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INFLUENCE OF MODIFIED ATMOSPHERE PACKAGING ON CHEMICAL AND SENSORY CHANGES OF BEEF

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Abstract

Contemporary tendencies of meat distribution is that it is packed in the production place and so wrapped up, in cold chain, transport to the place for sale. This ensures that keeping the store showcases, be exposed to direct observations of the customer, and achieved its longer shelf life. As part of a comprehensive investigation of the project III 46009, according activities which refers to the extension of shelf life of meat, it was investigated influence of modified atmosphere packaging on chemical and sensory changes of beef.

In the experiment were used beef tenderloin (Simmental cattle slaughtered in abattoir), and after cooling a portion were packed in a modified atmosphere, while the other remained unwrapped, and then, in the cold chain, transported to the place of storage. Packaging conditions were: composition of the gas mixture - 70% O_2 , 20% CO_2 and 10% N_2 ; gas evacuation - 8 mbar; pressure gas mix - 1175 mbar; container - polystyrene, top foil welded at 130 °C. Meat was stored at temperatures below 4 °C. Dynamic of sample testing was: day 1 (after packaging), 7, 14 and 21. Customize sensory properties were conducted using quantitative - descriptive test, on the scale 1 - 5, by 5 evaluators. Chemicaly were examined $a_{w'}$ pH, peroxide number, acid number, TBA, TVB-N.

The results obtained indicate that the prolonged shelf life for beef tenderloin packed in a modified atmosphere were achieved. For unpackaged samples shelf life was 5 - 7days, while for samples packaged in modified atmosphere 12 - 15 days.

Key words: Beef, Modified atmosphere packaging, Shelf life.

1. Introduction

The consumer has an obvious interest in purchasing meat that shows no signs of spoilage. Packers have an interest in increasing the shelf-life as much as possible allowing them to market a product with a high quality and reducing costs for logistic. Extending the shelf life of fresh meat is very important consideration for both consumers and meat packers. The storage life of fresh meat can be prolonged by limiting the extent of discoloration, lipid oxidation and microbiological contamination.

Modified-atmosphere packaging (MAP) is a type of packaging that completely removes the air, after which the resulting vacuum is filled by one gas or mixture of gases. The gases that are mainly used in modified atmosphere packaging technology are carbon dioxide (CO_2) , oxygen (O_2) and nitrogen (N_2) [1]. They are used in different combinations, and each of them has its own role. While other gases, such as nitrogen oxide, sulphur dioxide, ethylene, chlorine, ozone and propylene oxide are used experimentally, they do not apply to MAP technology because of safety, regulations and cost of packaging [2].

Modified-atmosphere packaging of meat uses mixtures of three gases: oxygen, carbon dioxide and nitrogen. Their roles in the modified atmosphere are very different. While N_2 is an inert gas whose task is to prevent the collapse of the packaging, CO_2 has antimicrobial activity whose mechanism has still not been fully explained. For foods that are packaged in an atmosphere with higher concentrations of carbon dioxide, in addition to reducing the number of microorganisms, there was a change in the type of microorganisms. Very often a shift from Gram-negative to Gram-positive bacteria oc-



curs, such as streptococci and lactobacilli. Oxygen plays an important role in MAP, especially in the packaging of fresh meat [1]. The presence of oxygen maintains the myoglobin in meat in oxygenized form, oxymyoglobin, and, thus, gives the meat bright red color, acceptable to the consumer.

So, depending on the mixture, these gases have different effects on the growth of bacteria on the meat surface and the state of the colour pigment, myoglobin. The two main gas compositions are: high oxygen, moderate carbon dioxide for short storage life of 5 - 12 days; and very low oxygen, high carbon dioxide for extended storage life of 6 weeks [3].

Modified atmosphere packaging (MAP) of foods has gained considerable popularity as a modern method for packaging fresh meat [4]. The combination of CO_2 , N_2 and O_2 in modified atmosphere (MA) packs is able to suppress the aerobic spoilage flora of perishable foods, such as meat, fish and related products, and to sustain their visual appearance [5, 6].

The paper presents the results of changes in chemical and sensorial parameters, obtained in the second year of the project III 46009, relating to the use of modified atmosphere packaging (MAP) for extension of shelf life of beef meat (tenderloin) in retail store, where is held under the usual conditions for fresh meat (refrigerated showcases). As control samples were used unpackaged fresh meat held under the same conditions.

The aim of this study was to make a comparison of chemical and sensorial parameters of quality unpacked and packed chilled beef meat (tenderloin) in the conditions prevailing in industrial cutting of fresh meat, fresh meat manipulation through the logistic chain from slaughterhouse to retail store and storage in conditions of retail and exposure of the product to consumers by season (spring, summer, autumn).

2. Materials and Methods

Beef tenderloin used in the experiment, derived from 3 Simmental young cattle of average weight of 400 kg, which were slaughtered in an industrial slaughterhouse (Yuhor - Jagodina).

Sample preparation

As material in this experiment is used beef tenderloin (*m.longissimus dorsi parcs thoracis*). The muscles were removed from the animal carcasses after two days of chilling. Steaks were uniform thickness of 2 - 3 cm and weight between 400 - 500 g. Meat from different animals was used under the same conditions of chilling and trimming. Meat was divided into two groups and stored at the temperature 0 - 4 °C. Meat from the first group was not packed than just covered by plastic foil

(unpacked meat) and meat from the second group was packed under modified atmosphere (packed meat).

When planning the experiment there was a strong need for extended shelf life in the market, and hence it was necessary to use a material with good barrier properties to oxygen. For packaging of beef (tenderloin with bones) it is used the upper foil F type LID HB-S (Spektar - Gornji Milanovac): Oxygen Transmission Rate $(OTR) < 15 cm^3/m^2$, 24h, atm (determined by ASTM F 1297- 98 i ASTM D 3985 -95, na 20 °C i 65 % RH); Water Vapour Transmission Rate (WVTR) < 15g/m² (determined by ASTM E 96-00) and polystyrene containers (HIPS, LDPE and XPS) with EVOH layer, black, size 290 x 215 x 40, country of origin Italy. Packaging was done on the apparatus CAVECO LC1. Composition of the gas mixture was: 70% O₂, 20% CO₂ and 10% N₂. Evacuation of gas before blowing the gas mixture was performed at 8 mbar. The pressure of the gas mixture was 1175 mbar. Temperature of the welding of upper foil was 130 °C. The temperature in the package room was 12 °C and pressure of 1012 mbar.

Meat cutting and packaging of samples was performed within 44h after slaughtering of the animals. Samples were regularly transported by a vehicle with Thermo King to central storage in plastic cassettes in the afternoon and the following day, in the morning, to a retail store. Unpacked meat was stacked in rows in separate plastic cassetes, wrapped and protected by stretch wrap as a regular procedure packs. In retail (Maxisupermarket, Beograd), the samples were stored in refrigerated showcases, with artificial light, at a temperature of 0 - 2 °C, in real life retail store. After ending working times samples were stored in a warehouse at a temperature of 0 - 4 °C. Samples were in the cold chain, delivered to the laboratory of the Institute of Meat Hygiene and Technology, where they performed chemical and sensory testing after 1, 5, 7, 9, 12 and 15 days. These testings were conducted in three separate time cycles (spring, summer, autumn).

Chemical and physical - chemical analysis

Temperature and pH of carcasses on the slaughtering line and carcass cutting was performed using combined puncturing pH / thermometer (Testo 205, the accuracy of the pH meter, \pm 0.02 pH units, the accuracy of the temperature readings \pm 0.4 °C). Calibration of the pH meter before use was conducted using a buffer solution Reagecon, Shannon Co., Clare, Ireland, Product no. 10705, Lot No: 710B1, traceable to NIST standards.

During the storage of the meat, parameters that show hydrolytic and oxidative rancidity were determined. Acid number was determined by standard method SRPS EN ISO 660:2011 [7], peroxide value by standard method SRPS EN ISO 3960:2011 [8], and thiobarbituric acid reactive substances (TBARS) by method according



to Tarladgis *et al.* [9] and Holland [10]. pH value of samples was measured by laboratotory pH-meter (EUTECH Instruments, Holandija), according to standard method SRPS ISO 2917:2004 [11], and a value was measured by higrometer (GBX Scientific Instruments) according to standard method ISO 21807:2004 [12]. TVBN (total volatile basic nitrogen) wad determined according to the method proposed by Official Journal of the European Union (2005) [13].

Sensory evaluation

Sensory tests were conducted using quantitative descriptive test (SRPS ISO 6658, 2002 [14]), on the numerically - descriptive scale (Table 1), with scores of 1 to 5. It were evaluated the following sensory characteristics: appearance of meat, color of the meat surface, color of the meat on intersection, consistency, smell of fresh meat, smell of meat after cooking, color of meat after cooking, meat consistency after cooking and taste of meat after cooking. A group of six assessors were made a panel for the evaluation of sensory properties of the tenderloin samples. Assessors have previously tested their senses using the test to determine the sense of taste (SRPS ISO 3972, 2002 [15]) test for the ability of assessors to distinguish between different concentrations of solution model - triangle test (SRPS ISO 4120, 2002 [16]) and test for the training of assessors in the detection and recognition of odors (SRPS ISO 5496, 2002 [17]).

Table 1. Quantitative descriptive scale for assessing sensory properties studied:

Numerical grades	Levels of quality			
5	Exceptionally acceptable			
4	Very acceptable			
3	Acceptable			
2	Low acceptable			
1	Non acceptable			

Examinations were performed for unpacked beef cuts 1 and 5 days and for packed beef tenderloin 1st, 5th, 7th, 9th and 12th (after packing).

Each parameter was determined in six replications and results are expressed as average value with standard deviation (SD).

3. Results and Discussion

The determined pH-values of carcasses slaughtered animals were ranged from 6.60 to 6.76, and the temperature of carcasses were between 29 - 30 °C. After 44 h and the cooling determined pH values ranged from 5.40 to 5.60 which is consistent with the literature data [18]. Carcasses temperature, after cooling, were 2.5 - 3.1 °C.

Chemical parameters (TVB-N, Peroxide number, Acid number, TBARS, pH and aW) of shelf life of unpackaged beef tenderloin and beef tenderloin packed in MAP were presented in Table 2.

Acid number (Table 2) slighly increased during unpacked meat storage and ranged from 1.62 ± 0.04 mg KOH/g at the first day of storage to 1.73 ± 0.02 mg KOH/g at the 5th day. In packed meat, acid number was permanently increased during the first 12 days of storage and ranged from 1.59 ± 0.03 mg KOH/g at the first day of storage up to 3.41 ± 0.01 mg KOH/g at 12th day of storage. Acid number is the parameter that shows the first step in degradation of meat lipids and sign hydrolytic changes of lipids. It can not be used as only one indicator for meat rancidity and its increasing during the storage is common appearance. Value of acid number is linked with the moisture content in meat which contributes to lipolysis reactions [19].

Table 2. Chemical	parameters of	unpackaged	and pack-
aged beef tenderlo	oin		

	BEEF TENDERLOIN - UNPACKAGED								
Day	TVB-N mg/100g	Peroxide number <i>mmol/kg</i>	Acid number <i>mg KOH/g</i>	TBARS mg MAL/kg	рН	aW			
1.	20.50±0.39	0.00	1.62±0.04	0.28±0.03	5.72±0.01	0.979±0.001			
5.	22.92±1.01	0.00	1.73±0.02	1.27±0.02	5.82±0.03	0.972±0.001			
	BEEF TENDERLOIN in MAP								
	TVB-N mg/100g	Peroxide number <i>mmol/kg</i>	Acid number <i>mg KOH/g</i>	TBARS mg MAL/kg	рН	aW			
1.	24.0±0.28	0.00	1.59±0.03	0.45±0.04	5.71±0.01	0.966±0.001			
5.	28.3±0.26	0.00	1.75±0.04	1.53±0.06	5.80±0.01	0.978±0.001			
7.	29.76±0.52	0.74±0.03	2.11±0.04	1.91±0.02	5.71±0.04	0.977±0.001			
9.	32.37±0.51	2.95±0.02	3.22±0.03	3.51±0.04	5.66±0.01	0.981±0.001			
12.	32.19±0.27	0.00	3.41±0.01	4.70±0.04	5.61±0.02	0.983±0.001			

Peroxide value (Table 2) was not detected at the first and 5th day of the storage in both unpacked and packed meat (0 mmol/kg). The highest peroxide value was detected at 9th day of storage of packed meat (2.95 \pm 0.02 mmol/kg). Peroxide value is connected mostly with pH value of meat. If pH value is closer to neutral point, it is favourable conditions for oxidation [20]. According to Khaksar *et al.* [21], the amount of hydroperoxide increased more rapidly at pH = 6.8 than pH = 3.

In unpacked meat, TBARS were 0.28 \pm 0.03 mg MAL/kg (1st day) and 1.27 \pm 0.02 mg MAL/kg (5th day). In packed meat, TBARS were permanently increasing and ranged from 0.45 \pm 0.04 mg MAL/kg (1st day) up to 4.70 \pm 0.04 mg MAL/kg (12th day), except at 7th day of storage, when it was decreased (1.91 \pm 0.02 mg MAL/kg).



The degree of oxidation of meat is generally assessed by measuring the content of secondary degradation products that arise from oxidation of polyunsaturated fatty acids. The analytical method uses substances that react with thiobarbituric acid (TBARS value) as a measure of the degree of this oxidation. A higher TBARS value indicates a greater degree of oxidation of meat. The highest content of TBARS of 4.70 ± 0.04 mg MAL/ kg in packed meat was detected in beef meat at 12th day of examination. TBARS is one of the products derived from peroxide decomposition and has the potential for reaction with other components [22]. Changes of TBARs are related to peroxide value. Simultaneous increase of TBARs and peroxide value at 9th day for packed meat was probably due to the partial decomposition of peroxide beside its formation, which resulted in an increase in TBARS and that is in accordance to results showed in the study of Khaksar et al. [21]. The highest content of TBARS of 4.70 \pm 0.04 mg MAL/kg in packed meat (12th day of examination) is probably in relationship with an intensive degradation of peroxides at the same time (12th day - peroxide number 0 mmol/kg). According to Wong et al. [23], the amounts of 3 mg MAL/kg is the critical value at which rancidity is detected. Increased levels of TBA-reactive substances (TBARS) were observed in samples of packed meat at 9th and 12th days of storage and that is probably in relationship with changes determined by sensory evaluation.

It is known that during storage the oxidative processes that occur in both lipid and protein fractions of meat are one of the major causes for changes in its quality parameters. Lipid oxidation is often responsible for quality loss via formation of rancid flavor and is affected by the duration and temperature of storage of meat [24] as well as the presence of oxygen. During chilled and frozen storage lipid oxidation is usually slow, but does not stop since the reactive species are soluble in the lipid fraction and stable at low temperature [25].

Lipid oxidation causes a rancid off-flavour and offodour in meat and it is initiated in muscle systems at the membrane level in the intracellular phospholipids fractions. Many factors affect lipid oxidations: light, temperature, oxygen concentration, degree of unsaturation of the fatty acids and the presence of enzymes [26]. Among these factors, fatty acid structure of muscle is the most important because it affects the number and the proportion of the produced hydroperoxides [27]. According to Zhao et al. [28], lipid oxidation has slower increasing than microbial growth and discoloration and it is not considered to be a limiting factor for shelf life of aerobic packed meat. Increased lipid oxidation has been reported for meat stored at elevated oxygen concentrations [29]. Lipid oxidation does not only contribute to off-flavour, but it is also essential to the typical aroma for many meat products [30].

Total volatile basic nitrogen (TVB-N) content is an important reference index for evaluating meat freshness. In unpacked meat, TVB-N were lower at the first day ($20.50 \pm 0.39 \text{ mg}/100$ g) and at the 5th day of examination ($22.92 \pm 1.01 \text{ mg}/100$ g) in comparison with packed meat ($24.0 \pm 0.28 \text{ mg}/100$ g and $28.3 \pm 0.26 \text{ mg}/100$ g, respectively). During the investigated study period, TVB-N in packed meat showed a permanent increase and ranged from $24.0 \pm 0.28 \text{ mg}/100$ g (12th day of storage) up to $32.19 \pm 0.27 \text{ mg}/100$ g (12th day of storage). Similary to results of Sunki *et al.* [31], obtained results in our study showed that the increase of TVB-N was more rapid during the later part of storage, indicating that the rate of protein degradation was faster as storage time was prolonged.

Recently TVB-N limit values of ca. 20 and 30 mg N/100 g for beef and pork (corresponding to 8 and 10 days of refrigerated storage, respectively) have been proposed as indicators of meat freshness and shelf-life [32]. Total volatile basic nitrogen is a product of bacterial spoilage and often used as a chemical index to assess the quality and shelf-life of seafood products [33]. Because ammonia production increases due to the de-amination of amino acids during spoilage, TVB-N has been proposed as an index of fresh meat quality and maximum acceptability limit values between of 20 and 30 mg N/100 g have been suggested for beef and pork, respectively [34]. But, in the same study [34] it was suggested that stored meat (beef, pork) is not necessarily unpalatable until the TVB-N value reaches 30 mg N/100 g.

The pH values increased during the first 5 day (5.80±0.01) and then decreased up to the end of storage (5.61 ± 0.02). Decrease in pH-values is resulted of high CO₂ content in the MAP meat packaging. The present CO₂ dissolves into the water and fat phases of the meat and associates with H+ to form carbonic acid [35] causing a pH decrease. On the other hand, the pH value can be affected by many factors; however, the lactic acid production, due to the lactic acid bacteria growth, is the major responsible factor for pH decrease in packaging.

The results of testing the sensory properties of beef tenderloin (packaged and unpackaged) are shown in Table 3. Results are expressed as the average score of all assessors, participants in the panel assessing. Evaluations of acceptability of samples was done in accordance with Article 25 of the Regulation of the meat quality of livestock for slaughtering, poultry and game [36].

As shown in Table 3 unpackaged beef samples after 7 days of storage, were evaluated as unacceptable, because of the very altered sensory properties. After 7 days on the surface of beef tenderloin samples is determined strange smell, with the changes that are indicative of the beginning of the fault. The surface of meat

Tested	U	npacked mea	at	Packed meat					
property	1st day	5th day	7th day	1stday	5th day	7th day	9thday	12th day	15th day*
Meat appearance	5.00±0.00	4.20±0.11	1.00±0.00	5.00±0.00	4.73±0.32	4.33±0.32	4.13±0.45	3,75±0.42	1.00±0.00 (2.80±0.45)
Meat surface color	5.00±0.00	4.30±0.33	1.00±0.00	5.00±0.00	4.60±0.28	4.40±0.28	3.87±0.52	3.45±0.52	1.00±0.00 (2.6±0.42)
Meat color on intersection	5.00±0.00	4.43±0.36	1.00±0.00	5.00±0.00	4.33±0.38	4.23±0.26	4.00±0.32	3.40±0.55	1.00±0.00 (2,6±0.42)
Consistency	5.00±0.00	4.87±0.25	1.00±0.00	5.00±0.00	4.90±0.16	4,87±0.33	4.10±0.42	4.00±0.47	1,00±0.00 (3.2±0.99)
Fresh meat smell	5.00±0.00	4.53±0.38	1.00±0.00	5.00±0.00	4.53±0.47	4.30±0.55	3.40±0.45	3.15±0.55	1.00±0.00 (2.5±0.35)
Meat smell after cooking	5.00±0.00	4.90±0.22	1.00±0.00	5.00±0.00	4.70±0.35	4.63±0.37	3.63±0.46	3.35±0.55	1.00±0.00 (2.8±0.27)
Meat color after cooking	5.00±0.00	4.83±0.24	1.00±0.00	5.00±0.00	4.80±0.27	4.57±0.39	4.03±0.56	3.50±0.43	1.00±0.00 (3.0±0.35)
Meat consistency after cooking	5.00±0.00	4.90±0.14	1.00±0.00	5.00±0.00	5.00±0.00	4.83±0.30	4.10±0.47	3.40±0.56	1.00±0.00 (3.1±0.90)
Meat taste after cooking	5.00±0.00	4.90±0.22	1.00±0.00	5.00±0.00	4.80±0.27	4.57±0.37	3.93±0.59	3.20±0.42	1.00±0.00 (2,8±0.27)

Table 3. The average value of the sensory properties of tested beef tenderloin

*in parentheses are given the values for the 15th day of testing in the third cycle

is a little bit sticky. After the probe cooking, the smell is atypical. Due to these changes evaluation of taste was not performed.

For samples of packed beef during the seven days of storage, sensory evaluation showed very high values, with an aggregate score of the samples even over 4.5, that mean exceptionally acceptable. Changes of conducted sensory parameters in packed samples of beef tenderloin are minimal, unlike the unpackaged samples that were eliminated 7th day of storage. Visible changes in sensory quality of packaged beef samples started after 9 days of storage, but even 12th day samples were still acceptable. Under the acceptability it is supposed that the surface of the sample, as in the central regions, has odor characteristic of beef, without impurities. Muscle tissue is red, associated fat is creamy white, characteristically consistency. After the rehearsal cooking, smell and taste are pleasant, characteristic for the type of meat.

In the 3rd test cycle (autumn to winter transition) it is achieved excellent results as they packed beef samples were acceptable after 15 days of storage. A possible explanation is that the handling and transport of samples occurred at relatively low air temperatures, which were further prevented (slowed) sensory changes.

4. Conclusions

- Acid number, peroxide value and TBARs, as well as TVB-N, pH value and aw value are important factors that influence meat shelf life and their highest values shows the moment when fresh meat both unpacked and packed is not more shelf stable, according to contribution of sensory evaluation.
- When the results obtained for the chemical pa-

rameters and sensory properties are taken into accounts, it can be concluded that the shelf life of the unpacked tenderloin was 5 days and for MAP packed tenderloin 12 days.

 Further studies are needed since limited information is available to date, with regard to establishing acceptability limit values of volatile amines, such as TVB-N that could be used as potential indicator in predicting the freshness and quality of beef meat.

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