FREE AMINO ACID CONTENT OF AGED BEEF FROM KOREAN HANWOO (*BOS TAURUS COREANAE*) STEERS DURING FREEZING

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Abstract – This research was conducted to estimate the free amino acid (FAA) content of aged beef (*M. longissimus lumborum*, LL) from Korean Hanwoo (*Bos taurus coreanae*) steers during freezing. The LL sample was aged at 2℃ for 0, 7, or 14 d and then frozen at -18℃ for 0, 3, 6, or 9 mon. Aging for 14 d significantly (P < 0.05) increased histidine, aspartate, arginine, threonine, alanine, proline, lysine, tyrosine, methionine, valine, isoleucine, leucine, phenylalanine, tryptophan, and total FAA contents. Regardless of aging time, freezing for 9 mon significantly (P < 0.05) decreased asparagine, glutamine, arginine, glycine, aspartate, glutamate, alanine, and cysteine and also reduced total FAA content. These findings suggest that long-term freezing has an adverse effect on quality of Hanwoo beef.

Key Words – free amino acid, freezing, aging, Hanwoo beef.

I. INTRODUCTION

In general, freezing has been utilized to preserve the shelf-life of fresh meat over a period of months or years (1). Particularly, in global meat export industry, freezing plays an important role in keeping the microbial safety of the meat products shipped all over the world (2). However, long-term freezing increases the size of ice crystals that damage the muscle structure or cells (3, 4). This process causes several serious problems, such as high thawing loss, loss of water-holding capacity, and high protein denaturation (5, 6). Moreover, Lagerstedt, Enfält, Johansson & Lundström (7) reported that freezing negatively affected the eating quality of beef, such as taste, juiciness, and tenderness.

The taste of beef is caused by various low molecular weight products, e.g., free amino acids (FAAs), sugars, and nucleotide–related compounds (8). FAAs contribute to sweet, bitter, and umami (monosodium glutamate-like) tastes (9). Koutsidis et al. (10) reported that aging significantly increased FAA content of beef. However, little information regarding the effect of freezing on FAA content of beef has been still reported.

Therefore, this research was conducted to estimate the FAA content of aged beef from Korean Hanwoo (*Bos taurus coreanae*) steers during freezing.

II. MATERIALS AND METHODS

A. Reagents and chemicals

AccQ-Tag Ultra Derivatization Kit (186003836), Eluent A Concentrate (186003838), and Eluent B Concentrate (186003839) were purchased from Waters Corporation (Milford, Massachusetts, USA). The other reagents were purchased from Sigma-Aldrich Co. LLC. (St. Louis, Missouri, USA). Deionized water was made with a Milli-Q Water Purifier (Millipore SAS, Molsheim, Alsace, France).

B. Sample preparation and design

Fresh striploins (quality grade of 1) from 5 Hanwoo (Korean native brown cattle, *Bos taurus coreanae*) steers were purchased from a local meat plant. At 2 d post-slaughter, each striploin was collected from the left side of each carcass. Following trimming, *M. longissimus lumborum* (LL) was cut and vacuum-packaged. The LL was aged at 2℃ for 0, 7, or 14 d before freezing at -18°C for 0, 3, 6, or 9 mon.

C. Extraction of free amino acids

FAAs were extracted from beef sample with the process reported by Tikk et al. (11). Sample was mixed with 0.6 M perchloric acid at 2,600 g for 1 min using an Ultra-Turrax (T25 Basic, Ika Werke GmbH & Co., KG, Staufen, Baden-Wüttenberg, Germany). The homogenate was centrifuged at 2°C and 5,000 g (Avanti J-E Centrifuge, Beckman Coulter, Inc., Fullerton, CA, USA) for 10 min before filtering with a Whatman filter paper No. 1. After neutralizing with both 0.8 M KOH and 0.2 M K_2CO_3, the filtrate was centrifuged, and the supernatant was filtered with a 0.20 μm syringe filter. The filtrate contained 125 μM (final concentration) norvaline as an internal standard.

D. Derivatization of free amino acids

According to the method reported by Waters (12), 200 μL of filtrate was transferred into a vial, and then 140 μl of borate buffer was added, followed by 40 μl of AQC reagent. Then, the mixture was heated in a 55°C water bath for 10 min.

E. Ultra-performance liquid chromatography analysis

As described by Waters (12), FAA derivatives were analyzed using an ultra-performance liquid chromatography (UPLC; Waters Corporation, USA) equipped with an Ultra C18 column (100 mm length × 2.1 mm i.d. × 1.7 μm particle size, Waters Corporation, USA). Two microliters of derivatized sample was injected and chromatographically separated in a 55°C column at a flow rate of 0.7 ml/min using a gradient elution of (A) 5% Eluent A Concentrate and (B) 100% Eluent B Concentrate as follows: 0-0.54 min, 99.9% A/0.1% B; 0.54-5.74 min, 99.9-90.0% A/0.1-10.0% B; 5.74-7.74 min, 90.9-78.8% A/10.0-21.2% B; 7.74-8.04 min, 78.8-40.4% A/21.2-59.6% B; 8.04-8.64 min, 40.4% A/59.6% B; 8.64-8.73 min, 40.4-99.9% A/59.6-0.1% B; 8.73-11.5 min, 99.9% A/0.1% B. Ultraviolet detection was run at 260 nm. The area of the resulted chromatographic peak was quantified as mg each FAA per 100 g meat.

F. Statistical analysis

The statistical analyses of all data were implemented using the SPSS (13) program. Using the general linear model, the effects of aging, freezing, and their interaction (aging × freezing) on FAA content were evaluated by two-way analysis of variance. Duncan's multiple range tests were conducted to compare significant differences among means when $P < 0.05$.

III. RESULTS AND DISCUSSION

The content of 21 each FAA of aged *M. longissimus lumborum* from Korean Hanwoo steers at -18℃ for 9 mon was presented in Table 1. Aging time had a remarkable effect on the contents of most of FAAs. The contents of histidine, aspartate, arginine, threonine, alanine, proline, lysine, tyrosine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophan were significantly ($P < 0.05$) increased at 14 d of aging. Moreover, total FAA content also appeared to increase significantly ($P < 0.05$) at the same aging time (Figure 1). These results are similar to previous findings of Koutsidis et al. (10) who observed an increase in FAA content of beef *M. longissimus lumborum* increased at 4℃ for 21 d. Also, Dashmaa, Yang, Ba, Ryu & Hwang (9) similarly reported that FAA content increased in *M. longissimus dorsi* from Hanwoo at 4℃ for 28 d. In fresh meat, FAAs are generated through three steps of proteolysis (14). In the first step, proteins were broken down into polypeptides by calpain, cathepsin, and multicatalytic proteinase complex. Then, by dipeptidyl peptidase, tripeptidyl peptidase, and carboxyipeptidase, polypeptides were degraded into the peptides of low molecular weight. Finally, AAs were liberated with the actions of dipeptidase, tripeptidase, and aminopeptidase.

As shown in Table 1, freezing time had a strong impact on the contents of FAAs of aged *M. longissimus lumborum* From Hanwoo steers. The contents of all FAAs tended to decrease at 9 mon of freezing. Particularly, the contents of asparagine, glutamine, arginine, glycine, aspartate, glutamate, alanine, and cysteine decreased significantly ($P < 0.05$) at the last freezing time regardless of aging time. Total FAA content also tended to decrease at 9 mon of freezing (Figure 1). Especially, non-aged or 14 d-aged *M. longissimus lumborum* showed a significant ($P < 0.05$) decrease in total FAA content. These results may be due to an increase in protein oxidation during freezing. In this research, freezing time increased

2-thiobarbituric acid reactive substances (TBARS) content of *M. longissimus lumborum*, measured as an index of lipid oxidation (data were not shown). Protein carbonyls, typical protein oxidation products in stored meat, are generated with the direct oxidation of side chain of AAs (15). Cho, Kang, Seong, Park & Kang (16) reported that protein carbonyls increased together TBARS in stored beef *M. longissimus lumborum*.

Table 1. Each free amino acid content (mg / 100 g meat) of aged *M. longissimus lumborum* from Korean Hanwoo (*Bos taurus coreanae*) steers during freezing at -18℃

Items	Aging time (d)	Freezing time (mon) 0	3	6	9
HIS	0	1.14^{aB}	0.97^{aC}	1.06^{a}	0.59^{bC}
	7	1.03^{abB}	1.39^{aB}	1.45^{a}	0.92^{bB}
	14	1.59^{A}	1.78^{A}	1.53	1.23^{A}
ASN	0	0.16^{cB}	0.68^{aC}	0.77^{aB}	0.46^{b}
	7	0.13^{cB}	1.00^{bB}	1.52^{aA}	0.83^{b}
	14	0.20^{cA}	1.29^{aA}	1.49^{aA}	0.73^{b}
SER	0	3.48^{aB}	2.63^{abC}	2.81^{abC}	1.69^{bB}
	7	3.23^{B}	4.66^{B}	5.23^{B}	4.27^{A}
	14	6.63^{abA}	6.16^{abA}	7.79^{aA}	5.01^{bA}
GLN	0	49.10^{bA}	40.47^{c}	55.53^{aA}	32.39^{d}
	7	37.16^{abB}	42.25^{a}	34.22^{abB}	30.38^{b}
	14	36.81^{bB}	41.77^{a}	38.49^{abB}	25.83^{c}
ARG	0	16.84^{aB}	7.67^{cB}	9.77^{b}	5.83^{c}
	7	15.70^{aB}	10.73^{bAB}	9.56^{bc}	7.35^{c}
	14	21.83^{aA}	12.77^{bA}	12.89^{b}	8.81^{c}
GLY	0	6.03^{b}	5.20^{bc}	7.42^{aA}	4.08^{c}
	7	4.99^{ab}	6.38^{a}	4.63^{abB}	3.78^{b}
	14	4.82^{a}	6.08^{a}	4.95^{aB}	3.24^{b}
ASP	0	0.73^{a}	0.30^{cB}	0.44^{b}	0.14^{d}
	7	0.71^{a}	0.45^{bA}	0.46^{b}	0.16^{c}
	14	0.88^{a}	0.53^{bA}	0.50^{b}	0.16^{c}
GLU	0	12.76^{a}	5.20^{bB}	6.35^{bB}	3.55^{cB}
	7	12.87^{a}	7.16^{bA}	6.45^{bcB}	4.90^{cAB}
	14	14.18^{a}	8.21^{bA}	8.66^{bA}	5.97^{cA}
THR	0	2.01^{aB}	1.92^{abC}	2.04^{aB}	1.74^{bB}
	7	2.01^{B}	2.29^{B}	2.46^{B}	2.09^{B}
	14	2.65^{A}	2.65^{A}	2.92^{A}	2.48^{A}
ALA	0	15.89^{aB}	12.85^{bC}	17.63^{aB}	10.16^{cB}
	7	14.88^{abB}	16.76^{aB}	15.86^{aB}	12.09^{bAB}
	14	18.69^{aA}	18.97^{aA}	20.56^{aA}	13.16^{bA}
PRO	0	3.46^{aB}	2.99^{abB}	3.35a	2.55b
	7	3.28^{B}	3.77^{A}	3.86	2.94
	14	4.56^{A}	4.25^{A}	4.08	3.30
ORN	0	1.54^{ab}	1.48^{ab}	1.62a	1.40^{b}
	7	1.59	1.64	1.51	1.46
	14	1.66	1.70	1.77	1.50
CYS	0	0.25^{b}	0.29^{bC}	1.40^{aA}	0.19^{bC}
	7	0.30^{b}	0.40^{bB}	1.06^{aAB}	0.33^{bB}
	14	0.73^{a}	0.60^{abA}	0.73^{aB}	0.47^{bA}
LYS	0	6.82^{abB}	5.51^{bB}	7.22^{aB}	4.07^{cB}
	7	6.29^{B}	7.86^{A}	8.58^{AB}	6.34^{AB}
	14	10.72^{abA}	8.39^{bA}	12.30^{aA}	8.39^{bA}
TYR	0	3.97^{B}	4.17^{C}	4.05^{B}	3.69^{B}
	7	4.03^{bB}	4.60^{abB}	5.36^{aA}	4.34^{bA}
	14	5.18^{A}	5.02^{A}	5.25^{A}	4.86^{A}
MET	0	2.36^{cB}	2.88^{aC}	2.70^{abB}	2.59^{bcB}
	7	2.46^{B}	3.17^{B}	3.51^{A}	3.09^{A}
	14	2.95^{bA}	3.51^{aA}	3.79^{aA}	3.43^{aA}
VAL	0	3.34^{B}	3.34^{C}	3.91^{B}	2.35^{B}
	7	3.33^{bB}	5.07^{abB}	6.72^{aA}	4.14^{bAB}
	14	6.64^{bA}	7.33^{abA}	9.14^{aA}	5.69^{bA}
ILE	0	1.85^{B}	2.00^{B}	2.29^{B}	1.94
	7	1.84^{bB}	2.36^{abB}	2.96^{aAB}	2.08^{b}
	14	2.85^{A}	3.00A	3.34^{A}	2.56
LEU	0	6.54^{B}	7.37^{B}	7.92^{B}	5.92^{B}
	7	6.55^{bB}	8.90^{bB}	12.29^{aA}	7.48^{bAB}
	14	11.53^{A}	12.01^{A}	13.37^{A}	9.66^{A}
PHE	0	3.66^{B}	4.65^{B}	5.14^{B}	4.66
	7	3.67^{bB}	5.29^{bB}	8.02^{aA}	4.32^{b}
	14	6.96^{A}	7.18^{A}	7.97^{A}	5.83
TRP	0	0.40^{B}	0.67	0.76	0.86
	7	0.38^{cB}	0.55^{b}	0.72^{a}	0.47^{bc}
	14	0.56^{A}	0.49	0.64	0.52

$^{a\text{-}d}$Means in the same row with different superscripts differ significantly ($P < 0.05$).

$^{A\text{-}C}$Means in the same column with different superscripts differ significantly ($P < 0.05$).

[1]HIS: histidine; ASN: asparagine; SER: serine; GLN: glutamine; ARG: arginine; GLY: glycine; ASP: aspartate; GLU: glutamate; THR: threonine; ALA: alanine; PRO: proline; ORN: ornithine; CYS: cystine; LYS: lysine; TYR: tyrosine; MET: methionine; VAL: valine; ILE: isoleucine; LEU: leucine; PHE: phenylalanine; TRP: tryptophan.

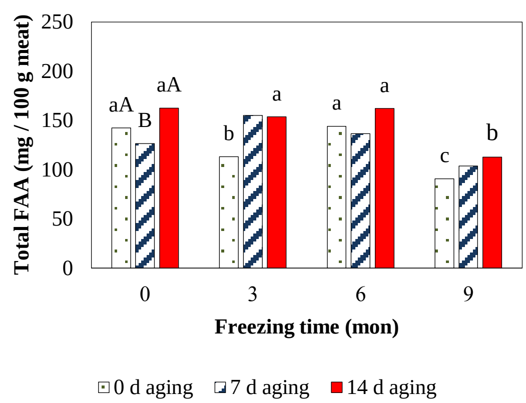

Figure 1. Total free amino acid (FAA) content (mg / 100 g meat) of aged *M. longissimus lumborum* from Korean Hanwoo (*Bos taurus coreanae*) steers during freezing at -18℃. These values are means. $^{a\text{-}c}$Different letters indicate significant differences among different freezing times within the same aging time ($P < 0.05$). $^{A\text{-}B}$Different letters indicate significant differences among different aging times within the same freezing time ($P < 0.05$).

IV. CONCLUSION

This research estimated the FAA content of aged beef from Korean Hanwoo steers at -18℃ for 9 mon. Increasing aging time enhanced the contents of most of FAAs. However, long-term freezing, more than 9 mon of freezing, markedly decreased FAA content regardless of aging time.

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