

## Effects of High-Oxygen Modified Atmosphere Packaging on the Microbiological Quality and Shelf Life of Tekirdag Kofte: A Turkish Type Meatball

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**Abstract:** Effects of different concentrations of O<sub>2</sub>/CO<sub>2</sub>/N<sub>2</sub> in modified atmosphere packaging on the microbiological quality and shelf-life of Tekirdag kofte (a Turkish type meatball) was investigated. For this purpose, meatballs were separately packaged under aerobic and various gas mixture conditions of 80:20:0, 60:20:20, 70:30:0 and 60:40:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>. Packages were stored at refrigerator temperature (4±1 °C) for 12 days and examined microbiologically comparing with pH and oxidative changes during storage. As a result, the quality and shelf-life of meatballs under various gas compositions were improved; microbial growth was delayed due to increasing level of CO<sub>2</sub> usage and shelf-life was increased by up to 8 days.

**Key words:** Meatball, shelf-life, modified atmosphere packaging, microbiological quality, growth, gas

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### INTRODUCTION

Meat and meat products are indispensable in human nutrition due to their valuable proteins. It contains high amounts of exogenous amino acids, minerals such as Fe, P, Zn, Cu, vitamins like B<sub>12</sub> and a certain amount of fat (Inal, 1992).

Because of the changing demand of consumers ready to cook meat products are widely consumed all over the world. Tekirdag, a Turkish type meatball is one of the most popular ground meat products in Turkey, produced from ground meat, toasted bread crumbs, salt, onion, garlic and various spices (Yilmaz *et al.*, 2002).

The shelf-life of meatballs depends on the microbiological quality of meat and other ingredients, especially spices used in its manufacturing, the hygienic precautions taken during production and finally the type of packaging and storage conditions (Inal, 1992). The safety of the product can easily be limited by microbial spoilage while it is minced and can be contaminated with pathogenic bacteria such as *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* sp. (Yilmaz *et al.*, 2002; Gokmen and Alisarli, 2003; Baskaya *et al.*, 2004; Cetin *et al.*, 2010; Ozturk *et al.*, 2010). Lipid oxidation and colour changes are also important factors affecting the quality and shelf-life of meatballs.

Oxidation of fats in meat products can adversely affect flavour and acceptability during storage (Insausti *et al.*, 2001; Berruga *et al.*, 2005).

Non-hygienic prior/during and post processing, including primary and secondary contaminations may shorten the shelf-life of meatballs (Koutsoumanis *et al.*, 2008; Temelli *et al.*, 2011). Meat and meat products stored under aerobic, chill conditions are generally exposed to spoilage by aerobic psychrophilic/psychrotrophic bacteria, mainly *Pseudomonas* sp. (Ercolini *et al.*, 2009).

Modified Atmosphere Packaging (MAP) is widely used in modern meat packaging technique which is intended to maintain the microbial and sensory quality of the product by inhibiting or retarding the growth of undesirable microflora by manipulating the meat microenvironment (Farber, 1991; Hotchkiss, 1988) is designed primarily to preserve the bright red appearance of meat (Taylor *et al.*, 1990) although, lipid oxidation and microbial growth are also important factors regarding shelf-life and consumer acceptance of fresh meat (Jakobsen and Bertelsen, 2000). MAP is inhibitory to some microorganisms and therefore increases the keeping quality of a variety of foods (Cutter, 2002). Elevated CO<sub>2</sub> and reduced O<sub>2</sub> levels in MAP can inhibit growth of various spoilage and pathogenic microorganisms (Jeremiah, 2001) and can also control

oxidative degradation and colour changes in the product. Although, high O<sub>2</sub> atmospheres (60-80% O<sub>2</sub>) are suggested for fresh beef to maintain bright red colour, it can cause oxidation depending on the fat level which can negatively affect the consumer preferences (Jakobsen and Bertelsen, 2004; Ozturk *et al.*, 2010).

The objective of this study was to investigate the effect of various concentration of high-oxygen modified atmosphere packaging on the microbiological quality and shelf-life of Tekirdag kofte by comparing pH and oxidative changes.

## MATERIALS AND METHODS

**Manufacturing of meatballs:** Well-matured and finely ground through a 3.2 mm plate in a meat grinder (Biro Meat Grinder Model 346, Biro, Marblehead, OH, USA) veal (*M. Longissimus dorsi*) was purchased from a local market in Avcilar, Istanbul and was used in experimental meatball production.

The samples were produced according to the following traditional recipe (Colak *et al.*, 2008). The ground veal (84%) which contains 10% fat was mixed with ground black pepper (0.1%), cumin (0.4%), red pepper (2.0%), onion rind (3.0%), garlic clove rind (0.5%), salt (2.0%) and toasted bread (made of wheat flour) crumbs (8.0%). The mix was kneaded for 30 min by hand (with sterile glove) to obtain homogeneous dough, then whole meatball dough was shaped by hand into 5 cm diameter meatballs with a weight of 40±5 g. The experimental meatball samples were manufactured in triplicate for each group in different dates.

**Packaging of meatballs:** Meatball samples were placed in low O<sub>2</sub> permeable (8-12 cm<sup>3</sup>/m<sup>2</sup>/24 h at STP) Polystyrene/Ethylvinylalcohol (EVOH)/Polyethylene (PE) trays and were heat-sealed with a Multivac packaging unit (Multivac A 300/16, Sepp Hagenmuller, D 87787 Wolfertschwenden, Germany) using a low O<sub>2</sub> permeable (3 cm<sup>3</sup>/m<sup>2</sup>/24 h) lidding film (20 mm of a laminate Orientated Polypropylene (OPP) and a co-extrusion layer (50 mm) of PE/EVOH/PE) (Wrap Film Systems Ltd., Shropshire, England) for aerobic and modified atmosphere packaging using a gas mixture of 80:20:0, 60:20:20, 70:30:0 and 60:40:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> with a headspace ratios of 1:1 (veal/gas).

Packages were stored at refrigerator temperature (4±1°C) for 12 days and examined at intervals of 0 (3 h after packaging), 2, 4, 6, 8, 10 and 12 day of storage.

**Gas analyses:** Gas analyses of the internal atmosphere were done in duplicate at analyzed days of storage before the packages were opened. Analyses for CO<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> within the packages were monitored by injecting 0.5 mL of

gas removed from the headspace with a syringe (B, Braun, Melsungen AG, Deutschland) into a PDI gas chromatograph (PBI-Dansensor A/B, Ronnedevaj 18, DK 410 Ringsted, Denmark) fitted with a thermal conductivity detector.

**Determination of pH:** The pH of the meatball samples was measured using a portable pH-meter (WTW pH 340i with a probe SenTix, Weilheim, Germany). The mean of three measures in each sample were evaluated as pH value (AOAC, 1995).

### Determination of Thiobarbituric Acid Reactive Substances (TBARS) value:

The meatball samples were first chopped thoroughly and then homogenous sample was made using the warring blender. About 20 g of the meatball sample was mixed with 50 mL of 20% trichloroacetic acid in 2 M phosphoric acid solution at 4°C and it was homogenized by ultra-turrax (ART Micra RT) for 1.5 min. It was diluted with deionised water to make 100 mL and then filtered. The 5 mL of the filtrate was mixed with 5 mL of freshly prepared 0.005 M thiobarbituric acid solution in a stopper fitted glass tube. It was mixed simply by inverting the tube several times and then kept in the dark for 15 h at room temperature. Finally, the absorbance of the colour developed was measured at 530 nm using UV visual spectrophotometer (Chebios Optimum-One). The TBARS value was calculated as following:

$$\text{TBARS value} = \left[ \frac{\text{Absorbance}-0.0121}{0.1379} \right] \times [72.06/94] \text{ mg MDA kg}^{-1} \text{ meatball}$$

Results were calculated according the percentage of Malondialdehyde (MDA) which has a molecular weight of 72.06 (Shrestha and Min, 2006).

**Microbiological analyses:** About 25 g of meatball sample from each group was transferred to a sterile bag with 225 mL sterile Peptone water (Oxoid, CM0009) and was homogenized for 90 sec using a stomacher (Lab Blender 400, Model BA6021, Steward Lab., London, UK). Serial decimal dilutions were prepared using the same diluent. A 0.1 or 1 mL inoculum of appropriate dilutions was spread on plate count agar (PCA, Oxoid, CM0325, Hampshire, UK); pour plates incubated at 35°C for 48 h for Total Aerobic Plate Counts (TAPC) and at 7°C for 10 days for total psychrophilic bacteria (Harrigan and McCance, 1968).

Lactic Acid Acteria (LAB) counts were determined by plating with overlay on de Man, Rogosa, Sharpe agar (MRS, Oxoid, CM0361) and incubating at 35°C for 48 h

(Davidson and Cronin, 1973); *Pseudomonas* sp. were enumerated on pseudomonas agar with Cetrimid, Fucidin, Cephaloridin supplement (PA with CFC, Oxoid, CM0559 and SR0103); on spread plates were incubated at 25-30°C for 48 h. Coliforms were examined in Violet Red Bile agar (VRB, Oxoid, CM0107) by using pour plates with overlay added before incubation with incubation at 35°C for 24 h. Yeast and mould were defined on Dichloran Rose Bengal Chloramphenicol agar with Chloramphenicol Selective supplement (DRBC, Oxoid, CM0727 and SR0078). Spread plates were incubated at 25°C for 3-5 days (Harrigan and McCance, 1968). All microbiological tests were carried out in duplicate and the results expressed as log CFU g<sup>-1</sup>.

**Statistical analyses:** Analysis of Variance (ANOVA) was conducted for each variable to investigate the effect of storage time and packaging type. The trial was performed in triplicate and microbial counts were expressed as log CFU g<sup>-1</sup>. The mean separations between and within the subjects were obtained using Duncan's multiple range tests of SPSS 13.0 where the significance of differences was defined as p<0.05 (SPSS, 2001).

**RESULTS AND DISCUSSION**

**Headspace composition:** The headspace compositions of aerobic and modified atmosphere packaged meatballs were almost constant during storage with a slight shift after 10 days of storage under MAP conditions similarly to the findings of Bingol and Ergun (2011). Jongberg *et al.* (2011) also emphasized that the headspace oxygen concentration in MA-packages containing oxygen decreased >9 days of storage by approximately 20% for untreated beef patties and 13% for white grape extract treated beef patties. The concentration of O<sub>2</sub> derivates with an increase in CO<sub>2</sub> concentration related to the

dynamic headspace with CO<sub>2</sub> dissolving in the meat and being formed by tissue and bacterial respiration with the consumption of O<sub>2</sub> (Gill, 1996). Similar results were indicated also by Coventry *et al.* (1998), Jakobsen and Bertelsen (2000), Daly and Acton (2004), Kennedy *et al.* (2004), Ozturk *et al.* (2010) and Esmer *et al.* (2011) where the initial increase in CO<sub>2</sub> was due to tissue utilisation of O<sub>2</sub> while the second increase corresponded to microbial growth (Daun *et al.*, 1971).

**pH:** The initial pH of meatball was 6.76 and decreased continuously during the storage time. Significant differences were observed between aerobic and modified atmosphere packaging (p<0.05) from day one with a higher amount of decrease in air package (Table 1). Meanwhile, increasing CO<sub>2</sub> concentration revealed lower pH values than other MA packages. Similarly, Jayasingh *et al.* (2002) stated that significant pH differences were detectable and air packaged ground beef dropped rapidly during storage while a slight decrease were observed in high-oxygen MAP over the same period.

Meat pH can be affected by many factors; however, growth of lactic acid bacteria resulting in lactic acid production is the major factor causing pH decreases in packaged meats (Gill, 1996). Lower pH values found in air packaged samples in the present study could support lactic acid bacteria outgrowth. Likewise, Yilmaz and Demirci (2010) determined that pH of meatball samples decreased significantly during storage due to microorganism activities which resulted with acidity development interacted with packaging methods. Temelli *et al.* (2011) also stated that MA packaged Inegol Kofte showed a decrease in terms of pH values during storage contrary to ambient packaging in agreement with Colak *et al.* (2008) who stored nisin and lactoferrin added Tekirdag kofte at 4°C for 12 days in air packaging.

Table 1: The pH and TBARS values of Tekirdag Kofte packaged under different conditions (air and modified atmosphere packaging of 80:20:0, 60:20:20, 70:30:0, 60:40:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> combinations) during storage at 4°C

Attribute	Groups	Storage time at 4°C (days)						p-values
		2	4	6	8	10	12	
pH	Air	6.71±0.006 <sup>cx</sup>	6.51±0.012 <sup>cy</sup>	6.48±0.018 <sup>cy</sup>	6.42±0.012 <sup>cz</sup>	6.32±0.012 <sup>ca</sup>	5.93±0.017 <sup>bp</sup>	***
	80:20:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	6.72±0.006 <sup>bcx</sup>	6.65±0.012 <sup>by</sup>	6.62±0.012 <sup>by</sup>	6.54±0.023 <sup>az</sup>	6.45±0.017 <sup>ca</sup>	6.25±0.029 <sup>ap</sup>	***
	60:20:20/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	6.75±0.006 <sup>ax</sup>	6.67±0.012 <sup>cy</sup>	6.63±0.018 <sup>cy</sup>	6.56±0.017 <sup>az</sup>	6.48±0.017 <sup>ca</sup>	6.27±0.040 <sup>ap</sup>	***
	70:30:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	6.73±0.006 <sup>bx</sup>	6.62±0.012 <sup>by</sup>	6.55±0.023 <sup>byz</sup>	6.49±0.012 <sup>bcz</sup>	6.43±0.017 <sup>ca</sup>	6.22±0.058 <sup>ap</sup>	***
	60:40:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	6.72±0.006 <sup>bcx</sup>	6.56±0.023 <sup>cy</sup>	6.54±0.023 <sup>byz</sup>	6.47±0.012 <sup>byz</sup>	6.4±0.058 <sup>bz</sup>	6.19±0.058 <sup>ca</sup>	***
	p-values	**	***	**	**	*	**	
TBARS	Air	0.578±0.003 <sup>ca</sup>	0.605±0.004 <sup>ca</sup>	0.628±0.003 <sup>abzq</sup>	0.689±0.004 <sup>abz</sup>	0.778±0.006 <sup>by</sup>	0.950±0.006 <sup>bcz</sup>	***
	80:20:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	0.500±0.002 <sup>bp</sup>	0.539±0.003 <sup>bp</sup>	0.661±0.004 <sup>ca</sup>	0.800±0.004 <sup>az</sup>	0.867±0.005 <sup>ay</sup>	1.161±0.006 <sup>ax</sup>	***
	60:20:20/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	0.400±0.002 <sup>dp</sup>	0.450±0.002 <sup>dp</sup>	0.578±0.004 <sup>ca</sup>	0.650±0.006 <sup>bcz</sup>	0.750±0.004 <sup>by</sup>	0.928±0.005 <sup>bcz</sup>	***
	70:30:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	0.439±0.002 <sup>ca</sup>	0.483±0.004 <sup>ca</sup>	0.639±0.004 <sup>abz</sup>	0.761±0.002 <sup>aby</sup>	0.800±0.004 <sup>by</sup>	1.033±0.006 <sup>bcz</sup>	***
	60:40:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	0.366±0.003 <sup>dp</sup>	0.400±0.002 <sup>dp</sup>	0.494±0.003 <sup>ca</sup>	0.539±0.005 <sup>caz</sup>	0.611±0.006 <sup>cy</sup>	0.844±0.012 <sup>ca</sup>	***
	p-values	***	***	**	**	**	**	

<sup>a-c</sup>Means within a column with different letters are significantly different (p<0.05); <sup>x-z</sup>Means within a row with different letters are significantly different (p<0.05); \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, NS: Not Significant

**Oxidative changes:** Storage time and gas composition were the significant factors affected TBARS values of meatballs ( $p < 0.01$ ). Meatballs packaged with air or with high  $\text{CO}_2$  concentrations had lower mean TBARS values during the 12 days of storage compared to meat in  $\text{O}_2$  packages (Table 1). Although, high  $\text{O}_2$  concentration led to higher lipid oxidation than other packaging used, the shelf-life of meatballs extended with increasing  $\text{CO}_2$  concentrations of modified atmosphere packaging. These findings agree with Ordonez and Ledward who stated that the concentration of oxygen in the atmosphere is the determining factor for the rate of lipid oxidation. Residual  $\text{O}_2$  levels in the range of 0-2% were found to be sufficient for lipid oxidation to occur (Berruga *et al.*, 2005). Besides, Jakobsen and Bertelsen (2000), Jayasingh *et al.* (2002), Kennedy *et al.* (2004), Yilmaz and Demirci (2010), Ozturk *et al.* (2010) and Esmer *et al.* (2011) emphasised that high oxygen MAP resulted the highest values of TBARS in meat environment which favours lipid oxidation. Additionally, Jakobsen and Bertelsen (2002) stated that  $\text{CO}_2$  decreased lipid oxidation rate in meat and this was attributed to pH reduction due to the absorption of  $\text{CO}_2$ .

The development of rancidity as a result of lipid oxidation has been defined as a limit of storage life of meat products (Yilmaz and Demirci, 2010). According to Ockerman (1976) meat products with up to 1 mg MDA  $\text{kg}^{-1}$  TBARS value could be considered as rancid. Due to this statement, even overall TBARS values increased with storage time ( $p < 0.01$ ), meatballs stored under 60:40:0/ $\text{O}_2$ : $\text{CO}_2$ : $\text{N}_2$  and 60:20:20/ $\text{O}_2$ : $\text{CO}_2$ : $\text{N}_2$  atmosphere were found acceptable till the end of storage time at 4°C.

**Microbiological examination:** Changes in microbial populations are shown in Table 2. Days of storage at 4°C and packaging conditions were the factors ( $p < 0.05$ ) affecting each bacterial group. Significant differences were determined between and within packaging systems ( $p < 0.05$ ) with a magnitude of lower microbial loads in MAP than in aerobic packaging. Microbial growth is more inhibitory in 60:40:0/ $\text{O}_2$ : $\text{CO}_2$ : $\text{N}_2$  atmosphere packages whereas bacterial inhibition was not generally different in the same packaging atmosphere (60-80%  $\text{O}_2$ ) during entire storage time.

Initial microbial counts at day 0 were  $5.85 \pm 0.3$  log CFU  $\text{g}^{-1}$  for total aerobic plate counts,  $6.42 \pm 0.2$  log CFU  $\text{g}^{-1}$  for total psychrophilic bacteria,  $3.31 \pm 0.3$  log CFU  $\text{g}^{-1}$  for lactic acid bacteria,  $4.63 \pm 0.4$  log CFU  $\text{g}^{-1}$  for pseudomonas,  $3.04 \pm 0.2$  log CFU  $\text{g}^{-1}$  for coliforms and  $3.56 \pm 0.2$  CFU  $\text{g}^{-1}$  for yeasts and moulds.

Air packaged samples showed higher aerobic plate counts than MA-packages during whole storage time

( $p < 0.01$ ) with approximately 1 log CFU  $\text{g}^{-1}$  higher than modified atmosphere. Initial counts for TAPC increased up to 7 log CFU  $\text{g}^{-1}$  at day 4 in air packaged samples which approach spoilage by off-odours and possible slime development (Inal, 1992; Jay, 1998) while MA-packages reached this critical level after 8 days of storage.

Counts of 7 log CFU  $\text{g}^{-1}$  is the approximate point at which meat would be unacceptable (Dainty and Mackey, 1992). Therefore, the shelf-life of meatballs stored under aerobic conditions would be 4 days while meatballs stored under modified atmospheres would be >6-8 days according to the gas composition used. Similarly, increased shelf life has been reported by Insausti *et al.* (2001), Kennedy *et al.* (2004), Ercolini *et al.* (2006), Koutsoumanis *et al.* (2008), Ozturk *et al.* (2010), Yilmaz and Demirci (2010), Esmer *et al.* (2011) and Temelli *et al.* (2011) for meat and meat products packaged under different modified atmospheres. Meatballs had greater numbers of total aerobes than beef meat indicating more primary and secondary processing contamination during their manufacturing.

Farber (1991) found that the overall effect of  $\text{CO}_2$  on microorganisms was an extension of the lag phase of growth and a decrease in growth rate during the logarithmic phase. Concerning this statement, higher  $\text{CO}_2$  concentrations reduced the number of aerobic bacteria during storage; counts in MAP samples were always lower ( $p < 0.01$ ) than in aerobic packaging whatever the storage time.

Although, psychrophilic bacteria counts showed differences ( $p < 0.01$ ) between aerobic and modified atmosphere packaging, an increase was recordable in all groups during storage with a final bacterial population of  $>8$  log CFU  $\text{g}^{-1}$  at the end of the storage. However, the growth of microorganisms was slowed among packaging systems by increasing  $\text{CO}_2$  concentrations in the packages, cold-resistant bacteria continued their activities at the low temperature indicating significant differences between and within groups ( $p < 0.05$ ). The highest bacterial counts were observed in air packaged meatball samples while the lowest ones were recorded in 60:40:0/ $\text{O}_2$ : $\text{CO}_2$ : $\text{N}_2$  packaged samples.

Lactic acid bacteria counts were found different ( $p < 0.05$ ) between aerobic and MA-packaged samples. LAB counts increased during storage time for all meatball samples while MA-packaged samples had lower counts than air packaged ones during entire storage. In addition, the increase in growth of LAB varied with the increase of  $\text{CO}_2$  concentration in MA-packaged samples by lower counts than in others. Likewise, meat products stored in high  $\text{CO}_2$  atmospheres had a mean count of LAB lower

**Table 2:** Microbial changes of Tekirdag Kofte packaged under different conditions (air and modified atmosphere packaging of 80:20:0, 60:20:20, 70:30:0, 60:40:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> combinations) during storage at 4°C

Microorganisms	Groups	Storage time at 4°C (days)						p-values
		2	4	6	8	10	12	
TAMB	Air	6.749±0.052 <sup>as</sup>	7.104±0.110 <sup>as</sup>	7.38±0.145 <sup>as</sup>	7.875±0.049 <sup>ay</sup>	8.048±0.108 <sup>ay</sup>	8.277±0.077 <sup>ax</sup>	***
	80:20:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	6.261±0.057 <sup>ba</sup>	6.785±0.057 <sup>ba</sup>	6.933±0.057 <sup>ba</sup>	7.23±0.115 <sup>by</sup>	7.477±0.115 <sup>by</sup>	7.601±0.115 <sup>ba</sup>	***
	60:20:20/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	6.255±0.115 <sup>ba</sup>	6.477±0.115 <sup>ca</sup>	6.788±0.115 <sup>ba</sup>	7.176±0.057 <sup>by</sup>	7.361±0.173 <sup>ba</sup>	7.576±0.173 <sup>ba</sup>	***
	70:30:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	6.123±0.057 <sup>ba</sup>	6.368±0.115 <sup>ca</sup>	6.637±0.173 <sup>by</sup>	7.113±0.057 <sup>ba</sup>	7.225±0.115 <sup>ba</sup>	7.348±0.173 <sup>ca</sup>	***
	60:40:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	6.075±0.043 <sup>ba</sup>	6.176±0.057 <sup>ca</sup>	6.584±0.173 <sup>by</sup>	6.953±0.115 <sup>ba</sup>	7.098±0.056 <sup>ca</sup>	7.113±0.063 <sup>ca</sup>	***
	p-values	***	***	**	***	**	**	
Psychrophilic bacteria	Air	8.097±0.115 <sup>as</sup>	8.301±0.052 <sup>ay</sup>	8.477±0.173 <sup>ay</sup>	8.602±0.115 <sup>ay</sup>	8.662±0.115 <sup>ay</sup>	8.889±0.057 <sup>ax</sup>	**
	80:20:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	7.698±0.115 <sup>ba</sup>	7.965±0.115 <sup>ca</sup>	8.023±0.115 <sup>ca</sup>	8.225±0.115 <sup>ca</sup>	8.397±0.115 <sup>ca</sup>	8.576±0.173 <sup>ba</sup>	**
	60:20:20/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	7.601±0.173 <sup>ba</sup>	7.882±0.057 <sup>ca</sup>	7.986±0.173 <sup>ca</sup>	8.113±0.057 <sup>ca</sup>	8.146±0.057 <sup>ca</sup>	8.301±0.115 <sup>ca</sup>	*
	70:30:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	7.301±0.173 <sup>ca</sup>	7.412±0.115 <sup>ca</sup>	7.516±0.173 <sup>ca</sup>	7.764±0.115 <sup>ca</sup>	7.881±0.057 <sup>ca</sup>	8.072±0.115 <sup>ca</sup>	*
	60:40:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	7.028±0.115 <sup>ca</sup>	7.222±0.128 <sup>ca</sup>	7.326±0.115 <sup>ca</sup>	7.477±0.173 <sup>ca</sup>	7.612±0.173 <sup>ca</sup>	7.826±0.057 <sup>ca</sup>	*
	p-values	**	***	**	**	***	***	
LAB	Air	4.602±0.173 <sup>P</sup>	4.778±0.115 <sup>NP</sup>	5.079±0.045 <sup>NP</sup>	5.447±0.115 <sup>st</sup>	6.041±0.115 <sup>sy</sup>	6.477±0.173 <sup>xt</sup>	***
	80:20:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	4.397±0.173 <sup>P</sup>	4.662±0.115 <sup>NP</sup>	4.863±0.056 <sup>st</sup>	5.255±0.115 <sup>st</sup>	5.778±0.057 <sup>sy</sup>	6.113±0.065 <sup>xt</sup>	***
	60:20:20/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	4.255±0.115 <sup>P</sup>	4.568±0.115 <sup>NP</sup>	4.778±0.115 <sup>st</sup>	5.113±0.065 <sup>st</sup>	5.447±0.173 <sup>sy</sup>	5.863±0.057 <sup>xt</sup>	***
	70:30:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	4.113±0.065 <sup>st</sup>	4.255±0.115 <sup>ca</sup>	4.447±0.115 <sup>st</sup>	5.041±0.023 <sup>sy</sup>	5.556±0.115 <sup>st</sup>	5.778±0.115 <sup>st</sup>	***
	60:40:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	4.041±0.023 <sup>st</sup>	4.113±0.057 <sup>ca</sup>	4.397±0.115 <sup>st</sup>	4.863±0.057 <sup>st</sup>	5.397±0.173 <sup>st</sup>	5.698±0.173 <sup>st</sup>	***
	p-values	NS	**	**	**	*	**	
<i>Pseudomonas</i> sp.	Air	6.079±0.115 <sup>ay</sup>	6.23±0.132 <sup>st</sup>	6.397±0.173 <sup>st</sup>	6.447±0.173 <sup>st</sup>	6.414±0.115 <sup>st</sup>	5.707±0.115 <sup>ay</sup>	*
	80:20:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	5.431±0.115 <sup>by</sup>	5.591±0.173 <sup>by</sup>	5.707±0.115 <sup>by</sup>	5.903±0.115 <sup>ba</sup>	5.812±0.057 <sup>by</sup>	4.903±0.173 <sup>ba</sup>	**
	60:20:20/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	5.342±0.115 <sup>ba</sup>	5.414±0.173 <sup>ca</sup>	5.591±0.173 <sup>ca</sup>	5.778±0.115 <sup>ba</sup>	5.662±0.115 <sup>ca</sup>	4.778±0.115 <sup>by</sup>	**
	70:30:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	5.255±0.115 <sup>ba</sup>	5.397±0.173 <sup>ca</sup>	5.477±0.115 <sup>ba</sup>	5.698±0.173 <sup>ba</sup>	5.414±0.115 <sup>ca</sup>	4.477±0.115 <sup>ca</sup>	**
	60:40:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	5.113±0.065 <sup>by</sup>	5.23±0.115 <sup>by</sup>	5.397±0.115 <sup>by</sup>	5.591±0.173 <sup>ba</sup>	5.278±0.115 <sup>ca</sup>	4.278±0.115 <sup>ca</sup>	***
	p-values	**	**	**	*	***	***	
Coliforms	Air	4.322±0.173 <sup>ay</sup>	5.113±0.065 <sup>st</sup>	5.146±0.057 <sup>st</sup>	4.929±0.173 <sup>st</sup>	4.397±0.173 <sup>sy</sup>	3.954±0.115 <sup>st</sup>	***
	80:20:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	3.602±0.173 <sup>ba</sup>	3.954±0.173 <sup>ca</sup>	4.653±0.115 <sup>st</sup>	4.477±0.173 <sup>by</sup>	4.176±0.057 <sup>sy</sup>	3.778±0.057 <sup>ay</sup>	**
	60:20:20/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	3.477±0.173 <sup>ba</sup>	3.602±0.173 <sup>ca</sup>	4.568±0.173 <sup>ca</sup>	4.397±0.173 <sup>ba</sup>	4.079±0.045 <sup>by</sup>	3.602±0.173 <sup>ay</sup>	**
	70:30:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	3.255±0.115 <sup>st</sup>	3.568±0.115 <sup>ca</sup>	4.176±0.057 <sup>ca</sup>	4.079±0.045 <sup>ba</sup>	3.832±0.057 <sup>by</sup>	3.477±0.230 <sup>ay</sup>	**
	60:40:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	3.176±0.057 <sup>by</sup>	3.414±0.173 <sup>ca</sup>	3.954±0.173 <sup>ca</sup>	4.079±0.045 <sup>ba</sup>	3.778±0.115 <sup>ca</sup>	3.00±0.057 <sup>ba</sup>	***
	p-values	**	***	**	**	**	**	
Yeast and mould	Air	4.844±0.057 <sup>ay</sup>	5.051±0.029 <sup>ay</sup>	5.112±0.057 <sup>ay</sup>	5.19±0.057 <sup>st</sup>	4.724±0.115 <sup>st</sup>	4.653±0.173 <sup>st</sup>	**
	80:20:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	4.477±0.173	4.653±0.173 <sup>b</sup>	4.806±0.057 <sup>ab</sup>	4.903±0.173 <sup>b</sup>	4.653±0.115	4.414±0.173	NS
	60:20:20/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	4.414±0.115	4.531±0.115 <sup>b</sup>	4.748±0.115 <sup>b</sup>	4.806±0.057 <sup>b</sup>	4.505±0.173	4.301±0.173	NS
	70:30:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	4.301±0.173	4.414±0.173 <sup>b</sup>	4.531±0.173 <sup>b</sup>	4.698±0.115 <sup>bc</sup>	4.342±0.173	4.255±0.115	NS
	60:40:0/O <sub>2</sub> :CO <sub>2</sub> :N <sub>2</sub>	4.255±0.057	4.301±0.057 <sup>b</sup>	4.477±0.115 <sup>b</sup>	4.414±0.115 <sup>c</sup>	4.301±0.057	4.113±0.057	NS
	p-values	NS	*	*	**	NS	NS	

<sup>as</sup>Means within a column with different letters are significantly different (p<0.05); <sup>ay</sup>Means within a row with different letters are significantly different (p<0.05); \*p<0.05. \*\*p<0.01. \*\*\*p<0.001. NS: Not Significant

than other packaging conditions (Esmer *et al.*, 2011; Temelli *et al.*, 2011). Kennedy *et al.* (2004) found that LAB counts increased throughout the storage period with different MAP combinations but slightly higher LAB counts were found in high oxygen packaging. Koutsoumanis *et al.* (2008) observed that until the 8th day of storage, LAB counts increased and then started to decrease in samples packaged with Low Permeability Films (LPF) where the produced carbon dioxide is maintained in the headspace. Ercolini *et al.* (2006) found lower counts for MA-packaged meat samples containing different amounts of O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> combinations with respect to air packaged samples. Esmer *et al.* (2011) stated that the lowest LAB counts were obtained in 50:50:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> and 50:30:20/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> gas combinations packaged minced beef samples with an increase until the 7th day of storage and then started to decrease till the end of storage whereas a slight increase for 30:70:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> and 30:30:40/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub>

combinations and a higher increase for 70:30:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> combination were remarkable till the end of the storage period. However, in the present study, a sustained increase was observed in LAB counts in both packaging conditions. Temelli *et al.* (2011) indicated that LAB counts of Inegol kofte stored under both aerobic and MAP conditions tended to increase but did not exceed the microbial spoilage limit of 7 log CFU g<sup>-1</sup> parallel to the present study.

Packaging under modified atmosphere conditions restricted and delayed the growth of *Pseudomonas* sp. during whole storage time. *Pseudomonas* counts increased both in aerobic and MAP conditions until the 8th day of storage and then started to decrease till the end of storage period with an approximately 1-1.5 log CFU g<sup>-1</sup> difference in the counts of bacteria for air and MA-packages. *Pseudomonas* growth correlated to the O<sub>2</sub> concentration in the package atmospheres while the increase was statistically different (p<0.05) between

MA-packages. *Pseudomonas* growth was restricted when the CO<sub>2</sub> concentration increased in the package and the lowest counts were obtained in 60:40:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> atmosphere.

Under aerobic conditions, predominant spoilage organisms are typically *Pseudomonas* (Gill, 1996). Esmer *et al.* (2011) and Temelli *et al.* (2011) determined that viable counts in aerobic packaging were higher than those of other MA-packaging and added that *Pseudomonas* sp. particularly were the dominant flora followed by lactic acid bacteria, Enterobacteriaceae and *B. thermosphacta* during the storage time while modified atmosphere packaging delayed and restricted the growth of these microorganisms depending on the gas composition used. Kennedy *et al.* (2004) determined that 60:40:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> was the most effective gas composition in inhibiting growth in agreement with the present study. In O<sub>2</sub> depleted atmospheres of N<sub>2</sub> or CO<sub>2</sub>, the anaerobic conditions prevent the growth of *Pseudomonas* (Gill, 1996). Ercolini *et al.* (2006), Koutsoumanis *et al.* (2008) and Temelli *et al.* (2011) also expressed that packaging with high CO<sub>2</sub> combination lowered the growth rate of *Pseudomonas* sp.

Coliforms showed steady growth during the 6th day of storage and reached to an average value of 4-5 log CFU g<sup>-1</sup> and then started to decrease till the end of storage depending on CO<sub>2</sub> concentration used. The higher inhibitory effect was recorded in 60:40:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> atmosphere packaged meatballs with a final count of 3 log CFU g<sup>-1</sup> while counts in aerobic packaging were almost 4 log CFU g<sup>-1</sup>. Temelli *et al.* (2011) stated that coliform bacteria counts in air packaged Inegol kofte markedly increased up to 5.57 log CFU g<sup>-1</sup> until 15 days of storage whereas counts for MAP remained almost the same during storage.

Yeast and moulds showed sustained growth during 10 days of storage and decreased slightly at the end of storage. Significant differences (p<0.05) were observed between air and MA-packages during 8 days of storage whereas no differences were determined within the same MA-packages during entire storage (p>0.05). Aerobic packaging of meatballs varied with the prolonged storage time (p<0.01) and a significant reduction in the growth rates of yeast and moulds were observed with increasing concentration of carbon dioxide. Similarly, Temelli *et al.* (2011) indicated that a slight increase was observed during the first 10 days of storage for yeast and mould counts, following a tendency to decline down to 5 log CFU g<sup>-1</sup> until the end of the storage period for both packaging conditions (30:40:30/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> and 60:30:10/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> atmospheres).

## CONCLUSION

The quality and shelf-life of Tekirdag Kofte packaged by various gas compositions of modified atmosphere were improved compared to aerobic packaging. MAP applications limited the growth of microorganisms and appeared as a substantial choice for the refrigerated storage of meatballs. The microbial growth in meatballs was delayed due to usage of various concentration of CO<sub>2</sub> and shelf-life was increased by 6-8 days. High CO<sub>2</sub> concentration performed better than increasing level of O<sub>2</sub> contents package environment thus the more effective inhibition on microbial growth was achieved by exposing longer to high CO<sub>2</sub> concentration. Additionally, the high CO<sub>2</sub> contents prevented increased lipid oxidation and tented to extend the shelf-life of the product by >10 days. In fact, the quality properties of meatballs during storage in the markets may be enhanced by MAP and longer display life may occur for the product without having to pay any attention.

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