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FUNGI FROM AGED BEEF: MOLECULAR IDENTIFICATION AND ENZYMATIC ACTIVITY

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I. INTRODUCTION

Meat aging involves resting beef or other meats under controlled temperature, relative humidity (RH), and airflow post-slaughter to enhance tenderness, flavor, and quality through enzymatic and biochemical changes [1]. There are mainly two types of meat aging: wet aging, where the meat is vacuum-sealed in packaging and stored under refrigerated conditions, and dry-aging, where the meat is stored without protective packaging, at controlled temperature, RH and airflow [2]. Dry-aged meat is gaining attention for its enhanced qualities and consumer willingness to pay a premium product [2, 3]. This is an especially useful strategy for valorizing endangered Pyrenean cattle breeds, like *Albera* and *Bruna dels Pirineus*, producing high-quality meat with distinctive flavors, thereby increasing its value and consistency for consumers [4]. During aging, endogenous enzymes like calpains and aminopeptidases degrade muscle proteins, increasing tenderness and contributing to flavor and aroma formation. Additionally, lipolysis also takes place, which generates compounds that will enhance flavor and aroma [2]. Various authors have suggested that yeasts and molds may contribute to the development of desirable characteristics in aged meat, and some tried to use them as starter cultures to improve the aging process [5, 6, 7]. This study, one of the first in Europe and Spain, investigates the fungal community in aged beef and aging chambers. It aims to (i) characterize the mycobiota of dry-aged and wet-aged beef from two endangered cattle breeds, -*Albera* and *Bruna dels Pirineus*- and their respective aging environments and (ii) assess fungal enzymatic (proteolytic and lipolytic) activity potentially contributing to meat quality.

II. MATERIALS AND METHODS

Meat samples (fresh meat and at 4 and 8 weeks of dry- and wet-aging meat cuts at IRTA premises) and chamber air (commercial and IRTA's dry-aging chambers) were collected to perform culturomics analysis for fungal isolation and identification. For meat samples (aged at 2 ºC and 75 % H_R), fungal colonies were isolated from the surface areas of the pieces (25 g) using Dichloran Rose Bengal Chloramphenicol Agar (25 ºC for 5 days). For aging chamber air, fungal colonies were isolated through plates of Potato Dextrose Agar and Yeast Extract Glucose Chloramphenicol Agar, which were placed inside the chambers and left open for 24h.To identify the fungal isolates, the internal transcribed spacer (ITS) region was amplified, sequenced and analyzed using Bionumerics (version 7.6), where dendrograms were generated through the UPGMA (unweighted pair group method with arithmetic mean) clustering method. Proteolytic activity was assessed on 10% Opaque Skim Milk Agar by measuring clear halos around wells on days 3 and 7. Lipolytic activity was evaluated on Tributyrin Agar (indicated the presence of esterase and lipase activity) and Rhodamine B agar by measuring clear halos on days 3, 7, and 10. Statistical analysis was performed using Microsoft® Excel® (Version 2409) and R Studio (R version 4.4.1). Chi-squared and Fisher's exact tests assessed differences in frequency distribution, with significance set at $p < 0.05$, using the stats package in R.

III. RESULTS AND DISCUSSION

A total of 291 fungi were isolated, 141 from meat samples and 150 from aging chambers. The isolates were nearly equally divided between yeasts (47.8%) and molds (52.2%). Their source differed significantly: 82.0% of the yeast isolates originated from meat samples, whereas 82.2% of the molds originated from aging chambers.

In meat samples, among the yeast isolates, *Candida* (47.4%) was predominant, followed by *Yarrowia* (29.8%), and *Debaryomyces* (14.9%). On the other hand, molds accounted for the minority of isolates (n=27) in meat samples, with *Penicillium* as the most frequently isolated genus (44.4%). Dry-aged meat exhibited slightly higher fungal diversity, with genus such as *Penicillium* and *Talaromyces* possibly originating from aging chamber environments.

Before aging, only *Candida zeylanoides*, *Debaryomyces* hansenii, and *Vishniacozyma victoriae* were isolated and the majority (>80%) of isolates were obtained after eight weeks of aging, indicating that the number and diversity of isolates increased progressively with aging.

In aging chambers, 83.3 % of fungal isolates corresponded to molds, primarily *Penicillium* genus (45.6 %), followed by *Cladosporium* (13.6 %). *Aspergillus*, *Beauveria* and *Talaromyces* genus were also isolated. Concerning yeasts in aging chambers, *Sporidiobolus metaroseus* was the most frequently isolated species (28.0%), followed by *Candida* (16.0%), *Yarrowia* (12.0%), *Tilletiopsis* (12.0%), *Rhodotorula* (8.0%), *Debaryomyces* (8.0%), *Sporobolomyces*, *Parengyodontum*, *Hypopichia* and *Filobasium* (4.0% each).
Among the isolates, 139 yeasts and 149 molds were submitted to the enzymatic activity assays, which revealed 67.1% of molds and 42.8% of yeasts displayed proteolytic activity, while 73.2% and 97.1% showed lipolytic potential. *Yarrowia* spp. showed strong proteolysis, and *Penicillium* spp. exhibited both high proteolytic and lipolytic activity, suggesting roles in flavor development. Despite detecting potentially mycotoxigenic species from *Aspergillus*, *Cladosporium*, *Penicillium*, controlled aging conditions (0–4°C, 65–85% RH) are likely sufficient to mitigate toxin production [7].

IV. CONCLUSION

The study highlights the interplay between environmental and meat-associated fungi in shaping dry-aged beef quality, emphasizing the need for controlled aging to balance safety and sensory attributes. Some isolated fungi may also contribute to enzymatic processes essential for flavor and texture development. These findings offer insights for developing starter cultures and refining post-mortem strategies to support and enhance local beef production.

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