Microbial Components for Dry Aging

A Guide for Dry Age Steak Suppliers

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https://www.debragga.com/dry-aged-beef/

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Abstract

https://www.swiftandsonschicago.com

Dry aging is a process of aging meat in a controlled, open-air environment that alters the flavor composition and the tenderness of the meat. Dry-aged steak suppliers will be able to gain insight into the process by which these physical properties occur. Suppliers will also be given important information on the safety of the dry-aged steak based on the composition of the microorganisms on the steak at any given time frame. This white paper will address the microbial communities present at different time points and give insight into the mechanisms of the dry aging process that leads to a difference in taste and texture. This paper will give readers insight into the intricacies of the dry age process, how to optimally dry age a steak, and the safest time frames for consumption.

Introduction of Microorganisms

Microorganisms play an important role in dry ag
The notable microorganisms can be used in the
dry-aging process to improve the quality of the n
Pilaira anomala, Debaryomyces hansenii, Tham.
sp. Each of these microorganisms affect the qual
the meat in terms of the meat's tenderness, and fl

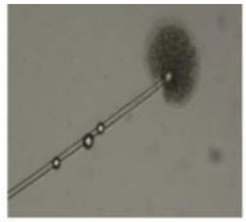

Figure 1a. Pilaira anomala taxonomic ID:
Eukaryota; Fungi; Fungi incertae sedis; Mucoromycota; Mucoromycotina; Mucoromycetes; Mucorales; Mucorineae; Mucoraceae; Pilaira
https://onlinelibrary.wiley.com/doi/full/10.1111/1750-3841.14813

Figure 1b. Debaryomyces hansenii taxonomic ID:
Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Debaryomycetaceae; Debaryomyces
https://www.jcm.riken.jp/cgi-bin/jcm/jcm_keyword?AN=Debaryomyces&BN=hansenii&CN=fabryi&DN=

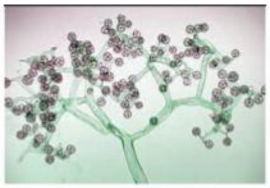

Figure 1c. Thamnidium sp. taxonomic ID
Eukaryota; Fungi; Fungi incertae sedis; Mucoromycota; Mucoromycotina; Mucoromycetes; Mucorales; Mucorineae; Mucoraceae; Thamnidium
http://tolweb.org/Zygomycota/20518/2004.12.21

Table 1: Description of microorganisms

Microorganisms	Description
Pilaira anomala (mold) [1]	- Predominant mold found on the dry-aged steak. - Impacts the tenderness and taste in the dry aged beef. - Showed protein and lipase degradation ability.
Debaryomyces hansenii (yeast) [1]:	- Shown to induce cheese and sausage fermentation. - Impacts the tenderness and taste in the dry aged beef. - Showed protein and lipase degradation ability.
Thamnidium sp. (mold) [2]:	- Appears as pale gray patches in the dry-aged steak known as "whiskers." - Impacts the tenderness and taste in the dry aged beef. - Growth starts to appear at around 3 weeks.

Analysis

Tenderness

There are multiple factors that impact the tenderness of the meat, but it first begins with the conversion from muscle to meat. After the animal is slaughtered, oxygen is immediately stopped, and the blood flow is ceased. The meat is in what is known as the pre-mortis phase, where residual O_2 in the muscles continue to maintain their structure due to the presence of ATP and phosphocreatine. After this stage, the muscles convert into the "young meat," or mortis phase, where ATP reserves and phosphocreatine levels are depleted, and anaerobic metabolism occurs *(Fig. 2),* [3] After some time, the meat goes through a tenderization process in which myofibrils are broken down via proteolysis.

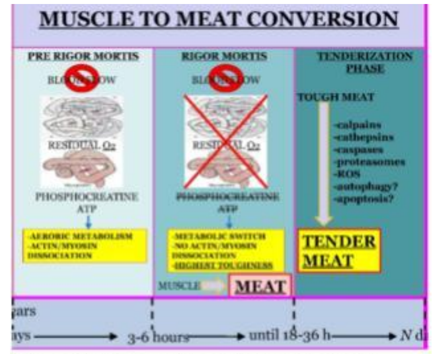

Figure 2. Conversion of Muscle to Meat. Timeline of the process from muscle to meat. After the cow is called, the muscles enter into the pre-rigor state. After O_2 is depleted, muscle transitions into rigor mortis, and tenderization begins.

The standard template. Science Direct. Lana A, Zolla L. (2016) www.sciencedirect.com/science/article/pii/S1874391916300331?via%3Dihub

Muscle Structure

The muscle structure consists of many repeating sarcomeric units, known as myofibrils. These sarcomeric units include the Z-line, I band, and A band (Fig. 3). Main components of the myofibril include filaments: thick filaments made of myosin, and thin filaments made of actin [3]. Broadly speaking, the myosin powerstroke releases P_i from the myosin cross bridges, allowing for contractions to occur as a result of the sliding of thin and thick filaments. When ADP is released, binding becomes tighter, and rigor mortis initiates [3].

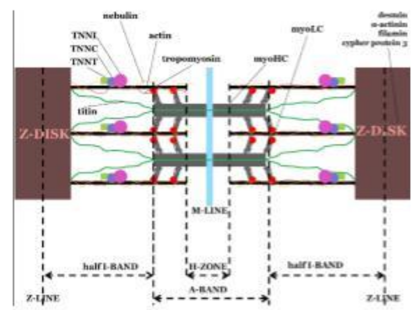

Figure 3: Sarcomeric Structure. Schematic of the sarcomeric structure highlighting key structures: the Z-Disk, I-Band, M-Line, and A-Band.

Schematic and simplified representation of the sarcomeric structure. Science Direct. Lana A, Zolla L. (2016) www.sciencedirect.com/science/article/pii/S1874391916300331?via%3Dihub

Tenderization

The process by which meat tenderizes is seen through proteolytic enzymes [3]. Myofibrils are broken down via proteases, which are enzymes that break down proteins and create collagenolytic enzymes [2]. Prior research has shown the microorganisms involved in the tenderization of meat. It can be seen from myofibril structures that show the effects that *P anomala* and *D. hansenii* have on tenderness. Transmission electron microscopy (TEM) shows that both *D.hansenii* and *P. anomala* causes myofibril breakdown via proteolysis [1], indicating improvement of tenderness. If present during the dry-aging process, can result in a more tenderized meat (Fig. 4).

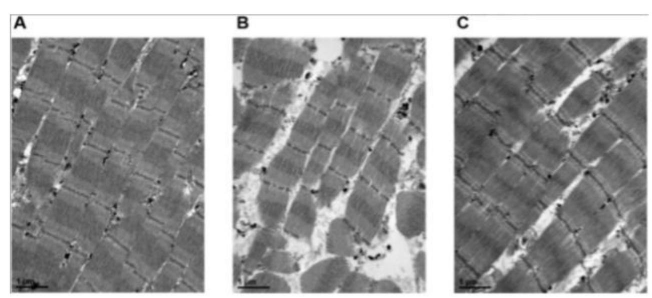

Figure 4: Myofibril ultrastructure of beef sirloin in (A) PBS (control), (B) Pilaira anomala, (C) Debaryomyces hansenii. Transmission Electron Microscopy (TEM) of the myofibril ultrastructure after 28 days of the dry-aging process. TEM analysis of *P. anomala* shows an increase breakdown of myofibril structure, with *D. hansenii* following.

The ultrastructure of beef sirloin. Journal of Food Science. Oh H, Lee, et. al (2019) https://doi.org/10.1111/1750-3841.14813

Shear force is a measure of force required to cut through meat. A decreased shear force in both *P. anomala* and *D. hansenii* indicates an increase in tenderness (*Fig. 5)* In addition, *Thamnidium* has also shown the ability to penetrate into the meat and release proteases. These create collagenolytic enzymes that help to break down muscles and connective tissues [2,4].

	Treatment	Dry-aging period (day) 0	14	21	28
Shear force (N)	Control	37.00 ± 7.38^{a}	19.61 ± 2.12^{cy}	15.89 ± 1.40^{dy}	25.19 ± 1.50^{bx}
	P. anomala	37.00 ± 7.38^{a}	19.66 ± 3.14^{cy}	13.04 ± 5.51^{dz}	27.44 ± 5.69^{bx}
	D. hansenii	37.00 ± 7.38^{a}	23.46 ± 3.93^{bx}	23.83 ± 1.97^{bx}	16.73 ± 1.56^{cy}

Figure 5: Shear force of dry aged beef. Shear force is the amount of force required to cut through meat. *P. anomala* has a lower shear force at 21 days, while *D. hansenii* has a lower shear force at 28 days. Lower shear force values indicate increased tenderness.

pH and shear force (mean ± SD) of dry‐aged beef inoculated with *Pilaira anomala* SMFM201611 and *Debaryomyces hansenii* SMFM201707. Journal of Food Science. Oh H, Lee, et. al (2019) https://doi.org/10.1111/1750-3841.14813

Flavor Compound

Dry-aging the steak comes with different flavor profiles throughout the process. As you reach the 14 - 21 dry-age period, there are notes of nuttiness and brown-roastes aromas. In addition, these flavors can transition from nuttiness, to complex flavors such as umami and mushroom [2]. Both free fatty acids and free amino acids play a role in this change.

Lipolytic activity - free fatty acids

Lipolysis involves the hydrolyzation of triglycerides and phospholipids to convert to free fatty acids (FFA) *(Fig. 6*). As the lipolysis occurs, there is a breakdown in fat content, which results in an intense nutty and beefy flavor. More specifically, oleic acid has been shown to improve beef quality [1,2]. Improvements in the flavor may be seen through the lipolytic activity that occurs, and subsequently, in the increase of oleic acid concentration in *P. anomala (Fig. 6).*

	Treatment	Dry-aging period (day) 0	14	21	28
Oleic acid (C18:1)	Control	1.85 ± 0.36^{c}	2.30 ± 0.10^{cy}	7.11 ± 1.41^{by}	9.14 ± 0.27^{ax}
	P. anomala	1.85 ± 0.36^{d}	5.62 ± 0.39^{cx}	31.77 ± 1.45^{ax}	7.71 ± 0.14^{by}
	D. hansenii	1.85 ± 0.36^{b}	2.52 ± 0.88^{by}	6.81 ± 0.94^{ay}	2.76 ± 0.22^{bz}

Figure 6: Oleic Acid content of sirloin. Measurement of lipolytic activity, which hydrolyzes triglycerides or phospholipids to free fatty acids (FFA). *P. anomala* shows the greatest oleic acid content at 21 days of dry-aging.

Free fatty acid content (mg/g) of dry‐aged beef sirloin. Journal of Food Science. Oh H, Lee, et. al (2019) https://doi.org/10.1111/1750-3841.14813

Maillard reaction - Free Amino Acids

Free amino acids are produced through the Maillard reaction, which involves the reduction of sugar and proteins by heat [1]. Certain amino acids produce different flavor profiles. While aliphatic amino acids like alanine glycine, isoleucine, leucine, proline, valine produce a sweet flavor, amino acids like asparagine and methionine produce an umami flavor [1,2]. *P. anomala* has the highest FFA value at 21 days of dry-aging (*Fig. 7)*

	Treatment	Dry-aging period (day) 0	14	21	28
Glutamic acid	Control	3.53 ± 0.16^{d}	9.42 ± 1.49^{cy}	17.28 ± 1.05^{by}	32.63 ± 2.21^{ax}
	P. anomala	3.53 ± 0.16^{c}	13.90 ± 4.25^{bcy}	22.65 ± 1.48^{ax}	23.00 ± 0.86^{ay}
	D. hansenii	3.53 ± 0.16^{c}	14.95 ± 1.23^{bx}	15.54 ± 1.34^{by}	19.78 ± 0.47^{az}
Methionine	Control	2.46 ± 0.21^{d}	5.86 ± 0.19^{cy}	10.61 ± 0.55^{by}	14.52 ± 0.42^{ax}
	P. anomala	2.46 ± 0.21^{d}	6.98 ± 0.14^{cy}	13.46 ± 0.47^{ax}	11.01 ± 0.11^{by}
	D. hansenii	2.46 ± 0.21^{d}	7.32 ± 0.21^{cx}	10.62 ± 0.30^{by}	11.54 ± 0.36^{ay}
Valine	Control	3.94 ± 0.14^{d}	9.80 ± 0.36^{cy}	18.24 ± 0.79^{by}	25.85 ± 0.46^{ax}
	P. anomala	3.94 ± 0.14^{d}	10.06 ± 0.17^{cy}	22.69 ± 0.99^{ax}	18.28 ± 0.06^{bz}
	D. hansenii	3.94 ± 0.14^{d}	11.83 ± 0.23^{cx}	16.92 ± 0.80^{by}	20.19 ± 0.92^{ay}
Phenylalanine	Control	4.38 ± 0.03^{d}	8.31 ± 0.39^{c}	14.33 ± 0.80^{by}	20.77 ± 0.42^{ax}
	P. anomala	4.38 ± 0.03^{c}	10.78 ± 3.34^{b}	18.44 ± 0.72^{ax}	15.34 ± 0.20^{ay}
	D. hansenii	4.38 ± 0.03^{d}	9.38 ± 0.38^{c}	13.95 ± 0.30^{by}	15.24 ± 0.53^{ay}
Isoleucine	Control	3.10 ± 0.05^{d}	6.84 ± 0.28^{cy}	13.11 ± 0.59^{by}	18.76 ± 0.28^{ax}
	P. anomala	3.10 ± 0.05^{d}	7.23 ± 0.19^{cy}	16.35 ± 0.79^{ax}	13.73 ± 0.16^{by}
	D. hansenii	3.10 ± 0.05^{d}	8.15 ± 0.21^{cx}	12.12 ± 0.32^{by}	14.00 ± 0.70^{ay}
Leucine	Control	5.91 ± 0.24^{d}	12.87 ± 0.37^{cy}	25.05 ± 1.16^{by}	35.15 ± 0.47^{ax}
	P. anomala	5.91 ± 0.24^{d}	13.62 ± 0.30^{cy}	29.61 ± 1.27^{bx}	23.61 ± 0.15^{az}
	D. hansenii	5.91 ± 0.24^{d}	15.55 ± 0.37^{cx}	21.84 ± 0.84^{bz}	25.56 ± 1.09^{ay}
Total	Control	23.33 ± 0.43^{d}	53.10 ± 2.92^{cy}	98.62 ± 4.81^{by}	147.69 ± 4.13^{ax}
	P. anomala	23.33 ± 0.43^{d}	62.57 ± 5.81^{cx}	123.19 ± 5.47^{ax}	104.97 ± 1.09^{by}
	D. hansenii	23.33 ± 0.43^{d}	67.19 ± 0.92^{cx}	90.98 ± 3.74^{by}	106.31 ± 3.78^{ay}

Figure 7: FAA values of amino acids with varying treatments. The Maillard reaction results from the reduction of sugars and amino acids by heat. This reaction produces free amino acids, which help produce different flavor profiles for the dry-aged meat. *P. anomala* has increased free amino acid content at 21 days of the dry-aging process.

Free fatty acid content (mg/g) of dry‑aged beef sirloin.Journal of Food Science. Oh H, Lee, et. al (2019) https://doi.org/10.1111/1750-3841.14813

Microbial and Fungal Compositions

Lactic Acid Bacteria

In the dry-aging process, lactic acid bacteria are the most dominant community until around 160 days of dry-aging (*Fig. 8)*. The presence of lactic acid bacteria in fermentation is beneficial in the dry-aging process as it inhibits pathogenic bacterial growth [4]. From 12 - 30 days, *Lactobacillus* is the most prevalent, but *Bifidobacterium* becomes more abundant after. This likely comes as a result of oxygen availability, where anaerobic lactic acid bacteria strains would thrive [4].

Potential Pathogens

One of the major pathogens found in dry-aging is *Pseudomonas psychrophila.* This pathogen may cause major spoilage, particularly in fish meats and food processing [4]. *Pseudomonas psychrophila* is present at 30 days, and 120 days (*Fig. 8)*. This may hinder the dry-aging process, as there may be deterioration of the beef [4].

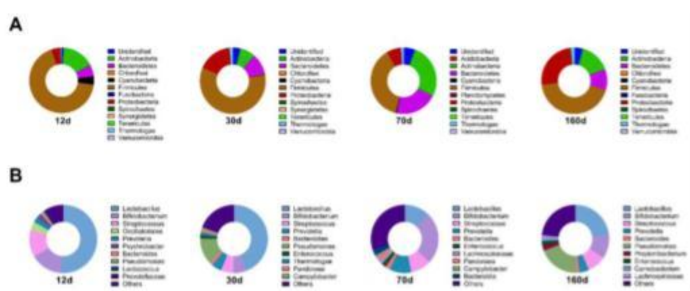

Figure 8: Relative abundance of microbial communities on the surface of dry-aged beef. A majority of the microbial communities found on the surface of the dry-aged beef are lactic acid bacteria until around 70 days. Notable pathogenic bacteria are found at 30 days, and 160 days.

Relative abundance (%) plots of the microbial communities on the surface of dry-aged beef with respect to the aging periods. Foods. Ryu S, Shin (2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7693710/

Conclusion

In conclusion, *P. anomala* should be considered as a starting culture when looking for short-term dry-ages to help with the tenderness, and the various flavor profiles. On the other hand, using *D. hansenii* for longer-term dry ages may be more beneficial. For all dry-aged beef, it is important to incorporate lactic acid bacteria culture in the process to prevent any spoilage and inhibit pathogenic bacterial growth. One thing to note is that *Thamnidium sp.* start to grow at around 3 weeks, so longer dry-ages would benefit from the growth of gray "whiskers" on the steak.

	Factors	Conclusion
Tenderness	*Pilaira anomala* - Shear force is lowest at 21 days *Debaryomyces hansenii* - Shear force is lowest at 28 days	*Pilaria anomala* should be considered when looking for short-term dry-ages of around 21 days. *Debaryomyces hansenii* should be considered when looking for long-term dry-ages of > 28 days.
Flavor	*Pilaira anomala* - Oleic concentration is greatest at 21 days - Free amino acid content is greatest at 21 days. *Debaryomyces hansenii* - Oleic concentration is relatively low compared to *P. anomala* - Free amino acid content is greatest at 28 days.	*Pilaria anomala* should be considered when looking for short-term dry-ages of around 21 days. *Debaryomyces hansenii* should be considered when looking for long-term dry-ages of > 28 days.
Microbial Communities (Time frame)	Lactic acid bacteria - Major bacterial community that is present until around 70 days. *Pseudomonas psychrophila* - Appears at 30 days, and 160 days.	May be safe to dry-age until around 70 days, but may require more care after that time frame. Be wary of pathogenic bacteria at around day 30 and 160.

References

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