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**Master's Thesis of Agriculture**

**Storage stability of vacuum-packaged dry-aged  
beef during refrigerated condition (4°C)**

**진공포장한 건식숙성 우육의 냉장 보관 (4°C)**

**중 저장안정성 규명**

**February 2019**

**By  
Seonjin Kim**

**Department of Agricultural Biotechnology  
Graduate School  
Seoul National University**

**Storage stability of vacuum-packaged dry-aged  
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Advisor: Prof. Cheorun Jo, Ph.D

**Submitting a Master's Thesis of Agriculture**

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Graduate School  
Seoul National University  
Department of Agricultural Biotechnology

**Seonjin Kim**

**Confirming the master's thesis written by  
Seonjin Kim**

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# 진공포장한 건식숙성 우육의 냉장 보관 (4°C) 중 저장안정성 규명

## Storage stability of vacuum-packaged dry-aged beef during refrigerated condition (4°C)

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위 원 장 \_\_\_\_\_ (인)

부위원장 \_\_\_\_\_ (인)

위 원 \_\_\_\_\_ (인)

# Summary

## **Storage stability of vacuum-packaged dry-aged beef during refrigerated condition (4°C)**

Seonjin Kim

Program in Animal Science and Biotechnology

Department of Agricultural Biotechnology

Graduate School of Seoul National University

Recently, the production of dry-aged beef has been increasing worldwide due to consumers' interest in the product. Therefore, appropriate condition for its distribution is important to supply dry-aged beef without quality deterioration. However, most producers are not aware of the changes in dry-aged beef with different packaging methods after completion of aging period and do not have any guideline for its storage. Therefore, the objective of this study was to investigate the storage stability of vacuum-packaged dry-aged beef based on the changes in microbial growth and

physicochemical and sensory properties during refrigerated condition (4°C). A total of nine sirloins were taken from nine beef carcasses (Holstein steer, quality grade 3) and dry aged for 28 days (temperature, 4°C; relative humidity, 75%; air flow velocity, 2.5 m/s). After the completion of dry aging, the samples were trimmed off the dried surface, vacuum-packaged, and stored for 21 days at refrigerated condition (4°C) for the analyses of microbial growth, pH, volatile basic nitrogen (VBN), 2-thiobarbituric acid reactive substances (TBARS), and sensory evaluation. As a result, the total aerobic bacterial (TAB) count was significantly increased until day 14 and exceeded 6 log CFU/g possibly at 11 days of storage. pH and VBN content were significantly changed between days 14 and 21, whereas TBARS value was constant ( $P < 0.05$ ). In the sensory evaluation juiciness and overall acceptability of vacuum-packaged dry-aged beef were significantly decreased at 14 and 21 days of storage, respectively, while others showed no difference. Therefore, the vacuum-packaged dry-aged beef could be stored 11 days at 4°C without any adverse effect on its microbial level and sensory quality.

Key words; Microbial growth, Meat quality, Dry-aged beef, Vacuum packaging

# Contents

<b>Summary .....</b>	<b>i</b>
<b>Contents.....</b>	<b>iii</b>
<b>List of Tables .....</b>	<b>iv</b>
<b>List of Figures .....</b>	<b>v</b>
<b>List of Abbreviations.....</b>	<b>vi</b>

## **Chapter I.**

### **General introduction**

1.1. Dry aging of beef .....	1
1.1.1. Aging type .....	1
1.1.1.1. Beef aging .....	1
1.1.1.2. Wet aging.....	2
1.1.1.3. Dry aging.....	3
1.1.2. Dry aging process condition .....	4
1.1.2.1. Aging time .....	4
1.1.2.2. Temperature.....	5
1.1.2.3. Air flow .....	6
1.1.2.4. Relative humidity .....	7
1.1.3. Microbiology in dry-aged beef.....	8

1.1.3.1. Microorganisms and their growth during dry aging process .	8
1.1.3.2. Limitation in shelf-life establishment of dry-aged beef.....	9
1.2. Shelf life of beef.....	12
1.2.1. Spoilage in beef.....	12
1.2.1.1. Microbial spoilage .....	12
1.2.1.2. Chemistry spoilage .....	12
1.2.2. Factors affecting spoilage .....	13
1.2.2.1. Packaging system .....	13
1.2.2.2. Temperature.....	14
1.2.3. Shelf-life of dry-aged beef.....	15
1.2.3.1. Current studies in the shelf-life of dry-aged beef.....	15

## **Chapter II.**

### **Storage stability of vacuum-packaged dry-aged beef during refrigerated condition (4°C)**

2.1. Introduction.....	18
2.2. Materials and methods.....	20
2.2.1. Dry-aging process and packaging conditions. ....	20
2.2.2. Microbial growth.....	21
2.2.3. Physicochemical properties .....	21
2.2.3.1. pH .....	21
2.2.3.2. Volatile basic nitrogen (VBN).....	22
2.2.3.3. 2-Thiobarbituric acid reactive substances (TBARS) value..	22

2.2.3.4. Instrumental color.....	23
2.2.3.5. Myoglobin (Mb) content.....	24
2.2.3.6. Texture profile analysis.....	24
2.2.3.7. Proteolysis index.....	24
2.2.4. Sensory property.....	25
2.2.5. Statistical analysis.....	26
2.3. Results and discussion.....	27
2.3.1. Microbial growth.....	27
2.3.2. Physicochemical properties.....	32
2.3.2.1. Spoilage indicator.....	32
2.3.2.2. TBARS.....	33
2.3.2.3. Instrumental color and Mb content.....	36
2.3.2.4. Texture profile analysis.....	42
2.3.2.5. Proteolysis index.....	44
2.3.3. Sensory property.....	46
2.4. Conclusion.....	49
<b>References.....</b>	<b>50</b>
<b>Summary in Korean.....</b>	<b>62</b>

# List of Tables

## Chapter I.

Table 1. Microbial regulation (CFU/g, cm <sup>2</sup> ) of recommended standards for safety in Korea .....	11
--	----

## Chapter II.

Table 2. The estimated shelf-life of wrap- and vacuum-packaged dry-aged beef for total aerobic bacteria with quality standards.....	31
Table 3. pH, volatile basic nitrogen , and 2-thiobarbituric acid reactive substances of vacuum-packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C) .....	35
Table 4. Instrumental color (CIE $L^*$ , $a^*$ , and $b^*$ ) and color difference of vacuum-packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C) .....	39
Table 5. Myoglobin content of vacuum packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C) .....	40
Table 6. Texture profile analysis of vacuum packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C) .....	43
Table 7. Sensory property of vacuum packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C) .....	47

Table 8. Sensory property of wrap-packaged dry-aged beef during 7 days of storage at refrigeration conditions (4°C) .....	48
---	----

# List of Figures

## Chapter II.

- Figure 1. Microbial growth (log CFU/g) of vacuum-packaged dry-aged beef during 21 days of storage time at refrigerated condition (4°C) .... 29
- Figure 2. Microbial growth (log CFU/g) of wrap-packaged dry-aged beef during 21 days of storage time at refrigerated condition (4°C) .... 30
- Figure 3. Combination of 2-thiobarbituric acid and malondialdehyde to make 2-thiobarbituric acid pigment ..... 34
- Figure 4. Redox reaction of myoglobin in fresh meat during storage. .... 38
- Figure 5. The absorbance curve of myoglobin (Mb) of vacuum-packaged dry-aged beef during 21 days of storage time at refrigerated condition (4°C)..... 41
- Figure 6. Proteolysis index (%) of vacuum-packaged dry-aged beef during 21 days of storage time at refrigerated condition (4°C) ..... 45

## List of Abbreviations

ATP	Adenosine triphosphate
DeoxyMb	Deoxymyoglobin
DDW	Deionized distilled water
LAB	Lactic acid bacteria
MAP	Modified atmosphere packaging
Mb	Myoglobin
MDA	Malondialdehyde
MetMb	Metmyoglobin
NPN	Non-protein nitrogen
OxyMb	Oxymyoglobin
RH	Relative humidity
TAB	Total aerobic bacteria
TBA	2-Thiobarbituric acid
TBARS	2-Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TN	Total nitrogen
VBN	Volatile basic nitrogen
YM agar	Yeast and mold agar

# **Chapter 1.**

## **Literature review**

### **1.1. Dry aging of beef**

#### **1.1.1. Aging type**

##### **1.1.1.1. Beef aging**

After slaughter, muscles of carcass experience the onset of rigor mortis and loss of extensibility (Lawrie, 1953). During muscle post-mortem, physical and chemical reaction have been reported by many researchers (Bate-smith and Bendall, 1949; Bendall, 1951). Among the reaction, decreasing ATP and pH value were closely related with the conversion of muscle to meat (Erdos et al., 1943). During the rigor mortis, anaerobic glycolysis increases lactic acid and H<sup>+</sup> ions in the muscle, resulting in pH decline. As post mortem glycolysis reached certain cessation and ATP depletion, the muscle became to shorten and loss its extensibility. Once muscle had critically lower value of ATP, the myosin head which affected on contraction and relaxation of muscle started permanently bound to actin filament which resulted in formation of actin-myosin complex (Pearce et al., 2011). Consequently, the muscle occurred quality change such as increasing toughness, and decreasing water holding capacity, and so on (Lawrie, 1998). After the rigor mortis, a chain of reactions occurs that affected the

tenderness and flavor of meat. Then, autolysis which was decomposed by the enzyme itself occurred. Consequently, degradation of actin-myosin complex and hydrolysis of protein by enzyme in carcass was proceeded during aging. Also, titin and desmin which were constituted the myofibrillar protein were decomposed, and affected the improvement of WHC, tenderness, and flavor of meat during aging (Pearce et al., 2011). Therefore, aging is important for final products and widely used in the meat industry to improve the meat quality such as flavor, tenderness, juiciness, and palatability (Campbell et al., 2001).

#### 1.1.1.2. Wet aging

Wet aging process is widely used for storage technology of cut meat with a sealed barrier packaging system (vacuum packaging) at refrigerated temperature (Gazalli et al., 2013). Wet aging, called vacuum aging, refers to extended the storage time for the fresh meat. Because meat is sealed by vacuum packaging, the wet-aged beef, in general, has higher yield, prevented microbial contamination and meat oxidation during wet aging process (Dikeman et al., 2013; Picouet et al., 2014). In addition, this aging method is very useful for storage and transportation due to packaging. However, the wet/vacuum-aged beef had more bloody, serummy, and metallic flavor compared to dry aged one (Laster et al., 2008). In addition, vacuum packaging caused the purple color of meat which was unattractive to consumers by reduction of oxyMb during wet aging process, and induced

decreasing the meat color stability (Troy et al., 2010). With this regard, packaging materials for wet aging were developed to prevent the color denaturation, protein and lipid oxidation of meat such as MAP system (Picouet et al., 2014). Like this, meat stability for color and oxidation has been reported in the meat industry.

#### 1.1.1.3. Dry aging

Dry aging is an ancient technology of meat to store fresh meat. In fact, for centuries, dry aging was a common method for meat market to tenderize beef without the vacuum packaging (Dashdorj et al., 2016). However, as vacuum packaging was developed, the dry aging technology in the meat industry had been disappeared (Savell et al., 2008). Recently, there has been increased interest for dry aging process in the United States and Australia (AMPC, 2010). Also, in the Asian countries such as Korea, Singapore, Japan, Hong Kong, and Taiwan, there has been increasing the interest for dry aging technology due to its unique flavor. In fact, Asian countries have served the dry-aged beef in their high end restaurant. In parallel, Korea meat industry has also experienced in rapidly increased demand of dry-aged beef for the high end niche food service market.

Dry aging process was performed using the unpackaged primal cuts under the controlled condition such as temperature, air flow velocity, and humidity during several weeks (Ahnstrom et al., 2006; Stenström et al., 2014). The environment was major characteristics for dry aging process (DeGeer et al.,

2009). Therefore, the storage condition during dry aging was influenced dry-aged beef quality. Especially, mold and yeast which formed on the dried surface, so called crust, by moisture evaporation in the meat affected the unique flavor (beefy and brown/roasted aroma) of dry-aged meat (Lee et al., 2017). However, dry-aged beef is costly because of lower yield due to higher trimming loss and weight loss by large amount of water evaporation as well as the risk of contamination of microorganisms during dry aging process (Li et al., 2013; Stenstrom et al., 2014). Although there are disadvantages described above in dry-aged beef, consumers prefer dry-aged beef than wet-aged one due to their enhanced palatability (Dikeman et al., 2013). In this circumstances, there are many studies on dry-aged beef to overcome the shortcomings for dry aging process recently and the investment and production of dry-aged beef has been increasing in the meat industry (Biswa Sinha et al., 2017).

### **1.1.2. Dry-aging process condition**

#### **1.1.2.1. Aging time**

Dry aging period was very controversy for acceptable consumption of the dry-age beef in the meat industry (Dashdorj et al., 2016). A large number of researchers have been reported that the most frequent period for dry aging process was between 14 and 40 days (Savell et al., 2007). Lepper-Bilie et al. (2012) reported that the acceptable aging was 21 days of aging period for

dry-aged products because there was no difference between 21 and 28 days of period for the production of dry-aged unique flavored and valued beef (DeGreer et al., 2009). Meanwhile, although the consumer preferred range from 28 to 55 days of dry-aged meat, USMEF (2014) suggested the aging period from 14 to 70 days of aging time. In addition, Perry et al. (2012) performed dry aging process using the 36-month-old Black Angus grass fed beef for 120 days of storage time. As a result, they reported dry-aged beef which aged 120 days of storage time did not increase the taste, juiciness, and their flavor compared to between 35 and 80 days of dry-aged beef. It has been reported after testing the meat with over 100 days of dry aging period that each consumer had different preference for the dry-aged beef. Also, this aging time might be varied depending on other aging condition such as temperature (Dashdorj et al., 2016).

#### 1.1.2.2. Temperature

Temperature is very important during dry aging process because of meat safety and quality. Dry aging has mainly processed at the optimum temperature of between 0°C and 4°C (from 32 to 39.2°F) (Ahnstrom et al., 2006; Savell et al., 2008). Aging temperature is important to dry aging process because the enzymatic process of microorganism affected to improve palatability (Dashdorj et al., 2016). However, higher temperature than optimal condition also accelerated more rapid microbial growth and contamination, resulting in off-odor of dry-aged beef. Therefore, dry aging

temperature performed as low as possible without freezing the carcass to prevent the microbial contamination (Dashdorj et al., 2016; Savell et al., 2008). USMEF (2013) recommended that the optimal temperature for dry aging process was from 0 and 4°C. If higher temperature more than recommended condition, it was caused excessive microbial growth resulting in product spoilage. In contrast, when the temperature was lower than required condition, aging process ceased as meat was frozen. Also, the dry aging room should have ante room or open to another refrigerated room to prevent inflow of outside air (Dashdorj et al., 2016).

#### 1.1.2.3. Air flow

Air flow provided air circulation without uncontacted spots or sites of high velocity during dry aging process (Khan et al., 2016). The USMEF (2013) recommended the optimal air flow range of 0.5 to 2 m/s for aging process. When air flow was not satisfied during aging period, although the air was fully in the dry aging room, the meat cannot release enough. Also, the dry-aged beef will dry out quickly and increase the weakness of dry-aged beef such as trimming and weight loss in the dry-aged final products (Dashdorj et al., 2016; Savell et al., 2008). In contrast, when the air flow was too low, the dry-aged beef had excessive microbial growth resulting in off-flavors by product spoilage (USEMF, 2013).

In the early stage of dry aging period, temperature and relative humidity had little effect on the drying rate and the air flow was the major factor of

drying in the meat surface (Nottingham et al., 1974). Therefore, the air flow and velocity should be maintained uniformly during the aging time and it is necessary that this condition adjusted at the start stage of aging period. In some cases, high air flow in dry aging room was used for faster dry-aging process in the market (Perry et al., 2012). Therefore, it is important to prevent microbial contamination while making contact to unconnected section with the air flow to ensure a great balance at drying of the beef.

#### 1.1.2.4. Relative humidity (RH)

Controlled RH was a crucial role during the dry aging process because RH was directly related to the final meat quality (Dashdorj et al., 2016). USMEF (2013) was recommended RH range of 80 to 85% for dry aging. When the RH value was higher than recommended condition, the dry-aged beef occurred excessive microbial growth resulting in off-flavors by product spoilage as well as had the sticky surface. In contrast, if the RH value was lower, grater water evaporation of meat was promoted during aging period. Consequently, the final dry-aged beef had not only excessive weight and trimming but also had less juiciness than normal one (Perry et al., 2012). Also, there are studies which processed at approximately 80% RH during dry aging (Ahnstrom et al., 2006; Parrish et al., 1991; Smith et al., 2008). Campbell et al. (2001) reported the dry-aged beef in a cooler at 75% RH and Warren et al. (1992) studied the range of 78% RH. However, there are still

limited reported studies which compared with the effects for different RH level during dry aging process (Dashdorj et al., 2016).

### **1.1.3. Microbiology in dry-aged beef**

#### 1.1.3.1. Microorganisms and their growth during dry-aging process

Concerns about microbial contamination during dry aging have persisted for several years (Ahnstrom et al., 2006). According to the previous studies, TAB count was significantly higher in dry-aged beef when compared to that in wet-aged one after 19 days of aging period (Li et al., 2014). Degeer et al. (2009) also reported the increase in TAB count of dry-aged beef from 6.6 to 9.4 log CFU/g after 28 days of dry aging ( $P < 0.05$ ; Degeer et al., 2009). However, there were many studies that have the opposite opinion for microorganism during dry aging (Dashdorj et al., 2016; Hulankova et al., 2018; Lee et al., 2017). They also reported that invasion of microorganisms into meat could be prevented by crust created to moisture evaporation outside of meat during dry aging. Also, they suggested that loss of water by evaporation might be affected on the delay of microbial growth. Based on these explanations, it is deemed that microbial contamination during dry aging can be prevented sufficiently if external surface microorganisms are not contaminated inside of the meat when raw meat is managed in good condition before dry aging and the crust is removed properly.

In addition, mold and yeast grow on the meat during dry aging. According to Lee et al. (2017), the population of mold and yeast growth were varied with

different air flow velocity during dry aging. And pathogens such as *Pseudomas* spp. and LAB were also affected on different air flow velocity. Changes in the composition of these microorganisms might be affected protein and fat degradation which changed meat quality after dry aging process and during distribution of dry-aged beef. In addition, *Pilaira anomala* and *Debaryomyces hansenii* were isolated by the dry-aged beef and identified mycotoxin of *P. anomala* in our previous study. According to other previous study, *P. anomala* was detected in the soybean paste in Korea and *D. hansenii* was already globally recognized as GRAS grade (Kim et al., 2009; Flores et al., 2015).

#### 1.1.3.2. Limitation in shelf-life establishment of dry-aged beef

Due to dry aging process which performed without vacuum packaging, microorganisms must be controlled the dry-aged beef during aging and distribution for safety. Establishment of expiration dates for livestock products includes microbiological safety, freshness and determination of decay level, and recommended standards is described in Table 1. The deterioration of meat by microbial growth in dry-aged beef caused an off-odor and off-taste, as well as meat color (e.g., rust due to the formation of  $H_2O_2$  or  $H_2S$ ) (Nychas et al., 2008). In addition, this is not generally harmful for human body, but must be considered in establishing an expiration date with the potential for pathogen existence (Kang et al., 2018). According to the microbial inspection guideline of the Ministry of Food and Drug Safety, Korea, the number of total aerobic acid and coliform in the meat is recommended less than 6 log CFU/g.

Also, the determination of freshness and deterioration of meat during the expiration date is a major criterion for measuring meat quality. pH, ammonia test, emulsified hydrogen detection test, Walkviewicz reactions, trimethylamine, and volatile basic nitrogen are used for determination of freshness and deterioration of meat (Jang et al., 2014). The pH and VBN are utilized as indicators for setting the distribution period of fresh meat and processed meat products. Currently, fresh meat is specified when the pH was less than 6.2 and VBN value was between 20 ~ 30 mg%. Also, KFRI (2018) selected as freshness indicators 10 types of volatile ingredients including 2,3-butanediol, 3-methylbutan-1-ol, acetoin, and 2-butanone. However, established regulation was only for fresh meat and processed meat, not for dry-aged meat and meat products. Also, there were a limitation of shelf-life stability for dry-aged meat and meat products study. Therefore, the importance of microbial control and other indicator of freshness in dry-aged beef during the storage has been reported (Campbell et al., 2011; Choe et al., 2018; Dashdorj et al., 2016).

**Table 1.** Microbial regulation (CFU/g, cm<sup>2</sup>) of recommended standards for safety in Korea

	Point	Total aerobic bacteria	Coliform
	At point of slaughter	$< 1 \times 10^5$	$< 1 \times 10^2$
Beef, lamb	At point of meat processing	$< 5 \times 10^6$	$< 1 \times 10^3$
	At point of sale	$< 5 \times 10^6$	$< 1 \times 10^3$
	At point of slaughter	$< 1 \times 10^5$	$< 1 \times 10^4$
Pork	At point of meat processing	$< 5 \times 10^6$	$< 1 \times 10^4$
	At point of sale	$< 5 \times 10^6$	$< 1 \times 10^4$

(MFDS, 2018)

## **1.2. Shelf life of beef**

### **1.2.1. Spoilage in beef**

#### 1.2.1.1. Microbial spoilage

Meat and meat products were abundant nutrient media for growth of microorganisms (Jay et al., 2005). The population of microflora in the meat depends on various factors. There are pre-slaughter control, age and sex of animal, handling during slaughter, temperature control during distribution and processing, and preservation method such as packaging system and so on (Dave and Ghaly, 2011). Bacteria as well as mold and yeast were also detected in the meat before spoilage (Garcia-Lopez et al., 1998). Cerveny et al. (2009) reported that *Pseudomonas* spp., *Psychrobacter* spp., *Acinetobacter* spp., and Gram-negative psychrotrophic was detected in the meat, especially *Enterobacteriaceae* are usually present under the refrigerate condition (4°C). The microbial spoilage decayed the meat and caused off-odor during aging and distribution of process. There were varied for inhibition of the microbial growth in the meat industry, and has been many reported (Kim et al., 2018; Yong et al., 2015). Therefore, control of microbial contamination was essential for good quality and safety of final products.

#### 1.2.1.2. Chemical changes in quality

Chemical changes included oxidation by combination of meat and oxygen,

warmed-over flavor, and off-odor (Huis in't Veld, 1996). The degree of fatty acid can vary depending on various factors such as heat, light and oxygen, as well as pre-slaughter condition and unsaturated fatty acid content. However, although there are no currently recommended standards, the meat had off- and warmed-over flavor in the meat after heating when the lipid oxidation in the meat is increased by more than a certain level. In addition, changes in color, texture, and nutrient loss, in particular, substances resulting from the oxidation of unsaturated fatty acids could be potentially toxic, which was one of the main factors to consider when setting shelf life (Gray et al., 1996; Ladikos and Lougovois, 1990). At present, domestic law recommends the measurement of lipid oxidation as an indicator of the distribution period (MFDS, 2017).

Also, glucose, lactic acid, nucleotides, urea, and water-soluble proteins were degraded by almost all bacteria in the meat (Nychas et al., 2007). For the microbial growth in the meat, these compounds were the vital energy source. Therefore, contamination of these microorganisms was directly affected rate of spoilage, consequently perceived precursors of microbial metabolites caused meat spoilage (Nychas et al, 2007). For these reasons, control of chemistry spoilage was important to persist for fresh meat during storage time.

## **1.2.2. Factors affecting spoilage**

### **1.2.2.1. Packaging system**

In the meat industry, MAP and vacuum packaging system were usually

used for meat packaging. Limitation of shelf life was affected by two main factors: 1) the chemical effect of O<sub>2</sub> and 2) growth of spoilage microorganisms. So, the shelf life of fresh meat was limited because the growth of bacteria such as aerobic bacteria (MFDS, 2014). Therefore, there are many studies to extend the shelf life of the meat by exchange air condition in the packaging. Gases for MAP usually used oxygen (O<sub>2</sub>), nitrogen (N<sub>2</sub>), and carbon dioxide (CO<sub>2</sub>) (Sivertsvik et al., 2002). These gas composition was varied accordance to food, preventing the oxidation and preserving safety during storage (Sivertsvik et al., 2002). MAP had many advantages: 1) extension of shelf-life for food by possibly 50 ~ 400%, 2) long distance distribution possible, 3) reduced economic losses because of longer shelf-life.

Among the MAP, vacuum-packaging system was usually used for meat packaging. Vacuum-packaging was commonly performed in industry, and was efficiently used to extend the shelf life and keep the fresh meat quality (Lambert et al., 1991). Seideman et al. (1976) demonstrated that a strong vacuum state was minimized the surface discoloration of the meat during storage, and improved the beef appearance by the vacuum-packaging system. Therefore, the packaging system assists not only protection of microbial contamination but also maintenance of meat quality during storage.

#### 1.2.2.2. Temperature

Bacteria generated during storage time, they usually inhibited by

temperature control (Adam et al., 1972; Clark et al., 1972). Also, the shelf-life of the eat stored at refrigerated condition was not only affected on types of spoilage bacteria, but also their population (Lambert et al., 1991). Thus, for extension of the meat shelf-life at chilled storage condition, an additional control was necessary to reduce the initial microbial number.

Also, inhibition temperature for microorganisms during storage was varied each microbial. Microorganisms were categorized as a psychrophile, psychrotroph, mesophile, and thermophile. Optimum temperature of their growth was different, and controlled the temperature during storage the food/meat was important to prevent spoilage. Therefore, establishment of temperature when storing the meat/food according to microorganisms and purpose of storage were essential part to maintain the meat quality and safety.

### **1.2.3. Shelf-life of dry-aged beef**

#### 1.2.3.1. Current studies in the shelf-life of dry-aged beef

Choe et al. (2018) reported microbial changes were observed during 21 days of storage time for safety analysis of dry-aged beef at different packaging system and temperature during storage (overwrapped at refrigeration temperature ( $3 \pm 2^{\circ}\text{C}$ ), vacuum-packaged refrigeration and freezing temperature ( $-23 \pm 2^{\circ}\text{C}$ ). As a result, the number of microorganisms showed no significant change between before and after dry

aging process. However, refrigerated wrap-packaging was exceeded the legal recommendation at day 7 of storage time. Therefore, Choe et al. (2018) confirmed that deterioration rate of dry-aged beef which packaged with wrap at refrigerated condition (4°C) was faster than other packaging system. On the other hand, dry-aged beef which stored with vacuum packaging at refrigerated and frozen temperature was maintained less than 7 log CFU/g of microbial count during 21 days of storage time. Also, in vacuum-packaging regardless of storage temperature, the dry-aged beef had no significance on lipid oxidation, and sensory evaluation during storage. Therefore, Choe et al. (2018) confirmed that vacuum-packaged dry-aged beef not only kept the microbial safety but also remained the meat quality, so vacuum-packaging was advantageous for extending the meat storage time.

Also, Lee et al. (2018) studied microbial and physicochemical analysis of dry-aged beef for setting the shelf-life under wrap-packaging at 4°C. After dry aging process, the number of total aerobic bacteria was 4.26 log CFU/g which was less than that reported by Choe et al. (2018), and Lee et al. (2018) reported the acceptable shelf-life of wrap-packaged dry-aged beef was less than 12.2 days. Also, there were significant changes in meat color after 3 days of storage time. The wrap-packaged dry-aged beef showed significantly decreased in flavor, taste, and overall acceptance, but remained the normal value (> 5 point) during 6.3 days of storage time. Therefore, based on Lee et al. (2018) result of microbial and quality analysis, it is

recommended that dry-aged beef was packaged for 6.3 days under refrigerated condition (4°C) (Lee et al., 2018).

The previous studies on the shelf-life of dry-aged beef are still insufficient to apply for industry. Establishing the quality indicator of dry-aged beef and studies for safety is essential and should continue.

Therefore, the objective of this study is establishment of the storage stability and shelf-life for vacuum-packaged dry-aged beef based on microbial growth, physicochemical properties, and sensory analysis.

## **Chapter 2.**

# **Storage stability of vacuum-packaged dry-aged beef during refrigerated condition (4°C)**

### **2.1. Introduction**

Dry aging is one of the aging techniques to enhance tenderness and flavor of meat (Dashdorj et al., 2016). It exposes the primal/sub cuts and/or whole carcasses of beef without packaging under the controlled condition such as temperature, RH, and air flow (Lee et al., 2017). However, its application had been limited due to its low salable yield (Dikeman et al., 2013). After that, in recent years, the consumption of dry-aged beef has been increasing worldwide, mainly by the increasing consumers' preference for its unique flavor (beefy and brown/roasted) (Dikeman et al., 2013; Smith et al., 2008).

However, due to the direct exposure of meat during the dry aging process, the consumers' concern for microbial contamination is higher in dry-aged beef than wet-aged one (Lee et al., 2017; Li et al., 2014). According to the previous studies, TAB count was significantly higher in dry-aged beef when compared to that in wet-aged one after 19 days of aging period (Li et al., 2014). Degeer et al. (2009) also reported the increase in TAB count of dry-aged beef from 6.6 to 9.4 log CFU/g after 28 days of dry aging ( $P < 0.05$ ; Degeer et al., 2009). In addition, the significant growth of mold and yeast in

the dry-aged beef has been reported (Lee et al., 2018; Li et al., 2013; Ryu et al., 2018). As the changes in microbial growth are critical during the dry aging process, it can affect the initial numbers of microorganisms at the beginning of storage. Then, it may increase the risk of microbial contamination and meat spoilage, resulting in the deteriorations of safety and quality in dry-aged beef (Dashdorj et al., 2016; Nychas et al., 2008). Therefore, the importance of microbial control in dry-aged beef during the storage has been referred by a lot of researchers (Campbell et al., 2011; Choe et al., 2018; Dashdorj et al., 2016). While the microbial quality of the outer surface of dry-aged beef cannot directly be incorporated into the expectation of quality or shelf-life of the products because the edible part of dry-aged beef is usually prepared after excising and trimming the crust completely. Therefore, the internal dry-aged beef for edible portion is less affected by the outer surface microorganism, unlike consumer concern. Rather, there was reported that mold and yeast produced in dry-aged beef were more effective flavor of meat. However, control of dry-aged beef was important in the meat industry because microbial growth of dry-aged beef was higher than wet/vacuum-aged beef.

The microbial control during the storage can be mainly attributed to packaging system (e.g. vacuum, wrap, and modified atmospheric packaging; Lambert et al., 1991). Among them, vacuum packaging might be more effective to inhibit microorganisms during the storage as it eliminates air, which is an important factor to microbial growth (Lopez-caballero et al.,

2000; Nychas et al., 2008). Also, this system can retard the lipid oxidation of meat during storage period due to the oxygen depletion (Mielnik et al., 2006). In consequence, vacuum packaging is widely used for improving shelf-life of meat and meat products (Giannuzzi et al., 1998; Nissen et al., 1996). It has been studied with various meat and meat products under the different conditions (Fuentes et al., 2010; Garcia-Esteban et al., 2004; Ansorena and Astiasaran, 2004), however, only little information is available for vacuum-packaged dry-aged beef. Therefore, the objective of this study was to investigate the changes in microbial growth and physicochemical and sensory properties of vacuum-packaged dry-aged beef to suggest its shelf-life at refrigerated condition (4°C).

## **2.2. Materials and methods**

### **2.2.1. Dry-aging process and packaging conditions**

A total of nine strip sirloins were taken from nine beef carcasses (Holstein, quality grade 3) (Kim et al., 2019) on three different slaughter days (three sirloins/trial) and dry aged for 28 days (temperature, 4°C; RH, approximately 75%; air flow, 2.5 m/s). After the completion of dry aging, the crusts were trimmed off of the samples and the sirloins were cut ( $12.7 \times 7.6 \times 2.54 \text{ cm}^3$ , length  $\times$  width  $\times$  height) for packaging. Then the samples were vacuum packaged in polyethylene bags ( $\text{O}_2$  permeability  $2.3 \text{ mL/m}^2/\text{d}$  at  $38^\circ\text{C}$ ) and stored at refrigeration temperature (4°C) for 21 days. During

the 21 days of storage, vacuum-packaged dry-aged beef was obtained at 0, 7, 14, and 21 days for further analysis.

### 2.2.2. Microbial growth

Five grams of dry-aged beef was blended with 45 mL of 0.85% saline solution for 2 min using a laboratory stomacher (BagMixer<sup>®</sup> 400, Interscience Ind., St. Nom, France). One-hundred microliters from each sample dilution was spread on the surface of agar plates. TAB, mold/yeast, and lactic acid bacteria (LAB) were enumerated using plate count agar (Difco Laboratories, Detroit MI, USA), yeast mold agar (Difco Laboratories), and de man, Rogosa and Sharpe agar (MRS; Difco Laboratories), respectively. After spreading the dilution on the agar, the agar plates for TAB and LAB were incubated at 37°C for 48 h and yeast mold agar plates were incubated at 25°C for 120 h, respectively. The number of colonies was enumerated and expressed as log CFU/g.

### 2.2.3. Physicochemical properties

#### 2.2.3.1. pH

Each beef sample (1 g) was homogenized with 9 mL of distilled deionized water (DDW) for 30 s (T10 basic, Ika Works, Staufen, Germany). The homogenates were centrifuged (Continent 512R, Hanil Co., Ltd., Incheon, Korea) at  $2265 \times g$  for 10 min. After centrifugation, each supernatant was filtered through filter paper (No. 4, Whatman PLC., Kent,

UK) and each filtrate was measured using a pH meter (SevenGo, Mettler-Toledo International Inc., Schwerzenbach, Switzerland) after calibration with standard buffers.

#### 2.2.3.2. Volatile basic nitrogen (VBN)

Three grams of each treatment sample was homogenized at 9500 rpm for 30 s (T25, Ika Works) followed by centrifugation (Continent 512R, Hanil Co., Ltd.) at  $2265 \times g$  for 10 min and filtration through filter paper (Whatman No. 1, Whatman PLC). One hundred microliters of each sample with 0.01 N boric acid and indicator solution [0.66% methyl red in ethanol:0.66% bromocresol green in ethanol = 1:1 (v/v)] was placed individually in the inner section of a Conway (Sibata Ltd., Sitama, Japan); then, 1 mL of sample and 50% potassium carbonate was added into the outer section of the conway, after which the lid was sealed immediately. Then, the conway was incubated at 37°C for 1 h and titrated with 0.01 N hydrogen chloride. The VBN value was calculated as follows:

$$\text{VBN (mg\%)} = \left[ \frac{1.4007 \times \text{concentration of HCl (N)} \times \{\text{titration volume of sample}(\mu\text{L}) - \text{titration volume of blank}(\mu\text{L})\}}{\text{sample weight (g)}} \right] \times 100$$

#### 2.3.3.3. 2-Thiobarbituric acid reactive substances (TBARS) value

Lipid oxidation was measured for the TABRS value using a

spectrophotometer (X-ma 3100, Human Co. Ltd., Seoul, Korea). Five grams of each sample was homogenized with 15 mL of DDW and 7.2% butylated hydroxyl toluene in ethanol at 9600 rpm for 30 s (T25, Ika Works). After homogenization, 2 mL of the homogenates was transferred to 15 mL Falcon® tubes and 4 mL of 20 mM 2-thiobarbituric acid in 15% trichloroacetic acid was added. The tubes were heated in a laboratory water bath at 90°C for 30 min, cooled, and centrifuged at  $2265 \times g$  for 15 min (HM-150IV, Hanil Co., Ltd.). The absorbance of the supernatant was measured at 532 nm. The TBARS value was expressed as mg malondialdehyde (MDA)/kg of meat sample.

#### 2.3.3.4. Instrumental color

After cutting and opening the package and allowing the beef to bloom for 30 min, lightness, redness, and yellowness of the meat were measured and expressed as CIE  $L^*$ ,  $a^*$ ,  $b^*$  values, respectively, using a spectrophotometer (CM-5, Konica Minolta Censing Inc., Osaka, Japan). The colorimeter was calibrated using a standard white and black plate before each measurement. Color difference ( $\Delta E$ ) was calculated as follows:

$$\Delta E = [(L^* - L_{\text{ref}}^*)^2 + (a^* - a_{\text{ref}}^*)^2 + (b^* - b_{\text{ref}}^*)^2]^{1/2}$$

where  $L_{\text{ref}}^*$ ,  $a_{\text{ref}}^*$ , and  $b_{\text{ref}}^*$  represents lightness, redness, and yellowness in vacuum-packaged dry-aged beef at day 0, respectively.

#### 2.3.3.5. Myoglobin (Mb) content

For Mb content and the composition of its related pigments, deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), and metmyoglobin (MetMb) were analyzed following the methods of Krzywicki (1979). Mb was extracted from 4 g beef samples with 20 mL of 0.4 M phosphate buffer (pH 6.8). Each sample was homogenized (T10 basic, Ika Works) at 13 000 rpm for 30 s and the homogenates were stabilized for 1 h at refrigeration conditions (4°C) with foil. After allowing to stand, the samples were centrifuged (Combi 514R, Hanil Co., Ltd.) at  $5000 \times g$  for 30 min. The filtrates were filtered with filter paper (Whatman No. 1, Whatman PLC) and the absorbance of the supernatant was measured at 525, 572, and 700 nm using a spectrophotometer (X-ma 3100, Human Co. Ltd.).

#### 2.3.3.6. Texture profile analysis

Texture profile (hardness, adhesiveness, springiness, chewiness, and cohesiveness) was analyzed with a texture analyzer (TA1, Lloyd Instruments Ltd., Fareham, UK). Ten grams of ground sample was placed into a petri dish ( $35 \times 10 \text{ mm}^2$ ), cooked in a laboratory water bath at 85°C for 15 min, and cooled. The conditions of the texture analyzer were set as follows: pre-load speed 10 mm/min, post-load speed 2 mm/s, maximum cell load 50 kg, compression level 60%.

#### 2.3.3.7. Proteolysis index

TN and NPN content were assessed by Kjeldahl method (ISO, 1978) using the Kjeltec 2200 (Dongjin, Gyeonggi-do, Korea). The grounded meat samples (1 g) were accessed for TN content and NPN content was extracted and analyzed as described by Pérez-Santaescolástica et al. (2018). The sample (2.5 g) was added with 25 mL of DDW and homogenized (T10 basic, Ika Works) for 30 sec. The homogenate was centrifuged at  $252 \times g$  for 20 min, followed by filtration (No.4, Whatman PLC). After filtration, 10 mL of 20% TCA was added for removing the protein nitrogen and stabilized at room temperature for 60 min. Then, the sample was centrifuged again at  $1734 \times g$  for 10 min and filtrated the supernatant. The supernatant used for NPN content analysis, following the same method as TN (ISO, 1978). The proteolysis index was calculated by the ratio of NPN and TN as follows:

$$\text{Proteolysis index (\%)} = \frac{\text{NPN}}{\text{TN}} \times 100$$

#### 2.4. Sensory property

Sensory evaluation was conducted with nine consumer panelists to determine the sensory properties of vacuum-packaged dry-aged beef during 21 days of storage (IRB no. 1810/003-001). There were three independent sensory tests for each storage day. The samples were cut into pieces of the same size ( $4 \times 2 \times 2.54 \text{ cm}^3$ ) and grilled until the core temperature reached  $72^\circ\text{C}$ . Sensory analysis was evaluated with a 9-point hedonic scale (1, extremely dislike; 9, extremely like) and scored for appearance, odor, taste,

tenderness, juiciness, and overall acceptability of beef.

## 2.5. Statistical analysis

All experiments were conducted in triplicate and averaged ( $n = 3$ ). Vacuum-packaged dry-aged beef samples at different storage days (0, 7, 14, and 21 days) were analyzed in each trial. A generalized linear model was used to perform the analysis using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and results were reported as mean values with standard error of the mean (SEM). Significant differences among the mean values were determined on the basis of Tukey's multiple comparison test at a significance level of  $P < 0.05$ .

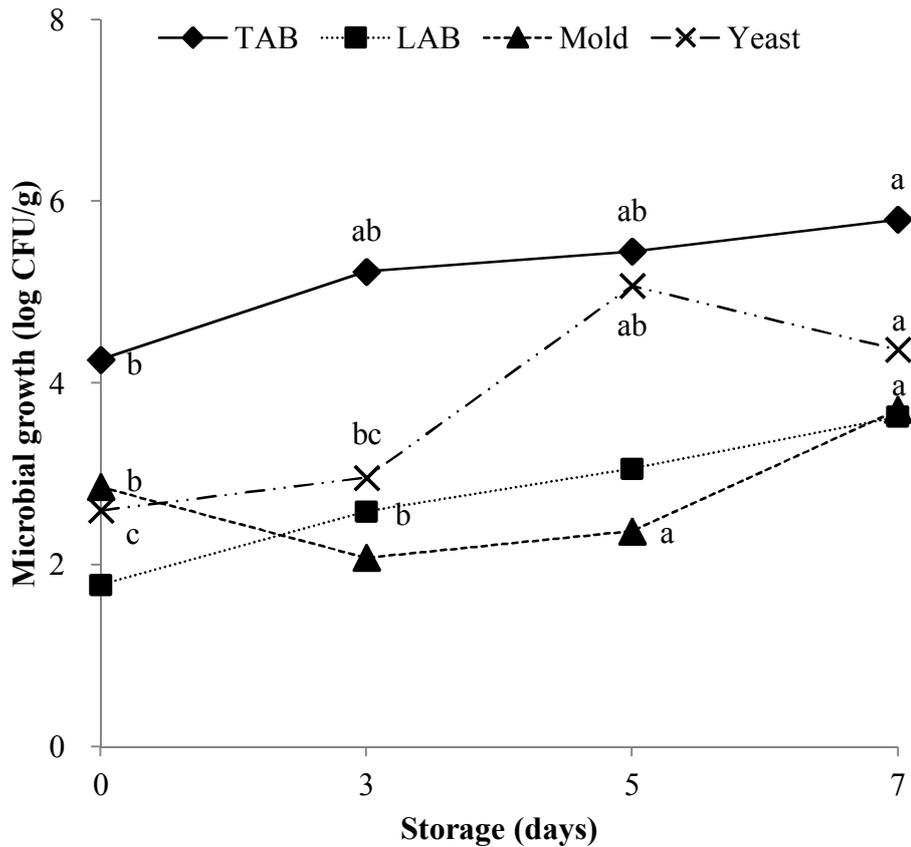
## 2.3. Results and discussions

### 2.3.1. Microbial growth

Microbial growth of meat depends on all environmental conditions during the slaughter and aging process, ultimately impacting meat spoilage and quality deterioration (Nychas et al., 2008). Therefore, the control of microbial growth (especially TAB) is important in meat during storage. In the Korean market, TAB count in meat and meat products is limited to  $< 6$  log CFU/g at the point of consumption (MFDS, 2018). In our previous study, the microbial growth of wrap-packaged dry-aged beef was observed for 7 days of storage refrigerated condition ( $4^{\circ}\text{C}$ ) (Fig. 1; Lee et al., 2018). Based on that, TAB count was assumed to exceed the present legal standard at 7.5 days of storage (Table 2). Meanwhile, in the present study, the initial numbers of TAB, LAB, mold, and yeast in vacuum-packaged dry-aged beef were 4.4, 2.4, 3.6, and 5.9 log CFU/g, respectively (Fig. 2). During 21 days of storage, TAB count steadily increased and exceeded the legal standard at day 14 (6.5 log CFU/g). Therefore, the shelf-life of vacuum-packaged dry-aged beef may be limited to less than 14 days of storage based on TAB count. The shelf-life of vacuum-packaged dry-aged beef was estimated as 11.5 days (Table 2). So, vacuum packaging may prolong the shelf-life of dry-aged beef, approximately 4 days, at refrigerated condition.

LAB count in vacuum-packaged dry-aged beef also increased over the first 7 days; these levels were maintained thereafter. However, given that the

growth of LAB did not affect meat spoilage during storage in both vacuum- and wrap-packaged beef (Lee et al., 2018a; Nychas et al., 2008), it was not considered a factor in the quality deterioration category. While mold count decreased significantly between days 14 and 21, possibly due to the depletion of oxygen—an element crucial for its growth (Kemp et al., 1983), yeast count fluctuated and reached its highest level at day 21 (Fig. 2). As the detection of mold and yeast is generally scarce in most meat and meat products, no recommendations for acceptable mold and yeast levels during storage are available. However, as the presence of mold and yeast has been consistently reported in dry-aged beef (Kim et al., 2018a; Lee et al., 2018a; Ryu et al., 2018), their impact on meat quality is currently being studied.

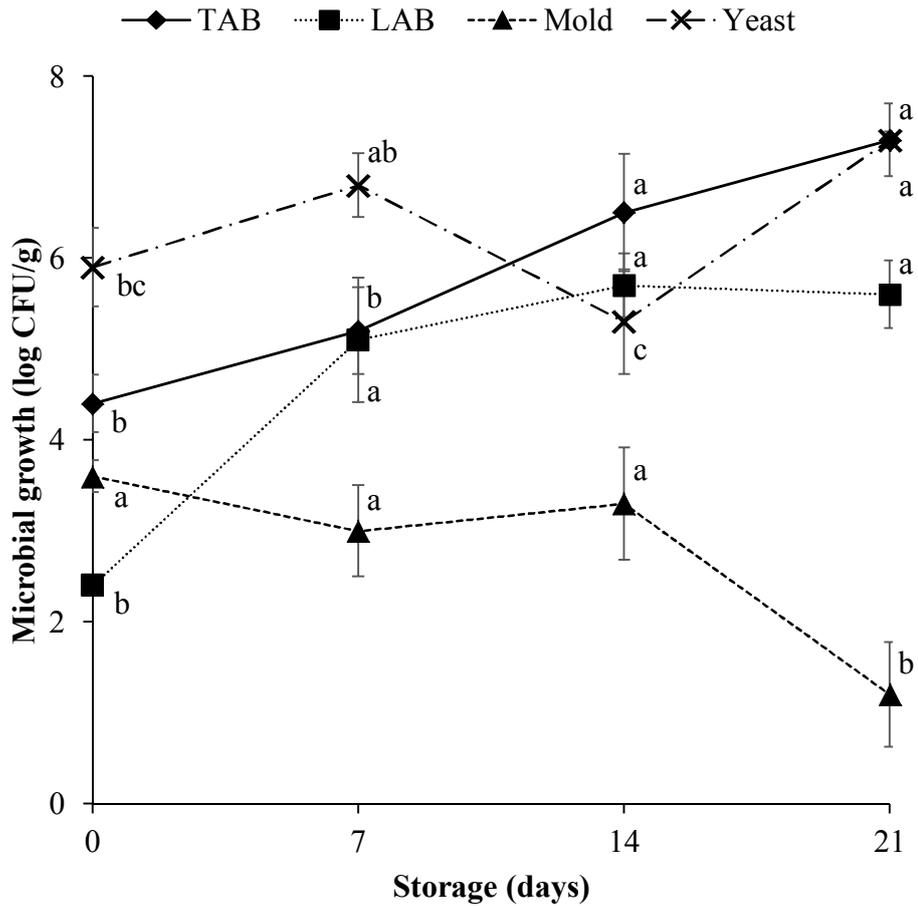


**Figure 1.** Microbial growth (log CFU/g) of wrap-packaged dry-aged beef during 7 days of storage at refrigeration condition (4°C) (mean ± standard deviation).

<sup>a-c</sup>Different letters indicate a significant difference within the same microorganisms during 21 days of storage time ( $P < 0.05$ ).

TAB, total aerobic bacteria; LAB, lactic acid bacteria.

(Adpated from Lee et al., 2018).



**Figure 2.** Microbial growth (log CFU/g) of vacuum-packaged dry-aged beef during 21 days of storage at refrigeration condition (4°C) (mean  $\pm$  standard deviation).

<sup>a-c</sup>Different letters indicate a significant difference within the same microorganisms during 21 days of storage time ( $P < 0.05$ ).

TAB, total aerobic bacteria; LAB, lactic acid bacteria.

**Table 2.** The estimated shelf-life of wrap- and vacuum-packaged dry-aged beef for total aerobic bacteria with quality standards

TAB	Shelf-life (days)	Regression equation	R <sup>2</sup>
Wrap-packaged dry-aged beef	< 7.5	$y = 0.2155x + 4.3768$	0.951
Vacuum-packaged dry-aged beef	< 11.5	$y = 0.1429x + 4.3500$	0.990

<sup>1)</sup>TAB, total aerobic bacteria.

Quality limit of TAB was legal standard (6.0 log CFU/g) from ‘Ministry of Food and Drug Safety’ (2018).

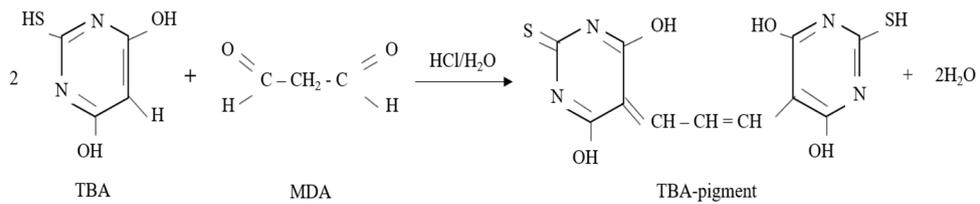
## 2.3.2. Physicochemical property

### 2.3.2.1. Spoilage indicator

pH and VBN have been used to evaluate meat freshness/spoilage during storage because these indicators have been closely associated with microbial growth (Byun et al., 2003). During storage, the generation of protein-derived basic products (VBN including amine and/or ammonia) by the proteolysis of microorganisms can cause increases in pH as well as VBN content in meat (Byun et al., 2003; Lee et al., 2018; Sunesen et al., 2003). In contrast, decreases in pH can be caused mainly by the generation of lactic acid by LAB growth (Dave and Ghaly, 2011). According to Lee et al. (2018), the quality limits of pH and VBN in Korea are 6.2 and 20 mg%, respectively, for fresh meat. In this study, the highest value of pH was 5.69 at day 14 and decreased thereafter (Table 3). In contrast, the VBN content of vacuum-packaged dry-aged beef did not change significantly until 14 days of storage; however, thereafter, it increased and exceeded its recommended level for fresh meat (20 mg%) at day 21. Consequently, the re-establishment of spoilage indicators for both dry- and wet-aged beef is necessary (Jang et al., 2014; Lee et al., 2018). Based on current recommendations, vacuum-packaged dry-aged beef may be considered fresh until day 14 at 4°C.

### 2.3.2.2. TBARS

Lipid oxidation of meat is very important as it can cause quality deterioration (e.g. color, flavor, texture, and nutritive value) to meat and meat products (Jakobsen and Bertelsen, 2000; Ladikos and Lougovois, 1990). It can be measured by the TBARS value (Fig. 3) and tends to be increased during the storage (Gok et al., 2008; Kim et al., 2004). However, in the present study, the TBARS value was significantly decreased after 14 days of storage time (Table 3), possibly as a result of excessive microbial growth in the vacuum-packaged dry-aged beef during that period (Fig. 2). According to Branen et al. (1978), the reaction of MDA and 2-thiobarbituric acid can be inhibited by the protein-derived amine, which is the resultant of microbial growth. Similarly, the TBARS value of raw pork was decreased during the storage (Kim et al., 2004). An et al. (2017) also reported the decrease in TBARS value of frozen pork during 7 days of storage time at degradation of MDA by the microbial growth. Due to the high initial value and no significant increase of the TBARS value, it could not represent the quality deterioration of vacuum-packaged dry-aged beef during the storage. Therefore, the result of TBARS may not be considered to determine the shelf-life of vacuum-packaged dry-aged beef, which is agreed with Lee et al. (2018) who reported no correlation between the TBARS value and quality attributes of dry-aged beef. However, further investigation of pH, VBN, and lipid oxidation may be necessary to clarify their changes in dry-aged beef during storage.



(Adapted from Fernandez, et al., 1997)

**Figure 3.** Combination of 2-thiobarbituric acid and malondialdehyde to make 2-thiobarbituric acid pigment.

TBA, 2-thiobarbituric acid; MDA, malondialdehyde.

**Table 3.** pH, volatile basic nitrogen, and 2-thiobarbituric acid reactive substances of vacuum-packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C)

Traits <sup>1)</sup>	Storage (days)				SEM <sup>2)</sup>
	0	7	14	21	
pH	5.62 <sup>c</sup>	5.65 <sup>b</sup>	5.69 <sup>a</sup>	5.55 <sup>d</sup>	0.004
VBN (mg%)	16.92 <sup>b</sup>	17.15 <sup>b</sup>	19.03 <sup>b</sup>	23.92 <sup>a</sup>	0.678
TBARS (mg MDA/kg meat)	0.98 <sup>ab</sup>	1.13 <sup>a</sup>	0.87 <sup>b</sup>	0.83 <sup>b</sup>	0.041

<sup>1)</sup>VBN, volatile basic nitrogen; TBARS, 2-thiobarbituric acid-reactive substance; MDA, malondialdehyde.

<sup>2)</sup>Standard error of means (n = 12).

<sup>a-c</sup>Different letters within the same row represent significant difference ( $P < 0.05$ ).

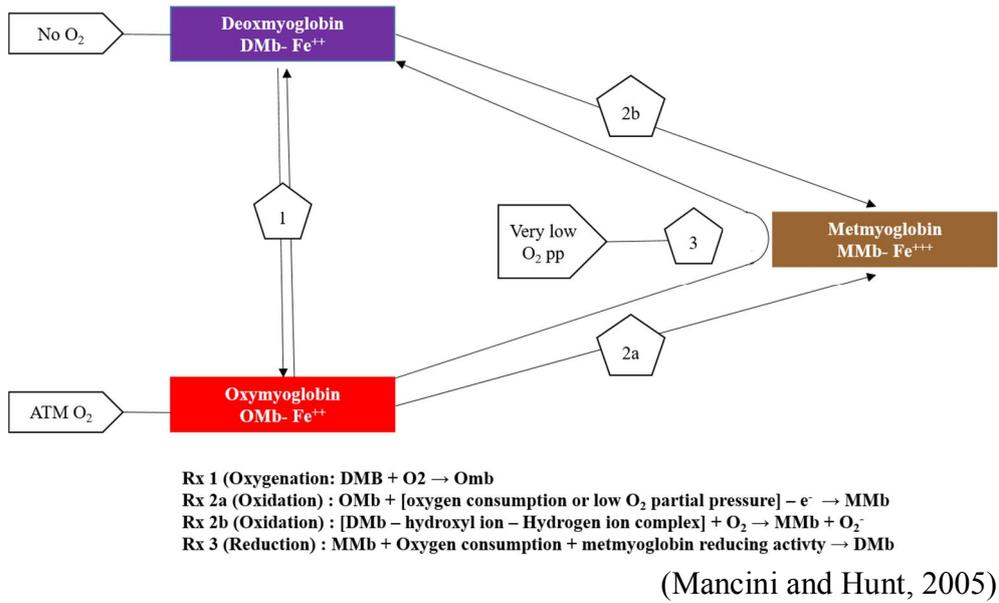
### 2.3.2.3. Instrumental color and Mb content

Meat color can affect acceptability by consumers when they purchase meat and meat products at the market (Yong et al., 2018). CIE  $L^*$ ,  $a^*$ , and  $b^*$  values are used to measure meat color; among them, CIE  $a^*$  may be important to consumers as it determines the redness of meat, which confers freshness at the market. Meat color is attributed to the chemical status of Mb (OxyMb, bright red color; MetMb, brown color; DeoxyMb, purple color; Fig 4) (Livingston and Brown, 1981; Yong et al., 2018). Therefore, the change in Mb content is a main determinant for color stability during the storage. Meat color and Mb content of vacuum-packaged dry-aged beef were shown in Tables 4 and 5, respectively. In the present study, significant increases in the composition of OxyMb was found between days 7 and 14, which was not expected, especially in the middle of vacuum packaging, as the generation of OxyMb is attributed exclusively to oxygen binding (Mancini and Hunt, 2005). However, Lee et al. (2018) also reported a sudden increase in OxyMb composition in wrap-packaged dry-aged beef at day three. Hence, regardless of packaging methods, the composition of OxyMb may change during the storage of dry-aged beef based on unknown factors that require further investigation of the chemical changes of myoglobin in dry-aged beef. In contrast, OxyMb composition in vacuum-packaged dry-aged beef decreased ( $P < 0.05$ ) after day 14 of storage, possibly via its oxidation to MetMb with a decrease in pH. Lower pH at day 21 may promote the oxidation of OxyMb to increase the content of MetMb

(Faustman et al., 2010).

During the storage of vacuum-packaged dry-aged beef, CIE  $L^*$  was significantly increased at day 7 and decreased thereafter, whereas CIE  $a^*$  and  $b^*$  resulted in a significant increase between days 7 and 14 and then decreased (Table 4). The change in CIE  $L^*$  may be related to microbial growth, especially TAB (Robach and Costilow, 1961). Meanwhile, the change in CIE  $a^*$  could be affected by OxyMb content (Table 5 and Fig. 5), which reached the highest value at day 14 and had similar tendency with CIE  $a^*$ . Also, as CIE  $b^*$  is positively correlated to CIE  $a^*$ , the tendency of CIE  $b^*$  in vacuum-packaged dry-aged beef during the storage was similar to that of CIE  $a^*$ . Also, the higher pH can contribute to the darker, redder, and more yellow color of meat by the increase in water holding capacity (Allen et al., 1997). In this study, the highest pH value of vacuum-packaged dry-aged beef at day 14 may resulted in high CIE  $a^*$  and CIE  $b^*$  on 14 days of storage time.

Total color difference ( $\Delta E$ ) of vacuum-packaged dry-aged beef was more significant until day 14 and decreased thereafter ( $P < 0.05$ ), meaning that there may be less change in meat color after 14 days of storage time. However, as CIE  $a^*$  of vacuum-packaged dry-aged beef reached the highest at day 14, the acceptability of consumer may also be higher on the same day when compared to the others.



**Figure 4.** Redox reaction of myoglobin in fresh meat during storage.

Mb, myoglobin; DeoxyMb, deoxymyoglobin; OxyMb, oxymyoglobin; MetMb, metmyoglobin.

**Table 4.** Instrumental color (CIE  $L^*$ ,  $a^*$ , and  $b^*$ ) and color difference of vacuum-packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C)

Traits	Storage (days)				SEM <sup>1)</sup>
	0	7	14	21	
CIE $L^*$	33.42 <sup>b</sup>	37.99 <sup>a</sup>	35.76 <sup>ab</sup>	35.59 <sup>b</sup>	0.524
CIE $a^*$	10.00 <sup>c</sup>	10.25 <sup>c</sup>	12.42 <sup>a</sup>	11.64 <sup>b</sup>	0.117
CIE $b^*$	6.35 <sup>c</sup>	6.35 <sup>c</sup>	9.50 <sup>a</sup>	7.83 <sup>b</sup>	0.054
$\Delta E$	-	6.11 <sup>a</sup>	5.53 <sup>ab</sup>	3.98 <sup>b</sup>	0.3664

<sup>1)</sup>Standard error of means (n = 12).

<sup>a-c</sup>Different letters within the same row represent significant difference ( $P < 0.05$ ).

**Table 5.** Myoglobin content of vacuum packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C)

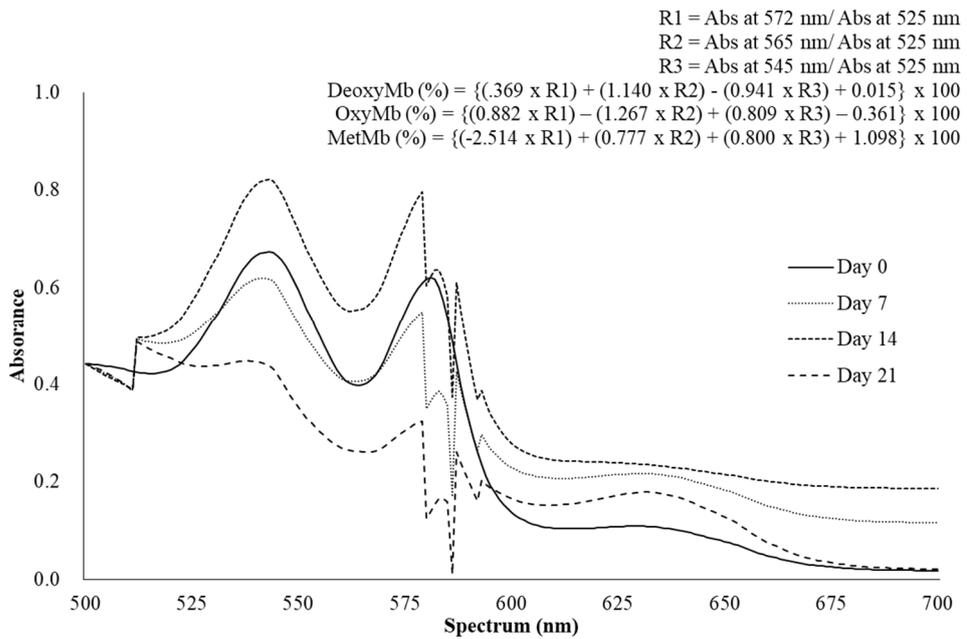
Traits	Storage (days)				SEM <sup>1)</sup>
	0	7	14	21	
DeoxyMb (%)	3.38 <sup>cz</sup>	5.88 <sup>bz</sup>	9.37 <sup>ay</sup>	5.77 <sup>bz</sup>	0.342
OxyMb (%)	57.91 <sup>bx</sup>	52.27 <sup>bx</sup>	86.20 <sup>ax</sup>	37.68 <sup>cy</sup>	1.531
MetMb (%)	38.71 <sup>by</sup>	41.85 <sup>by</sup>	4.43 <sup>cz</sup>	56.55 <sup>ax</sup>	1.675

<sup>1)</sup>Standard error of means (n = 12).

<sup>a-c</sup>Different letters within the same row represent significant difference ( $P < 0.05$ ).

<sup>x-z</sup>Different letters within the same column represent significant difference ( $P < 0.05$ ).

Mb, myoglobin; DeoxyMb, deoxymyoglobin; OxyMb, oxymyoglobin; MetMb, metmyoglobin.



**Figure 5.** The absorbance curve of myoglobin (Mb) in of vacuum-packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C)  
 Mb, myoglobin; DeoxyMb, deoxymyoglobin; OxyMb, oxymyoglobin;  
 MetMb, metmyoglobin.

#### 2.3.2.4. Texture profile analysis

Texture profile analysis (e.g. hardness, springiness, chewiness, and cohesiveness) is useful to predict sensory texture of cooked meat (De Huidobro et al., 2005) and adhesiveness can show its texture defect like slime (Pérez-Santaescolástica et al., 2018). In this study, the adhesiveness of vacuum-packaged dry-aged beef was not significantly changed during 21 days of storage time (Table 6), meaning that the deterioration in texture was not observed in vacuum-packaged dry-aged beef during the storage. On the other hand, the values of other parameters were significantly decreased at day 7, possibly due to the protein degradation of microbial growth during the storage (Fig. 2). Then, it was maintained thereafter, except for hardness and springiness (first and second bites of hardness; De Huidobro et al., 2005) (Table 6). Hardness was decreased at day 7 ( $P < 0.05$ ), similar to the other parameters, however, increased slightly but significantly at day 14, whereas springiness was decreased only at day 21 ( $P < 0.05$ ). Considering all results from texture profile analysis, it was assumed that the texture of vacuum-packaged dry-aged beef may not be entirely different after 7 days of storage.

**Table 6.** Texture profile analysis of vacuum packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C)

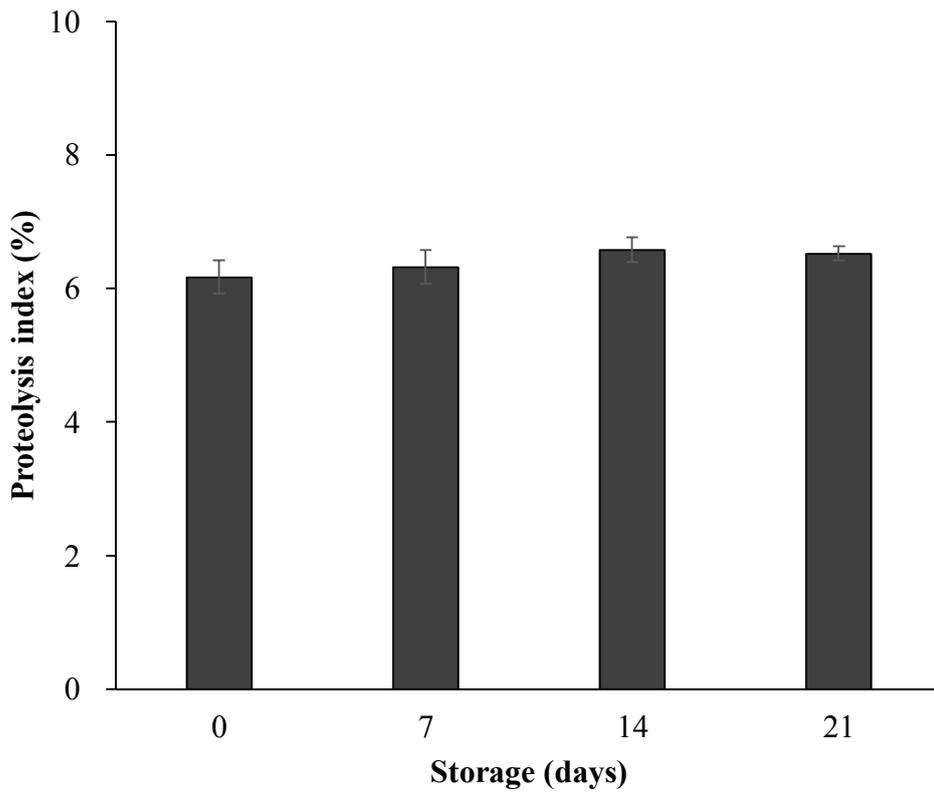
Traits	Storage (days)				SEM <sup>1)</sup>
	0	7	14	21	
Hardness (N)	389.91 <sup>a</sup>	310.65 <sup>c</sup>	333.25 <sup>b</sup>	322.19 <sup>bc</sup>	4.022
Adhesiveness (kgf.mm)	-0.01	-0.03	0.01	0.02	0.022
Springiness	0.58 <sup>ab</sup>	0.61 <sup>a</sup>	0.55 <sup>bc</sup>	0.55 <sup>c</sup>	0.006
Chewiness (N)	147.98 <sup>a</sup>	104.69 <sup>b</sup>	93.58 <sup>b</sup>	98.01 <sup>b</sup>	5.874
Cohesiveness	0.82 <sup>a</sup>	0.56 <sup>b</sup>	0.50 <sup>d</sup>	0.52 <sup>c</sup>	0.004

<sup>1)</sup>Standard error of means (n = 12).

<sup>a-c</sup>Different letters within the same row represent significant difference ( $P < 0.05$ ).

#### 2.3.2.5. Proteolysis index

Proteolysis is main biochemical reaction by the endogenous proteolytic enzymes and contributes to the texture change in meat and meat products during dry processing (Jurado et al., 2007; Pérez-Santaescolástica et al., 2018). Dry-cured/processed meat and meat products with long drying period can occur the excessive proteolysis, resulting in the texture defect of final products such as pastiness, softness, and slime (Costa-Corredor et al., 2009; Harkouss et al., 2015; Pérez-Santaescolástica et al., 2018). As vacuum-packaged dry-aged beef was aged and stored for approximately 40 days, proteolysis index was measured to determine the occurrence of texture defect in the present study (Fig. 6). As a result, proteolysis index of vacuum-packaged dry-aged beef was not significantly changed during the storage at refrigerated condition (4°C), which is agreed well with the adhesiveness (Table 6). The results from adhesiveness and proteolysis index indicate that vacuum-packaged dry-aged beef at refrigerated condition (4°C) did not show any texture defect during 21 days of storage time.



**Figure 6.** Proteolysis index (%) of vacuum-packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C) (mean  $\pm$  standard deviation).

### 2.3.3. Sensory property

In our previous study in wrap-packaged dry-aged beef, the scores of appearance and odor were not changed ( $P < 0.05$ ), whereas taste and overall acceptability were significantly decreased at day 7 (Table 7). In the present study, sensory property (appearance, odor, taste, tenderness, juiciness, and overall acceptability) of vacuum-packaged dry-aged beef was evaluated at 7 days interval of 21 days (Table 8). All parameters showed no significant changes during the entire storage time, except for juiciness and overall acceptability ( $P < 0.05$ ). Juiciness was significantly decreased at day 14, while overall acceptability had no change for intake until during 14 days of storage, and significantly decreased thereafter.

When considered the changes in the sensory property, (especially overall acceptability), of wrap- and vacuum-packaged dry-aged beef, vacuum-packaged dry-aged beef can maintain its sensory property (at least 21 days) longer than wrap-packaged one (6.2 days; Lee et al., 2018). So, it can prolong the shelf-life of dry-aged beef until 11.5 days (based on TAB count) without any adverse its sensory property.

**Table 7.** Sensory property of vacuum-packaged dry-aged beef during 21 days of storage at refrigeration conditions (4°C)<sup>1)</sup>

Traits	Storage (days)				SEM <sup>1)</sup>
	0	7	14	21	
Appearance	6.30	6.04	6.07	5.63	0.223
Odor	6.19	6.26	6.11	5.70	0.140
Taste	6.59	6.04	6.00	5.63	0.220
Tenderness	6.33	5.70	5.74	6.22	0.260
Juiciness	6.33 <sup>a</sup>	5.85 <sup>ab</sup>	5.55 <sup>b</sup>	5.59 <sup>ab</sup>	0.169
Overall acceptability	6.44 <sup>a</sup>	5.96 <sup>ab</sup>	5.78 <sup>ab</sup>	5.41 <sup>b</sup>	0.183

<sup>1)</sup>1, extremely dislike; 5, neither dislike nor like 9, extremely like.

<sup>2)</sup>Standard error of means (n = 12).

<sup>a,b</sup>Different letters within the same row represent significant difference ( $P < 0.05$ ).

**Table 8.** Sensory property of wrap-packaged dry-aged beef during 7 days of storage at refrigeration conditions (4°C)<sup>1)</sup> (Lee et al., 2018)

Traits	Storage (days)				SEM <sup>2)</sup>
	0	3	5	7	
Appearance	6.22	5.84	5.55	5.81	0.286
Odor	5.72 <sup>a</sup>	5.45 <sup>ab</sup>	4.95 <sup>b</sup>	5.11 <sup>ab</sup>	0.143
Taste	6.50 <sup>a</sup>	5.78 <sup>ab</sup>	5.08 <sup>b</sup>	5.36 <sup>b</sup>	0.231
Overall acceptability	6.11 <sup>a</sup>	5.28 <sup>b</sup>	5.11 <sup>b</sup>	5.06 <sup>b</sup>	0.130

<sup>1)</sup>1, extremely dislike; 5, neither dislike nor like 9, extremely like.

<sup>2)</sup>Standard error of the means (n = 12).

<sup>a,b</sup>Different letters within the same row represent significant difference ( $P < 0.05$ ).

(Adpated from Lee et al., 2018).

## **2.4. Conclusion**

Considering microbial safety and sensory property, the shelf-life of wrap-packaged dry-aged beef was limited until 6.2 days at refrigerated condition. Meanwhile, vacuum-packaged dry-aged beef could prolong its shelf-life, approximately 4 days, until at 11 days. However, further investigation in pH, VBN, and lipid oxidation may be in needs to clarify their changes in dry-aged beef during the storage.

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# Summary in Korean

## 진공포장한 건식숙성 우육의 냉장 보관 (4° C) 중 저장안정성 규명

김선진

서울대학교 대학원

농생명공학부 동물생명공학전공

본 연구의 목적은 미생물 성장 변화와 물리화학적 및 관능적 평가를 기반으로 하여 진공포장된 건식숙성 우육의 저장안정성과 유통기한을 확인하고자 한다. 총 9마리의 소 도체(3등급 홀스타인)에서 28일 동안 4° C, 75% 상대습도, 2.5 m/s 풍속의 조건으로 건식숙성을 완료한 채끝을 본 실험을 위해 사용하였다. 건식숙성 우육의 바깥 부분(크러스트)을 제거한 뒤 실험을 위해 진공포장 하여 21일간 냉장온도에서 보관하였으며, 미생물 성장, pH, 휘발성 염기태 질소(VBN), 지방산화 및 관능평가를

실시하였다. 그 결과, 식품의 유통안정성을 위해 검출량이 6 log CFU/g 이하로 규제되어 있는 총 호기성 미생물은 진공포장된 건식숙성 우육의 저장 7일차와 14일 사이에서 그 수준을 넘어섰다. 하지만 회귀방정식을 통해 진공포장된 건식숙성 우육을 11.5일동안 안전하게 저장할 수 있음을 확인하였다. 또한 식품의 부패 지표가 되는 pH 및 VBN은 저장 14일에서 21일 사이에 유의적으로 변화하는 반면, 지방산패도를 나타내는 2-thiobarbitruic acid reactive substances(TBARS) 값은 감소하는 값을 나타내었다. 진공포장된 건식숙성 우육의 냉장 저장 중 점착성을 제외한 경도, 탄성, 씹힘성 및 응집성과 같은 물리화학적 변화는 유의적으로 변하는 반면, 식육의 외관, 향, 맛, 그리고 연도에 저장 21일차동안 유의적인 변화가 없었다. 반면에, 진공포장된 건식숙성 우육의 다즙성은 저장 14일에 유의적인 감소를 보였고, 전체적인 기호도 또한 21일차에서 유의적으로 감소하였다. 따라서 진공포장된 건식숙성 우육의 유통기한은 총 호기성 미생물 수의 수준을 넘지 않고 품질에 부정적인 영향을 미치지 않는 냉장 조건 하에서 11일동안 유통 가능할 것으로 사료된다.