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Microbiological status of minced pork meat in vacuum and modified atmosphere packaging

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A b s t r a c t: The aim of this study was to evaluate the effectiveness of different packaging conditions (vacuum and modified atmosphere) on the microbiological status (total viable count, lactic acid bacteria and Enterobacteriaceae), total volatile basic nitrogen (TVB-N) and pH in minced pork meat. Pork mince was packaged in vacuum, modified atmosphere with 20% O_2, 50% CO_2 and 30% N_2 (MAP 1) or modified atmosphere with 20% O_2, 30% CO_2 and 50% N_2 (MAP 2), refrigerated at 3±1°C and examined on the days 0, 3, 6, 9 and 12 of storage. The average total viable counts and total Enterobacteriaceae counts in vacuum packaged mince were statistically significantly higher ($p<0.01$; $p<0.05$) than in modified atmosphere packaged mince with both combinations of gases, on different days of storage. The largest decrease of total viable count and Enterobacteriaceae count was noted in modified atmosphere packaged mince with the higher concentration (50%) of CO_2.

Keywords: *Lactic acid bacteria, Enterobacteriaceae, total viable count, minced pork meat.*

Introduction

Modern meat packaging techniques are intended to maintain microbial and sensory quality of the product (*Seydim et al.*, 2006). The use of modified atmosphere packaging (MAP) to extend the shelf life of meat has been recognized for many years (*Martínez et al.*, 2005). It is well known that the composition of different gases in modified atmosphere systems can be an effective means to inhibit growth of aerobic spoilage organisms and some food-borne pathogens (*Ivanovic et al.*, 2015; *Ivanovic et al.*, 2014). Oxygen and carbon dioxide are common in gas mixtures used for MAP of raw meat. The presence of O_2 is very important in the storage of raw meats, as it maintains the meat pigment myoglobin in its oxygenated form, oxymyoglobin, which gives raw meat its bright red colour. The CO_2 is responsible for the bacteriostatic effect in modified atmospheres. This bacteriostatic effect is influenced by the concentration of CO_2, the age and load of the initial bacterial population, the storage temperature and the type of meat being packaged (*Martínez et al.*, 2005). Increased levels of CO_2 (20 to 40%) in refrigerated storage have been shown to inhibit microbial populations, and especially the growth of gram negative bacteria, by increasing their lag phase (*McMillin*, 2008).

Nitrogen is an inert gas and no chemical interaction with substances with which it comes to contact. N_2 is used to prevent contraction of coatings for packaging, prevent lipid oxidation and prevent insect attack, etc. (*Ivanovic*, 2014).

In addition to the microbiological status of minced pork meat, pH is also an important parameter used to define pork quality. It is well known that changes in some meat quality traits can affect many other meat quality attributes and pork quality overall (*Dokmanovic et al.*, 2015).

The aim of this study was to examine the microbiological status of minced pork meat in vacuum and MAP with two different levels of CO_2, and to examine the changes in total volatile basic N_2 (TVB-N) and pH in the mince, during storage at 3±1°C over a period of 12 days.

Materials and Methods

Preparation of meat, packaging materials and storage

Raw pork meat was obtained from a slaughterhouse and minced in a sterile grinder (Meat Grinder TB-300E, Thunderbird Food Machinery,

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Dallas, USA). The minced pork meat was collected (in 100±5 g amounts) in sterile plastic bags. A packaging machine, VARIOVAC (Variovac Primus, Zarrentin, Germany), was used to package the minced meat in a foil (oriented polyamide/ethylene vinyl alcohol/polyethylene dynapack foil; Polimoon, Kristiansand, Norway) with low permeability to gas. The degree of permeability of this foil was O_2: 3.2 cm^3 m^{-2} day^{-1}; N_2: 1 cm^3 m^{-2} day^{-1}; CO_2: 14cm^3 m^{-2} day^{-1} at 23°C; and water vapour: 15 g m^{-2} day^{-1} at 38°C. The minced meat was packaged in vacuum or in the modified atmospheres MAP 1 or MAP 2. MAP 1 consisted of 20% O_2, 50% CO_2 and 30% N_2; MAP 2 consisted of 20% O_2, 30% CO_2 and 50% N_2. The packaged minced pork meat was stored at 3±1°C for 12 days. Six packs subjected to each packaging method were analysed on each of days 0, 3, 6, 9 and 12 of storage. The mince pH was measured with a pH meter (TESTO 205; Lenzkirch, Germany), and every three days during storage, the TVB-N was examined according to *Goulas and Kontominas* (2007).

Microbiological and chemical analysis

From each analysed pack of minced meat, 10 g was transferred to a Stomacher bag (Stomacher 400 Classic Bags, Belgrade, Serbia, VICOR), 90 mL of maximum recovery diluent (Merck, Germany) was added and the content was homogenized for 1 min with a stomacher blender (Stomacher 400 Circulator, Seward, UK). Microbial determinations were performed according to the following analytical methods: *ISO* (2008), total viable count; *ISO* (1998b), lactic acid bacteria count, and; *ISO* (2009), *Enterobacteriaceae* count. Chemical analyses were performed according to the following analytical methods: *ISO* (1998a), moisture content; *ISO* (1992b), lipid content; *ISO* (1992a), protein content, and; *ISO* (1999), total ash.

Statistical analysis

Statistical analysis of the results was conducted using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). All parameters were described by means and standard deviation (SD). One-way ANOVA and post hoc Tukey's test were performed to assess the significance of differences among various groups. Values of $p<0.05$ and $p<0.01$ were considered significant.

Results and Discussion

The moisture content of raw minced pork meat at the beginning of study was 70.31±0.22%, the lipid content was 18.68±0.32%, protein content was 18.68±0.32% and total ash content was 1.01±0.03%.

The total viable counts in minced pork meat during 12 days of storage are presented in Table 1. At the beginning of the study, the total viable count in the minced meat was 8.10±0.16 log CFU g$^{-1.}$ The average total viable count varied from 7.66±0.14 log CFU g^{-1} (vacuum) to 7.84±0.23 log CFU g^{-1} (MAP 1) on day 3 of storage. On day 3, there was no statistically significant difference ($p>0.05$) between the average total viable counts in the mince subjected to the three packaging regimes. Comparing the storage days, a statistically significant difference in the total viable count was found on days 6 and 9 of storage. The average total viable count in vacuum packaged mince was significantly higher ($p<0.01$) than the average total viable count in both MAP 1 and MAP 2 on day 12 of storage.

Analysis of the minced meat on days 0 and 12 showed that there was a statistically significant difference ($p<0.01$; $p<0.05$) in total viable counts under the same packaging conditions (Figure 1). The statistically significant differences were found in vacuum ($p<0.05$) and MAP 1 ($p<0.01$).

Kakouri and Nychas (1994) and *Bell et al.* (1995) found that mesophilic aerobic bacteria needed

Table 1. Total viable count (log CFU g^{-1}) in minced raw pork meat during 12 days storage in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	8.10±0.16	7.74±0.29	7.77^{A}±0.08	8.42^{AB}±0.05	8.41^{AB}±0.09
MAP 1	8.10±0.16	7.84±0.23	8.16^{AB}±0.16	8.57^{AC}±0.05	7.66^{AC}±0.07
MAP 2	8.10±0.16	7.66±0.14	7.70^{B}±0.08	8.03^{BC}±0.10	8.15^{BC}±0.11

Legend: $^{A, B, C}$ Within a column, means with a common superscript letter are significantly different, p< 0.01

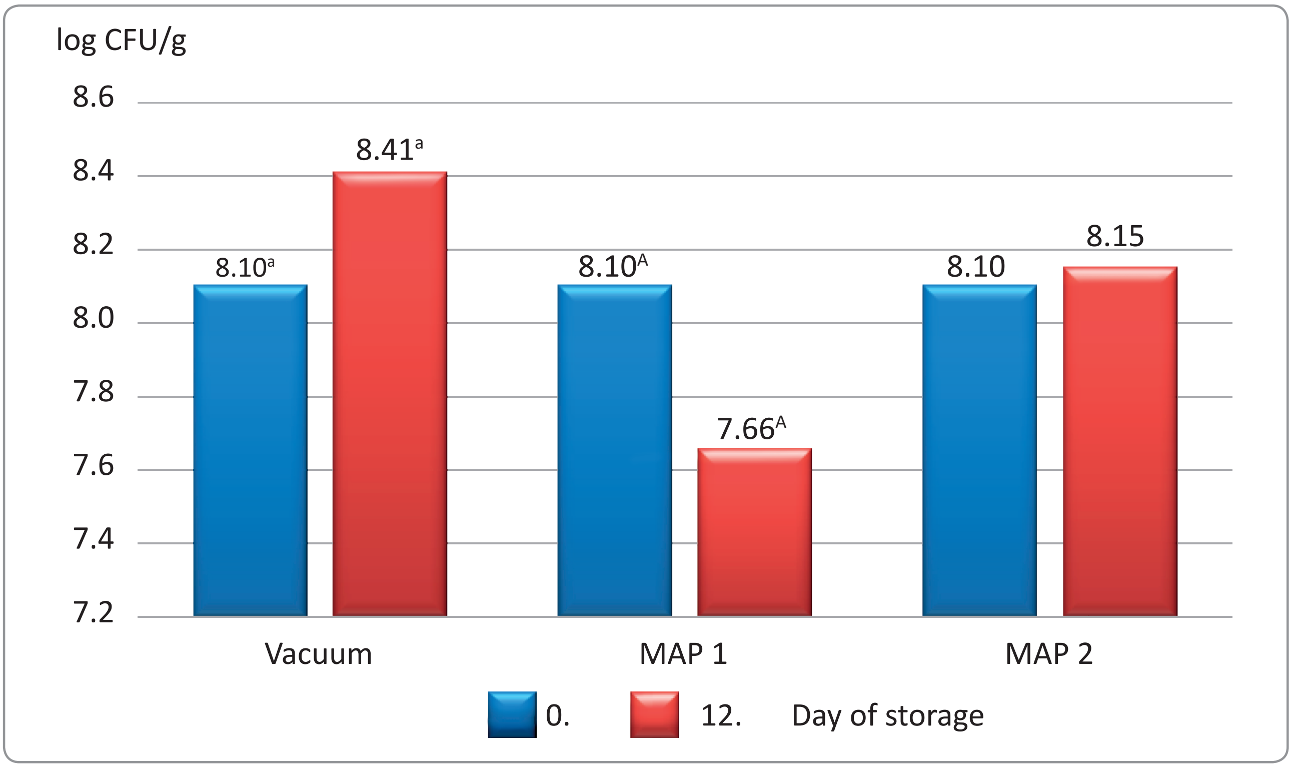

Figure 1. Total viable count at the beginning and at the end of the study ([a] $p<0.05$; [A] $p<0.01$)

approximately 10 days to achieve these counts (7.7 to 8.4 log CFU g^{-1}) in the same atmosphere in pork meat. *Zeitoun et al.* (1994) detected decreased total aerobic bacteria packaged in MAP with 90% CO_2 and 10% O_2. According to Table 1, total aerobic bacteria in our study grew slower under MAP 2. An explanation for this situation could be the high concentration of CO_2 which has bacteriostatic effects (*Arashisar et al.*, 2004). It is known that antimicrobial effect of CO_2 increases depending on the solubility, which is increased by the low water temperature, and CO_2 prolongs lag phase of bacterial growth and increases generation time (*Arashisar et al.*, 2004).

During the storage period, the average total *Enterobacteriaceae* count varied from 7.16±0.06 log CFU g^{-1} (day 0) to 7.72±0.05 log CFU g^{-1} (day 12) in vacuum packaged minced meat, up to 7.60±0.08 log CFU g^{-1} in MAP 1 mince and up to 7.66±0.05 log CFU g^{-1} in MAP 2 mince (Table 2). A significant difference ($p<0.01$) was found on day 6 between the average total *Enterobacteriaceae* count in vacuum and MAP mince.

The average total *Enterobacteriaceae* count on day 0 in vacuum was significantly lower ($p<0.01$) than the average total *Enterobacteriaceae* count in vacuum packaged mince at the end of study. Also, similar results were found in MAP 1 and MAP 2 mince (Figure 2).

The presence of *Enterobacteriaceae* in meat and meat products is determined in order to assess the general hygienic status of meat. Some

Table 2. Counts of *Enterobacteriaceae* (log CFU g^{-1}) in minced raw pork meat during 12 days storage in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	**Day of storage**				
	0	**3**	**6**	**9**	**12**
Vacuum	7.16±0.06	7.17±0.26	7.63^{AB}±0.05	7.62±0.04	7.72±0.05
MAP 1	7.16±0.06	7.10±0.07	7.62^{A}±0.05	7.65±0.04	7.60±0.08
MAP 2	7.15±0.05	7.16±0.06	7.46^{B}±0.04	7.62±0.05	7.66±0.05

Legend: $^{A, B}$ Within a column, means with a common superscript letter are significantly different, $p<0.01$

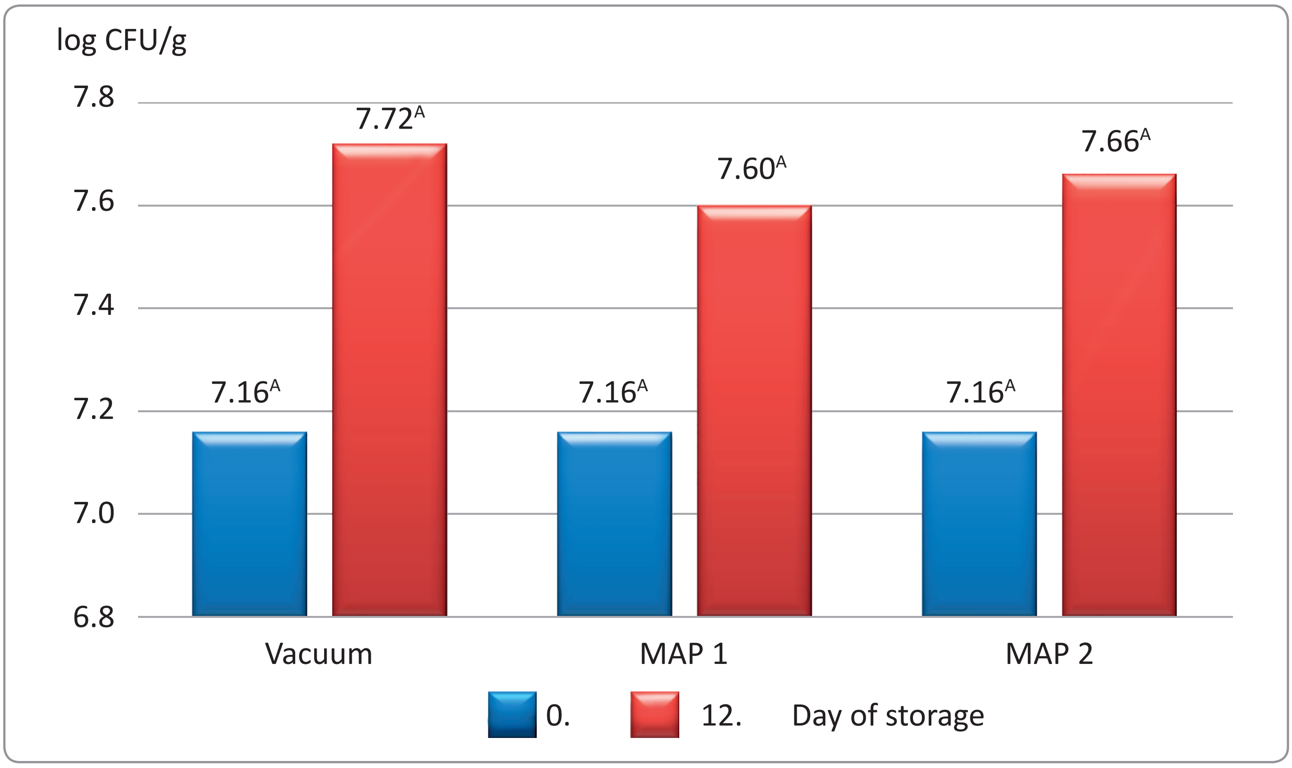

Figure 2. *Enterobacteriaceae* at the beginning and at the end of the study (^{A} p< 0.01)

authors recommend that the average number of *Enterobacteriaceae* can be used as criteria in assessing the sustainability of meat (*Ivanovic et al.*, 2014; *Djordjevic et al.*, 2016). Some of the *Enterobacteriaceae* are of interest to public health while others have commercial importance because of their ability to cause deterioration of meat and meat products during storage at refrigeration temperatures (*Ivanovic et al.*, 2014). CO_2 causes damage to bacterial cell membranes and causes changes in its functions, including the effect on the absorption processes and transport of nutrients through the membrane. This gas also penetrates into the bacterial cells, which leads to decreases in intracellular pH (acidification), and causes direct changes in the proteins' physico-chemical properties (*Siverstvik et al.* 2002; *Goulas and Kontominas*, 2007; *Cornforth and Hunt*, 2008). The average total *Enterobacteriaceae* count in our vacuum packaged mince was higher than the average total *Enterobacteriaceae* count in mince in both types of MAP from day 6 of storage. *Djordjevic et al*. (2016) found that the largest decrease in average total *Enterobacteriaceae* count was observed in minced meat packed in modified atmosphere with 50% CO_2. On day 12 of storage, significantly lower average total *Enterobacteriaceae* counts were measured in mince from MAP with 50% CO_2 than in mince from MAP with 30% CO_2 (*Djordjevic et al.*, 2016).

Table 3. Counts of lactic acid bacteria in raw minced pork meat during 12 days storage (log CFU g^{-1}) in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	5.78±0.06	5.80±0.08	5.94^{a}±0.04	6.01^{AB}±0.06	6.31^{A}±0.06
MAP 1	5.78±0.06	5.81±0.03	5.82^{aA}±0.05	6.31^{AC}±0.03	6.34^{B}±0.04
MAP 2	5.78±0.06	5.82±0.05	5.97^{A}±0.07	5.91^{BC}±0.05	6.13^{AB}±0.04

Legend: $^{A, B, C}$ Within a column, means with a common superscript letter are significantly different, p<0.01
^{a} Within a column, means with a common superscript letter are significantly different, p<0.05

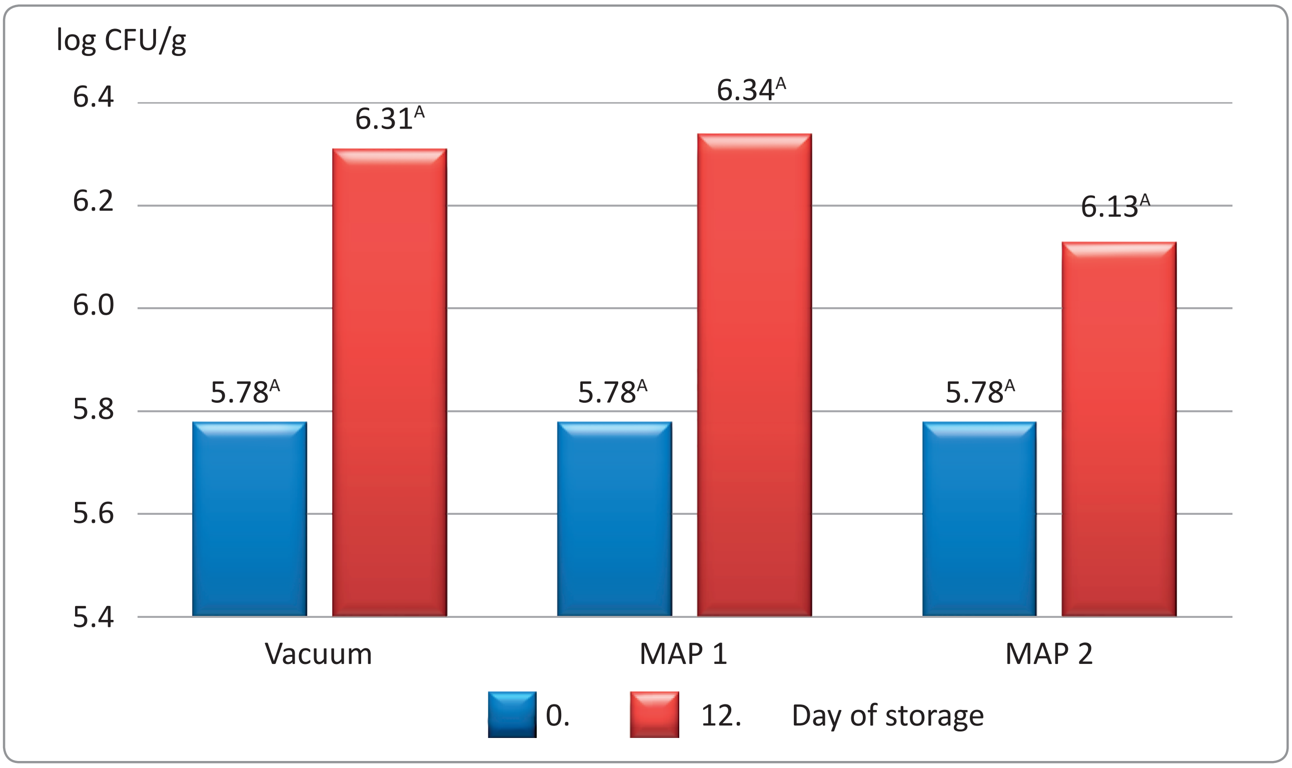

Figure 3. Lactic acid bacteria at the beginning and at the end of the study (A $p<0.01$)

The average lactic acid bacteria count in minced raw meat was 5.78±0.06 log CFU g^{-1} at the beginning of the study (Table 3). On days 6, 9 and 12, statistically significant differences ($p<0.05$; $p<0.01$) were found between the average lactic acid bacteria counts. During the storage period, the average lactic acid bacteria count increased from day 0 to 6.31±0.06 log CFU g^{-1} (day 12) in vacuum packaged minced meat, up to 6.34±0.04 log CFU g^{-1} in MAP 1 mince and up to 6.13±0.04 log CFU g^{-1} in MAP 2 mince.

Significant differences ($p<0.01$) were found in lactic acid bacteria numbers in the mince on day 0 and day 12 in vacuum, MAP 1 and MAP 2 (Figure 3).

Lactic acid bacteria are considered to be specific spoilage organisms that contribute to meat spoilage stored under packaging conditions in which the concentration of CO_2 is increased (*Nychas and Skandamis*, 2005). *Lactobacillus* spp., *Leuconostoc* spp. and *Carnobacterium* spp. are among the most frequently encountered genera on vacuum or modified atmosphere packaged meat and play an important role in the spoilage of refrigerated raw meat (*Parente et al.,* 2001; *Nychas and Skandamis*, 2005). *Daly and Acton* (2004) reported that fresh 92% lean ground beef in high O_2 packaging atmospheres initially had a mean count of 3.72 log CFU g^{-1} of lactic acid bacteria with outgrowth to 5.4 log CFU g^{-1} in 9 days at 4.4°C, similar to the outgrowth found in the current study for minced pork meat in the same time period.

The TVB-N values increased in the mince during storage in vacuum packaging (9.83±0.27 mg N $100g^{-1}$ and 38.79±1.65 mg N $100g^{-1}$ on days 0

Table 4. TVB-N values (mg N $100g^{-1}$) in raw minced pork during 12 days storage in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	9.83±0.27	13.41±0.47	21.18±0.70	30.47±0.77	38.79±1.65
MAP 1	9.83±0.27	13.25±0.63	20.54±0.86	29.91±0.71	37.36±1.06
MAP 2	9.83±0.27	13.44±0.25	20.70±0.95	30.31±0.68	37.77±1.48

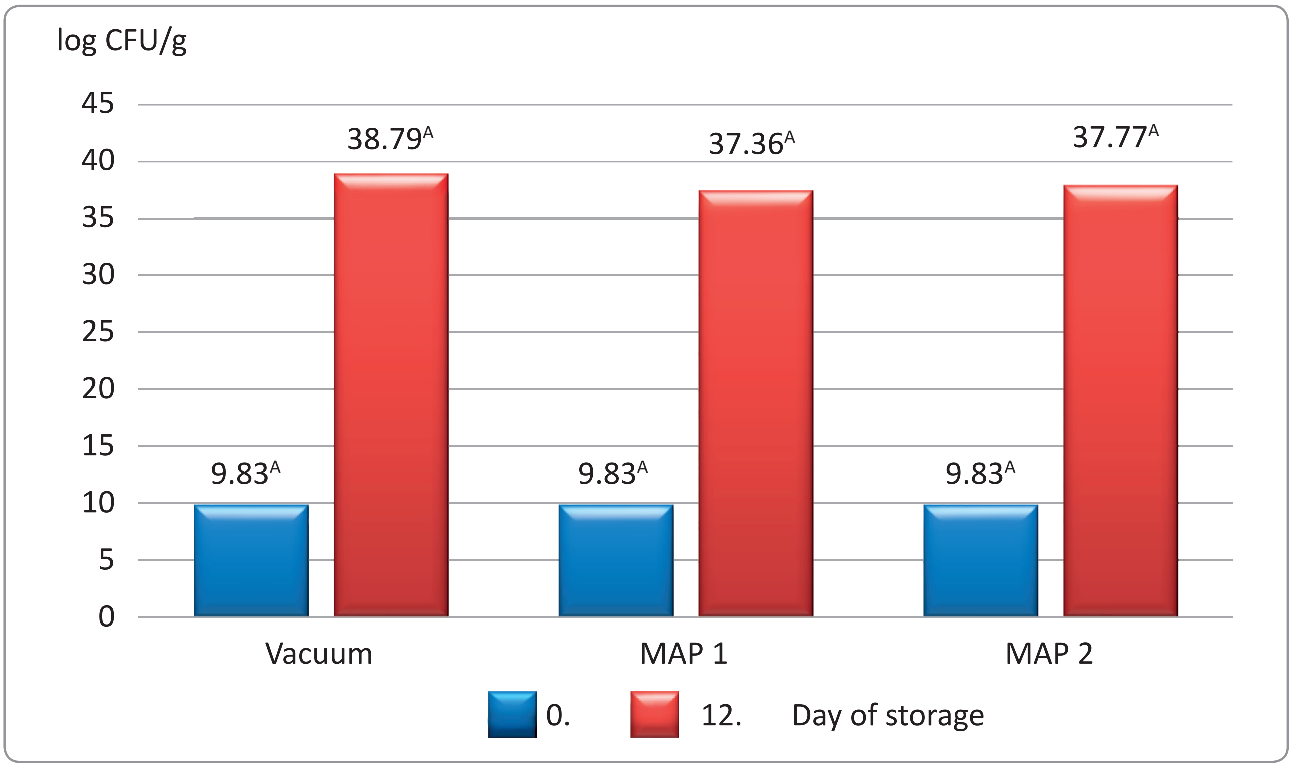

Figure 4. TVB-N values at the beginning and at the end of the study ($^{A-}$ $p<0.01$)

and 12, respectively; Table 4). In MAP 1, TVB-N values increased during storage time (9.83±0.27 mg N 100g^{-1} and 37.36±1.06 mg N 100g^{-1} on days 0 and 12, respectively; Table 4). TVB-N in MAP 2 was 9.83±0.27 mg N 100g^{-1} and 37.77±1.48 mg N 100g^{-1} on day 0 and 12, respectively. No significant differences in TVB-N values were detected between vacuum, MAP 1 and MAP 2 stored pork minced meat during the storage period. However, although differences were not significant, TVB-N values in pork were numerically highest in vacuum packaging on the end of the study (day 12).

Storage time was a significant factor for TVB-N value increases, regardless of the packaging effect (Figure 4). Significant differences ($p<0.01$) were found in vacuum, MAP 1 and MAP 2 at the beginning and at the end of the study (Figure 4).

The TVB-N value is used as a chemical indicator for assessing the freshness of pork (*Ivanovic et al.*, 2015). The TVB-N value measures the concentrations of compounds responsible for the occurrence of unpleasant smell and taste of meat (ammonia, dimethylamine, trimethylamine, amines). The prescribed limit value for TVB-N content of pork meat is 30 mg N 100g^{-1} (*Connell*, 1990). In this study, TVB-N values on day 12 were above the prescribed limit. TVB-N values for pork meat in vacuum packages on days 9 and 12 of storage were very high (>30 mg N 100g^{-1}) as compared to beef (*Brewer and Wu*, 1993), poultry (*Ulu*, 2004), and rabbit meat (*Fernandez-Espla and O'Neill*, 1993).

Table 5. The pH of raw minced pork meat during 12 days storage in vacuum, MAP 1 (20% O_2, 50% CO_2 and 30% N_2) and MAP 2 (20% O_2, 30% CO_2 and 50% N_2) (Mean±SD)

Group	Day of storage				
	0	3	6	9	12
Vacuum	6.04±0.01	6.02AB±0.01	5.97A±0.02	5.90AB±0.01	5.79AB±0.01
MAP 1	6.04±0.01	5.92A±0.01	5.95B±0.03	5.97A±0.01	5.94A±0.01
MAP 2	6.04±0.01	5.90B±0.01	5.83AB±0.02	5.97B±0.01	5.92B±0.01

Legend: $^{A, B}$ Within a column, means with a common superscript letter are significantly different, $p<0.01$

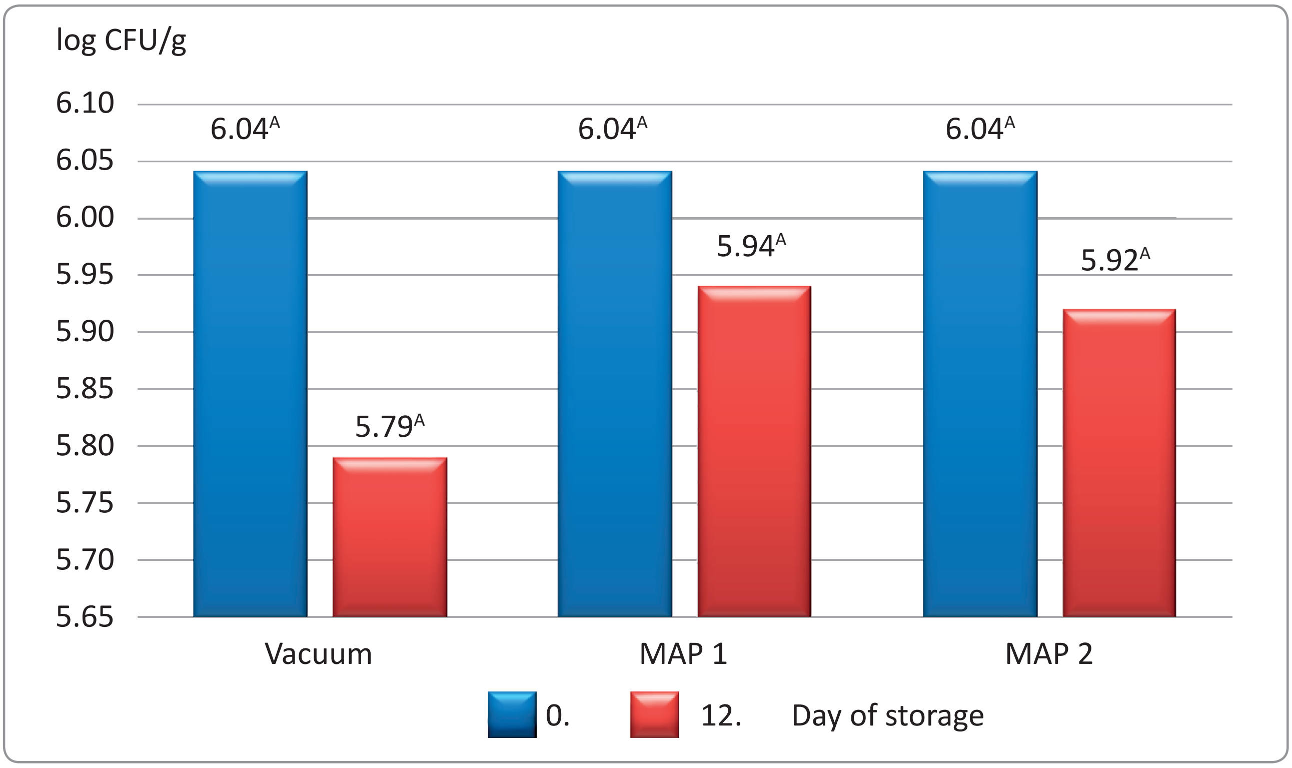

Figure 5. The pH values at the beginning and at the end of the study (A p<0.01)

The initial pH of the minced pork was 6.04±0.01 in all packaging types and decreased during the storage period (pH values were 5.79±0.01 in vacuum, 5.94±0.01 in MAP 1 and 5.92±0.01 in MAP 2 on day 12; Table 5). No significant differences occurred in pH between mince packed in vacuum, MAP 1 and MAP 2 during the first three days, but significant differences in pH were detected on days 3, 6, 9 and 12 of storage ($p<0.01$).

At the beginning of the study, the average pH of vacuum, MAP 1 and MAP 2 mince was statistically lower than the average pH of the same groups of minced meat at the end of the study (Figure 5).

In minced meat packaged in MAP, a slight decrease of average pH was noticed. In vacuum packaged minced meat, a slight decrease in average pH value occurred, which can be explained by the absence of CO_2. Similar results were reported by *Nissen et al*. (2000), *Juncher et al*. (2001), *Masniyom et al*. (2002), *Arashisar et al*. (2004), *Martínez et al*. (2005), *Yilmaz and Demirci* (2010), *Djordjevic et al*. (2016) and *Ivanovic et al*. (2015)

Conclusions

This study has shown that MAP with a high concentration of CO_2 is the better choice for packaging fresh pork meat than vacuum and packaging with a lower concentration of CO_2. The microbiological status (total viable count, *Enterobacteriaceae* and lactic acid bacteria) of raw minced pork meat packaged in MAP was acceptable compared to the vacuum samples. TVB-N value was acceptable until the day 12 of the storage period.

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