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Effect of carnosine preblending on the quality of ground buffalo meat

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Abstract

A study was conducted on carnosine preblending at 0%, 0.5%, 1.0% and 1.5% levels with ground buffalo meat obtained from spent, adult, male Murrah buffalo carcasses, to identify the level of carnosine required for improving the quality of the meat during refrigerated storage at 4 ± 1 °C. It was observed that meat samples containing 1.0% and 1.5% carnosine significantly inhibited metmyoglobin formation and brown colour development. Carnosine also improved meat pH, and water-holding capacity and lowered cooking loss and 2-thiobarbituric acid-reacting substances (TBARS) values as compared to control sample. Carnosine also improved desired visual colour and odour, and gave higher LTCU 'R' and chroma of meat samples. Visual colour was inversely correlated with metmyoglobin, aerobic mesophiles and psychrotrophs plate count, and odour was inversely correlated with TBARS values. Use of 1.0% carnosine for preblending extended the shelf life of ground buffalo meat up to 8 days under refrigerated storage.

Keywords: Carnosine; Ground buffalo meat; Preblending; Meat quality

1. Introduction

As a world leader in buffalo population, India possesses about 96.9 million animals which is about 58% of the world population (FAO, 2004). More than 60% of meat produced in India is from buffalo and cattle, and is producing more than 45.56% of global buffalo meat. Buffalo meat is coarse and tough as it is produced from aged and spent animals. Development of various comminuted meat products offers a profitable utilization of such tough meat (Anjaneyulu, Lakshmanan, Sharma, & Kondaiah, 1990). But such ground meat tends to become rancid and brown more rapidly, due to pigment and lipid oxidation. An oxidative reaction in muscle foods leads to degradation of lipid and proteins, resulting in deterioration of flavour, texture and nutritive value, and is considered as one of the major problems in the development of new convenient meat products and processes (Gray & Pearson, 1987). Moreover, mechanisms for the control of lipid oxidation in meats have become increasingly important with the rise in popularity of pre-cooked and convenience foods. Many substances have been investigated as potential antioxidants to prevent such lipid oxidation. These include chemical substances such as butylated hydroxyl anisole, propyl gallate, butylated hydroxytoluene, phosphates, citric acid and nitrite, nutritive antioxidants (α-tocopherol, β-carotene and vitamin C) and spice extracts (Chan, Decker, Lee, & Butterfield, 1994; King, Uijttenboogaart, & Vries, 1995; Sahoo & Anjaneyulu, 1997; Schaefer, Liu, Faustman, & Yin, 1995).

Since the use of synthetic antioxidants has become less acceptable in recent years, the interest in the application of naturally-occurring antioxidants in muscle foods has increased. Carnosine is a naturally occurring muscle dipeptide (Djenane & Roncales, 2004; Yanai,

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Shiotani, Mizuno, Nabetani, & Nakajima, 2004) and has excellent potential for food use as natural antioxidant in processed foods (Das, Dey, & Sharma, 2003; Decker & Faraji, 1990). Carnosine inhibits the catalysis of lipid oxidation by a wide range of pro-oxidants (Bekhit et al., 2004) and acts as a free radical-scavenger, metal chelator and hydrogen donor (Chan & Decker, 1994). Carnosine retains its antioxidative effectiveness over a pH range of 5.1–7.1 and in heated aqueous solution (Decker & Faraji, 1990). Some workers have attempted to use it to improve quality of salted ground pork (Decker & Crum, 1991), chicken meat (O'Neill, Galvin, Morrissey, & Buckley, 1998) and ground beef (Lee, Hendricks, & Cornforth, 1999) but its effects in ground buffalo meat have not been explored. The objective of the present study was to optimize the concentration of carnosine required for preblending of ground buffalo meat, to minimize pigment and lipid oxidation and also to improve meat quality during refrigerated storage.

2. Materials and methods

2.1. Source of meat samples

The samples, comprising mostly *Semitendinosus*, *Semimembranosus*, *Biceps fimoris* and *Qudriceps* muscles of spent, adult, male (of about 10–12 years age) Murrah buffalo carcasses, slaughtered according to traditional halal method at the buffalo slaughter house of Bareilly Municipal Corporation, were collected within 5 h of slaughter, packed in low-density polyethylene (LDPE) bags and brought to the Meat Technology Laboratory of the Livestock Products Technology Division, Indian Veterinary Research Institute, Izatnagar. The samples were placed, within 20 min, in a refrigerator at 4 ± 1 °C for about 24 h for conditioning.

2.2. Sample preparation

The meat chunks, after conditioning, were trimmed of separable fat and loose connective tissue, cut into small cubes and minced with a Seydelmann meat grinder (Model WD 114, Germany) using 8 mm (coarse) and 3 mm (fine) plates, simultaneously, to obtain ground buffalo meat (GBM). The test solution was added at 0%, 0.5%, 1.0% and 1.5% to the ground buffalo meat and blended for one minute in a Hobart food mixer. The pH of the test solution was not adjusted. The test solution pH value of carnosine (10%) before treatment was 8.4. 1.4 kg ground buffalo meat was preblended, separately, for each experimental group and divided into 200 g aliquots, packaged in LDPE bags, sealed and stored in a refrigerator at 4 ± 1 °C. The samples (both control and treated) were examined at two day intervals, over a 12 day storage period, to study various physico-chemical and microbiological quality parameters.

2.3. Analytical methods

pH was determined by dipping a combined glass electrode of a digital pH meter (Elico, Model LI 127, India) into the meat suspension, prepared by blending 10 g GBM with 50 ml of distilled water for 1 min (Trout et al., 1992). Water-holding capacity (WHC) of meat was estimated by a centrifugation method (Wardlaw, McCaskill, & Acton, 1973). Cooking loss was determined (Anjaneyulu, Sharma, & Kondaiah, 1989) by heating approximately 25 g of meat sample in a polypropylene bag at 80 °C for 20 min using a thermostatically controlled water bath. Colour score was determined (Sahoo & Anjaneyulu, 1997) by using a 5-point scale, where 1 = pale pink, 2 = pink, 3 = pinkish red, 4 = bright red and 5 reddish brown. Lovibond Tintometer red (LTCU 'R'') and yellow (LTCU 'Y') colour units were recorded using a Lovibond Tintometer (model E, UK). The sample colour was matched by adjusting red (a) and yellow (b) units, while keeping the blue units fixed at 1.0. The hue and chroma of meat were determined by using the formula $(\tan^{-1})^{b/a}$ and $(a^2 + b^2)^{1/2}$, respectively, where a = red units, b = yellow units (Froechlich, Gullet, & Usborne, 1983; Little, 1975). Total meat pigment and metmyoglobin % were estimated, following the procedures described by Arganosa and Hendrickson (1969) and Trout (1989), respectively. Sensory score for the meat odour was obtained using a 5-point scale, where 1 = very unpleasant, 2 = moderately unpleasant, 3 = moderately pleasant, 4 = pleasant and 5 = very pleasant. The distillation method described by Tarladgis, Watts, Younathan, and Dugan (1960) was followed to measure 2-thiobarbituric acid-reacting substances (TBARS) number of the meat. To study the microbiological quality during storage, aerobic mesophiles count (AMC) and psychrotrophs plate count (PPC) were calculated using Hi-Media plate count agar as in the standard procedure (APHA, 1984).

2.4. Statistical analysis

The total experiment was replicated thrice. Observed data up to 10 days of storage were statistically analyzed using a randomized block design. The data were subjected to analysis of variance (Snedecor & Cochran, 1994) and Duncan's new multiple range test (Steel & Torrie, 1981) and interpreted. Correlation coefficients (r-value) between different quality parameters were calculated. A regression line was drawn to predict correlation of metmyoglobin percent and TBARS number with the help of the statistical software package, MIECOSTAT on an IBM-compatible personal computer.

3. Results and discussion

3.1. pH, water-holding capacity and cooking loss

The pH, water-holding capacity (WHC) and cooking loss (CL) of the carnosine-treated ground buffalo meat samples stored in refrigerated storage (4 ± 1 °C) are presented in Table 1. It was found that the pH values of the carnosine-treated samples were significantly ($p < 0.05$) higher than those of the control sample. The pH showed inconsistency as the carnosine level differed. There were significant effects ($p < 0.05$) of carnosine treatment and treatment × storage interaction on pH of meat. We used carnosine commercially obtained from Sigma. Moreover, the pH of the test solutions (8.4) to investigate an increase in meat pH was not adjusted. Addition of 1.0% carnosine to ground buffalo meat increased pH by about 0.5 of a unit on day 0. The pH ranged from 5.74 to 6.13 in the treated samples whereas it was 5.60 in control samples. Furthermore, pH of the meat samples significantly increased beyond 6 days of storage. A similar finding was reported by Lee et al. (1999) who observed such results in ground beef.

Water-holding capacity was significantly ($p < 0.05$) affected by carnosine treatment and its interaction with storage period. All treated samples showed significantly higher water-holding capacity (13.87–14.22) than control (11.73) sample whereas, within treatment group, there were no significant differences of WHC. The results of the present study were in accordance with the finding of Lee et al. (1999). The higher water-holding capacity values in ground beef can also be partly explained by the increased meat pH (Bernthal, Booren, & Gray, 1991). As pH values are increased above the isoelectric pH of proteins, there is an increase in water-holding capacity. The above results thus indicated that carnosine treatment improved the functional properties of muscle proteins.

Carnosine decreased cooking loss % significantly ($p < 0.05$). The cooking loss of meat samples containing 1.0% and 1.5% carnosine showed the lowest loss (33.99–34.60), significantly below the 0.5 level of carnosine (35.46) and the control batch (36.65). There is a clear tendency for the water-holding capacity to increase with increasing meat pH (Thomsen & Zeuthen, 1988). The increase in meat pH by carnosine probably accounts for the observed decrease in cooking loss.

3.2. Visual and instrumental colour of meat

The visual colour scores (CS), i.e. Lovibond Tintometer colour units (LTCU 'R' for red and LTCU 'Y' for yellow) of the carnosine-treated ground buffalo meat samples are presented in Table 2. Colour is an important criterion for consumer acceptability. A significant ($p < 0.05$) improvement was found in respect of visual and instrumental colour of ground buffalo meat containing carnosine, as identified by higher colour scores and higher Lovibond Tintometer red (LTCU 'R') and yellow (LTCU 'Y') colour units, respectively (Table 2).

Table 1
Effect of carnosine level on pH, water-holding capacity and cooking loss of GBM during refrigerated storage (4 ± 1 °C)

Treatments ($n = 3$)	Storage periods (days)						Treatment [mean ± SD ($n = 18$)]
	0	2	4	6	8	10	
pH							
Control	5.54	5.56	5.59	5.60	5.67	5.70	$5.60^{c} \pm 0.07$
0.5% Carnosine	5.67	5.69	5.71	5.72	5.74	5.83	$5.74^{b} \pm 0.09$
1.0% Carnosine	6.02	6.03	6.06	6.09	5.96	6.29	$6.07^{a} \pm 0.39$
1.5% Carnosine	5.99	6.01	6.10	6.20	6.16	6.34	$6.13^{a} \pm 0.33$
Storage	5.81^{b}	5.82^{b}	5.87^{b}	5.90^{ab}	5.87^{b}	6.05^{a}	
Mean ± SD	±0.35	±0.34	±0.33	±0.35	±0.33	±0.35	
Water-holding capacity (%)							
Control	12.56	10.84	11.61	8.48	12.43	14.46	$11.73^{b} \pm 1.90$
0.5% Carnosine	14.73	14.50	14.49	13.11	13.28	14.11	$14.04^{a} \pm 0.78$
1.0% Carnosine	15.06	14.45	14.67	15.13	12.62	11.32	$13.87^{a} \pm 1.75$
1.5% Carnosine	15.88	15.03	15.73	14.08	12.71	11.91	$14.22^{a} \pm 1.70$
Storage	14.55^{a}	13.71^{b}	14.13^{b}	12.70^{c}	12.76^{c}	12.96^{c}	
Mean ± SD	±1.29	±1.81	±1.60	±2.64	±0.99	±1.80	
Cooking loss (%)							
Control	35.54	37.58	37.44	37.52	34.29	36.52	$36.65^{a} \pm 1.27$
0.5% Carnosine	35.80	35.55	35.39	35.33	35.19	35.51	$35.46^{b} \pm 0.45$
1.0% Carnosine	34.85	34.64	34.52	34.67	34.05	34.86	$34.60^{c} \pm 0.83$
1.5% Carnosine	34.68	34.26	33.91	33.53	33.12	34.43	$33.99^{d} \pm 0.93$
Storage	35.47^{a}	34.51^{a}	35.32^{a}	35.26^{a}	34.16^{b}	35.33^{a}	
Mean ± SD	±1.01	±1.49	±1.52	±1.61	±1.03	±0.92	

[a–c] Means in a row or column with different superscripts are different ($p < 0.05$), SD = Standard deviation.

Table 2
Effect of carnosine level on visual and instrumental colour of GBM during refrigerated storage (4 ± 1 °C)

Treatments ($n = 3$)	Storage periods (days)						Treatment [mean ± SD ($n = 18$)]
	0	2	4	6	8	10	
Colour (5 point)							
Control	4.27	4.67	3.99	3.26	2.16	1.61	$3.41^{c} \pm 1.22$
0.5% Carnosine	4.80	4.72	4.49	3.75	2.73	1.82	$3.71^{b} \pm 1.60$
1.0% Carnosine	4.54	4.84	4.54	3.94	2.98	2.11	$3.83^{ab} \pm 1.06$
1.5% Carnosine	4.74	4.95	4.62	4.03	3.05	1.97	$3.89^{a} \pm 1.11$
Storage	4.70^{a}	4.80^{a}	4.41^{b}	3.74^{c}	2.73^{d}	1.88^{e}	
Mean ± SD	±0.42	±0.17	±0.33	±0.42	±0.49	±0.34	
Lovibond Tintometer colour units (red)							
Control	10.43	10.39	8.29	7.92	6.52	4.51	$8.01^{d} \pm 2.15$
0.5% Carnosine	11.16	10.58	9.37	9.01	8.09	4.98	$8.86^{c} \pm 2.05$
1.0% Carnosine	10.46	11.01	10.22	10.43	9.79	6.58	$9.74^{b} \pm 1.50$
1.5% Carnosine	10.91	11.55	11.63	10.61	10.98	9.60	$10.88^{a} \pm 0.78$
Storage	10.74^{a}	10.88^{a}	9.88^{b}	9.49^{c}	8.85^{d}	6.42^{e}	
Mean ± SD	±0.42	±0.52	±1.31	±1.46	±1.76	±2.08	
Lovibond Tintometer colour units (yellow)							
Control	9.11	8.80	6.94	7.05	5.67	4.12	$6.95^{d} \pm 1.76$
0.5% Carnosine	9.22	8.62	8.29	8.10	6.64	4.35	$7.54^{c} \pm 1.67$
1.0% Carnosine	8.99	8.82	9.92	9.59	9.43	8.25	$9.17^{a} \pm 0.71$
1.5% Carnosine	9.48	9.08	9.20	8.34	8.36	7.81	$8.71^{b} \pm 0.73$
Storage	9.20^{a}	8.83^{b}	8.59^{c}	8.27^{d}	7.52^{e}	6.13^{f}	
Mean ± SD	±0.48	±0.47	±1.17	±0.97	±1.53	±1.97	

[a–f] Means in a row or column with different superscripts are different ($p < 0.05$), SE = Standard deviation.

Addition of 1.0% and 1.5% of carnosine contributed the highest colour scores (3.83, 3.89), LTCU 'R' (9.74, 10.88) and LTCU 'Y' (9.17, 8.71). Samples treated with carnosine maintained a reddish colour for the 6–8 day storage period. This is probably, in part, due to the increased meat pH, which is associated with darker reddish colour (Egbert & Cornforth, 1986). However, Lee et al. (1999) and Djenane et al. (2004) also showed a colour-stabilizing effect by carnosine without an increase in meat pH when the carnosine solution was adjusted to the initial meat pH. There was no difference in the colour scores between 0 and 2 days of storage. Subsequently as the storage period increased, there was significant ($p < 0.05$) decrease of LTCU 'R' from the fourth day and LTCU 'Y' from day 2 of refrigerated storage. All the samples lost visual appeal at the eighth day except for the 1.0% and 1.5% levels. Colour scores of meat showed negative correlation ($r = -0.28$) with pH and positive correlation with LTCU 'R' and LTCU 'Y' (Table 4). Colour-stabilizing effects of carnosine in pork and beef were also reported by Decker and Crum (1991) and Lee et al. (1999). Hue of the meat samples was significantly affected by the treatments and period of storage. All the treated samples had significantly higher chroma than control (data not presented). Further, the 1.5% carnosine level produced the highest (13.94) chroma. There was significant change of chroma until day 2 of storage but it decreased gradually as the storage period increased. The probable reason for the higher chroma value might be the effect of preblending with carnosine.

3.3. Total meat pigment and metmyoglobin

The total meat pigment (data not presented) in the present study varied from 0.37% to 0.46%, depending on treatment and period of storage, which is almost similar to the findings of Kulkarni (1989). The colour of meat varies, depending on the state of myoglobin. Ground meat tends to become brown and rancid more rapidly than whole retail cuts. In this study, ground buffalo meat was blended with the test solutions. This process not only increases air contact, but also may accelerate loss of intracellular reductants, thereby resulting in higher metmyoglobin formation. However, treatment of samples with carnosine significantly ($p < 0.05$) inhibited metmyoglobin formation (Table 3). The meat samples treated with 1% carnosine showed the lowest (56.29%) metmyoglobin content. Retardation in the formation of the metmyoglobin in beef, due to the effect of carnosine, was also reported by previous researchers (Bekhit et al., 2004; Lee et al., 1999). The metmyoglobin content increased significantly from 56.1% to 62.2% during 10 days of refrigerated storage. Highly significant effects of the storage period on increase in metmyoglobin in ground buffalo meat were also reported by Sahoo and Anjaneyulu (1997). Signs of brownish discoloration were observed when at least 60% of unstable, reduced myoglobin pigments in a particular area of meat become oxidized to metmyoglobin (Armstrong, 1993). Although many factors can influence meat colour stability, metmyoglobin formation by free radicals is predominant (Renerre & Labas, 1987). Carnosine can also act as a

Table 3
Effect of carnosine level on pigment oxidation of ground buffalo meat during refrigerated storage (4 ± 1 °C)

Treatments ($n = 3$)	Storage periods (days)						Treatment [mean ± SD ($n = 18$)]
	0	2	4	6	8	10	
Metmyoglobin (%)							
Control	57.9	62.6	62.2	64.5	65.4	68.3	$63.5^{a} \pm 3.27$
0.5% Carnosine	56.4	61.6	61.5	61.9	62.1	62.2	$60.1^{b} \pm 2.31$
1.0% Carnosine	54.8	55.1	57.4	56.0	56.1	58.4	$56.3^{c} \pm 1.45$
1.5% Carnosine	55.3	58.7	56.4	61.7	59.0	60.1	$58.5^{d} \pm 2.28$
Storage	56.1^{d}	59.5^{c}	59.4^{c}	61.0^{b}	60.7^{b}	62.2^{a}	
Mean ± SD	±1.35	±3.10	±2.71	±3.25	±3.57	±3.89	
Odour score (5 point)							
Control	4.91	4.72	3.89	3.22	2.23	1.42	$3.39^{c} \pm 1.47$
0.5% Carnosine	4.65	4.89	4.69	3.97	3.41	1.91	$3.92^{b} \pm 1.11$
1.0% Carnosine	4.95	4.77	4.70	4.62	3.86	2.89	$4.23^{a} \pm 0.88$
1.5% Carnosine	4.93	4.89	4.52	4.41	3.16	2.33	$4.04^{ab} \pm 0.99$
Storage	4.86^{a}	4.82^{a}	4.42^{b}	4.05^{c}	3.19^{d}	2.04^{e}	
Mean ± SD	±0.42	±0.29	±0.82	±0.65	±0.73	±0.48	
TBARS number (mg malonaldehyde/kg)							
Control	0.48	0.59	0.68	1.15	1.12	1.27	$0.88^{a} \pm 0.53$
0.5% Carnosine	0.34	0.51	0.69	0.71	0.93	1.15	$0.72^{b} \pm 0.29$
1.0% Carnosine	0.29	0.48	0.48	0.69	0.76	0.92	$0.60^{c} \pm 0.23$
1.5% Carnosine	0.31	0.46	0.60	0.65	0.81	0.97	$0.63^{c} \pm 0.25$
Storage	0.36^{f}	0.51^{e}	0.61^{d}	0.80^{c}	0.90^{b}	1.08^{a}	
Mean ± SD	±0.36	±0.14	±0.14	±0.27	±0.17	±0.21	

[a–f] Means in a row or column with different superscripts are different ($p < 0.05$), SD = Standard deviation.

reductant. The ability of carnosine to donate an electron to free radicals is generally related to its antioxidant activity (Kohen, Yamamoto, Cundy, & Ames, 1988). Hence the results of the study indicated that the effect of carnosine on meat colour was beneficial, due to inhibition of metmyoglobin formation. In the present study, 1.0% carnosine in ground buffalo meat contributed to its acceptable fresh meat colour over more than 8 days of refrigerated storage. Metmyoglobin % showed significant ($p < 0.01$) positive correlation ($r = 0.66$) with TBARS values (Table 4). The reason behind such positive correlation may be that metmyoglobin act as primary initiator of lipid oxidation in meat (Johns, Birkinshaw, & Ledward, 1989). To predict the TBARS number, a regression equation ($Y = -2.775 + 0.0583X$)

Table 4
Correlation coefficient (r-value) of pH, colour, metmyoglobin and odour with different quality characters of carnosine-treated ground buffalo meat during refrigerated storage (4 ± 1 °C)

	pH	Colour	Metmyoglobin	Odour
Colour	−0.28	–	–	–
LTCU (red)	0.18*	0.79**		
LTCU (yellow)	0.13	0.72**	–	–
Metmb	−0.25**	−0.51**	–	–
Odour	–	0.67**	–	–
TBARS	–	–	0.66**	−0.78**
TPC	0.21*	−0.56**	–	−0.52**
PC	0.21*	−0.62**	–	−0.54**

LTCU = Lovibond Tintometer colour units.
* $p < 0.05$.
** $p < 0.01$.

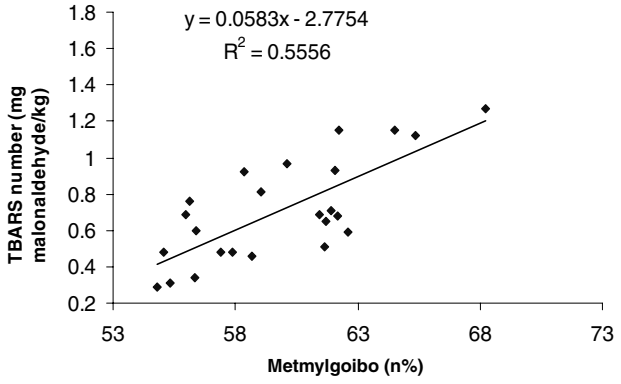

Fig. 1. Relationship of metmyoglobin accumulation and lipid oxidation in ground buffalo meat preblended with carnosine during refrigerated storage (4 ± 1 °C).

was established, where Y = TBARS number and X = metmyoglobin % (MMb) (Fig. 1). A similar finding in ground beef was also reported by Faustman, Specht, Malkus, and Kinsman (1992).

3.4. Odour and TBARS number

Odour, like colour of fresh meat is also an important criterion for its acceptability. TBARS number shows the oxidation changes of the meat lipids. In the present study, the meat samples treated with carnosine had significantly ($p < 0.05$) higher odour scores and lower TBARS numbers than the control. Among the treated samples, the 1.0% carnosine level showed highest

(4.23) odour score and lowest (0.60) TBARS number (Table 3). Lee, Hendricks, and Cornforth (1998) reported that carnosine (0.1–5 mM) treatment to pre-rigor beef or cooked beef significantly inhibited lipid peroxidation during 9 days of storage. Decker and Crum (1991) also reported that carnosine (0.5% and 1.5%) more effectively inhibited lipid peroxidation in frozen pork stored for up to 6 months compared with sodium tripolyphosphate and other lipid-soluble antioxidants, such as α-tocopherol and butylated hydroxytoluene.

The desirable meat colour in the present study started declining significantly ($p < 0.05$) from the fourth day of refrigerated storage. Previous workers reported that carabeef had a shelf life of 5 days and spoilage started on the sixth day, manifested by off-odour, slime formation and greenish discoloration due to microbial proteolysis (Sison et al., 1980), especially by psychrotrophilic spoilers such as pseudomonads in aerobic storage (McDowell, Hobson, Strain, & Owens, 1986). The off-odour development in the meat samples in the present study was well in agreement with the findings of the above researchers. There was a significant negative correlation of odour scores with TBARS number, aerobic mesophiles count and psychrotroph plate count (Table 4). This result indicates that the acceptable odour of meat is dependent on several quality parameters.

The higher TBARS values of the control sample at certain storage days might be due to an interaction between the natural substances (for example, polyunsaturated fatty acids) and catalysts (for example, iron ion) from the meat tissue during storage (Decker & Hutlin, 1990; Kim, Godber, & Prinaywiwatkul, 2000). Such storage would eventually increase the degradation of heme compounds and release the low-molecular-weight iron compounds in beef, which is hypothesized to be responsible for lipid peroxidation (Kenner & Harel, 1985; Kim et al., 2000). There was a significant linear increase of TBARS number from 0.36 at 0 day to 1.08 at the end of 10 days of refrigerated storage of ground buffalo meat. TBARS number was previously found to be 0.20 (Anjaneyulu, 1988) and 0.38 (Kesava Rao, 1988; Sahoo & Anjaneyulu, 1997) in fresh buffalo meat, which is comparable with the present study. TBARS numbers in treated samples were much lower than the control sample. The inhibitory effect of carnosine on lipid oxidation might be due to scavenging of free radicals and chelating of transition metals (Chan et al., 1994; Lee & Hendricks, 1997; Niki, 1991). As carnosine is capable of inactivating a wide range of aqueous and lipid-phase-generated prooxidants, it may be expected to exert a strong antioxidant effect in meats (O'Neill et al., 1998).

3.5. Microbial quality

The aerobic mesophilic count (AMC) and psychrotrophic plate count (PPC) of ground buffalo meat were significantly affected by carnosine treatment. Higher microbial load occurred on meat samples treated with carnosine than control sample. Higher pH values of carnosine-treated meat samples might favour the microbial growth, leading to such an observation. The AMC and PPC in control samples were log 5.38/g and log 4.33/g, respectively, while, in treated samples, it ranged from log 5.61 to log 5.71/g and log 4.59/g to log 4.68/g, respectively. AMC and PPC did not significantly change until the ends of the sixth and eighth day of storage, respectively, but increased during later storage. The overall mean of AMC in the present study increased from log 5.35/g at 0 day to log 6.54/g at 10 days' storage, while PPC increased from log 4.13/g to log 5.44/g. The findings of the present study were comparable with those of previous workers (Agnihotri, 1988; Anjaneyulu, 1988). They reported that standard plate count of ground buffalo meat ranged from log 5.46/g to log 7.06/g during refrigerated storage. They also observed that PPC increased up to log 6.62/g in ground buffalo meat after 10 days of refrigerated storage. The samples in the present study were well within the level of the incipient spoilage of meat, i.e. log 7.0/g (Hytiainen, Pohja, & Niskanen, 1975) at the end of the storage period. AMC and PPC were negatively correlated ($p < 0.01$) with colour score and odour score, and positively correlated with metmyoglobin (MMb) and TBARS number. This indicates that, as the pigment and lipid oxidation increased, the discolouration and off-odour of the meat also increased. These changes were due to increase in bacterial load of the meat samples. It was also observed that, as AMC increased during the storage period, the metmyoglobin content also increased (Fig. 2), indicating a relationship between bacterial load and discolouration problem in the meat. In this context, Schweigert (1956) found that the meat discolouration problem was due to increase in bacterial population, which in turn reduces the oxygen tension on the surface of meat. In the present study, the aerobic mesophiles increased

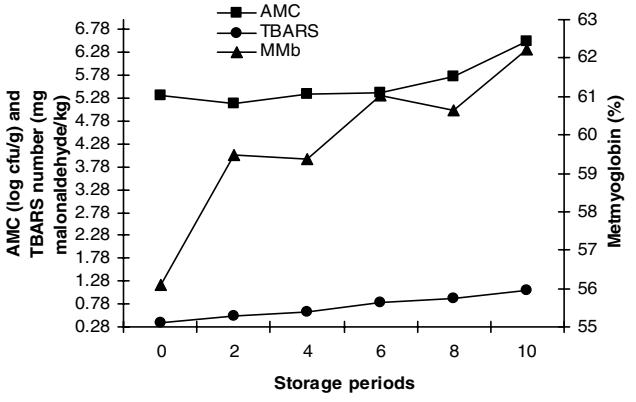

Fig. 2. Changes in metmyoglobin content, TBARS number and microbial load in ground buffalo meat preblended with carnosine during refrigerated storage (4 ± 1 °C).

significantly, which might have used up the available oxygen, thus reducing the oxygen tension and causing surface discolouration. In the present study it was also found that there was a significant relation between AMC and TBARS number (Fig. 2). As the bacterial load increased in meat samples, the TBARS number also increased, causing off-flavour and off-odour. Earlier workers also reported that some bacteria, namely *Pseudomonas ovalis*, *Micrococcus freudenseichii* and strains of *Streptomyces*, bring about oxidation by production of peroxides, carbonyls, aldehydes and ketones or similar compounds (Smith & Alford, 1969).

4. Conclusion

Carnosine is a well known antioxidant. It can inhibit metmyoglobin formation as well as lipid peroxidation of ground buffalo meat by several mechanisms, including their reducing, free radical-scavenging and/or chelating activities. Based on the above findings, it was concluded that the shelf life of ground buffalo meat treated with 1.0% carnosine could be extended to 8 days in refrigerated storage conditions, without any undesirable changes in colour or odour, whereas the control sample could be kept for up to 6 days only. So, carnosine, as a food or meat additive, may be useful, due to its strong effects in preventing off-flavour formation and also it may increase shelf life of meat and meat products to a limited extent when compared with the untreated groups.

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