

Lipid and Color Stability as Affected by Combination of Sodium Ascorbate and α-Tocopherol Acetate in Minced Buffalo Meat during Refrigerated Storage

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ABSTRACT

The target of this study was to evaluate the inhibition of oxidative changes of minced buffalo meat during refrigerated storage at 4°C for 6 days by adding a combination of vitamin C and E salts at two different blend levels on lipid and color stability as well as some other quality parameters. A significant difference was observed between the percentage antioxidant activity as a result of these suggested additions. The minced buffalo meat sample B blended with 600-mg/l sodium ascorbate + 5 mg/l α -tocopherol acetate exhibited higher antioxidant activity (P < 0.05) than sample A blended with 400 mg/l sodium ascorbate + 10 mg/l α -tocopherol acetate during the storage period. Both the suggested blend levels, especially in sample B, can help to minimizing TBARS and met-myoglobin accumulation during the storage period. Also, the same sample at the end of the storage period had higher redness (a*) color values, more acceptable visual color, moderately pleasant odor score and lower cooking loss compared to other samples. Thus, a combination of 600 mg/l sodium ascorbate + 5 mg/l α -tocopherol acetate could be utilized effectively for enhancing the shelf life of minced buffalo meat.

Keywords: buffalo meat quality parameters, natural antioxidants, oxidative stability, shelf life of meat, vitamin C and E salts

INTRODUCTION

Buffalo meat production is growing very rapidly in all buffalo-producing countries (Devatkal *et al.* 2004). Buffalo meat, being comparatively cheaper, has additional advantages over other meats (Sachindra *et al.* 2005). Buffalo meat is the chief foreign exchange earner in meat sector (Kandeepan and Biswas 2007). Buffalo meat can be profitably utilized by development of various comminuted meat products (Sahoo 1989; Anjaneyulu *et al.* 1990). Although buffaloes are slaughtered mainly for meat, the by-products available from slaughtered animals are of good value (Anna Anandh *et al.* 2008). Devatkal *et al.* (2004) explored the possibilities of commercial utilization of buffalo liver in comminuted meat products.

Buffalo meat has great potential to provide nutritional security to a considerable population of the world. Salient characteristics of buffalo meat make it more suitable for processing into meat products. Buffalo meat is coarse and tough as it is often produced from aged and spent animals (Das *et al.* 2006). This meat has the potential to produce high-grade meat and meat products and can increase tenderness compared to beef owing to its higher calpain activity in early post-mortem (Neath *et al.* 2007). Various post-mortem treatments can appreciably improve tenderness and other market-oriented quality attributes of meat. Prolonged storage of buffalo meat in refrigerators significantly increases the tenderness (Kandeepan and Biswas 2005).

Development of various comminuted meat products offers a profitable utilization of such tough meat (Anjaneyulu *et al.* 1990). However, such ground meat tends to become rancid and brown more rapidly, due to pigment and lipid oxidation (Das *et al.* 2006). Lipid oxidation in buffalo meat affects the quality of raw and cooked meat and leads to a deterioration in flavor, color, odor quality and nutritive value (Descalzo *et al.* 2008). Moreover, mechanisms to control 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 (Das *et al.* 2006).

Oxidative stability is a central parameter in the estimation of meat quality because of the susceptibility of meat products to oxidative degeneration, which is one of the main causes of spoilage. In meat products oxidative reactions are affected by several factors, including lipid composition and processing, and could be delayed by endogenous or exogenous antioxidants (Sacchetti *et al.* 2008).

Lipid oxidation is an autocatalytic process that occurs in foods and biological membranes. Lipid oxidation is a major cause of muscle food deterioration as it decreases the nutritional properties of foods since it involves the loss of essentially fatty acids, vitamins and the generation of potentially toxic reaction products such as malonaldehyde and cholesterol oxidation products (Tang *et al.* 2001). In addition, lipid oxidation affects essential sensory traits of meat products, causing flavor, color and texture deterioration (Estevez *et al.* 2005). The shelf life of meat is related to lipid oxidation reactions, which could affect its sensory properties, causing rancidity, as well as its nutritional characteristics through the formation of potentially toxic compounds (Sacchetti *et al.* 2008).

Moreover, oxidative processes are also associated with discoloration of meat products as lipid oxidation results in the formation of pro-oxidants which are capable of reacting with oxy-myoglobin and lead to formation of met-myoglobin (Frankel 1998). Therefore, color and lipid stability in meat are very important quality characteristics, which influence consumer acceptability and the problem of lipid oxidation has extensive economic importance for the meat industry.

Minced meats undergo oxidative changes and develop rancidity more quickly than intact muscle since grinding exposes more of the muscle surface to air and microbial contamination (Mitsumoto *et al.* 2005). Grinding meat leads to rapid formation of met-myoglobin, undesirable brown color and oxidative rancidity (Sahoo and Anjaneyulu 1997), seriously affecting consumer acceptance. Also, oxy-myoglobin and lipid oxidation appear to be interrelated in meat (Anton *et al.* 1993). With prolonged storage, oxy-myoglobin oxidizes to met-myoglobin and gives meat an unattractive brown color (Djenane *et al.* 2002).

An antioxidant is a substance that delays oxidation by inhibiting initial free radical formation or by preventing them from producing more free radicals, which can perpetuate the reaction (Fennema 1996). Several research studies indicated that lipid oxidation in meat can be effectively controlled or at least, minimized by adding antioxidants (Djenane *et al.* 2004).

Meat products containing natural antioxidants, as opposed to synthetic derivatives, are more desirable from a consumer viewpoint (Liu et al. 2010). It is well known that many herbs and spices contain antioxidant components and some vitamins (ascorbic acid and vitamin E) exhibit antioxidative activity. The antioxidative potential of natural antioxidants, such as Millard reaction products, ascorbic acid and spices in sheep, beef and pork in comparison with synthetic antioxidants was established by Jayathilakan et al. (2007). Abd El-Aal (2005) reported that the use of an antioxidant like ascorbic acid had a significant effect in reducing the oxidation of pigments and lipids of ground buffalo meat. Sepe et al. (2005) found that sodium ascorbate and sodium erythorbate more effectively maintained red color and myoglobin in the reduced state in cooked ground beef patties than ascorbic acid and ascorbyl palmitate.

Tocopherols are effective natural antioxidants for lipidcontaining foods. α -Tocopherol (Vitamin E) is generally regarded as an acceptable 'consumer friendly' supplement that can prolong color stability of beef and lipid stability of fresh pork (Liu *et al.* 2010). α -Tocopherol behaves like a chain-breaking electron donor antioxidant by competing with the substrate for chain-carrying peroxyl radicals and has been associated with retarding the decomposition of hydro-peroxides (Frankel 1998). In meat quality studies, α tocopherol has received considerable attention and it is the primary lipid-soluble antioxidant in biological systems, which act by disrupting the chain of lipid oxidation in cell membranes, thus preventing lipid hydro-peroxides (Haak *et al.* 2008).

Block and Langseth (1994) highlighted the beneficial effects of the three major antioxidant nutrients, vitamin C, E and carotenoids, in preventing and delaying cancer, cardio-vascular disease and cataracts. The use of cocktails of antioxidants may have superior effects compared to single antioxidants. Two or more antioxidants together can act synergistically (Haak *et al.* 2008). The combined use of antioxidants for meat represents a realistic and attractive strategy to increase the shelf life of fresh meat (Giese 1996).

The objective of the present research was to evaluate the inhibition of oxidative changes of minced buffalo meat during refrigerated storage at 4°C for 6 days by adding a combination of vitamin C and E salts at two different blend levels to minced buffalo meat and to study the effects on lipid and color stability as well as some other quality parameters. Thiobarbituric acid reactive substances (TBARS), antioxidant activity (AOA%), odor score, visual color score, met-myoglobin content, color measurements, pH value, water holding capacity (WHC) and cooking loss % were determined.

MATERIALS AND METHODS

Materials

Top round (*semimembranosus* muscle) from buffalo carcasses was obtained from a local slaughterhouse. After washing, removing all visible fat and loose connective tissue, the lean meat was minced twice using a meat grinder (Sanyo Meat Grinder MG 2000, Sanyo Electric Co. Ltd., Japan) to obtain minced buffalo meat.

Sodium ascorbate, α -tocopherol acetate and other chemicals were of "AnalaR" grade obtained from Sigma Chemical Co (St. Louis, MO).

Preparation of meat samples

The required concentrations of sodium ascorbate and α -tocopherol acetate were prepared by dissolving 400 and 600 mg sodium ascorbate in 4 and 6 ml distilled water, respectively and by dissolving 10 and 5 mg α -tocopherol acetate in 10 and 5 ml pure white mineral oil, respectively. Then, the freshly prepared sodium ascorbate and α -tocopherol acetate were added to the minced buffalo meat samples as follows:

1) Control sample was prepared without any additives.

2) Sample A was prepared by adding sodium ascorbate at 400 mg/l + α -tocopherol acetate at 10 mg/kg of minced buffalo meat.

3) Sample B was prepared by adding sodium ascorbate at 600 mg/l $+ \alpha$ -tocopherol acetate at 5 mg/kg of minced buffalo meat.

Each treated sample was blended well to homogeneity, divided into 200-g aliquots then packaged in polyethylene bags, sealed and stored in a refrigerator $(4 \pm 1^{\circ}C)$ for 6 days. The samples were analyzed at intervals of zero, two, four and six days for various quality parameters. The analyses were carried out in triplicate for each sample.

Analytical methods

1. TBARS

TBARS values were measured spectrophotometrically according to Byun *et al.* (2001). TBARS extraction was carried out according to Ahn *et al.* (2000) with some modifications. Homogenized minced buffalo meat samples (2 g) were taken and TBARS were extracted twice with 0.4 M perchloric acid (10 ml). The collected extracts were made up to 25 ml with 0.4 M perchloric acid then centrifuged for 5 min at $1790 \times g$. After centrifugation, 1 ml of the extract was poured into a glass test-tube with a stopper. TBARS reagent (5 ml) was added and the extract was heated in a boiling water bath for 35 min. After cooling in tap water, the absorbance of the sample was read against an appropriate blank at 538 nm. A standard curve was prepared using 1,1,3,3-tetraethoxypropane (TEP).

2. Antioxidant activity

The antioxidant potential expressed in terms of percentage of antioxidant activity (AOA %) was calculated by the following equation (Wijewickreme and Kitts 1998):

AOA % =

 $\frac{[\text{TBARS value of the control - TBARS of the test sample]}{\text{TBARS value of the control}} \times 100$

3. Odor score

The sensory score for meat odor was obtained by 10 panelists using a 5-point scale, where: 1 = very unpleasant, 2 = moderately unpleasant, 3 = moderately pleasant, 4 = pleasant and 5 = very pleasant (Das *et al.* 2006).

4. Visual color score

Visual color score of meat samples were determined by 10 panelists using a 5-point scale where: 1 = pale pink, 2 = pink, 3 = pinkish red, 4 = bright red and 5 = reddish-brown (Das*et al.*2006).

5. Met-myoglobin content

Met-myoglobin percentages of the tested samples were estimated according to the method of Trout (1990).

6. Color measurements

The color of each tested minced meat sample was measured using a Hunter Lab. scan XE color-meter (Hunter Lab. Inc., Reston, VA, USA) calibrated with a white standard tile: (X = 77.26, Y = 81.94 and Z = 88.14). Commission International d'Eclairage (CIE): L^* (lightness), a^* (redness) and b^* (yellowness) saturation index were measured. Reflectance measurements were collected at 10-nm increments using illuminate A (Podolak *et al.* 1997) and three random readings per sample were recorded.

Chroma of meat was calculated by using the formula $(a^2 + b^2)^{\frac{1}{2}}$, where a = red unit and b = yellow unit according to Bochi *et al.* (2008). The redness index (a^{\ast}/b^{\ast}) was determined as described by Chen *et al.* (1997).

7. pH value

Each minced buffalo meat sample (10 g) was blended with distilled water (50 ml) for 1 min (Trout *et al.* 1992) and pH values were determined in the prepared meat suspension using a digital pH-meter (HANNA, HI 902 meter, Germany).

8. Water holding capacity

Water-holding capacity (WHC) was measured by centrifugation according to the method described by Wardlaw *et al.* (1973). The centrifugation method was performed as follows. The meat samples were weighed and placed in tubes with a fiber (pore size 90 mm) in the bottom of the tubes to separate the meat from the expelled liquid. The samples were then centrifuged at 40 g for 1 h at 4°C. After centrifugation, the samples were weighed again, and the centrifugation loss was calculated as the difference in weight before and after centrifugation. Five replications were made on each meat sample

9. Cooking loss %

The percentage cooking loss was estimated by recording the weight of meat samples before and after cooking by heating a meat sample (~25 g) packed in a polypropylene bag at 80°C for 20 min, using a thermo-statistically controlled water bath. Samples were cooled for 15 min, dried with filter paper and weighed. Cooking loss was expressed as the percentage loss relative to the initial weight (Das *et al.* 2006).

Statistical analysis

All measurements were carried out in triplicate and the data were presented as mean \pm SD. The effects of adding vitamin C and E salts and storage period were analyzed and the data obtained was subjected to one-way analysis of variance (ANOVA) and least significant differences (LSD) at P < 0.05 were calculated using PC-Stat Version IA procedures (PC-Stat 1985).

RESULTS AND DISCUSSION

TBARS

Any process causing disruption of the muscle membrane system, such as grinding, cooking and de-boning, accelerates the development of oxidative rancidity. Meat mincing/ grinding is one of the most commonly employed techniques in meat processing and refers to the passing of fresh meat through a meat mincer/grinder, as a result meat and connective tissue are broken up. Mechanical treatments of meat tissue by blade tenderization (BT) or by mincing/grinding are well recognized and accepted (Anna Anandh et al. 2008). Processing of meat products with application of BT and various particle size reduction techniques like mincing/ grinding have been successfully applied (Raharjo et al. 1995). Both processes are commonly used to disrupt the muscle structure and to release myofibrillar proteins, which results in greater solubilisation of muscle proteins and thus may lead to an improved tenderness and cook yield (Pietrasik and Shand 2004; Trout and Schmidt 1984). Oxidative rancidity and auto-oxidation are serious problems occurring during the storage of meat and meat products.

Table 1 shows the effect of adding a combination of sodium ascorbate and α -tocopherol acetate at two different

blend levels on TBARS values of the minced buffalo meat samples (A, B and the control) during refrigerated storage at 4°C. The data reveals that the addition of these compounds resulted in a significant (P < 0.05) reduction of TBARS values. In addition, TBARS values of minced buffalo meat samples increased as refrigerated storage period increased.

At zero day TBARS values of the control, A and B samples were 0.115, 0.103 and 0.091 mg malonaldehyde/kg, respectively. TBARS values of samples A and B during the second, fourth and sixth days of storage were lower than the control, and the reduction in TBARS values was ranked as follows: sample B > sample A > control. Presumably, adding sodium ascorbate at 600 mg/l + α -tocopherol acetate at 5 mg/l mixture to minced buffalo meat (sample B) could be recommended for lowering TBARS values and hence for inhibiting lipid oxidation as well for preventing meat rancidity up to 6 days of refrigerated storage. Sahoo and Anjaneyulu (1997) indicated that sodium ascorbate at 500 mg/l was the optimum level for pre-blending which could extend shelf life of ground buffalo meat from 4 to 8 days under refrigerated storage. Djenane et al. (2002) reported that a combination of vitamins E + C was effective in inhibiting lipid oxidation and all of the antioxidant combinations used in their research exerted a significant (P < 0.05) inhibitory effect on the formation of TBARS, although not with the same intensity.

Antioxidant activity

The total antioxidant capacity or activity has been generally recognized as a tool to test the antioxidant potential of a pure compound or a food extract (Aruoma 1996). The AOA of a food could be a useful index to predict oxidative stability (Sacchetti *et al.* 2008). Data on the antioxidant potential or activity of sodium ascorbate and α -tocopherol as antioxidants in the minced meat samples A and B refrigerated at 4°C and stored for 6 days are depicted in **Table 1**. Within the tested samples, a significant difference between the AOA % as a result of adding the two different blend levels of sodium ascorbate and α -tocopherol acetate during storage for 6 days was observed. Also, it was noticed that minced meat sample B (containing sodium ascorbate at 600 mg/l + 5 mg/l α -tocopherol) exhibited a higher AOA (P < 0.05) than sample A during the same storage period.

Jayathilakan et al. (2007) indicated that natural antioxidants, e.g. Millard reaction products, cloves and ascorbic acid (AA) had antioxidant potential almost similar to the synthetic antioxidants TBHQ, BHA and PG respectively, and could be effectively employed as their substitutes for enhancing the shelf life of meat products by controlling lipid oxidation. AA is a chelating agent that binds metal ions and also scavenges free radicals and acts as a reducing agent. The enzymatic modifications of antioxidant vitamins C and E were discussed by Torres et al. (2008). L-Ascorbic acid (vitamin C) is the major water-soluble natural antioxidant acting as a free radical scavenger, and plays an important role in regenerating vitamin E. However, due to the low miscibility of ascorbic acid with α -tocopherol, it is necessary to use ascorbyl fatty acid derivatives. Thus, esters of Lascorbic acid with long-chain fatty acids (esp. palmitic or stearic) are employed as additives (E-304) in foods rich in lipids. The enzymatic synthesis of acyl L-ascorbates offers some advantages such as its high regioselectivity and the moderate reaction conditions. Vitamin E enhances the oxidative stability of polyunsaturated fatty acids from peroxidation acting as a free radical and generally administered in the form of *all-rac*- α -tocopheryl acetate or succinate to increase its stability. Several approaches have been described for the enzyme-catalyzed synthesis of vitamin E acetate, based on the trans esterification of vinyl acetate with vitamin E, or the regioselective hydrolysis of α -isophorone followed by reaction with isophytol. The above vitamin C and E derivatives may have impact not only as food preservatives but also as components of functional

Table 1 Thiobarbituric acid reactive substances (TBARS), antioxidant activity (AOA) and odor score of minced buffalo meat samples blended with sodium ascorbate and a-tocopherol acetate during refrigerate storage.

Minced meat samples	Storage period (days)			
	0	2 Mean ± SD	4 Mean ± SD	6 Mean ± SD
	Mean ± SD			
Control	$0.115 \pm 0.000 \ aC$	$0.172\pm0.004~aB$	$0.180\pm0.002\;aA$	$0.182 \pm 0.003 \text{ aA}$
Sample A	$0.103\pm0.015\ abC$	$0.140\pm0.010~bB$	$0.150\pm0.001\;bAB$	$0.160\pm0.002~bA$
Sample B	$0.091\pm0.001\ bB$	$0.117 \pm 0.002 \text{ cA}$	$0.121 \pm 0.002 \ cA$	$0.124 \pm 0.012 \text{ cA}$
Antioxidant activity (%) o	f sodium ascorbate and α-to	copherol acetate in minced m	eat samples during storage at	4°C
Control	-	-	-	-
Sample A	$10.435 \pm 0.000 \ bD$	$18.605 \pm 0.000 \text{ bA}$	$16.667 \pm 0.000 \text{ bB}$	$12.088 \pm 0.000 \ bC$
Sample B	$20.869 \pm 0.000 \; aD$	$31.977 \pm 0.000 \text{ aB}$	$32.778 \pm 0.000 \text{ aA}$	$31.868 \pm 0.000 \ aC$
Odor score (%) of minced	meat samples during storag	e at 4°C		
Control	$4.727 \pm 0.035 \text{ bA}$	$4.427\pm0.033~\text{cB}$	$3.650 \pm 0.150 \text{ cC}$	$2.683 \pm 0.021 \text{ cD}$
Sample A	$4.813\pm0.025\;aA$	$4.770\pm0.026\ aB$	$4573 \pm 0.015 \ aC$	$4.031 \pm 0.010 \; aD$
Sample B	$4.761\pm0.020\ abA$	$4.653\pm0.015\ bB$	$4.080 \pm 0.001 \text{ bC}$	$3.330\pm0.010\ bD$

All values determinations ± standard deviation (SD) are mean of triplicate

Sample A = minced meat with combination of ascorbic acid at 400 mg/l plus α -tocopherol at 10 mg/l

Sample B = minced meat with combination of ascorbic acid at 600 mg/l plus α -tocopherol at 5 mg/l Means within column with different small letters are significantly different (P < 0.05) according to according to one-way analysis of variance (ANOVA) and least significant differences (LSD) (PC-Stat 1985).

Means within row with different capital letters are significantly different (P < 0.05) according to according to one-way analysis of variance (ANOVA) and least significant differences (LSD) (PC-Stat 1985).

foods. In Min et al. (2009) study, hexane-insoluble and hexane-soluble fractions were separated from 100% methanolic extract from purple rice bran (RBE-HI and RBE-HS, respectively). RBE-HI showed higher total phenolics content, DPPH scavenging capability, and oxygen radical absorbance capacity (ORAC) value, but lower iron chelating capability (ICC), compared to RBE-HS. On the other hand, both fractions showed similar antioxidant activity in raw and cooked catfish patties and maintained better textural properties of cooked patties during 12 days of storage, compared to the control. Ashwell et al. (2010) clarified that the antioxidant capacities of phenolic compounds have been long recognized for their strong chain-breaking actions and ability to scavenge radicals, thereby protecting cells against the detrimental effects of reactive oxygen species (ROS). Due to the benefits of antioxidants, food and pharmaceutical products are normally supplemented with synthetic antioxidants. Natural antioxidants from plant products may be more effective in reducing ROS levels compared to synthetic single dietary antioxidants due to the synergistic actions of a wide range of biomolecules such as vitamins C and E, phenolic compounds, carotenoids, terpenoids and phytomicronutrients. Ferreira et al. (2011) reported that yerba maté (Ilex paraguariensis St. Hil.) ethanolic extracts were effective against lipid peroxidation in beef hamburger when added at 0.1%. Furthermore, adding these natural antioxidants to hamburger did not result in sensory loss. TBARs and hexanal levels, fatty acid profile and cholesterol oxides were evaluated as oxidation parameters in beef hamburgers during 90 days' storage. The addition of 0.01% yerba maté ethanolic extracts proved inefficient in restraining the lipid peroxidation while the addition of 0.1% yerba maté resulted in efficient antioxidant activity.

At high levels (> 1000 mg/kg), AA inhibits oxidation while at low levels (< 100 mg/kg) it can catalyze oxidation and warmed-over-flavour (WOF) development (Ahn and Nam 2004). AA acts as singlet oxygen quencher and functions as a synergist when used in combination with other antioxidants by promoting antioxidant effects (Elliott 1999). Furthermore, vitamin C regenerates vitamin E for protection against free radical attacks in vitro (Kinsella et al. 1993).

Thus, in the present study a combination of sodium ascorbate at 600 mg/l + α -tocopherol acetate at 5 mg/l could be utilized effectively for enhancing the shelf life of minced buffalo meat (52.70%) by minimizing lipid oxidation.

Odor score

The odor of fresh meat is an important criterion for its acceptability. From the data in **Table 1**, the odor of all studied minced buffalo meat samples was rated as pleasant at zero and 2 days of refrigerated storage. On day 4, the same evaluation was given by panelists for samples A, B and the odor score of control sample was moderately pleasant. At the end of the refrigerated storage period the odor scores were moderately unpleasant, pleasant and moderately pleasant for the control, samples A and B, respectively. Sahoo and Anjaneyulu (1997) indicated that the odor score (OS) increased as the concentration of sodium ascorbate (SA) in ground buffalo meat increased up to 500 mg/l while a further increase of SA to 600 mg/l decreased the OS indicating the optimum antioxidant effect at 500 mg/l. Also, they added that the shelf-life of ground buffalo meat treated with 10 mg/l α -tocopherol acetate could be extended to 8 days in refrigerated storage conditions without any undesirable changes in color, odor or microbial load.

Das et al. (2006) found that the shelf-life of ground buffalo meat treated with 1.0% carnosine could be extended to 8 days in refrigerated storage without any undesirable changes in color or odor whereas the control sample could be kept for up to 6 days only. The same authors indicated that the acceptable OSs of ground buffalo meat depended on several quality parameters such as TBARS number, aerobic mesophile count and psychrotroph plate count.

Naveena et al. (2006) illustrated that dipping buffalo meat steaks in lactic acid (LA) + clove or LA + clove + vitamin C had significant advantages over dipping with LA/distilled water alone with respect to maintaining shelflife. Treatment with LA + clove synergistically reduced the TBARS values and microbial counts without affecting color and odor, compared to control and LA-treated buffalo meat steaks. Further, addition of vitamin C to LA + clove significantly (P < 0.05) increased the CIE a^* values along with an improvement in sensory color and odor of the meat.

The chilled storage (i.e. $4 \pm 1^{\circ}$ C) of buffalo meat decreased the OS and flavor score values during prolonged storage (Biswas et al. 2009).

Fresh goat meat with curry leaf powder (CLP) had acceptable odor up to 5 days whereas in control sample it was up to 3 days as reported by Das et al. (2011). The CLP treated sample was acceptable up to 5 days, odor score being 3.35 but the control sample was below the acceptable level (3.00) on day 5. Also, Das et al. (2006) reported similar trend of odor score in ground buffalo meat during refrigerated storage.

Table 2 Visual Color Score and Metmyoglobine content of the minced buffalo meat blended with sodium ascorbate and α -tocopherol acetate during refrigerated storage.

	Color Score			
Minced meat samples	Storage period (days)			
	0 Mean ± SD	2 Mean ± SD	4 Mean ± SD	6 Mean ± SD
Sample A	$4.69\pm0.032\;aA$	$4.64\pm0.020\;aA$	$3.75\pm0.141\ aB$	$3.40\pm0.019\ bC$
Sample B	$4.73\pm0.025\;aA$	$4.70\pm0.009~aA$	$3.77\pm0.907~aB$	$3.68\pm0.030\;aB$
Met-myoglobin %				
Control	$49.60 \pm 0.027 \; aD$	$54.67\pm0.022~aC$	$55.78\pm0.083~aB$	$61.83 \pm 0.236 \text{ aA}$
Sample A	$47.70\pm0.016~bD$	$51.85\pm0.129\ bC$	$52.66\pm0.000\ bB$	$53.88\pm0.031\ bA$
Sample B	$46.64 \pm 0.035 \text{ cD}$	$50.40 \pm 0.016 \text{ cC}$	$52.43\pm0.000\ cB$	$53.20 \pm 0.083 \text{ cA}$

All values determinations \pm standard deviation (SD) are mean of triplicate

Sample A = minced meat with combination of ascorbic acid at 400 mg/l plus α -tocopherol at 10 mg/l Sample B = minced meat with combination of ascorbic acid at 600 mg/l plus α -tocopherol at 5 mg/l

Means within column with different small letters are significantly different (P < 0.05) according to according to one-way analysis of variance (ANOVA) and least significant differences (LSD) (PC-Stat 1985).

Means within row with different capital letters are significantly different (P < 0.05) according to according to one-way analysis of variance (ANOVA) and least significant differences (LSD) (PC-Stat 1985).

Visual color score

The color of fresh meat is one of the most important factors for its acceptability and has a considerable effect on purchase decision. Visual color scores of the tested minced buffalo meat samples A and B were rated, by the panelists, higher scores relative to the control (Table 2). Visual color score in the tested samples tended to decrease as the storage period increased. Throughout the storage period, the highest visual color score was given for sample B. By the sixth day of storage, the color was pink (2.95) for the control and pinkish-red for meat samples A and B (3.40 and 3.68, respectively), which indicated that the visual color was more acceptable for sample B. Das et al. (2006) illustrated a significant (P < 0.05) improvement in visual color of ground buffalo meat containing carnosine as identified by higher color scores in which the addition of 1.0 and 1.5% carnosine contributed the highest color scores (3.83 and 3.89, respectively). Samples treated with carnosine maintained a reddish color for the 6-8 day storage period. There was no difference in the color scores between 0 and 2 days of storage. Meanwhile, the desirable meat color started to decline significantly (P < 0.05) from the fourth day of refrigerated storage. Color evaluation studies by Naveena et al. (2006) revealed that treatment of buffalo meat with LA + clove and LA + clove + vitamin C did not show any discoloration throughout the display period, whereas the control and LA-treated samples caused brownish discoloration after 6 and 9 days of display, respectively. The reducing activity of AA played a role in maximizing muscle color stability through metmyoglobin reduction (Lee et al. 1999).

Met-myoglobin content

Met-myoglobin is responsible for the undesirable brown color of fresh meat. The color of meat varies depending on the state of myoglobin. Ground buffalo meat tends to become brown and rancid more rapidly than whole retail cuts (Das et al. 2006). Generally, the present investigation indicated that met-myoglobin percentages of the control sample were higher than those of the tested samples A and B during the refrigerated storage period (Table 2). As the refrigerated storage period increased, met-myoglobin accumulation in the meat samples increased consistently. It is worthy to note that on day 6 of storage sample B contained lower met-myoglobin content than sample A and the control. Highly significant effects of the storage period by increasing metmyoglobin in ground buffalo meat were also reported by Sahoo and Anjaneyulu (1997). The same authors indicated that ground buffalo meat (GBM) was preblended with sodium ascorbate (SA) at 0, 300, 400, 500 and 600 mg/l and was examined for its quality changes during refrigerated storage at $4 \pm 1^{\circ}$ C. The addition of 500 mg/l SA

contributed to significantly lower (43.7) MMb% compared with 300 mg/l (50.0) and 400 mg/l (46.7) levels during the storage period. Further, increasing SA to 600 mg/l had no added advantage for lowering the MMb content. As the refrigerated storage period increased, the MMb accumulation in the meat tissue increased consistently.

Djenane et al. (2002) clarified in their study that a combination several antioxidants effectively delayed met-myoglobin formation in beef steaks. Djenane et al. (2002) clarified that The surface application of natural antioxidants (vitamin C 500 mg/l), taurine (50 mM), rosemary (1000 mg/l) and vitamin \tilde{E} (100 mg/l), the three latter in combination with 500 mg/l of Vitamin C) prior to modified atmosphere packaging and stored at $1 \pm 1^{\circ}$ C for 29 days resulted in an effective delay of oxidative deterioration of fresh beef steaks. Shelf life was extended beyond that of control, according to evaluation of sensory attributes. Both combinations of vitamin C with either rosemary extract or taurine extended the shelf life of fresh beef steaks by about 10 days. Rosemary combination with vitamin C was the most effective in delaying myoglobin oxidation and lipid oxidation. The combination of vitamins E and C was significantly less effective than any other in delaying meat oxidation, though its effect was more intense than that of vitamin C alone. Das et al. (2006) observed that the ground buffalo meat samples containing 1.0% and 1.5% carnosine significantly inhibited metmyoglobin formation, and the use of 1.0% carnosine for preblending extended the shelf life of ground buffalo meat up to 8 days under refrigerated storage.

Myoglobin oxidation in a liposome model was greatly reduced by its combination of vitamin E with ascorbate (Yin *et al.* 1993).

Accordingly, blending minced buffalo meat with vitamin C + vitamin E salts could help to minimize metmyglobin accumulation in the studied minced buffalo meat samples throughout the tested storage period.

Color measurements

Data presented in **Table 3** shows that redness (a^*) color parameter values of treated samples A and B at day zero of storage were higher than the corresponding value of the control sample. Both initial (a^*) and (b^*) values at any time of storage were relatively higher for sample B than for both samples A and control. Armstrong (1993) found that vitamin E could preserve the initial color of pork chops and remained stable for 10 days at 4°C. Sánchez-Escalante *et al.* (2001) noticed that samples of beef patties with AA, carnosine and rosemary alone also gave significant higher a^* values (P < 0.05) than the control during the first 12 days of storage. Sahoo and Anjaneyuld (1997) indicated that redness and yellowness color parameters of ground buffalo meat containing sodium ascorbate at 500 mg/l maintained

The b Color measurements of the inneed burnant meat brended with solution as color	scopheror accure during reingerated storage
Table 3 Color measurements of the minced buffalo meat blended with sodium ascorbate and α -t/	ocopherol acetate during refrigerated storage

<u></u>		Keuness (<i>a⁻</i>)				
Minced meat samples	Storage period (days)					
	0	2	4	6		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
Control	$7.75\pm0.146~cB$	$8.21 \pm 0.085 \text{ cA}$	$6.77 \pm 0.081 \text{ cC}$	$6.55 \pm 0.009 \text{ cD}$		
Sample A	$9.30\pm0.157~bA$	9. 46 ± 0.199 bA	$8.\ 83\pm0.038\ bB$	$845\pm0.195\ bC$		
Sample B	$9.60 \pm 0.031 \text{ aD}$	$9.88 \pm 0.011 \text{ aC}$	$10.50 \pm 0.025 \text{ aA}$	$10.41 \pm 0.015 \text{ aB}$		
Yellowness(b*)						
Control	$5.65\pm0.021~cB$	$6.64 \pm 0.015 \text{ cA}$	$5.52 \pm 0.027 \text{ cC}$	$5.30\pm0.026~\text{cD}$		
Sample A	$7.20\pm0.099~bC$	$7.42\pm0.009~bB$	$7.73 \pm 0.019 \text{ bA}$	$6.88\pm0.027~bD$		
Sample B	$8.03\pm0.019\;aB$	$8.22\pm0.020~aA$	$7.84\pm0.032~aC$	$7.64 \pm 0.020 \text{ aD}$		
Chroma						
Control	$9.60\pm0.120~\text{cB}$	$10.56 \pm 0.057 \text{ cA}$	$8.74\pm0.046~\mathrm{cC}$	$8.43 \pm 0.009 \text{ cD}$		
Sample A	$11.76\pm0.170\ bAB$	$12.02 \pm 0.161 \text{ bA}$	$11.74\pm0.033~bB$	$10.90 \pm 0.156 \ bC$		
Sample B	$12.52\pm0.018~aD$	$12.85 \pm 0.007 \ aC$	$13.099 \pm 0.016 \text{ aA}$	$12.92\pm0.022~aB$		
Redness Index						
Control	$1.37 \pm 0.023 \text{ aA}$	$1.24\pm0.016~bB$	$1.23\pm0.020~bB$	$1.24\pm0.008~bB$		
Sample A	$1.29\pm0.017~bA$	$1.28\pm0.028~aA$	$1.14 \pm 0.005 \text{ cC}$	$1.23\pm0.028~bB$		
Sample B	$1.20\pm0.006~\text{cC}$	$1.20\pm0.004\ bC$	$1.34\pm0.008\ aB$	$1.36\pm0.011~aA$		

All values determinations ± standard deviation (SD) are mean of triplicate

Sample A = minced meat with combination of ascorbic acid at 400 mg/l plus α -tocopherol at 10 mg/l

Sample B = minced meat with combination of ascorbic acid at 600 mg/l plus α -tocopherol at 5 mg/l Means within column with different small letters are significantly different (P < 0.05) according to according to one-way analysis of variance (ANOVA) and least significant

differences (LSD) (PC-Stat 1985).

Means within row with different capital letters are significantly different ($P \le 0.05$) according to according to one-way analysis of variance (ANOVA) and least significant differences (LSD) (PC-Stat 1985).

Table 4 pH values, Water holding capacity (WHC) and Cooking loss of the minced buffalo blended with sodium ascorbate and α -tocopherol acetate during refrigerated storage.

Minced meat samples	Storage period (days)			
	0 Mean ± SD	2 Mean ± SD	4 Mean ± SD	6 Mean ± SD
Sample A	$5.54\pm0.153~\mathrm{B}$	$5.56\pm0.021~\mathrm{B}$	$5.59\pm0.025~A$	$5.62\pm0.015~A$
Sample B	$5.53\pm0.010\ C$	$5.57\pm0.015~\mathrm{B}$	$5.60\pm0.020~B$	$5.64\pm0.015~A$
Water Holding Capacity	(WHC)			
Control	9.48 ± 0.021 a	$9.50 \pm 0.009 \ a$	9.52 ± 0.029 a	9.53 ± 0.030 a
Sample A	$8.33\pm0.091\ bC$	$9.03\pm0.069\ bB$	$9.10\pm0.100\ bB$	$9.40\pm0.210\ bA$
Sample B	$6.65 \pm 0.009 \text{ cD}$	$7.72\pm0.020~cC$	$8.22\pm0.009~bB$	$8.53 \pm 0.021 \text{ cA}$
Cooking Loss (%)				
Control	$37.58 \pm 0.071 \ aC$	$38.37\pm0.022\ aB$	$38.44\pm0.016\ aB$	$38.86 \pm 0.016 \text{ aA}$
Sample A	$35.75\pm0.140\ bD$	$36.22\pm0.022\ bC$	$36.64\pm0.011\ bB$	$37.40\pm0.000\ bA$
Sample B	$34.50 \pm 0.019 \text{ cC}$	$34.52 \pm 0.029 \text{ cC}$	$35.64 \pm 0.019 \text{ cB}$	$36.57 \pm 0.016 \text{ cA}$

Sample A = minced meat with combination of ascorbic acid at 400 mg/l plus α -tocopherol at 10 mg/l

Sample B = minced meat with combination of ascorbic acid at 600 mg/l plus α -tocopherol at 5 mg/l

Means within column with different small letters are significantly different (P < 0.05) according to according to one-way analysis of variance (ANOVA) and least significant differences (LSD) (PC-Stat 1985).

Means within row with different capital letters are significantly different ($P \le 0.05$) according to according to one-way analysis of variance (ANOVA) and least significant differences (LSD) (PC-Stat 1985).

higher values than day 0 and throughout the 10-day storage period.

It can be concluded that by using the two combinations of sodium ascorbate and a-tocopherol acetate led to significant differences (P < 0.05) (i.e. improvements) relative to the control. At the end of the storage period (6 days), the control sample acquired lower (a^*) values than samples A and B, reflecting a sharp difference in redness index among them.

The chroma is an expression of the saturation or intensity and clarity of the color (Bochi et al. 2008). In the present study, chroma values indicated an increase in saturation for all samples. Chroma was observed, generally, to be of lowest value in control samples and highest in sample B during the 6-day storage period (Table 3). Adding a combination of sodium ascorbate and α -tocopherol acetate at the two suggested levels could help to maintain the chroma of the studied meat samples (A and B) higher throughout the storage period than the control sample. Sample B appeared optimal in improving and maintaining the intensity of meat color during refrigerated storage.

The redness index $(a^*/b^* \text{ ratio})$ of the control and sam-

ple A decreased when the storage period increased (Table 3) while on days 4 and 6 the redness index of sample B increased and remained higher than the values of sample A and the control. This ratio was used as an index of apparent change in redness. Chen et al. (1997) and Faustman et al. (1992) reported that the saturation of red color in meat was related to myoglobin concentration. Changes in the redness index can be used as an index of pigment changes and the discoloration was mainly caused by oxidation of myoglobin in sardine and mackerel muscles (Chaijan et al. 2005).

pH values

Generally, the pH value increased gradually with increasing storage time (Table 4). Abd El-Aal (2005) and Das et al. (2011) also reported that an increase in pH during the storage period may be due to growth of Gram-negative bacteria and due to accumulation of metabolites by bacterial action on protein and amino acids.

Water holding capacity

WHC is one of the major quality properties of fresh meat as it affects some major characteristics of meat such as potential drip loss, technological quality, appearance and sensory properties (Das *et al.* 2011).

In the present study, WHC was influenced by adding sodium ascorbate and α -tocopherol acetate. As shown in Table 4, there was a significant decrease in WHC of meat samples A and B compared to the control at 0 day. This might be due to the pre-blending effect of sodium ascorbate, especially of sodium ions. Regarding the effect of storage period, WHC increased throughout the storage period for all samples. Meat sample B had significantly lower WHC % than the control and sample A. Sahoo and Anjaneyulu (1997) found that ground buffalo meat sample containing 600 mg/l sodium ascorbate had significantly lower WHC % than the control and other treated samples. Obviously, the selected concentration of vitamin C + vitamin E salts used for blending with minced buffalo meat sample B appeared to be superior in terms of increasing WHC, than that blended with sample A. Trout and Schmidt (1984) emphasized that the effect of meat additives on pH and ionic strength was probably the most important reason for improving the WHC of beef.

Cooking loss

In the present study, cooking loss % of the control sample at day zero was higher than the corresponding percentages of the other tested minced buffalo meat samples (Table 4). The lowest cooking loss was found in sample B. This may be due to an increase in Na⁺ ions in the meat. Sahoo and Anjaneyulu (1997) reported that as the concentration of sodium ascorbate increased, there was a decrease in cooking loss of ground buffalo meat. At the end of refrigerated storage cooking loss % of the tested minced buffalo meat samples increased significantly up to 38.86, 37.40 and 36.57 for the control, A and B samples, respectively. Clarke et al. (1987) and Hamm (1977) indicated that some meat additives might have the ability to affect pH value and solubilize the protein matrix, which is capable of holding greater quantities of water resulting in lower cooking loss and owing to a decrease in the solubility of proteins during storage and post-mortem enzymatic hydrolysis of ATP.

CONCLUSION

From the present investigation it was concluded that adding combination of the vitamin C and E salts as antioxidants at the two different suggested blend levels to minced buffalo meat inhibit lipid oxidation, retard development of rancidity and discoloration, enhance color quality and minimize, as possible, met-myoglobin formation in meat during the applied storage period. The suggested blend at 600 mg/l sodium ascorbate + 5 mg/l α -tocopherol acetate could be employed as a substitute for synthetic antioxidants for enhancing the shelf life of minced buffalo meat.

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