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RESEARCH NOTE

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Correlation between Meat Color and L-Carnitine Content in Livestock Meats

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Abstract In this study, the correlation between color of redness and L-carnitine content in meats was investigated using microplate enzymatic assays. The L-carnitine levels and its storage stabilities of domestic and imported livestock products in Korean markets were also studied. The results showed a high correlation (r=0.9764) between L-carnitine content and redness values of homogenized meat solution. Korean native cattle ('Hanwoo') meat showed the highest L-carnitine content (3.64±0.14 μmol/g) in meat samples analyzed in this study. The L-carnitine level of the meats decreases during periods of storage in cold and freezing conditions, and the level of decrease was more significant at 4°C than at −20°C, which suggests that the storage stability of L-carnitine is related to its storage temperature. This study gives reliable data about correlation between meat color of redness and L-carnitine content, and gives useful information to determine the characteristics of 'Hanwoo'.

Keywords: correlation, L-carnitine, meat color, 'Hanwoo', storage stability

Introduction

L-Carnitine (β-hydroxy-gamma-trimethyl-amino-butyrate) is known to be found widely in nature, and is believed to be present in all animals. The molecule is essential for β-oxidation of fatty acids. It is synthesized primarily in the liver and the kidneys, and must be transported to other tissues. L-Carnitine plays an important role in energy production by chaperoning activated fatty acids (acyl-CoA) into the mitochondrial matrix for metabolism, and chaperoning intermediate compounds out of the mitochondrial matrix to prevent them from accumulating (1,2). Our recent research has indicated that, besides its well-known functions in metabolic process, L-carnitine also has a function in programmed cell death (apoptosis), with several specific pathways (3). A sufficient level of L-carnitine in the body is beneficial for maintaining human health, but a deficiency of L-carnitine may reduce the rate of fatty acid oxidation and result in muscle weakness, primarily in heart and skeletal muscle (4). For old people, pregnant women, and infants, L-carnitine is considered an essential nutrient. Most L-carnitine concentrates are found in tissues that use fatty acids, such as skeletal and cardiac muscle (5). In tissues and plasma, L-carnitine is present in free and esterified forms, with free carnitine comprising approximately 80% of total carnitine under normal circumstances (6). L-Carnitine concentrations vary widely in different species and among tissues, and its levels are influenced by factors such as age and lifestyle (7). L-Carnitine can be supplied to the human body through dietary sources. Meat, poultry, fish, and dairy products are the richest sources of L-carnitine (6,8,9). Variation in L-carnitine contents has been observed in meats and dairy products (10).

Since L-carnitine was first discovered in 1905 by Russian chemists Gulewitsch and Krimberg, it has been assayed by a number of different methods. In recent years, more analytical techniques for L-carnitine in plasma and urine, based on chromatography methods such as gas chromatography (11), high pressure liquid chromatography (HPLC) (12-14), and radio-enzymatic assay (15,16), and mass spectrometry (17,18) have been reported. These methods, however, suffer from the disadvantage that they are too time-consuming and labor-intensive to be implemented in experiments in a high-throughput context (19). Currently, the use of enzymatic methods such as a rapid assay method has been reported in some research. Compared to the methods mentioned above, the enzymatic method is considered practical for its reliable, sensitive, low cost, and short analysis time requirement. The enzymatic method has recently become a popular technique in food ingredients analysis.

A recent study has shown that the L-carnitine concentration in muscle is related to myoglobin concentration (6). From this information, it could be thought that the color value of meat is closely related to L-carnitine contents. However, there have been no reports about the relationship between L-carnitine and a* values as indices for the red color of meat. Additionally, few studies about the storage stability of L-carnitine are available. In this study, the correlation between meat color and L-carnitine content in livestock meats by using microplate enzymatic assays was investigated. L-Carnitine levels and storage stabilities with several domestic and imported livestock products in Korean markets were also determined.

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Materials and Methods

Reagents L-Carnitine, carnitine acetyltransferase (CAT, EC2.3.1.7), acetylcoenzyme A, 5,5'-dithiobis (2-nitro-

Table 1. Values of L*, a*, and b * at the surface of muscle and in homogenized muscle solution

Species	Color of surface of meat			Color of homogenized meat solution		
	L*1	a*1	b*1	L*2	a*2	b*2
Chicken	44.16±1.32$^{a1)}$	9.82±1.28^{a}	6.43±1.32^{a}	59.28±0.92^{a}	1.09±0.17^{a}	2.23±0.14^{a}
Pork	39.18±2.18^{b}	10.19±0.50^{ba}	3.65±0.38^{bd}	52.13±0.07^{bc}	3.37±0.07^{b}	2.92±0.06^{b}
Imported Australian beef	37.14±0.45^{cb}	22.1±0.82^{c}	9.12±0.41^{c}	53.34±1.71^{c}	10.2±0.29^{c}	7.23±0.09^{cd}
'Hanwoo' beef	24.24±0.55^{d}	15.22±0.20^{d}	3.34±0.34^{d}	45.88±1.84^{d}	12.94±0.32^{d}	7.48±0.16^{d}

[1)]Mean±SD (n=3); Significant differences between the species for each individual color index are indicated by different letters (p<0.01).

benzoic acid) (DTNB), ethylenediamine tetraacetic acid (EDTA), and perchloric acid (PCA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of the purest grade available.

Sample preparation The same parts of tissues (liver, heart, and muscle of top round) from chicken (broiler), pig ('Long white'×'Duroc'×'Yorkshire'), and cattle ('Hanwoo', domestic 'Holstein', and imported Australian cattle), were commercially obtained in triplicate, and each tissue was prepared as 6 pieces. Fresh tissues were frozen immediately in nitrogen, and were stored at –70°C until processing.

L-Carnitine analysis L-Carnitine was measured according to the method of Shimada *et al.* (6) and Xia and Folkers (20) with some modifications. In brief, 5 g frozen samples were homogenized in 5 volumes of 0.3 M PCA in a homogenizer (AM-11; Kaisha, Tokyo, Japan) at low temperature. After centrifugation at 3,000×g for 10 min, the pellet was suspended in 4 volumes of PCA, followed by centrifugation. The supernatants were pooled and filtered through a 0.2-μm glass microfibre filter, and the volume was adjusted to 50 mL with PCA. Eight mL of tissue extracts were gradually neutralized with approximately 2 mL of 1.2 M K_2CO_3. After centrifugation at 8,000×g for 10 min, the supernatants were pooled and filtered through a Millipore 0.45-μm filter. One-hundred μL of tissue extract compounded with 100 μL buffer containing DTNB, acetyl CoA, EDTA, and Tris-HCl (pH7.5) were seeded into each well of the microplate at 37°C. The compounds were newly prepared and then thoroughly shaken by a Sunrise microplate mixer (Tecan, Vienna, Austria). After incubation at 37°C for 10 min, the blank absorbance of the compounds was measured with a microplate reader at 413 nm. Fifty μL of CAT were added to catalyze the enzyme reaction, and the compounds were then incubated at 37°C for 10 min. Finally, the optical density (OD) was again assayed at 413 nm. The differences in absorbance readings (before and after addition of the enzyme) were used to calculate the carnitine concentrations.

Color analysis The color analysis was performed basically according to the method described previously (21). The meat color was measured at the surface of the meat using a reflectance spectrophotometer (CR-300; Minolta, Osaka, Japan) 30 min after the package had been opened in order to allow for the stabilization of color following exposure to air. L*, a*, and b* represent lightness, redness, and yellowness, respectively. In order to measure the color value of homogenized meat solution, 5 g of muscle sample were homogenized in 5 volumes of distilled water, and were then deposited at 30 min. The same procedures were to assay the color of supernatants of homogenized meat solution, and parameters were recorded.

Storage traits testing The meats were separately packaged and stored at 4 and –20°C. The L-carnitine levels of meat at 1, 7, 14, and 21 days at 4°C, and 0, 15, and 30 days at –20°C were determined.

Statistical analysis Data were evaluated using SPSS program for Windows Version 12.0 and are presented as mean±standard deviation (SD). Testing was performed using one-way analysis of variance (ANOVA). Differences were considered significant with p values <0.01.

Results and Discussion

Calibration standards Firstly, a calibration standard for the L-carnitine assay was obtained according to the previously described method. In order to obtain the calibration curve, assays for each concentration were independently repeated with 4 different standard solutions. The changes of absorbance at 413 nm were plotted according to the L-carnitine concentrations. The equation of curves was y=0.0202x+0.0493, and the correlation coefficient (r) was 0.9969 and most standards assays had low values of coefficients of variation (data not shown), indicating the high accuracy and repeatability of the microplate enzymatic analysis.

Correlation between L-carnitine levels and the color of muscle The L*, a*, and b* values are shown in Table 1. Two kinds of redness (a* value) of muscle were detected, and the muscles of chicken, pork, 'Hanwoo' beef, and imported Australian beef were randomly selected as samples. The a* value1 and a* value2 were representative of a* values measured at the surface of muscle and in homogenized muscle solution, respectively. Table 1 shows that a* values measured with homogenized meat solutions of 4 different species were significantly different (p<0.01), whereas the a* values measured at the surface of chicken and pork did not show significant differences. These findings indicate that the results estimated using homogenized meat solution could more reasonably reflect the myglobin content of meat, which is closely related to its L-carnitine level. The correlation of a* values and L-carnitine content values is shown in Fig. 1. There was low correlation between the L-carnitine content values and the a* values of the muscle surfaces (r=0.5988). However, a* values of

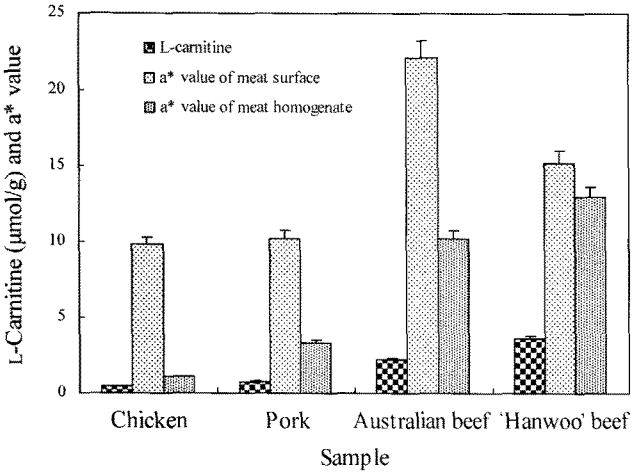

Fig. 1. Overview of the relationship between the L-carnitine contents and 2 kinds of a* (redness) of meat. a*1, a*2 indicate the redness of the surface of muscle and of homogenized muscle solution, respectively. Data are expressed as the mean±SD of 3 independent experiments.

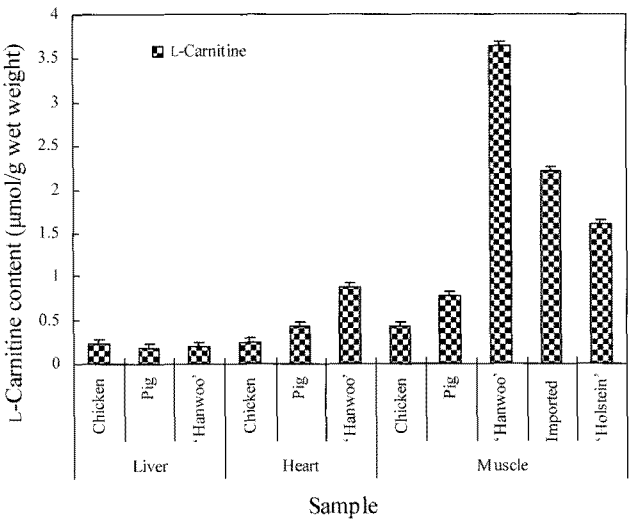

Fig. 2. Overview of the contents of L-carnitine in main livestock products from Korean markets. Data are expressed as the mean±SD of 3 independent experiments.

homogenized muscle solution were found to be highly correlated (r=0.9764) with L-carnitine content values (data not shown). These results could be explained by the degree of dispersion of myoglobin, which was equally released into solution following the homogenization of meat. Myoglobin mainly contributes to the color of meat (80-90% of the total pigment of the muscles) (22,23). In general, the level of myoglobin content is higher in beef and mutton than in pork, poultry, and fish (6,8,9,24). The present results could suggested that higher a* values of homogenized meat solution indicate greater L-carnitine content in meat. A previous study showed that, compared to white meats, red meats are rich in myoglobin, which gives them a red color, and in mitochondria, which the main cell structure that contains L-carnitine (25). Additionally, we also evaluated the correlation between the L-carnitine level and the L* value, which was the best instrumental color indicator of sensory redness (26), although this relationship was not significant (data not shown).

Determination of L-carnitine levels in main livestock products available from Korean markets In this investigation, the L-carnitine contents in the left side of fresh liver, heart, and leg muscles from chicken, pig, and cattle that were commercially obtained from Korean markets were measured. The contents of L-carnitine in main livestock products of Korean markets are shown in Fig. 2. In each sample, the variation of L-carnitine content was listed in order as follows: muscle>heart>liver. In the muscle, the highest L-carnitine content was found in the 'Hanwoo' with a 3.64±0.14 μmol/g wet weight (w.w.), followed by imported Australian beef (2.2±0.13 μmol/g w.w.), 'Holstein' (1.8±0.14 μmol/g w.w), pork (0.78 ±0.05 μmol/g w.w.), and chicken (0.42±0.07 μmol/g w.w). In the heart, L-carnitine concentrations in chicken, pig, and 'Hanwoo' were 0.24±0.16, 0.43±0.26, and 0.87±0.36 μmol/g w.w., respectively. In the liver, L-carnitine concentrations in chicken, pig, and 'Hanwoo' were 0.23±0.19, 0.18±0.21, and 0.2±0.12 μmol/g w.w., respectively. The lowest L-carnitine content was found in the liver of pig (0.18 μmol/g); this level was 19.2-fold lower than the level of 'Hanwoo' muscle. These results suggested that muscle showing high myoglobin content could be a main contributor to the L-carnitine content compared to other tissues of livestock. These findings support the results reported by Alhomida *et al.* (7), who showed that the concentrations of free L-carnitine in the skeletal muscle, heart, liver, and kidney of camel were 5.17±0.10, 1.45± 0.13, 0.59±0.60, and 0.59±0.20 μmol/g w.w., respectively. Seline and Johein (4) also reported that the concentrations of L-carnitine in the liver and kidney were lower than in skeletal muscle. The prominent differences in L-carnitine content among animal species and different tissues are well known. However, the reasons for this are not completely understood. There are some factors that contribute to this phenomenon. One is the myoglobin content in meat, and the other is animal species showing high L-carnitine levels from birth. In order to investigate the specific intrinsic L-carnitine contents in different kinds of beef, further studies on same conditions with many samples are needed.

Changes of intrinsic L-carnitine levels of meats at different storage temperature and time The results of stability of L-carnitine in several meats during their storage time are shown in Fig. 3. The L-carnitine level of the meats decreased according to the storage period in cold and freezing conditions, and the level of decrease was greater at 4°C than at −20°C. The L-carnitine contents of chicken, pork, 'Hanwoo', Australian beef, and 'Holstein' during the storage at 4°C were decreased by 42.9, 37.5, 35.9, 18.6, and 13.3% compared to their day 0 levels, while they were decreased by 30.4, 9.7, 8.5, 18.2, and 11.2% by the end of 30 days at −20°C, respectively, which suggests that the storage stability of L-carnitine is related to its storage temperature. To date, there have been no reports showing the storage stability of L-carnitine at different storage temperatures and times. This result showed that the decrease of L-carnitine content was more significant at 4°C

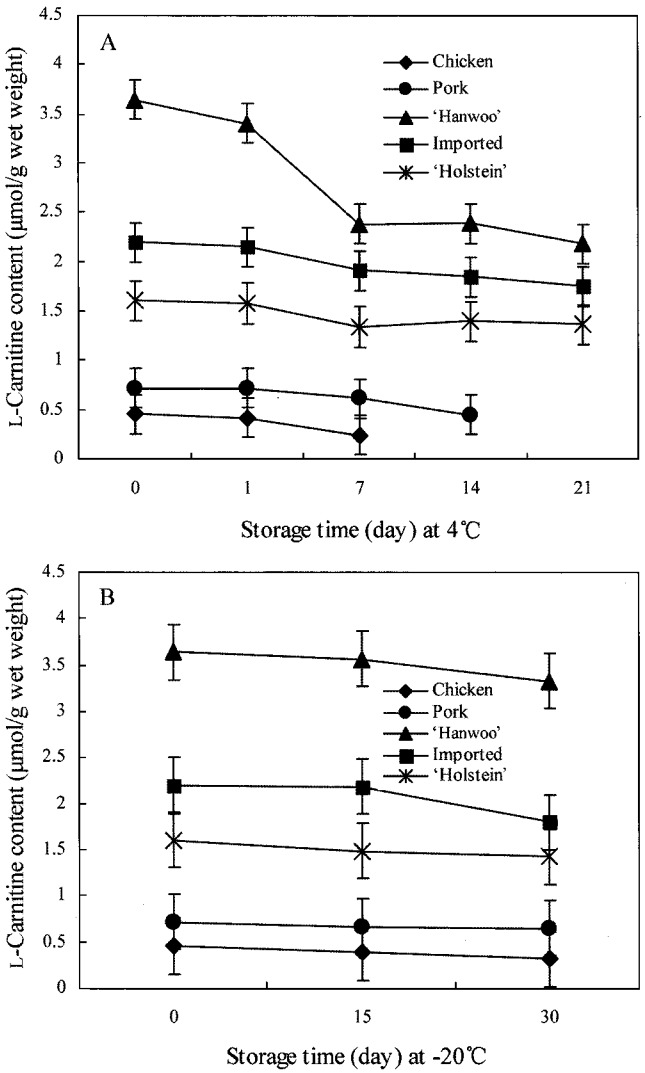

Fig. 3. The changes of levels of L-carnitine at different storage temperatures and times. (A) Contents of L-carnitine change at 4°C, (B) contents of L-carnitine change at −20°C.

than at −20°C (Fig. 3). These results could be explained by the postmortem aging of muscle which was more rapid at 4°C than at −20°C. During the postmortem aging, the muscle proteins were degraded by certain proteolytic enzymes such as Ca_2+-dependent proteases, which remain active in postmortem storage (27). This degradation of muscle fiber could cause an increase of muscle tenderness and liquid flux from muscle. Simultaneously, some of the free L-carnitine was also accompanied by the liquid flux from muscle. However, when postmortem aging was allowed to proceed at −20°C, autolysis of meat proceed more slowly, and the liquid-holding capability of muscle was maintained for a longer period of time.

In conclusion, The L-carnitine level and a* value, as an index of the redness of homogenized muscle solution, were highly correlated. The L-carnitine content varied in different species and tissues. The highest L-carnitine content was found in the 'Hanwoo' in meat samples including imported beefs commercially purchased from Korean markets. L-Carnitine content of the meats is changeable with storage conditions. The L-carnitine level of the meats decreased according to the storage period in cold and freezing conditions, and the level of decrease was more significant at 4°C than at −20°C, which suggests that the storage stability of L-carnitine is related to its storage temperature. This study is the first report about the correlation between meat color and L-carnitine content, and gives useful information with which to determine the characteristics of 'Hanwoo'.

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