THE ASSESSMENT OF BIOACTIVE COMPOUNDS IN LOIN AND RUMP OF BEEF DURING AGING AT 4°C

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Abstract –The aim of this study was to assess the bioactive compounds in loin and rump of beef during 21 days of aging at 4°C. Twenty eight-month-old Hanwoo steers were used for analysis of creatine, dipeptide (carnosine and anserine), betaine, and L-carnitine contents. The highest concentrations of creatine and carnosine measured on day 7. Concentration of anserine was significantly higher in loin than rump. Rump had significantly higher concentrations of betaine and L-carnitine during aging period than loin. Concentration of betaine in loin was increased with the aging time. Bioactive compounds in beef may be varied with different cuts resulting in different responses to aging.

Key Words – bioactive compounds, beef, aging

I. INTRODUCTION

In Korea, meat consumption has been expended based on barbecue culture. There are economical losses as consumers' preferences are on some specific cuts, which are more suitable for barbecue, rather than the other cuts. For beef, loin is the most preferred cuts while rump is the worst. So to overcome its drawbacks in rump, a lot of efforts have been made to improve tenderness and flavor through aging.

On the other hand, meat is known to contain abundant bioactive compounds such as creatine, carnosine, anserine, betaine, and L-carnitine, and each of them has their biological roles. Creatine performs an important role in the energy metabolism of skeletal muscle. Carnosine and anserine have pH buffering, antioxidative, and antiaging roles. Betaine acts as an osmolyte to preserve osmotic equilibrium and also interacts with fat metabolism resulting in fat reduction. L-carnitine combines with long chained fatty acids forming L-carnitine esters and fat combustion takes place though β-oxidation in mitochondria [1]. As consumers' interests are moving to functional properties of food, bioactive compounds can be a turning point to the non-preferred meat in Korea. However, no study was reported for concentration of bioactive compounds in different cut of beef and their changes during aging.

Therefore, the aim of this study was to assess the bioactive compounds in loin and rump of beef during 21 days of aging at 4°C.

II. MATERIALS AND METHODS

Sample preparation

Twenty eight-month-old Hanwoo steers were slaughtered at a slaughter house (Seoul, Korea) and loin and rump were transported in ice condition (4°C) to a laboratory the day after slaughtering. The samples were vacuum-packaged, aged at 4°C for 21 days, and analyzed on day 1, 7, 14, 21. A mini chopper (CH180, Kenwood, China) was used to mince the samples for 30 sec and connective tissue and visible fat were removed before weighing.

Creatine, carnosine, and anserine contents

The content of creatine, anserine, and carnosine contents in the samples were determined [2]. Minced meat sample (2.5 g) were homogenized (T10 basic, Ika Works) with 7.5 mL of 0.01 N HCl at 13,500 rpm for 1 min. The homogenate was centrifuged at 3,000 rpm for 30 min (Union 32R, Hanil Co., Ltd., Korea), and 1

mL of the supernatant was transferred into a microtube and centrifuged at 10,000 rpm for 10 min (HM-150IV, Hanil Co., Ltd., Korea). After centrifugation, 0.5 mL of the supernatant was mixed with 1.5 mL of acetonitrile, and the mixture was centrifuged at 10,000 rpm for 10 min (HM-150IV, Hanil), and the supernatant was filtered through a membrane filter (0.2 μm) into a glass vial. The samples were injected into a high performance liquid chromatography (HPLC; Ultimate 3000, Thermo Fisher Scientific Inc., USA) system under the gradient condition of two mobile phases; mobile phase A was 0.65 mM ammonium acetate in distilled water and acetonitrile (25:75 v/v, pH 5.5), and mobile phase B was 4.55 mM ammonium acetate in distilled water and acetonitrile (70:30 v/v, pH 5.5). The analytical conditions for HPLC was set up as described: injection volume, 10 μL; column, Atlantis HILIC silica column, 4.6 × 150 mm, 3 μm (Waters Corp., USA); flow rate, 1.2 mL/min. A detector was used at 214 nm to determine the creatine, anserine, and carnosine contents. The contents of the compounds were calculated using a standard curve obtained from the standard (Sigma, USA) of each compound.

Betaine and L-carnitine contents

Betaine and L-carnirine contents were quantified [3]. Five grams of each meat sample was added with 10 mL of acetonitrile-methanol (9:1) solution and homogenized (T10 basic, Ika Works) at 13,500 rpm for 30 s. The homogenate was then centrifuged at 3,100 rpm for 5 min at 4°C (Union 32R, Hanil), and the supernatant was filtered into a 20-mL volumetric flask through a funnel plugged with glass wool. The remaining filtrate was again mixed with 10 mL of acetonitrile-methanol solution and centrifuged (Union 32R, Hanil) under the same conditions. The resulting supernatant was collected in the same volumetric flask which was then filled with acetonitrile-methanol solution. Subsequently, 2 mL of this sample was transferred to a 15-mL tube and then 810 mg of Na_2HPO_4 and 90 mg of Ag_2O (9:1 w/w) were added. After vortex-mixing the solution, the sample tubes were dried by shaking without their caps in a shaking machine for 20 min and then centrifuged at 3,100 rpm for 5 min (Union 32R, Hanil). A 0.5-mL aliquot of each supernatant sample was then mixed with 0.5 mL of derivative reagent (0.066 g of 18-crown-6 and 1.39 g of bromoacetophenone in 100 mL of acetonitrile) in a 15-mL tube, voltexed, and heated in a water bath at 80°C for 60 min. After cooling under running water, this mixture was filtered through a membrane filter (0.2 μm) and analyzed in a HPLC system (Ultimate 3000, Thermo Fisher Scientific Inc.) to determine betaine and L-carnitine contents. Two mobile phases (A, 25 mM ammonium acetate in which pH was adjusted to 3.0 using formic acid; B, acetonitrile) were used and the analytical condition for HPLC was set up as described: injection volume, 10 μL; column, Atlantis HILIC silica column, 4.6 × 150 mm, 3 μm (Waters Corp.); Flow rate, 1.4 mL/min; detector was used at 254 nm. Standard curves were obtained using the standard (Sigma) for each compound and then used for calculation of betaine and L-carnitine contents.

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) to estimate the changes in creatine, carnosine, anserine, betaine, and L-carnitine contents (mg/100 g) in loin and rump of beef during 21 days of aging at 4°C. The significant differences between the mean values were identified with Duncan multiple range test using SAS software at a confidence level of $p<0.05$ (SAS 9.3, SAS Institute Inc., USA).

III. RESULTS AND DISCUSSION

Creatine, carnosine, and anserine contents

The results regarding creatine, anserine and carnosine contents are shown in Table 1. It is evident that both loin and rump and aging period significantly affected the bioactive compounds of beef. On day 1, higher concentration of creatine and carnosine were observed in rump and anserine concentration was higher in loin. Amount of creatine of loin was increased from day 1 to day 7 and decreased in day 14 and again increased in day 21. The highest concentration of creatine measured on day 7.

Table 1. The changes in creatine and dipeptide contents (mg/100 g) in loin and rump of beef during 21 days of aging at 4°C.

	Aging (day)	Loin	Rump	SEM[1]

Creatine	1	261.32^{cy}	312.40^{x}	3.186
	7	322.35^{ax}	308.00^{y}	1.435
	14	307.77^{b}	300.73	4.114
	21	251.37^{dy}	302.00^{x}	3.078
	SEM[2]	2.04	3.890	
Carnosine	1	128.54^{c}	160.75	10.018
	7	163.88^{a}	158.83	8.403
	14	138.83^{b}	164.55	13.34
	21	104.35^{dy}	162.61^{x}	14.296
	SEM[2]	0.639	16.621	
Anserine	1	65.93^{ax}	26.90^{y}	1.561
	7	36.74^{cx}	28.19^{y}	1.921
	14	36.46^{cx}	27.71^{y}	1.600
	21	44.27^{bx}	27.42^{y}	2.090
	SEM[2]	0.217	2.546	

[1]Standard error of the means (n=6), [2](n=12).
[a-d]Values with different letters within the same column differ significantly ($p<0.05$).
[x,y]Values with different letters within the same row differ significantly ($p<0.05$).

Similarly, the highest concentration of carnosine was also detected on day 7. Concentration of anserine was significantly higher in loin than rump. And on day 1, highest concentration of anserine observed in loin.

Betaine and L-carnitine contents

Table 2 describes the changes in betaine and L-carnitine contents in loin and rump of beef during 21 days of aging at 4°C. Rump had significantly higher concentration of betaine during aging period than loin. Concentration of betaine in loin was increased with the aging time, but no significant difference was observed in betaine concentration of rump with aging time. Concentration of L-carnitine was significantly high in rump. The concentration of L-carnitine was increased with the aging time but no significant difference evident in rump with the time.

Table 2. The changes in betaine and L-carnitine contents (mg/100 g) in loin and rump of beef during 21 days of aging at 4°C.

	Aging (day)	Loin	Rump	SEM[1]
Betaine	1	4.93^{dy}	10.39^{x}	0.515
	7	5.47^{cy}	10.93^{x}	0.521
	14	5.83^{by}	10.83^{x}	0.065
	21	5.98^{ay}	11.75^{x}	0.689
	SEM[2]	0.039	0.712	
L-carnitine	1	14.77^{cy}	113.04^{x}	2.418
	7	16.89^{cy}	114.91^{x}	16.476
	14	24.04^{by}	113.40^{x}	3.893
	21	30.05^{ay}	137.73^{x}	7.952
	SEM[2]	0.671	13.319	

[1]Standard error of the means (n=6), [2](n=12).
[a-d]Values with different letters within the same column differ significantly ($p<0.05$).
[x,y]Values with different letters within the same row differ significantly ($p<0.05$).

IV. CONCLUSION

In this study, concentration of bioactive compounds was shown significant difference in loin and rump of beef cuts. Therefore, bioactive compounds in beef may be varied with different cuts resulting in different responses to aging. A further study to investigate the nutritional quality according to the aging of beef cuts is desirable..

ACKNOWLEDGEMENTS

This work was supported from Radiation Technology R&D program (2013M2A6043308) through the National Research Foundation of Korea funded by Ministry of Science, ICT & Future Planning.

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