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Genotype and gender differences in carnosine extracts and antioxidant activities of chicken breast and thigh meats

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Abstract

The aim of this work was to investigate the effects of genotypes and gender of chickens on carnosine contents and their antioxidant activities. The carnosine content of fresh meat from Thai indigenous and hybrid native chickens differed between breeds ($p < 0.01$) and genders ($p < 0.01$). Regardless of these differences, breast meat contained 2–4-fold higher carnosine than thigh meat. After water and heat extraction at 80 °C and ultrafiltration, the carnosine content of meat extracts had the same distribution as in fresh meat. No relationship between total iron and carnosine content on antioxidant activity of the extract was detected. However, when compared in the extracts on the basis of mM carnosine in oxidation system, the extracts of chicken meat showed greater antioxidant activity than pure carnosine ($p < 0.05$). Furthermore, at equal concentrations, thigh meat extract had higher effective inhibiting ability than breast extract.

Keywords: Thai indigenous chicken; Chicken meat; Carnosine; Antioxidant

1. Introduction

There are many functional compounds found in skeletal muscle of vertebrate animals, such as free amino acids, dipeptides, and non-protein nitrogenous compounds. Some of these compounds have been implicated and purposed as the active compounds for biological activities in vertebrates, such as dipeptides including carnosine (Wu & Shiau, 2002). Carnosine (β-alanylhistidine) plays important role in physiological functions, such as a potent intracellular pH-buffer, inhibiting of oxidation, and neurotransmitter function (Chan & Decker, 1994; Wu & Shiau, 2002). From the broad-spectrum antioxidant activities, carnosine was studied for its ability of inhibiting oxidation in various food systems and in medical practices (Calvert & Decker, 1992; Decker & Crum, 1993; Jordan, 1999; Kyriazis, 1999; Lee, Hendricks, & Cornforth, 1999). Carnosine content can be affected by muscle types of animals which are influenced from breed, gender, age and breeding (Abe & Okuma, 1995). Muscle fiber types can be divided into three main groups: white glycolytic muscles rich in type IIB fibers, red oxidative muscles rich in type I and IIA fibers and intermediate muscles (Cornet & Bousset, 1999). Plowman and Close (1988) reported that white meat, such as chicken meat, had higher carnosine content than dark meats.

Nagai et al. (1996) studied effects of chicken essence on the recovery from fatigue caused by mental workload. They found that the students who consumed chicken essence felt more active and less fatigued during the workload and their performance to perform a mental arithmetic test and a short term memory test were also improved. They concluded that the chicken essence has a potential to metabolize stress-related substance in blood oxidants and to recover from mental fatigue. Carnosine and anserine are the major dipeptides in the

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chicken essence being responsible for recovery from physical and mental stress and regarded as a candidate for the cortisol metabolism accelerator. In 140 ml of the tested chicken essence, Brand's Essence, contained several hundred milligram of these dipeptides. In addition, Wu and Shiau (2002) investigated carnosine content in six types of commercial chicken essences. Carnosine content ranged from 8 to 162 mg/100 g essence indicating that the essence with the lowest amount of carnosine might be made from chicken bones instead of edible meat.

As the essential compound in chicken extracts, carnosine content depends on breed, gender and age of animals, better knowledge of its content and extraction from different breeds of chicken meats would lead to more understanding of carnosine in various chicken breeds. Hence, the purposes of this study were to investigate carnosine contents in fresh breast and thigh of Thai indigenous, 4-lines cross breed and 5-lines cross breed chicken meats, and produce a natural carnosine-containing antioxidant extract from those meats using heat treatment combined with ultrafiltration. These extracts were also evaluated for their antioxidant activities. In addition, protein and total iron contents were determined due to their important involvement in antioxidant activity of the extracts.

2. Materials and methods

2.1. Chemicals

Carnosine, *o*-phthaldehyde (OPA), soybean phosphatidylcholine, β -mercaptoethanol (BME), bovine serum albumin (BSA), and Folin Ciocalteu's phenol reagent were purchased from Sigma Chemical (St. Louis, MO, USA). Standard Fe (1000 mg/mL) was obtained from Merck KgaA (Darmstadt, Germany). Methanol and acetonitrile were HPLC grade, and all other chemicals were reagent grade or purer.

2.2. Raw materials

Three types of chickens were investigated including Thai indigenous, 4-lines cross breed, and 5-lines cross breed chickens. The Thai indigenous chickens conventionally fed ad libitum were obtained from small holder in Nakhon Ratchasima Province. The 4-lines (Bar Plymouth Rock × Rhode Ireland Red × Shianghai × Thai indigenous) and 5-lines (Bar Plymouth Rock × Rhode Ireland Red × Shianghai × unknown from Australia × Thai indigenous) cross breed chickens obtained from commercial poultry farms in central region of Thailand. All cross breed chickens were fed ad libitum with commercial formula feed in 2 stages, i.e., for grower (0–3 weeks) with 3200 kcal/kg metabolizable energy and 23.0% concentrated protein (CP) and for finisher (4 weeks up) with 3200 kcal/kg and 21.0% CP. The experiment was performed using 5 male and 5 female chickens with live weight of 1.8 kg in two replications. Statistic comparison was done among breeds and between gender of the chickens within the breast and thigh meats group, separately. All chickens were slaughtered at the university farm at the same time. Breast and thigh meats were separated debonded and minced using a domestic meat chopper (Moulinex 327, Spain) then vacuum packed and stored at −20 °C until used.

2.3. Chemical composition analysis of fresh meat

Fresh meat samples were analyzed for moisture and ash content according to AOAC (1997), protein content by Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) using BSA as a standard, carnosine content by HPLC (Cornet & Bousset, 1999; Gopalakrishnan, Decker, & Means, 1999), and total iron content by atomic absorption (AA) spectrophotometry (James, 1995) using an AAnalyst 100 (Perkin–Elmer, Norwalk, CT, USA).

2.4. Preparation and total carnosine content in fresh meats

The meat extracts from both breast and thigh were prepared according to the procedure of Aristoy and Toldra' (1991) with slight modification. Frozen minced meat sample was thawed under running tap water (27 ± 1 °C). One part of this minced meat was homogenized in 4 parts of precooled (4 °C) 0.1 N HCl in an AM-8 homogenizer (Nihonseiki, Kashi Ltd., Tokyo, Japan) for 8 min in an ice chamber. The homogenate was centrifuged at 10,000*g* using a Sorvall RC-5C Plus (Dupont, Delaware, USA) at 4 °C for 20 min. The supernatant was filtered through Whatman #4 filter paper, and the filtrate was freeze–dried 72 h using a HETO FD3 (Heto-Holten A/S, Allerod, Denmark). Subsequently, the dried filtrate was analyzed for total carnosine content present in chicken meat by HPLC.

2.5. Extraction of carnosine by heat treatment and ultrafiltration

The procedure was modified from Chan, Decker, and Means (1993) and Gopalakrishnan et al. (1999). Frozen minced meat was thawed under running tap water (27 ± 1 °C), homogenized in 2 volumes of precooled (4 °C) deionized distilled water in an AM-8 homogenizer in an ice chamber for 8 min. The homogenate was centrifuged at 20,000*g* for 30 min at 4 °C using a Sorval RC-5C Plus, and then, the supernatant was filtered through Whatman #4 filter paper. The filtrate was

heated in a water bath to 80 °C for 10 min and cooled in an ice bath. Heated extract was centrifuged to remove precipitated proteins at 5000*g* for 20 min at 4 °C using a Sorval RC-5C Plus. The supernatant was passed through a 5000 molecular weight cut off ultrafiltration membrane (PES) (Vivaflow50, Vivascience, Sartorius AG, Goettingen, Germany). The ultrafiltration permeate was freeze–dried using a HETO-FD3. The freeze–dried extract (FD-Ext) was analyzed for carnosine, protein, and total iron content, and evaluated for antioxidant activity.

2.6. Analysis of extract composition

Protein content. Protein content in FD-Ext was analyzed by the Lowry method (Lowry et al., 1951). FD-Ext (2.0000–3.0000 mg) was reconstituted with 1 ml phosphate buffer (5 mM, pH 7.0) prior to analysis.

Total iron content. The total iron content in fresh meats and FD-Exts were analyzed by means of AA spectrophotometry using an AAnalyst 100 (Perkin–Elmer, Norwalk, CT, USA). The sample (3.0000 g for fresh meat and 0.2500 g for FD-Ext) was ashed in a muffle furnace (Carbolite CSF1200, Cheffield, England) at 500 °C until a white or light gray ash was obtained. Five ml of concentrated hydrochloric acid was added to the crucible containing the ash, boiled for 5 min, transferred to a beaker, adjusted to 40 ml and boiled for 10 min. The mixture was cooled, filtered through Whatman #1 filter paper into a 50-ml volumetric flask, brought to volume with deionized water, and absorbance measured at 248.3 nm. The iron concentrations were calculated from a standard curve generated using known concentration of iron (0–10 μg/ml) (James, 1995).

Carnosine content. Carnosine content in fresh meat and FD-Exts was analyzed using HPLC, derivatizing the extract by OPA working solution. The OPA working solution was prepared 24 h before use by dissolving 27 mg of OPA in 500 μl absolute alcohol. Five mL of 0.1 M sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) (pH 9.5) was added, followed by adding 50 μl of BME. The mixture was thoroughly mixed, stored in a tightly closed container, and kept in the dark. The reagent could be kept for several days (Antoine, Wei, Littell, & Marshall, 1999).

Sample preparations for HPLC analysis were performed based on the methods of Gopalakrishnan et al. (1999) and Cornet and Bousset (1999) with slight modification. The FD-Ext, 100 mg reconstituted in 2 ml of 5 mM phosphate buffer, pH 7.0, was mixed with 2 ml of 0.4 M perchloric acid and boiled for 5 min to precipitate proteins, and centrifuged at 2000*g* for 20 min by a Labofuge 400R #8179 (Heraeus Instruments, Germany). The supernatant was filtered through a 0.45 μm membrane filter. The filtrate (10 μl) was derivatized with 10 μl of OPA working solution just prior to injection. The derivatized samples were analyzed on an Agilent 1100 series HPLC with an autosampler (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). OPA derivatized carnosine was separated using a mobile phase of 0.3 M sodium acetate (pH 5.5), methanol and acetonitrile (75:15:10) at flow rate of 0.5 ml/min with Eclipse XDB-C18 (4.6 × 150 mm, 5 μm) column. Fluorescence detector was set at an excitation wavelength of 310 nm and emission wavelength of 375 nm. A standard curve was prepared using pure carnosine solution, 10–1000 ppm. Carnosine concentration was calculated by Chemstation Rev.A.09.03 (1417) (Agilent Technologies 1990–2002).

2.7. Evaluation of antioxidant activity

Antioxidant activity of FD-Ext was determined by its ability to inhibit iron/ascorbate catalyzed oxidation of phosphatidylcholine liposome, modified from Decker and Faraji (1990), Kansci, Genot, Meynier, and Gandemer (1997), and Gopalakrishnan et al. (1999). Lipid oxidation was performed in a 2.0 ml model system containing 1 mg phosphatidylcholine liposome/ml of 5 mm phosphate buffer (pH 7.0) plus lipid oxidation catalyst and also various concentration of FD-Ext.

Preparation of reagents. Sodium ascorbate and $FeCl_3$ solution, acted as the catalysts, were separately prepared in degassed ultra pure water freshly before use (Kansci et al., 1997). The MDA standard was prepared according to Botsoglou et al. (1994). 1,1,3,3-Tetraethoxypropane (TEP) was weighed (100 mg) in a screwed-capped test tube, diluted with 10 ml of 0.1 N HCl, immersed into a boiling water bath for 5 min, and cooled immediately under tap water. Stock solution of MDA (100 μg/ml) was prepared by transferring the hydrolyzed TEP solution into a 100-ml volumetric flask and diluting to volume with water.

Analysis of thiobarbituric acid reactive substances (TBARS). Lipid oxidation was monitored by measuring TBARS. The pure carnosine or extract was dissolved in phosphatidlycholine liposome suspension to give a various concentration of 0–12.5 mg/ml. After 10 min, phospholipid oxidation was induced by adding sodium ascorbate and $FeCl_3$ solutions to a final concentration of 45 μM of each. The oxidation mixture was incubated in a 37 °C water bath for 30 min, followed by adding 2 ml of TBA reagent (0.02 M in water). The tightly closed tube was heated in boiling water bath for 15 min, cooled and centrifuged at 4000*g* for 15 min. The absorbance of the supernatant was measured at 532 nm using UV/VIS 916 Spectrophotometer (GBC Scientific Equipment, Ltd., Vic., Australia). Concentration of MDA in the oxidation system was calculated from the MDA standard curve (0–7 μg/ml). The ability of inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{(\text{MDA in absence of Ext}) - (\text{MDA in presence of Ext})}{\text{MDA in absence of Ext}} \times 100.$$

The oxidation activity of FD-Ext was expressed as amount of FD-Ext or the concentration of carnosine used in system that could inhibit 50% of oxidation (EC50). The EC50 of FD-Ext was also compared with EC50 of pure carnosine.

2.8. Statistical analysis

All experiments were performed in duplicate. Each replicate was chemically analyzed in triplicate samples. Statistical analysis was evaluated by factorial in completely randomized design (CRD) with statistical analysis system (SAS Institute, Inc., 1993). Analysis of variance (ANOVA) was analyzed and comparison of means was done by Duncan's multiple range test (DMRT).

3. Results and discussion

3.1. Chemical composition of fresh chicken meat

The individual compositions in fresh meat are presented in Tables 1 and 2 for breast and thigh meats, respectively. There were no significant differences among breeds and between gender of chickens in protein content ($p > 0.05$). Protein contents of breast and thigh were ranging 21–24% and 19–21%, respectively.

Total iron content of breast was lower than that of thigh meat. This result agreed with other studies (Hazell, 1982; Kongkachuichai, Napatthalung, & Charoensiri, 2002; McCormick, 1994; Pearson & Young, 1989) that red meat is more supplied with blood vessels and contains more myoglobin and hemoglobin than light meat. Therefore, it would be expected to have higher amounts of iron. In breast meat group (Table 1), total iron content was not significantly different among breeds, and between gender ($p > 0.05$), whereas it was significantly different among breeds ($p < 0.05$) and between genders ($p < 0.01$) in thigh meat group (Table 2). This could be due to breast meats contained more than 90% of white (fast glycolytic) fibers with less amounts of myoglobin (Lengerken, Maak, & Wicke, 2002; Smith & Fletcher, 1988) and thigh meats composed of red and intermediate fibers with varying amounts of myoglobin (Illingworth, 2004; Meyor, 2003).

For all breeds and gender, fresh chicken breast meats had 2–4-fold higher carnosine content compared with those of thigh meats. However, carnosine contents were not significantly different among thigh meats ($p > 0.05$) comparing between the interaction of breeds and gender, but there was significant difference among breast meats ($p < 0.01$). This result agreed with many previous studies (Cornet & Bousset, 1999; Davey, 1960; Decker, 1995) that white muscle had higher carnosine content than dark muscle. Davey (1960) reported that high carnosine content associated with white muscles which maintain their energy-rich phosphate ester supplies by anaerobic means. Therefore, in view of the power of carnosine, it becomes a factor of great importance to act as buffer in physiological range of pH affected from lactic acid accumulation.

Comparing between gender, it was found that carnosine content of female meat was higher than that of male meat ($p < 0.01$). At the same live weight (LW), female chicken is older than male chicken (Putnum, 1991). In our study regardless of age, the LW of chicken used was the same at 1.8 kg, meaning that female was older than male. Thus, results showed higher amount of carnosine contents found in female meats. It agreed with Chan and Decker (1994) who reported carnosine content of poultry skeletal muscle increased with age. At the same LW, the native chickens had growth time about twice longer than the cross breed chickens, even the 4-lines and 5-lines cross breed chickens were improved in order to obtain their meat qualities similar to that of the native chicken. The faster growth also depended on feed quality (Haitook, Tawfik, & Zöbisch, 2003). The 4-lines cross and 5-lines cross breeds were fed with commercial feed ad libitum, whereas the native chicken was fed with conventional feed by allowing scavenge during the day time in the simple sheds or farm house. Thus, according to the results, feed quality might

Table 1
Chemical composition of fresh breast meats from different breeds (Bd) and gender (S) of chickens

Composition	N		K		T		SEM	Effect		
	F	M	F	M	F	M		Bd	S	Bd*S
Moisture (%)	73.82^{bc}	73.23^{c}	73.24^{c}	74.89^{a}	74.53^{ab}	74.67^{a}	0.21	0.01	ns	0.01
Protein (%)	23.96^{a}	23.75^{a}	23.83^{a}	23.43^{a}	21.69^{a}	22.46^{a}	0.36	ns	ns	ns
Carnosine (μg/g wet meat)	993.23^{b}	818.55^{c}	1200.05^{a}	684.82^{c}	929.03^{b}	621.23^{d}	35.18	0.01	0.01	0.01
Total iron (μg/g wet meat)	11.94^{ab}	10.87^{bc}	12.91^{a}	9.78^{c}	9.71^{c}	11.89^{ab}	0.39	ns	ns	0.01

N = native, K = 4-lines cross breeds, and T = 5-lines cross breeds chickens; F = Female and M = Male. Effect = level of statistical differences by breeds, sexes and interaction between breeds and sexes: 0.01 = highly significant, 0.05 = significant, and ns = not significant differences. SEM = standard error of means. Means with different letters in the same row are significantly different ($p < 0.05$).

Table 2
Chemical composition of fresh thigh meats from different breeds (Bd) and gender (S) of chickens

Composition	N		K		T		SEM	Effect		
	F	M	F	M	F	M		Bd	S	Bd*S
Moisture (%)	76.24^{bc}	76.79^{ab}	75.59^{c}	77.58^{a}	76.34^{bc}	76.96^{ab}	0.21	ns	0.01	ns
Protein (%)	21.17^{a}	19.50^{a}	19.67^{a}	19.98^{a}	20.16^{a}	19.92^{a}	0.22	ns	ns	ns
Carnosine (μg/g wet meat)	311.93^{bc}	271.01^{c}	304.88^{c}	279.57^{c}	429.76^{a}	363.06^{b}	11.62	0.01	0.01	ns
Total iron (μg/g wet meat)	14.54^{b}	16.22^{a}	12.76^{c}	15.72^{ab}	15.84^{a}	15.90^{a}	0.38	0.05	0.01	0.05

N = native, K = 4-lines cross breeds, and T = 5-lines cross breeds chickens; F = Female and M = Male. Effect = level of statistical differences by breeds, sexes and interaction between breeds and sexes: 0.01 = highly significant, 0.05 = significant, and ns = not significant differences. SEM = standard error of means. Means with different letters in the same row are significantly different ($p < 0.05$).

have more effect on carnosine content than age of the birds since, the older native chickens had no remarkably higher carnosine content compared with cross breeds chickens in the same gender. This result was also in agreement with Wattanachant, Benjakul, and Ledward (2004) who reported that different feeding affected on the composition of chicken meat, such as amino acids and fatty acids. Hence, the commercial feed should be more enrichment than conventional feed, resulting in high concentration of amino acids, including histidine which is the precursor of carnosine synthesis.

3.2. Chemical composition of carnosine extracts (Ext) from chicken breeds

Chemical compositions of the Ext from breast and thigh meat are shown in Tables 3 and 4, respectively. Similar to fresh meats, that extracts from breast (Br-Ext) had 2–4-fold carnosine contents higher compared with the extracts from thigh meat (Th-Ext). Carnosine contents in the Br-Ext and Th-Ext were 18498.32–32874.11 and 6387.66–10766.02 ppm in Ext, respectively. These results agreed with the previous studies of Davey (1960), Decker (1995), and Cornet and Bousset (1999) that there were higher carnosine content in white fibers than in red fibers. Consequently, similar results would be observed in the meat extracts.

Differences within the contents of each Ext component were observed among breeds ($p < 0.01$) and between gender ($p < 0.01$) for both types of meats. The female-Exts contained greater amount of carnosine compared with the male-Exts. Furthermore, not only fiber type affected carnosine content, Chan and Decker (1994) reported that species and age of animal could be a factor as well.

Br-ext and Th-ext had approximately 3–6-fold and 6–10-fold total iron content higher compared with their fresh meats, respectively. Total iron contents in Br-Exts were ranging 41.02–83.95 ppm (Table 3) while in Th-Exts were ranging 82.11–150.86 ppm (Table 4). Total iron contents of Th-Exts were greater compared with those of Br-Exts, thus, it conformed to the total iron content of fresh meats shown in Tables 1 and 2. Regarding to the remarkably high total iron content in Th-Exts, it would be suggested that iron was released from myoglobin molecules into the extract during heating at 80 °C. According to Schricker and Miller (1983), and Chen, Pearson, Gray, Fooladi, and Ku (1984), these released irons would be the compounds with lower molecular weight than 5000 Da. Non-heme iron content in heated samples was found ranging from less than 10% to more than 100% depending on the length and severity of heat treatment. Heat treatment caused the oxidative cleavage of porphyrin ring followed by releasing of the iron.

3.3. Antioxidant activity of carnosine extracts (Ext)

TBARS value was measured for antioxidant activity of the Ext and expressed in percentage of inhibition, calculated from the reduction of MDA in oxidation system. Greater antioxidant activity was observed when higher amount of the Ext was used in oxidation system for both Br-Ext and Th-Ext (Fig. 1). These results agreed with the previous studies of Decker, Crum, and Calvert (1992), Chan et al. (1993), Decker, Chan, Livisay, But-

Table 3
Chemical composition of carnosine extracts (Ext) of breast meats from different breeds (Bd) and gender (S) of chickens

Component	N		K		T		SEM	Effect		
	F	M	F	M	F	M		Bd	S	Bd*S
Carnosine (μg/g Ext)	32874.11^{a}	30332.11^{b}	31297.39^{ab}	22978.72^{c}	23355.16^{c}	18498.32^{d}	928.48	0.01	0.01	0.01
Protein (mg/g Ext)	79.79^{a}	72.57^{c}	76.30^{b}	79.40^{a}	74.99^{bc}	72.71^{c}	0.76	0.01	0.01	0.01
Total iron (μg/g Ext)	54.12^{c}	48.42^{cd}	45.16^{de}	41.02^{e}	67.28^{b}	83.95^{a}	3.16	0.01	0.01	0.01

N = native, K = 4-lines cross breeds, and T = 5-lines cross breeds chickens; F = Female and M = Male. Effect = level of statistical differences by breeds, sexes and interaction between breeds and sexes: 0.01 = highly significant, 0.05 = significant, and ns = not significant differences. SEM = standard error of means. Means with different letters in the same row are significantly different ($p < 0.05$).

Table 4
Chemical composition of carnosine extracts (Ext) of thigh meats from different breeds (Bd) and gender (S) of chickens

Component	N		K		T		SEM	Effect		
	F	M	F	M	F	M		Bd	S	Bd*S
Carnosine (μg/g Ext)	9168.31[b]	7910.12[c]	10159.37[ab]	7872.40[c]	10766.02[a]	6387.66[d]	227.67	0.01	0.01	ns
Protein (mg/g Ext)	36.53[d]	46.55[b]	33.09[e]	40.31[c]	50.77[a]	41.27[c]	1.46	0.01	0.01	0.01
Total iron (μg/g Ext)	82.11[d]	96.40[c]	111.04[b]	115.20[b]	119.96[b]	150.86[a]	4.53	0.01	0.01	0.01

N = native, K = 4-lines cross breeds, and T = 5-lines cross breeds chickens; F = Female and M = Male. Effect = level of statistical differences by breeds, sexes and interaction between breeds and sexes: 0.01 = highly significant, 0.05 = significant, and ns = not significant differences. SEM = standard error of means. Means with different letter in the same row are significantly different ($p < 0.05$).

terfield, and Fausman (1995), and Gopalakrishnan et al. (1999). Comparison of antioxidant activities among the extracts is illustrated in Fig. 1. Antioxidant activities in term of amount of the Br-Ext and Th-Ext used (mg/ml) in oxidation system are presented in Figs. 1(A) and (C), respectively. Figs. 1(B) and (D) present antioxidant activities of the Br-Ext and Th-Ext in term of carnosine concentration in the extracts (mM), used in oxidation system, respectively.

Similar results of % inhibitions among Br-Exts and among Th-Exts, in term of amount of Ext used in the system, were observed that the higher amount of Ext used, the greater antioxidant activity obtained (Figs. 1(A) and (C)). On the other hand, when antioxidant activities compared in term of carnosine concentration presented in the extracts used in the system, different activities among Br-Exts and among Th-Exts (Figs. 1(B) and (D)) were found. When compared between Br-Exts and Th-Exts, the activities of the Th-Exts were greater than those of Br-Exts at the same levels of carnosine concentration presented in the system. In addition, the antioxidant activity was interpreted in term of amount of the Exts and carnosine concentration used in oxidation system that could reduce EC50 compared with pure carnosine as shown in Figs. 2 and 3. When compared in term of amount of the Ext used in the system, pure carnosine showed obviously greater antioxidant activity compared with the Br-Exts (Fig. 2(A)) and Th-Exts (Fig. 3(A)). The EC50 of pure carnosine was 3.54 mg/ml while EC50s of the Br-Exts and Th-Exts

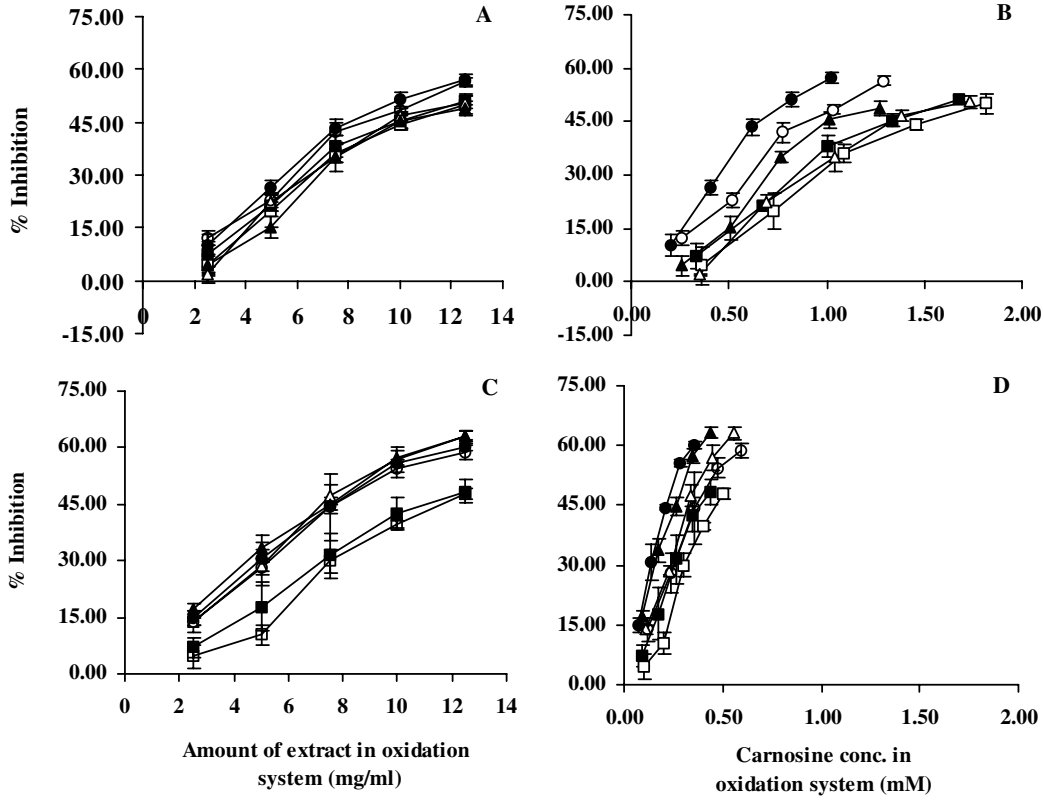

Fig. 1. Antioxidant activities of chicken meat extracts, expressed in term of % inhibition. (A) and (B) Systems contained breast extracts; (C) and (D) system contained thigh extracts; (A) and (C) comparison in term of amount of extract used; (B) and (D) comparison in term of carnosine concentration in oxidation system (□, NF; ■, NM; △, KF; ▲, KM; ○, TF; ●, TM).

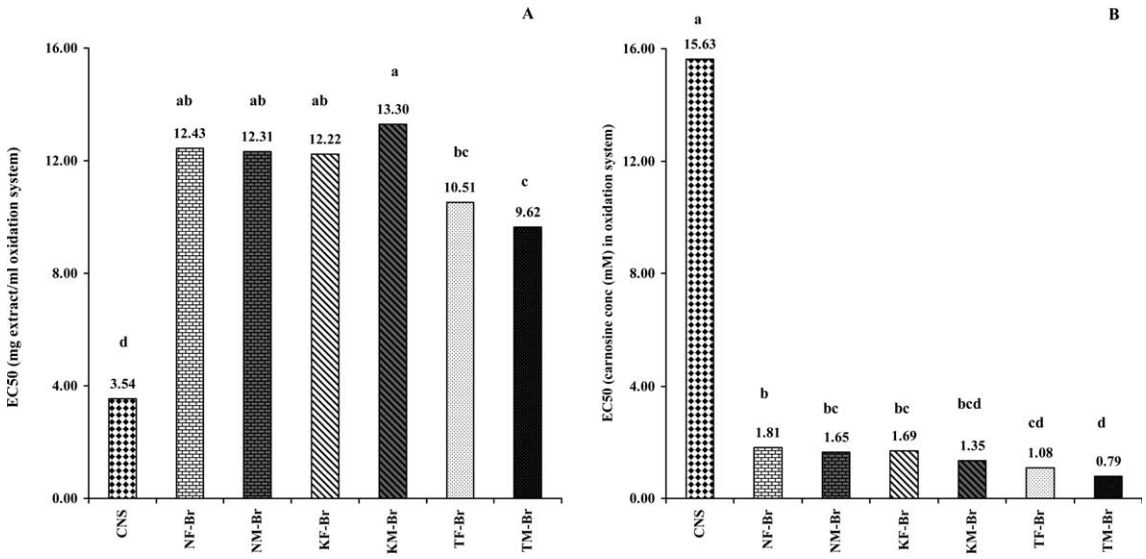

Fig. 2. EC50 of breast (Br)-extract or carnosine concentration in Br-extracts used in oxidation system. (A) Comparison of EC50s in term of amount of extract used. (B) Comparison of EC50s in term of carnosine concentration used in system. EC50s with different letters are significantly different ($p < 0.05$).

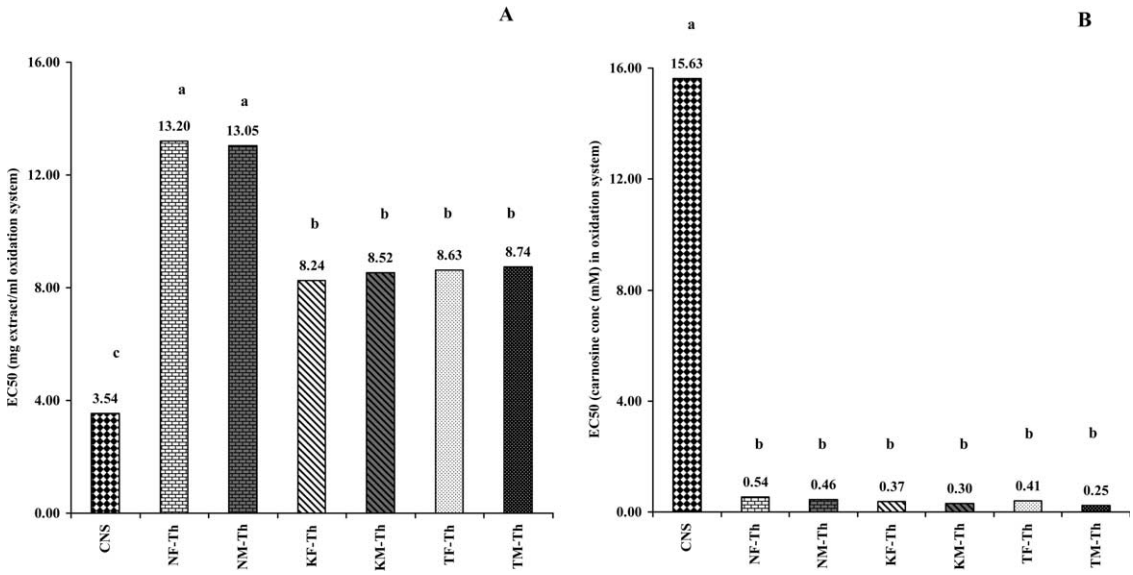

Fig. 3. EC50 of amount of thigh (Th)-extract or carnosine concentration in Th-extracts used in oxidation system. (A) Comparison of EC50s in term of amount of extract used. (B) Comparison of EC50s in term of carnosine concentration used in system. EC50s with different letters are significantly different ($p < 0.05$).

were 9.62–13.30 and 8.24–13.20 mg/ml, respectively. In contrast, pure carnosine showed definitely lower activity compared with the Br-Exts (Fig. 2(B)) and Th-Exts (Fig. 3(B)) when compared in term of carnosine concentration presented in oxidation system. The EC50 of pure carnosine was 15.63 mM whereas the EC50s of Br-Exts and Th-Exts were 0.79–1.81 and 0.25–0.54 mM, respectively. Higher efficiency of antioxidant activity of Exts than pure carnosine might be due to the presence of other antioxidants such as phosphates, and amino acids and low molecular weight peptides which released during heat treatment (Chan et al., 1993). In addition, there is some anserine in chicken muscle which play role similar to carnosine (Boldyrev, Dupin, Pindel, & Severin, 1988; Chan & Decker, 1994; Cornet & Bousset, 1999; Wu, Shiau, Chen, & Chiou, 2003). Thus, it would take part as a synergist in antioxidant activity of carnosine in the extracts.

Furthermore, Maillard reaction products (MRP) formed during heat treatment could add synergistic effect to the extracts as well. The MRP may have occurred by the reaction of amino acids and ribose from nucleic acids or other carbonyl compounds (Hultin, 1985; Pokorny', 1981). Eichner (1981) and Bailey and Um (1992)

also revealed that Maillard reaction intermediates (MRI) formed from Amadori compounds such as reductone-like compounds shown to be excellent antioxidants by inactivating radical-producing hydroperoxides and chelating metal ions such as copper and iron. Th-Ext is expected to contain more MRI products since red meat has higher pH than white meat (Davey, 1960). In addition, higher amount of myoglobin in thigh meat would give the Th-Ext upon heating with higher non-heme compounds and small protein fractions, such as histidine residue released from heme molecule, which provide better synergistic effects in the extracts. This evidence is agreed with the finding of Decker and Faraji (1990) who reported that inhibition of lipid oxidation of carnosine was highest in the system containing H_2O_2-hemoglobin comparing with system containing Fe-ascorbate, singlet oxygen and lipoxidase.

4. Conclusions

This study demonstrated that carnosine contents in fresh meat of both breast and thigh meats were significantly different among breeds and between genders of chickens ($p < 0.01$). Breast meats contained higher carnosine contents compared with thigh meats. Carnosine contents of female chicken meats were higher compared with those of male chicken meats. Breeds and gender were suggested to have influence on carnosine content. Similar results were obtained in their extracts. When compared with pure carnosine at the same concentration of carnosine (mM carnosine) presented in the extract in oxidation system, chicken extracts exhibited higher antioxidant activities ($p < 0.05$). Having high of other synergistic antioxidant compounds, the extracts of thigh meats gave higher inhibition efficiency compared with those of breast meats even though they contained lower amounts of carnosine.

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