71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

MICROBIAL DYNAMICS AND QUALITY OPTIMIZATION OF *BRUNA DELS PIRINEUS* BEEF: EFFECTS OF DRY AND WET AGING

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I. INTRODUCTION

The consumption of beef in Europe has declined due to changing consumer preferences and increased attention to health, environmental, and ethical concerns [1]. In response, interest has grown in traditional production systems and local breeds, such as the *Bruna dels Pirineus*, valued for their role in biodiversity, sustainability, and rural development [1, 2]. This native Spanish breed, classified as endangered [3], remains underutilized despite its potential for high-quality beef production. Post-mortem ageing significantly affects beef sensory traits, with dry and wet ageing influencing physicochemical and microbiological properties in different ways [4–6]. While microbial dynamics during ageing have been explored in general [7–9], data on *Bruna dels Pirineus* beef remain scarce. This study aimed to assess the sensory characteristics and microbial communities of *Bruna dels Pirineus* beef aged under dry- and wet- aging conditions for 4 and 8 weeks, to support its quality optimization and valorization.

II. MATERIALS AND METHODS

Sirloins from 11 *Bruna dels Pirineus* cattle were aged under dry and vacuum conditions for 4 or 8 weeks at 1.5 ± 0.6 °C and 86.3 ± 8.47 H_R. Fresh samples (T0) were also collected. Technological and sensory traits were assessed at each time point. Analyses included pH, color (CIELAB), texture (Warner-Bratzler), a_w,. Sensory evaluation followed standardized protocols. Microbiological analyses included culture-based enumeration of Enterobacteriaceae, lactic acid bacteria (LAB), Gram-positive catalase-positive cocci, *Staphylococcus aureus*, *Pseudomonas* spp., sulfite-reducing clostridia, total viable count (TVC) and yeast and molds. Additionally, real-time PCR for the detection of *Salmonella* spp., *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* (STEC) in 25g of meat was performed. High-throughput sequencing of 16S rRNA (V3–V4 region) was conducted on selected samples (n=18). Sequence data were processed using QIIME2 and analyzed through compositional data analysis with centered log-ratio (CLR) transformation. Alpha and beta diversity, as well as differential abundance analyses, were performed in R. Meat quality data were analyzed using the GLM procedure while microbial data comparisons used PERMANOVA, Kruskal-Wallis, Dunn's test, and Mann-Whitney tests, with significance set at $p < 0.05$.

III. RESULTS AND DISCUSSION

1. Technological and sensory characteristics

The interaction between aging method and time showed no significant effect on most of the technological traits, except for lightness, which varied depending on the aging method. Overall, aging time significantly affected instrumental color, and toughness. As aging progressed (0 to 8 weeks) lightness and redness increased, and meat tenderness improved markedly, with very tender meat achieved after 4 weeks. Sensory analysis by a trained panel revealed no significant differences in odor attributes across aging times or methods. However, the texture-related attributes (e.g., toughness, chewiness) evolved with time, with sensory hardness being lower in wet-aged meat at 4 weeks but similar across methods at 8 weeks, as expected. As aging progressed, meat became less juicy. Regarding flavor, some attributes were affected by time or method: overall flavor intensity decreased, while aged notes became stronger with aging. Dry-aged meat exhibited greater flavor intensity, but a lower perception of aged flavor compared to wet-aged meat.

2. Microbial counts

Microbial enumeration revealed significant differences between the two aging methods. TVC were higher in dry-aged samples compared to wet-aged ones, both with a progressive increase over time. LAB exhibited a significant increase during wet-aging, whereas their growth was minimal in dry-aging environment. *Pseudomonas* spp. displayed a significant opposite trend, increasing during dry-aging while decreasing during wet-aging.

Dry-aged samples exhibited consistent growth of yeasts and molds, significantly greater compared to wet-aged ones Enterobacteriaceae showed a trend towards a greater increase in wet-aged compared to dry-aged samples, with the latter showing higher variability. Gram-positive catalase-positive cocci remained stable across treatments, while

key food safety indicators, including sulfite-reducing clostridia, *E. coli*, and *S. aureus*, were either undetectable or present at negligible levels, confirming overall microbiological safety under the tested conditions. No foodborne pathogens of concern, such as *L. monocytogenes*, *Salmonella* spp., or STEC were detected in any of the samples of both aging methods.

3. Microbiome analysis

Microbiome profiling through 16S rRNA gene sequencing allowed for a detailed characterization of bacterial community structure and dynamics during aging. Alpha diversity analysis using the Shannon index revealed no significant differences between the dry- and wet-aged groups, nor between the 4- and 8-week time points in our study. Differently, beta diversity analysis showed a clear and statistically significant separation between the dry-aged and wet-aged microbial communities. However, consistent with the alpha diversity results, no significant clustering based on aging duration was observed. In wet-aged samples, the core microbiome was dominated by LAB, mainly *Lactobacillus, Leuconostoc* and *Carnobacterium*. *Pseudomonas* (*P. fragi* and other species) and *Brochothrix thermosphacta* were core members present in all samples but were predominant and characterized the dry-aged samples.

IV. CONCLUSION

This study highlights how dry- and wet-aging influence the microbiological, technological, and sensory characteristics of *Bruna dels Pirineus* beef. These results demonstrate that both dry- and wet-aging methods can enhance meat quality through controlled microbial and physicochemical transformations, while maintaining safety throughout the process. In line with the objective of supporting quality optimization and valorization of this local product, further research is needed to explore the impact of microbial communities on the technological and sensory properties of aged meat. Additionally, further research should be conducted to better understand the relationship between the sensory profile and chemical composition regarding volatile compounds, fatty acid profiles, etc.

ACKNOWLEDGEMENTS

This work was supported by the grant JDC2023-052485-I, funded by MICIU/AEI/10.13039/501100011033 and, “ESF+” and by the project POCTEFA EFA144/16/DIETAPYR2, which has been 65% cofinanced by the European Regional Development Fund (ERDF) through the Interreg V-A Spain-France-Andorra programme (POCTEFA 2014-2020). POCTEFA aims to reinforce the economic and social integration of the French–Spanish–Andorran border. Its support is focused on developing economic, social and environmental cross-border activities through joint strategies favouring sustainable territorial development.

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