CONCENTRATIONS OF ANSERINE AND CARNOSINE IN POULTRY MEAT EXTRACTS TREATED WITH DEMINERALIZATION AND PAPAIN

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Background.

The dipeptides, carnosine (β-alanyl-methyl histidine), and anserine (β-alanyl-L-1-methyl histidine) are found in the skeletal muscle tissue of most vertebrates. Concentrations of carnosine and anserine found in skeletal muscle are capable of inhibiting iron-catalyzed lipid oxidation (Decker and Faraji, 1990). Chan *et al.* (1993) found the beef extract with high carnosine and low proxidants could have potential as a natural antioxidant. Lai and Kuo (1999) found that salted ground pork added with demineralized chicken breast meat extracts had lower TBA values than the control. The antioxidative activity of demineralized chicken breast meat extracts was superior to undemineralized treatments.

Tinbergen and Slump (1976) reported that chicken meat could be identified in luncheon meats by an analysis of anserine and carnosine. Carnegie *et al.* (1982) reported that carnosine/anserine ratios were highly specific in meat and could be useful in estimation of meat species in processsed meat products. Olsman and Slump (1981) also reported that the histidine dipeptides could be useful for identification of species incorported into meat products.

Objective.

The purposes of this study were to produce a natural antioxidant from poultry (chicken, duck and turkey) meat extracts using a demineralization technique and a proteolytic enzyme (papain). The carnosine/anserine ratios of poultry meat extracts were also investigated which probably could be used to identify the meat species in meat products.

Methods.

The method of Knecht and Chang (1986) was used to determine carnosine and anserine concentrations in chicken breast meat extracts. Concentrations of carnosine and anserine were calculated by using 0-600 ppm of pure carnosine (Sigma, U.S.A.) or anserine (Sigma, U.S.A.) as a standard.

The method of Schricker *et al.* (1982) was used to determine non-heme iron. The procedure involved using 2-5 g samples of ground meat which were incubated in the presence of 15 ml of an acid mixture containing equal volumes of 6 N HCl and 40% trichloracetic acid at 65 °C for 20 hr in a loosely stopper 50 ml centrifuge tube. After incubating, transferreded 1 ml of solution from the mixture (room temperature) and added with 5 ml of the color reagent [including 20 parts distilled and deionized water, 20 parts saturated sodium acetate, and one part sulfonated bathophenanthroline reagent (0.162 g of sulfonated bathophenanthroline per 100 ml of distilled water plus 1 ml of thioglycolic acid)]. After 10 min later the solution was measured against a reagent blank at 540 nm (Spectronic Genesys 5, Milton Roy, U.S.A.).

Total iron concentration (CNS 12638-N6227, 1989) of the samples was measured (248.3 nm) by atomic absorption spectrophotometry (Shimadzu Z-8000, Japan). Heme iron was calculated by subtracting the non-heme iron from the total iron.

Results and Discussion.

In this study, it was found that undemineralized breast meat (white muscle) extracts of chicken, duck and turkey contained higher ($p<0.05$) concentrations of anserine and carnosine than thigh meat (dark muscle) extracts (Table 1). Crush (1970) reported that white muscle fibers generally had a higher concentration of anserine and carnosine than red muscle fibers. Carnegie *et al.* (1982) proposed the ratios of carnosine/anserine could be used for estimation of the meat species in processed meat products. They found that the carnosine/anserine ratios appeared to be highly specific in fresh meat. Tinbergen and Slump (1976) also reported that chicken meat could be identified in processed luncheon meats by analyzing carnosine and anserine, and these histidine dipeptides were useful for identification of species used in meat products. In Table 1, the ratios of carnosine/anserine of chicken breast and thigh meat extracts were 0.62 and 0.80, respectively; the ratio was slightly higher in thigh meat extracts. The ratios of carnosine/anserine of duck breast and thigh meat extracts were between 0.75-0.77; and the ratios of carnosine/anserine of turkey breast and thigh meat extracts are 0.15 and 0.16, respectively.

Demineralized (Table 2) poultry meat extracts (chicken, duck and turkey) had lower ($p<0.05$) concentrations of anserine and carnosine than undemineralized (Table 1) poultry meat extracts. The demineralization process decreased non-heme iron by 22-59%, 15-32% and 21-22%, heme iron by 28-90%, 50-69% and 40-50%, and total iron by 47-70%, 45-60% and 32-37% in chicken, duck and turkey meat extracts, respectively (Table 1 and Table 2). However, demineralization also removed 6-44% of the anserine and 4-36% of the carnosine in chicken, duck or turkey meat extracts (breast and thigh meat).

The ratios of carnosine/anserine of demineralized chicken, duck and turkey meat extracts were 0.71-0.80, 0.73-0.75 and 0.15-0.16, respectively (Table 2). In this study, it was found that the ratios of carnosine/anserine were very specific in chicken, duck and turkey meat extracts (breast and thigh); and the differences in carnosine/anserine ratios between undemineralized and demineralized poultry meat extracts (chicken, duck and turkey) were not significant. This research suggested that carnosine/anserine ratios in both undemineralized and demineralized poultry meat extracts especially turkey meat extracts, could be used to estimate the meat species in uncooked or cooked meat products.

The concentrations of anserine and carnosine in chicken breast meat extracts treated with papain for 30-120 min were higher than those in the controls (no papain added). Due to papain activity, more myofibrillar proteins of chicken breast meat were degraded and higher concentrations of anserine and carnosine were extracted from chicken breast muscle. However, the concentrations of anserine and carnosine were not significantly different as the reaction time for papain increased. Chicken breast meat extracts treated with papain contained higher concentrations of total, heme and non-heme iron than the controls (Table 3). This is probably due to degradation of chicken myofibrillar protein by papain, and more free iron (non-heme iron) and heme-containing compounds (hemin, metmyoglobin, methemoglobin) were released from the muscles, resulting in higher concentrations of total, heme and non-heme iron in chicken breast meat extracts. As the reaction time for papain increased (30, 60, 90 and 120 min.), the concentrations of total, heme and non-heme iron in demineralized chicken breast meat extracts increased. The carnosine/anserine ratios decreased from 0.71 to 0.35-0.37

in demineralized chicken breast extracts treated with papain (Table 3). The concentrations of both anserine and carnosine increased in meat extracts treated with papain. However, the percent increase of anserine was larger than that of carnosine; therefore, the carnosine/anserine ratios decreased.

Conclusions.

Undemineralized poutry meat extracts contained a larger amount of anserine, carnosine, heme and non-heme iron than demineralized poultry meat extracts. Concentrations of anserine, carnosine, heme and non-heme iron in chicken breast meat extracts increased with the addition of 1% of papain to the meat mixture before extraction. The ratios of carnosine/anserine were very specific in the chicken, duck and turkey meat extracts (breast and thigh); and carnosine/anserine ratios between undemineralized and demineralized meat extracts were not significantly different (p>0.05). This suggests that the carnosine/anserine ratios of undemineralized chicken (0.62-0.80), duck (0.75-0.77) and turkey (0.15-0.16) meat extracts could be used to estimate the single meat species in uncooked or cooked meat products.

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Table 1. Concentrations of anserine, carnosine and iron in undemineralized poultry meat extracts

Species	Muscles	Anserine (ppm)	Carnosine (ppm)	Ratio of carnosine / anserine	Non-heme iron (ppm)	Heme iron (ppm)	Total iron (ppm)
Chicken	Breast	1,687^{c}	1,053^{a}	0.62^{a}	0.68^{d}	0.43^{e}	1.11^{c}
	Thigh	911^{e}	724^{b,c}	0.80^{a}	1.22^{a}	2.94^{b}	4.16^{b}
Duck	Breast	1,024^{d}	787^{b}	0.77^{a}	1.14^{b}	2.97^{b}	4.11^{b}
	Thigh	896^{e}	668^{c}	0.75^{a}	1.01^{c}	4.76^{a}	5.77^{a}
Turkey	Breast	3,085^{a}	450^{d}	0.15^{b}	0.56^{e}	0.76^{d}	1.32^{c}
	Thigh	2,600^{b}	406^{d}	0.16^{b}	0.64^{d}	1.11^{c}	1.75^{c}

$^{a, b, c, d, e}$ Means in the same column with different superscripts are significantly different ($p < 0.05$).

Table 2. Concentrations of anserine, carnosine and iron in demineralized poultry meat extracts

Species	Muscles	Anserine (ppm)	Carnosine (ppm)	Ratio of carnosine / anserine	Non-heme iron (ppm)	Heme iron (ppm)	Total iron (ppm)
Chicken	Breast	952^{c}	674^{a}	0.71^{a}	0.28^{f}	0.31^{d}	0.59^{d}
	Thigh	853^{d}	683^{a}	0.80^{a}	0.95^{a}	0.29^{d}	1.24^{b}
Duck	Breast	885^{c,d}	644^{a}	0.73^{a}	0.78^{c}	1.49^{a}	2.27^{a}
	Thigh	855^{d}	639^{a}	0.75^{a}	0.86^{b}	1.46^{a}	2.32^{a}
Turkey	Breast	2,677^{a}	404^{b}	0.16^{b}	0.44^{e}	0.46^{c}	0.90^{c}
	Thigh	2,171^{b}	316^{c}	0.15^{b}	0.50^{d}	0.60^{b}	1.10^{c}

$^{a, b, c, d, e, f}$ Means in the same column with different superscripts are significantly different ($p < 0.05$).

Table 3. Concentrations of anserine, carnosine and iron in demineralized chicken breast meat extracts as affected by papain[a]

Reaction time for papain[a] (min)	Anserine (ppm)	Carnosine (ppm)	Ratio of carnosine / anserine	Non-heme iron (ppm)	Heme iron (ppm)	Total iron (ppm)
0	952^{c}	674^{c}	0.71^{b}	0.28^{d}	0.31^{d}	0.59^{d}
30	3,155^{b}	1,117^{b}	0.35^{c}	1.58^{c}	0.30^{d}	1.88^{d}
60	3,174^{b}	1,108^{b}	0.35^{c}	1.68^{c}	0.47^{c}	2.15^{c}
90	2,984^{b}	1,103^{b}	0.37^{c}	1.68^{c}	0.80^{b}	2.48^{c}
120	2,893^{b}	1,080^{b}	0.37^{c}	2.41^{b}	0.93^{b}	3.34^{b}

a One percent of papain was added to the chicken breast meat mixture (pH=6.0) before extraction .

b, c, d Means in the same column with different superscripts are significantly different ($p < 0.05$).