DISCOLORATION AND MICROBIAL GROWTH OF BEEF STORED UNDER AEROBIC CONDITIONS

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Abstract - The objective of this study was to investigate changes in meat organoleptic characteristics, microbial flora and their relationship when beef was stored aerobically for 10 days. Beef steaks wrapped with oxygen permeable film were stored in air at 4 °C. Beef pH, color (*L, *a**, *b**), and microbial counts were measured. Combination of T-RFLP and cloning of bacterial 16S rDNA was applied to study bacterial community structure and identity. Results showed that redness (*a**) decreased significantly from day 7 to day 10, with the formation of metmyoglobin. Total bacterial counts reached 8.4 $\log_{10}$ CFU/cm^2 at day 10, in which *Pseudomonas spp.* and *Brochothrix spp.* were the two dominant species. By PCA analysis, a close relationship between bacterial growth and meat discoloration was found. Those findings indicate that *Pseudomonas spp.* and *Brochothrix spp.* have a potential influence on meat discoloration.**

Key Words – Beef color, Microbiology, Spoilage, T-RFLP

I. INTRODUCTION

Spoiled meat is characterized by discoloration, strong off-odors and the development of slime which make the meat unacceptable to consumers. There are various factors involved in meat spoilage, such as microbial growth, lipid oxidation, and production of toxic substances. Microbial growth is the most common cause of meat deterioration [1].

Shelf life date (best before date, expire date or use by date) is important information, which affects purchase of meat products [2]. However, these data are not sufficiently defined and there is very limited knowledge on how meat organoleptic characteristics, palatability and nutritional value are altered after the best before date. There are little scientific data on how long after "best before date" meat still can be stored without negative effects. Depending on different storage conditions, shelf life of meat differs and different bacterial groups potentially contribute to meat decomposition [3]. To control meat safety and reduce waste caused by spoilage, it is important to characterize the spoilage microbiota at different storage conditions. Recently, terminal restriction fragment length polymorphism (T-RFLP) has been applied to investigate the bacterial communities in modified atmosphere packaged meat [4]. T-RFLP is a robust and powerful method to study bacterial community structures.

The objective of this study was to investigate changes in meat organoleptic characteristics, microbial flora in beef under aerobic storage at 4°C for up to 10 days.

II. MATERIALS AND METHODS

Six heifers of Hereford breed (22-23 months, 252±24.2 kg) were slaughtered on the same day according to standard routines at a commercial slaughterhouse. On day 6 post mortem, the muscle *longisimus dorse* (LD) was dissected, and collected from one side of each carcass and vacuum packaged. On day 7 post mortem, each LD was cut into 8 steaks (approximate 5.5 cm thick slices) after removal of fat tissues, the steaks were wrapped with oxygen-permeable PVC film (NORM PACK 115 45-1, Tempac AB, Tyresö, Sweden). The steaks were randomly divided into four groups consisted of two steaks from each LD (12 steaks per group). The four groups were randomly assigned to either of four storage time (0, 4, 7, 10 days). During storage period they were

kept at 4 °C in darkness. At designed storage time (0, 4, 7, 10 days), meat samples with 2.5 cm diameter and 2 mm thickness were aseptically taken from each steak of corresponding group.

Meat pH and color (CIE *L*, a*, b** color score) was measured as previously described [5]. The relative content of deoxymyoglobin (Mb), oxymyoglobin (OxyMb) and metmyoglobin (MetMb) were estimated and transformed as described before [6, 7].

Aerobic plate counts were enumerated on Plate Count Agar (PCA; Oxoid, Unipath Ltd., Basingstoke, UK) incubated at 30 °C for 72h; *Enterobacteriaceae* was enumerated on Violet Red Bile Glucose Agar (Difco, Sweden); lactic acid bacteria was enumerated on Man Rogosa Sharpe medium Agar (Oxoid, Unipath Ltd., Basingstoke, UK) after anaerobic incubation at 25 °C for 5 days; moulds and yeasts were enumerated on Sabouraud Dextrose Agar (Difco, Sweden) after incubation at 25 °C for 5 days. After incubation, plates with 30–300 colonies were counted. Results were expressed as $\log_{10}$ CFU/cm^2. DNA was extracted form samples according to the protocol described by the manufacturer of MoBio PowerFood Microbial DNA isolation Kit (MoBio, Solana Beach, CA). Cloning and sequencing of 16S rDNA from DNA extracted from beef was performed according to Dicksved, Flöistrup [8].
T-RFLP of bacterial 16S rDNA isolated from beef and clones was carried out according to Dicksved, Flöistrup [8].

The statistical analysis was carried out using Statistical Analysis System (Version 9.3, SAS Institute, Cary, NC, USA). The MIXED procedure was used with storage time as fixed factor and animal as random factor.

III. RESULTS AND DISCUSSION

Beef pH increased (P<0.05) from day 7 to 10 (Table 1), which could be related to buffering caused by base substances produced by bacteria, such as ammonia.

Lightness (*L**) increased during the entire storage period (P<0.05). Variations in the *L** value have been attributed to the meat structural changes and water loss [9]. There was a significant decrease in redness (*a**) between 7 and 10 days of storage (P<0.05). It was also observed that the relative content of metmyoglobin increased over the time, the relative content of oxymyoglobin decreased between 7 and 10 days (P<0.05). It is known that undesirable meat discoloration during storage is due to the accumulation of metmyoglobin [10].

Both total bacterial counts (TBC) and yeast counts increased significantly throughout the whole storage period. TBC counts reached the highest level of 8.4 $\log_{10}$ CFU/cm^2 on the day 10 (Table 2). Once the surface population of bacteria has reached 10^8CFU/cm^2, the supply of simple carbohydrates has been exhausted and recognizable off-odors developed [11]. There was no significant change in *Enterobacteriaceae* (ENT) and lactic acid bacteria (LAB) counts during up to 7 days of storage. On the day 10, both counts increased. The lower LAB counts may be due to suppression by aerobic environment.

Table 1 pH changes and color stability of beef stored for 10 days at 4 °C (Least squares means)

	Day 0	Day 4	Day 7	Day10	SE	*P*-value
pH	5.71^{b}	5.74^{b}	5.71^{b}	5.80^{a}	0.052	0.017
*L**	29.51^{c}	33.67^{ab}	32.24^{b}	34.52^{a}	0.910	<0.001
*a**	11.99^{a}	13.91^{a}	12.27^{a}	9.33^{b}	1.185	<0.001
*b**	10.00^{b}	11.94^{a}	11.37^{a}	9.06^{b}	0.714	<0.001
Chroma	15.61^{b}	18.34^{a}	16.86^{ab}	13.04^{c}	1.283	<0.001
Hue	0.83^{a}	0.86^{a}	1.01^{a}	1.01^{a}	0.083	0.149
Mb	0.64^{a}	0.55^{c}	0.57^{bc}	0.61^{ab}	0.019	0.011
MbO_2	0.62^{a}	0.67^{a}	0.64^{a}	0.54^{b}	0.031	0.005
MetMb	0.62^{c}	0.78^{b}	0.86^{ab}	0.93^{a}	0.050	<0.001

Different letters within the same row indicate significant differences (P<0.05). SE: standard error.

The relative abundance of defined T-RFs are presented in Fig 1. The T-RFLP profiles of day 0 differed largely from the other days in terms of diversity of bacterial species as the number of TRFs decrease over time. Seventeen T-RF peaks were present in the samples on day 0, 12 on day 4, 7 on day 7, and 6 on day 10. On day 0, T-RF 37

was present in each individual sample with a relatively high abundance. After 4 days of storage, T-RF 310 dominated in the T-RFLP profiles in most of the samples while TRF 33 only became dominant in 4 samples. T-RF 33 and 310 were the dominant T-RFs on the days 7 and 10, whereas the relative abundances of the remaining T-RFs were lower levels.

Table 2 Microbial counts (log_{10} CFU/cm^2) (Least squares means) of beef stored for 10 days at 4 °C

	Day 0	Day 4	Day 7	Day 10	SE	*P*-value
TBC	2.1^{d}	4.2^{c}	6.2^{b}	8.4^{a}	0.31	<0.001
ENT	0.3^{b}	0.5^{b}	1.1^{b}	2.8^{a}	0.57	0.002
LAB	1.8^{b}	1.0^{b}	0.8^{c}	2.7^{a}	0.37	<0.001
MOL	0.6^{a}	0.2^{a}	0.3^{a}	0.01^{a}	0.20	0.284
YEA	0.3^{d}	1.2^{c}	2.8^{b}	4.3^{a}	0.23	<0.001

TBC, total bacterial counts; ENT, *Enterobacteriaceae* counts; LAB, lactic acid bacteria counts; MOL, mold counts; YEA, yeast counts. Data of <log 1.0 was adjusted to 0.01. Different letters within the same row indicate significant differences (P<0.05). SE: standard error.

To identify the bacterial species that corresponded to the dominant T-RFs, the T-RFLP patterns of clones were compared to those of the corresponding bacterial community. From the sequencing results, it was revealed that T-RF 33 was generated from 16S rDNA closely related to *Pseudomonas spp*. and T-RF 310 to *Brochothrix spp*. It is generally believed that *Pseudomonas spp.* is mainly responsible for meat spoilage. Previous reports indicated that *P. fluorescens* could accelerate beef discoloration; oxygen consumption by *P. fluorescens* decreased oxygen pressure and accelerated oxymyoglobin oxidation [12]. Papadopoulou and Doulgeraki [13] reported that *Brochothrix thermosphacta* contribute to meat spoilage by producing a number of compounds (acetoin, methylbutyric, isovaleric acids, lactic acid, carbon dioxide and ethanol) under aerobic conditions.

In our study, based on the PCA analysis performed on the T-RF relative abundance data of corresponding storage time, two distinct clusters were found (Fig.2). One cluster contained the T-RFLP profiles of Day 0 samples with T-RF 37 as the dominant T-RF, whereas the other cluster consisted of day 10 samples which were dominated by T-RF 33 and 310. It was revealed that significant changes in bacterial community structure occurred during the storage of beef and two species, *Pseudomonas spp.* and *Brochothrix spp.* became predominant at day 10.

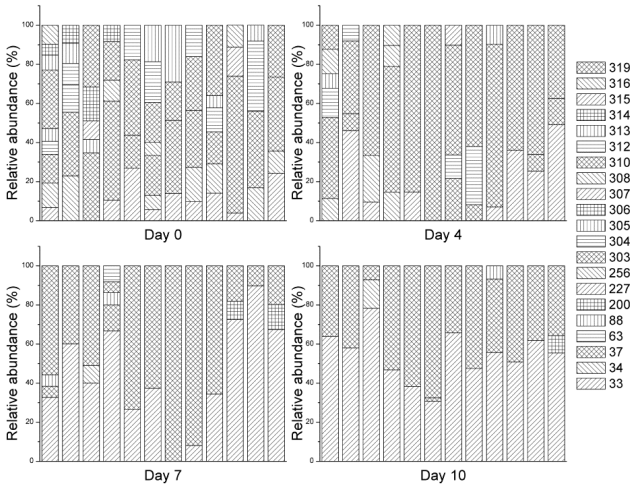

Figure 1. Histograms of relative abundance of T-RFs in all samples. Each column represents an individual sample.

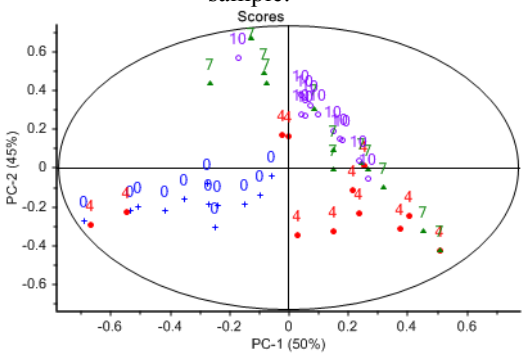

Figure 2. PCA score plot of relative abundance data of T-RFs at different storage times.

Overall, in order to better understand data structure consisting of all the measurements and their relationship, PCA analysis was applied on all data sets derived from different storage times. Beef of day 0 and beef of day 10 could be clearly classified into two clusters (Fig.2), Variables TBC counts, yeast counts, ENT counts, MetMb, oxyMb, *L*, a*, b**, and chroma were of great importance in the discrimination of the two clusters (Fig.3a). Moreover, microbial counts including TBC, ENT were negatively related to variables *L*, a*, b** and chroma and yeast while positively related to MetMb (Fig.3b). It confirms that bacterial growth has a close relationship with meat discoloration.

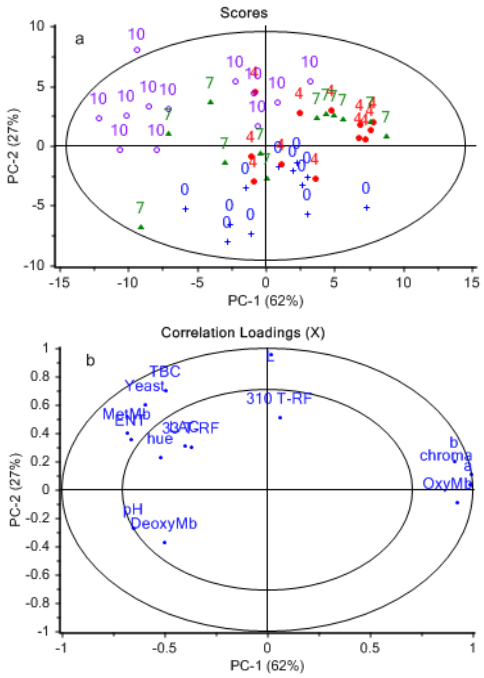

Figure 3. PCA score plot (a) with PC1 (62%) and PC2 (27%) and loading plot (b) of pH, color stability and microbial counts, and their relationships.

IV. CONCLUSION

Pseudomonas spp. and *Brochothrix spp.* are the two dominant species when beef is stored under aerobic conditions, and their colonization on meat could have a potential influence on meat discoloration.

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