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Effects of Sodium Tripolyphosphate and Modified Atmosphere Packaging on the Quality Characteristics and Storage Stability of Ground Beef

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Summary

The effect of sodium tripolyphosphate (STP; 0.25 and 0.50 % by mass), modified atmosphere packaging (MAP; 80 % O_2+20 % CO_2), and storage time (14 days) on the colour, lipid stability and microbial growth during storage ((2.0±0.5) °C) of ground beef was studied. During storage, total aerobic mesophilic, psychrotrophic, lactic acid bacteria, *Pseudomonas* sp. and *Enterobacteriaceae* sp. counts, thiobarbituric acid reactive substances (TBARS) and colour values of ground beef were determined. There was significant ($p<0.01$) difference in the microbial counts in ground beef among the treatment groups. The lowest mesophilic, psychrotrophic bacteria and *Pseudomonas* counts were determined in the 0.25 % STP+MAP group. Compared to aerobic control, the number of *Pseudomonas* was decreased approx. 2 log units in all other treatment groups. Other than MAP alone, 0.50 % STP+MAP resulted in a significant reduction of lipid oxidation of ground beef. The red colour value *a** of STP+MAP groups was more stable than that of aerobic and MAP controls. The values of *a** (redness) and *b** (yellowness) of the external surface of ground beef were higher than those of the internal surface. Using only MAP with high O_2 is unsuitable for ground beef because it increases lipid oxidation. Thus, the quality of ground beef can be protected using STP and MAP with 80 % O_2+20 % CO_2.

Key words: ground beef, sodium tripolyphosphate, MAP, lipid oxidation, shelf life, colour

Introduction

Polyphosphates are used in meat industry mostly in the form of sodium tripolyphosphate (STP) because of various functions such as improvement of textural properties by increasing water-holding capacity and inhibition of microbial growth. Sodium phosphate increases the water-binding capacity of the protein, leading to a stabilization of the myofibrils, and retards oxidative rancidity. It also binds heavy metals and thus helps protect against the microbes that need these metals (*1-5*).

Colour affects both the consumer's perception of meat freshness and the decision to purchase, and it is an important component of the visual appeal of meat. The muscle protein myoglobin and its different forms such as deoximyoglobin, oxymyoglobin and metmyoglobin are primarily responsible for the colour of beef. Mincing of beef meat facilitates oxygen penetration and leads to extensive myoglobin oxygenation. Myoglobin oxidizes to metmyoglobin and the resulting brown discolouration is associated with a lack of freshness in meat. The use of modified atmosphere packaging (MAP) can extend the colour stability of fresh meat (*6,7*). MAP usually uses carbon dioxide to inhibit microbial growth (*8*). Seyfert *et al.* (*9*) reported that 80 % of oxygen and 20 % of carbon dioxide provided a brighter, more cherry red and stable colour even during extended display periods, compared to 80 % of nitrogen and 20 % of carbon dioxide for injection-enhanced beef round muscles. On the other hand, oxygen is necessary to maintain oxymyo-

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globin in fresh beef, but it promotes lipid oxidation (*6,10*). Also, the disruption of muscle cell structure exposes lipid components sensitive to oxygen, thus lipid oxidation is an important problem in ground beef. Therefore, the addition of sodium tripolyphosphate (STP) to ground beef as a means of enhancing colour and lipid stability, and the effect of various levels of STP (0.25 and 0.50 %) and modified atmosphere packaging (MAP) on the acceptability of ground beef, *i.e.* both colour and lipid stability, and microbial quality on days 0, 3, 6, 8, 11 and 14 of storage at (2.0±0.5) °C were investigated.

Material and Methods

Material

Turkey beef was obtained from EBK Slaughterhouse, Erzurum, Turkey, ground through a 3-mm diameter mincer under hygienic conditions and stored for 24 h at 0–4 °C and 85–90 % relative humidity until use. Polyamide/polyethylene (PA/PE) 3-seal bags (GB 70, 15×25 cm) were used as packaging material. The properties of PA/PE material were as follows: O_2 permeability 40 $cm^3/(m^2 \cdot Pa \cdot day)$ at 23 °C, N_2 permeability 24 $cm^3/(m^2 \cdot Pa \cdot day)$ at 23 °C, CO_2 permeability 1454 $cm^3/(m^2 \cdot Pa \cdot day)$ at 23 °C and water vapour permeability <3 $g/(m^2 \cdot Pa \cdot day)$ at 23 °C.

Preparation of ground beef samples and the addition of STP

A mass of 12 kg of ground beef was used in each experiment. Samples were divided into 200-gramme samples for each treatment group (treatment 1: aerobic control; treatment 2: MAP control, 80 % O_2+20 % CO_2; treatment 3: 0.25 % STP+80 % O_2+20 % CO_2; treatment 4: 0.50 % STP+ 80 % O_2+20 % CO_2). Sodium tripolyphosphate was mixed with ground beef manually.

Packaging and storage of samples

For modified atmosphere packaging (80 % O_2+20 % CO_2), Multivac gas packaging unit (Multivac 300/16 Sepp. Haggenmuller, Wolfertschwenden, Germany) was used. Except for aerobic control, all other samples were packed in modified atmosphere. All samples were stored at (2.0±0.5) °C for 14 days.

Microbiological analysis

A mass of 25 g of ground beef was placed aseptically in a stomacher bag, 225 mL of physiological solution (NaCl 0.85 %) were added and then homogenized in a stomacher for 1 min at normal speed. Serial dilutions were prepared and surface plate method was used for enumeration. For total aerobic mesophilic bacteria, plate count agar (PCA; Merck, Darmstadt, Germany) was used and plates were incubated aerobically at 30 °C for 48 h. Psychrotrophic bacteria were enumerated on the PCA after incubation at 10 °C for 7 days. *Pseudomonas* were enumerated on cetrimide fucidin cephaloridine (CFC) agar (*Pseudomonas* Agar Base CM0559, Oxoid, Basingstoke, UK) with selective agar supplement (SR0103, Oxoid) after incubation at 25 °C for 48 h. To enumerate lactic acid bacteria, de Man Rogosa Sharpe agar (MRS, Oxoid) was used and the plates were incubated anaerobically at 30 °C for 48 h. *Enterobacteriaceae* were also incubated anaerobically on violet red bile dextrose agar (VRBD, Oxoid) at 30 °C for 2 days (*11*). All bacterial counts were determined on days 0, 3, 6, 8, 11 and 14 of storage and expressed as colony forming units per gram of sample (CFU/g).

Determination of thiobarbituric acid reactive substances (TBARS)

For the determination of thiobarbituric acid reactive substances (TBARS) values, 1 g of ground beef sample was taken from each treatment after 0, 3, 6, 8, 11 and 14 days of storage and 6 mL of trichloroacetic acid (TCA) solution were added (7.5 % TCA, 0.1 % EDTA, and 0.1 % 1-propyl gallate, which was dissolved in 3 mL of ethanol). The mixture was filtered through Whattman no. 1 paper after homogenizing for 15–30 s. Then, 1 mL of 0.02 M thiobarbituric acid solution was added to 1 mL of filtrate. This mixture was kept in boiling water bath for 40 min, then cooled and centrifuged at 2000 rpm for 5 min. Finally, absorbance was measured at 532 nm (UV 160, Shimadzu, Kyoto, Japan) and TBARS values were determined as μmol of malondialdehyde (MDA) per kg.

Determination of colour values

Colour was measured on both internal and external surface of ground beef samples on days 0, 3, 6, 8, 11 and 14 of storage. For colour readings of internal surfaces, the meat was sliced and the cut (internal) surface was measured. Measurements were done immediately after opening the package. A Minolta colorimeter (CR-200, Minolta Co, Osaka, Japan) was used for the measurements of darkness/lightness and colour values: L^*=0, darkness; L^*=100, lightness; $+a^*$=red, $-a^*$=green and $+b^*$=yellow, $-b^*$=blue.

Statistical analysis

The results were analyzed by SPSS (*12*), and completely random block design was used in the statistical analysis. Comparisons of mean values were made using the Duncan's multiple range test ($p<0.05$). The model included treatment (aerobic control, MAP control, 0.25 % STP+MAP and 0.50 % STP+MAP) and storage period (0, 3, 6, 8, 11 and 14 days) as main effects, and all their interactions. Experiments were replicated twice. The results of the statistical analysis are shown in tables as mean values±standard error.

Results and Discussion

Microbiological changes

Treatment had significant effect ($p<0.01$) on total aerobic mesophilic bacteria counts. The highest counts were determined in the aerobic control, while the lowest were determined in MAP control and 0.25 % STP+MAP samples ($p<0.05$) (Table 1). Both storage time and the interaction of treatment with storage time had a significant effect ($p<0.01$) on total aerobic mesophilic bacteria counts. Total counts increased rapidly between 6 and 14 days of storage in the aerobic control (Fig. 1a), which is above the acceptable level according to the Turkish

Table 1. Microbiological counts and TBARS values of ground beef meat stored at (2.0±0.5) °C for 14 days

Treatment	Total aerobic mesophilic bacteria log CFU/g	Psychrotrophic bacteria log CFU/g	*Pseudomonas* log CFU/g	Lactic acid bacteria log CFU/g	TBARS/MDA μmol/kg
aerobic control	5.00a	5.09a	4.50a	3.44a	47.62b
MAP control	3.93c	4.14b	2.97b	3.23c	54.76a
0.25 % STP+MAP	4.05c	3.92c	2.63c	3.37ab	29.90c
0.50 % STP+MAP	4.19b	4.18b	2.94b	3.25c	19.15d
standard error	0.040	0.044	0.050	0.057	1.837
significance	**	**	**	*	**
Storage time/day					
0	3.28e	3.18e	2.42f	2.47e	7.21e
3	3.41e	3.32e	2.58f	2.55e	19.25d
6	3.61d	3.62d	2.84e	3.15d	24.87d
8	4.31c	4.24c	3.17c	3.41c	43.79c
11	5.03b	5.31b	3.86b	3.81b	62.55b
14	6.11a	6.32a	4.70a	4.54a	69.46a
standard error	0.049	0.054	0.062	0.069	2.250
significance	**	**	**	**	**
Interactions					
Treatment×storage time	**	**	**	n.s.	**

STP=sodium tripolyphosphate, MAP=modified atmosphere packaging (80 % O_2+20 % CO_2), n.s.=not significant, *p<0.05, **p<0.01
Mean values in the same column and in the same section having the same letters are not significantly different at p>0.05

Ground Meat Standard of $5 \cdot 10^6$ CFU/g (*13*). These results show that in order to preserve the quality of ground meat for longer time, it needs to be packaged and stored at low temperatures. Mincing increases the risk of microbiological spoilage, also chemical changes can occur during storage and shelf life. Therefore, the addition of antioxidant and the use of MAP can inhibit a wide group of microorganisms, extend the shelf life, and preserve the quality properties of ground meat. Alp and Aksu (*14*) determined that ground beef packed in modified atmosphere (80 % O_2+20 % CO_2) and stored at (2.0±0.5) °C for 14 days had lower total aerobic mesophilic bacteria counts than the packed aerobic control samples. Similarly, Değirmencioğlu *et al.* (*15*) reported that the total aerobic mesophilic bacteria counts can be decreased by packing ground beef samples in modified atmosphere (70 % O_2+30 % CO_2). Carbon dioxide is highly soluble in meat, and its presence in the MAP restricts the growth of aerobic spoilage bacteria. Gill (*16*) and Jeremiah (*17*) reported that the growth of aerobic bacteria is not prevented by moderate carbon dioxide concentrations in modified atmospheres containing a high oxygen concentration but it is retarded, and near maximum inhibition of aerobic florae is achieved with carbon dioxide concentration below 25 %. Also, authors reported that increasing the storage temperature progressively reduced the inhibitory effects of carbon dioxide, and decreasing the carbon dioxide concentration below 20 % permitted significantly more rapid growth. However, McMillin (*18*) reported that levels of 20–60 % carbon dioxide are required for inactivation of aerobic spoilage organisms, but Gill and Tan (*19*) observed that carbon dioxide above 50–60 % has little or no effect.

Psychrotrophic bacteria counts for four different treatments and storage time are also given in Table 1. Significant differences in the psychrotrophic bacteria counts were recorded among treatment groups ($p<0.01$). The highest count was determined in aerobic control, while the lowest was determined in 0.25 % STP+MAP ($p<0.05$). Also, storage time (Table 1) and the interaction of treatment with storage time (Fig. 1b) had a significant effect ($p<0.01$) on psychrotrophic bacteria counts. The initial (on day 0) population of psychrotrophic bacteria was low (3.18 log CFU/g), while on day 14 of storage it was 6.32 log CFU/g (Table 1). The use of MAP and a combination of STP+MAP resulted in the stability of psychrotrophic bacteria count until day 14 of storage (except for days between 11 and 14 using 0.50 % STP+80 % O_2+20 % CO_2). Aerobic control resulted in an increase of psychrotrophic bacteria counts during storage, and the highest increase was determined after day 6 of storage (Fig. 1b). Christopher *et al.* (*20*) determined that lower psychrotrophic bacteria count had been observed in meat stored in 40 % carbon dioxide.

Significant difference in the *Pseudomonas* count was determined among treatment groups ($p<0.01$). The highest count was determined in aerobic control, while the lowest was determined in 0.25 % STP+MAP ($p<0.05$). Also, storage time (Table 1) and the interaction of treatment with storage time (Fig. 1c) had significant effect ($p<0.01$) on *Pseudomonas* counts. The counts increased faster in aerobically packaged beef samples, in comparison with the other three groups (Fig. 1c). However, after 14 days, *Pseudomonas* counts were not different between the MAP and 0.50 % STP+MAP groups. The initial population of *Pseudomonas* was 2.41 log CFU/g of

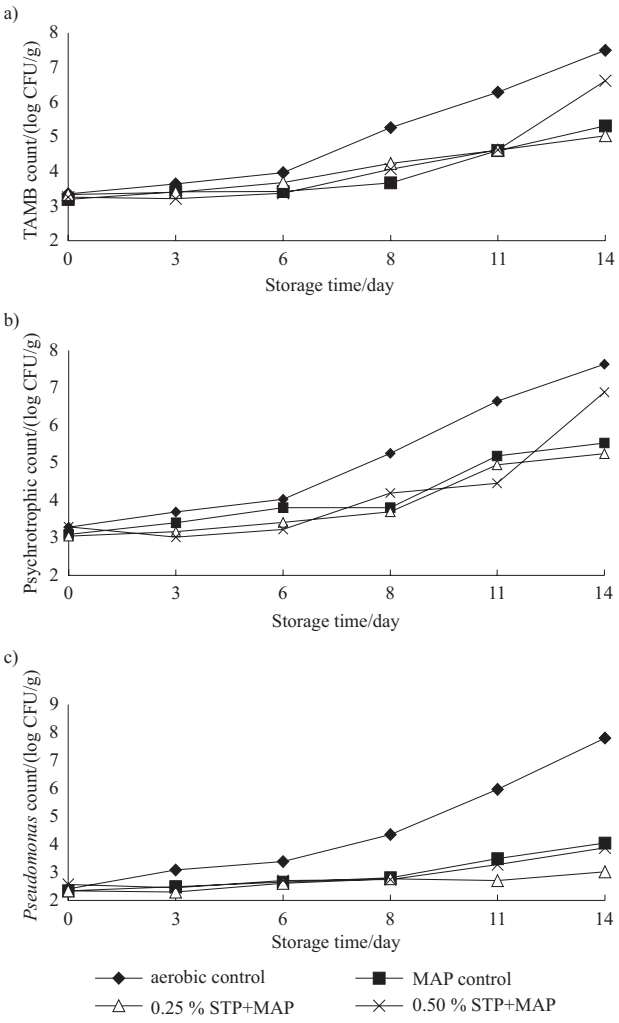

Fig. 1. Changes in: a) total aerobic mesophilic bacteria, b) psychrotrophic bacteria and c) *Pseudomonas* counts in MAP control (80 % O_2+20 % CO_2), aerobic control, and ground beef samples with 0.25 and 0.50 % sodium tripolyphosphate (STP) at (2.0±0.5) °C for 14 days

aerobic control samples, but on day 14 of storage, it was determined to be 7.81 log CFU/g. The use of MAP and/or combination of STP+MAP resulted in the stability of *Pseudomonas* count until day 14 of storage (Fig. 1c). This finding is in agreement with the results of Alp and Aksu (*14*), who found that ground beef packaged in 80 % O_2+20 % CO_2 and stored at (2.0±0.5) °C for 14 days had lower *Pseudomonas* count than the aerobically packaged samples. Several authors have reported that the survival and growth of spoilage microorganisms are affected by MAP (*18,21*), and the count of *Pseudomonas* can increase in meat in atmosphere with high O_2 (*8,22*). Kennedy *et al.* (*23*) also determined that the gas composition of 80 % O_2, 20 % CO_2 and 0 % N_2 and the product/gas ratio of 2:1 was the most effective in decreasing the count of *Pseudomonas* sp. in lamb meat stored at 4 °C for 12 days.

Treatment had significant effect ($p<0.05$) on lactic acid bacteria count. The highest count was determined in aerobic control and 0.25 % STP samples (Table 1), which is in agreement with the results of Alp and Aksu (*14*). Storage time also had a significant effect ($p<0.01$) on lactic acid bacteria count. The initial lactic acid bacteria count was 2.47 log CFU/g (on day 0) and reached 4.54 log CFU/g on day 14 of storage. Alp and Aksu (*14*) reported that the increase of LAB counts was approx. 2 log CFU/g during storage at (2.0±0.5) °C for 14 days in raw ground beef packaged in 80 % O_2+20 % CO_2.

The initial *Enterobacteriaceae* counts in ground beef were <2.00–2.47 log CFU/g. Their number increased to approx. 1–2 log CFU/g at the end of storage in all treatment groups. *Enterobacteriaceae* grew only under MAP and STP+MAP conditions at a slower rate than under aerobic packaging conditions (data not shown). They were below the detectable level (<2.00 log CFU/g) by day 8 of storage only under MAP and STP+MAP conditions, and by day 6 of storage in aerobically packaged samples. Alp and Aksu (*14*) reported that *Enterobacteriaceae* were below the detectable level until day 8 of storage period in modified atmosphere-packaged ground beef.

Thiobarbituric acid reactive substances values

Thiobarbituric acid reactive substances (TBARS) values changed significantly ($p<0.01$) depending on the treatment and storage time (Table 1). As seen in Fig. 2, the highest value was determined in MAP control, while the sample with 0.50 % STP+MAP showed the lowest value. In addition, the interaction of treatment with storage time had a significant effect ($p<0.01$) on TBARS values. Oxygen is necessary to maintain oxymyoglobin in fresh beef, but it promotes lipid oxidation (*6,10*). Also, the exposure of labile lipid components to oxygen is caused by the disruption of muscle cell structure, which represents a serious problem in ground beef. On the other hand, ground beef patties fortified with n-3 oil were incorporated with sodium tripolyphosphate, sodium citrate and sodium erythorbate in several combinations. Although STP tended to decrease the lipid oxidation ($p<0.05$), there was no difference between STP and STP+sodium erythorbate ($p>0.05$) (*24*). One of the most frequently observed chemical changes in meat and meat products is the lipid oxidation, especially in ground beef, which due to its adiposeness is more liable to lipolytic

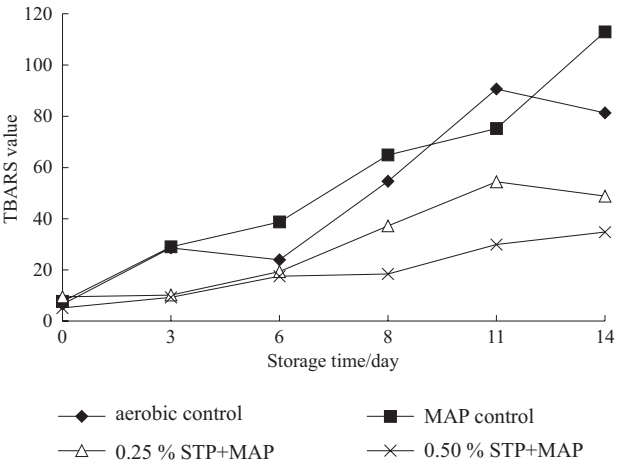

Fig. 2. Changes in TBARS values in MAP control (80 % O_2+20 % CO_2), aerobic control, and ground beef samples with 0.25 and 0.50 % sodium tripolyphosphate (STP) at (2.0±0.5) °C for 14 days

reactions with its wide surface area than the other fresh meat and meat products (*18*). Also, lipid oxidation causes rancid flavour. Some of the degradation products of lipid oxidations such as malondialdehyde had been reported as a carcinogenic factor (*25*). Cheng and Ockerman (*25*) determined that the addition of 0.5 % sodium tripolyphosphate maintained the oxidative stability of precooked roast beef better than 0.40 or 0.25 % sodium tripolyphosphate. Jayasingh and Cornforth (*26*) reported that TBA numbers were found to be low ($p<0.01$) in cooked pork patties treated with milk mineral or STP when compared to controls or BHT (butylated hydroxytoluene) treatments. Also, Vissa and Cornforth (*27*) reported that metal chelating antioxidants such as STP were more effective than vitamin E for inhibition of lipid and pigment oxidation in raw ground beef in MAP with 80 % O_2.

Colour values

Colour values for all of the four different treatments and their interactions are given in Table 2. Effects of treatments and storage time on L^*, a^* and b^* values are shown in Figs. 3 and 4. Both treatment ($p<0.01$) and storage time ($p<0.05$) affected the L^* value of the ground beef. The highest L^* value, which refers to lightness, was determined in aerobic and MAP control samples, while the lowest value was determined in STP+MAP samples ($p<0.05$). A significant ($p<0.05$) increase in the L^* values was recorded during 14 days of storage (Table 2). Also, the interactions of treatment with storage time (Fig. 3a) and of external and internal surfaces with storage time (Fig. 4a) had significant effect ($p<0.01$) on the L^* values. The lowest L^* value during storage period was determined in 0.25 % STP+MAP group (Fig. 3a). The L^* values of the external and internal surfaces were not different after day 6 of storage (Fig. 4a). Significant changes in colour values were recorded for the a^* values, which refer to redness. Treatment ($p<0.01$), external and internal surfaces ($p<0.01$) and storage time ($p<0.01$) had a significant effect on the changes of a^* values in ground beef (Table 2). The effect of interactions of treatment with storage time (Fig. 3b) and of external and internal surfaces with storage time (Fig. 4b) on a^* value was found to be significant ($p<0.01$). The difference in terms of a^* values of STP+MAP groups was not significant before

Table 2. The L^*, a^* and b^* values of ground beef meat stored at (2.0±0.5) °C for 14 days

Treatment	L^*	a^*	b^*
aerobic control	40.314a	20.542c	9.424
MAP control	40.111a	21.674b	9.720
0.25 % STP+MAP	38.304b	22.978a	9.532
0.50 % STP+MAP	38.353b	23.344a	9.557
standard error	0.377	0.288	0.152
significance	**	**	n.s.
External or internal surface			
external surface	39.592	23.218a	10.358a
internal surface	38.949	21.051b	8.758b
standard error	0.267	0.204	0.107
significance	n.s.	**	**
Storage time/day			
0	38.118b	25.157a	9.017c
3	38.876ab	25.216a	9.524abc
6	39.177ab	22.430b	10.070a
8	39.743a	21.775b	9.698ab
11	39.733a	18.109d	9.330bc
14	39.977a	20.120c	9.710ab
standard error	0.462	0.353	0.0186
significance	*	**	**
Interactions			
External or internal surface×storage time	**	**	**
External or internal surface×treatment	n.s.	**	n.s.
Treatment×storage time	**	**	**

STP=sodium tripolyphosphate, MAP=modified atmosphere packaging (80 % O_2+20 % CO_2), n.s.=not significant, *$p<0.05$, **$p<0.01$
Mean values in the same column and in the same section having the same letters are not significantly different at $p>0.05$

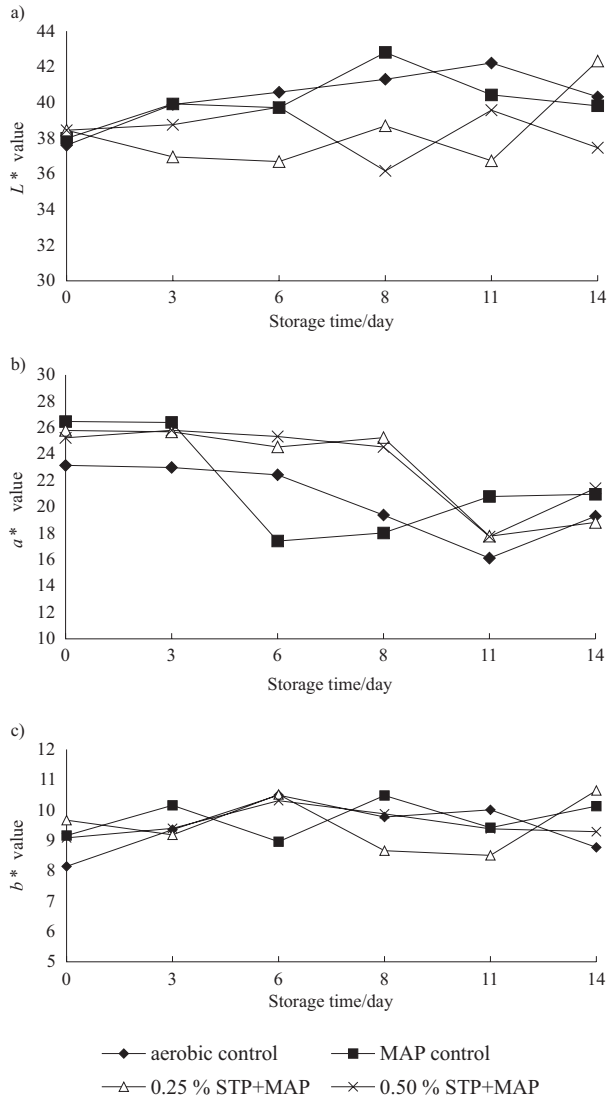

Fig. 3. Changes in: a) L^*, b) a^* and c) b^* values of MAP control (80 % O_2+20 % CO_2), aerobic control, and ground beef samples with 0.25 and 0.50 % sodium tripolyphosphate (STP) at (2.0± 0.5) °C for 14 days

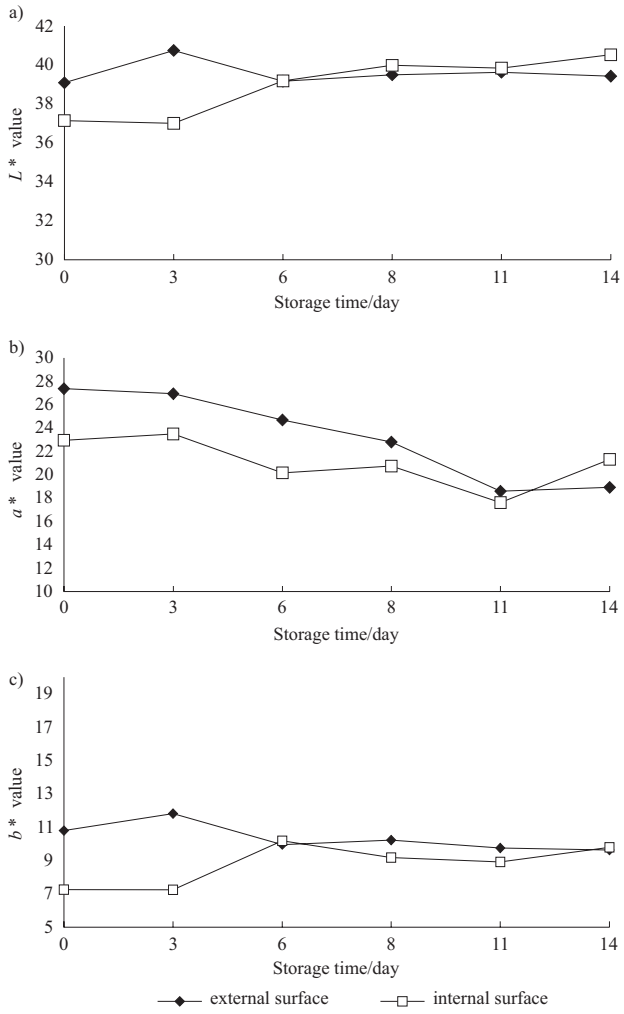

Fig. 4. Effects of storage time on: a) L^*, b) a^* and c) b^* values of the external and internal surfaces of ground beef samples at (2.0±0.5) °C for 14 days

day 8 of storage, when it decreased considerably (Fig. 3b). The a^* values of the external surface of ground beef were higher than those of the internal surface, and a^* values of both external and internal surfaces were generally low during storage period (Fig. 3b). Although both external and internal surfaces and storage time had a significant effect on the b^* values of the ground beef ($p<0.01$), there was no significant difference ($p>0.05$) among treatment groups (Table 2). Also, the interactions of treatment with storage time (Fig. 3c) and of external and internal surfaces with storage time (Fig. 4c) had a significant effect ($p<0.01$) on the b^* values. The use of modified atmosphere packaging (MAP) extended the colour of fresh meat during shelf life (*7*,*18*), and high concentrations of O_2 are used in MAP to maintain the meat pigment myoglobin in the oxygenated state (*28*). Thus, modified atmosphere packaging (MAP) is increasingly used for retail ready meat packaging. The process of mincing facilitates oxygen penetration into the meat and leads to extensive myoglobin oxygenation. Myoglobin oxidises to metmyoglobin and a brown discolouration associated with a lack of freshness of meat occurs. Lee *et al*. (*29*) determined that there was high correlation between Hunter a^* and metmyoglobin values, and that the addition of 0.5 % sodium tripolyphosphate to restructured beef rolls significantly increased a^* values, but significantly decreased L^* and b^* values. Similarly, as seen in Fig. 5, the effect of interaction of external and

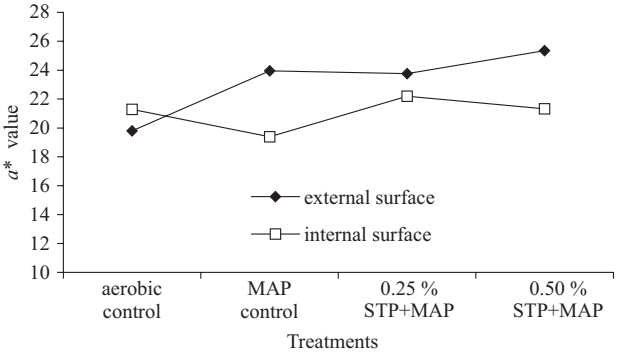

Fig. 5. Effects of treatments on a^* values of the external and internal surfaces of ground beef samples at (2.0±0.5) °C for 14 days

internal surfaces with treatment had a significant effect on the a^* values of the ground beef ($p<0.01$). There was significant difference in a^* values of the external and internal surfaces of ground beef stored for 14 days among treatment groups, and difference between MAP control and 0.50 % STP+MAP groups were higher than that of aerobic and 0.25 % STP+MAP groups (Fig. 5). Jakobsen and Bertelsen (*30*) determined that temperature and time are very important factors in retaining meat colour, and a good meat colour is stable between 55 and 80 % O_2 for samples of fresh beef muscle stored at 2–8 °C for 10 days. Also, Kennedy *et al*. (*23*) found that the gas composition of 80 % O_2, 20 % CO_2 and 0 % N_2 was the most effective packaging for maintaining and prolonging the attractive red colour of lamb meat stored at 4 °C for 12 days. The obtained results for a^* values during storage are in agreement with those of Vissa and Cornforth (*27*).

Conclusion

The results of this study show that the quality characteristics of ground beef during storage may be improved by using sodium tripolyphosphate (STP) and modified atmosphere packaging (MAP). It was observed that *Pseudomonas* sp. and psychrotrophic bacteria can be inhibited by STP and MAP combinations. Lipid oxidation can be prevented by using sodium tripolyphosphate, and the use of 0.50 % of STP decreased the TBARS values. In addition, colour values, especially a^* values, of ground beef during storage may be improved by STP and MAP. Contrary to aerobic control samples, the samples of ground beef with STP and MAP with 80 % O_2+20 % CO_2 showed acceptable properties at (2.0±0.5) °C for 14 days. In conclusion, based on the results of this study, the combination of sodium tripolyphosphate with modified atmosphere packaging containing 80 % O_2+20 % CO_2 can be used in order to maintain the quality of ground beef meat.

Acknowledgements

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