CHARACTERIZATION OF DRY-AGED MEAT FLAVOR PRECURSORS AND LIBERATION MECHANISM THROUGH A METABOLOMICS APPROACH

by

Derico Setyabrata

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Animal Sciences

West Lafayette, Indiana

December 2021

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Yuan H. Brad Kim, Chair

Department of Animal Sciences

Dr. Paul Ebner

Department of Animal Sciences

Dr. Timothy Johnson

Department of Animal Sciences

Dr. Stacy Zuelly

Department of Animal Sciences

Dr. Jerrad F. Legako

Department of Animal and Food Science,
Texas Tech University, Lubbock, TX, USA

Approved by:

Dr. Zoltan Machaty

Dedicated to my beloved family, friends and God, for which their support and love pushed me forward for success

ACKNOWLEDGMENTS

First and foremost, I would like to praise and thank God for His love and guidance throughout my Ph.D. journey. Without Him, I will not even get the chance to start this incredible journey where I met numerous outstanding mentors, loving friends and excellent education.

I would also like to extend my sincere gratitude to my major Ph.D. advisor, Dr. Brad Kim. Thank you for your constant encouragement and trust in me during this journey. I am very blessed that I could meet, work and learn from a very outstanding mentor like you for the last seven years. I will be forever grateful for the guidance, supports and life lessons for my future career.

Many thanks to my graduate committee members, Dr. Paul Ebner, Dr. Timothy Johnson, Dr. Stacy Zuelly and Dr. Jerrad Legako. Thank you for all the time you spent answering my questions and for your guidance in becoming a better researcher and teacher. I really appreciate all the time you dedicated to serving on my graduate committee. Additionally, I would also extend my appreciation to Dr. Bruce Cooper for his guidance.

I am also very grateful to past and present members of the meat science and muscle biology laboratory. I especially like to thank Dr. HyunWook Kim for his exceptional mentorship when I just started in the lab. I would also like to thank my labmates Jacob Tuell, Mariah Nondorf, Maha Abdelhaseib, Allison Trigg and Dr. Danyi Ma for their friendships, supports and encouragement throughout my doctoral journey. Likewise, I want to thank my colleagues: Yufei Guo, Mindi Dennis, Ayodeji Aderibigbe, ChanSol Park, Carmen Wickware, Eunice Centeno, Saud ur Rehman, Abdul Hannan and many others that I could not list for their help in research and lively conversation. Additionally, I would like to extend my appreciation to our meat laboratory staff, Blaine Brown and Gary Waters, and all the Department of Animal Sciences staff for their assistance.

Lastly, I would like to thank my parents and sister for supporting me and my life choices. Thank you to all my friends, Stefanus Dennis, Adriel Mulia, Kresentia Amelia, Sebastian Kenny, Jessica Kurniawan, Maureen Wiryadi and Niken Permana, for their continued motivation throughout my doctoral journey. Special thanks to my wife, Karina Desiree. I am forever grateful for your love, support and patience, which helped me through these past years.

Thank you to the USDA – National Institute of Food and Agriculture for funding this research through the Agriculture and Food Research Initiative Grant#2017-67017-26475.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Within the last decade, the popularity and interest in dry-aging have constantly increased among both consumers and producers. Dry-aging is a natural value-adding process where meat is exposed to a controlled refrigerated environment without any protective barrier during the aging process. This process leads to the development of unique flavors in the final meat product. Although the prevalence of this process is increasing, there are inconsistent reports regarding the impacts of dry-aging on meat sensory attributes, especially on the flavor aspect. Given that flavor generation is dependent on the composition and availability of flavor precursors, the presence or absence of these precursors may contribute to the inconsistency observed. Thus the main objective of the research described here was to characterize the flavor precursors in dry-aged meat and elucidate potential factors or mechanisms favoring to their production.

To achieve this objective, metabolomics analysis was conducted in conjunction with various chemical analyses (free amino acids, fatty acids, sugar content and volatile analysis), microbiome profiling and meat quality analysis (tenderness, water holding capacity, color stability, oxidative stability, microbial attributes and sensory analysis) to identify the essential flavor precursors and their production process. In addition, similar analyses were conducted using multiple meat sources (grass-fed beef loins, cull cow beef loins and pork loins) aged by wet-aging (WA), conventional dry-aging (DA), dry-aging in bag (DWA) and UV-light dry-aging (UDA) to elucidate the impact of the different aging treatments on meat quality, sensory attributes and flavor precursor availability.

Regardless of the meat source, the results demonstrated that dry-aging altered the meat flavor precursor compositions, primarily by increasing the presence of protein-derived precursors (e.g., free amino acids and dipeptides), especially glutamine and glutamate compounds. Additionally, nucleotide and carbohydrate-derived compounds such as adenosine and reducing sugars were greatly increased after the dry-aging process. While the fatty acid profile was minimally affected, metabolomics analysis revealed a decrease in sterol and terpenoid lipids following dry-aging, which could potentially reduce off-flavors development in the meat. Other compounds such as vitamin B and vitamin C were also detected in the dry-aged product, which potentially could contribute to the flavor development.

Analysis of the liberation mechanisms demonstrated that dehydration played a role in increasing the concentration of the flavor precursors in the dry-aged product, potentially promoting greater (e.g., Maillard reaction) during cooking. Furthermore, microorganisms might be responsible for further increasing the availability of flavor precursors in dry-aged meat, especially free amino acids, along with the dehydration process. Microbiome profiling found that *Pseudomonas* spp. are the most prominent bacterial species in microbial communities found on dry-aged meat which could affect the precursor release in dry-aged meat. Metabolomics analysis also indicated increased glutathione metabolism during dry-aging, which could lead to the liberation of glutamine-related compounds. The analysis also identified other compounds such as porphyrin rings (iron-related) and shikimic acid (bacterial metabolism), providing further examples of how metabolomics can identify dry-aged flavor precursors and reveal other potential mechanisms related to flavor development mechanisms.

These outcomes demonstrate that dry-aging alters meat flavor precursor composition, mainly by increasing the availability of protein-, nucleotide- and carbohydrate-derived compounds. Such results indicate that the Maillard reaction is likely be the main mechanism in flavor generation in dry-aged meat. The current results provided more insights into the dry-aging flavor development, especially highlighting important flavor precursor such as glutamate and glutamine containing products, likely to contribute to the dry-aged flavor. Future study to identify the impact of different microorganism (especially mold and yeast) on dry-aging flavor development would be of interest. Additionally, impact of different cooking process should also be studies to maximize the dry-aged flavor potential from the product.

CHAPTER 1. INTRODUCTION

Meat palatability is a vital factor in ensuring consumers' satisfaction and future willingness to purchase meat products (Smith et al., 2008). Postharvest processing, especially postmortem aging, has been well documented to enhance the meat palatability attributes, including tenderness, juiciness and flavor (Kim et al., 2018). As such, the application of postmortem aging has become a standard practice in the meat industry to improve the palatability of fresh meat products. In the United States, the National Beef Tenderness Survey has demonstrated a constant increase in the aging period applied to beef products, showing an average aging time of 25.9 days for retail and 31.5 days for food service in the most recent survey (Martinez et al., 2017). Among those palatability characteristics, tenderness has often been reported as the single most influential factor driving consumers' satisfaction (Miller et al., 2001; Savell et al., 1987). However, with the continuous increase in postmortem aging period, a shift in palatability attribute preferences have been continuously reported (Vierck et al., 2018; Wilfong et al., 2016; Lucherk et al., 2016), demonstrating a greater interest in meat flavor.

Currently, there are two aging methods practiced in the meat industry, namely wet-aging and dry-aging. Wet-aging is a widely practice aging process in the meat industry. In this process, meat cuts are vacuum packed and stored in a refrigerated condition to improve the palatability attributes. On the other hand, dry-aging is a traditional aging process in which the carcasses or meat cuts are exposed to a controlled refrigerated environment without any protective packaging. Generally, both methods have been shown to generate significant enhancement in all palatability traits. However, dry-aging specifically has been prized for the unique flavor developed through the process. Flavor descriptors such as beefy, roasted nut, sweet and buttery are often used to characterize the unique dry-aged flavor (Berger et al., 2018; Kim et al., 2016). The unique flavor and increased interest in flavor by consumers may create an opportunity for the beef producers to successfully utilize dry-aging to enhance consumer liking of meat products.

While the flavors are desirable, the impact of dry-aging on final product flavor is still inconsistent, with some studies reporting improvement (Kim et al., 2016; Lepper-Blilie et al., 2016; Li et al., 2014; Campbell et al., 2001) and some reporting no differences among the aging methods (Dikeman et al., 2013; DeGeer et al., 2009; Laster et al., 2008; Sitz et al., 2005). In general, the flavor generation process in food and meat products is dependent on the availability of flavor

precursors (Mottram, 1998). Compounds such as amino acids, sugars, fatty acids and nucleotides are some of the common flavor precursors responsible for the perceived meat flavor (Calkins and Hodgen, 2007). However, flavor generation is a complex process, and the presence of different precursor species could also play a role in determining the final meat flavor (Aaslyng and Meinert, 2017). Although recent studies have reported increase in amino acids and nucleotides availability in dry-aged products (Hanagasaki and Asato, 2018; Kim et al., 2016), the involvement and impact of other flavor precursors compounds in the dry-aged meat remain undetermined. Additionally, the factors that affected the liberation of these flavor precursors during the dry-aging process are still unclear and warrant further investigation to fully comprehend the influence of dry-aging on the flavor generation process. Therefore, this dissertation aims to characterize the presence and functions of the different flavor precursors following the dry-aging process and to provide some novel insights into the mechanisms involved in the liberation of those flavor compounds during the dry-aging process. A complete understanding of this process may better-equip producers with the necessary knowledge to generate consistent dry-aged products to guarantee consumers' demand for better meat flavor.

1.1 Literatures Cited

Aaslyng, M.D., and L. Meinert. 2017. Meat flavour in pork and beef – From animal to meal. Meat Sci. 132:112–117. doi:10.1016/j.meatsci.2017.04.012.

Berger, J., Y.H.B. Kim, J.F. Legako, S. Martini, J. Lee, P. Ebner, and S.M.S. Zuelly. 2018. Dry-aging improves meat quality attributes of grass-fed beef loins. Meat Sci. 145:285–291. doi:10.1016/j.meatsci.2018.07.004.

Calkins, C.R., and J.M. Hodgen. 2007. A fresh look at meat flavor. Meat Sci. 77:63–80. doi:10.1016/j.meatsci.2007.04.016.

Campbell, R.E., M.C. Hunt, P. Levis, and E. Chambers IV. 2001. Dry-aging effects on palatability of beef longissimus muscle. J. Food Sci. 66:196–199. doi:10.1111/j.1365-2621.2001.tb11315.x.

DeGeer, S.L., M.C. Hunt, C.L. Bratcher, B.A. Crozier-Dodson, D.E. Johnson, and J.F. Stika. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Sci. 83:768–774. doi:10.1016/j.meatsci.2009.08.017.

Dikeman, M.E., E. Obuz, V. Gök, L. Akkaya, and S. Stroda. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef Longissimus lumborum steaks. Meat Sci. 94:228–233. doi:10.1016/j.meatsci.2013.02.002.

Hanagasaki, T., and N. Asato. 2018. Changes in free amino acids and hardness in round of Okinawan delivered cow beef during dry- and wet-aging processes. J. Anim. Sci. Technol. 60:1–9. doi:10.1186/s40781-018-0180-x.

Kim, Y.H.B., R. Kemp, and L.M. Samuelsson. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Sci. 111:168–176. doi:10.1016/j.meatsci.2015.09.008.

Kim, Y.H.B., D. Ma, D. Setyabrata, M.M. Farouk, S.M. Lonergan, E. Huff-Lonergan, and M.C. Hunt. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. Meat Sci. 144:74–90. doi:10.1016/j.meatsci.2018.04.031.

Laster, M.A., R.D. Smith, K.L. Nicholson, J.D.W. Nicholson, R.K. Miller, D.B. Griffin, K.B. Harris, and J.W. Savell. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer sensory attribute evaluations of steaks from ribeyes, strip loins, and top sirloins from two quality grade groups. Meat Sci. 80:795–804. doi:10.1016/j.meatsci.2008.03.024.

Lepper-Blilie, A.N., E.P. Berg, D.S. Buchanan, and P.T. Berg. 2016. Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. Meat Sci. 112:63–68. doi:10.1016/j.meatsci.2015.10.017.

Li, X., J. Babol, W.L.P. Bredie, B. Nielsen, J. Tom??nkov??, and K. Lundstr??m. 2014. A comparative study of beef quality after ageing longissimus muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. Meat Sci. 97:433–442. doi:10.1016/j.meatsci.2014.03.014.

Lucherk, L.W., T.G. O'Quinn, J.F. Legako, R.J. Rathmann, J.C. Brooks, and M.F. Miller. 2016. Consumer and trained panel evaluation of beef strip steaks of varying marbling and enhancement levels cooked to three degrees of doneness. Meat Sci. 122:145–154. doi:10.1016/j.meatsci.2016.08.005.

Martinez, H.A., A.N. Arnold, J.C. Brooks, C.C. Carr, K.B. Gehring, D.B. Griffin, D.S. Hale, G.G. Mafi, D.D. Johnson, C.L. Lorenzen, R.J. Maddock, R.K. Miller, D.L. VanOverbeke, B.E. Wasser, and J.W. Savell. 2017. National Beef Tenderness Survey–2015: Palatability and Shear Force Assessments of Retail and Foodservice Beef. Meat Muscle Biol. doi:10.22175/mmb2017.05.0028.

Miller, M.F., M.A. Carr, C.B. Ramsey, K.L. Crockett, and L.C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79:3062–3068. doi:10.2527/2001.79123062X.

Mottram, D.S. 1998. Flavour formation in meat and meat products: A review. Food Chem. doi:10.1016/S0308-8146(98)00076-4.

Savell, J.W., R.E. Branson, H.R. Cross, D.M. Stiffler, J.W. Wise, D.B. Griffin, and G.C. Smith. 1987. National Consumer Retail Beef Study: Palatability Evaluations of Beef Loin Steaks that Differed in Marbling. J. Food Sci. 52:517–519. doi:10.1111/j.1365-2621.1987.tb06664.x.

Sitz, B.M., C.R. Calkins, D.M. Feuz, W.J. Umberger, and K.M. Eskridge. 2005. Consumer sensory acceptance and value of domestic, Canadian, and Australian grass-fed steaks. J. Anim. Sci. 83:2863–2868. doi:10.2527/2005.83122863x.

Smith, G.C., J.D. Tatum, and K.E. Belk. 2008. International perspective: Characterisation of United States Department of Agriculture and Meat Standards Australia systems for assessing beef quality. Aust. J. Exp. Agric. 48:1465–1480. doi:10.1071/EA08198.

Vierck, K.R., J.M. Gonzalez, T.A. Houser, E.A.E. Boyle, and T.G. O'Quinn. 2018. Marbling Texture's Effects on Beef Palatability. Meat Muscle Biol. 2:127. doi:10.22175/mmb2017.10.0052.

Wilfong, A.K., K. V. McKillip, J.M. Gonzalez, T.A. Houser, J.A. Unruh, E.A.E. Boyle, and T.G. O'Quinn. 2016. The effect of branding on consumer palatability ratings of beef strip loin steaks,. J. Anim. Sci. 94:4930–4942. doi:10.2527/JAS.2016-0893.

CHAPTER 2. LITERATURE REVIEW

The content presented in this chapter is partially reprinted from our previously published work titled "Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies". Some edits have been made to produce a cohesive dissertation chapter. The published work reference is as follow: Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., & Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. Meat Science, 144, 74-90. Copyright © 2020 Elsevier Ltd

Before discussing the investigation on the role of flavor precursors and mechanisms surrounding the dry-aged flavor generation process, it is important to address the current state of knowledge regarding the dry-aging process and its impact on meat palatability and quality. This literature review will first aim to describe the dry-aging process and the parameters potentially influencing the final product outcome. Next, we will address the current known impact of dry-aging on meat palatability, quality and safety, including the current knowledge on the composition of flavor precursors in the product. Finally, we will highlight the metabolomics technologies potentially beneficial in uncovering dry-aged products' flavor precursor composition and possible mechanisms responsible for their liberation.

2.1 Postmortem aging types and parameters

2.1.1 Aging types

Postmortem aging in fresh meat products has been shown to greatly influence meat palatability and quality. During this process, substantial biochemical changes occur due to the activity of endogenous enzymes naturally present within the meat (Huff Lonergan et al., 2010). These enzymes degrade the muscle myofibrillar structures and other large molecules, contributing to the sensory improvement observed in the meat products. Currently, there are two types of aging methods, namely dry-aging and wet-aging. These are the most commonly practiced aging methods used by local processors, large packers, and food retailers in the meat industry.

Wet-aging

Wet-aging is an aging process where sub-primal cuts are vacuum packaged and held in a refrigerated environment for a range of 3 to 83 days to create the desired quality changes. This time range covers from packing plants/local meat processors to the retail level (Voges et al., 2007). Wet-aging is the most predominant method of postmortem aging and is dependent on good vacuum packaging of cuts and excellent temperature control at -1 to 2 °C. Advantages associated with wet-aging are: 1) significant reductions in product weight loss, 2) less trim loss, 3) less refrigeration space required, 4) extended shelf-life without sacrificing palatability traits, 5) less operation facility cost, and 6) adaptability to automation, efficient product flow. Wet-aging of many carcass cuts improves tenderness; however, some negative flavor characteristics, such as bloody, serumy, metallic, and sour, may occur with wet-aging (Warren and Kastner, 1992). In wet-aging, temperature and aging time are the main factors to control without damaging other properties or traits in a negative way.

Dry-aging

Dry-aging, in contrast, is a traditional process where carcasses, primals, and/or sub-primals are stored in a cold room without protective packaging at 0-3 °C for several weeks (Savell, 2008). Dry-aging is an expensive process mainly due to the higher product loss (due to shrinkage and moisture loss) and the intensive time and labor cost associated with the aging processes (Savell, 2008; Parrish et al., 1991). Dry-aging is most typically practiced by small to medium-sized meat processors and meat purveyors for upscale hotels, restaurants, and gourmet markets. In general, dry-aging is known to enhance the palatability attributes of meat, especially a unique, "dry-aged flavor," yielding descriptive determinants such as brown-roasted, beefy/brothy, buttery, nutty, roasted nut, and sweet (Warren and Kastner, 1992; Campbell et al., 2001; O'Quinn et al., 2016; Kim et al., 2016).

There are currently two primary dry-aging methods in the meat industry; conventional whole carcass hanging and sub-primal dry-aging. Although most dry-aging involves beef sub-primals in the modern practice, conventional carcass dry-aging (by hanging whole carcass sides in a cooler for 10 to 35 days) is still practiced by many local meat processors as a value-adding process to attract local customers (Richardson et al., 2008; Jeremiah and Gibson, 2001).

Conversely, sub-primal dry-aging is a more common form of aging practiced by local niche/gourmet markets and high-end restaurants, where sub-primals (particularly the carcass middle portion) are stored in coolers engineered with a high degree of environmental control of factors such as temperature, air purity, humidity, air movement and limited access by people. Some of these chambers are designed to be "all in and all out" operations meaning that the unit is loaded with product, aged for a specified time and then emptied for cleaning and preparation for the next batch.

An integrated aging system has been introduced to the meat industry that combined both wet- and traditional dry-aging attributes. This system is known as "dry-aging in a bag" that is highly permeable to water vapor. Bags typically consist of a 2 mm thermoplastic elastomer made of a flexible polymer and rigid polyamide. Water vapor transmission rates are about 8000 g/15 μ/m2/24 h at 38 °C with 50% relative humidity and an oxygen transmission rate of 2.3 mL/m2/d at 38 °C (UMAi Dry®, Wayzata, MN). Several studies found that beef sub-primals that were dry-aged in the special bag system had similar sensory traits as traditionally dry-aged counterparts while having substantially higher saleable yield (due to lower weight loss during aging and trim loss after aging) and lower microbial contamination (Stenström et al., 2014; Dikeman et al., 2013; DeGeer et al., 2009; Ahnström et al., 2006)

2.1.2 Dry-aging parameters

As dry-aging exposes fresh meat products directly into a cooling chamber/room, the "balance" of environmental conditions are critical factors affecting the overall quality of the final meat product.

Aging temperature

Storage temperature is a critical factor in dry-aging, as it relates to microbial growth and proteolytic enzyme activity in meat. A lower temperature will slow the activity of the endogenous enzyme, significantly reducing the tenderizing process and flavor-related changes. Lower temperatures also retard microbial growth and thus prevent further flavor development induced by microbial enzymes. Higher temperatures would not be suitable for extended aging, as it creates a larger risk for microbial spoilage and possible pathogenic contamination of meat. Although it

varies between studies, dry-aging is mostly conducted at a temperature of 0-4 °C, similar to the temperature of regular wet-aging, as summarized in Table 2.1. Variation of temperature across the dry-aging period may have advantages.

Aging time

The length of aging and storage is often related to tenderness improvement and the extent of desirable flavor compound formation. Campbell et al. (2001) reported that storage of loins in vacuum for 7 or 14 prior to dry aging did not affect any dry-aging traits. They also reported that dry-aging for just 7 days was not sufficient for the development of desirable dry-aged traits. Most research reports common day-aging times ranging from 14 to 35 days (Table 2.1. For dry-aging, the meat starts to show the desirable dry-aged meat quality as early as 14 days (Savell, 2008; Campbell et al., 2001), although multiple studies found 21 days were needed for noticeable flavor development (Li et al., 2013; DeGeer et al., 2009; Richardson et al., 2008; Campbell et al., 2001). No difference in dry-aged flavor was observed by Iida et al. (2016) for beef dry-aged to either 30 days or 60 days, indicating a limited benefit of an extended aging period in dry-aging flavor development processes. Interestingly, Campbell et al. (2001) also reported that additional vacuum storage following dry-aging did not deteriorate the dry-aging up to 16 days of vacuum storage. Those authors suggested that the flavor intensity in the dry-aged product peaked at 9 days of vacuum storage.

Airflow

Air-flow is also one of the essential dry-aging parameters, since sufficient/proper air circulation in the dry-aging cooler results in uniform drying on meat surfaces and prevents spoilage and off-odor development (Savell, 2008). However, few studies have provided the airflow information used (Kim et al., 2017b; Lepper-Blilie et al., 2016; Parrish et al., 1991), showing airflow variation ranging from 0.2 – 2.5 m/s utilized in those studies (Table 2.1). While the specific airflow impacts on dry-aged meat quality have now been reported, a recent study from Kim et al. (2016) authors reported that no significant impact was observed on the overall yield when the products were aged using either 0.2 m/s or 0.5 m/s airflows. The authors, however, determined that the different airflow

regimes coupled with different aging temperatures affected the humidity of the dry-aging cooler and thus influencing the final meat quality attributes.

Relative humidity

Controlling relative humidity (RH) is an important element for dry-aging. In this respect, excessively high RH adversely impacts flavor (off-odors/off-flavors) due to spoilage, primarily resulting from bacterial growth (Savell, 2008). Conversely, if RH is too low, the yield loss of meat products will be increased due to shrinkage. Various ranges of RH for dry-aging, from 49% to 87%, have been evaluated in controlled scientific studies, as summarized in Table 2.1. Among these studies, it should be noted that no significant dry-aging effects on palatability attributes were observed when the RH was higher than 80%. In contrast, positive results from dry-aging have been observed in stored beef sub-primals when RH was lower than 78%, regardless of any major animal background factors (e.g., USDA quality grade).

Microorganism

Although the presence of microorganisms is often undesirable in meat products, recent reports suggested that the presence of microorganisms during the dry-aging process could be vital for flavor development (Oh et al., 2019b; Lee et al., 2019c; Hulánková et al., 2018; Dashdorj et al., 2016). Various microorganisms, such as *Thamnidium* sp. and *Debaromyces hansenii*, were previously identified and suggested to be responsible for the dry-aging flavor development (Lee et al., 2019c). Similarly, *Pseudomonas* sp. and *Lactobacillus* sp. were also identified following dry-aging, and thus was suggested to play a role in the dry-aging process (Ribeiro et al., 2021a; Hulánková et al., 2018). While it is still not clear, those authors attributed the release of proteolytic and lipolytic enzymes from the microorganism to greater flavor. It was speculated that the activity of such enzymes would further promote meat degradation, liberating more free amino acids and free fatty acids to participate in the flavor generation process. More research, however, is needed as the presence of microorganisms could lead to food safety issues in the product.

2.2 Dry-aging impacts on meat palatability, quality and safety

2.2.1 Meat Palatability

For meat products, palatability is often associated with tenderness, juiciness and flavor attributes perceived from the meat. It has been well known that postmortem aging significantly enhances the aged meat's palatability outcome (Kim et al., 2014; Huff Lonergan et al., 2010; Huff-Lonergan and Lonergan, 2005). The sensory improvement could be attributed to the activity of multiple endogenous proteolytic enzymes (such as calpains, caspases and cathepsins), which degraded the muscle proteins (Lepper-Blilie et al., 2016; Huff-Lonergan and Lonergan, 2005). This activity weakens the meat structures and releases small molecular compounds, which are often attributed to the increase of tenderness, juiciness, and flavor perceived from aged meat products.

Several groups have previously reported positive impacts on eating quality attributes in dry-aged meat, as summarized in Table 2.1. A recent study from Li et al. (2014) found that beef loins dry-aged for 21 days had greater scores on sensory results (e.g., tenderness, juiciness and umami flavor) than the wet-aged beef loins. Berger et al. (2018) also demonstrated that consumer panels determined greater flavor and tenderness preferences in steaks from dry-aged beef loins for 28 days compared to steaks from wet-aged counterparts. While tenderness is considered as the most important palatability characteristic for consumers (Miller et al., 2001; Savell et al., 1987), recent reports have indicated a shift in consumers' sensory preferences from tenderness to flavor (Nyquist et al., 2018; Vierck et al., 2018; Wilfong et al., 2016; Lucherk et al., 2016). When tenderness is deemed acceptable, flavor becomes the primary factor influencing beef palatability (Behrends et al., 2005a; b; Killinger et al., 2004; Goodson et al., 2002). Similarly, findings from a multi-city study show that, when tenderness was held constant, consumers considered flavor to be the greatest factor influencing meat purchasing decisions (Sitz et al., 2005).

In general, the primary reason for the application of dry-aging is to promote the development of unique/natural flavor rather than direct tenderness improvement of fresh meat (Savell, 2008). Brown/roasted flavor, nutty and beefy/umami are some of the sensory flavors often associated with "dry-aged flavor" (Savell, 2008; Campbell et al., 2001; Warren and Kastner, 1992). Conflicting results, however, exist in the literature in regards to the flavor improvement following dry-aging. Several studies (Dikeman et al., 2013; DeGeer et al., 2009; Laster et al., 2008) reported that no

flavor differences were observed between the dry-aged and wet-aged products by consumers. While the lack of perceived differences could be the result of unfamiliarity of consumers with the product, this lack of differences could also be attributed to the variability of the dry-aging processes. It is possible that these differences lead to different liberation of flavor precursors and thus lower ability for flavor generation. The factors and/or flavor-related chemical compounds that positively affect the development of dry-aging flavor are unclear and thus require further research.

Improving tenderness is not a primary reason for dry-aging by the meat industry. Most studies found that tenderness is likely affected by the duration of aging regardless of the type of aging applied. Interestingly, however, several studies reported that consumer panels found dry-aged beef loins to be more tender than wet-aged beef loins, although no difference ($P > 0.05$) in the shear force values between the different aging treatments was found (Berger et al., 2018; Kim et al., 2015; Li et al., 2014). In addition, it has been known that consumer beef flavor liking is well-correlated with overall acceptance and other eating quality attributes (Corbin et al., 2015). Thus, this phenomenon may be attributed to the synergistic effects of the improvements in other eating quality characteristics, such as juiciness and/or flavor, which subsequently result in an increase in perceived tenderness (Berger et al., 2018).

Dry-aging can have an impact on sensory juiciness (Berger et al., 2018; Kim et al., 2015; Li et al., 2014), despite the fact that dry-aging is typically accompanied by a considerable amount of moisture loss. It is assumed that while the moisture is lost, the fat is also being concentrated during the aging, increasing the fat to moisture ratio of the product. This is suspected to give the perception of better juiciness (Campbell et al., 2001). Higher fat content is also suggested to induce saliva production, giving the illusion of higher sensory juiciness of the product (Campbell et al., 2001).

2.2.2 Color and oxidative stability

The application of postmortem aging generally could negatively influence the color and oxidative quality of meat (Ma et al., 2017; King et al., 2012; Kim et al., 2011). The impact of dry-aging, with or without a bag, on meat color and color stability has not been fully explored yet. Since the dry-aged product is considered a high-end specialty product that is available in local niche/gourmet markets and restaurants, the retail display color is not primarily considered in the meat industry. There are few published studies that actually reported the effects of dry-aging on

an initial (bloomed) meat color. There is a general agreement that dry-aged beef steaks were slighter darker and had lower redness values compared with wet-aged counterpart steaks (Kim et al., 2016; Dikeman et al., 2013). The darker color in the dry-aged beef is mostly due to moisture loss and surface drying during aging (Kim and Hunt, 2011).

More recently, the color stability of steaks from dry-aged beef loins was assessed over a 7 day retail display simulation (Ribeiro et al., 2021b). In this study, steaks from dry-aged beef were reported to have similar color quality ($P>0.05$) compared to its wet-aged counterpart from day 1 to day 3 of the simulated display. However, the dry-aged steaks developed more discoloration ($P<0.05$) from day 4 until the end of the display. Likewise, the same authors also reported that higher lipid oxidation ($P<0.05$) was measured on dry-aged beef directly after the aging process and following the aerobic display. Both lipid and protein (myoglobin) oxidation are often linked together and suggested to exacerbate the intensity of the oxidation in the product. In meat, this oxidation has been suggested to not only influence the meat quality but also meat palatability, especially in dry-aged products (DeGeer et al., 2009). Further understanding of the influence of dry-aging on color and oxidative stability would be beneficial to highlight potential economic impact through retail merchandising and identifying its impact on flavor development during dry-aging.

2.2.3 Food Safety

Although the growth of microorganisms during the dry-aging processing has been speculated to be beneficial, a guarantee of safety is still required for any food produced and made available to the public. The presence of pathogenic bacteria cell could lead to mortality and could not be tolerated in the food industry. However, with the environmental exposure required for the dry-aging process, pathogen contamination is a constant concern. It is critical to ensure that the dry-aging process is safe and that the product generated is still wholesome following the processing. Multiple studies have presented the effect of dry-aging on microorganism growth or presence during the dry-aging process. However, only a handful of reports are currently available discussing the effect of dry-aging on safety and pathogenic microbial growth.

A study by Algino et al. (2007) surveyed multiple different meat processors and the effectiveness of their intervention techniques. It was demonstrated that the reduction of *E. coli*, coliforms, Enterobacteriaceae prevalence could be observed as early as 4 days into the carcass dry-

aging process. Tittor et al. (2011) also found a reduction in pathogens concentration through their inoculation studies, showing a reduction of up to 4 logs by the end of the 28 days aging period for both *Escherichia coli* O157:H7 and *Salmonella*. Those authors also highlighted that the reduction of the pathogenic bacteria was significantly greater in dry-aging compared to wet-aging, although both practices decreased the pathogen concertation throughout the aging period. Similarly, Knudsen et al. (2011) also reported a significant decrease of multiple *Salmonella* strains after 14 days of dry-aging treatment. While the extent of the decrease was different between the strains, a significant reduction was still observed before and after the aging application. In general, these results demonstrated that dry-aging could minimize the presence of pathogenic bacteria during the dry-aging process. While more studies are still required to confirm the results, it was proposed that extensive removal of moisture from the surface could be the main contributor in limiting the pathogenic bacteria growth. It was expected that the drying process created an environmental barrier and thus reducing microbial growth.

In addition to the environmental barrier, it is possible that the presence of other microorganism communities in the meat during dry-aging also contribute to inhibiting the pathogenic microorganism growth. Pathogenic bacteria are often opportunistic in nature and would only grow in favorable environmental conditions (Aujoulat et al., 2012). It is possible that native microorganisms present in the meat prior to the surface drying suppress the pathogenic microorganism and prevent further pathogen growth. Further studies are still required to support this speculation.

2.3 Dry-aging flavor compounds

Significant compositional changes occurred during postmortem aging, mainly due to the fact that the muscle cells are degraded through the process. The degradation of muscle components resulted in small molecular compounds (such as free amino acids, nucleotides, free fatty acids and sugars) being released from the cells and accumulated in the product. These compounds are known as flavor precursors and have been shown to influence the overall flavor perceived from food products by directly influencing flavor intensity or participating in Maillard reaction and thermal degradation during the cooking process (Diez-Simon et al., 2019; Frank et al., 2016, 2017). The greater flavor perceived in dry-aging is suggested to occur due to a unique flavor precursor composition developed in the products.

Free amino acids

In dry-aged products, the presence of free amino acids has been suggested to play a significant role in flavor development (Dashdorj et al., 2016; Iida et al., 2016; Koutsidis et al., 2008). Available reports indicated that a greater total free amino acids concentration was measured in products treated with dry-aging when compared to wet-aging (Lee et al., 2019b; c; Kim et al., 2016) and mainly increased with a longer aging period (Iida et al., 2016). Amino acids such as tryptophan, phenylalanine, valine, tyrosine, glutamate, isoleucine, and leucine have been shown to be present in greater concentration in the dry-aged product than in the wet-aged (Kim et al., 2016). As the majority of the free amino acids carry a specific taste (Zhao et al., 2016), the greater abundance of those free amino acids could potentially directly influence the flavor intensity of the dry-aged product. Some amino acids, such as glutamate and aspartate, are suggested to promote umami taste which is often associated with the dry-aged product and thus have been the main focus for numerous dry-aging studies (Dashdorj et al., 2016; Koutsidis et al., 2008). Although the amino acids carry a unique taste, participation in the Maillard reaction during cooking has been suggested as the main means of flavor production from free amino acids. With greater abundance, aroma volatiles could be generated in higher concentrations and thus contributing to the unique flavor perceived in dry-aged products.

The release of free amino acids is expected during the aging process due to the native aminopeptidase/degradation enzyme's activity in the muscle (Toldrá and Flores, 2010; Moya et al., 2001; Feidt et al., 1996). While those enzyme activities degraded the protein and liberated free amino acids, the greater increase of amino acids observed was suggested due to moisture evaporation. It is commonly accepted that the dry-aging process allowed moisture loss, which concentrated the flavor precursors and, therefore, created the unique flavor (Lee et al., 2019c; Kim et al., 2016; Savell, 2008). More recent reports also introduced the possible involvement of microorganisms in releasing free amino acids and other flavor precursors. Several microbes strains such as *P. anomala*, *D. hansenii*, *Thamnidium* spp., *Pseudomonas* spp. and *Lactobacillus* spp., have been reported to be influential in the flavor development (Ribeiro et al., 2021a; Lee et al., 2019c; Hulánková et al., 2018; Ryu et al., 2018). Those microorganisms were suggested to produce proteolytic enzymes, which could then further accelerate the muscle degradation and liberation of flavor precursors.

Additionally, the activity of the proteolytic enzymes (endogenous or exogenous) could potentially lead to the release of dipeptides and short peptides. It was suggested by Lee et al. (2019b) that exogenous microbial enzymes were more active in degrading large protein molecules to smaller peptide chains, indicating incomplete breakdown of the proteins to the amino acids. Zhao et al. (2016) reported that greater short peptide abundance was observed when *Penicillium* sp. or *Bacillus* sp. was present in the product. Interestingly, depending on the amino acid sequence, the peptides generated could also produce a taste similar to those of the amino acids (Ramalingam et al., 2019). Furthermore, a study conducted by Zou et al. (2018) also highlighted that the peptide-based Maillard reaction would generate a greater concentration of volatiles compared to the amino acid-based Maillard reaction, thus potentially affecting the overall flavor perceived from the product.

Nucleotides

The involvement of nucleotides and their derivatives are of interest in understanding the unique dry-aged flavor. The 5′-monophosphate nucleotides groups have been the main focus of flavor research, especially due to their ability as a flavor enhancer. Of those, guanosine 5′-monophosphate (GMP) and inosine 5′-monophosphate (IMP) have been identified as major flavor precursors associated with umami taste characteristics (Dashdorj et al., 2016; Kim et al., 2016; Khan et al., 2015; Koutsidis et al., 2008). Additionally, a strong synergistic interaction between GMP and IMP with glutamine and aspartate was exhibited in a previous study (Nishimura and Kato, 1988), highlighting the potential of these compounds to further intensify the umami taste of meat in presence of other flavor precursors groups.

Interestingly, nucleotide concentration decreased with aging time and decreased at a faster rate with the application of dry-aging. Previous reports indicated that lower IMP concentration was observed in dry-aged products compared to wet-aged products when both were aged for the same aging period (Lee et al., 2019b; c; Kim et al., 2016). This suggested the potential limitation of nucleotide participation in flavor development and possible optimization of aging time to maximize the synergistic effects between nucleotide and free amino acid concentrations for dry-aged flavor development. While the loss of strong flavor enhancers such as IMP and GMP might be detrimental to flavor intensity, degradation of nucleotides has been known to produce other flavor precursors that could generate flavor through other processes. Following nucleotide

degradation, the liberation of bitter compounds (xanthine and hypoxanthine) and reducing ribose sugar are identified, highlighting another potential mechanism for nucleotides to participate in dry-aged flavor generation.

Reducing sugars

In terms of reducing sugars, only limited information is available in the literature on the alteration following the dry-aging process. While it is limited, most of the studies did indicate that the reducing sugars concentration increased with a longer aging period, and significantly higher concentrations were observed in the dry-aged product (Foraker et al., 2020; Mungure et al., 2020; Lee et al., 2019b). In the flavor generation process, reducing sugar is usually acts as a substrate for the Maillard reaction. However, the extent and results of the Maillard reaction were reported to be affected by the type of reducing sugar utilized during the reaction (Hamid et al., 2020). Among the reducing sugars, several sugars, including glucose, ribose, mannose and myo-inositol, are often greater following dry-aging application (Foraker et al., 2020; Mungure et al., 2020) and consequently could influence the dry-aged flavor perceived. In muscle, ribose was suggested to be the most important sugar as it could be generated from the degradation of nucleotides (Mottram, 1998). While it is true, Foraker et al. (2020) observed a greater glucose concentration than ribose sugar in dry-aged meat compared to wet-aged meat. This indicated that although available, ribose sugar might not be the main sugar involved in the Maillard reaction. The mechanisms for reducing sugar generation in meat, especially dry-aged meat, are still unclear and warrant more investigation to elucidate their role further.

Fatty acids

Among the different flavor precursors generated during the dry-aging process, fatty acids and lipids are the least studied and discussed. From the available reports, the effect of dry-aging on the free fatty acid profile changes is still not clear and well understood yet. Conflicting results are observed in the literature, with some reported alteration following dry-aging (Passetti et al., 2019; Kim et al., 2019a; Gredell et al., 2018) and others did not (Foraker et al., 2020). Furthermore, the extent of alteration also varied depending on the study. It was reported by Kim et al. (2019) that a significant reduction in monounsaturated fatty acids (MUFAs) was observed following dry-

aging. Different results, however, were reported by Passetti et al. (2019), where they found greater accumulation of saturated fatty acids (SFAs) with longer dry-aging.

The availability of lipid-derived compounds and fatty acids has been identified as a crucial factor influencing the final product flavors. Those compounds have been well known as a major volatile producer and, therefore, could influence the flavor perceived from the products (Calkins and Hodgen, 2007; Mottram, 1998; Whitfield and Mottram, 1992). Generally, both lipids and fatty acids could directly induce an odor or participate in lipid oxidation and thermal degradation during the cooking process to release the volatile compounds (Calkins and Hodgen, 2007). The generated volatiles will greatly depend on the composition of the lipids and fatty acids in the meat product.

While it is unclear how dry-aging conditions alter the free fatty acids profile, several reports indicated that the presence of microorganisms could greatly influence the extent of modification and release of free fatty acids. Lee et al. (2019) reported that a significant change in free fatty acid concentration was detected during the later stage of dry-aging when greater microbial growth was also observed. Similarly, Oh et al. (2019) also reported that dry-aged samples inoculated with *P. anomala* mold had greater free fatty acid increase compared to inoculated samples and those inoculated with yeast (*D. hansenii*). This indicated that the different dry-aging conditions might not directly influence the changes. However, the different dry-aging conditions might promote the growth of different microorganisms and hence could potentially alter the fatty acids composition.

Other compounds

The involvement of other compounds groups such as minerals, vitamins and acids in dry-aged product flavor is still unclear. While limited, those compounds have been shown to influence the final flavor perceived in meat products. For example, the presence of vitamins, such as B vitamins, has been associated with greater meaty flavor from the product, mainly from the generation of thiazole compounds during thermal degradation (Ramalingam et al., 2019; Khan et al., 2015). Similarly, the presence of minerals (such as iron) was previously suggested to induce greater metallic flavor from the products. It was proposed that as the myoglobin degraded, iron was released to the meat matrix, causing the greater perception of metallic flavor when consumed (Yancey et al., 2006). The availability of these compounds, however, is not yet identified in dry-aged products and warrants further research to fully understand the impact of dry-aging on flavor development.

2.4 Metabolomics

The application of high-throughput omics analyses, such as metabolomics, proteomics and lipidomics, has led to a deeper understanding of biological processes. The metabolomics analysis, specifically, allows the identification of small molecular compounds (known as metabolites) present in the cells and tissues to understand the biological systems further. Metabolites are generated through biological activity and could be present as the substrate, intermediate and final products of those activities (Johanningsmeier et al., 2016).

In general, there are two main approaches in metabolomics analysis, targeted and untargeted analysis (Cevallos-Cevallos et al., 2009). For targeted analysis, selective extraction was conducted to identify a specific group of metabolites. On the other hand, untargeted analysis utilizes common extraction and separation, mainly aiming to simultaneously identify all metabolites present in the samples. Along with these approaches, depending on the objectives of the research, metabolomics analysis could be divided into discriminative (separating group based on metabolite composition to identify differences), informative (identification of specific metabolites responsible for an intrinsic alteration) and predictive (utilization of metabolomics profile to estimate changes on an attribute of interest).

Following the metabolite extractions, those metabolites will undergo separation and detection. Both gas chromatography (GC) and liquid chromatography (LC) are some of the most commonly utilized high throughput separation techniques utilized for metabolomics analysis (Cevallos-Cevallos et al., 2009; Wishart, 2008). In recent advancements, high-performance and ultra-performance LC systems have been developed and exhibited greater compounds separation capability. Detection of the metabolites is commonly performed using either nuclear magnetic resonance (NMR) or mass spectrometry (MS). In general, it is generally accepted that MS systems have greater sensitivity when compared to the NMR system, allowing more accurate detection and identification of the compounds (Es-Safi et al., 2005). Furthermore, the utilization of tandem MS/MS system is now gaining popularity as the system significantly increases the resolution and detection accuracy (Waridel et al., 2001).

In the last decade, various metabolomics analysis approaches have been employed in numerous studies to further understand mechanisms involved in producing various high-quality foods (Mazzei and Piccolo, 2012; Bianchi et al., 2011; Sun et al., 2011). In meat science, the utilization of metabolomics has been constantly increasing and has played a pivotal role in

understanding factors involved in the tenderization process (Kodani et al., 2017; Lana et al., 2015; Muroya et al., 2014; Graham et al., 2010), color and oxidative stability (Ma et al., 2017; Abraham et al., 2017; Subbaraj et al., 2016), water holding capacity (Welzenbach et al., 2016; Straadt et al., 2014; Bertram et al., 2010) and safety/regulation aspects (K. Trivedi et al., 2016; Zanardi et al., 2015; Cevallos-Cevallos et al., 2011; Xu et al., 2010).

Only limited metabolomics studies have been conducted to identify the flavor metabolites in fresh meat products with respect to meat flavor. Previous research by Kim et al. (2016) identified greater free amino acids (glutamine, leucine, isoleucine, and tryptophan) in dry-aged beef loins when compared to its wet-aged counterpart using ^{1}H NMR metabolomics analysis. Those authors suggested that the presence of the greater free amino acid, especially glutamine, likely contributed to improving the meat flavor. Similarly, a study by Kodani et al. (2017) also employed the ^{1}H NMR metabolomics system to identify flavor-related metabolites in beef products aged up to 10 weeks. The authors reported that the presence of acetic acid, alanine, glutamic acid, isoleucine, leucine, phenylalanine, tyrosine and valine increased as aging time progressed, with the highest concentration observed in 10 weeks aged beef. While limited, these reports demonstrated the capability of metabolomics analysis to provide more information regarding the flavor precursor changes in meat products. The results revealed a good opportunity for further application of metabolomics to reveal meat flavor precursors, potentially employing the high specificity LC-MS based metabolomics analysis.

2.5 Objective

In this review, we have identified a shift in consumer palatability preferences toward flavor. In this regard, the dry-aging process has the potential to fulfill the changing demands observed from the consumers due to the fact that dry-aging is prized for its unique and desirable flavor. However, as discussed in the review, several published studies also reported no positive impacts of dry-aging on palatability attributes, which could be attributed to the lack of uniformity in dry-aging practices, leading to changes on flavor precursor availability. Furthermore, there is a significant knowledge gap in dry-aged flavor precursors, and thus further knowledge is needed to ensure the production of dry-aged meats with consistent eating quality attributes. Therefore, the primary objectives of the research described here were to identify the alteration of flavor precursors profile following dry-aging process using novel metabolomics approach and to evaluate the

process involved in liberating those flavor precursors. Multiple different meat sources (grass-fed, cull cow and pork) were utilized this study to assist in uncovering the general impact following the dry-aging process.

2.6 Table

Table 2.1. Summary of dry-aging parameters used and major findings reported in scientific studies. Positive dry-aging impact is determined based on sensory evaluation results reported in the studies. DA: Dry-aging; DWA: Dry-aging in bag; SDA: Stepwise Dry-aging; CDA: Carcass Dry-aging; WA: Wet-aging; FAA: Free amino acid; FFA: Free fatty acid; LAB: Lactic Acid Bacteria. No info: Indicates that no data is reported/available in the study.

Sources	Species	Muscle	Dry-aging Form	Aging Parameters					Positive Dry-aging Impact	Major findings			
				Aging Time (days)	Temperature (°C)	Relative Humidity (%)	Air-flow (m/s)	UV Light		Sensory Palatability	Color	Shear Force	Microbial Properties
(Oreskovich et al., 1988)	Beef	Striploin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging	2 d PM + 7	2	No info	No info	No info	No	No detectable dry-aging impacts	No info	No difference in tenderness	No info
(Parrish et al., 1991)	Beef	Striploin, Ribs(Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging	21	0 to1	80 to 85	0.5-2.5	No info	No	WA: Higher overall acceptability	No info	No difference in tenderness	WA: Higher LAB, Mesophile and Psycotrophs
(Sitz et al., 2005)	Beef	Striploin, Ribs(Longissimus Thoracis et Lumborum)	Dry-aging (No specific info)	37	1	No info	No info	No info	No	No detectable dry-aging impacts	No info	No info	No info

Table 2.1 Continued

	(Laster et al., 2008)	Beef	Striploin, Ribeye (Longissimus Thoracis et Lumborum), Top Sirloin Butt (Gluteus medius)	Sub-primal Dry-aging	9 d PM + 14, 21, 28, or 35	-0.6	78 ± 9.3	No info	No info	No	Difference depending on muscle type	No info	Difference depending on muscle type	No info
	(Smith et al., 2008)	Beef	Shortloin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging	2 d PM + 14, 21, 28, or 35	1 to 2	83 ± 11	No info	No info	No	No detectable dry-aging impacts	No info	No difference in tenderness	No info
	(DeGeer et al., 2009)	Beef	Striploin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging, Sub-primal Dry-aging in bag	21 or 28	2.2	50	No info	Ceiling mounted continuous UV light	No	No detectable dry-aging impacts	No info	No difference in tenderness	DAB: Higher aerobic bacteria for shell loin No difference in microbial load for others

Table 2.1 Continued

(Juárez et al., 2011)	Pork	Loin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging in bag	2, 7 or 14	1	No info	No info	No info	No	No detectable dry-aging impacts	No difference in color	No difference in tenderness	No info
(Dikeman et al., 2013)	Beef	Shortloin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging, Sub-primal Dry-aging in bag	8 d PM + 21	2.2	No info	Minimal air movement	No info	No	No detectable dry-aging impacts	DAB: lighter in color	No difference in tenderness	No info
(Smith et al., 2014)	Beef	Rib eye (Longissimus Thoracis), Top Sirloin (Gluteus Medius)	Sub-primal Dry aging	35	WA: 3 DA: 4	98.1	Fan is used to circulate air	UV light is used to prevent mold	No	DA: Lower juiciness, brown roasted and beef flavor	No info	No info	No info
(Gudjónsdóttir et al., 2015)	Beef	Loin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging, Sub-primal Dry-aging in bag	7,14 or 21	4	No info	No info	No info	No	No info	DA: Lightness is lower	No difference in tenderness	DA: Higher total aerobic bacteria, yeast and mold WA: Higher LAB

Table 2.1 Continued

(Iida et al., 2016)	Beef	Loin (Longissimus Thoracis et Lumborum)	Carcass Dry-aging	4, 11, 20, 30, 40 ,50 or 60	1 to 4	80 to 90	No info	No Info	No	No detectable dry-aging impacts	No info	No difference in tenderness	No info
(Kim et al., 2017b)	Beef	Shortloin (Longissimus Thoracis et Lumborum)	Carcass Dry-aging, Stepwise Dry-aging	17	1	78	1.5	No info	No	No detectable dry-aging impacts	No difference in color	SDB: Higher tenderness	No info
(Hulánková et al., 2018)	Beef	Loin (Longissimus Thoracis et Lumborum)	Carcass Dry-aging	15 or 27	1	85	0.5	No info	No	No Info	No difference in color	Dry-aging: Higher tenderness	No info
(Ryu et al., 2018)	Beef	Loin (Longissimus Thoracis et Lumborum), Bottom Round (Bicep Femoris)	Carcass Dry-aging	3, 25, 40, 50 or 60	1 to 4	80 to 90	No info	No info	No	No info	No info	No info	DA: Higher total bacteria, yeast, mold, no pathogen detected, specific species were detected

Table 2.1 Continued

(Kim et al., 2019c)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	28	4	75	2.5	No info	No	No differences	No practical differences	No info	DA: Aerobic bacteria, mold and yeast higher
(Lee et al., 2019a)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	0, 7, 14, 21, 28, 42, 63	4	Varied based on company	Varied based on company	No info	No	No info	DA: Lipid oxidation increase with time	No info	DA: Aerobic bacteria increases with time
(Callahan et al., 2019)	Beef	Bottom round (Bicep femoris)	Sub-primal Dry-aging	16	1.1	82	No info	No info	No	No info	DA: Greater a* DA: Higher lipid oxidation	No info	No info
(Vilella et al., 2019)	Beef	Ribeye (Longissimus thoracis)	Sub-primal Dry-aging, Stepwise aging	14, 28	2	73	No airflow	No info	No	No detectable dry-aging impacts	No info	No differences in tenderness	No info
(Park et al., 2019)	Pork	Loin (Longissimus lumborum)	Sub-primal Dry-aging	0, 7, 14	4 and -1	No info	5	No info	No	No info	No differences in color/oxidative quality	No info	No info
(Bernardo et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	21	2	70	2.5	No info	No	No info	No differences in color/oxidative quality	No info	No info

Table 2.1 Continued

(Hwang and Hong, 2020)	Pork	Loin (Longissimus lumborum)	Sub-primal Dry-aging bag	21	1	No info	No info	No info	No	DAB: Volatile decreased	DAB: Color reduced	No info	DAB: Lower total bacteria and lactic acid bacteria
(Jin and Yim, 2020)	Pork	Loin (Longissimus lumborum)	Sub-primal Dry-aging	0,7,14	8	85	No info	No info	No	No info	DA: Higher lipid oxidation	No info	No info
(Park and Kim, 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	0, 7, 14, 21, 28, 35, 70	4 and -1	No info	5	No info	No	No info	DA: Numerically impractical	No info	DA: Greater microbial
(Shi et al., 2020)	Beef	Ribeye (Longissimus thoracis)	Sub-primal Dry-aging, dry-aging in bag	0, 7, 14	2	85	1.5	No info	No	No info	DA: L* and B* lower	DAB: Lower MFI	DA: Higher aerobic bacteria, mold and yeast
(Ribeiro et al., 2021b)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	42	2	50, 70, 85	No info	No info	No	No info	DA: Lower in all color traits DA: Higher lipid oxidation	No info	No info

Table 2.1 Continued

(Warren and Kastner, 1992)	Beef	Striploin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging	3d PM + 11	3.1 to 3.6	78 ± 3	air circulation every 30min	UV light air filtration	Yes	DA: Higher beef flavor and more brown/roasted flavor	No info	No difference in tenderness	No info
(Campbell et al., 2001)	Beef	Striploin, Shortloin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging	7 or 14 PM + 7, 14, or 21	2	75	No info	No info	Yes	DA: Longer aging significantly increase all palatability attributes	No info	No difference in tenderness	DA: Higher aerobic plate count
(Ahnström et al., 2006)	Beef	Striploin, Ribs(Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging, Sub-primal Dry-aging in bag	11 d PM + 14 or 21	2.5	87 ± 2.6	Limited to regular coolers	No UV lights were used	Yes	DA, DAB: Rated highly for tenderness, aged-beef, beefy, brown-roasted flavor	No info	No difference in tenderness	DA: Lower LAB, Higher yeast
(Richardson et al., 2008)	Beef	Fore Rib (Longissimus Thoracis et Lumborum)	Carcass Dry-aging	21	1	No info	No info	No info	Yes	DA: Higher tenderness/juiciness	No info	No info	No info

Table 2.1 Continued

(Li et al., 2013)	Beef	Top Sirloin (Gluteus medius)	Sub-primal Dry-aging in bag	21	2.9	91	No info	No UV lights were used	Yes	DAB: Higher preference, tenderness and juiciness	No difference in color	No difference in tenderness	DAB: Higher total bacterial count and yeast WA: Higher LAB
(Li et al., 2014)	Beef	Loin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging, Sub-primal Dry-aging in bag	7 d PM + 21	5.1	75	No info	No info	Yes	DA, DAB: Higher umami, butter-fried, salty, fatty flavor WA: Higher sour, metallic, liver, animal flavor	No difference in color	No info	DA: Higher total bacterial count, enterobacteria, yeast WA: Higher LAB
(Stenström et al., 2014)	Beef	Loin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging, Sub-primal Dry-aging in bag	2 d PM + 13	1.6	No info	No info	No info	Yes	DA: Higher tenderness compared to WA DAB: Higher tenderness, juiciness compared to WA	No info	No info	No info

Table 2.1 Continued

(O'Quinn et al., 2016)	Beef	Striploin (Longissimus Thoracis et Lumborum)	Dry-aging (No specific info)	16 PM + 30	2	77	No info	No info	Yes	DA: Higher brothy, browned, grilled, buttery, nutty flavor	No info	No info	No info
(Kim et al., 2016)	Beef	Shortloin (Longissimus Thoracis et Lumborum)	Sub-primal Dry aging	2 d PM + 21	1 or 3	49, 55, 73 or 79	0.2 or 0.5	No info	Yes	DA: Higher flavor and overall liking	DA: Lower L* and a*	No difference in tenderness	No info
(Lee et al., 2016)	Pork	Loin (Longissimus Thoracis et Lumborum)	Carcass Dry-aging	40	2	80	No info	No info	Yes	DA: Higher overall acceptance on sensory palatability	No info	DA: Higher tenderness	No difference in microbial load
(Lepper-Blilie et al., 2016)	Beef	Striploin, Shortloin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging	14,21,28,35,42 or 49	1	70	air circulation, 5.66 m3/h	UV light air filtration	Yes	DA: Higher aged flavor, especially in boneless	No info	No difference in tenderness	No info

Table 2.1. Continued

(Kim et al., 2017a)	Beef	Shank, Top Round (Semimembranosus)	Sub-primal Dry-aging	20 or 40	1	80 to 85	0.5 to 1.5	No info	Yes	Dry-aging: higher bitterness, astringency, umami and saltiness	Dry-aging: darker color	No info	No info
(Lee et al., 2017)	Beef	Sirloin (Gluteus Medius)	Sub-primal Dry-aging, Sub-primal Dry-aging in bag	28	1 or 2	75 or 85	No info	No info	Yes	DA in 75%RH: Higher overall acceptance on sensory palatability	No info	No difference in tenderness	DAB: Higher total aerobic bacteria
(Berger et al., 2018)	Beef	Loin (Longissimus Thoracis et Lumborum)	Sub-primal Dry-aging, Sub-primal Dry-aging in bag	4d PM + 28	2	78	<0.2 m/s	No info	Yes	DA, DAB: Higher flavor-liking, tenderness, juiciness	No info	No difference in tenderness	DAB: Higher aerobic bacteria DA, DAB: Higher yeast WA: Higher LAB
(Lee et al., 2019b)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	0, 7, 14, 21, 28	4	75	2.5	No info	Yes	DA: Higher FAA, IMP and reducing sugars	No info	No info	DA: Higher microbial indicator (trimethylamine)

Table 2.1 Continued

(Lee et al., 2019c)	Beef	Rumps (Gluteus medius)	Sub-primal Dry-aging	14, 28	4	75	0, 2.5, 5	No info	Yes	DA: Higher FAA, reducing sugar, FFA	No info	No info	DA:Greater mold concentration
(Ha et al., 2019)	Beef	Bone-in ribeye (Longissimus thoracis), Top round (Semimembranosus), Rumps (Gluteus medius)	Sub-primal Dry-aging	0, 20, 40, 60	2-4	65-85	No info	No info	Yes	DA: Higher volatiles (aldehydes and alcohols)	No info	No info	No info
(Oh et al., 2019a)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	0, 14, 21, 28	4	75	No info	No info	Yes	DA: Higher FAA and FFA	No info	No info	No info
(Kim et al., 2019a)	Beef	Sirloin (Gluteus Medius)	Sub-primal Dry-aging	40	1	80-85	0.2	No info	Yes	DA: Higher FAA DA: More Juicy	DA: lower color	No info	No info
(Oh et al., 2019b)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	0, 7, 14, 21, 28, 35	2	75	2.5	No info	Yes	DA: Consumer found higher flavor and tenderness	No info	No info	DA: Higher mold

Table 2.1 Continued

(Hwang et al., 2019)	Pork	Belly and Shoulder blade	Sub-primal Dry-aging	21	2	80	No info	No info	Yes	DA: Higher FAA, hypoxanthine and inosine	No info	No info	No info
(Kim et al., 2019b)	Beef	Butts, rumps and sirloins	Sub-primal Dry-aging	28	1	85	2	No info	Yes	DA: Higher acceptance by consumer	DA: Increase all color traits	No info	No info
(Bischof et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	28	2	75	No info	No info	Yes	DA: Higher FAA, IMP, creatine, betain, carnosine, lactic acid	No info	No info	No info
(Foraker et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging, Stepwise aging	21, 14+21	3	70-90	No info	No info	Yes	DA: Higher reducing sugar DA: Minimal differences by trained panel	No info	No info	No info
(Jose et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	28	2	87	0.33	No info	Yes	No info	No info	DA: Decreased shear force	No info

Table 2.1 Continued

(Kim et al., 2020a)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	7,14,21,28	4	75	2.5	No info	Yes	DA: Higher FAA	No info	No info	No info
(Kim et al., 2020b)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	30	1	85	0.5	No info	Yes	No info	No info	DA: Increased protein degradation	No info
(Mungure et al., 2020)	Venison	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	21	4	80	1.5	No info	Yes	DA: Higher FAA, reducing sugar and alcohol volatile	No info	No info	No info
(Utama et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	0, 20, 24, 40, 50	2	85	2	No info	Yes	DA: Higher volatile, MUFA decrease	No info	No info	No info
(Zhang et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging bag	21, 21+7	2	75	0.5,1.5, 2.5	No info	Yes	DAB: FAA increased	DAB: Lipid and carbonyl not affected	No info	DA: Identified multiple mold and bacterial strains
(Ribeiro et al., 2021a)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	42	2	50	0.8	No info	Yes	DA: Higher flavor acceptability	DA: Lower L* and a*	No info	DA: Lower microbial concentration

Table 2.1 Continued

(Capouya et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	45	2	80	No info	Only in 1 location	Other-Microbial identification	No info	No info	No info	DA: Identified multiple mold and bacterial strains
(Ryu et al., 2020)	Beef	Ribeye (Longissimus thoracis), Bicep femoris	Carcass dry-aging	12, 30, 70, 160	1	80	No info	No info	Other-Microbial identification	No info	No info	No info	CDA: LAB dominant, mold did not change much
(Choe et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	28	4	75	2.5	No info	Other-Crust addition	DA: Higher peptide concentration	No info	No info	No info
(Lee and Kim, 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	28	4	70	5	No info	Other-Crust addition	DA: Higher FAA	No info	No info	No info
(Park et al., 2020)	Beef	Striploin (Longissimus lumborum)	Sub-primal Dry-aging	28	4	80	5	No info	Other-Crust addition	DA: Crust addition increase taste and acceptability	DA: Crust addition lower color	No info	No differences in microbial load
(Witte et al., 2020)	Beef	Sirloin (Gluteus Medius)	Sub-primal Dry-aging	21, 28	0.5	75	No info	No info	Other-Applying HPP	DA: trimming increased taste	No info	No info	No info

2.7 Literatures Cited

Abraham, A., J.W. Dillwith, G.G. Mafi, D.L. VanOverbeke, and R. Ramanathan. 2017. Metabolite Profile Differences between Beef Longissimus and Psoas Muscles during Display. Meat Muscle Biol. 1:18. doi:10.22175/mmb2016.12.0007.

Ahnström, M.L., M. Seyfert, M.C. Hunt, and D.E. Johnson. 2006. Dry aging of beef in a bag highly permeable to water vapour. Meat Sci. 73:674–679. doi:10.1016/j.meatsci.2006.03.006.

Algino, R.J., S.C. Ingham, and J. Zhu. 2007. Survey of antimicrobial effects of beef carcass intervention treatments in very small state-inspected slaughter plants. J. Food Sci. 72:M173–M179. doi:10.1111/j.1750-3841.2007.00386.x.

Aujoulat, F., F. Roger, A. Bourdier, A. Lotthé, B. Lamy, H. Marchandin, and E. Jumas-Bilak. 2012. From Environment to Man: Genome Evolution and Adaptation of Human Opportunistic Bacterial Pathogens. Genes 2012, Vol. 3, Pages 191-232. 3:191–232. doi:10.3390/GENES3020191.

Behrends, J.M., K.J. Goodson, M. Koohmaraie, S.D. Shackelford, T.L. Wheeler, W.W. Morgan, J.O. Reagan, B.L. Gwartney, J.W. Wise, and J.W. Savell. 2005a. Beef customer satisfaction: USDA quality grade and marination effects on consumer evaluations of top round steaks1. J. Anim. Sci. 83:662–670. doi:10.2527/2005.833662x.

Behrends, J.M., K.J. Goodson, M. Koohmaraie, S.D. Shackelford, T.L. Wheeler, W.W. Morgan, J.O. Reagan, B.L. Gwartney, J.W. Wise, and J.W. Savell. 2005b. Beef customer satisfaction: Factors affecting consumer evaluations of calcium chloride-injected top sirloin steaks when given instructions for preparation. J. Anim. Sci. 83:2869–2875. doi:10.2527/2005.83122869X.

Berger, J., Y.H.B. Kim, J.F. Legako, S. Martini, J. Lee, P. Ebner, and S.M.S. Zuelly. 2018. Dry-aging improves meat quality attributes of grass-fed beef loins. Meat Sci. 145:285–291. doi:10.1016/j.meatsci.2018.07.004.

Bernardo, A.P.S., C.R. Calkins, F. Ribeiro, and sergio bertelli pflanzer. 2020. Bone and Subcutaneous Fat Influence on Yield, Physicochemical Traits, and Color Stability of Dry-Aged Loin From Grass-Fed Nellore Bulls. Meat Muscle Biol. 4:31–32. doi:10.22175/mmb.11141.

Bertram, H.C., M.R. Weisbjerg, C.S. Jensen, M.G. Pedersen, T. Didion, B.O. Petersen, J.Ø. Duus, M.K. Larsen, and J.H. Nielsen. 2010. Seasonal Changes in the Metabolic Fingerprint of 21 Grass and Legume Cultivars Studied by Nuclear Magnetic Resonance-Based Metabolomics. J. Agric. Food Chem. 58:4336–4341. doi:10.1021/JF904321P.

Bianchi, F., A. Mangia, M. Mattarozzi, and M. Musci. 2011. Characterization of the volatile profile of thistle honey using headspace solid-phase microextraction and gas chromatography–mass spectrometry. Food Chem. 129:1030–1036. doi:10.1016/J.FOODCHEM.2011.05.070.

Bischof, G., F. Witte, N. Terjung, E. Januschewski, V. Heinz, A. Juadjur, and M. Gibis. 2020. Analysis of aging type- and aging time-related changes in the polar fraction of metabolome of beef by 1H NMR spectroscopy. Food Chem. doi:10.1016/j.foodchem.2020.128353.

Calkins, C.R., and J.M. Hodgen. 2007. A fresh look at meat flavor. Meat Sci. 77:63–80. doi:10.1016/j.meatsci.2007.04.016.

Callahan, Z.D., J. V. Cooper, and C.L. Lorenzen. 2019. Aging Condition and Retail Display Lighting Impact Retail Display Life and Lipid Oxidation of Beef Biceps Femoris Steaks. Meat Muscle Biol. 3. doi:10.22175/mmb2019.02.0005.

Campbell, R.E., M.C. Hunt, P. Levis, and E. Chambers IV. 2001. Dry-aging effects on palatability of beef longissimus muscle. J. Food Sci. 66:196–199. doi:10.1111/j.1365-2621.2001.tb11315.x.

Capouya, R., T. Mitchell, D.L. Clark, D.I. Clark, and P. Bass. 2020. A survey of microbial communities on dry-aged beef in commercial meat processing facilities. Meat Muscle Biol. 4:5–6. doi:10.22175/mmb.10373.

Cevallos-Cevallos, J.M., M.D. Danyluk, and J.I. Reyes-De-Corcuera. 2011. GC-MS Based Metabolomics for Rapid Simultaneous Detection of Escherichia coli O157:H7, Salmonella Typhimurium, Salmonella Muenchen, and Salmonella Hartford in Ground Beef and Chicken. J. Food Sci. 76:M238–M246. doi:10.1111/j.1750-3841.2011.02132.x.

Cevallos-Cevallos, J.M., J.I. Reyes-De-Corcuera, E. Etxeberria, M.D. Danyluk, and G.E. Rodrick. 2009. Metabolomic analysis in food science: a review. Trends Food Sci. Technol. 20:557–566. doi:10.1016/J.TIFS.2009.07.002.

Choe, J., B. Park, H.J. Lee, and C. Jo. 2020. Potential Antioxidant and Angiotensin I-converting Enzyme Inhibitory Activity in Crust of Dry-aged Beef. Sci. Rep. 10. doi:10.1038/s41598-020-64861-0.

Corbin, C.H., T.G. O'Quinn, A.J. Garmyn, J.F. Legako, M.R. Hunt, T.T.N. Dinh, R.J. Rathmann, J.C. Brooks, and M.F. Miller. 2015. Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. Meat Sci. 100:24–31. doi:10.1016/j.meatsci.2014.09.009.

Dashdorj, D., V.K. Tripathi, S. Cho, Y. Kim, and I. Hwang. 2016. Dry aging of beef; Review. J. Anim. Sci. Technol. 58:20. doi:10.1186/s40781-016-0101-9.

DeGeer, S.L., M.C. Hunt, C.L. Bratcher, B.A. Crozier-Dodson, D.E. Johnson, and J.F. Stika. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Sci. 83:768–774. doi:10.1016/j.meatsci.2009.08.017.

Diez-Simon, C., R. Mumm, and R.D. Hall. 2019. Mass spectrometry-based metabolomics of volatiles as a new tool for understanding aroma and flavour chemistry in processed food products. Metabolomics. 15. doi:10.1007/s11306-019-1493-6.

Dikeman, M.E., E. Obuz, V. Gök, L. Akkaya, and S. Stroda. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef Longissimus lumborum steaks. Meat Sci. 94:228–233. doi:10.1016/j.meatsci.2013.02.002.

Es-Safi, N.E., L. Kerhoas, J. Einhorn, and P.H. Ducrot. 2005. Application of ESI/MS, CID/MS and tandem MS/MS to the fragmentation study of eriodictyol 7-O-glucosyl-(1→2)-glucoside and luteolin 7-O-glucosyl-(1→2)-glucoside. Int. J. Mass Spectrom. 247:93–100. doi:10.1016/J.IJMS.2005.10.002.

Feidt, C., A. Petit, F. Bruas-Reignier, and J. Brun-Bellut. 1996. Release of free amino-acids during ageing in bovine meat. Meat Sci. 44:19–25. doi:10.1016/S0309-1740(96)00088-5.

Foraker, B.A., D.R. Woerner, D. Gredell, J.D. Tatum, J.F. Legako, K.E. Belk, and R.D. Stevens. 2020. Flavor, Tenderness, and Related Chemical Changes of Aged Beef Strip Loins. Meat Muscle Biol. 4. doi:10.22175/mmb.11115.

Frank, D., S.-T. Joo, and R. Warner. 2016. Consumer Acceptability of Intramuscular Fat. Korean J. Food Sci. Anim. Resour. 36:699. doi:10.5851/KOSFA.2016.36.6.699.

Frank, D., K. Kaczmarska, J. Paterson, U. Piyasiri, and R. Warner. 2017. Effect of marbling on volatile generation, oral breakdown and in mouth flavor release of grilled beef. Meat Sci. 133:61–68. doi:10.1016/J.MEATSCI.2017.06.006.

Goodson, K.J., W.W. Morgan, J.O. Reagan, B.L. Gwartney, S.M. Courington, J.W. Wise, and J.W. Savell. 2002. Beef customer satisfaction: factors affecting consumer evaluations of clod steaks. J. Anim. Sci. 80:401–408. doi:10.2527/2002.802401x.

Graham, S.F., T. Kennedy, O. Chevallier, A. Gordon, L. Farmer, C. Elliott, and B. Moss. 2010. The application of NMR to study changes in polar metabolite concentrations in beef longissimus dorsi stored for different periods post mortem. Metabolomics 2010 63. 6:395–404. doi:10.1007/S11306-010-0206-Y.

Gredell, D.A., J.H. McHenry, D.R. Woerner, J.F. Legako, T.E. Engle, J.C. Brooks, J.D. Tatum, K.E. Belk, D.A. Gredell, J.H. McHenry, D.R. Woerner, J.F. Legako, T.E. Engle, J.C. Brooks, J.D. Tatum, and K.E. Belk. 2018. Palatability Characterization of Fresh and Dry-Aged Ground Beef Patties. Meat Muscle Biol. 2. doi:10.22175/MMB2018.04.0007.

Gudjónsdóttir, M., M.D. Gacutan, A.C. Mendes, I.S. Chronakis, L. Jespersen, and A.H. Karlsson. 2015. Effects of electrospun chitosan wrapping for dry-ageing of beef, as studied by microbiological, physicochemical and low-field nuclear magnetic resonance analysis. Food Chem. 184:167–175. doi:10.1016/j.foodchem.2015.03.088.

Ha, Y., I. Hwang, H. van Ba, S. Ryu, Y. Kim, S.M. Kang, J. Kim, Y. Kim, and S. Cho. 2019. Effects of Dry- And Wet-ageing on Flavor Compounds and Eating Quality of Low Fat Hanwoo Beef Muscles. Food Sci. Anim. Resour. 39:655–667. doi:10.5851/kosfa.2019.e58.

Hamid, A.H., A.F. Nurrulhidayah, M.S.A. Sani, N.W.F. Muhammad, R. Othman, and A. Rohman. 2020. Discrimination of porcine and bovine gelatines based on reducing sugar types on maillard reaction. Food Res. 4:301–306. doi:10.26656/fr.2017.4(2).297.

Huff-Lonergan, E., and S.M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71:194–204. doi:10.1016/j.meatsci.2005.04.022.

Huff Lonergan, E., W. Zhang, and S.M. Lonergan. 2010. Biochemistry of postmortem muscle — Lessons on mechanisms of meat tenderization. Meat Sci. 86:184–195. doi:10.1016/J.MEATSCI.2010.05.004.

Hulánková, R., J. Kameník, A. Saláková, D. Závodský, and G. Borilova. 2018. The effect of dry aging on instrumental, chemical and microbiological parameters of organic beef loin muscle. LWT - Food Sci. Technol. 89:559–565. doi:10.1016/j.lwt.2017.11.014.

Hwang, S.I., and G.P. Hong. 2020. Effects of high pressure in combination with the type of aging on the eating quality and biochemical changes in pork loin. Meat Sci. 162:108028. doi:10.1016/j.meatsci.2019.108028.

Hwang, Y.H., N. Sabikun, I. Ismail, and S.T. Joo. 2019. Changes in sensory compounds during dry aging of pork cuts. Food Sci. Anim. Resour. 39:379–387. doi:10.5851/kosfa.2019.e29.

Iida, F., Y. Miyazaki, R. Tsuyuki, K. Kato, A. Egusa, H. Ogoshi, and T. Nishimura. 2016. Changes in taste compounds, breaking properties, and sensory attributes during dry aging of beef from Japanese black cattle. Meat Sci. 112:46–51. doi:10.1016/j.meatsci.2015.10.015.

Jeremiah, L.E., and L.L. Gibson. 2001. The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. Food Res. Int. 34:815–826. doi:10.1016/S0963-9969(01)00104-1.

Jin, S.K., and D.G. Yim. 2020. Comparison of effects of two aging methods on the physicochemical traits of pork loin. Food Sci. Anim. Resour. 40:844–851. doi:10.5851/KOSFA.2020.E22.

Johanningsmeier, S.D., G.K. Harris, and C.M. Klevorn. 2016. Metabolomic Technologies for Improving the Quality of Food: Practice and Promise. http://dx.doi.org/10.1146/annurev-food-022814-015721. 7:413–438. doi:10.1146/ANNUREV-FOOD-022814-015721.

Jose, C.G., R.H. Jacob, and G.E. Gardner. 2020. Alternative cutting methods and dry aging reduce the shear force of hot boned beef striploin in Bos indicus cattle. Meat Sci. 163. doi:10.1016/j.meatsci.2019.108036.

Juárez, M., W.R. Caine, M.E.R. Dugan, N. Hidiroglou, I.L. Larsen, B. Uttaro, and J.L. Aalhus. 2011. Effects of dry-ageing on pork quality characteristics in different genotypes. Meat Sci. 88:117–121. doi:10.1016/j.meatsci.2010.12.011.

K. Trivedi, D., K. A. Hollywood, N.J. W. Rattray, Holli Ward, D. K. Trivedi, Joseph Greenwood, D. I. Ellis, and Royston Goodacre. 2016. Meat, the metabolites: an integrated metabolite profiling and lipidomics approach for the detection of the adulteration of beef with pork. Analyst. 141:2155–2164. doi:10.1039/C6AN00108D.

Khan, M.I., C. Jo, and M.R. Tariq. 2015. Meat flavor precursors and factors influencing flavor precursors-A systematic review. Meat Sci. 110:278–284. doi:10.1016/j.meatsci.2015.08.002.

Killinger, K.M., C.R. Calkins, W.J. Umberger, D.M. Feuz, and K.M. Eskridge. 2004. Consumer sensory acceptance and value for beef steaks of similar tenderness, but differing in marbling level1. J. Anim. Sci. 82:3294–3301. doi:10.2527/2004.82113294x.

Kim, H.C., K.H. Baek, Y.J. Ko, H.J. Lee, D.G. Yim, and C. Jo. 2020a. Characteristic Metabolic Changes of the Crust from Dry-Aged Beef Using 2D NMR Spectroscopy. Molecules. 25. doi:10.3390/molecules25133087.

Kim, J.H., M.Y. Jeon, and C.H. Lee. 2019a. Physicochemical and sensory characteristics of commercial, frozen, dry, and wet-aged Hanwoo sirloins. Asian-Australasian J. Anim. Sci. 32:1621–1629. doi:10.5713/ajas.18.0610.

Kim, J.H., D.H. Kim, D.S. Ji, H.J. Lee, D.K. Yoon, and C.H. Lee. 2017a. Effect of aging process and time on physicochemical and sensory evaluation of raw beef top round and shank muscles using an electronic tongue. Korean J. Food Sci. Anim. Resour. 37:823–832. doi:10.5851/kosfa.2017.37.6.823.

Kim, J.H., T.K. Kim, D.M. Shin, H.W. Kim, Y.B. Kim, and Y.S. Choi. 2020b. Comparative effects of dry-aging and wet-aging on physicochemical properties and digestibility of Hanwoo beef. Asian-Australasian J. Anim. Sci. 33:501–505. doi:10.5713/ajas.19.0031.

Kim, M., J. Choe, H.J. Lee, Y. Yoon, S. Yoon, and C. Jo. 2019b. Effects of aging and aging method on physicochemical and sensory traits of different beef cuts. Food Sci. Anim. Resour. 39:54–64. doi:10.5851/kosfa.2019.e3.

Kim, S., H.J. Lee, M. Kim, J.W. Yoon, D.J. Shin, and C. Jo. 2019c. Storage stability of vacuum-packaged dry-aged beef during refrigeration at 4°C. Food Sci. Anim. Resour. 39:266–275. doi:10.5851/kosfa.2019.e21.

Kim, Y., and M. Hunt. 2011. Advance technology to improve meat color. Control meat Qual. 661:31–60.

Kim, Y.H.B., M. Frandsen, and K. Rosenvold. 2011. Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. Meat Sci. 88:332–337. doi:10.1016/j.meatsci.2010.12.020.

Kim, Y.H.B., R. Kemp, and L.M. Samuelsson. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Sci. 111:168–176. doi:10.1016/j.meatsci.2015.09.008.

Kim, Y.H.B., C. Liesse, R. Kemp, and P. Balan. 2015. Evaluation of combined effects of ageing period and freezing rate on quality attributes of beef loins. Meat Sci. 110:40–5. doi:10.1016/j.meatsci.2015.06.015.

Kim, Y.H.B., B. Meyers, H.W. Kim, A.M. Liceaga, and R.P. Lemenager. 2017b. Effects of stepwise dry/wet-aging and freezing on meat quality of beef loins. Meat Sci. 123:57–63. doi:10.1016/j.meatsci.2016.09.002.

Kim, Y.H.B., R.D. Warner, K. Rosenvold, Y.H.B. Kim, R.D. Warner, and K. Rosenvold. 2014. Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. Anim. Prod. Sci. 54:375–395. doi:10.1071/AN13329.

King, D.A., S.D. Shackelford, N. Kalchayanand, and T.L. Wheeler. 2012. Sampling and aging effects on beef longissimus color stability measurements. J. Anim. Sci. 90:3596–3605. doi:10.2527/jas.2011-4871.

Knudsen, G.M., H.M. Sommer, N.D. Sørensen, J.E. Olsen, and S. Aabo. 2011. Survival of Salmonella on cuts of beef carcasses subjected to dry aging. J. Appl. Microbiol. 111:848–854. doi:10.1111/J.1365-2672.2011.05094.X.

Kodani, Y., T. Miyakawa, T. Komatsu, and M. Tanokura. 2017. NMR-based metabolomics for simultaneously evaluating multiple determinants of primary beef quality in Japanese Black cattle. Sci. Rep. 7:1–13. doi:10.1038/s41598-017-01272-8.

Koutsidis, G., J.S. Elmore, M.J. Oruna-Concha, M.M. Campo, J.D. Wood, and D. Mottram. 2008. Water soluble precursor of beef flavor: I. Effect of diet and breed. Meat Sci. 79:124–130. doi:10.1016/j.meatsci.2007.08.008.

Lana, A., V. Longo, A. Dalmasso, A. D'Alessandro, M.T. Bottero, and L. Zolla. 2015. Omics integrating physical techniques: Aged Piedmontese meat analysis. Food Chem. 172:731–741. doi:10.1016/J.FOODCHEM.2014.09.146.

Laster, M.A., R.D. Smith, K.L. Nicholson, J.D.W. Nicholson, R.K. Miller, D.B. Griffin, K.B. Harris, and J.W. Savell. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer sensory attribute evaluations of steaks from ribeyes, strip loins, and top sirloins from two quality grade groups. Meat Sci. 80:795–804. doi:10.1016/j.meatsci.2008.03.024.

Lee, C.W., J.R. Lee, M.K. Kim, C. Jo, K.H. Lee, I. You, and S. Jung. 2016. Quality improvement of pork loin by dry aging. Korean J. Food Sci. Anim. Resour. 36:369–376. doi:10.5851/kosfa.2016.36.3.369.

Lee, H., M. Jang, S. Park, J. Jeong, Y.S. Shim, and J.C. Kim. 2019a. Determination of indicators for dry aged beef quality. Food Sci. Anim. Resour. 39:934–942. doi:10.5851/kosfa.2019.e83.

Lee, H.J., J. Choe, K.T. Kim, J. Oh, D.G. Lee, K.M. Kwon, Y. Il Choi, and C. Jo. 2017. Analysis of low-marbled Hanwoo cow meat aged with different dry-aging methods. Asian-Australasian J. Anim. Sci. 30:1733–1738. doi:10.5713/ajas.17.0318.

Lee, H.J., J. Choe, M. Kim, H.C. Kim, J.W. Yoon, S.W. Oh, and C. Jo. 2019b. Role of moisture evaporation in the taste attributes of dry- and wet-aged beef determined by chemical and electronic tongue analyses. Meat Sci. 151:82–88. doi:10.1016/j.meatsci.2019.02.001.

Lee, H.J., J.W. Yoon, M. Kim, H. Oh, Y. Yoon, and C. Jo. 2019c. Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. Meat Sci. 153:152–158. doi:10.1016/j.meatsci.2019.03.019.

Lee, J.A., and H.Y. Kim. 2020. Physicochemical properties of crust derived from dry-aged Holstein and Hanwoo loin. J. Anim. Sci. Technol. 62:692–701. doi:10.5187/jast.2020.62.5.692.

Lepper-Blilie, A.N., E.P. Berg, D.S. Buchanan, and P.T. Berg. 2016. Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. Meat Sci. 112:63–68. doi:10.1016/j.meatsci.2015.10.017.

Li, X., J. Babol, W.L.P. Bredie, B. Nielsen, J. Tom??nkov??, and K. Lundstr??m. 2014. A comparative study of beef quality after ageing longissimus muscle using a dry ageing bag, traditional dry ageing or vacuum package ageing. Meat Sci. 97:433–442. doi:10.1016/j.meatsci.2014.03.014.

Li, X., J. Babol, A. Wallby, and K. Lundström. 2013. Meat quality, microbiological status and consumer preference of beef gluteus medius aged in a dry ageing bag or vacuum. Meat Sci. 95:229–234. doi:10.1016/j.meatsci.2013.05.009.

Lucherk, L.W., T.G. O'Quinn, J.F. Legako, R.J. Rathmann, J.C. Brooks, and M.F. Miller. 2016. Consumer and trained panel evaluation of beef strip steaks of varying marbling and enhancement levels cooked to three degrees of doneness. Meat Sci. 122:145–154. doi:10.1016/j.meatsci.2016.08.005.

Ma, D., Y.H.B. Kim, B. Cooper, J.-H. Oh, H. Chun, J.-H. Choe, J.P. Schoonmaker, K. Ajuwon, and B. Min. 2017. Metabolomics Profiling to Determine the Effect of Postmortem Aging on Color and Lipid Oxidative Stabilities of Different Bovine Muscles. J. Agric. Food Chem. 65:6708–6716. doi:10.1021/acs.jafc.7b02175.

Mazzei, P., and A. Piccolo. 2012. 1H HRMAS-NMR metabolomic to assess quality and traceability of mozzarella cheese from Campania buffalo milk. Food Chem. 132:1620–1627. doi:10.1016/J.FOODCHEM.2011.11.142.

Miller, M.F., M.A. Carr, C.B. Ramsey, K.L. Crockett, and L.C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79:3062–3068. doi:10.2527/2001.79123062X.

Mottram, D.S. 1998. Flavour formation in meat and meat products: A review. Food Chem. doi:10.1016/S0308-8146(98)00076-4.

Moya, V.J., M. Flores, M.C. Aristoy, and F. Toldrá. 2001. Pork meat quality affects peptide and amino acid profiles during the ageing process. Meat Sci. 58:197–206. doi:10.1016/S0309-1740(00)00152-2.

Mungure, T.E., M.M. Farouk, E.J. Birch, A. Carne, M. Staincliffe, I. Stewart, and A.E.D.A. Bekhit. 2020. Effect of PEF treatment on meat quality attributes, ultrastructure and metabolite profiles of wet and dry aged venison Longissimus dorsi muscle. Innov. Food Sci. Emerg. Technol. 65:102457. doi:10.1016/j.ifset.2020.102457.

Muroya, S., M. Oe, I. Nakajima, K. Ojima, and K. Chikuni. 2014. CE-TOF MS-based metabolomic profiling revealed characteristic metabolic pathways in postmortem porcine fast and slow type muscles. Meat Sci. 98:726–735. doi:10.1016/J.MEATSCI.2014.07.018.

Nishimura, T., and H. Kato. 1988. Taste of free amino acids and peptides. Food Rev. Int. 4:175–194. doi:10.1080/87559128809540828.

Nyquist, K.M., T.G. O'Quinn, L.N. Drey, L.W. Lucherk, J.C. Brooks, M.F. Miller, and J.F. Legako. 2018. Palatability of beef chuck, loin, and round muscles from three USDA quality grades. J. Anim. Sci. 96:4276–4292. doi:10.1093/JAS/SKY305.

O'Quinn, T.G., D.R. Woerner, T.E. Engle, P.L. Chapman, J.F. Legako, J.C. Brooks, K.E. Belk, and J.D. Tatum. 2016. Identifying consumer preferences for specific beef flavor characteristics in relation to cattle production and postmortem processing parameters. Meat Sci. 112:90–102. doi:10.1016/j.meatsci.2015.11.001.

Oh, H., H.J. Lee, J. Lee, C. Jo, and Y. Yoon. 2019a. Identification of Microorganisms Associated with the Quality Improvement of Dry-Aged Beef Through Microbiome Analysis and DNA Sequencing, and Evaluation of Their Effects on Beef Quality. J. Food Sci. 84:2944–2954. doi:10.1111/1750-3841.14813.

Oh, J., H.J. Lee, J.W. Yoon, J. Choe, and C. Jo. 2019b. Electrical resistance and mold distribution on beef surface as indicators of dry aging. J. Food Process Eng. 42. doi:10.1111/jfpe.13122.

Oreskovich, D.C., F.K. McKeith, T.R. Carr Jan Novakofski, and P.J. Bechtel. 1988. Effects of Different Aging Procedures on the Palatability of Beef. J. Food Qual. 11:151–158. doi:10.1111/j.1745-4557.1988.tb00875.x.

Park, S.Y., and H.Y. Kim. 2020. Electric field induced super-cooling system for long term dry-aged beef loin. Food Sci. Anim. Resour. 40:286–296. doi:10.5851/kosfa.2020.e14.

Park, S.Y., H.Y. Kim, and J. Choe. 2019. Application of an electric field refrigeration system on pork loin during dry aging. Food Sci. Anim. Resour. 39:668–676. doi:10.5851/kosfa.2019.e59.

Park, S.Y., K.H. Seol, and H.Y. Kim. 2020. Effect of dry-aged beef crust levels on quality properties of brown sauce. Food Sci. Anim. Resour. 40:699–709. doi:10.5851/KOSFA.2020.E45.

Parrish, F.C., J.A. Boles, R.E. Rust, and D.G. Olson. 1991. Dry and Wet Aging Effects on Palatability Attributes of Beef Loin and Rib Steaks from Three Quality Grades. J. Food Sci. 56:601–603. doi:10.1111/j.1365-2621.1991.tb05338.x.

Passetti, R.A.C., I.N. do Prado, F. de Assis Fonseca de Macedo, A.J.C. Teixeira, C.A.L. Cardoso, G.R. de Arruda Santos, and C.A. Santos. 2019. Physicochemical characteristics of dry aged beef from younger Nellore bulls slaughtered at different body weights. Trop. Anim. Health Prod. 51:2635–2640. doi:10.1007/s11250-019-01944-9.

Ramalingam, V., Z. Song, and I. Hwang. 2019. The potential role of secondary metabolites in modulating the flavor and taste of the meat. Food Res. Int. 122:174–182. doi:10.1016/J.FOODRES.2019.04.007.

Ribeiro, F.A., S.K. Lau, R.A. Furbeck, N.J. Herrera, M.L. Henriott, N.A. Bland, S.C. Fernando, J. Subbiah, G.A. Sullivan, and C.R. Calkins. 2021a. Ultimate pH effects on dry-aged beef quality. Meat Sci. 172:309–1740. doi:10.1016/j.meatsci.2020.108365.

Ribeiro, F.A., S.K. Lau, S.B. Pflanzer, J. Subbiah, and C.R. Calkins. 2021b. Color and lipid stability of dry aged beef during retail display. Meat Sci. 171:108274. doi:10.1016/j.meatsci.2020.108274.

Richardson, R.I., G.R. Nute, and J.D. Wood. 2008. Effect of wet vs . dry ageing on eating quality of beef from traditional breeds. Proc. 54th ICoMST. 1993–1995.

Ryu, S., M.R. Park, B.E. Maburutse, W.J. Lee, D.J. Park, S. Cho, I. Hwang, S. Oh, and Y. Kim. 2018. Diversity and characteristics of the meat microbiological community on dry aged beef. J. Microbiol. Biotechnol. 28:105–108. doi:10.4014/jmb.1708.08065.

Ryu, S., M. Shin, S. Cho, I. Hwang, Y. Kim, and S. Oh. 2020. Molecular Characterization of Microbial and Fungal Communities on Dry-Aged Beef of Hanwoo Using Metagenomic Analysis. Foods. 9:1571. doi:10.3390/foods9111571.

Savell, J.W. 2008. Dry-Aging of Beef. Beef Checkoff. 16.

Savell, J.W., R.E. Branson, H.R. Cross, D.M. Stiffler, J.W. Wise, D.B. Griffin, and G.C. Smith. 1987. National Consumer Retail Beef Study: Palatability Evaluations of Beef Loin Steaks that Differed in Marbling. J. Food Sci. 52:517–519. doi:10.1111/j.1365-2621.1987.tb06664.x.

Shi, Y., W. Zhang, and G. Zhou. 2020. Effects of different moisture-permeable packaging on the quality of aging beef compared with wet aging and dry aging. Foods. 9. doi:10.3390/foods9050649.

Sitz, B.M., C.R. Calkins, D.M. Feuz, W.J. Umberger, and K.M. Eskridge. 2005. Consumer sensory acceptance and value of domestic, Canadian, and Australian grass-fed steaks. J. Anim. Sci. 83:2863–2868. doi:10.2527/2005.83122863x.

Smith, A.M., K.B. Harris, D.B. Griffin, R.K. Miller, C.R. Kerth, and J.W. Savell. 2014. Retail yields and palatability evaluations of individual muscles from wet-aged and dry-aged beef ribeyes and top sirloin butts that were merchandised innovatively. Meat Sci. 97:21–26. doi:10.1016/j.meatsci.2013.12.013.

Smith, R.D., K.L. Nicholson, J.D.W. Nicholson, K.B. Harris, R.K. Miller, D.B. Griffin, and J.W. Savell. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. Meat Sci. 79:631–639. doi:10.1016/j.meatsci.2007.10.028.

Stenström, H., X. Li, M.C. Hunt, and K. Lundström. 2014. Consumer preference and effect of correct or misleading information after ageing beef longissimus muscle using vacuum, dry ageing, or a dry ageing bag. Meat Sci. 96:661–666. doi:10.1016/j.meatsci.2013.10.022.

Straadt, I.K., M.D. Aaslyng, and H.C. Bertram. 2014. An NMR-based metabolomics study of pork from different crossbreeds and relation to sensory perception. Meat Sci. 96:719–728. doi:10.1016/j.meatsci.2013.10.006.

Subbaraj, A.K., Y.H.B. Kim, K. Fraser, and M.M. Farouk. 2016. A hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-MS) based metabolomics study on colour stability of ovine meat. Meat Sci. 117:163–172. doi:10.1016/j.meatsci.2016.02.028.

Sun, Q., M.J. Gates, E.H. Lavin, T.E. Acree, and G.L. Sacks. 2011. Comparison of Odor-Active Compounds in Grapes and Wines from Vitis vinifera and Non-Foxy American Grape Species. J. Agric. Food Chem. 59:10657–10664. doi:10.1021/JF2026204.

Tittor, A.W., M.G. Tittor, M.M. Brashears, J.C. Brooks, A.J. Garmyn, and M.F. Miller. 2011. Effects of simulated dry and wet chilling and aging of beef fat and lean tissues on the reduction of escherichia coli O157:H7 and salmonella. J. Food Prot. 74:289–293. doi:10.4315/0362-028X.JFP-10-295.

Toldrá, F., and M. Flores. 2010. The Role of Muscle Proteases and Lipases in Flavor Development During the Processing of Dry-Cured Ham. http://dx.doi.org/10.1080/10408699891274237. 38:331–352. doi:10.1080/10408699891274237.

Utama, D.T., Y.J. Kim, H.S. Jeong, J. Kim, F.H. Barido, and S.K. Lee. 2020. Comparison of meat quality, fatty acid composition and aroma volatiles of dry-aged beef from Hanwoo cows slaughtered at 60 or 80 months old. Asian-Australasian J. Anim. Sci. 33:157–165. doi:10.5713/ajas.19.0205.

Vierck, K.R., J.M. Gonzalez, T.A. Houser, E.A.E. Boyle, T.G. O'Quinn, K.R. Vierck, J.M. Gonzalez, T.A. Houser, E.A.E. Boyle, and T.G. O'Quinn. 2018. Marbling Texture's Effects on Beef Palatability. Meat Muscle Biol. 2:127.

Vilella, G. de F., C.L. Gomes, C.T. Battaglia, M.T.B. Pacheco, V.S.N. da Silva, A. Rodas-González, and S.B. Pflanzer. 2019. Effects of combined wet-and dry-aging techniques on the physicochemical and sensory attributes of beef ribeye steaks from grain-fed crossbred zebu steers. Can. J. Anim. Sci. 99:497–504. doi:10.1139/cjas-2018-0127.

Voges, K.L., C.L. Mason, J.C. Brooks, R.J. Delmore, D.B. Griffin, D.S. Hale, W.R. Henning, D.D. Johnson, C.L. Lorenzen, R.J. Maddock, R.K. Miller, J.B. Morgan, B.E. Baird, B.L. Gwartney, and J.W. Savell. 2007. National beef tenderness survey – 2006: Assessment of Warner–Bratzler shear and sensory panel ratings for beef from US retail and foodservice establishments. Meat Sci. 77:357–364. doi:10.1016/J.MEATSCI.2007.03.024.

Waridel, P., J.L. Wolfender, K. Ndjoko, K.R. Hobby, H.J. Major, and K. Hostettmann. 2001. Evaluation of quadrupole time-of-flight tandem mass spectrometry and ion-trap multiple-stage mass spectrometry for the differentiation of C-glycosidic flavonoid isomers. J. Chromatogr. A. 926:29–41. doi:10.1016/S0021-9673(01)00806-8.

Warren, K.E., and C.L. Kastner. 1992. A comparison of dry-aged and vacuum-aged beef strip loins. J. Muscle Foods. 3:151–157. doi:10.1111/j.1745-4573.1992.tb00471.x.

Welzenbach, J., C. Neuhoff, C. Looft, K. Schellander, E. Tholen, and C. Große-Brinkhaus. 2016. Different Statistical Approaches to Investigate Porcine Muscle Metabolome Profiles to Highlight New Biomarkers for Pork Quality Assessment. PLoS One. 11:e0149758. doi:10.1371/JOURNAL.PONE.0149758.

Whitfield, F.B., and D.S. Mottram. 1992. Volatiles from interactions of Maillard reactions and lipids. Crit. Rev. Food Sci. Nutr. 31:58. doi:10.1080/10408399209527560.

Wilfong, A.K., K. V. McKillip, J.M. Gonzalez, T.A. Houser, J.A. Unruh, E.A.E. Boyle, and T.G. O'Quinn. 2016. The effect of branding on consumer palatability ratings of beef strip loin steaks,. J. Anim. Sci. 94:4930–4942. doi:10.2527/JAS.2016-0893.

Wishart, D.S. 2008. Metabolomics: applications to food science and nutrition research. Trends Food Sci. Technol. 19:482–493. doi:10.1016/J.TIFS.2008.03.003.

Witte, F., S. Smetana, V. Heinz, and N. Terjung. 2020. High-pressure processing of usually discarded dry aged beef trimmings for subsequent processing. Meat Sci. 170:108241. doi:10.1016/j.meatsci.2020.108241.

Xu, Y., W. Cheung, C.L. Winder, and R. Goodacre. 2010. VOC-based metabolic profiling for food spoilage detection with the application to detecting Salmonella typhimurium-contaminated pork. Anal. Bioanal. Chem. 397:2439–2449. doi:10.1007/s00216-010-3771-z.

Yancey, E.J., J.P. Grobbel, M.E. Dikeman, J.S. Smith, K.A. Hachmeister, E.C. Chambers IV, P. Gadgil, G.A. Milliken, and E.A. Dressler. 2006. Effects of total iron, myoglobin, hemoglobin, and lipid oxidation of uncooked muscles on livery flavor development and volatiles of cooked beef steaks. Meat Sci. 73:680–686. doi:10.1016/J.MEATSCI.2006.03.013.

Zanardi, E., A. Caligiani, L. Palla, M. Mariani, S. Ghidini, P.A. Di Ciccio, G. Palla, and A. Ianieri. 2015. Metabolic profiling by 1H NMR of ground beef irradiated at different irradiation doses. Meat Sci. 103:83–89. doi:10.1016/J.MEATSCI.2015.01.005.

Zhang, R., M.J.Y. Yoo, and M.M. Farouk. 2020. Oxidative stability, proteolysis, and in vitro digestibility of fresh and long-term frozen stored in-bag dry-aged lean beef. Food Chem. 128601. doi:10.1016/j.foodchem.2020.128601.

Zhao, C.J., A. Schieber, and M.G. Gänzle. 2016. Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations – A review. Food Res. Int. 89:39–47. doi:10.1016/J.FOODRES.2016.08.042.

Zou, T., J. Liu, H. Song, and Y. Liu. 2018. Discovery of Amadori-Type Conjugates in a Peptide Maillard Reaction and Their Corresponding Influence on the Formation of Pyrazines. J. Food Sci. 83:1588–1595. doi:10.1111/1750-3841.14156.

CHAPTER 3. ELUCIDATING MECHANISMS INVOLVED IN FLAVOR GENERATION OF DRY-AGED BEEF LOINS USING METABOLOMICS APPROACH

The content presented in this chapter is reprint of our previously published work titled "Elucidating mechanisms involved in flavor generation of dry-aged beef loins using metabolomics approach." Some edits have been made to produce a cohesive dissertation. The published work reference is Setyabrata, D., Cooper, B. R., Sobreira, T. J., Legako, J. F., Martini, S., & Kim, Y. H. B. (2021). Elucidating mechanisms involved in flavor generation of dry-aged beef loins using metabolomics approach. Food Research International, 139, 109969.

3.1 Abstract

The present study was conducted to identify flavor-related chemical compounds and to elucidate beef flavor development in response to dry-aging. Paired grass-fed beef loins (n=18) were obtained at 7 d postmortem, cut into two sections and assigned to 3 aging methods: conventional dry-aging (DA), vacuum packaged wet-aging (WA) and dry-aging in a bag (DW) for 28 days. Following aging, samples were analyzed for UPLC-MS metabolomics, volatile, fatty acid profiling, and consumer sensory comment analysis. A greater number of proteins and nucleotides-derived metabolites were liberated in dry-aged samples compared to WA ($P<0.05$). In particular, the liberation of gammaglutmayl peptides and glutamine metabolites through glutathione metabolism were identified. While fatty acid profile was not affected by treatments ($P>0.05$), higher concentrations of volatile compounds were found in the dry-aged ($P<0.05$). The dry-aging process decreased the presence of terpenoid and steroid lipid groups, which could possibly result in reducing the undesirable flavor of grass-fed beef.

Keywords: Dry-aging, Metabolomics, Beef, Flavor, Volatile compounds, Grass-fed, Free fatty acid

3.2 Introduction

Postmortem aging is a widely implemented process in the meat industry to improve meat quality attributes. Extended postmortem aging has been known to improve meat palatability factors, such as tenderness, juiciness and flavor, through the endogenous proteolytic enzyme activity (Kim

et al., 2018). Wet-aging or storing vacuum-packaged meat at refrigerated temperatures postmortem is the most common aging approach. However, dry-aging has increased in popularity in a niche market for consumers seeking unique, high-quality products (Savell, 2008). Dry-aging is a traditional butchery process, where fresh meat is aged without any protective packaging barrier under a controlled environment. While controversial, dry-aging has been generally known to improve meat palatability, especially generating a unique flavor, which lends to the perception that dry-aged meats are premium products.

In general, the development of the meat flavor has been tightly related to the presence of flavor precursors, such as free fatty acids, sugars, nucleotides, and free amino acids (Mottram, 1998). Some of the compounds can be derived from muscle degradation and oxidation, which occurs during the postmortem aging process. However, little research has been undertaken to determine the underlying mechanisms by which flavor-related chemical compounds can be naturally liberated through different postmortem aging types, especially dry-aging. Although some recent studies reported the relevance of a high abundance of free amino acids and nucleotides in dry-aged products (Hanagasaki & Asato, 2018; Kim, Kemp, & Samuelsson, 2016), the development of beef flavor is a complex interaction among components in meat, involving various chemical species. Therefore, there is a need to characterize palatability-related flavor precursors, which can be generated through dry-aging to provide further understanding and practical insights to better utilize this practice.

The recent development of high-throughput mass spectrometry tools has greatly improved our understanding of chemical compounds related to meat quality attributes. Metabolomics, in particular, is an emerging technique where small molecular weight molecules (i.e., sugars, free amino acids, nucleotides) could be detected and profiled (Ma et al., 2017). For the meat research application, metabolomics profiling has been used to further understand the postmortem aging in improving the meat quality, especially in meat tenderness (King et al., 2019) and meat color stability (Ma et al., 2017; Subbaraj et al., 2016). However, despite the potential benefit, metabolomics has not been fully employed to decipher underlying mechanisms by which postmortem aging can affect meat flavor development by identifying different metabolic pathways and precursors responsible for the production of volatile compounds associated with dry-aged beef flavor.

Taken together, the main objective of this study was to identify flavor-related chemical compounds and thus elucidate the flavor generation process by dry-aging, utilizing a metabolomics approach coupled with other chemical analyses. For this purpose, this study is a further elaboration of our recent study (Berger et al., 2018), where a significant improvement in eating quality attributes (e.g., tenderness, juiciness and flavor) was observed in the dry-aged grass-fed beef loins rated by a consumer panel. In this present follow-up study, detailed comments collected from the consumer panelists were further analyzed to provide additional information about the impact of dry-aging on sensory eating quality attributes. The findings from this study will advance knowledge and understanding of how palatability attributes of beef can be enhanced with postmortem aging, namely dry-aging.

3.3 Materials and methods

3.3.1 Sample collection, preparation and processing

The sampling and processing procedure used in this study was described in the previous study by Berger et al. (2018). In brief, paired bone-in loins from 9 animals were collected from a grass-fed beef packing plant and transported to the Purdue Meat Science Laboratory. Each loin was divided into 2 sections (totaling to 36 loin sections; 9 carcasses x 2 sides x 2 sections) and assigned into a pre-determined aging treatment arrangement (n = 12; 36 loin sections/3 aging treatments). The aging treatments include wet-aging (WA), conventional dry-aging (DA), and dry-aging in a water-permeable bag (DW). The WA samples were vacuum packaged using individual vacuum pouches (Clarity Vacuum Pouches, Bunzl Processor Division, Riverside, MO, USA.). The DW loins were packed into a commercial dry-aging bag (UMAi Dry, Minneapolis, MN, USA.) following the manufacturer's guidelines. The DA sections were exposed to the environment during the aging process, following the standard practice of dry-aging. All sections were placed in the same room subjected to 28 days of aging at 2 °C, 78% relative humidity, and minimal airflow (<0.2 m/s). At the end of the aging period, dehydrated surfaces and bones of each section were trimmed and removed. Sections were then cut into steaks (1cm thick) for metabolomics profiling and biochemical analyses.

3.3.2 Volatile compound analysis

Volatile compound analysis was conducted following the method by Gardner & Legako (2018). In brief, samples were cooked to an internal temperature of 65 °C, and five cores (1.27 cm diameter) were then cut perpendicular to the cooked steak surface and minced. Five grams of the minced cooked meat were weighed into GC vials, and 10 µL of 1,2-dichlorobenzene was added to each vial as an internal standard. The vials were capped and loaded to a Gerstel agitator for incubation (5 minutes, 65 °C) prior to 20 minutes extraction via headspace solid-phase microextraction. The extracted compounds were then injected into a capillary column, and selective ion monitoring scan mode was utilized for data collection. The compounds were identified by comparing them to an external standard.

3.3.3 Fatty acid analysis

The fatty acid analysis was performed following protocols by Chail et al. (2016). Fatty acids methyl ester (FAME) were prepared following the methylation technique by O'Fallon et al. (2007). Following the methylation, one gram of the homogenate was put into a glass vial, and an internal standard was added (tridecanoic acid, 0.5 mg/mL in methanol). Vials were then incubated at 55°C, and FAME were extracted using Hexane prior to analysis. Hexane extracts were evaluated by an Agilent 6890N gas chromatographer (GC) equipped with a flame ionization detector (FID). A total of one microliter of the sample was injected into the GC inlet maintained at 250 ºC and with a 50:1 split ratio. An HP-88 capillary column (100 m × 250 µm × 0.2 µm) was used for separation. Helium served as the carrier gas with a flow rate of 2.5 mL/min. The oven was held at 35 ºC for 2 minutes before increasing to 170 ºC at 4 ºC/min. After holding at 170 ºC for 4 minutes, the oven temperature was increased at a rate of 3.5 ºC/min up to 240 ºC and held for 7 minutes. The FID was maintained at 280 ºC with a hydrogen flow rate of 60 mL/min, air flow rate of 300 mL/min, and helium column/make up flow rate of 35 mL/min. The fatty acids were identified by comparing the retention time to GC reference standards (Nu-Chek Prep, Inc, Elysian, MN, USA.). The concentrations were calculated relative to initial wet weight and presented in mg/g sample.

3.3.4 Protein oxidation

The protein oxidation was determined by measuring the carbonyl content of the samples, following the method described by Vossen and De Smet (2015). The beef samples collected after trimming were used for the carbonyl content measurement. The carbonyl content was expressed as nmol carbonyls/mg of protein.

3.3.5 Metabolomics profiling

Sample preparation and extraction

Of the 12 samples, 5 samples were randomly selected from each group for the metabolomics profiling. The samples were powdered by submerging samples into liquid nitrogen and immediately homogenized in a blender (Waring Products, CT, USA). Protein removal and sample extraction were performed using the Bligh-Dyer extraction protocol (Bligh and Dyer, 1959). Chloroform (300 µL) mixed with an equal volume of methanol was added to 100 mg of meat powder. Samples were extracted in a Precellys 24 tissue homogenizer. Water (300 µL) was added, mixed and centrifuged at 16,000 × *g* for 8 minutes. The upper methanol and water phase contained the polar metabolites, which was transferred to separate vials and evaporated to dryness in a SpeedVac Concentrator (Thermo Scientific, Waltham, MA, USA). The dried polar fraction was reconstituted in 75 µL of a diluent composed of 95% water and 5% acetonitrile, containing 0.1% formic acid.

UPLC-MS Analysis

The UPLC-MS analysis was conducted following the method by Ma et al. (2020) with modification. Chromatographic separations were performed using an Agilent 1290 Infinity II UPLC system (Agilent Technologies, Palo Alto, CA, USA) using a Waters Acquity HSS T3 (2.1 x 100 mm x 1.8 µm) separation column (Waters, Milford, MA, USA) with a HSS T3 (2.1 x 5 mm x 1.8 µm) guard column. Sample injections were 5 µL. A binary mobile phase consisting of solvent systems A and B were used, where A was 0.1% formic acid (v/v) in ddH2O and B was 0.1% formic acid (v/v) in acetonitrile. Initial conditions of 100:0 A:B were held for 1 minute, followed by a linear gradient to 70:30 over 15 minutes, followed by a linear gradient to 5:95 over 5 minutes, with a 5:95 hold for 1.5 minutes. Column re-equilibration was performed by returning to the initial

starting conditions of 100:0 over 1 minute, with a hold for 5 minutes. The total run time was 28.5 minutes. The mobile phase flow rate was 0.45 mL/min, and the column was maintained at 40 °C. Following chromatographic separation, the column effluent was introduced by positive electrospray ionization (ESI) into an Agilent 6545 quadrupole time-of-flight (Q-TOF) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). ESI capillary voltage was 3.5 kV, nitrogen gas temperature was set to 325 °C, drying gas flow rate was 8.0 L/min, nebulizer gas pressure was 30 psig, fragmentor voltage was 130 V, skimmer was 45 V, and OCT RF was 750 V. Mass data (from m/z 70-1000) were collected and analyzed using Agilent MassHunter B.06 software (Agilent Technologies, Santa Clara, CA, USA). Mass accuracy was improved by infusing Agilent Reference Mass Correction Solution (G1969-85001; Agilent Technologies, Santa Clara, CA, USA).

Peak deconvolution was performed using Agilent ProFinder (v B.08). Peak annotations were performed based on mass assignment and retention behavior using the HMDB (www.hmdb.ca) and METLIN (www.metlin.scripps.edu) metabolite databases, with a mass error of less than 30 ppm.

Metabolic pathway analysis

Following identification and statistical analysis of the metabolites, significantly different metabolites from each treatment were then imported to MetaboAnalyst 4.0 (https://www.metaboanalyst.ca/) and subjected to pathway analysis following the method described by Chong et al. (2019). The metabolites were matched to the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway library for *Bos taurus*.

3.3.6 Consumer sensory comments evaluation

The sensory evaluation results and procedures of consumer panelists (n=120) were previously reported in our recent published work by Berger et al. (2018). In brief, the consumer sensory evaluations were conducted at the Utah State University, Department of Nutrition, Dietetics and Food Science and were approved by the Utah State Institutional Review Board (#7315). The beef samples were cooked until the internal temperature reached 65 °C using an electric griddle (Griddler GR-150, Cuisinart, Glendale, AZ, USA). After reaching the designated temperature, visible external fat and connective tissues were removed from the steaks. Steaks were

then cut into cubes (2.5cm x 2.0cm x 2.0cm), placed in sample cups with lids and served warm to the consumer panelists. The panelists were requested to evaluate the overall liking, aroma, flavor, juiciness, and texture of the samples provided on a scale of 0 to 100 points. Following the evaluation of each palatability trait, the panelists were provided with a comment box and asked to provide additional comments for each of the palatability traits. The consumer comments were collected and further analyzed to determine its efficacy and relevance to be used as additional sensory information namely potential descriptive comments from the actual consumer group.

The comments provided were analyzed following the two-cycle coding process described by Saldaña (2013) to quantify the frequency of similar comments given to each palatability trait.
The first cycle of the analysis was conducted using a general descriptor and used to extract the comments that mentioned a neutral/positive experience and/or any specific descriptor of the attributes. A more in-depth separation was then performed in the second cycle of the analysis. In the second cycle, only the extracted comments from the first cycle were used and separated into further groups to provide a deeper understanding of the data and identification of patterns. Prior to creating the descriptor, some keywords, phrases and/or themes that could be used to get clusters were identified. The descriptor details were gathered into a codebook (Table 3.1) and were used as a guide to cluster the comments. The comments were analyzed independently by 2 people, and the averaged results were presented as a percentage.

3.3.7 Statistical analysis

The experimental design of this study was an incomplete balanced block design with a full saturated arrangement considering anterior and posterior and left and right sides of each carcass. Each animal serves as a block and random effect. The different aging treatments were set as the fixed effect. The data for protein oxidation, volatile compounds, and fatty acid analysis were analyzed using the PROC mixed procedure of SAS 9.4, where least-square means for all traits were separated using the PDIFF options (F test, $P \leq 0.05$).

The metabolomics data were analyzed in R software. The peak area of the metabolites was normalized using log2 transformation. Individual sample within each group was compared to each other to check the presence of extreme variance. ANOVA ($P<0.05$) was used to identify the metabolites significantly affected by the aging treatments. An unsupervised principal component

analysis was performed to aid in the visualization of the data. The pathway analysis of the metabolites was conducted using the hypergeometric test and relative-betweenness centrality.

3.4 Results

3.4.1 Volatile compounds, fatty acid analysis and protein oxidation

A total of 35 volatiles were measured and analyzed. Those compounds consisted of 12 aldehydes, 7 ketones, 3 sulfur-containing compounds, 4 carboxylic acids, 2 alkanes, 2 esters, 2 alcohols and 3 heterocyclic compounds. Out of the 35 volatiles, 5 compounds, including 2,3-pentanedione, 2-heptanone, methanethiol, octane, and ethanol, were found to be significantly affected by the different aging treatments (Table 3.2). DA had higher concentrations of methanethiol, octane, and ethanol compared to the other treatments ($P<0.05$). The concentration of 2,3-pentanedione was also higher in DA compared to DW ($P<0.05$), however, the concentration in WA was not different from both DA and DW ($P>0.05$). The 2-heptanone concentration was lower in WA ($P<0.05$), compared to both DA and DW, where they are not significantly different from each other.

For fatty acid profiling, a total of 27 fatty acids was identified and quantified, ranging from C10 to C24 (Table 3.3). Among the fatty acids measured, 2 fatty acids (docosopentanoic acid (C22:5) and tetracosahexaenoic acid (C22:6) were influenced by aging treatment ($P<0.05$). The concentration for docosopentanoic acid was higher in DW ($P<0.05$) compared to the other treatments. For the tetracosahexannoic acid, DW also had a higher concentration compared to DA ($P<0.05$), but WA was not different from both DW and DA ($P>0.05$). The concentrations of cumulative saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and total unsaturated fatty acids (UFA) were not affected by different aging treatments ($P>0.05$).

No treatment effect was observed ($P>0.05$, Figure 3.1) for the carbonyl content (indication of protein oxidation) of the beef loins.

3.4.2 Metabolomics profiling

A total of 1666 metabolites was detected from the untargeted UPLC-MS metabolomics analysis used in this study. Based on the ANOVA F-test analysis, 86 metabolites were found to be

affected by the treatments ($P<0.05$). A heat map plot accompanied by hierarchical clustering analysis (HCA) was generated to help visualize the presence of the metabolites among the observed treatments (Figure 3.2). Based on the HCA, it could be identified that both dry-aging treatments (DA and DW) had more comparable metabolites profiles, distinctly different from the WA counterpart.

Utilizing the significant metabolites as a subset, principle component analysis (PCA) was conducted. The PCA plot clearly exhibited a separation of metabolites between the three aging methods (Figure 3.3). A clear separation between the WA and both dry-aging methods (DA and DW) was observed across the PC1 axis. The PC1 explained 55.1% of the variation in metabolite changes between samples, indicating that major variation occurred due to the application of wet-aging or dry-aging treatment. Furthermore, both DA and DW were grouped into separate clusters across the PC2 axis, which could explain 13.2% of the variation observed, indicating differences between the two dry-aging methods.

Of the 86 metabolites, 63 metabolites were able to be annotated and loosely categorized into several major groups, as presented in Table 3.4. Among the identified metabolites, the protein-derived metabolites (primarily dipeptides and several amino acids) were observed in both DA and DW, mainly comprised of glutamine, glutamic acid, and aspartic acid-related compounds. The DA samples were observed to have higher pyroglutamic acid abundance. The DW samples were observed to have higher amino acid (keto-glutaramic) and dipeptides (glutaminyl-aspartic acid, asparaginyl-glutamic acid, gammaglutamyl-ornithine and glutaminyl-serine) abundance.

Other than the protein metabolites, a higher abundance of nucleotides, such as thymidine monophosphate and queuine, were observed in DA. Similarly, hexanoyl adenosine monophosphate and methyl adenosine were also identified in DW. A greater abundance of glucoside compounds (isopentenyladenine glucoside, digalloyl glucoside, and xanthoxol glucoside, nerolidol glucopyranoside) and glycoside compound (hydroxyriluzole glucuronide) were detected in WA compared to both DA and DW ($P<0.05$). Additionally, carnitines compounds were also observed to be in greater abundance in WA samples compared to both dry-aged samples.

For lipid-derived metabolites, 15 metabolites were identified from the samples, in which 11 were detected to be in greater abundance in WA compared to the others. Those metabolites could be classified into several groups, which were terpenoids (taraxastanediol, lutein, homofukinolide, and boviquinone 4), steroids (cholestryl ester, cholestryl ester, and perulactone), fatty acyls

(muricatin A and diacyl glycerol), and glycerolipids (hydroxypropyl undecanoate and docosanoyl glycerophosphate). Other compounds, such as vitamins, were also identified. The WA group had a greater abundance of calcitroic acid (vitamin D derivates) and pyridoxine phosphate (vitamin B derivates) compared to both dry-aged treatments. Only ascorbyl stearate (vitamin C derivates) was identified and more abundant in DA. A porphyrin compound (hematoporphyrin) was also identified and was in greater abundance in DW compared to other treatments ($P<0.05$).

3.4.3 Sensory Comments Evaluation

The descriptive comments from consumer panelists were collected and then subjected to further analyses, separated by the specific descriptors described in the codebook (Table 3.1). While the detailed results were summarized in Table 5, for the overall liking of the samples, greater percentages of positive comments were found for both DA (75.8%) and DW (76.7%) when compared to WA (66.7%). Among those comments, 46.3%, 51.4% and 56% of the comments were related to flavor for WA, DA and DW, respectively. Only 8.8% of comments were attributed to juiciness in WA compared to 22.1% in DA and 32.6% in DW. More consumer comments related to texture were observed in DA (40.9%) and DW (39.7%) compared to WA (25%).

Considering the comments related to the aroma, 76.7% of consumer responses were positive comments for DW, while 81.7% of consumer responses were positive comments for both DA and WA. Out of those comments, the number of comments categorized as "neutral," "good," "meaty," and "smoky" were similar across the different aging methods. However, there were more "other" comments in DW (42.4%) compared to the other treatments, mentioning aromas such as gamey, unfamiliar, savory.

As of the flavor-related comments, 74.2% of consumers provided positive comments for DA, while both DW and WA had 65% and 61.7% positive comments, respectively. More comments describing samples having "good" and "meat/beefy" flavor were observed in DA (69.6%) and DW (67.9%) compared to WA (59.5%). The WA had more "other" comments compared to other aging treatments, indicating flavors such as fatty, liver, sour and natural. In the texture attributes, more comments mentioning "tender" were observed in DA (73%) compared to both DW (57.6%) and WA (69.2%). A higher percentage for "very tender" was found in DW (39.4%) compared to DA (19%) and WA (22%). For Juiciness, 57.5% of consumer panelists commented more positive notes on DA (66.7%) and DW (70%) than WA (57.5%).

3.5 Discussion

3.5.1 Non-volatile compounds

The protein-derived metabolites in DA and DW could be further classified to dipeptides and several amino acids, mainly belonging to glutamic acid and aspartic acid, which have been well known to produce beefy and/or umami flavor in beef (Ramalingam et al., 2019; Dinh et al., 2018). The presence of these compounds in dry-aged beef has been suggested as one of the main contributors in providing greater flavor in the product (Lee et al., 2019; Kim et al., 2016). Interestingly, pathway analysis of the significant metabolites present in each treatments groups revealed that DA treatment was linked to glutathione metabolism (Table 6). Glutathione is an antioxidant often synthesized from cysteine, glycine and glutamate and has been well known to play a crucial role as an antioxidant in the cell (Andrisic et al., 2018). The identification of this pathway exhibited a potential response of the muscle cell to the environmental exposure. It is possible that muscles over synthesize glutathione to support the oxidative system and limit oxidation. This response could potentially play a role in the flavor development process, generating a greater abundance of the observed amino acids as the antioxidant was degraded in the DA treatment, and thus possibly explaining the higher scores in flavor-liking reported in our previous study by Berger et al (2018) and more positive descriptive comments in flavor from the consumer panel.

A greater abundance of dipeptides was observed, especially in DW compared to WA and DA (Table 3.4). The involvement of dipeptides and peptides in the Maillard reaction was previously reported by Zou et al. (2018). The authors suggested that the dipeptide could directly be involved through the dipeptide Maillard reaction to produce more flavor and volatile compounds compared to regular amino acids related to the Maillard reaction. Among those peptides, gammaglutamyl peptides were previously found in cheese or soy sauce products generated from microbial activities such as *Penicillium* spp. or *Bacillus* spp. (Zhao et al., 2016). A potential explanation for the given result would be likely attributed to the increase in microbial concentration in the DW beef sample. In fact, in our previous published study, higher levels of aerobic bacteria, lactic acid bacteria and yeast present in the DW sample were found compared to others (Berger et al., 2018). The recent study by Lee et al. (2019) also demonstrated greater liberation of amino acids when mold was present during the dry-aging process. The current results

suggest that other compounds such as short peptides could also take part in developing final dry-aged flavor, possibly generated through microbial involvements during aging.

The WA samples were observed to contain a higher abundance of glycine-containing peptides. Glycine has been suggested to give a sweet taste to meat, making it more desirable (Dinh et al., 2018). However, there was also a higher abundance of tyrosine, lysine, and arginine containing compounds, which contributes to sour and bitter taste on meat (Ramalingam et al., 2019), thus potentially overriding the desirable flavors and adding to the sour flavor as mentioned in the comments observed from the panelists. The abundance of amino acids in meat is an important factor in flavor development as they are highly involved in the Maillard reaction to produce desired beef flavor (Mottram, 1998).

Additionally, the nucleotide derived flavor precursors, such as adenosine, have often been associated with umami and beefy flavor development in meat (Aaslyng and Meinert, 2017). These compounds subsequently release ribose phosphate and free ribose, which would induce more flavor formation through participation in Maillard reaction as reducing sugar (Aaslyng and Meinert, 2017). Perhaps, the release of reducing sugars from nucleotide degradation alongside the greater availability of amino acids from protein degradation would create an environment promoting more Maillard reaction to occur in both DA and DW compared to WA.

The glucoside, glycoside, and carnitines compounds have been recognized as an antioxidant and often found in different food products (Ma et al., 2017; Elisia et al., 2007). The greater presence of these compounds in WA could explain the lipid metabolites identified in the WA samples, potentially from protecting the lipid from further oxidative degradation. Only one sugar compound, feruloylglucose, was observed to be abundant in DA. Glucose has been identified to be a reducing sugar, which would then participate in Maillard reaction to produce meat flavor. While reducing sugar is an important factor in flavor generation in meat, only one sugar and/or carbohydrate-derived metabolite was identified to be in greater abundance in either DA or DW. It is possible that the reducing sugar role might be more related to ribose sugar released from nucleotide, as more nucleotides abundance were observed in DA and DW.

3.5.2 Volatile compounds

The presence of lipid and fatty acids in meat has been identified as a major source of volatile compounds (Calkins and Hodgen, 2007; Forss, 1973). However, the effect of dry-aging on fatty

acids profile has not been well-understood, as there is very little published information in the literature. Passetti et al. (2019) reported that a higher concentration of SFA in the *Longissimus thoracis* from Nellore bull carcasses was accumulated with extended dry-aging periods. Gredell et al. (2018) found that beef patties manufactured from 42 days dry-aged shoulder clod had a lower SFA, MUFA and PUFA contents when compared to patties from unaged shoulder clod collected at 4 days postmortem, in exception of stearic acid, gamma-linolenic acids and trans-vaccenic acids. Both studies, however, had a different result compared to this study, where no difference in overall fatty acids contents was observed (Table 3.3). The discrepancies between the studies may be attributed to the fact that grass-fed beef was used in the present study. Several studies have reported a higher level of antioxidative enzymes (e.g., superoxide dismutase and catalase) in meat from grass-finished cattle (De la Fuente et al., 2009; Descalzo et al., 2005), which could minimize the extent of lipid oxidation occurred during aging. Similarly, the protein oxidation was not different across all the treatments when measured by quantifying the amount of carbonyl content, supporting the low oxidation observed in the current study.

While the overall fatty acid compositions were not affected by the treatments, differences were observed in the lipid-derived metabolites (Table 3.4). Both fatty acyls and glycerollipids are common forms of lipids found in meat products and would be involved in lipid oxidation and degradation during the cooking process to produce lipid-derived volatiles, such as aldehydes, alcohols, and ketones, giving the animal-like odor detected in meat (Mottram, 1998). The steroid lipid groups, however, have been identified to negatively affect the flavor, producing animal-like flavor in meat products (Vesely, 1973).

Terpenoids originated from plants and could be transferred to the animal fat through direct digestion (Valdivielso et al., 2017). While the exact function of terpenoids in meat flavor development has not been well-elucidated, terpenoids are volatile compounds often responsible for the fragrance detected in plants or flowers (Arroo, 2007). Terpenoids presence has also been known to contribute to the floral and vegetal odor to the flavor of cheese and milk originated from grazing animals (Carpino et al., 2004), and thus potentially will also contribute to the plant flavor often observed in meat, especially in grass-fed meat products used in this study. The less abundance of those compounds in dry-aged, however, exhibited that the dry-aging process might potentially reduce the presence of terpenoid lipid, and thus potentially reducing the grassy flavor and increasing acceptability of the grass-fed products. In fact, in our previous study (Berger et al.,

2018), consumer panelists did indicate that dry-aged grass-fed beef (DA and DW) had superior flavor-liking scores compared to WA counterparts, supporting this postulation. There has also been evidence suggesting that terpenoids exerting some antioxidant properties (Barroso et al., 2011), which could possibly limit the extent of lipid oxidation in the WA samples.

Interestingly, while more lipid metabolites were found in WA samples, more volatiles were significantly greater in concentration in the dry-aged samples (Table 3.2). Given volatile compounds were produced from the interaction of Maillard reaction and lipid oxidation product (Whitfield and Mottram, 1992). The greater abundance of dipeptides and peptides along with nucleotide-derived products in DA and DW could potentially promote more Maillard reaction products generation. This would then allow more interaction with lipid degradation products, generating more volatiles concentration following cooking compared to WA. In the current study, dry-aging resulted in greater liberation of 5 volatile compounds that were identified to emit aroma commonly associated with dry-aged beef, such as buttery, beefy, cheesy and sweet. Both 2,3-pentanedione and 2-heptanone are part of the ketones group and have been previously identified as a product of lipid oxidation (Gardner and Legako, 2018; Mottram, 1998). According to Machiels et al. (2003), 2,3-pentanedione was also measured in Irish beef and was identified to have a buttery sweet aroma when measured using a gas chromatography-olfactometry. The same study also found that 2-heptanone was associated with having a gravy aroma. Out of the sulfur-containing group, only methanethiol was observed to be significantly different. This compound was reported to generate meat and cheese-like aroma. Of all the aging methods, DW has the highest number of comments mentioning "other" aroma. It is possible that while the consumers detected the unique aroma from dry-aging, describing the presence of a specific aroma could be challenging for the non-trained consumer panelists. Our observation, however, still showed that consumer panelists could provide some extents of descriptive comments and inputs (although in untrained manners), which could be further utilized along with the general acceptance/preference evaluation to provide complementary information about the palatability attributes of fresh beef.

3.5.3 Other compounds

Several vitamin products were also identified through the UPLC-MS metabolomics analysis. In addition to having antioxidant capability, vitamins contribute to flavor development following thermal degradation. The B vitamins degradation was identified to produce thiazole groups, which

has been repeatedly correlated to the meaty flavor in the meat. Similarly, vitamin C degradation could also produce flavor compounds such as thiazole and pyrazine when heated in the presence of amino acids such as cysteine (Yu and Zhang, 2010), producing a meat-like aroma and flavor as well.

A porphyrin compound (hematoporphyrin) was also identified in the samples. Porphyrin rings are commonly present in meat as part of hemoglobin and myoglobin. While the impact of the porphyrin ring on meat flavor has not been clearly identified, both hemoglobin and myoglobin have been suggested to promote metallic flavor due to the presence of heme iron in the structure. However, it is of interest to observe that the compounds were present in greater abundance in both dry-aged samples rather than WA. No clear explanation why DW and DA had a greater presence of porphyrin rings compared to WA, especially knowing that WA has often been associated with more metallic flavor (Campbell et al., 2001; Warren and Kastner, 1992). Dry-aging could possibly concentrate the metal-containing compounds without generating the metallic flavor. Besides, it is possible that the metallic flavor would be masked by other flavors or affected by different metal oxidation states. More studies looking at the presence of total iron content and myoglobin abundance in dry-aged beef would be beneficial to further understand the development of metallic flavor in the meat.

3.6 Conclusions

Metabolomics profiling of dry-aged beef samples revealed that a higher abundance of flavor precursors such as glutamate/glutamine containing dipeptide and nucleotide derived metabolites were identified in dry-aged beef samples (both DA and DW) compared to WA, potentially generating the unique flavor associated with dry-aged products upon cooking. In particular, the liberation of gammaglutmayl peptides from the involvement of microbial during the dry-aging process and glutamine metabolites through the glutathione metabolism were newly identified. The results of the current study also indicate that dry-aging possibly reduced negative aroma and flavor by minimizing the presence of lipid metabolites such as terpenoids and steroids, which were present in greater abundance in WA samples. More antioxidant-related compounds such as carnitine and glucoside were observed in WA, potentially reducing the flavor formation from thermally induced lipid oxidation process. This study also showed that while limited, consumer comments could provide further insights into the sensory attributes of the meat product. The

consumer panelists were found to be capable of detecting the sensory changes and provide some descriptive comments to a certain extent. Additional sensory analysis, such as the application of check all that apply technique, might be useful to gain the full benefits of those comments from untrained consumer panelists. Future studies to observe the impacts of microbial and the identification of those microbial involved during the dry-aging process would be of interest. In particular, the microbe identification coupled with employing metabolomics technology could potentially provide a deeper understanding behind the flavor generation, characterizing both compounds and specific mechanisms involved in the dry-aging process.

3.7 Tables and Figures

Table 3.1. Codebook of descriptor used during consumer sensory panelist comment evaluation for both first and second cycle of coding.

Descriptor	Descriptor description
Overall	
Positive	Positive is considered as all comments that include the word good/great (including any word with similar meaning such as yummy, more, OK, etc.) Including neutral and acceptable to eat (no disgust/negative feeling)
Aroma	The comment marked as positive is referring to the aroma/smell of the sample
Flavor	The comment marked as positive is referring to the flavor of the sample
Juiciness	The comment marked as positive is referring to the juiciness of the sample
Texture	The comment marked as positive is referring to the texture of the sample
Other	The comment marked as positive is referring to an attribute other than aroma, flavor, juiciness and texture of the sample
Aroma	
Positive	Positive is considered as all comments that include the word good/great (including any word with similar meaning such as yummy, more, OK, etc.) Any aroma related descriptive word is included. Neutral/non offensive smell is included.
Neutral	The comment marked as positive have "neutral", "acceptable/ok", "Normal" or something similar. Like steak
Good	The comment marked as positive have "good", "very" "great", "better", "strong", "sharp", "more flavor", "yummy"
Meat/Beefy	The comment marked as positive have "meaty", "beefy", "like steak"
Smoky/BBQ	The comment marked as positive have "smoky", "Barbecue/BBQ"
Other	The comment marked as positive have other related word such as "off", "old", "nature", "woody", "fresh, "familiar"
Flavor	
Positive	Positive is considered as all comments that include the word good/great (including any word with similar meaning such as yummy, more, OK, etc.) Any flavor related descriptive word is included. Neutral/non offensive comment is included.
Neutral	The comment marked as positive have "neutral", "acceptable/ok", "Normal" or something similar. Like steak
Good	The comment marked as positive have "good", "very" "great", "better", "strong", "sharp", "more flavor", "yummy"
Meat/Beefy	The comment marked as positive have "meaty", "beefy", "like steak"
Other	The comment marked as positive have other related word such as "off", "gamey", "sweet", "grassy", "fatty", "fishy"

Table 3.1 Continued

Texture	
Positive	Positive is considered as all comments that include the word good/great (including any word with similar meaning such as yummy, more, OK, etc.), tender and soft. Any comment that not referring to tough/chewy is included.
Tender	The comment marked as positive have "tender", "easy", "good"
Very Tender	The comment marked as positive have "very" "great", "strong", "sharp", "more", "better"
Juiciness	
Positive	Positive is considered as all comments that include the word good/great (including any word with similar meaning such as yummy, more, OK, etc.), juicy and very juicy. Any comments that refer that the meat is juicy and not dry is included.
Little Juiciness	The comment marked as positive have "little", "acceptable/ok", "just enough", "somewhat", "quite", "decent"
Normal Juiciness	The comment marked as positive have "good", "normal", "Juicy", "neutral"
Very Juiciness	The comment marked as positive have "very" "great", "strong", "more ", "better"

Table 3.2. Effect of different aging method [Wet aging (WA), Dry aging (DA) and Dry aging in a water permeable bag (DW)] on volatile compound profiles of low marbled grass-fed beef loins (M. *longissimus lumborum*)

Volatile Name, (ng/g sample)	WA	DA	DW	SEM	P-value
n-aldehydes					
Acetaldehyde	10.10	12.00	10.31	1.55	0.620
Butanal	10.76	10.70	10.13	2.01	0.967
Pentanal	2.18	2.08	3.38	0.51	0.121
Hexanal	63.09	39.00	98.40	21.25	0.108
Heptanal	9.08	12.68	15.76	2.41	0.138
Octanal	10.01	17.68	14.92	2.39	0.080
Nonanal	10.27	15.48	12.61	1.89	0.152
Strecker aldehydes					
3-Methylbutanal	2.22	1.64	1.62	0.60	0.685
2-Methylbutanal	1.84	1.49	1.36	0.55	0.795
Methional	0.45	0.30	0.40	0.06	0.255
Benzaldehyde	3.21	3.53	3.29	0.50	0.888
Phenylacetaldehyde	0.67	0.66	0.61	0.05	0.653
Ketones					
3-Hydroxy-2-butanone (Acetoin)	10.09	9.07	9.14	1.00	0.661
2-Propanone	71.99	51.92	74.94	17.08	0.323
2,3-Butanedione	42.99	36.90	29.61	2.06	0.439
2-Butanone	10.81	12.83	11.88	1.98	0.720
2-Pentanone	0.17	0.16	0.18	0.03	0.806
2,3-Pentanedione	0.06^{ab}	0.09^{b}	0.04^{a}	0.01	0.032
2-Heptanone	0.37^{a}	0.53^{b}	0.57^{b}	0.05	0.008

Table 3.2. Continued

Sulfur containing					
Dimethyl sulfide	3.63	3.20	2.91	0.58	0.500
Carbon disulfide	15.90	14.85	20.00	3.63	0.550
Methanethiol	0.43[a]	1.01[b]	0.58[a]	0.14	0.025
Carboxylic acids					
Acetic acid	6.04	5.39	5.71	0.50	0.651
Butanoic acid	16.69	18.71	19.61	2.72	0.714
Hexanoic acid	6.58	8.66	8.27	0.98	0.290
Octanoic acid	10.06	11.03	9.13	1.42	0.616
Alkanes					
Pentane	9.44	5.58	11.85	1.85	0.056
Octane	3.26[a]	4.64[b]	3.03[a]	0.48	0.039
Esters					
Butanoic acid methylester	3.89	3.87	3.88	0.01	0.212
Hexanoix acid methylester	3.30	2.73	4.03	0.98	0.555
Alcohol					
1-Penten-3-ol	0.84	0.91	0.96	0.05	0.143
Ethanol	5.34[a]	22.11[b]	7.95[a]	3.22	0.002
Heterocyclic					
Toluene	3.53	3.70	3.04	0.52	0.629
p-Xylene	0.98	4.18	1.36	2.02	0.454
D-limonene	1.55	7.69	0.77	4.11	0.404

[a-b] Different superscript letter indicated a significant difference between the different aging methods ($P<0.05$)

Table 3.3. Effect of different aging [Wet aging (WA), Dry aging (DA) and Dry aging in a water permeable bag (DW)] on fatty acid profiles of low marbled grass-fed beef loins (M. *longissimus lumborum*).

Fatty acids, mg/g wet sample	**Type**	**Common Name**	**WA**	**DA**	**DW**	**SEM**	**P-value**
C10:0	SFA	Capric acid	0.040	0.033	0.036	0.005	0.269
C12:0	SFA	Lauric acid	0.052	0.044	0.042	0.006	0.271
C14:0	SFA	Myristic acid	2.083	1.813	1.710	0.276	0.345
C14:1	MUFA	Myristoleic acid	0.969	0.902	0.782	0.118	0.221
C15:0	SFA	Pentadecylic acid	0.415	0.371	0.385	0.042	0.689
C16:0	SFA	Palmitic acid	22.307	20.352	19.625	2.266	0.519
C16:1 (*trans*-9)	MUFA	Palmitelaidic acid	0.246	0.239	0.253	0.027	0.790
C16:1 (trans-9)	MUFA	Palmitoleic acid	2.795	2.569	2.439	0.358	0.452
C17:0	SFA	Margaric acid	0.082	0.740	0.718	0.080	0.586
C17:1	MUFA	Heptadecenoic acid	0.747	0.678	0.663	0.071	0.530
C18:0	SFA	Stearic acid	12.617	11.958	11.313	1.235	0.711
C18:1 *(trans*-9/11)	MUFA	Elaidic and trans-vaccenic acids	1.579	1.569	1.392	0.265	0.752
C18:1 (*n*-9/7)	MUFA	Oleic and vaccenic acids	48.606	44.860	43.026	4.881	0.556
C18:2 *(trans*-9/12)	PUFA	Linoelaidic acid	0.144	0.136	0.125	0.017	0.647
C18:2 *(cis*-9/12)	PUFA	Linoleic acid	3.685	3.534	3.711	0.254	0.663
C18:3 (*n*-3)	PUFA	Alpha-linolenic acid	2.312	2.194	2.310	0.259	0.744
C18:3 (*n*-6)	PUFA	Gamma-linolenic acid	0.019	0.018	0.018	0.002	0.568

Table 3.3. Continued

C20:0	SFA	Arachidic acid	0.091	0.086	0.082	0.008	0.676
C20:1 (*n*-11)	MUFA	Gadoleic acid	0.671	0.632	0.584	0.087	0.658
C20:2	PUFA	Eicosadienoic acid	0.066	0.064	0.068	0.008	0.894
C20:3 (*n*-6)	PUFA	Dihomo-gamma-linolenic acid	0.112	0.112	0.119	0.007	0.478
C20:4 (*n*-6)	PUFA	Arachidonic acid	0.429	0.421	0.445	0.029	0.619
C20:5	PUFA	Eicosapentaenoic acid	0.055	0.041	0.066	0.014	0.439
C22:3	PUFA	Docosatrienoic acid	0.236	0.246	0.294	0.026	0.360
C22:5 (*n*-3)	PUFA	Docosapentaenoic acid	1.111[a]	1.098[a]	1.363[b]	0.075	0.033
C22:6	PUFA	Tetracosahexaenoic acid	0.403[ab]	0.380[a]	0.506[b]	0.037	0.050
C24:1	MUFA	Nervonic acid	0.192	0.191	0.244	0.028	0.308
Total SFA			38.450	35.323	33.951	3.679	0.573
Total MUFA			55.818	51.623	49.386	5.581	0.558
Total PUFA			8.568	8.232	8.976	0.407	0.358
Total UFA			64.401	59.835	58.368	5.813	0.619
UFA:SFA			1.683	1.711	1.728	0.022	0.322

[a-b] Different superscript letter indicated a significant difference between the different aging methods ($P<0.05$)

Table 3.4. Effect of different aging method [Wet aging (WA), Dry aging (DA) and Dry aging in a water permeable bag (DW)] on metabolomics profiles of low marbled grass-fed beef loins (M. *longissimus lumborum*). P-value < 0.05.

Mass	RT	HMDB/Metlin ID	Putative Compounds	Highest Abundance	Peak Area		
					WA	DA	DW
Peptides/Amino Acids							
310.1813	19.2213	HMDB0028963	Lysyl-Tyrosine	WA	20.340^{c}	20.071^{a}	20.145$^{\mathbf{b}}$
187.0666	5.6200	HMDB0061890	Pyroglutamyl-Glycine	WA	26.458^{b}	26.371^{a}	26.371^{a}
232.1439	2.0487	HMDB0028709	Arginyl-Glycine	WA	19.877^{b}	19.174^{a}	19.469^{ab}
298.2662	19.9460	HMDB0013648	Palmitoleoyl Ethanolamide	DA	19.173^{a}	19.410^{b}	19.243^{a}
152.0357	4.1353	HMDB0000267	Pyroglutamic Acid	DA	20.601^{a}	20.939^{b}	20.738^{ab}
168.0301	1.4540	HMDB0001552	Keto-Glutaramic Acid	DW	18.042^{a}	19.700^{b}	19.815^{b}
284.0785	4.1387	HMDB0028793	Glutaminyl-Aspartic Acid	DW	18.403^{a}	18.443^{a}	18.673^{b}
284.0775	4.1353	HMDB0028730	Asparaginyl-Glutamic Acid	DW	19.251^{a}	19.242^{a}	19.606^{b}
262.137	7.1553	HMDB0002248	Gammaglutamyl-Ornithine	DW	19.523^{a}	19.494^{a}	19.915^{b}
234.1024	5.7860	HMDB0028806	Glutaminyl-Serine	DW	19.067^{a}	19.241^{ab}	19.304^{b}
Nucleotides							
208.1504	19.5593	HMDB0030354	Arenaine	WA	19.033^{c}	18.593^{a}	18.740^{b}
345.0403	1.2693	HMDB0062560	Thymidine Monophosphate	DA	18.176^{a}	18.682^{b}	18.598^{b}
278.1286	4.0627	HMDB0001495	Queuine	DA	19.357^{a}	19.690^{b}	19.660^{ab}
446.1494	6.0167	75448	Hexanoyl Adenosine Monophosphate	DW	18.238^{a}	19.093^{b}	19.162^{b}
446.1498	5.8553	75448	Hexanoyl Adenosine Monophosphate	DW	19.674^{a}	20.606^{ab}	20.730^{b}
282.1249	3.7500	HMDB0004044	Methyl Adenosine	DW	20.027^{a}	20.133^{ab}	20.250^{b}

Table 3.4. Continued

Carbohydrates							
386.1895	19.4453	HMDB0012240	Isopentenyladenine Glucoside	WA	19.547^{b}	18.692^{a}	18.827^{a}
673.1505	1.3407	HMDB0039312	Digalloyl Glucoside	WA	19.514^{b}	19.042^{a}	19.093^{a}
427.0296	1.2013	2328	Hydroxyriluzole Glucuronide	WA	20.339^{b}	18.334^{a}	17.929^{a}
845.4378	19.5413	HMDB0040847	Nerolidol Glucopyranoside	WA	18.338^{b}	17.418^{a}	17.925^{ab}
365.0905	4.5353	HMDB0038626	Xanthotoxol Glucoside	WA	19.175^{b}	18.358^{a}	18.637^{a}
379.1088	3.4480	HMDB0036938	Feruloylglucose	DA	18.741^{a}	19.579^{b}	19.428^{b}
Carnitines							
268.148	1.0707	HMDB0000378	Methylbutyroyl Carnitine	WA	18.867^{b}	11.999^{a}	11.999^{a}
392.2825	19.3807	HMDB0002014	Tetradecenoyl Carnitine	WA	21.297^{c}	19.928^{a}	20.401^{b}
420.3137	19.3807	HMDB0006463	Stearidonyl Carnitine	WA	20.908^{c}	20.349^{a}	20.581^{b}
284.1133	2.5233	HMDB0013133	Methylmalonyl Carnitine	WA	20.989^{b}	19.944^{a}	20.104^{a}
Lipids							
467.3867	21.3227	HMDB0035930	Taraxastanediol	WA	19.831^{b}	19.057^{a}	19.166^{a}
643.4888	21.2480	HMDB0112215	Cholestryl Ester	WA	19.413^{b}	18.706^{a}	18.722^{a}
569.4511	21.6787	HMDB0003233	Lutein	WA	20.591^{b}	19.861^{a}	19.792^{a}
613.4777	21.6580	HMDB0029964	Muricatin A	WA	20.452^{b}	19.692^{a}	19.687^{a}
599.4646	21.2667	HMDB0055996	Diacyl Glycerol	WA	19.783^{b}	19.207^{a}	19.169^{a}
657.5045	21.6373	HMDB0112226	Cholestryl Ester	WA	20.011^{b}	19.226^{a}	19.240^{a}
431.2353	19.5447	HMDB0034659	Homofukinolide	WA	21.027^{b}	20.482^{a}	20.698^{a}

Table 3.4. Continued

357.2921	18.8800	HMDB0034031	Hydroxypropyl Undecanoate	WA	20.376^{b}	19.617^{a}	19.924^{ab}
519.3364	19.9693	HMDB0034392	Perulactone	WA	20.681^{b}	19.745^{ab}	19.209^{a}
413.2672	4.4287	HMDB0030057	Boviquinone 4	WA	21.760^{b}	21.422^{a}	21.256^{a}
495.3368	20.3933	HMDB0062314	Docosanoyl Glycerophosphate	WA	21.577^{b}	19.893^{a}	19.786^{a}
330.2439	19.0860	HMDB0030936	Trihydroxyoctadecenoic acid	DA	19.452^{a}	19.852^{b}	19.636^{a}
447.2352	1.3200	HMDB0062318	Heptadecanoyl Glycerophosphate	DA	19.824^{a}	20.264^{b}	20.125^{b}
547.2448	7.1407	HMDB0041048	Physagulin B	DA	18.023^{a}	18.802^{b}	18.503^{b}
287.198	1.4067	HMDB0240268	Farnesyl Acetate	DW	18.933^{a}	19.949^{b}	20.041^{b}
Aromatic							
234.1288	19.4947	HMDB0040033	Heptyl Benzothiazole	WA	20.672^{c}	20.332^{a}	20.501^{b}
525.4262	21.6927	HMDB0040736	Flavidulol D	WA	20.518^{b}	19.748^{a}	19.637^{a}
427.0311	1.4513	HMDB0126050	Oxidanesulfonic Acid	WA	22.510^{b}	21.063^{a}	20.991^{a}
133.0745	0.7947	HMDB0033173	Methylpyrrolo Pyrazine	WA	20.520^{b}	20.311^{a}	20.336^{a}
109.0641	1.1057	HMDB0033895	Anisole	WA	21.921^{b}	21.612^{ab}	21.559^{b}
166.032	20.1573	HMDB0032580	Hydroxybenzyl Isothiocyanate	DA	18.343^{a}	19.028^{c}	18.872^{b}
347.0635	1.2920	HMDB0033936	Bisjuglone	DA	20.541^{a}	22.775^{b}	22.091^{b}
407.2257	21.5413	HMDB0033523	Pyrifolidine	DA	21.429^{a}	21.535^{b}	21.510^{b}
407.2263	21.5413	HMDB0033523	Pyrifolidine	DA	21.410^{a}	21.530^{b}	21.509^{b}
155.0716	1.4569	HMDB0037724	Propyl Furoate	DA	18.264^{a}	19.009^{b}	18.772^{b}
132.0448	4.1360	HMDB0035882	2 Acetylpyrrole	DW	17.788^{a}	17.918^{a}	18.164^{b}

Table 3.4. Continued

333.1356	7.2667	HMDB0040308	Dihydroxytrimethoxyflavan	DW	19.302 [a]	19.618[b]	19.680[b]
155.074	3.4340	HMDB0037728	Furanylmethyl Propanoate	DW	18.853 [a]	19.003[b]	19.018[b]
480.2362	6.4733	HMDB0030203	Fumitremorgin B	DW	19.053 [a]	19.565[ab]	19.638[b]
Vitamins							
375.2561	19.3800	HMDB0006472	Calcitroic Acid	WA	21.452[c]	20.325[a]	20.712[b]
250.0439	21.5413	HMDB0001319	Pyridoxine Phosphate	WA	20.723 [b]	20.640[a]	20.691[ab]
443.306	20.6900	HMDB0038242	Ascorbyl Stearate	DA	18.360[a]	18.908[b]	18.579[a]
Other							
367.4227	21.0973	HMDB0038309	Methylpentacosane	WA	21.700[b]	21.619[a]	21.691[a]
239.2267	18.3980	HMDB0039429	Canavalmine	WA	19.520[c]	18.439[a]	18.796[b]
316.2114	4.4273	HMDB0040383	Pandamarine	WA	20.409[b]	20.060[a]	19.903[a]
312.2339	19.0780	HMDB0014948	Biperiden	DA	18.374[a]	18.712[b]	18.561[a]
621.268	7.8071	HMDB0000668	Hematoporphyrin	DW	19.161[a]	19.446[b]	19.533[b]

[a-c] Different superscript letter indicated a significant difference between the different aging methods ($P<0.05$)

Table 3.5. Consumer sensory panelist comments evaluation (n=120) of low marbled grass-fed beef loins (M. longissimus lumborum) aged using different aging method [Wet aging (WA), Dry aging (DA) and Dry aging in a water permeable bag (DW)].

Attributes		Aging Treatments					
		WA		DA		DW	
		n	%	n	%	n	%
Overall							
Factors	Positive	80	66.7	90.5	75.8	92	76.7
	Aroma	2	2.5	4	4.4	5.5	6.0
	Flavor	37	46.3	46.5	51.4	51.5	56.0
	Juiciness	7	8.8	20	22.1	30	32.6
	Texture	20	25.0	37	40.9	36.5	39.7
	Other	2	2.5	5	5.5	6.5	7.1
Aroma							
Factors	Positive	98	81.7	98	81.7	92	76.7
	Neutral	24	24.5	23	23.5	29	31.5
	Good	45	45.9	49	50.0	48	52.2
	Meaty/Beefy	18	18.4	22	22.4	14	15.2
	Smoky/BBQ	17	17.3	18	18.4	16	17.4
	Other	29	29.6	29	29.6	39	42.4
Flavors							
Factors	Positive	74	61.7	89	74.2	78	65.0
	Neutral	30	40.5	29	32.6	25	32.1
	Good	37	50.0	52	58.4	44	56.4
	Meaty/Beefy	7	9.5	10	11.2	9	11.5
	Other	25	33.8	21	23.6	15	19.2
Texture							
Factors	Positive	91	75.8	100	83.3	99	82.5
	Tender	63	69.2	73	73.0	57	57.6
	Very tender	20	22.0	19	19.0	39	39.4
Juiciness							
Factors	Positive	69	57.5	80	66.7	84	70.0
	Little Juiciness	26	37.7	32	40.0	26	31.0
	Normal Juiciness	31	44.9	33	41.3	37	44.0
	High Juiciness	10	14.5	15	18.8	21	25.0

Table 3.6. Potential metabolomics pathways involved in different aging processes [Wet aging (WA), Dry aging (DA) and Dry aging in a water permeable bag (DW)] based on metabolite matching with KEGG database.

Treatment	Identified Pathway
WA	Metabolism of terpenoids and polyketides
WA	Vitamin B6 metabolism
DA	Glutathione metabolism
DW	Alanine, aspartate and glutamate metabolism

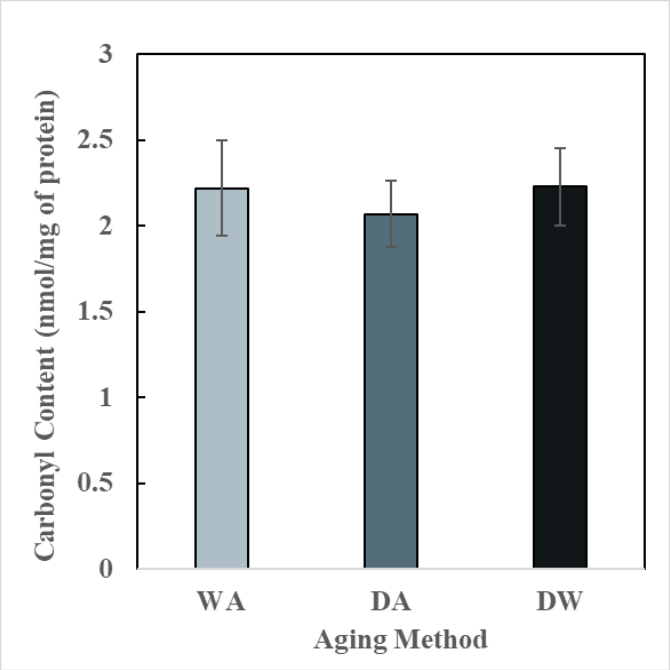


Figure 3.1. Carbonyl content of low marbled grass-fed beef loins (M. *longissimus lumborum*) aged with different aging methods [Wet aging (WA), Dry aging (DA) and Dry aging in a water permeable bag (DW)].

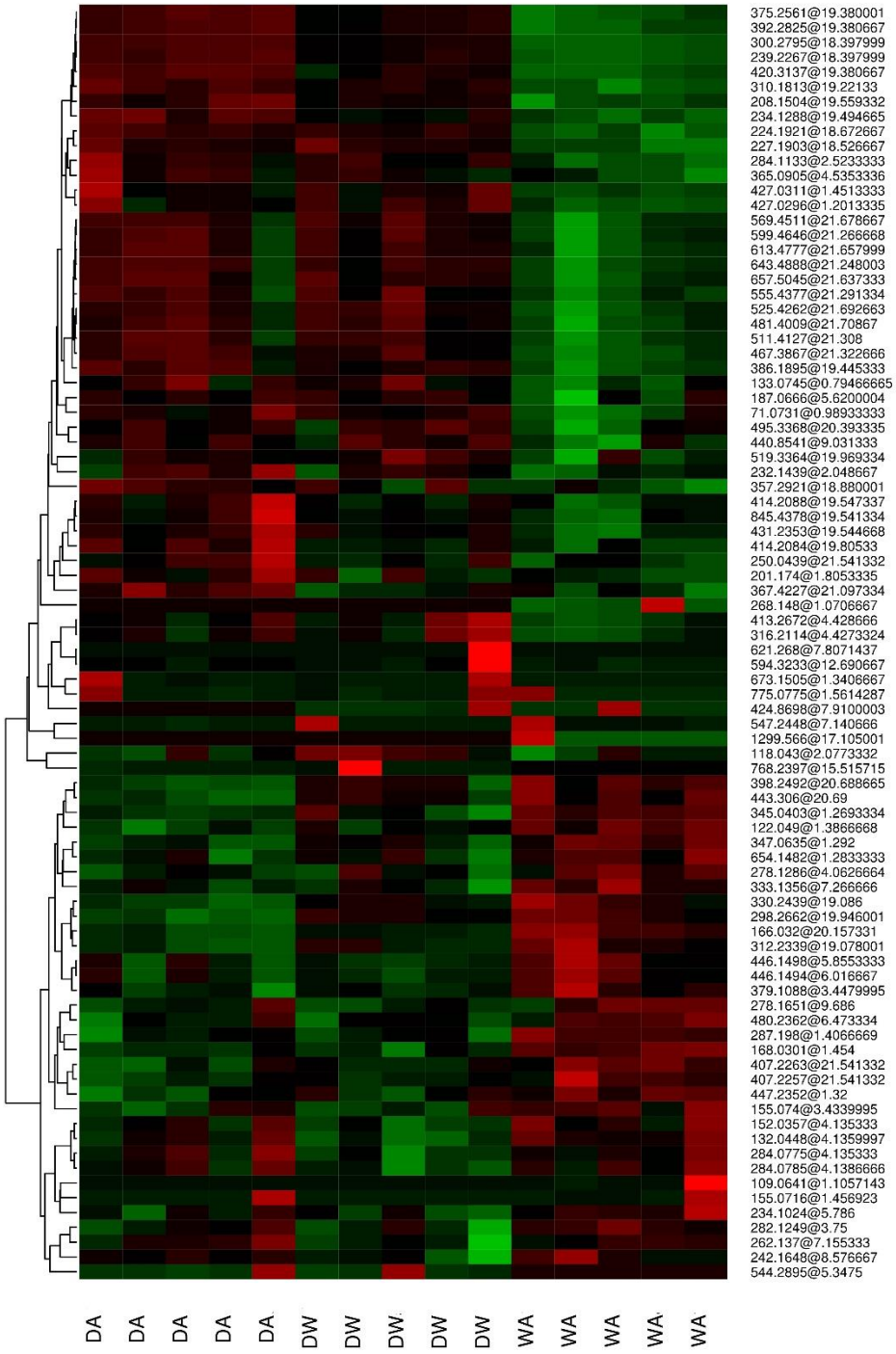


Figure 3.2. Heat map exhibiting the pattern of metabolites changes in grass-fed beef loins (M. *longissimus lumborum*) aged with different aging methods [Wet aging (WA), Dry aging (DA) and Dry aging in a water permeable bag (DW)]. The red color indicates a lesser abundance and green color indicates a greater abundance of the metabolites.

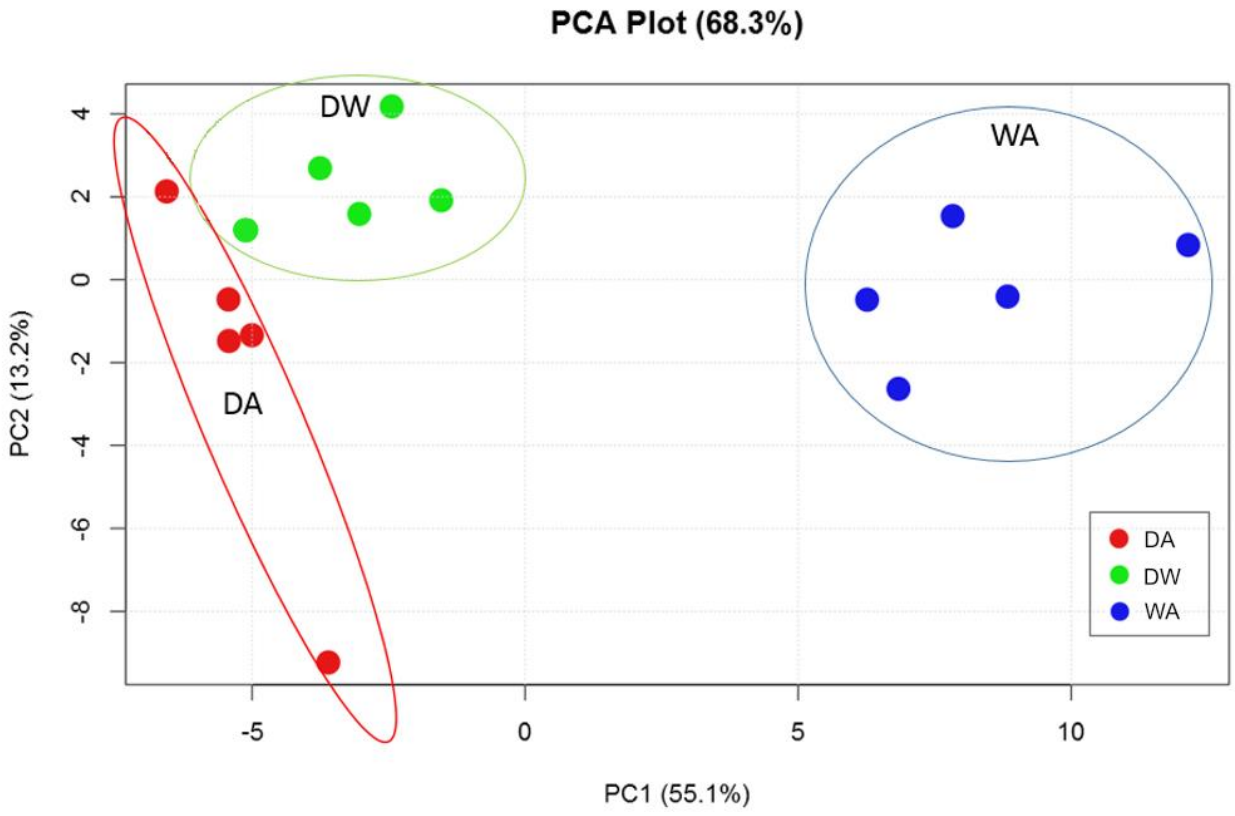


Figure 3.3. Principle component analysis (PCA) of significant metabolites from grass-fed beef loins (M. *longissimus lumborum*) aged with different aging methods [Wet aging (WA), Dry aging (DA) and Dry aging in a water permeable bag (DW)].

3.8 Literatures Cited

Aaslyng, M.D., and L. Meinert. 2017. Meat flavour in pork and beef – From animal to meal. Meat Sci. 132:112–117. doi:10.1016/j.meatsci.2017.04.012.

Andrisic, L., D. Dudzik, C. Barbas, L. Milkovic, T. Grune, and N. Zarkovic. 2018. Short overview on metabolomics approach to study pathophysiology of oxidative stress in cancer. Redox Biol. 14:47–58. doi:10.1016/j.redox.2017.08.009.

Arroo, R. 2007. Eberhard Breitmaier. Terpenes—flavors, fragrances, pharmaca, pheromones. Wiley-VCH, 2006, 214 pp ISBN 3-527-31786-4. Appl. Organomet. Chem. 21:377–377. doi:10.1002/aoc.1209.

Barroso, M.F., J.P. Noronha, C. Delerue-Matos, and M.B.P.P. Oliveira. 2011. Flavored Waters: Influence of Ingredients on Antioxidant Capacity and Terpenoid Profile by HS-SPME/GC-MS. J. Agric. Food Chem. 59:5062–5072. doi:10.1021/jf1048244.

Berger, J., Y.H.B. Kim, J.F. Legako, S. Martini, J. Lee, P. Ebner, and S.M.S. Zuelly. 2018. Dry-aging improves meat quality attributes of grass-fed beef loins. Meat Sci. 145:285–291. doi:10.1016/j.meatsci.2018.07.004.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911–917. doi:https://doi.org/10.1139/o59-099.

Calkins, C.R., and J.M. Hodgen. 2007. A fresh look at meat flavor. Meat Sci. 77:63–80. doi:10.1016/j.meatsci.2007.04.016.

Campbell, R.E., M.C. Hunt, P. Levis, and E. Chambers IV. 2001. Dry-aging effects on palatability of beef longissimus muscle. J. Food Sci. 66:196–199. doi:10.1111/j.1365-2621.2001.tb11315.x.

Carpino, S., J. Horne, C. Melilli, G. Licitra, D.M. Barbano, and P.J. Van Soest. 2004. Contribution of native pasture to the sensory properties of Ragusano cheese. J. Dairy Sci. 87:308–315. doi:10.3168/jds.S0022-0302(04)73169-0.

Chail, A., J.F. Legako, L.R. Pitcher, T.C. Griggs, R.E. Ward, S. Martini, and J.W. MacAdam. 2016. Legume finishing provides beef with positive human dietary fatty acid ratios and consumer preference comparable with grain-finished beef1. J. Anim. Sci. 94:2184–2197. doi:10.2527/jas.2015-0241.

Chong, J., D.S. Wishart, and J. Xia. 2019. Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. Curr. Protoc. Bioinforma. doi:10.1002/cpbi.86.

Descalzo, A.M., E.M. Insani, A. Biolatto, A.M. Sancho, P.T. García, N.A. Pensel, and J.A. Josifovich. 2005. Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. Meat Sci. 70:35–44. doi:10.1016/j.meatsci.2004.11.018.

Dinh, T.T.N., J.F. Legako, M.F. Miller, and J.C. Brooks. 2018. Effects of USDA quality grade and cooking on water-soluble precursors of beef flavor. Meat Sci. 146:122–130. doi:10.1016/j.meatsci.2018.08.008.

Elisia, I., C. Hu, D.G. Popovich, and D.D. Kitts. 2007. Antioxidant assessment of an anthocyanin-enriched blackberry extract. Food Chem. 101:1052–1058. doi:10.1016/j.foodchem.2006.02.060.

Forss, D.A. 1973. Odor and flavor compounds from lipids. Prog. Chem. Fats Other Lipids. 13:177–258. doi:10.1016/0079-6832(73)90007-4.

Gardner, K., and J.F. Legako. 2018. Volatile flavor compounds vary by beef product type and degree of doneness. J. Anim. Sci. 96:4238–4250.

Gredell, D.A., T.G. O'Quinn, J.F. Legako, J.C. Brooks, and M.F. Miller. 2018. Palatability and Biochemical Factors of Beef from Mature Cattle Finished on a Concentrate Diet Prior to Harvest. Meat Muscle Biol. 2:111. doi:10.22175/mmb2017.09.0046.

Hanagasaki, T., and N. Asato. 2018. Changes in free amino acids and hardness in round of Okinawan delivered cow beef during dry- and wet-aging processes. J. Anim. Sci. Technol. 60:1–9. doi:10.1186/s40781-018-0180-x.

Kim, Y.H.B., R. Kemp, and L.M. Samuelsson. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Sci. 111:168–176. doi:10.1016/j.meatsci.2015.09.008.

Kim, Y.H.B.B., D. Ma, D. Setyabrata, M.M. Farouk, S.M. Lonergan, E. Huff-Lonergan, and M.C. Hunt. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. Meat Sci. 144:74–90. doi:10.1016/J.MEATSCI.2018.04.031.

King, D.A., S.D. Shackelford, C.D. Broeckling, J.E. Prenni, K.E. Belk, and T.L. Wheeler. 2019. Metabolomic Investigation of Tenderness and Aging Response in Beef Longissimus Steaks. Meat Muscle Biol. 3:76. doi:10.22175/mmb2018.09.0027.

De la Fuente, J., M.T. Díaz, I. Álvarez, M.A. Oliver, M. Font i Furnols, C. Sañudo, M.M. Campo, F. Montossi, G.R. Nute, and V. Cañeque. 2009. Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. Meat Sci. 82:331–337. doi:10.1016/j.meatsci.2009.02.002.

Lee, H.J., J.W. Yoon, M. Kim, H. Oh, Y. Yoon, and C. Jo. 2019. Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. Meat Sci. 153:152–158. doi:10.1016/j.meatsci.2019.03.019.

Ma, D., Y.H.B. Kim, B. Cooper, J.-H. Oh, H. Chun, J.-H. Choe, J.P. Schoonmaker, K. Ajuwon, and B. Min. 2017. Metabolomics Profiling to Determine the Effect of Postmortem Aging on Color and Lipid Oxidative Stabilities of Different Bovine Muscles. J. Agric. Food Chem. 65:6708–6716. doi:10.1021/acs.jafc.7b02175.

Ma, D., Q. Yu, V.E. Hedrick, B.R. Cooper, T.J. Paschoal Sobreira, J.H. Oh, H. Chun, and Y.H.B. Kim. 2020. Proteomic and metabolomic profiling reveals the involvement of apoptosis in meat quality characteristics of ovine M. longissimus from different callipyge genotypes. Meat Sci. 166:108140. doi:10.1016/j.meatsci.2020.108140.

Machiels, D., S.M. Van Ruth, M.A. Posthumus, and L. Istasse. 2003. Gas chromatography-olfactometry analysis of the volatile compounds of two commercial Irish beef meats. Talanta. 60:755–764. doi:10.1016/S0039-9140(03)00133-4.

Mottram, D.S. 1998. Flavour formation in meat and meat products: A review. Food Chem. doi:10.1016/S0308-8146(98)00076-4.

O'Fallon, J. V., J.R. Busboom, M.L. Nelson, and C.T. Gaskins. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 85:1511–1521. doi:10.2527/jas.2006-491.

Passetti, R.A.C., I.N. do Prado, F. de Assis Fonseca de Macedo, A.J.C. Teixeira, C.A.L. Cardoso, G.R. de Arruda Santos, and C.A. Santos. 2019. Physicochemical characteristics of dry aged beef from younger Nellore bulls slaughtered at different body weights. Trop. Anim. Health Prod. 51:2635–2640. doi:10.1007/s11250-019-01944-9.

Ramalingam, V., Z. Song, and I. Hwang. 2019. The potential role of secondary metabolites in modulating the flavor and taste of the meat. Food Res. Int. 122:174–182. doi:10.1016/J.FOODRES.2019.04.007.

Savell, J.W. 2008. Dry-Aging of Beef. Beef Checkoff. 16.

Subbaraj, A.K., Y.H.B. Kim, K. Fraser, and M.M. Farouk. 2016. A hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-MS) based metabolomics study on colour stability of ovine meat. Meat Sci. 117:163–172. doi:10.1016/j.meatsci.2016.02.028.

Valdivielso, I., M. de Renobales, N. Aldai, and L.J.R. Barron. 2017. Changes in terpenoid composition of milk and cheese from commercial sheep flocks associated with seasonal feeding regimens throughout lactation. J. Dairy Sci. 100:96–105. doi:10.3168/jds.2016-11761.

Vesely, A. 1973. Fatty acids and sterois affecting flavor and aroma of meat from ram, cryptorcthid, and wether lambs. Can. J. Anim. Sci. 53:673–678. doi:https://doi.org/10.4141/cjas73-106.

Vossen, E., and S. De Smet. 2015. Protein Oxidation and Protein Nitration Influenced by Sodium Nitrite in Two Different Meat Model Systems. J. Agric. Food Chem. 63:2550–2556. doi:10.1021/jf505775u.

Warren, K.E., and C.L. Kastner. 1992. A comparison of dry-aged and vacuum-aged beef strip loins. J. Muscle Foods. 3:151–157. doi:10.1111/j.1745-4573.1992.tb00471.x.

Whitfield, F.B., and D.S. Mottram. 1992. Volatiles from interactions of Maillard reactions and lipids. Crit. Rev. Food Sci. Nutr. 31:58. doi:10.1080/10408399209527560.

Yu, A.N., and A.D. Zhang. 2010. Aroma compounds generated from thermal reaction of l-ascorbic acid with l-cysteine. Food Chem. 121:1060–1065. doi:10.1016/j.foodchem.2010.01.049.

Zhao, C.J., A. Schieber, and M.G. Gänzle. 2016. Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations – A review. Food Res. Int. 89:39–47. doi:10.1016/J.FOODRES.2016.08.042.

Zou, T., J. Liu, H. Song, and Y. Liu. 2018. Discovery of Amadori-Type Conjugates in a Peptide Maillard Reaction and Their Corresponding Influence on the Formation of Pyrazines. J. Food Sci. 83:1588–1595. doi:10.1111/1750-3841.14156.

CHAPTER 4. IMPACT OF VARIOUS DRY-AGING METHODS ON MEAT QUALITY AND PALATABILITY ATTRIBUTES OF BEEF LOINS (M. *LONGISSIMUS LUMBORUM*) FROM CULL COW

The content presented in this chapter is a reprint of our previously published work titled "Impact of various dry-aging methods on meat quality and palatability attributes of beef loins (M. Longissimus lumborum) from cull cow." Some edits have been made to produce a cohesive dissertation. The published work reference is Setyabrata, D., Xue, S., Vierck, K.R., Legako, J. F., Ebner, P., Zuelly, S., & Kim, Y. H. B. (2021). Impact of various dry-aging methods on meat quality and palatability attributes of beef loins (M. Longissimus lumborum) from cull cow. Meat and Muscle Biology. Accepted.

4.1 Abstract

The objective of this study was to determine the effect of various dry-aging methods on meat quality and palatability attributes of cull cow beef loins. Paired bone-in loins (*M. longissimus lumborum*) from 13 cull cow carcasses (Holstein, 42+ mo) were obtained at 5d postmortem, divided into four equal sections and randomly assigned into 4 aging methods (wet-aging [WA], conventional dry-aging [DA], dry-aging in water-permeable bag [DWA], and UV-light dry-aging [UDA]). The beef sections were aged for 28d at 2 °C, 65% relative humidity and 0.8 m/s airflows. Following aging, surface crusts and bones were removed, and loin samples were collected for the meat quality, microbiological and sensory analyses. Results indicated that all dry-aged loins had greater moisture and trimming loss compared to WA ($P<0.05$), while DWA had lower loss than DA and UDA ($P<0.05$). No differences in shear force, cook loss and both lipid and protein oxidation across all treatments were observed ($P>0.05$). Among all treatments, DWA exhibited the least color stability indicated by rapid discoloration observed in the sample, while UDA had color attributes comparable to WA throughout the whole display. Microbial analysis indicated that UDA had a lower microbial concentration on the surface than the other samples ($P<0.05$). The consumer panel analysis found that all loins were acceptable, and the trained panel analysis indicated that DA loins decreased sourness and animal fat flavor ($P<0.05$) and had a trend of decreasing oxidized flavor ($P = 0.07$). The results indicate that dry-aging can potentially be utilized as an effective natural process by nullifying some of the well-known off-flavor attributes

associated with cull cow beef while not compromising other meat quality attributes or microbiological shelf-life.

Keywords: Dry-aging, Cull Cow, UV light, Sensory attributes, Microbial attributes

4.2 Introduction

Cull cows account for up to 19% of the total beef cattle harvested in the United States (USDA, 2019), playing an important role in fulfilling the beef demands in the United States (about 10% of beef supply). As cows are often culled from the herd at an older age (42 months or higher), beef from mature animals is known to have inferior palatability, such as decreased tenderness and undesirable flavor (Gredell et al., 2018). Additionally, cull cow diets are often based on low-energy forages, decreasing the flavor desirability by the consumer (Woerner, 2010). As such, the majority of cull cow beef, however, was utilized as ground beef or manufacturing products rather than retail whole muscle cuts (Xiong et al., 2007), thus placing the products in a low-value beef category compared to conventional beef from young animals.

Multiple post-harvest techniques, such as salt injection (Diles et al., 1994; Morgan et al., 1991) and blade tenderization (Obuz et al., 2014), have been developed and are currently utilized as a post-harvest intervention to negate these palatability issues. However, the current post-harvest processes maybe less favorable to consumers, as there is an increasing demand for natural and minimally processed meat products (Verbeke et al., 2010). Thus, there is a need for developing natural/value-adding post-harvest processes to improve cull cow meat palatability.

Postmortem aging is a common practice which improves meat quality attributes, where considerable improvements in tenderness, juiciness and/or flavor take place through naturally occurring endogenous enzymatic activities (Kim et al., 2018). In general, aging can be performed in two different types; wet-aging (utilizing vacuum packaging) and dry-aging (without packaging materials in a highly controlled environment). Dry-aging specifically has been known to generate unique flavors such as “brown-roasted,” “beefy,” and “buttery” flavors (Setyabrata et al., 2021; Campbell et al., 2001). In addition to the unique flavors, positive impacts of dry-aging on improvements in tenderness and juiciness have also been reported, making the product more desirable by consumers (Campbell et al., 2001). Furthermore, a recent study by Berger et al. (2018)

reported that palatability improvements from dry-aging were identified in low marbled grass-fed beef, suggesting the potential feasibility of utilizing dry-aging as a natural value-adding process for low quality/low-value products, such as cull-cow beef.

Given that dry-aging conditions require exposure of meat to the environment without a protective barrier, the potential risk of microbial contamination of the meat exists. The application of moisture-permeable bags for dry-aging was reported to be effective in minimizing contamination and moisture loss while providing similar dry-aged beef characteristics (Li et al., 2013; Ahnström et al., 2006). Furthermore, UV lights have been demonstrated as an effective and affordable method to limit microbial growth (Chun et al., 2010) and could be applied during the dry-aging processing, either through direct exposure of the product to UV light (Smith et al., 2014; DeGeer et al., 2009) or by sterilizing incoming air within the air filter (Lepper-Blilie et al., 2016; Warren and Kastner, 1992). While UV light application has previously been shown to successfully retard the growth of microbial in various applications (Yeh et al., 2018; Ganan et al., 2013; Chun et al., 2010), its impact on dry-aged meat quality development has not been fully studied. Therefore, the objective of this study was to evaluate the effect of dry-aging and different aging methods on meat quality, microbiological shelf-life, and palatability of beef from cull cows.

4.3 Materials and methods

4.3.1 Sample collection, preparation and processing

Paired bone-in beef loins from 13 cull cow carcasses (42+ months old, C maturity, Holstein, NAMP:175, *M. longissimus lumborum*) were collected from a commercial beef plant at 5 d postmortem. The samples collected were all categorized as USDA utility grade by the plant worker and had a fat cover < 2 cm in the loin area. Beef loins were vacuum-packed and stored in ice chests during transportation to the Purdue Meat Science and Muscle Biology Laboratory. The samples were immediately processed following arrival in the facility. Prior to any processing, initial microbiological and biochemical samples (INI) were individually excised from the loin eye area (anterior portion) from one side of the loins. The loins were then divided into 2 equal-length sections, totaling 4 sections from each animal. The sections were then randomly assigned to 4 different aging methods: wet-aging (WA; Clarity Vacuum Pouches, Bunzl Processor Division, Riverside, MO, USA.), conventional dry-aging (DA), dry-aging in water-permeable bag (DWA;

UMAi Dry® Short Loin [Large], UMAI Dry, Minneapolis, MN, USA) and UV-light dry-aging (UDA).

The initial pH value and weight were measured for all beef sections, followed by 28-days of aging at 2 °C, 65% relative humidity (RH) and 0.8 m/s airflows. The UV-light treatments were applied twice per day to UDA samples, with a dose of 5 J/m^2 for each UV treatment (5 minutes of exposure per treatment, Phillip TUV T8 UVC light, Eindhoven, Netherlands). The UV lights were mounted 30 cm above the samples. The beef sections were rotated weekly to minimize any location variation within the cooler. At the end of aging treatment, the sections were weighed, deboned and trimmed, separating the dehydrated surfaces (crust) from the inner lean portions. The trimmed sections were then weighed again to calculate the final yield. Samples from both the surface crust and lean portions were collected for microbiological analyses. Post-aging pH values were measured, and sections were cut into steaks (2.4 cm thick) for further meat quality analyses, including Warner-Bratzler shear force, color stability, water-holding capacity, oxidative stability and sensory analysis. Except for the samples assigned for color and drip-loss analyses, all steaks samples collected were individually vacuum packed and stored in a -80 °C freezer until use.

4.3.2 pH measurement

The pH measurement was performed before and after the aging treatment using a portable meat pH meter (HANNA HI 99163, Hanna Instrument, Inc., Warner, NH, USA) by directly inserting the probe into two different locations of the meat. The pH meter was calibrated to both pH 4 and 7 standards (Thermo Scientific™ Orion™, Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's guidelines.

4.3.3 Aging loss, processing loss and saleable yield

The weights for each section were collected prior to and after the aging regime to estimate the shrink/water loss during aging. The final weights of the trimmed lean portions were recorded to calculate the final saleable yield of the products. The losses were presented as percent loss, measuring the loss over the aged sample weight.

4.3.4 Water-holding capacity measurement

The water-holding capacity (WHC) was analyzed by measuring both drip loss and cook loss of each sample. All losses were expressed as percent weight loss, measuring the change between the initial and final weight of the samples. The drip loss measurement was conducted using the Honikel drip loss method following the modification by Kim et al. (2017).

The cook loss was performed by cooking the sample until the internal temperature reached 65 °C using a clamshell grill (Griddler GR-150, Cuisinart, Glendale, AZ, USA). The samples were blotted dry using paper towels and weighed before and after the cooking process. The internal temperature was monitored using a T-type thermocouple (Omega Engineering, Stamford, CT, USA) connected to an OctTemp 2000 data logger (Madge Tech, Inc., Warner, NH, USA). Following cooking, samples were wrapped using aluminum foil and kept at 4 °C overnight for Warner-Bratzler shear force (WBSF) measurement.

4.3.5 Proximate analysis

Proximate analysis was performed following the protocol described in the AOAC official guidelines (AOAC, 2007). All proximate contents are presented on a wet matter base (%). Fat content was calculated using the following formula:

$$Fat\ content\ (\%) = 100 - (Moisture\ content + Protein\ content + Ash\ content)$$

4.3.6 Display color stability

At the end of the aging process, one steak from each section was randomly assigned for 7 days simulated display under continuous light at 2 °C. The samples were placed on a Styrofoam tray with a drip soaking pad and overwrapped using oxygen-permeable PVC film (Reynolds Food Service Packaging, Richmond, VA, USA). The steaks were displayed for 7 days under fluorescent light (1800 lx, color temperature = 3500 K, OCTRON® T8 Lamps, Osram Sylvania LTD., Canada), and the color and color stability were evaluated daily by both trained color panelist and instrumental colorimeter.

The panelists (n= 8) were trained following the American Meat Science Association meat color guidelines (AMSA, 2012) on lean surface color and discoloration. The panelists were required to pass the Farnsworth-Munsell 100 Hue Test and trained on multiple sessions prior to

the study (AMSA, 2012). The surface lean color was scored with a scale of 1 to 8 (1 = extremely dark brown red; 2 = dark brown red; 3 = moderately dark/brown red; 4 = slightly dark/brown red; 5 = slightly bright red; 6 = moderately bright red; 7 = bright red; 8 = extremely bright red) over the display. At the same time, surface discoloration was also scored using a scale of 1 to 7 (1 = no discoloration; 2 = 1–19% discoloration; 3 = 20–39% discoloration; 4 = 40–59% discoloration; 5 = 60–79% discoloration; 6 = 80–99% discoloration; 7 = 100% discoloration).

Following the trained panel observation, the Hunter MiniScan EZ colorimeter (Hunter, Reston, VA, USA) was utilized to collect the instrumental color by measuring the CIE L*, a* and b* on three random locations on the surface of the steak, parallel to the fiber direction. The instrument was calibrated following the manufacturer's guidelines and equipped with a 25mm (diameter) opening prior to any data collection. The illuminant A was used, and the observer was set to standard 10°. Hue angle and Chroma value were calculated using the following formulas; hue angle = $\tan^{-1}(b^*/a^*)$ and Chroma = $(a^{*2} + b^{*2})^{½}$ (AMSA, 2012). At the end of the display, steaks were vacuum packaged individually and stored at -80 °C for lipid and protein oxidation determination.

4.3.7 Lipid Oxidation

The extent of lipid oxidation for the samples was determined by measuring the lipid oxidation level of the steaks before and after the color display following the aging process. The lipid oxidation was measured through the 2-thiobarbituric reactive substances (TBARS) assay described by Setyabrata and Kim (2019). The absorbance was read at 531 nm using Epoch™ Microplate Spectrophotometer (BioTek Instrument Inc., Winooski, VT, USA), and TBARS value was expressed as mg malondialdehyde/kg meat.

4.3.8 Protein Oxidation

The degree of protein oxidation was measured through the estimation of carbonyl content, using the same samples utilized for the observation of lipid oxidation. The measurement was performed following the method described by Vossen and De Smet (2015), and the carbonyl content was expressed as nmol carbonyls/mg protein.

4.3.9 Microbial analysis

The total aerobic bacteria (aerobic plate count; APC), lactic acid bacteria (LAB), yeast, and mold concentrations were determined for all treatments and samples collected from lean and crust portions at the end of the aging period. Microbial analyses were then conducted following the method described by Berger et al. (2018) with modification. In brief, 5 g of sample were aseptically collected, placed into a stomacher bag (WhirlPak, Madison, WI, USA) with 50 mL 0.1% peptone water (BD Difco™, Sparks, MD, USA), and hand stomached for 1 minute. The rinsate was collected, serially diluted and plated for enumeration of viable bacteria. The APC concentration was determined using plate count agar (BD Difco™, Sparks, MD, USA) which were incubated for 48 h at 37 °C following inoculation. LAB were quantified using de Man, Rogosa and Sharpe agar (BD Difco™, Sparks, MD, USA) which were incubated for 72 h at 37 °C under anaerobic conditions generated using anaerobic packs (Oxoid™ AnaeroGen, Waltham, MA, USA) following inoculation. Both yeast and mold concentration were calculated using Yeast and Mold films (Petrifilm™, 3M Microbiology Products, St. Paul, MN), which were incubated at 25 °C for 120 h following inoculation. After each incubation, colonies were counted, and the microbial concentration was expressed as $\log_{10}$ CFU/mL of rinsate. For both APC and LAB measurement, plates with colonies count below 25 colonies on the lowest dilution were considered as having bacterial concentration below the detection limit (BDL). For the yeast and mold petrifilms, the detection limit was set at 15 colonies per the manufacturer's recommendation.

4.3.10 Sensory analysis

Sensory evaluation was conducted using both trained and consumer panelists at Texas Tech University. The research protocol was approved by the Texas Tech Institutional Review Board (IRB# 2017-721). The steak samples collected for the sensory analyses were frozen at -40 °C and were shipped using overnight shipping in a Styrofoam cooler. The steaks were thawed for 24 h at 4 °C in preparation for the sensory session. All samples were cooked on a clamshell grill (Griddler GR-150, Cuisinart, Glendale, AZ, USA) until the internal temperature reached 65 °C. Following cooking, steaks were cut into 2.4 × 1 × 1 cm cubes, and two cubes were served to each panelist. The samples were held in a warmer (Cambro Ultra Heated Holding Pan Carrier, 214UPCH400, Webstaurant Store, Lititz, PA) for no longer than 30 minutes prior to serving. Samples were served

under red incandescent light. Panelists were supplied with distilled water, apple juice and unsalted saltine crackers as a palate cleanser between each sample, as well as an expectorant cup, toothpick, fork, knife, and napkin.

Trained sensory panelist

A total of 11 panelists were recruited and trained to detect various beef flavor and palatability characteristics according to the AMSA Sensory Guidelines (AMSA, 2016). The panelists were trained and tested for 4 weeks prior to participating in panel evaluation. Panelists were trained to identify the following traits: beef flavor identity, brown/roasted, bloody/serumy, fat-like, liver-like, oxidized, metallic, fishy, buttery, nutty, earthy/musty, umami, salty, bitter, sour, overall juiciness, and overall tenderness. Each scale was anchored at each endpoint and had a neutral midpoint (e.g. 0 = extremely bland/dry/tough; 50 = neither tough/dry nor tender/juicy; 100 = extremely tender/juicy/intense). The sensory samples were randomly assigned to each sensory session, ensuring that each treatment was represented in each panel. A total of 7 panel sessions was conducted, each having 8 beef samples except for the final panel, where only 4 samples were served. Samples were prepared and served following the condition previously described.

Consumer sensory panelist and survey

The consumer sensory evaluation was conducted using 130 panelists recruited from the Lubbock, Texas area. The evaluation was conducted in 1 h session with 20 consumer panelists per session, except the final session with only 10 panelists. The steak samples were prepared and served following the process previously mentioned. During each session, panelists received an electronic ballot (Qualtrics, Provo, UT, USA) consisting of a demographics questionnaire, five sample ballots, and a final survey to determine the panelist's familiarity with dry-aging, beef from dairy cattle, and their willingness to pay for the dry-aged product. Each panelist evaluated one warm-up sample, followed by one sample of each treatment (n = 5). Samples were evaluated for flavor, tenderness, juiciness, and overall liking on unstructured 100-point line scales. Scales were verbally anchored at each endpoint and midpoint (0 = extremely dislike/extremely tough/extremely dry; 50 = neither dislike nor like/neither tough nor tender/neither dry nor juicy; 100 = extremely like/extremely tender/extremely juicy). Additionally, each panelist was also asked to rate each trait as acceptable or unacceptable and to designate each sample as unsatisfactory,

everyday, better than everyday, or premium quality. The term dairy cattle beef was utilized in the survey to represent beef from mature cull cows since it is commonly perceived that dairy cattle are older, and therefore the term might be more familiar to the consumers (Moreira et al., 2021).

4.3.11 Statistical analysis

This study was a randomized complete block design with four different aging treatments as the fixed effect and animal as the random effect. In the microbial analysis, sample source (lean or curst) was added as a fixed effect to consider potential location differences. For the sensory evaluation, panelists and sessions were added as the random effects in the model during analysis. The meat processing and quality data were analyzed using the PROC MIXED procedure from SAS 9.4 software (SAS Institute Inc., Cary, NC). Both trained and consumer sensory panel data were analyzed using PROC GLIMMIX procedures from SAS 9.4 software (SAS Institute Inc., Cary, NC). Least square means for all traits were separated, and the significance level was defined at the level of $P<0.05$.

4.4 Results

4.4.1 Processing loss and saleable yield

Lower shrink/purge loss was observed for WA samples compared to the dry-aged beef samples, such as DA, DWA and UDA, as expected ($P<0.05$, Table 4.1). Within the dry-aged beef samples, DWA loins had less aging loss than both DA and UDA loins ($P<0.05$). Correspondingly, more dehydrated lean surface crusts were trimmed from both DA and UDA loins when compared to DWA loins ($P<0.05$). As expected, all dry-aging treatments had a greater total loss compared to WA samples ($P<0.05$). Of the dry-aging treatments, the total loss in UDA and DA loins was not different from each other ($P>0.05$) but was greater compared to DWA loins ($P<0.05$). Accordingly, both DA and UDA loins also had a lower total yield compared to all treatments ($P<0.05$).

4.4.2 pH, shear force, proximate composition and WHC

No differences were observed in initial pH values across all treatments (P>0.05, data not shown). A significant treatment effect was observed on the final product pH following the different aging treatments (P<0.05, Table 4.2).

Different aging treatment processes did not affect the WBSF values of the samples (P>0.05). All the samples were observed to have a shear force value of less than 30 *N*, indicating that the products could be considered as moderately tender (Smith et al., 2008).

Proximate compositions of the meat samples were found to be significantly affected by the treatment applied (Table 4.2). The dry-aging treatments (DA and UDA) induced excessive dehydration, having lower moisture contents than WA treatments (P<0.05). The DWA samples, however, had similar amounts of moisture to WA samples (P>0.05). Higher protein, fat and ash (mineral) contents were observed in both DA and UDA (P<0.05) compared to WA and DWA, potentially due to greater moisture loss, concentrating the content of the product.

No difference was observed in cook loss among the different treatments (P>0.05). All the products exhibited a similar cook loss, ranging from 12.88% to 13.83% (Table 4.2). For the drip loss, greater loss (P<0.05) was identified in UDA loins compared to all other treatments

4.4.3 Display color stability

No differences in color and color stability of beef samples from different aging treatments were found until 5 d of the display, where the majority of the instrumental color attributes indicate significant changes between the samples (P>0.05; Figure 4.1). The WA samples displayed a greater lightness (P<0.05) starting from day 5 until the end of the display compared to all dry-aging treatments. Among the dry-aging methods, UDA loins maintained similar redness, yellowness, hue angle and chroma value when compared to WA loins throughout the display (P>0.05). Both DA and DWA samples had a comparable color stability (P>0.05), except for redness and hue angle, where the DWA samples had lower redness and higher hue angle values (P<0.05) compared to DA samples and all other treatments.

The results of the visual color observation by trained panelists were in agreement with the instrumental observation, where a significant interaction was observed between aging treatment and display period. A decrease was observed along the display period for lean color (P<0.05).

Similar discoloration and lean color were observed at the beginning of display up to day 4 among all samples ($P>0.05$). A significant decrease in lean color was observed in DWA samples, which were scored the lowest starting from day 5 of the display. At the end of the display, WA loins had the greatest lean color score (indicating greater brightness and redness), followed by UDA, DA and DWA loins having the lowest lean color score ($P<0.05$, Figure 4.2). For the discoloration score, a similar result to hue angle result was found, where a significant increase was observed from day 5 until the end of display. Both UDA and WA loins had the lowest visual discoloration score, followed by DA and DWA loins having the highest score ($P<0.05$, Figure 4.2).

4.4.4 Oxidative stability

No significant aging treatment and interaction between aging treatment and display time on both lipid and protein oxidation was found. However, both protein and lipid oxidation were affected by display time ($P<0.05$, Table 4.3), shown by increased TBARS and carbonyl values over the display.

4.4.5 Microbial Analysis

Initial microbial concentrations prior to any aging treatments for all microbial groups measured were below detection limits (data not shown), indicating similar initial microbial concentrations across the samples. Following 28 days of aging treatments, a significant interaction between treatment and location was observed on all microbial groups measured, indicating different microbial growth patterns in the lean and crust portions (Table 4.4).

The APC and mold concentration were found to be greater in DWA samples, for both crust (6.40 and 5.99 $\log_{10}$ CFU/mL, respectively) and lean (4.80 and 4.65 $\log_{10}$ CFU/mL, respectively) portions compared to all other treatments ($P<0.05$). WA samples had the greatest concentrations of LAB in both crust (5.90 $\log_{10}$ CFU/mL) and lean (3.79 $\log_{10}$ CFU/mL) portions compared to other treatments ($P<0.05$). The yeast was only detected in the crust portion of DWA and WA samples, in which DWA had greater yeast concentrations compared to WA samples ($P<0.05$). For the lean, DA samples had a higher yeast count compared to WA samples ($P<0.05$). No yeast was detected in the lean portion of DWA and UDA samples. Across all treatments, UDA crust

consistently had a lower concentration of microbial groups compared to all other treatments ($P<0.05$).

4.4.6 Demographic and survey data

The demographic information of the consumer panel is presented in Table 4.5. When consuming meat, 76.2% of the participants preferred beef products compared to other meat products and 76.9% of participants reported consuming beef 1-5 times/week. Participants were split for the most important palatability attribute when eating beef steak, with 46.2% selecting tenderness and 42.3% selecting flavor. Most of the participants in the study preferred that their beef steak cooked to Medium-Rare (35.4%), followed by Medium-Well (26.2%) and Medium (22.3%) doneness.

After evaluating the samples, the panelists were given a series of questions regarding dairy cattle beef, dry-aging and their willingness to pay for the product (Table 4.6). Only 39.2% of the consumer were familiar with dairy beef or cull cow beef, however, 62.8% of participants reported having a positive perception of dairy beef or cull cow beef. Of all the panelists, 44.6% had previously consumed dry-aged beef products. Of those participants who had consumed dry-aged beef, 43.3% reported obtaining dry-aged beef in a restaurant, while 43.1% reported obtaining dry-aged beef from a local butcher or supermarket. The postmortem meat aging process itself was perceived as a positive term by the majority of the participants (86.9%). About 39.2% of the panelist were not sure about the safety of the dry-aged beef product, while 37% of participants perceived the safety of dry-aged as similar to other beef products, 16.2% of participants perceived it as safer and 6.9% perceived it as less safe compared to other beef products. When asked about willingness to pay for dry-aged beef products, 59.2% of the consumer were willing to pay $1.00 more per 1 lb. (0.45 Kg) of dry-aged beef. The willingness slightly decreased to 50% if the dry-aged beef originated from dairy beef.

4.4.7 Sensory panel evaluation

Overall, results from the consumer panel showed that different aging methods resulted in similar sensory attributes ($P>0.05$, Table 4.7). Consumers rated similar scores in flavor, tenderness, juiciness and overall liking for steak samples from the different aging methods. Similarly, when

asked for the acceptability of each trait, consumers found the majority of the products to be acceptable regardless of the aging treatments (P>0.05). When requested to select perceived flavor from a pre-selected list, a greater percentage of participants assigned a beefy flavor of WA and DWA samples compared to DA and UDA (P<0.05). No significant difference was found for all other flavor attributes across all treatments (P>0.05).

For the trained sensory panel evaluation, however, a significant treatment effect was found in fat-like flavor, sour flavor and overall juiciness (P<0.05, Table 4.8). Greater fat-like flavor and overall juiciness were observed in the WA samples compared to all dry-aged treatments (P<0.05). Both WA and UDA samples were more sour, while DA samples were the least sour (P<0.05). The DWA loins were not different in sourness when compared to all the treatments (P>0.05). Samples from UDA tended to have greater oxidized flavor among all the treatments (P=0.0767).

4.5 Discussion

4.5.1 Processing loss and saleable yield

Dry-aging has been known to induce a considerable amount of aging loss due to the moisture evaporation during the process. In conjunction, the dehydrated surface crust forms as the result of dry-aging, which requires removal prior to sale and/or consumption of the product (Savell, 2008), further increasing the yield loss. In the present study, the lower loss in purge, trim and total loss exhibited by DWA compared to other dry-aging methods is in agreement with previous studies reported by Ahnström et al. (2006) and Berger et al. (2018). The lower total loss potentially occurred due to additional protection from the dry-aging bag, which may have limited the rate of moisture transfer during the aging process. While dry-aging produced a greater loss, the crust generated from the process was demonstrated to have antioxidant and emulsifying capability in other reports (Xue et al., 2021; Choe et al., 2020), indicating the potential of crust as a novel food ingredient and therefore recouping the loss from the dry-aging process.

4.5.2 pH, shear force, proximate composition and WHC

Among all the treatments, DWA samples were observed to have the lowest pH (P<0.05). It is possible that the moisture-permeable bag in DWA treatment provided a flourishing environment that could allow microbial growth, as indicated with the high microbial count in DWA loins in the

current study. It has been suggested that lactic acid bacteria have a symbiotic relationship with aerobic bacteria and yeast to promote fermentation (Edeghor et al., 2016; Horiuchi and Sasaki, 2012) and thus potentially decrease the product pH due to acid production.

In regards to the WBSF value, similar results were also reported previously where similar shear force values were observed regardless of the aging treatments (Berger et al., 2018; Kim et al., 2016; Dikeman et al., 2013). This observation indicated that the different aging processes within a given extended aging period might not alter the extent of proteolysis, and thus the instrumental tenderness improvement would be more affected by the total duration period of aging.

Similar proximate composition results were previously observed by Berger et al. (2018), suggesting that the dry-aging bag could provide additional protection to limit moisture loss during dry-aging, which is in line with the previous observation in aging/shrink loss. It is of interest to note that greater concentration of protein, fat and ash, along with lower moisture contents, were observed in UDA samples compared to the other treatments. While very little information is available regarding UV application and its subsequent impacts on fresh beef quality, a previous study on salmon fillets showed that following UV treatments, salmon fillets had a significant temperature increase, likely due to heat introduced by UV light (Ozer and Demirci, 2006). Therefore, it may be possible that heat generated from UV-light would induce further moisture loss during the aging.

In terms of WHC, similar results were observed and reported in previous studies, where no significant differences were observed in water-holding capacity (including cook loss) of meat aged with different aging methods (Berger et al., 2018; Kim et al., 2016; Dikeman et al., 2013; Laster et al., 2008). For the drip loss, while significant changes was observed among the aging treatments, the magnitude of the differences was very small (<1%) and thus would be practically less meaningful.

4.5.3 Display color stability

The results of the simulated color display in the current study provide an additional insight into the color stability of dry-aged products, where dry-aged beef products can be displayed in the retail condition without any noticeable discoloration up to 4 days. Among the dry-aged samples, DWA exhibited lower color stability, determined as the most discolored at the end of the display by both trained panel and instrumental measurement. It could be speculated that the additional

protection from the dry-aging bag interfered with the drying of the meat surface, slowing the generation of the dehydrated crust on the surface as a physical barrier. This would then allow oxidation to occur through the aging process and thus reduce the color stability. Interestingly, while UV light has been suggested to induce oxidation through photo-oxidation (Jongberg et al., 2017), UDA samples were observed to have color comparable to WA samples. The UDA samples also showed greater color traits among all dry-aged treatments, suggesting that microbial presence could contribute more to the reduction in color stability of dry-aged beef loins.

4.5.4 Oxidative stability

The result of both lipid and protein oxidation exhibited that dry-aged beef potentially would have a comparable oxidative stability to wet-aged meat product. This could be the result of the dehydrated surface crust that formed on dry-aged beef loins (Berger et al., 2018), which provide some level of protection against the oxidative environment, limiting oxygen transfer and light penetration to the product. However, it is of our interest to note that there was a trend ($P=0.068$) of an increase in carbonyl content in the UDA samples following the display, compared to other treatments. This could indicate that although the surface crust might work as a protective layer, a continuous exposure to UV light may still lead to adverse impacts on products and thus, higher dose levels of UV light application could be undesirable for oxidative stability and subsequent off-flavor development.

4.5.5 Microbial Analysis

For the microbial concentration, the LAB concentrations was greater in WA samples for both the interior and surface portion, which would be likely due to an anaerobic environment preferred by the lactic acid bacteria (Ahnström et al., 2006). Among the dry-aging treatments, DWA was identified to possess the highest microbial load in the crust and more APC and mold concentration within the lean portion. A similar result was also observed for beef samples packaged in dry-aging bags, where greater concentrations of microbial were found in both the lean (Berger et al., 2018) and the crust portion (DeGeer et al., 2009). It was surmised that the utilization of dry-aging bag hindered the crust formation, generating an environment suited for more microbial growth (Berger et al., 2018; DeGeer et al., 2009). The excessive microbial presence and growth in meat could

potentially lead to a shorter shelf-life of the product. Previous studies showed that higher microbial concentrations induce more discoloration in meat during display (Li et al., 2013), which could be possibly related to the extent of discoloration and color stability observed in the DWA samples in the current study.

In general, greater microbial concentrations were detected on the crusts of all samples compared to the inner portion, with the exception of UDA samples. This result was expected as microbes are commonly found on the surfaces of the dry-aged meat. In the UDA treatment, however, the UV light application clearly suppressed the growth of microbes. This observation suggests that the UV light can be applied as an affordable and practical intervention method to minimize microbial presence in dry-aged meat. However, while UV light application could be beneficial for food, sterilization of the exposed surface of meat may affect meat palatability as the presence of some bacteria and mold that may be associated with the development of dry-aged beef palatability. Previous reports showed that the presence of mold during aging liberated more free amino acid in the dry-aged product, affecting the flavor potential of the product (Lee et al., 2019; Hanagasaki and Asato, 2018). Both bacteria and mold are well known to have proteolytic and lipolytic activity, which could then enhance the production of flavor precursors through protein and lipid degradation, contributing to the development of unique dry-aged flavor. Further research, therefore, is still needed to fully understand the function and the effects of different microbial groups and species on dry-aged flavor development.

4.5.6 Demographic and survey data

The current consumer survey indicated that while tenderness is an important factor for beef palatability, consumers are now also starting to consider the flavor aspects of beef products at almost an equivalent level. It was previously reported that when tenderness was held constant, flavor became the second most important factor influencing beef satisfaction (Killinger et al., 2004). As most fresh beef currently have acceptable tenderness due to branding requirements and longer postmortem aging application (Martinez et al., 2017), it is possible that consumers are now shifting their focus to flavor, thus explaining the increasing interest in dry-aging process. Compared to a previous study by Berger at al. (2018), a greater percentage of participants reported familiarity with dry-aging process and had consumed dry-aged beef (25.8% compared to 44.6% in the current study). While it could potentially be due to survey location differences, the growing

interest in dry-aged beef could potentially reflect the survey results, as more consumers seek for flavor enhanced fresh beef products (O'Quinn et al., 2016; Kim et al., 2016; Campbell et al., 2001).

4.5.7 Sensory panel evaluation

Generally, beef originated from older animals tends to have more intense flavor (Stelzleni et al., 2007). In the current study, a greater percentage of WA and DWA samples were rated beefy by consumers when compared to the DA and UDA samples. The trained panelists, however, did not find any discernable differences in beefy flavor among treatments. This could potentially indicate that the consumer description of beefy flavor might be different from the beefy flavor described by the trained panelist. It is possible that the beefy flavor the consumer perceived is more related to the wet-aging process since they are more accustomed to the product, which could then lead to higher beefy ratings for WA and DWA loins. Moreover, it is important to note that DA samples resulted in a decrease in sour flavor compared to WA and DWA samples, indicating conventional dry-aging effectively diminished one of major off-flavor attributes of mature cull-cow beef.

The increase in juiciness could potentially be attributed to greater moisture retention in WA following the aging process. However, it is not clear why lower fat-like flavor was observed in all dry-aged treatments since greater fat content was observed in actual dry-aged samples. The fat flavor is often associated with the species-specific flavor that identifies meat as beef and is related to the cooked beef fat aromatic (Boylston et al., 2012). While it might be desirable, the increase of this flavor might not be beneficial in mature cow products. As previous reports indicated, mature cow beef often has an intense flavor resulting from increased physiological age and the extended pasture diet (Corbin et al., 2015; Woerner, 2010). It is possible that the intense flavor from both the meat and fat portion generated an overwhelming flavor, making the flavor undesirable for the consumer.

4.6 Conclusions

The results of the current study indicated that dry-aging could be utilized as a natural value-adding process for mature cow beef by improving the eating quality with minimal negative impact. The application of DA could potentially increase the product quality by minimizing the prevalence

of negative flavors such as sour, oxidized and intense animal flavor without any detrimental impact on meat quality. Likewise, the application of UV light limited microbial growth during the aging process with no immediate detrimental impact on meat quality, while there were some trends of increases in oxidized flavor and protein oxidation, as well as sour flavor in the product. The usage of dry-aging bag, while limiting the processing loss, significantly increased the microbial concentration as well as reduced color stability. Additionally, the consumer survey results confirmed an increase in consumer interest in dry-aging, however, consumers might not be familiar with the actual dry-aged beef flavor. Further research to identify the alteration of chemical compounds following dry-aging and the underlying mechanism of flavor development from the different aging methods would be warranted.

4.7 Tables and Figures

Table 4.1. Effect of different aging treatments on shrink/purge loss, trim loss, total loss and total saleable yield of cull cow beef M. *longissimus lumborum* after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

Treatments	**Shrink/Purge loss (%)**	**Trim Loss (%)**	**Total Loss (%)**	**Total Loss w/o trim (%)**	**Total Yield (%)**
WA	1.17[c]	0.00[c]	47.78[c]	47.78[b]	52.22[a]
DA	12.09[a]	5.58[a]	56.05[a]	50.47[a]	43.95[c]
DWA	7.59[b]	3.99[b]	50.68[b]	46.69[b]	49.32[b]
UDA	12.44[a]	6.31[a]	54.37[a]	46.23[b]	45.63[c]
Standard Error of Means	0.682	0.318	1.494	1.562	1.494
P-value	<0.0001	<0.0001	<0.0001	0.0227	<0.0001

[a-c] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)

Table 4.2. Effect of different aging treatments on pH value, Shear force, Proximate content and Water holding capacity (WHC) of cull cow beef M. *longissimus lumborum* after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

Treatments	**pH**	**Shear Force (N)**	**Moisture content (%)**	**Protein content (%)**	**Fat content (%)**	**Ash content (%)**	**Cook Loss (%)**	**Drip Loss (%)**
WA	5.75^{a}	26.85	67.75^{a}	24.11^{c}	7.24^{b}	0.90^{d}	13.42	1.08^{b}
DA	5.74^{a}	27.99	60.12^{b}	29.48^{b}	9.02^{a}	1.38^{b}	13.83	0.90^{b}
DWA	5.64^{b}	26.74	66.33^{a}	25.00^{c}	7.51^{b}	1.16^{c}	13.73	0.74^{b}
UDA	5.74^{a}	29.70	52.89^{c}	35.24^{a}	10.28^{a}	1.59^{a}	12.88	1.45^{a}
Standard Error of Means	0.016	1.178	1.340	0.820	1.179	0.043	0.592	0.116
P-value	<0.0001	0.0760	<0.0001	<0.0001	<0.0001	<0.0001	0.5750	<0.0001

$^{a-c}$ Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)

Table 4.3. Effect of different aging treatments on Thiobarbituric acid reactive substance (TBARS) and Carbonyl content before and after 7 days of color display on cull cow beef M. *longissimus lumborum* aged for 28 days. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

Treatments	**TBARS (mg MDA/Kg of meat)**		**Carbonyl Content (nmol/mg protein)**	
	D1	**D7**	**D1**	**D7**
WA	0.944	1.373	9.344	15.690
DA	1.007	1.391	9.672	14.471
DWA	1.039	1.375	9.042	14.557
UDA	0.979	1.421	9.063	17.254
Standard Error of Means	0.059	0.039	0.997	1.421
P-value	0.4565	0.2500	0.7270	0.0684

Table 4.4. Effect of different aging treatments on Total aerobic bacteria (APC), Lactic acid bacteria (LAB), Mold and Yeast concentration on crust (surface) and lean portion of cull cow beef M. *longissimus lumborum* after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

Location	**Treatment**	**APC (log10 CFU/mL of rinsate)**	**LAB (log10 CFU/mL of rinsate)**	**Mold (log10 CFU/mL of rinsate)**	**Yeast (log10 CFU/mL of rinsate)**
Lean	WA	3.90[c]	3.79[c]	2.23[e]	1.81[c]
	DA	3.79[c]	3.15[d]	3.34[c]	3.95[ab]
	DWA	4.80[b]	3.14[d]	4.65[b]	BDL[1]
	UDA	3.84[c]	2.78[d]	2.81[d]	BDL
Crust	WA	5.96[a]	5.90[a]	3.75[c]	3.29[b]
	DA	3.95[c]	1.88[e]	3.33[c]	BDL
	DWA	6.40[a]	5.23[b]	5.99[a]	4.43[a]
	UDA	2.24[d]	1.84[e]	1.82[e]	BDL
Standard Error of Means		0.27	0.28	0.19	0.49
P-value	Treatment	<0.0001	<0.0001	<0.0001	0.0032
	Location	0.0066	0.0027	0.0023	0.0016
	Interaction	<0.0001	<0.0001	<0.0001	<0.0001

[a-e] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)

[1]Below Detection Limit

Table 4.5. Demographic characteristics of consumers (n = 130) participated in the consumer sensory panels.

Demographic Questions	Response options	Frequency (%)
Gender	Male	50.0
	Female	50.0
Household Size	1	11.5
	2	37.7
	3	12.3
	4	19.2
	5	13.9
	6	5.4
Marital Status	Single	35.4
	Married	64.6
Age	<20 years old	4.6
	20-29 years old	14.6
	20-39 years old	23.9
	40-49 years old	17.7
	50-59 years old	20.0
	>60 years old	19.2
Ethnic Origin	African-American	10.0
	Asian	0.0
	Caucasian	56.2
	Hispanic	29.2
	Native American	0.0
	Mixed Race	1.5
	Other	3.1

Table 4.5. Continued

Annual Household Income	< $25,000	7.7
	$25,000-$34,000	14.6
	$35,000-$49.999	21.5
	$50,000-$74,000	20.0
	$75,000-$99,000	12.3
	$100,000-$149,000	18.5
	$150,000-$199,999	3.1
	>$199,999	2.3
Highest Level of Education Completed	Non-High School Graduate	0.0
	High School Graduate	22.3
	Some College / Technical School	39.2
	College Graduate	26.9
	Post-College Graduate	11.5
When eating beef, what palatability trait is the most important to you?	Flavor	42.3
	Juiciness	11.5
	Tenderness	46.2
When eating beef steaks, what degree of doneness do you prefer?	Very Rare	2.3
	Rare	4.6
	Medium-Rare	35.4
	Medium	22.3
	Medium-Well	26.2
	Well-Done	7.7
	Very Well-Done	1.5

Table 4.5. Continued

What is your preferred meat product for flavor?	Beef	76.2
	Chicken	13.1
	Fish	2.3
	Lamb	0.8
	Mutton	0.0
	Pork	2.3
	Shellfish	2.3
	Turkey	0.8
	Veal	0.0
	Venison	2.3
How many times per week do you consume beef?	1-5 Times/week	76.9
	6-10 Times/Week	13.9
	11-15 Times/Week	7.7
	>16 Times /Week	1.5

Table 4.6. Consumer panelist perceptions on dairy cow, dry-aging and willingness to pay (n = 130).

End Survey Questions	Response options	Frequency (%)
Are you familiar with beef from dairy cattle?	Yes	39.2
	No	60.8
Do you perceive beef from dairy cattle as positive or negative?	Positive	62.8
	Negative	2.0
	Not Sure	35.3
Have you ever eaten dry-aged beef?	Yes	44.6
	No	24.6
	Not Sure	30.8
If you have eaten dry-aged beef, where did you purchase the product from?	Restaurant	48.3
	Local butcher store	17.2
	Local retail/super market	25.9
	Others	8.6
Is aging a positive or negative term?	Positive	86.9
	Negative	13.1
Do you think dry-aged beef is safe?	Safer	16.2
	Less safe	6.9
	Same as other beef	37.7
	Not Sure	39.2
Would you be willing to pay $1.00 more per 1 lb for dry-aged beef?	Yes	59.2
	No	40.8
Would you be willing to pay $1.00 more per 1 lb for dry-aged beef from dairy cattle?	Yes	50.0
	No	50.0

Table 4.7. Effect of different aging treatments on Consumer sensory panel (n=130) likeness, acceptability, perceived quality, and perceived flavor of cull cow beef M. *longissimus lumborum* after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

Traits	WA	DA	DWA	UDA	SEM	P-value
Likeness						
Flavor	60.24	61.50	62.78	61.39	4.28	0.9371
Tenderness	61.98	64.45	66.79	65.31	3.56	0.5948
Juiciness	65.38	64.89	68.42	68.01	3.17	0.6575
Overall	60.53	60.84	62.72	60.47	3.84	0.9311
Acceptability (%)						
Tenderness Acceptability	83.92	85.73	85.98	84.57	4.16	0.9620
Juiciness Acceptability	89.81	89.83	91.95	85.47	4.56	0.3720
Flavor Acceptability	82.62	84.71	98.50	81.05	4.66	0.8830
Overall Acceptability	83.80	84.87	83.98	81.25	5.06	0.8752
Perceived Quality (%)						
Unsatisfactory Quality	13.85	14.90	14.51	16.49	4.94	0.9408
Everyday Quality	45.13	39.85	38.11	43.61	4.53	0.6501
Better Than Everyday Quality	29.10	32.52	26.63	24.27	4.51	0.5073
Premium Quality	9.52	10.03	18.05	13.00	3.39	0.1597
Check all that apply flavor (%)						
Beefy	80.47[a]	66.90[b]	78.356[a]	67.95[b]	4.35	0.0282
Brown/Roasted	16.41	15.31	12.52	19.66	4.84	0.4782
Nutty	4.94	6.81	5.29	5.98	2.21	0.9188
Buttery	10.35	13.23	16.17	15.43	3.25	0.5290
Sour	3.63	3.09	3.85	3.02	1.55	0.9723
Metal	6.65	5.47	6.44	3.58	2.37	0.6977
Rancid	1.24	0.64	2.00	4.50	1.20	0.1627
Other	3.76	7.19	4.73	9.90	3.62	0.1538

[a-b] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)

[1]Standard Error of Means

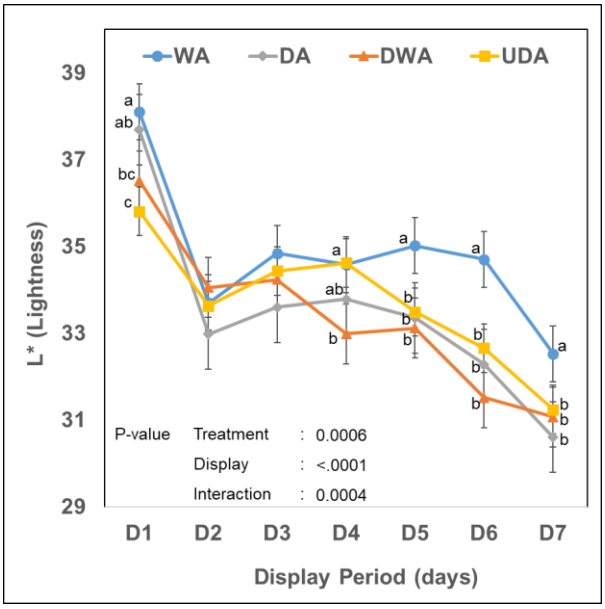
Table 4.8. Effect of different aging treatments on Trained panel rating (n=11) of cull cow beef M. *longissimus lumborum* after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

Traits	WA	DA	DWA	UDA	SEM[1]	P-value
Beefy	46.00	47.97	47.57	48.34	1.70	0.2863
Brown/Roasted	39.20	43.43	41.16	41.61	2.82	0.2583
Bloody	15.36	13.13	16.65	14.65	2.16	0.2743
Fat	17.41[a]	15.08[b]	14.63[b]	15.60[b]	1.17	0.0301
Liver	6.87	5.40	5.71	5.04	0.71	0.2390
Oxidized	4.50	3.72	4.35	5.52	0.57	0.0767
Metallic	8.87	7.60	8.25	8.27	0.60	0.2934
Fishy	6.83	5.77	6.35	5.96	0.75	0.7218
Buttery	11.35	10.07	8.42	8.89	1.14	0.2260
Nutty	8.57	8.32	7.43	8.44	0.98	0.7563
Earthy	14.14	14.50	14.37	14.33	1.19	0.9960
Umami	14.28	14.82	14.23	14.12	0.87	0.9102
Salty	3.45	3.33	4.29	4.15	0.66	0.2057
Bitter	3.15	2.68	2.76	2.83	0.42	0.7395
Sour	6.14[a]	4.43[b]	5.29[ab]	6.15[a]	0.45	0.0113
Overall Juiciness	63.30[a]	57.46[b]	58.56[b]	57.91[b]	1.49	0.0067
Overall Tenderness	60.31	60.76	60.65	57.28	1.81	0.2053

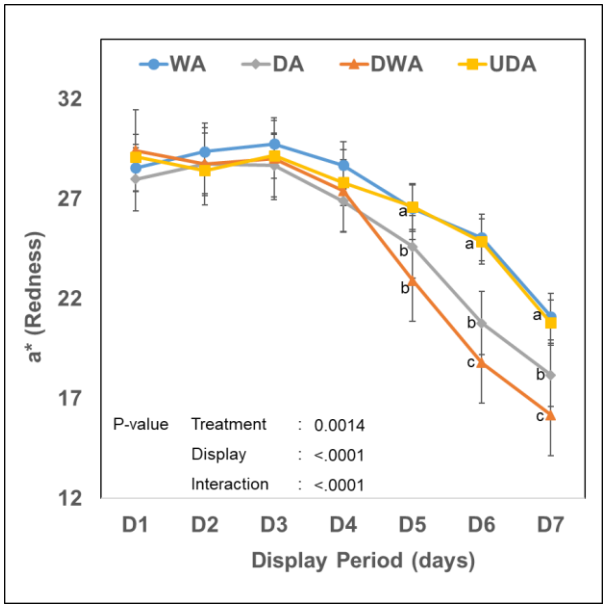
[a-b] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)

[1]Standard Error of Means

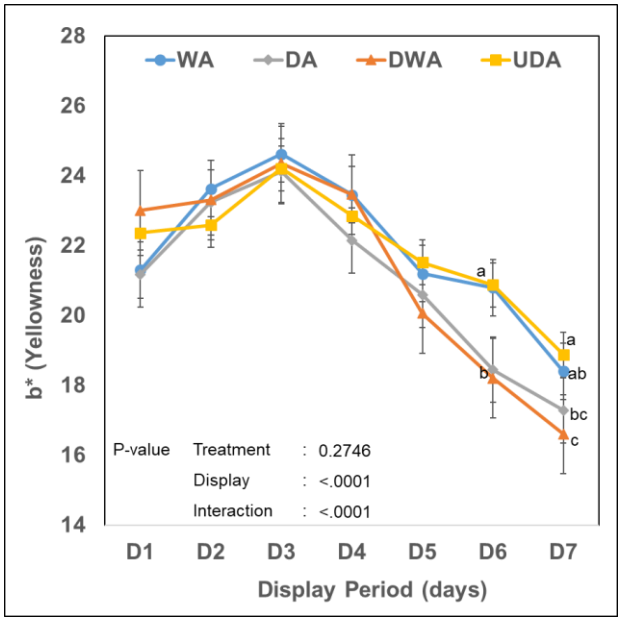
(a) CIE L*



(b)CIE a*



(c) CIE b*



(d) Hue angle

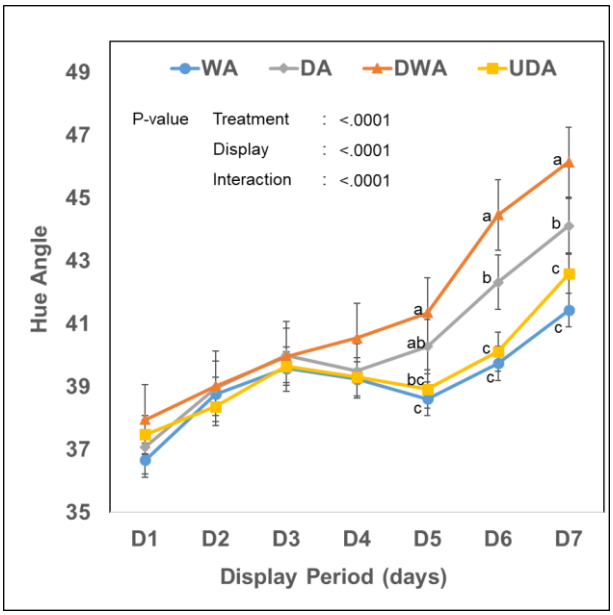
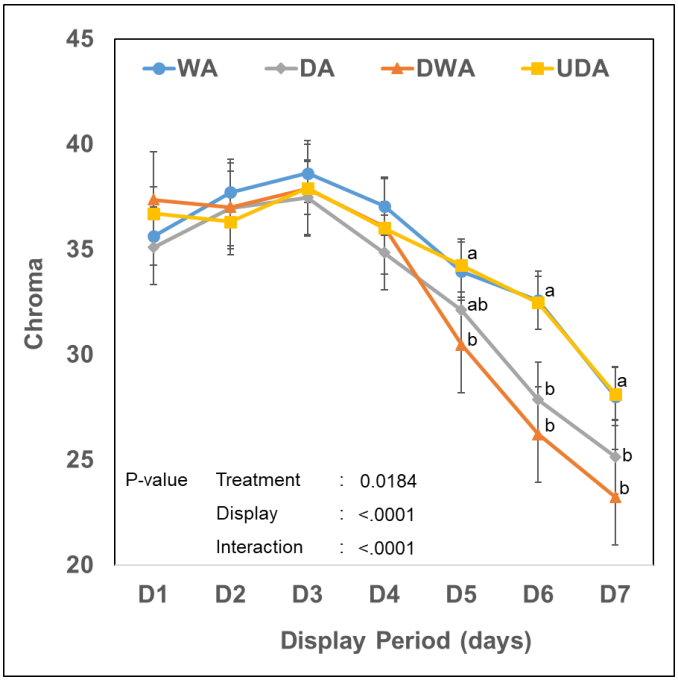


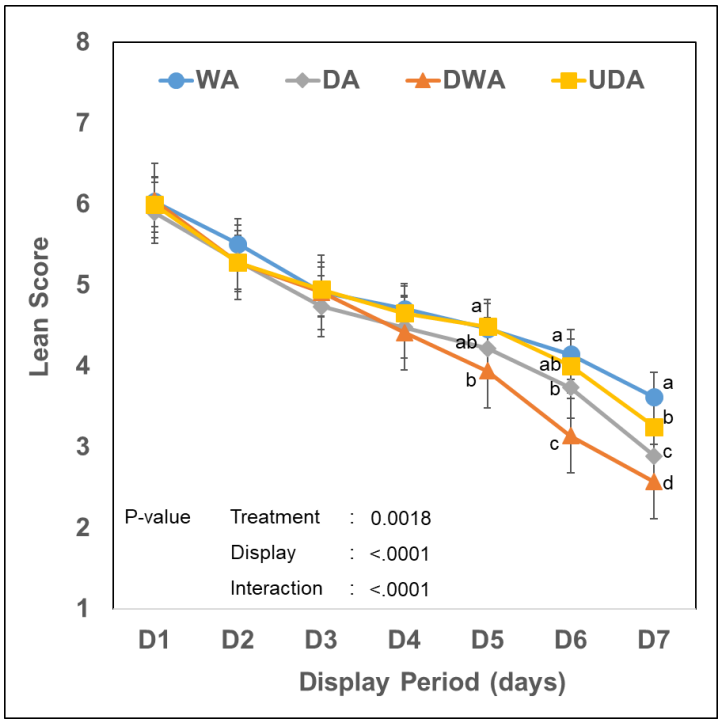
Figure 4.1. Effect of different aging treatments on instrumental color characteristic changes of cull cow beef M. *longissimus lumborum* during 7 days of display period. WA (Wet-aging); DA (Conventional Dry-aging); DWA (Dry-aging in water permeable bag); UDA (UV-light dry-aging). [a-c] Means with different letters indicates significant differences within the same display day (P<0.05)

Figure 4.1. Continued

(e) Chroma



(a) Lean color Score



(b) Discoloration Score

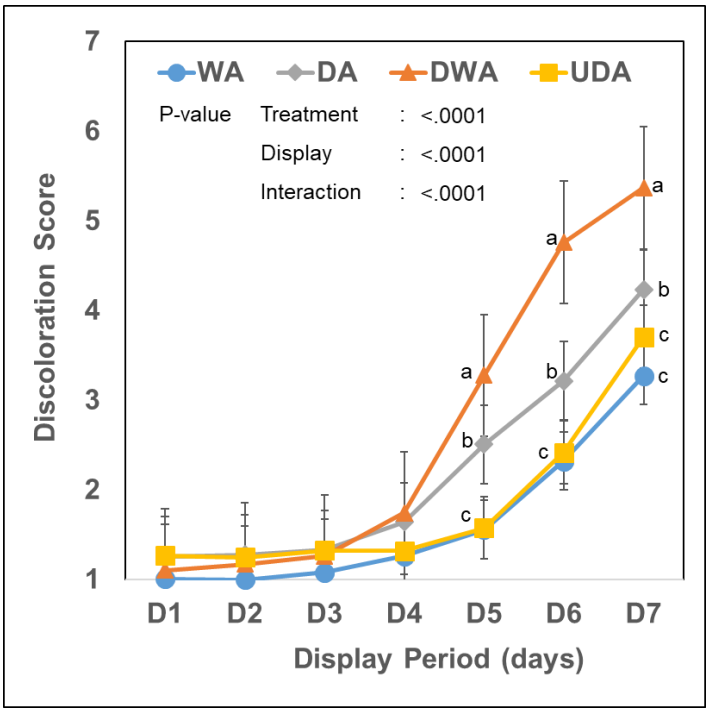


Figure 4.2. Effect of different aging treatments on visual color characteristic changes of cull cow beef M. *longissimus lumborum* during 7 days of display period. WA (Wet-aging); DA (Conventional Dry-aging); DWA (Dry-aging in water permeable bag); UDA (UV-light dry-aging). [a-d] Means with different letters indicates significant differences (P<0.05)

4.8 Literatures Cited

Ahnström, M.L., M. Seyfert, M.C. Hunt, and D.E. Johnson. 2006. Dry aging of beef in a bag highly permeable to water vapour. Meat Sci. 73:674–679. doi:10.1016/j.meatsci.2006.03.006.

AMSA. 2012. Meat Color Measurement Guidelines.

AMSA. 2016. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat.

AOAC. 2007. Official Methods of Analysis of AOAC International (18 Edition, Second revision).

Berger, J., Y.H.B. Kim, J.F. Legako, S. Martini, J. Lee, P. Ebner, and S.M.S. Zuelly. 2018. Dry-aging improves meat quality attributes of grass-fed beef loins. Meat Sci. 145:285–291. doi:10.1016/j.meatsci.2018.07.004.

Boylston, T., F. Chen, P. Coggins, G. Hydlig, and L. McKee. 2012. Handbook of meat, poultry and seafood quality. Wiley-Blackwell, New Jersey.

Campbell, R.E., M.C. Hunt, P. Levis, and E. Chambers IV. 2001. Dry-aging effects on palatability of beef longissimus muscle. J. Food Sci. 66:196–199. doi:10.1111/j.1365-2621.2001.tb11315.x.

Choe, J., B. Park, H.J. Lee, and C. Jo. 2020. Potential Antioxidant and Angiotensin I-converting Enzyme Inhibitory Activity in Crust of Dry-aged Beef. Sci. Rep. 10. doi:10.1038/s41598-020-64861-0.

Chun, H.H., J.Y. Kim, B.D. Lee, D.J. Yu, and K.B. Song. 2010. Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. Food Control. 21:276–280. doi:10.1016/j.foodcont.2009.06.006.

Corbin, C.H., T.G. O'Quinn, A.J. Garmyn, J.F. Legako, M.R. Hunt, T.T.N. Dinh, R.J. Rathmann, J.C. Brooks, and M.F. Miller. 2015. Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. Meat Sci. 100:24–31. doi:10.1016/j.meatsci.2014.09.009.

DeGeer, S.L., M.C. Hunt, C.L. Bratcher, B.A. Crozier-Dodson, D.E. Johnson, and J.F. Stika. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Sci. 83:768–774. doi:10.1016/j.meatsci.2009.08.017.

Dikeman, M.E., E. Obuz, V. Gök, L. Akkaya, and S. Stroda. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef Longissimus lumborum steaks. Meat Sci. 94:228–233. doi:10.1016/j.meatsci.2013.02.002.

Diles, J.J.B., M.F. Miller, and B.L. Owen. 1994. Calcium chloride concentration, injection time, and aging period effects on tenderness, sensory, and retail color attributes of loin steaks from mature cows. J. Anim. Sci. 72:2017–2021. doi:10.2527/1994.7282017x.

Edeghor, U., J.A. Lennox, B.E. Agbo, and D. Aminadokiari. 2016. Bread Fermentation using Synergistic Activity Between Lactic Acid Bacteria (Lactobacillus bulgaricus) and Baker's Yeast (Sacchromyces cerevisae). Pakistan J. Food Sci. 26:2226–5899.

Ganan, M., E. Hierro, X.F. Hospital, E. Barroso, and M. Fernández. 2013. Use of pulsed light to increase the safety of ready-to-eat cured meat products. Food Control. 32:512–517. doi:10.1016/j.foodcont.2013.01.022.

Gredell, D.A., T.G. O'Quinn, J.F. Legako, J.C. Brooks, and M.F. Miller. 2018. Palatability and Biochemical Factors of Beef from Mature Cattle Finished on a Concentrate Diet Prior to Harvest. Meat Muscle Biol. 2:111. doi:10.22175/mmb2017.09.0046.

Hanagasaki, T., and N. Asato. 2018. Changes in free amino acids and hardness in round of Okinawan delivered cow beef during dry- and wet-aging processes. J. Anim. Sci. Technol. 60:1–9. doi:10.1186/s40781-018-0180-x.

Horiuchi, H., and Y. Sasaki. 2012. Short communication: Effect of oxygen on symbiosis between Lactobacillus bulgaricus and Streptococcus thermophilus. J. Dairy Sci. 95:2904–2909. doi:10.3168/jds.2011-5147.

Jongberg, S., M.N. Lund, and L.H. Skibsted. 2017. Protein oxidation in meat and meat products. Challenges for antioxidative protection. *In* Global Food Security and Wellness. Springer New York. 315–337.

Killinger, K.M., C.R. Calkins, W.J. Umberger, D.M. Feuz, and K.M. Eskridge. 2004. Consumer sensory acceptance and value for beef steaks of similar tenderness, but differing in marbling level1. J. Anim. Sci. 82:3294–3301. doi:10.2527/2004.82113294x.

Kim, Y.H.B., R. Kemp, and L.M. Samuelsson. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Sci. 111:168–176. doi:10.1016/j.meatsci.2015.09.008.

Kim, Y.H.B., B. Meyers, H.W. Kim, A.M. Liceaga, and R.P. Lemenager. 2017. Effects of stepwise dry/wet-aging and freezing on meat quality of beef loins. Meat Sci. 123:57–63. doi:10.1016/j.meatsci.2016.09.002.

Kim, Y.H.B.B., D. Ma, D. Setyabrata, M.M. Farouk, S.M. Lonergan, E. Huff-Lonergan, and M.C. Hunt. 2018. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. Meat Sci. 144:74–90. doi:10.1016/J.MEATSCI.2018.04.031.

Laster, M.A., R.D. Smith, K.L. Nicholson, J.D.W. Nicholson, R.K. Miller, D.B. Griffin, K.B. Harris, and J.W. Savell. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer sensory attribute evaluations of steaks from ribeyes, strip loins, and top sirloins from two quality grade groups. Meat Sci. 80:795–804. doi:10.1016/j.meatsci.2008.03.024.

Lee, H., M. Jang, S. Park, J. Jeong, Y.S. Shim, and J.C. Kim. 2019. Determination of indicators for dry aged beef quality. Food Sci. Anim. Resour. 39:934–942. doi:10.5851/kosfa.2019.e83.

Lepper-Blilie, A.N., E.P. Berg, D.S. Buchanan, and P.T. Berg. 2016. Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. Meat Sci. 112:63–68. doi:10.1016/j.meatsci.2015.10.017.

Li, X., J. Babol, A. Wallby, and K. Lundström. 2013. Meat quality, microbiological status and consumer preference of beef gluteus medius aged in a dry ageing bag or vacuum. Meat Sci. 95:229–234. doi:10.1016/j.meatsci.2013.05.009.

Martinez, H.A., A.N. Arnold, J.C. Brooks, C.C. Carr, K.B. Gehring, D.B. Griffin, D.S. Hale, G.G. Mafi, D.D. Johnson, C.L. Lorenzen, R.J. Maddock, R.K. Miller, D.L. VanOverbeke, B.E. Wasser, and J.W. Savell. 2017. National Beef Tenderness Survey–2015: Palatability and Shear Force Assessments of Retail and Foodservice Beef. Meat Muscle Biol. 1:138. doi:10.22175/mmb2017.05.0028.

Moreira, L.C., G.J.M. Rosa, and D.M. Schaefer. 2021. Beef production from cull dairy cows: a review from culling to consumption. J. Anim. Sci. 99:1–18. doi:10.1093/jas/skab192.

Morgan, J.B., R.K. Miller, F.M. Mendez, D.S. Hale, and J.W. Savell. 1991. Using calcium chloride injection to improve tenderness of beef from mature cows1. J. Anim. Sci. 69:4469–4476. doi:10.2527/1991.69114469x.

O'Quinn, T.G., D.R. Woerner, T.E. Engle, P.L. Chapman, J.F. Legako, J.C. Brooks, K.E. Belk, and J.D. Tatum. 2016. Identifying consumer preferences for specific beef flavor characteristics in relation to cattle production and postmortem processing parameters. Meat Sci. 112:90–102. doi:10.1016/j.meatsci.2015.11.001.

Obuz, E., L. Akkaya, V. Gök, and M.E. Dikeman. 2014. Effects of blade tenderization, aging method and aging time on meat quality characteristics of Longissimus lumborum steaks from cull Holstein cows. Meat Sci. 96:1227–1232. doi:10.1016/j.meatsci.2013.11.015.

Ozer, N.P., and A. Demirci. 2006. Inactivation of Escherichia coli O157:H7 and Listeria monocytogenes inoculated on raw salmon fillets by pulsed UV-light treatment. Int. J. Food Sci. Technol. 41:354–360. doi:10.1111/j.1365-2621.2005.01071.x.

Savell, J.W. 2008. Dry-Aging of Beef. Beef Checkoff. 16.

Setyabrata, D., B.R. Cooper, T.J.P. Sobreira, J.F. Legako, S. Martini, and Y.H.B. Kim. 2021. Elucidating mechanisms involved in flavor generation of dry-aged beef loins using metabolomics approach. Food Res. Int. 139:109969. doi:10.1016/j.foodres.2020.109969.

Setyabrata, D., and Y.H.B. Kim. 2019. Impacts of aging/freezing sequence on microstructure, protein degradation and physico-chemical properties of beef muscles. Meat Sci. 151:64–74. doi:10.1016/J.MEATSCI.2019.01.007.

Smith, A.M., K.B. Harris, D.B. Griffin, R.K. Miller, C.R. Kerth, and J.W. Savell. 2014. Retail yields and palatability evaluations of individual muscles from wet-aged and dry-aged beef ribeyes and top sirloin butts that were merchandised innovatively. Meat Sci. 97:21–26. doi:10.1016/j.meatsci.2013.12.013.

Smith, R.D., K.L. Nicholson, J.D.W. Nicholson, K.B. Harris, R.K. Miller, D.B. Griffin, and J.W. Savell. 2008. Dry versus wet aging of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. Meat Sci. 79:631–639. doi:10.1016/j.meatsci.2007.10.028.

Stelzleni, A.M., L.E. Patten, D.D. Johnson, C.R. Calkins, and B.L. Gwartney. 2007. Benchmarking carcass characteristics and muscles from commercially identified beef and dairy cull cow carcasses for Warner-Bratzler shear force and sensory attributes1. J. Anim. Sci. 85:2631–2638. doi:10.2527/jas.2006-794.

USDA. 2019. Livestock Slaughter Record High Red Meat and Pork Production in November.

Verbeke, W., F.J.A. Pérez-Cueto, M.D. d. Barcellos, A. Krystallis, and K.G. Grunert. 2010. European citizen and consumer attitudes and preferences regarding beef and pork. Meat Sci. 84:284–292. doi:10.1016/j.meatsci.2009.05.001.

Vossen, E., and S. De Smet. 2015. Protein Oxidation and Protein Nitration Influenced by Sodium Nitrite in Two Different Meat Model Systems. J. Agric. Food Chem. 63:2550–2556. doi:10.1021/jf505775u.

Warren, K.E., and C.L. Kastner. 1992. A comparison of dry-aged and vacuum-aged beef strip loins. J. Muscle Foods. 3:151–157. doi:10.1111/j.1745-4573.1992.tb00471.x.

Woerner, D.R. 2010. Beef from market cows. White paper product enhancement research. Centennial, CO .

Xiong, Y.L., O.E. Mullins, J.F. Stika, J. Chen, S.P. Blanchard, and W.G. Moody. 2007. Tenderness and oxidative stability of post-mortem muscles from mature cows of various ages. Meat Sci. 77:105–113. doi:10.1016/j.meatsci.2007.04.012.

Xue, S., D. Setyabrata, C.C. Bonham, and Y.H.B. Kim. 2021. Evaluation of functional and chemical properties of crust from dry-aged beef loins as a novel food ingredient. Meat Sci. 173:108403. doi:10.1016/j.meatsci.2020.108403.

Yeh, Y., F.H. de Moura, K. Van Den Broek, and A.S. de Mello. 2018. Effect of ultraviolet light, organic acids, and bacteriophage on Salmonella populations in ground beef. Meat Sci. 139:44–48. doi:10.1016/j.meatsci.2018.01.007.

CHAPTER 5. ELUCIDATION OF FLAVOR PRECURSOR AND LIBERATION MECHANISMS RELATED TO DRY-AGING PROCESS THROUGH METABOLOMICS AND MICROBIOME APPROACHES

5.1 Abstract

The objective of this study was to characterize the dry-aging flavor precursors and their liberation mechanisms. Thirteen paired loins were collected at 5d postmortem, divided into 4 sections and randomly assigned into 4 aging methods (wet-aging [WA], conventional dry-aging [DA], dry-aging in water-permeable bag [DWA], and UV-light dry-aging [UDA]). All sections were aged for 28d at 2 °C, 65% RH and 0.8 m/s airflow, before trimming and sample collection for chemical, metabolomics, and microbiome analyses. Higher concentrations of free amino acids and reducing sugars were observed in all dry-aging samples ($P<0.05$). Similarly, metabolomics revealed greater short-chain peptides in the dry-aged beef ($P<0.05$). Microbiome profiling identified that *Pseudomonas* spp. are the most prominent bacterial species in dry-aged meat, potentially contributing to the greater accumulation of flavor precursors concentration in addition to the dehydration process during the dry-aging. Minor bacterial species might also contribute to releasing precursors related to unique dry-aged flavor.

Keywords Dry-aging, Cull Cow, Metabolomics, Microbiome, Amino acids, Reducing sugars

5.2 Introduction

Dry-aging of beef is a traditional aging method that has recently seen renewed interest from both consumers and producers (Dashdorj et al., 2016). Unlike wet-aging (where meat is aged by storing in vacuum packaging), the dry-aging process exposes the meat to a highly controlled environment without any protective packaging materials. These particular aging conditions have been associated with the development of unique flavors such as "beefy", "buttery", "nutty", and "brown-roasted", making the final product highly desirable (Kim et al., 2016). However, while those flavors have often been associated with the dry-aged meat, there is inconsistency in the current literature with respect to the impact of dry-aging on flavor development, where some

studies have reported improvement (Berger et al., 2018; Kim et al., 2016) and some studies have not (Dikeman et al., 2013; DeGeer et al., 2009). It is well-established that the presence of flavor precursors (e.g., free amino acids, fatty acids, sugars, nucleotides) plays a crucial role in determining the final meat flavor perceived by consumers (Mottram, 1998). Therefore, the identification of the flavor precursor composition, which is integral to the dry-aging process, could provide beneficial information and practical insights to produce dry-aged beef products with consistent eating quality attributes.

In recent years, the advancement in high-throughput analyses, such as metabolomics, have been adopted to determine the multiple biological systems, including the biochemical changes in meat products. The metabolomics analysis allowed the profiling of small compounds (metabolites), elucidating the molecular changes responsible for meat quality development (Ma et al., 2017; Abraham et al., 2017). While limited, the utilization of metabolomics analysis has also been employed to identify compounds related to the meat flavor changes, showing increased concentration in small molecular flavor precursors (e.g., amino acids and nucleotides) in dry-aged products (Setyabrata et al., 2021a; Kim et al., 2016). A recent study by Setyabrata et al. (2021a) using mass spectrometry-based metabolomics also reported a decrease in off-flavor related metabolites in dry-aged beef, such as terpenoids, when using mass spectrometry-based metabolomics, demonstrating the capability of metabolomics to identify diverse flavor precursor species. These studies exhibited the potential of metabolomics analysis for use in profiling the flavor precursor composition of dry-aged meat products and identifying other flavor precursors such as vitamins, acids and minerals.

Although a greater abundance of flavor precursors are often reported for dry-aged meat compared to their wet-aged counterparts (Setyabrata et al., 2021a; Mungure et al., 2020; Kim et al., 2016), the mechanism responsible for the release and accumulation of these precursors is still unclear. Previous research by Setyabrata et al. (2021a) speculated that elevated glutathione metabolism might be responsible for increased flavor precursors during dry-aging treatment based on the metabolites profile. Additionally, microbial activity during the dry-aging process has been suggested to contribute to the flavor precursor liberation. For example, a previous study by Lee et al. (2019b) revealed that the greater presence of *Pilaira anomala* and *Debaryomyces hansenii* during dry-aging led to the greater abundance of free amino acids in the products. Those authors suggested that the microorganisms potentially released exogenous proteolytic and lipolytic

enzymes, accelerating muscle breakdown during aging. However, the increased microbial activity found during dry-aging potentially affect the flavor precursor development during the aging process. Therefore, characterization of the microbial community may be crucial to understanding the role of microbial presence and growth in the release of flavor precursors.

Thus, the main objective of this study was to characterize flavor precursors and the liberation mechanisms in beef aged under different aging conditions utilizing metabolomics and microbiome analyses coupled with multiple chemical analyses, such as free amino acids, fatty acid, sugars and volatile compounds content. This study is a further elaboration of our previously published study, where significant changes in sensory palatability attributes were reported following the application of dry-aging in cull cow beef loins (Setyabrata et al., 2021b).

5.3 Materials and methods

5.3.1 Sample collection, preparation and processing

The sample collection process was described in our parallel study (Setyabrata et al., 2021b). In brief, paired bone-in beef loins were collected from 13 carcasses (42+ months old, C maturity, Holstein, NAMP:175, M. *longissimus lumborum*). Prior to any processing, initial (INI) samples were individually excised from the loin eye area of one side of the loins for microbiome profiling. The loins were then split into 4 equal sections and randomly assigned into 4 different aging treatments: wet-aging (WA; Clarity Vacuum Pouches, Bunzl Processor Division, Riverside, MO, USA.), conventional dry-aging (DA), dry-aging in water-permeable bag (DWA; UMAi Dry® Short Loin (Large), UMAI Dry, Minneapolis, MN, USA) and UV-light dry-aging (UDA). All samples were aged for 28 days at 2°C and 65% relative humidity with 0.8 m/s airflow. The UDA samples were treated with UV-light twice per day (Phillip TUV T8 UVC light, Eindhoven, Netherlands). The UV lights were mounted 30 cm above the samples and turned on for a total of 5 minutes per treatment (totaling a dose of 5 J/m^2 for each UV treatment). At the end of aging, samples were trimmed of the dehydrated surfaces (crust) and deboned. Following the trimming process, the crust/surface samples were collected and lean portion samples were excised from the section for microbiome profiling. The sections were then cut into steaks (2.4 cm thick) and collected for further biochemical analysis. All samples were individually vacuum packaged and stored in a -80°C freezer until further analyses.

5.3.2 Free amino acid analysis

The free amino acid analysis was performed using the method described by Vierck et al. (2020). The samples were first prepared and extracted following the technique used by Koutsidis et al. (2008). In brief, 3 g of homogenized sample was added to conical tube containing 10 mL of cold water (deionized and autoclaved) and 300 µL of rhamnose (2 mg/ml) and was shaken for 10 minutes. The sample was then centrifuged, and the supernatant was collected. The pellet was then resuspended in 5 mL of cold water and recentrifuged following the same procedure. The supernatant from both extractions was combined and filtered through a 0.2 µm disc filter to remove any fat and/or tissue particles. The filtered sample was then derivatized using the EZ-Faast amino acids kit (Phenomenex, Torrance, CA, USA), following the manufacturer's guidelines.

The free amino acid content was measured using a GC-MS (Agilent Technologies, Palo Alto, CA, USA) with electron impact mode with a 3:1 split ratio. The derivatized sample was separated using Zebron EZ-AAA Amino Acid GC Column (10 m × 0.25 mm × 0.15 mm; Phenomenex, Torrance, CA, USA) with helium as the carrier gas. Both internal standards and authentic standards for each amino acid were utilized to identify and quantify the free amino acids from the samples. Concentrations were then reported as millimoles per kilogram of initial wet sample.

5.3.3 Sugar content analysis

Prior to the sugar analysis, meat samples were extracted following the method by Koutsidis et al. (2008) as described in the previous section. After the extraction process, the liquid extract was freeze-dried, and the final dried product was added to a solution containing dimethyl sulfoxide, hexamethyldisilazane, trimethylchlorosilane and cyclohexane. The sample was sonicated and incubated at room temperature for 24 hours. Following incubation, the organic layer was separated and injected to GC-MS (Agilent Technologies, Palo Alto, CA, USA) in electron impact mode. The gas chromatograph was set to splitless mode, and the injector temperature was set to 250°C. The oven temperature was initially set at 60°C for 1 minute, increased to 130°C for 2 minutes, followed by 2°C/minute increase until 170°C and finally adjusted to 300°C by gradually increasing the temperature 4°C/minute. The separation was performed using the DB-17ms capillary column (30 m × 0.25 mm; 0.25-µm film thickness) coupled with 1.5 mL of deactivated methylsilicone fused silica capillary retention gap. Helium was utilized as the carrier gas. Authentic standards (Sigma-

Aldrich, Bellefonte, PA) were used to identify peaks. The sugar concentration was reported in millimoles per kilogram of initial wet sample.

5.3.4 Fatty acid analysis

The fatty acid content was analyzed using the protocol described by Chail et al. (2016). The fatty acids methyl esters (FAME) were prepared by incubating 1 g of homogenized sample at 55°C with internal samples (tridecanoic acid; 0.5 mg/mL in methanol) following the method described by O'Fallon et al. (2007). Hexane was then added to the vial and the sample was centrifuged to extract the FAME.

The extract (1 µL) was injected into GC equipment for the analysis. The inlet was maintained at 250 °C with a 50:1 split ratio. Separation was performed using an HP-88 capillary column (100 m × 250 µm × 0.2 µm), and helium was used as a carrier gas with a flow rate set to 2.5 mL per minute. All fatty acids were identified by comparing their retention time to GC reference standards ((Nu-Chek Prep, Inc, Elysian, MN, USA.). The fatty acid concentration was presented as the percent of total fatty acids.

5.3.5 Volatile compound analysis

The volatile compounds were profiled using the method outlined by Gardner and Legako, (2018). Briefly, samples were cooked until the internal temperature reached 63°C, and six cores (1.27 cm in diameter) perpendicular to the muscle fibers were collected. The cores were minced using a coffee grinder (Mr. Coffee, Sunbeam Corporation, Boca Raton, FL, USA), and 5 grams of the minced sample were transferred into vials. An internal standard (1,2-dichlorobenzene) was added to each vial and incubated for 5 minutes at 65°C in gerstel automatic sampler (Gerstel Inc., Linthicum Heights, MD, USA) followed by 20 minutes of extraction via headspace solid-phase microextraction. The volatile compounds extracted from the headspace were injected into a VF-5 MS capillary column (30 m × 0.25 mm × 1.0 µm; Agilent Technologies, Inc., Santa Clara, CA, USA) for separation and identification. Identified volatiles were compared to authentic standards (Sigma-Aldrich, St. Louis, MO, USA) for validation. The volatile compounds concentrations were reported in nanogram per gram of initial wet sample.

5.3.6 Metabolomics profiling

Sample extraction

A total of 6 samples were randomly selected from each of the aging treatments for the metabolomics analysis. The samples were homogenized by submerging the samples into liquid nitrogen and powdered using a blender (Waring Products, CT, USA). The metabolites were then extracted using the method described by Setyabrata et al. (2021a). In brief, 100 mg of each sample was extracted using an equal amount of chloroform (300 µL) and methanol (300 µL) in a Precellys 24 tissue homogenizer (Bertin Instruments, Bretonneux, France). The homogenizer extraction was conducted in 3 cycles of 30 seconds at 6500 rpm with 30 seconds rest. After the homogenization process, water was added, and the mixture was centrifuged at 16,000 x *g* for 8 minutes. The upper layer was collected and dried for chromatographic separation.

Ultra performance liquid chromatography – Mass spectrometer analysis

The chromatographic separation was conducted according to the procedure described by Setyabrata et al. (2021a). The dried samples were first reconstituted into an aqueous solution containing 95% water, 5% acetonitrile and 0.1% formic acid. The reconstituted samples were then assayed using an Agilent 1290 Infinity II UPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Waters Acquity HSS T3 (2.1 × 100 mm × 1.8 µm) separation column (Waters, Milford, MA, USA) for separation. The column was maintained at 40 °C with the binary mobile phase flow set at 0.45 mL/minute. The binary mobile phase consisted of solvent A (0.1% formic acid (v/v) in ddH2O) and solvent B (0.1% formic acid (v/v) in acetonitrile). Initial conditions of 100:0 A:B were held for 1 minute, followed by a linear gradient to 70:30 over 15 minutes, changed to a linear gradient of 5:95 over 5 minutes, and 5:95 hold for 1.5 minutes.

Following the separation, sample was identified using Agilent 6545 quadrupole time-of-flight (Q-TOF) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with positive electrospray ionization (ESI) mode for data collection. HPLC-MS scans were collected over a range of 70 – 1000 m/z. HPLC-MS-MS data was collected to aid in compound identification. The collected data were analyzed using Agilent MassHunter B.06 software (Agilent Technologies, Santa Clara, CA, USA), and the mass accuracy was improved by infusing Agilent Reference Mass Correction Solution (G1969-85001; Agilent Technologies, Santa Clara, CA, USA). The peak

deconvolution was conducted using Agilent ProFinder (Agilent Technologies, Santa Clara, CA, USA) and annotated using the HMDB (www.hmdb.ca) metabolite database.

5.3.7 Microbiome analysis

Sample preparation and DNA extraction

Meat samples (5 g) were aseptically collected, in a stomacher bag (WhirlPak, Madison, WI, USA) containing 50 mL of sterile 0.1% peptone water and stomached by hand for 1 minute. The rinsate was then collected and centrifuged at 3200 x *g* for 40 minutes. After the centrifugation, the supernatant was removed, and the pellet was resuspended in 1.5 mL of sterile 0.1% peptone water before centrifuging the samples at 21000 x *g* for 10 minutes. Following the second centrifugation, the supernatant was removed and the pellet was stored at -80°C until further processing. Total DNA extraction was conducted using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Germantown, MD, USA), following the manufacturer's guidelines.

16S library preparation and sequencing

The library was constructed using by PCR using barcode indexed amplification product from the V4 region of the 16S rRNA using AccuPrime™ Pfx SuperMix (Thermo Scientific, Waltham, MA, USA) as described by Kozich et al. (2013). The PCR amplicon quality was then checked via gel electrophoresis. The amplified DNA was then normalized using the SequalPrep™ Normalization Plate Kit (Thermo Scientific, Waltham, MA, USA) following the manufacturer's guidelines. Finally, samples were pooled by collecting 5 μL of the amplified DNA from each sample for amplicon sequencing via Illumina MiSeq sequencing platform (2x250 paired-end; Illumina Inc., San Diego, CA, USA).

Bioinformatics analysis

The raw sequences obtained were analyzed using Quantitative Insight Into Microbial Ecology (QIIME2) v.2020.2. The samples were denoised using DADA2 step (Callahan et al., 2016) with both the forward and reverse sequences trimmed at position 0 and truncated at position 245 to obtain sequences with quality of > Q30. All the sequence reads were clustered into Amplicon Sequence Variants (ASVs) with 100% similarity to identify unique microbiome variants. The sequences were then rarefied with a sampling depth of 2391 for both alpha and beta diversity

calculation. Both alpha and beta diversity metrics were estimated using the QIIME2 pipeline. The taxonomy was assigned by matching to the SILVA 13_8, 515F/806 region database.

5.3.8 Statistical analysis

This study utilized a randomized complete block design with the different aging treatments as the fixed effect and animals as the random effect. Source location (crust/surface and lean) was added as an additional fixed effect for the microbiome analysis to consider potential location effect. The fatty acid, free amino acid, reducing sugar and volatile compounds concentrations were analyzed using the PROC GLIMMIX procedure of SAS 9.4 software (SAS Institute Inc., Cary, NC). The least-square means for all traits were separated, and the statistical significance level was defined at the level of $P<0.05$.

The metabolomics data were analyzed using RStudio (Boston, MA, USA). The metabolite peaks were normalized using log 2 transformation and were checked for the presence of extreme variance within the group. The metabolites were also analyzed using ANOVA to identify features significantly affected by the aging treatment. Significance was defined at $P<0.05$ and adjusted using the false discovery rate (FDR) method. An unsupervised principal component analysis was performed to aid in the visualization of the data.

The alpha and beta diversity of the microbiome data were visualized using RStudio (Boston, MA, USA). The permutational multivariate analysis of variance (PERMANOVA; $P \leq 0.05$) and multivariate homogeneity analysis to test the difference in beta diversity were performed using the vegan package (Oksanen et al., 2020). The significance was set at $P<0.05$ and adjusted using the false discovery rate (FDR) method. The co-occurrence analysis was also performed to identify ASV-metabolites pairs most prevalent within each aging treatment. The significant metabolites and all identified ASVs were utilized in the co-occurrence analysis. Significant ASV-metabolites pairs were determined at $P<0.05$ and $R^2>0.8$. Linear discriminant analysis effect size (LEFSE) and Analysis of Compositions of Microbiomes (ANCOM) was also performed to identify potential microbial marker unique to the different aging methods. Differences were considered statistically significance at $P<0.05$ and adjusted using FDR method.

5.4 Results

5.4.1 Free amino acid and sugar concentration

Most of the amino acids concentrations were significantly affected by the treatments ($P<0.05$), except for aspartate, hydroxyproline and cystine ($P>0.05$, Table 5.1). Of those significant amino acids, 8 amino acids (alanine, cysteine, glycine, histidine, methionine, phenylalanine, tyrosine, tryptophan) were in greater concentrations across all dry-aged samples; 6 amino acids (glutamine, isoleucine, lysine, ornithine, proline, valine) were in highest concentrations in both DA and DWA samples; 2 amino acids (beta-alanine and leucine) were identified to be greatest in both DA and UDA samples; 4 amino acids (asparagine, glutamate, serine, threonine) were found in greater concentrations in DWA samples. No amino acids were found to be greater in the WA treatment compared to the dry-aged counterparts. The total free amino acids concentrations were significantly different among the samples ($P<0.05$) with greater concentration in all dry-aged samples compared to WA samples. When expressed using the dry-matter basis, DWA samples had the greatest concentration of total free amino acids ($P<0.05$), followed by DA samples. In contrast, UDA and WA samples had the lowest total free amino acid concentrations ($P<0.05$) which were not significantly different from each other ($P>0.05$).

The sugar content was generally increased in the dry-aging treatment compared to the WA treatment (Table 5.2). Total sugar and reducing sugar content were increased in both DA and DWA steaks compared to WA steaks ($P<0.05$), while UDA steaks were not different compared to all treatments ($P>0.05$). Of the 10 sugars identified, ribose, glucose and myoinositol were the only sugars identified to be significantly altered following the aging process. The ribose content greatest in DA samples and lowest in WA samples ($P<0.05$), while DWA and UDA samples had intermediate ribose concentrations and were not different from both DA and WA samples ($P>0.05$). Similarly, myoinositol content was most abundant in DA steaks and lowest in WA steaks ($P<0.05$). The glucose concentration was significantly higher in all dry-aged samples compared to the WA samples ($P<0.05$). Analysis of the total sugars on a dry matter basis demonstrated a strong trend ($P=0.0535$) of increasing sugar concentration in both DA and DWA loins compared to UDA and WA loins.

5.4.2 Fatty acid and volatile content analysis

Following fatty acid profiling, 34 fatty acids were identified and quantified, ranging from C10 to C24 (Table 5.3). Of those, 7 fatty acids (C13:1, C15:0, C17:1, C18:2trans, C18:3n3, C20:2 and C20:5) were affected by the aging treatments applied ($P<0.05$). Significantly higher proportions of polyunsaturated fatty acids (C18:2trans, C18:3n3, C20:2 and C20:5) in DWA and/or UDA samples were found ($P<0.05$). The overall percentage of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), however, was not affected by the different aging treatments ($P>0.05$). Total fatty acids content was not affected when expressed in either wet- or dry-matter basis ($P>0.05$). However, a trend ($P=0.0689$) for lower fatty acid concentration was observed in UDA samples when expressed on a dry matter basis.

A total of 52 volatile compounds was detected including 11 aldehydes, 6 alcohols, 6 ketones, 9 hydrocarbons, 4 pyrazines, 1 furan, 1 lactone, 6 sulfur-containing compounds and 8 carboxylic acids (Table 5.4). Of those, concentrations of 31 compounds were significantly affected by the different aging treatments ($P<0.05$). In general, greater volatile compounds concentrations were observed in all dry-aging samples compared to WA samples. The DWA samples consistently had the highest volatile compounds concentration among the dry-aged samples, although the significance varied depending on the compounds. Additionally, the aging treatments appeared to significantly affect hydrocarbon, alcohol, aldehydes and ketone group, as indicated by greater significant volatile compounds originating from those groups.

5.4.3 Metabolomics analysis

The metabolomics profiling was conducted via the UPLC-MS platform. The analysis detected 1405 metabolite features across all the treatments. Of those metabolites, 60 metabolites were found to be significantly affected by the aging treatment applied ($P<0.05$, $FDR<0.05$) and were utilized for further analysis. The principal component analysis (PCA) of the metabolites exhibited separation of the metabolite profile based on their treatments (Figure 5.1). A notable separation between all the dry-aging treatments and wet-aging treatment could be observed across the PC1 axis, explaining 22% of the variation observed. Additionally, the dry-aging treatments were further separated along the PC2 axis, with 16.02% of the variation being explained. The PC2

showed that DA samples were isolated from both DWA and UDA samples, indicating a distinct metabolites profile in the DA treatment.

From the 60 significant features, 42 were able to be annotated through mass comparison with the HMDB database and were then loosely categorized into protein-derived, carbohydrate-derived, lipid-derived, organic acids and others (Table 5.5). The majority of the features identified was belong to the protein-derived group as amino acids and dipeptides. Most of these features were also presented in greater abundance in the dry-aging treatments than WA counterparts. Among the dry-aging treatments, the DA sample had more amino acids/dipeptides metabolites present when compared to DWA and UDA samples. Similarly, a higher abundance of carbohydrate and organic acids species was identified in the dry-aging treatments compared to the WA treatment. On the other hand, the WA samples were observed to have an elevated concentration of lipid metabolites compared to the dry-aging treatments.

5.4.4 Microbiome analysis

Sequence quality and contamination

Following the 16S rRNA gene sequencing, a total of 7,080,966 sequences were identified and were reduced to 6,270,992 sequences after the denoising step via DADA2. The sequences were then able to be clustered into a total of 565 ASVs in the study. Comparison to PCR negative control samples (PCR-grade water used as DNA template) indicated potential contamination of with bacterial genus identified as *Escherichia-Shigella*. The relative abundance of the genus was observed to be more than 95% in the negative control samples and therefore was considered as a contaminant. As such, all members of the genus were removed from the samples. All samples were then rarified to a sampling depth of 2391 to minimize sample removal due to low sequence reads and used for subsequent microbiome analyses.

The initial samples were excluded from the analysis as most of the samples had very low sequences following the contaminant removal. While *Escherichia-Shigella* might be naturally present in meat products, microbial quantification through conventional plate culture in our parallel study (Setyabrata et al., 2021b) demonstrated that the initial samples had microbial concentration below the detection limit, explaining the low sequence read. The *Escherichia-Shigella* relative abundance in most of the initial samples was greater than 90% and therefore was concluded to be a contaminant. After the removal, the initial microbial composition was found to

consist mainly of Firmicutes and Proteobacteria, with the top genera belonging to *Brochtothrix* and *Pseudomonas* (data not shown).

Diversity measures

Alpha diversity is a measure of richness (number of unique ASVs identified) and evenness (percentage of each identified ASVs) within an environment. In the current study, the alpha diversity was estimated using multiple measures, including Chao1 index for richness, Pielou index for evenness and Faith phylogenetic diversity index for phylogenetic diversity estimation. A significant treatment effect was observed for both Chao1 and Pielou index measures ($P<0.05$, Figure 5.2) and a significant source effect was observed for the Faith diversity measure ($P<0.05$, Figure 5.2). No significant interaction between treatment and source was observed across all alpha diversity measures ($P>0.05$). WA samples had a greater richness (Chao1) when compared to the DWA samples ($P<0.05$), while DA and UDA samples were similar with both WA and DA samples ($P>0.05$). Similarly, WA samples also had significantly higher evenness than DA samples ($P<0.05$) but not from DWA and UDA samples. The phylogenetic diversity was only influenced by the samples source with greater diversity in the crust samples when compared to lean samples ($P<0.05$).

Beta diversity is utilized to estimate community structure dissimilarity between environments. The Bray-Curtis Dissimilarity index and Weighted UniFrac were calculated to estimate beta diversity in this study. Similar results could be observed from the principal coordinate analysis (PCoA) of both measures, showing a clear separation between the dry-aging treatments and wet aging treatment community separation (Figure 5.3). Homogeneity analysis did not identify significant differences across all effects ($P>0.05$), indicating similar sample dispersion within the same treatments. Therefore, any community dissimilarity observed could be attributed to separation of treatment group centroids. PERMANOVA of the community structure based on the Bray-Curtis index revealed a significant aging treatment and source interaction ($P<0.05$). When phylogenetic relations were taken into consideration in the Weighted Unifrac index, significant aging treatment effect ($P<0.05$) and source effect ($P<0.05$) were observed from the PERMANOVA of the community structure. Pairwise analysis of the Bray-Curtis index revealed significant community differences between both lean and crust samples of all dry-aged treatments when compared to both lean and crust portions of WA treatment ($P<0.05$). The DWA lean community structure was also found to be significantly different from the DWA crust, UDA lean and UDA

crust community structure. Pairwise analysis of the Weighted Unifrac index demonstrated that DA and DWA communities were similar ($P>0.05$) and were different from the WA community ($P<0.05$). The DWA treatment was also found to have a significantly different bacterial community when compared to the UDA treatment ($P<0.05$).

Relative abundances, microbial marker and co-occurrence

The 10 ASVs with the highest relative abundances are presented in Figure 5.4, showing the identification on both phylum and genus levels. At the phylum level, 6 ASVs were identified as Proteobacteria, 3 as Firmicutes, and 1 as unclassified bacteria. When observed at the genus level, the Proteobacteria ASVs could be further identified as unclassified Yersiniaceae (2 ASVs) and *Pseudomonas* (4 ASVs). The Firmicutes ASVs were identified as unclassified Lactobacillales (1 ASV), *Carnobacterium* (1 ASV) and *Brochothrix* (1 ASV). No pathogens were identified in the current study through the taxonomy identification.

Similar ASVs compositions were observed between the crust and lean portion of the same treatment, although greater consistency between replicates could be observed within the lean portion when compared to the crust portion. The WA microbial community composition in both crust and lean portions was dominated by unclassified Lactobacillales, *Brochothrix* and unclassified Yersiniaceae, having more than 50% of the total microbial abundances. Conversely, the crust and lean of the dry-aged samples were mainly comprised of *Pseudomonas* spp., with the genera presenting more than 50% of the total bacterial abundances.

The microbial marker analysis using LEFSE and ANCOM (Table 5.6) identified common microbes, which could potentially indicate their influence during the aging process. The *Pseudomonas* ASV1 was shown to be greatly enriched in the DA treatment, while unclassified Yersiniaceae ASV1, *Carnobacterium*, unclassified Lactobacillales and *Brochothrix* were enriched in the WA treatment. Co-occurrence analysis between the microbial ASVs and the significant metabolites also showed greater numbers of unique ASVs-metabolites pairs in the DA treatment than other treatments (Table 5.7). Among the correlated metabolites, majority of the compounds were identified to belong in the protein-derived group.

5.5 Discussion

5.5.1 Flavor precursors and flavor generation

Flavor is a complex sensory attribute involving the combination of aroma and taste perceived from a food product. The presence, composition and concentration of flavor precursors influence the final meat aroma and taste, and thus determine the final flavor perceived by consumers (Diez-Simon et al., 2019). Among the different flavor precursors, the availability of free amino acids has been suggested as the most integral aspect in meat flavor generation, mainly due to their involvement in Maillard reaction to generate flavor volatiles (Dashdorj et al., 2015). In the current study, greater concentrations of free amino acids were identified in the dry-aging treatments compared to WA treatment through free amino acid analysis. Multiple studies have constantly reported similar results in meats from different animal species (Mungure et al., 2020; Lee et al., 2019a; Kim et al., 2016), where free amino acids increase as a result of dry-aging treatment and such increase is likely essential for final dry-aged flavor generation. Further, similar to the current result, those studies also reported that a greater increase in umami-related amino acids (i.e., glutamate and glutamine) was observed, indicating the importance of such amino acids in developing the unique dry-aging flavor.

The contribution of the different amino acid groups to dry-aged flavor, however, is still unclear. A higher abundance of both cysteine and methionine in the dry-aged treatment was observed in the current study. Those amino acids were previously reported to generate meat-like aroma volatile and positively correlated with beefy/meaty flavor in meat (Calkins and Hodgen, 2007). These results, however, were contradictory to the trained and consumer sensory analysis reported in our parallel study, which reported no difference in beefy flavor was observed by the trained panel and more beefy flavor was observed in the WA samples by consumers (Setyabrata et al., 2021b). In addition to generating flavor volatile, free amino acids have been proposed to be a taste-active compound and, therefore, could also alter the aromas and taste perceived by the consumers (Ramalingam et al., 2019).

The untargeted metabolomics analysis also revealed several dipeptides and short peptides to be present in greater abundance in the DA treatments. The role of peptides in meat flavor generation, especially in the dry-aged product, is still not well studied. A previous study suggested that peptide-based Maillard reaction will generate more volatile compounds compared to amino

acid-based Maillard reaction (Zou et al., 2018). Likewise, peptides could also be a taste-active compound, generating a similar taste profile to its amino acid compositions. Several short peptides were also abundant in the WA treatments, mainly isoleucine, leucine, proline, hydroxyproline, and phenylalanine. The majority of these amino acids, with the exception of proline and hydroxyproline, were identified to produce a bitter (Dashdorj et al., 2015). Similar observations were previously identified and reported by Setyabrata et al. (2021a), where the authors identified a greater abundance of short peptides in dry-aged grass-fed beef following the dry-aging process compared to the wet-aged counterpart.

The concentration of reducing sugars available in meat will also play a major role in the flavor development as they participate in the Maillard reaction. Similar to the current results, several studies also reported a greater abundance of reducing sugar after the dry-aging process (Foraker et al., 2020; Mungure et al., 2020; Lee et al., 2019a). More ribose, fructose, mannose, glucose and myoinositol were observed in beef loins dry-aged for 21 days compared to those wet-aged for 28 days (Foraker et al., 2020). In the current study, only ribose, glucose, and myoinositol were significantly affected by the aging treatments, although a general trend of increased sugar concentration in all dry-aging treatments was observed compared to the WA treatment equivalent. Among the sugars, ribose was often considered as the primary sugar source involved in the Maillard reaction in meat products since this type of sugar could be released through degradation of nucleotides such as inosine and adenosine (Koutsidis et al., 2008; Baek, 2007). However, in the present study, glucose was available in significantly higher concentrations when compared to ribose and other sugars (~0.66 mmol/Kg of wet meat compared to ~15.81 mmol/Kg of wet meat for average ribose and glucose concentration, respectively). The current observation could indicate that glucose still plays a significant role in the Maillard reaction in dry-aged products, likely due to its relatively high abundance. It was suggested by Dinh et al. (2018) that ribose sugar was more unstable and rapidly degraded when compared to glucose, thus potentially further explaining the lower ribose concentration observed in the current study. The elevated concentration of free amino acids and reducing sugars observed in the dry-aging treatments could promote more Maillard reactions during the cooking process. Supporting this speculation, more Maillard reaction-based volatile compounds such as strecker aldehydes (2-methylbutanal and 3-methylbutanal), pyrazine (methyl-pyrazine) and sulfur-containing volatile compounds (carbon disulfide, dimethyl sulfide

and methanethiol) were identified and present in greater abundance in the dry-aging treatments, especially in the DWA samples.

The extent of flavor volatile production is often dependent on the fatty acid profile of the meat products. In regards to the fatty acid profile, limited information is available in respect to dry-aging in the current literature. Available studies reported no changes or minimal alteration on the fatty acid profile following the dry-aging application (Setyabrata et al., 2021a; Foraker et al., 2020; Kim et al., 2019). In the present study, no differences were found in the total concentration of the fatty acid among the different treatments ($P>0.05$). However, differences were identified when observing the proportion of the fatty acids between the aging treatments, indicating potential alteration of free fatty acid composition through the application of dry-aging. A higher proportion of PUFAs was observed in DWA and UDA treatments when compared to both WA and DA treatments. Likewise, greater concentrations of volatile compounds were released from DWA and UDA samples after the cooking process. More lipid-based volatile compounds, such as hydrocarbons, alcohols, n-aldehydes and ketones, were present in higher abundance in both DWA and UDA samples when compared to both WA and DA samples. The greater lipid volatile compounds production could be attributed to the greater proportion of unsaturated fatty acids in those treatments. Unsaturated fatty acids are more readily oxidized and degraded and thus more active during the lipid thermal oxidation and degradation during cooking (Min and Ahn, 2005).

Although greater concentrations of lipid volatile compounds were observed in the current study, lipid volatile compounds tend to have a higher detection threshold for influencing final meat flavor (Mottram, 1998). Interestingly, the interaction between Maillard reaction and lipid thermal oxidation/degradation may further enhance the abundance and variation of flavor volatiles generated during the cooking process (Whitfield and Mottram, 1992). It has been proposed that the lipid thermal oxidation/degradation products (e.g., aldehydes, acids) could participate in the Maillard reaction by acting as a substrate to generate unique meat flavor volatiles such as pyrazines and thiazoles (Dinh et al., 2021; Whitfield & Mottram, 1992). Thus, it would be reasonable to postulate that the higher production of lipid volatile compounds along with greater Maillard reaction ability could affect the flavor potential of the dry-aged product. The increased interaction of both flavor production mechanisms might be translated to greater concentration and variation of volatile compounds produced in the dry-aged product compared to the WA treatments, thus explaining the more desirable flavor often perceived from the dry-aged product.

The untargeted metabolomics analysis also revealed more organic acids metabolites in the dry-aging treatments. Organic acids are often generated from lipid hydrolysis and microbial activity in dry-cured meat products (Pugliese et al., 2015). However, the effect of organic acids in meat flavor development is still not clear. A previous study suggested that organic acids could introduce sour and/or sweet notes into the meat product (Ramalingam et al., 2019). In our parallel study (Setyabrata et al., 2021b), the trained panel reported lower sourness in the final dry-aged products when compared to the WA products, thus demonstrating that dry-aging was effective in reducing the presence of undesirable flavor from cull cow beef.

5.5.2 Flavor precursors generation mechanisms

Dehydration

Dehydration has been considered as a major mechanism responsible for the flavor development in dry-aged meat products, mainly from the moisture loss during the aging process, which subsequently concentrated the flavor precursors in the product. The extensive moisture loss during dry-aging is inevitable and has been shown to reach up to 35% loss depending on the length of the dry-aging (Dashdorj et al., 2016). In our parallel study (Setyabrata et al., 2021b), both DA and UDA treatments had the highest aging shrinkage, showing 12.09% and 12.44% moisture loss from the aging process, respectively. The DWA loins had an intermediate moisture loss, averaging to 7.59%. Likewise, lower moisture content in trimmed lean portions was reported for both DA and UDA compared to WA and DWA. This decrease in moisture content could be partially responsible for the observed increase in free amino acids and reducing sugar concentration found in the current study, subsequently affecting the dry-aged flavor development. Although no increases in the overall concentration of the flavor-precursors were found, the dehydration process will increase the relative abundance of the precursors, thus potentially promoting an environment suitable for more Maillard reactions during cooking. This observation, therefore, confirms the postulation regarding the significance of dehydration in the flavor generation process of dry-aged meat.

Moreover, it is of interest to note that amino acid abundance and reducing sugar concentration were constantly influenced by the dry-aging treatments, where concentrations of both precursor groups increased following dry-aging. The total fatty acid concentration was not impacted by dry-aging when compared to WA. Further study to elucidate the impact of dry-aging

on fat acid profile alteration would be of interest to understand the dry-aging impact on the liberation of lipid-based flavor precursors.

Microbial involvement

The involvement of microorganisms (bacteria, yeast and mold) have been suggested to participate in the liberation of flavor precursors. Previous reports suggested the involvement of microorganisms in the liberation of flavor precursors via release of exogenous proteolytic and lipolytic enzymes (Ribeiro et al., 2021; Capouya et al., 2020; Lee et al., 2019b). The current microbiome analysis demonstrated that the phylum Proteobacteria has the highest relative abundance in all dry-aging treatments. This phylum has been previously reported as the most prominent microbial phyla in dry-aged meat by Ribeiro et al. (2021) and Capouya et al. (2020), more specifically the *Pseudomonas* genus. Likewise, a greater abundance of the Firmicutes phylum was also reported to be the dominant bacteria group in wet-aged samples by Ribeiro et al. (2021), similar to the current observation. The Firmicutes present in meat are often more specifically identified as *Lactobacillus* spp., which are anaerobic bacteria often observed in vacuum packaged meat (Pothakos et al., 2015). While the relative abundances depended on the environmental conditions, the aforementioned microbe groups have been identified as spoilage bacteria in meat products by degrading available nutrients such as proteins and lipids to other products, including free amino acids, fatty acids, organic acids, esters and aldehydes (Casaburi et al., 2015). However, it is reasonable to postulate that the presence of members of the Proteobacteria phylum are more influential in the liberation of flavor precursors such as free amino acids and reducing sugars, as those compounds were enhanced in the dry-aging treatments, due to the greater abundance of this phylum.

As previously discussed, the dry-aging treatments had distinct flavor precursor compositions compared to the wet-aging counterpart. The metabolomics profiling exhibited an apparent clustering between the dry-aged and wet-aged samples, demonstrating differences between the aging methods. While this separation could be attributed to the environmental factors during the aging process, the dehydration process might not necessarily increase the flavor precursors' total availability in dry-aged meat. When presented on a dry matter basis, a greater abundance of free amino acids and reducing sugars were observed in both DA and DWA samples, indicating the involvement of other mechanisms in liberating those flavor pre-cursors. The UDA samples were

found to have a concentration similar to WA samples when the flavor precursors were presented on a dry matter basis. This observation may indicate that the elevated liberation of flavor precursors could be attributed to the participation of microorganisms during the dry-aging process as the UV light substantially decreased and suppressed the presence of microbes, as reported in our parallel study (Setyabrata et al., 2021b).

Interestingly, a similar separation pattern was observed in the microbiome composition based on the beta diversity analysis. The microbiome analyses identified distinct clustering patterns separating the dry-aging and wet-aging samples, which could help explaining the differences in the observed flavor precursors composition. While UDA samples had a similar microbiome profile to both DA and DWA samples, the microbiome analysis did not account for the activity of the bacteria. The microbiome analysis used recovered DNA materials and did not distinguish between active, dead or injured bacteria (Cangelosi and Meschke, 2014). Therefore, while a similar profile was observed, it is possible that the UV-light suppressed the microbial activity, which could result in no considerable impacts on the flavor precursor changes.

More protein-derived metabolites were identified to be correlated to more unique ASVs, indicating that the microbes might play a significant role in the protein degradation, such as the glutaminyl peptides. These peptides were previously identified to be released through the activity of *Bacillus* sp. (Zhao et al., 2016), which were also identified as a the unique ASV-metabolites pair in the DA treatment. This could indicate that the activity of less abundant microbes (such as *Bacillus*) present in the meat during dry-aging could also contribute to the overall flavor precursors development in the meat. Perhaps, the major microorganism groups would increase the flavor precursor without any distinction, while the minor microorganism groups could be more specific and liberate more particular flavor precursors. This could lead to the generation of unique compounds that could potentially influence the final perceived flavor, such as the release of glutamnyl peptides by the *Bacilllus* spp. as observed in the current study. Future study on specific bacteria metabolism would be of interest to provide insight to the liberation of unique metabolites.

The role of mold and yeast was not analyzed in this study. However, a previous study has identified the involvement of mold (*Pilaira anomala*) and yeast (*Debaryomyces hansenii*) in liberating the flavor precursors (Lee et al., 2019b). Furthermore, our parallel study observed greater mold and yeast content in the DWA samples (Setyabrata et al., 2021b), indicating their potential activity in explaining the difference in free amino acids and free fatty acids profile

observed in the DWA samples compared to DA. Future studies to further identify this relation will be of interest to fully understand the impact of microorganisms in dry-aging flavor development.

5.6 Conclusions

The results of the current study demonstrated that dry-aging increased the abundance of flavor precursors, such as free amino acids, short peptides and reducing sugars, which are key chemical compounds related to Maillard reactions. While only limited impact was observed in the free fatty acid profiled, more PUFAs were identified in DWA and UDA samples, potentially contributing to the greater lipid volatile compounds as a result of those treatments. Greater flavor volatiles were observed in the dry-aging samples, especially in DWA samples, likely due to greater chemical interactions between compounds related to the Maillard and lipid chemical reactions. Two major dry-aging flavor generation mechanisms, compounds concentration through dehydration and microbes-induced flavor, were characterized in the current study. While dehydration played a role in increasing overall flavor precursors concentration, it did not influence their relative abundance. Microbiome analysis revealed that microbial groups, especially Proteobacteria, might contribute to the increased availability of the flavor precursors in dry-aged treatments. The microbiome co-occurrence analysis also identified minor microbial groups which could potentially release unique metabolites that contribute to the overall dry-aged flavor. Future studies to identify the role of the mold and yeast will be of interest to identify their role in dry-aged flavor development.

5.7 Tables and Figures

Table 5.1. Effect of different aging methods on free amino acids content of cull cow beef loins (M. *longissimus lumborum*) after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water-permeable bag (DWA) and UV-light dry-aging (UDA)

Free amino acids (mmol/Kg of wet meat)	WA	DA	DWA	UDA	SEM[1]	P-value
Alanine	3.899[b]	6.477[a]	5.730[a]	5.601[a]	0.345	<.0001
Asparagine	0.218[c]	0.323[ab]	0.348[a]	0.269[bc]	0.027	0.0021
Aspartate	0.103	0.153	0.165	0.145	0.022	0.0622
Beta-Alanine	0.241[b]	0.349[a]	0.315[ab]	0.386[a]	0.040	0.0498
Cysteine	1.056[b]	1.578[a]	1.685[a]	1.398[a]	0.129	0.0008
Cystine	0.015	0.016	0.016	0.014	0.003	0.9738
Glutamate	0.920[c]	1.586[b]	2.278[a]	1.287[b]	0.139	<.0001
Glutamine	0.002[c]	0.012[a]	0.011[a]	0.006[b]	0.001	<.0001
Glycine	1.329[b]	2.153[a]	1.961[a]	1.859[a]	0.140	0.0004
Histidine	1.050[b]	2.773[a]	3.675[a]	3.164[a]	0.352	<.0001
Hydroxy Proline	0.041	0.047	0.048	0.057	0.005	0.1702
Isoleucine	0.846[b]	1.134[a]	1.130[a]	1.059[ab]	0.095	0.0415
Leucine	1.065[b]	1.396[a]	1.256[ab]	1.340[a]	0.103	0.0462
Lysine	0.477[c]	1.353[a]	1.308[a]	1.002[b]	0.115	<.0001
Methionine	0.379[b]	0.606[a]	0.523[a]	0.520[a]	0.052	0.0048
Ornithine	0.048[b]	0.118[a]	0.110[a]	0.083[ab]	0.013	0.0017
Phenyl Alanine	0.508[b]	0.749[a]	0.721[a]	0.720[a]	0.062	0.0057
Proline	0.356[b]	0.483[a]	0.476[a]	0.415[ab]	0.033	0.0206
Serine	1.336[c]	2.070[b]	2.726[a]	2.029[b]	0.213	0.0001
Threonine	0.767[c]	1.159[ab]	1.394[a]	1.113[b]	0.103	0.0007
Tyrosine	0.389[b]	0.840[a]	0.825[a]	0.769[a]	0.074	<.0001
Tyrptophan	0.047[b]	0.102[a]	0.087[a]	0.083[a]	0.009	0.0004
Valine	1.409[b]	1.875[a]	1.865[a]	1.729[ab]	0.148	0.0454

Table 5.1. Continued

Total Free Amino Acid	16.308[b]	27.351[a]	28.652[a]	24.522[a]	1.806	<.0001
Total Free Amino Acid Dry Basis (mmol/Kg dry meat)	50.663[c]	69.480[b]	85.513[a]	53.074[c]	5.226	<.0001

[a-c] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)
[1]Standard Error of Means

Table 5.2. Effect of different aging methods on sugar concentration of cull cow beef loins *(*M. *longissimus lumborum*) after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water-permeable bag (DWA) and UV-light dry-aging (UDA)

Reducing sugars (mmol/Kg of wet meat)	**WA**	**DA**	**DWA**	**UDA**	**SEM[1]**	**p-value**
Ribose	0.5173[b]	0.7881[a]	0.6672[ab]	0.6808[ab]	0.0690	0.0418
Fructose	0.8581	1.3355	1.1871	1.1818	0.1743	0.2260
Mannose	1.8872	2.7303	2.7838	2.5197	0.2789	0.0734
Glucose	11.1989[b]	19.4053[a]	16.1550[a]	16.4701[a]	1.4932	0.0017
Myoinositol	0.3815[c]	0.6911[a]	0.6224[ab]	0.4802[bc]	0.0703	0.0046
Ribose 5-phosphate	0.0467	0.0875	0.1674	0.0680	0.0343	0.0771
Fructose 6-phosphate	1.6241	2.0178	2.7800	1.7763	0.5367	0.4410
Mannose 6-phosphate	0.9645	1.4169	1.4313	1.4415	0.3009	0.5632
Glucose 6-phosphate	7.2096	13.1020	10.3266	8.7132	1.8726	0.1276
Maltose	0.0492	0.0603	0.0438	0.0468	0.0152	0.8184
Total Sugars	24.7371[b]	41.6349[a]	36.1647[a]	33.3785[ab]	3.8001	0.0222
Total Reducing Sugars	24.3556[b]	40.9438[a]	35.5423[a]	32.8983[ab]	3.7877	0.0251
Total Sugars Dry Basis (mmol/Kg of dry meat)	78.9235	105.82	108.33	73.6774	11.2584	0.0535

[a-c] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)
[1]Standard Error of Means

Table 5.3. Effect of different aging methods on free fatty acid profiles composition of cull cow beef loins (M. *longissimus lumborum*) after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water-permeable bag (DWA) and UV-light dry-aging (UDA)

Fatty Acid (%FA/total FA)	Type	WA	DA	DWA	UDA	SEM[1]	P-value
C10:0	SFA	0.045	0.043	0.047	0.048	0.003	0.6764
C12:0	SFA	0.056	0.052	0.059	0.060	0.003	0.3028
C13:1	MUFA	0.016^{c}	0.048^{b}	0.167^{a}	0.153^{ab}	0.039	0.0184
C14:0	SFA	2.643	2.434	2.790	2.824	0.160	0.2895
C14:1n5	MUFA	1.103	0.873	1.117	1.040	0.115	0.4254
C15:0	SFA	0.273^{a}	0.263^{a}	0.326^{a}	0.182^{b}	0.024	0.0009
C16:0	SFA	28.148	28.541	28.480	29.274	0.489	0.4313
C16:1trans	MUFA	0.017	0.036	0.031	0.024	0.008	0.3438
C16:1n7	MUFA	5.509	4.756	5.603	5.016	0.269	0.0982
C17:0	SFA	0.698	0.699	0.658	0.676	0.026	0.6366
C17:1	MUFA	0.790^{a}	0.688^{b}	0.710^{b}	0.649^{b}	0.027	0.0049
C18:0	SFA	11.083	12.056	11.137	11.899	0.377	0.1642
C18:1trans	MUFA	0.352	0.447	0.369	0.376	0.056	0.4618
C18:1n9	MUFA	42.591	42.012	40.852	40.480	0.615	0.0669
C18:1n7	MUFA	1.789	1.625	1.851	1.794	0.138	0.6879
C18:2trans	PUFA	0.061^{bc}	0.128^{b}	0.196^{a}	0.051^{c}	0.021	0.0021
C18:2n6	PUFA	2.820	3.225	3.373	3.189	0.236	0.3802
C18:3n3	PUFA	0.061^{c}	0.069^{ab}	0.062^{bc}	0.070^{a}	0.003	0.0371
C18:3n6	PUFA	0.258	0.245	0.255	0.242	0.011	0.6406
C19:0	SFA	0.119	0.102	0.106	0.098	0.006	0.1008
C19:1	MUFA	0.023	0.023	0.022	0.027	0.003	0.7092
C20:0	SFA	0.150	0.191	0.152	0.175	0.014	0.1581
C20:1n9	MUFA	0.072	0.071	0.060	0.063	0.005	0.2623
C20:1n11	MUFA	0.427	0.343	0.377	0.341	0.028	0.0621
C20:2	PUFA	0.034^{ab}	0.025^{b}	0.037^{a}	0.037^{a}	0.003	0.0294

Table 5.3. Continued

C20:3n6	PUFA	0.094	0.107	0.122	0.132	0.015	0.3124
C20:4n6	PUFA	0.532	0.573	0.710	0.716	0.096	0.4286
C20:5	PUFA	0.030[b]	0.042[ab]	0.046[a]	0.058[a]	0.006	0.0136
C22:3	PUFA	0.006	0.007	0.006	0.009	0.001	0.129
C22:4	PUFA	0.004	0.004	0.010	0.004	0.003	0.4551
C22:5n3	PUFA	0.122	0.150	0.180	0.166	0.021	0.2425
C22:6n3	PUFA	0.004	0.006	0.011	0.009	0.002	0.0759
C24:0	SFA	0.003	0.006	0.004	0.005	0.001	0.1229
C24:1n9	MUFA	0.107	0.119	0.138	0.139	0.020	0.6374
SFA%		44.143	43.599	43.611	45.235	0.741	0.3808
MUFA%		51.342	52.089	51.678	50.144	0.712	0.2858
PUFA%		4.515	4.312	4.711	4.656	0.354	0.5164
Total Free Fatty Acids Wet Basis (mg/g wet meat)		82.04	83.09	73.71	72.03	13.14	0.6804
Total Free Fatty Acids Dry Basis (mg/g dry meat)		260.26	208.29	213.35	154.21	38.62	0.0689

[a-c] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid[1]Standard Error of Means

Table 5.4. Effect of different aging methods on volatile compound profiles of cull cow beef loins (M. *longissimus lumborum*) after 28 days of aging. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water-permeable bag (DWA) and UV-light dry-aging (UDA)

Volatile Name (ng/g sample)	WA	DA	DWA	UDA	SEM[1]	P-value
n-aldehydes						
Acetaldehyde	9.12^{c}	19.22^{c}	53.58^{a}	36.99^{b}	5.18	<.0001
Butanal	4.67^{c}	22.02^{b}	44.26^{a}	44.04^{a}	5.54	<.0001
Heptanal	8.99	8.70	17.06	7.72	3.13	0.1397
Hexanal	119.12	79.55	175.56	88.48	48.16	0.4940
Nonanal	6.74^{ab}	4.4^{b}	10.96^{a}	2.72^{b}	1.93	0.0245
Octanal	1.69^{b}	2.32^{b}	4.48^{a}	2.16^{b}	0.44	0.0002
Pentanal	40.86	3.41	8.28	4.21	19.87	0.4931
Strecker aldehydes						
2-methylbutanal	8.00^{c}	49.59^{b}	110.07^{a}	106.20^{a}	16.23	<.0001
3-methylbutanal	10.29^{c}	67.42^{b}	144.84^{a}	143.90^{a}	21.82	<.0001
Benzaldehyde	27.55	19.14	23.35	21.99	5.26	0.7209
Phenylacetaldehyde	2.94	1.74	2.31	1.45	0.56	0.2062
Alcohols						
1-Hexanol	1.11^{c}	3.54^{b}	5.60^{a}	3.78^{ab}	0.76	0.0007
1-Octanol	2.90^{b}	2.38^{b}	6.12^{a}	1.06^{b}	0.99	0.0061
1-Octen-3-ol	2.68^{b}	5.63^{ab}	7.74^{a}	5.70^{ab}	1.28	0.0262
1-Pentanol	12.83	14.89	23.77	26.65	6.20	0.3014
1-penten-3-ol	0.10^{b}	0.64^{ab}	1.12^{a}	0.98^{a}	0.21	0.0043
Ethanol	59.84	89.84	277.57	136.94	59.87	0.0635

Table 5.4. Continued

Ketone						
2,3-butanedione	8.17[b]	54.37[a]	70.71[a]	65.68[a]	15.03	0.0198
2,3-pentanedione	0.04[b]	0.14[a]	0.19[a]	0.14[a]	0.02	0.0002
2-heptanone	1.69	2.25	2.89	2.44	0.48	0.3274
2-pentanone	0.41[c]	1.28[bc]	2.79[a]	1.79[ab]	0.35	0.0002
2-Propanone	42.12[b]	63.01[b]	131.49[a]	114.52[a]	13.86	<.0001
3-hydroxy-2-butanone	10.88[b]	60.72[ab]	169.82[a]	133.57[a]	39.62	0.0223
Hydrocarbon						
Alpha-pinene	0.00[c]	0.13[cb]	0.35[a]	0.25[ab]	0.05	<.0001
Benzene	0.99[b]	2.07[a]	2.58[a]	2.65[a]	0.31	0.0011
D-limonene	10.67[b]	30.01[a]	37.29[a]	30.31[a]	3.81	<.0001
Ethyl benzene	0.37[b]	0.86[a]	1.06[a]	0.79[a]	0.13	0.0025
p-Xylene	0.97[b]	2.07[ab]	2.96[a]	2.22[a]	0.41	0.0083
Styrene	1.24[b]	2.38[a]	2.90[a]	2.46[a]	0.31	0.0028
Toluene	6.59[b]	17.03[a]	21.87[a]	20.13[a]	2.15	<.0001
Octane	2.13[c]	6.13[b]	10.71[a]	7.96[ab]	1.02	<.0001
Pentane	3.85[b]	5.46[b]	13.48[a]	10.56[a]	1.63	0.0003
Pyrazine						
2,5-dimethylpyrazine	5.69	4.89	6.64	5.25	2.04	0.9229
2-ethyl-3,5/6-dimethylpyrazine	1.48	1.41	2.08	1.56	0.45	0.6246
Methyl-pyrazine	0.97[b]	2.77[a]	3.91[a]	3.26[a]	0.78	0.0107
Trimethylpyrazine	1.08	1.30	2.35	1.58	0.46	0.1949
Furans						
2-Pentyl furan	0.92	0.45	0.69	0.38	0.28	0.5207
Lactone						
Butyrolactone	2.09[b]	15.94[a]	24.46[a]	18.11[a]	3.54	0.0004

Table 5.4. Continued

Sulfur-containing						
2-methyl thiophene	0.59	0.47	0.59	0.51	0.05	0.2327
Carbon disulfide	10.33[b]	12.38[b]	21.11[a]	13.36[b]	2.57	0.0232
Dimethyl sulfide	3.74[c]	7.37[cb]	14.82[a]	10.82[ab]	1.43	<.0001
Dimethyl-disulfide	0.03	0.03	0.06	0.05	0.01	0.1275
Methanethiol	2.88[bc]	1.54[c]	5.22[a]	4.12[ab]	0.77	0.0107
Methional	3.17	2.36	3.58	2.50	1.20	0.8636
Carboxylic acid						
Acetic acid	9.30[c]	11.25[cb]	23.11[a]	18.27[ab]	2.52	0.0008
Butanoic acid	20.30[c]	110.38[b]	202.04[a]	164.57[ab]	26.03	0.0001
Butanoic acid, methyl ester	3.55	0.98	1.55	2.01	1.67	0.6963
Heptanoic acid, methyl ester	0.17	0.24	0.37	0.27	0.07	0.2516
Hexanoic acid, methyl ester	3.51	4.57	6.70	6.70	1.53	0.3502
Hexanoic acid, methyl ester	3.11	4.57	6.70	6.70	1.53	0.2768
Nonanoic acid, methyl ester	0.79	0.46	0.53	0.34	0.13	0.0861
Octanoic acid, methyl ester	1.32	1.48	1.84	1.09	0.20	0.0690

[a-c] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)
[1]Standard Error of Means

Table 5.5. Effect of different aging methods on metabolomics profile of cull cow beef loins (M. *longissimus lumborum*) after 28 days of aging. (P-value <0.05, FDR<0.05). Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

Mass	RT	Highest Abundant	HMDB ID	Putative Name	WA	DA	DWA	UDA
Peptides								
115.0633	1.21	WA	HMDB0000162	Proline	18.32^{a}	16.23^{b}	16.92^{b}	16.74^{b}
244.1774	6.35	WA	HMDB28910	Isoleucyl-Isoleucine	18.37^{a}	16.21^{b}	16.25^{b}	16.49^{b}
357.2623	9.86	WA	HMDB0094648	Leucine-Leucine-Leucine	18.02^{a}	16.30^{b}	16.26^{b}	16.65^{b}
244.1067	7.38	WA/DA	HMDB0028864	Hydroxyprolyl-Hydroxyproline	17.91^{a}	17.83^{a}	17.06^{b}	17.25^{ab}
312.1437	5.47	WA	HMDB0131468	Phenylalanyl-Phenylalanine	18.07^{a}	17.94^{ab}	16.98^{c}	17.23^{bc}
239.0794	7.21	DA/WA	HMDB0131468	Aspartic Acid	18.11^{a}	18.12^{a}	17.21^{b}	17.53^{b}
284.1122	3.95	DA/WA	HMDB0028821	Glutamyl-Histidine	19.11^{a}	19.16^{a}	18.19^{b}	18.27^{b}
204.1112	2.23	DA	HMDB0029136	Valyl-Serine	18.11^{b}	18.50^{a}	17.55^{c}	17.59^{bc}
236.0465	4.78	DA	HMDB0028750	Aspartyl-Cysteine	17.66^{ab}	17.96^{a}	17.07^{b}	17.24^{b}
133.0196	4.78	DA	HMDB0062164	Thioproline	17.25^{ab}	17.49^{a}	16.62^{b}	16.81^{b}
218.1259	4.01	DA/DWA/UDA	HMDB0029042	Seryl-Isoleucine	20.92^{b}	21.69^{a}	21.40^{a}	21.51^{a}
284.1100	1.71	DA	HMDB0028884	Histidyl-Glutamic acid	22.40^{ab}	22.73^{a}	21.74^{b}	21.72^{b}
174.1032	1.43	DA	HMDB0028854	Theanine	17.97^{b}	18.33^{a}	17.84^{b}	17.66^{b}
188.1165	3.43	DA/DWA	HMDB0000446	Acetyl-Lysine	22.50^{b}	23.08^{a}	23.08^{a}	22.56^{ab}
127.0632	8.22	DA/WA	HMDB0029434	Methyleneproline	17.53^{a}	17.76^{a}	16.83^{b}	17.25^{ab}

Table 5.5. Continued

115.0634	0.86	DWA/WA	HMDB0000162	Proline	19.35^{a}	19.10^{b}	19.36^{a}	18.85^{b}
257.1022	0.82	DWA/WA	HMDB0039229	Glutaminyl-Glutamine	17.44^{a}	16.99^{b}	17.67^{a}	17.13^{ab}
155.0693	3.26	UDA/DWA/DA	HMDB0000177	Histidine	17.42^{b}	17.99^{a}	18.10^{a}	18.32^{a}
343.1257	1.85	UDA/DWA/DA	HMDB0037845	Deoxyfructosyl Tyrosine	20.48^{b}	20.36^{a}	20.86^{a}	20.96^{a}
Carbohydrates								
464.2283	12.43	WA	HMDB0031367	Linalooloxide apiosylglucoside	21.06^{a}	20.74^{ab}	19.64^{c}	20.21^{bc}
284.1211	0.72	DA	HMDB0029819	Phenylethyl glucopyranoside	17.82^{b}	18.28^{a}	18.15^{b}	18.19^{ab}
379.1063	3.34	UDA/DA	HMDB0001066	Lactoylglutathione	19.40^{b}	19.86^{a}	19.68^{ab}	20.22^{a}
Lipids								
565.4201	16.36	WA	HMDB0011497	Lysophosphatidylethanolamine	20.43^{a}	19.18^{b}	19.25^{b}	19.47^{b}
452.3357	14.23	WA/DWA/UDA	HMDB0037065	Oxoursadienoate	20.29^{a}	18.96^{b}	19.09^{a}	19.42^{a}
452.3361	14.04	WA	HMDB0035888	Tyromycic acid	21.83^{a}	20.82^{b}	20.82^{b}	21.13^{ab}
284.1073	1.56	WA/DA	HMDB0030694	Demethoxymatteucinol	21.66^{a}	21.56^{a}	20.89^{b}	20.88^{b}
232.1129	2.2	WA	HMDB0036189	Tetrahydrofurfuryl cinnamate	19.17^{a}	18.81^{ab}	18.43^{b}	18.69^{b}
286.1532	16.3	DA	HMDB0060085	Estradiol quinone	19.62^{ab}	19.63^{a}	18.64^{c}	19.00^{bc}
266.1728	12.08	UDA/DWA/DA	HMDB0030356	Didehydrocondyfolan	16.30^{b}	17.32^{a}	17.79^{a}	18.04^{a}
407.0982	4.11	UDA	HMDB0030257	Erysothiopine	19.98^{ab}	19.46^{b}	20.00^{ab}	20.33^{a}
132.0946	1.59	UDA/DWA	HMDB0029641	Cymenene	22.03^{b}	22.06^{b}	22.28^{a}	22.29^{a}

Table 5.5. Continued

Organic acids								
365.0897	4.89	DA	HMDB0062198	Glutathionyl acetate	21.88^{b}	22.24^{a}	21.23^{b}	21.42^{b}
298.1283	3.01	DA/WA	HMDB06101	Enterolactone	17.90^{a}	17.95^{a}	16.93^{b}	17.06^{ab}
276.1212	3.05	DWA/DA	HMDB0034263	Triethyl citrate	22.12^{b}	22.46^{a}	22.51^{a}	22.27^{ab}
118.0277	3.59	UDA/DA/WA	HMDB0031204	Hydroxyoxobutanoic acid	18.57^{a}	18.63^{a}	18.44^{b}	18.65^{a}
164.0469	2.34	UDA	HMDB0001713	Coumaric acid	21.85^{b}	21.77^{b}	21.32^{c}	22.13^{a}
Other								
113.0843	6.55	WA	HMDB0031199	Trimethyloxazoline	21.64^{a}	20.32^{b}	21.13^{ab}	20.32^{b}
301.1637	4.59	WA/DA	HMDB0032654	Futoamide	19.15^{a}	19.10^{a}	18.43^{b}	18.59^{ab}
194.1156	5.62	DA/DWA/UDA	HMDB0094708	Tetraethylene glycol	22.17^{b}	22.74^{a}	22.56^{a}	22.69^{a}
132.0949	1.6	DWA	HMDB0032303	Heptanethiol	22.96^{b}	23.12^{a}	23.19^{b}	23.11^{b}
94.0395	1.53	DWA	HMDB0000228	Phenol	18.66^{b}	18.76^{b}	18.96^{a}	18.71^{b}
327.1884	4.55	UDA	HMDB0038645	Piperamide	20.47^{ab}	19.89^{b}	20.74^{ab}	21.03^{a}

[a-c] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$)

Table 5.6. Potential microbial marker correlated with changes observed in cull cow beef loins (M. *longissimus lumborum*) after 28 days of aging using different aging methods identified through both LEFSE (P<0.05, FDR<0.05) and ANCOM (P<0.05, FDR<0.05) analyses. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

ASVs	Enriched Treatment
Pseudomonas1	DA
Unclassified Yersiniaceae1	WA
Carnobacterium	WA
Unclassified Lactobacillales	WA
Brochothrix	WA

Table 5.7. Co-occurrence analysis results showing unique ASVs (genus level) and metabolites pairs identified from cull cow beef loins (M. *longissimus lumborum*) aged using the different aging methods for 28 days. (P-value <0.05, Rho > 0.8). Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA), Dry-aging in water permeable bag (DWA) and UV-light dry-aging (UDA)

Treatment	**Metabolites**	**ASV**
WA	Acetyl-Lysine	*Acinetobacter* ASV1
	Deoxyfructosyl Tyrosine	*Carnobacterium, Lactobacillus, Psychromonas*
	Glutaminyl-Glutamine	*Aeromonas*
	Heptanethiol	*Aeromonas*
	Proline	*Flavobacterium* ASV2, *Massilia, Rothia, Shewanella,* Unclassified Gammaproteobacteria
	Tetraethylene glycol	*Acinetobacter* ASV1
	Triethyl citrate	*Carnobacterium, Lactobacillus, Psychromonas*
DA	Estradiol quinone	*Micrococcaceae, Mucor, Noccaea*
	Futoamide	*Micrococcaceae, Mucor, Noccaea*
	Glutaminyl-Glutamine	*Acinetobacter* ASV6, *Flavobacterium* ASV1
	Glutamyl-Histidine	*Actinomyces, Bacillus* ASV1, *Bacillus* ASV2, *Bacillus* ASV3, *Bifidobacterium, Chlorobi, Comamonadaceae* ASV1, *Comamonadaceae* ASV2, *Enterococcus* ASV2, *Enterococcus* ASV3, *Ferruginibacter, Geobacillus, Mesorhizobium, Metagenome* ASV1, *Metagenome* ASV2, *Microbacteriaceae, Proteiniphilum, Streptococcus* ASV2
	Linalooloxide apiosylglucoside	*Flavobacterium* ASV1, *Flavobacterium* ASV2,*Flavobacterium* ASV3, *Pedobacter* ASV2, *Staphylococcus, Vagococcus*
	Methyleneproline	*Acinetobacter* ASV6, *Flavobacterium* ASV1, *Pedobacter* ASV1
	Proline	*Actinomyces, Bacillus* ASV1, *Bacillus* ASV2, *Bacillus* ASV3, *Bifidobacterium, Chlorobi, Comamonadaceae* ASV1, *Comamonadaceae* ASV2, *Enterococcus* ASV2, *Enterococcus* ASV3, *Ferruginibacter, Geobacillus, Mesorhizobium, Metagenome* ASV1, *Metagenome* ASV2, *Microbacteriaceae, Proteiniphilum, Streptococcus* ASV2
	Phenylalanyl-Phenylalanine	*Micrococcaceae, Mucor, Noccaea*
	Seryl-Isoleucine	*Micrococcaceae, Mucor, Noccaea*

Table 5.7. Continued

DWA	Heptanethiol	*Chryseobacterium* , Unclassified Enterobacteriaceae
	Hydroxyprolyl-Hydroxyproline	*Chryseobacterium* , Unclassified Enterobacteriaceae
	Oxoursadienoate	*Chryseobacterium* , Unclassified Enterobacteriaceae
	Tyromycic acid	*Chryseobacterium* , Unclassified Enterobacteriaceae
UDA	Glutaminyl-Glutamine	*Acinetobacter* ASV2, *Acinetobacter* ASV3, *Acinetobacter* ASV4, *Acinetobacter* ASV5, *Acinetobacter* ASV7, *Corynebacterium* ASV1, *Corynebacterium* ASV2, *Enterococcus* ASV1, *Granulicatella, Leuconostoc, Neisseria,* Unclassified Pasteurellaceae, *Phyllobacterium, Rothia, Streptococcus* ASV1, *Streptococcus* ASV3
	Lactoylglutathione	*Acinetobacter* ASV2, *Acinetobacter* ASV3, *Acinetobacter* ASV4, *Acinetobacter* ASV5, *Acinetobacter* ASV7, *Corynebacterium* ASV1, *Corynebacterium* ASV2, *Enterococcus* ASV1, *Granulicatella, Leuconostoc, Neisseria,* Unclassified Pasteurellaceae, *Phyllobacterium, Rothia, Streptococcus* ASV1, *Streptococcus* ASV3

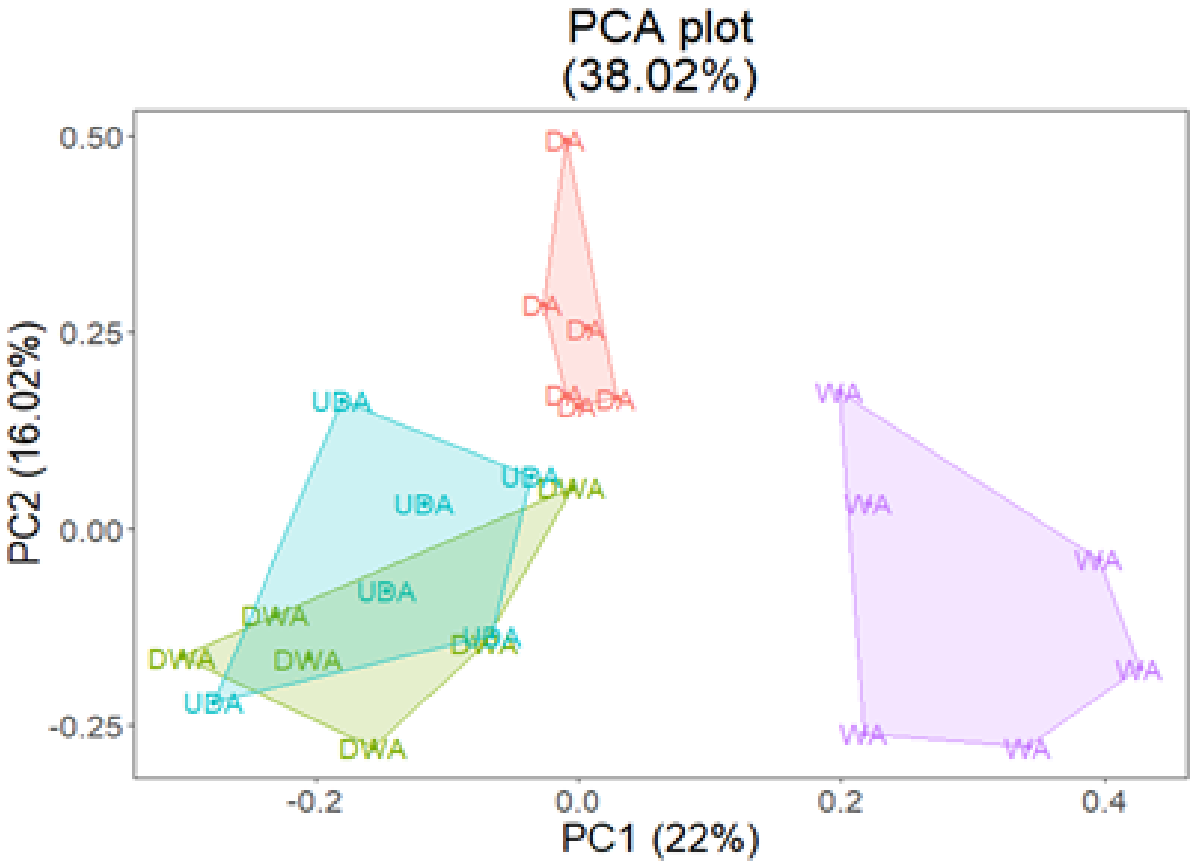
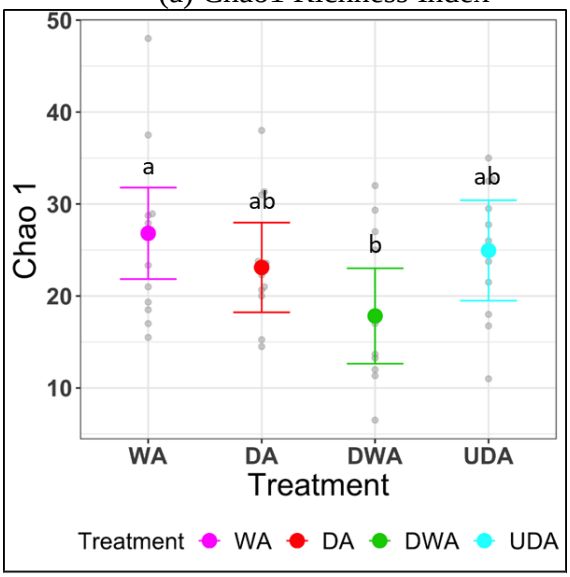


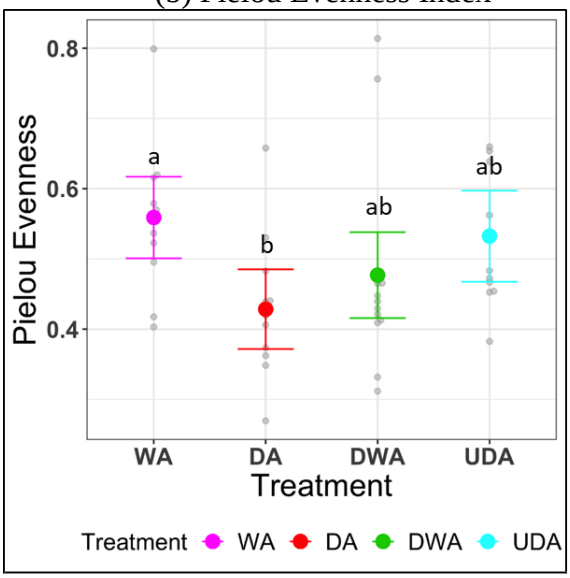
Figure 5.1 Principle component analysis (PCA) of significant metabolites from cull cow beef loins (M. *longissimus lumborum*) aged with different aging methods [Wet aging (WA), Dry aging (DA), Dry aging in water-permeable bag (DW) and UV-light dry-aging (UDA)].

(a) Chao1 Richness Index



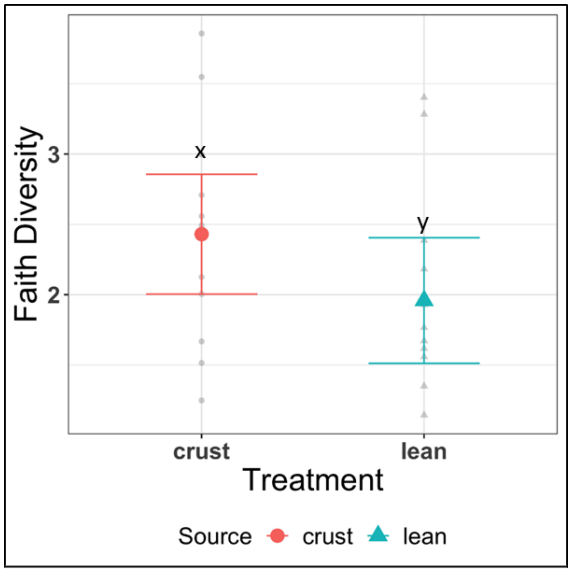
	Effect	P-value
Chao1	Treatment	0.0284
	Source	0.3039
	Treatment x Source	0.0549

(b) Pielou Evenness Index



	Effect	P-value
Pielou Evenness	Treatment	0.0099
	Source	0.8893
	Treatment x Source	0.4027

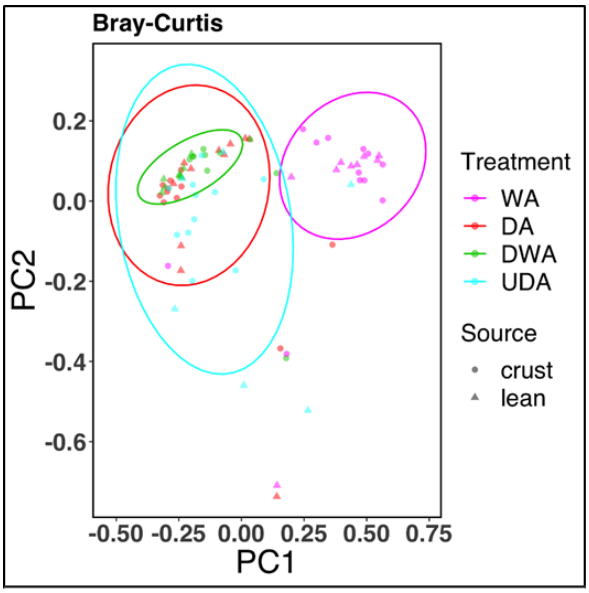
(c) Faith Diversity Index



	Effect	P-value
Faith Diversity	Treatment	0.1969
	Source	0.0117
	Treatment x Source	0.1109

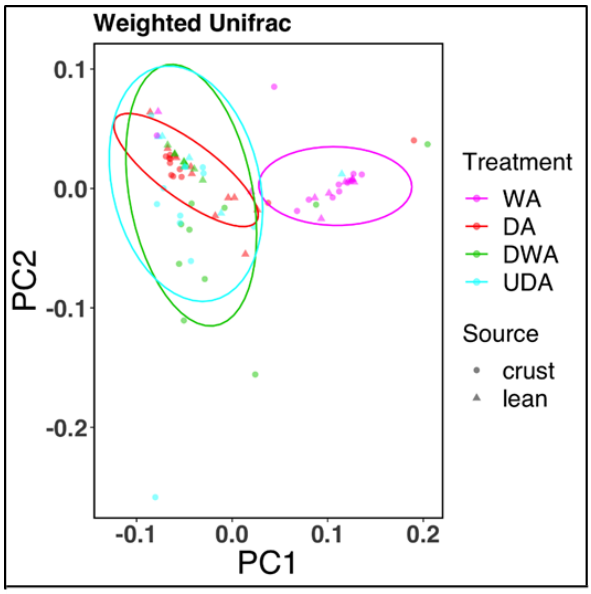
Figure 5.2. Microbiome alpha diversity index on microbial community collected from cull cow beef loins (M. *longissimus lumborum*) aged with different aging methods [Wet aging (WA), Dry aging (DA), Dry aging in water-permeable bag (DW) and UV-light dry-aging (UDA)].[a-b] Means with different letter indicates significant differences (P<0.05). [a-b] Means with different letter indicates significant treatment effect (P<0.05). [x-y] Means with different letter indicates significant source effect (P<0.05)

(a) Bray-Curtis Dissimilarity



	Effect	P-value
Homogeneity Test	Treatment x Source	0.467
PERMANOVA Test	Treatment	0.001
	Source	0.092
	Treatment x Source	0.003

(b) Weighted Unifrac Distance



	Effect	P-value
Homogeneity Test	Treatment x Source	0.059
PERMANOVA Test	Treatment	0.001
	Source	0.024
	Treatment x Source	0.478

Figure 5.3. Microbiome beta diversity measures on microbial community collected cull cow beef loins (M. *longissimus lumborum*) aged with different aging methods [Wet aging (WA), Dry aging (DA), Dry aging in water-permeable bag (DW) and UV-light dry-aging (UDA)].

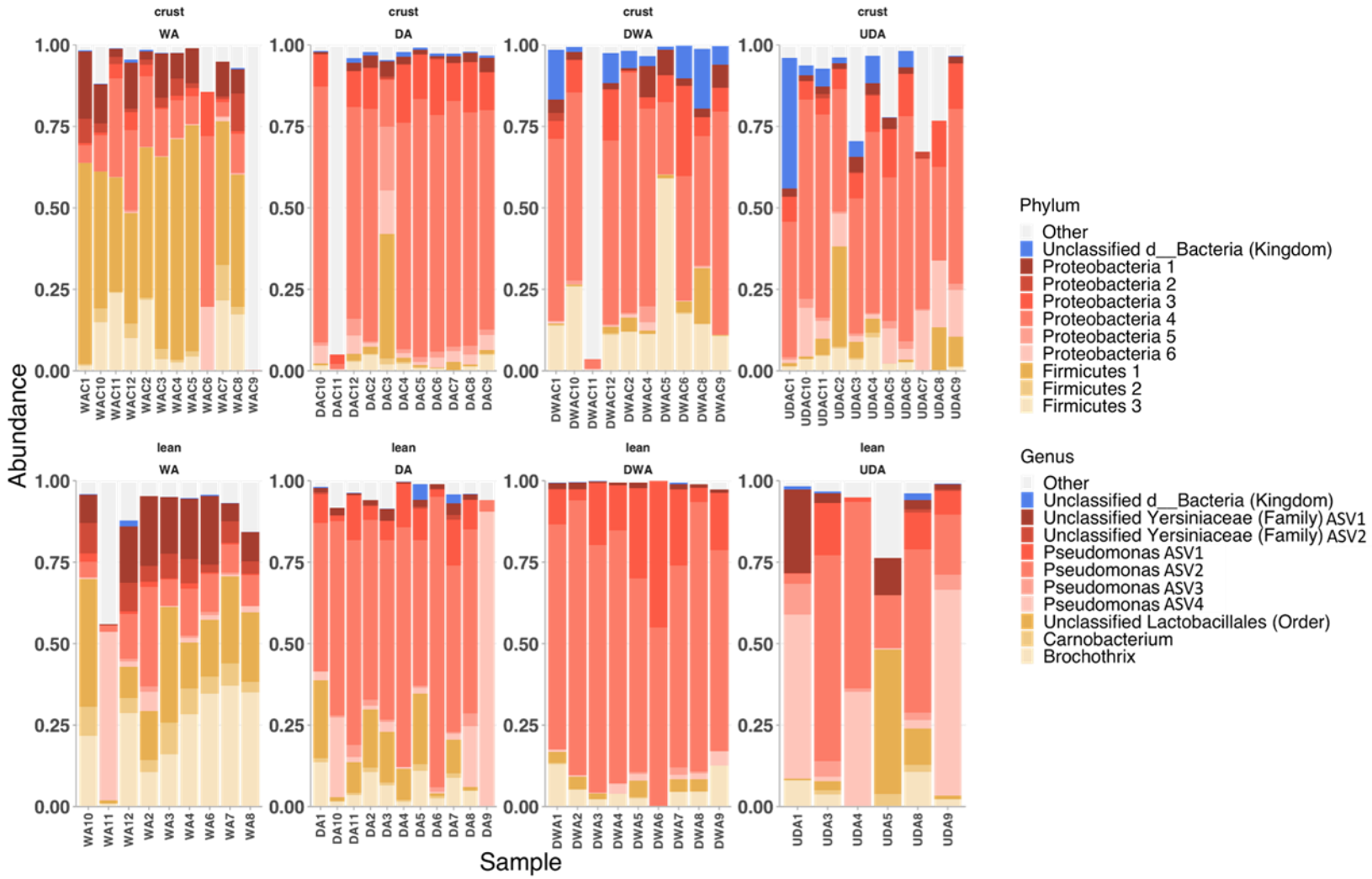


Figure 5.4. Relative abundances of top 10 bacterial ASVs from cull cow beef loins (M. *longissimus lumborum*) aged with different aging methods [Wet aging (WA), Dry aging (DA), Dry aging in water-permeable bag (DW) and UV-light dry-aging (UDA)].

5.8 Literatures Cited

Abraham, A., J.W. Dillwith, G.G. Mafi, D.L. VanOverbeke, and R. Ramanathan. 2017. Metabolite Profile Differences between Beef Longissimus and Psoas Muscles during Display. Meat Muscle Biol. 1:18. doi:10.22175/mmb2016.12.0007.

Baek, H.H. 2007. Process Flavors. In Handbook of Meat, Poultry and Seafood Quality. Blackwell Publishing. 151–162.

Berger, J., Y.H.B. Kim, J.F. Legako, S. Martini, J. Lee, P. Ebner, and S.M.S. Zuelly. 2018. Dry-aging improves meat quality attributes of grass-fed beef loins. Meat Sci. 145:285–291. doi:10.1016/j.meatsci.2018.07.004.

Calkins, C.R., and J.M. Hodgen. 2007. A fresh look at meat flavor. Meat Sci. 77:63–80. doi:10.1016/j.meatsci.2007.04.016.

Callahan, B.J., P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, and S.P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 2016 137. 13:581–583. doi:10.1038/nmeth.3869.

Cangelosi, G.A., and J.S. Meschke. 2014. Dead or alive: Molecular assessment of microbial viability. Appl. Environ. Microbiol. 80:5884–5891. doi:10.1128/AEM.01763-14.

Capouya, R., T. Mitchell, D.L. Clark, D.I. Clark, and P. Bass. 2020. A survey of microbial communities on dry-aged beef in commercial meat processing facilities. Meat Muscle Biol. 4:5–6. doi:10.22175/mmb.10373.

Casaburi, A., P. Piombino, G.J. Nychas, F. Villani, and D. Ercolini. 2015. Bacterial populations and the volatilome associated to meat spoilage. Food Microbiol. 45:83–102. doi:10.1016/j.fm.2014.02.002.

Chail, A., J.F. Legako, L.R. Pitcher, T.C. Griggs, R.E. Ward, S. Martini, and J.W. MacAdam. 2016. Legume finishing provides beef with positive human dietary fatty acid ratios and consumer preference comparable with grain-finished beef1. J. Anim. Sci. 94:2184–2197. doi:10.2527/jas.2015-0241.

Dashdorj, D., T. Amna, and I. Hwang. 2015. Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: an overview. Eur. Food Res. Technol. 241:157–171. doi:10.1007/s00217-015-2449-3.

Dashdorj, D., V.K. Tripathi, S. Cho, Y. Kim, and I. Hwang. 2016. Dry aging of beef; Review. J. Anim. Sci. Technol. 58:20. doi:10.1186/s40781-016-0101-9.

DeGeer, S.L., M.C. Hunt, C.L. Bratcher, B.A. Crozier-Dodson, D.E. Johnson, and J.F. Stika. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Sci. 83:768–774. doi:10.1016/j.meatsci.2009.08.017.

Diez-Simon, C., R. Mumm, and R.D. Hall. 2019. Mass spectrometry-based metabolomics of volatiles as a new tool for understanding aroma and flavour chemistry in processed food products. Metabolomics. 15. doi:10.1007/s11306-019-1493-6.

Dikeman, M.E., E. Obuz, V. Gök, L. Akkaya, and S. Stroda. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef Longissimus lumborum steaks. Meat Sci. 94:228–233. doi:10.1016/j.meatsci.2013.02.002.

Dinh, T.T.N., J.F. Legako, M.F. Miller, and J.C. Brooks. 2018. Effects of USDA quality grade and cooking on water-soluble precursors of beef flavor. Meat Sci. 146:122–130. doi:10.1016/j.meatsci.2018.08.008.

Dinh, T.T.N., K.V. To, and M.W. Schilling. 2021. Fatty Acid Composition of Meat Animals as Flavor Precursors. Meat Muscle Biol. 5. doi:10.22175/mmb.12251.

Foraker, B.A., D.R. Woerner, D. Gredell, J.D. Tatum, J.F. Legako, K.E. Belk, and R.D. Stevens. 2020. Flavor, Tenderness, and Related Chemical Changes of Aged Beef Strip Loins. Meat Muscle Biol. 4. doi:10.22175/mmb.11115.

Gardner, K., and J.F. Legako. 2018. Volatile flavor compounds vary by beef product type and degree of doneness. J. Anim. Sci. 96:4238–4250.

Kim, J.H., M.Y. Jeon, and C.H. Lee. 2019. Physicochemical and sensory characteristics of commercial, frozen, dry, and wet-aged Hanwoo sirloins. Asian-Australasian J. Anim. Sci. 32:1621–1629. doi:10.5713/ajas.18.0610.

Kim, Y.H.B., R. Kemp, and L.M. Samuelsson. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Sci. 111:168–176. doi:10.1016/j.meatsci.2015.09.008.

Koutsidis, G., J.S. Elmore, M.J. Oruna-Concha, M.M. Campo, J.D. Wood, and D.S. Mottram. 2008. Water-soluble precursors of beef flavour. Part II: Effect of post-mortem conditioning. Meat Sci. 79:270–277. doi:10.1016/J.MEATSCI.2007.09.010.

Kozich, J.J., S.L. Westcott, N.T. Baxter, S.K. Highlander, and P.D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Appl. Environ. Microbiol. 79:5112–5120. doi:10.1128/AEM.01043-13.

Lee, H.J., J. Choe, M. Kim, H.C. Kim, J.W. Yoon, S.W. Oh, and C. Jo. 2019a. Role of moisture evaporation in the taste attributes of dry- and wet-aged beef determined by chemical and electronic tongue analyses. Meat Sci. 151:82–88. doi:10.1016/j.meatsci.2019.02.001.

Lee, H.J., J.W. Yoon, M. Kim, H. Oh, Y. Yoon, and C. Jo. 2019b. Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. Meat Sci. 153:152–158. doi:10.1016/j.meatsci.2019.03.019.

Ma, D., Y.H.B. Kim, B. Cooper, J.-H. Oh, H. Chun, J.-H. Choe, J.P. Schoonmaker, K. Ajuwon, and B. Min. 2017. Metabolomics Profiling to Determine the Effect of Postmortem Aging on Color and Lipid Oxidative Stabilities of Different Bovine Muscles. J. Agric. Food Chem. 65:6708–6716. doi:10.1021/acs.jafc.7b02175.

Min, B., and D.U. Ahn. 2005. Mechanism of Lipid Peroxidation in Meat and Meat Products -A Review. Food Sci. Biotechnol. 14:152–163.

Mottram, D.S. 1998. Flavour formation in meat and meat products: A review. Food Chem. doi:10.1016/S0308-8146(98)00076-4.

Mungure, T.E., M.M. Farouk, E.J. Birch, A. Carne, M. Staincliffe, I. Stewart, and A.E.D.A. Bekhit. 2020. Effect of PEF treatment on meat quality attributes, ultrastructure and metabolite profiles of wet and dry aged venison Longissimus dorsi muscle. Innov. Food Sci. Emerg. Technol. 65:102457. doi:10.1016/j.ifset.2020.102457.

O'Fallon, J. V., J.R. Busboom, M.L. Nelson, and C.T. Gaskins. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 85:1511–1521. doi:10.2527/jas.2006-491.

Oksanen, J., F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, P.R. Minchin, R.B. O'hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs, and H.W. Maintainer. 2020. Package "vegan" Title Community Ecology Package Version 2.5-7.

Pothakos, V., F. Devlieghere, F. Villani, J. Björkroth, and D. Ercolini. 2015. Lactic acid bacteria and their controversial role in fresh meat spoilage. Meat Sci. 109:66–74. doi:10.1016/J.MEATSCI.2015.04.014.

Pugliese, C., F. Sirtori, M. Škrlep, E. Piasentier, L. Calamai, O. Franci, and M. Čandek-Potokar. 2015. The effect of ripening time on the chemical, textural, volatile and sensorial traits of Bicep femoris and Semimembranosus muscles of the Slovenian dry-cured ham Kraški pršut. Meat Sci. 100:58–68. doi:10.1016/J.MEATSCI.2014.09.012.

Ramalingam, V., Z. Song, and I. Hwang. 2019. The potential role of secondary metabolites in modulating the flavor and taste of the meat. Food Res. Int. 122:174–182. doi:10.1016/J.FOODRES.2019.04.007.

Ribeiro, F.A., S.K. Lau, R.A. Furbeck, N.J. Herrera, M.L. Henriott, N.A. Bland, S.C. Fernando, J. Subbiah, G.A. Sullivan, and C.R. Calkins. 2021. Ultimate pH effects on dry-aged beef quality. Meat Sci. 172:309–1740. doi:10.1016/j.meatsci.2020.108365.

Setyabrata, D., B.R. Cooper, T.J.P. Sobreira, J.F. Legako, S. Martini, and Y.H.B. Kim. 2021a. Elucidating mechanisms involved in flavor generation of dry-aged beef loins using metabolomics approach. Food Res. Int. 139:109969. doi:10.1016/j.foodres.2020.109969.

Setyabrata, D., S. Xue, K.R. Vierck, J.F. Legako, P. Ebner, and S.M. Zuelly. 2021b. Impact of Various Dry-Aging Methods on Meat Quality and Palatability Attributes of Beef Loins from Cull Cow. Meat Muscle Biol. Accepted-.

Vierck, K.R., J.F. Legako, J.K. Kim, B.J. Johnson, and J.C. Brooks. 2020. Determination of Package and Muscle-Type Influence on Proteolysis, Beef-Flavor-Contributing Free Amino Acids, Final Beef Flavor, and Tenderness. Meat Muscle Biol. 4:1–14. doi:10.22175/mmb.10933.

Whitfield, F.B., and D.S. Mottram. 1992. Volatiles from interactions of Maillard reactions and lipids. Crit. Rev. Food Sci. Nutr. 31:58. doi:10.1080/10408399209527560.

Zhao, C.J., A. Schieber, and M.G. Gänzle. 2016. Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations – A review. Food Res. Int. 89:39–47. doi:10.1016/J.FOODRES.2016.08.042.

Zou, T., J. Liu, H. Song, and Y. Liu. 2018. Discovery of Amadori-Type Conjugates in a Peptide Maillard Reaction and Their Corresponding Influence on the Formation of Pyrazines. J. Food Sci. 83:1588–1595. doi:10.1111/1750-3841.14156.

CHAPTER 6. EFFECT OF DRY-AGING ON QUALITY AND PALATABILITY ATTRIBUTES AND FLAVOR-RELATED METABOLITES OF PORK LOINS

The content presented in this chapter is a reprint of our previously published work titled "Effect of Dry-Aging on Quality and Palatability Attributes and Flavor-Related Metabolites of Pork Loins." Some edits have been made to produce a cohesive dissertation. The published work reference is Setyabrata, D., Wagner, A. D., Cooper, B. R., & Kim, Y. H. B. (2021). Effect of Dry-Aging on Quality and Palatability Attributes and Flavor-Related Metabolites of Pork Loins. Foods, 10(10), 2503.

6.1 Abstract

This study evaluated the effect of dry-aging on quality, palatability, and flavor-related compounds of pork loins. Ten pork loins were obtained at 7 days postmortem, divided into three equal portions, randomly assigned into three different aging methods (wet-aging (W), conventional dry-aging (DA), and UV-light dry-aging (UDA)), and aged for 21 days at 2 °C, 70% RH, and 0.8 m/s airflow. The results showed similar instrumental tenderness values across all treatments ($P>0.05$), while DA and UDA had a greater water-holding capacity than WA ($P<0.05$). Both DA and UDA were observed to have comparable color stability to WA up to 5 days of retail display ($P>0.05$). Greater lipid oxidation was measured in both DA and UDA at the end of display compared to WA ($P<0.05$). The UV light minimized microorganisms concentration on both surface and lean portions of UDA compared to other treatments ($P<0.05$). The consumer panel was not able to differentiate any sensory traits and overall likeness between the treatments ($P>0.05$). Metabolomics analysis, however, identified more flavor-related compounds in dry-aged meat. These findings suggested that dry-aging can be used for pork loins for value-seeking consumers, as it has a potential to generate unique dry-aged flavor in meat with no adverse impacts on meat quality and microbiological attributes.

Keywords: Dry-aging, Loin, Pork, Metabolomics, Consumer sensory, microbial attributes

6.2 Introduction

The pork industry's focus on growth efficiency has led to the production of leaner and heavier pigs (Lonergan et al., 2001). While improvements in feed efficiency and growth performance have increased the yields and profitability of the swine industry, quality issues in the final products, such as inferior tenderness, juiciness, and flavor, have been reported (Hoa et al., 2019; Channon et al., 2017; Fortin et al., 2005). Providing high-quality (palatability) meat products is a vital factor for consumer satisfaction and, in the long term, for the profitability and sustainability of the pork industry (Miller, 2020). In order to meet consumer expectations for high-quality meat products, post-harvest enhancement techniques, such as brine injection and marination with non-meat ingredients, are often applied to pork products (Baublits et al., 2006; Sheard and Tali, 2004; Prestat et al., 2002). Although the application of these techniques has been proven to improve eating quality attributes, there is growing demand for more natural and minimally processed meat products among consumers (Verbeke et al., 2010).

Postmortem aging is a natural value-adding process extensively practiced by the meat industry. The application of postmortem aging has been well documented to further improve the sensory attributes of meat, increasing the tenderness, juiciness, and flavor perceived from the products (Kim et al., 2018b). Across the industry, wet-aging (aging by storing meat in vacuum packaging) is the most commonly utilized aging method. Recently, however, there has been an increasing interest in fresh meat products from dry-aged carcasses or subprimals from value-seeking consumers (Park et al., 2019). Dry-aging is a traditional aging method, where meat is aged without any protective packaging material in a highly controlled environment. In addition to the improvement in both tenderness and juiciness, the application of dry-aging has been reported to generate unique flavors such as "sweet," "buttery," and "brown-roasted" in beef, making the final products more desirable (Kim et al., 2016; Campbell et al., 2001). The generation of desirable meat flavors has been known to be dictated by the availability of flavor precursors such as amino acids, sugars, nucleotides, and fatty acids in the meat product (Mottram, 1998).

The development of high-throughput analysis, such as metabolomics, has enabled comprehensive understanding of biological function through the chemical and biochemical profiling of small compounds (metabolites) in a biological sample. Recently, there has been a growing interest in adopting metabolomics in meat research to gain insights into biochemical and molecular changes of postmortem muscle and their concomitant impacts on meat quality attributes

(Ma et al., 2017; Abraham et al., 2017; Kim et al., 2016; D'Alessandro et al., 2012). By utilizing mass spectrometry (MS)-based metabolomics analysis, greater abundance of free amino acids, nucleotides, and sugars were reported in dry-aged beef, potentially explaining the greater flavor observed from dry-aged products (Setyabrata et al., 2021a; Kim et al., 2016). Moreover, reduction in off-flavor-related metabolites such as terpenoids and hormones coupled with observed sensory detection were reported, revealing the additional flavor development mechanism following the dry-aging process (Setyabrata et al., 2021a). These results indicate metabolomics as a novel approach to elucidate and profile flavor-related compounds in meat products.

Currently, dry-aging has been extensively studied in beef products, and only limited research has reported the impacts of dry-aging on pork loin quality attributes (Hwang and Hong, 2020; Jin and Yim, 2020; Kim et al., 2018a; Juárez et al., 2011). While some levels of conventional chemical analyses along with trained sensory evaluation were conducted, the alteration of flavor precursors and flavor-related metabolites in dry-aged pork loin products have never been profiled. Moreover, given the nature of dry-aging, the presence of microorganisms during the process is inevitable. Consequently, UV lights are often employed by the processors in order to prevent any growth of spoilage bacteria and minimize the microorganism contamination in meat during aging (Lepper-Blilie et al., 2016; Smith et al., 2014). In recent reports, however, it was suggested that the presence of some microorganism could be vital for the development of dry-aging flavor, potentially through the release of proteolytic and lipolytic enzymes into the meat, allowing greater liberation of flavor precursors (Lee et al., 2019; Ryu et al., 2018). While UV light application has been shown to be effective in reducing spoilage bacteria and pathogens in various meat applications (Ganan et al., 2013; Chun et al., 2010), the impact of UV light on dry-aging flavor development is still unclear. Hence, the objectives of this study were to determine the meat quality and consumer acceptance of dry-aged pork loin products and to investigate the flavor precursor differences between dry- and wet-aged pork loins using a novel metabolomics approach.

6.3 Materials and methods

6.3.1 Sample collection, preparation and processing

At 7 days postmortem, bone-in and skin-on loins (*M. longissimus thoracis et lumborum,* from 11^{th}–21^{st} vertebrae) were obtained from one side of 10 market-weight pork carcasses (left side, live

weight = 117.3 ± 1.7 Kg, crossbreed Landrace x Large White x Duroc, National Pork Board marbling score = 1.4) from Purdue University Meat Laboratory harvest facility. Prior to processing, initial microbiological samples were excised from the lean meat portion of the loin eye (anterior side) of each loin sample, placed in sterile sample bags, and stored in −80 °C until analyses. All loins were then divided into three equal sections (~15 cm) using a band saw and randomly assigned into three aging methods: wet-aging (WA; Clarity Vacuum Pouches Bunzl Processor Division, Riverside, MO, USA), conventional dry-aging (DA), and UV-light dry-aging (UDA).

All sections were measured for initial pH and weight prior to 21 days of aging at 2 °C, 70% relative humidity, and 0.8 m/s airflow. The samples were placed on food-safe racks (Uline, Pleasant Praire, WI, USA) for the aging process. The UDA samples were exposed to UV-light treatment twice each day with a dose of 5 J/cm^2 per treatment. The UV lights (Phillip TUV T8 UVC light, Eindhoven, Netherlands) were mounted 30 cm above the loins and turned on for 5 minutes per treatment. Sections were rotated weekly to reduce location variation during aging. At the end of aging, sections were measured for final weight. All sections were then skinned, deboned, trimmed of any dehydrated surface, and weighed for the final yield estimation. Microbial samples were collected from trimmed dehydrated surfaces and lean portion of each loin by immediately excising the inner lean meat portion following trimming. The samples were placed in sterile sample bags and stored in −80 °C until analyses, similar to the initial microbiological samples. The trimmed loins were then measured for their pH and cut into multiple chops for further meat quality (2.54 cm thick) and biochemical analyses (1.27 cm thick). Except for the chops assigned for the color display and drip loss analyses, all chops were vacuum-packed individually and stored in a −80 °C freezer until analyses.

6.3.2 Aging Loss, Processing Loss, and Saleable Yield

The aging loss was measured by calculating the weight differences before and after the aging treatments to observe the shrink/water loss during aging. Final weights were collected for the sections to calculate trimming loss and final yield following the trimming process. All of the losses were presented as percentage loss.

6.3.3 pH measurement

The pH was measured using a hand-held meat pH meter (HANNA HI 99163, Hanna Instrument, Inc., Warner, NH, USA) before and after the aging treatments. The probe was inserted directly into the meat in two different locations. The pH meter was calibrated according to the manufacturer's guidelines before any measurement.

6.3.4 Water-holding capacity measurement

The water-holding capacity (WHC) was measured by measuring drip loss, display loss, freeze/thaw loss, and cook loss. All measurements were expressed as percent loss, measuring the weight changes between the initial and final weight of the samples following each procedure. All samples were blotted dry using paper towels prior to any weight measurement.

The drip loss was measured using the Honikel method (Honikel, 1998) with the modification described by Kim et al. (Kim et al., 2017). In brief, 40 g of meat was collected from each sample. The cubes (about 2.54 × 2.54 × 2.54 cm) were trimmed of any visible connective tissues and fat and were then suspended using netting for 48 h in airtight containers at 2 °C. Immediately after, the final weights of the samples were measured to calculate the drip loss (%).

The display loss was measured on the chops designated for color display simulation. Chops were weighed before display and were then re-weighed following the 7 days color display.

For freeze/thaw loss, samples designated for cook loss and Warner–Bratzler shear force (WBSF) were utilized. The frozen samples were thawed at 2 °C for 24 h prior to final weight measurement. The loss was determined by calculating the differences between the weight before and after the freezing and thawing process.

The cook loss was observed by cooking the sample to an internal temperature of 71 °C using a clamshell grill (dual-sided grill) (Griddler GR-150, Cuisinart, Glendale, AZ, USA) and monitored using a T type thermocouple (Omega Engineering, Stamford, CT, USA) connected to an OctTemp 2000 data logger (Madge Tech, Inc., Warner, NH, USA). When the internal temperature was reached, samples were removed from the griddle and rested for 10 minutes prior to weighing for the final weight. Samples were then wrapped using aluminum foil and kept in a 4 °C refrigerator overnight for WBSF measurement.

6.3.5 Warner–Bratzler Shear Force Measurement

The shear force was measured by collecting a total of 8 cores parallel to the muscle fiber direction from each chop. The cores (1.27 cm diameter) were cut perpendicular to the muscle fiber using TA-XT Plus Texture Analyzer (Stable Micro System Ltd., Godalming, UK) using the V-shaped blade attachment for the WBSF measurement. The crosshead speed was set to 3 mm/second, and a 2.5 Kg load cell was utilized during the measurement. The average peak shear force (*N*) from the cores was calculated.

6.3.6 Display color stability

One chop from each section was collected for simulated color display. The chops were placed on Styrofoam trays with drip pad, overwrapped using PVC film (Reynolds Food Service Packaging, Richmond, VA, USA), and displayed for 7 days under light (1800 lx, color temperature = 3500 K, OCTRON® T8 Lamps, Osram Sylvania LTD., Canada) at 2 °C. The samples were evaluated daily for color using Hunter MiniScan EZ colorimeter (Hunter, Reston, VA, USA), measuring the CIE L*, a*, and b* on three random locations on the surface of the chops. The instrument was calibrated following the manufacturer's guidelines and equipped with a 25 mm (diameter) port opening prior to any data collection. Illuminant A was used, and the observer was set to standard 10°. Hue angle and Chroma value were calculated using the following formulas: hue angle = tan − 1(b*/a*) and Chroma = (a*2 + b*2)½ (AMSA, 2012).

6.3.7 Lipid Oxidation

Prior to the analysis, the whole fresh pork chops assigned for biochemical analysis were minced, submerged into liquid nitrogen, and pulverized using a blender (Waring Products, CT, USA). The lipid oxidation extent of the samples was measured through the 2-thiobarbituric reactive substances (TBARS) assay described by Buege and Aust (1978) with modification by Setyabrata and Kim (2019). In brief, 5 g of the pulverized samples was homogenized in 15 mL of distilled water and 50 µL of 10% butylated hydroxyl anisole. Following homogenization, 1 mL of the homogenate was added to 2 mL of 20 mM 2-thiobarbituric acid solution in 15% tricholoroacetic acid solution. The samples were then mixed and heated in an 80 °C water bath for 15 minutes. The samples were removed and cooled in ice water for 10 minutes prior to

centrifugation at 2000× *g* for 10 minutes. After centrifugation, the supernatant was filtered through a Whatman Filter Paper No. 4 (Cytiva, Marlborough, MA, USA). The samples' absorbance was then read at 531 nm using Epoch™ Microplate Spectrophotometer (BioTek Instrument Inc., Winooski, VT, USA). The TBARS value was calculated using a molecular extinction coefficient ($1.56 \times 105\ M^{-1}\ cm^{-1}$) and expressed as mg malondialdehyde/Kg meat. Lipid oxidation was measured on samples collected before and after the display.

6.3.8 Microbial analysis

The microbial analyses were conducted following the method described by Setyabrata et al., (2021b). The microbial analysis was performed on the initial (prior to aging), surface, and lean portions from each sample collected after aging treatments. The samples were thawed for 6 h prior to the analyses. In brief, 5 g of sample was aseptically collected and placed into a stomacher bag (WhirlPak, Madison, WI, USA) with 50 mL 0.1% peptone water (BD Difco™, Sparks, MD, USA). The samples were then hand stomached for 1 minute. The rinsate was collected and serially diluted using a 1:10 dilution factor with the dilution range of 10^0 to 10^{-5}. All dilutions were then plated in duplicate into plate count agar (BD Difco™, Sparks, MD, USA) for total aerobic bacteria plate count (APC); de Man, Rogosa, and Sharpe agar (BD Difco™, Sparks, MD, USA) for lactic acid bacteria (LAB); and Yeast and Mold films (Petrifilm™, 3M Microbiology Products, St. Paul, MN, USA) for both yeast and mold enumeration. After inoculation, the APC plates were incubated at 37 °C for 48 h. The LAB plates were incubated under anaerobic conditions generated using anaerobic packs (Oxoid™ AnaeroGen, Waltham, MA, USA) for 72 h at 37 °C. The yeast and mold films were incubated at 25 °C for 120 h. After the designated incubation period, colonies were counted, and the final concentration was expressed as log10 CFU/mL of rinsate. For APC and LAB measurement, plates with colonies count below 25 colonies on the lowest dilution were considered to have bacterial concentration below detection limit (BDL). For the yeast and mold petrifilm, the detection limit was set at 15 colonies per the manufacture's recommendation.

6.3.9 Consumer Sensory Analysis

The consumer sensory evaluation was conducted at Purdue University, and the exemption was approved by Purdue University Institutional Review Board (#IRB-2019-16). The consumer

sensory evaluation was conducted using 120 panelists recruited from the community surrounding the West Lafayette, Indiana area.

The samples collected for the sensory analysis were thawed at 2 °C overnight before the sensory session. All samples were cooked using a clamshell grill (Griddler GR-150, Cuisinart, Glendale, AZ, USA) until the internal temperature reached 71 °C. Following the cooking process, chops were trimmed from any visible fat and connective tissues. The chops were then cut into 1 cm × 1 cm × 2.54 cm cubes, placed into a sample cup with a lid and kept in a warmer held at 60 °C for no longer than 15 minutes prior to serving. The samples were served under a red incandescent light, and panelists were supplied with water and unsalted saltine crackers as a palate cleanser. A starter chop (wet-aged, 2 weeks) was also served and evaluated first prior to performing the actual samples to help the panelist adjust to the evaluation process.

Prior to the sample evaluation, a basic demographic survey was conducted. For the sensory evaluation, samples were scored using an unstructured hedonic test with a scale of 0 to 100 points (0 as dislike extremely, 50 as neither like or dislike, and 100 as like extremely) to observe the flavor, tenderness, juiciness, and overall liking. Additionally, the panelists were also asked to rate the acceptability (acceptable or unacceptable) of each attribute tested and each sample's perceived quality (unsatisfactory quality, everyday quality, better than everyday quality, and premium quality). After all samples were evaluated, a questionnaire regarding the dry-aging process, pork dry-aging, and willingness to pay was provided as an end survey.

6.3.10 Metabolomics Analysis

Metabolite Extraction

A total of 5 samples were randomly selected from each treatment for the metabolomics analysis. The metabolomics analysis was conducted using the previously homogenized biochemical samples, as described in Section 2.7. Briefly, 200 mg of homogenized sample was mixed with 300 µL of methanol in a tube containing ceramic beads. The samples were then extracted using a Precellys 24 tissue homogenizer (Bertin Instruments, Bretonneux, France). A total of 3 cycles was used to extract the sample, each running for 30 seconds at 6500 rpm with 30 seconds rest in between the cycles. Following the extraction, 300 µL of chloroform was added to the tube and mixed for 10 seconds. Water (300 µL) was then added, and the tubes were placed on a shaker for 15 minutes at 4 °C. The tubes were then centrifuged at 1000× g for 5 minutes to

separate the layers. The upper layer was then collected, transferred to a vial, and dried using a SpeedVac Concentrator (Thermo Scientific, Waltham, MA, USA).

Ultra-Performance Liquid Chromatography-Mass Spectrometer Analysis

Prior to chromatographic separation, the dried samples were reconstituted in an aqueous solution (95% water with 5% acetonitrile) containing 0.1% formic acid. The samples were then separated using similar conditions described by Setyabrata et al. (2021a). The reconstituted samples were separated using an Agilent 1290 Infinity II UPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Waters Acquity HSS T3 (2.1 × 100 mm × 1.8 um) separation column (Waters, Milford, MA, USA) and an HSS T3 (2.1 × 5 mm × 1.8 um) guard column. The sample injection volume was set to 5 μL. The binary mobile phase consisted of solvent A (0.1% formic acid (*v*/*v*) in ddH2O) and solvent B (0.1% formic acid (*v*/*v*) in acetonitrile). The column was maintained at 40 °C with the mobile phase flow kept at 0.45 mL/min. Initial conditions of 100:0 A:B were held for 1 minute, followed by a linear gradient to 70:30 over 15 minutes, followed by a linear gradient to 5:95 over 5 minutes, with a 5:95 hold for 1.5 minutes. Column re-equilibration was performed by returning to initial starting conditions of 100:0 over 1 minute, with a hold for 5 minutes. Following the separation, the sample was identified using Agilent 6545 quadrupole time-of-flight (Q-TOF) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA), with positive electrospray ionization (ESI) mode applied for mass spectral (70–1000 m/z) data collection. The collected data were analyzed using Agilent MassHunter B.06 software (Agilent Technologies, Santa Clara, CA, USA), and the mass accuracy was improved by infusing Agilent Reference Mass Correction Solution (G1969-85001; Agilent Technologies, Santa Clara, CA, USA). Peak deconvolution was performed using Agilent ProFinder (v B.08). Peak identification was improved by applying data-dependent MS/MS collection on composite samples with 10 eV, 20 eV, and 40 eV collision energy. The metabolites were identified by comparing them to the human metabolome database (HMDB; www.hmdb.ca, accessed 01 September 2021), with a tolerance of 0.1 Da for MS1 and 0.5 Da for MS2.

6.3.11 Statistical analysis

The experimental design of the current study was a randomized complete block design. The animal was considered as the random effect, and the different aging treatments were considered as

the fixed effect in the model. Sample location source was added as a fixed effect during the microbiological analysis to identify potential location effect. The period effect was also added as a fixed effect for the color and oxidative analyses. Both panelists and sessions were added as a random effect for the sensory evaluation. The data were analyzed using PROC GLIMMIX procedure from SAS 9.4 software (SAS Institute Inc., Cary, NC). The least-square means were separated, and the significance level was defined at the level of $P<0.05$.

Metabolomics analysis were conducted using MetaboAnalyst 5.0 (Pang et al., 2021). Metabolite features with missing values were given a small value using half of the minimum value in the original samples. The data were then normalized by log transformation and were scaled using the auto-scaled option (mean-centered and divided by the standard deviation). The metabolomics data were then subjected to ANOVA with Tukey post hoc testing, principal component analysis (PCA), and hierarchical cluster analysis (HCA) with ward clustering methods.

6.4 Results

6.4.1 Processing loss and saleable yield

6.5 Greater processing loss was observed from both DA and UDA loins compared to WA loins ($P<0.05$, Tables and Figures

Table 6.1), leading to a higher total yield for WA treatments compared to the other treatments ($P<0.05$). The processing loss consisted of shrink/purge loss, crust loss, fat/skin loss, and bone loss. No differences were observed for fat/skin loss and bone loss for all the treatments ($P>0.05$). Both DA and UDA samples, however, had more shrink/purge loss compared to WA samples ($P<0.05$). Consequently, more crust loss was also observed from both DA and UDA loins compared to WA loins ($P<0.05$).

6.5.1 pH, Water-Holding Capacity, and Shear Force

Higher pH ($P<0.05$, Table 6.2) was measured in UDA (5.62) compared to WA and DA (5.58 and 5.59, respectively) samples.

For water-holding capacity (WHC), it was found that WA chops had reduced WHC compared to both DA and UDA chops, indicated by higher drip loss, display loss, and freeze/thaw

loss measured in WA chops (P<0.05; Table 6.2). Based on the current results, it could be postulated that the greater moisture loss during the dry-aging process decreased the available moisture in the product, hence limiting the meat water loss in subsequent processes. No difference was found for cook loss among all the treatments (P>0.05).

The different aging treatments did not affect the WBSF of the samples (P>0.05, Table 6.2), having comparable shear force values of 26.41, 25.08, and 27.05 N for WA, DA, and UDA loins, respectively.

6.5.2 Display color stability

A significant treatment and period interaction was observed on all instrumental color traits measured, except for b* (yellowness) and Chroma, during the 7-day retail display (Figure 6.1). The lightness (L*) was found to be generally lower (P<0.05) for both DA and UDA chops throughout the retail display compared to WA chops. The redness (a*) was initially comparable (P>0.05); however, significantly lower redness was then identified in both DA and UDA chops on days 4, 5, and 7 of the display. More discoloration was detected in both DA and UDA samples from day 5 until the end of the display compared to WA samples (P<0.05), indicated by the hue angle values.

6.5.3 Oxidative stability

A significant period and aging treatment interaction was identified for lipid oxidation of the samples based on the TBARS analysis (Figure 6.2). The lipid oxidation was increased over the simulated display regardless of the treatments (P<0.05). Prior to the display, no difference was observed across all treatments (P>0.05). Following the display, however, greater lipid oxidation (P<0.05) in DA and UDA samples was measured when compared to WA samples.

6.5.4 Microbial Analysis

The initial samples were found to have microbial concentration below the detection limit for all microbial groups measured (data not shown), indicating a microbial load in the product comparable to prior to the aging process. A significant aging treatment and location interaction, however, was observed following the aging across all of the treatments (Table 6.3).

The WA surface was found to have the greatest concentration of APC (2.69 log10 CFU/mL, $P<0.05$), followed by DA crust (1.37 log10 CFU/mL), while UDA crust and lean portions had a similar APC concentration ($P>0.05$). WA surface was also identified to have the highest LAB concentration (2.33 log10 CFU/mL) compared to all other treatments ($P<0.05$). UDA crust and DA lean had concentrations below detection limit for LAB. Both WA and DA crust had no significant differences for mold concentration (1.82 log10 CFU/mL and 1.39 log10 CFU/mL, respectively); however, both were higher compared to other treatments ($P<0.05$). Yeast was only detected on the surface crust of WA and DA treatments, and there was found to be no difference between the two treatments ($P>0.05$).

6.5.5 Demographic and survey data

The consumer demographic data is available in Table 6.4. The panelists were mainly between 20–29 years old (70%). The consumer panelists responded that they mainly consumed pork 1–5 times/week (87.5%). A total of 45.8% of the panelists considered flavor the most important palatability trait when consuming pork products, while both juiciness and tenderness shared a similar percentage (26.7% and 27.5%, respectively). In addition, the panelists in this study indicated that they preferred pork cooked to medium doneness (31.7%), followed by a split between medium-well (25.8%) and well done (26.7%) degree of doneness. Following the sample evaluation, panelists were presented with survey questions involving dry-aging and their willingness to pay for dry-aged pork (Table 6.5). From all of the panelists, 52.5% had previously tried and consumed dry-aged products. It was indicated that restaurant was the primary method to obtain the dry-aged products (39.7%), followed by the local supermarket (23.8%). Most of the panelists agree that meat aging is a positive term (85.8%), and the dry-aging process itself is perceived to generate products with similar safety with other meat products (65.8%). When asked about the willingness to pay for the dry-aged pork product, 55.8% of the consumer panel were willing to pay $1.00 more per 1 lb. (0.45 Kg) of dry-aged pork.

6.5.6 Consumer panel evaluation

The consumer panelists found that the different aging methods generated products with comparable sensory attributes ($P>0.05$, Table 6.6). Similar scores were given by the panelists for

flavor, tenderness, juiciness, and overall liking, regardless of the treatments. The products were also rated to have similar acceptability in all the traits tested ($P>0.05$), with all considered to have acceptable tenderness, juiciness, flavor, and overall acceptability. For the perceived quality, the majority of the samples were considered as everyday quality by the consumer panelists. More panelists, however, rated WA to have premium quality compared to DA ($P<0.05$), while UDA was not different from both WA and DA ($P>0.05$).

6.5.7 Metabolomics Analysis

A total of 1839 metabolite features were observed via the untargeted UPLC-MS analysis. Following the statistical analysis, 197 metabolites were found to be significantly influenced by the aging treatments ($P<0.05$, FDR < 0.05) and were then utilized as a subset for further analysis. Principle component analysis (PCA) of the metabolites revealed a clear clustering of all treatments (Figure 6.3). A distinct separation between dry-aging treatments and wet-aging was exhibited along the PC1 axis, explaining 65.9% of the variation observed between the aging types. Further separation within the dry-aging treatments was observed across the PC2 axis, explaining 11.5% of the variation and demonstrating metabolite profile difference between the DA and UDA treatments. Likewise, HCA (Figure 6.3) presented a more comparable metabolite profile between DA and UDA loins when compared to WA loins.

Of the 197 metabolites, 27 features were identified through MS/MS spectral matching with the HMDB database. A total of 13 metabolites were found to be significantly more abundant in either DA or UDA treatments, 10 metabolites were greatly abundant in the WA treatment, and 4 metabolites were found to be abundant in both WA and DA treatments. Those metabolites could be loosely separated into 4 different groups and presented in Table 6.7. More protein/amino-acid-derived metabolites were found in the dry-aged treatments compared to the WA treatment, including histidine, nitrotyrosine, methylcrotonylglycine, and phenylalanine. Likewise, more nucleotide-derived metabolites (dihydrothymine, thymidine, cyclic AMP, IMP, hypoxanthine, and cytidine) were identified and observed to be in higher abundance for both DA and UDA samples compared to the WA samples. Interestingly, greater concentrations of antioxidant compounds (hydroquinone, niacinamide, and pelargonidin) were observed in WA and DA samples compared to UDA samples.

6.6 Discussion

6.6.1 Processing loss and saleable yield

A decrease in product yields from dry-aged treatments has been constantly reported in previous studies (though predominantly dry-aged beef) (Kim et al., 2016, Kim et al., 2018a, Berger et al., 2018). The substantial decrease in yield is expected, mainly due to the moisture evaporation during the dry-aging process and the removal of the dehydrated surfaces (crust) following the aging process. Our current results were in agreement with Berger et al. (2018), where the authors reported no significant treatment differences in both bone and fat losses for grass-fed beef loins aged using WA, DA, and dry-aging in water-permeable bag methods. In the current study, the skin was kept intact for all treatments during the aging process. It was previously suggested that adding a barrier, such as a moisture-permeable bag, helped minimize the moisture loss during aging (Li et al., 2013; Ahnström et al., 2006). For pork dry-aging, thus, it could be surmised that the presence of the skin during the aging process could act as an additional barrier to limit moisture loss and environmental exposure. Further study to identify the functionality of the skin during dry-aging would be beneficial to increase profit and yield from the process.

6.6.2 pH, Water-Holding Capacity, and Shear Force

Currently, there is still inconsistency in the literature in regards to pH changes following dry-aging treatment in pork. A previous study by Hwang et al., (2018) reported an increase in pH following dry-aging process when compared to wet-aging, showing pH values of 5.91 and 5.73, respectively. Similarly, Kim et al. (2018a) also reported a higher pH value in DA pork compared to WA pork, showing pH values of 5.51 and 5.41, respectively. On the other hand, Jin and Yim (2020) found the pH was not affected by dry-aging application in pork compared to WA, showing pH values of 5.70 and 5.74, respectively. It was suggested that the presence of microbes during the process contributed to the change of pH in the product, whether through the generation of acid or the release of nitrogen products (Lee et al., 2019; Edeghor et al., 2016; Horiuchi and Sasaki, 2012). While the pH was significantly affected in this study, the changes observed were numerically minimal (<0.05 unit difference), and thus its impacts on meat quality attributes would be practically less meaningful.

Similar results were reported by Hwang and Hong (2020), where the shear force values of unpressurized DA pork loins aged for 21 days were not different from those of its WA counterpart, and by Juárez et al. (2011), where the shear force of DA and WA pork loins were not different following 14 days of aging. Likewise, Kim et al. (2016), Berger et al. (2018), and Dikeman et al. (2013) also reported that DA beef exhibited no difference in shear force and texture profile compared to its WA counterpart. This indicated that different aging treatments would not affect the extent of proteolysis in the product, while the length of the aging period might be more influential on product tenderness.

6.6.3 Display color stability

The lower lightness in dry-aged products could be associated with the greater moisture loss in the product. Lower moisture has been associated with less surface moisture availability, leading to a decrease in light reflection and thus resulting in a darker appearance (Kim et al., 2016). These findings are in agreement with previous pork dry-aging studies, where higher initial lightness in wet-aged products compared to dry-aged products was reported (Jin and Yim, 2020; Kim et al., 2018a; Hwang et al., 2018). While changes in color and color stability could be detrimental, studies have reported that pork consumers preferred darker-colored chops over lighter-color chops (Richardson et al., 2018; Mancini and Hunt, 2005; Brewer et al., 2001), suggesting potential dry-aging benefits during retail settings. Although there has been consistency in terms of meat lightness following the dry-aging process, conflicting results were reported for a* value. Previous studies by Jin and Yim (2020) and Hwang et al. (2018) reported that dry-aged pork had a higher a* value compared to the wet-aged counterpart. On the other hand, similar to the current study, Kim et al. (Kim et al., 2018a) reported a decrease in a* value of dry-aged pork compared to wet-aged pork. This discrepancy could potentially be attributed to the pH of the meat samples utilized between the studies. Both Jin and Yim (2020) and Hwang et al. (2018) reported a higher pH value (~5.9) compared to the value measured in the current study and the study by Kim, Kim, et al. (2018) (~5.6). Meat with lower ultimate pH is often observed to have inferior color stability when compared to high–ultimate pH meat (Brewer et al., 2006), mainly due to a decrease in the redox stability of myoglobin (Richardson et al., 2018).

In terms of the color stability of dry-aged meat, only limited information is currently available in the literature. Previous studies in beef reported a significant increase in sensory

discoloration (Ribeiro et al., 2021; Setyabrata et al., 2021b) and instrumental discoloration (Hue angle) (Setyabrata et al., 2021b) in dry-aged beef compared to wet-aged beef during simulated retail display. Similarly, an increase in instrumental discoloration in dry-aged treatments was also identified in the current study, starting from day 5 of the display until the end of the display.

6.6.4 Oxidative stability

The results of the current study indicated that dry-aging altered the oxidative stability of the product. Although no immediate effect was observed, dry-aged products were more susceptible to oxidation, demonstrated by the greater extent of lipid oxidation following the retail display period. Corroborating the current TBARS observation, greater discoloration was also observed on both DA and UDA chops following the color display, indicating a general loss of reducing capability in the meat. It is possible that during the dry-aging process, the environmental exposure initiated the oxidation process in the meat and began the accumulation of radical oxygen species (ROS). While limited, the presence of the ROS could then accelerate further oxidation (Papuc et al., 2017), decreasing the oxidative stability following the dry-aging process.

Interestingly, no difference was observed in the oxidative stability between DA and UDA loin samples. It was previously suggested that UV light application would further induce the extent of oxidation through photo-oxidation (Jongberg et al., 2017). Similar results were previously reported by Setyabrata, Xue, et al. (2021) for dry-aged beef lipid oxidation. Those authors found that the UDA treatment had a similar color to both WA and DA treatments both before and after 7 days of aerobic display storage. It was suggested that the presence of the dehydrated surface acted as a barrier to limit the extent of oxygen transfer and light penetration, therefore minimizing the oxidative impact of UV light.

6.6.5 Microbial Analysis

Generally, greater microbial concentration was measured in the crust portion of the sample and was reduced following the trimming process. Within the crust portion, UDA had the lowest microbial concentration compared to the other treatments, indicating that UV light suppressed microbial growth. Following trimming, however, minimal microbial concentration was detected in the lean portion of the UDA group. Similar results were previously presented by Li et al., (2013),

where a higher concentration of both APC and LAB count was observed in the inner portion when compared to the surface portion after the trimming process. No explanation, however, was provided by those authors. While it is still unclear, it has been suggested that the attached microbes could penetrate into the meat utilizing the gaps between muscle fibers generated during the aging process, thus contaminating the inner portion of the meat (Shirai et al., 2017; Maxcy, 1981; Elmossalami and Wassef, 1971). Additionally, microbe-induced proteolysis was suggested to increase the extent of penetration and allow more microbes to migrate into the inner portion (Shirai et al., 2017). Furthermore, UV light can only affect the areas exposed to the light, and thus still allows microbial growth in unexposed areas (e.g., within meat fold, knife cuts).

6.6.6 Demographic and survey data

The majority of the panelists rated flavor as the most important palatability attribute when consuming pork products compared to tenderness and juiciness. Both juiciness and tenderness are often considered as the main palatability attributes critical for pork acceptability, motivating researchers to work on minimizing these sensory issues (Klehm et al., 2018; Wilson et al., 2017; Moeller et al., 2010). While there are studies focusing on pork flavor, most of those studies focused on the reduction off-flavor development and were not yet looking at the different precursors of desirable pork flavors (Fan, 2021; Miller, 2020; Borrisser-Pairó et al., 2017; Panella-Riera et al., 2016; Font-i-Furnols et al., 2012). The current results indicate that there might be a potential shift in consumer preferences in pork palatability as more improvements are observed in both juiciness and tenderness attributes of pork products. Supporting the current observation, a previous consumer perception survey in Italy also reported higher preferences of potential purchase for dry-aged pork loins, further indicating the change in interest among pork consumers (Canavari et al., 2018). Additionally, similar shifts showing an increased focus in flavor by the consumers have also been repeatedly reported in beef products (Nyquist et al., 2018; Vierck et al., 2018; Wilfong et al., 2016; Lucherk et al., 2016), demonstrating a general increase in flavor interest by the consumers.

6.6.7 Consumer panel evaluation

While sensory evaluation is routinely reported for dry-aged beef, only limited information regarding dry-aged pork is available in the literature. A previous study by Lee et al. (2016) reported that experienced panelists scored 40-days-dry-aged pork, which had higher taste, flavor, texture, and overall acceptability scores when compared to unaged pork products. Similarly, Kim et al. (2018b) also reported that the trained panel found greater aroma, higher juiciness, and lower off-flavor in dry-aged pork compared to the wet-aged counterpart aged to both 7 and 14 days. Although positive dry-aged sensory improvements were observed by trained panel evaluation in previous studies, the current consumer panel results do not show any significant differences for all sensory traits between dry-aged and wet-aged pork. This observation could potentially be attributed to the unfamiliarity of consumers with the dry-aged pork taste (Canavari et al., 2018). To our knowledge, this is the first study to report consumer likeness of dry-aged pork products. Additional research, including trained panel evaluation to profile descriptive sensory attributes of dry-aged pork, would be necessary to determine the impacts of dry-aging on specific organoleptic properties of pork loins.

6.6.8 Metabolomics Analysis

Greater abundance of amino acids and nucleotides have been suggested to positively influence meat flavor, mainly by acting as flavor precursors involved in Maillard reaction during the cooking process (Koutsidis et al., 2008; Calkins and Hodgen, 2007). However, in the current study, the consumer panel did not find any differences in sensory traits between different aging methods. It might be due to the fact that although dry-aged meat contained a higher abundance of flavor precursors, the flavor volatiles might not be adequately generated during the cooking process to influence flavor perception. Previous studies found that the volatile generation from Maillard reaction is not only dependent on the concentration of the substrates but also on the environmental condition such as pH, water activity, and temperature (Li et al., 2021; Jousse et al., 2002). Reports had also indicated that consumers rated higher for the flavor attribute when the pork product had a higher pH (>5.8) and was cooked to a lower degree of doneness (Wilson et al., 2017; Moeller et al., 2010), providing flavor descriptors such as sweet and less acidic (Bryhni et al., 2003), potentially due to greater volatile generation in the product. The samples in the current

study, however, were cooked to an internal temperature of 71 °C (medium doneness) and were observed to have a pH range of around ~5.6. It was suggested that lower pH increases the presence of protonated amino groups, decreasing the reactivity during Maillard reaction and therefore influencing the final volatile concentration (Martins et al., 2000). Additionally, the lower pH condition was also reported to decrease the presence of pyrazines, thiazoles, and furans volatiles, which have been known to contribute to the meaty and roasted flavors (Li et al., 2021; Raza et al., 2020; Aaslyng and Meinert, 2017; Mottram, 1998) often associated with dry-aged products. Similarly, changes in the water activity and cooking temperature have also been suggested to alter the rate and type of Maillard reaction in the product (Martins et al., 2000; Hartman et al., 1984), impeding the volatile production during the cooking process. Subsequent studies to expand the effect of different meat conditions on dry-aging flavor production would be crucial to understand further the mechanism involved in the flavor production.

Other than flavor precursors, hydroquinone, niacinamide, and pelargonidin were present in a greater abundance in both WA and DA samples compared to UDA. These metabolites have been previously identified to display antioxidant capability (Chepeleva et al., 2021; Kwak et al., 2015; Noda et al., 2001). The loss of antioxidant availability in UDA could potentially be attributed to the application of UV light during the dry-aging process. The reduction of antioxidant compounds in UDA treatment was expected as the samples were exposed to UV light, which is known to induce oxidation through photo-oxidation (Jongberg et al., 2017).

Shikimic acid was also identified in the samples through metabolomics analysis. This metabolite was previously reported to act as an intermediate compound involved in the biosynthesis of aromatic amino acids (L-phenylalanine, L-tryptophan, and L-tyrosine) by microorganisms through the shikimate pathway (Ghosh et al., 2012). Interestingly, this pathway is only observed in plants and microorganisms and is not observed to be present in animal metabolism. Currently, the role of microorganisms during the dry-aging process is still obscure. Microorganisms have been well known to release proteolytic and lipolytic enzymes to further promote muscle degradation. The observation of this compound, however, suggested that microorganisms could also participate in flavor development by directly producing the flavor precursor and are not limited to muscle degradation activity.

6.7 Conclusions

In the current study, dry-aging of fresh pork loins resulted in similar instrumental tenderness, greater WHC, and lower microbial concentrations compared to conventional WA samples. The application of UV lights during dry-aging was also identified to further minimize the presence of microorganisms with minimal impact on meat quality. Untargeted UPLC-MS metabolomics analysis determined that a greater abundance of flavor-related precursors (amino acids and nucleotides) were liberated in both dry-aging treatments compared to conventional WA products. While this result could suggest potential development of unique dry-aged flavor in the dry-aged pork loins, the consumer panel was not able to find sensory trait differences across all aging treatments. Hence, additional studies utilizing a trained (focus group) panel to conduct descriptive sensory analysis along with other volatile chemical analysis would be of interest to further elucidate the dry-aging flavor volatile generation and their impact on the dry-aged pork's organoleptic properties.

6.8 Tables and Figures

Table 6.1. Effect of different aging treatments on shrink/purge loss, crust loss, fat/skin loss, bone loss, total loss, and total saleable yield of pork loins (*M. longissimus thoracis et lumborum*) aged for 21 days. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA) and UV-light dry-aging (UDA)

Treatments	Shrink/Purge Loss (%)	Crust Loss (%)	Fat/Skin Loss (%)	Bone Loss (%)	Total Processing Loss (%)	Total Yield (%)
WA	3.20[b]	0.00[b]	34.57	21.02	45.04[b]	54.96[a]
DA	16.13[a]	8.52[a]	34.23	23.50	58.54[a]	41.46[b]
UDA	16.47[a]	8.29[a]	32.35	22.21	59.12[a]	40.88[b]
Standard Error of Means	0.65	0.53	2.18	1.52	1.78	1.78
P-value	<0.0001	<0.0001	0.7439	0.1996	<0.0001	<0.0001

[a-b] Different superscript letter indicated a significant difference between the different aging methods (P<0.05)

Table 6.2. Effect of different aging treatments on pH, water-holding capacity measurements, and shear force of pork loins (*M. longissimus thoracis et lumborum*) aged for 21 days. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA) and UV-light dry-aging (UDA)

Treatments	**pH**	**Drip Loss (%)**	**Display Loss (%)**	**Freeze/thaw Loss (%)**	**Cook Loss (%)**	**Shear Force (N)**
WA	5.58[b]	1.42[a]	4.37[a]	2.86[a]	21.92	26.41
DA	5.59[b]	0.85[b]	3.57[b]	1.92[b]	22.83	25.08
UDA	5.62[a]	0.77[b]	3.48[b]	1.79[b]	21.98	27.05
Standard Error of Means	0.0117	0.1611	0.2562	0.2426	0.7548	1.3044
P-value	0.0311	0.0159	0.0285	0.0083	0.5573	0.3274

[a-b] Different superscript letter indicated a significant difference between the different aging methods ($P<0.05$)

Table 6.3. Effect of different aging treatments on total aerobic bacteria (APC), lactic acid bacteria (LAB), and mold and yeast con-centration on the crust (surface) and the lean portion of pork loins (*M. longissimus thoracis et lumborum*) aged for 21 days. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA) and UV-light dry-aging (UDA)

Location	**Treatment**	**APC (log10 CFU/mL of rinsate)**	**LAB (log10 CFU/mL of rinsate)**	**Mold (log10 CFU/mL of rinsate)**	**Yeast (log10 CFU/mL of rinsate)**
	WA	0.72 [c]	0.40 [b]	BDL	BDL
Lean	DA	0.54 [c]	[1] BDL	0.13 [b]	BDL
	UDA	0.29 [c]	0.14 [b]	0.17 [b]	BDL
	WA	2.69 [a]	2.33 [a]	1.82 [a]	0.24
Crust	DA	1.37 [b]	0.10 [b]	1.39 [a]	0.64
	UDA	0.15 [c]	BDL	BDL	BDL
Standard Error of Means		0.25	0.13	0.26	0.19
P-value	Treatment	0.0004	<0.0001	0.0136	0.2379
	Location	<0.0001	<0.0001	0.0004	0.0644
	Interaction	<0.0001	<0.0001	0.0007	0.2379

[a-c] Different superscript letter indicated a significant difference between the different aging methods ($P<0.05$)

[1]Below Detection Limit

Table 6.4. Demographic characteristics of consumers (n = 120) participated in the consumer sensory panels.

Demographic Questions	Response options	Frequency (%)
Gender	Female	63.3
	Male	35.8
	Other	0.9
Household Size	1 person	33.3
	2 people	23.3
	3 people	16.7
	4 people	13.3
	5 people	5.9
	>5 people	5.0
	Prefer not to disclose	2.5
Marital Status	Married	29.2
	Single	70.0
	Prefer not to disclose	0.8
Age	<20 years old	4.2
	20 to 29 years old	70.0
	30 to 39 years old	12.5
	40 to 49 years old	3.3
	50 to 59 years old	6.7
	>60 years old	3.3
Ethnic Origin	African-American	3.3
	Asian	30.0
	Caucasian/White	60.8
	Hispanic	2.5
	Mixed Race	2.5
	Other	0.9

Table 6.4. Continued

Annual Household Income	<$25,000	35.8
	$25,000 to $34,999	7.5
	$35,000 to $49,999	4.2
	$50,000 to $74,999	7.5
	$75,000 to $99,999	10.0
	$100,000 to $149,999	10.8
	$150,000 to $199,999	5.0
	>$199,999	5.8
	Prefer not to disclose	13.4
When eating pork, what palatability trait is the most important to you?	Flavor	45.8
	Juiciness	26.7
	Tenderness	27.5
When eating pork, what degree of doneness do you prefer?	Medium-Rare	13.3
	Medium	31.7
	Medium-Well	25.8
	Well Done	26.7
	Not sure	2.5
How many times per week do you consume pork?	1 Time/week	28.33
	2 Times/Week	25.83
	3 Times/Week	15.83
	4 Times/Week	9.17
	5 Times/Week	5.83
	6-10 Times/Week	10.0
	>10 Times /Week	2.5

Table 6.5. Consumer panelist perceptions on dry-aging and willingness to pay (n = 120).

End Survey Questions	Response Options	Frequency (%)
Have you ever eaten dry-aged products?	Yes	52.5
	No	14.2
	Not Sure	33.3
If you have eaten dry-aged product, where did you purchase the product from?	Local butcher store	19.1
	Local retail/supermarket	23.8
	Restaurant	39.7
	Other	17.5
If you answered "Other" in the previous question, where did you get the product from?	Personally made	45.5
	Research panels/projects	36.4
	School events	18.2
Is aging a positive or negative term?	Positive	85.8
	Negative	14.2
Do you think dry-aged product is safe?	Safer	10.8
	Less Safe	2.5
	Same as other product	65.8
	Not sure	20.8
Would you be willing to pay $1.00 more per 1 lb. of dry-aged pork?	Yes	55.8
	No	44.2

Table 6.6. Effect of different aging treatments on consumer sensory panel (n = 120) for likeness, acceptability, and perceived quality of pork loins (*M. longissimus thoracis et lumborum*) aged for 21 days. Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA) and UV-light dry-aging (UDA)

Traits	WA	DA	UDA	SEM	P-Value
Likeness					
Flavor	63.79	62.15	61.03	2.43	0.6184
Tenderness	61.53	61.80	60.78	3.04	0.9621
Juiciness	66.02	65.31	67.31	2.38	0.7876
Overall	62.99	62.72	63.89	2.60	0.9315
Acceptability					
Tenderness Acceptability	85.26	87.52	88.14	3.59	0.7950
Juiciness Acceptability	76.29	77.70	79.14	4.52	0.8762
Flavor Acceptability	86.26	82.33	84.14	3.77	0.7152
Overall Acceptability	82.14	83.62	85.09	3.62	0.8366
Perceived Quality					
Unsatisfactory Quality	13.82	15.47	13.82	3.48	0.9146
Everyday Quality	48.22	50.85	48.22	4.90	0.8981
Better Than Everyday Quality	25.25	30.68	30.39	4.36	0.5861
Premium Quality	8.00 [a]	1.23 [b]	4.49 [ab]	3.05	0.0416

[a–b] Different superscript letters indicated a significant difference between the different aging methods ($P<0.05$).

[1]Standard Error of Means

Table 6.7. Effect of different aging treatments on metabolomics profile of pork loins (*M. longissimus thoracis et lumborum*) aged for 21 days (p-value < 0.05 and FDR < 0.05). Different aging treatments: Wet-aging (WA), Conventional dry-aging (DA) and UV-light dry-aging (UDA)

Mass	RT	HMDB ID	Name	Highest Abundance	WA	DA	UDA
Protein-derived							
155.0350	0.69	HMDB0000177	L-Histidine	DA/UDA	4.43 [b]	4.85 [a]	4.73 [a]
226.0959	5.76	HMDB0001904	3-Nitrotyrosine	DA/UDA	5.20 [b]	5.37 [a]	5.32 [a]
157.1467	19.41	HMDB0000459	3-Methylcrotonylglycine	UDA/DA	5.10 [b]	5.17 [a]	5.18 [a]
165.1162	4.41	HMDB0000159	L-Phenylalanine	UDA	6.20 [b]	6.21 [b]	6.31 [a]
129.0425	0.88	HMDB0000267	Pyroglutamic acid	WA	5.73 [a]	5.63 [b]	5.61 [b]
145.1101	0.79	HMDB0003464	4-Guanidinobutanoic acid	WA	6.18 [a]	6.00 [b]	6.01 [b]
181.1018	2.31	HMDB0000158	L-Tyrosine	WA/DA	6.07 [a]	6.02 [a]	5.96 [b]
Carbohydrate-derived							
260.1372	4.86	HMDB0000124	Fructose 6-phosphate	DA/UDA	5.11 [b]	5.21 [a]	5.18 [a]
164.0475	2.31	HMDB0000174	L-Fucose	WA	7.34 [a]	7.28 [b]	7.23 [c]
Nucleotide-derived							
128.1316	0.52	HMDB0000079	Dihydrothymine	DA/UDA	6.41 [b]	6.46 [a]	6.45 [a]
242.1268	5.40	HMDB0000273	Thymidine	DA/UDA	5.68 [b]	5.85 [a]	5.83 [a]
329.1949	7.60	HMDB0000058	Cyclic AMP	DA/UDA	5.49 [b]	5.68 [a]	5.66 [a]
348.0591	1.70	HMDB0000175	Inosine monophosphate	DA/UDA	5.23 [b]	6.17 [a]	6.06 [a]
136.0387	4.12	HMDB0000157	Hypoxanthine	UDA/DA	7.45 [b]	7.53 [a]	7.58 [a]

Table 6.7. Continued

243.1835	18.87	HMDB0000089	Cytidine	UDA/DA	5.55 [b]	5.61 [a]	5.62 [a]
79.0424	1.58	HMDB0000926	Pyridine	WA	6.00 [a]	5.78 [b]	5.78 [b]
135.0665	1.65	HMDB0000034	Adenine	WA	5.41 [a]	5.20 [b]	5.15 [b]
252.1108	4.17	HMDB0000071	Deoxyinosine	WA	5.17 [a]	4.74 [b]	4.63 [b]
Others							
85.0892	4.00	HMDB0002039	2-Pyrrolidinone	DA/UDA	6.11 [b]	6.24 [a]	6.20 [a]
212.0800	1.05	HMDB0014814	Benzyl benzoate	DA	4.88 [b]	5.00 [a]	4.94 [ab]
132.0247	1.28	HMDB0001844	Methylsuccinic acid	WA	7.14 [a]	7.05 [b]	7.02 [b]
84.0213	0.68	HMDB0001184	Methyl propenyl ketone	WA	4.87 [a]	4.72 [b]	4.75 [b]
110.9992	0.51	HMDB0002434	Hydroquinone	WA/DA	6.16 [a]	6.15 [a]	6.13 [b]
122.0371	2.31	HMDB0001406	Niacinamide (vitb3)	WA/DA	6.17 [a]	6.12 [a]	6.07 [b]
174.1133	0.63	HMDB0003070	Shikimic acid	WA	5.42 [a]	5.31 [b]	5.26 [b]
226.1075	0.65	HMDB0000245	Porphobilinogen	WA	6.92 [a]	6.88 [b]	6.85 [b]
271.1644	1.05	HMDB0003263	Pelargonidin	WA/DA	5.15 [a]	5.12 [a]	4.92 [b]

[a-c] Different superscript letter indicated a significant difference between the different aging methods ($P<0.05$)

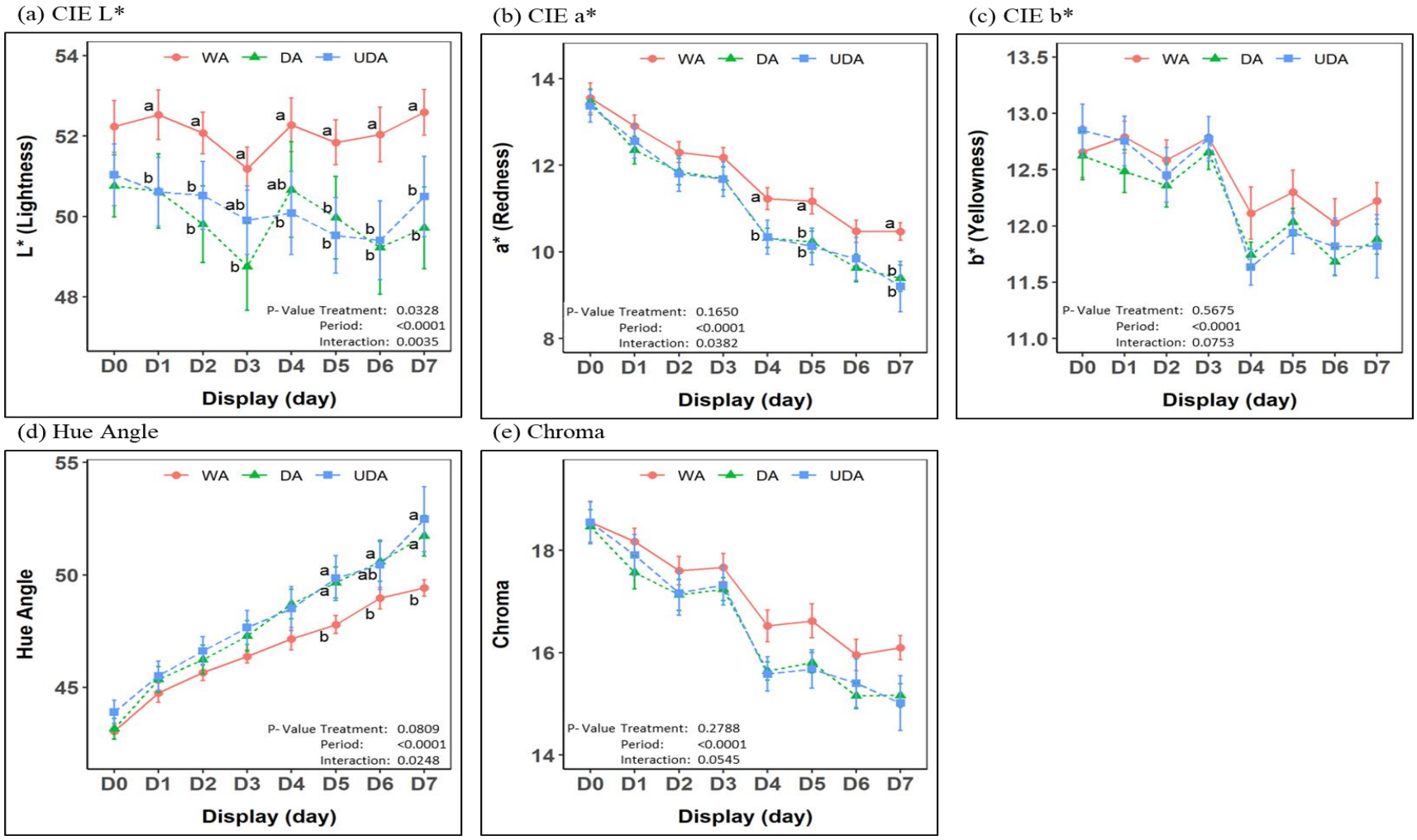


Figure 6.1. Effect of different aging treatments on the instrumental color characteristic of pork loins (*M. longissimus thoracis et lumborum*) aged for 21 days during 7 days of display period. Different aging treatments: wet-aging (WA), conventional dry-aging (DA), and UV-light dry-aging (UDA). [a–b] Means with different letters indicate significant differences within the same display day ($P<0.05$)

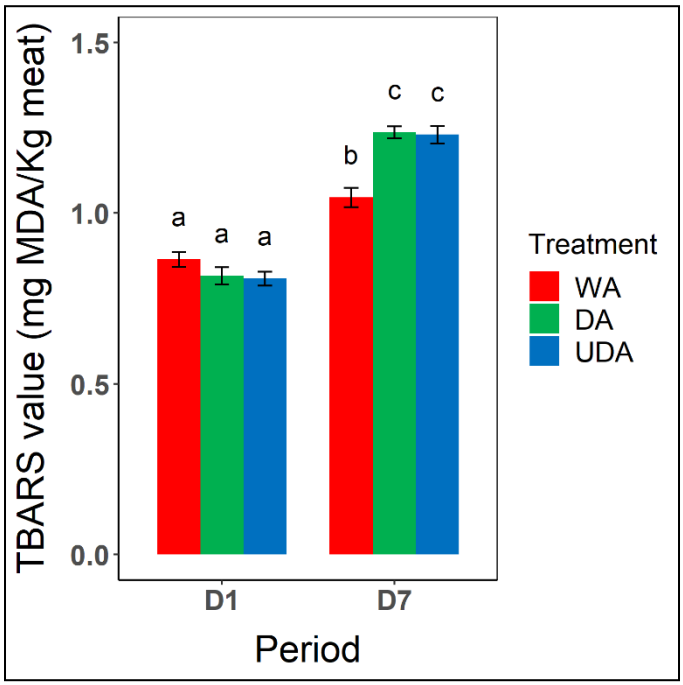


Figure 6.2. Effect of different aging treatments on lipid oxidation of pork loins (*M. longissimus thoracis et lumborum*) aged for 21 days. Different aging treatments: wet-aging (WA), conventional dry-aging (DA), and UV-light dry-aging (UDA). $^{a–c}$ Means with different letters indicates significant differences within the same display day ($P<0.05$).

(a) PCA plot

(b) HCA plot

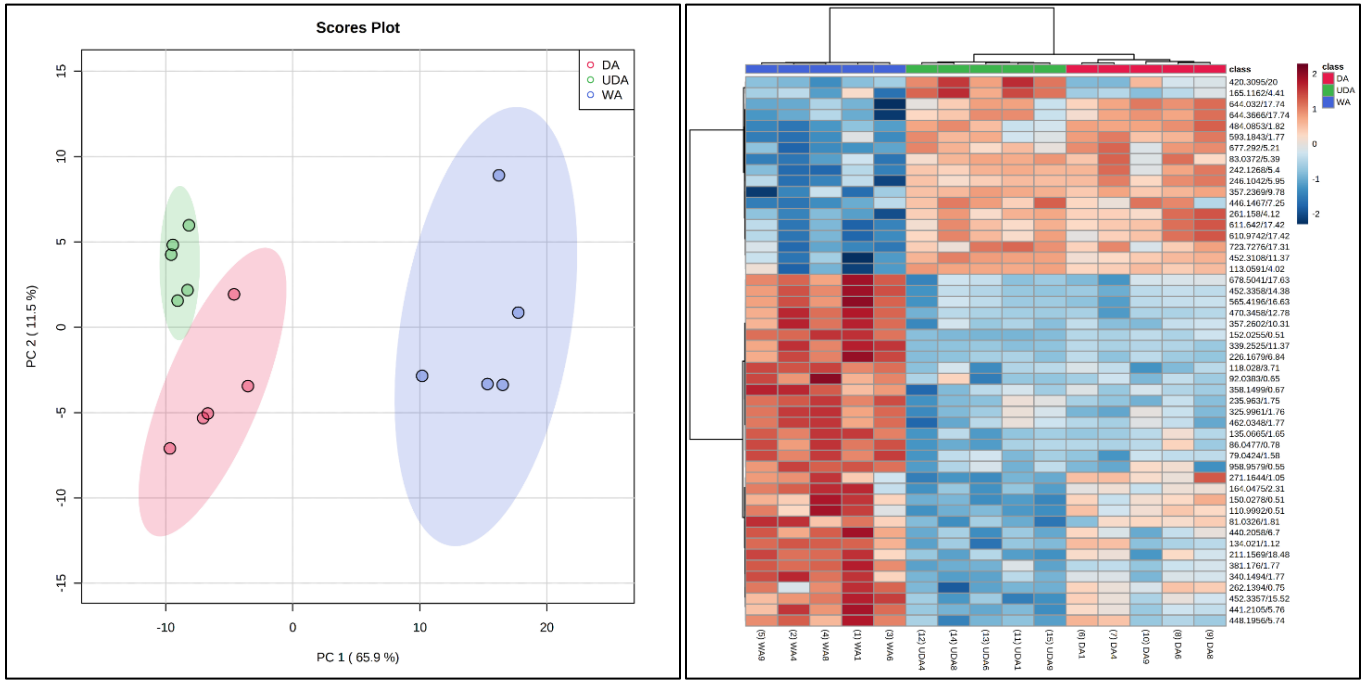


Figure 6.3. Principle component analysis (PCA, (a)) and hierarchical clustering analysis (HCA, (b)) of significant metabolites from pork loins (*M. longissimus thoracis et lumborum*) aged for 21 days with different aging treatments: wet-aging (WA), conventional dry-aging (DA), and UV-light dry-aging (UDA).

6.9 Literatures Cited

Aaslyng, M.D., and L. Meinert. 2017. Meat flavour in pork and beef – From animal to meal. Meat Sci. 132:112–117. doi:10.1016/j.meatsci.2017.04.012.

Abraham, A., J.W. Dillwith, G.G. Mafi, D.L. VanOverbeke, and R. Ramanathan. 2017. Metabolite Profile Differences between Beef Longissimus and Psoas Muscles during Display. Meat Muscle Biol. 1:18. doi:10.22175/mmb2016.12.0007.

Ahnström, M.L., M. Seyfert, M.C. Hunt, and D.E. Johnson. 2006. Dry aging of beef in a bag highly permeable to water vapour. Meat Sci. 73:674–679. doi:10.1016/j.meatsci.2006.03.006.

AMSA. 2012. Meat Color Measurement Guidelines.

Baublits, R.T., J.F. Meullenet, J.T. Sawyer, J.M. Mehaffey, and A. Saha. 2006. Pump rate and cooked temperature effects on pork loin instrumental, sensory descriptive and consumer-rated characteristics. Meat Sci. 72:741–750. doi:10.1016/J.MEATSCI.2005.10.006.

Berger, J., Y.H.B. Kim, J.F. Legako, S. Martini, J. Lee, P. Ebner, and S.M.S. Zuelly. 2018. Dry-aging improves meat quality attributes of grass-fed beef loins. Meat Sci. 145:285–291. doi:10.1016/j.meatsci.2018.07.004.

Borrisser-Pairó, F., N. Panella-Riera, M. Gil, Z. Kallas, M.B. Linares, M. Egea, M.D. Garrido, and M.A. Oliver. 2017. Consumers' sensitivity to androstenone and the evaluation of different cooking methods to mask boar taint. Meat Sci. 123:198–204. doi:10.1016/J.MEATSCI.2016.10.006.

Brewer, M.S., J. Novakofski, and K. Freise. 2006. Instrumental evaluation of pH effects on ability of pork chops to bloom. Meat Sci. 72:596–602. doi:10.1016/J.MEATSCI.2005.09.009.

Brewer, M.S., L.G. Zhu, and F.K. McKeith. 2001. Marbling effects on quality characteristics of pork loin chops: consumer purchase intent, visual and sensory characteristics. Meat Sci. 59:153–163. doi:10.1016/S0309-1740(01)00065-1.

Bryhni, E.A., D. V. Byrne, M. Rødbotten, S. Møller, C. Claudi-Magnussen, A. Karlsson, H. Agerhem, M. Johansson, and M. Martens. 2003. Consumer and sensory investigations in relation to physical/chemical aspects of cooked pork in Scandinavia. Meat Sci. 65:737–748. doi:10.1016/S0309-1740(02)00276-0.

Buege, J.A., and S.D. Aust. 1978. Microsomal lipid peroxidation. F. S. and P. L, editors. Academic Press, New York. 302–310 pp.

Calkins, C.R., and J.M. Hodgen. 2007. A fresh look at meat flavor. Meat Sci. 77:63–80. doi:10.1016/j.meatsci.2007.04.016.

Campbell, R.E., M.C. Hunt, P. Levis, and E. Chambers IV. 2001. Dry-aging effects on palatability of beef longissimus muscle. J. Food Sci. 66:196–199. doi:10.1111/j.1365-2621.2001.tb11315.x.

Canavari, M., D. Biasco, and R. Wongprawmas. 2018. Is Dry Aging for Pork Relevant to Consumers? J. Int. Food Agribus. Mark. 30:291–303. doi:10.1080/08974438.2017.1413613.

Channon, H.A., D.N. D'Souza, F.R. Dunshea, H.A. Channon, D.N. D'Souza, and F.R. Dunshea. 2017. Guaranteeing consistently high quality Australian pork: are we any closer? Anim. Prod. Sci. 57:2386–2397. doi:10.1071/AN17266.

Chepeleva, A.D., A.M. Grobov, A. V. Sirik, and E.M. Pliss. 2021. Antioxidant Activity of Hydroquinone in the Oxidation of 1,4-Substituted Butadiene. Russ. J. Phys. Chem. A 2021 955. 95:1077–1079. doi:10.1134/S0036024421050071.

Chun, H.H., J.Y. Kim, B.D. Lee, D.J. Yu, and K.B. Song. 2010. Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. Food Control. 21:276–280. doi:10.1016/j.foodcont.2009.06.006.

D'Alessandro, A., S. Rinalducci, C. Marrocco, V. Zolla, F. Napolitano, and L. Zolla. 2012. Love me tender: An Omics window on the bovine meat tenderness network. J. Proteomics. 75:4360–4380. doi:10.1016/j.jprot.2012.02.013.

Dikeman, M.E., E. Obuz, V. Gök, L. Akkaya, and S. Stroda. 2013. Effects of dry, vacuum, and special bag aging; USDA quality grade; and end-point temperature on yields and eating quality of beef Longissimus lumborum steaks. Meat Sci. 94:228–233. doi:10.1016/j.meatsci.2013.02.002.

Edeghor, U., J.A. Lennox, B.E. Agbo, and D. Aminadokiari. 2016. Bread Fermentation using Synergistic Activity Between Lactic Acid Bacteria (Lactobacillus bulgaricus) and Baker's Yeast (Sacchromyces cerevisae). Pakistan J. Food Sci. 26:2226–5899.

Elmossalami, E., and N. Wassef. 1971. Penetration of some Microorganisms in Meat. Zentralblatt für Veterinärmedizin R. B. 18:329–336. doi:10.1111/J.1439-0450.1971.TB01605.X.

Fan, Y. 2021. The flavor chemistry of pork broth: A review. *In* IOP Conference Series: Earth and Environmental Science.

Font-i-Furnols, M., N. Tous, E. Esteve-Garcia, and M. Gispert. 2012. Do all the consumers accept marbling in the same way? The relationship between eating and visual acceptability of pork with different intramuscular fat content. Meat Sci. 91:448–453. doi:10.1016/J.MEATSCI.2012.02.030.

Fortin, A., W.M. Robertson, and A.K.W. Tong. 2005. The eating quality of Canadian pork and its relationship with intramuscular fat. Meat Sci. 69:297–305. doi:10.1016/J.MEATSCI.2004.07.011.

Ganan, M., E. Hierro, X.F. Hospital, E. Barroso, and M. Fernández. 2013. Use of pulsed light to increase the safety of ready-to-eat cured meat products. Food Control. 32:512–517. doi:10.1016/j.foodcont.2013.01.022.

Ghosh, S., Y. Chisti, and U.C. Banerjee. 2012. Production of shikimic acid. Biotechnol. Adv. 30:1425–1431. doi:10.1016/J.BIOTECHADV.2012.03.001.

Hartman, G.J., J.D. Scheide, and C.T. Ho. 1984. Effect of Water Activity on the Major Volatileś Produced in a Model System Approximating Cooked Meat. J. Food Sci. 49:607–613. doi:10.1111/J.1365-2621.1984.TB12480.X.

Hoa, V.B., P.-N. Seong, S.-H. Cho, S.-M. Kang, Y.-S. Kim, S.-S. Moon, Y.-M. Choi, J.-H. Kim, and K.-H. Seol. 2019. Quality characteristics and flavor compounds of pork meat as a function of carcass quality grade. Asian-Australasian J. Anim. Sci. 32:1448–1457. doi:10.5713/ajas.18.0965.

Honikel, K.O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49:447–457. doi:10.1016/S0309-1740(98)00034-5.

Horiuchi, H., and Y. Sasaki. 2012. Short communication: Effect of oxygen on symbiosis between Lactobacillus bulgaricus and Streptococcus thermophilus. J. Dairy Sci. 95:2904–2909. doi:10.3168/jds.2011-5147.

Hwang, S.I., and G.P. Hong. 2020. Effects of high pressure in combination with the type of aging on the eating quality and biochemical changes in pork loin. Meat Sci. 162:108028. doi:10.1016/j.meatsci.2019.108028.

Hwang, Y.H., N. Sabikun, I. Ismail, and S.T. Joo. 2018. Comparison of meat quality characteristics of wet- and dry-aging pork belly and shoulder blade. Korean J. Food Sci. Anim. Resour. 38:950–958. doi:10.5851/kosfa.2018.e27.

Jin, S.K., and D.G. Yim. 2020. Comparison of effects of two aging methods on the physicochemical traits of pork loin. Food Sci. Anim. Resour. 40:844–851. doi:10.5851/KOSFA.2020.E22.

Jongberg, S., M.N. Lund, and L.H. Skibsted. 2017. Protein oxidation in meat and meat products. Challenges for antioxidative protection. *In* Global Food Security and Wellness. Springer New York. 315–337.

Jousse, F., T. Jongen, W. Agterof, S. Russell, and P. Braat. 2002. Simplified kinetic scheme of flavor formation by the Maillard reaction. J. Food Sci. 67:2534–2542. doi:10.1111/J.1365-2621.2002.TB08772.X.

Juárez, M., W.R. Caine, M.E.R. Dugan, N. Hidiroglou, I.L. Larsen, B. Uttaro, and J.L. Aalhus. 2011. Effects of dry-ageing on pork quality characteristics in different genotypes. Meat Sci. 88:117–121. doi:10.1016/j.meatsci.2010.12.011.

Kim, J.H., J.H. Kim, D.K. Yoon, D.S. Ji, H.J. Jang, and C.H. Lee. 2018a. A comparison of dry and wet aging on physicochemical and sensory characteristics of pork loin with two aging times. Food Sci. Biotechnol. 27:1551–1559. doi:10.1007/s10068-018-0418-x.

Kim, Y.H.B., R. Kemp, and L.M. Samuelsson. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Sci. 111:168–176. doi:10.1016/j.meatsci.2015.09.008.

Kim, Y.H.B., B. Meyers, H.W. Kim, A.M. Liceaga, and R.P. Lemenager. 2017. Effects of stepwise dry/wet-aging and freezing on meat quality of beef loins. Meat Sci. 123:57–63. doi:10.1016/j.meatsci.2016.09.002.

Kim, Y.H.B.B., D. Ma, D. Setyabrata, M.M. Farouk, S.M. Lonergan, E. Huff-Lonergan, and M.C. Hunt. 2018b. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. Meat Sci. 144:74–90. doi:10.1016/J.MEATSCI.2018.04.031.

Klehm, B.J., D.A. King, A.C. Dilger, S.D. Shackelford, and D.D. Boler. 2018. Effect of packaging type during postmortem aging and degree of doneness on pork chop sensory traits of loins selected to vary in color and marbling. J. Anim. Sci. 96:1736–1744. doi:10.1093/jas/sky084.

Koutsidis, G., J.S. Elmore, M.J. Oruna-Concha, M.M. Campo, J.D. Wood, and D. Mottram. 2008. Water soluble precursor of beef flavor: I. Effect of diet and breed. Meat Sci. 79:124–130. doi:10.1016/j.meatsci.2007.08.008.

Kwak, J.Y., H.J. Ham, C.M. Kim, and E.S. Hwang. 2015. Nicotinamide Exerts Antioxidative Effects on Senescent Cells. Mol. Cells. 38:229. doi:10.14348/MOLCELLS.2015.2253.

Lee, C.W., J.R. Lee, M.K. Kim, C. Jo, K.H. Lee, I. You, and S. Jung. 2016. Quality improvement of pork loin by dry aging. Korean J. Food Sci. Anim. Resour. 36:369–376. doi:10.5851/kosfa.2016.36.3.369.

Lee, H.J., J.W. Yoon, M. Kim, H. Oh, Y. Yoon, and C. Jo. 2019. Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. Meat Sci. 153:152–158. doi:10.1016/j.meatsci.2019.03.019.

Lepper-Blilie, A.N., E.P. Berg, D.S. Buchanan, and P.T. Berg. 2016. Effects of post-mortem aging time and type of aging on palatability of low marbled beef loins. Meat Sci. 112:63–68. doi:10.1016/j.meatsci.2015.10.017.

Li, L., C. Belloch, and M. Flores. 2021. The Maillard Reaction as Source of Meat Flavor Compounds in Dry Cured Meat Model Systems under Mild Temperature Conditions. Mol. 2021, Vol. 26, Page 223. 26:223. doi:10.3390/MOLECULES26010223.

Li, X., J. Babol, A. Wallby, and K. Lundström. 2013. Meat quality, microbiological status and consumer preference of beef gluteus medius aged in a dry ageing bag or vacuum. Meat Sci. 95:229–234. doi:10.1016/j.meatsci.2013.05.009.

Lonergan, S.M., E. Huff-Lonergan, L.J. Rowe, D.L. Kuhlers, and S.B. Jungst. 2001. Selection for lean growth efficiency in Duroc pigs influences pork quality. J. Anim. Sci. 79:2075–2085. doi:10.2527/2001.7982075x.

Lucherk, L.W., T.G. O'Quinn, J.F. Legako, R.J. Rathmann, J.C. Brooks, and M.F. Miller. 2016. Consumer and trained panel evaluation of beef strip steaks of varying marbling and enhancement levels cooked to three degrees of doneness. Meat Sci. 122:145–154. doi:10.1016/j.meatsci.2016.08.005.

Ma, D., Y.H.B. Kim, B. Cooper, J.-H. Oh, H. Chun, J.-H. Choe, J.P. Schoonmaker, K. Ajuwon, and B. Min. 2017. Metabolomics Profiling to Determine the Effect of Postmortem Aging on Color and Lipid Oxidative Stabilities of Different Bovine Muscles. J. Agric. Food Chem. 65:6708–6716. doi:10.1021/acs.jafc.7b02175.

Mancini, R. a., and M.C. Hunt. 2005. Current research in meat color. Meat Sci. 71:100–121. doi:10.1016/j.meatsci.2005.03.003.

Martins, S.I.F.S., W.M.F. Jongen, and M.A.J.S. Van Boekel. 2000. A review of Maillard reaction in food and implications to kinetic modelling. Trends Food Sci. Technol. 11:364–373. doi:10.1016/S0924-2244(01)00022-X.

Maxcy, R.B. 1981. Surface Microenvironment and Penetration of Bacteria into Meat. J. Food Prot. 44:550–552. doi:10.4315/0362-028X-44.7.550.

Miller, R. 2020. Drivers of Consumer Liking for Beef, Pork, and Lamb: A Review. Foods 2020, Vol. 9, Page 428. 9:428.

Moeller, S.J., R.K. Miller, T.L. Aldredge, K.E. Logan, K.K. Edwards, H.N. Zerby, M. Boggess, J.M. Box-Steffensmeier, and C.A. Stahl. 2010. Trained sensory perception of pork eating quality as affected by fresh and cooked pork quality attributes and end-point cooked temperature. Meat Sci. 85:96–103. doi:10.1016/J.MEATSCI.2009.12.011.

Mottram, D.S. 1998. Flavour formation in meat and meat products: A review. Food Chem. doi:10.1016/S0308-8146(98)00076-4.

Noda, Y., T. Kaneyuki, A. Mori, and L. Packer. 2001. Antioxidant Activities of Pomegranate Fruit Extract and Its Anthocyanidins: Delphinidin, Cyanidin, and Pelargonidin. J. Agric. Food Chem. 50:166–171. doi:10.1021/JF0108765.

Nyquist, K.M., T.G. O'Quinn, L.N. Drey, L.W. Lucherk, J.C. Brooks, M.F. Miller, and J.F. Legako. 2018. Palatability of beef chuck, loin, and round muscles from three USDA quality grades. J. Anim. Sci. 96:4276–4292. doi:10.1093/JAS/SKY305.

Panella-Riera, N., M. Blanch, Z. Kallas, P. Chevillon, A. Garavaldi, M. Gil, J.M. Gil, M. Font-i-Furnols, and M.A. Oliver. 2016. Consumers' segmentation based on the acceptability of meat from entire male pigs with different boar taint levels in four European countries: France, Italy, Spain and United Kingdom. Meat Sci. 114:137–145. doi:10.1016/J.MEATSCI.2015.12.017.

Pang, Z., J. Chong, G. Zhou, D. Anderson De Lima Morais, L. Chang, M. Barrette, C. Gauthier, P.-´ E. Jacques, S. Li, and J. Xia. 2021. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Res. 49. doi:10.1093/nar/gkab382.

Papuc, C., G. V. Goran, C.N. Predescu, and V. Nicorescu. 2017. Mechanisms of Oxidative Processes in Meat and Toxicity Induced by Postprandial Degradation Products: A Review. Compr. Rev. Food Sci. Food Saf. 16:96–123. doi:10.1111/1541-4337.12241.

Park, S.Y., H.Y. Kim, and J. Choe. 2019. Application of an electric field refrigeration system on pork loin during dry aging. Food Sci. Anim. Resour. 39:668–676. doi:10.5851/kosfa.2019.e59.

Prestat, C., J. Jensen, F.K. McKeith, and M.S. Brewer. 2002. Cooking method and endpoint temperature effects on sensory and color characteristics of pumped pork loin chops. Meat Sci. 60:395–400. doi:10.1016/S0309-1740(01)00150-4.

Raza, A., H. Song, J. Raza, P. Li, K. Li, and J. Yao. 2020. Formation of beef-like odorants from glutathione-enriched yeast extract via Maillard reaction. Food Funct. 11:8583–8601. doi:10.1039/D0FO01946A.

Ribeiro, F.A., S.K. Lau, S.B. Pflanzer, J. Subbiah, and C.R. Calkins. 2021. Color and lipid stability of dry aged beef during retail display. Meat Sci. 171:108274. doi:10.1016/j.meatsci.2020.108274.

Richardson, E.L., B. Fields, A.C. Dilger, and D.D. Boler. 2018. The effects of ultimate pH and color on sensory traits of pork loin chops cooked to a medium-rare degree of doneness. J. Anim. Sci. 96:3768–3776. doi:10.1093/jas/sky258.

Ryu, S., M.R. Park, B.E. Maburutse, W.J. Lee, D.J. Park, S. Cho, I. Hwang, S. Oh, and Y. Kim. 2018. Diversity and characteristics of the meat microbiological community on dry aged beef. J. Microbiol. Biotechnol. 28:105–108. doi:10.4014/jmb.1708.08065.

Setyabrata, D., B.R. Cooper, T.J.P. Sobreira, J.F. Legako, S. Martini, and Y.H.B. Kim. 2021a. Elucidating mechanisms involved in flavor generation of dry-aged beef loins using metabolomics approach. Food Res. Int. 139:109969. doi:10.1016/j.foodres.2020.109969.

Setyabrata, D., and Y.H.B. Kim. 2019. Impacts of aging/freezing sequence on microstructure, protein degradation and physico-chemical properties of beef muscles. Meat Sci. 151:64–74. doi:10.1016/J.MEATSCI.2019.01.007.

Setyabrata, D., S. Xue, K.R. Vierck, J.F. Legako, P. Ebner, and S.M. Zuelly. 2021b. Impact of Various Dry-Aging Methods on Meat Quality and Palatability Attributes of Beef Loins from Cull Cow. Meat Muscle Biol. Accepted-.

Sheard, P.R., and A. Tali. 2004. Injection of salt, tripolyphosphate and bicarbonate marinade solutions to improve the yield and tenderness of cooked pork loin. Meat Sci. 68:305–311. doi:10.1016/J.MEATSCI.2004.03.012.

Shirai, H., A.K. Datta, and S. Oshita. 2017. Penetration of aerobic bacteria into meat: A mechanistic understanding. J. Food Eng. 196:193–207. doi:10.1016/J.JFOODENG.2016.10.012.

Smith, A.M., K.B. Harris, D.B. Griffin, R.K. Miller, C.R. Kerth, and J.W. Savell. 2014. Retail yields and palatability evaluations of individual muscles from wet-aged and dry-aged beef ribeyes and top sirloin butts that were merchandised innovatively. Meat Sci. 97:21–26. doi:10.1016/j.meatsci.2013.12.013.

Verbeke, W., F.J.A. Pérez-Cueto, M.D. d. Barcellos, A. Krystallis, and K.G. Grunert. 2010. European citizen and consumer attitudes and preferences regarding beef and pork. Meat Sci. 84:284–292. doi:10.1016/j.meatsci.2009.05.001.

Vierck, K.R., J.M. Gonzalez, T.A. Houser, E.A.E. Boyle, T.G. O'Quinn, K.R. Vierck, J.M. Gonzalez, T.A. Houser, E.A.E. Boyle, and T.G. O'Quinn. 2018. Marbling Texture's Effects on Beef Palatability. Meat Muscle Biol. 2:127.

Wilfong, A.K., K. V. McKillip, J.M. Gonzalez, T.A. Houser, J.A. Unruh, E.A.E. Boyle, and T.G. O'Quinn. 2016. The effect of branding on consumer palatability ratings of beef strip loin steaks,. J. Anim. Sci. 94:4930–4942. doi:10.2527/JAS.2016-0893.

Wilson, K.B., M.F. Overholt, C.M. Shull, C. Schwab, A.C. Dilger, and D.D. Boler. 2017. The effects of instrumental color and extractable lipid content on sensory characteristics of pork loin chops cooked to a medium-rare degree of doneness. J. Anim. Sci. 95:2052–2060. doi:10.2527/JAS.2016.1313.

CHAPTER 7. SUMMARY AND FUTURE RESEARCH

7.1 Summary

7.1.1 Flavor precursors

Through the research described here, we demonstrated that dry-aging altered the flavor precursor composition of grass-fed beef, cull cow beef and pork. Furthermore, flavor precursor analyses utilizing metabolomics and multiple chemical analyses (free amino acids analysis, sugar content analysis and fatty acid profiling) allowed characterization of different flavor precursor compositions and concentration between the dry-aged and wet-aged samples. A diagram summarizing the dry-aged flavor precursor alteration could be observed on Figure 7.1.

In regards to protein-derived flavor precursors (e.g., free amino acids, dipeptides, short peptides), both metabolomics and free amino acids analyses constantly demonstrated that the application of dry-aging increased the concentration of those compounds. Interestingly, the increase in glutamine and glutamate compounds was often observed, indicating that those amino acids play a significant role in dry-aging flavor generation. Elevated concentrations of other amino acids, such as aspartate, cysteine, methionine and histidine, were also observed in the dry-aged products, which could result in the generation of desirable meat volatiles during the cooking process. Metabolomics analyses also identified higher abundances of peptides (e.g., glutaminyl peptides) in dry-aged meats, although more study is still needed to fully understand the impact peptides may have in meat flavor production.

Similarly, significant changes in nucleotides-derived and carbohydrate-derived compound availability were identified after dry-aging application. The nucleotide-derived compounds were identified in greater abundance in dry-aged meat compared to wet-aged meat. Nucleotide products, especially, adenosine-based compounds were identified through metabolomics analysis in chapter 3 and 6. Additionally, other types of nucleotides products such as thymidine and cytidine were also observed following dry-aging, although their role in meat flavor is still unclear. In terms of carbohydrate-based products, increased availability of reducing sugars was observed in dry-aging when analyzed using reducing sugar analysis. Metabolomics analyses only detected minimal differences in reducing sugar presence between the different aging treatments. The analysis in

chapter 3, however, showed different types of carbohydrate-based compounds such as glucoside and glucuronide. Those compounds were present in greater abundance in WA samples and could potentially be related to antioxidant activity. Conversely, the lower abundance in dry-aged treatment could indicate the further degradation of the compounds and thus potentially affect the availability of sugar compounds in the product.

With respect to lipid-based compound composition, dry-aging had minimal impacts on the fatty acid composition of the products. Fatty acids profiling exhibited no changes in the overall concentration of the fatty acids following aging. However, changes in the fatty acid proportions were observed in both dry-aging in bag and UV-light dry-aging, showing greater polyunsaturated fatty acids. The results, however, were still inconsistent between the current studies and other available reports. Hence, more studies are needed to fully understand the impact of dry-aging on fatty acid changes and the effects such changes could have on flavor alteration. Through metabolomics analysis, unique lipid compounds such as steroids and terpenoids were identified in grass-fed beef and could potentially be related to the reduction of off-flavor after the dry-aging process. Similar to fatty acids, the results between the two studies conducted in the current dissertation were inconsistent and unpredictable, warranting more studies to comprehend the impact of dry-aging on lipid-based compound alteration. Metabolomics analysis also identified an increase of vitamins as a results of aging, with dry-aging exhibited a greater abundance of vitamin C and vitamin B.

Taking all these together, dry-aged meat flavor precursors could be characterized with increased protein-derived flavor precursors along with elevated availability of sugar and nucleotide-based precursors. While changes on lipid-derived precursors could be expected, the impact of dry-aging on those products might not be as apparent. Therefore it could be suggested that the changes in free amino acids, reducing sugars and nucleotides are the main alteration following dry-aging and could be the primary source influencing the dry-aged flavor.

7.1.2 Liberation Mechanisms

We also identified several mechanisms responsible for dry-aged flavor development. These mechanisms are presented schematically in Figure 7.2.

Dehydration was identified as an initial mechanism influencing the overall concentration of flavor precursors in meat. Therefore, due to moisture loss, the dry-aged meat could be expected to have more concentrated flavor precursors. Such concentration could lead to the generation of more flavor-related chemical compounds particularly related to the Maillard reaction.

Microbes are also recognized as an essential part of the flavor precursor liberation in dry-aged meat. It was shown that the presence of microbes could affect the flavor precursor availability after the dry-aging process. The microbiome analysis found that Proteobacteria, especially *Pseudomonas* spp., had the highest relative abundance in dry-aged production and could be responsible for the increased of flavor precursor via degradation of meat during the dry-aging process. In addition, some unique bacterial species (such as *Bacillus* spp.) were also identified in the dry-aging treatment. These unique bacterial species could also contributed to the release of distinctive flavor compounds that can influence the final dry-aged flavor as well.

Through metabolomics profiling, glutathione metabolism was identified as another potential influence on flavor precursor composition as glutathione metabolites were constantly observed in dry-aged products. The metabolomics also identified uncommon metabolites such as porphyrin ring and shikimic acid in dry-aged meat. While those two compounds are not believed to be directly related to meat flavor, the presence could shed light on other processes influencing the meat composition. For example, the porphyrin ring is often found in globin moieties, and its presence could be correlated to iron availability and metallic flavor in the meat. Likewise, shikimic acid is part of the shikimate pathway in bacterial amino acid production. Thus, the identification of this compound could indicate bacterial participation in dry-aging flavor through muscle degradation and production of secondary metabolites.

7.2 Future research directions

As flavor is a complex attribute, more research is still needed to fully comprehend the impact of dry-aging on flavor precursor release and their role in generating the dry-aged flavor (Figure 7.3). In this respect, future work should be warranted to address the role of peptides and free fatty acids on dry-aged flavor generation. While our research suggested that dry-aged products had greater abundance of flavor volatiles, only limited understanding is currently available on peptide functions in meat flavor production, especially under different aging conditions. Given the

fact that not all protein-derived compounds will be degraded to the amino acids, as shown by the multiple peptides identified in the studies, future studies to understand their impact will be beneficial in understanding dry-aging flavor. Likewise, the impact of dry-aging on lipid-derived compounds and free fatty acids profiles is still unclear. Despite the current studies found no major changes in lipid profile upon aging, lipids are still important flavor compounds that contribute the cooked meat flavor production through thermally-induced lipid oxidation and degradation. Moreover, lipid oxidation could contribute to off-flavor development, which is detrimental for meat products. Further research to reveal the impact of lipids in dry-aged meat flavor will be of interest. A potential lipid study using a lipidomics approach might be especially beneficial.

Microorganism involvement in flavor generation was identified in dry-aging process. However, since the dry-aging process exposed the meat to the ambient environment, different microorganisms, including yeast and mold, will also be present. In the current study, only bacterial identification and analysis were conducted concerning flavor precursor release. Both mold and yeast have also been identified to participate in flavor generation in various food products, and thus their presence and activity will also influence dry-aged products. Future research to elucidate the role of both mold and yeast will be of interest. Additionally, as previously mentioned, microorganisms could participate in flavor generation through degradation and by producing secondary metabolites. An investigation to identify particular flavor precursors might be of interest to generate a distinct dry-aged product.

Finally, the availability and presence of flavor precursor is only a part of the flavor generation process. The cooking process plays a significant role in promoting the production of meat flavor. Therefore, determining impacts of different meat conditions (e.g., pH, moisture content) and cooking conditions (e.g., grilling, frying) on meat palatability (flavor, in particular) will be beneficial to maximize the positive impacts of dry-aging on meat flavor generation.

7.3 Figures

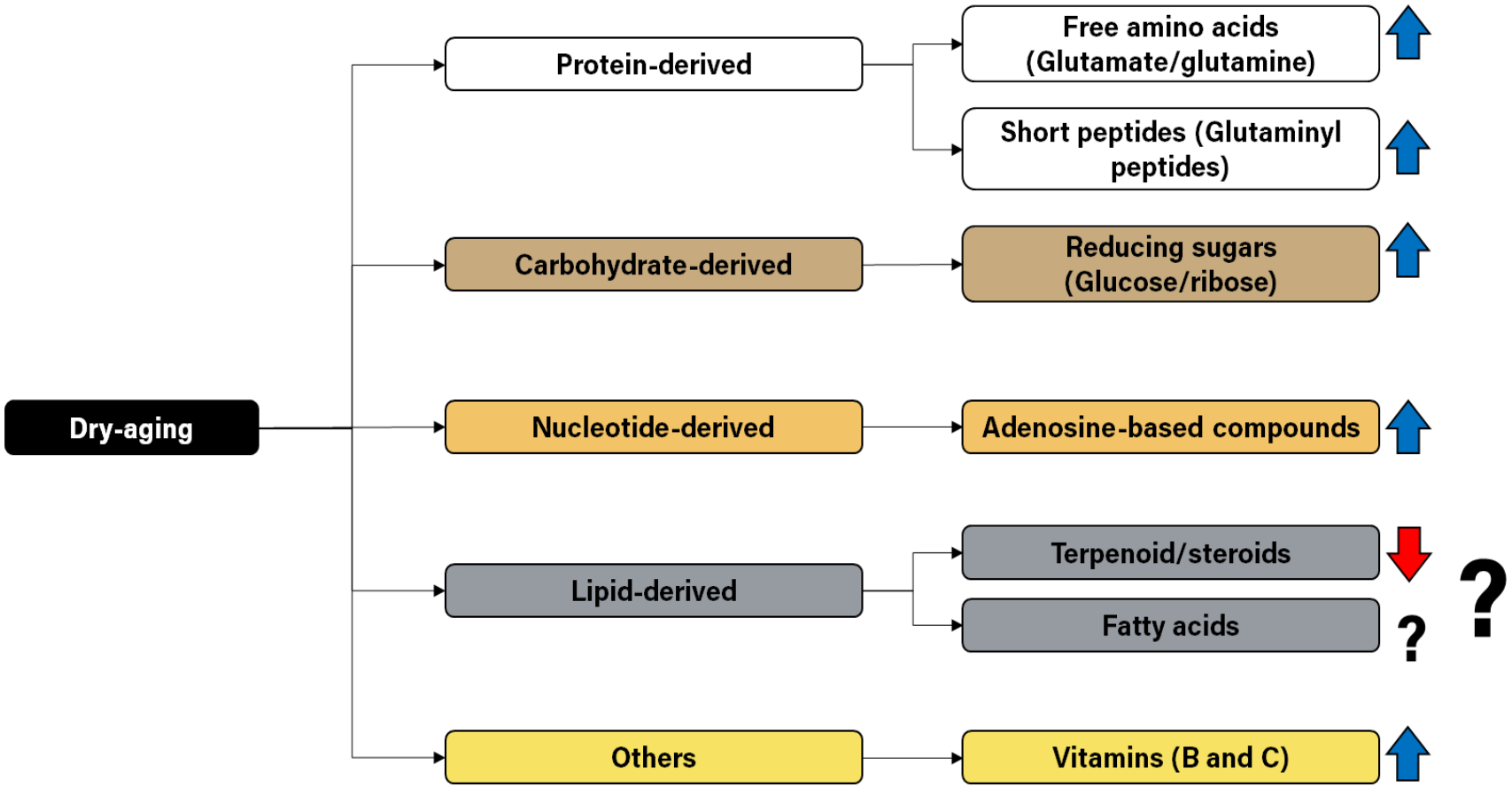


Figure 7.1. Summary of identified flavor precursor alteration following dry-aging application

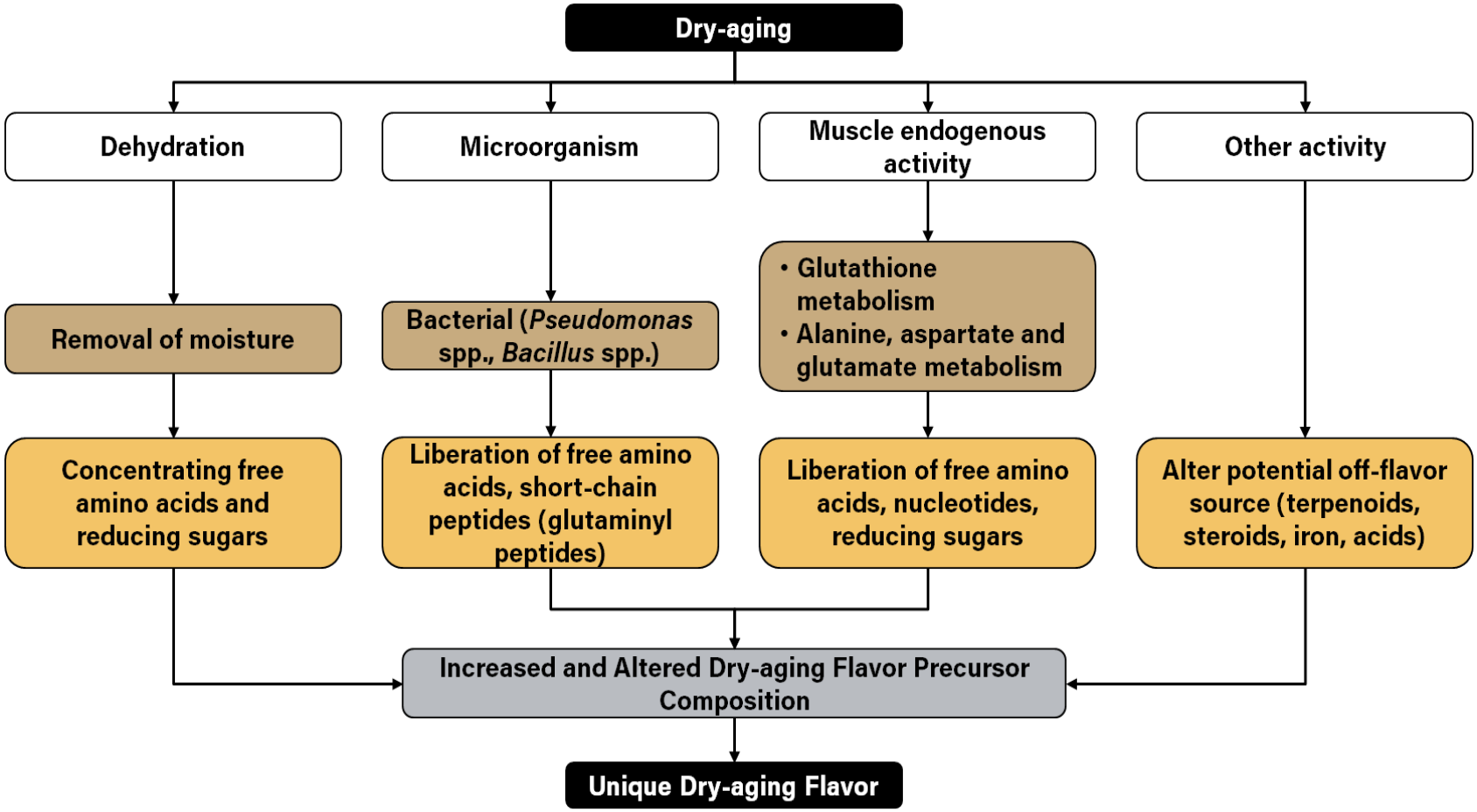


Figure 7.2. Summary of identified flavor precursor liberation mechanism during dry-aging application

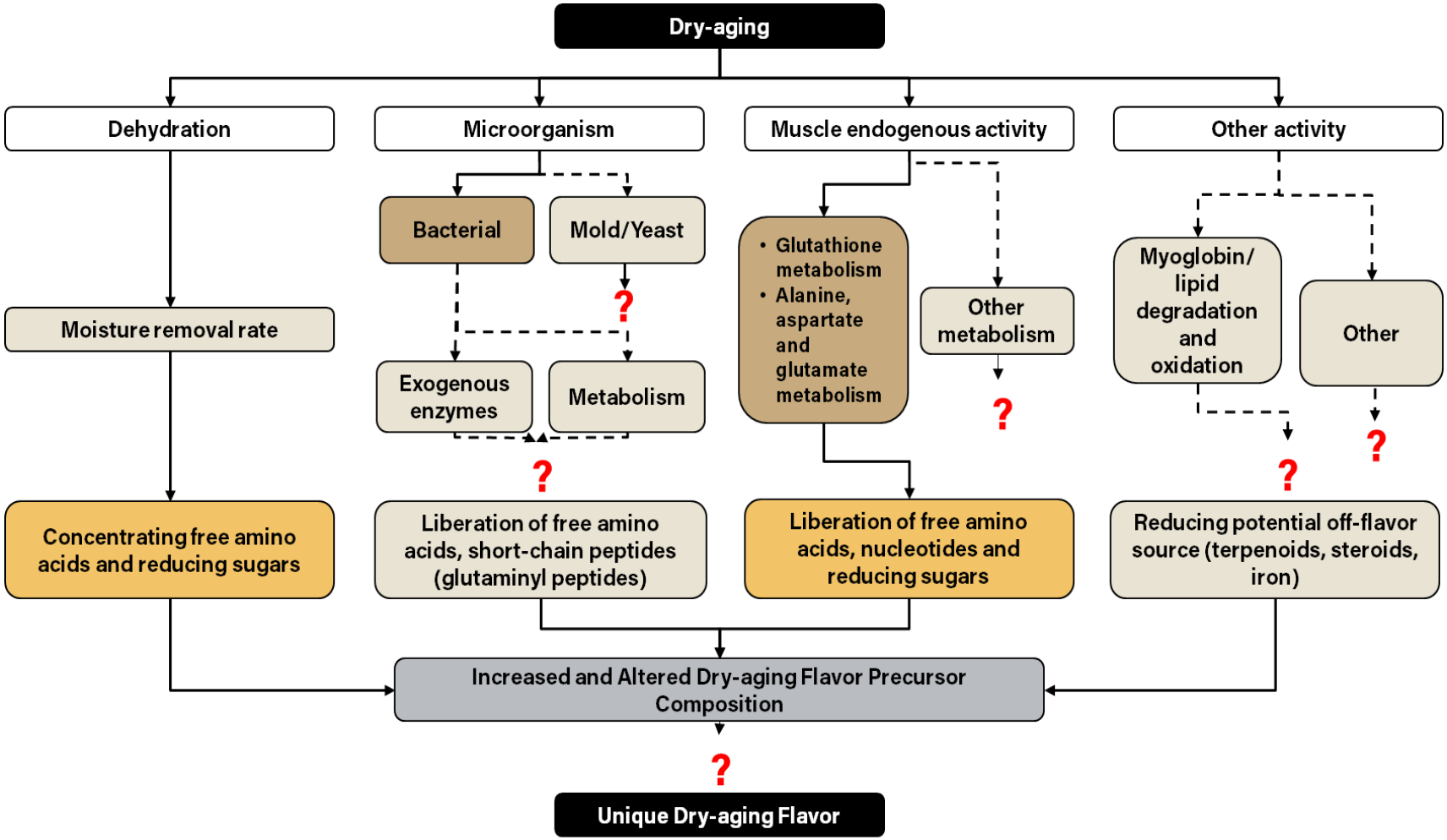
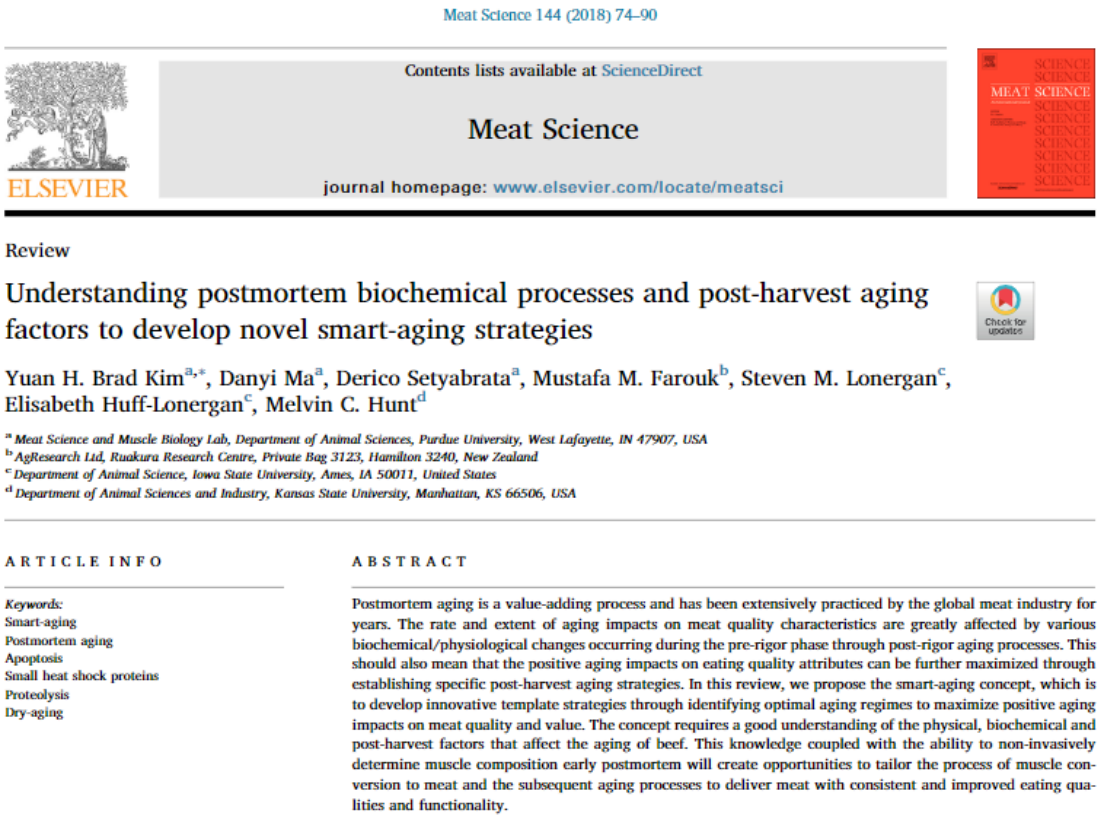


Figure 7.3. Potential area for future study to understand impact of dry-aging on flavor generation

APPENDIX A. PUBLISHED STUDIES



Meat Science 144 (2018) 74–90

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Review

Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies

Yuan H. Brad Kim[a,*], Danyi Ma[a], Derico Setyabrata[a], Mustafa M. Farouk[b], Steven M. Lonergan[c], Elisabeth Huff-Lonergan[c], Melvin C. Hunt[d]

[a] Meat Science and Muscle Biology Lab, Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA
[b] AgResearch Ltd, Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand
[c] Department of Animal Science, Iowa State University, Ames, IA 50011, United States
[d] Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506, USA

ARTICLE INFO

Keywords:
Smart-aging
Postmortem aging
Apoptosis
Small heat shock proteins
Proteolysis
Dry-aging

ABSTRACT

Postmortem aging is a value-adding process and has been extensively practiced by the global meat industry for years. The rate and extent of aging impacts on meat quality characteristics are greatly affected by various biochemical/physiological changes occurring during the pre-rigor phase through post-rigor aging processes. This should also mean that the positive aging impacts on eating quality attributes can be further maximized through establishing specific post-harvest aging strategies. In this review, we propose the smart-aging concept, which is to develop innovative template strategies through identifying optimal aging regimes to maximize positive aging impacts on meat quality and value. The concept requires a good understanding of the physical, biochemical and post-harvest factors that affect the aging of beef. This knowledge coupled with the ability to non-invasively determine muscle composition early postmortem will create opportunities to tailor the process of muscle conversion to meat and the subsequent aging processes to deliver meat with consistent and improved eating qualities and functionality.

Figure A.1. Published study used in Chapter 2.

Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., & Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. Meat Science, 144, 74-90.

Food Research International 139 (2021) 109969

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Elucidating mechanisms involved in flavor generation of dry-aged beef loins using metabolomics approach

Derico Setyabrata [a], Bruce R. Cooper [b], Tiago J.P. Sobreira [b], Jerrad F. Legako [c], Silvana Martini [d], Yuan H. Brad Kim [a,*]

[a] Meat Science and Muscle Biology Lab, Department of Animal Sciences, Purdue University, West Lafayette, IN, USA
[b] Bindley Bioscience Center, Purdue University, West Lafayette, IN, USA
[c] Department of Animal and Food Science, Texas Tech University, Lubbock, TX, USA
[d] Department of Nutrition, Dietetics, and Food Science, Utah State University, Logan, UT, USA

ARTICLE INFO

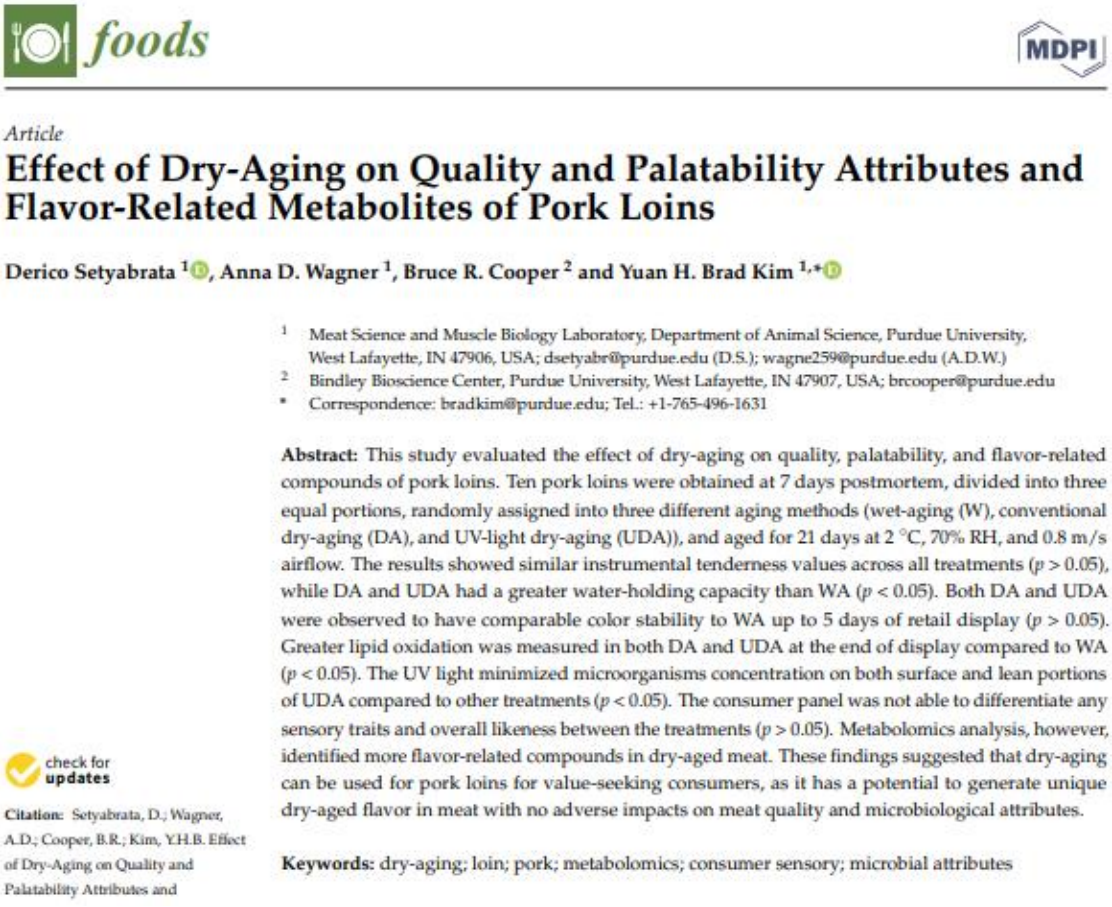
Keywords:
Dry-aging
Metabolomics
Beef
Flavor
Volatile compounds
Grass-fed
Free fatty acid

ABSTRACT

The present study was conducted to identify flavor-related chemical compounds and to elucidate beef flavor development in response to dry-aging. Paired grass-fed beef loins (n = 18) were obtained at 7 d postmortem, cut into two sections and assigned to 3 aging methods: conventional dry-aging (DA), vacuum packaged wet-aging (WA) and dry-aging in a bag (DW) for 28 days. Following aging, samples were analyzed for UPLC-MS metabolomics, volatile, fatty acid profiling, and consumer sensory comment analysis. Greater number of proteins and nucleotides derived metabolites were liberated in dry-aged samples compared to WA ($P < 0.05$). In particular, the liberation of gammaglutmayl peptides and glutamine metabolites through the glutathione metabolism were identified. While fatty acid profile was not affected by treatments ($P > 0.05$), higher concentrations of volatile compounds were found in the dry-aged ($P < 0.05$). Dry-aging process decreased the presence of terpenoid and steroid lipid group, which could possibly result in reducing undesirable flavor of grass-fed beef.

Figure A.2. Published study used in Chapter 3.

Setyabrata, D., Cooper, B. R., Sobreira, T. J., Legako, J. F., Martini, S., & Kim, Y. H. B. (2021). Elucidating mechanisms involved in flavor generation of dry-aged beef loins using metabolomics approach. Food Research International, 139, 109969.



foods

MDPI

Article

Effect of Dry-Aging on Quality and Palatability Attributes and Flavor-Related Metabolites of Pork Loins

Derico Setyabrata [1], Anna D. Wagner [1], Bruce R. Cooper [2] and Yuan H. Brad Kim [1,*]

[1] Meat Science and Muscle Biology Laboratory, Department of Animal Science, Purdue University, West Lafayette, IN 47906, USA; dsetyabr@purdue.edu (D.S.); wagne259@purdue.edu (A.D.W.)
[2] Bindley Bioscience Center, Purdue University, West Lafayette, IN 47907, USA; brcooper@purdue.edu
* Correspondence: bradkim@purdue.edu; Tel.: +1-765-496-1631

Abstract: This study evaluated the effect of dry-aging on quality, palatability, and flavor-related compounds of pork loins. Ten pork loins were obtained at 7 days postmortem, divided into three equal portions, randomly assigned into three different aging methods (wet-aging (W), conventional dry-aging (DA), and UV-light dry-aging (UDA)), and aged for 21 days at 2 °C, 70% RH, and 0.8 m/s airflow. The results showed similar instrumental tenderness values across all treatments ($p > 0.05$), while DA and UDA had a greater water-holding capacity than WA ($p < 0.05$). Both DA and UDA were observed to have comparable color stability to WA up to 5 days of retail display ($p > 0.05$). Greater lipid oxidation was measured in both DA and UDA at the end of display compared to WA ($p < 0.05$). The UV light minimized microorganisms concentration on both surface and lean portions of UDA compared to other treatments ($p < 0.05$). The consumer panel was not able to differentiate any sensory traits and overall likeness between the treatments ($p > 0.05$). Metabolomics analysis, however, identified more flavor-related compounds in dry-aged meat. These findings suggested that dry-aging can be used for pork loins for value-seeking consumers, as it has a potential to generate unique dry-aged flavor in meat with no adverse impacts on meat quality and microbiological attributes.

Keywords: dry-aging; loin; pork; metabolomics; consumer sensory; microbial attributes

check for updates

Citation: Setyabrata, D.; Wagner, A.D.; Cooper, B.R.; Kim, Y.H.B. Effect of Dry-Aging on Quality and Palatability Attributes and

Figure A.3. Published study used in Chapter 6.

Setyabrata, D., Wagner, A. D., Cooper, B. R., & Kim, Y. H. B. (2021). Effect of Dry-Aging on Quality and Palatability Attributes and Flavor-Related Metabolites of Pork Loins. Foods, 10(10), 2503.