ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(4): 658-668
doi: 10.25083/rbl/24.4/658.668

Received for publication, October, 2, 2017
Accepted, April, 3, 2018

Original paper

Spoilage-related bacteria of pork and beef minced meat under vacuum and modified atmosphere

JASNA DJORDJEVIĆ[1]*, MARIJA BOŠKOVIĆ[1], IVANA BRANKOVIĆ LAZIĆ[2], VESNA DJORDJEVIĆ[2], TATJANA BALTIĆ[2], MILICA LAUDANOVIĆ[1], MILAN Ž. BALTIĆ[1]

[1]University of Belgrade, Department of Food Hygiene and Technology, Belgrade, Serbia
[2]Institute of Meat Hygiene and Technology, Belgrade, Serbia

Abstract Changes of spoilage-related microbiota of pork and beef minced meat during 12 days at 3°C under vacuum (VP) and modified atmosphere (MAP) with initial headspace containing 20%O2/50%CO2/30%N2-MAP1 and 20%O2/30%CO2/50%N2-MAP2 were studied. Samples were analysed for total viable count (TVC), Enterobacteriaceae count and lactic acid bacteria (LAB) count. TVC and LAB count increased during storage, with significantly ($P<0.01$) higher TVC in VP (except 9th and 6th day for TVC and LAB count, respectively), while Enterobacteriaceae count decreased with significantly lower value ($P<0.01$ and $P<0.05$) in MAP1, compared to VP. MAP samples were more acceptable in terms of color and odor than VP.

Keywords Beef, pork, modified atmosphere, vacuum, microbiological status, headspace composition.

To cite this article: DJORDJEVIĆ J, BOŠKOVIĆ M, LAZIĆ IB, DJORDJEVIĆ V, BALTIĆ T, LAUDANOVIĆ M, BALTIĆ MZ. Spoilage-related bacteria of pork and beef minced meat under vacuum and modified atmosphere. *Rom Biotechnol Lett*. 2019; 24(4): 658-668. DOI: 10.25083/rbl/24.4/658.668

*Corresponding author: JASNA DJORDJEVIĆ, University of Belgrade, Department of Food Hygiene and Technology, Belgrade, Serbia
E-mail: jasna.djordjevic@vet.bg.ac.rs

Introduction

Products (e.g. hamburgers, cevapcici, sausages) containing minced pork and beef are consumed widely in Europe (KOPPEL & al [1]). In the Republic of Serbia, as well as in other Balkan and some Mediterranean countries, minced meat is an inseparable part of traditional dishes (e.g. moussaka), which is why mince meat safety and quality is an important issue. Due to the mincing process, which disrupts fibrillar structures (myofibrils and connective tissue), meat gets a more porous structure while tissue fluids are released and minced meat becomes a highly nutritious medium for bacterial growth (IRKIN & al [2]). Favouring microbial growth to the unacceptability limits significantly contributes to the meat spoilage, making it unsuitable for human consumption (DOULGERAKI & al [3]). Although microorganisms play an important role in the development of meat spoilage, final assessment of the changes during storage is based on sensory evaluation. During storage, meat develops a characteristic odour due to degradation of different substances, especially carbohydrates, by the microorganisms and the release of various volatile (aromatic) compounds such as carbonyl and sulphuric acids, various organic acids, alcohols or ammonia (ELLIS & GOODACRE [4]; ERCOLINI & al [5]). Because of this, minced meat is considered to be a very perishable food, which should be packaged and chilled, during storage and transport, to the temperature up to 2°C (Regulation (EC) 853/2004) (EFSA [6]).

It was established that storage temperature, as well as different applied treatments, such as, various chemical preservatives, vacuum packaging (VP) or modified atmosphere packaging (MAP) influence the spoilage-related microbiota, and consequently the spoilage of meat (NYCHAS & al [7]; ERCOLINI & al [8]; PENNACCHIA & al [9]). Different new packaging systems, in order to delay the spoilage and extend the shelf life of meat and meat products were developed in the last decade. MAP which contains a mixture of different gas combinations (oxygen, carbon dioxide, nitrogen, carbon monoxide, etc.) is very popular for fresh meats and frequently used because it suppresses bacterial spoilage. Depending on the type of meat or meat product, the gases in the packaging can be used individually or can be combined in different proportions in order to achieve desirable effect. The most frequently used gasses in MAP are carbon dioxide, which possesses strong antimicrobial activity, especially on gram-negative bacteria by extending the lag phase, and thus the extension of generation time of the bacterial cell life cycle; oxygen, which inhibits the growth of anaerobic bacteria and affects the meat colour (meat retains a red colour) and nitrogen, with a role in preventing the fat oxidation and package collapse (SKANDAMIS & NYCHAS [10]; BERRUGA & al [11]; ZHANG & SUNDAR [12]; NICOLALDE & al [13]; STETZER & al [14]; SINGH & al [15]).

Although, there are numerous studies on the microbial status of meat during storage, studies are generally done with different meat pieces and less with minced meat, especially mixed pork and beef. Thus, the aim of the study was to compare the changes in spoilage-related microbiota of minced pork and beef during refrigerated storage packaged under vacuum and modified atmosphere.

Materials and Methods

Meat sample preparation, packaging and storage conditions

Pork and beef used in the experiment (muscles from pork and beef leg), 48 h post-slaughter, were provided in the local slaughterhouse. External fat and connective tissue were removed and the meat was minced separately in a sterile grinder (4 mm hole diameter in the meat grinder plate), mixed in a ratio of 50:50 (pork: beef), and transported refrigerated at 3±1°C to the laboratory within an hour.

Meat was divided into three equal parts and packaged (six sample packages per group, per day of examination) in vacuum packaged and modified atmosphere packages containing 20%O_2/50%CO_2/30%N_2- MAP1 20%O_2/30% CO_2/50%N_2- MAP2, with ratio between the volume of gas and weight of food product (G/P ratio) of 3:1 (v/w). A Variovac machine (Variovac Primus, Zarrentin, Germany) was used for sample packaging. Samples were packaged in an OPA/EVOH/PE foil (oriented polyamide/ethylene vinyl alcohol/polyethylene Dynopack, POLIMOON, Kristiansand, Norway), with low gas permeability (O_2 - 3.2 cm^3/m^2/ day at 23°C, N_2 - 1 cm^3/m^2/day at 23°C, CO_2 - 14 cm^3/m^2/day at 23°C, water vapour - 15 g/m^2/day at 38°C). All minced meat samples weighed 100±5 g and were refrigerated at 3±1°C for 12 days.

Microbiological analyses

All meat samples were analysed for total viable count (TVC-mesophiles, 30°C), *Enterobacteriaceae* count and lactic acid bacteria count (LAB) immediately after packaging and on 3rd, 6th, 9th and 12th day of storage. For bacterial enumeration, approximately 10 g of meat were weighted out aseptically after package opening, transferred into sterile Stomacher bags and 90 ml of Buffered Peptone Water (BPW) (Merck, Germany) was added to each sample. Samples were homogenized in Stomacher blender (Stomacher 400 Circulator, Seward, UK) for 2 min. Serial decimal dilutions were prepared and 1ml of appropriately diluted samples was inoculated directly on the surface of the appropriate media for enumeration of the different bacteria. TVC-mesophiles were enumerated according to ISO 4833: 2003 [16], on Plate Count Agar (PCA, Merck, Germany) and incubated at 30°C for 72 h,

Enterobacteriaceae count according to ISO 21528-2:2004 [17], on Violet Red Bile Glucose Agar (VRBGA, Merck, Germany) after incubation at 37°C for 24 h, while LAB were enumerated according to ISO 15214:1998 [18], on MRS (Merck, Germany) following incubation at 30°C for 72 h. After incubation, plates were examined visually for typical colony types and morphological characteristics associated with each growth medium, a number of the colony were count and results were recorded as colony forming units per g (CFU/g).

pH measurement

The pH value was measured after 10 minutes at room temperature using hand-held pH meter Testo 205 (Testo AG, Lenzkirch, Germany) equipped with a penetrating glass electrode, calibrated with standard buffers of pH 4.0 and 7.0. The pH meter was rinsed with distilled water after every reading and re-calibrated after every fourth reading.

Sensory evaluation

Ten trained panellists, evaluated odor and color of the meat samples immediately after packaging and on the 3rd, 6th, 9th and 12th day of storage. Before evaluation, meat was kept at room temperature for 20 min. The samples were tested in a sensory laboratory designed according to SRPS ISO 8589: 2012 [19]. Sensory assessment, which included evaluation of the odor and color acceptability, was carried out using an assessment sheet with a scale ranging from 1 to 7 for each property.

Headspace

Measurement of headspace composition in minced meat packaging was done using a tester for gas composition, Oxybaby (GASETECHNIK WITT – Germany). The measurement range of the instrument is 0-100% by volume (vol) for oxygen (O_2) and carbon dioxide (CO_2). The nitrogen content is calculated as the difference from 100% when deducting the measured values of oxygen and carbon dioxide. The accuracy of the device is 0.1% for oxygen and carbon dioxide.

Statistical analysis

The experiment was conducted in a completely randomized design, six repetitions were carried out for each treatment and the treatments were arranged in a 3 x 5 factorial design (3 treatments, 5 ageing periods). Numbers of microorganism were transformed into logarithms (log). Statistical analyses of the results were conducted using the software GraphPad Prism version 6.00 (GraphPad Software, San Diego, California USA, www.graphpad.com). The results were expressed as the mean ± the standard error of the mean and reported in tables. The effects of different treatments during storage period were appraised by one-factor analysis of variance-ANOVA with Tukey's multiple comparison test at 95% confidence level (difference considered significant if $P<0.05$). Linear regression was used to establish the statistical relationship between the number of microorganisms in different packaging and ageing period.

Results and Discussion

Total viable count (TVC)

After slaughter, TVC on the carcass ranged between 2 and 4 log CFU/cm^2, which is explained by the fact that during the slaughter process, muscles (that are initially sterile) become contaminated with microorganisms from the carcass, as well as from the environment. The initial number of microorganisms also depends on the animal species used for slaughter and literature data shows that the number of bacteria generally is the highest on the pig carcasses (BORCH & al [20]; PLAZONIĆ & al [21]; DE FILIPPIS & al [22]). In addition, minced meat is especially susceptible because of the mincing process, during which meat becomes contaminated. The biggest proportions of the initial microbiota on fresh meat are mesophilic and psychrotrophic bacteria, and this latter group of bacteria is mainly responsible for meat spoilage. For these reasons, TVC is used as an important microbiological quantitative indicator of production process hygiene, and for safety evaluation, as well as a spoilage indicator of raw meat (JAY [23]; MOTARJEMI [24]; TAO & PENG [25]). TVC in minced meat higher than 7 log CFU/g is unsatisfactory from a hygienic point of view and indicates poor hygienic practice (Regulation (EC) 2073/2005 and 94/65/EEC) (EFSA [6]).

In this study, initial TVC in minced meat samples packaged in all types of packages was 4.59 log CFU/g which indicates good quality of the meat used (Table 1). From the start of experiment till the 12th day of storage, TVC increased and the highest increase was noted in the minced meat samples packaged in modified atmosphere on the 9th day of storage (8.47±0.014 log CFU/g in MAP1 and 8.47±0.054 log CFU/g in MAP2), while in the vacuum packaged meat samples was noticed faster growth and increase of TVC. From the 3rd day of storage TVC in the vacuum packaged samples was significantly higher ($P<0.01$) than TVC in meat samples packaged in modified atmosphere, except on the 9th day of storage, when TVC in vacuum packaged samples was significantly lower ($P<0.01$) then TVC in meat samples in both modified atmospheres packaging (MAP1 and MAP2). There were no significant differences ($P>0.05$) between TVC in samples packaged in modified atmosphere with different gas concentrations during the whole storage period. TVC was already higher than the recommended limit of 7 log CFU/g by the 6th day in the vacuum packaged meat samples (8.16±0.036 log CFU/g), while TVC in meat samples packaged in modified atmosphere was higher than the recommended limit at the 9th day of storage (8.47±0.014 log CFU/g in MAP1 and 8.47±0.054 log CFU/g in MAP2). IRKIN & al [2] and BERRUGA & al [11] also reported

variation in the TVC during storage of minced beef and rabbit meat, while KHALED & al [26] presented results which showed spoilage of chicken meat stored under vacuum after 5 days. Same as in the present study, BOSKOVIC & al [27] reported lower TVC in minced pork packaged under MAP than in vacuum packaged mince during the whole storage period. The number of micro-organisms including bacteria depends on the intrinsic and extrinsic factors including pH, meat surface morphology, O2 availability, temperature and the presence and development of other bacteria (ERCOLINI & al [28]). Changes in these factors and bacterial competition could influence the changes in the TVC in these studies and in the current study. At the end of experiment, lower TVC was present in meat samples packaged with modified atmosphere (7.65±0.204 log CFU/g in MAP1 and 7.82±0.032 log CFU/g in MAP2) compared to TVC in vacuum packaged meat samples (8.42±0.005 log CFU/g), which can be attributed to antibacterial effect of modified atmosphere, especially carbon dioxide. The TVC within packaging groups was significantly different during storage (P<0.01 and P<0.05) (Table 1). Results from the present study are in accordance with those obtained by BALAMATSIA & al [29], which showed that TVC reached 8.2 log CFU/g on the 11th day of storage in samples packaged under MAP with 30% CO_2, as well as results of WANG & al [30], who reported initial TVC 4.94 log CFU/g in the lamb meat samples, which significantly increased until 14th day of storage to the value of about 7.60 log CFU/g and significantly lower TVC in the samples packaged with middle amount of carbon dioxide (60%) compared to vacuum and low (20%) CO_2 MAP.

Table 1. Change in total viable count (log CFU/g) in packaged minced meat samples stored at 3±1°C (mean± SEM)

Day of storage	Group of sample		
	Vacuum	**MAP1**	**MAP2**
0^{th}	4.59^{ABCD} ±0.016	4.59^{ABC} ±0.016	4.59^{ABCD} ±0.016
3^{rd}	$6.87^{\alpha AEF}$ ±0.224	$5.43^{\alpha DEF}$ ±0.021	6.34^{AEF} ±0.052
6^{th}	$8.16^{\alpha\beta BEG}$ ±0.036	$6.68^{\alpha ADGa}$ ±0.025	$6.59^{\beta BGH}$ ±0.081
9^{th}	$6.66^{\alpha\beta CGH}$ ±0.031	$8.47^{\alpha BEG}$ ±0.014	$8.47^{\beta CEGI}$ ±0.054
12^{th}	$8.42^{\alpha DFH}$ ±0.005	7.65^{CFa} ±0.204	$7.82^{\alpha DFHI}$ ±0.032

Legend: the same letter within row indicates significant differences $^{\alpha, \beta}$ P<0.01; the same letter within column indicates significant differences $^{A\text{-}I}$ P<0.01; ^{a} P<0.05

YILMAZ & DEMIRCI [31] recorded a growth of TVC, by about 2 log CFU/g, in minced beef with added spices during storage at 4°C. In their study the total number of aerobic mesophilic bacteria grew to over the recommended limit on the 7^{th} day of storage in the samples packaged in vacuum and the 9^{th} day in the samples packaged in modified atmosphere (65%N_2/35%CO_2). IRKIN & al [2] also found faster growth and the higher number of bacteria in ground beef samples packaged in vacuum (the TVC was higher than the recommended limit on the 9^{th} day of storage), compared to the meat samples packaged in the modified atmosphere, whereas the TVC was higher than the recommended limit on the 14^{th} day of storage, wherein the bacteria count was lower in meat samples packaged in modified atmosphere with higher carbon dioxide concentration. KARABAGIAS & al [32] also reported growth of this bacterial group in lamb packaged in modified atmosphere with 80% CO_2 and 20% N_2, stored at 4°C.

Enterobacteriaceae count

The presence of *Enterobacteriaceae* in meat and meat products is very important from the hygienic and commercial aspect because of the many pathogenic bacteria from this family and because of their ability to cause meat spoilage when their number reaches 7 log CFU/g of meat, even at refrigeration temperatures. In spoilage, their role is significant because they degrade amino acids to sulphur compounds and diamines, volatile, malodorous components (SADE & al [33]; EL-GENDY & al [34]). The initial number of *Enterobacteriaceae* which will be present in meat is mostly affected by hygiene during the slaughter and production process, but also the types and forms of meat.

In this study, *Enterobacteriaceae* count decreased during storage (Table 2). The largest decrease and the lowest (P<0.01) *Enterobacteriaceae* count were observed in meat samples packaged in a modified atmosphere with 30% carbon dioxide (MAP2) on the 9^{th} day of storage

(3.25±0.021 log CFU/g). From the 6th day of storage significantly lower (P<0.01 and P<0.05) *Enterobacteriaceae* counts in meat samples packaged in MAP1 compared to vacuum packaged meat samples were noticed. Only on the 6th day of storage was there significantly different (P<0.05) Enterobacteriaceae count in meat samples packaged in modified atmosphere with 50% of carbon dioxide (MAP1) (3.26±0.092 log CFU/g) compared to *Enterobacteriaceae* count in meat samples packaged in modified atmosphere with 30% carbon dioxide (MAP2) (3.71±0.035 log CFU/g). *Enterobacteriaceae* counts did not differ significantly (P>0.05) during storage within groups of packages, which is in accordance with results of WANG & al [30] which also reported relatively stable *Enterobacteriaceae* counts compared to other bacteria, within groups of vacuum and MAP packaged lamb meat.

Table 2. Change in *Enterobacteriaceae* count (log CFU/g) in packaged minced meat samples stored at 3±1°C (mean± SEM)

Day of storage	Group of sample		
	Vacuum	**MAP1**	**MAP2**
0th	3.93±0.027	3.93±0.027	3.93±0.027
3rd	3.62±0.145	3.72±0.110	3.53 ±0.212
6th	3.83^{α}±0.008	$3.26^{\alpha a}$±0.092	3.71^{a}±0.035
9th	$3.60^{\alpha\beta}$±0.011	3.30^{α}±0.022	3.25^{β}±0.021
12th	$3.86^{a\alpha}$±0.014	3.46^{a}±0.064	3.49^{α}±0.011

Legend: the same letter within row indicates significant differences $^{\alpha,\beta}$ P<0.01; ^{a} P<0.05

Results from this study can be explained by the previously mentioned literature (BERRUGA & al [11]; ZHANG & SUNDAR [12]), as a package with modified atmosphere slows or suppresses the *Enterobacteriaceae* growth, which mostly depends on gases and their concentrations. Beside the antibacterial effect of carbon dioxide and the sensitivity especially of gram-negative bacteria, which includes *Enterobacteriaceae*, carbon dioxide is absorbed into the meat, which contributes to pH value decreasing to the values unsuitable for bacterial growth.

Similar results were reported by ESMER & al [35], who examined the influence of aerobic and packaging with modified atmosphere with the different concentrations of oxygen, carbon dioxide and nitrogen on the shelf life of ground beef during 14 days of storage at 4°C. At the end of storage, the *Enterobacteriaceae* count was the highest in the meat samples in the aerobic packaging while the lowest was in the packaging with modified atmosphere with 50%O_2/30%CO_2/20% N_2.

Lactic acid bacteria count (LAB count)

After the inhibition of aerobic bacteria growth, LAB become the dominant bacterial species, because of their ability to growth in the absence of oxygen and high resistance, even at low pH value, and due to this, they may cause spoilage of meat and meat products (DOULGERAKI & al [3]; POTHAKOS & al [36]). The literature emphasizes the role of psychrotrophic LAB in the meat spoilage, when their number exceeds 107 CFU/g of meat, which leads to development of sour odour and taste, resulting in the meat unacceptability (POTHAKOS & al [37]).

In this study, at the beginning of the storage LAB count in meat samples was 3.18±0.35 log CFU/g (Table 3). The LAB count increased and the highest load (P<0.01) was detected on the 6th day of storage in samples packaged in modified atmosphere with the lower concentration of carbon dioxide (5.13±0.024 log CFU/g). It was noted that LAB count in the meat samples packaged in modified atmosphere with 50% carbon dioxide was lower from the 6th day of storage, but were significantly different (P<0.01) only compared to the vacuum packaged meat samples, except on the 9th day of storage where LAB count of the meat samples packaged in MAP1 was significantly different (P<0.01) compared to the meat samples packaged in MAP2. The LAB count increased significantly with time (P<0.01 and P<0.05) in all groups of packages (Table 3).

Table 3. Change in lactic acid bacteria count (log CFU/g) in packaged minced meat samples stored at 3±1°C (mean± SEM)

Day of storage	Group of sample		
	Vacuum	**MAP1**	**MAP2**
0th	3.18^{ABC} ±0.035	3.18^{ABaC} ±0.035	3.18^{ABCD} ±0.035
3rd	4.15^{Aa} ±0.011	3.93^{Ab} ±0.037	3.88^{AEF} ±0.035
6th	$3.67^{\alpha\beta DE}$ ±0.031	$4.36^{\alpha\gamma BbD}$ ±0.001	$5.13^{\beta\gamma BEG}$ ±0.024
9th	$4.91^{\alpha BaD}$ ±0.026	$3.63^{\alpha\beta aCDE}$ ±0.021	$4.76^{\beta CFa}$ ±0.002
12th	$4.69^{\alpha\beta CE}$ ±0.066	$4.33^{\alpha FE}$ ±0.011	$4.26^{\beta DGa}$ ±0.007

Legend: the same letter within row indicates significant differences $^{\alpha, \beta, \gamma}$ $P<0.01$; the same letter within column indicates significant differences $^{A\text{-}G}$ $P<0.01$; $^{a, b}$ $P<0.05$

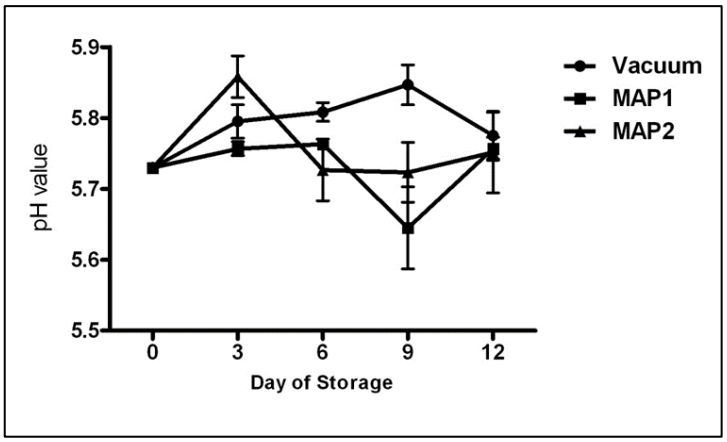

Figure 1. Change in pH value in packaged minced meat samples stored at 3±1°C (mean± SEM).

The increase of the LAB count can be explained by the high resistance of this group of bacteria to the antibacterial effect of carbon dioxide, which these bacteria also produce themselves during the respiratory processes, and also by the fact that carbon dioxide from the packaging with modified atmospheres dissolves better at low temperatures at which the samples are stored and, due to which leads to inhibition of a large number of micro-organisms, especially gram-negatives, and subsequent growth of competitive microorganisms including facultative anaerobes such as lactic acid bacteria (WANG & al [30]; POTHAKOS & al [36]).

PEXARA & al [38], SANTOS & al [39], MARTINEZ & al [40] and RUIZ-CAPILLAS & JIMENEZ-COLMENERO [41], also reported increases of LAB counts. In the study of PETROU & al [42] the number of lactic acid bacteria also increased in the chicken breasts stored at 4°C and packaged in the modified atmosphere with 30% CO_2 and 70% NO_2, while in the studies of BOSKOVIC & al [27] and ŁOPACKA & al [43] during whole storage period LAB count in pork and beef meat, respectively, packaged in MAP were lower compared to vacuum packaged samples.

pH value

One of the meat quality parameters is pH value. In addition, the pH value is one of the parameters responsible for bacterial growth.

In present study, at the beginning of storage, pH value was 5.73±0.004 and increased to 5.85±0.004 on the 9th day of storage in the vacuum packaged samples, while in MAP1 a slight increase was noted, but only to pH value of 5.76±0.004, and it stayed at that level on the 6th day (5.76±0.001), after which the pH value decreased to 5.65±0.009 on the 9th day of storage. The pH value of MAP2 was the highest on the 3rd day of storage (5.86±0.011), after which it decreased to 5.72±0.004 on the 9th day of storage. On the 12th day of storage, pH values were similar in all packaging types (5.78±0.006 in vacuum packaging, 5.76±0.009 in MAP1 and 5.75±0.003 in MAP2), with significant differences between the pH value of vacuum and MAP2 ($P<0.01$) and MAP1 and MAP2 packaging ($P<0.05$).

Figure 1 shows the slight increase in the pH value in the vacuum packaged meat samples as a result of microorganism activity, due to proteolytic process, but also

as a result of native enzyme action in the meat during storage. These processes create alkali compounds (ammonia, trimethylamine, dimethylamine) that increase pH values (BALTIĆ [44]; KARABAGIAS & al [32]).

A large number of authors stated that different factors affect the pH value of the meat, but growth of bacteria that produce lactic acid is the main cause of decreases in the pH value of the packaged meat (MILIJAŠEVIĆ & al [45]; FERNANDEZ-LOPEZ & al [46]; GOK & al [47]; IRKIN & al [2]). KARABAGIAS & al [32] reported the increase in pH value during storage in lamb packaged in the modified atmosphere with 60% CO_2, which is explained by the solubility of carbon dioxide in meat and activity of lactic acid bacteria.

Sensory evaluation

Although microorganisms play an important role in meat spoilage, final assessment of the changes are based on the sensory analysis. Volatile (olfactory) ingredients such as carbonyl and sulphur compounds, various organic acids, alcohols and ammonia which affect meat odor are formed in the meat and meat products during storage as a result of oxidation and bacterial activity (ELLIS & GOODACRE [4]). In present study, the score of minced meat odor acceptability was 6.77±0.018 (maximum possible score was 7) at the beginning of storage, then it decreased, until the 12th day when it was below the level of acceptability (score 3.5) and ranged from 3.46±0.04 (vacuum packaging) to 3.58±0.05 (MAP1) and 3.51±0.04 (MAP2). There were no significant differences between odor acceptability of different packaged minced meat samples (Figure 2). These results are similar to those of NISSEN & al [48] which showed that all samples of minced beef packed in air and modified atmospheres (with a high concentration of CO_2, or a high concentration of O_2) had, by the 8th day of storage, odor below acceptable limits. ERCOLINI & al [5] reported that after 14 days storage, beef samples packaged in air and modified atmosphere showed signs of deterioration and were unacceptable from the sensory aspect.

The addition of O_2 into MAP leads to formation of oxymyoglobin, a red form of myoglobin, which gives meat desirable red color. Additionally, O_2 can have a negative influence on oxidative stability of the meat, leading to rancidity and decreasing tenderness (ZAKRYS & al [49]; ZAKRYS & al [50]). Thus, the amount of oxygen in the package should be balanced in order to maintain the color stability and obtain acceptable taste and tenderness of the meat, which is why in the present study, 20% O_2 was added to the MAP. The color of samples did not differ at the beginning of the experiment and color scores ranged from 6.2 to 6.3. During storage, color scores significantly decreased in all samples, reaching the limit of acceptability (3.5) only in the samples packaged in vacuum on the 12th day. While the colour of the vacuum packaged samples had the lowest score during the experiment, there was no difference between samples packaged in different atmospheres, which could be explained by the fact that equal concentrations of O_2 were added to both MAP (Table 4).

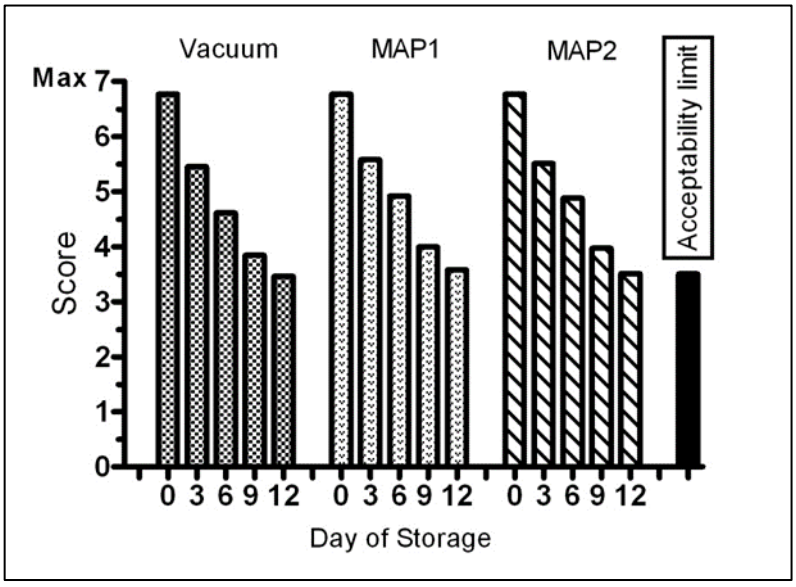

Figure 2. Changes in odor acceptability scores of packaged minced meat samples stored at 3±1°C.

Table 4. Changes in color acceptability scores in packaged minced meat samples stored at 3±1°C (mean± SEM)

Day of storage	Group of sample		
	Vacuum	**MAP1**	**MAP2**
0^{th}	6.2±0.070	6.2±0.070	6.3±0.070
3^{rd}	5.6^{a}±0.105	5.9±0.081	6.0^{a}±0.073
6^{th}	4.8^{aA}±0.081	5.2^{a}±0.104	5.3^{A}±0.116
9^{th}	4.1^{AB}±0.107	4.6^{A}±0.029	4.7^{B}±0.128
12^{th}	3.4^{AB}±0.058	3.9^{A}±0.129	4.0^{B}±0.058
Legend: the same letter within row indicates significant differences $^{A, B}$ $P<0.01$; ^{a} $P<0.05$			

Headspace

Figure 2 shows concentrations of gasses in the headspace of packaged minced meat. The initial amount of gases in MAP1 packaging was 50% CO_2, 20% O_2 and 30% N_2, while in MAP2 it was 30% CO_2 and 50% N_2, with the same amount of O_2 (20%). Oxygen concentration decreased in both MAP packaging to values of 11.4 in MAP1 and 11.1 in MAP2, while carbon dioxide concentration increased, after an initial decrease until the 3rd day of storage (to 37.2 in MAP1 and 22.3 in MAP2) to values of 47.5 in MAP1 and 27.7 in MAP2. Nitrogen concentration increased until the ninth day of storage to values of 46.7 in MAP1 and 61.4 in MAP2, after which slight decreases were noted to values of 40.8 (in MAP1) and 61.2 (in MAP2).

The ratio of gases added into a modified atmosphere is not constant and changes during storage as a result of the respiratory processes in the meat (muscle), but also as a result of the metabolic processes of microorganisms (WANG & al [30]; HAN [51]; ESMER & al [35]). The highest amount of carbon dioxide from the modified atmosphere is absorbed in the water and lipid fraction of meat until a balance is achieved and that is why the preservative effect of this gas is reached with addition above the saturation level. According to literature data, the saturation and absorption of carbon dioxide happen in the first two days of storage (AHVENAINEN [52]; MCMILLIN [53]). This is in accordance with results of STOOPS & al [54] and this study which showed decreases in carbon dioxide amounts until the third day of storage, when the concentration of this gas started to grow, until the end of the experiment in both packaging types with modified atmosphere (Figure 3).

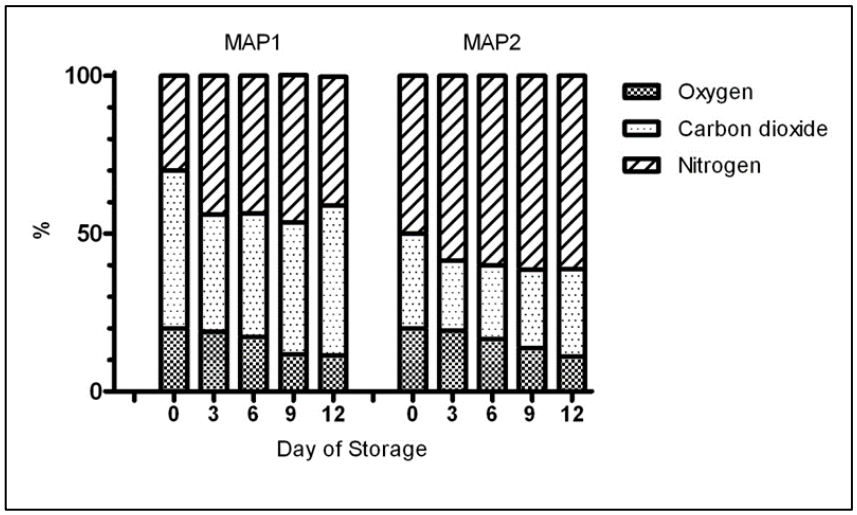

Figure 3. Changes of CO_2, O_2, and N_2 concentrations in headspace of minced meat stored at 3±1°C (%).

Increase in carbon dioxide concentration can be attributed to the activity of bacteria such as lactic acid bacteria which produce carbon dioxide in metabolic processes (ESMER & al [35]). Reductions of oxygen seen in this study can be attributed to the use of oxygen by the aerobic bacteria, whose growth was noted in samples

packaged in modified atmosphere packaging with both combinations of gases (JEREMIAH [55]). Decrease of carbon dioxide at the beginning and increase on the last day, as well as a decrease of oxygen during storage, were reported by IRKIN & al [2] and ESMER & al [35] in ground beef packaged in the modified atmosphere with different concentrations of carbon dioxide and oxygen.

Conclusion

Present study showed greatest influence of modified atmosphere packaging compared to vacuum packaging in the term of antimicrobial effect (especially modified atmosphere with 50% of CO_2) and sensory properties, particularly in the term of meat color.

Acknowledgements

This paper was supported by the Ministry of Education, Science and Technological development, Republic of Serbia, through the funding of the Project – Selected biological hazards to the safety/quality of food of animal origin and the control measures from farm to consumer (No 31034).

References

1. R. KOPPEL, A. EUGSTER, J. RUF, J. RENTSCH. Quatification of Meat Proportions by Measuring DNA Contents in Raw and Boiled Sausages Using Matrix-Adapted Calibrators and Multiplex Real-Time PCR. *J. AOAC Int*., 95(2): 494-499 (2012).
2. R. IRKIN, O.K. ESMER, N. DEGIRMENCIOGLU, A. DEGIRMENCIOGLU. Influence of packaging conditions on some microbial properties of minced beef meat at 4°C storage. *Bulg. J. Agric. Sci*., 17(5): 655-663 (2011).
3. A.I. DOULGERAKI, D. ERCOLINI, F. VILLANI, G.J.E. NYCHAS. Spoilage microbiota associated to the storage of raw meat in different conditions. *Int. J. Food Microbiol*., 157(2): 130-141 (2012).
4. D.I. ELLIS, R. GOODACRE. Rapid and quantitative detection of the microbial spoilage of muscle foods: current status and future trends. *Trends Food Sci. Technol*., 12(11): 414- 424 (2001).
5. D. ERCOLINI, F. RUSSO, E. TORRIERI, P. MASI, F. VILLANI. Changes in the Spoilage-Related Microbiota of Beef during Refrigerated Storage under Different Packaging Conditions. *Appl. Environ. Microbiol.*, 72(7): 4663-4671 (2006).
6. EFSA. Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 2 (minced meat from all species). *EFSA J* 12(7): 3783 (2014).
7. G-J.E. NYCHAS, P.N. SKANDAMIS, C.C. TASSOU, K.P. KOUTSOUMANIS. Meat spoilage during distribution. *Meat Sci.*, 78(1): 77-89 (2008).
8. D. ERCOLINI, I. FERROCINO, A. NASI, M. NDAGIJIMANA, P. VERNOCCHI, La A. STORIA. L. LAGHI, G. MAURIELLO, M.E. GUERZONI, F. VILLANI, Monitoring of microbial metabolites and bacterial diversity in beef stored in different packaging conditions. *Appl. Environ. Microbiol*., 77(20): 7372-7381 (2011).
9. C. PENNACCHIA, D. ERCOLINI, F. VILLANI. Spoilage- related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiol*., 28(1): 84-93 (2011).
10. P.N. SKANDAMIS, G-J.E. NYCHAS. Preservation of fresh meat with active and modified atmosphere packaging conditions. *Int. J. Food Microbiol*., 79(1): 35-45 (2002).
11. M.I. BERRUGA, H. VERGARA, M.B. LINARES. Control of microbial growth and rancidity in rabit carcasses by modified atmosphere packaging. *J. Sci. Food Agr.*, 85(12): 1987-1991 (2005).
12. B.M. ZHANG, S. SUNDAR. Effect of oxygen concentration on the shelf life of fresh pork packed in a modified atmosphere. *Packag. Technol. Sci*., 18(4): 217-222 (2005).
13. C. NICOLALDE, A.J. STETZER, E.M. TUCKER, F.K. MCKEITH, M.S. BREWER. Antioxidant and modified atmosphere packaging prevention of discoloration in pork bones during retail display. *Meat Sci*., 72(4): 713-718 (2006).
14. A.J. STETZER, R.A. WICKLUD, D.D. PAULSON, E.M. TUCKER, B.J. MACFARLANE, M.S. BREWER. Effect of carbon monoxide and high oxygen modified atmosphere packaging (MAP) on quality characteristics of beef strip steaks. *J. Muscle Foods*, 18(1): 56-66 (2007).
15. P. SINGH, A.A. WANI, S. SAENGERLAUB, H.C. LANGOWSKI. Understanding critical factors for the quality and shelf-life of MAP fresh meat: a review. *Crit. Rev. Food Sci. Nutr*., 51(2): 146- 177 (2011).
16. ISO 4833:2003 – Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 degrees C.
17. ISO 21528-2:2004 - Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae – Part 2: Colony count method.
18. ISO 15214:1998 – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony count technique at 30 degrees C.
19. SRPS ISO 8589: 2012 – Sensory analysis – General guidance for the design of test rooms.

20. E. BORCH, M.L. KANT-MUERMANS, Y. BLIXT. Bacterial spoilage of meat and cured meat product. *Int. J. Food Microbiol.,* 33(1): 103-120 (1996).
21. Z. PLAZONIĆ, B. MIOKOVIĆ, B. NJARI. Pakiranje mesa u modificiranoj atmosferi. *Meso,* 12(1): 45-48 (2010).
22. F. DE FILIPPIS, A. LA STORIA, F. WILLAMI, D. ERCILLINI. Exploring the Sources of Bacterial Spoilers in Beefsteaks by Culture-Independent High-Throughput Sequencing. PLoS One 8(7):e70222, 2013. doi: 10.1371/journal.pone.0070222.
23. J.M. JAY, eds., Modern Food Microbiology, 5th Aspen Publisher Inc., Gaithersburg, Marylend, USA, 1998.
24. Y. MOTARJEMI, G. MOY, E. TODD. eds., Encyclopedia of Food Safety, Academic Press, Elsevier, San Diego, USA, 2014.
25. F. TAO, Y. PENG. A non-destructive Method for Prediction of Total Viable Count in Pork Meat by Hyperspectral Scattering Imaging. *Food Bioprocess Technol.,* 8: 17-30 (2015).
26. H. KHALED, A. AZIZIAH, A. MARII. Effect of oregano extract on shelf-life, microbiological quality of chilled chicken carcasses. *Int. Food Res. J.,* 23(3): 1296-1299 (2016).
27. M. BOSKOVIC, J. DJORDJEVIC, J.IVANOVIC, J. JANJIC, N. ZDRAVKOVIC, M. GLISIC, N. GLAMOCLIJA, B. BALTIC, V. DJORDJEVIC, M. BALTIC. Inhibition of Salmonella by thyme essential oil and its effect on microbiological and sensory properties of minced pork meat packaged under vacuum and modified atmosphere. *Int. J. Food Microbiol*., 258: 58-67 (2017).
28. D. ERCOLINI, F. RUSSO, A. NASI, P. FERRANTI, F. VILLANI. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. *Appl. Environ. Microbiol*., 75(7): 1990-2001 (2009).
29. C.C. BALAMATSIA, E.K. PALEOLOGOS, M.G. KONTOMINAS, I.N. SAVVAIDIS. Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4°C: possible role of biogenic amines as spoilage indicators. *Antonie van Leeuwenhoek*, 89(1): 9-17 (2006).
30. T. WANG, L. ZHAO, Y. SUN, F. REN, S. CHEN, H. ZHANG, H. GUO. Changes in the microbiota of lamb packaged in a vacuum and in modified atmospheres during chilled storage analysed by high-throughput sequencing. *Meat Sci*. 121: 253-260 (2016).
31. I. YILMAZ, M. DEMIRCI. Effect of Different Packaging Methods and Storage Temperature on Microbiological and Physicochemical Quality Characteristics of Meatball. *Food Sci. Tech. Int.,* 16(3): 259-265 (2010).
32. KARABAGIAS, A. BADEKA, M.G. KONTO-MINAS. Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. *Meat Sci.,* 88(1): 109-116 (2011).
33. E. SADE, A. MURROS, J. BJÖRKROTH. Predominant enterobacteria on modified-atmosphere packaged meat and poultry. *Food Microbiol*., 34(2): 252-258 (2013).
34. N.M. EL-GENDY, H.A. IBRAHIM, N.A. AL-SHABASY, I.A. SAMAHA. Enterobacteriaceae in beef products (Luncheon, Pasterma, Frankfurter and Minced meat) from Alexandria retail outlets. *Alexandria J. Veterinary Sci.,* 41(1): 80-86 (2014).
35. O.K. ESMER, R. IRKIN, N. DEGIRMENCIOGLU, A. DEGIRMENCIOGLU. The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. *Meat Sci.,* 88(2): 221-226 (2011).
36. V. POTHAKOS, F. DEVLIEGHERE, F. VILLANI, J. BJÖRKROTH, D. ERCOLINI. Lactic acid bacteria and their controversial role in fresh meat spoilage. *Meat Sci.,* 109: 66-74 (2015).
37. V. POTHAKOS, S. SAMAPUNDO, F. DEVLIE-GHERE. Total mesophilic counts underestimate in many cases the contamination levels of psychrotrophic lactic acid bacteria (LAB) in chilled-stored food products at the end of their shelf-life. Food Microbiol., 32(2): 437-443 (2012).
38. E.S. PEXARA, J. METAXOPOULOS, E.H. DROSINOS. Evaluation of shelf life of cured, cooked, sliced turkey fillets and cooked pork sausages—'piroski'—stored under vacuum and modified atmospheres at +4 and +10°C. *Meat Sci.,* 62(1): 33-43 (2002).
39. E.M. SANTOS, A.M. DIEZ, C. GONZÁLEZ-FERNÁNDEZ, I. JAIME, J. ROVIRA. Microbiological and sensory changes in "Morcilla de Burgos" preserved in air, vacuum and modified atmosphere packaging. *Meat Sci.,* 71(2): 249-255 (2005).
40. L. MARTINEZ, D. DJENANE, I. CILLA, J.A. BELTRAN, P. RONCALES. Effect of varying oxygen concentrations on the shelf-life of fresh pork sausages packaged in modified atmosphere. *Food Chem.*, 94(2): 219-225 (2006).
41. C. RUIZ-CAPILLAS, F. JIMÉNEZ-COLMENERO. Effect of an argon-containing packaging atmosphere on the quality of fresh pork sausages during refrigerated storage. *Food Control,* 21(10): 1331-1337 (2010).
42. S. PETROU, M. TSIRAKI, V. GIATRAKOU, I.N. SAVVAIDIS. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *Int. J. Food Microbiol.*, 156(3): 264-271 (2012).
43. J. ŁOPACKA, A. POŁTORAK, A. WIERZBICKA. Effect of MAP, vacuum skin-pack and combined

packaging methods on physicochemical properties of beef steak stored up to 12 days. *Meat Sci.*, 119: 147-153 (2016).
44. T. BALTIĆ. Influence of marinating on *Salmonella* spp. growth in broiler meat. PhD Thesis, Faculty of Veterinary Medicine, University of Belgrade (2014).
45. M. MILIJAŠEVIĆ, B. VELEBIT, J. JOVANOVIĆ. The effect of different gas mixtures on colour and microbiological compliance of beef packaged in protective atmosphere. International 54th meat Industry Conference, Vrnjačka Banja, Serbia 2007, pp. 93, 2007.
46. J. FERNANDEZ-LOPEZ, E. SENDRA, E. SAYAS-BARBERÁ, C. NAVARRO, J.A. PÉREZ-ALVAREZ. Physico-chemical and microbiological profiles of "salchichón" (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Sci.*, 80(2): 410-417 (2008).
47. V. GOK, E. OBUZ, L. AKKAYA. Effects of packaging method and storage time on the chemical, microbiological, and sensory properties of Turkish pastirma – A dry cured beef product. *Meat Sci*. 80(2): 335-344 (2008).
48. H. NISSEN, O. ALVSEIKE, S. BREDHOLT, A. HOLCK, T. NESBAKKEN. Comparison between the growths of Yersinia entrocolitica, Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella spp. in ground beef packed by three commercially used packaging techniques. *Int. J. Food Microbiol.*, 59: 211-220 (2000).
49. P.I. ZAKRYS, S.A. HOGAN, M.G. O'SULLIVAN, P. ALLEN, J.P. KERRY. Effect of oxygen concentrations on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. *Meat Sci.*, 79: 648-655 (2008).
50. P.I. ZAKRYS, M.G. O'SULLIVAN, P. ALLEN, J.P. KERRY. Consumer acceptability and physiochemical characteristics of modified atmosphere packed beef steaks. *Meat Sci.*, 81: 720-725 (2009).
51. J.H. HAN. Innovations in Food Packaging, Academic Press, London, UK, 2005.
52. R. AHVENAINEN. ed., Novel food packaging techniques, Academic Press, Elsevier, San Diego, USA, 2003.
53. K.W. MCMILLIN. Where is MAP going? A review and future potential of modified atmosphere packaging for meat. *Meat SCi.*, 80(1): 43-65 (2008).
54. J. STOOPS, S. RUYTERS, P. BUSSCHAERT, R. SPAEPEN, C. VERRETH, J. CLAES, B. LIEVENS, L. VAN CAMPENHOUT. Bacterial community dynamics during cold storage of minced meat packaged under modified atmosphere and supplemented with different preservatives. *Food Microbiol.*, 48: 192-199 (2016).
55. L.E. JEREMIAH. Packaging alternatives to delivery fresh meats using short- or long-term distribution. *Food Res. Int.*, 34(9): 749-772 (2001).