EFFECT OF CALPAIN SYSTEM ON VOLATILE FLAVOR COMPOUNDS OF BEEF LONGISSIMUS MUSCLE

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Abstract – The effect of calpain system was identified on the formation volatile compounds of Hanwoo beef. In first experiment, LD muscle blocks were injected with solutions containing 50mM $CaCl_2$, 50mM $ZnCl_2$ or 154mM NaCl and aged for 7d. The second was done with powdered samples, incubated with aforenamed solutions containing cathepsin inhibitor for 7d. Totally 51 volatile compounds were identified by using the SPME-GC. The enhancements with Ca^{2+} resulted in greatly ($p<0.05$) increased content of pyrazines and sulfuric volatile compounds that was coincided with higher rate of protein degradation in comparison with the control group.

Key Words – beef, calpain system, calcium ions, protein degradation, volatiles

I. INTRODUCTION

When meat is treated with calcium ion, myofibrillar proteolysis takes place and produce breakdown of Z-line proteins [1]. Myofibrillar and sarcoplasmic proteins undergo autolysis of proteolyses resulting in formation of small peptides and free amino acids [2] which are great determinants of both palatable meat taste and flavor by generating volatile compounds through Maillard reactions and Strecker degradations [3]. The calcium ion treatment enhances calpain autolysis while zinc ion treatment inhibits calpain autolytic activity [4]. Given the studies the generation of lower molecular flavor precursor compounds greatly influenced by post-mortem proteolysis in muscle tissue ([2; 5]. We hypothesized that elevated calpain activity with supplementation of calcium ions would enhance generation of volatile flavor compounds. To our best knowledge, there is very limited accessible scientific information regarding the volatile compounds of Hanwoo beef as a function of the calpain system. The current study was designed to identify the effect of the calpain system on the formation of favorable volatile flavor compounds of Hanwoo m. *longissimus*.

II. MATERIALS AND METHODS

Experimental design and sampling; In the first experiment (*in vivo*), *m. longissimus dorsi* were divided into three portions of around 250 g. Randomized blocks were injected with solutions (50mM $CaCl_2$, 50mM $ZnCl_2$ or 154mM NaCl) by using multiple arranged syringes and chiller aged at 4°C during 7 days. For the second experiment (*in vitro*), 5g of powdered in liquid nitrogen samples was incubated with aforenamed solutions containing cathepsin inhibitor in the ratio of 1:3(w/v). Then 100 mM EDTA was added to stop incubation

SDS-Page-Western blot were determined by following method of Wheeler (2010)

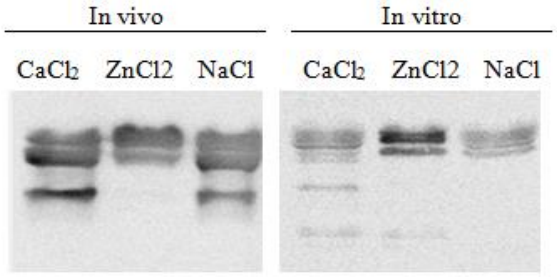
Volatile compounds; A 1 g aliquot of ground meat samples was placed in 40 mL headspace vials then heated at 121°C for 20 min in an autoclave and cooled. The volatile compounds were extracted using solid phase micro-extraction (SPME) and analyzed by GC/MS following the method of Ba et al., [5].

III. RESULTS AND DISCUSSION

Proteolysis as a function of chloride salt ions; The postmortem proteolyses stimulated degradation of skeletal regulatory protein was observed *in vivo*. SDS-PAGE results showed different proteolysis speed in two experiments; calpains showed different protein degradation patterns than whole protein degradation (data not shown). Calpains intensively degraded the majority of proteins in relatively few fragments [6], as calpain is activated, the 80 kDa catalytic subunit progressively autolyzed to the 78-76 kDa form

[7]. The *in vitro* had highest intensity of Tn-T degradation product (30 kDa) than *in vivo* (Fig.1). The breakdown of Tn-T product is well known as indicator protein of tenderness and major degradation product that is often seen in beef is polypeptide of approximately 38 kDa [6]. Then 28 kDa subunit of enzyme is reduced to 18 kDa (myosin light chain) *in vitro*. Un-degraded form of $ZnCl_2$ showed inhibited calpain activity by zinc ion treatment [4].

Figure 1. Troponin-T degradation patterns by proteases activated and inhibited by Ca^{2+} and Zn^{2+} ions



Effect of the calpain system on the volatile compounds; Totally 51 volatile compounds were identified in Hanwoo beef, however Table 1 showed quantity of important compounds as significantly affected by calcium induced calpain system.

Generally more than 1000 volatile compounds have been identified in meat. However relatively few volatiles have been shown key contribution to the flavor of cooked meat, especially sulfur and nitrogen containing compounds are considered to be the principal contributors to meat desirable flavor [3; 5]. In present study, amount of total furans greatly ($p<0.05$) increased by endogenous proteinases *in vivo*. The furans with sweet, caramelic notes were produce generously from the Maillard reaction between reducing sugars and amino acids. Strecker aldehyde 2-methylbutanal detected in Ca^{2+} treated beef and in control whereas did not detect in Zn^{2+} treated samples *in vitro*. The formation 3-methylbutanal and 2-methylbutanal with meaty, oily notes [8] is probably due to some of amino acid level of isoleucine and leucine, which released from calcium activated protein degradation. The released amino acids produce important intermediate products such as sulfur and nitrogen radicals undergo Strecker degradation, which can further react with each other or interact with other compounds to yield volatiles such as pyrazins and thiazoles in Maillard reaction [3; 5].

The pyrazines such as methylpyrazine, dimethylpyrazine, 2-ethyl-2.5-dimethylpyrazine and sulfuric compounds such as dimethyldisulfide and 2-acetylthiazole were significantly ($p<0.05$) greater in Ca^{2+} enhanced samples than control in both experiments. The pyrazines described generally have pleasant roasted, nutty flavor characteristics and low odor thresholds [3]. In our study 2-methylbutanal, octylfiran, and sulfuric compounds did not detect $ZnCl_2$ treated samples *in vitro*. This may related to postmortem calpain proteolyses inhibited with zinc ions. Interestingly, the most of aldehydes coming from lipid oxidation were greater detected *in vitro* especially in Zn^{2+} treated samples than *in vivo*. Possible explanation is the using of chloride salts could affect the activity of lipolytic enzymes changing the fatty acid profile and consequently affecting the generated flavor compound's profile [9].

CONCLUSION

The current data implied that Ca^{2+} induced calpain play a major role in beef tenderization, and involved formation of important favorable volatiles such as pyrazines and sulfuric volatile compounds of cooked beef. Increased level of some aldehydes likely coming from lipid oxidation involved in enhancement of $ZnCl_2$. Except inhibiting postmortem calpain proteolysis, $ZnCl_2$ solution may affect activity of lipolytic enzyme.

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Table 1. Quantity (µg/g) of important volatile compounds out of 51 compounds detected in Hanwoo beef as significantly affected by calcium induced calpain system

Compounds	LRI[1)]	In vivo			In vitro			SEM	F value		
		NaCl	$CaCl_2$	$ZnCl_2$	NaCl	$CaCl_2$	$ZnCl_2$		Inj. treat	Exp[2)]	Inj. treat* Exp.
3-methylbutanal	≤800	0.02[b]	0.09[a]	0.06[b]	nd	nd	nd	0.06	4.14*	-	3.26
2-metbutanal	≤800	0.03	0.04	0.02	0.01	0.02	nd	0.15	0.59	4.26*	0.35
Hexanal	816	0.5[a]	0.35[b]	0.46[a]	0.13[b]	0.14[b]	0.53[a]	0.28	2.39*	6.57*	0.3
Heptanal	920	0.28[b]	0.16[b]	0.36[a]	0.07[b]	0.03[b]	0.3[a]	0.18	5.22	6.38*	19.33**
Benzacetylaldehyde	1189	0.04	0.05	0.07	0.19[a]	0.09[b]	0.08[b]	0.03	2.81	8.42**	5.60*
Decanal	1235	0.03	0.02	0.02	0.06	0.03	0.12	0.01	2.88	52.5[***]	19.32**
(E)-2-decenal	1277	0.18[a]	0.1[b]	0.15[a]	0.08[a]	0.04[b]	0.14[a]	0.07	4.32*	4.29*	8.01*
(E,E)-2.4-decadienal	1313	0.03[b]	0.1[a]	0.06[ab]	0.12[a]	0.04[b]	0.03[b]	0.05	3.49*	3.53*	3.34
Undecanal	1583	0.06	0.05	0.04	0.1[a]	0.07[b]	0.05[b]	0.03	5.2*	6.28*	24.49**
Tridecanal	1745	0.01[b]	0.03[a]	nd	0.02	0.03	0.02	0.01	8.33*	0.6	5.64
Hexadecanal	1825	0.08	0.02	0.04	0.06[a]	0.01[b]	0.02[ab]	0.04	5.22*	5.77*	0.79
Total aldehydes		3.70	2.9	3.49	2.56	2.51	4.49	1.55	0.69	1.1	1.87
Dimethyldisulfide	≤802	0.12[a]	0.22[a]	0.02[b]	nd	nd	nd	0.28	3.63*	-	27.04*
Dimethyltrisulfide	1864	0.05	0.01	nd	0.02	0.02	nd	0.03	0.001	0.45	3.51
2-acetylthiazole	1030	0.02[b]	0.03[a]	0.02[b]	0.03	0.02	nd	0.02	5.04*	6.81*	0.15
Total sulfuric		0.19	0.24	0.07	0.05	0.04	-	0.2	1.2	0.11	0.38
Methylpyrazine	868	0.01[b]	0.02[a]	0.01[c]	0.05[ab]	0.08[a]	0.01[b]	0.03	2.83*	4.32	4.72*
Dimethylpyrazine	943	0.03[b]	0.06[a]	0.05[b]	0.06[a]	0.05[a]	0.01[b]	0.12	9.75*	2.86	0.39
2-ethyl-2.5-dimethylpyrazine	1079	0.07[b]	0.09[a]	0.01[c]	0.05	0.06	0.01	0.04	4.13*	0.33	1.77
Total pyrazines		0.11[b]	0.12[a]	0.07[b]	0.16[a]	0.19[a]	0.03[b]	0.15	6.09*	3.16	0.37
Pentylfuran	1007	0.15	0.13	0.21	0.11	0.07	0.15	0.12	0.36	1.59	5.17*
Hexylfuran	1110	0.12	0.08	0.06	0.12	0.08	0.04	0.06	0.86	0.72	5.69*
Heptylfuran	1615	0.04	0.04	0.05	0.06	0.02	0.03	0.02	0.84	1.78	0.28
Octylfuran	1317	0.05	0.03	0.08	0.01	0.04	nd	0.05	0.56	0.56	0.29
Total furans		0.36	0.28	0.40	0.3[a]	0.21[ab]	0.22[b]	0.19	1.52	5.43*	0.01

[a-c] Means within a row with different superscripts differ ($p<0.05$); nd; not detected ; * $p<0.05$; ** $p<0.01$, *** $p<0.001$

[1)] LRI; linear retention index, calculated by applying a series of n-alkanes (C_8-C_{20}); [2)] Exp; Experiment

REFERENCES

1. Hopkins, D.L (2014) Tenderizing mechanisms, in Encyclopedia of meat science, 3:431-437
2. Toldra, F & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. Critical Reviews in Food Sci. 38:331–352.
3. Mottram, D.S. (1994.) Some aspects of the chemistry of meat flavor. In the flavor of meat and meat products, Ed. F. Shahidi. Chapman and Hall, London
4. Ilian,M.A., El-Din.A, Bekhit, A., Stevenson, B., Morton, J. D., Isherwood, P., Bickerstaffe, R. (2004). Up- and down-regulation of *longissimus* tenderness parallels changes in the myofibril-bound calpain 3 protein Meat Science. 67:433–445
5. Ba,H.V., Amna,T & Hwang,I.H. (2013). Significant influence of unsaturated fatty acids and pH on the volatile compounds in meat-like model systems. Meat Science. 94:480–488
6. Huff-Lonergan. E (2014) Enzymatic, in Encyclopedia of meat science, 3:438-442
7. Goll, D.E., Thompson, V.F., Li, H., Wei, W. & Cong, J. (2003). The calpain system. *Physiol. Reviews.* 83, 731-801
8. Machiels, D., van Ruth, S.M., Posthumus, M.A., stasse, L. (2003). GC-olfactometry analysis of the volatile compounds of two commercial Irish beef meats. *Talanta*, 60-755.
9. Ripollés,S., Campagno,PCB, Armenteros, M. Aristoy, M.C.& Toldrá, F (2011). Influence of partial replacement of NaCl with KCl, $CaCl_2$ and $MgCl_2$ on lipolysis and lipid oxidation in dry-cured ham. Meat Science. 89, 58–64