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Effect of alpha-tocopherol acetate preblending on the quality of ground buffalo meat

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Ground buffalo meat (GBM) was preblended with alpha-tocopherol acetate (TA) @ 0, 5, 7.5, 10 and 12.5 ppm level and was examined for its quality changes during refrigerated storage at 4±1°C. It was observed that the meat samples containing 10 ppm TA had significantly lengthened desired visual colour and odour, higher LTCU 'R' and chroma and lower cooking loss, metmyoglobin content and TBARS number as compared to other treated samples. Visual colour was inversely correlated with metmyoglobin, aerobic mesophiles and psychrotrophs plate count, and odour was inversely correlated with TBARS number. Use of 10 ppm TA for preblending extended the shelf life of GBM from 6 to 8 days under refrigerated storage. © 1997 Elsevier Science Ltd

INTRODUCTION

India ranks first and possesses about 50 per cent of world buffalo population. Buffalo meat contributes to about 85 per cent of total meat being exported from our country. Generally the meat is coarse and tough as it is produced from old and unproductive animals. Such tough meat can be profitably utilized by development of various comminuted meat products (Sahoo, 1989; Anjaneyulu *et al.*, 1990). Ground meat tends to become brown and rancid more rapidly than whole muscle retail cuts. Such changes are due to pigment and lipid oxidation. Lipid oxidation in meats leads to the development of off-flavour, loss of colour and nutritive value (Pearson *et al.*, 1983) and is a major problem in the development of new convenience meat products and processes (Gray & Pearson, 1987). Previous researchers had shown that use of chemical substances in meat such as propyl gallate, butylated hydroxy anisole, butylated hydroxytoluene, citric acid, polyphosphates and nitrites prevent lipid oxidation. However, consumers all over the world like to avoid chemical additives in fresh meat. They would prefer natural substances to increase shelf life. Some workers have attempted to improve the quality of beef by use of natural antioxidants such as vitamin C and E (Mitsumoto *et al.*, 1991; Arnold *et al.*, 1993).

Little information is available about buffalo meat. The present study was aimed at optimising the concentration of alpha-tocopherol acetate required for preblending of ground buffalo meat to minimise pigment and lipid oxidation and to improve quality during refrigerated storage.

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MATERIALS AND METHODS

Source of meat samples

The muscles, comprising mostly Semitendinosus, Semimembranosus, Biceps femoris and Quadriceps muscles of spent, adult, female (of about 10 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 within 20 min. The muscle samples were immediately kept for conditioning in a refrigerator at 4±1°C for about 24 h.

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). Alpha-tocopherol acetate (Evion 100 mg pearl, Merck) solution (1 mg/ml) was freshly prepared by dissolving it in 100 ml DHARA refined mustard oil. The solution was added @ 0, 5, 7.5, 10 and 12.5 ppm to GBM and blended for a minute in a Hobart food mixer. 1.4 kg GBM 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 were examined at two day intervals up to a 12-day storage period for various physicochemical and microbiological quality parameters.

Analytic methods

pH was determined by dipping a combined glass electrode of a digital pH meter (Century model CP901) into the meat suspension prepared by blending 10 g GBM with 50 ml distilled water for 1 min (Trout *et al.*, 1992). Water holding capacity (WHC) of meat was estimated by a centrifugation method (Wardlaw *et al.*, 1973). Cooking loss was determined (Anjaneyulu *et al.*, 1989) by heating approx. 25 g meat sample in a polypropylene bag at 80°C for 20 min using a thermostatically controlled water bath. Colour score was determined 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, U.K.). 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 unit, b = yellow unit (Little, 1975; Froehlich *et al.*, 1983). Total meat pigments and metmyoglobin per cent were estimated following the procedure described by Arganosa and Henrickson (1969) and Trout (1989), respectively. Sensory score for meat odour was obtained by following 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 *et al.* (1960) was followed to measure 2-thiobarbituric acid-reacting substances (TBARS) number of the meat. To discover the microbiological quality during storage, aerobic mesophiles count (AMC) and psychrotrophs plate count (PPC) were calculated using Hi-Media plate count agar as per the standard procedure (APHA, 1984).

Statistical analysis

The experiment was replicated thrice. Observed data up to 10 days' storage were statistically analyzed using a randomized block design. The data were subjected to analysis of variance (Snedecor & Cochran, 1994) and Duncans 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 per cent and TBARS number with the help of a statistical software package, MICROSTAT on an IBM-compatible personal computer.

RESULTS AND DISCUSSION

pH, WHC and cooking loss

The pH and WHC (ml/100 g) ranged from 5.54 to 5.61 and 12.85 to 13.89 in alpha-tocopherol acetate (TA)-treated samples, whereas they were 5.58 and 11.32, respectively in the control batch. Neither pH and WHC were significantly affected by treatment on its interaction with storage period. However, the pH of meat samples increased significantly after 6 days of storage, which might be due to increase in microbial growth. There was a nonsignificant decrease of WHC during the refrigerated storage period. The cooking loss (CL) of the meat samples containing 10 and 12.5 ppm TA did not differ significantly, but was lower than other levels of TA and the control batch.

Visual and instrumental colour of meat

Colour is an important criterion for consumer acceptability of fresh meat. A significant ($P<0.05$) improvement was found in respect of visual and instrumental colour of GBM containing TA as evidenced by high colour scores (CS) and higher Lovibond tintometer red (LTCU 'R') and yellow (LTCU 'Y') colour units, respectively (Table 1). Addition of 10 ppm TA contributed to highest CS (3.78), LTCU 'R' (10.83) and LTCU 'Y' (9.07). There was a significant decrease of CS and LTCU 'R' from fourth day and LTCU 'Y' from the sixth day of refrigerated storage. All the treated samples lost visual appeal at the eighth day except for the 10 ppm level. CS of meat showed negative correlation ($r=-0.85$) with pH and positive correlation with LTCU 'R' and LTCU 'Y' (Table 3). Previous researchers reported that vitamin E could preserve the initial colour of meat. The colour of pork chops treated with vitamin E remained stable for 10 days at 4°C under fluorescent light (Armstrong, 1993). The desirable colour of beef from cattle supplemented with vitamin E was extended by 2 to 5 days over the control samples (Klis, 1993).

Hue of the meat samples was not significantly affected by treatments and period of storage. On the other hand, the treated samples had significantly higher chroma than control. Further, the 10 ppm TA level produced the highest (14.13) chroma. There was no significant change of chroma until 4 days of storage, but it decreased gradually as the storage period increased. The probable reason for the increase of chroma might be the effect of preblending with TA.

Table 1. Effect of α-tocopherol acetate level on visual and instrumental colour of ground buffalo meat during refrigerated storage (4 ± 1°C)

Treatments ($n=3$)	Storage period (days)						Treatment mean ± SE ($n=18$)
	0	2	4	6	8	10	
Colour score (5 pt.)							
Control	4.73	4.70	4.00	3.23	2.20	1.53	$3.40^{d} \pm 1.25$
5 PPM	4.77	4.73	4.07	3.73	2.60	1.57	$3.58^{c} \pm 1.19$
7.5 PPM	4.83	4.87	4.20	3.73	2.63	1.67	$3.66^{b} \pm 1.21$
10 PPM	4.87	4.93	4.33	3.87	2.70	2.00	$3.78^{a} \pm 1.14$
12.5 PPM	4.90	4.93	4.37	3.80	2.60	1.90	$3.75^{ab} \pm 1.18$
Day mean	4.82^{a}	4.83^{a}	4.19^{b}	3.67^{c}	2.55^{d}	1.73^{e}	
± SE	±0.13	±0.12	±0.17	±0.27	±0.21	±0.29	
Livibond Tintometer colour units (Red)							
Control	10.60	10.43	8.13	7.90	6.63	4.60	$8.05^{d} \pm 2.35$
5 PPM	11.03	10.47	9.37	9.00	8.07	5.03	$8.83^{c} \pm 2.34$
7.5 PPM	10.43	11.00	10.30	10.43	9.80	6.60	$9.76^{b} \pm 1.92$
10 PPM	10.63	11.23	11.37	10.57	11.20	9.97	$10.83^{a} \pm 0.81$
12.5 PPM	10.37	10.67	10.50	9.80	10.37	9.37	$10.18^{ab} \pm 0.65$
Day mean	10.61^{a}	10.76^{a}	9.93^{ac}	9.54^{bc}	9.21^{bc}	7.11^{d}	
± SE	±0.58	±0.56	±1.22	±1.40	±2.48	±2.38	
Lovibond Tintometer colour units (Yellow)							
Control	9.07	8.73	6.90	7.00	5.57	4.07	$6.89^{c} \pm 1.97$
5 PPM	9.17	8.67	8.17	8.10	6.57	4.27	$7.49^{b} \pm 1.94$
7.5 PPM	8.37	8.57	9.00	8.73	7.90	4.67	$7.87^{b} \pm 1.74$
10 PPM	8.93	8.77	9.83	9.50	9.37	8.00	$9.07^{a} \pm 0.71$
12.5 PPM	9.27	9.00	9.03	8.43	8.70	7.87	$8.72^{a} \pm 0.67$
Day mean	8.96^{a}	8.75^{a}	8.59^{a}	8.35^{a}	7.62^{b}	5.77^{c}	
± SE	±0.58	±0.37	±1.11	±1.32	±1.90	±1.88	

SE = Standard error

$^{a-e}$ Means in a row or column with different superscripts are different ($P < 0.05$).

Total meat pigments and metmyoglobin

The quantity of total meat pigments (TMP) in GBM was not significantly changed by addition of TA and subsequent refrigerated storage. The TMP% varied from 0.36 to 0.45 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. Metmyoglobin (MMb), the oxidized meat pigment, is responsible for the undesirable brown colour of fresh meat. In the present study the MMb% of the treated samples was significantly lower than the control batch. The meat samples incorporated with 10 ppm TA showed the lowest (56.8%) metmyoglobin content (Table 2). Retardation in the formation of MMb in beef due to the effect of vitamin E was also reported by previous researchers (Mitsumoto *et al.*, 1991). The MMb content increased significantly from 56.7 to 63.3% during 10 days of refrigerated storage. Highly significant effects of the storage period on increase of MMb in ground beef were also reported by Mitsumoto *et al.* (1991). Signs of brownish discolouration were observed when at least 60% of unstable, reduced myoglobin pigment in a particular area of meat, become oxidized to MMb (Armstrong, 1993). In the present study, 10 ppm TA in GBM contributed to its acceptable fresh meat colour up to 8 days of refrigerated storage. MMb% showed significant ($P < 0.01$) positive correlation ($r = 0.66$) with TBARS number (Table 3). The reason may be due to the fact that MMb act as primary initiator of lipid oxidation in meat (Rhee *et al.*, 1987; Johns *et al.*, 1989). To predict TBARS number, a regression equation ($Y = -2.677 + 0.057X$) was established, where Y = TBARS number and X = MMb%. A similar finding in ground beef was also reported by Faustman *et al.* (1992).

Odour and TBARS number

Like colour in fresh meat, odour is also an important criterion for its acceptability. TBARS number shows the oxidative changes of the meat lipids. In the present study, the meat samples treated with TA had significantly higher odour score (OS) and lower TBARS number than the control. Among the treated samples, the 10 ppm TA level showed highest (4.05) OS and lowest (0.628) TBARS number (Table 2). Mitsumoto *et al.* (1991) also concluded that 10 ppm vitamin E was sufficient to retard lipid oxidation of ground beef during illuminated display at 4°C for 7 days. It was also

Table 2. Effect of α-tocopherol acetate level on pigment and lipid oxidation of ground buffalo meat during refrigerated storage (4 ± 1°C)

Treatments ($n=3$)	Storage period (days)						Treatment mean ± SE ($n=18$)
	0	2	4	6	8	10	
Metmyoglobin (%)							
Control	58.1	63.5	62.0	65.0	65.4	69.1	$63.9^{a} \pm 4.15$
5 PPM	56.7	60.2	61.4	63.0	60.2	63.1	$60.8^{b} \pm 2.90$
7.5 PPM	56.2	62.0	61.2	61.7	58.9	61.5	$60.3^{b} \pm 2.89$
10 PPM	55.4	55.5	57.4	59.0	53.8	59.7	$56.8^{c} \pm 2.90$
12.5 PPM	57.0	61.7	60.1	62.5	59.1	63.0	$60.6^{b} \pm 2.95$
Day mean	56.7^{d}	60.6^{bc}	60.4^{c}	62.2^{ab}	59.5^{c}	63.3^{a}	
± SE	± 1.90	± 3.45	± 2.75	± 2.95	± 4.29	± 4.00	
Odour Score (5 pt.)							
Control	4.80	4.63	3.73	3.27	2.43	1.47	$3.39^{c} \pm 1.22$
5 PPM	4.80	4.70	4.20	4.10	3.23	1.57	$3.77^{b} \pm 1.19$
7.5 PPM	4.77	4.77	4.63	4.33	3.33	1.53	$3.89^{ab} \pm 1.23$
10 PPM	4.93	4.87	4.77	4.53	3.10	2.10	$4.05^{a} \pm 1.11$
12.5 PPM	4.97	4.90	4.47	4.40	3.07	1.90	$3.95^{ab} \pm 1.16$
Day mean	4.85^{a}	4.77^{a}	4.36^{b}	4.13^{c}	3.03^{d}	1.71^{e}	
± SE	± 0.11	± 0.13	± 0.41	± 0.51	± 0.56	± 0.34	
TBARS number (mg malonaldehyde/kg meat)							
Control	0.383	0.613	0.696	1.165	1.107	1.26	$0.871^{a} \pm 0.342$
5 PPM	0.319	0.654	0.663	0.792	1.037	1.15	$0.769^{b} \pm 0.287$
7.5 PPM	0.302	0.561	0.694	0.881	1.064	1.03	$0.755^{b} \pm 0.289$
10 PPM	0.250	0.467	0.554	0.705	0.844	0.95	$0.628^{c} \pm 0.251$
12.5 PPM	0.307	0.585	0.687	0.819	0.941	1.03	$0.729^{b} \pm 0.256$
Day mean	0.312^{f}	0.576^{e}	0.659^{d}	0.872^{c}	0.998^{b}	1.09^{a}	
± SE	± 0.063	± 0.091	± 0.108	± 0.176	± 0.123	± 0.137	

SE = Standard error

$^{a\text{-}f}$Means in a row or column with different superscripts are different ($P < 0.05$).

observed that a high concentration of vitamin E in a model system produced a prooxidant effect on lipid oxidation (Mahoney & Graf, 1986).

The desirable meat odour in the present study started declining significantly 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 discolouration due to microbial proteolysis (Sison *et al.*, 1980), especially by psychrophilic spoilers such as Pseudomonads in aerobic storage (McDowell *et al.*, 1986). The off-odour development in the meat samples of the present study was well in agreement with the findings of the above researchers. There was a significant negative correlation of OS with TBARS number, aerobic mesophiles count and psychrotrophs plate count (Table 3). This indicates that the acceptable odour of meat is dependent on several quality parameters.

There was a significant linear increase of TBARS number from 0.312 at 0 day to 1.086 at the end of 10 days refrigerated storage of GBM. TBARS number was found to be 0.20 (Anjaneyulu, 1988) and 0.38 (Kesava Rao, 1988) in fresh buffalo meat which is comparable with the present study. Other researchers were also of the opinion that oxidative rancidity as shown by TBA number increases rapidly in raw ground meat during refrigerated storage (Greene, 1969; Sato & Hegarty, 1971).

Table 3. Correlation coefficient (*r*-value) of pH, colour, metmyoglobin and odour with different quality characters of α-tocopherol acetate-treated ground buffalo meat during refrigerated storage (4 ± 1°C).

Characters	pH	Colour score	Metmyoglobin	Odour score
Colour score	−0.85**	—	—	—
Lovibond tintometer colour units (Red)	−0.54**	0.73**	—	—
Lovibond tintometer colour units (Yellow)	−0.57**	0.74**	—	—
Total meat pigments	−0.50**	0.53**	—	—
Metmyoglobin	0.48**	−0.45**	—	—
Odour score	−0.82**	0.46**	—	—
TBARS number	—	—	0.66**	−0.85**
Aerobic mesophiles count	0.69**	−0.79**	—	−0.88**
Psychrotrophs plate count	0.70**	−0.79**	—	−0.84**

*$P < 0.05$ **$P < 0.01$

Microbiological quality

The aerobic mesophiles count (AMC) and psychrotrophic plate count (PPC) of GBM were not significantly affected by TA treatment. The AMC and PPC in control samples were log 5.46/g and log 5.2/g, respectively while in treated samples it ranged from log 5.36 to 5.53/g and log 4.94 to 5.12/g, respectively. AMC and PPC did not significantly change till the end of the sixth and eighth day of storage, respectively, but increased during the latter period. The overall mean of AMC in the present study increased from log 5.19/g at 0 day to log 6.48/g at 10 days' storage, while PPC increased from log 4.87 to 5.81/g. The findings of the present study were comparable with those of previous workers (Agnihotri, 1988; Anjaneyulu, 1988; Murthy, 1988) who reported that standard plate counts of GBM ranged from log 5.46 to 7.06/g during refrigerated storage. They also observed that PPC increased up to log 6.62/g in GBM after 10 days of refrigerated storage. The samples in the present study were well below the level of incipient spoilage of meat, i.e. log 7.0/g (Hytiainen *et al.*, 1975) at the end of the storage period.

CONCLUSIONS

Based on the above findings, it was concluded that the shelf-life of GBM treated with 10 ppm alpha-tocopherol acetate could be extended to 8 days in refrigerated storage conditions, without any undesirable changes in colour, odour or microbial load, whereas, the control sample could be kept up to 6 days only.

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