Master's Thesis of Agriculture

Antioxidant and angiotensin I converting enzyme inhibitory activity of the crust from dry-aged beef and its utilization as flavor enhancer in beef patty

건식숙성 소고기 크러스트의 항산화 및 ACE 저해활성과 패티 제조시 풍미증진제로서의 활용

August, 2018

By

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이 논문을 농학석사 학위논문으로 제출함

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Overall Summary

Antioxidant and angiotensin I converting enzyme inhibitory activity of the crust from dry-aged beef and its utilization as flavor enhancer in beef patty

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The objective of present experiments was 1) to determine the antioxidant and angiotensin I–converting enzyme (ACE) inhibitory activity of the crust of dry-aged beef, and 2) to find the way of utilizing the crust as a flavor enhancer in the manufacturing beef patty.

Experiment I. Evaluation of antioxidant and ACE inhibitory activity of crust derived from dry aged beef

Moisture evaporation of meat surface in dry aging process inevitably produces crust which is cut and discarded before consumption. Antioxidant activity, ACE inhibitory activity, and protein profile of the crust were evaluated compared with un-aged, wet-, and dry-aged beef. The antioxidant activity was

determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) radical scavenging, ferric reducing antioxidant power (FRAP), and ferrous ion chelating activity. The crust samples showed the greatest (P<0.05) antioxidant activity resulting from the 3 different mechanisms of action (radical scavenging, non-radical redox potential activity, and metal cheating) as antioxidant and ACE inhibitory activity among the treatment. Protein bands with small molecular weight indicating potent bioactivity were appeared in myofibrillar protein profile of the crust sample. The lowest (P<0.05) ACE inhibitory activity was observed in un-aged beef. Based on results from this study, it could be suggested the crust, usually recognized as discarded portion of dry-aged beef, can be utilized in various areas as functional ingredient possessed antioxidant and ACE inhibitory activity.

Experiment II. Application of the crust from dry-aged beef on beef patty and its quality assessment

The aim of this study was to find the way of utilizing the crust in meat products to enhance sensory characteristic and to reduce expensive waste. Total of four sirloins were dry-aged for 28 days at 4°C (75% relative humidity). The crust was obtained from surface of the dry-aged beef and prepared as powdered form after freeze-drying. Patties were prepared with 75% ground beef, 20% beef fat, 0.3% salt, and the crust [0 (control) and 5%; w/w] The patties were packaged with aerobic method and stored at 4°C for 4 or 6 days with 2-day interval, respectively. The patties with the crust showed higher score in flavor, tenderness, and acceptability by sensory panel compared to control. In addition, different profile was observed in electronic nose analysis between with and without crust group. In conclusion, the crust from dry-aged beef could be used as flavor

enhancer in meat products by providing beefy and palatable flavor without long period of dry aging time as meat industry practiced commonly.

Keywords: ACE inhibitory activity, Antioxidant activity, Beef patty, Crust, Dry-aged beef, Electronic nose, Sensory evaluation

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Contents

Chapter III. Application of the crust from dry-aged beef on beef patty and its quality assessment

List of Tables

Chapter II.

Chapter III.

List of Figures

Chapter I.

Chapter II.

Chapter III.

List of Abbreviations

ABTS	:	2,2'-Azino-di-(3-ethylbenz-thiazoline sulphonate), scavenging activity (%)
ACE	:	Angiotensin I - converting enzyme (ACE) inhibitory activity
Chelating	:	Ferrous metal irons chelating activity, chelating activity (%)
DPPH	:	2,2-Diphenyl 1-picrylhydrazyl, scavenging activity (%)
DW	:	Distilled water
FRAP	:	Ferric reducing antioxidant power, reducing power
HCL	:	Hydrogen chloride
HHL	:	Hippuryl-L-histidyl-L-leucine
RH	:	Relative humidity
SDS-PAGE	:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TBARS	:	2-Thiobarbituric acid reactive substances
TEAC	:	Trolox equivalent antioxidant capacity
TPTZ	:	2,4,6-tris-2,4,6-tripyridyl-2-triazine

CHAPTER I.

General introduction

Aging is defined as storing meat for a period of time to improve meat tenderness, flavor, and juiciness (Campbell et al., 2001). Aging methods are divided into wet and dry, which are the most common methods of postmortem aging practiced on beef products.

Wet-aged meat is sealed in highly moisture-impermeable vacuum packaged and stored at refrigerated temperature during the storage days (Gudjónsdóttir et al., 2015; Smith et al., 2008). The advantages of wet aging are known as minimal weight loss, reduced bacterial growth, and prolonged shelf life compared to dry aging. Therefore, wet aging is the aging method that most commonly practiced in the beef industry, which is used by about 95% of beef (DeGeer et al., 2009; Dikeman et al., 2013; Laster et al., 2008).

On the other hand, dry-aged meat is produced unpackaged, controlled at designated temperature and relative humidity. According to the DeGeer et al. (2009), dry aging is a process used to produce unique flavor and added the value of beef. A greater proportion of ester and heptane in the volatile compounds were produced from dry- than wet-aged beef that may be related to flavor development (King et al., 1995). In addition, Gallego et al. (2018) and Seol et al. (2018) reported several bioactive peptides are generated from meat and meat products during dry fermentation or aging with protease treatment. Therefore,

dry-aged meat is also expected to have these bioactive peptides generated by endogenous proteolytic enzymes in meat.

Meanwhile, dry aging of beef is a costly procedure because of decreased yields due to greater weight and trim losses (trim off the curst) compared with vacuum aging (Dikeman et al., 2013).

Crust (Fig. 1) is the surface of the dry-aged meat and trimmed off before consumption (Fig. 2). Most of the produced crust are discarded without utilization because of the unique characteristics; hard, dry, and a large number of microorganisms. The wasted cost by crust is approximately 73.9 billion won per year in meat industry of South Korea. It can be calculated as percentage of dry-aged beef multiplied by total amount of beef production and 30% of loss (Korea Institute for Animal Products Quality Evaluation, KAPE, 2016). Despite the heavy economic losses, the utilization of crust has never been studied yet.

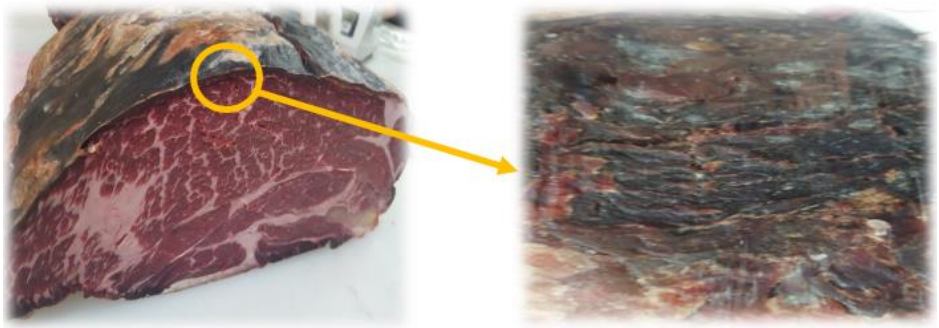

Fig. 1. Aspect of dry-aged beef (left) and expansion of crust (right)

Therefore, two experiments (Fig. 3) were conducted 1) to determine the antioxidant and ACE inhibitory activity of the crust of dry-aged beef and 2) to find the way of utilizing the crust as a flavor enhancer in the manufacturing beef patty.

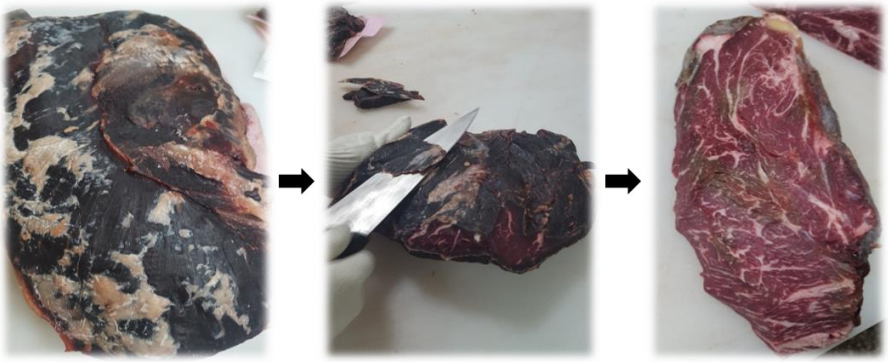

Fig. 2. Procedure of crust trimming on dry-aged beef

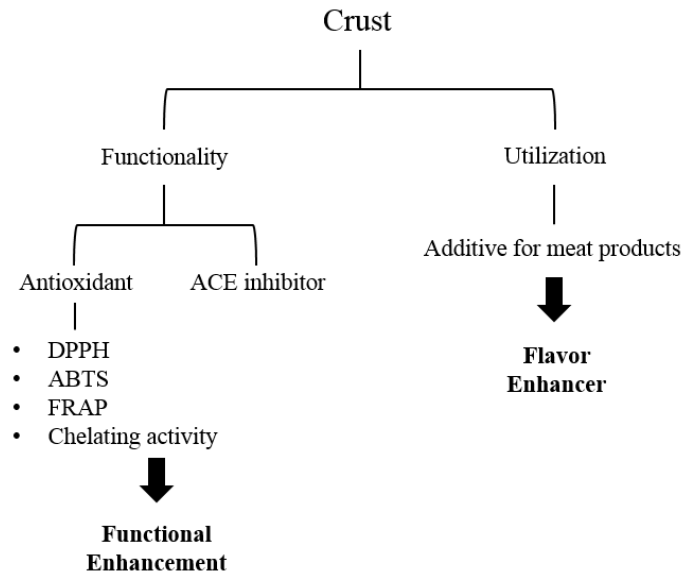

Fig. 3. The scheme of experiments using the crust of dry-aged beef

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CHAPTER II.

Evaluation of antioxidant and ACE inhibitory activity of crust derived from dry aged beef

2.1. Introduction

Postmortem aging induces enhancement in tenderness and juiciness of meat as activating proteolysis by endogenous proteolytic enzymes (Kim et al., 2004). In principle, there are two methods of aging: wet aging (stored under vacuum packaging) and dry aging [stored under controlled temperature, relative humidity (RH) and airflow with exposure to air] (Lee et al., 2018). During dry aging, air exposure in meat lead to high weight loss (20-45%) due to the moisture evaporation and generation of crust (trimming portion) on the meat's surface, while exhibit intensively concentrated beefy flavor compared to wet-aged meat (Dashdorj et al., 2016; Khan et al., 2016). Furthermore, decomposed protein by microbial protease during dry aging could possess bioactivity including antioxidant and angiotensin I - converting enzyme (ACE) inhibitory activities (Rajapakse et al., 2005). Recently, studies on generation of several bioactive peptides containing antioxidant activity and ACE inhibitory activity from meat and meat products during dry fermentation or aging with protease treatment have been reported (Gallego et al., 2018). The muscle based protein contains unique amino acids including methylhistidine and hydroxymethyllysine, which have

bioavailability (Wang & Shahidi, 2018), and functional dipeptides including carnosine (β-Ala-His) and anserine [β-Als-His(3-Me)], which exhibit antioxidant activity as chelating metal ions or scavenging free radical (Ambigaipalan et al., 2015; Mora et al., 2008). However, functional properties including antioxidant and ACE inhibitory activity of the crust produced by dry aging process, which accounts for approximately 35% of meat but completely unutilized, have never been studied yet.

This study hypothesized that the crust of dry-aged beef may possess antioxidant and ACE inhibitory activity by the production of peptides during aging. Therefore, the objective of this study was to determine the antioxidant and ACE inhibitory activity of the crust (trimming portion of dry-aged beef) compared to un-aged, wet-aged, and dry-aged beef samples. Furthermore, difference in protein profile was determined between un-aged beef and crust samples by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2.2. Materials and Methods

2.2.1. Meat Sample preparation

At 24 h postmortem, loins (M. *longissimus,* n = 4) were obtained from 23-month-old castrated Holstein bulls (quality grade 3, n = 2) slaughtered at a local slaughterhouse. Each loin was cut into two sections and the eight sections randomly assigned into 3 treatments; (1) un-aged (fresh) (2) aged for 28 days under vacuum packaging (temperature, 4°C; wet aging) (3) dry-aged for 28 days (temperature, 4°C; RH, 75%; airflow, 2.5 m/s). The crust sample was obtained

from external surface of the dry-aged meat. The crust was trimmed off approximately 1 cm from external surface of the dry-aged beef by a professional butcher. There were no identified pathogens found in the crust based on result of 16S rDNA sequencing (data not shown). All samples (crust, the fresh of dry-aged, wet-aged, and fresh beef) were removed of visible fat and connective tissue and cut into cubes then minced using a blender (HMF-985, Hanil electronic, Korea) for 30 s. The blended meat samples were immediately frozen in liquid nitrogen and subsequently lyophilized (PVTFD-10K, Ilshin, Korea). The lyophilized samples were ground to a fine powder in a mortar and pestle, screened through a 10-mesh sieve, and kept at -70 °C until use.

2.2.2. Extraction

The whole muscle protein extracts of beef sample were prepared following the procedure as described in Kim et al. (2010). Briefly, 1 g of each beef sample was homogenized in 10 ml of sodium phosphate buffer containing 2% SDS (w/v) and homogenized for 30 s. The homogenate was centrifuged (Continent 512R, Hanil, Korea) at 1,500 ×g for 15 min at 25 ℃. The supernatant was collected and used for analysis of antioxidant and ACE inhibitory activity.

2.2.3. Antioxidant activity

The antioxidant activity of meat extracts obtained from four different beef samples were determined by four different methods due to different mechanisms of action as below.

2.2.3.1. DPPH assay

The meat extract was diluted 50 times with distilled water (DW), and 2 ml of the diluted samples were mixed with 2 ml of 0.2 mM DPPH in methanol (Blois, 1958). After vigorous vortexing, the mixture was left in dark for 20 min at room temperature, and centrifuged (Continent 512R, Hanil, Korea) at 2,268 ×g for 10 min at 4 ℃. Absorbance of the supernatant was measured at 517 nm using a spectrophotometer (X-ma 3100, Human Corp., Korea) and antioxidant trolox was used as an antioxidant standard. The absorbance was converted to equivalent activity of Trolox per ml based on a standard curve and expressed as Trolox equivalent antioxidant capacity (TEAC, TEAC/ml extract).

DPPH radical scavenging activity (%) = [1 – (Absorbance of sample at 517 nm/Absorbance of control at 517 nm)] × 100

2.2.3.2. ABTS assay

ABTS capacity of each muscle extract was measured by degree of suppression of ABTS radical cation ($ABTS^{\bullet+}$) produced by reaction of ABTS with potassium sulfate (Erel, 2004). To obtain the $ABTS^{\bullet+}$ solution, a mixture of 7.0 mM ABTS and 2.45 mM potassium persulfate (final concentration) was prepared and stored in darkness for 16 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Fresh $ABTS^{\bullet+}$ solution was prepared for each analysis. The reaction mixture consisted of 3 ml of the $ABTS^{\bullet+}$ working solution and 20 μL sample, placed in a cuvette incubated for 5 min at 30 ℃. The mixture was centrifuged (Continent 512R, Hanil, Korea) at 2,268 ×g for 5 min. Absorbance of the supernatant was measured at 734 nm using a

spectrophotometer (X-ma 3100, Human Corp., Korea). The percentage $ABTS^{\bullet+}$ inhibition was calculated by the equation of radical cation scavenging activity (%) = $[(A_B–A_S)/ A_B] \times 100$; where A_B = the absorbance of the blank; A_S = the absorbance of the muscle extract. Antioxidant trolox was used as an antioxidant standard with the same method at 2.3.1.1.

2.2.3.3. FRAP assay

As a non-radical redox potential based method, the reducing power of each muscle extract was carried out according to Benzie and Strain. (1996) with slight modifications. For FRAP say, 300 mM acetate buffer (pH 3.6), TPTZ (2,4,6-tris-2,4,6-tripyridyl-2-triazine; 10 mM in 40 mM HCl), and 20 mM ferric chloride solution were prepared in a portion of 10:1:1 (v/v/v). The mixture was incubated in a water bath maintained at 37 ℃ for 30 min. A 3 ml of FRAP was added to 100 μL of each 4 times diluted sample with DW and incubated for 5 min. The mixture was centrifuged (Continent 512R, Hanil, Korea) at 2,268 ×g for 5 min. Absorbance of the supernatant was measured at 593 nm using a spectrophotometer (X-ma 3100, Human Corp., Korea). An increase in the absorbance was considered as the reducing power.

2.2.3.4. Ferrous ion chelating activity assay

The chelating activity of each muscle extract was determined using a method reported by Gil et al. (2017) and monitored by measuring the ferrous ion-ferrozine complex at 562 nm. Briefly, 0.5 ml of muscle extract sample was added to 50 μL of $FeCl_2$ (2 mM). The reaction was initiated by the addition of 5

mM ferrozine (0.1 ml) and 3.2 ml of ethanol. The mixture was vortexed vigorously and left in dark at room temperature for 10 min. The mixture was centrifuged (Continent 512R, Hanil, Korea) at 2,268 ×g for 5 min. Absorbance of the supernatant was measured at 562 nm using a spectrophotometer (X-ma 3100, Human Corp., Korea). The inhibition (%) of ferrous ion-ferozzine complex formation was calculated by the formula below:

Chelating activity (%) = [1 – (A_B –A_S)/A_B] × 100; where A_B = the absorbanceof the blank; A_S = the absorbance of the muscle extract.

2.2.4. ACE inhibitory activity

ACE inhibitory activity was determined according to Arihara. (2001) with some modifications. Ten microliter of each muscle extract was incubated with 30 μL of hippuryl-L-histidyl-L-leucine (HHL, 12.5 mM in 0.1 M sodium borate buffer) was incubated at 37℃ for 10 min. Distilled water instead of sample was used as a blank and control. After incubation, 10 μL of ACE (peptidyldipeptide hydrolase from rabbit lung acetone extract) were added and the mixture incubated at 37℃ for 30 min. The enzymatic reaction was stopped by adding 50 μL of 1 N HCl. The hippuric acid generated by the action of the angiotensin-converting enzyme on HHL was extracted from the acidified solution into 300 μL ethyl acetate by vortex mixing for 15 s. This was centrifuged at 1,000 ×g for 5 min at 4°C, and a 250 μL aliquot of each ethyl acetate layer was transferred to clean tubes and evaporated by heating at 70℃ for 1 h in a water bath. The hippuric acid was redissolved in 300 μL of DW, and the amount formed was determined by absorbance at 228 nm. ACE inhibitory activity (%) was

determined using the equation:

$$\text{ACE inhibitory activity (\%)} = [1 - (A_S - A_{SB})/(A_C - A_{CB})] \times 100$$

where A_S was the absorbance of the sample, A_{SB} was the absorbance of the sample blank, A_C was the absorbance of the control, and A_{CB} was the absorbance of the control blank. In this assay, enalapril maleate salt (0.3 mg/ml) was used as control.

2.2.5. SDS-PAGE

Myofibrillar proteins for SDS-PAGE analysis were prepared from each beef samples according to the procedure described by Grabski and Burgess. (2001), using 0.03 M phosphate buffer (pH 7.4) and 0.1 M phosphate buffer potassium containing 0.7 M potassium iodide and 0.02% sodium azide as isolating medium. Protein concentration of isolated beef samples was determined by Lowry method (1951). The isolated myofibrillar proteins were analyzed using SDS-PAGE method described by Laemmli. (1970) with some modifications. The stacking gel and separating gel contained 4.5% and 12.5% polyacrylamide, respectively and 20 μL of meat extract was loaded onto the gel. Protein standards (Precision Plus Protein™ Unstained Standards, Bio-Rad, CA, USA) were included in each electrophoretic run to determine molecular size. Electrophoresis was performed using a Mini-Slab Size Electrophoresis System AE-6531 (Atto Corporation, Tokyo, Japan) at 20 mA for 70 min. Gels were stained for 30 min in 0.1% Coomassie Brilliant Blue R-250 solution, containing 30% methanol and 10% acetic acid. After staining, gels were destained for 90 min using a solution containing 30% and 10% acetic acid.

2.2.6. Statistical analysis

The experimental design was completely randomized. The data obtained from three replications with two observations per replicate. Statistical analysis was performed using the SAS statistical package (version 9.3, SAS Institute Inc., NC, USA). For all analysis, animal was included as a random effect. Significant differences among the treatments were determined using the Student–Newman–Keuls multiple comparison test at a level of $P<0.05$.

2.3. Results and Discussion

2.3.1. Antioxidant activity of meat extracts obtained from four different beef samples

2.3.1.1. Radical scavenging activity

The DPPH radical scavenging activity was affected ($P<0.05$) by processing methods and portion of beef (Table 1). The protein extract from the crust exhibited the greatest values ($P<0.05$; 44.7% and 0.48 TEAC/ml protein extract) while wet-aged beef extract showed the lowest values ($P<0.05$; 40.8% and 0.43 TEAC/ml extract) in DPPH radical scavenging activity, respectively. According to Kim et al. (2016), more abundant aromatic amino acids including phenylalanine, tyrosine, and tryptophan were exhibited in dry-aged beef when compared to wet-aged beef. The aromatic amino acids have been known as effective radical-scavengers by donating proton to electron-deficient radicals (Thiansilakul et al., 2007). However, in this study, protein extract from the dry-aged beef (fresh) showed similar value to un-aged one in DPPH radical scavenging activity and lower ($P<0.05$) value compared to that of the crust one. The greatest value of the protein extract from the crust in DPPH radical scavenging activity may be due to amino acids or peptides produced by proteolytic enzymes of microorganisms which present on surface (crust) directly during dry aging. Dry aging of beef is prone to microbial contamination and the growth of mold/yeast is often observed on the curst (Lee et al., 2017). It is expected that both endogenous enzymes and exogenous proteolytic enzymes produced from microorganism act together, resulting in greater level of meat protein degradation and production of different peptides than fresh, wet-aged, and dry-aged beef. According to Toldrá et al. (2018), naturally generated

peptides by microorganisms could induce biological activity as producing the peptides of different size and various free amino acids. In addition, previous studies reported that fermentation process with microorganisms led to generation of bioactive peptides having antioxidant activity from muscle-based foods (Kim et al., 2004).

Similar results to DPPH radical scavenging activity were shown in ABTS radical cation scavenging activity of four protein extracts tested (Table 1). The greatest ABTS radial cation scavenging activity (69.7%, 43% higher compared with the lowest value) was observed in protein extract from the crust and followed by that of dry-aged beef. This result could be due to the same reason described previously. The protein extracts from wet-aged and un-aged beef showed the lowest (P<0.05) in ABTS radical cation scavenging activity and no significant difference was observed between them.

Table 1. Antioxidant activity of fresh (un-aged), wet-aged, dry-aged beef and the crust of the dry-aged beef

Treatment	DPPH	ABTS	FRAP (μmol Fe^{2+}/g)	Chelating
Fresh (Un-aged)	42.4[b]	37.8[c]	336[bc]	65.2[c]
Wet-aged	40.9[b]	39.0[c]	322[c]	69.0[c]
Dry-aged	41.3[b]	44.3[b]	360[b]	77.7[b]
Crust	45.4[a]	70.2[a]	495[a]	83.9[a]
SEM[1)]	0.45	0.50	8.49	1.33

[1)]Standard error of the means (n=12).

[a-c]Values with different letters within the same column differ significantly (P<0.05).

2.3.1.2. Ferric ions reducing power

Reducing agents act as electron donor to the reduced species and a higher value in absorbance was indicated as greater reducing capacity. The greatest value (P<0.05; 0.34) in absorbance at 593 nm was observed in protein extract from the crust among the treatment (Table 1). Based on the result, direct action of microorganisms on the surface of dry-aged meat (crust) could contribute the generation of reducing power capacity. Previous study reported that the protein extracted from fermented sausages had 2 or 3 times higher antioxidant activity at the end of ripening than at the initial processing stage (Vaštag et al., 2010). The difference in absorbance among protein extracts from un-aged, wet-aged, and dry-aged beef (ranged from 0.23 to 0.25) seemed not meaningful even though there was a significant difference between wet- and dry-aged beef.

2.3.1.3. Metal chelating activity

In current study, the difference in metal chelating activity was clearly shown among the samples (Table 1). The protein extract from the crust showed the greatest value (P<0.05; 83.9%) and followed by dry-aged one (77.6%) in chelating activity. The lowest chelating activity (P<0.05) was observed in protein extracts from un-aged and wet-aged beef (65.2 and 68.9%, respectively). Previous studies reported that dry-aged beef had higher amount of methionine, sulfur-containing amino acid, which may have a role in increase of metal chelating activity (Kim et al., 2016), in comparison with wet-aged counterpart. Consequently, types of amino acids or peptides produced by proteolysis might be dependent on the endogenous enzymes and those from microorganisms contacted and grown on the surface of beef during aging period.

2.3.2. ACE inhibitory activity

Fig. 1 exhibited that ACE inhibitory activity of protein extract was affected by aging method (wet or dry aging) and section of dry-aged meat [internal or external (crust) section]. The greatest and lowest value (P<0.05) in ACE inhibitory activity was observed for protein extract from the crust and un-aged beef, respectively. Similarly, Gallego et al. (2014) reported that peptides derived from dry-cured ham as acting of endo- and exogenous enzymes (microorganisms) showed ACE inhibitory activity. The protein extract from wet-aged beef showed similar ACE inhibitory activity to un-aged one. According to Lafarga and Hayes (2014), generation and/or identification of peptides with ACE inhibitory activity by endogenous proteases is limited due to uncontrollability and irregularity of endogenous protease. A recent report demonstrated that injection of thermolysin to beef loin during marination process resulted in approximately 500 times higher ACE inhibitory activity than control (IC_{50} of 1,206.0 vs 2.3 μg/mL) with inhibitory effect on cancel cell viability after 3 days of storage (Seol et al.,2018). Generation of small peptide through proteolysis by various enzymes (either/both endo- or/and exogenous enzymes) can lead to increase in potent ACE inhibitory activity as producing peptides with unspecified cleavage sites (Liu et al., 2017; Toldrá et al., 1997). Generally, crust has higher chance of protein decomposition by the enzymes described above because the meat surface layer can be affected by microorganisms directly during dry aging. Thus, this postulation should be confirmed in a further study by investigating the effect of microorganism (microbial enzymes) grown on the surface of beef on the production of ACE inhibitory peptides during dry aging.

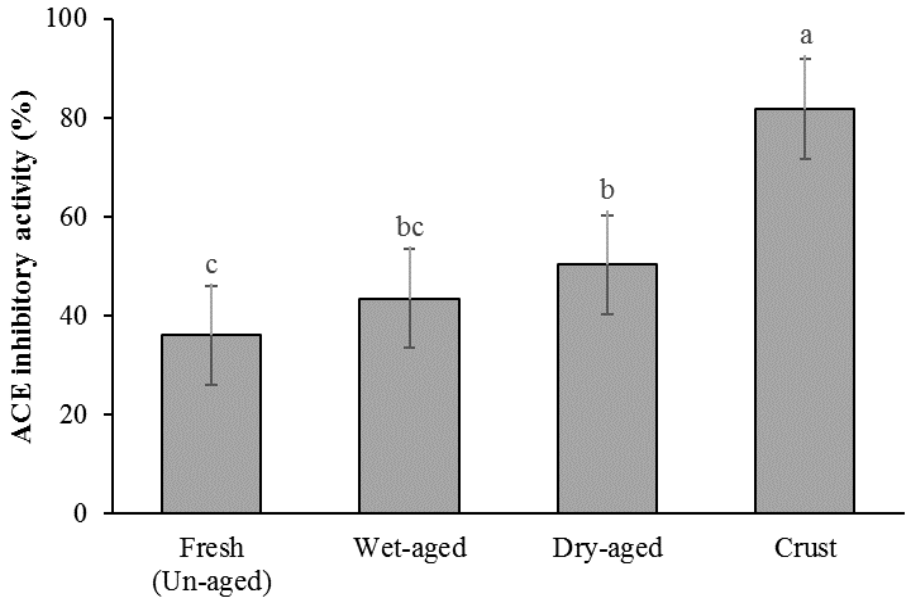

Fig. 1. Angiotensin I - converting enzyme (ACE) inhibitory activity of fresh, wet-aged, dry-aged beef, and the crust of the dry aged beef. [a-c]Values with different letters on the bar are significantly different ($P<0.05$).

2.3.3. Protein profile using SDS-PAGE

Greater extents in protein bands with relatively low molecular weight (< 15 kDa) were observed in crust samples compared to un-aged one (Fig. 1). Sun et al. (2009) found peptides with 5-10 kDa molecular weight in dry sausages showed up to 86% of DPPH radical scavenging activity. In addition, Aluko (2015) reported that low-molecular-weight peptides generated from protein by protease treatment exhibited higher ACE inhibitory activity than low-molecular-weight peptides from treated protein one. Taken together, in this study, the generation of protein with low molecular weight induced the greater antioxidant and/or ACE inhibitory activity of crust due to the proteolysis during dry aging.

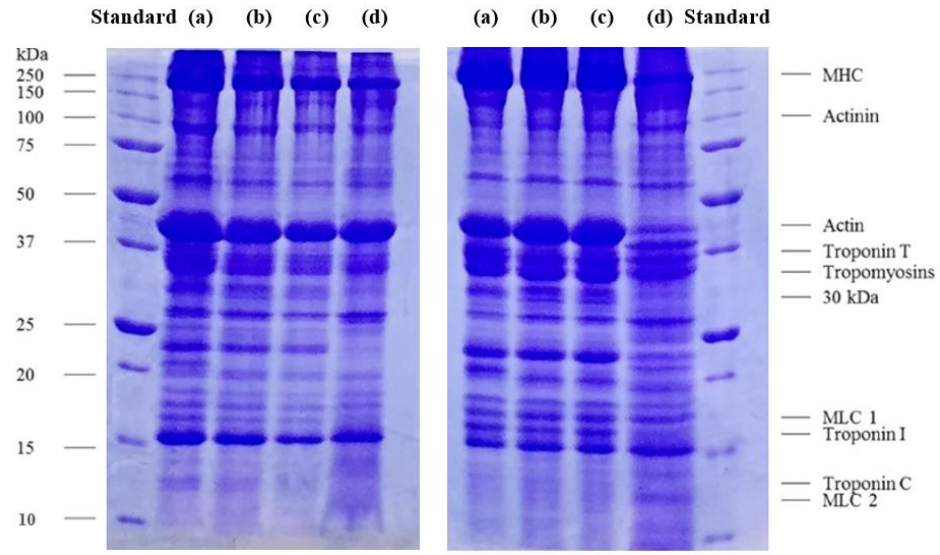

Fig. 2. SDS-PAGE about myofibrillar protein of fresh (un-aged, control) (a), wet-aged (b), dry-aged beef (c), and the crust (d) of the dry aged beef. Range from 250 to 10 kDa size

2.4. Conclusion

This study exhibited potential functionality of the crust form dry-aged beef with confirmed antioxidant and ACE inhibitory activities, which is generally unutilized portion during the production of dry-aged meat. The identification of peptides or amino acids from the crust with bioactivities is worth to investigate further. The effect of microorganisms on proteolysis and lipolysis in crust and edible portion of meat and the relationship with the generation of bioactive peptides or characteristic flavor are under investigation in our laboratory. These further study based on the present results could make crust portion applicable for industry as a functional source of food material.

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CHAPTER III.

Application of the crust from dry-aged beef on beef patty and its quality assessment

3.1. Introduction

In recent years, beef consumption is continuously increased around the worlds and the demand for high quality beef and beef products are also rapidly increased (Myers and Kent, 2003; Zhang et al., 2017). In these trends, consumers are interested in dry-aged beef with a premium value due to its characteristic aged flavor (beefy and roasted) and its high tenderness (Oh et al., 2017). Dry aging is well known method to enhance the meat quality especially for flavor, and which have been successfully used in some high-end restaurants (Smith et al., 2008). It has been reported that US consumers were willing to pay more for a dry-aged beef than wet-aged counterpart (Parrish et al., 1991; Richardson et al., 2008).

Dry-aged beef is commonly produced when the beef is stored in a refrigerated condition (approximately 2-4 °C) for 1-5 weeks without any packaging (Hodges et al., 1974). Thus, the meat surface is directly contacted with air and easily dried (Dikeman et al., 2013; Kim et al., 2016). The surface of dry-aged meat called crust is generally wasted during the trimming process due to its unique characteristics; hard, dry, large number of microorganisms (Smith

et al., 2008). According to the DeGeer et al. (2009), trim loss of dry-aged beef strip loin was more than 34% after 28 days of aging time. It is expected that the amount of discarded crust will increase as the amount of production and consumption of dry-aged beef increases.

In the previous chapter, it was reported that the crust from dry-aged beef contained greater antioxidative and ACE inhibitory activity than unaged, wet-aged, and dry-aged beef. Meanwhile, crust has more aged flavor compared to dry-aged beef according to our experience. Flavor is one of the important quality attributes in meat and meat products (Gorraiz et al., 2002). Thus, it is hypothesized that crust could be used as aged flavor enhancer in the meat products. In other words, aged flavored meat product could be made with the addition of the crust without using an expensive dry-aged meat. It is also economically valuable to use curst because it is considered as a by-product of dry aged meat in meat industry.

However, no scientific information is available on the utilization of crust in meat products. Therefore, the objective of present study was to evaluate the quality properties, especially sensorial quality, of beef patties added with the crust in order to find the way of utilizing it.

3.2. Materials and Methods

3.2.1. Preparation of patties

Crust was obtained from dry-aged beef sirloins which was aged for 28 days at 4°C freezer. Then, the crust were dried in a lyophilizer (PVTFD-10K, Hanil Co., Ltd., Korea) during 17 days. The fully dried samples were then pulverized with mortar and passed through 2-mm aperture sieve (Testing sieve, Chung Gye Sang Sa, Korea) to eliminate remainders.

In order to manufacture beef patties, eye of rounds were purchased from a commercial butcher (Korea) and ground using a meat grinder with 6 mm plate. The ground beef was mixed with prepared crust sample (0 and 5%) as mentioned in Table 1. Then, the mixture was ground again with a grinder using a 3 mm pore size plate. The ground mixture was then made beef patty (130 g) using a round patty pressure (15 cm diameter). The manufactured beef patties were packaged in a polyethylene bag (38.5 × 30.0 cm) with an aerobic condition. The packaged beef patties were stored for 0, 2, 4, and 6 days to analyze the lipid oxidation, electronic nose, and total aerobic bacterial count, while those were stored for 0, 3, and 7 days to analysis the texture. All samples were stored in a freezer (4°C) until the experiment.

Table 1. Formulation of beef patties (g)

Addition of crust (%)	Lean meat	Fat	Salt	Water	Crust
0	2,400	600	9	-	-
5	2,250	600	9	105	45

3.2.2. Sensory evaluation

For the sensory evaluation, patty samples were cooked with an oven (ML32UW, LG electronics, Korea). The oven was preset 180℃ for 20 min and internal temperature (the core of the sample) was monitored using a digital thermometer (YF-160A Type-K; YFE, Taiwan); the samples were turned over after 10 min of cooking (internal temperature of approximately 60℃) and removed from the oven when the preset time was finished (internal temperature of approximately 80℃). For sensory evaluation, the cooked beef patty was cut into 8 pieces as fan shape. These sample were placed on the white plate which was randomly 3-digit-coded the name of sample on the side and served together with water. Nine semi-trained panelists (five men, 23-31 years old and four women, 23-33) evaluated the cooked sample 2 times as replication for appearance, odor, taste, flavor, tenderness, juiciness, and acceptability based on a 9-point hedonic scale (from extremely dislike=1 to extremely like = 9).

3.2.3. Electronic nose

Difference of flavor and odor which affected by the storage days and different concentration of crust (%) in patty were performed by electronic nose analysis. Ground meat (5 g) was place in a 20 ml vial and volatiles from the headspace were injected to a gas chromatography-type electronic nose (Heracles II, Alpha MOS, France) equipped with dual columns (MXT-5 and 1701, Restek, USA). The analytical conditions were as follows: 10 min headspace generation at 80°C; 5 ml injection volume; 40°C and 240°C the initial and final trap temperature; and flame ionization detector. The column oven temperature was initially hold 40°C for 5 s, increased to 150°C by 0.5°C/s, 260°C by 5°C/s, and

hold for 30 s. The peak area was integrated using Alpha Soft program (Alpha MOS).

3.2.4. Texture analysis

In present study, the method for texture analysis was followed by Claus. (1995). Beef patties were cooked by oven and the cooking time and temperature were the same as the patty for sensory evaluation. Those patties were stored at room temperature for an hour to cool. Samples were then cut to obtain the same size (35 mm diameter, 25 mm height). The center of each sample was compressed twice to 60% of their original height using a texture analyzer (TA1, Lloyd Instruments Ltd, UK) attached with a compression probe (7.5 cm diameter) at a test speed of 2 mm/s and a trigger force of 1 N. Hardness (N), springiness (mm), gumminess (N), chewiness (Nmm), and cohesiveness were measured and recorded.

3.2.5. Total aerobic bacterial counts

Total aerobic bacterial counts of uncooked and cooked beef patties were analyzed by method of Yong et al (2017). Sample (3 g) was blended with 27 ml of sterile saline (0.85%) for 2 min using a laboratory blender (BagMixer® 400 P, Interscience, France). Then, appropriate dilutions were prepared in sterile saline and spread on plate count agar (Difco Laboratories, USA). The agar plates were incubated at 37°C for 48 h and microbial counts were calculated. The results were expressed as Log numbers of colony-forming units per gram (Log CFU/g). Identification of microorganisms in crust of the dry-aged beef was carried out by using the 16S rDNA sequencing method (Kim et al., 2016). Each single colony

from the purified isolates on the plate count agar plates were transferred to 10 ml tryptone soy broth (Difco Laboratories, USA), and the cells were grown at 37°C during overnight. The chromosomal DNA of isolated stain was separated by using the BioFact Genomic DNA prep kit (BioFact, Korea). The DNA extracts were used for the polymerase chain reaction (PCR) with the universal primers [27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3')]. PCR was carried out in a programmable therma cycler (BioFact LAMP-Taq, Korea), according to the following steps: one cycle of denaturation at 95°C for 15 min, followed by 30 cycles of 95°C for 20s, 50°C for 40 s, and 72°C for 90 s. The final extension was carried out at 72°C for 5 min. The purified PCR product obtained by using a BioFact PCR purification kit (BioFact, Korea) was used for sequencing by Basic Local Alignment Search Tool (BLAST) search of the National Center for Biotechnology Information (NCBI) (Maidak et al., 2001).

3.2.6. Lipid oxidation

As a lipid oxidation value, TBARS value was analyzed according to the Jung et al. (2011). Each beef patty sample (3 g) was homogenized with 9 ml of distilled water using a homogenizer (T10 basic). The homogenate (2 ml) was transferred to a test tube and mixed with 4 ml of thiobarbituric acid (0.02 M)/trichloroacetic acid (15%) solution. Then, the test tubes were heated at 90ºC in a water bath for 30 min, cooled for 30 min in ice water, and centrifuged (Continent 512R, Hanil Co., Ltd., Korea) at 2,265 × *g* for 10 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (X-ma 3100). TBARS values were reported as mg

malondialdehyde per kg of sample.

3.2.7. Statistical analysis

All experimental procedures were repeated in three individual trials. Statistical analysis was performed by one-way Analysis of Variance (ANOVA) with a completely randomized design using the General Linear Model procedure. Significant differences were identified with the Student-Newman-Keuls multiple-range test using Statistical Analysis System software (SAS 9.3, SAS Institute Inc., Cary, NC, USA) at a confidence level of $P<0.05$.

3.3. Results and Discussion

3.3.1. Sensory evaluation and electronic nose

In sensory evaluation, the score of taste, flavor, tenderness, and acceptability of beef patty with 5% crust were significantly higher than those of patty without crust (0%) (Table 1, $P<0.05$). Meanwhile, appearance and juiciness score of beef patty with 5% crust showed no significant difference compared to those of beef patty without crust. The different sensory evaluation values between beef patty with 5% crust and those of patty without crust were to be expected due to the curst, which is the surface of dry-aged beef with a strong and unique flavor.

According to Kim et al. (2016), isoleucine, leucine, methionine, tryptophan, and valine were increased during dry aging periods, which may suggest the rate of protein hydrolysis. The surface of dry-aged meat has advantage as a flavor is excellent as a result of increased concentration of taste-related substance including the free amino acids due to water evaporation and endogenous enzyme reaction. This improve the flavor of meat (Rye et al., 2018). Furthermore, some of microorganisms such as *Penicillium camemberti* and *Debaryomyces hansenii* contributed to the flavor development of dry-aged beef (Lessard et al., 2012). This enhanced flavor by concentration of substances and action of microorganisms may be much stronger in the crust portion when compared with inner flesh portion of dry-aged beef.

The data from electronic nose also showed different patterns between patty with 0 and 5% of crust during storage days (Fig. 1). Especially, ethanol, 2-methylfuran, and methylcyclopentane were detected more in patty with 5% crust than those of control during whole storage periods (data not shown).

Table 2. Sensory evaluation of beef patties added with the crust of dry-aged beef

Addition of crust (%)	Appearance	Odor	Taste	Flavor	Tenderness	Juiciness	Acceptability
0	5.63	5.44^{y}	5.70^{y}	5.56^{y}	5.48^{y}	5.19	5.78^{y}
5	6.00	6.19^{x}	6.85^{x}	7.04^{x}	6.48^{x}	6.44	7.00^{x}
SEM[1)]	0.159	0.133	0.107	0.209	0.222	0.324	0.231

[1)]Standard error of the means (n=6).

^{x,y}Values with different letters within the same column differ significantly (P<0.05).

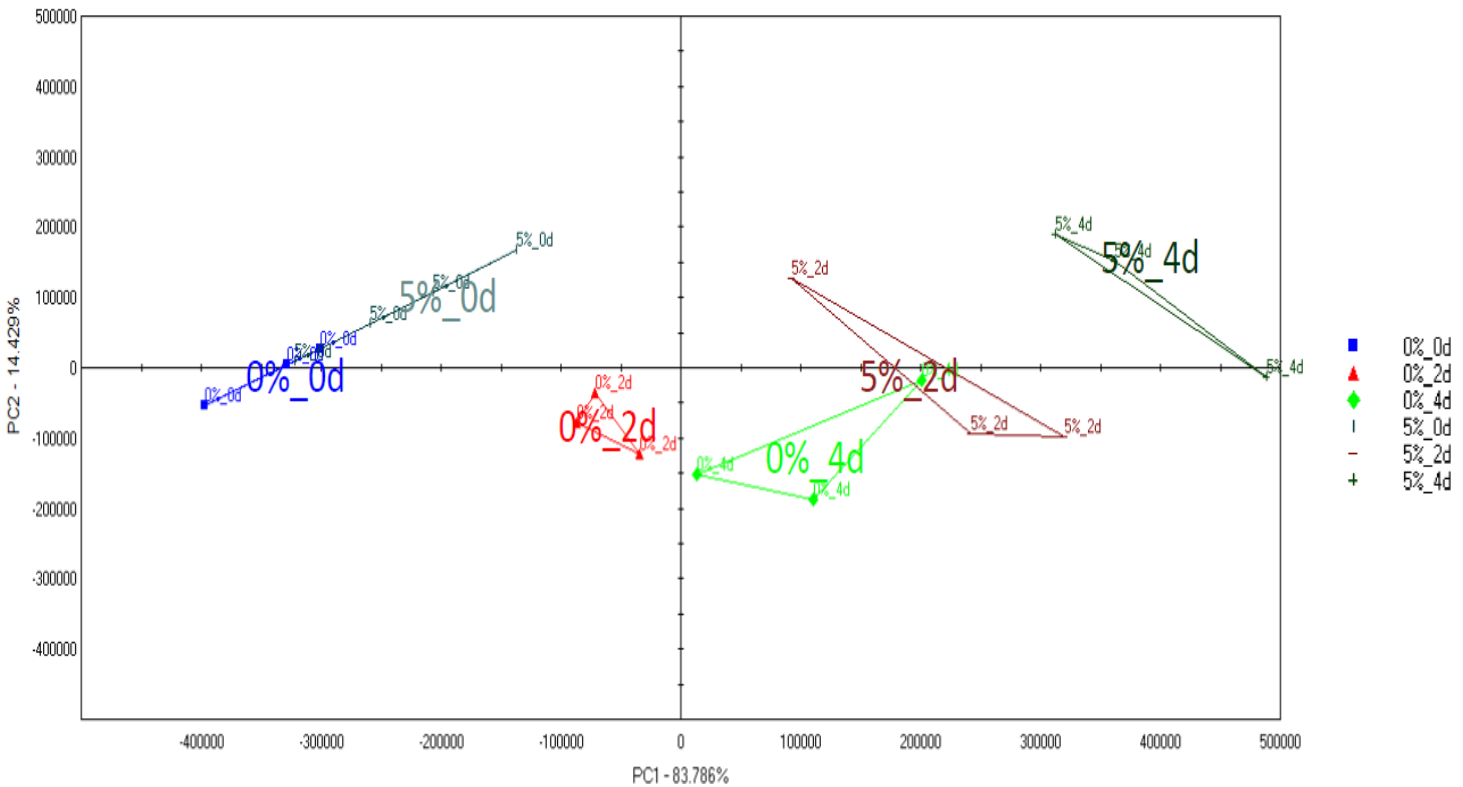

Fig. 1. Result from electronic nose of cooked beef patties with and without crust during storage

3.3.2. Texture analysis

Tenderness of beef patty is one of the major sensory traits by the consumer (An et al., 2017). In present study, beef patty with 5% crust did not differ from patty without crust in terms of texture such as hardness, springiness, gumminess, chewiness, and cohesiveness in initial day of storage (Table 3). On the contrary, beef patty with crust had lower hardness, gumminess, and chewiness than those without crust (P<0.05) at 3 and 7 day of storage.

Rui et al. (2010) demonstrated that protein oxidation led to an increase of hardness in burger patties through the formation of protein carbonyls, the loss of protein functionality, and formation of cross links between proteins. Similarly, the increased hardness, gumminess, and chewiness in the patty without crust may be come from the protein oxidation discussed above. However, the patty with crust seemed to be less influenced by the protein oxidation due to the antioxidant activity possessed by the curst as mentioned in Experiment I and the lipid oxidation section in Experiment II.

In addition, the texture of meat patty depends on various factors; fat and water content, raw meat condition, the type of additives, and degree of protein denaturation by the heat temperature (Kim et al., 2015). The patty without crust was consisted only of a lean meat, which may have more adhesive property because the crust used in this study was added in the form of powder. To prevent the difference of this adhesive property in samples, the amount of protein and water ratio was calculated when the formulation of patty was set.

Table 3. Texture of cooked patties with and without crust during storage

Texture parameters	Addition of crust (%)	Storage (days)			
		0	3	7	SEM[1)]
Hardness (N)	0	159.14	199.00^{x}	197.89^{x}	18.182
	5	134.25	139.57^{y}	111.59^{y}	8.754
	SEM[2)]	19.523	9.374	11.907	
Springiness (mm)	0	0.68	0.77	0.68	0.031
	5	0.73	0.77	0.74	0.034
	SEM[2)]	0.040	0.026	0.030	
Gumminess (N)	0	69.35	71.46^{x}	72.14^{x}	6.211
	5	58.40^{a}	51.36^{ay}	41.12^{by}	2.994
	SEM[2)]	7.191	3.319	2.928	
Chewiness (Nmm)	0	45.44	55.11^{x}	49.01^{x}	4.046
	5	42.45^{a}	39.55^{ay}	30.05^{by}	2.350
	SEM[2)]	3.670	3.364	2.837	
Cohesiveness	0	0.45^{a}	0.36^{b}	0.37^{b}	0.017
	5	0.44	0.37	0.37	0.019
	SEM[2)]	0.018	0.020	0.017	

[1)]Standard error of the means (n=18), [2)](n=12).

[a,b]Values with different letters within the same row differ significantly (P<0.05).

[x,y]Values with different letters within the same column differ significantly (P<0.05).

3.3.3. Total aerobic bacterial counts

The high levels of aerobic bacteria were detected in uncooked patty with 5% crust (4.5 Log CFU/g) than that without crust (0%) (2.5 Log CFU/g) in initial storage (Fig. 2). The number of total aerobic bacteria in uncooked patty with crust was continuously increased to 8.4 Log CFU/g at 6 days of storage (P<0.05).

The higher number of aerobic bacteria in the patty with 5% crust during storage might be attributed to the crust, which is the surface of dry-aged meat and greatly affected by microorganisms in air. This phenomenon agrees with the report by Ryu et al. (2018) about bacteria and fungi/yeast characteristics on dry aged beef. The patty with crust also showed rapid increase in the number of total aerobic bacteria on 6 days of storage. Li et al. (2018) indicated that the population of total bacteria and yeast count were rapidly increased in dry-aged samples. In addition, the number of total bacteria was increased up to 5 Log at 28 days of dry aging process (Lee et al., 2017).

Although the total aerobic bacterial counts of uncooked patty with crust on 6 days of storage were approximately 8 Log CFU/g, pathogenic microorganisms had not been found from preliminary studies (Table 4). However, the upper microbial limit for distribution of fresh meat in the market is 7 Log CFU/g (ICMFS, 1986), thus, the research for controlling the number of microorganisms will be needed in further studies.

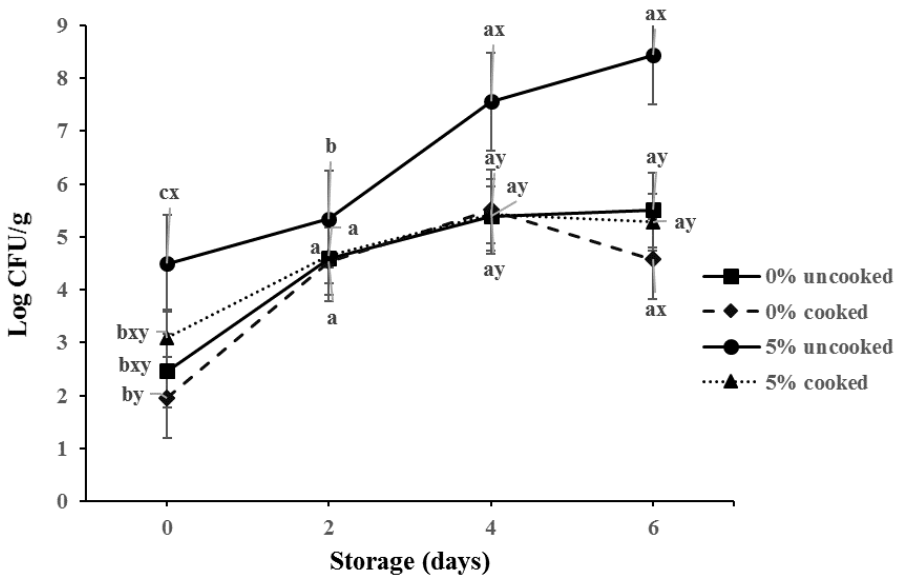

Fig. 2. Total aerobic bacterial counts (Log CFU/g) of uncooked and cooked beef patties with and without crust during storage. [a-c]Values with different letters within treatment combination differ significantly (P<0.05). [x,y]Values with different letters within the same storage day differ significantly (P<0.05).

Table 4. Identification of microorganisms in crust of the dry-aged beef

Microorganisms
Burkholderia lata, Leclearia adecarboxylate, Serratia grimesii, Carnobacterium divergens, Cutaneotrichosporon curvatus, Candida zeylanoides, Rhodotorula mucilaginosa

3.3.4. Lipid oxidation

The malondialdehyde detected in the beef patty indicates the level of lipid oxidation, which can be determined by TBARS method (Kim et al., 2013). TBARS values were not different between the patties tested at day 0 (Table 5). However, significant differences were observed after 2 days of storage ($P<0.05$). In addition, TBARS values were gradually increased during 6 days of storage in both patties. Furthermore, the TBARS values of patty without crust (0%) was higher than that of added crust at day 6.

The above results could be due to the interaction between microorganisms and malonaldehyde. The MDA removal/losses might occur through direct microbial utilization of malonaldehyde and other TBARS or through reactions between these and the amine compounds products by bacterial metabolisms (Branen, 1978; Rhee et al., 1997) or both. In the result previously mentioned (Fig. 2), the patty with 5% crust showed higher number of aerobic bacteria than that without crust (0%) during the 6 days of storage.

In addition, less increased the TBARS value for beef patty with 5% crust than those for patty with 0% crust might be partially explained by antioxidant activity of the crust. According to the previous studies, several bioactive peptides are generated from meat and meat products during dry fermentation or aging with protease treatment (Gallego et al., 2018; Seol et al., 2018). The previous study in Experiment I, it is also observed that the crust has antioxidant activity. It is well known that addition of antioxidant activity materials can reduce TBARS value in beef patty (Rojas and Brewer, 2007) and the curst could be one of them. However, this is not conclusive because no research was conducted to find the functionality of the crust of dry-aged beef.

Table 5. TBARS (mg malondialdehyde/kg) of uncooked beef patty with and without crust during storage

Addition of crust (%)	Storage (days)				
	0	2	4	6	SEM[1)]
0	1.00^{d}	1.16^{cx}	1.38^{b}	1.54^{ax}	0.028
5	0.95^{c}	1.04^{by}	1.33^{a}	1.35^{ay}	0.027
SEM[2)]	0.032	0.017	0.024	0.033	

[1)]Standard error of the means (n=8), [2)](n=16).

[a-d]Values with different letters within the same row differ significantly (P<0.05).

[x,y]Values with different letters within the same column differ significantly (P<0.05).

3.4. Conclusions

Sensory evaluation of beef patty with 5% crust resulted significantly higher taste, flavor, and tenderness than control. This difference in sensory can also be discriminated by electronic nose and texture analysis.

Therefore, the crust from dry-aged beef could be used as a flavor enhancer in meat products by providing beefy and palatable flavor without long period of dry aging as meat industry commonly practiced. However, microbial safety of the crust should be reconfirmed prior to utilization in different dry-aging practices.

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CHAPTER IV.

Overall Conclusion

In present study, it was confirmed that the crust from dry-aged beef possessed antioxidant and ACE inhibitory activities. Furthermore, the beef patty made with 5% crust scored higher in taste, flavor, and tenderness than control patty in sensory evaluation. Taken together, it can be concluded that the crust of dry-aged beef could be used both as a functional source of food material and a flavor enhancer in meat products to provide beefy and roasted flavor without dry aging.

Summary in Korean

건식숙성 소고기 크러스트의 항산화 및 ACE 저해활성과 패티 제조시 풍미증진제로서의 활용

박범진

농생명공학전공

서울대학교 대학원

식육의 숙성은 식육의 품질을 개선시키기 위해 산업에서 널리 사용되고 있는 방법으로, 크게 습식 숙성과 건식 숙성으로 나뉜다. 습식 숙성이란 진공 포장한 식육을 냉장 온도(0-4℃)에서 3주까지 저장하며 연도를 증진시키는 방법이다. 반면에 건식 숙성이란 육 표면을 공기중에 노출시킨 상태에서 습도, 온도, 풍속, 및 기간을 설정하여 숙성하는 방법이다. 이에 따라 많은 연구에서는 건식숙성육이 습식숙성육보다 특유의 향이 강하다고 보고하고 있다.

한편 건식숙성의 경우, 육 내 수분의 증발과 Trim loss를 통한 수율 감소와 그에 따른 생산 가격 상승이 단점으로 꼽히며 대략 전체 무게의 30%가 감소하는 것으로 연구되었다. 크러스트란 trim loss를 통해 제거되는 부분이며 대부분의 손실이 크러스트를 통해 발생된다. 이는 건식 숙성의 가격 상승을 야기할 뿐만 아니라 단백질 자원의 손실을 의미한다. 따라서 건식 숙성 후, 버려지는 크러스트를 연구, 개발할 시, 육가공 산업에서 활용할 수 있는 새로운 첨가물의 개발 및 부가가치 창출이 가능하다. 그러므로 본 연구에서는 소고기를 28일간

건식 숙성하여 생성된 크러스트의 항산화력 및 항고혈능력에 대해 평가하며 크러스트를 소고기 패티 제조에 활용하여 관능평가 하였을 때, 기호도의 변화에 대해 알아보았다.

실험 결과, 크러스트는 숙성을 하지 않은 소고기와 건식 숙성을 한 소고기 및 습식 숙성을 한 소고기보다 항산화력 및 항고혈능력이 큰 것으로 나타났다. 또한 SDS-PAGE를 통해 확인해본 결과, 크러스트는 15 ~ 10 kDa의 펩타이드가 숙성을 하지 않은 소고기, 건식 숙성을 한 소고기 및 습식 숙성을 한 소고기보다 진한 것을 확인할 수 있었다. 또한 크러스트를 소고기 패티 제조에 활용하여 관능평가 하였을 때, 소고기만으로 제조한 패티보다 향, 맛, 연도 및 전체적인 기호도가 향상됨을 확인하였으며 전자코 결과를 통해서도 두 패티가 나타내는 향의 차이가 있음을 확인하였다. 조직감은 크러스트를 첨가한 소고기 패티의 경도, 검성, 씹힘성이 소고기만으로 제조한 패티보다 유의적으로 감소하였다. 이를 종합해보았을 때, 건식 숙성을 통해 얻어진 크러스트는 항산화력 및 항고혈능력을 가짐과 동시에 육제품의 기호도 향상에도 도움을 주는 것으로 평가되었다. 따라서 앞으로의 육가공 산업에서 크러스트를 이용할 시, 기존에 이용되지 않고 버려지던 단백질 자원의 부가가치 창출 및 향미 증진을 위한 첨가물로의 활용이 가능할 것으로 판단된다.

주요어: 건식숙성, 관능평가, 소고기패티, 전자코, 조직감, 크러스트, 항고혈능력, 항산화력
학번: 2016-24641

감사의 글

관악산의 녹음이 짙은 여름입니다. 큰 꿈을 안고 시작한 대학원 석사과정의 마무리에서 그동안의 시간을 회상하며 감사의 인사를 전하고자 합니다.

어린 시절, 장래희망 조사 답란에는 항상 동물학자를 적었었습니다. 감사의 글을 작성하며 문득 어릴 적의 꿈에 한 발 다가간 것 같은 기분에 기쁨과 설렘이 공존합니다.

서울대학교에서의 석사과정은 다양한 수업과 연구를 통해 폭넓은 사고를 가능케했습니다. 이 모든 것을 경험할 수 있었던 석사과정이 저에게는 매우 뜻깊었으며 큰 영광이었다고 생각합니다.

무엇보다도 부족한 저에게 큰 가르침 주신 조철훈 교수님께 감사의 인사를 올립니다. 교수님께서 지도해주신 석사과정 동안 식육 과학과 관련된 다양한 지식을 함양할 수 있었으며 많은 연구를 통해 폭넓은 사고가 가능하였습니다. 이는 제가 앞으로의 학업을 이어나가는 데 큰 밑거름이 될 것이라 확신하며 다시 한 번 베풀어주신 관심과 애정에 진심으로 감사드립니다. 또한 학문에 흥미를 가질 수 있게 도와주시고 응원해주신 충북대학교 최양일 교수님, 힘든 순간순간마다 훌륭한 조언으로 큰 힘이 되어주신 최향순 교수님, 앞으로의 진로를 확고하게 세울 수 있게 도와주신 충남대학교 정사무엘 교수님께 감사의 인사를

올립니다. 이와 더불어 학문적 지식을 함께 공유하며 서로에게 큰 도움이 되어준 실험실 분들께도 감사하다는 말씀을 드리고 싶습니다.

매 순간, 묵묵히 지켜봐 주시며 응원해주신 가족에게 감사의 인사를 올립니다. 특히 아버지께서 말씀해주신 '극한속에 여유를' 이라는 문장은 매 순간 저에게 큰 위로가 되어주었습니다. 이를 가슴 깊이 새겨 앞으로의 학업 과정 및 삶에 고난이 다가와도 현명하고 태연하게 헤쳐나갈 수 있는 사람이 되겠습니다.

이 밖에도 감사의 글에서 직접 인사를 전해드리지 못 하였지만 큰 힘이 되어주신 모든 분들께 감사의 인사를 올립니다.

서울대학교에서의 석사 학위는 앞으로 나아가기 위한 첫걸음이 되었음을 깨달으며 인간과 동물이 함께 할 수 있는 세상을 위해 더욱 정진할 수 있는 연구자가 되도록 노력하겠습니다. 감사합니다.

2018 년 8 월

박범진 올림