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ScienceDirect
Food Chemistry 104 (2007) 665–679
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidative activity of carnosine in gamma irradiated ground beef and beef patties

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Received 3 April 2006; received in revised form 3 December 2006; accepted 6 December 2006

Abstract

The activity of carnosine as a natural antioxidant in gamma irradiated ground beef and beef patties was studied. Samples of ground beef, in the absence and presence of 0.5% or 1.0% carnosine, as well as raw and cooked beef patties prepared with 1.5% salt (NaCl), in the absence and presence of 0.5% or 1.0% carnosine, were gamma irradiated at doses of 0, 2, and 4 kGy. The extent of oxidation in irradiated and non-irradiated samples of ground beef and raw beef patties was then determined during refrigerated (4 ± 1 °C) and frozen (−18 °C) storage, while determined for cooked beef patties during refrigerated storage only. Moreover, the determination of metmyoglobin (MetMb) accumulation and sensory evaluation for the visual color were carried out for samples of ground beef and raw patties. The results indicated that salt or salt and cooking accelerated the oxidative processes and significantly increased the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) in the prepared non-irradiated samples. However, salt slowed down the accumulation of MetMb in raw patties. Irradiation treatments and storage in the absence of carnosine significantly ($P < 0.05$) increased the PV and TBARS in samples, at higher rates in salted or salted and cooked beef. Moreover, irradiation and storage significantly ($P < 0.05$) increased the formation of MetMb in ground beef and raw patties in the absence of carnosine. Addition of carnosine significantly ($P < 0.05$) reduced the oxidative processes and MetMb formation (proportionally to the used concentration) in samples post-irradiation and during storage. Furthermore, carnosine exerted significant efficacy in maintaining an acceptable visual red color post-irradiation and during storage of ground beef and raw patties. These results demonstrate that carnosine can be successfully used as a natural antioxidant to increase the oxidative stability in gamma irradiated raw and cooked meat products.

Keywords: Irradiation; Carnosine; Antioxidant; Oxidation; Ground beef; Beef patties; Metmyoglobin

1. Introduction

It is known that neither traditional meat inspection nor supposedly good manufacturing practices can really assure the attainment and maintenance of high hygienic standards for meat with respect to contamination with pathogenic or spoilage bacteria (Gill, 1998). On the other hand, there is an increasing growth in the demand for convenience ready-to-cook/eat minimally processed meat products in both developed and developing countries (Kanatt, Chander, & Sharma, 2005; Lee et al., 2005). However, their hygienic quality can be threatened by the growth of foodborne pathogens (Thayer, Boyed, Kim, Fox, & Farrell, 1998). Although such meat products are manufactured and frozen in the processing establishments, distributed, and sold in the frozen condition (Tsutomu, 1990), frozen foods are not always safe as freezing does not eliminate pathogens (Kanatt et al., 2005). In addition, the demand for chilled meat products continues to increase (Hagyard, Keiller, Cummings, & Chrystall, 1993). Thus, it is important to maintain the microbiological safety of these meat products and extend their shelf-life, with particularly reference to those foodborne pathogens that are capable of growth at refrigeration or frozen temperatures (Farkas et al., 1998).

Irradiation, as a method of meat preservation, has excellent potential in the elimination of pathogenic and spoilage

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0308-8146/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2006.12.015

microorganisms from meat and meat products, and has been viewed, by most food safety officials and scientists, as an effective critical control point in a hazard analysis and critical control points (HACCP) system established for meat processing (Badr, 2004; Farkas, 1998; Rao, Nair, & Sakhare, 1998; Satin, 2002). Meanwhile, the acceleration of lipid oxidation and off-odor production caused by irradiation processing in raw and cooked meat products has been reported and the rate of oxidation increased in a dose-dependant manner (Ahn, Nam, Du, & Jo, 2001; Gomes, da Silva, do Nascimento, & Fukuma, 2003; Kim, Nam, & Ahn, 2002; Nam & Ahn, 2003). The development of oxidative off-flavors has long been recognized as a serious problem during the holding or storage of meat products (Gray, Gomaa, & Buckley, 1996; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Also it is well known that lipid oxidation is positively correlated with pigment oxidation affecting the meat color, which is a main factor affecting meat product acceptability (Bekhit, Geesink, Ilian, Morton, & Bickerstaffe, 2003).

The use of antioxidants is one of the effective control methods against oxidation (Frankel, 1996; Nam & Ahn, 2003). However, special attention has been recently given to the use of natural antioxidants because of the worldwide trend to avoid or minimize the use of synthetic food additives (Bekhit et al., 2003; Frankel, 1996). There has been some recent interest in the antioxidant potential of carnosine in processed meat products. It is a naturally occurring skeletal muscle dipeptide composed of β-alanine and histidine and exerts its antioxidant effect by a number of mechanisms (Chan, Decker, & Means, 1993; Sánchez-Escalante et al., 2003; Zhou & Decker, 1999). Carnosine has been shown to be an effective antioxidant in model systems and meats (Decker, Crum, & Calvert, 1992; Lee & Hendricks, 1997; O'Neill, Galvin, Morrissey, & Buckley, 1999; Sánchez-Escalante et al., 2001; Sánchez-Escalante et al., 2003). Therefore, the objective of the present study was to examine the antioxidant effect of carnosine in gamma irradiated ground beef and beef patties during storage.

2. Materials and methods

2.1. Ground beef

Beef muscles were excised from three beef carcasses at a butcher's shop (after 2 h of slaughtering and dressing) and used separately as replications for the preparation of samples (three separate replicates). Interest in hot-boning has arisen as a result of demonstrated improvements in the functional and color properties and hence the quality of all processed meat products (Pearson & Gillett, 1996; Pisula & Tuburcy, 1996; Sammel et al., 2002; Seyfert et al., 2004). After the removal of the surface fat and connective tissue, each of the obtained beef muscles were chopped into small pieces, ground using a meat grinder (National, MK-G20NR, Japan) through a plate with 4-mm holes, well mixed, and divided into two batches for the preparation of samples under investigation.

2.2. Preparation of ground beef samples

The first batch of each of the observed ground beef (for each carcass separately) was sub-divided into three portions. The first portion kept without addition of carnosine, while carnosine of 99% purity (Fluka, Switzerland) was added in the dry form at a final concentration of 0.5% and 1.0% (w/w) to the second and third portions, respectively. Ground beef was well mixed in a meat mixer after the addition of carnosine to ensure uniform distribution of the antioxidant in samples. Then each of the three prepared portions was subdivided into ~50 g samples and aerobically packaged in sealed polyethylene pouches.

2.3. Preparation of raw and cooked beef patties

To the second batch of the observed ground beef, salt (NaCl) was added at a final concentration of 1.5% (w/w). After well mixing in the meat mixer, the ground meat was sub-divided into three portions for the addition of carnosine at a final concentrations of 0%, 0.5%, and 1% (w/w) as mentioned above in the preparation of the ground beef samples. After well mixing, patties were formed (as described by O'Neill et al. (1999)) with a diameter of 10 cm and thickness of 0.6 cm (~60 g). Half of the observed patties (for each portion) was kept raw and aerobically packaged in sealed polyethylene pouches (individually). While the other half of the observed patties was cooked in an oven (160 °C) to an internal temperature of 75 °C for 30 min, cooled down to room temperature, and packaged as mentioned for the raw patties.

2.4. Irradiation of samples

Packaged samples of ground beef and beef patties were transported (immediately after their preparation) and gamma irradiated at doses of 0, 2, and 4 kGy. Irradiation was carried out at room temperature using an experimental Co-60 source providing a dose rate of 5.937 kGy/h (Isslevovatel, Tenex, Russia) located at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The dose rate was established using reference PNL (Physical National Laboratory, UK) dichromate dosimeter (ISO/ASTM, 2002).

2.5. Storage and sampling

Irradiated and non-irradiated samples of each of the prepared ground beef and raw beef patties were divided into two parts that required for the refrigeration storage at 4 ± 1 °C and frozen storage at −18 °C (except samples for the day zero analysis). Meanwhile samples of irradiated and non-irradiated cooked patties were kept only at refrigerated temperature of 4 ± 1 °C. The periodical sampling

for analysis was carried out at 3 and 15 days intervals for refrigerated and frozen samples, respectively. Frozen samples were thawed overnight at 4 ± 1 °C before analysis.

2.6. Measurements of oxidation

The extent of oxidation in samples was assessed through the determination of peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). For the determination of PV, lipids were extracted from samples under investigation using 2:1 (v/v) chloroform/methanol solvent (Folch, Lee, & Sloane-Stanley, 1957), and then the PV was determined in the recovered lipids according to the Official Methods of AOCS (1998). Meanwhile the contents of TBARS were determined in samples of ground beef and patties using the method of Alasnier, Meynier, Viau, and Gandmer (2000). Two grams of ground beef (or beef patties) were mixed with butylated hydroxytoluene (BHT) in ethanol (4 μg BHT) and 16 mL of 5% trichloroacetic acid. Samples were homogenized for 20 s and filtered through Whatman filter paper No. 40, then 2 mL portion of the filtrate was added to 2 mL thiobarbituric-acid solution (20 mM) in tightly closed tube. Tubes were heated at 70 °C for 30 min using a water bath and the absorbance was read against water using a Pye-Unicam spectrophotometer.

2.7. Metmyoglobin (MetMb %)

The contents of MetMb in ground beef and raw beef patties were determined as described by Bekhit et al. (2003). Briefly, 5 g samples were homogenized in 25 mL ice-cold 40 mM phosphate buffer (pH 6.8) for 10 s. The homogenate was allowed to stand for 1 h at 4 °C and centrifuged at 4500*g* for 30 min at 4 °C. The supernatant was filtered through Whatman No. 1 filter paper and the absorbance was measured at 572, 565, 545 and 525 nm using a Pye-Unicam (Unicam Ltd., UK) spectrophotometer. Then the percentages of MetMb were calculated based on these absorbance values according to Krzywicki (1982) using the following formula:

$$\%\ \text{MetMb} = \{-2.51(A_{572}/A_{525}) + 0.777(A_{565}/A_{525}) + 0.8(A_{545}/A_{525}) + 1.098\} \times 100$$

The determination was run only on samples of ground beef and raw patties since cooking denatures the meat pigments.

2.8. Sensory evaluation

Samples of ground beef and raw beef patties under investigation were subjected to visual color evaluation post-irradiation and during refrigeration and frozen stor-

Table 1
Effects of salt addition or salt and cooking on oxidation processes and metmyoglobin accumulation during refrigeration storage of the prepared non-irradiated beef patties

Storage (days)	Peroxide value (milliequivalents of peroxide/kg lipid)			Thiobarbituric acid reactive substances (mg malonaldhyde equivalents/kg lipid)			Metmyoglobin accumulation (%)	
	Ground* beef	Raw beef patties	Cooked beef patties	Ground* beef	Raw beef patties	Cooked beef patties	Ground* beef	Raw beef patties
0	0.72^{P}	0.79^{O}	2.03^{J}	0.17^{OP}	0.19^{O}	0.89^{G}	10.71^{J}	7.79^{K}
3	1.35^{N}	1.45^{M}	2.61^{H}	0.26^{N}	0.35^{M}	1.81^{E}	24.20^{H}	16.31^{I}
6	1.51^{L}	1.94^{K}	2.82^{G}	0.39^{L}	0.51^{K}	3.17^{D}	34.51^{F}	26.18^{G}
9	2.07^{I}	2.82^{G}	3.78^{D}	0.50^{K}	0.69^{J}	4.36^{C}	39.52^{E}	39.82^{E}
12	R	R	4.86^{B}	R	R	4.98^{B}	R	R
15	–	–	R	–	–	R	–	–

Means with different subscript within each determination are significantly different ($P < 0.05$).
R, rejected due to the deterioration of odor and their values were discarded after statistical analysis.
* 0.0% salt.

Table 2
Effects of salt on oxidation processes and metmyoglobin accumulation during frozen storage of the prepared non-irradiated raw beef patties

Storage (days)	Peroxide value (milliequivalents of peroxide/kg lipid)		Thiobarbituric acid reactive substances (mg malonaldhyde equivalents/kg lipid)		Metmyoglobin accumulation (%)	
	Ground* beef	Raw beef patties	Ground* beef	Raw beef patties	Ground* beef	Raw beef patties
0	0.71^{G}	0.80^{I}	0.18^{I}	0.19^{I}	10.51^{I}	7.78^{J}
15	1.05^{H}	1.27^{G}	0.31^{H}	0.37^{G}	17.92^{G}	10.03^{H}
30	1.41^{F}	1.68^{E}	0.57^{F}	0.66^{E}	26.72^{E}	20.93^{F}
45	2.32^{D}	2.77^{C}	0.76^{D}	0.83^{C}	31.46^{C}	31.02^{D}
60	3.34^{B}	4.01^{A}	0.97^{B}	1.39^{A}	43.33^{A}	39.55^{B}

Means with different subscript within each determination are significantly different ($P < 0.05$).
* 0.0% salt.

age. The panelists consisted of a 10-member untrained individuals, while the color attributes were rated using a 5-point descriptive scale according to Djenane, Martínez, Sánchez-Escalante, Beltrán, and Roncalés (2003). Scores for color, referred to the degree of pinkness/redness, were as follows: 5 = extent; 4 = moderate; 3 = small; 2 = slight;

Table 3
Effects of carnosine addition on the peroxide value (milliequivalents of peroxide/kg lipid) of lipids separated from irradiated and non-irradiated ground beef, raw and cooked beef patties during refrigeration storage

Beef product	Storage (days)	Concentration of carnosine (%)/irradiation dose (kGy)								
		0.0%			0.5%			1.0%		
		0.0 kGy	2.0 kGy	4.0 kGy	0.0 kGy	2.0 kGy	4.0 kGy	0.0 kGy	2.0 kGy	4.0 kGy
Ground beef	0	0.72^{j}	1.53^{U}	2.43^{K}	0.72^{j}	0.97^{f}	1.63^{T}	0.71^{j}	0.79^{h}	1.35^{X}
	3	1.35^{X}	1.72^{RS}	2.31^{L}	0.96^{f}	1.18^{b}	1.75^{R}	0.81^{i}	0.91^{g}	1.44^{V}
	6	1.51^{U}	2.07^{N}	3.45^{F}	1.11^{d}	1.31^{Y}	2.02^{O}	0.88^{h}	1.02^{e}	1.53^{U}
	9	2.07^{N}	1.92^{P}	3.76^{D}	1.35^{X}	1.40^{VW}	2.32^{L}	1.01^{e}	1.16^{bc}	1.71^{S}
	12	R	2.77^{I}	4.58^{B}	R	1.76^{R}	2.72^{J}	R	1.27^{Z}	1.81^{Q}
	15	–	3.93^{C}	4.91^{A}	–	2.10^{MN}	2.92^{H}	–	1.40^{VW}	2.12^{M}
Raw beef patties	0	0.79^{l}	1.92^{Z}	2.87^{O}	0.78^{l}	1.32^{f}	1.91^{Z}	0.78^{l}	1.01^{i}	1.24^{g}
	3	1.45^{d}	2.31^{U}	3.41^{K}	1.03^{i}	1.70^{a}	2.41^{T}	0.87^{k}	1.17^{h}	1.62^{c}
	6	1.94^{Z}	3.02^{M}	3.91^{H}	1.23^{g}	2.10^{X}	2.88^{O}	0.96^{j}	1.40^{e}	1.92^{Z}
	9	2.82^{P}	3.91^{H}	4.61^{E}	1.62^{c}	2.71^{Q}	3.31^{L}	1.25^{g}	1.67^{b}	2.49^{S}
	12	R	4.88^{D}	5.52^{B}	R	3.32^{L}	3.90^{H}	R	2.13^{W}	2.91^{N}
	15	–	5.42^{C}	6.15^{A}	–	3.51^{J}	4.43^{F}	–	2.53^{R}	3.53^{J}
Cooked beef patties	0	2.03^{Y}	3.47^{Q}	4.52^{I}	1.48^{bc}	2.23^{X}	3.06^{S}	1.02^{e}	1.37^{c}	2.11^{XY}
	3	2.61^{VW}	3.92^{LM}	4.91^{H}	1.82^{Z}	2.65^{UVW}	3.48^{PQ}	1.21^{d}	1.68^{a}	2.57^{VW}
	6	2.82^{T}	4.38^{IJ}	5.41^{F}	2.21^{X}	3.03^{S}	3.90^{LM}	1.37^{c}	2.17^{XY}	2.95^{S}
	9	3.78^{MN}	4.84^{H}	6.10^{D}	2.76^{TU}	3.62^{OP}	4.18^{K}	1.54^{b}	2.68^{TUV}	3.61^{OP}
	12	4.86^{H}	5.43^{E}	6.72^{B}	3.32^{R}	3.98^{L}	4.88^{H}	2.10^{XY}	3.10^{S}	4.04^{L}
	15	R	6.31^{C}	7.53^{A}	R	4.31^{JK}	5.32^{FG}	R	3.55^{PQ}	4.52^{I}

Means with different subscript within each beef product are significantly different ($P < 0.05$).
R, rejected due to the deterioration of odor and their values were discarded after statistical analysis.

Table 4
TBARS values (mg malonaldhyde equivalents/kg lipid) of refrigerated stored irradiated and non-irradiated ground beef, raw and cooked beef patties as affected by carnosine addition

Beef product	Storage (days)	Concentration of carnosine (%)/irradiation dose (kGy)								
		0.0%			0.5%			1.0%		
		0.0 kGy	2.0 kGy	4.0 kGy	0.0 kGy	2.0 kGy	4.0 kGy	0.0 kGy	2.0 kGy	4.0 kGy
Ground beef	0	0.17^{b}	0.37^{RS}	0.56^{N}	0.17^{b}	0.24^{XY}	0.38^{RS}	0.17^{b}	0.20^{Z}	0.31^{U}
	3	0.26^{WX}	0.44^{Q}	0.69^{K}	0.20^{Za}	0.29^{UV}	0.45^{Q}	0.18^{ab}	0.25^{X}	0.39^{R}
	6	0.39^{RS}	0.57^{MN}	0.81^{H}	0.28^{VW}	0.38^{RS}	0.56^{N}	0.23^{V}	0.30^{UV}	0.44^{Q}
	9	0.50^{O}	0.71^{J}	1.10^{D}	0.34^{T}	0.45^{Q}	0.72^{J}	0.29^{UV}	0.36^{ST}	0.59^{M}
	12	R	0.93^{E}	1.41^{B}	R	0.57^{MN}	0.88^{F}	R	0.48^{OP}	0.70^{JK}
	15	–	1.33^{C}	1.73^{A}	–	0.79^{I}	1.10^{D}	–	0.63^{L}	0.86^{G}
Raw beef patties	0	0.19^{de}	0.41^{X}	0.66^{O}	0.18^{e}	0.28^{b}	0.46^{VW}	0.17^{e}	0.23^{c}	0.38^{Y}
	3	0.35^{Z}	0.53^{RS}	0.78^{L}	0.26^{b}	0.36^{YZ}	0.54^{R}	0.21^{cd}	0.31^{a}	0.47^{V}
	6	0.51^{ST}	0.65^{O}	0.93^{J}	0.37^{YZ}	0.44^{W}	0.66^{O}	0.31^{a}	0.37^{Y}	0.57^{Q}
	9	0.69^{N}	0.81^{K}	1.31^{E}	0.50^{TU}	0.55^{QR}	0.94^{J}	0.42^{X}	0.46^{VW}	0.78^{L}
	12	R	1.10^{G}	1.74^{B}	R	0.75^{M}	1.22^{F}	R	0.62^{P}	1.01^{H}
	15	–	1.42^{D}	2.11^{A}	–	0.98^{I}	1.47^{C}	–	0.82^{K}	1.10^{G}
Cooked beef patties	0	0.57^{o}	0.78^{k}	0.95^{h}	0.46^{q}	0.59^{o}	0.73^{l}	0.40^{r}	0.53^{p}	0.63^{n}
	3	1.05^{f}	1.16^{e}	1.22^{c}	0.79^{k}	0.89^{i}	0.99^{g}	0.68^{m}	0.72^{l}	0.84^{j}
	6	2.28^{N}	2.41^{L}	2.91^{F}	1.32^{a}	1.47^{Z}	1.80^{U}	1.16^{d}	1.27^{b}	1.53^{Y}
	9	2.47^{K}	2.75^{G}	3.14^{E}	1.88^{T}	1.90^{S}	2.39^{L}	1.57^{X}	1.66^{W}	1.96^{R}
	12	2.74^{G}	3.14^{E}	3.47^{C}	2.15^{P}	2.34^{M}	2.63^{I}	1.74^{V}	1.91^{S}	2.07^{Q}
	15	R	3.76^{B}	4.25^{A}	R	2.60^{J}	2.71^{H}	R	2.15^{P}	2.20^{O}

Means with different subscript within each beef product are significantly different ($P < 0.05$).
R, rejected due to the deterioration of odor and their values were discarded after statistical analysis.

and 1 = none. Decreasing of the recorded scores indicates the increase of brownness. Fresh raw ground beef samples were used as a reference for fresh pinkness/redness to help the panelists in evaluating the changes in the color during storage. In addition, the panelists were also asked to detect the odors associated with spoilage of meat in all refrigerated samples just to define the end of their cold storability within the chosen period of study.

2.9. Statistical analysis

All analyses were performed using three pouches per each separate replicate. Then data were statistically analyzed by using the generalized linear model procedure of the SAS software (SAS Institute, 1998), and mean values were reported. The differences among means (at $P < 0.05$) were compared by using Duncan's multiple range test.

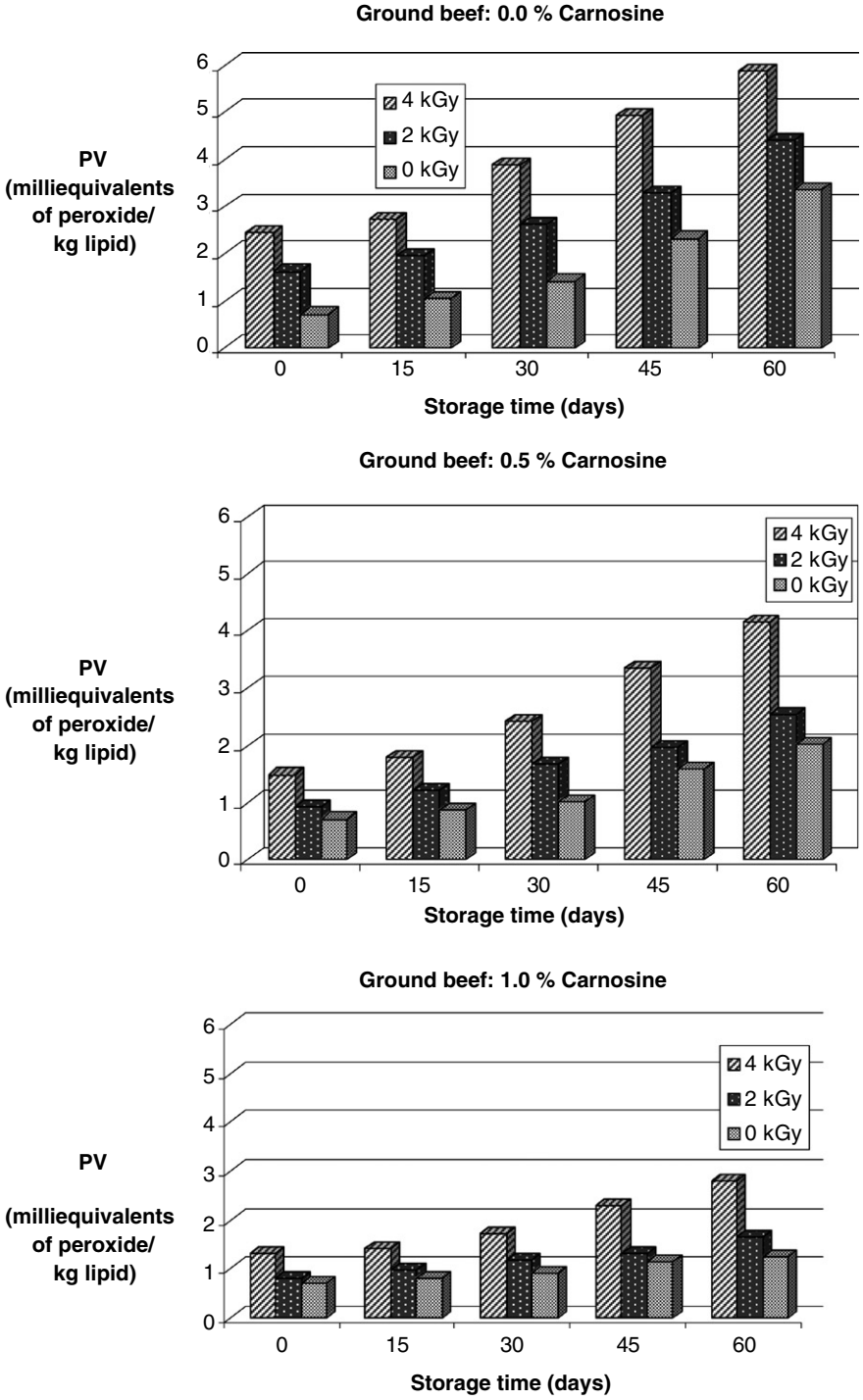

Fig. 1a. Effects of carnosine addition on the peroxide value (PV) of lipids separated from irradiated and non-irradiated ground beef during frozen storage.

3. Results and discussion

3.1. Oxidation and metmyoglobin formation in the prepared ground beef and beef patties as affected by grinding, salt and cooking

As shown in Table 1, the initial PV, TBARS and MetMb percent in samples of raw non-irradiated ground beef were 0.72 meq/kg lipid, 0.17 mg malonaldehyde equivalents/kg meat and 10.17%, respectively, on day zero of refrigeration storage. It was previously shown that fresh raw meat may contain some TBARS (Hampson, Fox, Lakritz, & Thayer, 1996). Rancidity in meat begins to develop soon after death and continues to increase in intensity until the meat becomes unacceptable. The biochemical changes that accompany post-slaughter metabolism and post-mortem aging give rice to conditions whereby the process of lipid oxidation is no longer tightly controlled and the balance of prooxidative factors/antioxidant capacity favors oxidation. Furthermore, any disruption of integrity of muscle membranes by deboning, grinding and restructuring facilitates the interactions of prooxidants with unsatu-

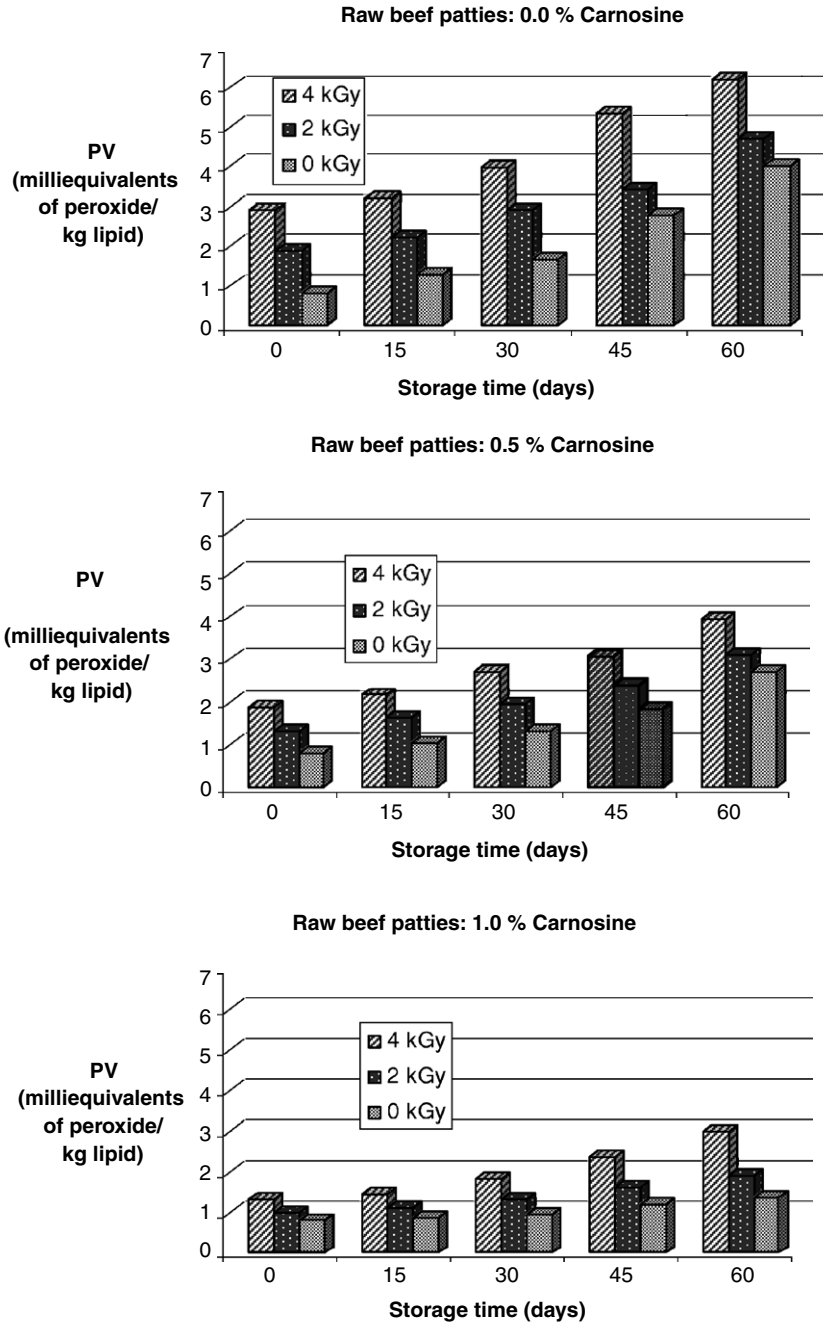

Fig. 1b. Effects of carnosine addition on the peroxide value (PV) of lipids separated from irradiated and non-irradiated raw beef patties during frozen storage.

rated fatty acids resulting in the propagation of oxidative reactions (Gray et al., 1996). On the other hand, it has been pointed out that grinding of meat also speeds up the oxidation of myoglobin (Torres, Pearson, Gray, Booren, & Shimokomaki, 1988).

Addition of salt and cooking significantly ($P < 0.05$) increased the PV and TBARS contents after preparation and during storage of raw and cooked beef patties, respectively. However, the addition of salt slowed down the accumulation of MetMb in raw beef patties (Tables 1 and 2). These results agree with the findings of O'Neill et al. (1999) and Torres et al. (1988). It is well documented that salt is a powerful prooxidant (Anderson & Skibsted, 1991; Kanner, Harel, & Jaffe, 1991) and cooking greatly accelerates the oxidative changes in meat (Ahn et al., 1998; Ahn et al., 2001). A possible mechanism explaining the effect of salt on increasing lipid oxidation with increased color stability can be illustrated by the findings of Torres et al. (1988). They demonstrated that pre-rigor grinding and salting reduced the pH decline leading to a lower content of MetMb (the ferric state) and higher ultimate pH (when compared with the case of post-rigor samples). On the other hand, lipid oxidation occurred rapidly in the presence of a greater proportion of heme proteins in the reduced, ferrous state. Ferrous ion can induce peroxidation of unsaturated fatty acids in mitochondrial and microsomal fractions, and can catalyze the decomposition of the preformed hydroperoxides into peroxyl radicals, which in turn

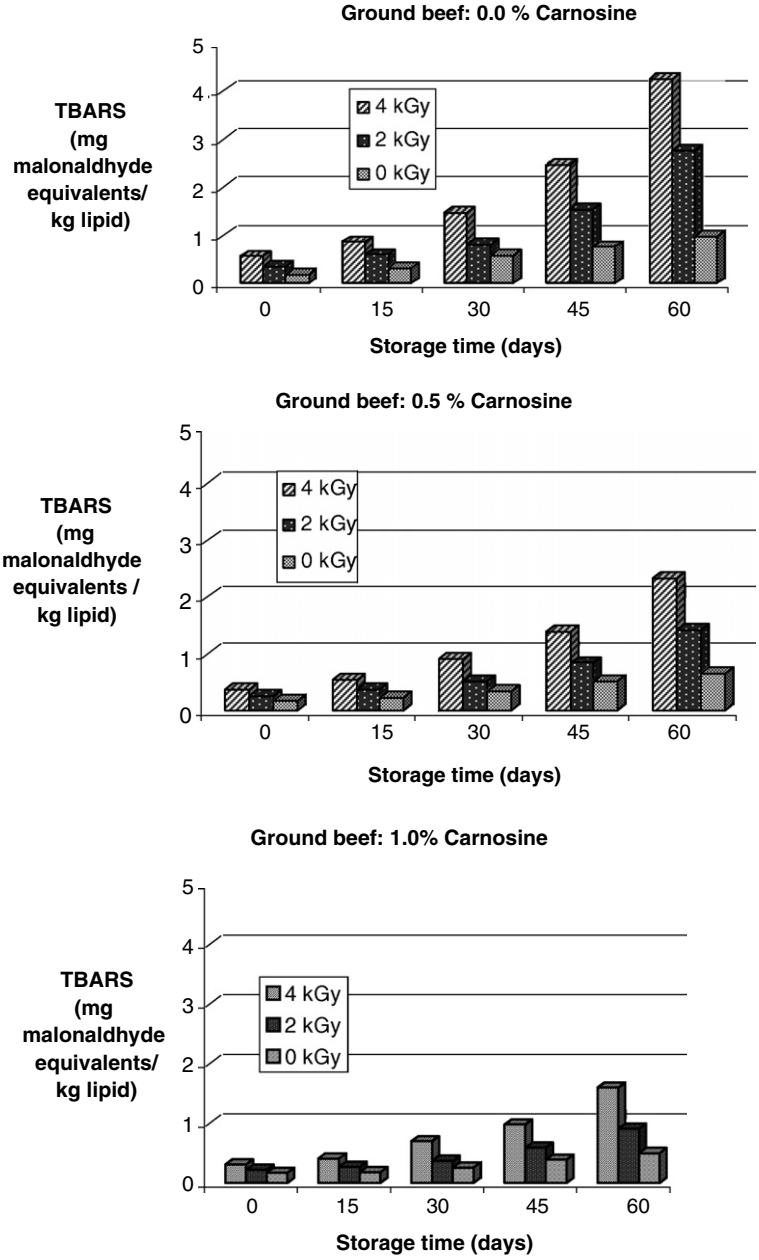

Fig. 2a. TBARS values of frozen stored irradiated and non-irradiated ground beef as affected by carnosine addition.

can remove a hydrogen atom from the unsaturated fatty acids and increase the rate of lipid oxidation (Gandemer, 1998).

3.2. Effects of carnosine addition on the formation of hydroperoxides and TBARS during refrigerated storage (4 ± 1 °C) of irradiated and non-irradiated ground beef and beef patties

The effects of carnosine on the oxidative processes, expressed as PV and TBARS values, during refrigerated storage of ground beef and beef patties under investigation are presented in Tables 3 and 4. Addition of carnosine at different concentrations showed no significant ($P > 0.05$) antioxidant effect during the preparation of raw non-irradiated ground beef and beef patties. However, carnosine addition significantly ($P < 0.05$) reduced the formation of hydroperoxides and TBARS, in a concentration-dependant manner, during cooking of beef patties. These results are in agreement with previous results which showed that carnosine was an effective antioxidant in cooked chicken thigh meat patties (O'Neill et al., 1999) and cooked pork (Decker & Crum, 1993). The antioxidant properties of aqueous solutions of carnosine were not inactivated when heated at 100 °C for 15 min (Decker & Faraji, 1990).

Irradiation of beef products in the absence of carnosine significantly ($P < 0.05$) increased the oxidative processes in samples and oxidation was more pronounced in the pres-

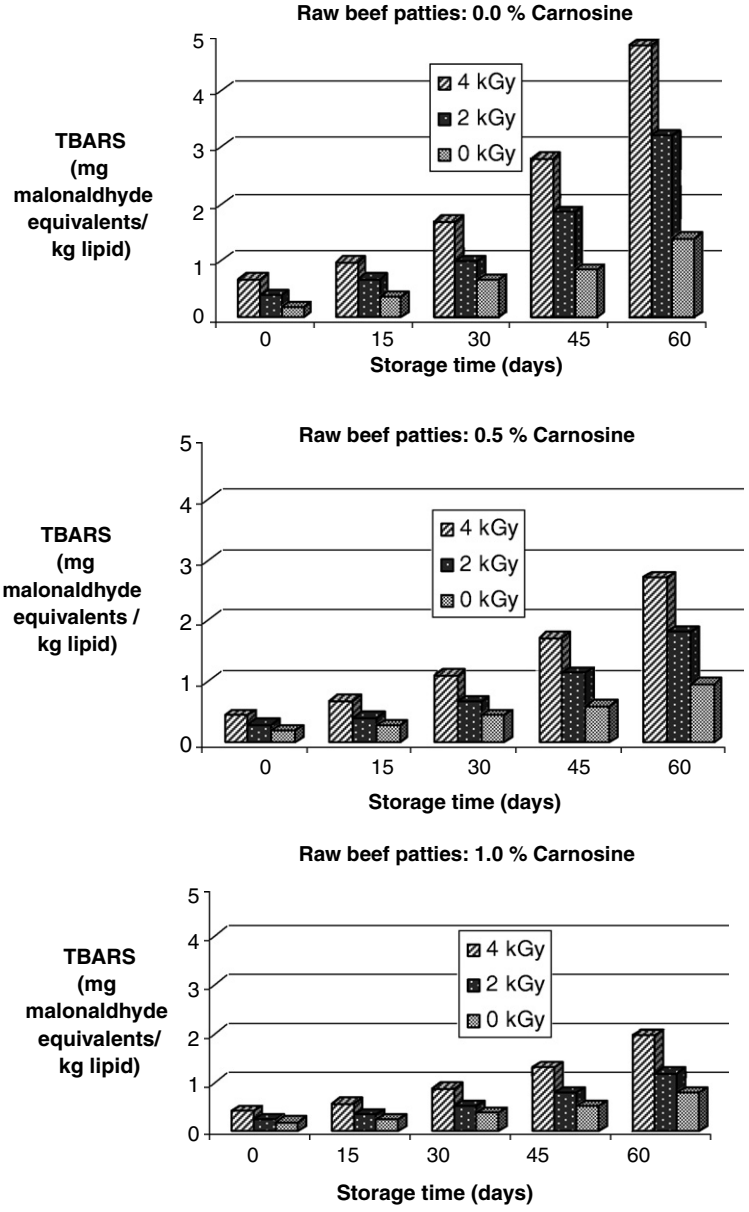

Fig. 2b. TBARS values of frozen stored irradiated and non-irradiated raw beef patties as affected by carnosine addition.

ence of salt, in raw patties, or salt and cooking, in the cooked patties (Tables 3 and 4). Irradiated meats are susceptible to lipid oxidation (Nam & Ahn, 2003) and oxidation is enhanced in the presence of oxygen (Lee et al., 2005). Carnosine exerted a significant ($P < 0.05$) antioxidant effect during irradiation of all samples and the formation of hydroperoxides and TBARS significantly ($P < 0.05$) decreased with increasing the concentration of carnosine.

During storage at 4 ± 1 °C, the primary and secondary products of oxidation also significantly ($P < 0.05$) decreased as a function of carnosine addition in both irradiated and non-irradiated samples. From Tables 3 and 4, it can be seen that presence of 0.5% carnosine reduced the formation of hydroperoxides and TBARS in samples of ground beef, raw patties and cooked patties subjected to the highest dose by 40.5%, 28% and 29% and 36.4%, 30% and 36% after 15 days of refrigeration storage, respectively. Meanwhile, presence of 1% carnosine reduced the formation of hydroperoxides and TBARS in the above-mentioned samples by 56.8%, 42.6% and 40% and 50.3%, 47.9% and 48% after 15 days of refrigeration storage, respectively. Hydroperoxides are the primary initial products of lipid oxidation. Although essentially odorless, they will decompose to a variety of volatile and non-volatile secondary products affecting the organoleptic quality (Gray et al., 1996; Lefebvre, Thibault, Charbonneau, & Piette, 1994). Moreover, decomposition of hydroperoxides increases the rate of lipid oxidation propagation because

Table 5
Influence of carnosine addition on the accumulation of metmyoglobin (%) during refrigeration storage of irradiated and non-irradiated ground beef and raw beef patties

Beef product	Storage (days)	Concentration of carnosine (%)/irradiation dose (kGy)								
		0.0%			0.5%			1.0%		
		0.0 kGy	2.0 kGy	4.0 kGy	0.0 kGy	2.0 kGy	4.0 kGy	0.0 kGy	2.0 kGy	4.0 kGy
Ground beef	0	10.71^{z}	16.82^{s}	20.74^{o}	10.70^{z}	13.11^{x}	16.61^{u}	10.71^{z}	11.01^{y}	14.21^{w}
	3	24.20^{k}	28.13^{f}	31.71^{a}	17.61^{r}	20.22^{p}	23.76^{l}	15.04^{v}	17.82^{q}	21.18^{n}
	6	34.51^{X}	37.91^{U}	40.37^{P}	25.01^{i}	27.02^{h}	30.11^{d}	22.11^{m}	20.63^{o}	27.11^{g}
	9	39.52^{R}	45.23^{M}	48.64^{H}	31.03^{c}	33.01^{Y}	36.32^{V}	24.88^{j}	28.80^{e}	32.73^{Z}
	12	R	56.72^{E}	60.02^{C}	R	42.02^{O}	45.62^{L}	R	35.74^{W}	39.62^{Q}
	15	–	66.87^{B}	69.23^{A}	–	50.42^{G}	53.76^{F}	–	43.50^{N}	47.02^{K}
Raw beef patties	0	7.79^{w}	12.38^{o}	15.26^{l}	7.74^{w}	9.64^{u}	12.22^{p}	7.78^{w}	8.09^{v}	10.46^{s}
	3	16.31^{i}	18.94^{g}	21.40^{d}	11.86^{r}	13.63^{n}	16.03^{j}	10.12^{t}	12.01^{q}	14.28^{m}
	6	26.18^{a}	29.03^{Y}	30.88^{W}	19.16^{f}	20.70^{e}	23.02^{c}	15.82^{k}	16.92^{h}	20.60^{e}
	9	39.82^{Q}	44.75^{L}	48.16^{J}	30.69^{X}	32.68^{U}	35.95^{S}	24.63^{b}	28.51^{Z}	32.41^{V}
	12	R	59.57^{E}	63.08^{D}	R	44.12^{M}	47.87^{K}	R	37.52^{R}	41.58^{O}
	15	–	74.23^{B}	76.86^{A}	–	55.95^{F}	59.68^{E}	–	48.24^{J}	52.17^{H}

Means with different subscript within each beef product are significantly different ($P < 0.05$).
R, rejected due to the deterioration of odor and their values were discarded after statistical analysis.

Table 6
Influence of carnosine addition on the accumulation of metmyoglobin (%) during frozen storage of irradiated and non-irradiated ground beef and raw beef patties

Beef product	Storage (days)	Concentration of carnosine (%)/irradiation dose (kGy)								
		0.0%			0.5%			1.0%		
		0.0 kGy	2.0 kGy	4.0 kGy	0.0 kGy	2.0 kGy	4.0 kGy	0.0 kGy	2.0 kGy	4.0 kGy
Ground beef	0	10.51^{o}	16.90^{e}	20.60^{W}	10.46^{p}	13.28^{i}	16.62^{d}	10.68^{n}	11.04^{m}	14.31^{h}
	15	17.92^{Z}	20.61^{W}	24.13^{R}	12.52^{k}	15.22^{g}	17.82^{a}	11.22^{l}	13.18^{j}	15.67^{f}
	30	26.72^{N}	30.18^{L}	33.64^{I}	20.03^{X}	21.61^{V}	24.10^{S}	17.63^{b}	16.51^{e}	21.66^{U}
	45	31.46^{K}	35.25^{G}	37.93^{E}	24.17^{Q}	25.51^{O}	27.96^{M}	19.16^{Y}	22.15^{T}	25.21^{P}
	60	43.33^{C}	49.92^{B}	53.40^{A}	33.97^{H}	37.38^{F}	40.60^{D}	27.96^{M}	31.82^{J}	35.24^{G}
Raw beef patties	0	7.78^{o}	12.37^{g}	15.23^{Z}	7.72^{p}	9.63^{l}	12.19^{h}	7.73^{p}	8.08^{n}	10.42^{k}
	15	13.03^{d}	15.16^{a}	17.12^{W}	9.50^{m}	10.91^{j}	12.83^{e}	8.09^{n}	9.61^{l}	11.50^{i}
	30	20.39^{T}	22.93^{R}	24.42^{P}	15.13^{b}	16.36^{X}	18.16^{V}	12.49^{f}	13.36^{c}	16.27^{Y}
	45	31.02^{K}	34.92^{G}	37.60^{D}	23.93^{Q}	25.47^{N}	28.05^{L}	19.21^{U}	22.24^{S}	25.28^{O}
	60	39.55^{C}	46.45^{B}	49.22^{A}	31.60^{J}	34.42^{H}	37.35^{E}	25.76^{M}	33.20^{I}	35.21^{F}

Means with different subscript within each beef product are significantly different ($P < 0.05$).

these radicals remove hydrogen at a faster rate than initial alkyl radicals (Gandemer, 1998). It is well known that free radicals possess strong chemical reactivity and can react with unsaturated fatty acids (McMillin, 1996). A distinct difference between carnosine and other free radical scavengers is that is water soluble. This property allows carnosine to inactivate lipid oxidation catalysts and free radicals in the aqueous phase of meat (Decker & Crum, 1993). The observed antioxidant effect of carnosine disagrees with the results of Nam and Ahn (2003) as they observed a small antioxidant effect of carnosine in irradiated pork homogenates. However, they indicated that is possibly because the added amount was so small.

3.3. Effects of carnosine addition on the oxidative processes during frozen storage (−18 °C) of irradiated and non-irradiated ground beef and raw beef patties

In the absence of carnosine, frozen storage (−18 °C) significantly ($P < 0.05$) slowed down, but did not inhibit, the formation of both primary and secondary products of oxidation in irradiated and non-irradiated ground beef and raw beef patties (Figs. 1 and 2). Much is known about the development of oxidative rancidity in frozen meat during storage, and the deleterious effect such reactions have on meat acceptability (Hagyard et al., 1993). In common with other chemical reactions initiated by free

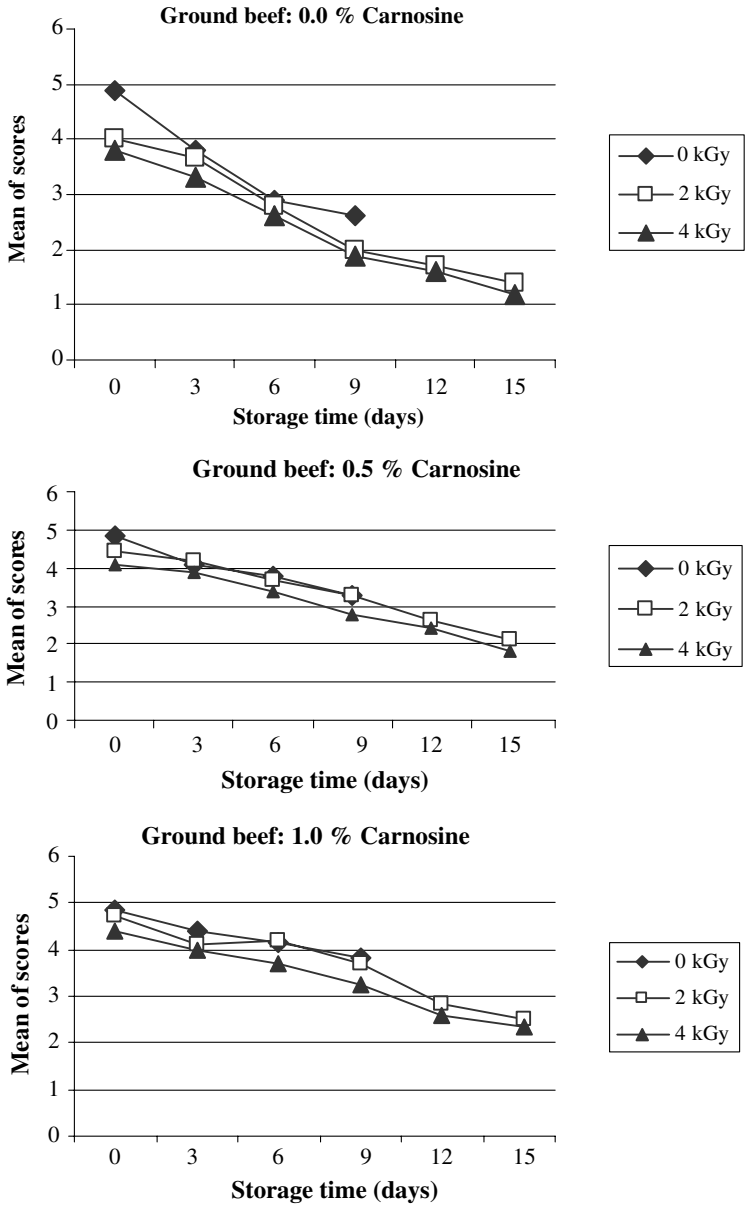

Fig. 3a. Influence of carnosine addition on the visual color of refrigerated stored irradiated and non-irradiated ground beef. Control non-irradiated samples were rejected after 9 days for the deterioration of odor and their values were discarded after statistical analysis.

radicals, rancidity development, once started, will be slowed only by extremely low temperatures (Hagyard et al., 1993).

As with refrigeration storage, carnosine addition significantly ($P < 0.05$) reduced the formation of both primary and secondary oxidation products during frozen storage of irradiated and non-irradiated ground beef and raw beef patties (Figs. 1 and 2). In the presence of 0.5% carnosine, the formation of hydroperoxides and TBARS decreased by 29% and 36.5% and 45% and 43.3%, respectively, after 60 days of frozen storage in ground beef and beef patties exposed to the highest radiation dose. Meanwhile, addition of 1% carnosine decreased the formation of hydroperoxides and TBARS by 52% and 51.5% and 62.4% and 58.8% after 60 days of frozen storage for the above-mentioned samples, respectively. Decker and Crum (1991) also found that carnosine effectively inhibited TBARS formation in frozen stored pork.

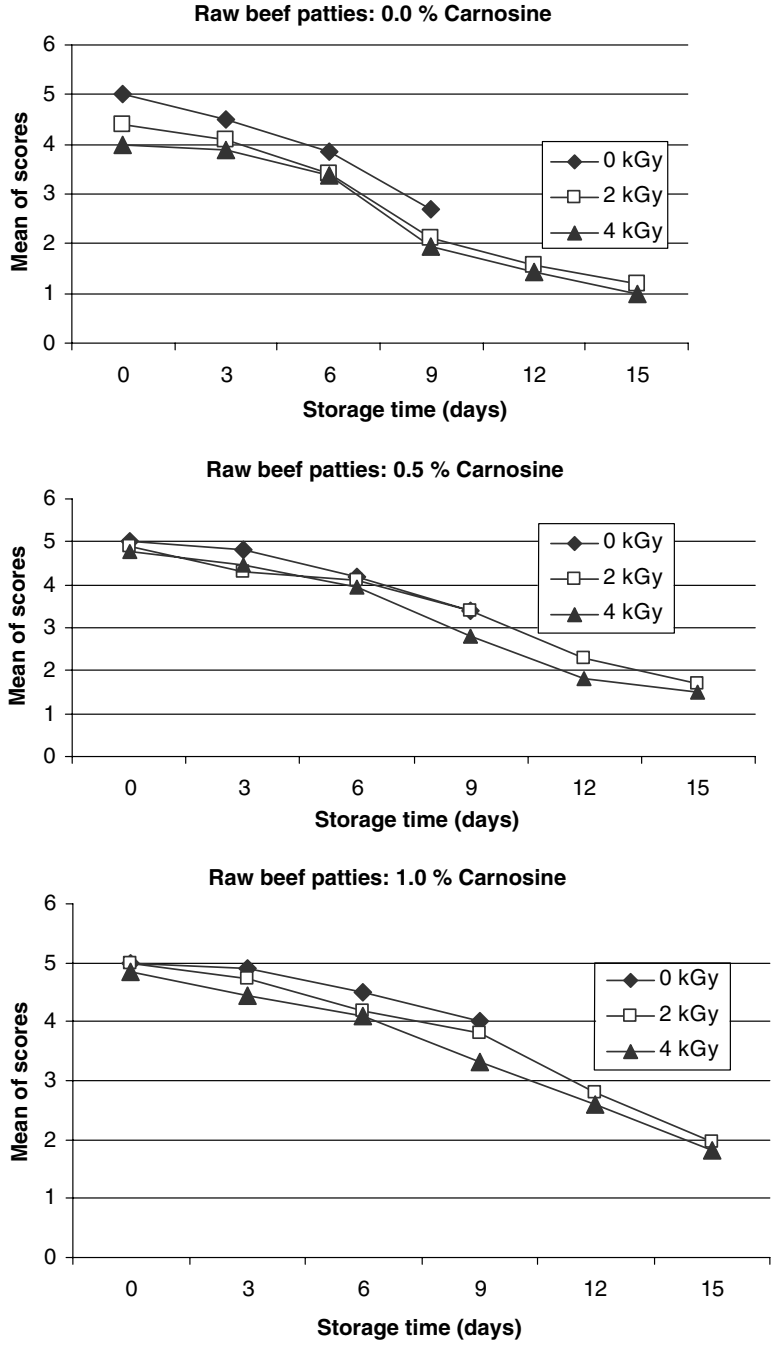

Fig. 3b. Influence of carnosine addition on the visual color of refrigerated stored irradiated and non-irradiated raw beef patties. Control non-irradiated samples were rejected after 9 days for the deterioration of odor and their values were discarded after statistical analysis.

3.4. Influence of carnosine on metmyoglobin accumulation in irradiated and non-irradiated ground beef and raw beef patties during refrigeration and frozen storage

On day zero, addition of carnosine showed no significant ($P > 0.05$) effects on the contents of MetMb in control non-irradiated ground beef and raw patties neither at concentration of 0.5% nor at 1% (Tables 5 and 6). Treatment of ground beef and raw beef patties by gamma irradiation in the absence of carnosine significantly ($P < 0.05$) increased the accumulation of MetMb in a dose-dependant manner. It has been reported that lipid oxidation and metmyoglobin accumulation show a close similarity in their progression (Chan, Faustman, & Decker, 1997; Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2001). Previous studies on meat discoloration have also indicated that meat heme pigments initiate and catalyze the oxidation of muscle tissue lipids, and then free radicals, produced during lipid oxidation, can oxidize and decompose the heme pigments causing discoloration of meat (Bekhit et al., 2003; Faustman & Cassens, 1990).

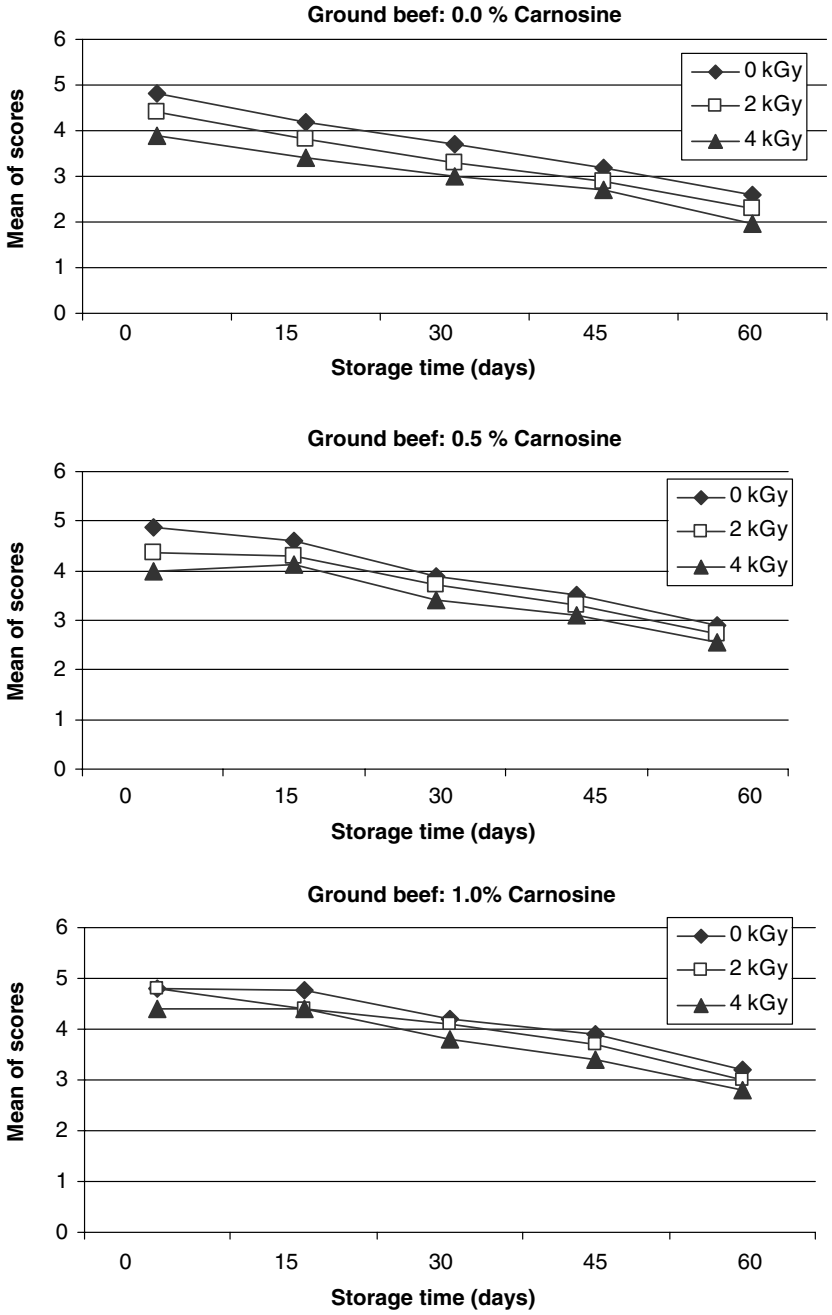

Fig. 4a. Influence of carnosine addition on the visual color of frozen stored irradiated and non-irradiated ground beef.

Presence of carnosine in ground beef and raw patties significantly ($P < 0.05$) decreased the accumulation of MetMb due to irradiation treatments with increasing the added concentration (Tables 5 and 6). Moreover, carnosine addition exerted further significant ($P < 0.05$) inhibitory effect on the MetMb accumulation during refrigeration and frozen storage of both irradiated and non-irradiated samples. After 15 days of refrigeration storage (4 ± 1 °C), presence of carnosine at 0.5% and 1% reduced the accumulation of MetMb by 22.3% and 32.1% and 22.4% and 32.1% in samples of ground beef and raw patties exposed to 4 kGy radiation dose, respectively. However, addition of carnosine at these concentrations decreased the contents of MetMb in the above-mentioned samples by 24% and 34% and 24% and 28.5%, respectively, after 60 days of frozen storage (−18 °C). The effectiveness of carnosine in delaying MetMb formation was previously reported by Sánchez-Escalante et al. (2001, 2003).

3.5. Visual color attributes

Figs. 3 and 4 represent the effects of carnosine addition on the visual color scores for irradiated and non-irradiated ground beef and raw beef patties during refrigeration

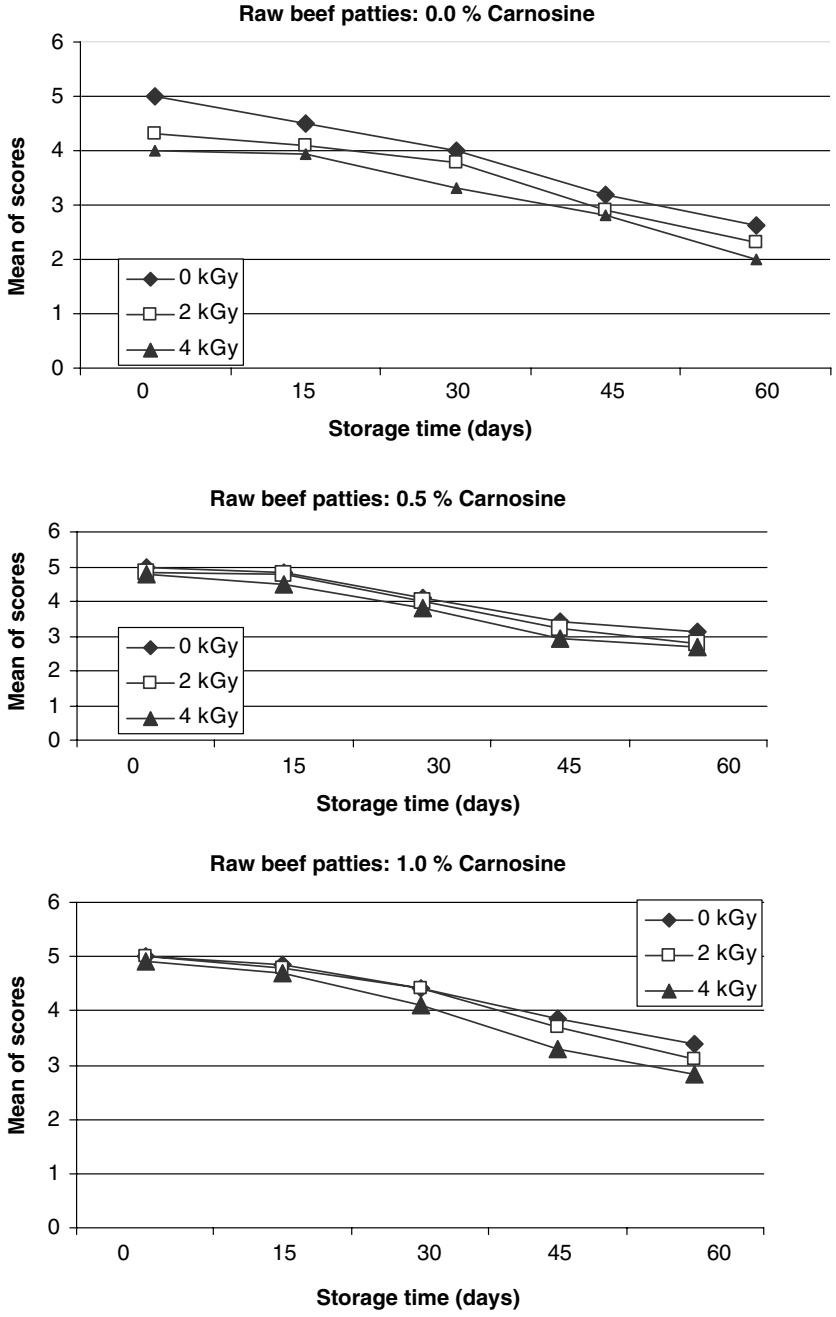

Fig. 4b. Influence of carnosine addition on the visual color of frozen stored irradiated and non-irradiated raw beef patties.

(4 ± 1 °C) and frozen (−18 °C) storage. As shown, presence of carnosine had a significant ($P < 0.05$) effect in maintaining an acceptable red color of irradiated and non-irradiated ground beef and raw patties during refrigeration and frozen storage. Visual color scores showed that irradiated samples without added carnosine were less red/more brown than those irradiated in the presence of carnosine. The results of sensory scores agreed with those of MetMb formation and both were similar to the findings of Greene, Hsin, and Zipser (1971). These authors reported that 40% metmyoglobin caused meat rejection by consumers. The efficacy of carnosine in maintaining meat color during storage was also reported by Lee, Hendricks, and Cornforth (1999) and Sánchez-Escalante et al. (2003). They illustrated that the color stabilizing effects of carnosine may be due to its ability to chelate transition metals involved in free radical generation and/or free radical scavenging, thereby delaying the oxidation of oxymyoglobin to metmyoglobin.

4. Conclusions

The results of this study indicated that carnosine significantly reduced the acceleration of oxidation due to irradiation and storage of ground beef and raw or cooked beef patties, and slowed down the formation of metmyoglobin post-irradiation and during storage of raw meat samples. These results demonstrated that carnosine has a good potential as a natural antioxidant in irradiated raw and cooked meat products.

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