Effects of Vacuum and Modified Atmosphere Packaging on Shelf Life Extention of Minced Meat Chemical and Microbiological Changes

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Abstract: In this study, the effects three different headspace conditions on the properties of canned minced beef meat were investigated by measuring pH changes at intervals of 1, 4 and 7 days. The three types of headspace conditions were a headspace of atmospheric air, Vacuum Packaging (VP) and Modified-Atmosphere Packaging (MAP), i.e., 70% O_2 and 30% CO_2 (MAP1) and 10% O_2, 30% CO_2 and 60% N_2 (MAP2). The properties assessed at each of the four different conditions were color properties, oxidation stability and microbiological properties of minced beef meat stored at 4°C. Compared with air packaged samples, the most effective packaging methods were vacuum packaging for inhibiting TV and psychrotrophs counts. Among the three different head space consitions, the best preservation of minced beef meat occurred when MAP1 was used whereas vacuum packaging maintained acceptable color and oxidation stability, except for the air packaged samples.

Key words: Color, microbiological properties, minced beef meat, modified atmosphere packaging, oxidation stability, vacuum packaging, Turkey

INTRODUCTION

Minced beef meat is popular because it is convenient to use. Unfortunately, its shelf life is limited because the large exposed surface area facilitates spoilage. The rate of deteriorative changes depends on the composition of the meat; hygienic practices during cutting, grinding and preparation and finally, storage conditions. The most important factor in controlling meat spoilage is microbial contamination which affects safety and color (Brooks et al., 2008).

The purposes of modern meat packaging techniques are to extend shelf life, enhance appearance and presentation, reduce the need for artificial preservatives and minimize waste (Faber, 1991; Gill, 1996; Nattress and Jeremiah, 2000; Seydim et al., 2006; Koutsoumanis et al., 2008; Kayim and Can, 2010). MAP is an effective food preservation method for meat and poultry products because it retains the fresh character of the products (Viana et al., 2005; Chouliara et al., 2008; Jin et al., 2010) maintains the attractive red color of the products (Church and Parson, 1995; Sorheim et al., 1999; Mancini and Hunt, 2005; Ercolini et al., 2006;

Paulsen et al., 2006; Seydim et al., 2006; Velzen and Linnemann, 2008; Limbo et al., 2010), limits rancidity due to oxidation (Rao and Sachindra, 2002) and reduces the spoilage of meat and meat products (Koutsoumanis et al., 2008; Limbo et al., 2010).

The shelf life of meat packaged in MAPs depends on gas concentrations and other factors including storage temperature, the degree of contamination of the initial carcass, the permeability of the packaging film to Oxygen (O_2) and Carbondioxide (CO_2) and the headspace volume in the package (Jimenez et al., 1997; Limbo et al., 2010). These methods of preservation include the use of MAP using gas mixtures that contain variable O_2 and CO_2 concentrations in order to inhibit the different spoilage-related bacteria and they are often associated with the use of low temperatures during storage (Faber, 1991; Bell, 2001; Limbo et al., 2010). Typical oxygen concentrations range from 70-80% with carbon dioxide making up the remaining percentage. Modified atmosphere packaging in a high concentration of oxygen allows the growth of aerobic microorganisms (Lauzurica et al., 2005; Koutsoumanis et al., 2008) but the shelf life of the product can be reduced due to oxidative

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rancidity in certain oxygen-sensitive products and to the growth of aerobic microflora (Rao and Sachindra, 2002). CO_2 has been found to have a bacteriostatic effect on gram-negative spoilage organisms and concentrations as low as 10-20% of CO_2 in the atmosphere were found to control the growth of putrefactive bacteria which are associated with meat spoilage, provided the stored meat is chilled (Rao and Sachindra, 2002; Lauzurica *et al.*, 2005; Koutsoumanis *et al.*, 2008). Nitrogen (N_2) prevents the collapse of the packaging, fills the packaging headspace and influences the shelf life of perishable foods indirectly by retarding the growth of aerobic organisms that cause spoilage (Gill and Jones, 1994; Rao and Sachindra, 2002; Del Nobile *et al.*, 2009; Karabagias *et al.*, 2011).

Vacuum packaging is defined as the packaging of a product in a high barrier package from which air is removed to prevent the growth of aerobic spoilage organisms, shrinkage, oxidation and color deterioration. The consumption of residual oxygen by microorganisms in the packages results in the production of carbon dioxide. Successful storage of vacuum-packaged meat products requires the following precautions, i.e., the use of oxygen-impermeable films; thorough evacuation; reduced addition of water; control of seam or slip closures; good hygiene; reduced storage of sliced products; appropriate storage temperatures and low illumination (Rao and Sachindra, 2002).

The trend to self-service merchandising of fresh meat requires a high standard of color presentation since consumers' judgment concerning the freshness of meat is related to the bright-red color of oxymyoglobin (Bell, 2001; Rao and Sachindra, 2002). During prolonged storage, oxymyoglobin is oxidized to metmyoglobin which gives meat an unattractive brown color (O'Grady *et al.*, 1998, 2000; Rao and Sachindra, 2002; Tang *et al.*, 2006; Ferioli *et al.*, 2008). The shelf life and color stability of meat stored in this gas mixture is still limited. Anaerobic conditions extend the shelf life of meat considerably compared to air and O_2 enriched atmospheres (Sorheim *et al.*, 1999). This condition is normally associated with the use of packaging materials that provide a barrier to the exchange of gases between the pack and the external atmosphere (Gill, 2003).

While oxygen is necessary to maintain oxymyoglobin in fresh beef, it also promotes lipid oxidation which can lead to flavor defects (Zhao *et al.*, 1994; Jakobsen and Bertelsen, 2000; O'Grady *et al.*, 2000; Cayuela *et al.*, 2004). Lipid oxidation is particularly pronounced in ground meats in which the disruption of the muscle cell structure exposes labile lipid components to oxygen (Sato and Hegarty, 1971). Previous studies have shown that a relationship exists between oxymyoglobin and lipid oxidation in muscle-based model systems and in minced beef stored in 80% O_2:20% CO_2 (O'Grady *et al.*, 1998; 2000; Lauzurica *et al.*, 2005). Also, it has been shown that a high-oxygen content in MAP can affect both the beef's tenderness and sensory attributes such as juiciness and flavor are affected negatively (Lagerstedt *et al.*, 2010).

Meat and meat products stored at aerobic conditions and low temperatures generally succumb to spoilage due to *Pseudomonads* sp. (Koutsoumanis *et al.*, 2008). Storage of fresh meat in modified atmospheres leads to the dominance of *Brochothrix thermosphacta*, psychrotolerant Lactic Acid Bacteria (LAB) and/or Enterobacteriaceae which are also capable of growth but they usually account for only a small proportion of the total flora (Borch *et al.*, 1996; Jones, 1999, 2004; Rodriquez-Calleja *et al.*, 2005; Koutsoumanis *et al.*, 2008; Ercolini *et al.*, 2009).

To date, numerous research efforts have been conducted to investigate vacuum packaging and modified-atmosphere packaging of fresh meat. However, most of these studies involved samples of whole muscle or other whole meat products and did not include minced meat. Minced meat is more sensitive to oxidation because of its porous structure and it has more susceptibility to microbial spoilage due to its initial microbiological load and the mincing process (Esmer *et al.*, 2011). Thus, the aim of this research was to determine the influences of vacuum packaging and of different gas compositions used in modified-atmosphere packaging on the microbiological properties, color and oxidation stability of minced meat at +4°C.

MATERIALS AND METHODS

Meat from pectoralis major and minor muscles of beef carcasses from 2 years old cattle, 36 h post-mortem was purchased from a local slaughterhouse in Susurluk, Turkey. The meat was kept at 2°C before further preparations and all of the following sample preparation steps were performed at 4°C. Meat samples were trimmed to separate exterior fat and loosened connective tissue and then they were minced in a sterilized mincer to a 3 mm size. All packages of minced meat weighed 250±0.1 g. The minced meat samples that were used as research material were packaged in the same factory.

Packaging parameters: Modified-atmosphere packaging was performed by using a Tiromat Compact M 380-3.11 packaging machine (Convenience Food Systems, Tiromat Kraemer+Grebe GmbH and Co. KG, Germany). The interior of Multilayer Polyvinyl Chloride (MLPVC) trays was covered with Polyvinylchloride/Ethylene-Vinyl alcohol

copolymer/Polyethylene (PVC/EVOH/PE) multilayer barrier film (POLYRAZ Plastics Industries, Israel), the minced meat samples were packed in the trays and a barrier made of polyethylene film Toplex HB 60 (PO_2 = 3.5 $cm^3/m^2/24$ h, 23°C, 0% RH; Plastopil Hazorea Company Ltd. Israel) was used to seal the package. An absorbent sheet was placed on the bottom of the tray to avoid excessive accumulation of exudates. Vacuum packaging was performed by using a VC-999 Multivac packaging machine (Switzerland) and minced meat samples were packed in FMXBK films (PO_2 = 15 $cm^3/m^2/24$ h/, 23°C, 75% RH, Flexopack S.A. Plastics Industry, Greece).

The minced meat samples were packed at ambient Air conditions (AP), Vacuum (VP), 70% O_2/30% CO_2 (MAP1) and 10% O_2/30% CO_2/60% N_2 (MAP2). The ratio between the volume of gas and weight of food product (G/P ratio) was 3:1 (vol/wt). All samples were stored in a refrigerator at a constant temperature (4°C) for 7 days. Analyses were conducted on the 1st, 4th and 7th days of storage. The entire experiment was conducted three separate times.

Gas composition analyses: The O_2 and CO_2 concentrations in the headspace of the packages were monitored periodically using a digital PBI Dansensor Check Pointer O_2/CO_2 analyzer (Ringsted, Denmark) and expressed as O_2 and CO_2%. The remaining gas was N_2.

Microbiological analyses: The samples to be analyzed were taken from all the treatment groups on the 1st, 4th and 7th days of storage. About 10 g samples of minced meat were weighed out aseptically, 90 mL of a sterile Maximum Recovery Dilution solution (1 g L^{-1} peptone and 8.5 g L^{-1} saline) were added and the mixture was homogenized in a Stomacher blender (Masticator, IUL Instruments, Spain) for 60 sec at room temperature. Decimal dilutions of the Maximum Recovery Dilution solution were prepared and duplicate 1 or 0.1 mL of at least three appropriate dilutions were mixed or spread on the following agar media: Plate Count Agar (PCA; Oxoid CMO325) for Total Viable (TV) and psychrotroph counts incubated at 35°C for 2 days and at 7°C for 7 days, respectively (Berruga *et al.*, 2005) de Man-Rogosa-Sharp medium (MRS; Oxoid CM0361) for lactic acid bacteria, overlaid with the same medium and incubated at 37°C for 48 h; Cetrimide Fucidin Cephalorodine (CFC; Oxoid CM 559 supplemented with SR 103) medium for Pseudomonads counts incubated at 25°C for 48 h (Mead and Adams, 1977), Rose Bengal Chloramphenicol agar (RBC; Oxoid CM 549 supplemented with SR 78) for yeasts and molds incubated at 25°C for 5 days (Chouliara *et al.*, 2007), Violet Red Bile Glucose Agar (VRBGA; Merck 1.10275) for Enterobacteriacea counts

incubated at 37°C for 24 h (Govaris *et al.*, 2007), McCONKEY Broth (Merck 1.05396) by the three Most Probable Number (MPN) method for total coliform counts (Bolling *et al.*, 2002) incubated at 37°C for 48 h and Streptomycin Thallous Acetate Actidione Agar (STAA, Oxoid CM0881 supplemented with SR0151) for Brochothrix thermosphacta incubated at 22-25°C for 48-72 h (Gardner, 1966; Gill and Badoni, 2003). All samples were analyzed in duplicate and the results were averaged for statistical analysis. Analyses were conducted separately on the materials from each package. All microbial counts were expressed as base-10 logarithms of colony forming (log CFU, g^{-1}).

Color: Surface meat color measurements were acquired using a Hunter Lab CFLX-45-2 colorimeter (Reston, VA, USA) by assessing L*, a* and b* values. Immediately after opening each package on each sampling day, the instrument was standardized using white and black standard plates. Four measurements per package were taken and the averages were calculated and used for the statistical analyses.

Oxidation stability: The extent of lipid oxidation was measured as Thiobarbituric Acid Reactive Substances (TBARS) in mg malonaldehyde equivalents/kg of meat as described by Tarladgis *et al.* (1960).

pH determination: The pH value was recorded by a pH meter (Hanna 210 pH meter), the glass electrode being immersed in the homogenate of minced meat after the end of microbiological analysis.

Statistical analyses: Data were analyzed statistically by means of ANOVA (Analysis of Variance) and Lowest Standard Deviation (LSD) tests.

RESULTS AND DISCUSSION

Microbiological analysis: The initial bacterial load of meat depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing and the temperature and other conditions of storage and distribution (Doulgeraki *et al.*, 2011). Thus, high numbers of microorganisms in meat before storage shortens the shelf life since the microorganism limit will be achieved more rapidly (Blixt and Borch, 2002).

After 7 days of storage, the air packaged samples showed objective signs of spoilage and were unacceptable sensorially. The results of viable counts on appropriate media of the meat spoilage target groups from

Table 1: Microbial populations[a] for minced meat packaged different atmospheres stored 4±1°C ($\log_{10}$ CFU g^{-1})

Packaging	Total viable	Pyscrotrophs	Lactic acid bacteria	Pseudomonads	*B. thermospacta*	Enterobacteriaceae	Total yeast mould	Total coliform
Day 1								
AP	4.18±0.08[a]	3.77±0.07[a]	2.94±0.11[a]	3.31±0.09[a]	2.75±0.04[a]	4.72±0.05[a]	2.45±0.04[a]	1.98±0.12[a]
MAP1	3.85±0.12[b]	2.76±0.06[c]	2.47±0.12[d]	2.96±0.10[c]	2.34±0.05[c]	3.03±0.12[d]	2.42±0.06[a]	1.65±0.13[b]
MAP2	3.14±0.13[d]	2.73±0.10[d]	2.78±0.09[b]	3.06±0.12[b]	2.65±0.09[b]	3.51±0.10[c]	2.44±0.08[a]	1.58±0.09[c]
Vacuum	3.22±0.11[c]	3.00±0.11[b]	2.61±0.08[c]	3.05±0.11[b]	2.30±0.08[d]	3.71±0.06[b]	2.36±0.06[b]	1.65±0.08[b]
Day 4								
AP	5.26±0.10[a]	5.10±0.03[a]	4.61±0.07[a]	6.39±0.12[a]	5.37±0.04[a]	5.56±0.07[a]	3.61±0.05[a]	2.66±0.11[a]
MAP1	5.10±0.12[b]	4.56±0.05[b]	4.34±0.08[c]	3.27±0.10[d]	3.08±0.05[d]	3.86±0.11[d]	2.88±0.08[b]	1.87±0.14[d]
MAP2	4.72±0.14[d]	4.47±0.07[c]	4.55±0.10[b]	3.37±0.08[c]	3.16±0.06[c]	4.48±0.06[c]	2.48±0.10[d]	1.97±0.12[c]
Vacuum	4.82±0.06[c]	4.56±0.08[b]	4.58±0.04[b]	3.54±0.06[b]	3.38±0.05[b]	4.91±0.08[b]	2.58±0.11[c]	2.46±0.08[b]
Day 7								
AP	7.68±0.05[a]	6.35±0.11[a]	5.67±0.05[a]	7.15±0.05[a]	6.26±0.08[a]	6.95±0.10[a]	4.55±0.10[a]	4.27±0.10[a]
MAP1	6.87±0.06[b]	5.77± 0.09[c]	5.03±0.05[b]	3.99±0.06[b]	3.56±0.09[d]	4.68±0.11[d]	3.77±0.11[b]	3.34±0.12[b]
MAP2	6.06±0.09[c]	5.81± 0.10[b]	5.33± 0.06[c]	3.67±0.07[c]	3.79±0.10[c]	5.52±0.12[c]	2.58±0.09[d]	2.27±0.14[d]
Vacuum	5.99±0.10[d]	5.49± 0.07[d]	5.27±0.08[c]	3.97±0.08[b]	4.17±0.12[b]	5.86±0.09[b]	2.96±0.08[c]	2.68±0.07[c]

[a]Each value is the mean of two batch production with two samples analyzed per batch (n = 4) and means with different lowercase letters in the same column are significantly different (p<0.05)

the minced meat samples are shown in Table 1. For day 1, the initial value of TV count was between 3.14 and 4.18 log CFU g^{-1} in different packaged minced meat samples and the TV count increased significantly with time, reaching in excess of 7 log CFU g^{-1} after 7 days for the air packaged samples. Some researchers have reported that microbial spoilage of meat occurs when the TV count reaches levels of 7-8 log CFU g^{-1} (Nassos *et al.*, 1983; Sadler and Swan, 1997; Insausti *et al.*, 2001; Jeremiah, 2001; Fernandez-Lopez *et al.*, 2008; Limbo *et al.*, 2010) as evidenced by off-odors and the smell of vacuum/gas packaged meat products (Rao and Sachindra, 2002). As reported by Brooks *et al.* (2008) some researchers have stated that microbial populations on raw beef must reach approximately 10^8 CFU g^{-1} to show tackiness when touched whereas others have claimed that proteolytic changes do not occur until bacterial populations >3.2×10^9 CFU cm^{-2} are reached.

As expected, the TV count increased during storage at 4°C, irrespective of the other storage conditions. Significant TV counts were obtained for combinations of vacuum-packed meat (p<0.05), MAP2-packed meat (p<0.05) and MAP1-packed meat (p<0.05), i.e., 5.99, 6.06 and 6.87 log CFU g^{-1} at the 7th day of storage, respectively however, in all MAP combinations and in VP, TV counts were still below the spoilage limit of red meat, except for the air packaged samples. As a result, it can be said that vacuum packaging (5.99 log CFU g^{-1}) is the most effective method for restricting the increase of TV count.

It is known that CO_2 has a great inhibitory effect on common spoilage microorganisms (Pastoriza *et al.*, 1996a, b; Pettersen *et al.*, 2004; Cheng *et al.*, 2007; Stamatis and Arkoudelos, 2007) by extending the lag phase and increasing the generation time of sensitive organisms (Stiles, 1991). Goulas (2008) showed that packaging that used a gas combination of 60% CO_2,

20% N_2 and 20% O_2 provided decreased TV count resulting in better quality retention and greater shelf life of mussels than vacuum packaging. Pastoriza *et al.* (1996b) and Goulas (2008) also expressed that the presence of high level of CO_2 in the MAP system can inhibit bacteriological growth while the Vacuum Packaging (VP) system allow to growth of facultative microorganisms because of not produce a complete anaerobic environment and in films with low permeability impedes the diffusion of oxygen to meat (Rao and Sachindra, 2002) however, it is assumed that vacuum packaging reduces the number of aerobic bacteria during storage, many aerobic microorganisms need only 0.5% O_2 to survive so that such packaging prevents only the logarithmic growth of many bacteria (Nowak *et al.*, 2006).

Psychrotrophs could have a visible growth at 7°C within 7-10 days (Ho *et al.*, 2003). In this research, psychrotrophs increased until the end of storage and they existed above the spoilage limit for the air, MAP1, MAP2 and VP samples, i.e., 6.35, 5.77, 5.81 and 5.49 log CFU g^{-1}, respectively on the 7th day of storage (Table 1). There were significant differences in the psychrotrophs between the air packaged group and other packaging groups (p<0.05) but the use of VP was significantly more effective in inhibiting psychrotrophs than the MAP applications (p<0.05) in that they found the highest counts in the air packaged samples with intermediate counts in VP. However, Rao and Sachindra (2002) reported that psychrotropic bacteria counts and lactobacilli counts for beef steaks or chops stored in CO_2/N_2 atmospheres were usually lower than those of VP samples.

Lactic acid bacteria, *Pseudomonads* sp. and *Brochothrix thermosphacta* are considered to be the main spoilage bacteria of low and high pH raw meat, stored at chill temperatures in aerobic or vacuum/MAP conditions (Koutsoumanis *et al.*, 2008; Nychas *et al.*, 2008;

Ercolini *et al.*, 2009). It is important to note that the final composition of the microbial flora eventually characterizes the type of spoilage (Nychas *et al.*, 2008). In other words, spoilage is the outcome of the imposed environmental conditions and microbial interactions (between facultatively anaerobic gram-positive and negative flora (Nychas *et al.*, 2008; Doulgeraki *et al.*, 2011).

Lactic Acid Bacteria (LAB) as facultative anaerobic bacteria can grow in high concentrations of CO_2 and in vacuum conditions as specific spoilage organisms so, they constitute a substantial part of the natural microflora that occur in VP or MAP meats (Govaris *et al.*, 2007; Pietrasik *et al.*, 2006; Vaikousi *et al.*, 2009; Doulgeraki *et al.*, 2010; Karabagias *et al.*, 2011). The use of CO_2 in MAP can improve storage by allowing the growth of LAB such as *Lactobacillus* sp. and *Leuconostoc* sp. and because of their antagonistic properties that allow them to compete more effectively than Enterobacteriaceae, *Pseudomonads* sp. and *Brochothrix thermosphacta* (Rao and Sachindra, 2002; Ercolini *et al.*, 2006) thereby inhibiting the growth of food pathogens more effectively than vacuum packaging (Ercolini *et al.*, 2006; Limbo *et al.*, 2010). In this study, starting from an initial level of 2.94 log CFU g^{-1} in raw fresh meat, LAB reached high populations of 5.67 CFU g^{-1} in the air-packaged samples. At the end of the storage, it was determined that the increases in the counts of LAB were similar in MAP2 (5.33 log CFU g^{-1}) and vacuum (5.27 log CFU g^{-1}) applications. The lactic acid bacteria count showed a lower increasing trend in MAP1 packaging than in the other applications by day 7 of storage at 4°C thus, it was thought that the package contained more oxygen than MAP2 and vacuum applications. The dominance of LAB in the CO_2 rich atmosphere has been reported repeatedly in the literature for meat and meat products (Devlieghere *et al.*, 1998; Sorheim *et al.*, 1999; Samelis *et al.*, 2000; Gok *et al.*, 2008; Nychas *et al.*, 2008; Vaikousi *et al.*, 2009). This is in contrast with the results as well as with the results of Ogihara *et al.* (1993) who reported no effect of MAP on LAB.

B. thermosphacta is a gram-positive, psychrotrophic, facultative anaerobe bacterium and has greater spoilage potential than other gram-positive bacteria. It has been recognized as one of the predominant bacterial species involved in the spoilage of meat and meat products even in refrigerated conditions and when packaged under vacuum, aerobically and/or MAP (Labadie, 1999; Erkmen, 2000; Rodriquez-Calleja *et al.*, 2005; Russo *et al.*, 2006; Koutsoumanis *et al.*, 2008; Pennacchia *et al.*, 2009; Ercolini *et al.*, 2009; Karabagias *et al.*, 2011). In the

absence of lactobacilli, *B. hermosphacta* may also form a dominant part of spoilage flora, depending on the permeability of the film and the residual oxygen that remains after the vacuum process (Erkmen, 2000; Rao and Sachindra, 2002; Rodriquez-Calleja *et al.*, 2005; Vermeiren *et al.*, 2005; Russo *et al.*, 2006).

On day 7th of storage, *B. thermosphacta* counts reached values of 6.26, 3.56, 3.79 and 4.17 CFU g^{-1} for the samples packaged aerobically, MAP1, MAP2 and vacuum, respectively. At the end of storage, a reduction of approximately 2 log CFU g^{-1} in minced meat stored under MAP1 at 4°C was observed as compared to aerobically packaged samples. *B. thermosphacta* are not competitive under anaerobic conditions and they are rapidly outgrown by lactobacilli in refrigerated, vacuum-packaged meat products (Vermeiren *et al.*, 2005). Nevertheless, MAP can be beneficial in the sense that the end products of LAB and/or *B. thermosphacta* are less offensive than are the typical spoilage odors produced by the Pseudomonads (Patsias *et al.*, 2006). Similar results were obtained in the research which showed that the levels of *B. thermosphacta* were lower in vacuum samples lower in the than air packaged samples. In the MAP2 samples, the counts of *Pseudomonads* sp. and *B. thermosphacta* were lower than the counts for vacuum-packaged, minced-meat samples. These findings are not in agreement with those of Paulsen *et al.* (2006) who reported a reduction of *Pseudomonads* sp. and *B. thermosphacta* by 1-2 log CFU g^{-1} under reduced pressure in beef. Generally, *B. thermosphacta* and *Pseudomonads* sp. represented the dominant spoilage flora. Because the distribution of these microorganisms in meat is generally rather heterogeneous. The results showed a great dissimilarity in microbial counts so, the growth and survival of these spoilage bacteria should be investigated further. For *B. thermosphacta*, the inhibitory effect at anaerobic conditions also depends on the combination of several intrinsic factors (i.e., pH, lactate, water activity and fat content) and extrinsic factors (i.e., residual oxygen and temperature) (Grau, 1981; Gill and Harrison, 1989; Jeremiah, 2001) which could possibly favor its growth.

CO_2 enriched atmospheres are able to suppress the aerobic spoilage microorganisms such as *Pseudomonads* sp. which are responsible for the off-odor and off-flavor development (Chouliara *et al.*, 2008; Karabagias *et al.*, 2011). Although, *Pseudomonads* sp. are strictly aerobic and sensitive to CO_2 as compared to air packaging, some researchers indicated that they were also found in the samples packaged under MAP (Rodriquez Calleja *et al.*, 2005; Chouliara *et al.*, 2007) perhaps because they have been observed to attach more readily to the surface of

meat than several other spoilage bacteria (Ellis and Goodacre, 2001). However, researchers found that the Pseudomonads counts were lower in the MAP2 samples (3.67 log CFU g^{-1}) than in the AP samples (7.15 log CFU g^{-1}). The differences in the Pseudomonads counts were not found to be significant between vacuum-packaged samples (3.97 log CFU g^{-1}) and MAP1-packaged samples (3.99 log CFU g^{-1}). The population of Pseudomonads to the arbitrary level of 10^7 CFU g^{-1} in the air packaged samples has been attributed to the formation of slime and off-odors (Table 1). However in practice, both of these characteristics become evident when the Pseudomonads exhaust the glucose and lactate present in meat and begin to metabolize nitrogenous compounds such as amino acids (Nychas *et al.*, 2008).

Koutsoumanis *et al.* (2006) determined the level of Pseudomonads in aerobically-stored, ground meat to be approximately 10^9 CFU g^{-1} when the meat reached the end of its shelf life. Other studies have reported that spoilage of aerobically-stored, chilled meat cuts occurs when Pseudomonads levels reach 10^7-10^8 CFU cm^{-2} or g^{-1} (Gill and Newton, 2007). The results are in agreement with the results earlier. In contrast, Pseudomonads hardly grows in vacuum-packaged foods due to the decrease of O_2 levels to <1% and the increase of CO_2 levels to about 20% (v/v) as a result of aerobic metabolism of muscular tissue (Dainty and Mackey, 1992; Church and Parson, 1995; Viana *et al.*, 2005). Pseudomonads also do not show any pronounced growth in meat packaged under atmospheres that have been modified by replacing oxygen with other gases (Viana *et al.*, 2005). In addition to reducing the concentration of oxygen, the lactic acid flora appear to inhibit the growth of proteolytic gram-negative spoilage flora, such as the *Pseudomonads* species (Blixt and Borch, 2002). The growth of common aerobic spoilage organisms such as Pseudomonads is inhibited when the oxygen supply is restricted by use of proper packaging material and LAB tend to become dominant (Rao and Sachindra, 2002). In this study with the exception of the AP samples, it was determined that the number of Pseudomonads decreased in the MAP2 and that more lactic acid bacteria were present.

Cold-tolerant Enterobacteriaceae (e.g., *Hafnia alvei, Serratia liquefaciens* and *Enterobacter agglomerans*) also occur on chilled meat that is stored aerobically (Nychas *et al.*, 2008) but they occur in small numbers and do not contribute to the microbial associations (Nychas *et al.*, 2008; Doulgeraki *et al.*, 2011). However, Enterobacteriaceae have been considered as indicators of food safety even though they rarely if ever, make a significant contribution to the spoilage flora on meat and meat products (Zeitoun *et al.*, 1994; Chouliara *et al.*, 2007; Nychas *et al.*, 2008). Also because of proteolytic

metabolism, Enterobacteriaceae may become a significant portion of the spoilage microbiota of refrigerated meats packaged under vacuum or in modified atmospheres (Penny and Bell, 1993; Insausti *et al.*, 2001). In this research, initial counts were determined to be between 3.03 and 4.72 CFU g^{-1} which is approximately the average quality for minced meat since, the counts increase to between 4.68 and 6.95 log CFU g^{-1} at the end of storage (Table 1). In the MAP1 samples, the numbers of Enterobacteriaceae after 7 days of storage were greater than those reported by Sheridan *et al.* (1997) their research showed values of 3.5 log$_{10}$ CFU g^{-1} in lamb meat packed under modified atmosphere (80% O_2 and 20% CO_2) stored at 0°C. Insausti *et al.* (2001) reported a high correlation between Enterobacteriaceae counts and the sensory evaluation of unacceptable odor in beef packed under modified atmosphere (60% O_2, 30% CO_2 and 10% N_2) at 2°C. They achieved values >4 log$_{10}$ CFU g^{-1} at 15 days of storage but these values are greater than those found in the present study.

Enterobacteriaceae and Pseudomonads grew at a slower rate under MAP conditions than under aerobic packaging due to their facultatively anaerobic character (Church and Parson, 1995; Doulgeraki *et al.*, 2011). These microorganisms are more sensitive to CO_2 than gram-positive bacteria such as LAB and *B. thermosphacta* (Rao and Sachindra, 2002; Karabagias *et al.*, 2011). Vacuum and MAP packages prevent the growth of high spoilage microorganisms that are potentially aerobic although, microbial spoilage can become apparent when the aerobic plate count reaches 10^6 CFU g^{-1} if high spoilage potential anaerobes such as Enterobacteriaceae are present as a high proportion of the microflora in anaerobic packs (Sadler and Swan, 1997). Sheridan *et al.* (1997) observed the parallel growth of *B. thermosphacta* and Enterobacteriaceae under vacuum conditions. Researchers found results that were similar to those of other studies but in the case, the bacteria were grown at lower levels in the MAP1-packaged minced meats samples than the other samples.

The established route of yeast and mold contamination from field and farm via the animal to the resultant carcass has been demonstrated. Psychrotropic yeasts are considered to be unable to compete with bacteria because of their slower growth rates (Rodriquez-Calleja *et al.*, 2005). The initial populations of total yeast and mold were low (2.36-2.45 CFU g^{-1}) while on day 7th of storage, counts between 2.58 and 4.55 CFU g^{-1} were recorded. Although, yeast/molds are strictly aerobic, they were also found in the microbial association of the minced meat however, significantly higher counts were recorded for minced meat samples stored in air than for those stored under MAP1,

MAP2 and vacuum conditions throughout the entire storage period under refrigeration. On a given storage day, MAP2 gave lower yeast and mould counts than other packaging methods (Table 1). This is in agreement with the results of Borch *et al.* (1996). These results show that the absence or scarcity of oxygen suppresses the growth of mold and yeast and it is thought that the growth of yeast and mold may be accelerated depending on the amount of oxygen available (Stiles, 1991). It has been well established that atmospheres containing CO_2, either alone or in combination with nitrogen, delay the growth of typically aerobic spoilage microflora by inhibiting the growth of aerobic gram-negative bacteria, such as *Pseudomonads* sp. and also yeast/molds (Borch *et al.*, 1996; Patsias *et al.*, 2006). Dermiki *et al.* (2008) reported that high CO_2 concentrations were very effective for inhibiting the growth of molds and yeasts. Also, Skandamis and Nychas (2001) showed that atmospheres of 100% CO_2 and 40% CO_2, 30% N_2 and 30% O_2 had inhibitory effects on yeasts compared to air packaging of minced meats. In the study, the atmosphere containing CO_2 and N_2 (MAP2) more effectively inhibited the growth of these microorganisms than the other packaging methods. Also, the relatively high initial numbers of different groups in minced beef can be attributed to the grinding process which contributes to the increase of total viable counts of meat including yeasts (Nychas *et al.*, 1991; Skandamis and Nychas, 2001).

Initial counts of total coliform bacteria that are <2.0 log CFU g^{-1} indicate adequate sanitary production of minced beef meat. In fact, some of the microorganisms originate from the animal's intestinal tract as well as from the equipment and the environment the animal contacted before, during or after slaughter (Koutsoumanis and Sofos, 2004).

This is evident in studies of the origin of the contaminants which have shown an association of the work surfaces with the presence of Enterobacteriaceae on meats. Other psychrotrophic bacteria are recovered from hides and work surfaces within an abattoir as well as from carcasses and butchered meat at all stages of processing (Nychas *et al.*, 2008).

The number of coliform bacteria increased slightly for MAP2 and vacuum packaging until the end of storage but the count was below the accepted limit (3 log CFU g^{-1}), except for the AP and the MAP1 samples (Table 1). At day 7th of storage, MAP2 packaging obtained the lowest count, i.e., 2.27 log CFU g^{-1} whereas the count for the AP samples was 4.27 log CFU g^{-1} on day 7th of storage. Coliform bacteria proliferated during storage both in ordinary and modified packaging conditions, suggesting

that MAP does not affect the growth of coliform bacteria to a great extent which has been reported in the literature (Seydim *et al.*, 2006).

Changes of colour: The two most important factors that limit the shelf life of fresh meat are color and microbial growth. Lipid oxidation is the third most important factor (Lauzurica *et al.*, 2005) causing changes in the color of the meat and increases in L-values which are closely associated that increased lipid and pigment oxidation (Buckley *et al.*, 1995; Pietrasik *et al.*, 2006) as well as with bacterial load (Brewer *et al.*, 2002; Lauzurica *et al.*, 2005; Linares *et al.*, 2007). Nevertheless, for meat packed under aerobic conditions, lipid oxidation is not a limitation for storage because it occurs at a slower rate than discoloration and microbial growth (Zhao *et al.*, 1994; Jakobsen and Bertelsen, 2000; Lauzurica *et al.*, 2005). At the same time, the oxygen permeability of the packaging film is likely to affect the developing microflora as well as color and flavor of the product (Rao and Sachindra, 2002). The color of fresh meat which affects the perception of freshness and wholesomeness (Faustman and Cassens, 1990; Zerby *et al.* 1999; O'Grady *et al.*, 2000; Mancini and Hunt, 2005; Limbo *et al.* 2010) is of utmost importance in the marketing of meat (Rao and Sachindra, 2002).

In this study, color values (L, a and b) for all minced meat treatments as a function of storage time are shown in Table 2.

Table 2: Changes L*, a*, b*, TBA values and pH[a] of minced beaf meat samples packaged under different packaging conditions with storage period

Characteristics	Time (days)		
	0	4	7
L*			
AP	50.31±0.78[Bb]	46.33±0.34[Aab]	42.46±0.23[Ba]
MAP1	44.53±0.34[Ab]	43.79±0.23[Ab]	38.84±0.58[Aa]
MAP2	44.27±0.65[Ab]	44.36±0.78[Ab]	39.42±0.97[ABa]
Vacuum	43.31±0.44[Aa]	43.54±0.60[Aa]	44.38±0.26[Ba]
a*			
AP	11.77± 0.65[Ab]	9.14±0.06[Aa]	7.81±1.00[Aa]
MAP1	17.59±0.54[Db]	17.37±0.55[Cb]	15.67±0.78[Ba]
MAP2	13.28±0.92[Bb]	8.89±0.09[Aa]	8.06±0.66[Aa]
Vacuum	15.62±0.72[Cb]	14.86±0.87[Ba]	14.93±0.34[Ba]
b*			
AP	15.64±0.38[Ab]	13.84±0.13[Aab]	13.13±0.98[Aa]
MAP1	17.25±0.42[Ba]	17.15±0.22[Ba]	18.52±1.25[Cb]
MAP2	15.71±0.45[Ab]	13.94±0.17[Ab]	12.56±1.10[Aa]
Vacuum	16.86±0.56[ABa]	15.76±0.38[ABa]	15.78±0.67[Ba]
TBARS			
AP	2.72±0.05[Ba]	5.52±0.28[Cb]	5.91±0.35[Cb]
MAP1	5.87±0.08[Ca]	5.81±0.25[Ca]	5.16±0.32[BCa]
MAP2	2.18±0.08[Ba]	4.52±0.16[Bb]	3.86±0.27[Bab]
Vacuum	0.50±0.06[Aa]	0.60±0.09[Aa]	1.45±0.68[Ab]
pH			
AP	5.62±0.45[Ba]	6.44±0.23[Cb]	6.51±0.25[Cb]
MAP1	5.66±0.32[Ba]	5.58±0.67[Bc]	5.46±0.32[Bb]
MAP2	5.59±0.42[Ac]	5.48±0.56[Bb]	5.10±0.27[Aa]
Vacuum	5.79±0.46[Cb]	5.38±0.45[Aa]	5.36±0.34[Ba]

[a]Each value is the mean of two batch production with two samples analyzed per batch (n = 4). Means with different lowercase letters in the same line are significantly different (p = 0.05). Means with different capital letters in the same column are significantly different (p = 0.05)

The L-values which refer to lightness, generally decreased with time in all sample groups by day 7th of storage. O'Keeffe and Hood (1982) reported that the most significant factor that influences the stability of the color of different muscles appeared to be enzymatic activity which determined the rate of autooxidation of oxymyoglobin. Renerre and Labas (1987) observed that muscles with the poorest color stability also had the greatest oxidative activity (O_2 consumption rates) and the highest rates of myoglobin autoxidation.

Probably, the most important color parameter for fresh meat is the redness value (a*). As meat loses its ability to reduce metmyoglobin to oxymyoglobin indicating the presence of oxygen that has gained access to the package, either through the film or a leak in the package during packaging or subsequent storage, the brownish color of metmyoglobin begins to appear on the surface of meats and a* values will decrease (Jeremiah, 2001; Rao and Sachindra, 2002; Friedrich *et al.*, 2008). For minced meat, the high a* values (redness) of the interior of the high O_2 samples indicate oxygenation and partial formation of oxymyoglobin. The a* values of the surface of the high O_2 samples were substantially reduced in the control samples during storage probably due to oxidation of oxymyoglobin and external accumulation of metmyoglobin (Kerry *et al.*, 2000; Vergara and Gallego, 2001; Kennedy *et al.*, 2005; Karabagias *et al.*, 2011). In addition, results were found for MAP2 samples that were similar to the results obtained for the air packaged samples.

In general, packaging films with O_2 permeability of 5-100 cm³/m²/atm/24 h, at 23-25°C and a RH of 0% are used for vacuum and MAP to prevent pigment oxidation and consequent browning of the meat colour (Gill and Molin, 1991; Jeremiah, 2001; Rao and Sachindra, 2002). Also, the packaging must be impermeable to oxygen to result in complete reduction of myoglobin to deoxymyoglobin to subsequently facilitate oxygenation to oxymyoglobin (Rao and Sachindra, 2002). Deoxymyoglobin is more susceptible to oxidation than oxymyoglobin causing meat to discolor most rapidly at low oxygen concentrations. Therefore, meat color can be stabilized either by increasing the oxygen concentration above atmospheric, or removing essentially all of the oxygen from the package (Gill, 1992) or using of low O_2-permeability packaging material (Rao and Sachindra, 2002).

The loss of redness due to oxidation of oxymyoglobin for meat packaged in the air packaged samples were expected due to the effects of the high pH (>6.0) of the meat. At higher pH values, mitochondrial enzyme systems (cytochrome, succinate and pyruvate-malate oxidase) do not shut down and have the ability to utilize available oxygen (Fernandez-Lopez *et al.*, 2008). Bendall and Taylor (1972) reported that the oxygen consumption rate of high-pH muscle is greater than the rate for normal-pH muscle. Bembers and Satterlee (1975) also noted that the rate of the conversion of oxymyoglobin to metmyoglobin was 1.5-2.0 times faster in high-pH systems than that of muscle having a more normal pH. In the present study, this rapid loss of redness in O_2 packages was likely due to these muscle characteristics.

Vacuum samples had intermediate a* values as a consequence of the presence of purple deoxymyoglobin. High-oxygen, modified-atmosphere packaging reduces the shelf life compared to vacuum packaging since, the presence of O_2 will produce off-flavors and allow growth of aerobic bacteria (Jeremiah, 2001). However, the purple deoxymyoglobin color of meat in vacuum package and the visible purge loss in the vacuum bag are thought to be unattractive to consumers so, vacuum packaging is used infrequently for retail displays (Mancini and Hunt, 2005; Lagerstedt *et al.*, 2010).

Changes of oxidative stability: The development of oxidative off-flavors (rancidity) has long been recognized as a serious problem during the storage of meat products. TBA and hexanal content are considered to be lipid oxidation markers in meat and meat products (Shahidi *et al.*, 1987). High oxygen atmosphere packaging is associated with increased TBA numbers during storage (O'Grady *et al.*, 2000) and any disruption of the integrity of muscle membranes, as in minced beef, facilitates the interactions of prooxidants with unsaturated fatty acids, resulting in the generation of free radicals and the propagation of oxidative reactions (Gray *et al.*, 1996; Patsias *et al.*, 2006; Limbo *et al.*, 2010).

TBA values for minced meat treatments are given in Table 2. The TBA values for all treatments varied between 0.50 and 2.87 mg MDA kg⁻¹ meat indicating a very low degree of lipid oxidation in the 1st day followed by increases every day thereafter. According to Houben *et al.* (2000) and to Houben *et al.* (2002), TBARS were at levels of 0.9-1.1 MDA kg⁻¹ of product in fresh minced meat beef stored under oxygen-enriched MAP. Other investigators have reported higher TBARS values in fresh beef, ranging from 1.8-10.4 mg MDA kg⁻¹ of meat (Formanek *et al.*, 1998; Ferioli *et al.*, 2008). In minced beef under MAP (O_2/CO_2, 80/20), Formanek *et al.* (1998) verified that TBARS rose from 2.3-5.6 MDA kg⁻¹ in minced beef under MAP (O_2/CO_2, 80/20) from the 2nd to the 8th day of refrigerated storage. Houben *et al.* (2000) reported that TBARS increased from

1.1-5.7 mg MDA kg^{-1} of meat in minced beef held under MAP with high oxygen level ($O_2/CO_2/N_2$, 65/25/10) during a week's storage at 7°C.

The concentration of oxygen in the atmosphere of the package is the determining factor for the rate of lipid oxidation (Kerry *et al.*, 2006; Pietrasik *et al.*, 2006; Fernandez Lopez *et al.*, 2008). Lipid oxidation under air and MAPs excluding O_2 may be explained by the presence of very high levels of O_2 in the packages (Smiddy *et al.*, 2002). Excluding or limiting the oxygen content in vacuum packaging atmospheres limited oxidation and thus, resulted in lower TBA values for these minced meat samples. Also, vacuum packaging protected the product effectively from the beginning of storage, keeping the TBA scores <2 mg MDA kg^{-1} meat until day 7 of storage. But it appears that meat with the poorest oxidative stability does not have the best color stability.

Lipid oxidation has been associated with off-odors and taste in stored meat products (Insausti *et al.*, 2001; Jeremiah, 2001; Limbo *et al.*, 2010). According to Insausti *et al.* (2001) TBA values equal to or greater than 5 mg MDA kg^{-1} meat comprise the threshold for detecting off-odors and off-taste for humans. Such high TBA values were recorded in the present study, except for the vacuum-packaged samples.

Changes of pH values: The pH values of all samples ranged from 5.59-5.79 initially (Table 2) and showed slight except for the air packaged samples with final pH values between 5.10 and 6.51 after 7 days. Fresh meats generally have pH values that range between 5.50 and 5.90 (Gill and Newton, 2007; Seymour *et al.*, 1994; Ellis and Goodacre, 2001) and the results are compatible with the reported values. Such as increase in pH reflects the degree of spoilage of the meat as a result of protein breakdown for the production of free amino acids which leads to the formation of NH_3 and amines, both of which are compounds that result from alkaline reactions (Aksu and Kaya, 2005; Karabagias *et al.*, 2011). Nevertheless in MAP-packaged and vacuum-packaged samples, the pH decreased for a period of 7 days due to the production of lactic acid through LAB metabolism which was favored by the low-oxygen environment (Stiles, 1991; Jeremiah, 2001; Blixt and Borch, 2002; Patsias *et al.*, 2006; Gok *et al.*, 2008; Karabagias *et al.*, 2011) the production of pseudomonads which grow the fastest and utilize glucose and other simple carbohydrates at refrigeration temperatures (Gill and Newton, 2007; Seymour *et al.*, 1994; Ellis and Goodacre, 2001) the dissolution of CO_2 into the aqueous phase of the meat (Stiles, 1991; Blixt and Borch, 2002; Patsias *et al.*, 2006; Fernandez Lopez *et al.*, 2008; Gok *et al.*, 2008; Karabagias *et al.*, 2011). The results were similar to the above-mentioned results.

Table 3: Changes in gas composition values[a] in package headspace during storage of minced meats

Gas composition	Days	O_2 (%)	CO_2 (%)	N_2 (%)
AP	1	16.40±1.50[c]	3.45±0.82[a]	80.15±1.00[a]
	4	15.25±1.45[b]	8.45±0.97[b]	76.30±0.85[a]
	7	14.30±1.24[a]	10.00±0.45[c]	75.70±1.25[a]
MAP1	1	72.30±0.25[c]	26.45±0.28[a]	1.25±0.06[a]
	4	69.20±0.20[b]	29.75±0.09[b]	1.25±0.06[a]
	7	68.15±0.09[a]	30.75±0.93[c]	1.15±0.08[a]
MAP2	1	10.35±0.49[c]	30.60±0.57[a]	59.05±0.45[b]
	4	9.85±1.12[b]	31.15±0.49[b]	59.00±0.67[b]
	7	9.75±0.94[a]	33.85±0.65[c]	56.40±0.38[a]

[a]Each value is the mean of two batch production with two samples analyzed per batch (n = 4). Means with different lowercase letters in the same column are significantly different (p<0.05)

Changes of gas composition: When a modified atmosphere is applied in the headspace of the package, the initial gas concentrations are altered during storage. The composition of the atmosphere is dynamic and changes occur due to muscle respiration, microbial metabolism, gas absorption into the meat and the permeability of the packaging material (Jakobsen and Bertelsen, 2002).

During the 7 days of storage, only a very limited variation in CO_2 concentration was observed in both types of modified storage (Table 3). This is in agreement with the results obtained by Djenane *et al.* (2003) and Friedrich *et al.* (2008). In general, a decrease in the concentrations of O_2 and N_2 and an increase in the concentration of CO_2 can be expected due to microbial activity (such as *B. thermosphacta* and lactic acid bacteria) and muscle respiration (Jakobsen and Bertelsen, 2002; Goulas, 2008; Koutsoumanis *et al.*, 2008). Nitrogen has a minimal effect on the metabolic reactions in the meat because it has low solubilities in water and lipids. However, anoxic atmospheres created by the use of N_2 and/or other gases create a selectively favorable environment for anaerobic, aero-tolerant microorganisms (McMillin, 2008). Even so, the results we obtained for changes in headspace gas compositions at the end of the shelf life of the samples did not suggest low microbial activity in the minced meat samples that we tested.

CONCLUSION

Although, all gas combinations investigated in in this research inhibited TVC and psychrotrophs in the minced beef meat samples, the most effective packaging method was vacuum packaging. However, MAP applications (MAP1 and MAP2) were more effective in retarding the rate of growth of lactic acid bacteria, Enterobacteriaceae, *B. thermosphacta*, Pseudomonads, yeast-molds and coliform bacteria growth on minced beef meat during storage than vacuum packaging. The oxidation stability of minced beef was best preserved in low O_2 environments; in addition, these environments produced results for

acceptable color that were similar to the results obtained using the MAP1 combination of gases in the headspace. Under the conditions of this study, the results indicated that vacuum packaging was superior to the modified atmospheres tested (MAP1: 70% O_2 and 30% CO_2; MAP2:10% O_2, 30% CO_2 and 60% N_2) with respect to rancidity and color stability in minced beef meat therefore vacuum packaging is thought to be an alternative to the MAP1 combination.

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