WHAT IS THE REAL IMPACT OF *HAFNIA ALVEI* DURING COLD-STORAGE OF VACUUM-PACKED BEEF?

Pierre Ledormand[1]*, Christophe Denoyelle[1], Matthieu Lemoine[2], Catherine Malayrat[2], Valerie Hardit[2], Nathalie Desmasures[3], Isabelle Legrand[1] and Clemence Bieche-Terrier[1]

[1] Institut de l'Elevage, Meat quality Department, France
[2] Institut de l'Elevage, Meat analysis and technology Laboratory, 14310 Villers-Bocage, France
[3] Normandie Univ, UNICAEN, UNIROUEN, ABTE, 14000 Caen, France
*Corresponding author email: pierre.ledormand@idele.fr

I. INTRODUCTION

The microbiota composition of vacuum-packed (VP) beef product is diversified and includes desirable and also undesirable bacteria. Among these bacteria, it has been shown in previous works that the psychrotrophic bacterium *Hafnia alvei* was often detected in high abundance in the microbiota of vacuum-packed beef during cold-storage, but no direct impact on the product shelf life was identified [1]. Other studies recognized *H. alvei* as potential spoilage agents in VP beef but with no demonstration of their spoiling activity [2]. *H. alvei* belonged to the former *Enterobacteriaceae* family (hygiene indicator) until that the *Enterobacteriales* order was refunded into seven families, of which the *Hafniaceae* fam. nov., a family in it's own right [3]. Thus, the objective of the study was to investigate the behaviour and the impact in meat spoilage of *Hafnia alvei* strains during cold-storage, growing in VP beef.

II. MATERIALS AND METHODS

To explore the impact of *H. alvei* in the meat shelf life, challenge tests and sensory analysis were performed in parallel. Challenge tests were realized using eye round (*M. semitendinosus*) muscles collected from three suppliers in the cutting workshop. These tests were realized on two types of eye round meat: a sterilized meat (cauterized matrix : CM) and a natural meat (uncauterized matrix : UM). Eye round muscles were cut in sterile conditions into slices and were then inoculated by using strains from the UCMA collection (University of Caen Normandy), either *Hafnia alvei* UCMA 14205 (S1) or *H. alvei* UCMA 17635 (S2) strains. Uninoculated CM and UM meat was used as control. Meat cuts were vacuum-packed and kept following two scenarios, a professional (industrial) circuit (named A) with a temperature of 4°C for 40 days and a consumer's circuit (named B) with a temperature of 4°C for 14 days and 8°C for 7 days. Experiments were accomplished in triplicate. At each sampling time, microbial enumeration (presumed *Enterobacteriaceae sensu lato* in NF ISO 21528-2 then aerobic mesophilic flora in NF ISO 4833-1) and sensory analysis tests according to a grid for the conservation status of beef (odour, colour, exudate, blowing and overall impression), were realized. The overview of the experiment is shown in Figure 1.

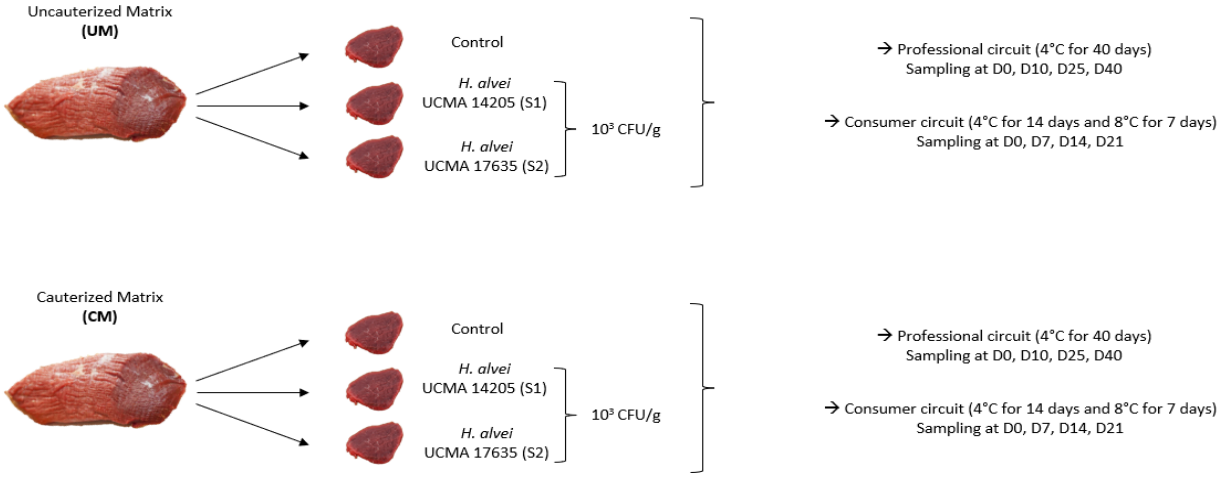

Figure 1 : Experimental design of the study.

To observe *H. alvei* behaviour within a natural microbiota, 16S metagenetic analyses were conducted on UM samples at the end of the conservation (D25 and D40 for circuit A and D14 and D25 for circuit B). Total DNA was extracted and the V3-V4 region of the 16S rDNA was amplified and sequenced on Illumina MiSeq. Bioinformatic and statistical treatment of data were performed in Galaxy solution [4].

III. RESULTS AND DISCUSSION

For the CM, the level of presumed *Enterobacteriaceae* increased over the conservation time both in the circuits A and B, as counts were around 10^6-10^7 CFU.g^{-1} at D40 and at D21 for the conditions inoculated with S1 and S2 strains, while counts were below the quantification threshold (10^1 CFU.g^{-1}) in the control condition. The results were globally the same for aerobic mesophilic flora, showing that the cauterization was effective at D0 (control condition) and that *H. alvei* was also enumerable with this method. Concerning the sensory analyses for the CM, no product was classified as unsatisfactory, some products were acceptable (10/72) and the majority were satisfactory (62/72) during the entire experiment. For the UM, the counts of presumed *Enterobacteriaceae* also increased along the conservation time but were slightly lower than in CM, as 10^5-10^6 CFU.g^{-1} counts were enumerated at the end of the conservation for the conditions inoculated with S1 and S2 strains. As expected in a natural matrix, aerobic mesophilic flora was detected at D0 around 10^1 CFU.g^{-1} and reached $10^{5\text{-}6}$ CFU.g^{-1} at the end of the conservation in both circuits. As for CM, no product was classified as unsatisfactory for the sensory analyses, some products were acceptable (9/72) and the majority were satisfactory (63/72) during the entire experiment. The 16S metagenetic analysis showed that Lactic Acid Bacteria dominated in the majority of the samples analyzed, with *Dellaglioa* and *Lactococcus* genera found in high proportions (often > 50% of abundance). The *Hafnia* genus was detected in proportions varying from 6% to 30%, principally at the end of the conservation process. Other genera, such as *Photobacterium*, *Carnobacterium* or *Latilactobacillus* were also detected. The statistical analyses of metagenetic also revealed that the origin of the meat impacts the evolution of bacterial composition.

IV. CONCLUSION

This study showed that *H. alvei* seems probably not correlated with beef spoilage. This work also showed the necessity to develop other methods to understand spoilage scenarios in meat, in order to have microbial indicators more informative of the state of conservation of the products. Globally, these issues still need to continue to be developed in order to reduce food waste and to ensure healthy fresh meat products to the consumer.

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