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The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat

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ABSTRACT

This paper reports the effects of modified atmosphere gas compositions with different concentrations of $CO_2/O_2/N_2$ on color properties (L*, a* and b* values), oxidation stability (TBARS value) and microbiological properties of minced beef meat stored at +4 °C. Sampling was carried out on the 1st, 3rd, 5th, 7th, 9th, 11th and 14th day of storage. The gas mixtures used were as follows: (i) %30O_2 + %70CO_2 (MAP1), (ii) %50O_2 + %50CO_2 (MAP2), (iii) %70O_2 + %30CO_2 (MAP3), (iv) %50O_2 + %30CO_2 + %20 N_2 (MAP4), and (v) %30O_2 + %30CO_2 + %40N_2 (MAP5). Control samples (AP) were packaged under atmospheric air. *Pseudomonas*, lactic acid bacteria, *Brochothrix thermosphacta*, and *Enterobacteriaceae* members were monitored. Among these five modified atmosphere gas compositions, the best preservation for minced beef meat was in MAP4 gas combination maintaining acceptable color together with oxidation stability and acceptable microbial loads until the end of storage period of fourteen days.

1. Introduction

The modified atmosphere packaging is a technique, which is widely used to extend the shelf-life and to improve the quality of perishable foods including meat and meat products stored at refrigeration temperatures or below. Color, lipid oxidation, and microbial criteria are the most important quality criteria for storage of fresh red meat. Therefore, the modified atmosphere packaging must stabilize both the color and oxidation, as well as retard the microbial growth. It is included that 20–30% CO_2 + 70–80% O_2 in conventional gas composition of modified atmosphere packaging of fresh red meat. Oxygen is required for myoglobin—the principle protein responsible for the meat color (Mancini & Hunt, 2005) to keep it in oxygenated form, which gives the bright cherry red color to meat. However, the presence of oxygen also increases the rate of lipid oxidation, which causes undesirable changes in color and flavor (Love & Pearson, 1971). While the use of high oxygen concentration is known to prolong the color stability by promoting the formation of oxymyoglobin, it is also expected to increase the rate of lipid oxidation (Zhao, Wells, & McMillin, 1994; Cayuela & Gil, 2004; O'Grady, Monahan, Burke, & Allen, 2000). Lipid oxidation is particularly pronounced in ground meats, where the disruption of muscle cell structure exposes label lipid components to oxygen (Sato & Hegarty, 1971).

The gas atmosphere also creates a selective pressure on the microflora of meats. Aerobic chilled storage favors the growth of gram-negative, aerobic rod-shaped bacteria including *Pseudomonas*. Many other bacteria are present but *Pseudomonas* spp. predominate and produce off-odors from protein break-down and amino-acid metabolism. Under anaerobic conditions of chilled storage with elevated levels of carbon dioxide, while the slower growing lactic acid bacteria are encouraged, the growth of aerobic spoilage microflora is discouraged. In the presence of oxygen, growth of *Brochothrix thermosphacta* occurs and can cause spoilage of meats.

In this research, it was aimed to determine the effects of five different modified atmosphere packaging gas compositions with different concentrations of $O_2/CO_2/N_2$ on the color properties, oxidation stability and microbiological properties of minced beef meat. To date, numerous researches have been carried out in regards to the modified atmosphere packaging of fresh meat. Most of these studies involved samples as a whole muscle or in the form of steak (Kennedy, Buckley, & Kerry, 2004; Jakobsen & Bertelsen, 2000; Zakrys, Hogan, O'Sullivan, Allen, & Kerry, 2008), not minced meat. However, minced meat is more sensitive to oxidation because of its porous structure and it has more susceptibility to microbial spoilage due to the grounding process. For this reason, in this research color, oxidation stability and microbiological properties were evaluated extensively for modified atmosphere packaging application of minced beef meat in order to determine the effects of different gas compositions on properties of minced beef meat.

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2. Materials and methods

2.1. Meat samples

Meat from *pectoralis major* and *minor* muscles of beef carcasses from 2-year-old cattle, after 48 h from postmortem were purchased from a local establishment in Bandirma, Turkey. The meat was trimmed, of all exterior fat and connective tissue, and minced in a sterilized meat mincer in 3 mm size. The samples were transported to the laboratory under refrigeration conditions. The fat content of the minced meat samples was 22%.

2.2. Packaging parameters

The minced meat samples were placed in "Poly Ethylene Terephtalate (PET)/Ethylene Vinyl alcohol (EVOH)/Low Density Polyethylene (LDPE)" trays in 750 μm thickness and sealed with a laminated film of "Oriented Poly Propylene (OPP)/Low Density Poly Ethylene (LDPE)/Ethylene Vinyl alcohol (EVOH)/Low Density Polyethylene (LDPE)" in 77 μm thickness, with an oxygen transmission rate of 3.5 cm^3/m^2/24 h (23 C, 0% RH). Modified-atmosphere packaging was carried out using Multivac R-230 (Multivac, Germany) packaging machine. 3/1 (vol/wt) ratio of headspace to meat was used in modified atmosphere packaging. Table 1 illustrates the modified atmosphere gas combinations used to package the meat samples. Control samples (Air packaged—AP) was also packaged with ambient air without giving any gas composition. The samples were stored in the dark under refrigeration conditions (+4 °C) for 14 days. Analyses were carried out on the 1st, 3rd, 5th, 7th, 9th,11th and 14th day of storage. The whole experiment was replicated twice.

2.3. Methods

- Gas composition
  The gas composition of headspace in package was measured using a digital PBI Dansensor Check Pointer O_2/CO_2 (Ringsted, Denmark) analyzer and expressed as $\%O_2$ and $\%CO_2$. The remaining gas was N_2.

- Color
  Color analysis was carried out using a Hunter Lab CFLX-45-2 (Reston, Virginia, USA) by assessing L*, a* and b* values. The instrument was calibrated with black and white standard plates before the analysis. The reported data are the mean of four determinations.

- Lipid oxidation
  The extent of lipid oxidation was determined through thiobarbituric acid reactive substances (TBARS) in mg malonaldehyde/kg of meat as described by Tarladgis, Watts, Younathan, and Dugan (1960).

- Microbiological analysis
  25 g of each sample was diluted in 225 ml sterile 0.1 g/100 ml peptone water and homogenized in a stomacher for 90 s at room temperature. A serial 10-fold dilution series was prepared in 0.1 g/100 g peptone water. *Pseudomonas* count was enumerated on *Pseudomonas* agar supplemented with CFC at 25 °C for 48 h (Mead & Adams, 1977). *Lactobacillus* were counted on double-layer pH 5.6 MRS agar (Oxoid) incubated at 30 °C for 48 h (Russo, Ercolini, Mauriello, & Villani, 2006). *Brochothrix thermosphacta* was enumerated on Streptomycin Thallous Acetate Agar (STAA) at 25 °C for 48 h (Gardner, 1966). *Enterobacteriaceae* members were determined on double-layer violet-red-bile-dextrose agar (VRBG, Oxoid) at 37 °C for 24 h (Govaris et al., 2007). Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g).

- Statistical analysis
  The data obtained from analyses were subjected to variance analysis in order to determine the effect of gas composition of modified atmosphere packaging and storage time on each variable. The analysis was performed by ANOVA one-way analysis, using SPSS 8.0. To identify the different groups, the Duncan's post hoc test was applied.

Table 1
Gas compositions of MAP samples.

	O_2	CO_2	N_2
MAP1	30	70	0
MAP2	50	50	0
MAP3	70	30	0
MAP4	50	30	20
MAP5	30	30	40

3. Results and discussion

3.1. Headspace composition

The gas composition of each package changed significantly within storage period ($p \leq 0.05$). It is known that gaseous environment within a modified atmosphere pack is not static. It may be the result of microbial growth, the permeability of packaging material, and respiration of the product or the gas absorption by the food.

As it is seen from Table 2, O_2 concentration decreased and CO_2 concentration increased in all samples starting from the 1st day of storage, but this change was more evident in AP, MAP1($O_2/CO_2/N_2$:30/70/0) samples which contain less O_2 concentrations than the other samples. As stated by O'Grady et al. (2000), relative changes in gaseous atmospheres within the modified atmosphere packs were higher at a lower oxygen level. Similar results related the changes in the headspace atmospheres were reported in other studies (O'Grady et al., 2000; Kennedy et al., 2004; Koutsoumanis, Stamatiou, Drosinos, & Nychas, 2008; Ercolini, Russo, Torrieri, Masi, & Villani, 2006). It is an expected result for CO_2 concentration to decline due to the absorption of CO_2 in meat (Jakobsen & Bertelsen, 2002), but this decline cannot be monitored by reason of microbial growth. During storage, the majority of microorganisms present in meat utilize available oxygen in the headspace while some members of the meat microflora such as B. *thermosphacta* and lactic acid bacteria (LAB) produce carbon dioxide as a metabolic product (Nychas, 1994).

3.2. Color

The evolution of L*, a* and b* values is shown in Table 3. The effect of gas compositions on L* value of minced meat samples were not statistically significant ($p > 0.05$), whereas the L* values were significantly affected by the storage time ($p \leq 0.01$). L* values showed a varying trend, irrespective of packaging treatments throughout the entire storage period. This result shows us that varying concentrations of CO_2 or O_2 gases in MAP applications does not affect the lightness of minced beef meat and this result is consistent with the findings of Soldatou, Nerantzaki, Kontominas, and Savvaidis (2009).

Both gas composition and storage period had a significant effect on the a* values (redness) of minced beef samples ($p \leq 0.01$). In 1st day of storage, a significant difference was not observed among samples but, after the 1st day a* values started to decrease. The lowest values were obtained for MAP1($O_2/CO_2/N_2$:30/70/0) and MAP2($O_2/CO_2/N_2$:50/50/0) samples where a* values were below 10 at the 9th day of storage for both samples. High CO_2 concentrations led to sharp decreases in the a* value

Table 2
Changes in gas composition values[a] of modified atmosphere packages.

Gas comp.	Days	O_2	CO_2	N_2 (%)
AP	1	17.70 ± 1.70^{c}	3.95 ± 0.78^{a}	78.35 ± 1.13^{a}
	3	16.35 ± 1.60^{bc}	7.35 ± 0.21^{b}	76.30 ± 1.81^{a}
	5	15.40 ± 1.95^{bc}	8.10 ± 1.13^{b}	76.50 ± 3.08^{a}
	7	14.50 ± 1.37^{b}	10.05 ± 0.49^{b}	75.45 ± 1.87^{a}
	9	13.65 ± 1.74^{b}	18.35 ± 0.35^{c}	68.00 ± 2.09^{a}
	11	12.30 ± 1.10^{b}	18.50 ± 0.42^{c}	69.20 ± 1.53^{a}
	14	7.60 ± 1.39^{a}	22.30 ± 0.57^{d}	70.10 ± 1.96^{a}
MAP1	1	31.60 ± 0.14^{f}	68.35 ± 0.07^{a}	0.05 ± 0.07^{a}
	3	29.80 ± 0.14^{e}	69.45 ± 0.35^{b}	0.75 ± 0.21^{a}
	5	28.15 ± 0.21^{d}	71.30 ± 0.00^{c}	0.55 ± 0.21^{a}
	7	26.50 ± 0.57^{c}	73.20 ± 0.28^{d}	0.30 ± 0.28^{a}
	9	26.25 ± 0.21^{c}	73.25 ± 0.21^{d}	0.50 ± 0.00^{a}
	11	24.75 ± 0.21^{b}	75.00 ± 0.14^{e}	0.25 ± 0.35^{a}
	14	21.15 ± 0.21^{a}	78.55 ± 0.49^{f}	0.30 ± 0.28^{a}
MAP2	1	50.40 ± 0.57^{c}	48.65 ± 0.35^{a}	0.95 ± 0.21^{a}
	3	49.20 ± 0.28^{abc}	49.95 ± 0.64^{b}	0.85 ± 0.36^{a}
	5	49.30 ± 0.28^{bc}	49.60 ± 0.14^{ab}	1.10 ± 0.42^{a}
	7	49.70 ± 0.57^{c}	49.50 ± 0.00^{ab}	0.80 ± 0.57^{a}
	9	48.10 ± 0.00^{ab}	51.50 ± 0.28^{c}	0.40 ± 0.28^{a}
	11	48.15 ± 0.07^{ab}	51.40 ± 0.14^{c}	0.45 ± 0.07^{a}
	14	48.00 ± 0.00^{a}	51.90 ± 0.14^{c}	0.10 ± 0.14^{a}
MAP3	1	72.80 ± 0.28^{c}	26.60 ± 0.28^{a}	0.60 ± 0.00^{a}
	3	70.10 ± 0.14^{b}	29.45 ± 0.07^{b}	0.45 ± 0.07^{a}
	5	69.35 ± 1.20^{ab}	30.05 ± 1.13^{bc}	0.60 ± 0.07^{a}
	7	69.50 ± 0.28^{ab}	29.90 ± 0.00^{bc}	0.60 ± 0.28^{a}
	9	68.25 ± 0.07^{a}	31.35 ± 0.35^{c}	0.40 ± 0.42^{a}
	11	68.10 ± 0.14^{a}	31.55 ± 0.07^{c}	0.35 ± 0.07^{a}
	14	68.10 ± 0.00^{a}	31.65 ± 0.49^{c}	0.25 ± 0.49^{a}
MAP4	1	55.60 ± 0.49^{c}	28.40 ± 0.57^{a}	16.00 ± 1.06^{a}
	3	54.25 ± 0.35^{bc}	28.30 ± 0.42^{a}	17.45 ± 0.07^{a}
	5	51.15 ± 1.48^{abc}	28.45 ± 0.35^{a}	20.40 ± 1.84^{ab}
	7	51.40 ± 1.84^{abc}	29.65 ± 0.35^{ab}	18.95 ± 2.19^{ab}
	9	45.70 ± 2.26^{a}	30.55 ± 1.34^{ab}	23.75 ± 0.92^{b}
	11	46.80 ± 2.40^{a}	31.95 ± 0.49^{b}	21.25 ± 1.91^{ab}
	14	47.80 ± 2.26^{ab}	31.85 ± 0.07^{b}	20.35 ± 2.19^{ab}
MAP5	1	34.55 ± 0.49^{b}	27.80 ± 0.85^{a}	37.65 ± 0.35^{a}
	3	32.10 ± 0.28^{ab}	28.15 ± 1.06^{a}	39.75 ± 0.78^{a}
	5	31.40 ± 0.14^{ab}	29.25 ± 0.78^{a}	39.35 ± 0.64^{a}
	7	31.65 ± 0.26^{ab}	29.60 ± 0.14^{a}	38.75 ± 2.12^{a}
	9	30.10 ± 0.00^{ab}	30.65 ± 0.07^{a}	39.25 ± 0.07^{a}
	11	27.80 ± 2.69^{a}	31.15 ± 0.49^{a}	41.05 ± 3.18^{a}
	14	28.65 ± 1.77^{a}	34.50 ± 1.70^{b}	36.85 ± 3.46^{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 ($p \leq 0.05$).

and rapid discoloration of meat in high-CO_2 atmospheres was also reported by Martinez, Djenane, Cilla, Beltran, and Roncales (2005) as an effect of the decrease in pH. The discoloration appeared earlier in MAP5 ($O_2/CO_2/N_2$:30/30/40) samples than MAP3($O_2/CO_2/N_2$:70/30/0) and MAP4($O_2/CO_2/N_2$:50/30/20) samples and the a* value was determined as 14.48 ± 2.75 on the 9th day of storage and declined to 8.22 ± 0.59 on the 14th day of storage. There were no significant statistical and visual differences between the a* values of MAP3($O_2/CO_2/N_2$:70/30/0) and MAP4($O_2/CO_2/N_2$:50/30/20) samples and the redness was kept until the 14th day of storage with these gas compositions. Jakobsen and Bertelsen (2000) also expressed the stability of the a* value between 55–80% O_2 and the color stability did not increase markedly after increasing the oxygen level from 55% to 80%.

It must be emphasized that high standard deviations were obtained for the a* values especially for MAP5($O_2/CO_2/N_2$:30/30/40) and AP samples towards the end of the storage period.

This situation depends on to the regional differences in the redness of samples because of the usage of oxygen during the storage and oxygen deficiency until the end of storage period.

The b* value changed significantly with the gas compositions of modified atmosphere packaging and the storage period ($p \leq 0.01$), and decreased throughout the whole storage period. The correlation between the a* and b* value was found to be significant (r^2: 0.908 and $p < 0.01$). This means that the decrease in the a* value, which stands for the loss of redness in color of the meat and transition of its color to brownish red by formation of metmyoglobin, leads to the decrease in the b* value. O'Sullivan et al. (2003) expressed that the b* value was more correlated to brown by sensory panelists.

3.3. Oxidative stability

Table 3 illustrates the TBA values of samples. It was found that the gas composition was not statistically significant ($p > 0.05$) for oxidative stability, whereas the storage time was significant ($p \leq 0.05$). Oxidative stability decreased for the whole storage period. The TBA values of all samples were about 2.5 except MAP1($O_2/CO_2/N_2$:30/70/0) on the 5th day of storage and the TBA values of both MAP1($O_2/CO_2/N_2$:30/70/0) and MAP2($O_2/CO_2/N_2$:50/50/0) samples were higher than the other samples ($p \leq 0.05$) on the 7th and 9th day of storage. This result shows us that high CO_2 concentration also leads to the decrease in oxidation stability together with discoloration. The MAP1($O_2/CO_2/N_2$:30/70/0) and MAP2($O_2/CO_2/N_2$:50/50/0) samples were not analyzed anymore because of the very high TBA values and discoloration. The TBA values of MAP3($O_2/CO_2/N_2$:70/30/0) samples were virtually higher than MAP4($O_2/CO_2/N_2$:50/30/20), MAP5($O_2/CO_2/N_2$:30/30/40) and AP throughout the whole storage period. At the end of storage period, the best sample was the MAP4($O_2/CO_2/N_2$:50/30/20) and AP, whereas the ordinary gas composition MAP3($O_2/CO_2/N_2$:70/30/0) was the worst case because of the higher oxygen concentration. Decrease in lipid oxidation stability with higher oxygen concentrations was also determined by various authors (Kennedy et al., 2004; Jakobsen & Bertelsen, 2000; O'Grady et al., 2000; Jayasingh, Cornforth, Brennand, Carpenter, & Whittier, 2002; Zakrys et al., 2008).

They both decreased the oxidation stability significantly to increase the carbon dioxide concentration more than 30% and increase the oxygen concentration from 50 to 70%. Among the five modified atmosphere packages with different gas composition, MAP4 ($O_2/CO_2/N_2$:50/30/20) gave the best results for oxidative stability. However, Jakobsen and Bertelsen (2000) did not find any significant effect of reducing the oxygen from 80 to 55% on the oxidation stability of meat.

3.4. Microbiological analyses

Fig. 1 illustrates the results of the viable counts of the targeted microbial groups from minced meat samples packaged under different gas compositions of MAP and AP. In aerobic packaging of ground meat, all the microbial groups showed viable counts higher than those of other MAP applications did and as stated in the literature (Ercolini et al., 2006). *Pseudomonas* spp. particularly were the dominant population in the first three days of storage for AP control samples followed by lactic acid bacteria, *Enterobacteriaceae* and *B. thermosphacta*. However, packaging under modified atmosphere delayed and restricted the growth of these microorganisms depending on the gas composition of package.

Packaging under MAP1($O_2/CO_2/N_2$:30/70/0) combination delayed the growth of LAB, *Enterobacteriaceae* family and completely inhibited the growth of *Pseudomonas* spp. and B. *thermosphacta*. MAP2($O_2/CO_2/N_2$:50/50/0) combination restricted the growth of LAB and delayed the growth of *Enterobacteriaceae* family and completely inhibited the growth of *Pseudomonas* spp. and *B. thermosphacta*. MAP3($O_2/CO_2/N_2$:70/30/0) combination favored the growth of LAB and *Enterobacteriaceae* family and restricted the growth of *Pseudomonas* spp. and *B. thermosphacta* in comparison with AP samples. MAP4($O_2/CO_2/N_2$:50/30/20) gas combination restricted all microorganisms and completely inhibited the growth of *Enterobacteriaceae* family at the end of eleven days of storage. MAP5($O_2/CO_2/N_2$:30/30/40) gas combination delayed the growth of LAB and *Enterobacteriaceae* family, but the

Table 3
Changes in L*, a*, b* and TBA values[a] of minced beaf meat samples packaged under different modified atmosphere packaging conditions with storage period.

		Times (days)						
		1	3	5	7	9	11	14
L*	AP	$42.92 \pm 0.44^{bc\ A}$	$43.88 \pm 0.73^{b\ A}$	$43.03 \pm 1.61^{bc\ A}$	$43.24 \pm 1.35^{a\ A}$	$42.45 \pm 0.35^{a\ A}$	$42.10 \pm 0.69^{a\ A}$	$43.18 \pm 1.15^{a\ A}$
	MAP1	$40.12 \pm 1.43^{a\ A}$	$40.26 \pm 0.30^{a\ A}$	$40.17 \pm 0.23^{ab\ A}$	$42.01 \pm 0.23^{a\ AB}$	$43.32 \pm 1.69^{a\ B}$	NA	NA
	MAP2	$41.19 \pm 1.22^{ab\ A}$	$39.31 \pm 2.09^{a\ A}$	$39.06 \pm 0.49^{a\ A}$	$41.10 \pm 3.30^{a\ A}$	$41.15 \pm 2.07^{a\ A}$	NA	NA
	MAP3	$40.70 \pm 1.02^{a\ A}$	$41.00 \pm 1.23^{ab\ AB}$	$43.65 \pm 2.62^{c\ AB}$	$43.67 \pm 1.52^{a\ AB}$	$44.70 \pm 1.24^{a\ B}$	$43.21 \pm 0.96^{a\ AB}$	$43.05 \pm 1.33^{a\ AB}$
	MAP4	$43.34 \pm 0.91^{c\ A}$	$43.59 \pm 1.14^{b\ A}$	$43.02 \pm 1.79^{bc\ A}$	$43.38 \pm 0.49^{a\ A}$	$44.48 \pm 1.65^{a\ A}$	$43.32 \pm 0.13^{a\ A}$	$43.88 \pm 0.88^{a\ A}$
	MAP5	$43.07 \pm 1.55^{bc\ A}$	$42.22 \pm 0.02^{ab\ A}$	$43.79 \pm 1.42^{c\ A}$	$42.35 \pm 0.63^{a\ A}$	$41.96 \pm 2.67^{a\ A}$	$42.16 \pm 1.51^{a\ A}$	$44.29 \pm 0.23^{a\ A}$
a*	AP	$27.20 \pm 1.16^{a\ E}$	$24.57 \pm 0.78^{ab\ DE}$	$21.50 \pm 1.39^{b\ CD}$	$19.56 \pm 2.05^{c\ BC}$	$14.41 \pm 3.42^{b\ A}$	$15.67 \pm 3.72^{a\ AB}$	$17.97 \pm 2.22^{b\ ABC}$
	MAP1	$28.10 \pm 0.79^{a\ E}$	$22.69 \pm 1.26^{a\ D}$	$13.14 \pm 0.03^{a\ C}$	$11.14 \pm 0.62^{a\ B}$	$8.14 \pm 0.28^{a\ A}$	NA	NA
	MAP2	$28.81 \pm 0.81^{a\ E}$	$25.71 \pm 1.59^{b\ D}$	$20.26 \pm 0.05^{b\ C}$	$13.92 \pm 0.92^{b\ B}$	$9.43 \pm 0.68^{a\ A}$	NA	NA
	MAP3	$28.92 \pm 0.65^{a\ D}$	$26.44 \pm 0.67^{b\ C}$	$26.13 \pm 1.25^{c\ C}$	$23.61 \pm 1.12^{e\ B}$	$22.76 \pm 0.45^{c\ B}$	$20.45 \pm 1.26^{a\ A}$	$18.29 \pm 0.00^{b\ A}$
	MAP4	$28.32 \pm 0.81^{a\ F}$	$26.03 \pm 0.66^{b\ E}$	$24.53 \pm 0.42^{c\ D}$	$22.49 \pm 0.35^{de\ C}$	$20.35 \pm 0.33^{c\ B}$	$19.93 \pm 0.42^{a\ B}$	$16.81 \pm 0.07^{b\ A}$
	MAP5	$29.09 \pm 1.42^{a\ D}$	$25.79 \pm 1.42^{b\ D}$	$21.55 \pm 1.28^{b\ C}$	$20.22 \pm 1.72^{cd\ C}$	$14.48 \pm 2.75^{b\ B}$	$12.22 \pm 4.95^{a\ B}$	$8.22 \pm 0.59^{a\ A}$
b*	AP	$23.42 \pm 0.61^{ab\ D}$	$22.55 \pm 0.48^{b\ CD}$	$20.78 \pm 0.76^{c\ BC}$	$20.01 \pm 1.42^{bc\ B}$	$16.73 \pm 1.69^{a\ A}$	$16.84 \pm 2.26^{a\ A}$	$17.77 \pm 1.26^{b\ A}$
	MAP1	$22.27 \pm 0.60^{a\ D}$	$20.88 \pm 0.56^{a\ C}$	$16.82 \pm 1.03^{a\ B}$	$16.53 \pm 0.10^{a\ AB}$	$15.29 \pm 0.03^{a\ A}$	NA	NA
	MAP2	$22.75 \pm 0.42^{a\ D}$	$22.20 \pm 0.04^{ab\ D}$	$18.14 \pm 0.35^{b\ C}$	$17.23 \pm 0.09^{a\ B}$	$15.56 \pm 0.54^{a\ A}$	NA	NA
	MAP3	$22.93 \pm 0.38^{a\ C}$	$22.89 \pm 0.13^{b\ C}$	$21.82 \pm 0.21^{cd\ C}$	$19.65 \pm 0.117^{b\ B}$	$16.38 \pm 1.19^{a\ A}$	$16.45 \pm 0.25^{a\ A}$	$16.45 \pm 0.59^{ab\ A}$
	MAP4	$24.13 \pm 0.59^{ab\ E}$	$22.84 \pm 0.66^{b\ D}$	$22.35 \pm 0.32^{d\ D}$	$21.49 \pm 0.18^{c\ C}$	$20.41 \pm 0.41^{b\ B}$	$20.03 \pm 0.04^{a\ B}$	$18.20 \pm 0.06^{b\ A}$
	MAP5	$24.89 \pm 0.76^{b\ E}$	$22.76 \pm 0.91^{b\ D}$	$20.97 \pm 0.92^{c\ C}$	$20.17 \pm 1.03^{bc\ C}$	$17.25 \pm 0.65^{a\ B}$	$16.62 \pm 1.79^{a\ AB}$	$15.45 \pm 0.81^{a\ A}$
TBA	AP	$1.14 \pm 0.078^{a\ A}$	$2.09 \pm 0.23^{a\ B}$	$2.62 \pm 0.39^{a\ C}$	$2.50 \pm 0.00^{a\ BC}$	$2.34 \pm 0.00^{a\ BC}$	$2.73 \pm 0.16^{ab\ CD}$	$3.12 \pm 0.00^{b\ D}$
	MAP1	$1.85 \pm 0.078^{c\ A}$	$2.34 \pm 0.16^{ab\ B}$	$3.43 \pm 0.00^{b\ C}$	$3.93 \pm 0.16^{c\ D}$	$6.32 \pm 0.23^{d\ E}$	NA	NA
	MAP2	$1.97 \pm 0.078^{c\ A}$	$2.57 \pm 0.16^{b\ B}$	$2.73 \pm 0.00^{a\ B}$	$3.67 \pm 0.00^{c\ C}$	$5.93 \pm 0.16^{c\ D}$	NA	NA
	MAP3	$1.93 \pm 0.078^{c\ A}$	$2.37 \pm 0.16^{ab\ B}$	$2.41 \pm 0.078^{a\ B}$	$2.96 \pm 0.00^{b\ C}$	$3.59 \pm 0.078^{b\ D}$	$3.67 \pm 0.23^{b\ D}$	$3.98 \pm 0.078^{c\ E}$
	MAP4	$1.40 \pm 0.078^{b\ A}$	$2.38 \pm 0.16^{ab\ B}$	$2.56 \pm 0.23^{a\ BC}$	$2.89 \pm 0.16^{b\ C}$	$2.65 \pm 0.16^{a\ BC}$	$2.57 \pm 0.00^{a\ BC}$	$2.73 \pm 0.078^{a\ BC}$
	MAP5	$1.25 \pm 0.078^{ab\ A}$	$2.28 \pm 0.00^{ab\ B}$	$2.64 \pm 0.16^{a\ B}$	$2.57 \pm 0.078^{a\ B}$	$2.65 \pm 0.078^{a\ B}$	$3.27 \pm 0.47^{b\ C}$	$3.40 \pm 0.31^{b\ C}$

[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 ($p \leq 0.01$).
Means with different capital letters in the same line are significantly different ($p \leq 0.01$).

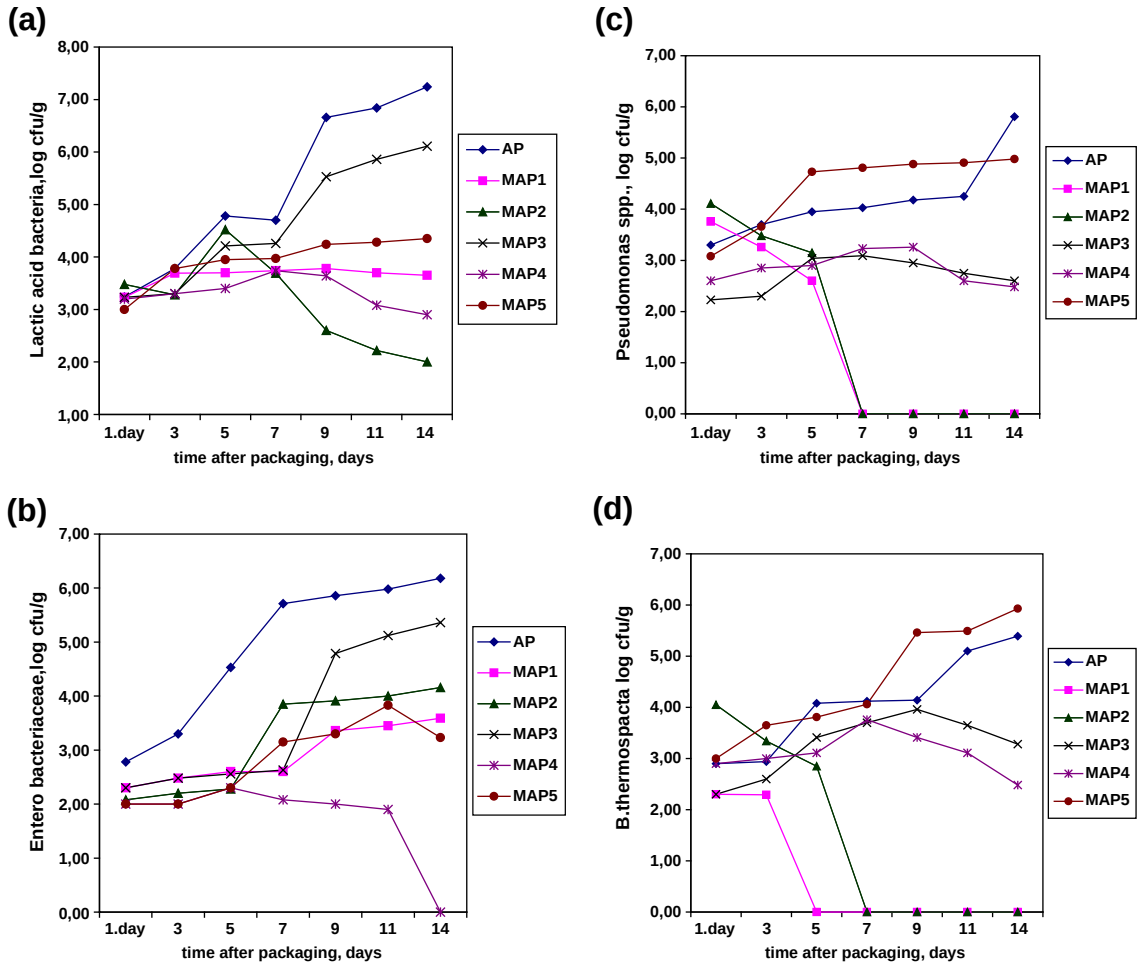

Fig. 1. (a) Lactic acid bacteria, (b) *Enterobacteriaceae*, (c) *Pseudomonas* spp, (d) *B. thermosphacta* counts of minced beef meat samples packaged under different modified atmosphere packaging conditions during storage at 4 °C.

numbers of *Pseudomonas* spp. and *B. thermosphacta* were very close to the microbial load of AP samples. The lowest numbers were obtained in MAP2($O_2/CO_2/N_2$:50/50/0) and MAP4($O_2/CO_2/N_2$:50/30/20) combination for LAB. Until the 7th day of storage LAB counts increased and then started to decrease for these two MAP combination, whereas for MAP1 ($O_2/CO_2/N_2$:30/70/0) and MAP5($O_2/CO_2/N_2$:30/30/40) combinations they slightly increased and for MAP3($O_2/CO_2/N_2$:70/30/0) the increase was higher by the end of the storage period. Enterobacteriaceae family were completely inhibited on the 14th day of storage in MAP4($O_2/CO_2/N_2$:50/30/20) combination.

MAP3($O_2/CO_2/N_2$:70/30/0) and MAP4($O_2/CO_2/N_2$:50/30/20) gas combinations restricted the growth of *Pseudomonas* spp. whereas *Pseudomonas* spp. were completely inhibited in MAP1($O_2/CO_2/N_2$:30/70/0) and MAP2($O_2/CO_2/N_2$:50/50/0) combinations after five days of storage The growth of *B. thermosphacta* was completely inhibited in MAP1($O_2/CO_2/N_2$:30/70/0) and MAP2($O_2/CO_2/N_2$:50/50/0) gas combinations after three days and after five days of storage respectively, while MAP4($O_2/CO_2/N_2$:50/30/20) restricted the growth of it.

As seen from our results, high-CO_2 concentration sample groups as 70%CO_2 and 50%CO_2 inhibited the growth of *B. thermosphacta* and *Pseudomonas* spp. Although the effect of carbon dioxide, higher than 20%, was specified in the literature for inhibition of *Pseudomonas* spp. (Koutsoumanis et al., 2008; Soldatou et al., 2009; Giatrakou, Kykkidou, Papavergou, Kontominas, & Savvaidis, 2008; Ntzimani, Paleologos, Savvaidis, & Kontominas, 2008; Ravi Sankar, Lalitha, Jose, Manju, & Gopal, 2008; Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007; Cutter, 2002; Skandamis & Nychas, 2002; Kennedy et al., 2004; Sheridan et al., 1997; Patsias, Chouliara, Badeka, Savvaidis, & Kontominas, 2006), there are some controversial results for inhibition of *B. thermosphacta* in carbon dioxide atmospheres. Koutsoumanis et al. (2008), expressed that packaging with low permeability films (LPF) lowered the growth rate of *B. thermosphacta* and *Pseudomonas* spp. due to the higher inhibitory effect of carbon dioxide and they observed a significant reduction in the growth rates of both microorganisms in minced pork packaged in LPF. Soldatou et al. (2009), Ercolini et al. (2006) and Sheridan et al. (1997) found that under high carbon dioxide atmosphere of 70%, 40% and 50% respectively, growth of *B. thermosphacta* and *Pseudomonas* spp was restricted. However, Berruga, Vergara, and Gallego (2005); Skandamis and Nychas (2002); Gill and Harrison (1989) and Patsias et al. (2006) determined an important growth of *B. thermosphacta* in carbon dioxide atmospheres.

It was explained that the behaviors of LAB were fully anticipated considering the fact that LAB are facultative anaerobic and are able to grow both in the presence and absence of oxygen (Patsias, Badeka, Savvaidis, & Kontominas, 2008). According to our results, while LAB counts for AP control samples increased throughout the storage, LAB counts of MAP2($O_2/CO_2/N_2$:50/50/0) and MAP4($O_2/CO_2/N_2$:50/30/20) samples, with the amount of oxygen as 50%, began to decrease after the 7th day of storage and by the end of the 14th day of storage, there was a decrease of about 1.48 log cfu/g and 0.30 log cfu/g for each samples respectively. LAB counts of MAP1($O_2/CO_2/N_2$:30/70/0) and MAP5($O_2/CO_2/N_2$:30/30/40) samples, in which the amount of oxygen started as 30%, increased slightly until the end of storage period, whereas there was an increase of about 0.42 log cfu/g for MAP1($O_2/CO_2/N_2$:30/70/0) sample and an increase of 1.35 log cfu/g for MAP5($O_2/CO_2/N_2$:30/30/40) sample. However, for MAP3($O_2/CO_2/N_2$:70/30/0) sample, which contains high amounts of oxygen, LAB counts increased by about 2.88 log cfu/g until the end of storage period. In agreement with our results, Kennedy et al. (2004) found that LAB counts increased throughout the storage period with different MAP combinations that have high oxygen and low carbon dioxide, but the MAP combinations that have the higher oxygen content gave slightly higher LAB counts. Koutsoumanis et al. (2008) found that until the 8th day of storage, LAB counts increased and then started to decrease in LPF samples, where the produced carbon dioxide is maintained in the headspace. Skandamis and Nychas (2002) found that 100% CO_2 decreased the growth rate of LAB compared with 40%CO_2/30%O_2/30%N_2 at 5 °C. Ercolini et al. (2006) found lower counts for MAP applications containing different amounts of oxygen, carbon dioxide and nitrogen with respect to air packaged samples. In the study of Patsias et al. (2006), high-CO_2 MAP application lowered the LAB counts compared to aerobic packaging. Aksu, Kaya, and Ockerman (2005) also showed the effects of high-CO_2 MAP application compared to aerobic packaging for inhibition of LAB counts. Contrary of our results, it was determined by some researchers that LAB can grow under high concentrations of CO_2 in MAP products as facultative anaerobic bacteria (Giatrakou et al., 2008; Ntzimani et al., 2008; Arkoudelos, Stamatis, & Samaras, 2007; Chouliara et al., 2007; Franzetti, Martinoli, Piergiovanni, & Gali, 2001).

MAP applications limited the growth of *Enterobacteriaceae* in comparison to AP samples in all groups irrespective of gas combinations applied. However, it was completely inhibited in MAP4 ($O_2/CO_2/N_2$:50/30/20) samples on the 14th day of storage. Compatible with our results, Berruga et al. (2005) found that *Enterobacteriaceae* counts in lamb meat increased throughout the storage period and the presence of CO_2 levels over 40% in packs limited the growth of *Enterobacteriaceae*. Soldatou et al. (2009) found the same trend for *Enterobacteriaceae* counts in lamb meat throughout the storage period in comparison with air packaging. Compatible with our results, Chouliara et al. (2007) determined that *Enterobacteriaceae* grew under MAP conditions at a slower rate than under aerobic packaging. However, in contrast to our results Ravi Sankar et al. (2008) found higher counts for the gas combination of 40%CO_2 + 30%O_2 + 30%N_2 than five gas combinations, which contain CO_2 concentration between 40–70% and O_2 concentration between 60–30%. They also found that the behavior of *Enterobacteriaceae* was different in MAP combinations with the same carbon dioxide concentration as we found for MAP3 ($O_2/CO_2/N_2$:70/30/0), MAP4($O_2/CO_2/N_2$:50/30/20) and MAP5($O_2/CO_2/N_2$:30/30/40). Patsias et al. (2008) did not find any difference for *Enterobacteriaceae* counts in chilled precooked chicken product between air packaging and MAP applications of 30%CO_2 + 70%N_2, 60%CO_2 + 40%N_2 and 90%CO_2 + 10%N_2. Goulas, Chouliara, Nessi, Kontominas, and Savvaidis (2005) also did not find any effect of MAP application on *Enterobacteriaceae* counts in mussels stored under modified atmospheres of 50%CO_2 + 50%N_2, 80%CO_2 + 20%N_2, 40%CO_2 + 30%O_2 + 30%N_2.

4. Conclusion

Increasing the concentration of CO_2 in modified atmosphere applications resulted with a decrease in oxidation stability and loss of redness due to the metmyoglobin formation, which means a decrease in the a* and b* values of samples, whereas the lightness of samples were not affected. The oxidation stability and the color of minced beef meat packaged in modified atmospheres were best preserved in atmospheres containing low CO_2 concentrations (30%) rather than high (70–50%) concentrations although high-CO_2 concentrations inhibited the growth of spoilage microorganisms.

However, the oxygen concentration is also an important factor in the shelf-life of fresh red meat. High concentrations of oxygen (50–70%) are effective in maintaining and prolonging the redness of meat, whereas it also leads to the decrease in lipid stability.

When we keep the carbon dioxide concentration as 30%, we determined that keeping the oxygen concentration as low as 30% was not enough to maintain the redness of minced beef meat throughout the storage period. When we compare the MAP groups having 50% and 70% oxygen concentration, we determined that oxidation stability is better with 50% than in 70% as well as microbial growth, whereas there was no significant difference in the redness of samples.

As a result, we concluded that, the best preservation for minced beef meat was in MAP4 gas combination maintaining acceptable color together with oxidation stability and acceptable microbial loads until the end of storage period of fourteen days.

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