S8P04.WP

THE ABILITY OF CARNOSINE, A SKELETAL MUSCLE DIPEPTIDE, TO INHIBIT LIPID OXIDATION IN MEATS

E.A. DECKER, A.D. CRUM, W.K.M. CHAN, L. MEI and J.T. CALVERT

Food Science Section, Dept. of Animal Sciences, University of Kentucky, Lexington, KY 40546-0215 United States

Please refer to Folio 64.
[Ed. note: Folio 64 incorrectly labelled S8P05.WP]

INTRODUCTION

Carnosine is a naturally occurring ß-alanine, histidine dipeptide found at concentrations ranging from 100 to 400mg/100g of skeletal muscle (Crush, 1970). The physiological role of carnosine and the related dipeptide, anserine (ß-alanine, 1-methylhistidine) is not completely understood. Since the dipeptides are found in greater concentrations in muscles which contain large proportions of white muscle fibres (Crush, 1970), anserine and carnosine have been postulated to protect against oxygen concentration and pH changes associated with anaerobic metabolism. The pK_a of the histidine moiety of anserine and carnosine is 7.04 and 6.83 respectively. Therefore, the dipeptides would have excellent buffering capacity at physiological pH. In addition, carnosine and anserine have been shown to inhibit lipid oxidation suggesting they could protect against oxidative damage caused by reperfusion of oxygen into muscle following anaerobic metabolism.

The ability of carnosine to inhibit lipid oxidation at physiological concentrations suggests that carnosine contributes to the overall antioxidant tone of skeletal muscle. In addition, carnosine has potential for use as a "natural" food additive to decrease the oxidative deterioration of muscle foods. The objective of this research was to determine the antioxidant mechanism of carnosine and to evaluate the antioxidant potential of carnosine in processed meats.

MATERIALS AND METHODS

Carnosine and soybean phosphatidylcholine were obtained from Sigma Chemical Co. (USA). D_2O and 5,5-dimethylpyrroline-N-oxide (DMPO) were obtained from Aldrich Chemical Co. (USA). All other chemical were reagent grade or purer. Pork boston butts were obtained from a local retailer and were used within 72 hours post-mortem.

Oxidation Model Systems

The ability of carnosine to inhibit lipid oxidation was tested in a soybean phosphatidylcholine liposome model system (Decker and Hultin, 1990). Lipid oxidation was catalyzed by either $FeCl_3$ (15uM) or $CuCl_2$ (30uM) plus ascorbate (100uM). All reactions were run at 37°C for 30 min. Lipid oxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS; 3) and lipid peroxides (Beuge and Aust, 1978). The effect of carnosine on iron- and copper-catalyzed ascorbate oxidation was monitored at 265nm in a model system consisting of 100uM ascorbate and 15uM of the respective metals (Davies *et al.*, 1986). Initial rates of ascorbate oxidation were obtained every minute for a total of five minutes. Percent inhibition for the oxidation studies were calculated as :

$$\left[(1 - \frac{\text{Activity in presence of inhibitor}}{\text{Activity in absence of inhibitor}}) * 100\right]$$

Nuclear Magnetic Resonance

^1H nuclear magnetic resonance spectra of carnosine and carnosine in combination with either copper or iron were performed on a Varian VXRS-400 Spectrometer operating at 400MHz. Carnosine (10mM) was dissolved in deuterium oxide containing trimethylsilylpropionic acid as the internal standard. $FeCl_3$, and $CuCl_2$ (10uM) were added immediately prior to analysis.

Electron Paramagnetic Resonance (EPR)

Hydroxyl radical generation was initiated by addition of $FeSO_4$ (10mM) to a mixture of H_2O_2 (5mM) and DMPO (10 or 100mM) in 0.1M phosphate buffer (pH7.4; Kadiiska *et al.*, 1986). Carnosine was added to the hydroxyl radical generation system after addition of H_2O_2 and DMPO and prior to FeSO . The reactive solution was mixed and transferred to a quartz flat cell which was centred in TM EPR resonant cavity for EPR analysis. Spectra were recorded at room temperature using a Bruker 300 EPR spectrometer with computerized data acquisition and analysis capabilities. Operation conditions of the spectrometer were 7.45mM microwave power, 0.98G modulation amplitude, 75G scan width, 1.28ms time constant, 9.79GHz microwave frequency.

Ground Pork Storage Studies

Trimmed and excised muscle was ground twice through 4.5mm plates (4°C). A modified Babcock method was used to determine fat content of the ground pork (Decker and Crumm, 1991) after which fat was mixed and reground into the muscle to give a final concentrations of either 10 or 30% fat. Salted ground pork was produced by mixing 2% NaCl with the ground meat for one minute using a Kitchen Aid mixer with a paddle attachment. Antioxidants were mixed into the ground pork (500g) for 30 seconds (4°C). Carnosine (0.5%) and sodium tripolyphosphate (0.25%) were added on a total weight basis, while mixed tocopherols (0.1%) and rosemary extract (0.1%) were added as a percentage of total fat. Control samples contained no antioxidants. Ground pork was cooked in 50mL polypropylene tubes to an internal temperature of 70°C and immediately cooled on ice. Ground pork samples were stored at four (cooked) or -15°C (uncooked) in 500mL Whirlpak bags. Oxidative changes in the ground pork was determined by measuring thiobarbituric acid reactive substances (TBARS; Sinnhuber, 1977).

RESULTS AND DISCUSSION

The antioxidant mechanism of carnosine could be due to either chelation and/or free radical scavenging. Table 1 shows carnosine (1-25 mM) is capable of inhibiting both iron- or copper-catalyzed lipid oxidation and copper-catalyzed ascorbate oxidation. Carnosine was found to be more effective against copper- than iron-catalyzed lipid oxidation especially at low concentrations (1 and 5mM). Carnosine was not observed to inhibit iron-catalyzed ascorbate oxidation. ^1H NMR (Figures 1a, b, c) indicate that the spectra of carnosine was unaffected by Fe^{+3} while Cu^{+2} caused loss of the peaks associated with carbon-2 and carbon-4 of the imidazole ring (6.9 and 7.7ppm respectively) and significant broadening of the peaks associated with the carbons in the α-chain of carnosine. The ability of carnosine to inhibit Cu-catalyzed oxidative reactions and the ability of Cu^{+2} to alter the ^1H-NMR spectra of carnosine indicate carnosine is capable of chelating copper. Lack of alterations of carnosine's ^1H- NMR spectra by Fe and the inability of carnosine to inhibit Fe-catalyzed ascorbate oxidation suggests that carnosine does not chelate iron. However, carnosine was still observed to inhibit Fe-catalyzed lipid oxidation suggesting that carnosine act as a free radical scavenger.

Electron paramagnetic resonance was used to determine if carnosine was capable of scavenging free radicals. Figures

2a and b show carnosine (10mM) decreased the intensity of the DMPO (100 mM)-hydroxyl radical adduct approximately 75% (note differences in attenuation). Decreasing the DMPO concentration to 10mM (Figure 2c) revealed a 6 line spectra which originates from a carbon-based radical on carnosine. These data indicate carnosine is capable of scavenging hydroxyl radicals in a manner which results in the formation of a carnosine radical.

Tables 2 and 3 show carnosine (0.5%) is capable of inhibiting lipid oxidation in cooked and salted, uncooked ground pork, respectively. General patterns of antioxidant activity were similar between in cooked and salted, uncooked pork except carnosine was more effective in the uncooked pork. General patterns of the antioxidant properties of carnosine included:

a) Carnosine was more effective in 30% than 10% fat pork, and

b) Combinations of carnosine and sodium tripolyphosphate (STP; 0.25%), mixed tocopherol isomers (0.1%) or rosemary extract (0.1%) resulted in improved antioxidant activity.

The combined antioxidants inhibited lipid oxidation in an additive manner suggesting the antioxidants were acting independently instead of synergistically. Carnosine (0.5%) was also found to retard loss of red color in salted, uncooked ground pork during frozen storage (Table 3). Combinations of carnosine with tocopherol or rosemary extract resulted in improved colour retention in pork containing 30% fat.

CONCLUSIONS

Carnosine inhibits lipid oxidation by a combination of both chelation and free radical scavenging. Carnosine (0.5%) is an effective antioxidant in ground pork and the antioxidant activity of carnosine can be improved by using it in combination with tocopherols or rosemary extracts. Carnosine is also effective at inhibiting loss of red color in salted, ground pork during frozen storage. Currently, carnosine is too expensive for practical use as a food additive. However, it is possible a carnosine-containing extract could be produced from low-value meats for use as a food additive.

ACKNOWLEDGEMENTS

This research was partially supported by the Pork Industry Group of the National Livestock and Meat Board.

REFERENCES

CRUSH, K.G. 1970. Carnosine and related substances in animal tissues. *Comp. Biochem. Physiol.* 34:3-30.

DECKER, E.A., and HULTIN, H.O. 1990. Factors influencing the catalysis of lipid oxidation by the soluble fraction of mackerel muscle. *J. Food Sci.* 55:947-950, 953.

McDONALD, R.E., and HULTIN, H.O. 1987. Some characteristics of the enzymic lipid peroxidation systems in the microsomal fraction of flounder muscle. *J. Food Sci.* 52:15-21, 27.

BEUGE, J.A., and Aust, S.D. 1978. Microsomal lipid peroxidation. *Methods Enzymol.* 52:302-310.

DAVIES, K.J., SEVANIAN, A., MUAKKASSAH-KELLY, S.F., and HOCHSTEIN, P. 1986. Uric acid-iron ion complexes. A new aspect of the antioxidant functions of uric acid. *Biochem J.* 235:747-754.

KADIISKA, M.B., MAPLES, K.P., MASON, R.P. 1989. A comparison of cobalt (II) and iron (II) hydroxyl and superoxide free radical formation. *Achiv. Biochem. Biophys.* 275:98-111.

DECKER, E.A., and CRUM, A. 1991. Inhibition of oxidative rancidity in salted ground pork by carnosine. *J. Food Sci.* 56:1179-81.

SINNHUBER, R.O., Yu, T.C. 1977. The 2-thiobarbituric acid reaction, an objective measure of the oxidative deterioration occurring in fats and oils. *J. Jap. Soc. Fish Sci.* 26(5):259-267.

DECKER, E.A., CRUM, A.D., and ALVERT, J.T. 1992. Differences in the antioxidant mechanism of carnosine in the presence of copper and iron. *J. Agric. Food Chem.* 40:756-759.

Table 1. Inhibition (%) of iron- and copper-catalyzed thiobarbituric acid reactive substances (TBARS) formation, lipid peroxide formation and ascorbate oxidation. (Adapted from Decker *et al.*, 1992).

Carnosine (mM)	TBARS % inhibition Fe	Cu	Peroxide % inhibition Fe	Cu	Ascorbate Oxidation % inhibition Fe	Cu
0	0	0	0	0	0	0
1	3.0	33.0	20.0	57.5	0.0	1.1
5	24.0	65.0	11.0	88.0	0.0	14.5
10	54.0	70.0	13.0	78.0	0.0	45.9
25	80.0	71.0	22.8	73.0	0.0	--

Table 2. Inhibition of TBARS (mg/kg muscle) formation in cooked ground pork containing various antioxidants after four days of storage at 4°C.

Treatment	TBARS 10% fat	TBARS 30% fat
Control	39.2 ± 2.9	39.2 ± 7.4
Carnosine	38.8 ± 3.2	30.6 ± 4.3
Rosemary	33.8 ± 4.1	28.4 ± 4.3
Tocopherol	26.6 ± 1.1	26.8 ± 3.4
STP	36.6 ± 2.4	17.1 ± 3.6
Carnosine + Rosemary	30.0 ± 1.6	26.8 ± 2.1
Carnosine + Tocopherol	24.9 ± 2.1	24.9 ± 3.6
Carnosine + STP	29.1 ± 1.6	20.4 ± 4.1

Table 3. Inhibition of TBARS (mg/kg muscle) formation and colour deterioration in salted (2%) uncooked pork containing various antioxidants after four weeks of frozen storage at -15°C. Colour deterioration was determined by sensory analysis using a 13.0cm scale anchored by the terms red (0cm) and brown (13.0cm).

	TBARS 10% fat	TBARS 30% fat	Colour (cm) 10% fat	Colour (cm) 30% fat
Control	15.5±1.0	22.0±6.2	9.2±0.3	10.2±0.2
Carnosine	7.1±1.5	8.5±3.2	2.6±0.2	2.6±0.2
Rosemary	7.3±2.7	7.8±2.5	6.3±0.2	6.3±0.3
Tocopherol	16.5±7.7	14.0±4.4	7.6±0.3	9.4±0.2
Carnosine + Rosemary	5.6±2.4	6.6±1.1	2.8±0.2	1.2±0.9
Carnosine + Tocopherol	4.5±0.5	8.7±1.9	3.4±0.2	1.5±0.1