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GROUND BUFFALO MEAT QUALITY AS AFFECTED BY COMBINED BLENDS OF THE ANTIOXIDANTS VIT.C AND VIT.E SALTS.

Ibrahim, Hayam M.A.
Food Technology and Dairy Dept., National Research Center, Dokki, Cairo, Egypt.

ABSTRACT

The effect of two combined levels of the biological antioxidants Vit.C and Vit.E salts (sodium ascorbate and alpha-tocopherol acetate) on some quality parameters of ground buffalo meat (GBM) was studied during refrigerated storage for six days at 4±1°C. GBM sample blended with 600 ppm sodium ascorbate plus 5 ppm alpha-tocopherol acetate had lower cooking loss, metmyoglobin (MetMb) and thiobarbituric acid (TBA) values. Also, the sample showed higher a* (redness) color values ,more acceptable visual color and odor scores as compared to GBM sample blended with 400 ppm sodium ascorbate plus 10 ppm alpha-tocopherol acetate and the control.

INTRODUCTION

Lipid oxidation, microbial spoilage and associated changes are a major cause of quality deterioration of meat during storage. Problems associated with lipid oxidation are of importance as they relate to flavor deterioration and loss of nutritional values, thereby affecting the acceptability of meat during storage (Gray and Monahan, 1992).

Consumers associate meat freshness with high concentration of oxymyoglobin, characterized by a bright, cherry-red color (Sprouls and Brewer, 1997).

Pigment and lipid stability in ground meat is very important for meat packers and consumers. Ground meat tends to become brown and rancid more rapidly than whole muscle retail cuts. When the color of ground meat changes from bright red (oxymyoglobin) to brown (metmyoglobin), consumers discriminate against the product (Mitsumoto *et al*., 1991).

Consumers like to avoid chemical additives in fresh meat. They would prefer natural substances to increase shelf life. Block and Longseth (1994) highlighted the beneficial effects of the three major antioxidant nutrients,Vitamin C, E and carotenoids, in preventing and delaying cancer, cardiovascular diseases and cataracts.

Vitamin E functions as a lipid-soluble antioxidant in cell membranes and its most potent form is α-tocopherol. It is capable of quenching free radicals and thus protects phospholipids and cholesterol against oxidation and subsequent breakdown to potentially harmful chemical reactive products (Linder, 1985).

Vitamin E content of meat is low; Piironen *et al.,* (1985) found that the α-tocopherol content of Finnish meat and meat products ranged from 0.16 to

0.84 mg/100g. The addition of Vitamin E inhibited lipid oxidation to some extent in ground beef meat when used at 50 ppm (Benedict *et al.,* 1975).

Vitamin C functioned as an antioxidant with some substrates by scavenging oxygen and inhibiting radical formation at double bonds (Cort, 1982). The natural Vitamin C content of fresh meat was usually considered negligible (~0.00 ppm)(Anderson,1985). Shivas *et al.,*(1984) has found that addition of Vitamin C increased pigment and lipid stability, maintained good color and retarded rancidity in ground beef.

Cheng and Cocoma (1989) indicated that when Vitamin C was used at low concentration under 176 ppm ; the addition of metal chelators would be needed to suppress pro-oxidant reactions. Since meat contain about 0.7 ppm Cu^{2}+ and 21.8 ppm Fe^{3}+ (Anderson *et al.,*1985) a range of 200-1000 ppm Vitamin C should be sufficient to act as antioxidant without use of chelators. Kowale *et al*., (1996) reported that the changes in TBA values were more pronounced during refrigerated than during frozen storage; indicating that the deteriorative changes were directly related to the increase in temperature of storage.

The combined addition of Vitamin E plus C has been found by Okayama *et al.,*(1987) to increase lipid stability in beefsteaks. Tappel *et al.,* (1961) suggested that mixture of Vitamin E and C acted synergistically; Vitamin E acting as the primary antioxidant and the resulting Vitamin E radical then reacting with Vitamin C to regenerate Vitamin E.

Several studies have been conducted on dietary supplementation with the biological antioxidants (Vit. C or Vit. E) and also the *in vitro* addition of each of these antioxidants in order to improve lipid and color stability of pork and beef meat. However, very little information are available on the combined blends of these antioxidants and their effect on lipid and color stability as well as other quality parameters of ground meat. Therefore, the aim of the present work was to investigate the effect of two different blend levels of the biological antioxidants Vitamin C plus Vitamin E salts on color and lipid stability as well as on some other quality properties of ground buffalo meat during refrigerated storage.

MATERIALS AND METHODS

1.Meat

Top round roasts (semimembranosus muscle) were obtained from El-Basatin local slaughterhouse. After removing all visible fat and loose connective tissue, the lean meat was minced twice using a meat grinder to obtain ground buffalo meat (GBM).

2. Samples preparation

Sample A: was prepared by adding sodium ascorbate at concentration of 400 ppm plus α-tocopherol acetate at 10 ppm per 1 kg ground buffalo meat and the mixture was blended to form homogenous blend. The required concentrations were freshly prepared by dissolving 400

mg sodium ascorbate in 4 ml distilled water; and dissolving 10 mg α-tocopherol acetate in 10 ml pure white mineral oil.

Sample B: was prepared by adding sodium ascorbate at concentration of 600 ppm plus α-tocopherol acetate at 5 ppm per 1kg ground buffalo meat and the mixture was blended to form homogenous blend. The required concentrations were freshly prepared by dissolving 600 mg sodium ascorbate in 6 ml distilled water; and dissolving 5mg α-tocopherol acetate in 5 ml pure white mineral oil.

Control sample: ground buffalo meat without antioxidant additives were blended to form a homogenous blend.

Each of the treated samples as well as the control was divided into 200g aliquots, then packaged in polyethylene bags, sealed and stored at 4±1 ° C in a refrigerator for six days. The samples were analyzed at intervals of zero, two, four and six days for various quality parameters. The analyses were carried out in triplicates for each sample.

3.Analytical methods

Meat samples (10g) were homogenized in distilled water (50ml), pH of the homogenized samples were measured using glass electrodes of Hi 9021 Micro processor pH meter (HNNA instruments).

Water holding capacity (WHC) was measured by a centrifugation method (Wardlaw et *al.,* 1973).

Cooking loss (CL) was estimated by recording the weights of meat before and after cooking then the cook loss calculated for 3 replicates per treatment as described by Hughes *et al.,* (1997).

Visual color was determined by ten panelists to score meat samples for redness scale where : 1 = pale pink, 2 = pink, 3 = pinkish red, 4 = bright red and 5 = red.

Color measurements of meat samples were conducted using a Hunter Lab. Scan XE colorimeter (Hunter Lab. Inc., Reston VA) with a wavelength 400-700nm. The instrument was standardized prior each use by a white tile. Values of the white standard tile were X=77.26, Y=81.94 and Z=88.14.Commission International d`Elclairage (CIE): L*(lightness), a*(redness) and b*(yellowness) saturation index were measured. Reflectance measurements were collected at 10 nm increment using illuminate A. Three random readings per sample were obtained and analyzed.

The hue and chroma of meat samples were calculated using the formula: Hue= (tan-¹)¾ and chroma= (a² +b²)½ where a =red value and b =yellow value (Little, 1975; Froehlich *et al.,* 1983).

Metmyoglobin percentages of the tested samples were determined as described by Kryzwicki (1982) using 4054 U/V visible spectrophotometer LKB-Biochrom for spectrophotometric analysis of extracts.

Thiobarbituric acid (TBA) value was determined as described by Pearson (1981).

Odor score for meat samples were rated using 5 point scale where: 1 = very unpleasant, 2 = moderately unpleasant, 3 = moderately pleasant, 4= pleasant and 5 = very pleasant.

RESULTS AND DISCUSSION

The pH value, water holding capacity (WHC) and cooking loss (CL) of refrigerated stored ground lean buffalo meat samples are presented in Table 1.

Table 1: Changes in pH, water holding capacity and cooking loss of ground buffalo meat (GBM) blended with Vit.C plus Vit.E salts and stored at 4±1°C.

GBM	Refrigerated storage (days)				Treatment mean*	
Samples	0	2	4	6	±SE	SD
			pH value:			
Control	5.56	5.57	5.61	5.55	5.74±0.07	0.003
A	5.53	5.55	5.59	5.57	5.54±0.01	0.066
B	5.53	5.55	5.44	5.37	5.47±0.02	0.008
Day mean	5.54	5.56	5.56	5.50		
±SE	±0.007	±0.005	±0.007	±0.033		
			Water holding capacity (ml/100g):			
Control	9.47	10.31	9.07	8.65	9.37±0.15	0.64
A	8.25	9.92	9.07	9.50	9.18±0.18	0.65
B	6.64	7.81	8.28	8.65	7.85±0.20	0.79
Day mean	8.12	9.35	8.81	8.93	8.80±0.11	0.97
±SE	±0.410	±0.390	±0.131	±0.143		
			Cooking loss(%):			
Control	37.65	38.37	38.23	38.86	38.28±0.3	0.45
A	35.77	35.65	35.50	37.23	36.04±0.1	0.73
B	35.81	35.54	35.40	37.05	35.95±0.0	0.68
Day mean	36.41	36.52	36.38	37.71	36.76±0.1	1.25
±SE	±0.311	±0.462	±0.463	±0.288		

Control: GBM sample without antioxidant additives.
A: GBM sample blended with 400 ppm sodium ascorbate plus 10ppm α-tocopherol acetate.
B: GBM sample blended with 600 ppm sodium ascorbate plus 5ppm α-tocopherol acetate.
*** Treatment means of six days storage. SE: Standard error. SD: Standard deviation.**

Values of pH at day zero were 5.56, 5.53 and 5.53 for the control, A and B samples respectively. Gradual increase in pH value of the control sample was noticed up to fourth day of refrigerated storage; thereafter a slight decrease occurred at six day. On the other hand, little increase in pH values of the treated samples A and B was observed at fourth and second days respectively; then a slight decrease occurred at fourth and sixth days of storage. The decrease in pH during meat storage may have been owing to the growth of gram positive bacteria; especially lactic acid bacteria (Jay and Shelef,1978).

There was an increase in WHC for the control and sample A at second day; then a marked decrease was noticed at fourth day. At sixth day; WHC of the control sample decreased to 8.65 and that of sample A increased to 9.50 ml/100g. In comparison, there was a gradual increase in WHC of sample B at second, fourth and sixth days of refrigerated storage. The

calculated changes in WHC values of the control, A and B samples at the end of storage period were -8.66, +15.27 and +30.27 % respectively. Obviously, the selected concentration of Vit. C plus Vit.E salts for blending with ground buffalo meat (sample B) appeared to be superior for increasing WHC than that blended with sample A. Trout and Schmidt (1984) emphasized that the effect of meat additive on pH and ionic strength was probably the most important reason for improving water holding capacity of beef

. Cooking loss (CL) percentage of the control sample at day zero was higher than the corresponding percentages of samples A and B containing the antioxidant additives (Table 1). The cooking loss mean values of the tested GBM samples at the end of refrigerated storage were 38.28±0.13, 36.04±0.21 and 35.95±0.20 for the control, A and B samples respectively. Clarke *et al.,*(1987) reported that some meat additives may have the ability to affect pH value and solubilize proteins and thus creating a fine protein matrix which is capable of holding greater quantities of water resulting in lower cooking loss.

Color is an extremely critical component of appearance of fresh red meat and has substantial influence on purchase decision. Visual color scores of samples A and B, containing the biologically antioxidant additives, rated by the panelists higher color scores relative to the control (Table 2).All-over the storage period the highest visual color score was given for sample B. The color scores at sixth day were 2.99(pink), 3.42(pinkish red) and 3.74(pinkish red) for the control, samples A and B respectively. This could indicate that a visual color improvement (i.e. appeared more acceptable) was clearly attained for the treated sample B.

Data presented in Table 2 showed that a*(redness) color parameter of samples A and B at day zero were of higher values (9.25 and 9.59 respectively) than the corresponding value of the control sample (7.77). It was interesting to observe that both initial a*-value and a*-value at any stage of storage were, relatively, higher for sample B than for both samples A and the control. Armstrong (1993) reported that Vit.E could
preserve the initial color of pork chops and remained stable for 10 days at 4°C.

Regarding the yellowness color parameter (Table 2) it was observed that b*-values of the treated A and B samples were higher than the control. During the storage period, the treated mean values of sample B (7.33) was higher than the corresponding values of the other samples (A and the control). Color score of meat samples showed significant ($p < 0.01$) negative correlation ($r = - 0.999$) with pH and showed significant ($p<0.01$) positive correlation wirth a* (redness) value ($r = 1.00$) and b* (yellowness) value ($r = 0.995$). (Table 4).

Hue values of sample B was higher than those of the control and sample A at second day of storage were. During fourth and sixth days the calculated hue values differed for all the investigated ground meat samples. Chroma parameter indicates the intensity of color. All-over the applied storage period the calculated chroma values of both sample A and the control were lower than the corresponding values of sample B.

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Table 2: Visual and instrumental color parameters of Ground buffalo meat (GBM) blended with Vit.C plus Vit.E and stored at 4±1°C.

GBM	Refrigerated storage (days)				Treatment mean*	
Samples	0	2	4	6	±SE	SD
			Color score:			
Control	4.56	4.35	3.51	2.99	3.90±0.190	0.67
A	4.71	4.66	3.76	3.42	4.14±0.170	0.58
B	4.76	4.72	3.80	3.74	4.30±0.150	0.51
Day mean	4.68	4.58	3.69	3.38	4.10±0.010	0.60
± SE	±0.031	±0.056	±0.045	±0.109		
			Color a*(redness) unit:			
Control	7.77	8.21	6.77	6.55	7.32±0.21	0.72
A	9.25	9.48	8.88	8.50	9.03±0.11	0.40
B	9.59	9.97	10.86	10.37	10.20±0.14	0.50
Day mean	8.87	9.22	8.84	8.47	8.85±0.22	1.31
± SE	±0.281	±0.263	±0.591	±0.552		
			Color b*(yellowness) unit:			
Control	5.70	6.54	5.37	5.25	5.72±0.15	0.53
A	7.18	7.51	7.39	6.64	7.18±0.10	0.35
B	8.01	8.16	7.76	7.40	7.33±0.09	0.30
Day mean	6.97	7.40	6.84	6.43	6.91±0.16	0.98
± SE	±0.340	±0.236	±0.371	±0.315		
			Hue:			
Control	36.23	38.44	38.43	38.70	37.95±0.30	1.04
A	37.86	38.44	39.75	37.92	38.50±0.22	0.80
B	39.87	39.32	35.46	35.51	37.54±0.62	2.15
Day mean	37.99	38.73	37.88	37.38	37.99±0.24	1.47
± SE	±0.526	±0.147	±0.633	±0.480		
			Chroma:			
Control	9.64	10.50	8.66	8.40	9.30±0.25	0.87
A	11.73	12.09	11.55	10.80	11.54±0.14	0.50
B	12.51	12.89	13.35	12.75	12.87±0.09	0.31
Day mean	11.29	11.83	11.18	10.65	11.23±0.27	0.60
± SE	±0.43	±0.351	±0.683	±0.628		

*** For abbreviations see Table 1.**

It is well known that the color of meat varies depending on the state of myoglobin. Generally, metmyoglobin percentages of the control sample were higher than their corresponding of the treated samples A and B during the refrigerated storage period (Table 3 and Fig.1). Sample B blended with 600 ppm sodium ascorbate plus 5 ppm α-tocopherol acetate was found to contain low MetMb content (50.92±0.91) than sample A (51.71±0.81) which blended with 400 ppm sodium ascorbate plus 10 ppm α-tocopherol acetate and also than the untreated control sample (55.80±1.44). Thus, it could be stated that blending of GBM with the antioxidant mixture of Vit.C plus Vit.E salts can help in minimizing metmyoglobin accumulation in meat tissues along the storage period. Mitsumoto *et al.,*(1991) found that sodium ascorbate (SA) at 500 ppm in ground beef minimized metmyoglobin formation during seven days illuminated display at 4 °C. Guidera *et al.,*(1997) has previously postulated a logical hypothesis which stated that α-tocopherol quenches free radicals originating from lipid oxidation and this in turn protects oxymyoglobin against oxidation. Metmyoglobin showed negative correlation with color score, a*(redness) and b*(yellowness) values (r = - 0.967, - 0.965 and - 0.988 respectively), while a positive correlation was shown between metmyoglobin and pH(Table 4).

Any process causing disruption of the muscle membrane system, such as grinding, cooking and deboning, accelerates development of oxidative rancidity. Oxidative rancidity and auto-oxidation are serious problems occurring during storage of meat and meat products for which thiobarbituric acid (TBA) has been used as an empirical measure.

Table 3: Changes in metmyoglobin, odor score and TBA values of ground buffalo meat (GBM) blended with Vit.C plus Vit. E and stored at 4±1 °C.

GBM	Refrigerated storage (days)				Treatment mean*	
Samples	0	2	4	6	±SE	SD
Metmyoglobin						
Control	49.65	54.67	55.87	62.99	55.80±1.440	4.98
A	47.59	51.44	52.85	54.99	51.71±0.812	2.81
B	46.06	50.88	52.64	54.11	50.92±0.910	3.17
Day mean	47.77	52.33	53.78	57.36	52.81±0.710	4.26
± SE	±0.520	±0.591	±0.521	±1.413		
Odor score						
Control	4.71	4.42	3.59	2.67	3.85±0.240	0.84
A	3.76	4.75	4.55	4.02	4.27±0.120	0.42
B	4.75	4.65	4.06	3.31	4.19±0.173	0.60
Day mean	4.41	4.66	4.07	3.33	4.10±0.108	0.65
± SE	±0.162	±0.048	±0.151	±0.195		
Thiobarbituric acid (TBA) value (mg malonaldehyde/kg meat)						
Control	0.17	0.16	0.17	0.11	0.15±0.008	0.0028
A	0.15	0.13	0.14	0.09	0.13±0.007	0.0025
B	0.11	0.09	0.11	0.08	0.10±0.004	0.0015
Day mean	0.14	0.13	0.14	0.09	0.13±0.005	0.0033
± SE	±0.010	±0.011	±0.010	±0.005		

*** For abbreviations see Table 1.**

Data presented in Table 3 and Fig 2 showed that TBA values of the control, A and B samples at day zero were 0.17, 0.15 and 0.11 mg malonaldehyde/kg meat respectively. Upon comparing TBA values of the studied samples during second, fourth and sixth days of storage; sample B was found to be of the lower TBA values. Presumably, mixing of ground buffalo meat with 600 ppm sodium ascorbate plus 5 ppm α-tocopherol acetate mixture could be recommended for lowering TBA value and hence for inhibiting lipid oxidation as well as for preventing rancidity to an extent up to six days of refrigerated storage. Okayama *et al.* (1987) have found that beefsteaks dipped in Vit.E plus Vit.C solution showed very low TBA values during 13 days after treatment. TBA number showed a positive correlation (r= 0.979) with pH and metmyoglobin; meanwhile, there was a negative correlation between TBA number and odor score
(r = - 0.664) (Table 4).

Table 4: Correlation coefficient of pH, color score, metmyoglobin and odor score with different quality parameters of ground buffalo meat (GBM) samples during refrigerated storage at 4±1 °C.

Parameter	pH	Color score	Metmyoglobin	Odor score
Color score	- 0.999*	---	- 0.967	---
A*(redness)	- 0.999*	1.00**	- 0.965	---
B*(yellowness)	- 0.988	0.995	- 0.988	---
Metmyoglobin	0.953	- 0.967	---	---
Odor score	- 0.802	0.832	---	---
TBA	0.979	---	0.871	- 0.664

*** p < 0.05** **** p < 0.01**

Odor of all the tested buffalo meat samples (Table 3) rated pleasant scores at zero and second days of refrigerated storage. At fourth day the same evaluation was given by the panelists for the treated samples A and B; meanwhile, the control sample odor score was moderately pleasant. At the end of refrigerated storage period the odor scores were moderately unpleasant, moderately pleasant and pleasant for the control, samples A and B respectively. There was negative correlation of odor score with pH (r = - 0.802) and positive correlation with color score (Table 4).

From the present investigation it was obvious that blending of GBM with mixture of the biological antioxidants Vit C plus Vit. E salts especially at the level: 600 ppm sodium ascorbate plus 5 ppm α-tocopherol acetate could inhibit lipid oxidation, retard development of rancidity & discoloration (enhance color quality) and minimize, as possible, metmyoglobin (MetMb) formation in meat tissues during the applied storage period.

Fig 1+2

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جودة لحم الجاموس المفروم وتأثرها بخلطات من أملاح فيتامين C وفيتامين E كمضادات للأكسدة

هيام محمد أحمد إبراهيم

قسم الصناعات الغذائية والألبان ، المركز القومى للبحوث ، الدقى ، جيزة ، مصر.

تم دراسة تاثير إضافة مستويين من خليط أملاح فيتمين C (أسكوربات الصوديوم) ، فيتامين E (خلات الألفاتوكوفيرول) – مواد حيوية مضادة للأكسدة – على بعض محددات جودة اللحم الجاموسى المفروم أثناء تخزينة مبرداً لمدة ستة أيام عند 4± 1 ^{5}م .

بمقارنة عينة اللحم الجاموسى المفروم والمخلوط بـ 600 جزء فى المليون أسكوربات الصوديوم + 5 جزء فى المليون من خلال الألفاتوكوفيرول بمثيلتها المخلوطة بـ 400 جزء فى المليون من أسكوربات الصوديوم + 100 جزء فى المليون من خلات الألفاتوكوفيرول وأيضاً بالعينة الكنترول (غير المخلوطة بمضادات الأكسدة الحيوية المذكورة) وجد أن :

عينة اللحم الأولى المخلوطة كانت أقل فى كل من قيم الفقد أثناء الطهى (Cooking loss) وتكوين الميتميوجلوبين (Metmyoglobin) ، حمض الثيوباربيتوريك (TBA) . وأيضاً بمقارنة العينة الأولى بالعينة الثنية المخلوطة والعينة الكنترول جاءت عينة اللحم الأولى أعلى فى قيمة مقياس اللون الأحمر a(rednes) ، وأكثر قابلية بالنسبة لتقييم اللون والرائحة حسياً (Visual color, Odor score).

وهكذا يتضح أن خلط اللحم الجاموسى المفروم بـ 600 جزء فى المليون من أسكوربات الصوديوم + 5 جزء فى المليون من خلات الألفاتوكوفيرول يمكن أن يؤدى إلى تأخير التزنخ فى الدهن والتغير فى اللون ويقلل بقدر الأمكان من تكوين الميتموجلوبين وبالتالى يؤدى إلى تحسن اللون وجودة اللحم المفروم خلال مدة التخزين.