Definition of predictor variables for MAP poultry filets stored under different temperature conditions\textsuperscript{1}

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

Key words: temperature, modified atmosphere packed, poultry filets, shelf life modeling, high-oxygen atmosphere

INTRODUCTION

Raw poultry meat is sensitive to microbial spoilage due to its physical and chemical properties. Therefore, packaging under modified atmosphere conditions is widely established to improve the quality attributes and shelf life (Rokka et al., 2004). The main gases used for packaging are oxygen, carbon dioxide, and nitrogen, combined in different mixtures (Phillips, 1996; Mullan and McDowell, 2003). Changes of the packaging conditions due to oxygen requirements of the bacteria have a selective effect on the microbial population (Farber, 1991; Labadie, 1999). Next to packaging, temperature is the most important influence factor on shelf life and quality of fresh meat and meat products during processing, distribution, and storage (Cox et al., 1998; McDonald and Sun, 1999; Labadie, 1999; Bruckner et al., 2012a,c). Thereby, modified atmosphere packed (MAP) treatment combined with the storage at chill temperatures leads to an extended shelf life and an improved storage stability of the food product (Leister, 1995). Thus, increasing temperature conditions results in an increase of microbial growth with a decrease in the lag phase and the generation time (Herbert and Sutherland, 2000). For example, an increase of the storage temperature from 2 to 4°C leads to a decrease in shelf life of fresh aerobic packed poultry by nearly 22% (Bruckner et al., 2012b). The spoilage of poultry under aerobic conditions is mainly caused by different species of psychrotrophic \textit{Pseudomonas} spp. (Barnes, 1976; Gill and Newton, 1977; Pooni and Mead, 1984; Gram et al., 2002; Koutsoumanis et al., 2006; Raab et al., 2008). The detailed knowledge about the growth behavior of these so-called specific spoilage organisms (SSOs) are the basis for the development of predictive shelf life models (Gram et al., 2002). Under modified atmosphere conditions, \textit{Brochothrix thermosphacta} (\textit{B. thermosphacta}) and \textit{Lactobacillus} spp. are often described as the main spoilage microorganisms on meat stored at cold temperatures (Dainty and Mackey, 1992; Davies, 1995; Limbo et al., 2010). Actually, inconsistent information regarding the identification of a main spoilage microorganism of MAP poultry is described in the scientific literature. Most studies are only focused on CO\textsubscript{2}-N\textsubscript{2} gas mixtures for the packaging of MAP poultry, but the German poultry industry packs the meat under high oxygen (50 to 70% O\textsubscript{2}) atmospheres (Rossaint et al., 2013, unpublished report). However, the spoilage process is mainly caused by a wide spectrum of microorganisms like \textit{B. thermosphacta}, \textit{Pseudomonas} spp., \textit{Enterobacteriaceae}, etc.
and Lactobacillus spp. (Borch et al., 1996; Saucier et al., 2000; Walsh and Kerry, 2000), and the growth is mainly influenced by the initial bacterial load, the gas mixture, the product-gas ratio, and the storage temperature (Sivertsvik et al., 2002). Jiménez et al. (1997) demonstrated good growth of Lactobacillus spp., Enterobacteriaceae, and B. thermosphacta on fresh chicken breast stored at 4°C in MAP under two atmospheric conditions (30% CO₂/70% N₂ and 30% N₂/70% CO₂). Smolonard et al. (2004) and Rajamäki et al. (2006) found that varying storage temperatures and MAP with 80% CO₂/20% N₂ most affected the growth of Enterobacteriaceae on poultry meat. In contrast, Balamatsia et al. (2007) identified B. thermosphacta and lactic acid bacteria as the main spoilage microorganisms on aerobic and MAP (30% CO₂/70% N₂) chicken fillets. Therefore, the reliable determination of remaining shelf life based on the definition of SSOs under changing extrinsic influence factors is challenging for the development of a predictive shelf life model. Consequently, the present study analyzed the spoilage process of poultry, packed in a gas atmosphere commonly used in Germany, at varying temperature conditions to identify the best predictor variable, which is most effective in predicting the deteriorative changes of MAP poultry during storage.

MATERIALS AND METHODS

Preparation of Meat Samples

Unsexed 42-day-old-broiler chickens (Ross 308/708) were slaughtered and air-chilled in a poultry processing plant in Germany. The skinless double-breast chicken fillets were transported from the poultry slaughter plant to a wholesaler in Bonn and forwarded to the laboratory under temperature-controlled conditions in isolated boxes with cooling packs. The first investigation started within 24 h after slaughtering. In the laboratory the double breast fillets were divided into single fillets using a sterile scalpel.

Packaging and Storage of Meat Samples

For modified atmosphere packed, the poultry breast fillets were placed in polypropylene trays (R. Fearch Plast A/S, Holstebro, Denmark). Tray volume was 680 mL and approximately 230 g meat samples were packaged to achieve a package headspace to meat ratio of nearly 2:1. The meat samples were packaged under an atmosphere containing 30% CO₂/70% O₂. Thereafter, the trays were heat-sealed with a polypropylene foil (Suedpack Verpackungen, Ochsenhausen, Germany; water vapor permeability <3.5 g/m²d at 23°C/85% relative humidity (RH); oxygen permeability <1.5 cm²/m²d bar at 23°C/35% RH) for 3 s/175°C using a tray sealer packaging machine (Trayssealer T200, Multivac Sepp Haggenmüller, Wolfertschwenden, Germany). Gas mixtures were prepared by a 4-component gas blender machine (KM 60-4 MEM SO, Witt Gasetechnik, Witten, Germany). The packaged meat samples were stored at 2, 4, 10, and 15°C in low-temperature high-precision incubators (Sanyo model MIR 153, Sanyo Electric, Ora-Gun, Gumma, Japan). The storage temperatures were monitored by a data logger (Escort Junior Internal Temperature Data Logger, Escort, Auckland, New Zealand) every 5 min. The microbiological, sensory, and chemical analyses were conducted at appropriate time intervals. Each measurement was repeated 3 times.

Microbiological Analyses

For microbiological analyses, the meat surfaces were removed aseptically (4 x 7 x 0.5 cm pieces) using a sterile scalpel. Each product sample (approximately 25 g) was transferred to a filtered sterile stomacher bag and 225 mL saline peptone diluent (0.85% NaCl with 0.1% peptone saline tablets, Oxoid BR0033G, Cambridge, United Kingdom) was added. Samples were blended with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. Ten-fold dilutions of the sample rinsates were prepared in saline peptone diluents. Total viable count (TVC), Pseudomonas spp., B. thermosphacta, Enterobacteriaceae, and Lactobacilli spp. in rinsates were enumerated. Total Viable Count was determined by pour plate technique on plate count agar (Merck, Darmstadt, Germany) incubated at 30°C for 72 h. Pseudomonas spp. were detected by spread plate technique on Pseudomonas agar with Cetrimide-Fucidin-Cephalosporin selective supplement (Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 h. B. thermosphacta was detected by drop plate technique and counted on Streptomyces inosit toluylene red agar according to Hechelmann (1981). Petri dishes were incubated at 25°C for 48 h. Enterobacteriaceae were identified by overlay treatment on violet red bile dextrose agar (Merck, Darmstadt, Germany) by incubation of the agar plates at 30°C for 48 hours. Lactobacilli spp. were detected by pour plate technique on de Man, Rogosa, Sharpe agar (Oxoid, Cambridge, United Kingdom). Plates were incubated aerobically at 37°C for 72 h. Counts of colony-forming units (CFUs) were expressed as log10CFU/g for each medium and sample.

pH Measurement

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

Concentrations of oxygen and carbon dioxide inside the trays were monitored over the storage period, using a hand-held gas analyzer (Oxybaby V O₂/CO₂, Witt Gasetechnik, Witten, Germany). Before starting the gas measurement inside the trays, the composition of air was analyzed to control the accuracy of the gas analyzer. Headspace in packages was sampled, using a syringe needle to withdraw 10 mL of headspace gas through a self-adhesive sealing pad in the package. Gas volume was absorbed in 15 s and the oxygen concentration was detected by an electrochemical sensor; carbon dioxide concentration was detected by IR absorption. Control packages containing no meat samples were stored as reference and the gas composition was also monitored over the entire storage period.

Sensory Evaluation

Sensory analyses were carried out by trained sensory panelists that were recruited from the Institute of Animal Science (University of Bonn) and experienced in poultry evaluation. During the trials, each sample was evaluated directly after opening the tray, using a developed sensory scheme according to the quality index method for fish evaluation (Bremner, 1985). A picture of fresh chicken breast fillets was used as reference during the sensory evaluations. Attributes were defined as general appearance (G), color (C), odor (O), texture (T), and drip loss (D). Changes of the attributes were expressed in a 5-point scoring system. The lower the score, the better the quality and freshness of the product. A weighted quality index (QI) was calculated by the following equation (Kreyenschmidt, 2003):

\[ QI = \frac{2G + 2C + 1T + 1D + 2O}{8} \]  

(1)

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

Primary Modeling

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

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

(2)

with \( N(t) \) = microbial count [log10cfu/g] at any time, \( A \) = lower asymptotic line of the growth curve (initial bacterial count), \( C \) = difference between upper asymptotic line of the growth curve (\( N_{\text{max}} \) = maximum population level) and the lower asymptotic line, \( B \) = relative maximum growth rate at time, \( M \) = time at which maximum growth rate is obtained (reversal point), and \( t \) = time.

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

The time when the bacterial count achieves the plateau (\( t_{\text{plateau}} \)) is calculated according to the following equation:

\[ t_{\text{plateau}} = M + \frac{1}{B} \]  

(3)

with the variables as defined for Eq. (2).

Statistical Analysis

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

RESULTS AND DISCUSSION

Development of Spoilage Microflora under Different Temperature Conditions on MAP Poultry Files

Figure 1 shows the development of TVC under different constant temperature conditions (2 to 15°C).
on poultry breast filets packed under MAP (70% O\(_2\)/30% CO\(_2\)). Figure 1 shows that increasing storage temperatures lead to a faster microbiological growth on fresh meat as also described by several researchers (Baranyi et al., 1995; Kreyenschmidt, 2003; Raab et al., 2008; Bruckner et al., 2012a).

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

\textit{B. thermosphacta} is often associated with spoilage under MAP conditions based on the improved tolerance to CO\(_2\) (Borch et al., 1996; Branscheid et al., 2007). During storage, \textit{B. thermosphacta} becomes the predominant spoilage microorganism as also emphasized in Table 1 with the highest growth rates during

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### Table 1. Calculated growth parameter for typical spoilage organisms (Gompertz function), stored under different temperature conditions (70% O\(_2\)/30% CO\(_2\)).

<table>
<thead>
<tr>
<th>Growth parameter</th>
<th>Temperature (°C)</th>
<th>2</th>
<th>4</th>
<th>10</th>
<th>15</th>
<th>2</th>
<th>4</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVC</td>
<td></td>
<td>181.25</td>
<td>38.25</td>
<td>9.81</td>
<td>4.56</td>
<td>0.017</td>
<td>0.022</td>
<td>0.040</td>
<td>0.080</td>
</tr>
<tr>
<td>\textit{Pseudomonas} spp.</td>
<td></td>
<td>274.90</td>
<td>27.17</td>
<td>11.50</td>
<td>19.47</td>
<td>0.009</td>
<td>0.016</td>
<td>0.038</td>
<td>0.096</td>
</tr>
<tr>
<td>\textit{B. thermosphacta}</td>
<td></td>
<td>236.10</td>
<td>33.92</td>
<td>19.74</td>
<td>23.23</td>
<td>0.018</td>
<td>0.029</td>
<td>0.038</td>
<td>0.120</td>
</tr>
<tr>
<td>\textit{Enterobacteriaceae}</td>
<td></td>
<td>250.65</td>
<td>47.37</td>
<td>54.79</td>
<td>9.60</td>
<td>0.009</td>
<td>0.021</td>
<td>0.042</td>
<td>0.079</td>
</tr>
<tr>
<td>\textit{Lactobacillus} spp.</td>
<td></td>
<td>345.76</td>
<td>203.68</td>
<td>94.33</td>
<td>33.95</td>
<td>0.009</td>
<td>0.007</td>
<td>0.056</td>
<td>0.082</td>
</tr>
</tbody>
</table>

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**Figure 2.** Comparison of spoilage microflora development during storage of MAP poultry filets (70% O\(_2\)/30% CO\(_2\)) under various temperature conditions.
storage at 2 to 4°C. The results show that B. thermosphacta is relatively resistant against refrigeration temperatures because of its psychrotropic properties (McClure et al., 1993). Also the used gas mixture in this study showed no effect in delaying the growth of B. thermosphacta, as stated also by Santé et al. (1994). Even though the microorganism is a facultative anaerobic competitor, it prefers to grow under oxygen atmospheres. Therefore the microorganisms are able to dominate the microflora, when oxygen is present (Gribble and Brightwell, 2013).

_Pseudomonas_ spp. are aerobic microorganisms and they grow preferably under oxygen conditions (Chouliara et al., 2007; Fraqueza and Barreto, 2009; Herbert et al., 2013). Besides the fact that _Pseudomonas_ spp. are psychrotrophic bacteria, which show better growth at nonrefrigeration temperatures, the results show that the growth of _Pseudomonas_ spp. is retarded at low storage temperatures between 2 and 4°C (Figure 2). This is presumably due to the fact that pseudomonads are sensitive to the antimicrobial component CO₂ (Saucier et al., 2000; Fraqueza and Barreto, 2009), which leads to an increase in the lag phase and the generation time (Kreyenschmidt and Ibald, 2012). At higher temperatures, _Pseudomonas_ spp. dominates the spoilage flora, accordingly to the lower solubility of CO₂ in water and fat, and the reduced antimicrobial activity.

_Enterobacteriaceae_ are facultative anaerobic microorganisms, which prefer to grow under oxygen conditions. The initial counts of _Enterobacteriaceae_ are approximately 2 log10CFU/g under all storage conditions, as also shown by Smolander et al. (2004). During storage, the growth of _Enterobacteriaceae_ is slowed down under refrigeration temperatures (2 to 4°C), whereas at higher temperatures (10 to 15°C), the growth is clearly accelerated (Table 1). This is due to the fact that these microorganisms prefer to grow under mesospheric temperatures and their growth is favored in comparison to the other microorganisms when temperature increases.

_Lactobacillus_ spp. are only dominant at the beginning of storage under refrigeration temperatures (2 to 4°C), as also observed by Santé et al. (1994) and Herbert et al. (2013). Their growth becomes not dominant over the entire storage period under all investigated temperatures and they play a minor role in the overall spoilage flora. This is based on the growth characteristics of _Lactobacillus_ spp., which belongs to a slow-growing group of microorganism with preferred growth under anaerobic conditions. Despite the fact that _Lactobacillus_ spp. are showing an enhanced tolerance to CO₂, the slow growth at 2 to 4°C is possibly related to cold temperatures, because _Lactobacillus_ spp. are also mesophilic bacteria (Huis in’t Veld, 1996; Jay et al., 2005). At storage temperatures between 10 and 15°C, the microorganisms growth is favored and possibly caused by the reduced solubility of CO₂ at higher temperatures (Gill, 1988) and the mesophilic properties.

Generally, temperature has the main influence on microbiological growth also under MAP conditions. The growth curves indicate some kind of synergistic effect between the improved solubility of CO₂ and refrigeration temperatures (2 and 4°C), while higher temperatures are reduce the solubility of CO₂ and favor the growth of each microorganism. These results are also in accordance to Devlighere et al. (1998), which established a significant interaction term between temperature and dissolved CO₂ on the growth of _Lactobacillus sake_. Regarding the effect of temperature on the development of microorganism growth curves, the results show that no specific spoilage microorganism could be observed as main spoilage originator under MAP conditions. Also Alfaro et al. (2013) showed for MAP fish products, that the spoilage microflora is changing by varying the storage temperature (0 to 20°C). Further on Coton et al. (2013) stated out that the packaging conditions have a further effect on the microbial ecosystem and lead to an increase in the bacterial diversity.

**Development of the Gas Atmosphere**

Figure 3 shows the development of the gas atmosphere (30% CO₂/70% O₂) under different constant temperature conditions with product inside the trays and without any sample as reference. In the beginning of storage, a small decrease of CO₂ could be detected in all test packages with product inside. This is due to the high solubility of carbon dioxide in the fat tissue and water on the meat surface (Bettis, 1995; Gill, 1988). Herbert et al. (2013) and Parra et al. (2010) reported similar results for modified atmosphere (MA) packed meat. The solubility of the antimicrobial component CO₂ is, besides the muscle tissue pH and the proportion and composition of the fat, also dependent on the storage temperature. The solubility of CO₂ in muscle tissue decreases with increasing temperature (Gill, 1988), which is reflected in the faster increase of CO₂ at 10 and 15°C (Figure 3). But the proportion which gets dissolved on the meat tissue cannot be quantified due to microbiological growth. During storage, the O₂ concentration decreases and the CO₂ concentration increases. This can be seen under MAP conditions due to bacterial activity and occurs faster with increasing temperature conditions due to the accelerated microbial growth. During the entire storage period, the O₂ concentration inside the trays shows a small decrease at lower temperature (2 to 4°C) and a rapid decrease with rising temperature condition (10 to 15°C). This is caused by microbiological consumption of O₂, the respiration of meat enzymes and gaseous exchanges between the gas composition inside the trays and the environment (Mullan and McDowell, 2003). Generally, changes in the gas atmosphere, especially at 10 and 15°C, were initiated when TVC reaches 7 log10CFU/g, which also corresponds with the sensory end of shelf life. Temperature has also an effect on the gas and water vapor permeability of
Development of the gas atmosphere (%O₂/CO₂) in the packages stored under different temperature conditions of MAP poultry filet (70% O₂/30% CO₂).

Figure 3. Development of the gas atmosphere (%O₂/CO₂) in the packages stored under different temperature conditions of MAP poultry filet (70% O₂/30% CO₂).

the packaging material, whereas the gas transmission rate increases with increasing temperatures (Kirwan and Stawbridge, 2003; Mullan and McDowell, 2003). Comparing the development of the O₂ and CO₂ concentration in the reference packs (without product), no significant change of the gas concentrations could be observed. Therefore, the changes in the gas proportions are caused by the increased microbial growth due to temperature increase.

Development of Meat pH

The initial broiler breast meat pH 24-h postmortem varies between 5.7 and 6.2 (data not shown), which is in a normal range for poultry meat (ICMSF, 1988; Lund and Eklund, 2000; Herbert et al., 2013). As reported by Devlighere et al. (1998) CO₂ gets partly dissolved in the water and fat phase of the product under formation of carbonic acid with a direct ionization, which results in a decrease of the surface meat pH. This effect is mainly influenced by the storage temperature; the CO₂ solubility decreases with increasing temperature (Gill, 1988; Walsh and Kerry, 2000). In contrast, the results show that the pH-value was not significantly influenced by any storage temperature (P > 0.05) over the entire storage periods. This is due to the buffer effect of the meat proteins which limits significant variations in pH while storing the meat under MAP, as also stated out by Gilka et al. (1980) and Dixon and Kell (1989).

Development of Sensory Parameter and Shelf Life Determination

The quality index increases for poultry, with increasing storage time for all temperatures. A quality index of 2.5 was taken as the lower limit of acceptability, corresponding to initial deteriorative changes regarding color, odor, texture, general appearance and drip loss. The increase of the storage temperature from 2 up to 4°C results in a shelf life reduction from 423 to 228 h, which reflects approximately 45% (Table 2). Generally, the decay of shelf life follows also an exponential function (Figure 4). Table 2 shows the bacterial counts of the different investigated bacteria at the end of sensory shelf life. The results show that with increasing temperature the variation of the bacteria which are influencing the spoilage process is increasing. At 2 and 4°C, B. thermosphacta dominates the flora at the end of the shelf
Table 2. Bacterial counts at the end of sensory shelf life during storage of poultry filets under different temperature conditions (70% O₂/30% CO₂).

<table>
<thead>
<tr>
<th>End of shelf life (QI = 2.5)</th>
<th>TVC</th>
<th>Pseudomonas spp.</th>
<th>B. thermosphacta</th>
<th>Enterobacteriaceae</th>
<th>Lactobacillus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2°C (423 h)</td>
<td>7.0</td>
<td>4.3</td>
<td>6</td>
<td>3.9</td>
<td>3.2</td>
</tr>
<tr>
<td>4°C (228 h)</td>
<td>7.0</td>
<td>4.4</td>
<td>6.1</td>
<td>5.2</td>
<td>3.2</td>
</tr>
<tr>
<td>10°C (107 h)</td>
<td>7.3</td>
<td>7.0</td>
<td>5.9</td>
<td>4.4</td>
<td>3.7</td>
</tr>
<tr>
<td>15°C (75 h)</td>
<td>7.5</td>
<td>7.6</td>
<td>6.7</td>
<td>6.7</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Figure 4. Development of the quality index of MAP poultry filet (70% O₂/30% CO₂) stored under different temperatures (left) and the development of sensory shelf life as a function of the storage temperature (right).

Table 3. Factor loadings (varimax normalized).

<table>
<thead>
<tr>
<th></th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. thermosphacta (plateau)</td>
<td>-0.994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas spp. (plateau)</td>
<td>-0.994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TVC (plateau)</td>
<td>-0.990</td>
<td>-0.149</td>
<td></td>
</tr>
<tr>
<td>QI</td>
<td>-0.986</td>
<td>-0.118</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae (plateau)</td>
<td>-0.975</td>
<td>0.219</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae (k)</td>
<td>0.935</td>
<td>0.156</td>
<td>-0.319</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.892</td>
<td>0.437</td>
<td>-0.118</td>
</tr>
<tr>
<td>TVC (k)</td>
<td>0.836</td>
<td>0.466</td>
<td>-0.289</td>
</tr>
<tr>
<td>TVC (N_max)</td>
<td>0.785</td>
<td>0.280</td>
<td>0.552</td>
</tr>
<tr>
<td>Pseudomonas spp. (k)</td>
<td>0.755</td>
<td>0.468</td>
<td>-0.460</td>
</tr>
<tr>
<td>Lactobacillus spp. (k)</td>
<td>0.739</td>
<td>0.654</td>
<td>0.162</td>
</tr>
<tr>
<td>B. thermosphacta (k)</td>
<td>0.727</td>
<td>0.455</td>
<td>-0.513</td>
</tr>
<tr>
<td>Enterobacteriaceae (N_max)</td>
<td>0.699</td>
<td>0.176</td>
<td>0.693</td>
</tr>
<tr>
<td>B. thermosphacta (N_0)</td>
<td>-0.190</td>
<td>0.995</td>
<td>0.228</td>
</tr>
<tr>
<td>B. thermosphacta (N_max)</td>
<td>-0.130</td>
<td>-0.949</td>
<td>0.289</td>
</tr>
<tr>
<td>Lactobacillus spp. (N_0)</td>
<td>0.599</td>
<td>0.797</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas spp. (N_max)</td>
<td>0.512</td>
<td>0.769</td>
<td>0.323</td>
</tr>
<tr>
<td>Lactobacillus spp. (plateau)</td>
<td>-0.617</td>
<td>-0.780</td>
<td>-0.103</td>
</tr>
<tr>
<td>Lactobacillus spp. (N_0)</td>
<td>-0.377</td>
<td>0.780</td>
<td>0.500</td>
</tr>
<tr>
<td>Enterobacteriaceae (N_0)</td>
<td>0.113</td>
<td></td>
<td>0.992</td>
</tr>
<tr>
<td>Pseudomonas spp. (N_0)</td>
<td>0.166</td>
<td></td>
<td>0.986</td>
</tr>
<tr>
<td>TVC (N_0)</td>
<td>-0.476</td>
<td>0.118</td>
<td>0.872</td>
</tr>
</tbody>
</table>

Values in bold font are the principle component.

Definition of Predictor Variable

As stated out in Table 2, the spoilage of MAP poultry under different temperature conditions is caused by a wide range of microorganisms and the bacterial counts at the end of sensory shelf life are varying. For the identification of predictor variables for a reliable shelf life prediction under MAP conditions, a stepwise regression and a principle component analyses were carried out. The results of the stepwise regression analyses shows a significant correlation ($P < 0.05$) between the sensory shelf life and the time when Pseudomonas spp. and TVC pass over into the stationary phase. Additionally, a principle component analyses was conducted. Table 3 shows the component matrix, where three main components could be identified: 1) the time when Pseudomonas spp. and B. thermosphacta reach the stationary phase, 2) the initial bacterial count of B. thermosphacta, and 3) the initial bacterial count of Enterobacteriaceae. As well as the stepwise regression analyses, the PCA indicates that the time when Pseudomonas spp. pass over into the stationary phase is an important variable on the shelf life. However, the PCA shows also that Brochothrix thermoshacta ($t_{plateau}$) has a main influence on the shelf life, because component one is life, and at 10 and 15°C dominates Pseudomonas spp., but at 15°C also the other bacteria have a main part on the microbial flora. Therefore, the definition of a specific spoilage organisms and common bacteria acceptance level for all temperature is challenging. Only the microbiological count of the TVC indicate that TVC counts are in the same range (7.0 to 7.5 log10CFU/g) under all investigated temperature conditions, which represents the upper microbiological acceptability limit of 7 log10CFU/g, which concord with the International Commission on Microbiological Specifications for Foods (ICMSF, 1978). Therefore, the microbiological spoilage regarding TVC 7 log10CFU/g is in compliance with the end of sensory shelf life defined at QI = 2.5.
mainly explained by $t_{\text{plateau}}$ of *Pseudomonas* spp. and $t_{\text{plateau}}$ of *B. thermosphacta*. Also Component 2 is influenced by *B. thermosphacta* ($N_0$). The results emphasize that no common variable could be identified that has the highest explanatory power for the data set regarding the identification of a specific spoilage organism.

**CONCLUSION**

Despite the fact that MAP results in a remarkable extension of the shelf life of meat and meat products in comparison to aerobic storage, the results emphasize also a strong influence of the storage temperature on the shelf life under modified atmosphere conditions. The shelf life reduction while increasing the temperature from 2 to 4°C is comparatively high (45%) due to storage under aerobic conditions (22%) (Bruckner et al., 2012b). No significant influence of the storage temperature on the development of the meat pH could be observed. The development of gas atmosphere was strongly influenced by the storage temperature due to an increase of CO$_2$ and a decrease of O$_2$. From the microbiological point of view, *B. thermosphacta* showed higher growth rates at 2 and 4°C, whereas the microflora changed under 10 and 15°C with a predominance of *Pseudomonas* spp. In contrast to aerobic storage where *Pseudomonas* spp. is the main spoilage microorganism, the spoilage microflora under MAP consists of a wide mixture of species and the contribution of each microorganism to the spoilage process strongly depends from the temperature. Therefore, the definition of an acceptance level based on SSO is not feasible. Also the results of the stepwise regression and PCA reflected that no single predictor variable could be identified as main spoilage organism. Therefore, the shelf life under high oxygen conditions is caused by several factors. In conclusion, TVC seems to be the best predictor variable for the prediction of remaining shelf life for MAP poultry based on the significant correlation between the development of the sensory decay and the development of the TVC and the number of TVC at the end of sensory shelf life for each single storage temperature. In dependence of the composition and variation of the microflora, the TVC is not always meaningful. Therefore, further research is needed to gather a detailed knowledge of the growth and the interaction of SSOs, under various packaging and temperature conditions, and the initial composition of the flora.

**REFERENCES**


