MICROBIOLOGICAL, CHEMICAL, AND PHYSICAL CHANGES IN REFRIGERATED GROUND BUFFALO MEAT TREATED WITH POTASSIUM SORBATE, SODIUM ASCORBATE, AND SODIUM TRIPOLYPHOSPHATE

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Abstract: Ground buffalo meat was preblended with either 0.3% potassium sorbate, 0.05% sodium ascorbate, 0.5% sodium tripolyphosphate, 0.3% potassium sorbate/0.05% sodium ascorbate, 0.3% potassium sorbate/0.5% sodium tripolyphosphate, 0.05% sodium ascorbate/0.5% sodium tripolyphosphate, and mixture of 0.3% potassium sorbate/0.05% sodium ascorbate/0.5% sodium tripolyphosphate and stored in refrigerator at 4°C ± 1°C. Color (L, a, b), pH, acidity, water holding capacity (WHC), cooking loss 2-Thiobarbituric acid reacting substances (TBARS) number, and total volatile bases, (TVB) free amino nitrogen (FAN) were determined. Aerobic plate counts (APC), Anaerobic bacterial count, psychrophilic bacterial count, total coliform and sensory properties were also determined. The results indicated that ground buffalo meat treated with 0.3% potassium sorbate/0.05% sodium ascorbate had the highest Hunter a value (redness). pH values, TBARS number, and TVB increased along with storage period. Samples treated with potassium sorbate alone or potassium sorbate mixed with other chemicals had lower anaerobic and psychrophilic bacterial counts than other samples and still accepted for panelist after 12 days of storage.

Key words: buffalo meat, potassium sorbate, sodium ascorbate, sodium tripolyphosphate

Introduction

Buffalo meat production in Egypt is 306.500 Mt contributes 20.52% of total meat production (FAO, 2005). The meat is produced mainly from very old unproductive animals which results in it being coarse and tough in texture and dark in color. Such meat is profitably utilised by comminuting and using in a variety of meat products (Sahoo and Anjaneyulu 1997a). Ground meat tends to become brown and rancid more rapidly than whole muscle retail cut since grinding exposes more of the muscle surface to air and microbial contamination (Mitsumoto et al., 2005). Such changes are attribute to rapid formation of metmyoglobin, the undesirable brown color and oxidative rancidity. Lipid oxidation in meats leads to the development of off-flavour, loss of color and nutritive value (Pearson et al., 1983). Microbial growth in fresh meat is the primary factors associated with meat quality reduction and spoilage. The off-odour compounds that characterize spoilage meat originate largely from the nonprotein nitrogen compounds. Spoilage flora attacks the nonprotein nitrogen components and produces amines and ammonia from these simple components (Jay and Shelef, 1978).

Extended shelf life and meat product safety require maintaining low microbial numbers during fabrication, packaging, and storage of meat at refrigeration temperature. A variety of additives which have the potential for inhibiting microorganisms associated with fresh meat products have been investigated. A concentration of 0.1% Potassium sorbate delayed the growth of the spoilage microflora, retarded growth of salmonellae, and Staphylococcus Aureus, and growth and toxin production by C. botulinum (Sofos and Busta, 1981; Robach and Sofos, 1982; Sofos 1989). Sorbate has also inhibited bacteria (i.e., total psychrotrophs, Pseudomonas spp., B. thermosphacta, Lactobacillus spp., Enterobacteriaceae, Salmonella and Staphylococcus aureus, Cl. botulinum yeast and molds) and extended the shelf life of raw beef (Robach and Ivey 1978; Zamora and Zarithzy 1987b; Zamora and Zarithzy 1987a Sofos 1989).

The use of antioxidant like ascorbic acid had a significant effect in reducing oxidation of pigments and lipids of ground beef and beef steaks (Greene et al., 1971; Shivis et al., 1984; Okayama et al., 1987; Mitsumoto et al., 2005). Sodium ascorbate (SA) at 500ppm retarded pigments and lipids oxidation and extended the shelf life of ground buffalo meat from 4 to 8 days under refrigerated storage at 4°C+1°C (Sahoo

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and Anjaneyulu 1997a). Phosphates are widely used in meat industry to improve binding, water-holding capacity and yield, and to retard oxidative rancidity of meat products (Li et al., 1993; Lin and Lin 2002). Sodium tripolyphosphate increases water holding capacity and juiciness of meat products (Molins 1991), prevents oxidative rancidity development (Unda et al., 1990) and may have an antimicrobial effect (Marcy et al., 1988). Extending the shelf life of fresh meat is very important consideration for both consumers and meat packers. The storage life of fresh meat can prolonged by limiting the extent of discoloration, lipid oxidation and microbiological contamination.

The objectives of this study were to evaluate effect of adding potassium sorbate, Sodium tripolyphosphate and sodium ascorbate used alone or in combination on the physicochemical, microbiological, and sensory quality of ground buffalo meat during refrigerated storage.

Materials and methods

Sample preparation
15 kg meat chunks of about 2 kg size from top round of spent female buffalo, about 10 years age were obtained within 4 hr of slaughter from local market in Minia city. Meat chunks were packed in polyethylene bags, transported to Food Science Department, Faculty of Agriculture, Minia University and kept for conditioning in a refrigerator at 4°C±1°C for about 24hr. The meat chunks were trimmed of separable fat and loose connective tissue, cut into small cubes and ground by using a meat grinder (Moulinex, HV2, Model A14, Moulinex, France) with a 8-mm hole plate with adding 20% fat. Ground buffalo meat 20% fat was divided into 8 portions and mixed with either 0.3% potassium sorbate (Sofos 1989), 0.05% sodium ascorbate was bought from Sigma Chemical Company (ST. Louis, MO, USA), (Sahoo and Anjaneyulu, 1997a). 0.5% sodium tripolyphosphate was obtained from (BK Labendury Corp, Cresskill, NJ, USA), (Sahoo and Anjaneyulu, 1997b). 0.3% potassium sorbate/0.05% sodium ascorbate, 0.3% potassium sorbate/0.5% sodium tripolyphosphate, 0.05% sodium ascorbate/0.5% sodium tripolyphosphate, and mixture of 0.3% potassium sorbate/0.05% sodium ascorbate/0.5% sodium tripolyphosphate and minced again with 4 mm hole plate for uniform dispersion of additives. Both control and treated ground buffalo meat were divided into 200g, placed in Styrofoam tray and overwrapped with stretch film (saran). All trays were stored in refrigerator at 4°C ±1°C for 14 days. The samples were examined for quality parameters at 4day intervals during storage.

Microbiological analysis
(APC), Anaerobic count, Psychrophilic count, and coliform count of the treated and the untreated ground buffalo meat were determined as (CFU/g) according to the methods described in the standard methods of (APHA, 1985; Vaderantz and Splittstoesser, 1992). BBLGasPak® anaerobic chamber with BBL GasPak CO₂ gas packs (Becton Dickinsin Microbiology System, Boston, MA) was used to create an anaerobic environment for incubation.

Color evaluation
Hunter color values (lightness L*, redness a*, and yellowness b*) were measured for treated and untreated ground buffalo meat at zero time and during storage period with a colorimeter (Color Tec PCM Color Meter Tec. NJ, USA). Four random measurement spots on each sample were made and the average data were recorded according to Holownia et al., (2003)

pH measurement
pH was determined by homogenizing 10g of ground meat in 90ml distilled water using a homogenizer (VIRTIS Model 6-105 AF, The VIRTIS Company, NY, USA) for 5 min and measuring the pH of the resulting slurry with a digital pH meter (Model 41250, ICM, OR, USA), standardized at pH 4 and 7 (Lee and Yoon, 2001 ). The average of three reading was recorded.

Determination of acidity
Acidity was determined by titration according to Keeton and Melton (1978).

Determination of expressible water (EW) and water holding capacity (WHC)
Expressible water was determined according to Alvarez et al., (1992), while water-holding capacity (WHC) was calculated.

Thiobarbituric acid reacting substances (TBARS) number
TBARS was determined following the distillation method described by Tarladgis et al., (1960).
Total volatile basic nitrogen (TVBN)
Total volatile basic nitrogen was measured according to Pearson, (1975).
Free amino nitrogen (FAN) was determined according to AOAC (1980).

Cooking loss
Meat samples (25g each) were tightly wrapped in polyethylene bags and cooked, totally immersed, in water bath at 80°C for 20 min. After cooking they were cooled, dried with paper towels and cooking losses were determined from the weights before and after cooking Anjaneyulu et al., (1989).

Sensory evaluation
Samples from each group were randomly assigned for sensory evaluation according to Sahoo and Anjaneyulu, (1997a). Twelve panel members with previous panel experience were chosen to evaluate ground meat buffalo odor and color discoloration during storage. Sensory score for odor 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 score for color discoloration was 1 = pale pink, 2 = pink, 3 = pinkish red, 4 = bright red and 5 = reddish-brown.

Statistical analysis
Data were analyzed with the GLM (General Linear Model) program using statistical analysis system (SAS, 1987). Mean values were compred by Duncan’s Multiple Range Test.

Results and discussion
Data in table 1 revealed that the L values (lightness) of all samples significantly (P<0.05) increased during storage time. The a value (redness) of all sample significantly (P<0.05) decreased after 4 days of storage except samples treated with sodium ascorbate (SA) only, SA and K-sorbate and mixture. No changes were found in the redness of samples treated with sodium ascorbate and K-sorbate and mixture after 12 days of storage. Sahoo and anjaneyulu (1997a) found that 500ppm treatment increased the lovibond tintometer red color units of ground buffalo during storage at 4°C. Redness of control and sample treated with K-sorbate only was sharply decreased after 4 days of storage.

Table 1. Effect of potassium sorbate, sodium tripolyphosphate and sodium ascorbate on the color (L*, a* and b*) of ground buffalo meat during refrigerated storage at 4°C.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage time days</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>42.12b</td>
</tr>
<tr>
<td>A</td>
<td>19.63a</td>
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<tr>
<td>b</td>
<td>10.27a</td>
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<tr>
<td>K-sorbate</td>
<td></td>
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<tr>
<td>L</td>
<td>45.44b</td>
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<tr>
<td>A</td>
<td>20.13a</td>
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<tr>
<td>b</td>
<td>8.69ab</td>
</tr>
<tr>
<td>Sodium tripolyphosphate</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>45.00a</td>
</tr>
<tr>
<td>A</td>
<td>20.17a</td>
</tr>
<tr>
<td>b</td>
<td>10.37a</td>
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<td>Sodium ascorbate</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>45.49a</td>
</tr>
<tr>
<td>A</td>
<td>23.56a</td>
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<tr>
<td>b</td>
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<tr>
<td>Mixture</td>
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</tr>
<tr>
<td>A</td>
<td>20.84a</td>
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<tr>
<td>b</td>
<td>9.29b</td>
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<td>K-sorbate+Sodium tripolyphosphate</td>
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</tr>
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<tr>
<td>L</td>
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<tr>
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</tr>
<tr>
<td>L</td>
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<tr>
<td>A</td>
<td>21.55a</td>
</tr>
<tr>
<td>b</td>
<td>10.38a</td>
</tr>
</tbody>
</table>

A bcd Mean values in the same row not followed by the same letter are significantly different (P< 0.05)
pH values of control, SA, and K-sorbate with SA treated samples were lower than other treatments at zero time (Figure 1).

![Figure 1: Effect of Potassium carbonate, sodium tripolyphosphate and sodium ascorbate on the pH value of ground buffalo meat during refrigerated storage at 4°C.](image)

Generally the pH was increased gradually with increased storage time. The highest values of pH were found in K-sorbate with STPP (5.92) followed by mixture treatment (5.80) at the fourth day, and STPP with SA (6.21 and control (6.17) at the eighth day of storage. Shelef and Jay (1970) reported that the difference between freshness and incipient spoilage ground beef usually do not exceed 0.3-0.5 of a pH unit during the first 4 days of storage. The increase of pH at zero time may have been owing to the STPP (Molius, 1991), and bacterial metabolic by-products, such as amino sugar during storage (Jay, 1992). Acidity of all samples decreased with increased the storage period (Figure 2).

![Figure 2: Effect of Potassium carbonate, sodium tripolyphosphate and sodium ascorbate on acidity (% as lactic acid) of ground buffalo meat during refrigerated storage at 4°C.](image)

Found that titratable acidity to be a relatively accurate method of determining spoilage in ground beef (Keeton and Melton 1978).
Water holding capacity (WHC) for all samples increased during storage time and the lowest values of WHC was found in the sample treated with K-sorbate and AS at 4, 8, and 12 days of storage (Figure 3).

![Graph](image)

Figure 3: Effect of Potassium sorbate, sodium tripolyphosphate and sodium acetate on the water holding capacity (WHC) % of ground buffalo meat during refrigerated storage at 4°C.

It is well known that lower WHC is associated with lower pH. Both increasing a pH as a result of ammonia production and amino sugar complex formation have the effect of increasing the WHC of meats during refrigerated storage (Jay and Shelef, 1978). Expressible water (EW) of samples treated with SA, K-sorbate and SA, and STPP and SA was higher than other sample at zero time. K-sorbate and SA had the highest EW during storage time this may attribute to its low pH values (Figure 4).

![Graph](image)

Figure 4: Effect of Potassium sorbate, sodium tripolyphosphate and sodium acetate on the expressible water % of ground buffalo meat during refrigerated storage at 4°C.

Cooking loss gradually decreased along with storage time. K-sorbate and K-sorbate and SA treated samples had higher values of cooking loss than those of other treated samples at 4 and 8 days of storage (Figure 5).
STPP and SA decreased the cooking loss during storage. This may be due to increase in Na+ ions in the meat (Sahoo and Anjaneyulu, 1997a) and STPP increase the water holding capacity of meat (Dziezak, 1990).

Free amino nitrogen (FAN) ranged from 299.31 to 260.72 mg/100g at zero time and ranged from 143.15 to 110.61 mg/100g at 4 days of storage. FAN for all samples sharply decreased after the first 4 days of storage then gradually decreased with storage period (Figure 6).

Jay (1992) reported that free amino acids and related simple nitrogenous compounds utilized by bacteria during the first days of refrigerated storage and the primary proteins are not attacked until the supply of simpler constituents has been exhausted. The total volatile bases (TVB) could be used as a quality indicator
for fish products and is associated with the amino acid decarboxylase activity of microorganisms during storage (Jay, 1992). Changes in TVB values during storage are shown in Figure 7.

Figure 5: Effect of Potassium carbonate, sodium triphosphate and sodium ascorbate on the total volatile basic nitrogen (TVBN, mg/100 g) of ground buffalo meat during refrigerated storage at 4°C.

TVBN values of all treatments increased with increasing storage time and K-sorbate, mixture, K-sorbate and STPP, and K-sorbate and SA had lower TVBN values than other treatments. Control, STPP, SA, and STPP and SA treatments remained at higher TVBN values suggesting greater bacterial populations and activity, which in agreement with microbial counts (Figures 9, 10, 11, and 12).

Figure 9: Effect of Potassium carbonate, sodium triphosphate and sodium ascorbate on the aerobic plate counts in ground buffalo meat during refrigerated storage at 4°C.
Only control sample had 34.56 mg/100g TVBN and became unacceptable after 8 days of storage. TBARS values increased over time for all samples. The increment was rapid for the control samples and the greatest changes occurring between the 8 and 14 days of storage. Samples treated with SA or STPP alone and mixture of SA and STPP had lower TBARS than other treatment (Figure 8).
Treatment of processed meat and poultry with polyphosphates has shown beneficial effects for inhibition of oxidative changes and flavor deterioration (Ang and Young 1989; Molins 1991). On the other hand, Reece et al., (1997) reported that TBARS were higher in antimicrobial treated samples, which suggested that microorganisms in the untreated meat may have removed malonaldehyde and other TBARS. Sahoo and Anjanevula, (1997a) reported that Sodium ascorbate at 500 ppm contributed to the lowest TBARS value (0.26 mg malonaldehyde/kg) in refrigerated ground buffalo meat indicating that it inhibited lipid oxidation.

The growth of microbes in meat is one of the main factors that cause discoloration and spoilage. Aerobic plate counts (APC) for samples treated with SA, STPP, and SA and STPP increased with increasing storage time and reached 6Log CFU/g after 8 days (Figure 9). However, APC for K-sorbate, K-sorbate and SA, K-sorbate and STPP, and mixture decreased after 4 and 8 days and then increased after 12 and 14 days of storage. According to the guidelines from the Meat Hygiene Manual (Canadian Food Inspection Agency) these maximum values are 7 and 3 log CFU/g for total aerobic mesophilic and coliform count (Saucier et al., 2000). Aerobic plate count of control sample was over the accepted limit on the day 12 but exhibited off-odor on the day 8. APC of samples treated with K-sorbate alone or K-sorbate mixed with other compounds were less than 5Log CFU/g after 12 days of storage. Aerobic plate count remained under the maximum value (7 log CFU/g) after 14 day of storage for all samples except the control, STPP and SA treated samples. Zamora and Zaritzky (1987a,b) reported that potassium sorbate treatment inhibited the bacterial growth and extended the shelf life of refrigerated beef slices. Sorbic acid is a lipophilic acid preservative with a short chain length and this kind of substances inhibits both gram positive and gram negative bacteria (Sofos and Busta, 1981).

Psychrophilic counts and anaerobic count of all samples increased with storage time (Figure 10 and 11). Refrigerated meat under aerobic conditions, the spoilage flora is dominated by pseudomonas spp. and under anaerobic condition by lactobacillus spp. (Marth, 1998). Coliform counts had the same trend of APC. Control sample had the highest number of coliform during storage time. Sample treated with K-sorbate alone or mixed with other compounds had lower coliform count than other treatments and its coliform counts less than 3Loge after 12 days of storage (Figure 12).
Color score of all samples slightly decreased after 4 days of storage (Figure 13).

Figure 13- Effect of Potassium sorbate, sodium tripolyphosphate and sodium ascorbate on the color score of ground buffalo meat during refrigerated storage at 4°C.
The control, K-sorbate, and SA treated samples had lower color score than other treatments on the day 8. Samples treated with K-sorbate mixed with SA had the highest color score during storage followed by samples treated with STPP, Mixture, K-sorbate and STPP, and K-sorbate and SA. Discoloration may be attributed to alteration or destruction meat pigments. Myoglobin may be oxidized to brown metmyoglobin, it may combined with H2S, produced by bacteria, to form sulphydmyoglobin (Lawrie, 1998). Rancid flavor and odors arise from oxidative changes occurring in the meat during refrigerated storage.

Odor score of control sharply decreased after 8 days of storage (Figure 12). Samples treated with STPP, SA, and STPP and SA had lower odor score than other treatments during storage time. The mixture and K-sorbate and SA treatments had higher odor scores than other treatment after 12 days of storage. The off-odor of meat may be due to the organisms attacked glucose initially and amino acids subsequently, producing hydrogen, carbon dioxide and ammonia (Jay, 1992). Van Laak, (1994) reported that off-odors become noticeable in chilled meat and poultry when bacterial numbers are between 7.0 and 7.5 log CFU/cm². Sahoo and Anjaneyulu, (1997a) found that 500 ppm sodium ascorbate extended the shelf life of ground buffalo meat from 4 to 8 days stored at 4°C. From these results it could be concluded that the shelf life of ground buffalo meat treated with potassium sorbate alone or mixed with other compounds (populations of microorganisms chemical and sensory quality) could be extended from 8 to 12 days. The SA, STPP, and SA and STPP treatment extended the shelf life of ground buffalo meat from 4 to 8 days under refrigerated storage.

MIKROBILOŠKE, HEMIJSKE I FIZIČKE PROMENE U HLADENOM MESU BIVOLA TRETIRANOG KALIJUMSORBATOM, NATRIJUMASKORBATOM I NATRIJUMTRIPOLOFOSFATOM

H. A. Abd El-Aal

Rezime

Mleveno meso bivola je tretirano prethodno u blenderu sa 0.3% kalijumsorbata, 0.05%, natrijumaskorbata ili 0.5% natrijumtrilopolifosfata, 0.3% kalijumsorbata /0.05% natrijumaskorbata, 0.3% kalijumsorbata /0.5% natrijumtrilopolifosfata, 0.05% natrijumaskorbata /0.5% natrijumtrilopolifosfata, i
kombinacijom 0.3% kalijumsorbata /0.05% natrijummaskorbata /0.5% natrijumtriplifosfata i čuvano u frizideru na 4°C ±1°C.

Boja (L, a, b), pH, kiselost, sposobnost zadržavanja vode (WHC), kalo kuvanja, TBARS broj i ukupni TVB, slobodni amino azot – FAN su određivani. Broj aerobnih čelija (APC), broj anaerobnih čelija, broj psihrofilnih čelija, ukupne koliformne i sensorne osobine su takođe određivane.

Rezultati su pokazali da mleveno meso bivola tretirano sa 0.3% kalijumsorbata/0.05% natrijummaskorbata imalo najveću Hunter-ovu vrednost (crvenilo). pH vrednosti, TBARS broj i TVB su se povećavali tokom perioda skladištenja mesa. Uzorci tretirani samo kalijumsorbatom ili kalijumsorbatom pomešanim sa ostalim hemikalijama su imali nižu vrednost broja anaerobnih I psihrofilnih čelija nego ostali uzorci.

**Ključne reči:** bivolje meso, kalijumsorbat, natrijummaskorbat, natrijumtriplifosfat

**References**

33. USA.