THE USE OF MALDI-TOF MS FOR MICROBIAL IDENTIFICATION OF DISCOLORED VACUUM-PACKAGED BEEF

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I. INTRODUCTION

A significant improvement in tenderness and palatability of beef can be achieved by storing sub-primal cuts in vacuum-packaging for around 7 and 21 days [1] at -1 to 2°C [2]. During this period, the proliferation of gram-positive bacteria replaces the typical gram-negative microflora that is present when meat is exposed to oxygen-rich environments [3, 4]. It has been described in the literature that the maturation of red-meat muscles can be particularly problematic if pH_u attains values higher than 5.9, increasing the probability to cause a superficial green discoloration [5]. Tissues of healthy animals are in principle sterile; however, enhanced contamination processes post-mortem can facilitate the multiplication and dissemination of bacteria in the meat, changing important quality traits like color and smell [6]. Therefore, it is important to evaluate microbial contamination not only quantitively, but also qualitatively to identify individual microbial populations. This can be achieved, for instance through matrix assisted laser desorption/ionization time-of-flight mass spectrometry MALDI-TOF MS, which is a fast and reliable method, that can be easily implemented in routine analysis [7]. A notable advantage of the MALDI-TOF MS method is the capability to identify all types of food-related microorganisms using the same protocol [7]. Alternative methods such as sequencing or metabarcoding might be used for the same purpose; however, they can be time consuming, and require expensive materials and trained personnel [8]. Moreover, a high level of agreement in the results between MALDI-TOF and 16S rRNA genomic sequencing has been established at a genus level [9].

II. MATERIALS AND METHODS

Six packages containing discolored beef pieces from a combination of *M. gluteobiceps, M. tensor fasciae latae*, and *M. lateral vastus* were identified 4 days post-mortem and collected from the storage facility of a cattle specialized slaughterhouse located in Niedersachsen, Germany. After breakage of the plastic film, meat pieces of around 10g were sterile cut from the part of the muscles where discoloration was notorious and were repackaged in vacuum as soon as possible, kept on refrigeration, and further used for total viable counts (TVC), anaerobic counts and MALDI-TOF (MS) identification. Only after the collection of the samples for the microbiological analysis, the meat color was determined using a ColorLite sph870 colorimeter (Katlenburg Lindau, Germany) set at 45°/0° measuring geometry, 8 mm aperture size, D65 illuminant and 10° aperture, in 15 different points of the muscles. Then, meat pH was performed by inserting a Testo 250 pH-meter (Lenzkirch, Germany) in three different points of the muscles. Both pH and meat color were recorded from the average values. The same procedure was repeated for six control samples. The statistical analysis of the data was carried out using SPSS software (version 23.0, IBM Corporation, NY, USA).

III. RESULTS AND DISCUSSION

Table 1 – Average quality parameters for discolored (n=6) and control (n=6) meat samples

Parameter	Discolored meat	Control
pH	5.60±0.48[a]	5.66±0.12[a]
L*	36.75±5.11[a]	20.27±4.27[b]
a*	17.42±2.51[a]	26.57±4.03[b]
b*	18.09±5.15[a]	16.54±2.38[a]
TVC (CFU/g)	3.7E+03	5.22E+03
Anaerobic counts (CFU/g)	1.61E+03	2.12+E03

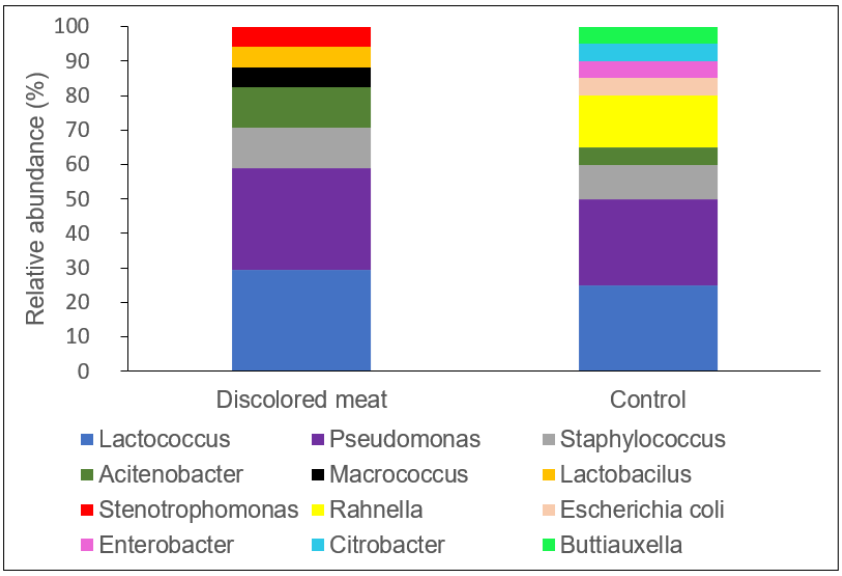

Figure 1: Results of the bacterial identification of discolored (n=6) and normal meat samples (n=6) using the MALDI-TOF (MS) method

It was found that pH values did not considerably vary between discolored meat and the control samples, even if there was an obvious superficial difference in color. The instrumental color measurement showed that *L** values were significantly higher and *a** values were significantly lower in discolored meat when compared to the control. Discolored beef and the control samples attained about the same TVC and anaerobic counts. The results of the bacterial identification using the MALDI-TOF method exposed that the bacterial communities growing in discolored beef and in the control after 4 days post-mortem were a mixture between gram-positive and gram-negative microorganisms.

IV. CONCLUSION

The microbiological and pH values both from discolored and control samples were low enough to assume that the difference in color between the two groups of muscles was not originated due to microbial contamination. The bacterial identification by MALDI-TOF showed that the microorganisms were usual bacteria that can grow under anaerobic conditions in vacuum-packaging, and that these microorganisms were not commonly related to discoloration processes in fresh bovine muscles. This project will be extended to a greater number of samples, and the microbial counts will also be tested before the expiration date, to

have a better picture of the development of the bacterial populations in discolored beef and in the control samples with the storage time.

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