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Heat and ultrafiltration extraction of broiler meat carnosine and its antioxidant activity

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Abstract

This study examined the effects of extraction and further ultrafiltration on the carnosine content, antioxidant activity and total iron content of chicken muscle extracts. Fresh breast meat had 7-fold higher carnosine than fresh thigh meat (2900 versus 419 μg/g meat, respectively). Carnosine extracts of breast and thigh were prepared by heating at 60, 80 and 100 °C, and ultrafiltration (UF) using a 5000 MW cut-off. At increasing temperatures, protein concentrations decreased while carnosine, total iron and antioxidant activity increased. Antioxidant abilities of the 80 and 100 °C-heated extracts were greater than that of the 60 °C extract ($p < 0.05$). The ultrafiltrate from the 80 °C-heated extract had approximately 20% higher carnosine, but 40% lower protein and 10–30% lower iron than the 80 °C-heated ultrafiltrate. However, compared in terms of carnosine concentration, the meat extracts had greater antioxidant activity than pure carnosine ($p < 0.05$).

Keywords: Broiler meats; Carnosine; Antioxidant; Heat extraction; Ultrafiltration

1. Introduction

Oxidative reactions have been implicated in the development of food deterioration affecting color, flavor, texture and nutritional values. The toxic substances formed by these reactions cause the development of numerous diseases including atherosclerosis and cancer (Decker, 1995; Kansci, Genot, Meynier, & Gandemer, 1997; Zhou & Decker, 1999). In recent years, there have been some interests in the antioxidant potential of carnosine in meats (Calvert & Decker, 1992; Decker & Hultin, 1992; Lee, Hendricks, & Cornforth, 1999; O'Neill, Galvin, Morrissey, & Buckley, 1999). Carnosine is a naturally occurring skeletal muscle dipeptide consisting of β-alanine and histidine. Carnosine shows the antioxidant activity by playing role as metal chelator and free-radical scavenger (Chan & Decker, 1994). In copper-catalyzed oxidation system, carnosine inhibits the oxidation by chelating copper, as shown by inhibiting the destruction of vitamin B_{12} in the presence of copper, and also by strongly inhibiting the peroxidation of copper-catalyzed oxidation in liposome (Lee & Hendricks, 1997; Takenaka, Sugiyama, Watanabe, Abe, & Tamura, 1997). But it only works as free radical scavenger in the iron-catalyzed oxidation system (Decker, Crum, & Calvert, 1992; Decker & Faraji, 1990). In biological system, carnosine is used in medical practices to prevent the oxidation which cause eye-cataract and aging. In addition, it has been shown to suppress the tumor growth in rat (Boissonneault et al., 1998; Jordan, 1999; Kyriazis, 1999). In previous experiments, carnosine was extracted from many skeletal muscle sources including beef and pork by using either heat treatment or ultrafiltration (Chan, Decker, & Means, 1993; Gopalakrishnan, Decker, & Means, 1999). The carnosine-containing extracts obtained were capable of inhibiting in vitro lipid oxidation and in meat products. However, the extracts

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contained high amount of pro-oxidants, such as free iron and iron-containing proteins.

Therefore, in order to obtain carnosine extract low in pro-oxidants, the muscle used should contain low amount of pro-oxidants and extraction procedures should be able to reduce more pro-oxidants while remaining in high carnosine. Chicken meat contains lower myoglobin content than beef and pork (Hazell, 1982), thus it should be a good alternate source of muscle for carnosine. The purpose of this study was to compare carnosine content and antioxidant activity of various carnosine extracts from chicken breast and thigh, as affected by hot water extraction at various temperatures and further purification by ultrafiltration to remove iron. The carnosine extracts were determined for carnosine, protein and total iron contents and their antioxidant activities were analyzed by TBARS analysis.

2. Materials and methods

2.1. Chemicals

Carnosine, *o*-phthaldehyde (OPA), soybean phosphatidylcholine, bovine serum albumin (BSA), β-mercaptoethanol (BME), 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

Broiler meats were purchased from Lotus Supercenter Store (Nakhon Ratchasima, Thailand). Breast and thigh meats were used for the experiment. The meats were ground using a domestic food chopper (Moulinex Moulinette, Model 327, Spain) then, vacuum packed in linear low density polyethylene (LLDPE) bag and stored at −20 °C until used.

2.3. Chemical composition analysis of fresh meats

Fresh breast and thigh meat samples were determined for moisture content according to AOAC (1997), protein content by Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) using BSA as a standard. Carnosine content was determined by HPLC (Cornet & Bousset, 1999; Gopalakrishnan et al., 1999), and total iron content by atomic absorption (AA) spectrophotometry (James, 1995).

2.4. Extraction of fresh meat for determining of total carnosine content

Meat extracts were prepared according to method of Aristoy and Toldra' (1991) with slight modification. The frozen minced meat was thawed in LLDPE bag under running tap water. 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 (2 min × 4) in the ice chamber. The homogenate was centrifuged at 10,000 × *g* by Sorvall RC-5C Plus (Dupont, Delaware, USA) at 4 °C for 20 min. The supernatant was filtered through Whatman #4 filter paper, and then, the filtrate was subjected to freeze–drying. Frozen (−45 °C) filtrate was freeze–dried for 72 h using HETO FD3 (Heto-Holten A/S, Allerod, Denmark). Subsequently, the dried filtrate was determined for total carnosine content present in fresh broiler meat.

2.5. Extraction of carnosine by heat treatment

Heat extraction of carnosine was performed following the procedure of Chan et al. (1993) and Gopalakrishnan et al. (1999) with slight modification. The frozen minced meat was thawed under running tap water. One part of minced meat was homogenized with 2 parts of precooled (4 °C) deionized distilled water in an AM-8 homogenizer in the ice chamber for 8 min. The homogenate was centrifuged at 20,000 × *g* for 30 min at 4 °C using Sorval RC-5C Plus, and then, the supernatant was filtered through Whatman #4 filter paper. The water-extracted filtrate was subjected to heat treatment at 60, 80 and 100 °C for 10 min in water bath and cooled in ice bath. The heated extract was centrifuged to remove precipitated proteins at 5000 × *g* for 20 min. Supernatant was filtered through Whatman #4 filter paper. The filtrate was freeze–dried using a HETO-FD3 for 72 h, and analyzed for carnosine, protein and total iron content and antioxidant activity. Temperature at which maximum carnosine content and low total iron content obtained was used to prepare the heated liquid extract prior to subsequently treated by ultrafiltration for further purification.

2.6. Ultrafiltration

The heated extract, prepared at selected temperature (80 °C), was subjected to ultrafiltration. The separation between low and high molecular weight fractions of the extract was obtained by passing liquid extracts through the 5000 molecular weight cutoff ultrafiltration membrane (PES) (Vivaflow 50, Vivascience, Sartorius AG, Goettingen, Germany). Permeate containing low molecular weight fraction was collected, freeze–dried and kept for further analyses.

2.7. Analysis of extract composition

Protein content. Protein contents of undried and dried extracts were determined by Lowry method (Lowry

et al., 1951) using BSA as a standard. The dried extracts (2.0–3.0 mg) were reconstituted with 1 ml phosphate buffer (5 mM, pH 7.0) then, further diluted for protein determination.

Total iron content. The total iron contents in fresh meats and dried extracts were measured by means of AA spectrophotometry using AAnalyst 100 (Perkin Elmer, Norwalk, CT, USA). Accurate weight of sample (3.0000 g for fresh meat and 0.2500 g for dried extract) 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 HCl was added to the crucible containing the ash, boiled for 5 min, transferred to a beaker, adjusted volume to about 40 ml and then, boiled for 10 min. The mixture was cooled, filtered through Whatman #1 filter paper into a 50-ml volumetric flask, made up to volume with deionized water, and 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 contents in fresh meats, undried and dried extracts were determined using HPLC, derivatizing the extracts by OPA working solution. The OPA working solution was prepared 24 h before use by dissolving 27 mg of OPA in 500 μl of absolute ethanol. 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 can be kept for several days (Antoine, Wei, Littell, & Marshall, 1999).

Sample preparation for HPLC analysis was based on the methods of Gopalakrishnan et al. (1999) and Cornet and Bousset (1999) with slight modification. For liquid extract, 2 ml of the extracts or 100 mg dried extract 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 $2000 \times g$ for 20 min by Labofuge 400R #8179 (Heraeus Instruments, Germany). The supernatant was filtered through 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 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 of 10–1000 μg/ml. Carnosine concentration was calculated by Chemstation Rev.A.09.03 (1417) (Agilent Technologies 1990–2002).

2.8. Evaluation of antioxidant activity

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

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 malonaldehyde (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 values. The pure carnosine or extract (Ext) 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 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 $4000 \times g$ for 15 min. The absorbance of supernatant was measured at 532 nm using UV/VIS 916 Spectrophotometer (GBC Scientific Equipment, Ltd., Victoria, Australia). Concentration of MDA in oxidation system was calculated from the MDA standard curve (0–7 g/ml). The percentage 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 antioxidant activity of extract was expressed as amount of freeze–dried (FD)-extract or the concentration of carnosine used in the system to obtain 50% inhibition of oxidation (EC50). The EC50 of extract was also compared with EC50 of pure carnosine.

2.9. Statistical analysis

All experiments were performed in 2 replicates. Each replicate was chemically analyzed in triplicate samples.

Statistical analysis was evaluated in completely randomized design (CRD) with statistical analysis system (SAS Institute, Inc., 1993). Analysis of variance (ANOVA) and comparison of means by Duncan's multiple range test (DMRT) were analyzed.

3. Results and discussion

3.1. Chemical composition of fresh meats

Carnosine and total iron content of fresh breast and thigh meats are presented in Table 1. The total iron content of thigh (17.2 μg Fe/g meat) was 1.3 fold higher compared with breast muscle (12.7 μg Fe/g meat) which agreed with Foegeding, Lanier, and Hultin (1996). Thigh meat has higher total iron content because it contains higher amount of myoglobin and darker (red) color than breast. The degree of redness is closely related to the amount of myoglobin and/or blood supply in those muscles. The darker muscle, the higher total iron content observed (Hazell, 1982; Pearson & Young, 1989). Carnosine content of the breast is approximately 5–7 times higher compared with that of thigh meat. This result agreed with the previous studies by Decker (1995) and Cornet and Bousset (1999). Chicken breast (white fiber) contains mainly fast-twitch glycolytic fiber (type IIB), while chicken thigh contains slow-twitch oxidative fiber (red fiber, type I) (Meyor, 2003). Type IIB fibers generate ATP by anaerobic fermentation of glucose to lactic acid due to limiting in capillary supply to deliver oxygen. Hence, it has a fast contraction and is easily fatigued resulting from accumulation of lactic acid. Type I fibers have good capillary supply for efficient gas exchange, and also high myoglobin. Thus, they are built for aerobic metabolism. They have a slow contraction and are highly resistant to fatigue (Illingworth, 2004).

One of the biological roles of carnosine is buffering. Carnosine acts as a buffer to provide pH-buffering capacity, by regulating pH which decreases due to lactic acid accumulation (Chan & Decker, 1994). This role of carnosine can explain why higher concentration was found in breast muscle where high levels of anaerobic metabolism are commonly occurrence. This is in good agreement with the study of Davey (1960) who reported that the aerobic capacity of muscle was inversely related to the dipeptide concentration measured as carnosine in muscle. Furthermore, Sewell, Harris, Marlin, and Dunnett (1992) found the strong positive correlation between carnosine content and type IIB fiber in the equine middle gluteal muscle. Their experiment showed that carnosine contributed as much as 46% to the buffering of H^+ produced by type IIB fibers, and interpreted the importance of this dipeptide in stabilizing the pH in anaerobic contraction.

3.2. Heat treatment

Composition of heated extracts. In comparison of undried (UD) – heated extracts within meat type, it was found that carnosine concentration increased with increasing temperature in the thigh, but not breast meat (Tables 2 and 3). Protein content of both breast and thigh extracts decreased with increasing temperatures. UD-80C and UD-100C of both breast and thigh groups had similar protein contents which were less than UD-60C ($p < 0.05$). Heating of extracts at 60, 80 and 100 °C could reduce protein approximately 58–60%, 87–90% and 88–92%, respectively, compared to the untreated extract. Freeze–dried–heated (FD) extract had similar trend to its UD extract, i.e. carnosine content increased with increasing temperature, but protein content decreased. Carnosine contents and protein contents of the FD-80C and FD-100C were similar. This result conformed to the previous study by Gopalakrishnan et al. (1999) who extracted carnosine from mechanically separated pork by varying temperatures at 60, 70 and 80 °C. They found that protein content in UD-extracts were significantly decreased ($p < 0.05$) with increasing temperatures.

Heat treatment for removing proteins from untreated extracts may cause protein denaturation by aggregation. An aggregation caused by folding of globular proteins lead to solubility reduction and then, can be precipitated by centrifugation (Belitz & Grosch, 1999). Although, the protein concentration of the UD-extracts significantly decreased ($p < 0.05$), there was no significant difference ($p > 0.05$) among carnosine contents of breast meat extracts. This could be due to loss of carnosine during concentration. This result was in agreement with Chan et al. (1993) in extraction of carnosine from beef muscle. They suggested that lack of complete recovery of carnosine might be due to the destruction of carnosine or

Table 1
Chemical compositions of fresh broiler meats

Sample	% Protein (wet basis)	% Moisture (wet basis)	Total Fe (μg/g meat)		Carnosine (μg/g meat)	
			Wet basis	Dry basis	Wet basis	Dry basis
Breast	22.1 ± 1.9	75.2 ± 0.2	12.7 ± 1.0	51.4 ± 4.1	718.7 ± 54.0	2900.1 ± 217.7
Thigh	18.7 ± 0.7	75.2 ± 0.1	17.2 ± 2.1	69.2 ± 8.6	104.2 ± 15.3	419.4 ± 61.4

$n = 6$.

Table 2
Composition of undried (UD) and freeze–dried (FD) broiler breast extracts which had been subjected to heat treatment

Treatments	% Yield (g/100 g meat)	Carnosine		Protein		Total iron
		UD (μg/ml UD)	FD (μg/g FD)	UD (mg/ml UD)	FD (mg/g FD)	FD (μg/g FD)
Br-unheated	ND	845.1 ± 13.7[a]	ND	21.9 ± 1.6[a]	ND	ND
Br-60C	3.4 ± 0.1[a]	857.7 ± 15.6[a]	33,200.8 ± 683.8[b]	8.7 ± 0.2[b]	415.3 ± 12.9[a]	98.0 ± 6.3[b]
Br-80C	2.5 ± 0.3[b]	874.5 ± 76.9[a]	38,434.0 ± 3348.7[a]	2.0 ± 0.0[c]	140.6 ± 10.9[b]	109.7 ± 4.0[a]
Br-100C	2.6 ± 0.3[b]	884.7 ± 52.7[a]	40,380.1 ± 3123.5[a]	1.8 ± 0.0[c]	121.8 ± 1.9[c]	113.8 ± 3.8[a]

Br = breast; 60C, 80C, and 100C represent the samples which had been extracted by heating to 60, 80 or 100 °C for 10 min, respectively. Numbers with different letters within the same column are significantly different ($p < 0.05$); ND = not determined.

Table 3
Composition of undried (UD) and freeze–dried (FD) broiler thigh extracts which had been subjected to heat treatment

Treatments	% Yield (g/100 g meat)	Carnosine		Protein		Total iron
		UD (μg/ml UD)	FD (μg/g FD)	UD (mg/ml UD)	FD (mg/g FD)	FD (μg/g FD)
Th-unheated	ND	261.9 ± 8.4[b]	ND	18.9 ± 0.9[a]	ND	ND
Th-60C	2.25 ± 0.2[a]	268.3 ± 0.2[b]	12,918.6 ± 942.2[a]	7.9 ± 0.2[b]	437.5 ± 19.6[a]	200.8 ± 11.4[b]
Th-80C	1.60 ± 0.1[b]	280.7 ± 6.7[a,b]	13,899.7 ± 1154.5[a]	2.3 ± 0.1[c]	159.6 ± 18.3[b]	215.7 ± 5.5[a]
Th-100C	1.70 ± 0.1[b]	297.6 ± 9.8[a]	14,104.5 ± 1257.4[a]	2.2 ± 0.1[c]	147.2 ± 7.4[b]	220.9 ± 7.6[a]

Th = thigh; 60C, 80C, and 100C represent the samples which had been extracted by heating to 60, 80 or 100 °C for 10 min, respectively. Numbers with different letters within the same column are significantly different ($p < 0.05$); ND = not determined.

association of carnosine with precipitated proteins during processing.

Total iron contents of meat extracts increased with increasing temperatures (Tables 2 and 3). There was no significant difference between FD-80C and FD-100C ($p > 0.05$) but these extracts were significantly different from the FD-60C ($p < 0.05$). The total iron content of the FD-Br-60C, FD-Br-80C, and FD-Br-100C were 98.0, 109.6, and 113.6 μg/g FD extract, respectively. The total iron content of the FD-Th-60C, FD-Th-80C, and FD-Th-100C were 200.8, 215.3, and 221.0 μg/g FD extract, respectively. If the total iron content was calculated in term of iron content in the FD extract/g of fresh meat, the data would show the decrease of total iron content when heating temperature was increased. The total iron contents of the FD-Br-60C, FD-Br-80C, and FD-Br-100C were 3.3, 2.7 and 3.0 μg/g meat, respectively. The total iron content of the FD-Th-60C, FD-Th-80C and FD-Th-100C was 4.5, 3.5, and 3.8 μg/g meat, respectively (data not shown). The total iron contents, in term of μg Fe/g meat, were in agreement with Chan et al. (1993). They found that heating extracts of beef muscle could remove iron-containing protein. That means after heating the extract, the iron-containing proteins were denatured, aggregated and removed by centrifugation. Thus, the higher temperature, the less total iron content would be observed.

On the other hand, if total iron contents were compared in term of μg Fe/g of FD-extract, the result would not agree with the above suggestion. It was found that total iron in the dried extract increased with decreasing temperature of heat treatment. Chan et al. (1993) reported this finding in dry-heated extract. Although, the total iron content decreased with increasing temperatures of extraction, the low-molecular-weight (LMW) iron would increase 2.6–2.9 folds compared with the untreated extract. This could be concluded from the previous reports by Igene, King, Pearson, and Gray (1979) Chen, Pearson, Gray, Fooladi, and Ku (1984) that heating beef muscle extract at 63–70 °C released protein-bound iron from the samples. Therefore, in our study, it can be concluded that the LMW iron should be the main component of total iron content in chicken extracts. However, in beef extracts, hemin is the main source of iron (Chan et al., 1993). Beef muscle contains about 89% myoglobin in total iron but chicken muscle has only 12% myoglobin. Myoglobin is a high-MW iron compound, with MW at least 17,000 Da. But chicken meat has higher amount of LMW-iron (<12,000 Da) than beef (Hazell, 1982). Hence, in our study, it can be suggested that most of iron in heated chicken meat extract should be LMW iron compounds since higher heating temperature increased the releasing of LMW-iron. Total iron contents (g/g FD extract) were in the order of Br-100C > Br-80C > Br-60C and Th-100C > Th-80C > Th-60C, for breast extracts and thigh extracts, respectively.

According to the results of carnosine, total iron and protein contents obtained above, heating at 80 °C for 10 min for preparation of the extract was used for further purification by ultrafiltration.

Antioxidant activity of heated extracts. From TBARS determination, antioxidant activity of the extracts was expressed in percentage of inhibition calculated from the reduction of MDA in oxidation system. The result showed that antioxidant activity increased with increas-

ing amount of FD-heated extract in the system for both breast and thigh extract groups (Fig. 1). This agreed with the previous studies of Decker et al. (1992), Chan et al. (1993), Decker, Chan, Livisay, Butterfield, and Fausman (1995) and Gopalakrishnan et al. (1999). The antioxidant activities of the broiler meat extracts were remarkably lower compared with pure carnosine (Fig. 2). As shown in Table 4, amount of pure carnosine between 7.5 and 12.5 mg/ml in oxidation system produced constant antioxidative activity at approximately 92% inhibition, whereas the activities of all heated extracts were from 39% to 77% inhibition. The final percentages of inhibition (at 12.5 mg Ext/ml) of the heated extracts were approximately 51–78% inhibition.

Comparisons of antioxidant activities among heated extracts are demonstrated in Fig. 1. Fig. 1A and C illustrated antioxidant activities of heated breast and thigh extracts in mg/ml oxidation system, respectively. The FD-80C and FD-100C extracts showed similar antioxidant activities which were greater compared with that of the FD-60C. In addition, Fig. 1B and D showed antioxidant activities of breast and thigh extracts in term of carnosine concentrations (mM) in the extracts used in oxidation system, respectively. Similar trend of % inhibitions was obtained as shown in term of amount of extract used in the system. For breast and thigh extracts, % inhibition of oxidation increased with increasing amount of the extract used.

In addition, the antioxidant activity was also interpreted as concentration of carnosine or amount of extract in oxidation system that could reduce 50% of oxidation (EC50) compared with pure carnosine (Fig. 2). Fig. 2A and B illustrated the EC50 of heated extracts from breast meat in term of amount of extract and in term of carnosine concentration in oxidation system, respectively. Similar pattern of comparison among heated extracts from thigh meat were shown in Fig. 2C and D, respectively. The result showed that there was no significant difference in EC50 between 80C and 100C extracts ($p > 0.05$). Considering the EC50 in of term of amount of extract used (mg/ml) in the oxidation system (Fig. 2A and C), pure carnosine had the greatest antioxidant activity with EC50 of 3.5 mg/ml. There was no significant difference in EC50s between the FD-80C and FD-100C extracts ($p > 0.05$), which were lower compared with that of the FD-60C extract ($p < 0.05$). When EC50s were compared in term of carnosine concentration present in the extracts used in oxidation system (Fig. 2B and D), EC50s of the extracts were much lower compared with that of pure carnosine. The EC50 of pure carnosine was 15.45 mM, whereas Br-heated extracts were 1.40–1.75 mM and

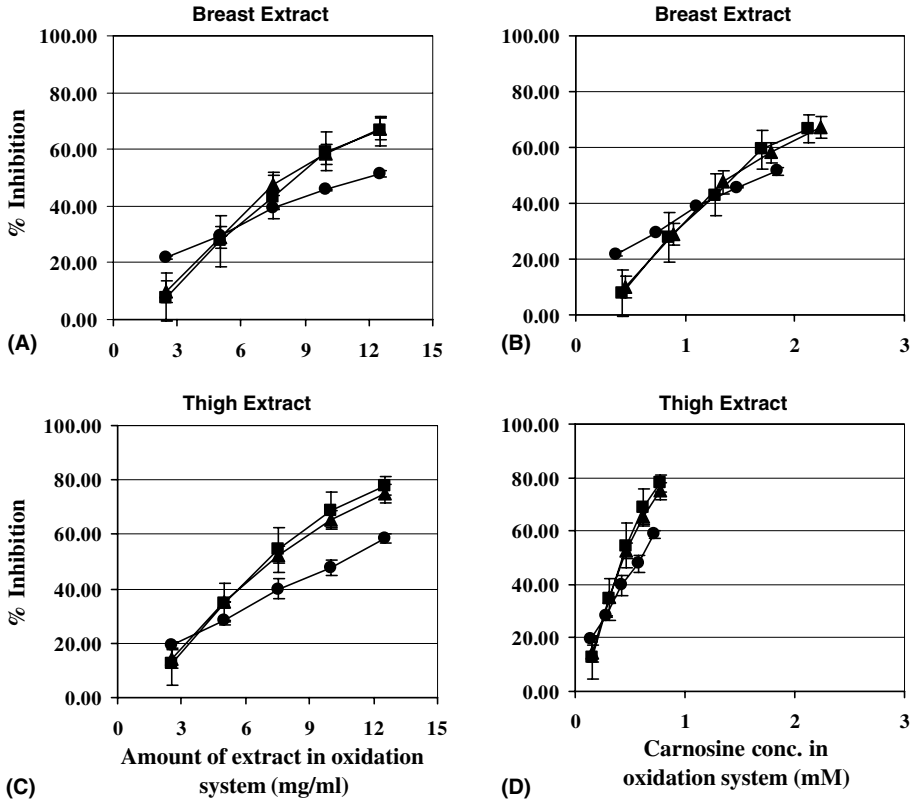

Fig. 1. Antioxidant activities of chicken meat extracts, expressed in term of % inhibition. A and B = systems contained breast extracts; C and D = system contained thigh extracts; A and C = comparison in term of amount of extract used; B and D = comparison in term of carnosine concentration in oxidation system. (● = 60C, ■ = 80C, ▲ = 100C).

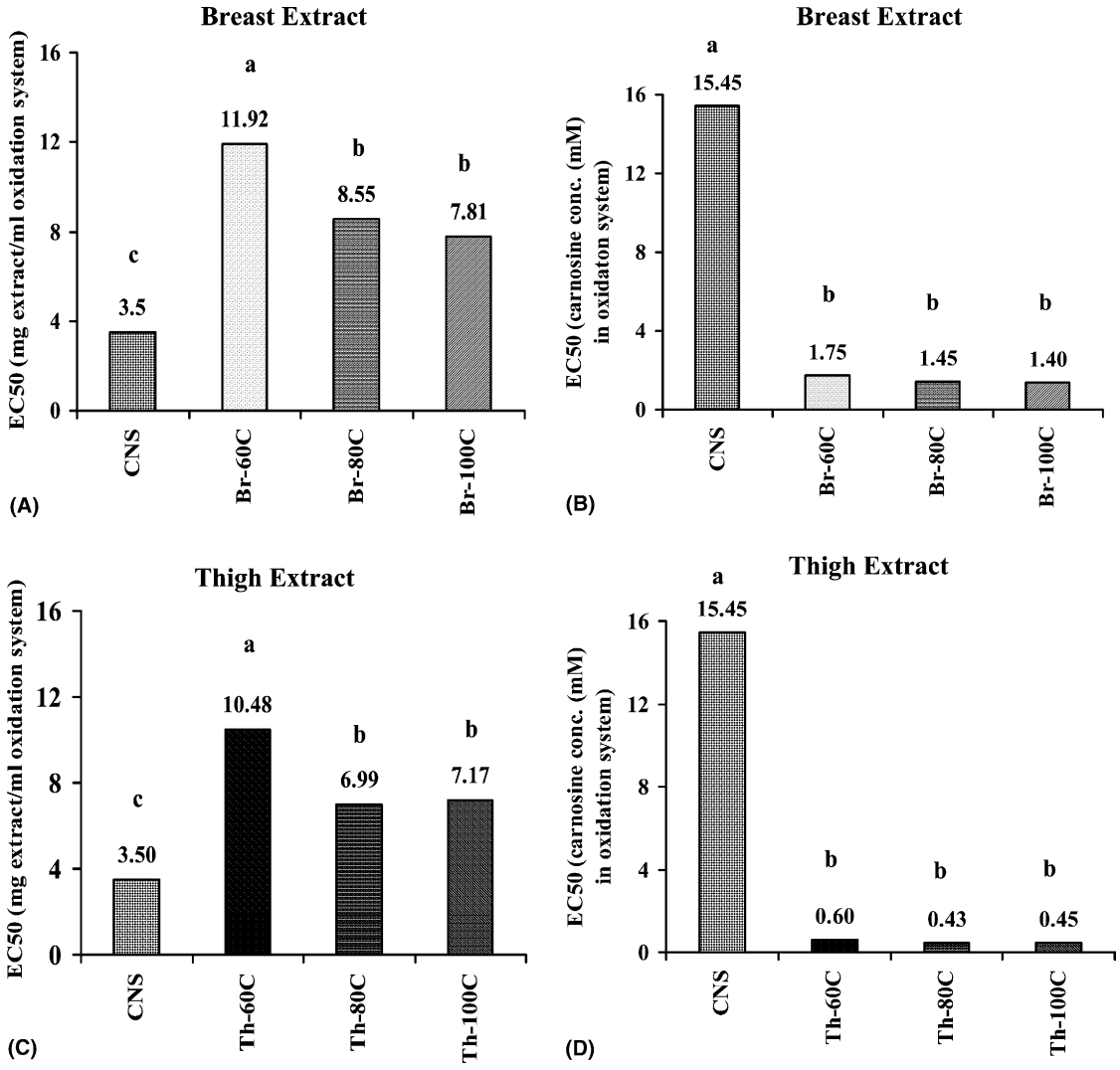

Fig. 2. Comparisons of EC50s among heated extracts and pure carnosine. A and B = systems contained breast extracts (Br-); C and D = system contained thigh extracts (Th-); A and C = comparison in term of amount of heated extract used; B and D = comparison in term of carnosine concentration in oxidation system. EC50s with different letters are significantly different ($p < 0.05$). (EC50 = amount of extract or carnosine concentration to give 50% inhibition of oxidation, CNS = pure carnosine, 60C, 80C and 100C = extracted at 60, 80 and 100 °C, respectively).

Th-heated extracts 0.43–0.60 mM of oxidation system. The antioxidant activities of the heated extracts increased with increasing temperatures of extraction, in the order of 100C $\geqslant$ 80C > 60C. It is obvious that pure carnosine had lower efficiency to reduce 50% of oxidation when compared with heated extracts ($p < 0.01$). Higher efficiency of antioxidant activity of the extracts might be due to the presence of other antioxidants such as phosphates, free amino acids (glycine and alanine) and low molecular weight peptides (anserine and glutathione) which released during heat treatment (Chan et al., 1993; Decker, Livisay, & Zhou, 2000; Wu, Shiau, Chen, & Chiou, 2003). In addition, there are some endogenous antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase in chicken muscle which play role similar to carnosine (Boldyrev, Dupin, Pindel, & Severin, 1988; Chan & Decker, 1994; Cornet & Bousset, 1999; Decker et al., 2000; Wu et al., 2003). These compounds would take part as synergists in antioxidant activity of carnosine in the extracts. However, heat extraction of meat to 80 °C results in complete inactivation of catalase and glutathione peroxidase, but does not inactivate superoxide dismutase (Gopalakrishnan et al., 1999). Therefore, the greater antioxidant activity of the heated extracts was likely due to nonenzymic antioxidants and/or superoxide dismutase.

Controversy was found between total iron content and antioxidant activity in both meats since the iron containing compounds act as oxidation catalysts. At higher extraction temperatures, higher amounts of iron were extracted from the meat (Tables 2 and 3). However, heating at higher temperatures could be more effective as well to remove other pro-oxidants, such as

Table 4
The ability of inhibiting oxidation by freeze–dried–heated extracts (FD-Ext) from broiler meat compared with pure carnosine

Amount of extract or pure carnosine (mg FD-Ext/ml oxidation system)	% Inhibition						
	Pure cns	Br-60C	Br-80C	Br-100C	Th-60C	Th-80C	Th-100C
2.5	28.1 ± 4.9	21.9 ± 0.7	7.9 ± 8.3	10.1 ± 3.8	19.4 ± 0.9	12.7 ± 8.0	14.2 ± 3.3
5.0	83.1 ± 5.0	29.5 ± 0.2	27.7 ± 9.0	28.9 ± 4.0	28.2 ± 0.3	34.5 ± 7.8	35.4 ± 0.1
7.5	90.7 ± 4.5	39.1 ± 0.5	43.0 ± 7.7	47.6 ± 4.2	39.8 ± 3.7	54.4 ± 8.3	52.5 ± 3.0
10.0	92.0 ± 4.4	45.8 ± 0.3	59.2 ± 7.0	58.3 ± 3.7	47.7 ± 3.1	69.0 ± 6.8	65.4 ± 3.3
12.5	91.7 ± 4.1	51.4 ± 3.3	66.6 ± 5.2	67.2 ± 4.0	58.7 ± 1.6	77.8 ± 3.3	74.9 ± 3.2

cns = carnosine; Br = breast and Th = thigh; 60C, 80C, and 100C extracts extracted by heating to 60, 80 and 100 °C for 10 min, respectively. % Inhibition was calculated as follow:

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

phospholipid and heme protein, and to lower protein contents. In addition, there might be other LMW antioxidants such as phosphates and amino acids which might be concentrated more with increasing temperatures of extraction (Chan et al., 1993). In addition, anserine (β-alanyl-l-1-methyl histidine) in skeletal muscle of vertebrates plays a biological role similar to carnosine (Boldyrev et al., 1988; Chan & Decker, 1994; Cornet & Bousset, 1999). Anserine shows antioxidant activities in muscle and brain (Kohen, Yamamoto, Cundy, & Ames, 1988) and in vitro oxidation systems (Wu et al., 2003). This dipeptide would be concentrated by heat treatment and contribute to antioxidant activity of carnosine in this study. In addition, carnosine contents in the 80C and 100C extracts were higher compared with the amount in the 60C extracts. Thus, these compounds could act as synergist in antioxidant activity of carnosine in the extracts. This suggestion might also support the EC50 of pure carnosine of which was higher value (lower antioxidant activity) compared with those of the 80C and 100C extracts, considering in term of carnosine concentration used in oxidation system (Fig. 2B and D).

3.3. Heated-ultrafiltration extraction

The UD-80C extract was subjected to ultrafiltration (UF) in attempt to remove LMW iron containing compounds, and get purer carnosine in UF permeate.

Composition of ultrafiltration permeate (UFP). Carnosine contents in UFP of both breast and thigh were obviously higher compared with the amount in heated extracts. It was significantly different ($p < 0.05$) (Table 5) with 47,816.9 and 18,206.3 μg/g FD extracts for Br-UFP and Th-UFP, respectively. Carnosine contents increased about 20%, and protein contents decreased about 40% for both UD- and FD-UFP compared with the 80C extract. Total iron contents of the Br-UFP and Th-UFP decreased 10% and 40%, respectively, compared with 80C extracts. These results showed good agreement with the previous study of Chan et al. (1993) and Gopalakrishnan et al. (1999) in beef and pork. Their studies showed higher removal of LMW iron compounds and other proteins by UF than did in this experiment. This may due to the naturally higher content of LMW iron compounds (less than 5000 Da) in broiler meat than in beef and pork.

Antioxidant activity of ultrafiltration permeate (UFP). As similar trend to the heated extracts, antioxidant activity of UFP increased with increasing concentration of carnosine or amount of extract in oxidation system (Fig. 3). The UFP showed less ability to inhibit oxidation compared with the 80C extract for both breast and thigh groups. The EC50 of UFP was higher compared with that of the 80C extract ($p < 0.05$) of both breast and thigh meat extracts (Fig. 4). When compared in term of carnosine concentration (mM) in the system, the EC50s of Br-UFP and Th-UFP were 2.25 and 0.67 mM of the

Table 5
Composition of undried (UD), freeze–dried (FD) of 80C-heated extracts and permeate of ultrafiltration (UFP) from breast and thigh meats

Treatment	% Yield (g/100 g meat)	Carnosine		Protein		Total iron
		UD (μg/ml UD)	FD (μg/g FD)	UD (mg/ml UD)	FD (mg/g FD)	FD (μg/g FD)
Breast extracts						
Br-80C	2.5 ± 0.3[a]	874.5 ± 76.9[a]	38,434.0 ± 3348.7[b]	2.0 ± 0.0[a]	140.6 ± 10.9[a]	109.7 ± 4.0[a]
Br-UFP	1.7 ± 0.1[b]	1105.9 ± 21.1[a,b]	47,816.9 ± 2823.7[a]	1.3 ± 0.0[b]	73.6 ± 5.4[b]	98.3 ± 1.5[b]
Thigh extracts						
Th-80C	1.6 ± 0.1[a]	280.7 ± 6.7[b]	13,899.7 ± 1257.4[b]	2.3 ± 0.1[a]	159.6 ± 18.3[a]	215.7 ± 5.5[a]
Th-UFP	1.0 ± 0.1[b]	367.6 ± 28.9[a]	18,206.3 ± 1260.0[a]	1.4 ± 0.0[b]	82.7 ± 9.1[b]	114.2 ± 4.7[b]

Br = breast: Th = thigh extracted; Numbers with different letters within the same column of each meat group are significantly different ($p < 0.05$).

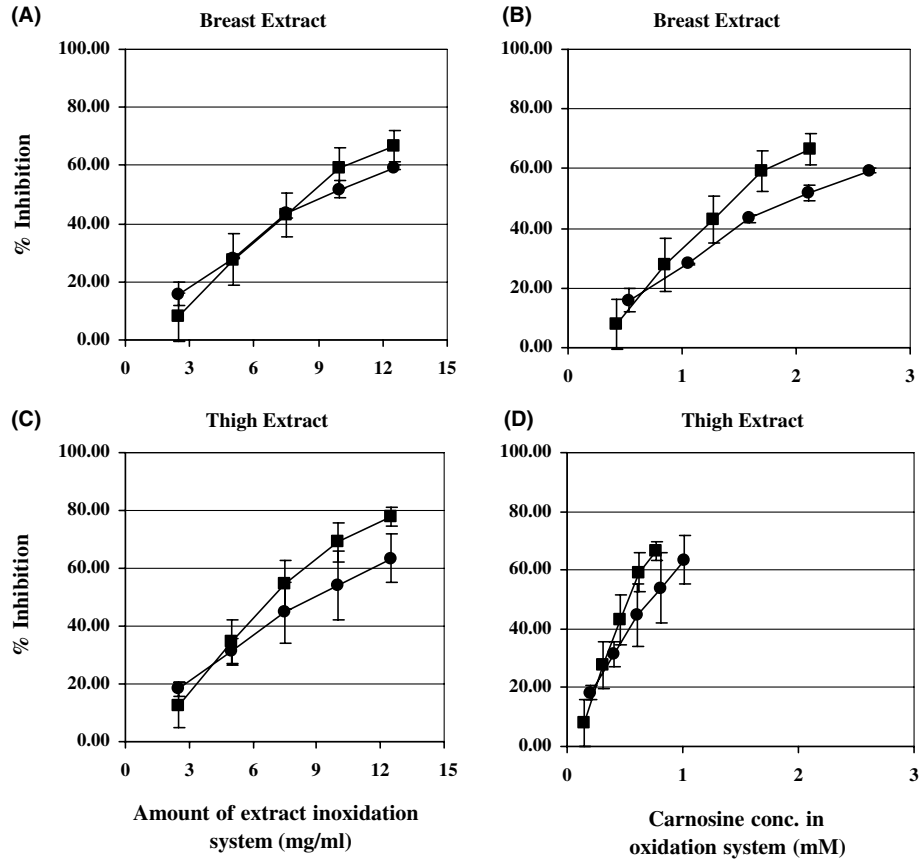

Fig. 3. Antioxidant activities of 80C-extracts and ultrafiltration permeate (UFP), expressed in term of % inhibition. A and B = systems contained breast extracts; C and D = system contained thigh extracts; A and C = comparison in term of amount of extract used; B and D = comparison in term of carnosine concentration in oxidation system. (■ = 80C, ● = UFP).

system, respectively. While the EC50s of Br-80C and Th-80C extracts was 1.45 and 0.45 mM, respectively (Fig. 4B and D). Therefore, the UFP had less antioxidant activity compared with that of 80C extract, even though the UFP had higher carnosine and lower total iron content than did the 80C extract. On the contrary, when compared in term of the amount of extract used (Fig. 4A and C), pure carnosine had the greatest antioxidant activity per unit weight. This evidence was similar to the comparison of EC50 among the heated extracts (Fig. 2). The lower activity of the UFP could be due to the loss of other synergistic antioxidant compounds in the remaining retentate of the UF membrane. Consequently, the ratio of antioxidant compounds (including carnosine) to other LMW pro-oxidants (including LMW iron compounds) could be decreased. Thus, there could be only carnosine or carnosine with small amount of other antioxidant compound in the UFP resulting in lower antioxidant activity of the UFP when compared with that of the 85C extract. However, the presence of other LMW antioxidant molecules in UFP caused greater antioxidant activity (Fig. 4B and D) compared with pure carnosine when compared in term of carnosine concentration presented in oxidation system.

4. Conclusions

Chicken breast had 7-fold higher carnosine content compared with thigh meat. In heat treatment extraction, increasing temperatures resulted in increased carnosine concentration and total iron content, and decreased protein content of the heated extracts. There were no significant differences in the amount of each component needed to give 50% inhibition of oxidation between 80C and 100C extracts, but their contents were different from that of the 60C extract ($p < 0.05$). After the 80C extract was passed through UF, carnosine content in the UFP increased. In addition, the UF was effective to remove LMW iron compounds. The antioxidant activities of all the extracts increased with higher amounts of extract in the oxidation system. Comparing on the weight basis among pure carnosine and the chicken extracts, pure carnosine showed higher antioxidant activity, i.e. the EC50 of pure carnosine in mg/ml oxidation system was lower compared with mg of chicken extract/ml in oxidation system ($p < 0.05$). However, when considered concentration of carnosine (mM) presented in the oxidation system, it was obvious that the chicken extracts had greater antioxidant activity compared with

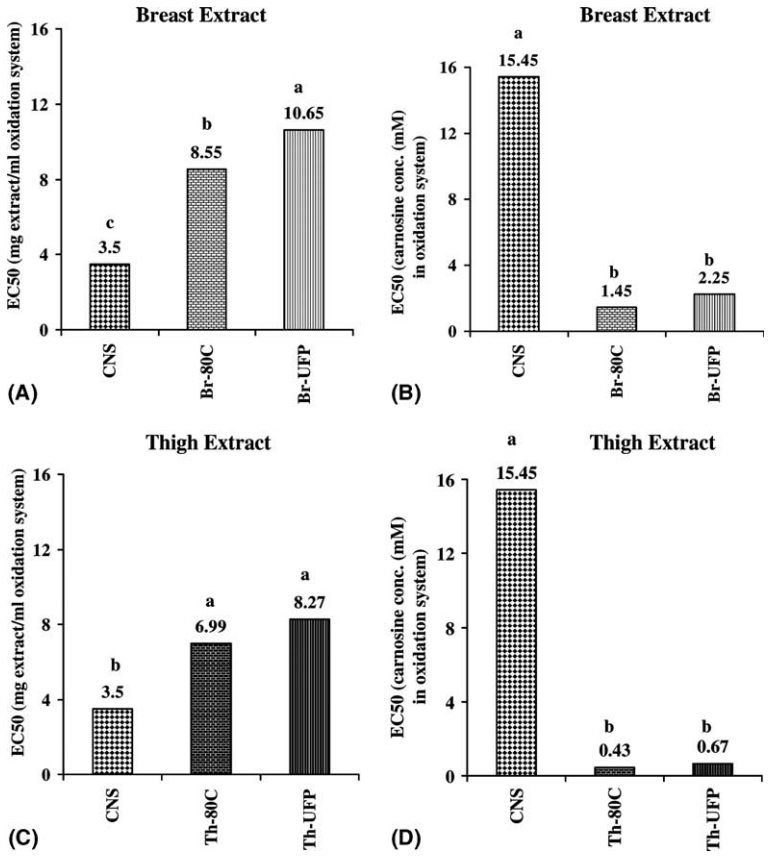

Fig. 4. Comparisons of EC50s among 80C-extract, ultrafiltration permeate (UFP) and pure carnosine used in oxidation system. A and B = systems contained breast extracts (Br-); C and D = system contained thigh extracts (Th-); A and C = comparison in term of amount of extract used; B and D = comparison in term of carnosine concentration in oxidation system. EC50s with different letters are significantly different ($p < 0.05$). (EC50 = amount of extract or carnosine concentration to give 50% inhibition of oxidation, CNS = pure carnosine and 80C extracted at 80 °C, respectively).

pure carnosine, i.e. the EC50s (calculated as mM carnosine in oxidation system) of the chicken extracts were lower ($p < 0.05$).

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