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The influence of modified atmosphere packaging on shelf life of ćevapčići

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Abstract. The aim of this study was to determine the microbiological, sensory and chemical changes of modified atmosphere packaged ćevapčići in a gas mixture consisting of 70% O₂ and 30% CO₂. Packaged ćevapčići were stored for 10 days at 3°C. Microbiological examination comprised determination of pathogenic microorganisms (*Salmonella* spp., coagulase positive staphylococci, *Escherichia coli* and *Listeria monocytogenes*) as well as indicators of hygiene and spoilage (total viable count, psychrotrophic bacteria, *Enterobacteriaceae*, lactic acid bacteria and *Brochothrix thermosphacta*). Using quantitative-descriptive test with grading scales from one to five, sensory properties of ćevapčići were assessed (color and odor in raw condition and odor, texture and taste after roasting) on the 1st, 4th, 6th, 8th and 10th days of storage. Regarding the chemical parameters, every day during the storage, pH was examined and on 4th and 8th days, acid value and peroxide number were established. On the basis of the results obtained and the recommended total viable count, which should not be higher than 7 log cfu/g, and taking into consideration sensory properties, we can conclude that ćevapčići packed in the modified atmosphere containing 70 % O₂ and 30% CO₂ had a shelf life of seven days.

1. Introduction

Due to its specific chemical composition and high water content, meat is one of the most perishable groceries. Spoilage can be defined as any change that makes food unacceptable for the consumer from a sensory point of view [1]. An unpleasant odor and taste can occur as a result of the growth of microorganisms, whereby intense sensory changes are associated with degradation of nutrients in meat and the emergence of undesirable volatile metabolites.

In fresh meat, about 7 log cfu/g of bacteria is responsible for the appearance of an unpleasant odor with a milky note, while a putrid odor is created by the decomposition of free amino acids when the bacteria population reaches 9 log cfu/g [2]. Under aerobic conditions, *Pseudomonas* spp. are the dominant species of microorganisms that cause meat spoilage, even at chill temperatures [3]. The ability of *Brochothrix thermosphacta* to grow in aerobic and anaerobic conditions classifies it in the group of microorganisms responsible for the appearance of unpleasant odors [4]. Bacteria of the genera *Serratia*, *Enterobacter*, *Proteus* etc., belonging to the family *Enterobacteriaceae*, as well as lactic acid bacteria, contribute to the spoilage process of chilled meat [5, 6]. Although microorganisms play a significant role in the occurrence of meat spoilage, the final assessment of changes is based on sensory analysis [3]. In assessing the shelf life of meat and meat products, it is necessary to apply a combination of microbiological, chemical and sensory analysis.

Modified atmosphere packaging (MAP) is used as an effective way to extend the shelf life and preserve the quality of fresh and minced meat [7, 8, 9, 10, 11, 12]. MAP is a type of packaging from



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which the air is completely removed, after which the newly formed vacuum is replaced with a single gas or mixture of gases. The most commonly used gases in a modified atmosphere are carbon dioxide (CO₂), oxygen (O₂) and nitrogen (N₂) [13]. They are used in various combinations, in which each of them has a role to play. Because of its ability to significantly extend the shelf life of the meat, MAP has become, over the last two decades, a significant and increasingly popular technology in the field of meat packaging for retail [7].

Color, oxidative changes of lipids and growth of microorganisms are the most important quality criteria to be evaluated regarding the storage of fresh meat. Packing meat in a modified atmosphere should prevent discoloration and oxidation of meat lipids, and at the same time, it should slow down the growth of microorganisms responsible for meat spoilage. That is why the most common modified atmosphere for packaging fresh meat consists of 70–80% O₂ and 20–30% CO₂ [14].

Use of high concentrations of oxygen inside packaging preserves meat's bright red color, which is the most important sensory characteristic of fresh meat in retail, but at the same time it can bring an increase in oxidative reactions and the occurrence of rancidity of fat in the meat, which causes an unpleasant odor and taste. Zakrys et al. (2008) [15] monitored the effect of packaging in a modified atmosphere on sensory properties of beef, and came to the conclusion that the meat packaged in a modified atmosphere with 50% O₂, 30% N₂ and 20% CO₂ has more desirable sensory properties than when other gas mixtures were used.

Carbon dioxide is a major antimicrobial factor in MAP, especially against Gram-negative bacteria, such as *Pseudomonas* spp. The effectiveness of this gas depends on its original and final concentration in packaging, the storage temperature and initial microbiota of the meat [7]. Higher CO₂ concentrations in gaseous mixtures favor the growth of lactobacilli, and reduce growth of *B. thermosphacta* [16].

In retail stores in Serbia, ćevapčići are very common as part of the range of meat products. They belong to the group of shaped minced meat products. However, ćevapčići packed in a modified atmosphere are rarely found on the Serbian market. The goal of this work was to establish the shelf life of packaged ćevapčići in a gas mixture consisting of 70% oxygen and 30% carbon dioxide. The microbiological, sensory and chemical characteristics of ćevapčići were monitored during cold storage.

2. Materials and Methods

Ćevapčići were made from beef and pork meat that were minced to a granulation of about 4 mm, with the addition of water, table salt, spices, emulsifying salts (sodium polyphosphate) and antioxidants (ascorbic acid). After shaping, ćevapčići were packaged in a modified atmosphere, ten pieces in one package. A Traysealer T-350 Multivac packaging machine was used, containers were Cryovac LidSys (Sealed, Air, USA) and top foil F-Type, Lid HB-S (manufactured by Spektar, Gornji Milanovac) with the following characteristics: degree of oxygen permeability (<15 cm³/m²/day, 20°C, 65% RH), moisture permeability (<50 g/m², 38°C, 90% RH). Packages were filled with a ready-made mixture of gases from the MESSER TEHNOGAS (70% O₂ and 30% CO₂). The amount of gas mixture in the package was 100–200 ml per 100 g of product. The same day, packaged ćevapčići were transported under cold chain conditions to the laboratory at the Institute of Meat Hygiene and Technology, where they were stored at 3°C for ten days.

2.1. Microbiological analysis

Samples of ćevapčići were microbiologically examined by the following methods:

- a) Total number of aerobic mesophilic and psychrophilic bacteria was determined by the SRPS EN ISO method 4833: 2008 (PCA, Merck) [17]
- b) Number of bacteria in the family *Enterobacteriaceae* was determined by the method SRPS ISO 21528-2: 2009 (VRBG, Merck) [18]
- c) The presence of *Salmonella* spp. was determined by the method SRPS ISO 6579: 2008 (BPW, MKTTN, RVS, XDL, Merck) [19]
- d) The number of *E. coli* was determined by the SRPS ISO method 16649-2: 2008 (TBX, Oxoid) [20]

e) The number of lactic acid bacteria was determined according to method ISO 15214: 1998 (MRS, Merck) [21]

f) The number of coagulase-positive staphylococci was determined according to method SRPS EN ISO 6888-2: 2009 (ETGP, Merck) [22]

g) The number of *Brochothrix thermosphacta* was determined by the method ISO 13722: 1999 (STAA agar, Oxoid) [23]

h) The number of *Listeria monocytogenes* was determined by the method SRPS EN ISO 11290: 2010 (Fraser broth base, Palcam agar, Oxoid) [24]

2.2. Evaluation of sensory properties

Sensory properties were evaluated using a quantitative descriptive test (SRPS ISO 6658, 2002) [25], with a scale from 1 to 5 (Table 1) (color and odor before thermal treatment, smell, texture and taste after thermal treatment) on the 1st, 4th, 6th, 8th and 10th days of the study. A group of five evaluators evaluated the sensory properties of the samples. Evaluators were previously chosen and trained by tests for determining the sense of taste (SRPS ISO 3972, 2002) [26] and training assessors in detection and recognition of odors (SRPS ISO 5496, 2002) [27].

Table 1. Quantitative-descriptive scale used for evaluation of sensory properties of čevapčići

Numerical score	Descriptive score
5	Exceptionally good
4	Very good
3	Acceptable
2	Barely acceptable
1	Unacceptable

2.3. Chemical analysis

The pH was determined by a standard method, SRPS ISO 2917/2004 (pH meter Cyber Scan 510) [28]. The pH of the samples was analyzed daily. The acid value and peroxide numbers were determined on the fourth and eighth days of the study, and the following methods were used:

a) SRPS ISO 660, 2000 – Oils and fats of vegetable and animal origin - Determination of acid number and acidity [29];

b) SRPS ISO 3960, 2001 – Oils and fats of vegetable and animal origin - Determination of peroxide number [30].

2.4. Statistical analysis

Test results (mean, measures variations, analysis of variance) were statistically processed using Microsoft Excel 2007.

3. Results and Discussion

Changes in the numbers of tested microorganisms in samples of čevapčići are shown in Figure 1.

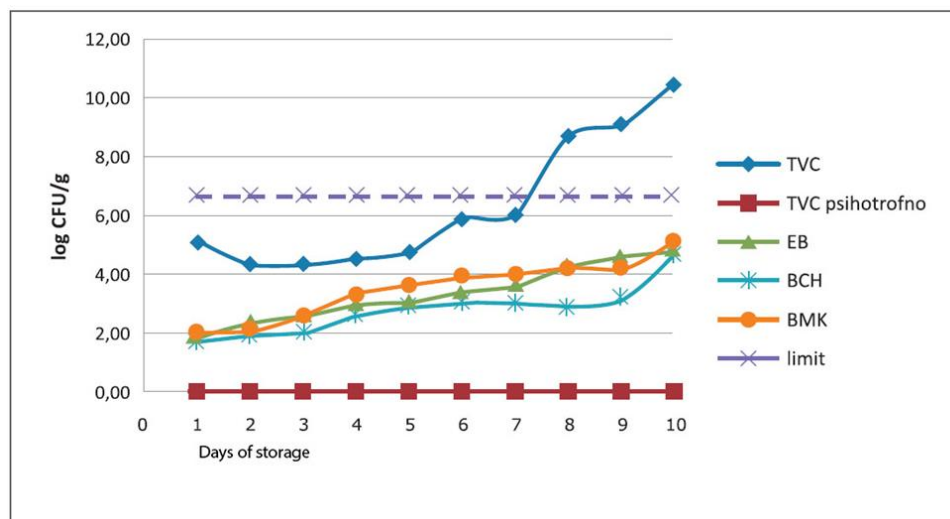


Figure 1. Changes of counts of various microorganisms in čevapčići during storage

TVC – Total count of aerobic mesophilic microorganism; TVC – Total count of aerobic psychrophilic microorganisms; EB – Bacteria of the *Enterobacteriaceae* family; BCH – *Brochothrix thermosphacta*; BMK – Lactic acid bacteria

In čevapčići during the storage period, no pathogenic microorganisms or growth of these organisms were detected (coagulase-positive *Staphylococcus*, *E. coli*, *L. monocytogenes*, *Salmonella* spp.). During storage of packaged čevapčići at 3°C for ten days, the number of aerobic mesophilic bacteria showed linear growth trend of 0.66 log cfu/g/day, where on the eighth day of storage, the growth became exponential and numbers exceeded the recommended eligibility limit of 7 log cfu/g (ICMSF, 1986) [31]. For *Enterobacteriaceae*, lactic acid bacteria and *B. thermosphacta*, gradual growth by the tenth day of storage was found. The total number of aerobic psychrophilic bacteria during the storage period did not exceed 1 log cfu/g. Ozlem et al. (2011) [12] found that modified atmosphere with 70% O₂ and 30% CO₂ favors the growth of lactobacilli and bacteria from the *Enterobacteriaceae* family in minced beef, and inhibits the growth of *Pseudomonas* spp. and *B. thermosphacta*. Ercolini et al. (2006) [32] studied growth of *Pseudomonas* spp., *B. thermosphacta*, lactobacilli and *Enterobacteriaceae* in fresh beef and found weaker growth of these microorganisms in samples packed in a modified atmosphere with 60% O₂ and 40% CO₂ relative to the samples stored in the air.

The results of the sensory evaluation of čevapčići are shown in Figure 2.

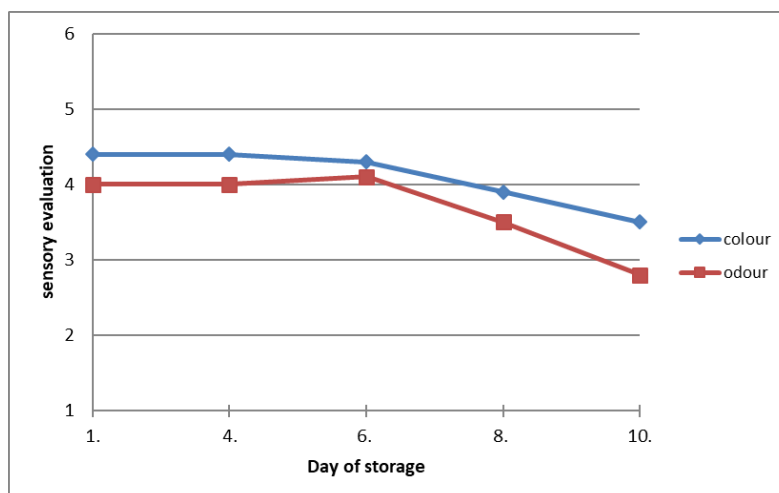
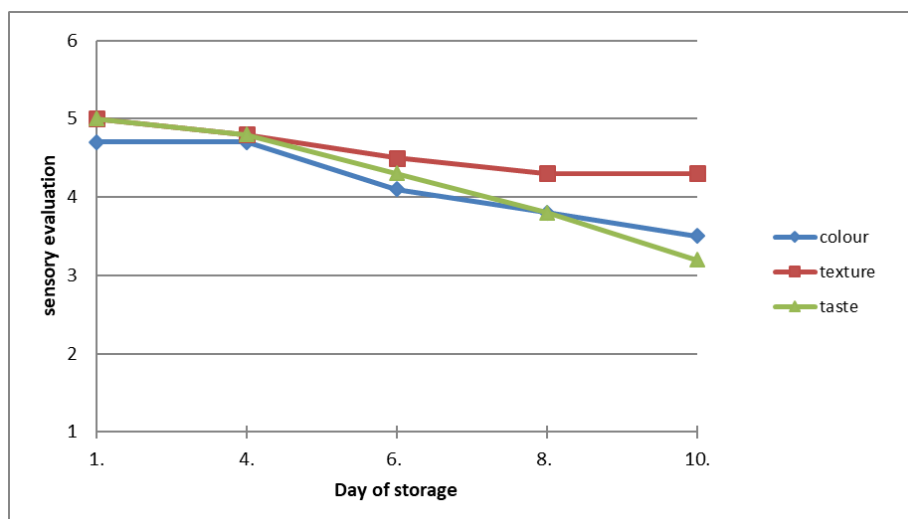


Figure 2. Changes of color and odor in ćevapčići during storage

The color and smell of ćevapčići were rated as very good (4.40 ± 0.22 and 4.00 ± 0.35 , respectively) at the start of storage. The smell of ćevapčići on the last day of storage was rated as acceptable (2.80 ± 0.22) while the color was rated higher (3.50 ± 0.41). Results of sensory evaluation of odor, texture and taste of ćevapčići after the thermal treatment are shown in Figure 3.

**Figure 3.** Changes of color, texture and taste in ćevapčići during storage

On the first and fourth day of the study, average odor grades after the thermal treatment were 4.70 ± 0.27 and 4.70 ± 0.22 , respectively. The odor was rated as exceptionally good. During the storage, the average odor grade decreased after the thermal treatment so that on the last day of storage, it was 3.40 ± 0.27 and was rated as acceptable. After the thermal treatment on the first, fourth and sixth days of storage, the texture of ćevapčići was rated high (5.00 ± 0.00 , 4.80 ± 0.27 and 4.50 ± 0.35 , respectively), while on the eighth and tenth days of storage, slightly lower grades were assigned for texture (4.30 ± 0.44 and 4.30 ± 0.41 , respectively). The taste of ćevapčići on the first and on the fourth days of storage was assessed as exceptionally good (5.00 ± 0.00 and 4.80 ± 0.27 , respectively), and on the sixth and eighth days, the taste scores were lower (4.30 ± 0.27 and 3.80 ± 0.27 , respectively). On the last day of storage, although rated lower (3.20 ± 0.25), the taste was still acceptable. Since the applied quantitatively descriptive scale defined grade 2.00 as the limit of acceptability, the taste scores obtained show the ćevapčići retained the desired sensory properties on the tenth day of storage.

The average pH of ćevapčići during the storage was 5.57 ± 0.08 with a coefficient of variation of 1.45%. Changes of pH in ćevapčići during the storage are shown in Figure 4.

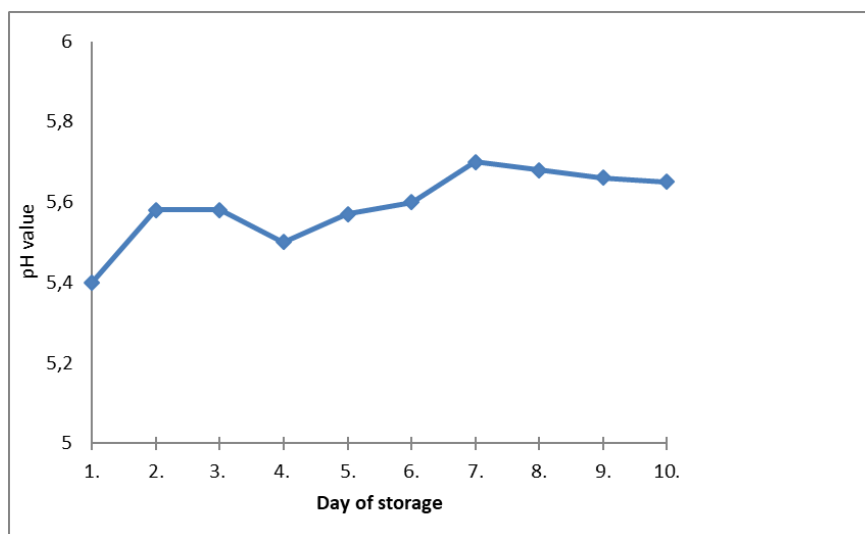


Figure 4. Changes of pH of ćevapčići during storage

In contrast to our results, Yilmaz and Demirci (2010) [10], for kebabs packaged in a modified atmosphere with 65% N₂ and 35% CO₂, found pH declined during the entire storage period. Acid value and peroxide number changes are shown in Table 2.

Table 2. Acid value and peroxide number

Examination day	Acid value	Peroxide number
4 th day	3.67 mg KOH/g	0.00 mmol/kg
8 th day	3.74 mg KOH/g	0.00 mmol/kg

On the eighth day of storage, the acid value indicated that no hydrolytic changes of lipids had occurred in samples relative to the fourth day of testing, while peroxide values showed that no oxidative changes had occurred in lipids. Ozlem et al. (2011) [12] concluded that packaging of minced beef in modified atmosphere with 50% O₂, 30% CO₂ and 20% N₂ achieves the lowest degree of lipid oxidation. Jakobsen and Bertelsen (2000) [33] found that temperatures below 4°C in fresh meat packed in a modified atmosphere prevent lipid oxidation, which is in accordance with our results.

4. Conclusion

The total number of aerobic psychrophilic bacteria during the storage period of ten days did not exceed 1 log cfu/g. Gradual growth of bacteria from the family *Enterobacteriaceae*, lactic acid bacteria and *B. thermosphacta* was found up to the tenth day of storage. Based on acid values and peroxide number, it can be concluded that no hydrolytic or oxidative changes of lipids occurred in ćevapčići during storage. Also, sensory evaluation did not identify any changes in terms of rancidity, and the ćevapčići were acceptable in terms of color, smell, taste and texture throughout storage. Based on the results, and especially based on the recommended total number of aerobic mesophilic bacteria, which should not exceed 7 log cfu/g, and on sensory characteristics, ćevapčići packaged in a modified atmosphere with 70% oxygen and 30% carbon dioxide were still acceptable after seven days' cold storage.

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