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INFLUENCE OF PACKAGING CONDITIONS ON SOME MICROBIAL PROPERTIES OF MINCED BEEF MEAT AT 4°C STORAGE

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Abstract

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In this study, the effect of initial head-spaces of atmospheric air, vacuum packaging and modified atmospheres packaging (MAP) containing 70% CO_2/30% O_2 (MAP1); 50% O_2/50% CO_2 (MAP2); 30% CO_2/70% O_2 (MAP3); 50% O_2/30% CO_2/20% N_2 (MAP4); 30% O_2/ 30% CO_2 /40% N_2 (MAP5) on some microbiological quality of minced beef meat stored at 4 °C were investigated with pH changes in time intervals (1, 3, 5, 7, 9, 11 and 14 d). At the results, total viable counts were different significantly ($P < 0.01$) for packaged with MAP2. Psychrotrophs, yeasts and molds counts were smaller for packaged with MAP1 than the others, packaging with MAP5 and MAP4 were also different significantly ($P < 0.01$) from the other samples for inhibiting coliform counts. Vacuum packaging was not different significantly ($P > 0.05$) for total viable counts and psychrotrophs counts.

Key words: minced meat; beef meat; packaging; modified atmosphere; vacuum package
Abbreviations: MAP- Modified Atmosphere Packaging; VP- Vacuum Packaging; AP- Air Packaging; CFU- Colony Forming Unit; G/P- Gas/Product Ratio; RH%- Relative Humidity; PCA- Plate Count Agar; TVC- Total Viable Count; PET- Polyethylene Terephtalate; EVOH- Ethylene Vinyl Alcohol; LDPE- Low Density Polyethylene; OPP- Oriented Poly Propylene; PA/ PE- Polyamide/Polyethylene

Introduction

Minced beef meat has very high economic value because of its nutritive quality and practically usage in almost every type of meals. But fresh meat and meat products are highly perishable foods. The acceptation limit for total aerobic count varies from 5×10^6, 1×10^7 to even 1×10^8 CFU/g in meats at various companies and this case causes short shelf life of traditionally produced minced meat products about 1–3 d (Velzen et al., 2008). High initial numbers of different groups of minced beef can be attributed to the grinding process, which contributes to the increase of total viable counts of meat including yeasts and other microorganisms (Skandamis and Nychas, 2001). Extrinsic parameters (e.g., temperature and packaging atmosphere) can affect meat spoilage. Microbial spoilage leads to

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the development of off-odors and slime formation which make the product undesirable for human consumption (Djenane et al., 2005; Koutsoumanis et al., 2006; Ercolini et al., 2006). Microbial flora of spoiled meat is very complex and this makes the spoilage very difficult to prevent and it is a limiting factor which determines the shelf-life of meat. The initial micro flora of meat is mesophilic and after carcass evisceration microbial load reaches to 10^2–10^4 bacteria per 1 cm^2. This load can vary with storage conditions (Saucier et al., 2000). It has been established that microbial levels of 6–7 log CFU/g are critical spoilage of meat (Djenane et al., 2005; Berruga et al., 2005).

In the last years a new packaging method as modified atmosphere packaging (MAP) using gas mixtures containing variable O_2, CO_2 and N_2 concentrations are widely used in order to inhibit the different spoilage bacteria associated with low storage temperatures. It was shown that the CO_2 affects microbial growth by extending the lag phase and increasing the generation time. Modified atmosphere packaging with CO_2 as the preservative gas is very popular for fresh meats (Berruga et al., 2005; Aksu and Kaya, 2005; Zhang and Sundar, 2005; Nicolalde et al., 2006; Stetzer et al., 2007).

Although, most of the studies have been performed for modified atmosphere packaging of fresh meat to date, there isn't any covered research about minced or ground beef meat (Skandamis and Nychas, 2002; Ercolini et al., 2006; Soldatou et al., 2009). However, minced meat is more sensitive to microbial spoilage because of its porous structure and due to the grounding process. The effects of MAP technology with different gas combinations on the spoilage microorganisms in minced beef are not so definite. For this reason, the aim of the study was to compare the micro floral patterns of minced beef meat packaged under modified atmosphere or vacuum followed by air packaging systems during +4 °C storage conditions.

Materials and Methods

Minced meat and packaging

Meat from *pectoralis major* and *minor* muscles of beef carcasses from 2-year-old cattle, after 48 h from postmortem was purchased from a local establishment in Bandirma, Turkey. Meat was trimmed of all exterior fat and connective tissue and minced in a sterilized mincer through 3 mm size. All packages of minced meat were portioned (250 ± 0.1) g each. Modified-atmosphere packaging was performed by using Multivac Model R-230 (Multivac, Sepp Haggenmüller GmbH and Co., Wolfertschwenden, Germany) packaging machine. Minced meat samples were packed in expanded "polyethylene terephtalate (PET)/ ethylene vinyl alcohol (EVOH)/low density polyethylene (LDPE)" trays of 750 µm thickness. A film of "oriented poly propylene (OPP)/low density polyethylene (LDPE)/ethylene vinyl alcohol (EVOH)/low density polyethylene (LDPE)" of 77 µm thickness and having an oxygen transmission rate of 3.0 cm^{3}/m^2 during 24h (23°C, 0% Relative Humidity-RH), carbondioxide transmission rate of 10 cm^{3}/m^2 during 24h (23°C, 0% RH) and water vapor transmission rate of 3 g/m^2 during 24 h (38°C, 0% RH) with antifog property was used as a sealing top.

Vacuum packaged was performed by using VC 999/K12NA (Verpackungssysteme A G., Herisau, Switzerland) packaging machine and minced meat samples were packed in Polyamide/Polyethylene (PA/PE) films.

The minced meat samples were packed under ambient air conditions (Control), vacuum (VP), 30% O_2/70% CO_2 (MAP1), 50% O_2/50% CO_2 (MAP2), 70% O_2/30% CO_2 (MAP3), 50% O_2/30% CO_2/20% N_2 (MAP4), 30% O_2/30% CO_2/40% N_2 (MAP5). The ratio between the volume of gas and weight of food product (G/P ratio) was 3:1 (v/w). All samples were stored in a refrigerator at a constant temperature (+4°C) for 2 weeks. O_2

and CO_2 concentrations in the package headspace were monitored periodically using a digital PBI Dansensor Check Pointer O_2/CO_2 (Ringsted, Denmark) analyzer.

Chemical analysis

All pH values were determined with Hanna Instruments (HI 221) Microprocessor pH meter (Hanna Instruments Inc., Woonsocket, Rhode-Island, US). Fat and moisture contents of minced meats determined according to AOAC (1980) and ISO (1997), respectively.

Microbial Analysis

The samples for analysis were taken from the control and treatment groups on 1., 3., 5., 7., 9., 11. and 14. days of storage. 10 g of each sample was diluted in 90 ml physiological saline solution (0.85% (w/v) NaCl+0.1% peptone (w/v)) and homogenized in a stomacher for 1 min. A serial 10-fold dilution series was prepared, total viable count (TVC) was enumerated on Plate Count Agar (PCA, Merck, Darmstadt, Germany) at 32°C after 48h (Berruga et al., 2005), psychrotrophs were counted on PCA (Merck, Darmstadt, Germany) at 7°C after 10 days (Soldatou et al., 2009), coliform bacteria was determined by Lauryl Sulfate Tryptose Broth (LST, Oxoid, Basingstoke, UK) at 35°C after 48 h (Bolling et al., 2002), moulds and yeasts were determined on Rose Bengal Chloramphenicol (RBC, Oxoid, Basingstone, UK) Agar at 25–28°C after 4–5 days (Soldatou et al., 2009). Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

Statistical analysis

Experiments were twice replicated on different occasions with different minced meat samples. Analysis were run in triplicate for each replicate ($N = 3 \times 2$). The data were statistically analyzed by ANOVA one way analysis by using SPSS 10.0 (Microsoft Corp. Chicago, Illinois, US). The Duncan's post hoc test was employed to identify the different groups at a significance level of 0.05 (Ozdamar, 2004).

Results and Discussion

Moisture content and fat content ± standard deviation of minced meats were determined as (72.3 ± 0.2) % and (1.12 ± 0.4) %, respectively.

The results of the present study showed that the tested combinations of modified atmosphere packaging for the minced beef meat could be used for extending the shelf-life of meat according to the results of studied microbial populations (Figure 1). The results of the viable counts showed that the spoilage groups had different trends depending on the packaging conditions.

Total viable count (TVC) of microorganisms

TVC counts of all MAP packages were significantly ($P<0.05$) different from the control groups. TVC increased significantly with time and reached over the 7 log CFU/g after 7 d for control and VP samples. TVC counts in VP were found insignificantly ($P > 0.05$) from the control samples. As it is seen from Figure 1a, the highest values were obtained for control samples, VP samples and for MAP groups having lower CO_2 and higher O_2 concentrations respectively. It was 8.36 log CFU/g for control samples and 7.01 log CFU/g for VP samples at 9. day of storage. The minimum TVC counts were obtained significantly for MAP2 ($P < 0.01$), MAP4 ($P < 0.05$) and MAP1 ($P < 0.05$) combinations and they were 4.05, 4.30 and 5.12 log CFU/g after 14. day of storage respectively whereas it was 6.92 log CFU/g for MAP3 and 6.62 log CFU/g for MAP5 and all MAP combinations were still below the spoilage limit of red meat. Higher concentrations of CO_2 gave better results for the inhibition of total viable counts in minced beef meat and VP did decreased the TVC count compared with control group but its effectiveness was lower than MAP groups. It is known that CO_2 has a great inhibitory effect on common spoilage microorganisms (Chen et al., 2007; Stamatis and Arkoudelos, 2007; Pettersen et al., 2004; Pastoriza et al., 1996a; Pastoriza et al., 1996b) by extending the lag phase and increasing the generation time

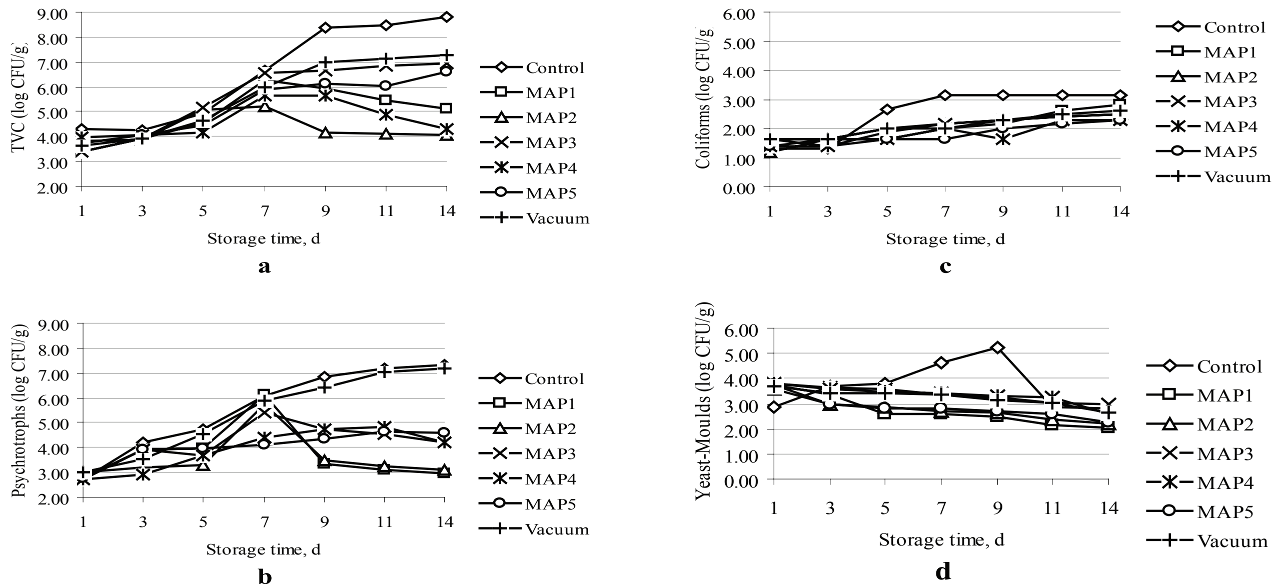

Fig. 1. Total viable counts (a), psychrotrophs (b), coliforms (c) and yeasts-moulds (d) counts of map, vacuum packaged and air packaged minced beef meat during +4ºC storage

of sensitive organisms (Stiles, 1991). In agreement with our results, Goulas (2008), Pastoriza et al. (1996a) also expressed that the presence of high level of CO_2 in the MAP system can inhibit bacteriological growth while the VP system allow to growth of facultative microorganisms. Fernandez-Lopez et al. (2008) found that 80% CO_2 in headspace significantly inhibited the aerobic plate counts of ostrich steaks and Patsias et al. (2006) determined that 90% CO_2 and 60% CO_2 gases in headspace were effective than 30% CO_2 and (Air Packaging) AP in inhibition of aerobic plate counts in precooked chicken. Chen et al. (2007) found that MAP application with 80% CO_2 suppressed the growth of aerobic bacteria in red claw crayfish when compared with AP and VP. The results of these studies were in accordance with our results related to the effect of higher CO_2 concentration in head space and VP application in minced beef meat.

Psychrotrophs

Psychrotrophs as the spoilage microorganisms that can grow at 7ºC within 7 to 10 d in meats (Ho et al., 2003). Psychrotrophs increased until the end of storage and their count was above the spoilage limit for VP and control samples which was 7.17 log CFU/g and 7.34 log CFU/g respectively at 14. day of storage (Figure 1b). Differences of psychrotrophs were not found significant between VP samples and control groups ($P > 0.05$). But MAP applications were more effective in inhibiting psychrotrophs than VP significantly ($P < 0.05$). The number of psychrotrophs increased until 7. day of storage and then continuously decreased until the end of storage for MAP1 and MAP2 combinations. But for MAP3, MAP4 and MAP5 they slightly increased until the end of storage. Especially a sharp decrease in psychrotroph numbers occurred after the 7. day in MAP1 and MAP 2. The numbers of the psychrotrophs at 14. day of storage for MAP1 and MAP2 were 2.95 log CFU/g and 3.11 log CFU/g respectively and were lower than the other combinations whereas the number of the psychrotrophs were 4.21, 4.20 and 4.59 log CFU/g at 14.day of storage for MAP3, MAP4 and MAP5 respectively. Our results are in agreement with Fernandez-Lopez et al. (2008) that

Table 1
Concentrations of CO_2, O_2 and N_2 in headspace of minced beef meat stored at 4°C

Storage time, days	Headspace combinations													
	Control		MAP1		MAP2		MAP3		MAP4			MAP5		
	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	N_2 (%)	O_2 (%)	CO_2 (%)	N_2 (%)
1	18.9 ± 0.2*	1.0 ± 0.1	37.7 ± 0.1	58.3 ± 0.8	60.8 ± 0.5	33.4 ± 0.1	73.0 ± 0.2	16.4 ± 0.7	56.2 ± 0.1*	18.8 ± 0.1	25.0 ± 0.4	34.9 ± 0.2	17.2 ± 0.4	47.9 ± 0.1
3	19.6 ± 0.4	0.9 ± 0.5	29.7 ± 0.9	65.7 ± 0.7	58.4 ± 0.7	35.5 ± 0.1	70.9 ± 0.6	19.4 ± 0.5	56.0 ± 0.3	18.6 ± 0.5	25.4 ± 0.5	32.3 ± 0.4	17.4 ± 0.3	50.3 ± 0.3
5	18.9 ± 0.1	5.3 ± 0.6	24.0 ± 0.5	74.3 ± 0.5	59.5 ± 0.5	34.7 ± 0.5	72.2 ± 0.1	19.7 ± 0.5	52.2 ± 0.3	18.7 ± 0.7	29.1 ± 0.1	31.5 ± 0.5	17.7 ± 0.1	50.8 ± 0.9
7	18.3 ± 0.9	6.2 ± 0.2	37.0 ± 0.4	55.8 ± 0.1	59.3 ± 0.1	34.5 ± 0.1	67.7 ± 0.3	19.9 ± 0.7	53.7 ± 0.2	24.9 ± 0.2	21.4 ± 0.1	33.3 ± 0.1	18.5 ± 0.4	48.2 ± 0.2
9	17.0 ± 0.1	8.1 ± 0.2	26.4 ± 0.1	69.7 ± 0.6	53.1 ± 0.1	41.7 ± 0.4	70.3 ± 0.5	19.6 ± 0.3	47.3 ± 0.6	25.6 ± 0.4	27.1 ± 0.5	33.1 ± 0.1	21.7 ± 0.5	45.2 ± 0.3
11	9.4 ± 0.4	10.6 ± 0.2	26.7 ± 0.5	67.8 ± 0.5	59.2 ± 0.2	35.8 ± 0.2	68.3 ± 0.3	19.5 ± 0.1	48.5 ± 0.2	27.6 ± 0.3	23.9 ± 0.8	29.7 ± 0.5	29.5 ± 0.2	40.8 ± 0.5
14	5.2 ± 0.5	12.5 ± 0.3	32.7 ± 0.2	68.5 ± 0.2	58.7 ± 0.4	34.4 ± 0.1	69.7 ± 0.2	19.4 ± 0.2	49.4 ± 0.8	26.3 ± 0.5	23.3 ± 0.5	27.4 ± 0.6	33.3 ± 0.4	39.3 ± 0.7

*Values represent the mean of 6 determinations ($N = 3×2$) ± standard deviation

MAP1: 30% O_2–70% CO_2; **MAP2**: 50% O_2–50% CO_2; **MAP3**: 70% O_2–30% CO_2 ;**MAP4**: 50% O_2-30% CO_2-20% N_2; **MAP5**: 30% O_2-30% CO_2-40% N_2

they found highest counts in AP samples, lowest counts in MAP of 80%CO_2/20%N_2 application and intermediate counts for VP samples. Although all MAP combinations were significantly effective in inhibition of psychrotrophic counts compared to VP and control samples ($P < 0.01$ and $P < 0.05$),

Table 2
Minced meat pH values during storage at 4°C

Storage time, days	Control	Vacum	MAP1	MAP2	MAP3	MAP4	MAP5
	pH	pH	pH	pH	pH	pH	pH
1	5.85 ± 0.5*	6.04 ± 0.6	5.95 ± 0.4	5.82 ± 0.5	6.01 ± 0.7	5.92 ± 0.5	5.89 ± 0.4
3	5.82 ± 0.4	5.74 ± 0.3	5.60 ± 0.6	5.75 ± 0.5	5.79 ± 0.5	5.92 ± 0.5	5.89 ± 0.6
5	6.04 ± 0.5	5.74 ± 0.1	5.85 ± 0.8	5.77 ± 0.3	5.87 ± 0.6	5.95 ± 0.8	5.92 ± 0.5
7	6.18 ± 0.3	5.84 ± 0.7	5.90 ± 0.7	5.86 ± 0.5	5.85 ± 0.7	5.97 ± 0.6	5.95 ± 0.5
9	6.22 ± 0.1	5.68 ± 0.5	5.52 ± 0.3	5.64 ± 0.6	5.75 ± 0.5	5.83 ± 0.7	5.89 ± 0.8
11	6.70 ± 0.2	5.62 ± 0.3	5.48 ± 0.6	5.62 ± 0.5	5.68 ± 0.2	5.63 ± 0.3	5.82 ± 0.6
14	7.00 ± 0.4	5.45 ± 0.2	5.43 ± 0.4	5.54 ± 0.3	5.60 ± 0.5	5.78 ± 0.5	5.91 ± 0.4

*Values represent the mean of 6 determinations $N = (3×2)$ ± standard deviation

MAP1: 30% O_2–70% CO_2; MAP2: 50% O_2–50% CO_2; MAP3: 70% O_2–30% CO_2 ;
MAP4: 50% O_2-30% CO_2-20% N_2; MAP5: 30% O_2-30% CO_2-40% N_2

the effectiveness of MAP1 and MAP2 was higher statistically ($P < 0.01$) than the other MAP combinations because of their higher CO_2 concentration as 70% and 50%. The effectiveness of low CO_2 concentration was shown by Calhoun et al. (1999) and Huang et al. (2005) that they found 80%O_2/20%CO_2 effectively inhibited the slime producing psychrotropic Gram-negative bacteria usually responsible for spoilage of refrigerated meat. And also Ho et al. (2003) stated that psychrotrophic bacteria inhibited by high level of CO_2 because the enzymatic decarboxylation metabolism of Gram-negative may be blocked.

Coliforms

Initial counts of coliforms (< 2.0 log CFU/g) indicate adequate sanitary production of minced beef meat. Coliforms show post contaminations especially from environment and equipments to the minced meat. The coliform counts of both MAP4 and MAP5 were statistically lower and different that the other samples ($P < 0.01$). Coliform counts for MAP3 and VP samples ($P < 0.05$) and coliforms in MAP2 ($P < 0.01$) were different statistically from the control samples. The number of coliforms slightly increased for all samples until the end of storage and was below the accepted limit (3 log CFU/g) except control samples (Figure 1c). The lowest numbers were obtained for MAP4 and MAP5 combinations as 2.30 log CFU/g for both at 14. day of storage whereas it was 3.15 log CFU/g for control samples at 14.day of storage. There are few papers discussing the effect of MAP gas composition on coliform bacteria counts. Chen et al. (2007) found 80%CO_2/10 %O_2/10%N_2 suppressed coliforms when compared with aerobic packaging and VP of red claw cray fish stored in 2 ºC for 14 d period. Also Pettersen et al. (2004) stated that high concentrations of CO_2 reduced the coliform counts in chicken breast fillets.

Yeasts and Moulds

Yeasts and moulds counts decreased through the storage period for all samples except control samples. This decrease was more evident and significantly ($P < 0.01$) at the 5. day especially for MAP1 than other samples (Figure 1d). At the end of storage period the lowest yeast and mold counts were obtained and they were different significantly for MAP1 ($P < 0.01$), MAP2 ($P < 0.01$) and MAP5 ($P < 0.01$) respectively and the highest counts were obtained for MAP3, MAP4 and VP and they were not different from control samples significantly ($P > 0.05$). This results show us increase in carbon dioxide suppresses the growth of mold and yeast as it was expressed by Stiles (1991) and it is thought that the amount of oxygen may be accelerated yeast and mold growth. Dermiki et al. (2008) reported that high CO_2 concentrations were very effective for the inhibition of moulds and yeasts. Also Skandamis and Nychas (2001) showed 100%CO_2 and 40%CO_2/ 30%N_2/ 30%O_2 had an inhibitory effects on yeasts with compared air packaging of minced meats.

The headspaces

The headspace data for all MAP samples are given in Table 1. For MAP samples, the O_2 and N_2 concentrations decreased and the CO_2 concentration increased during the storage.

Such a decrease in O_2 concentration can be attributed to the growth of aerobic bacteria and microbial respiration, which utilize O_2 and produce CO_2 and accelerated the spoilage. Goulas (2008), Koutsoumanis et al. (2008) also observed increasing in CO_2 concentrations in MAP packaged products depend on aerobic bacteria growth. N_2 has minimal effects on metabolic reactions in the meat, being lowly soluble in water and lipid, but anoxic atmospheres created by the use of N_2 and/or other gases will select for anaerobic, aero tolerant microorganisms.

There must be an optimal ratio of headspace to meat volume for fresh beef and the headspace, CO_2 varies with different concentrations of CO_2 in the gaseous mixture and with storage temperatures (McMillin, 2008).

pH values

The pH values show differences due to the packaging conditions (Table 2). The pH values decreased during storage time for all storage conditions. Initial pH values slowly increased in air packaged samples, but at a faster rate on further storage when some of the spoilage bacteria e.i. lactic acid bacteria reached their maximal counts. The pH of meat can be affected by many factors; however, growth of lactic acid bacteria resulting in lactic acid production is the major factor in pH decrease in packaged meats (Fernandez-Lopez et al., 2008; Gok et al., 2008; Patsias et al., 2006). It was stated that dissolving of carbon dioxide in the meat tissues causes pH decreasing in MAP packaged minced beef samples (Stiles, 1991). The pH values of the control and MAP5 samples showed increasing after 14 days period. Proteolysis may have produced nitrogenous compounds which may have caused increase in the pH values (Aksu and Kaya, 2005).

Conclusions

The most effective gas combinations are MAP1 and MAP2 in inhibiting psychrotrophs, mould-yeast growths and TVC in minced beef meat samples although all gas combinations inhibited their growths. MAP application was more effective in retarding the rate of TVC, psychrotrophs, coliforms, yeast and mold growths on minced beef meat during storage compared with vacuum and air packages. Control groups of minced meat spoiled after 7. day but in the other tested package's shelf-life extended up to the 14. days. Especially the microorganism numbers of TVC in MAP2 packages were lower than the other groups, but MAP1 package gave better results for psychrotrophs and yeast-mold counts. Coliform growth was inhibited in MAP5 and MAP4 packages effectively. These results shows that higher carbon dioxide concentrations in the packages are more effective for TVC, psychrotrophs and yeast-mould counts and extend the shelf-life more in MAP of minced beef meat whereas it doesn't affect the coliform count. High CO_2 concentrations with MAP technology can reduce microbial growth and therefore may extend the shelf-life of minced beef. In the future researches, oxygen and CO_2 concentration combinations in the packages should be decided according to the sensory properties especially bright red appearance of minced beef meat, also.

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