Fresh pork and poultry are highly perishable commodities due to their nutritional composition. The main cause for their loss of freshness during storage is microbial growth (Singh & Anderson, 2004), especially the growth of the specific spoilage organism (SSO) *Pseudomonas* sp. (Blickstad & Molin, 1983; Pooni & Mead, 1984; Gallo et al., 1988; Coates et al., 1995; Kreyenschmidt et al., 2007; Liu et al., 2006b; Raab et al., 2008).

During storage, the growth of the SSO is affected by several factors, which are divided into four groups according to Mossel (1971): (i) intrinsic factors, which are the expression of physical and chemical properties of the food itself (e.g. water activity, nutrients, structure), (ii) extrinsic factors, which are parameters of the storage environment of the food (e.g. storage temperature, gas atmosphere), (iii) processing factors, which are physical or chemical treatments during processing of the food (e.g. heat treatment) and (iv) implicit factors, which describe synergistic or antagonistic influences among the primary selection of organisms (e.g. specific rate of growth). Factors which are considered as being relevant for fresh meat are the intrinsic factors initial number of psychotrophs present on the meat surface, water activity ( $a_w$ ), inherent pH of the meat surface and nutritional content as well as the extrinsic factors storage temperature and oxygen availability (McDonald & Sun, 1999; Cervený et al., 2009).

Changes of these parameters influencing microbiological growth during storage have been investigated by several authors. Huff-Lonergan et al. (2002) analysed different quality traits including intrinsic parameters, such as pH-value, glycolytic potential and their mutual correlations in fresh pork, but without considering their relationship to microbiological growth and thus shelf life. More emphasis has been laid on improving these characteristics by rearing in the pig meat industry (Hovenier et al., 1993). Byun et al. (2003) compared different parameters in fresh pork and beef during storage and found high correlations between D- glucose as well as L-lactate and bacterial counts (total plate count and psychotrophic bacterial count) in fresh pork. Correlations between pH and bacterial counts were only of medium magnitude and the SSO were not investigated in their study. Allen et al. (1997) described significant correlations between the psychotrophic plate count and the pH as well as the capacitance detection time (for enumeration of *Pseudomonas fluorescens*) and pH in poultry breasts, but these correlations were very low. In a later study, no significant correlations between these parameters were observed (Allen et al., 1998). Despite all these studies it is unclear which factors have the greatest influence on the shelf life of fresh pork and poultry and whether these factors are the same or different for fresh pork and poultry meat.

Therefore, the objective of the study was to investigate different intrinsic parameters during storage in fresh pork and poultry to attempt to establish similarities as well as differences between fresh pork and poultry and to identify relevant factors influencing the growth of *Pseudomonas* sp. Additionally, the influence of the extrinsic parameter temperature was analysed.

## 2.2 Material and methods

### 2.2.1 Sample description and experimental design

Pork loins (*M. longissimus dorsi*) were transported from local butchers and slaughterhouses to the laboratory under temperature-controlled conditions. In the laboratory, every loin was divided into 150 - 200 g slices (chops) under sterile conditions. Skinless chicken breast fillets (150 - 170 g) were transported from a poultry slaughtering and processing plant in Germany to a wholesaler and forwarded to the laboratory under temperature-controlled conditions. Each pork chop and each chicken breast fillet was placed in an individual tray and over-wrapped with a low density polyethylene (LDPE) film (aerobe packaging). For all storage experiments, high precision low temperature incubators (MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan) were used. Time between slaughtering and the first investigation was 24 h for both meat types.

In the first step of the study, the microbiological growth data from previous investigations (Raab et al., 2008) were taken as a basis and data from new measurements were added to identify the influence of the extrinsic parameter temperature on *Pseudomonas* sp. growth and hence shelf life. Altogether, microbiological data of 147 pork chops and 124 poultry fillets were considered, which were stored at five different isothermal temperatures (2, 4, 7, 10 and 15°C). Three samples were analysed for total viable count (TVC), *Pseudomonas* sp. count and sensory characteristics at appropriate time intervals.

In the second step, loins of 5 pig carcasses (25 chops) and 25 skinless chicken breast fillets were prepared and packaged as described previously. Samples were stored at 4°C for about 14 days. Five samples of each meat type were analysed for microbial counts (TVC and *Pseudomonas* sp.), sensory characteristics and the intrinsic parameters pH-value,  $a_w$ -value, D-glucose, L-lactic acid and Warner-Bratzler shear force (WBSF) at five sample points during storage. Sample points were chosen based on the spoilage processes at 4°C determined in step 1 of the study. For pork, chops from the same loins were chosen at all sample points, which means that parameter changes in one loin was tracked during storage. Additionally, 5 poultry fillets and 5 pork chops were frozen at -20°C at day 0 of the study for analysis of

initial fat and protein content. The storage experiment was repeated twice, so that a total of 90 pork chops and 90 chicken breast fillets were investigated.

### 2.2.2 Microbiological analysis

For microbial analysis, a representative product sample of 25 g was extracted using a cork borer and transferred to a filtered stomacher-bag, which was filled with saline peptone diluents (0.85 % NaCl with 0.1 % peptone; Oxoid, Basingstoke, United Kingdom) to a final weight of 250 g. The contents were homogenised for 60 s using a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany). A 10-fold dilution series of the homogenate was prepared using saline peptone diluents. Appropriate dilutions were transferred to the following media: plate count agar (PCA, Oxoid, Basingstoke, United Kingdom) for TVC, incubated at 30°C for 72 h as well as *Pseudomonas* Agar Base (Oxoid Basingstoke, United Kingdom) plus CFC supplement (Oxoid, Basingstoke, United Kingdom) for *Pseudomonas* sp., incubated at 25°C for 48 h. TVC was enumerated using the pour plate technique, for *Pseudomonas* sp. the spread plate technique was used.

### 2.2.3 Sensory analysis

Sensory evaluation of each sample was assessed by a trained sensory panel. Odour, texture and colour were evaluated using a 3-point scoring system where 3 = very good and 1 = unacceptable. A weighted sensory index (*SI*) was calculated using the following equation 2.1.

$$SI = \frac{2 \cdot C + 2 \cdot O + 1 \cdot T}{5} \quad (2.1)$$

with *SI*: sensory index, *C*: colour, *O*: odour, *T*: texture

Sensory acceptance was described as a function of time by linear regression. The meat was considered “spoiled” when the *SI* reached 1.8 (Kreyenschmidt, 2003). From the shelf life times obtained based on the decrease in sensory acceptance, a spoilage level for *Pseudomonas* sp. was derived to specify the end of shelf life.

### 2.2.4 Measurement of physical and chemical properties

The pH-value was measured on three different points in each sample by lancing a pH-meter (Testo 206; Testo, Lenzkirch, Germany) directly in the product. From these three measurements, an average pH value was calculated for each poultry fillet and each pork chop. The water activity ( $a_w$ -value) was measured with the AquaLab CX-3 TE (Decagon Devices Inc., Pullman, USA). The sample dish was filled with sample materials according to the instructions. Measurements were conducted at room temperature (20°C) and repeated

three times. An average  $a_w$ -value was calculated from these three measurements for each sample.

For the measurement of the WBSF, five cores were removed from each poultry fillet and each pork chop using a cork borer to ensure comparability of the samples. Each core was placed in a Texture Analyser TA.XT plus (Stable Micro System, Surrey, UK) with a notched Warner-Bratzler-Shear blade. Assay parameters were: pre-test speed: 2 mm/s, test speed: 2 mm/s, post-test speed: 10 mm/s, down stroke distance: 20 mm and trigger force: 250 g. After a manual start, the measurement itself and its documentation was performed with the related software Texture Exponent 32. Peak shear force (kg) was recorded for each core. An average WBSF value for every meat sample was then calculated from the five cores per sample. After the investigation of microbial and physicochemical parameters, remaining samples were frozen at  $-20^{\circ}\text{C}$  for D-glucose and L-lactic acid analyses.

### 2.2.5 Measurement of nutrients

D-glucose and L-lactic acid concentrations were assayed with the respective enzyme kit for the determination of these concentrations in foodstuffs and other materials (D-glucose: Test-Combination 10 716 251 035, r-biopharm, Darmstadt, Germany; L-lactic acid: Test-Combination 10 139 084 035, r-biopharm, Darmstadt, Germany). Remaining samples from previous investigations were thawed at  $4^{\circ}\text{C}$  in the refrigerator for 24 h prior to the investigation. 5 g of the thawed sample was weighed in a stomacher bag, 35 ml double distilled water was added and the mixture homogenised for 10 min using a Stomacher (IUL, Königswinter, Germany). After Carrez clarification of the sample solution, pH was adjusted to 8.0 – 8.5 by using sodium chloride. 100 ml double distilled water was added, the suspension was shaken well and filtered (Whatman filter type 595; Whatman Int. Ltd., Maidstone, UK). The filtrate was used for the enzymatic analysis of D-glucose and L-lactic acid according to the instructions of the enzyme kits. Extinctions were measured with a UV-Vis photometer (Genesys 6, Thermo Scientific, Waltham, USA) at a wavelength of 340 nm. Measurements were performed in duplicate.

Pork chops and poultry fillets being examined for fat and protein analysis were thawed in a refrigerator at  $4^{\circ}\text{C}$  and homogenised in a mixer (Moulinex/Groupe SEB, Ecully Cedex, France). Four samples of 1 g and 5 g each were forwarded to external institutes for fat and protein content analysis. Analysis of the protein content was conducted according to the official analytical methods specified in the German Food Law (Lebensmittel- und Futtermittelgesetzbuch; LFGB), which is based on the nitrogen determination by Kjeldahl (§ 64 LFGB, L06.00-7). Fat content was determined according to Weibull-Stoldt, which is also specified among the official analytical methods (§ 64 LFGB, L06.00-6)

### 2.2.6 Statistical methods

The microbiological growth data were transformed to  $\log_{10}$  values and then fitted using nonlinear regression (Levenberg-Marquardt algorithm) by the statistical software package Origin 8.0G (OriginLab Corporation, Northampton, USA). The Gompertz model was used to describe the growth of microorganisms with time (equation 2.2) (Gibson et al., 1987):

$$N(t) = A + C \cdot e^{-e^{-B \cdot (t-M)}} \quad (2.2)$$

with  $N(t)$ : microbial count [ $\log_{10}$  cfu/g] at time  $t$ ,  $A$ : 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 growth rate at time  $M$  [1/h],  $M$ : time at which maximum growth rate is obtained (reversal point),  $t$ : time [h].

Data of microbial and intrinsic parameters were analysed using SPSS statistics 17 (SPSS Inc., Chicago, USA). Due to the sample size, normality was checked for using Shapiro-Wilk-Test (Janssen & Laatz, 2007). Based on the results, correlations were calculated with Spearman's Rho. Evaluation of these correlations was made according to the classification of Bühl (2008):  $r \leq |0.2|$  = very low correlation,  $|0.2| < r \leq |0.5|$  = low correlation,  $|0.5| < r \leq |0.7|$  = medium correlation,  $|0.7| < r \leq |0.9|$  = high correlation and  $r > |0.9|$  = very high correlation. Furthermore, the Mann-Whitney-U-Test was used to compare parameters and significance which was established at  $p < 0.05$ . For parameter comparison of pork samples from the same loin during storage, the Wilcoxon-Test was used.

## 2.3 Results & Discussion

### 2.3.1 Influence of the extrinsic parameter temperature

Figure 2.1 shows the growth of *Pseudomonas* sp. on fresh pork and poultry at constant storage temperatures from 2 – 15°C fitted with the Gompertz model. A genetic strain identification revealed that the *Pseudomonas* sp. were dominated by *Ps. putida* (about 90 %). Less frequently occurring was *Ps. fluorescens*. Initial observed *Pseudomonas* sp. counts were slightly higher for fresh poultry than for pork (mean values: pork 3.5  $\log_{10}$  cfu/g; poultry 4.1  $\log_{10}$  cfu/g). But at the end of storage, the maximum number of *Pseudomonas* sp. did not show relevant differences between both meat types (about 9 – 10  $\log_{10}$  cfu/g), independent of temperature and initial bacterial count. This was also observed by Giannuzzi et al. (1998), Koutsoumanis (2001) and Fujikawa et al. (2004). Increasing temperature led to a faster growth with fresh pork and poultry as also described in several studies (e.g. Baranyi et al., 1995; Moore & Sheldon, 2003; Raab et al., 2008; Kreyenschmidt et al., 2010). A comparison of growth on fresh pork and poultry revealed that growth was faster on poultry than on pork.

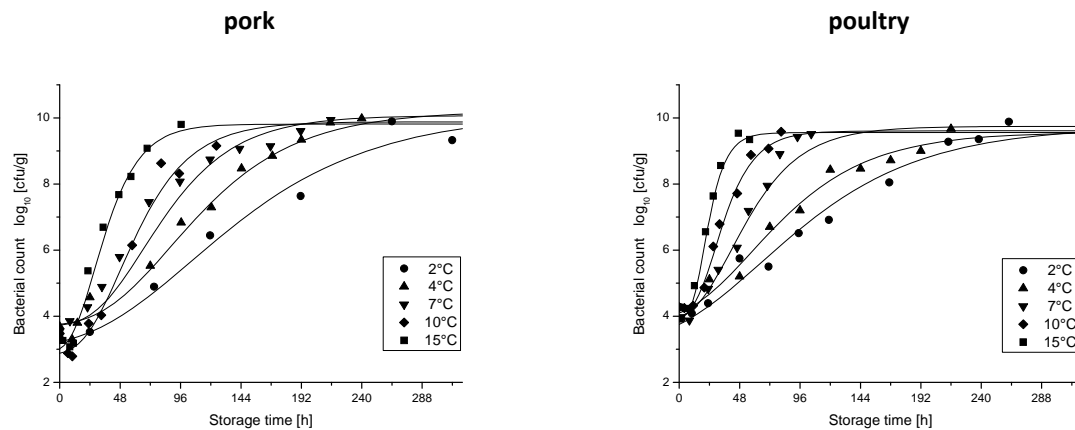


Figure 2.1: Growth of *Pseudomonas* sp. on fresh pork (left) and poultry (right) at constant storage temperatures fitted with the Gompertz model

The sensory index (SI) decreased linearly for fresh pork and poultry. With increasing storage temperature a faster decrease of the SI was observed (Figure 2.2). As for microbial growth, the SI declined faster for fresh poultry than for fresh pork.

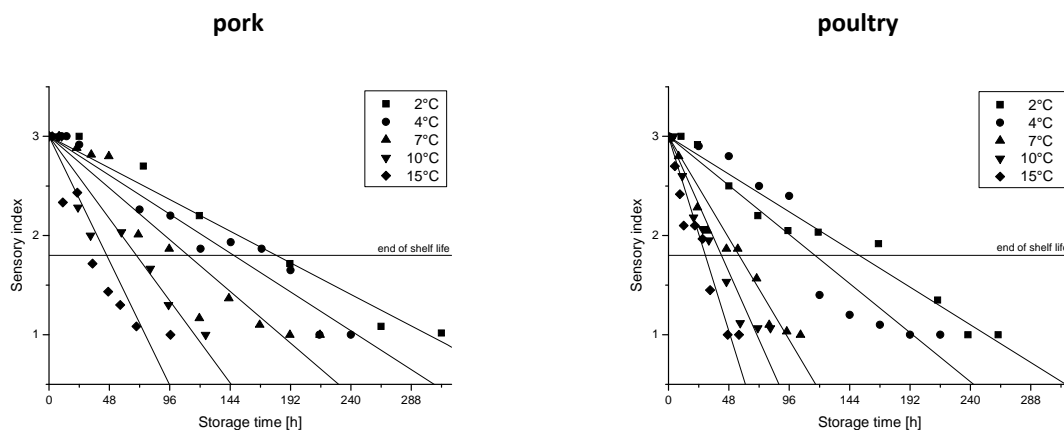


Figure 2.2: Sensory index for fresh pork (left) and poultry (right) at different constant storage temperatures (end of shelf life at  $SI \leq 1.8$ )

At all investigated constant storage temperatures, very high significant correlations ( $p < 0.05$ ) were obtained between the count of *Pseudomonas* sp. and the SI for fresh pork ( $r = -0.902$  to  $-0.989$ ) as well as poultry ( $r = -0.930$  to  $-0.997$ ). These correlations underline the applicability of the *Pseudomonas* sp. count as a freshness and hence shelf life indicator for fresh pork and poultry as the definition and assessment of spoilage relies on sensory evaluation (Gram et al., 2002). Dainty & Mackey (1992) and Nychas et al. (2008) reported that spoilage based on the growth of *Pseudomonas* sp. leads to off-odours and slime-production when the bacterial numbers reach  $7 - 8 \log_{10}$  cfu/cm<sup>2</sup> or cfu/g. In this study, the enlargement of the data of previous investigation (Raab et al., 2008) allowed the determination of a common spoilage level for both meat types based on sensory characteristics at a *Pseudomonas* sp. count of  $7.5 \log_{10}$  cfu/g. The agreement between



estimated microbial and sensory shelf lives for fresh pork and poultry is good, with a maximum discrepancy of 25.1 h for poultry at 2°C and 24.7 h for pork at 4°C (Table 2.1). Differences were greater at lower temperatures.

**Table 2.1: Microbial and sensory determined shelf lives of fresh pork and poultry at different constant storage temperatures**

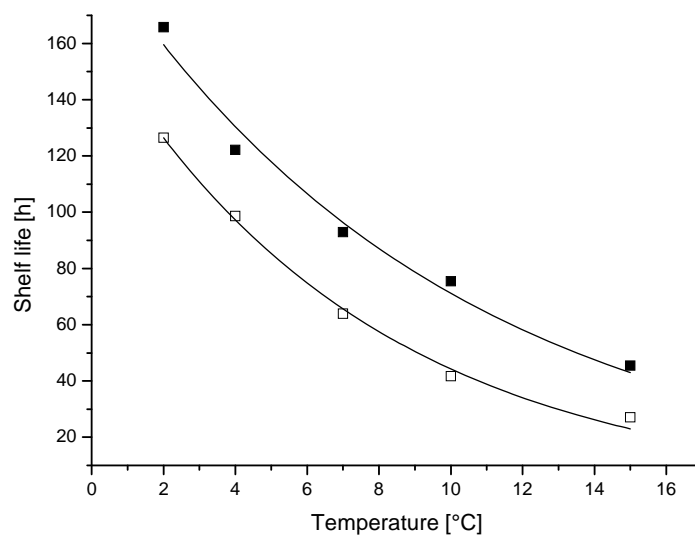
Temperature [°C]	Pork		Poultry		Difference between Pork and Poultry	
	Microbial shelf life <sup>a)</sup> [h]	Sensory shelf life <sup>b)</sup> [h]	Microbial shelf life [h]	Sensory shelf life [h]	Microbial shelf life [h]	Sensory shelf life [h]
2	165.8	180.7	126.4	151.5	39.4	29.2
4	122.2	146.9	98.6	116.4	23.6	30.5
7	92.9	110.5	63.9	56.1	29.0	54.4
10	75.4	69.5	41.5	42.1	33.9	27.4
15	45.5	45.9	27.1	29.3	18.4	16.6

Microbial and sensory shelf life was estimated from time point zero of the laboratory investigations, which means 24h after slaughtering.

<sup>a)</sup> Evaluated by count of *Pseudomonas* sp.: End of shelf life:  $7.5 \log_{10}$  cfu/g

<sup>b)</sup> Evaluated by sensory index: End of shelf life:  $SI \leq 1.8$

At each constant storage temperature level, shelf life of fresh pork was longer than shelf life of fresh poultry. The decay of shelf life with increasing temperature can be described exponentially (Figure 2.3). Differences between shelf life of fresh pork and fresh poultry were between 18.4 h and 39.4 h for microbial shelf life and 16.6 h and 54.4 h for sensory shelf life, respectively.



**Figure 2.3: Microbial shelf life of fresh pork (■) and poultry (□) at different constant storage temperatures**

### 2.3.2 Influence of intrinsic parameters

Table 2.2 shows the mean values and standard deviations of the analysed intrinsic parameters in fresh pork and poultry at different sample points during storage, as well as the total mean value.

**Table 2.2: Intrinsic parameters (mean values  $\pm$  standard deviation) during storage in fresh pork and poultry meat at 4°C**

Parameter	Meat type	Sample points					Mean value
		I	II	III	IV	V	
pH	pork	5.48a <sup>a)</sup> $\pm$ 0.14	5.61a $\pm$ 0.14	5.66a $\pm$ 0.24	5.76a $\pm$ 0.24	5.73a $\pm$ 0.15	5.65a $\pm$ 0.21
	poultry	6.02b $\pm$ 0.16	6.16b $\pm$ 0.15	6.16b $\pm$ 0.17	6.08b $\pm$ 0.27	6.23b $\pm$ 0.17	6.13b $\pm$ 0.20
a <sub>w</sub>	pork	0.990a $\pm$ 0.001	0.990a $\pm$ 0.001	0.990a $\pm$ 0.001	0.992a $\pm$ 0.002	0.990a $\pm$ 0.002	0.990a $\pm$ 0.002
	poultry	0.991b $\pm$ 0.001	0.991b $\pm$ 0.001	0.991b $\pm$ 0.001	0.990b $\pm$ 0.001	0.990a $\pm$ 0.001	0.990b $\pm$ 0.001
D-glucose [g/100g]	pork	0.236a $\pm$ 0.120	0.241a $\pm$ 0.110	0.234a $\pm$ 0.151	0.187a $\pm$ 0.120	0.135a $\pm$ 0.111	0.207a $\pm$ 0.127
	poultry	0.043b $\pm$ 0.027	0.022b $\pm$ 0.012	0.028b $\pm$ 0.016	0.033b $\pm$ 0.033	0.014b $\pm$ 0.009	0.028b $\pm$ 0.023
L-lactic acid [g/100g]	pork	0.939a $\pm$ 0.139	0.874a $\pm$ 0.081	0.878a $\pm$ 0.116	0.820a $\pm$ 0.099	0.731a $\pm$ 0.153	0.849a $\pm$ 0.137
	poultry	0.828b $\pm$ 0.081	0.763b $\pm$ 0.095	0.760b $\pm$ 0.086	0.774a $\pm$ 0.113	0.762a $\pm$ 0.085	0.778b $\pm$ 0.094
WBSF [kg]	pork	3.14a $\pm$ 0.52	3.38a $\pm$ 0.61	3.22a $\pm$ 0.60	3.20a $\pm$ 0.52	3.18a $\pm$ 0.49	3.22a $\pm$ 0.54
	poultry	1.19b $\pm$ 0.28	1.15b $\pm$ 0.25	1.12b $\pm$ 0.32	1.02b $\pm$ 0.32	1.13b $\pm$ 0.22	1.12b $\pm$ 0.28
Fat <sup>b)</sup> [g/kg]	pork	116.04a $\pm$ 84.94	-	-	-	-	116.04a $\pm$ 84.94
	poultry	13.85b $\pm$ 3.97	-	-	-	-	13.85a $\pm$ 3.97b
Protein <sup>b)</sup> [g/kg]	pork	208.33a $\pm$ 24.35	-	-	-	-	208.33a $\pm$ 24.35
	poultry	237.73b $\pm$ 6.69	-	-	-	-	237.73b $\pm$ 6.69

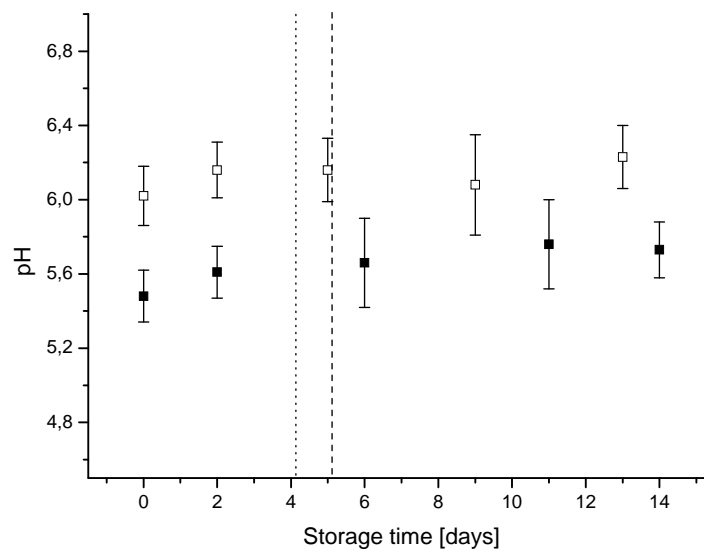
Sample points: I = day 0; II = day 2; III = day 5 (poultry)/day 6 (pork); IV = day 9 (poultry)/day 11 (pork); V = day 13 (poultry)/day 14 (pork)

WBSF = Warner-Bratzler shear force

<sup>a)</sup> Means with different small letters in the same column represent significant differences at  $p < 0.05$  for the parameter

<sup>b)</sup> Fat and protein content were only analysed at sample point I

During storage at 4°C the pH-value was increasing for pork (from 5.48 to 5.73) as well as poultry (from 6.02 to 6.23) (Figure 2.4). The difference between initial and end value was significant ( $p < 0.05$ ) for both meat types. The results of Allen et al. (1997) also indicated an increasing pH for fresh poultry during storage. In the study of Byun et al. (2003) the pH of fresh pork decreased first and then increased again but in total it was lower at the beginning (5.40) than at the end (5.51).



**Figure 2.4: Changes in mean values ( $\pm$  standard deviation) for pH in fresh pork (■) and poultry (□) during storage at 4°C. (---) microbial end of shelf life pork, (· · ·) microbial end of shelf life poultry**

Initial pH-value (24 h after slaughtering) for pork ( $5.48 \pm 0.14$ ) was consistent with generally observed pH values for fresh pork meat in the literature (5.4 – 5.8) (Blickstad & Molin, 1983; Klont et al., 1999). Initial pH for poultry was higher than for pork at the beginning ( $6.02 \pm 0.16$ ), as described previously (Newton & Gill, 1981) and also slightly higher than reported values for poultry breast fillets in other studies (Barnes, 1976; Fletcher, 1999). Comparisons showed significant differences ( $p < 0.05$ ) between pH of pork and poultry at every sample point with higher pH-values for poultry. Higher pH-values have been associated with a faster microbial spoilage of meat (Borch et al., 1996). However in contrast to this, Gill & Newton (1982) have demonstrated that the growth rate of *Pseudomonas* sp. on fresh meat was the same at a pH of 5.5 as well as 6.4 which is consistent with the findings of McMeekin & Ross (1996), who also observed no effect of the pH on the growth rates of *Pseudomonas* sp. in the pH range of 5.3 – 7.8. In this study, these results were confirmed by the correlations between pH-value and *Pseudomonas* sp. in fresh pork (Table 2.3) and fresh poultry (Table 2.4). Correlations were significant ( $p < 0.05$ ) but their magnitudes were low (pork:  $r = 0.4687$ ; poultry:  $r = 0.301$ ). Allen et al. (1997) also reported significant but low correlations between pH and bacterial counts. Furthermore, when *Ps. putida* isolated from the investigated meat samples in this study was inoculated in nutrient broth with three different pH values (5.3, 5.8 and 6.3) and stored at 4°C, growth data of *Ps. putida* showed no difference at the three investigated pH values (Bruckner et al., 2009). Therefore, pH as an intrinsic factor can be disregarded concerning its influence on *Pseudomonas* sp. and hence shelf life for fresh pork as well as poultry.

**Table 2.3: Correlations between intrinsic and microbiological parameters as well as sensory index for fresh pork at 4°C**

	TVC	<i>Pseu. sp.</i>	pH	$a_w$	D-Glucose	L-Lactic acid	WBSF	Sensory index	Fat content
<i>Pseudomonas sp.</i>	<b>0.852</b>								
pH	<b>0.608</b>	<b>0.467</b>							
$a_w$	<b>0.554</b>	<b>0.391</b>	<b>0.427</b>						
D-Glucose	<b>-0.560</b>	<b>-0.364</b>	<b>-0.773</b>	<b>-0.384</b>					
L-Lactic acid	<b>-0.645</b>	<b>-0.505</b>	<b>-0.667</b>	<b>-0.375</b>	<b>0.672</b>				
WBSF	-0.094	0.021	<b>-0.239</b>	-0.066	0.147	0.155			
Sensory index	<b>-0.909</b>	<b>-0.818</b>	<b>-0.525</b>	<b>-0.395</b>	<b>0.402</b>	<b>0.546</b>	0.154		
Fat content	<b>0.654</b>	<b>0.611</b>	<b>0.754</b>	<b>0.590</b>	<b>-0.689</b>	<b>-0.239</b>	-0.059	-0.346	
Protein content	<b>-0.601</b>	<b>-0.625</b>	<b>-0.810</b>	<b>-0.668</b>	<b>0.680</b>	0.322	0.222	0.421	<b>-0.912</b>

Significant correlations ( $p < 0.05$ ) are written in bold numbers

TVC = total viable count; *Pseu.* = *Pseudomonas*; WBSF = Warner-Bratzler shear force

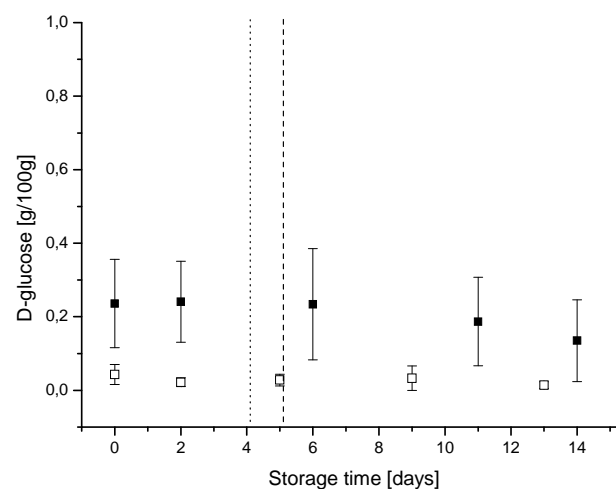
**Table 2.4: Correlations between intrinsic and microbiological parameters as well as sensory index for fresh poultry at 4°C**

	TVC	<i>Pseu. sp.</i>	pH	$a_w$	D-Glucose	L-Lactic acid	WBSF	Sensory index	Fat content
<i>Pseudomonas sp.</i>	<b>0.971</b>								
pH	<b>0.307</b>	<b>0.301</b>							
$a_w$	<b>-0.289</b>	<b>-0.317</b>	-0.115						
D-Glucose	<b>-0.412</b>	<b>-0.376</b>	<b>-0.813</b>	0.057					
L-Lactic acid	<b>-0.306</b>	<b>-0.302</b>	<b>-0.719</b>	0.070	<b>0.720</b>				
WBSF	-0.148	-0.170	0.020	0.130	-0.008	0.076			
Sensory index	<b>-0.905</b>	<b>-0.908</b>	<b>-0.270</b>	<b>0.353</b>	<b>0.380</b>	<b>0.269</b>	<b>0.265</b>		
Fat content	0.004	-0.398	-0.080	0.514	0.079	0.148	0.054	0.055	
Protein content	-0.280	-0.112	0.047	-0.199	-0.010	0.113	-0.099	-0.319	<b>-0.540</b>

Significant correlations ( $p < 0.05$ ) are written in bold numbers

TVC = total viable count; *Pseu.* = *Pseudomonas*; WBSF = Warner-Bratzler shear force

Decreasing D-glucose concentrations for pork as well as poultry were reported in earlier studies (Byun et al., 2003; Nychas & Tassou, 1997; Nychas et al., 1998). In this study D-glucose values for pork were also constantly decreasing during storage (about 0.101 g/100 g). For fresh poultry they were not constantly decreasing but lower at the end (0.014 g/100 g) than at the beginning (0.043 g/100g) (Figure 2.5). Differences between initial and end concentration were significant for both meat types ( $p < 0.05$ ).

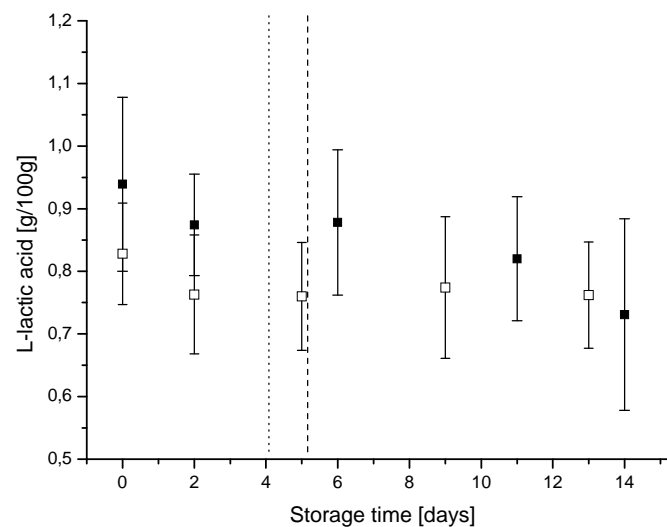


**Figure 2.5: Changes in mean values ( $\pm$  standard deviation) for D-glucose in fresh pork (■) and poultry (□) during storage at 4°C. (---) end of shelf life pork, (-·-) end of shelf life poultry**

Byun et al. (2003), Nychas & Tassou (1997) as well as Nychas (1998) reported lower initial and lower end values at a storage temperature of 3 – 4°C than observed in this study. For example, initial values were about twice as high for pork and four times higher for poultry than in the mentioned studies. But the values were comparable when considering the standard deviation in these investigations, which was not mentioned in the other studies. Especially for poultry, observed standard deviations were very high compared to the mean value, which can be attributed to a greater variability in poultry samples as well as their independence from each other. For poultry, at every sample point individual breast fillets were analysed, so that the greater variability between animals played a role. Pork chops analysed at each sample point were always from the same loin, thus the same pig. So, the changes of glucose content in loins from several animals were tracked during storage. However, the high variation in glucose content in meat is well known (Gill, 1983; Belitz et al., 2009). This is easily understood as the amount of D-glucose is strongly influenced by the condition of the animal before slaughter e.g. age, nutritional state, stress, exercise levels (Nychas et al., 1988; Gill, 1983; Dainty & Mackey, 1992; Belitz et al., 2009). The results of the study also show that glucose contents were significantly ( $p < 0.05$ ) lower for poultry than for pork (about ten times) which is consistent with the findings of Nychas & Tassou (1997) and Nychas et al. (1998). In the literature, the depletion of glucose in meat is related to the onset of spoilage (Boers et al., 1994; Nychas et al., 2008). However, only low significant correlations could be observed between the *Pseudomonas* sp. counts and the D-glucose concentration in this study (pork:  $r = -0.364$ ; poultry:  $r = -0.376$ ). Correlations between the TVC and D-glucose were in the low to medium range (pork:  $r = -0.560$ ; poultry:  $r = -0.412$ ). In contrast, Byun et al. (2003) reported high negative correlations ( $\geq -0.9$ ) between the total plate count as well as the psychotrophic plate count and D-glucose content but without giving information about the significance of these correlations. However, the results in this study show that the D-glucose concentration has only a minor influence on growth of *Pseudomonas* sp. and thus shelf life in fresh pork as well as in fresh poultry.

L-lactic acid values were also decreasing for both meat types. The decrease was not constant during storage, but end values were significantly lower ( $p < 0.05$ ) than initial values for both meat types (decrease: pork: 0.208 g/100 g; poultry: 0.066 g/100 g) which is consistent with data from other studies (Nychas & Tassou, 1997; Byun et al., 2003). However, differences between initial and end values in the literature are much higher for pork with 0.563 g/100 g (Byun et al., 2003) as well as poultry with 0.361 g/100 g (Nychas & Tassou, 1997). As for the amount of D-glucose, variations of L-lactic acid are usually present and influenced by the nutritional state as well as the handling of the animals before slaughtering (e.g. stress, exercise levels) (Nychas et al., 1988; Gill, 1983; Dainty & Mackey, 1992; Belitz et al., 2009).

In contrast to the glucose content, lactic acid values were in the same range for both meat types at all sample points when considering the standard deviation with no significant difference ( $p > 0.05$ ) at sample points IV and V (Figure 2.6).



**Figure 2.6:** Changes in mean values ( $\pm$  standard deviation) for L-lactic acid in fresh pork (■) and poultry (□) during storage at 4°C. (---) microbial end of shelf life pork, (· · ·) microbial end of shelf life poultry

The decrease during storage can be explained by the metabolism of *Pseudomonas* sp.: the bacteria utilise lactate after the depletion of glucose during storage (Nychas et al., 2008). The amount of lactic acid in the beginning of storage depends on the glycogen content at the time of slaughtering. After slaughter, the degradation of glycogen leads to the accumulation of lactic acid and a decrease of the meat pH to about 5.5 (Newton & Gill, 1981). These relations were confirmed in significant medium to high correlations between lactic acid and pH-value (pork:  $r = -0.667$ , poultry:  $r = -0.719$ ) in this study. However, correlations between *Pseudomonas* sp. count and lactic acid concentration were significant, but their magnitudes were low (pork:  $r = -0.505$ ; poultry:  $r = -0.302$ ) which is why the L-lactic acid concentration can be considered as having little relevance for the growth of *Pseudomonas* sp. and thus shelf life of fresh pork and poultry.

The  $a_w$ -value was comparable at all sample points for both meat types (around 0.990 - 0.992) and similar to the value of 0.993 reported by Rödel & Krispien (1977) and 0.99 reported by Ingham et al. (2009) for fresh meat. As for the pH-value, correlations with *Pseudomonas* sp. were significant but low (pork:  $r = 0.391$ ; poultry:  $r = -0.317$ ). The minimum  $a_w$ -value for growth of *Pseudomonas* sp. is determined at 0.97 (Singh & Anderson, 2004). With minimum measured  $a_w$ -values of 0.985 for pork and 0.986 for poultry, optimal growth conditions for *Pseudomonas* sp. existed during the whole study. Therefore, no influence on shelf life of the  $a_w$ -value can be assumed for fresh pork and poultry.

Because sensory characteristics are a valuable indicator of the shelf life status of fresh meat (as also proven by high and very high significant correlations between *Pseudomonas* sp. count and sensory index in this study (pork:  $r = -0.818$ ; poultry:  $r = -0.908$ )), the WBSF was investigated as an objective measurement for the sensory characteristic texture. No clear trend in the development of the WBSF could be observed during storage for both meat types. The average WBSF for pork was twice as high as for poultry at every sample point at a significance level of  $p < 0.05$ . No significant correlations could be found between the WBSF and the sensory index as well as between WBSF and microbial parameters for fresh pork. For fresh poultry a significant correlation was observed for WBSF and sensory index but the magnitude was very low ( $r = 0.265$ ). Measurement of WBSF is often used for evaluating tenderness of cooked meat (e.g. Cavitt et al., 2005; Brooks et al., 2009). In this study, raw meat was analysed whereas the natural heterogeneity of raw meat as a product complicated a standardised sampling with the cork borer, especially for poultry breasts. Thus, WBSF could not be related to microbial parameters and thus shelf life for both meat types in this study.

The average fat content was significantly ( $p < 0.05$ ) higher in pork ( $116.04 \pm 84.94$  g/kg) than in poultry ( $13.85 \pm 3.97$  g/kg), but the standard deviation was also larger for pork. This is likely to be due to the natural variation in fat content in pork. In this study, three pork loins had a fat content of more than 200 g/kg whereas the majority of the samples had a fat content  $< 100$  g/kg. Lambert et al. (1991) also reported higher fat contents for pork (6%) than for poultry (3%). Because the fat content was only analysed at sample point I, correlations could only be calculated for this point. For pork, several significant ( $p < 0.05$ ) medium to high correlations were observed (fat content with TVC, *Pseudomonas* sp., pH,  $a_w$  and D-glucose:  $r \geq |0.59|$ ), correlations in fresh poultry were substantially lower and not significant. Data of the study of Blickstad & Molin (1983) suggest that there is no difference in microbiological growth on fat and lean surfaces. In contrast, Gill & Newton (1980) reported a faster spoilage of moist adipose tissues but also stated that this is not of great importance for commercially chilled carcasses as drying of the surfaces according to normal good practice will prevent bacterial growth. Because of this and the fact that *Pseudomonas* sp. do not utilise fat as a growth substrate, no influence of the fat content on shelf life on fresh pork and poultry is assumed.

Protein content in pork was significantly different from protein content in fresh poultry ( $p < 0.05$ ) with  $208.33 \pm 24.35$  g/kg (pork) and  $237.73 \pm 6.69$  g/kg (poultry), respectively. Lambert et al. (1991) reported a slightly higher value for pork (22%) than for poultry (21%), but values are comparable to the ones obtained in this study. No significant correlations for bacterial counts and protein content in fresh poultry were observed. For pork, a medium significant correlation was obtained between protein content and *Pseudomonas* sp. count

( $r = -0.625$ ). According to Nychas et al. (2008) *Pseudomonas* sp. metabolise nitrogenous compounds (e.g. amino acids) as energy substrates not until the exhaustion of glucose, lactate and other low molecular substrates. At the point when nitrogenous compounds (e.g. amino acids) are utilised, overt spoilage in the form of off-odours and slime already occurs. Therefore, the protein content is not relevant for the shelf life of fresh meat.

In summary, several storage trials with pork chops and chicken breast fillets were conducted for the characterisation and comparison of the spoilage processes of fresh pork and poultry. Especially the relevant factors influencing the growth of *Pseudomonas* sp. as specific spoilage organism (SSO) of fresh pork and poultry were attempted to be identified. The findings of this study shall provide a more elementary and better understanding of the spoilage processes than has been researched up to the present, which gives a solid basis for the improvement of quality management and shelf life prediction in the food industry. The results showed that the growth of *Pseudomonas* sp. was clearly dependent on temperature, with faster growth at higher temperatures as described in the literature previously. *Pseudomonas* sp. grew more rapidly on fresh poultry than on fresh pork which led to shorter shelf lives at constant temperatures from 2 - 15°C for fresh poultry. The highly significant correlations ( $p < 0.05$ ) with the sensory index underlined the applicability of *Pseudomonas* sp. as a freshness indicator for pork and poultry. At almost all sample points during storage investigated intrinsic factors (pH-value,  $a_w$ -value, D-glucose, L-lactic acid, WBSF, fat content, protein content) were significantly different for fresh pork and poultry except for  $a_w$ -value at sample point V and L-lactic acid at sample points IV and V. More significant correlations were obtained between *Pseudomonas* sp. counts and intrinsic parameters in pork than in poultry with also mostly higher magnitudes in pork. But altogether, magnitudes were only very low to medium (pork:  $r \leq |0.625|$ ; poultry:  $r \leq |0.376|$ ) which indicates only minor influences of the investigated parameters on shelf life of fresh pork and poultry.

These results suggest that the incorporation of other factors than temperature in a common predictive shelf life model for fresh aerobically packed pork and poultry could be neglected. This is in agreement with the premise that models should be kept as simple as possible with a sufficient accuracy of prediction (Bernaerts et al., 2004; Zwietering & den Besten, 2010). Therefore, it is more important that shelf life models can precisely predict the growth of *Pseudomonas* sp. and hence shelf life under dynamic temperature conditions, because temperature was identified as most important influencing factor in this study and temperatures often vary greatly during transportation and storage of fresh meat (Nychas et al., 2008; Raab & Kreyenschmidt, 2008). For this reason, the investigation and comparison of the influence of dynamic temperature conditions on the growth of *Pseudomonas* sp. should be evaluated in further studies both for fresh pork and poultry.



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**CHAPTER 3**

**INFLUENCE OF COLD CHAIN INTERRUPTIONS  
ON THE SHELF LIFE OF FRESH PORK AND  
POULTRY**

### 3.1 Introduction

Temperature is the most important environmental influence factor on microbial growth and thus on shelf life of fresh meat as shown in chapter 2. The higher the temperature during transportation and storage, the faster the rate of microbial growth. To prevent spoilage and to prolong the shelf life of fresh meat it is important to maintain the cold chain during the entire supply chain of fresh meat. This is stipulated amongst others in the “Regulation on the hygiene of foodstuffs” (Regulation (EC) No 853/2004), which demands, that the cold chain is not interrupted. But “limited periods outside temperature control are permitted”.

Several investigations have shown that temperatures in real food supply chains often vary greatly from the mandatory or recommended temperature. For example, Raab & Kreyenschmidt (2008) recorded environmental temperatures in a truck during transportation in a German poultry supply chain ranging between  $-3^{\circ}\text{C}$  and  $+15^{\circ}\text{C}$  in the summer and between  $-2.5^{\circ}\text{C}$  and  $7.5^{\circ}\text{C}$  in the winter. In the study of Koutsoumanis et al. (2010) of a Greek milk chain, the temperature in the trucks ranged from  $+3.6^{\circ}\text{C}$  to  $+10.9^{\circ}\text{C}$  during transportation. To estimate the influence of these temperature variations on shelf life, the behaviour of *Pseudomonas* sp. as specific spoilage organism (SSO) of fresh meat (e.g. Gill & Newton 1977; Pooni & Mead, 1974; Coates et al., 1995; Raab et al., 2008) under dynamic temperature conditions is of substantial importance (Shimoni & Labuza 2000; Koutsoumanis et al., 2006; Nychas et al, 2008).

Several storage trials at fluctuating temperature conditions have been conducted during the last years. Koutsoumanis (2001) investigated the growth of *Pseudomonas* sp. on gilt-head sea bream under five different dynamic scenarios. In another study, the growth of several microorganisms (*Pseudomonas* sp., *Brochothrix thermosphacta*, lactic acid bacteria and *Enterobacteriaceae*) with different, periodically changing, temperature scenarios in ground pork was observed (Koutsoumanis et al., 2006). In both studies, the data were used for the validation of a predictive model. But microbiological growth, and thus shelf life, at fluctuating temperatures was not related to shelf life at a comparable constant storage temperature. Almonacid-Merino & Torres (1993) developed a computer-based tool which combined a microbiological growth model developed in liquid media and a heat transfer model. A simulation of dynamic temperature conditions predicted shelf life reductions of 20 – 30 %, if the fraction of the total storage time at an undesirable room temperature was only 2 – 3 %. These findings were confirmed by Simpson et al. (2003) who combined the shelf life model for modified atmosphere-packed (MAP) fish (Pacific Hake) of Dalgaard et al. (1997) with a heat transfer model and simulated temperature abuse conditions. Simulated storage at  $0^{\circ}\text{C}$  with 4 temperature abuses for 3 h at  $15^{\circ}\text{C}$  led to shelf life reductions of 3 days compared to storage at  $0^{\circ}\text{C}$ . This means, that 4.3 % of the total storage time with an abusive

temperature led to a shelf life reduction of 25 %. However, in both studies these findings were only predictions made by the model which were not validated by microbiological growth data in real food.

Therefore, the objective of the present study was to analyse the influence of cold chain interruptions on the growth of *Pseudomonas* sp., and thus on shelf life, focusing on a comparison of the influence of temperature abuses on fresh pork and poultry. Additionally, the effects of the duration of the abuse as well as the amplitude of abusive temperature at different time points during storage were analysed.

## 3.2 Material and methods

### 3.2.1 Sample description

Pork loins (*M. longissimus dorsi*) were purchased at a local butcher in Bonn, Germany and forwarded to the laboratory under temperature controlled conditions. Pork loins were cut with a knife into 150 - 200 g chops under sterile conditions. Chickens were slaughtered and divided into 150 - 170 g skinless chicken breast filets at a poultry slaughtering and processing plant in Germany. After processing, the filets were transported to a wholesaler under temperature-controlled conditions and forwarded to the laboratory. Each pork chop and each chicken breast fillet was packed into individual trays and over-wrapped with a low density polyethylene (LDPE) film (aerobic packaging). The time between slaughtering and the first investigation was 24 hours for both meat types.

### 3.2.2 Experimental design

Altogether four storage trials (A, B, C and D) were conducted under dynamic temperature conditions (Table 3.1). Each trial was conducted in the same way. Three batches of pork and three batches of poultry were stored with three different temperature scenarios:

- one control scenario with a constant storage temperature of 4°C (scenario 0)
- one dynamic scenario with a basic storage temperature of 4°C and temperature shifts to 7°C (scenario 1) and
- one dynamic scenario with a basic storage temperature of 4°C and temperature shifts to 15°C (scenario 2).

Temperature shifts in scenario 1 and 2 were made at the same point in time with the same duration in each trial. On the one hand, in trial A shifts were made continuously during storage, which means mainly in the exponential microbiological growth phase. On the other hand, in trials B – D shifts were conducted at the beginning of storage, i.e. mainly in the microbiological lag phase, with different numbers and durations of the shifts in the trials.

The effective temperatures of all scenarios in all 4 trials were similar with a maximum difference of 0.5°C. The fraction of the total storage time while an abusive temperature occurred was between 3.3 and 4.8 %.

**Table 3.1: Dynamic temperature scenarios for fresh pork and poultry**

Trial	Name of scenario	Description of scenario	Time at abusive temperature [%]	
			Pork	Poultry
<i>Continuous temperature abuse during storage (trial A)</i>				
Trial A	A0	Control (no shifts, 4°C constant)	0	0
	A1	4 shifts for 4 hours from 4°C to 7°C	4.8	4.8
	A2	4 shifts for 4 hours from 4°C to 15°C	4.8	4.8
<i>Temperature abuse at the beginning of storage (trial B, C and D)</i>				
Trial B	B0	Control (no shifts, 4°C constant)	0	0
	B1	3 shifts for 4 hours from 4°C to 7°C	4.1	3.9
	B2	3 shifts for 4 hours from 4°C to 15°C	4.1	3.9
Trial C	C0	Control (no shifts, 4°C constant)	0	0
	C1	2 shifts for 6 hours from 4°C to 7°C	3.3	3.9
	C2	2 shifts for 6 hours from 4°C to 15°C	3.3	3.9
Trial D	D0	Control (no shifts, 4°C constant)	0	0
	D1	1 shift for 12 hours from 4°C to 7°C	3.6	3.6
	D2	1 shift for 12 hours from 4°C to 15°C	3.6	3.6

In the first trial (trial A), four temperature shifts were performed with a duration of 4 h each. The shifts started after 48 h of storage with time periods around 2 days between one and the next shift and ended after 196 h of storage. The total time at an abusive storage temperature was 16 h (4.8 % of the total storage time). In the next three trials (trial B - D) temperature shifts were conducted only at the beginning of storage, which means they started and ended during the first 60 h of storage. Afterwards samples were stored at a constant temperature of 4°C until the end of the trials. The total time at an abusive temperature was 12 h in these three trials (3.3 – 4.1 % of the total storage time), but the trials varied in the number of shifts. In trial B three temperature shifts with a duration of 4 h each were made. In trial C two shifts lasting 6 h each were conducted whereas there was only one shift for 12 h in trial D.

All storage experiments were performed in high precision low temperature incubators (Sanyo model MIR 153; Sanyo Electric Co., Ora-Gun, Gumma, Japan). The air temperature within the incubators was controlled by data loggers every 5 minutes (ESCORT JUNIOR Internal Temperature Data Logger; Escort, New Zealand). During storage, samples of pork and poultry were analysed for the total viable count (TVC), the number of *Pseudomonas* sp. and sensory changes at appropriate time intervals. Every measurement was repeated 3 times.



### 3.2.3 Sample preparation and microbiological analysis

For the microbiological analysis, a representative product sample of 25 g was transferred to a stomacher-bag and filled with saline peptone diluents (0.85 % NaCl with 0.1 % peptone; Oxoid, Basingstoke, United Kingdom) up to a final weight of 250 g. The contents were homogenised using a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. A 10-fold dilution series of the homogenate was prepared using saline peptone diluents. TVC was determined by the pour plate technique on Plate Count Agar (Merck, Darmstadt, Germany) after incubation at 30°C for 72 h. Levels of *Pseudomonas* sp. were determined by the spread plate technique using Pseudomonas Agar Base (Oxoid Basingstoke, United Kingdom) plus CFC supplement (Oxoid, Basingstoke, United Kingdom). Petri dishes were aerobically incubated at 25°C for 48 h.

### 3.2.4 Sensory analysis

Sensory characteristics of each sample were assessed by a trained sensory panel. Odour, texture and colour were evaluated using a 3-point-scoring-system from very good (3) to unacceptable (1). A weighted sensory index (SI) was calculated using equation 3.1. Sensory acceptance was described as a function of time by linear regression. The meat was considered “spoiled” when the SI reached 1.8 (Kreyenschmidt, 2003).

$$SI = \frac{2 \cdot C + 2 \cdot O + 1 \cdot T}{5} \quad (3.1)$$

with: SI: sensory index, C: colour, O: odour, T: texture.

### 3.2.5 Statistical analysis

The growth data from the enumeration of TVC and *Pseudomonas* sp. were fitted using nonlinear regression (Levenberg-Marquardt algorithm) by the statistical software package Origin 8.0G (OriginLab Corporation, Northampton, USA). The Gompertz model was used to describe the growth of microorganisms with time (equation 3.2) (Gibson et al., 1987):

$$N(t) = A + C \cdot e^{-e^{-B \cdot (t-M)}} \quad (3.2)$$

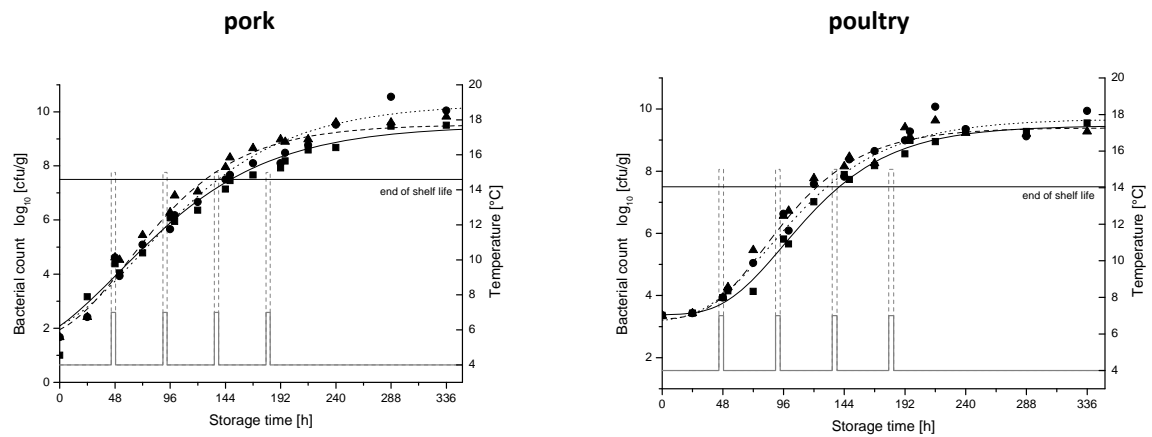
with N(t): microbial count [ $\log_{10}$  cfu/g] at time t, A: lower asymptotic line of the growth curve (initial bacterial count), C: difference between upper asymptotic line of the growth curve ( $N_{\max}$ = maximum population level) and the lower asymptotic line; B: relative growth rate at time M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

The observed shelf life was defined by the time t when the *Pseudomonas* sp. reached 7.5  $\log_{10}$  cfu/g which was determined as the spoilage level in chapter 2.

### 3.3 Results and Discussion

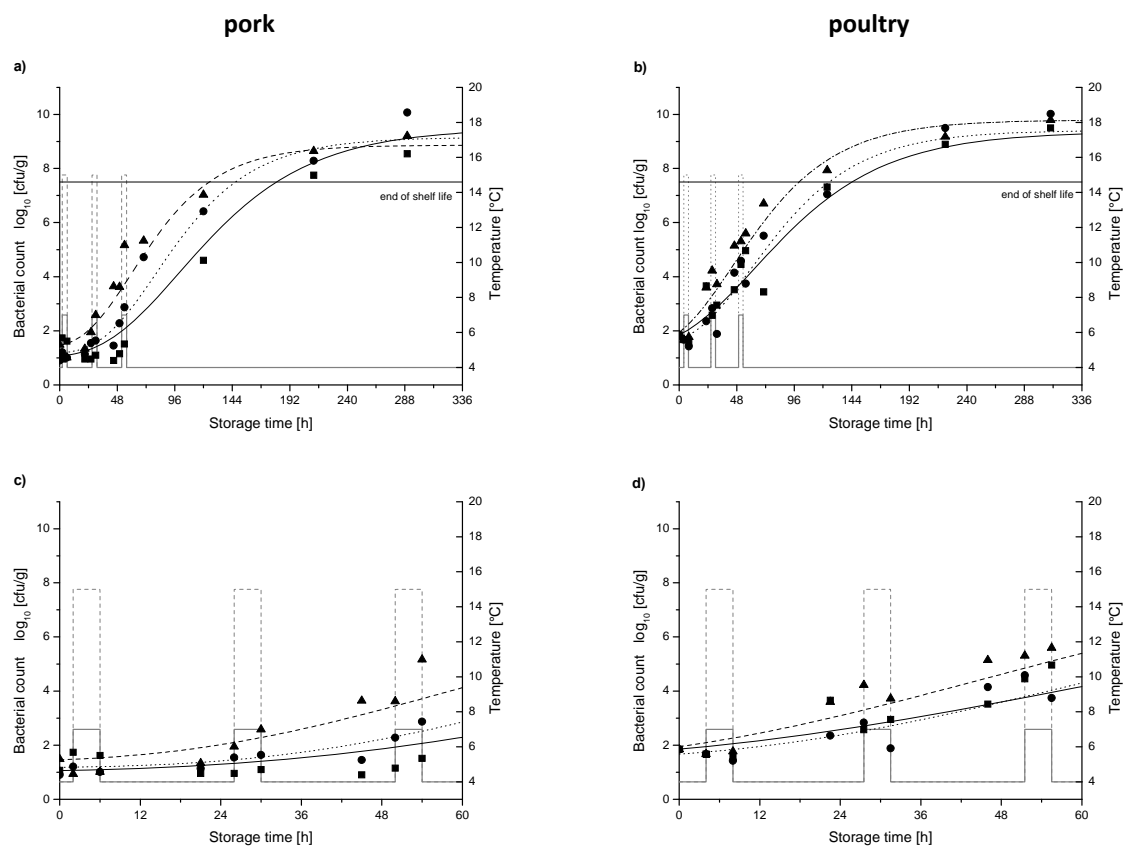
In all conducted storage trials changes in TVC and *Pseudomonas* sp. counts were comparable for all constant and dynamic temperature scenarios for fresh poultry (data not shown). For pork, greater differences were observed at the beginning of storage, but the counts of *Pseudomonas* sp. rapidly converged to the TVC when the exponential growth phase started as has already been reported (Gill & Newton, 1982; Blickstad & Molin, 1983; Coates et al., 1995; Lebert et al., 2000; Olsson et al., 2003; Lebert et al., 2005). Furthermore, significant ( $p < 0.05$ ) high correlations were observed between the count of *Pseudomonas* sp. and the sensory index ( $r > -0.85$ ) at all conducted dynamic temperature scenarios. This confirmed the applicability of *Pseudomonas* sp. as freshness indicator also under dynamic temperature conditions. Therefore, only the counts of *Pseudomonas* sp. are shown and discussed in this chapter.

Figure 3.1 shows the growth of *Pseudomonas* sp. on fresh pork and on fresh poultry for all three temperature scenarios in storage trial A. Independent of the temperature scenario and the initial bacterial count, the maximum population density of *Pseudomonas* sp. was in the same range for both meat types with values between 9.5 and 10.0  $\log_{10}$  cfu/g for fresh pork and between 9.3 and 9.9  $\log_{10}$  cfu/g for fresh poultry. Similar maximum bacterial counts were observed in trials B – D for both meat types. These values were also in agreement with those obtained at constant storage temperatures (chapter 2). The absence of a relationship between the maximum bacterial count and temperature as well as initial bacterial count was confirmed by the results of earlier studies (Giannuzzi et al., 1998; Koutsoumanis, 2001; Fujikawa et al., 2004). During storage, *Pseudomonas* sp. counts were comparable at almost all sample points of trial A for both meat types, with a maximum difference between the three scenarios of 1.1  $\log_{10}$  cfu/g for pork and 1.3  $\log_{10}$  cfu/g for poultry.



**Figure 3.1:** Growth of *Pseudomonas* sp. in trial A fitted with the Gompertz model: a) on pork, b) on poultry; (■ —) scenario A0 at 4°C constant, (● ... ) scenario A1 with shifts to 7°C, (▲ --) scenario A2 with shifts to 15°C (solid grey line: temperature profile A1, dashed grey line: temperature profile A2).

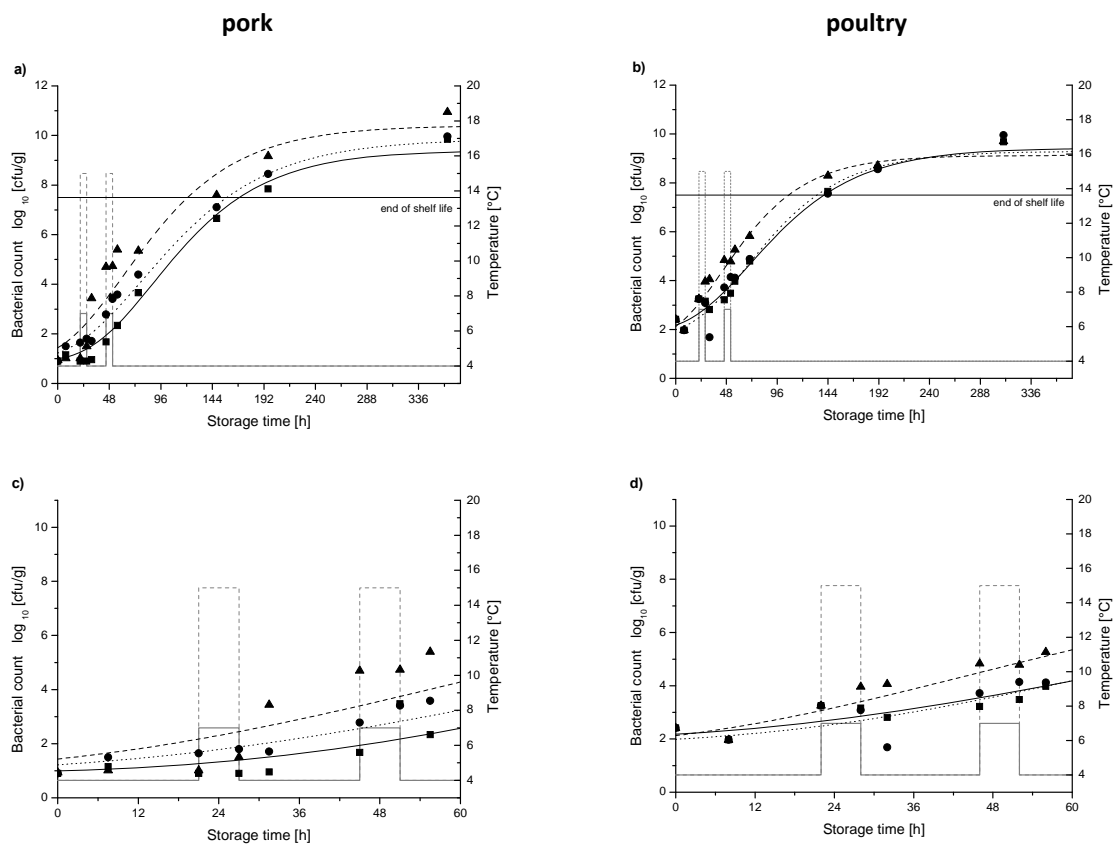
Growth of *Pseudomonas* sp. on pork and poultry in trials with abusive temperature periods in the first 60 h of storage (trial B – D) are shown in Figure 3.2 – 3.4.



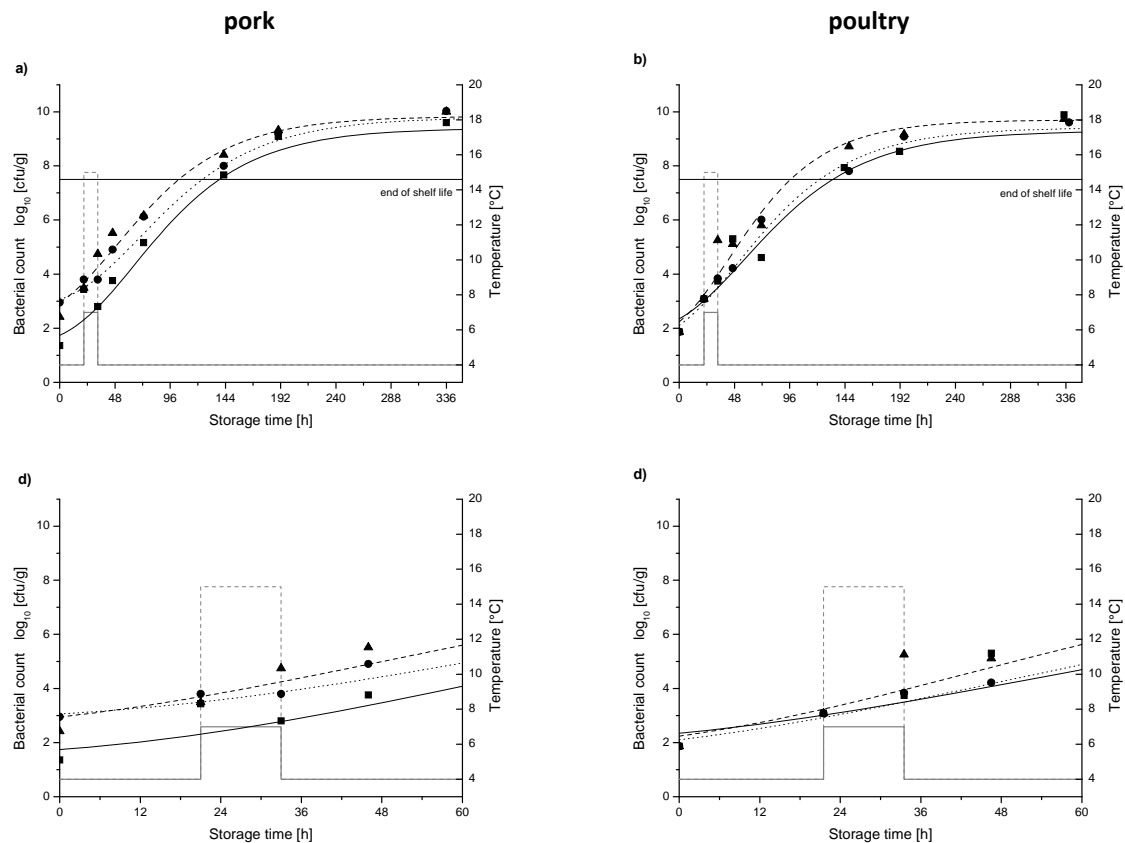
**Figure 3.2:** Growth of *Pseudomonas* sp. in trial B fitted with the Gompertz model on pork (left) and poultry (right), a) and b): during the complete storage, c) and d): during the first 60 h of storage; (■ —) scenario B0 at 4°C constant, (● ... ) scenario B1 with shifts to 7°C, (▲ --) scenario B2 with shifts to 15°C (solid grey line: temperature profile N1, dashed grey line: temperature profile B2).

In trial B, the *Pseudomonas* sp. counts increased faster in scenario B2 (shifts to 15°C) than in scenario B1 (shifts to 7°C) in the first 60 h for fresh pork and poultry. This observation was

more pronounced for fresh pork than for fresh poultry, which is possibly due to the greater variability of initial counts in the single poultry samples. At each sample point individual poultry breast fillets were analysed, but pork chops were always from the same loin, thus from the same pig for each scenario. Additionally, counts of *Pseudomonas* sp. in both dynamic scenarios (B1 and B2) increased faster than in the control scenario at 4°C (scenario B0) for fresh pork. For fresh poultry, counts in scenarios B1 and B0 were close together. In trial C and D, the same changes for *Pseudomonas* sp. counts at the different scenarios were observed for fresh pork and poultry. However, the counts in all three scenarios converged again when approaching the end of shelf life (7.5 log<sub>10</sub> cfu/g) in trial B, C and D.



**Figure 3.3:** Growth of *Pseudomonas* sp. in trial C fitted with the Gompertz model on pork (left) and poultry (right), a) and b): during the complete storage, c) and d): during the first 60 h of storage; (■ —) scenario C0 at 4°C constant, (● ...) scenario C1 with shifts to 7°C, (▲ --) scenario C2 with shifts to 15°C (solid grey line: temperature profile C1, dashed grey line: temperature profile C2).



**Figure 3.4: Growth of *Pseudomonas* sp. in trial D fitted with the Gompertz model on pork (left) and poultry (right), a) and b): during the complete storage, c) and d): during the first 60 h of storage; (■ —) scenario D0 at 4°C constant, (● ...) scenario D1 with shifts to 7°C, (▲ - -) scenario D2 with shifts to 15°C (solid grey line: temperature profile D1, dashed grey line: temperature profile D2).**

Shelf life for pork at control scenarios revealed differences up to 42 h (scenario A0, B0, C0 and D0) which could be mainly attributed to the long shelf life in scenario B0 (Table 3.2). The shelf life of fresh poultry was comparable for all control scenarios at 4°C (133.5 - 140.2 h). One possible explanation are the different processes of different pork suppliers. Whereas all the poultry breast fillets came from the same slaughtering and processing plant in this study, pork loins were indeed purchased from the same local butcher, but the butcher was supplied with pork meat from different slaughterhouses and cutting plants. According to Augustin & Minvielle (2008), the contamination of pork loins with different bacteria is varying and depends mainly from the cutting plant. Other bacteria present on the pork loins after slaughtering and cutting could have suppressed *Pseudomonas* sp. at the beginning of storage in trial B, which led to a slower growth in the beginning and thus to a longer shelf life. Additionally, there was no information available about the time-temperature history during storage and transportation of the pork loins within the first 24 h which can also differ between the various slaughterhouses and cutting plants and result in varying shelf lives. As in the different constant storage scenarios in chapter 2, shelf life of fresh pork was always longer than shelf life of fresh poultry in the constant control scenarios.

**Table 3.2: Calculated shelf life times and shelf life reductions for fresh pork and fresh poultry in different dynamic storage trials**

Storage trial	Scenario <sup>a)</sup>	Number of shifts	Pork			Poultry		
			Shelf life <sup>b)</sup>	Shelf life reduction <sup>c)</sup>	Shelf life reduction	Shelf life	Shelf life reduction	Shelf life reduction
			[h]	[h]	[%]	[h]	[h]	[%]
<i>Continuous temperature abuse during storage (trial A)</i>								
Trial A	A0	0	148.6	-	-	140.0	-	-
	A1	4	144.2	4.4	3.0	130.5	9.5	6.8
	A2	4	126.5	22.1	14.9	122.4	17.6	12.6
<i>Temperature abuse in the beginning of storage (trial B, C and D)</i>								
Trial B	B0	0	180.9	-	-	138.4	-	-
	B1	3	146.6	34.3	19.0	125.0	13.4	9.7
	B2	3	124.7	56.2	31.1	100.0	38.4	27.7
Trial C	C0	0	169.1	-	-	140.2	-	-
	C1	2	157.5	11.6	6.9	133.5	6.7	4.8
	C2	2	121.1	48.0	28.4	106.7	33.5	23.9
Trial D	D0	0	138.9	-	-	133.5	-	-
	D1	1	124.0	14.9	10.7	122.1	11.4	8.5
	D2	1	103.5	35.4	25.5	102.9	30.6	22.9

Shelf life was estimated from time point zero of the laboratory investigations, which means 24 h after slaughtering.

<sup>a)</sup> Scenarios as described in Table 3.1

<sup>b)</sup> Evaluated by count of *Pseudomonas* sp.: End of shelf life: 7.5 log<sub>10</sub> cfu/g

<sup>c)</sup> In relation to shelf life at 4°C (Scenario 0 in each trial)

In trial A with continuous temperature abuses during the entire storage time, shelf life reductions for fresh pork as well as for poultry were comparable. Whereas the shifts to 7°C (scenario A1) led to minor shelf life reductions of 4.4 and 9.5 h, respectively, shifts to 15°C (scenario A2) led to reductions of 22.1 for fresh pork and 17.6 h for fresh poultry. Altogether, shelf life reductions were less than a day for both meat types at all dynamic scenarios in this trial (< 15 %).

The comparison of shelf life reductions in trials B – D showed that the absolute reductions were up to 20.9 h higher for pork than for poultry (scenario B1). But this resulted in comparable relative reductions of less than 5 % with the exception of scenario B1: (9.3 %). Altogether, the shifts to 7°C in the first 60 h of storage resulted in shelf life reductions of less than 15 h (less than 11 %) for fresh pork and for fresh poultry except for pork at scenario B1 with 34.3 h. But the shifts to an abusive temperature of 15°C always reduced the shelf life by more than 30 h (> 20 %) for fresh pork as well as for fresh poultry, even if the storage time with this abusive temperature was less than 5 % of the total storage time. These findings are in agreement with the predictions reported by Almonacid-Merino & Torres (1993) and Simpson et al. (2003). In both studies, temperature abuses during storage were simulated and shelf lives predicted by a model. The predictions showed shelf life reductions of over 20 % induced by storage times with an abusive temperature lasting less than 5 % of the total storage time.

In this study, shifts to 15°C led to higher shelf life reductions than shifts to 7°C for both meat types in all conducted storage trials, which was expected as microbial growth is faster with

increasing temperatures (Barnes, 1976; Baranyi et al., 1995; Moore & Sheldon, 2003; Kreyenschmidt et al., 2010).

When the shifts to 7°C were conducted within the first 60 h of storage, no clear trend could be observed regarding the influence of the number of temperature shifts (one, two or three) on shelf life for both meat types. But such a trend was observed for the shifts to 15°C for both meat types: the larger the number of shifts, the higher was the shelf life reduction for pork as well as poultry. Shelf life reduction caused by three temperature shifts to 15°C was more than 5 % higher than reduction caused by just one temperature shift, even if the overall time at an abusive temperature was the same (12 h).

Shelf lives of control scenarios determined by sensory characteristics were mainly in good agreement with the microbial determined shelf lives (difference less than 23 h) which was already observed at different constant storage temperatures (Chapter 2). Exceptions were the scenarios B0 and C0 for fresh poultry with higher differences. In the dynamic scenarios, the sensory indices often decreased rapidly and linear at the beginning which was followed by a slow and long decrease until the end of storage for both meat types. This led to large discrepancies in shelf life times from the real observed sensory shelf life when the decrease of the sensory index was described linearly during the entire storage period. The fast decrease at the beginning was mainly caused by a change of colour which could be due to the change of the oxymyoglobin to metmyoglobin in fresh meat. Whereas the oxymyoglobin is responsible for the red colour which is associated with fresh meat, metmyoglobin is responsible for an undesirable brown colour. With increasing temperatures, the stability of oxymyoglobin decreases and thus discolourations occur (Lawrie, 1998; Belitz, 2009).

Altogether the results of the study showed that short temperature abuses during storage and distribution of fresh pork and poultry led to remarkable shelf life reductions, especially when temperature abuses took place at the beginning of storage (in the first 60 h of storage). Reductions of up to two days were observed for both meat types even when the time with an abusive temperature was less than 5 % of the total storage time. Absolute shelf life reductions were just slightly higher for fresh pork than for fresh poultry with a maximum difference of 20.9 h and a minimum difference of 3.5 h between both meat types. Reductions were comparable, with a difference of less than < 10 % at each trial, when considering the relative shelf life reductions.

In summary, this study revealed similar spoilage patterns for fresh pork and fresh poultry under dynamic temperature conditions, even though only short temperature abuses simulating cold chain interruptions in real meat chains were conducted. As temperature has already been identified as the main influencing factor on the growth of *Pseudomonas* sp. and thus shelf life of fresh pork and fresh poultry (chapter 2), the development of a common

predictive shelf life model for the estimation of shelf life under different temperature conditions based on the growth of *Pseudomonas* sp. is thinkable. Additionally, the results emphasize the need for a continuous temperature monitoring as well as the exchange of temperature data in meat supply chains to obtain the complete temperature history of the meat because this is a prerequisite for the prediction of remaining shelf life as already highlighted by Raab et al. (2010).

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## **CHAPTER 4**

# **MODEL FOR SHELF LIFE PREDICTION AS A TOOL FOR QUALITY MANAGEMENT IN PORK AND POULTRY CHAINS**

## 4.1 Introduction

Unexpected spoilage of meat can lead to food waste and thereby economic losses as well as the loss of consumer confidence (Nychas et al., 2007). Thus, the exact definition of shelf life and remaining shelf life is of high relevance for companies at all stages of the meat supply chain. It allows them to optimise their storage management and thus to reduce these losses by the supply of meat of high quality and with an adequate shelf life (Koutsoumanis et al., 2005; Kreyenschmidt et al., 2008; Raab et al., 2008).

But the determination of microbial growth and thereby shelf life with traditional microbiological challenge tests is expensive and time-consuming. An alternative is the concept of predictive microbiology, which uses mathematical models to predict microbiological growth and thus to estimate shelf life (McMeekin, 1993; Walker, 1994; Roberts, 1995; Whiting, 1995; McMeekin & Ross, 1996). The successful development of such a shelf life model requires a detailed knowledge of the spoilage process based on the growth of the specific spoilage organism (SSO) that is responsible for spoilage within a certain range of environmental conditions (McMeekin et al., 1993; Blackburn, 2000; Gram & Dalgaard, 2002; Gram et al., 2002). Especially, information is required concerning the population level of the SSO at which spoilage occurs (spoilage level) and the range of environmental conditions over which a particular SSO is responsible for spoilage (Dalgaard, 1995). The growth of the SSO and thus the validation of the model under dynamic temperature conditions is also important (Shimoni & Labuza, 2000; Nychas et al., 2008), since temperatures usually vary in real food supply chains (Raab & Kreyenschmidt, 2008; Koutsoumanis et al., 2010). Additionally, the validation should be conducted in real food products because models based on microbiological growth data collected in laboratory media often overestimate microbiological growth in real food (Pin et al., 1999)

Several predictive models have been developed in recent years, but most of them were based on and validated with microbiological growth data resulting from experiments in laboratory media (e.g. Baranyi et al., 1995; Mitchell et al., 1994, 1995). Only a few were validated using real meat and meat products under dynamic temperature conditions (Neumeyer et al., 1997a, b; Bovill et al., 2000). Models which were developed using microbiological growth data generated in real meat and meat products as well as validated in real meat and meat products under dynamic temperature conditions are rare. Examples are the model of Koutsoumanis et al. (2006) for the growth of *Pseudomonas* sp. in ground meat, the model of Gospavic et al. (2008) for *Pseudomonas* sp. in poultry and the models of Mataragas et al. (2006) as well as Kreyenschmidt et al. (2010a) for lactic acid bacteria in modified atmosphere-packed (MAP) cooked sliced ham. All these models delivered good predictions compared to observations under non-isothermal temperature conditions. But

they were only developed and validated for just one type of meat or meat product. No predictive models exist that are applicable to estimate the shelf life of different types of fresh meat (fresh pork and fresh poultry) and that have been validated under dynamic temperature conditions.

Therefore, the objective of the present study was to develop a common predictive shelf life model that is generally applicable for fresh pork as well as for fresh poultry, based on the growth of *Pseudomonas* sp. as SSO. The temperature dependency at constant storage temperatures was determined with the data presented in chapter 2. Microbiological growth data of previous investigations (Raab et al., 2008, chapter 3) was used to validate the model under dynamic temperature conditions.

## 4.2 Materials and methods

### 4.2.1 Experimental description

Storage trials with pork chops (150 – 200 g) and chicken breast fillets (150 – 170 g) were conducted under aerobic conditions at different isothermal storage temperature scenarios (2, 4, 7, 10 and 15°C) as described previously (chapter 2). The count of *Pseudomonas* sp. was measured in pork and poultry samples at appropriate time intervals. A detailed description for the preparation of the samples and the microbiological is given chapter 2 and 3.

For the validation of the model under non-isothermal temperature conditions, a scenario with periodically changing temperatures of previous investigations was used (Raab et al., 2008). The temperature cycle was 4 h at 12°C, 8 h at 8°C and 12 h at 4°C and the scenario was named trial E in this study. Additionally, growth data of *Pseudomonas* sp. at different non-isothermal temperature scenarios with short temperature abuses from chapter 3 were used (trial A, B, C and D). These trials consisted of three scenarios: one control scenario at a constant storage temperature of 4°C (scenario 0) as well as two dynamic temperature scenarios with a basic storage temperature at 4°C and short temperature shifts to 7°C (scenario 1) and 15°C (scenario 2), respectively. The trials differed in the number and duration of temperature shifts in scenario 1 and 2. All dynamic scenarios used for the validation of the model are listed in Table 4.1).

**Table 4.1: Non-isothermal temperature scenarios used for model validation (modified after chapter 3)**

Trial	Scenario <sup>a)</sup>	Description	Source
Trial A	A1	4 shifts for 4 hours from 4°C to 7°C	Chapter 3
	A2	4 shifts for 4 hours from 4°C to 15°C	Chapter 3
Trial B	B1	3 shifts for 4 hours from 4°C to 7°C	Chapter 3
	B2	3 shifts for 4 hours from 4°C to 15°C	Chapter 3
Trial C	C1	2 shifts for 6 hours from 4°C to 7°C	Chapter 3
	C2	2 shifts for 6 hours from 4°C to 15°C	Chapter 3
Trial D	D1	1 shift for 12 hours from 4°C to 7°C	Chapter 3
	D2	1 shift for 12 hours from 4°C to 15°C	Chapter 3
Trial E	E	4h at 12°C, 8h at 8°C and 12h at 4°C (cycle)	Raab et al. (2008)

<sup>a)</sup> Trial A–D additionally included a control scenario (A0, B0, C0 and D0) with a constant storage temperature of 4°C.

#### 4.2.2 Statistical analysis and modelling

The growth data of *Pseudomonas* sp. were fitted using nonlinear regression (Levenberg-Marquardt algorithm) by the statistical software package Origin 8.0G (OriginLab Corporation, Northampton, USA). The Gompertz model was used as the primary model to describe the growth of microorganisms with time (equation 4.1) (Gibson et al., 1987):

$$N(t) = A + C \cdot e^{-B \cdot (t-M)} \quad (4.1)$$

with N(t): microbial count [ $\log_{10}$  cfu/g] at time t, 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 growth rate at time M [1/h], M: time at which maximum growth rate is obtained (reversal point), t: time [h].

The influence of temperature on the relative growth rate  $B$  at time  $M$  was assessed by using the Arrhenius equation as secondary model. Therefore the function in equation 4.2 was fitted to the  $B$  values, which were obtained with the Gompertz function at different constant temperature scenarios.

$$\ln(B) = \ln F - \left( \frac{E_a}{R \cdot T} \right) \quad (4.2)$$

with B: relative growth rate at time M [1/h], F: pre-exponential factor [1/h],  $E_a$ : activation energy for bacterial growth [kJ/mol], R: gas constant [8.314 J/mol K], T: absolute temperature [K]

The accuracy of the fits was evaluated with the adjusted coefficient of determination ( $\overline{R^2}$ ). The variable  $\overline{R^2}$  is written as  $R^2$  in the following text.

As described by Kreyenschmidt et al. (2010a) the primary and the secondary model were combined to predict the growth of *Pseudomonas* sp. under non-isothermal conditions. The combined model predicts the microbial growth within intervals. Therefore, the time-temperature history of the product was divided into several assumed time-temperature

intervals with constant storage temperature. Microbial growth could then be predicted by using the Gompertz function. Therefore, besides  $B$  the unknown parameters  $A$ ,  $C$  and  $M$  had to be determined. The parameter  $C$  was calculated from the maximum bacterial count ( $N_{\max}$ ) with equation 4.3:

$$C = N_{\max} - A \quad (4.3)$$

$N_{\max}$  was set to the mean of the observed values for constant temperature scenarios: 10.0  $\log_{10}$  cfu/g for pork and 9.8  $\log_{10}$  cfu/g for poultry. The observed initial bacterial counts ( $N_0 = A$ ) of the dynamic scenarios varied greatly in this study (pork: 0.9 – 3.0  $\log_{10}$  cfu/g, poultry: 1.9 – 4.1  $\log_{10}$  cfu/g). Therefore,  $N_0$  was set to the real observed initial bacterial count in each scenario.

Because the observed bacterial count was lower for the second sample point than for the first in some scenarios, the starting point for these scenarios was computed from the minimal observed bacterial count with the Gompertz model by inserting  $N_{\min}$  and  $t_{\min}$  from sample point two of the observations (equation 4.4).

$$A = N_{\min} - C \cdot e^{-e^{-B \cdot (t_{\min} - M)}} \quad (4.4)$$

with  $N_{\min}$ : minimum microbial count [ $\log_{10}$  cfu/g] at time  $t_{\min}$ ,  $A$ : lower asymptotic line of the growth curve ( $N_0 =$  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  $M$  [1/h],  $M$ : time at which maximum growth rate is obtained (reversal point),  $t$ : time [h].

In every interval a new reversal point  $M$  had to be calculated as current microbial counts and thus  $M$  are changing with fluctuating temperatures due to the accelerated or decelerated microbial growth.  $M$  could be computed from the model parameters for the interval by converting the Gompertz formula. With every new  $M$  the bacterial count at the end of the interval  $N(t_e)$  could be calculated.

For the first interval in the non-isothermal temperature scenarios a proper  $M$  was derived from the linear regression of  $M$  against temperature in the isothermal experiments. With this  $M$  and  $B$ , the bacterial count at the end of the first interval could be calculated. The further interval calculations were conducted with the equations 4.5 and 4.6:

$$N(t_e) = A + C \cdot e^{-B_T \cdot (t-M)} \quad (4.5)$$

with  $N(t_e)$ : the bacterial count [ $\log_{10}$  cfu/g] at the end of the interval;  $e = 1 \dots n$ : number of intervals;  $A$ : 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  $A$ ,  $B_T$ : relative growth rate estimated by secondary modelling [1/h],  $M$ : reversal point computed for the interval,  $t$ : time [h].

$$M = \frac{\ln(-\ln(\frac{N(t_{e-1}) - A}{C}))}{B_T} + t \quad (4.6)$$

with  $M$ : reversal point computed for the interval,  $N(t_{e-1})$ : the bacterial count [ $\log_{10}$  cfu/g] at the end of the previous interval,  $e = 1 \dots n$ : number of intervals;  $A$ : 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  $A$ ,  $B_T$ : relative growth rate estimated by secondary modelling,  $t$ : time [h].

An absence of intermediate lag phases at temperature changes was assumed because a new adaptation phase is only necessary when large temperature shifts occur which are outside the normal physiological growth range of the respective microorganism (Ng et al. 1962; Bovill et al., 2000; Bernaerts et al., 2002).

Furthermore, adjustments of the model parameters had to be made for *Pseudomonas* sp. data of fresh poultry at trial A, B, C and D. These will be presented and explained in detail in the results and discussion section.

Bias factor ( $B_f$ ) and accuracy factor ( $A_f$ ) (equations 4.7 and 4.8) were determined according to Ross (1996) to evaluate the precision of the model by comparing predicted and observed microbial counts.

$$B_f = 10^{(\sum \log(\text{predicted}_i / \text{observed}_i) / n)} \quad (4.7)$$

with  $\text{observed}_i$ : observed values,  $\text{predicted}_i$ : predicted values,  $n$ : number of observations.

$$A_f = 10^{(\sum |\log(\text{predicted}_i / \text{observed}_i)| / n)} \quad (4.8)$$

with  $\text{observed}_i$ : observed values,  $\text{predicted}_i$ : predicted values,  $n$ : number of observations.

Bias and accuracy factor for the model were calculated with the predicted and observed values of *Pseudomonas* sp. count. If the bias factor is 1.00, the model shows an exact agreement with the observed microbiological count. An underestimation of microbial counts would lead to a bias factor above 1.00, an overestimation to a bias factor below 1. As for the bias factor, an accuracy factor of 1.00 shows a perfect agreement between observed and



predicted values. The larger the accuracy factor, the less accurate the mean values which are estimated (Ross 1996).

### 4.3 Results and discussion

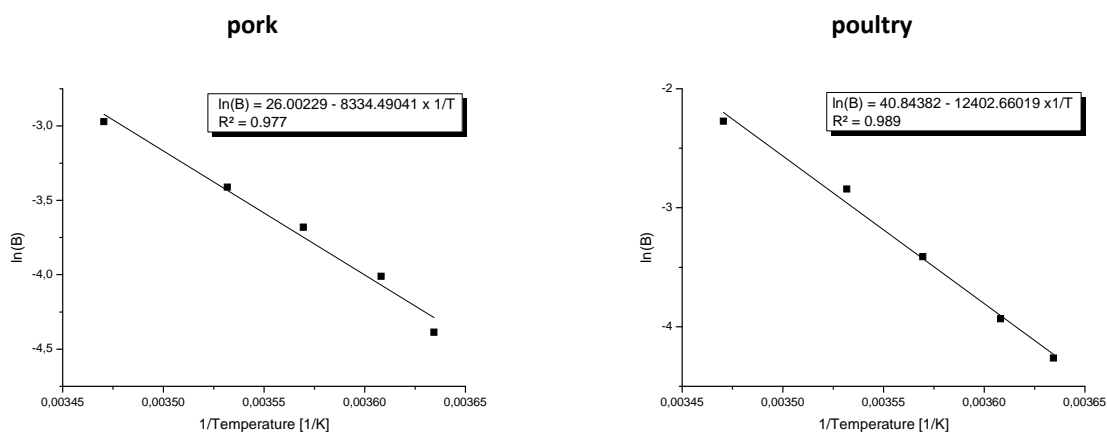
The calculated relative growth rates of *Pseudomonas* sp. on fresh pork and poultry obtained with the Gompertz model as well as the accuracy of the Gompertz fits at constant storage temperatures from 2 – 15°C are listed in Table 4.2. Growth of *Pseudomonas* sp. was described well with the Gompertz function which can be seen in  $R^2$  values of  $\geq 0.926$ . As expected,  $B$  (the relative growth rate at time  $M$ ) was increasing with increasing temperatures.  $B$  values were higher for poultry as for pork in every constant temperature scenario. Additionally, values were increasing faster for poultry than for pork.

**Table 4.2: Growth parameters obtained with the Gompertz model for *Pseudomonas* sp. on fresh pork and poultry at different isothermal storage temperatures**

Temperature [°C]	Pork		Poultry	
	B [1/h]	$R^2$	B [1/h]	$R^2$
2	0.012	0.955	0.014	0.941
4	0.018	0.970	0.020	0.971
7	0.025	0.942	0.033	0.926
10	0.033	0.965	0.058	0.960
15	0.051	0.967	0.103	0.961

$B$  = relative growth rate at time  $M$  (reversal point),  $R^2$  = adjusted coefficient of determination

Figure 4.1 shows the  $\ln(B)$  values versus  $1/T$  for fresh pork and poultry modelled with the Arrhenius equation. The Arrhenius equation described the temperature dependency well as shown by  $R^2$  values of 0.977 (pork) and 0.989 (poultry).



**Figure 4.1: Modelling temperature dependency of the relative growth rate  $B$  with the Arrhenius equation for fresh pork (left) and fresh poultry (right)**

The linear fit of  $M$  values obtained at isothermal temperatures against temperature is shown in Figure 4.2.  $R^2$  values of 0.974 (pork) and 0.943 (poultry) show a good linear description of

the temperature dependency which enables the calculation of an adequate  $M$  value for the first interval in non-isothermal storage scenarios.

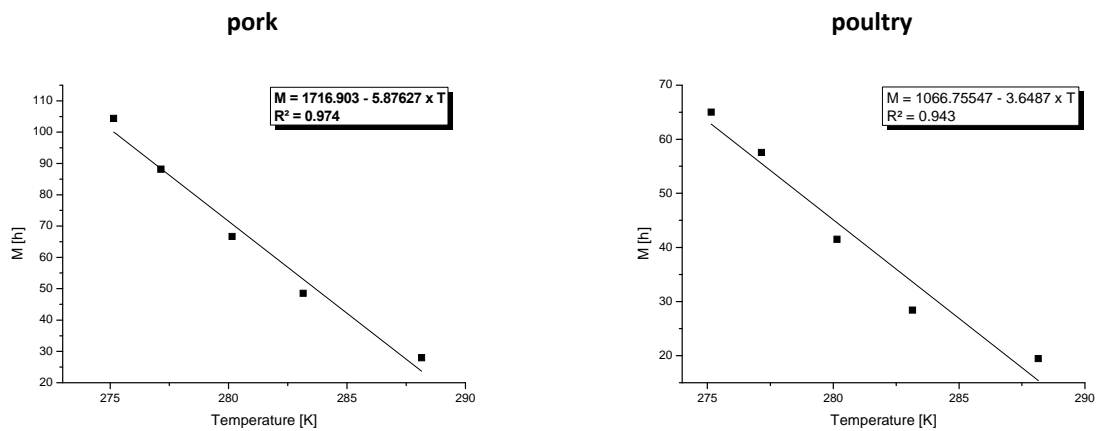


Figure 4.2: Linear fit of reversal point  $M$  against temperature for fresh pork (left) and fresh poultry (right)

$B$  and  $M$  values for poultry could be related to pork values by linear fitting (Figure 4.3). The fits were good with  $R^2$  values of 0.979 (for  $B$ ) and 0.998 (for  $M$ ) which made it possible to predict the growth of *Pseudomonas* sp. for fresh poultry based on the kinetics of fresh pork.

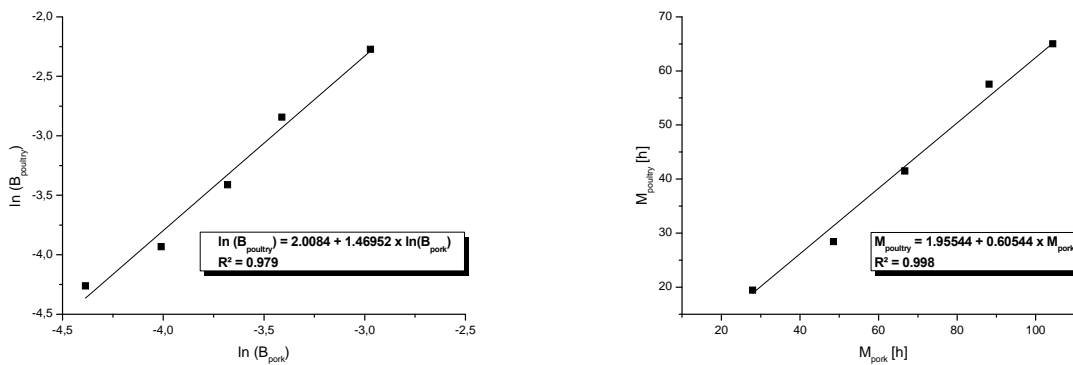
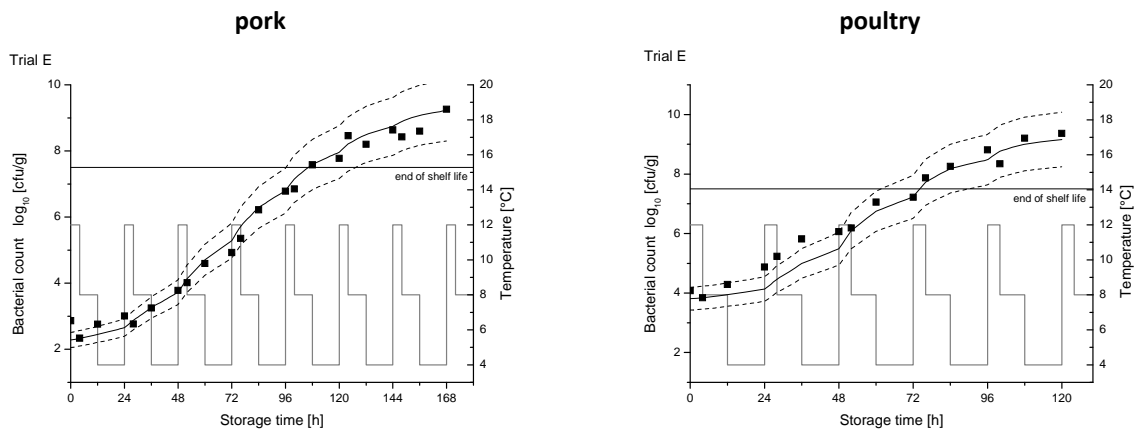


Figure 4.3: Linear fit for  $\ln(B_{\text{poultry}})$  versus  $\ln(B_{\text{pork}})$  (left) as well as for  $M_{\text{poultry}}$  versus  $M_{\text{pork}}$  (right)

Figure 4.4 shows the observed bacterial count as well as the model predictions for trial E (periodically changing temperature: 4 h at 12°C, 8 h at 8°C and 12 h at 4°) for fresh pork and poultry. The model predicted the growth of *Pseudomonas* sp. well for pork during the whole storage. For poultry, a slight underprediction could be seen in the first 50 h. But at the determined end of shelf life, when the count of *Pseudomonas* sp reached 7.5  $\log_{10}$  cfu/g (chapter 2), the predictions of both meat types matched to the observations.



**Figure 4.4: Observed and predicted growth of *Pseudomonas* sp. on fresh pork (left) and poultry (right) under dynamic temperature conditions in trial E; (■) observed growth, (—) predicted growth; (---)  $\pm 10\%$ , (grey line: temperature profile).**

Observed and predicted counts of *Pseudomonas* sp. on fresh pork of all other dynamic temperature scenarios (trial A, B, C and D) are shown in Figure 4.5. Generally, a slight underprediction by the model could be observed at the beginning of the exponential phase. But at the end of shelf life the model predictions were in good agreement with the observed growth data of *Pseudomonas* sp.

pork

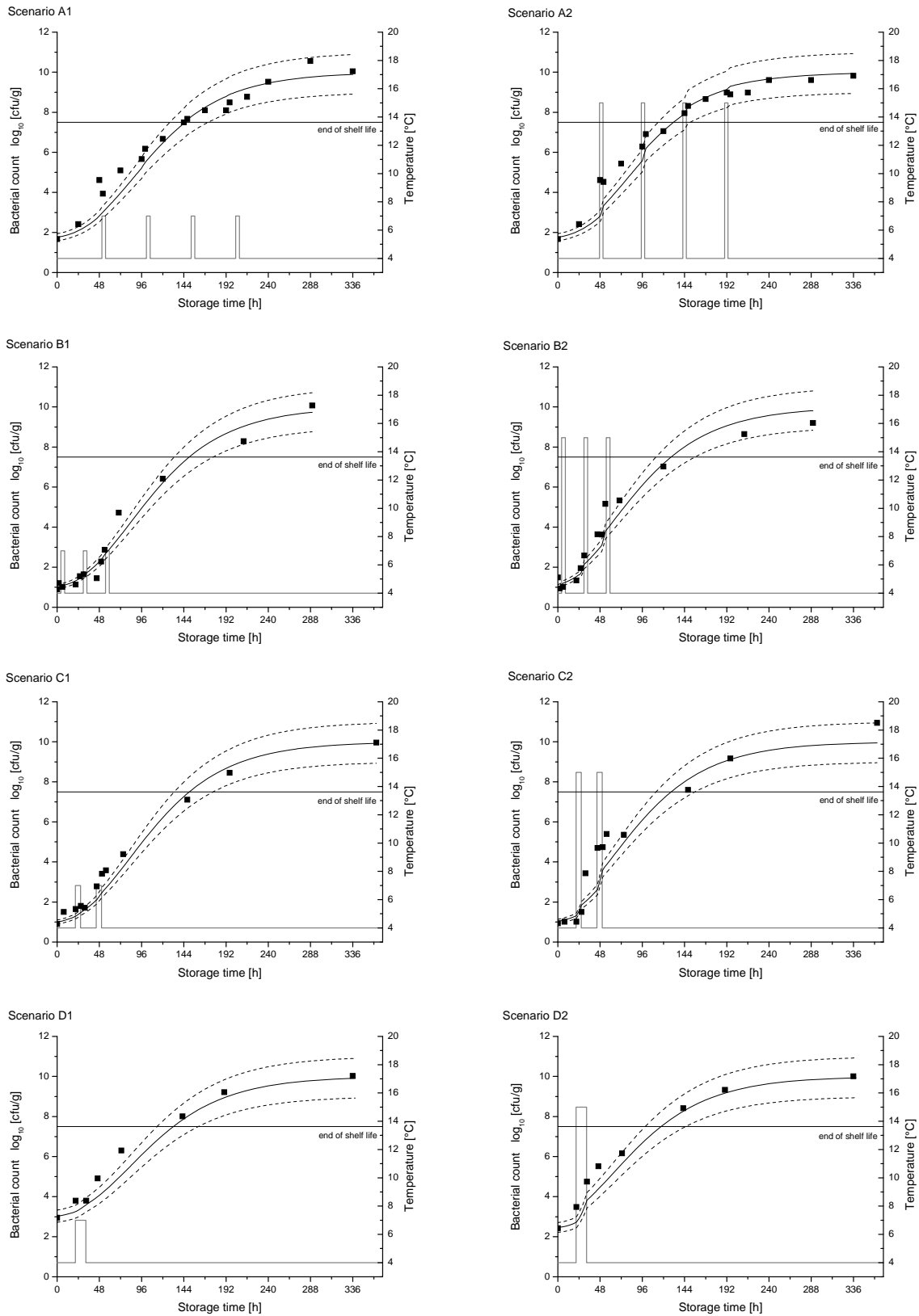


Figure 4.5: Observed and predicted growth of *Pseudomonas* sp. on fresh pork under dynamic temperature conditions (trial A – D); (■) observed growth, (—) predicted growth; (---)  $\pm 10\%$ , (grey line: temperature profile).

For the further dynamic temperature scenarios with fresh poultry (trial A – D), adjustments had to be made for the parameter  $M$  in the model settings due to changes in the spoilage kinetic of fresh poultry. Between the trials at constant temperature scenarios as well as at the periodically changing temperature scenario (trial E) and the storage trials with short temperature abuses (trial A - D), the poultry processing company in Germany optimised their slaughter and processing line by an improvement of the hygienic conditions. This resulted in an extension of the shelf life of fresh chicken breast fillets of about 2 days which was confirmed by the poultry company (personal communication). In comparison, the observed shelf life at the constant storage scenario of 4°C was 98.6 h (chapter 2) whereas the observed shelf lives in the constant control scenarios (4 °C) of trial A – D were between 133.5 h and 140.2 h (chapter 3). Therefore, the  $M$  value for the model in the first interval at the dynamic temperature scenarios in trial A – D was calculated by averaging the  $M$  values obtained at the four control scenarios (A0, B0, C0 and D0) instead of computing it from the  $M$  values for pork. This could be done, because the scenarios of trial A – D all started with a temperature interval at 4°C. All other parameter settings for the model ( $N_0$ ,  $N_{max}$ ,  $B$ ) were kept as described previously. With these settings the predicted growth of *Pseudomonas* sp. on fresh poultry under dynamic temperature conditions was in good agreement with the observed growth data as shown in Figure 4.6.

poultry

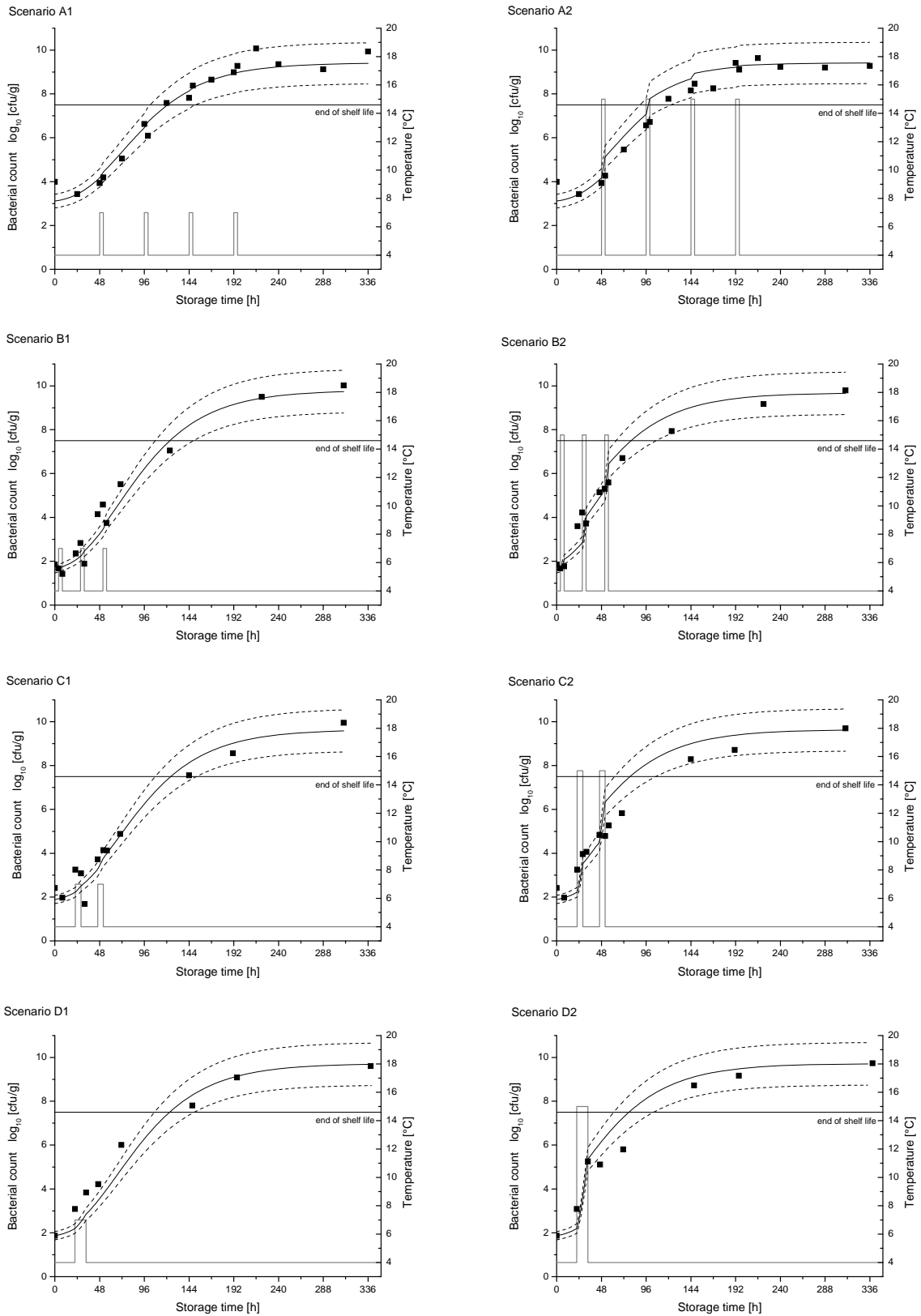


Figure 4.6: Observed and predicted growth of *Pseudomonas* sp. on fresh poultry under dynamic temperature conditions (trial A – D); (■) observed growth data (—) predicted growth; (---)  $\pm 10\%$ , (grey line: temperature profile).

To evaluate the performance of the model, the bias and the accuracy factor according to Ross (1996) were calculated for microbial counts of each dynamic temperature profile by considering each data point as a separate observation. So, bias and accuracy factors can be calculated from a single curve. The bias factors in this study ranged from 0.87 to 1.01 with a mean value of 0.93 for pork and from 0.89 to 1.04 with a mean value of 0.97 for poultry (Table 4.3). Average values < 1 for both meat types mean, that the model has to be considered as “fail-dangerous” (Ross 1996). The model predicts lower counts of *Pseudomonas* sp. as observed for both meat types. But as already mentioned no underprediction occurred at the end of shelf life which was confirmed by similar predicted and observed shelf life times (Table 4.4). Koutsoumanis (2001) also compared observed and predicted microbial counts and received slightly better bias factors for the prediction of *Pseudomonas* sp. growth on gilt-head seabream under dynamic temperature conditions. Bias factors were between 0.97 – 1.17 with 4 out of 5 in the “fail-safe” range (> 1.00). Neumeyer et al. (1997b) obtained a bias factor of 0.94 when validating their model, which was built in broth for *Pseudomonas* sp., in different food products (e.g. milk, minced beef) under dynamic temperature conditions. But they compared the time to each observed viable count to the time predicted to reach the same cell density as that observed. This means a bias factor < 1.00 shows an overprediction of the model and the model can be classified as “fail-safe”.

**Table 4.3: Bias and accuracy factor for the developed model at different non-isothermal temperature scenarios for fresh pork and fresh poultry**

Storage trial	Scenario <sup>a)</sup>	Pork		Poultry	
		B <sub>f</sub>	A <sub>f</sub>	B <sub>f</sub>	A <sub>f</sub>
Trial A	A1	0.93	1.11	0.99	1.05
	A2	0.93	1.10	1.04	1.07
Trial B	B1	1.01	1.13	0.95	1.15
	B2	0.93	1.18	0.97	1.12
Trial C	C1	0.87	1.19	0.95	1.15
	C2	0.91	1.24	1.00	1.16
Trial D	D1	0.92	1.09	0.89	1.15
	D2	0.90	1.11	1.02	1.11
Trial E		1.00	1.05	0.95	1.06
Mean		0.93	1.13	0.97	1.11

<sup>a</sup> Scenarios as described in Table 4.1

The mean accuracy factor was slightly higher for pork (1.13), varying between 1.05 and 1.24, than for poultry (1.11), with variations from 1.05 to 1.16. This means the predictions varied from the observations between 5 and 24 % for fresh pork and between 5 and 16 % for fresh poultry. These accuracy factors are comparable to the ones obtained with the models of Neumeyer et al. (1997b) and Koutsoumanis (2001).

Furthermore, the observed and predicted shelf lives in the different dynamic scenarios were compared to evaluate the applicability of the model (Table 4.4).

**Table 4.4: Observed and predicted shelf lives for fresh pork and fresh poultry at different non-isothermal temperature scenarios**

Storage trial	Scenario	Pork				Poultry			
		SL <sub>obs</sub> <sup>a)</sup> [h]	SL <sub>pred</sub> [h]	D [h]	%D	SL <sub>obs</sub> <sup>*</sup> [h]	SL <sub>pred</sub> [h]	D [h]	%D
Trial A	A1	144.2	145.1	-0.9	-0.6	130.5	121.0	9.5	7.3
	A2	126.5	131.4	-4.9	-3.8	122.4	98.3	24.1	19.7
Trial B	B1	146.6	150.2	-3.6	-2.5	125.0	125.6	-0.6	-0.5
	B2	124.7	128.7	-4.0	-3.2	100.0	79.8	20.2	20.2
Trial C	C1	157.5	150.2	7.3	4.6	133.5	124.3	9.2	6.9
	C2	121.1	128.7	-7.6	-6.3	106.7	78.8	27.9	26.2
Trial D	D1	124.0	132.1	-8.1	-6.5	122.1	123.0	-0.9	-0.7
	D2	103.5	117.3	-13.8	-13.3	102.9	77.4	25.5	24.8
Trial E		114.8	106.3	8.5	7.4	71.1	74.1	-3.0	-4.2

SL<sub>obs</sub>: shelf life observed; SL<sub>pred</sub>: shelf life predicted, D: difference between observed and predicted shelf life [SL<sub>obs</sub> - SL<sub>pred</sub>]; %D: percental difference between observed and predicted shelf life [%D = (100/SL<sub>obs</sub>) x D]

<sup>a)</sup> Data for observed shelf life from chapter 3 (except trial E), calculated by fitting microbiological growth data with the Gompertz model. End of shelf life at a count of *Pseudomonas* sp. of 7.5 log<sub>10</sub> cfu/g

For pork, a slight overestimation of shelf life was obtained with the model in most of the conducted scenarios (except for scenario C1 and trial E) with a maximum difference of 13.8 h between observed and predicted shelf life. In contrast, the predicted shelf lives for poultry were mainly underestimated by the developed model up to 27.9 h (26.2 %). Generally, the predictions for poultry were better for scenarios with temperature abuses to 7°C with differences less than 10 h, than for scenarios with shifts to 15°C with differences of about one day. The prediction for trial E with original model settings showed a difference of just 3 h (4.2 %). Altogether, the comparison of observed and predicted shelf life of fresh pork showed a difference of -2.7 % in average whereas for poultry it was 11.1 %. These differences are similar to the results of Koutsoumanis (2001) (5.8 % in average for gilt-head seabream) and Koutsoumanis et al. (2006) (13.1 % in average for ground meat). Kreyenschmidt et al. (2010a) observed differences of 0.4 and 17 % when comparing observed with predicted shelf lives of cooked sliced MAP ham at two different dynamic temperature scenarios. Koutsoumanis (2001) and Kreyenschmidt et al. (2010a) defined the observed shelf life as the shelf life determined by sensory characteristics whereas in this study, the time when counts of *Pseudomonas* sp. reached 7.5 log<sub>10</sub> cfu/g was used. But this definition for the end of shelf life is also based on sensory characteristics (see chapter 2).

The higher shelf life differences and the underprediction of shelf lives of fresh poultry can be assumed to be caused by the kinetic data of poultry at constant storage temperatures on which the model is based. As described previously the optimisation of hygienic conditions in the poultry company before trials A – D extended the shelf life at 4°C around 2 days which led to an adjustment of the initial *M* value for the model. A more precise prediction for



poultry can probably be obtained by repeating the constant storage trials at 2 – 15°C for fresh poultry and by adapting the calculation of  $M$  and  $B$  values for the first interval with new kinetic data for poultry at constant storage temperatures.

Altogether, the results showed that relevant microbial growth parameters for fresh poultry ( $M$ ,  $B$ ) could be related to the corresponding parameters for fresh pork which enabled the development of a common shelf life model applicable for both meat types. Other parameters besides temperature were not incorporated into the model because previous investigations have shown that several investigated intrinsic parameters (pH-value,  $a_w$ -value, D-glucose, L-lactic acid, fat content, protein content and Warner-Bratzler shear force) had only a minor or no influence on the growth of *Pseudomonas* sp. and thus shelf life (chapter 2). Microbial interaction could also be neglected as *Pseudomonas* sp. is not influenced by the growth of other microorganisms (Gill & Newton, 1977; Pin & Baranyi, 1998). In this study, model predictions for *Pseudomonas* sp. counts and shelf lives at dynamic temperature conditions were good for fresh pork as well as for fresh poultry. Even when short cold chain interruptions occurred, the model delivered reliable shelf life predictions, which shows the general applicability of the model for different meat types in real supply chains.

Although the model delivers good predictions, it has been shown in this study that changes in the supply chain (e.g. improvement of hygienic conditions by modernisation of the processing line) can change the kinetics of the product and thereby influence the precision of the model. Therefore, the continuous improvement and validation of the model is of fundamental importance which is also a basic principle in quality management. Janevska et al. (2010) also emphasise the importance of a continuous verification and validation of shelf life prediction tools used in quality management. The problem of the varying initial bacterial count, which was observed in this study, is also well known (Dogbevi et al., 1999; Krause et al., 2003; Ingham et al., 2009). But for an accurate shelf-life prediction, it is necessary to have a reliable indication of initial numbers of spoilage organisms (Pooni & Mead, 1984). A possible solution could be the approach of Giannakourou et al. (2001). They estimated the initial bacterial count from a database which was built for each batch of products from online rapid microbial measurements at the production site.

For the prediction of shelf life and remaining shelf life with the developed model an effective and continuous temperature monitoring during the entire supply chain is essential, as the combination of the predictive model with new temperature monitoring systems is a prerequisite for its implementation in real meat supply chains. The incorporation of the model in a user-friendly computer program (the so-called tertiary model) can allow the calculation of remaining shelf life of the product at strategic control points along the chill chain. This leads to better informed decisions of actors in the supply chain about optimal

handling as well as optimal stock rotation of the product. Thus, a change in the storage management principle from the FIFO concept (First In, First Out) to the LSFO concept (Least Shelf life, First Out) can be achieved, which reduces product waste and thus economic losses and enables the supply of a product within a narrow quality spectrum at the point of sale (Giannakourou et al., 2001; Koutsoumanis et al., 2005). Additionally, the confidence of the consumer in meat chains can be strengthened and can lead to financial benefits for the actors in the supply chain (Nychas et al., 2007)

But at the moment, a continuous and exact temperature monitoring throughout the entire meat supply chain is largely absent and the implementation of optimal temperature control systems in meat supply chains is difficult as discussed in detail by Raab et al. (2010). A possible solution could be the combination of the predictive model with new temperature monitoring devices as e.g. Radio frequency identification tags (RFIDs) combined with temperature sensors. The RFID technology is a wireless communication technology, which can deliver real-time information in the supply chain. Its use in the meat supply chain offers several potential benefits, e.g. traceability, inventory management, labour saving costs and security (Mousavi et al., 2002). The incorporation of a shelf life model in RFID tags combined with a temperature sensor allows the collection of real-time temperature data and thus the immediate estimation of shelf life and remaining shelf life. Another solution is so-called time temperature integrators (TTIs). TTIs are small, inexpensive devices which change their colour dependent on temperature: these are based on enzymatic, chemical, mechanical, electrochemical or microbiological reactions (Taoukis & Labuza, 2003). To use the TTI as a freshness indicator, the kinetics of the TTI has to match the kinetics of the food product, which allows its use during the whole supply chain from slaughtering to the consumer (Taoukis & Labuza, 1989a, b). Several TTIs, which are applicable for specific food products, have been developed during the last few years (Smolander et al., 2004; Ellouze et al., 2008; Vaikousi et al., 2008, 2009; Kreyenschmidt et al., 2010b) and have already been combined with shelf life prediction tools e.g. in the Safety Monitoring and Assurance System (SMAS) (Koutsoumanis et al., 2005; Tsironi et al., 2008). Similar approaches are the Shelf Life Decision System (SLDS) by Giannakourou et al. (2001) and a Decision Support Tool (DST) proposed by Kreyenschmidt et al. (2007).

In the above mentioned SMAS and the DST, Quantitative Microbial Risk Assessment (QMRA) was also incorporated, as ignoring spoilage in risk assessment could lead to a significant overestimation of risk (Koutsoumanis, 2009). Janevska et al. (2010) proposed an approach which also considers the integration of the existing Hazard Analysis and Critical Control Point (HACCP) approach with the Quantitative Microbial Risk Assessment (QMRA) as well as a shelf life predictor (SLP). They suggest using the HACCP in order to implement the SLP in an

efficient way so that no separate system for shelf life prediction has to be added to the supply chain.

In summary, the developed model delivers good predictions for the growth of *Pseudomonas* sp. and thus shelf life of fresh pork and fresh poultry. For the implementation in real meat chains, it is necessary to adapt the model to the specific product and supply chain characteristics. Then the model can be considered to be an effective tool, (in combination with adequate temperature monitoring solutions) for the improvement of quality management within the meat supply and distribution chain.

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# **CHAPTER 5**

## **SUMMARY**

Fresh meat with a high moisture content, a moderate pH and readily available sources of energy, carbon and other nutrients provides an ideal matrix for microbiological growth. Its shelf life is mainly limited by the growth of *Pseudomonas* sp. as specific spoilage organism (SSO). Thus, estimation of the growth of *Pseudomonas* sp. and thereby shelf life and remaining shelf life is of high relevance in meat chains as it allows companies to optimise their storage management and thereby reduce economic losses. An alternative to traditional microbiological challenge tests is the concept of predictive microbiology which uses mathematical models to predict microbiological growth and thus shelf life. But until now only a few models were developed for fresh meat or meat products, which are also applicable under dynamic temperature conditions. However, these models were only developed and validated for just one type of meat or meat product. Predictive shelf life models which are applicable for different types of fresh meat (e.g. fresh pork and poultry) are missing.

Therefore, the objective of this thesis was the development of a common predictive shelf life model for fresh pork and fresh poultry which led to the definition of three research questions.

The first research question focussed at the characterisation and comparison of the spoilage processes of fresh pork and poultry with the identification of the main influencing factors on shelf life. Storage tests were conducted at different isothermal temperatures (2, 4, 7, 10 and 15°C) to investigate the influence of the extrinsic factor temperature on the growth of *Pseudomonas* sp. and thus shelf life of fresh pork and poultry. Furthermore, the intrinsic factors pH,  $a_w$ , Warner-Bratzler shear force (WBSF), D-glucose, L-lactic acid, fat and protein were analysed concerning their effect on *Pseudomonas* sp. during storage at 4°C.

The applicability of *Pseudomonas* sp. as a freshness indicator for fresh pork and poultry has been confirmed under all constant temperature scenarios which was shown by high significant correlations ( $r > -0.900$ ;  $p < 0.05$ ) with the sensory characteristics for both meat types. Based on these sensory characteristics a common spoilage level, which indicates the end of shelf life, could be established at a *Pseudomonas* sp. count of  $7.5 \log_{10}$  cfu/g for both fresh pork and fresh poultry. The growth of *Pseudomonas* sp. was clearly dependent on temperature, with faster growth at higher temperatures for fresh pork and fresh poultry. In comparison, *Pseudomonas* sp. grew more rapid on fresh poultry than on fresh pork resulting in shorter shelf lives at all constant storage temperatures for fresh poultry. The differences in shelf life between fresh pork and fresh poultry at constant storage temperatures were about 18.4. – 39.4 h.

Almost all of the investigated intrinsic factors were significantly different ( $p < 0.05$ ) for fresh pork and poultry at all sample points during storage (except for  $a_w$ -value and L-lactic acid).



The *Pseudomonas* sp. count could be correlated significantly ( $p < 0.05$ ) to all investigated parameters in pork except WBSF and to four parameters in poultry (pH-value,  $a_w$ -value, D-glucose, L-lactic acid). However, magnitudes of these correlations were only very low to medium ( $r < 0.630$ ) which indicates only minor influences on the shelf life of fresh pork and poultry. The incorporation of the investigated intrinsic factors was therefore neglected in the development of the shelf life model.

The second research question aimed at the investigation of the influence of cold chain interruptions on the growth of *Pseudomonas* sp. and thus on shelf life of fresh pork and poultry. Four different dynamic temperature trials were performed, each consisting of one isothermal control scenario at 4°C and two dynamic temperature scenarios with a basic storage temperature at 4°C including short temperature shifts to 7°C and 15°C, respectively. The dynamic scenarios in the four trials differed in number and duration as well as starting point of the temperature shifts. Shelf lives at dynamic temperature scenarios were compared to shelf lives at the control scenarios to calculate shelf life reductions.

High significant correlations between the counts of *Pseudomonas* sp. and the sensory indices ( $r > -0.85$ ;  $p < 0.05$ ) for fresh pork as well as fresh poultry verified the use of *Pseudomonas* sp. as freshness indicator also under dynamic temperature conditions. Concerning the influence of temperature abuse on the growth of *Pseudomonas* sp. and thus shelf life, the results showed that especially short temperature abuses at the beginning of storage led to remarkable shelf life reduction for both meat types. Reductions were up to two days (up to over 30 %) although the storage time with an abusive temperature was less than 5 % of the total storage time. As expected, scenarios with shifts to 15°C led to higher shelf life reductions than scenarios with shifts to 7°C for both meat types. Shifts to 15°C conducted at the beginning of storage led to shelf life reductions between 30.6 - 38.4 h for poultry and 35.4 - 56.2 h for pork. The reductions caused by shifts to 7°C at the beginning of storage were only between 11.6 and 34.3 h for pork and between 6.7 and 13.4 h for poultry. Although absolute shelf life reductions were higher for fresh pork than for fresh poultry, the reductions for both meat types were comparable when considering the relative shelf life reductions (differences between pork and poultry < 10 %).

The final research question aimed at the development and validation of a common predictive shelf life model for fresh pork and fresh poultry based on the growth of *Pseudomonas* sp. The growth data of *Pseudomonas* sp. at different constant storage temperatures were modelled with the Gompertz function (primary model). The Arrhenius equation was used as secondary model to describe the temperature dependency of the relative growth rate  $B$  for both meat types. These two models were combined for the common predictive shelf life model. The previously described dynamic temperature

scenarios were used for the validation of the model. Additionally, the model was validated with growth data from a scenario with periodically changing temperature conditions (4h at 12°C, 8h at 8°C and 12h at 4°C).

It was possible to relate relevant microbiological growth parameters for fresh poultry to the corresponding parameters for fresh pork. This enabled the development of a common predictive shelf life model applicable for both meat types. The model predictions for *Pseudomonas* sp. growth as well as shelf life under dynamic temperature conditions were in good agreement with the observations for fresh pork as well as poultry. Whereas for pork a slight overestimation of shelf life occurred (mean difference between observed and predicted shelf life: -2.7 %), the shelf lives for poultry were rather underestimated (mean difference: 11.1 %). The good shelf life predictions at real chill chain conditions with short temperature abuses showed the general applicability of the model for different meat types in real supply chains.

It has to be mentioned that for reliable shelf life predictions the continuous improvement and validation of the model is essential as the modification of processes in the supply chain can lead to changes in shelf life kinetics of fresh pork and poultry. For example, hygienic improvements in the poultry slaughtering and processing company in this study led to changes in the shelf life kinetics for fresh poultry with longer shelf life times at constant storage temperatures. This shows also the necessity of the adaptation of the model to supply chain and product specific characteristics when the model should be implemented in decision support systems for quality management in different meat supply chains.

By incorporating the developed model in user-friendly software (tertiary model) and combining it with an effective and continuous temperature monitoring, the software will act as a shelf life prediction tool. With this tool, it is possible to calculate the remaining shelf life of fresh pork and poultry at strategic control points of the chill chain resulting in better informed decisions of actors in meat supply chains. The handling of the product as well as the stock rotation can be optimised in companies in the meat chain and thus economic losses and product waste can be reduced. Furthermore, the combination of a shelf life prediction tool based on the developed model with the HACCP as well as the QMRA approach is thinkable, leading to the improvement of quality management in the entire meat supply chain.

## List of Publications

### 2009

Bruckner, S., Raab, V. & Kreyenschmidt, J. (2009). Concept for the implementation of a generic model for remaining shelf life prediction in meat supply chains. Extended Abstracts of the "6th International Conference on Predictive Modelling in Foods", 09. - 12. September 2009, Washington DC/USA, online available:

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### 2008

Bruckner, S., Gymnich, S., Petersen, B., Schmitz, T., van der Roest, J., Kägi, A., Crespo, P. & Bertschinger, L. (2008). Monitoring strategies in food supply chains. In MUNLV, GIQS (eds.): Food quality and safety in international Food Chains – Technical reports of the Interreg IIIC Initiative PromSTAP, 189-197.

Bruckner, S. & Kreyenschmidt, J. (2008). Development of a generic shelf life model for pork and poultry. Poster and Abstract in the Programme and Abstract Book of the Food Micro 2008 – The 21st International ICFMH Symposium "Evolving microbial food quality and safety", 01. - 04. September 2008, Aberdeen/Scotland, 226.

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Kampmann, Y., Bruckner, S., Kohn, S., Kloft, K. & Kreyenschmidt, J. (2008). Investigation of microbiological contamination in domestic refrigerators and an analysis of appropriate methods for reduction of contamination in private households. Proceedings of "The International Conference of Refrigeration" IIF. 15. - 17. October 2008, Poznan/Poland, 309-318.

Kreyenschmidt, J., Hansen, T.B., Kampmann, Y., Christensen, B.B., Aabo, S., Lettmann, T., Bruckner, S., Kostov, I., Van Beek, P. & Petersen, B. (2008). Innovative Systems in the field of food quality and safety. In MUNLV, GIQS (eds.): Food quality and safety in international Food Chains – Technical reports of the Interreg IIIC Initiative PromSTAP, 152-165.

Raab, V., Bruckner, S., Beierle, E., Kampmann, Y., Petersen, B. & Kreyenschmidt, J. (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.

Best paper award in the category "Chain Management" at the 8th International Conference on Management in Agrifood Chains and Networks, 29. – 30. Mai 2008, Wageningen/NL

## **2007**

Bruckner, S., Gymnich, S., Schmitz, T., van der Roest, J., Bertschinger, L., Crespo, P. & Petersen, B. (2007). MonStratFood: Monitoring strategies in food supply chains. Abstract Book of the 2nd Annual Congress "Promoting the table to stable approach" (PromSTAP), 04. - 05. Oktober 2007, Vidin/Bulgaria, 35-37.

Kreyenschmidt, J., Aabo, S., Bruckner, S., Christensen, B.B., Gkisakis, V., Hansen, T.B., Kampmann, Y., Lettmann, T., Raab, V., van Beek, P. & Petersen, B. (2007). Development of a „Decision Support Tool“ (DST) for the pork supply chain. Poster and short paper in the proceedings of the "5th International Conference on Predictive Modelling in Foods", 16. - 19. September 2007, Athens/Greece, 519-522.

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