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ARTICLE

Physicochemical Meat Quality, Fatty Acid and Free Amino Acid Composition of Strip loin, Chuck Tender, and Eye of Round Produced by Different Age Groups of Hanwoo Cow

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Abstract

This study was performed to investigate the influence of age on the physicochemical properties of strip loin (*m. longissimus lumborum)*, chuck tender (*m. supraspinatus*), and eye of round (*m. semitendinosus*) of Hanwoo cows. Hanwoo cows (n=126; 24-194 mon; live weight, 270-500 kg) were slaughtered and three muscles were obtained according to 3 age groups (G1, < 5 years old; G2, 6-8 years old; G3, > 9 years old). The chuck tender had significantly higher protein contents in G3 than in G1 or G2 (p<0.05). For strip loin and chuck tender, G1 had significantly higher intramuscular fat contents than G3 (p<0.05). The chuck tender had significantly higher b* (yellowness) values for G2 than for G1 (p<0.05). The three muscles had significantly higher cooking loss (%) and lower WHC (%) in G3 than in G1 or G2 (p<0.05). WBS values of strip loin were significantly higher in G3 than in the other groups (p<0.05). The three muscles had higher C18:1n9 in G1 than that in the other groups. The total content of saturated fatty acids was significantly higher in G3 than in G1 for all 3 muscles (p<0.05). Regarding free amino acid contents, G1 had significantly higher contents of threonine, alanine, valine, methionine, phenylalanine, leucine, and lysine in the strip loin than G2 or G3 (p<0.05). In conclusion, young cow beef were higher in the WHC, intramuscular fat and free amino acids contents, whereas old cow beef had higher cooking loss and WBS (p<0.05).

Key words: Hanwoo cow, meat quality, fatty acids, free amino acids

Introduction

In 2012, approximately 842,771 heads of Hanwoo cattle were slaughtered in Korea. Mature Hanwoo cows represent a significant meat source for the Korean beef industry and they accounted for 51.8% (436,977 heads) of the total beef cattle slaughtered in Korea (KAPE, 2012). Several factors influence the quality attributes of mature cows in the beef cattle group. Beef from physiologically old carcasses is generally less tender than that from young animals because of the chemical nature of muscle-fiber fragmentation and connective tissue collagen (i.e., type and degree of cross-linking) (Lawrie, 1998; Shorthose and Harris, 1990). Furthermore, the oxidative stability of meat may be affected by animal age. Lipid oxidation that occurs in post-mortem meat can be a major impediment to the successful marketing of cow meat (Xiong *et al*., 2007); the discoloration resulting from myoglobin oxidation could negatively affect the utility of the meat. Boccard *et al*. (1979) reported that increased chronological age in cattle resulted in dark-colored lean meat. Further, Stadtman (2006) reported that increasing age increased the susceptibility of muscle cells to oxidizing agents in small animals and humans because age-related loss in redox potential could predispose post-mortem muscles to higher rates of oxidation in old cows. A limited number of studies have been conducted to investigate the possible effects of animal age on the post-mortem progression of proteolysis in relation to meat tenderness (Huff-Lonergan *et al*., 1995; Koohmaraie *et al*., 1984; Parrish, 1981). These studies showed a reduced rate of post-mortem myofibrillar protein degradation in muscle from physiologically mature (E maturity) cattle compared to muscle from young (A maturity) carcasses and the variability in beef tenderness resulted from the different physiological ages of cattle. The lack of literature showing the potential differences in post-mortem

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muscles from mature cows of various ages, coupled with the abundant supply of mature cows as a meat source in the market, indicates the need to further explore factors that may be responsible for the overall quality of post-mortem meat. The objective of the present study was to investigate the physicochemical meat quality and compositional properties of strip loin (*m. lumborum*), chuck tender (*m. supraspinatus*), and eye of round (*m. semitendinosus*) from different age groups of Hanwoo cow.

Material and Methods

Sample preparation

Hanwoo cows (n=126; age, 24-194 mon old; live weight, 270-500 kg) were reared at the Daekwanryung experimental station of National Institute of Animal Science (NIAS) of RDA. Animals were transported to the NIAS abattoir, Suwon, Korea, and fasted for approximately 12 h but with access to water prior to slaughter. The left side of each carcass was deboned and trimmed to domestic fabrication. Strip loin (*m. longissimus dorsi*), chuck tender (*m. supraspinatus*), and eye of round (*m. semitendinosus*) were separated, vacuum packaged, and stored at 2°C for 7 d. All beef samples were divided into 3 age groups: Group 1 (G1), < 5 years old; Group 2 (G2), 6-8 years old; Group 3 (G3) > 9 years old. The means of carcass weights for each age group were G1 372, G2 365, G3 367 kg, respectively. Each beef sample was vacuum-packaged and stored at -20°C until the analysis was conducted.

Chemical and meat quality analysis procedures

Protein, fat, moisture, and collagen content were analyzed using the Food Scan^{TM} Lab 78810 (Foss Tecator Co., Ltd., DK), according to the method of the Association of Official Analytical Chemists (AOAC, 2006). Water-holding capacity (WHC) was measured using the method of Ryoichi *et al.* (1993). Color values on a freshly cut surface of the Warner-Bratzler shear force (WBSF) block were measured using a CR-301 chroma meter (Minolta Co., Osaka, Japan) for CIE standard lightness (L*), redness (a*), and yellowness (b*) after a 30 min blooming at 2°C (CIE, 1986). The cooking loss (%) was calculated as the percent of weight change during cooking for the WBS measurement. For cooking loss determination, the samples were freshly cut into blocks (50×50×25 mm) and weighed (initial weight). The individual meat blocks were placed in polyvinylchloride film bag and cooked at 80°C for 40 min in a water-bath (Waterbath BS-21. Jeiotech Co., Korea). The samples were removed from the water-bath, cooled in cold water, and weighed. The cooking loss was expressed as a percentage of the initial sample weight (Honikel, 1998). WBSF was measured on cooked steaks (25 mm thick) according to the method of Wheeler *et al.*, (2000) using an Instron Universal Testing Machine (Model 5543, UK).

Fatty acids analysis

Total lipids of beef samples were extracted by using chloroform-methanol (2:1, v/v) according to the procedure of Folch *et al*. (1957). An aliquot of total lipid extract was methylated as described by Morrison and Smith (1964). Fatty acid methyl esters were analyzed by a gas chromatograph (Varian 3800) fitted with a fused silica capillary column, omegawax 205 (30 m × 0.32 mm I·D, 0.25 μm film thickness). The injection port was at 250°C and the detector was maintained at 300°C. Results were expressed as percentages based on the total peak area.

Free amino acids analysis

The free amino acid contents in beef sample were analyzed using a RP-HPLC 1200 system (Agilent Technologies Inc., USA) equipped with a diode array detector (DAD). HPLC conditions for free amino acids followed as described by Woodward and Henderson (2007). A Rapid Resolution HT Eclipse XDBC18 column (4.6 mm × 150 mm column packed with 5 μm particles Agilent Technologies Inc., USA) was used for separation of amino acids. Two hundred microlitres of the supernatant were derivatized with OPA for the primary amino acids and FMOC for the secondary amino acids; furthermore a 0.4 N borate buffer was used. Briefly, OPA-derivatized amino acids were monitored at 338 nm and FMOC-derivatized amino acids were monitored at 262 nm. The column temperature at 40°C; flow rate was 2 mL/min, using a gradient between two solvents. Solvent A was 10 mM Na_2HPO_4: 10 mM $Na_2B_4O_7$: 0.5 mM NaN_3 at pH 8.2 and Solvent B was acetonitrile: methanol: water (45:45:10 v/v). For the calibration curves 17 primary amino acids, plus 4 extended amino acids are combined with fixed amounts of internal standards and final concentration of standard solution were 90, 225 and 900 pM. The primary amino acids were quantified by using external standard procedures or by using norvaline as an internal standard and the secondary amino acids were Extraction Methods and Free Amino Acids 371 quantified using sarcosine as an internal standard. Individual free amino acid values were expressed as μmol/g.

Statistical Analysis

Each animal within the same slaughtered age group was treated as a replicate. Data were analyzed by the Student-Newman-Keuls' multiple comparison using the General Linear Model Procedure of the SAS program (2005). The significance level was set at $p<0.05$.

Results and Discussion

Chemical composition

The moisture, protein, fat, and collagen contents of the strip loin, chuck tender and eye of round muscles from Hanwoo cows are shown in Table 1. No significant differences were observed in protein contents among the age groups for strip loin and eye of round muscles; however, chuck tender muscles had significantly higher protein contents in G3 than in G1 and G2 ($p<0.05$). The moisture contents were not significantly different among the 3 age groups for strip loin, chuck tender, and eye of round muscles ($p>0.05$). For strip loin and chuck tender muscles, G1 showed significantly higher intramuscular fat content than that in G3 ($p<0.05$).

Visible intramuscular fat (IMF) or marbling is an important meat characteristic that is appreciated by the consumer because of its positive effects on taste, juiciness, and tenderness (Platter *et al*., 2005). Galli *et al*. (2008) reported that 12-year-old Hereford cows had the least amount of intramuscular fat in 4 age groups: 3, 4-5, 6-8, and 12 years. Reagan *et al*. (1976) showed different results in a study of 3 age categories (9-34, 44-96, and 119-323 months). No differences were observed in intramuscular fat content among the maturity classes and only moisture content differed between the groups. Pflanzer and de Felício (2011) found that a high and negative correlation coefficient of -0.92 ($p<0.05$) between moisture and fat contents. In the present study, the young maturity group had a higher ($p<0.05$) intramuscular fat contents than that in the older maturity group. This could be due to Korean consumers preferred young Hanwoo cows (< 5 years old) to old cows in the beef market and most young cows were fattened for 6-12 mon at the farm before slaughter. However, no significant differences were observed in collagen contents among the 3 age groups ($p>0.05$) (Table 1).

Meat quality

Color is the first quality attribute that influences a consumer's purchasing intent (Risvik, 1994), while toughness is the attribute that will determine whether a consumer will re-purchase the product. Regarding meat color, L* and a* values of strip loin, chuck tender, and eye of round muscles were not significantly different among the 3 age groups; however, chuck tender muscles had significantly lower b* values for G2 than for G1 ($p<0.05$) (Table 2). Meat color (a*) values increased as the age increased from 3 to 15 mon of young Qinchuan cattle in China (Li *et al.*, 2011). Xiong *et al.* (2007) reported that there were no significant differences between the 2-4, 6-8, and 10-12 year age groups for raw semitendinosus (ST) or raw semimembranosus (SM) patties for any of the surface color parameters (CIE L*, a*, and b*). The 3 muscles had significantly higher cooking loss (%) and lower WHC (%) in G3 than in G1 and G2 ($p<0.05$) (Table 2). Regarding WBS, values for strip loin muscles were significantly higher in G3 than in the other groups ($p<0.05$), but chuck

Table 1. Chemical compositions and collagen contents of striploin, chuck tender and eye of round by different age groups of Hanwoo cows

Cut	Age group*	Moisture (%)	Protein (%)	Fat (%)	Collagen (%)
Striploin					
	1	62.42±0.65**	19.63±0.19	13.88±0.55^{a}	1.87±0.04
	2	63.79±0.47	20.17±0.13	12.17±0.50^{b}	1.87±0.03
	3	63.59±0.72	19.88±0.24	11.39±0.52^{b}	1.85±0.04
Chuck tender					
	1	68.15±0.43	19.69±0.10^{b}	8.66±0.36^{a}	1.88±0.04
	2	67.87±0.36	19.68±0.10^{b}	8.40±0.34^{a}	1.80±0.03
	3	69.20±0.30	20.10±0.13^{a}	6.92±0.33^{b}	1.88±0.04
Eye of round					
	1	70.42±0.48	21.06±0.16	5.64±0.48	1.83±0.02
	2	69.64±0.38	20.80±0.09	5.87±0.30	1.81±0.03
	3	69.65±0.48	20.89±0.16	5.89±0.47	1.86±0.04

*Group 1(G1), < 5 years old; Group 2 (G2), 6-8 years old; Group 3(G3) > 9 years old.

**Mean±standard error.

$^{a-b}$Means in the same column within the same category with different letters are significantly different ($p<0.05$).

Table 2. CIE meat color (L*, a*, b*), cooking loss (CL, %), sarcomere length (SL, μm), Warner-Bratzler shear force (WBS, kg) and water holding capacity (WHC, %) of striploin, chuck tender and eye of round by different age groups of Hanwoo cows

Cut	Age group*	CIE			CL (%)	WHC (%)	WBS (kg)	SL (μm)
		L*	a*	b*				
Striploin								
	1	33.96±0.47**	19.88±0.48	9.07±0.33	23.65±0.43[c]	55.34±0.34[a]	3.99±0.12[b]	2.48±0.03
	2	34.33±0.48	19.44±0.35	8.86±0.26	25.50±0.46[b]	54.54±0.30[a]	3.85±0.11[b]	2.49±0.02
	3	33.30±0.64	18.97±0.42	9.82±0.19	27.17±0.52[a]	52.54±0.37[b]	4.62±0.13[a]	2.54±0.02
Chuck tender								
	1	35.12±0.52	20.88±0.44	9.94±0.29[a]	26.54±0.57[b]	54.94±0.27[a]	5.03±0.18	2.63±0.03[a]
	2	34.55±0.41	19.85±0.29	8.98±0.23[b]	27.23±0.53[b]	53.79±0.40[b]	4.73±0.11	2.55±0.02[b]
	3	33.78±0.48	20.41±0.37	9.70±0.17[ab]	29.24±0.67[a]	51.35±0.49[c]	5.24±0.15	2.52±0.03[b]
Eye of round								
	1	34.97±0.46	20.88±0.48	10.03±0.36	26.93±0.53[b]	54.77±0.29[a]	4.97±0.15	2.91±0.03
	2	34.71±0.45	19.50±0.37	9.21±0.28	26.97±0.49[b]	53.79±0.45[a]	4.75±0.11	2.90±0.03
	3	33.86±0.45	19.51±0.45	9.90±0.24	28.96±0.58[a]	52.43±0.39[b]	5.20±0.20	2.84±0.05

*Group 1(G1), < 5 years old; Group 2 (G2), 6-8 years old; Group 3(G3) > 9 years old.
**Mean±standard error.
[a-c]Means in the same column within the same category with different letters are significantly different (p<0.05).

tender and eye of round muscles showed no significant differences in WBS values among the 3 groups (p>0.05) (Table 2). The sarcomere length (SL) was significantly longer for G1 of chuck tender muscle than for the other groups (p<0.05), and there were no significant differences in strip loin and eye of round muscles. The sarcomere was the repeating structural unit of the myofibril and also the basic unit of the muscle's contraction-relaxation cycle occurred. Although the influences of the sarcomere length on the tenderness by different age groups was need to further study, Pflanzer and de Felício (2011) reported that sarcomere length was not affected (p>0.05) by maturity. Several endogenous muscle enzyme systems with cathepsins and calpains were reported in the post-mortem tenderization of meat (Goll *et al*., 1983; Rowe *et al*., 2004). Huff-Lonergan *et al*. (1995) postulated that LD samples from old cattle probably contained higher calpastatin activity that inhibited calpain-mediated protein degradation in post-mortem beef. Xiong *et al*. (2007) postulated that older cows exhibited increased toughness because they probably had reduced calpain activity in muscles with greater oxidative stress compared to younger cattle. During post-mortem storage of beef muscles, the degradation of myofibrillar and cytoskeletal proteins depends on muscle type and animal age. Changes of titin T1 in the fraction of washed myofibrillar proteins observed on SDS-PAGE were small. Further pronounced changes occurred in the 30-kDa fraction, i.e., the Tn-T degradation product. The appearance was slower in cow muscles than in heifer and calf muscles (Kolczak *et al*., 2003).

Fatty acid composition

The fatty acid compositions (%) of strip loin, chuck tender, and eye of round muscles are shown in Tables 3-5. The strip loin muscles had significantly higher contents of C16:0, and eye of round muscles had significantly higher contents of C18:0 in G3 than in G1. The total contents of saturated fatty acids were significantly higher in

Table 3. Fatty acid compositions (%) of striploin by different age groups of Hanwoo cows

Fatty acids (%)	Age groups*		
	1	2	3
C14:0	2.90±0.08**	3.08±0.09	3.02±0.10
C16:0	28.04±0.40[b]	29.28±0.35[a]	29.69±0.41[a]
C18:0	11.04±0.27	11.11±0.32	11.37±0.46
C16:1n7	5.04±0.18	5.16±0.15	4.84±0.23
C18:1n7	0.51±0.04	0.44±0.03	0.40±0.04
C18:1n9	50.26±0.50	49.16±0.47	48.86±0.73
C18:2n6	1.58±0.08	1.46±0.08	1.45±0.07
C18:3n3	0.04±0.00[c]	0.09±0.01[b]	0.13±0.02[a]
C18:3n6	0.02±0.00[b]	0.03±0.00[ab]	0.04±0.00[a]
C20:1n9	0.13±0.02	0.10±0.01	0.09±0.02
C20:4n6	0.08±0.01	0.10±0.02	0.11±0.02
SFA	41.98±0.61[b]	43.46±0.51[ab]	44.08±0.74[a]
MUFA	56.30±0.60[a]	54.86±0.50[ab]	54.19±0.79[b]
PUFA	1.73±0.09	1.68±0.09	1.73±0.08
n3	0.04±0.00[c]	0.09±0.01[b]	0.13±0.02[a]
n6	1.68±0.09	1.59±0.09	1.60±0.08

*Group 1(G1), < 5 years old; Group 2 (G2), 6-8 years old; Group 3(G3) > 9 years old.
**Mean±standard error.
[a-c]Means in the same row within the same category with different letters are significantly different (p<0.05).

Table 4. Fatty acid compositions (%) of chuck tender by different age groups of Hanwoo cows

Fatty acids (%)	Age groups*		
	1	2	3
C14:0	2.22±0.07**	2.37±0.07	2.30±0.11
C16:0	25.97±0.77	27.06±0.36	27.43±0.49
C18:0	12.24±0.42	13.10±0.28	13.57±0.54
C16:1n7	3.79±0.16	3.82±0.12	4.35±0.69
C18:1n7	0.40±0.03[a]	0.32±0.02[b]	0.29±0.03[b]
C18:1n9	50.15±1.14	49.01±0.63	47.08±1.10
C18:2n6	3.40±0.55	3.33±0.45	3.58±0.29
C18:3n3	0.17±0.07	0.14±0.01	0.27±0.09
C18:3n6	0.09±0.04	0.04±0.00	0.12±0.07
C20:1n9	0.16±0.02	0.13±0.01	0.15±0.03
C20:4n6	1.34±1.00	0.69±0.17	0.78±0.27
SFA	39.89±1.26[b]	42.53±0.53[ab]	43.30±0.86[a]
MUFA	52.93±1.69	53.28±0.68	51.87±1.12
PUFA	5.49±1.32	4.19±0.49	4.82±0.74
n3	1.92±1.68	0.14±0.01	0.34±0.16
n6	6.30±1.89	4.05±0.49	4.49±0.60

*Group 1(G1), < 5 years old; Group 2 (G2), 6-8 years old; Group 3(G3) > 9 years old.
**Mean±standard error.
[a-b]Means in the same row within the same category with different letters are significantly different ($p<0.05$).

Table 5. Fatty acid compositions of eye of round by different age groups of Hanwoo cows

Fatty acids (%)	Age groups*		
	1	2	3
C14:0	2.52±0.11**	2.75±0.06	2.55±0.13
C16:0	28.22±0.46	29.15±0.34	29.62±0.42
C18:0	9.88±0.40[b]	10.93±0.27[ab]	11.83±0.50[a]
C16:1n7	5.76±0.26[a]	5.28±0.21[ab]	4.67±0.25[b]
C18:1n7	0.63±0.05[a]	0.48±0.03[b]	0.42±0.04[b]
C18:1n9	49.45±0.82	48.66±0.47	47.51±0.87
C18:2n6	2.36±0.24	2.00±0.12	2.19±0.27
C18:3n3	0.17±0.08	0.12±0.01	0.22±0.09
C18:3n6	0.12±0.07	0.04±0.00	0.11±0.07
C20:1n9	0.13±0.02	0.10±0.01	0.09±0.02
C20:4n6	0.61±0.24	0.48±0.07	0.73±0.28
SFA	40.62±0.63[b]	42.82±0.49[a]	44.00±0.61[a]
MUFA	55.98±0.98[a]	54.54±0.48[ab]	52.69±0.98[b]
PUFA	3.39±0.71	2.64±0.18	3.32±0.76
n3	0.25±0.14	0.12±0.01	0.29±0.16
n6	3.14±0.58	2.52±0.18	3.03±0.60

*Group 1(G1), < 5 years old; Group 2 (G2), 6-8 years old; Group 3 (G3) > 9 years old.
**Mean±standard error.
[a-b]Means in the same row within the same category with different letters are significantly different ($p<0.05$).

G3 than in G1 for all 3 muscles ($p<0.05$). The chuck tender and eye of round muscles had significantly higher contents of C18:1n7 in G1 than in G3 ($p<0.05$). The total content of monounsaturated fatty acids was significantly higher in G1 than in G3 for strip loin and eye of round muscles ($p<0.05$). Fatty acid composition has an important effect on firmness or softness of the fat in meat, especially for the subcutaneous and intramuscular fat. The effect of fatty acids on firmness is a result of the different melting points of the fatty acids in meat. Unsaturated fatty acids with more than two double bonds are important in regulating the shelf life of meat (rancidity and color deterioration) because of their rapid oxidation and in flavor development during cooking (Wood *et al*., 2003). Although it has been suggested that dietary fatty acids influence tenderness and juiciness, these are more likely to be affected by the total amount of fatty acids rather than by individual ones. In very fat cattle, the fat is soft and oily mainly due to an increase in 18:1 relative to 18:0 and 16:0 (Leat, 1975). Wood (1984) recorded a value for 18:0 for 14.7% of total fatty acids in a young heifer and 2.7% in an 11-year-old fat steer. However, in the present study, no significant differences were observed in the contents of C18:0 among the 3 age groups, except in the eye of round muscle. Sensory-panel scores were negatively associated with total saturated fatty acids, mainly stearic and palmitic acids, which were the highest in grass-fed animals, and positively associated with total unsaturated fatty acids, mainly because of the high levels of oleic acid in grain-fed animals (Wood, 1984). In addition, different SFAs have been found to have different effects on the serum cholesterol concentrations, and stearic acid (18:0) was shown to have no effect on or even reduce serum cholesterol level.

Free amino acids

Feidt *et al*. (1996) reported that the final protein degradation products such as peptides and free amino acids were involved in the enzymatic reactions, and they played an important role in flavor development (Kato *et al*., 1989; Aristoy and Toldrá, 1995; Nishimura, 1998). In the comparison of the free amino acids contents among the 3 age groups of strip loin muscle, G1 had significantly higher contents of threonine, alanine, valine, methionine, phenylalanine, leucine, and lysine than those in G2 and G3 ($p<0.05$) (Table 6). It had been reported that the degradation of myofibrillar proteins depends on animal age (Lonergan *et al*., 1995; Xiong *et al*., 2007). During post-mortem storage of beef muscles, the muscle from old cattle less susceptible to degrade due to it contained higher calpastatin activity with greater oxidative stress compared to younger cattle. An increase in amino acids in beef during the ageing period has been elucidated in

Table 6. Free amino acids (FAA) compositions of strip loin by different age groups of Hanwoo cows

FAA	Age groups*		
	1	2	3
Glutamate	0.75±0.02**	0.71±0.03	0.68±0.04
Asparagine	0.10±0.01	0.09±0.01	0.11±0.01
Serine	0.65±0.02	0.60±0.03	0.62±0.02
Glutamine	4.66±0.17	4.79±0.15	4.75±0.18
Histidine	0.36±0.01	0.33±0.01	0.34±0.01
Glycine	0.40±0.05	0.72±0.05	0.88±0.05
Threoinine	0.56±0.03[a]	0.43±0.02[b]	0.46±0.02[b]
Arginine	0.60±0.03	0.54±0.02	0.55±0.02
Alanine	15.89±0.37[a]	13.68±0.28[b]	13.76±0.33[b]
Tyrosine	0.41±0.02	0.35±0.01	0.34±0.03
Cysteine	0.09±0.01	0.13±0.02	0.12±0.02
Valine	0.51±0.02[a]	0.44±0.02[b]	0.45±0.02[ab]
Methionine	0.70±0.03[a]	0.63±0.03[b]	0.63±0.02[b]
Phenylalanine	0.87±0.02[a]	0.74±0.03[b]	0.69±0.01[b]
Isoleucine	0.93±0.01[ab]	0.86±0.02[b]	0.98±0.01[a]
Leucine	0.66±0.02[a]	0.54±0.01[b]	0.55±0.01[b]
Lysine	0.78±0.02[a]	0.63±0.03[b]	0.66±0.03[b]
Proline	0.87±0.04	0.98±0.05	0.90±0.04

*Group 1(G1), < 5 years old; Group 2 (G2), 6-8 years old; Group 3 (G3) > 9 years old.
**Mean±standard error.
[a-b]Means in the same row within the same category with different letters are significantly different (p<0.05).

previous studies that showed that proteolysis contributes to the breakdown of myofibrillar protein and the action of calpains and cathepsins on major myofibrillar proteins, generating protein fragments and intermediate size polypeptides; furthermore, polypeptides and peptides generate free amino acids (Toldrá, 2006). An increase in particular amino acids caused by ageing was shown in previous studies. Field *et al*. (1971) found that large increases in alanine, glutamine, serine, valine, leucine, and lysine are obtained by ageing beef for 5 d. Batzer *et al*. (1960) showed that amino acids participated as the precursors of the taste and flavor of cooked meat. The specific meat flavor could not develop with only one component; rather it was developed through the combination of many types of amino acids and sugars. The balance in the quantities of different peptides and amino acids from the breakdown of proteins was the key for palatability of meat. Feidt *et al*. (1996) reported that the contents of free amino acids and peptides degraded from meat protein were related to the cut and aging condition and that they increased with increasing numbers of aging days. The increase in peptides is because of the action of proteinases such as calpains and cathepsins, while the increase of free amino acids is because of the action of aminopeptidases, which are enzymes whose optimal pH is close to neutral (Flores *et al*., 1993, 1996).

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