

Diversity and Characteristics of the Meat Microbiological Community on Dry Aged Beef

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Beef was dry aged for 40–60 days under controlled environmental conditions in a refrigerated room with a relative humidity of 75%–80% and air-flow. To date, there is little information on the microbial diversity and characteristics of dry aged beef. In this study, we explored the effect of change in meat microorganisms on dry aged beef. Initially, the total bacteria and LAB were significantly increased for 50 days during all dry aging periods. There was an absence of representative foodborne pathogens as well as coliforms. Interestingly, fungi including yeast and mold that possess specific features were observed during the dry aging period. The 5.8S rRNA sequencing results showed that potentially harmful yeasts/molds (*Candida* sp., *Cladosporium* sp., *Rhodotorula* sp.) were present at the initial point of dry aging and they disappeared with increasing dry aging time. Interestingly, *Penicillium camemberti* and *Debaryomyces hansenii* used for cheese manufacturing were observed with an increase in the dry aging period. Taken together, our results showed that the change in microorganisms exerts an influence on the quality and safety of dry aged beef, and our study identified that fungi may play an important role in the palatability and flavor development of dry aged beef.

Keywords: Dry aged beef, meat microorganisms, *Penicillium*, *Debaryomyces*

With respect to beef consumption, in the case of first grade beef, consumer preference is high, whereas second- and third-grade beef are less preferred. Although the prevalence of consumption of second- and third-grade beef accounts for approximately 35% [1], consumption inequality occurs as a result of the low consumer preference, leading to a large price difference between different grades of beef and causing economic loss for the farmers. Therefore, in order to solve these problems, it is necessary to improve the sensory quality of low-grade beef, increase the value added, and balance consumption. There are representative methods of improving the palatability and flavor of beef.

Ripening can be classified into wet ripening and dry ripening depending on the method [2]. Wet ripening is a method of vacuum packing the beef and aging it at a low

temperature, thereby alleviating yield reduction from moisture evaporation and inhibiting microbial growth. Dry aging has a disadvantage, as the yield is reduced by evaporation of water and removal of the cured surface by aging of the subducting material or partial meat without exposing the material to air [3]. However, it has an advantage as the flavor is excellent [4] as a result of increased concentration of taste-related substances due to water evaporation [5] and has an effect on improving the palatability and flavor of meat [6]. On a commercial basis, dry aging of beef is performed by storing for 40–60 days under controlled environmental conditions in a refrigerated room (0°–4°C) and with a relative humidity of 75%–80% and air-flow to improve several traits of beef that affect consumer satisfaction, such as tenderness and flavor [3].

According to Li *et al.* [7], wet and dry ripening of beef fillets showed that the flavor of dry aged beef loin was superior to that of wet aged beef loin. Therefore, we can improve the sensory quality of grade 2 and grade 3 dry aged beef that has a low content of muscle fat. However, there are few studies assessing the quality characteristics of low-grade beef that has been dry aged and matured. In particular, to date, there is very little information on the microbiological diversity, characteristics, and safety of dry aged beef [3]. In this study, we explored the effects of the characteristics of meat microorganisms, including bacteria (especially representative foodborne pathogens) and fungi/yeast, on dry aged beef.

Eight carcasses (grade 1st Hanwoo cattle) were selected and aged at 1°C–4°C and a relative humidity of 80%–90% until 60 days after slaughter. On 3, 25, 40, 50, and 60 days postmortem during aging, a single 5.0-cm-thick longissimus thoracis (LT) and biceps femoris (BF) section was taken from the surface of each carcass. Samples from the dry aged beef were transported to the laboratory at 4°C within 3 h after being cut, without being vacuum packed.

Initially, 25 g of dry aged beef samples were aseptically removed from the packages and placed into whirlpak bags containing 225 ml of 0.1% peptone water. Samples were then stomached for 2 min and serial dilutions were made. According to the manufacturer's instructions, subsequent triplicate spread plating was performed on Petrifilm aerobic plate count (APC) plates, Petrifilm coliform plate count plates, and Petrifilm yeast and mold count plates. The APC and coliform plate count plates, Petrifilm lactic acid bacteria count plates, and Petrifilm yeast and mold count plates were incubated aerobically at 25°C for 72 h in an aerobic incubation chamber, respectively. Counts were recorded as colony forming units per gram (CFU/g).

Next, the detection of pathogens was performed according to the methods of the Korea Food & Drug Administration [8]. Briefly, for qualitative enrichment of *Escherichia coli* O157:H7 populations, stomached samples were initially added to 225 ml of mTSB broth supplemented with novobiocin (8.0 µg/ml). These enrichments were incubated at 37°C for 24 h. After incubation, MacConkey agar with sorbitol, cefixime, and tellurite (TC-SMAC; Oxoid, UK) plates were inoculated respectively and incubated at 37°C for 24 h. For qualitative enrichment of *Salmonella* spp., samples were added to 225 ml of buffered peptone water and incubated at 37°C for 24 h. After incubation, 0.1 ml of enrichment broth was added to 10 ml of Rappaport-Vassiliadis (RV; Oxoid) broth and incubated for an additional 24 h at 42°C. After incubation, the samples were inoculated

onto XLT4 (Oxoid) plates respectively and incubated at 37°C for 24 h, and then colonies were identified from yellow to red colonies with a black center. Enrichment of *Listeria monocytogenes* was carried out in *Listeria* enrichment broth (Oxoid). The suspensions were homogenized and incubated at 30°C for 24 h. After incubation, 1 ml was transferred to 10 ml of Fraser broth and incubated at 30°C for 24 h. The enrichment culture was inoculated onto PALCAM (Oxoid) agar and cultured at 30°C for 48 h. To identify *Staphylococcus aureus*, samples were added to 225 ml of 10% NaCl-added Tryptic Soy Broth and incubated at 37°C for 24 h. The enrichment culture was inoculated onto Baird Parker (Oxoid) agar and cultured at 37°C for 24 h. For *Bacillus cereus* assay, samples were added to 225 ml of 0.1% peptone water and serial dilutions were spread on Mannitol Egg Yolk Polymyxin agar plates and colonies were enumerated after incubation for 24 h at 35°C.

In addition, during dry aging, the featured fungi samples on the surface were collected. For isolation of fungi from dry aged beef, 1 g of samples were serially diluted in 9 ml of a NaCl aqueous solution (0.85% (w/v)) to a final concentration of 10⁶ and spread on Rose Bengal or Dichloran-Glycerol (DG18) agar (Oxoid). As soon as the colonies developed on dry aged beef, they were transferred to fresh Rose Bengal agar. After isolation, single colonies were stored on Potato Dextrose Agar at 4°C. To identify these colonies, genomic DNA was extracted using a ZR Fungal/Bacterial DNA MicroPrep Kit according to the manufacturer's instructions and amplified using specific primers targeting internal transcribed spacers, including the 5.8S rRNA gene. The PCR products were sequenced using an automated DNA sequencer (Macrogen, Korea) and analyzed with the BLAST program provided by the National Center for Biotechnology Information to confirm the fungal species.

In this study, we determined the microbial characteristics of dry aged beef using plate counting with a selective medium. As expected, total bacteria and yeast/mold were significantly increased in both LT and BF samples for 50 days during all dry aging periods, whereas their growth was significantly slower after 50 days. Consistent with our results, previous results have indicated that total bacteria increase up to 5 log at 28 days of the dry aging process [9]. Moreover, Li *et al.* [10] have reported that the population of total bacteria and yeast count were rapidly increased in dry aged samples, and they could tolerate the low water activity and transmission of oxygen during the dry aging process. In addition, coliforms were not detected on the surface of samples obtained from dry aged beef. Interestingly, lactic acid bacteria (LAB) were also found in both LT and

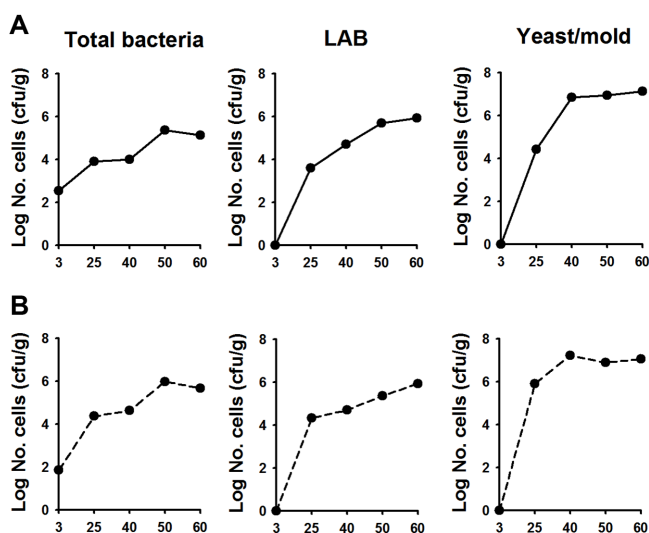


Fig. 1. Enumeration (number of CFU/g) of the total bacteria, lactic acid bacteria (LAB), and yeast/mold on the surface of dry aged beef samples of longissimus thoracis (A) and biceps femoris (B) during ripening for 60 days.

Each experiment was repeated with two independent biological replicates. Error bars show standard deviations.

BF samples with an increase in aging time (up to 6 log; Fig. 1). Unfortunately, few reports have indicated the importance of LAB in meat. In salami, various *Lactobacillus* spp., including *L. sakei* and *L. plantarum*, were detected on the surface of meat and they strongly influenced the flavor during the ripening period. Thus, our ongoing study is evaluating the novel functionality of LAB for flavor development in dry aged beef, using GC-MS/MS analysis. During dry aging for 60 days, there was no development of food-borne pathogens, including *B. cereus*, *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* sp. (data not shown). Taken together, we consider that dry aged samples during 60 days were acceptable in terms of food safety.

Furthermore, we explored the effects of the characteristics and identification of yeast/mold on dry aged beef. As expected, a number of yeast/mold species that possess specific features (colony shape and color) were specifically observed during the dry aging period (Fig. 2). The 5.8S rRNA sequencing results showed that potentially harmful yeasts and molds (*Candida* sp., *Cladosporium* sp., *Rhodotorula glutinis*, and *Rhodotorula mucilaginosa*) were present at the initial point of dry aging (~25 days). Fortunately, these strains disappeared after extending the dry aging period (60 days). It has been known that these spoilage fungi are the most important contaminants in meat products because

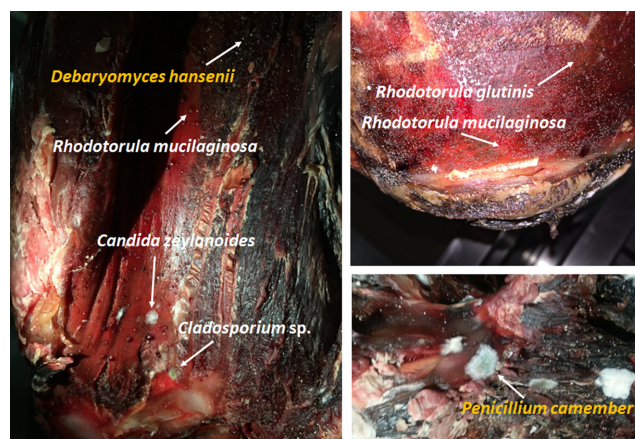


Fig. 2. Identified strains of fungi on the surface of dry aged beef.

The featured colonies were isolated on Rose Bengal or DG18 agar and were identified by comparing with the sequences obtained from the GenBank database (BLASTN freeware from <http://www.ncbi.nlm.nih.gov/BLAST>) after sequencing internal transcribed spacers, including the 5.8S rRNA gene.

of their high prevalence and their potential toxicologic properties in the fermented meat production environment [11, 12], but, to date, there are no reports of them on dry aged beef. To the best of our knowledge, this is the first report that has assessed the presence of mycobiota on the surface of dry aged beef. In addition, *Penicillium camemberti* and *Debaryomyces hansenii* used in cheese manufacturing were observed with an increase in the dry aging period (40–60 days). The most common commercial ripening strains used to produce Camembert-type cheese are *P. camemberti* and *D. hansenii* and they are the major contributors to the sensory properties of dairy foods [13]. In particular, *D. hansenii* has been used as a strategy to produce volatile compounds [14] and improve aroma [15] in animal foods. According to Matsuishi *et al.* [16], the process of dry aging produced a sweet (Glu and Asp associated with the umami taste), milk, or cheese-like aroma, which improved the flavor; hence we considered that *P. camemberti* and *D. hansenii* contributed to the flavor development of dry aged beef.

In conclusion, our results suggested that the change in microorganisms exerts an influence on the quality and safety of dry aged beef, and our study identified that yeasts/fungi may play an important role in the palatability and flavor development of dry aged beef. Future research with microbiological analysis of dry aging should focus on the application of newly identified strains of yeast/fungi to reduce the aging time and achieve the desirable features.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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