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ARTICLE

Carnosine and Anserine in Chicken: Distribution, Age-dependency and their Anti-glycation Activity

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Abstract

The imidazole dipeptide carnosine and its methylated anserine analogues are the major histidine containing dipeptides in vertebrate tissue, especially in skeletal muscle, the heart, and the central nervous system. In this study, the carnosine and anserine content in chicken from different parts and of differing ages was determined and their physiological activities were compared. Anserine was more dominant than carnosine in these tissues and both of them significantly decreased with aging in all parts of chicken muscles. Chicken breast muscle showed the highest content of carnosine and anserine than drumstick and wing. Advanced glycated end-product (AGE) formation was inhibited up to 60% by the extract from 20 wk chicken breast and decreased with aging (90 wk). Anti-oxidation activity was also significantly reduced from 61.2% to 52.9% with aging. As results, anti-glycation and anti-oxidation activity of carnosine and anserine extract from chicken muscle increased proportionally to the amount of those peptides in the muscle, while these decreased with the aging process.

Key words: carnosine, anserine, advanced glycated end-product, anti-glycation activity, chicken

Introduction

Carnosine is a natural dipeptide containing of β-alanine and L-histidine. The imidazole dipeptide carnosine and its methylated analogues anserine are major histidine containing dipeptides in vertebrate animal (chicken, pork, cattle and so on) tissue, especially in skeletal muscle, the heart, and the central nervous system (Mora *et al*., 2008; Tian *et al*., 2007). It has been known to play a role in quite a number of physiological functions in vertebrates. Carnosine's role includes those of physiological buffer in vertebrate's muscle to offset the production of lactic acid during exercise, inhibition of oxidative reaction in hydroxyl-radical and singlet oxygen-scavenging and lipid peroxidation (Intarapichet and Maikhunthod, 2005; Lee *et al*., 1998), inhibition of advanced glycated end-product (AGE) formation (Dukic-Stefanovic *et al*., 2001), neurotransmitter in brain (Hipkiss and Brownson, 2000), regulation of enzyme activity, and chelate prooxidative metals (Decker *et al*., 2000). Since the first isolation of carnosine from beef muscle extract in 1900s, quantitative determination of carnosine from muscle food has been a focus of interest and the potential of carnosine as a natural food supplement for those with poor health has been getting an interest as well (Hipkiss *et al*., 2000; Mora *et al*., 2008). For example, carnosine and anserine have been known as good anti-oxidants, preventing of diseases resulting from lipid oxidation in skeletal muscle (Chan and Decker, 1994). Their physiological activities were exhibited through buffering capacity (Kohen *et al*., 1988), reducing chelating pro-oxidant metals (Decker *et al.*, 2000), singlet oxygen (Dahl *et al.*, 1988) and peroxy radical (Kohen *et al.*, 1988).

Carnosine has been found with higher concentration in white muscle than red muscle tissue (Boldyrev *et al.*, 2004; Brown., 1981; Hipkiss, 2005; Plowman and Close, 1988). Chicken is a representative animal with higher carnosine content among the white muscle tissue animals (Boldyrev and Severin, 1990). However, no systemic investigation was attempted on the changes of carnosine and anserine levels in chicken muscle with aging. In this study, we compared the content of carnosine and anserine from different parts and ages of chicken muscles along with their physiological (anti-glycation and anti-oxidation) activities.

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Materials and Methods

Chemicals and reagents

All the reagents used in this study were of the highest purity available. Acetonitrile (ACN), methanol, bovine serum albumin (BSA), methylglyoxal, L-ascorbic acid, α,α-diphenyl-β-picrylhydrazyl (DPPH), 35% hydrochloric acid, sodium chloride, boric acid, *o*-phthaldialdehyde (OPA), L-carnosine, and L-anserine were purchased from Sigma Aldrich Co. (USA).

Extraction of carnosine and anserine from chicken muscle

Chicken samples (20 and 90 wk laying hen) were received from Department of Animal Science and Technology, Chung-Ang University, Korea. At the end of each week, chickens were sacrificed by cervical dislocation, a method approved by Animal Care Committee of Chung-Ang University. Carnosine and anserine was extracted according to Auh *et al.* (2010) with slight modification. Muscle samples from three different parts (Breast, Drumstick, and Wing) were taken and freeze-dried for 72 h. Dried muscle samples were finely ground and 0.5 g of ground muscle was suspended in 12 mL of distilled water and incubated for 2 h at 25°C with moderate shaking. Subsequently, the mixture was centrifuged at 14000 g for 30 min and 300 μL of supernatant was mixed with 900 μL of cold ethanol, then kept for 20 min at 4°C. Supernatant taken after centrifugation at 14000 g for 30 min was filtered through membrane syringe filter (MCE type, 0.45 mm, Adavantec, MFS Inc., Japan) and freeze dried. Lyophilized samples were kept at -40°C until analysis.

HPLC analysis

Carnosine and anserine concentration was determined according to Aristoy *et al.* (2004) and Auh *et al.* (2010). The HPLC system (Gilson Medical Electronics, USA) equipped with a fluorescence detector (Agilent, USA), a column oven and a Spherisorb SCX column (4.6×250 mm, Waters Co., USA) was used for the analysis. Ten μL of the sample solution (10%, w/v) was mixed with 100 μL of OPA solution and placed for 2 min at room temperature under dark. Then, 20 μL of derivatized samples was injected into HPLC. Mobile phase was composed of 80% of phase A (20% acetonitrile in 6.6 mM hydrochloric acid) and 20% of phase B (phase A containing 0.8 M sodium chloride), eluted as 0.6 mL/min for 30 min at 40°C. For the fluorescence detection, 338 and 445 nm were used as excitation and emission wavelengths, respectively.

Anti-glycation activity (AGE inhibition)

Anti-glycation activity of carnosine containing extract from chicken breast (20 and 90 wk laying hen) was measured by % inhibition of advanced glycated end-product (AGE) formation according to Li *et al.* (2008) with modification. Extract was mixed with bovine serum albumin (BSA, 50 mg/mL) and methylglyoxal (3 mM) in 50 mM sodium phosphate buffer (pH 7.4) and incubated for 24 h in dark condition. The formation of AGE was quantified by measuring the fluorescence intensity (excitation at 350 nm, emission 450 nm) using Spectrofluorimeter RF-1500 (Shimadzu, Japan). All the measurement was conducted in triplicates.

Anti-oxidation activity (DPPH radical scavenging effect)

Anti-oxidation of carnosine containing extract from chicken breast (20 and 90 wk laying hen) extracts on α,α-diphenyl-β-picrylhydrazyl (DPPH) radicals was measured with a method described by Wu *et al*. (2003) with modification. Samples were mixed with 0.2 mM DPPH (in 95% methanol), kept for 30 min at room temperature and the absorbance at 517 nm was measured and anti-oxidation activity was calculated. All the measurements were conducted in triplicates.

Statistical analysis

All the experimental data were analyzed using the ANOVA procedure of SAS (SAS Institute Inc., USA) and the significance was defined at $p<0.05$.

Results and Discussion

Determination of carnosine and anserine in chicken

Carnosine and anserine in chicken were determined by HPLC after OPA pre-column derivatization. The carnosine and anserine content of chicken from different parts and ages were summarized in Table 1. In general, anserine was more dominant than carnosine and both of them significantly decreased with aging in all parts of chicken muscles. Chicken breast parts showed the highest content of carnosine and anserine followed by drumstick and wing. It was in good agreement with previous report by Intarapichet and Maikhunthod (2005) stated that chicken breast muscle had more carnosine than other parts of chicken. Carnosine and anserine in drumstick muscle was seriously affected by aging, but no significant decrease

Table 1. Concentration of carnosine and anserine in chickens from different parts and ages

	Parts	Carnosine concentration (mg/kg)	Anserine concentration (mg/kg)
20 wk laying hen	Breast	712.64±22.38[a]	3367.71±90.62[A]
	Drumstick	395.97±17.40[c]	1165.23±49.51[E]
	Wing	384.19±32.52[c]	2594.99±92.41[C]
90 wk laying hen	Breast	553.55±13.81[b]	2915.54±12.01[B]
	Drumstick	142.79±11.23[d]	693.04±18.91[F]
	Wing	359.17±11.37[c]	2264.86±15.84[D]

[A-F, a-d]Values with the same letters are not significantly different at $p<0.05$.

was observed in the wing muscle. It implied that the decrease of carnosine was proportional to the ratio of white to red muscle in the samples. More decrease of carnosine content was observed in white muscle (breast) than red muscle (wing).

Anti-glycation and anti-oxidation activity (AGE inhibition)

Anti-glycation activity of chicken breast extract was measured by % inhibition of advanced glycated end-product (AGE) formation. AGE formation is increasing with aging as well as in diabetes mellitus, which play an important role in the development of chronic diabetic complications (Ahmed, 2005). Although numerous agents for AGE inhibition have been developed, side effects and cost are limiting the application of the inhibiting agents in practice. The chicken breast extract containing carnosine and anserine showed nice AGE inhibition and the results are summarized in Fig. 1. AGE formation was inhibited up to 60% with the extract from 20 wk chicken breast and decreased with aging (90 wk), which meant the more accumulation of AGE in muscle as aging proceed. Anti-oxidation activity of carnosine containing peptide mixture derived from chicken breast was measured by DPPH radical scavenging effect (Fig. 1). The activity significantly decreased from 61.2% to 52.9% with aging, which might be influenced by the reduction of carnosine and anserine content in the muscle. Although anserine and carnosine are interrelated in physiologically, more research has been done on carnosine to date. But, Wu *et al.* (2003) indicated that anserine had similar radical scavenging effect and higher reducing power compared to carnosine. Therefore, anserine content should be considered along with carnosine for the anti-oxidation activity. As a result, anti-glycation and anti-oxidation activity of carnosine and anserine extract from chicken muscle increase proportionally to

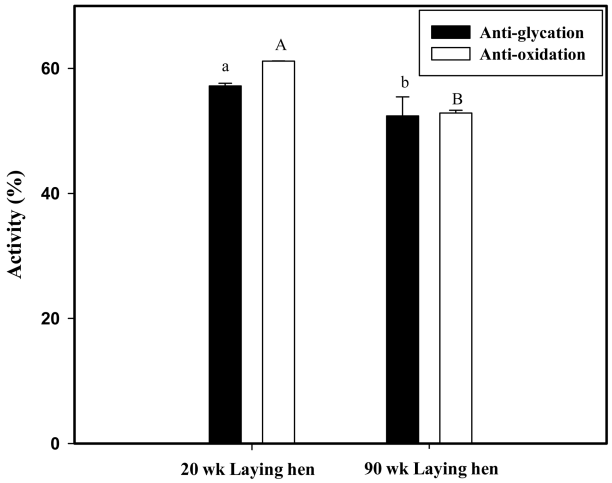

Fig. 1. Anti-glycation and anti-oxidation activities of the carnosine and anserine extract from chicken breast from different ages.

the amount of those peptides in the muscle.

In conclusion, we revealed that the carnosine and anserine content in chicken decreased with aging and the degree of reduction increased in proportion to the ratio of white muscles in each tissue. Although the chicken breast muscle was reported as a good source of carnosine (Intarapichet and Maikhunthod, 2005), anserine was more predominant than carnosine as indicated by Auh *et al.* (2010) and Huang and Kuo (2000). Anti-glycating and anti-oxidation, typical physiological activities of carnosine and anserine, significantly decreased by aging, however, they exhibited sufficient levels of activities even in 90 wk old. This is the first report regarding the distribution of carnosine and anserine in chicken and the effect of aging on it. This study would be a solid basement for the development of specialized functional meat product by the physiological functions of carnosine and anserine.

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