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# MICROBIOLOGICAL STABILITY OF SELECTED BEEF ELEMENTS, SUBJECTED TO TECHNOLOGICAL PROCESSES AND STORED UNDER AEROBIC AND VACUUM CONDITIONS AT 5°C

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**Introduction.** The longer storage period is required to obtain beef meat of an adequate tenderness. Its microbiological condition is determined by such factors as type of the meat, the packaging method, as well as the applied technological processes. The aim of this paper was to compare the concentration of microorganisms (aerobic bacteria, lactic acid bacteria and bacteria of the family *Enterobacteriacea*) in the *longissimus dorsi* muscle (rump cut) and in the *semimembranosus dorsi* muscle (heel of round), stored at 5°C and in both aerobic and vacuum conditions. The brine containing 1% NaCl or 1% NaCl and 0.3% pentasodium triphosphate E 451 was applied to improve the meat tenderness.

Material and methods. The types of beef meat: rump cut (R) and the heel of round (L) were used as the materials in this experiment. The meat was cured either using the brine A, containing 1% NaCl in total weight, or the brine B, containing 1% NaCl and 0.3% pentasodium triphosphate E 451 (including 56% P<sub>2</sub>O<sub>5</sub>). The samples were either vacuum packaged (P) or left in open containers (T) and cold stored (5°C) for 1, 3, 7, 10 and 15 days. The total amount of mesophilic aerobic bacteria, the amount of lactic acid bacteria, the amount of bacteria of the family *Enterobacteriaceae* (including coliform bacteria and *E. coli*) and pH value were determined after each period of the storage.

**Results.** Comparing to aerobic storage, the vacuum storage confined the growth of microflora in the beef meat, with the biggest effect found in case of aerobic bacteria. The higher concentration of lactic acid bacteria was found in the period up to 10th day of the storage in comparison with the beef stored in aerobic conditions. In the longer period, the differences depend on the type of the muscle and the type of the applied brine. Higher concentration of bacteria of the family *Enterobacteriacea* in vacuum packed meat was found in the heel of round than in the rump cut.

**Conclusions.** The microbiological contamination of beef stored at 5°C is statistically significantly affected by not only the time of storage, but also the type of the meat elements, as well as the applied technological processes.

Key words: beef, salting, vacuum packaging, microbiological stability

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#### INTRODUCTION

The beef is produced first of all for consumption purposes, with the tenderness being one of the most important quality attributes evaluated by the consumer. It is modified by many factors both during the animal's life and by post-slaughter changes, occurring during meat ageing [Houbak et al. 2008, Kołczak 2008, Iwanowska et al. 2010, Iwanowska and Pospiech 2010]. A longer storage time under cold storage conditions (10-14 days) [Krasnowska et al. 2005] is required to obtain an adequate tenderness of beef. This is connected with a potential increase of the microbiological contamination. At a temperature of 0-5°C microorganisms proliferate at a slower rate (a longer generation time) and the composition of the microflora changes from the mesophilic to psychrotrophic and psychrophilic one. The spoilage of meat and meat products is caused by bacteria from the genus Pseudomonas, which are typically predominant under these conditions and form lipases and proteinases. These enzymes cause irreversible changes of the quality, which become evident when the bacterial count is approx. 10<sup>7</sup>-10<sup>9</sup> cfu/g [Rosiak and Kołożyn-Krajewska 2005]. Bacteria from the genus *Pseudomonas* belong to aerobic microorganisms, the growth of which may be reduced by the elimination of oxygen from the medium. A commonly applied method of vacuum packaging limits the access of oxygen to meat during the storage. The stability of vacuum packaged meat depends on many factors, among others microbial contamination before packaging process, the size of packaged elements, pH value, the level of vacuum or the storage temperature. In some cases, next to the oxygen removal, other preserving procedures are also applied, such as e.g. an addition of the lactic acid or lactic acid bacteria and therefore the different shelf life lengths for vacuum packaged beef is given by different authors, ranging from approx. a dozen days to approx. a dozen weeks [Blixt and Borch 2002, Signorini et al. 2006, Crowley et al. 2010, Bilska 2011].

The aim of the presented study was to compare the concentration of microorganisms (aerobic bacteria, lactic acid bacteria and bacteria from the family *Enterobacteriacea*) in the *longissimus dorsi* muscle – rump cut, and in the *semimembranosus dorsi* muscle – heel of round stored at a temperature of 5°C under aerobic and vacuum storage conditions. In order to improve meat tenderness brine was introduced, containing 1% NaCl, as well as 1% NaCl and 0.3% pentasodium triphosphate E 451i.

## MATERIAL AND METHODS

Material for analyses comprised beef: rump cut (R) and heel of round (L). Meat was cured either with the brine A, containing 1% NaCl in the total weight or the brine B, containing 1% NaCl, 0.3% pentasodium triphosphate E 451i (including 56%  $P_2O_5$ ). The brine was introduced at 20% in relation to raw material weight. Samples were left in open containers (aerobic conditions) or vacuum packaged using a Multivac type A 300/16 device, generating 98% vacuum. The following sample variants were applied:

- LAT: heel of round with the brine A, stored under aerobic conditions
- LAP: heel of round with the brine A, stored under vacuum conditions
- LBT: heel of round with the brine B, stored under aerobic conditions
- LBP: heel of round with the brine B, stored under vacuum conditions
- RAT: rump cut with the brine A, stored under aerobic conditions

- RAP: rump cut with the brine A, stored under vacuum conditions
- RBT: rump cut with the brine B, stored under aerobic conditions
- RBP: rump cut with the brine B, stored under vacuum conditions.

Samples were stored under cold storage conditions (5°C) for 1, 3, 7, 10 and 15 days and the following parameters were determined after each period of the storage:

- the total amount of mesophilic aerobic bacteria on nutrient agar medium according to PN-A-82055-6:1994
- the amount of lactic acid bacteria on MRS-agar medium according to PN-A--82055-17:1997
- the amount of bacteria from the family *Enterobacteriaceae*, including coliform bacteria and *E. coli* on Violet Red Bile Glucose Agar according PN-A-04023: 2001
- pH value.

The media were produced by BTL Company in Łódź, Poland. All analyses were performed in triplicate.

The results were analysed statistically. The interpretation of the results was performed at the significance level  $\alpha = 0.05$ . The three-variant analysis of variance ANOVA and Tukey's multiple comparison test were performed. All calculations were conducted using STATISTICA 8.0 and Excel software.

#### RESULTS AND DISCUSSION

Experiments were conducted on two muscles collected from beef carcasses and typically used for eating purposes. The muscles used in experiments were of different location in the carcass and of the different function performed in the musculature. During the animal's lifetime the rump cut is responsible for the maintenance of balance, while the heel of round is a dynamic muscle. The type of muscle, from which meat cut originates and the functions played by it during the animal's lifetime, determine its chemical composition. For this reason, differences may be expected in the rate of microbial growth during the meat storage.

### Changes in the total count of aerobic bacteria

Table 1 presents results of determinations of total amounts of aerobic bacteria in the analysed samples. After one day, the log amount of mesophilic aerobic bacteria ranged from 3.71 to 5.63, while in case of the heel of round, bigger numbers of aerobic bacteria were recorded in vacuum packaged samples than in samples stored without venting, with the differences being statistically significant, irrespective of the type of the applied brine. In turn, no such differences were shown between the log amount of aerobic bacteria determined in the rump cut stored under oxygen access conditions and under vacuum conditions. The dynamics of proliferation for aerobic bacteria in the heel of round stored under aerobic conditions was similar, irrespective of the fact whether the meat sample contained the brine A or the brine B. Only after 7 days, the LBT sample was more contaminated with the analysed microorganisms than LAT, while during the other storage periods (the 3rd, 10th and 15th days) no statistically significant differences were found.

	Time of storage, days / conditions										
Sample	1		3		7		10		15		
	T	P	T	P	T	P	T	P	T	P	
LA	4.11 <sup>bc</sup> ±0.00	4.42 <sup>d</sup> ±0.02	6.46 <sup>hi</sup> ±0.09	5.93g ±0.04	7.34 <sup>n</sup> ±0.00	$6.88^{jkl} \\ \pm 0.13$	9.25 <sup>p</sup> ±0.03	6.90 <sup>jkl</sup> ±0.07	10.11 <sup>s</sup> ±0.00	6.66 <sup>ij</sup> ±0.11	
LB	$3.71^{a} \pm 0.02$	$^{5.63^{\rm f}}_{\pm 0.04}$	$6.50^{i} \pm 0.13$	$5.63^{j} \pm 0.04$	7.91° ±0.02	$^{6.93^{kl}}_{\pm 0.04}$	$9.18^{p} \pm 0.10$	$7.02^{lm} \\ \pm 0.04$	$9.95^{rs} \pm 0.01$	$\begin{array}{c} 6.71^{ijk} \\ \pm 0.10 \end{array}$	
RA	$4.23^{bcd} \\ \pm 0.16$	$^{4.29^{cd}}_{\pm 0.09}$	$^{6.87^{jkl}}_{\pm 0.10}$	5.20° ±0.01	$9.11^{p}$ $\pm 0.00$	$^{5.71^{fg}}_{\pm 0.03}$	$9.79^{r} \pm 0.07$	$6.22^{h} \pm 0.03$	$10.10^{s} \pm 0.03$	$6.53^{i} \pm 0.03$	
RB	$4.24^{bcd} \pm 0.02$	4.03 <sup>b</sup> ±0.03	$7.33^{n} \pm 0.04$	5.11e ±0.04	$7.25^{mn} \pm 0.00$	$5.87^{fg} \pm 0.04$	8.00° ±0.03	$6.54^{i}$ $\pm 0.03$	$9.87^{rs} \pm 0.06$	$^{6.87^{ikl}}_{\pm 0.01}$	

Table 1. Total aerobic bacteria count ( $\log_{10}$  cfu·g<sup>-1</sup>) in beef stored at 5°C (x ±s), N = 3

Explanations: L - heel of round, R - rump cut, A - brine containing 1% NaCl, B - brine containing 1% NaCl and 0.3% pentasodium triphosphate E 451i, T - sample storaged in aerobic conditions, P - sample storaged in vacuum conditions, x - mean value from three replicates, s - standard deviation, a-s - the same letter symbols indicate no significant differences according to Tukey's test (p = 0.05).

The pattern of changes in the amount of aerobic bacteria in the rump cut was different: between the 1st and 3rd day bacteria multiplied faster in the sample with brine B, in the successive periods (the 7th and 10th days) – in the sample with brine A, while after 15 days the statistical analysis of results of microbiological analyses did not show statistically significant differences between the amount of aerobic bacteria in 1 g rump cut with the brine A and B.

Due to the low oxygen content (2%), the rate of proliferation of aerobic bacteria in vacuum packaged samples was slower and, as a result 3 days after the packaging the amount of these microorganisms in vacuum stored samples was always lower than under aerobic conditions, irrespective of the type of the meat and the brine. Results of microbiological analyses indicate that, there were more aerobic bacteria in 1 g of the heel of round than in 1 g of the rump cut, irrespective of the type of the applied brine, while statistically significant differences were not shown only as late as after the 15th day of storage. Bacteria multiplied at a slower rate (from 5.63 log cfu/g after 1 day to 6.71 log cfu/g after the 15th day) In the heel of round with the brine B than in that with the brine A (from 4.42 log cfu/g after the 1st to 6.66 log cfu/g after the 15th day). The growth of aerobic bacteria in the rump cut was similar throughout the entire storage period, irrespective of the type of the applied brine: a difference was found only after the 10th day, when the RBP sample contained statistically significantly more of these microorganisms than RAP.

Equations are presented below for straight lines, describing the rate of growth for bacterial amounts in individual samples, on the basis of which value d was established, i.e. time (days) during which the bacterial amount in 1 g sample increased ten times.

- 1.  $y_{LAT} = 0.4061x + 4.5304$ ,  $R^2 = 0.916$ , d = 2.462.  $y_{LAP} = 0.1409x + 5.1437$ ,  $R^2 = 0.5634$ , d = 7.103.  $y_{LBT} = 0.4121x + 4.4829$ ,  $R^2 = 0.8706$ , d = 2.434.  $y_{LBP} = 0.0966x + 5.6885$ ,  $R^2 = 0.5985$ , d = 10.35

- 5.  $y_{RAT} = 0.3950x + 5.1763$ ,  $R^2 = 0.80$ , d = 2.53

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6. y_{RAP} = 0.1504x + 4.5071, R^2 = 0.8999, d = 6.65
7. y_{RBT} = 0.3274x + 4.9806, R^2 = 0.8131, d = 3.05
8. y_{RBP} = 0.1945x + 4.2835, R^2 = 0.9013, d = 5.14.
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Conducted analyses showed that the vacuum packaging, in comparison to the storage under aerobic conditions, reduced the growth rate for aerobic bacteria in the meat (equations 1-8).

The number of bacteria under aerobic conditions increased ten-fold after a roughly identical storage time: d = 2.43 - 3.05 days (equations 1, 3, 5, 7). A ten-fold increase in the count of aerobic bacteria under vacuum storage conditions in 1 g rump cut occurred in time d = 6.65 days (rump cut with brine A – equation 7) and d = 5.14 days (rump cut with brine B – equation 8), i.e. faster than in the heel of round, in which the values d amounted to 7.10 (heel of round with the brine A – the equation 2) and 10.35 (the heel of round with the brine B – equation 4).

The investigations conducted by Crowley et al. [2010] also showed a slower proliferation of aerobic bacteria in the beef stored in vacuum conditions in comparison to that stored under aerobic conditions. However, in comparison to the results recorded in the presented study, the log value of total amounts of aerobic bacteria during storage at 5°C was lower both in case of aerobic conditions and in vacuum conditions, amounting to 5.09 and 2.49 (the 7 day) and 7.16 and 3.91 (the 10th day), respectively. In turn, investigations conducted by Blixt and Borch [2002] showed that after two weeks of the storage of the comminuted beef (entrecôte and beef strip loin) at a temperature of 4°C under vacuum conditions the amount of aerobic bacteria varied depending on the type of retail cuts and it amounted to 6-7 log cfu/g. The high contamination of the beef with aerobic bacteria was also found in a study by Khalafalla et al. [2010], in which the beef contained  $4 \times 10^8$  (aerobic conditions) and  $7 \times 10^7$  (vacuum packaged conditions) of these bacteria in 1 g after 11 days of storage at a temperature of 5°C.

### Changes in counts of lactic acid bacteria

The lactic acid bacteria in fresh meat are found in small numbers, whereas they become predominant microflora under vacuum conditions and their amount is at least  $10^7$  cfu/g. On the one hand, it is believed that lactic acid bacteria have a slight effect on sensory changes and vacuum packaged meat may be kept for 3-4 weeks at  $0^{\circ}$ C [Labadie 1999]; on the other hand, bacteria belonging to this group are considered to be the main reason of the spoilage of vacuum packaged meat and poultry through the formation of the slime and an undesirable change of taste [Huis in't Veld 1996].

The results of determinations of lactic acid bacteria amounts in the beef are presented in Table 2. These microorganisms belong to the class of facultative anaerobes and grow very well at a limited availability of the oxygen, thus a higher amount of these bacteria is found in vacuum packaged samples. Despite the fact that the amount of bacteria in 1 g vacuum packaged heel of round after 1 day of storage was statistically significantly different depending on the type of the applied brine, during the successive periods of storage such differences were not found. In case of rump cut no statistically significant differences were also found between the concentration of lactic acid bacteria in samples containing the brine A and B. The statistical analysis showed that after 15 there were more lactic acid bacteria days in 1 g heel of round than in 1 g rump cut.

	Time of storage, days / conditions										
Sample	1		3		7		10		15		
	T	P	T	P	T	P	T	P	T	P	
LA	1.00 <sup>a</sup> ±0.00	4.42 <sup>fg</sup> ±0.03	2.15 <sup>bc</sup> ±0.15	5.23 <sup>ijkl</sup> ±0.11	4.00 <sup>fg</sup> ±0.00	6.1 <sup>mnop</sup> ±0.07	5.26 <sup>ijkl</sup> ±0.20	6.74 <sup>prsl</sup> ±0.07	7.10 <sup>st</sup> ±0.18	7.87 <sup>t</sup> ±0.99	
LB	$^{1.00^a}_{\pm 0.00}$	$\begin{array}{c} 5.4^{jklm} \\ \pm 0.09 \end{array}$	$^{1.50^{ab}}_{\pm 0.71}$	$\begin{array}{c} 5.4^{jklm} \\ \pm 0.17 \end{array}$	$^{3.65^{ef}}_{\pm 0.06}$	5.9 <sup>lmno</sup> ±0.06	$\begin{array}{c} 5.18^{ijkl} \\ \pm 0.02 \end{array}$	$6.85^{rs} \pm 0.07$	$\begin{array}{c} 6.1^{mnopr} \\ \pm 0.02 \end{array}$	$7.86^{t} \\ \pm 0.99$	
RA	$^{1.00^a}_{\pm 0.00}$	$3.67^{ef} \pm 0.05$	$^{1.50^{ab}}_{\pm 0.71}$	$\begin{array}{c} 4.64^{ghi} \\ \pm 0.04 \end{array}$	$^{2.58^{cd}}_{\pm 0.28}$	$\begin{array}{c} 5.11^{hijk} \\ \pm 0.01 \end{array}$	$3.93^{fg} \pm 0.05$	$\begin{array}{c} 6.2^{nopr} \\ \pm 0.01 \end{array}$	$\begin{array}{c} 5.72^{lmn} \\ \pm 0.02 \end{array}$	6.58 <sup>oprs</sup> ±0.01	
RB	$^{1.00^a}_{\pm 0.00}$	$^{3.82^{\rm f}}_{\pm 0.03}$	$2.09^{bc} \pm 0.07$	$\begin{array}{c} 4.90^{hij} \\ \pm 0.04 \end{array}$	$^{2.97^{de}}_{\pm 0.010}$	$5.4^{jklm} \pm 0.01$	$\begin{array}{c} 4.37^{fgh} \\ \pm 0.02 \end{array}$	6.55 <sup>oprs</sup> ±0.03	$6.56^{oprs} \\ \pm 0.02$	$6.72^{prs} \\ \pm 0.01$	

Table 2. Lactic acid bacteria count ( $\log_{10} \text{ cfu} \cdot \text{g}^{-1}$ ) in beef stored at 5°C (x ±s), N = 3

Explanations – as in Table 1.

The heel of round with the brine A stored with no limitation to oxygen availability was characterised by a higher concentration of lactic acid bacteria than rump cut with this brine throughout the entire period of the storage (no differences were found only after 3 days). The heel of round with brine B was more contaminated with lactic acid bacteria after 10 days of storage than rump cut. Studies showed that up to day 10 the amount of lactic acid bacteria both in the heel of round and in the rump cut was not statistically significantly varied depending on the type of the brine. In turn, after the 15th day the LA sample was more contaminated than LB, while the RB sample was more contaminated than RA.

The variation in the contamination of the vacuum stored beef, depending on the type of retail part, was also showed in a study by Blixt and Borch [2002]. In this case the amount of lactic acid bacteria in entrecôte was 6 log cfu/g after 2 weeks at a temperature of 4°C, while in beef strip loin it was 7 log cfu/g. A slower growth rate of lactic acid bacteria was shown in case of comminuted goat meat, stored in vacuum under cold storage conditions, as the amount of these bacteria after 2 weeks was 3.8 log cfu/g [Babii et al. 2000].

The lactic acid bacteria in vacuum packaged samples proliferated at different rates in comparison to those stored under aerobic conditions. A list of equations of straight lines is presented below, describing growth rate of amounts of lactic acid bacteria for individual samples and thus established values d (days):

- 9.  $y_{LAT} = 0.4116x + 1.0263$ ,  $R^2 = 0.9966$ , d = 2.43
- 10.  $y_{LAP} = 0.1621x + 4.6565$ ,  $R^2 = 0.8365$ , d = 6.17
- 11.  $y_{LBT} = 0.3879x + 0.7205$ ,  $R^2 = 0.943$ , d = 2.58
- 12.  $y_{LBP} = 0.1848x + 4.9596$ ,  $R^2 = 0.9544$ , d = 5.41
- 13.  $y_{RAT} = 0.3586x + 0.2948$ ,  $R^2 = 0.9928$ , d = 2.79
- 14.  $y_{RAP} = 0.2037x + 3.7735$ ,  $R^2 = 0.9314$ , d = 4.91
- 15.  $y_{RBT} = 0.3811x + 0.6631$ ,  $R^2 = 0.9777$ , d = 2.62
- 16.  $v_{RBP} = 0.2036x + 4.0119$ ,  $R^2 = 0.8951$ , d = 4.91.

The ten-fold increase in the amounts of lactic acid bacteria in 1 g of the analysed samples under aerobic conditions took approximately the same amount of time, with

values d amounting to 2.43-2.79 days (equations 9, 11, 13, 15). A faster proliferation of lactic acid bacteria in rump cut was recorded under vacuum conditions, d = 4.91 days (rump cut with brine A and B – equations 14, 16) than in heel of round, for which values d were 6.17 (the heel of round with the brine A – the equation 13) and 5.41 (the heel of round with the brine B – the equation 12).

# Changes in amounts of bacteria from the family Enterobacteriaceae

Microbiological analyses included also the determinations of amounts of bacteria from the family *Enterobacteriaceae*. Results of these analyses are presented in Table 3. The higher contamination by the above mentioned group of microorganisms in samples stored in the atmosphere with an unlimited access to oxygen after one day was observed in case of the rump cut and the heel of round with the brine B than with the brine A. A faster growth of bacteria during the 3rd day of storage was found in heel of round samples than in the rump cut, while no statistically significant differences were observed depending on the fact whether samples contained the brine A or B. In case of the application of the brine A the amount of bacteria from the family *Enterobacteriaceae* was higher in the rump cut than in the heel of round during further storage periods (the 7th, 10th, 15th day). Such a result was also recorded after 10 days for samples containing the brine B. The effect of the type of brine on microbiological contamination after 15 days was recorded in the analysed meat samples: both the heel of the round and rump cut, to which the brine A was introduced, contained more bacteria from the family *Enterobacteriaceae* in 1 g than samples with the brine B.

Table 3. Bacteria of the family *Enterobacteriaceae* count ( $log_{10}$  cfu·g<sup>-1</sup>) in beef stored at 5°C (x ±s), N = 3

	Time of storage, days / conditions										
Sample	1		3		7		10		15		
	T	P	T	P	T	P	T	P	T	P	
LA	1.00 <sup>a</sup> ±0.00	3.17 <sup>cde</sup> ±0.00	2.91 <sup>cd</sup> ±0.13	4.07 <sup>i</sup> ±0.10	3.48 <sup>efg</sup> ±0.20	$4.74^{jk} \pm 0.04$	7.56 <sup>pr</sup> ±0.13	6.11 <sup>mn</sup> ±0.03	7.31 <sup>op</sup> ±0.08	7.07° ±0.08	
LB	$\begin{array}{c} 1.00^a \\ \pm 0.00 \end{array}$	$^{4.07^{i}}_{\pm 0.11}$	$2.83^{c} \pm 0.06$	$^{4.07^{i}}_{\pm 0.06}$	$\begin{array}{c} 3.85^{ghi} \\ \pm 0.03 \end{array}$	$5.03^{k} \\ \pm 0.01$	$7.61^{pr} \\ \pm 0.03$	$\begin{array}{c} 5.75^{lm} \\ \pm 0.06 \end{array}$	$6.30^{\rm n} \\ \pm 0.27$	$7.15^{\circ} \pm 0.03$	
RA	$\begin{array}{c} 1.00^a \\ \pm 0.00 \end{array}$	$2.88^{cd} \pm 0.03$	2.39 <sup>b</sup> ±0.15	$^{3.43^{ef}}_{\pm 0.03}$	$^{4.04^{hi}}_{\pm 0.00}$	$\begin{array}{c} 3.26^{def} \\ \pm 0.00 \end{array}$	$\begin{array}{c} 8.24^{st} \\ \pm 0.29 \end{array}$	$^{4.56^{j}}_{\pm 0.06}$	$\begin{array}{c} 7.84^{rs} \\ \pm 0.03 \end{array}$	$6.44^{n} \pm 0.04$	
RB	$1.00^{a} \pm 0.00$	$^{2.88^{cd}}_{\pm 0.10}$	$2.37^{b} \pm 0.17$	$\begin{array}{c} 3.46^{efg} \\ \pm 0.07 \end{array}$	$\begin{array}{c} 3.85^{ghi} \\ \pm 0.00 \end{array}$	$\begin{array}{c} 3.65^{\text{fgh}} \\ \pm 0.06 \end{array}$	$8.30^{t} \pm 0.18$	$\begin{array}{c} 3.65^{fgh} \\ \pm 0.03 \end{array}$	$5.59^{1}$ $\pm 0.02$	$6.52^{n} \pm 0.03$	

Explanations – as in Table 1.

The storage of samples with the brine B under vacuum conditions resulted in a situation when throughout the entire storage period the heel of round contained more bacteria from the family *Enterobacteriaceae* than the rump cut. In case of samples with the brine A such a trend was observed as late as only after three days of storage.

The analyses performed after one day showed that both in case of both the heel of round and the rump cut, the samples with the brine B were more contaminated. No differences in the concentration of bacteria from the family *Enterobacteriaceae* were found in the successive periods of storage depending on the type of the applied brine. The only exception in this respect was found for the rump cut after the 10th day, where a statistically significantly higher amount of bacteria was detected in sample RA than RB.

Similarly, equations of straight lines for bacteria from the family *Enterobacteriaceae* were established, describing the growth rates for bacterial amounts in individual samples and their basis values d (days) were calculated:

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17. y_{LAT} = 0.421x + 1.6309, R^2 = 0.7488, d = 2.38

18. y_{LAP} = 0.2769x + 3.0383, R^2 = 0.9771, d = 3.61

19. y_{LBT} = 0.3372x + 2.1973, R^2 = 0.604, d = 2.97

20. y_{LBP} = 0.2287x + 3.5677, R^2 = 0.9758, d = 4.37

21. y_{RAT} = 0.5015x + 1.2397, R^2 = 0.7807, d = 1.99

22. y_{RAP} = 0.2411x + 2.3783, R^2 = 0.8708, d = 4.15

23. y_{RBT} = 0.325x + 2.1833, R^2 = 0.4164, d = 3.08

24. y_{RBP} = 0.224x + 2.419, R^2 = 0.7698, d = 4.46.
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Recorded values d indicate that the storage under vacuum conditions in comparison to the storage under aerobic conditions slowed down the growth rate of the analysed bacteria and the biggest effect was found in case of the rump cut with the brine A, with value d of 1.99 (aerobic conditions – the equation 21) and 4.15 (vacuum conditions – the equation 22).

Bacteria from the family *Enterobacteriaceae* are capable to grow in vacuum packaged meat at a temperature of 0-10°C, but they do not constitute the dominant microflora, since at low temperatures their metabolism is slowed down [Labadie 1999]. In this study after 15 days at 5°C a relatively high amount of *Enterobacteriaceae* (of 6.44-7.15 log cfu/g) was found, despite the dynamic growth of lactic acid bacteria in these samples. A slower proliferation of *Enterobacteriaceae* was reported in a study by Blixt and Borch [2002]: in the comminuted beef the amount of these bacteria increased from 1 log cfu/g on the first day of measurement to 4 (beef strip loin) and 5 (entrecôte) after 2 weeks of storage at 4°C.

# Changes in pH values

Table 4 presents results of measurements for pH values. On their basis it may be stated that the type of the meat and the brine, as well as the storage time, did not change statistically significantly the values of pH in analysed samples. Such a difference was recorded only in case of the heel of round with the brine A, stored for 15 days under conditions of free oxygen access. The pH value of this sample was statistically significantly higher than after 1 day.

However, it needs to be considered that changes in pH values during the storage, apart from the type of the meat, the brine and storage conditions of samples, were also influenced by the microbiological status. Results presented in Tables 1-3 indicate that during the 15 days of storage, a dynamic growth of aerobic bacteria and bacteria from the family *Enterobacteriaceae* was found, which is connected with the meat spoilage and an increased pH value and the growth of lactic acid bacteria, producing lactic acid and thus reducing pH.

	Time of storage, days / conditions										
Sample	1		3		7		10		15		
	Т	P	T	P	T	P	T	P	T	P	
LA	5.97 <sup>ab</sup> ±0.01	6.09 <sup>abc</sup> ±0.25	6.04 <sup>abc</sup> ±0.00	6.34 <sup>abc</sup> ±0.35	5.99 <sup>abc</sup> ±0.00	6.16 <sup>abc</sup> ±0.53	6.08 <sup>abc</sup> ±0.00	6.38 <sup>abc</sup> ±0.49	6.79° ±0.01	6.36 <sup>abc</sup> ±0.47	
LB	$5.93^{ab} \\ \pm 0.00$	$6.00^{abc} \pm 0.21$	$\begin{array}{c} 6.03^{abc} \\ \pm 0.01 \end{array}$	$\begin{array}{c} 6.14^{abc} \\ \pm 0.16 \end{array}$	$\begin{array}{c} 6.06^{abc} \\ \pm 0.01 \end{array}$	$6.07^{abc} \\ \pm 0.19$	$^{6.15^{abc}}_{\pm 0.01}$	$\begin{array}{c} 6.22^{abc} \\ \pm 0.33 \end{array}$	$^{6.62^{bc}}_{\pm 0.01}$	$\begin{array}{c} 6.30^{abc} \\ \pm 0.25 \end{array}$	
RA	$5.92^{ab} \pm 0.03$	$5.81^{a} \pm 0.06$	$\begin{array}{c} 5.97^{ab} \\ \pm 0.00 \end{array}$	$\begin{array}{c} 5.87^{ab} \\ \pm 0.03 \end{array}$	$5.94^{abc} \\ \pm 0.03$	$5.77^{a} \pm 0.23$	$\begin{array}{c} 6.08^{abc} \\ \pm 0.00 \end{array}$	$5.75^{a} \pm 0.33$	$\begin{array}{c} 6.42^{abc} \\ \pm 0.00 \end{array}$	$5.81^{a} \pm 0.16$	
RB	$5.93^{ab} \\ \pm 0.01$	$5.98^{ab} \\ \pm 0.23$	$\begin{array}{c} 5.90^{ab} \\ \pm 0.02 \end{array}$	$\begin{array}{c} 6.02^{abc} \\ \pm 0.04 \end{array}$	$\begin{array}{c} 5.97^{ab} \\ \pm 0.01 \end{array}$	$5.91^{ab} \pm 0.01$	$5.99^{abc} \pm 0.01$	$5.83^{ab} \\ \pm 0.03$	$\begin{array}{c} 6.31^{abc} \\ \pm 0.06 \end{array}$	$\begin{array}{c} 5.87^{ab} \\ \pm 0.04 \end{array}$	

Table 4. pH value of beef stored at 5°C (x  $\pm$ s), N = 3

 $Explanations-as \ in \ Table \ 1.$ 

The presented information indicates that vacuum packaging of beef extends its shelf life, but at the determination of the shelf life for such a product, many factors need to be taken into consideration, such as e.g. the type of the packaged cut, applied technological conditions, the size of the cut and the level of vacuum. The storage temperature is also highly significant. The recommendations "store under cold storage conditions" or "store at a temperature below 5°C" are inadequate, since within the narrow range of cooler temperatures (0 to 5°C) for microorganisms each degree Celsius is crucial. Moreover, during the distribution or storage of the packaged meat in a household refrigerator the temperature may increase, thus high expectations are connected with the application of nanotechnologies for the more quantification and rapid detection of bacteria and in the food packaging process. This packaging system may adequately adapt its parameters to changes in the medium (such as a temperature or the moisture content) and supply consumers with information of possible spoilage of the food. However, nanotechnologies and nanomaterials require legal regulations and an evaluation in terms of their safety, since certain such new materials have never been tested in this respect [Otles and Yalcin 2008 a, 2008 b, 2010, Ozimek et al. 2010].

#### CONCLUSIONS

The vacuum packaging in comparison to the storage under aerobic conditions slows down the growth of the microflora in the beef, with the biggest effect being found in case of aerobic bacteria.

Up to the 10th day of storage at 5°C a higher concentration in vacuum packaged meat was found for lactic acid bacteria in comparison to the meat stored under aerobic conditions. Differences after a longer time depend on the type of the muscle and the type of the applied brine.

A higher concentration of bacteria from the family *Enterobacteriacea* in vacuum packaged meat was found in the heel of round than in the rump cut.

#### REFERENCES

Babji Y., Murthy T.R.K., Anjaneyulu A.S.R., 2000. Microbial and sensory quality changes in refrigerated minced goat meat stored under vacuum and in air. Small Rum. Res. 36, 75-84.

- Bilska A., 2011. Packaging systems for animal origin food. LogForum 7, 1, 4.
- Blixt Y., Borch E., 2002. Comparison of shelf life of vacuum-packed pork and beef. Meat Sci. 60, 371-378.
- Crowley K.M., Prendergast D.M., Sheridan J.J., McDowell D.A., 2010. The influence of storing beef aerobically or in vacuum packs on the shelf life of mince. J. Appl. Microbiol. 109, 1319-1328.
- Houbak M.B., Ertbjerg P., Therkildsen M., 2008. In vitro study to evaluate the degradation of bovine muscle proteins post-mortem by proteasome and μ-calpain, Meat Sci. 79, 77-85.
- Huis in't Veld J.H.J., 1996. Microbial and biochemical spoilage of foods: an overview. Int. J. Food Microbiol. 33, 1-18.
- Iwanowska A., Pospiech E., 2010. Comparison of slaughter value and muscle properties of selected cattle breeds in Poland Review. Acta Sci. Pol., Technol. Aliment. 9 (1), 7-22.
- Iwanowska A., Pospiech E., Łyczyński A., Rosochacki S., Grześ B., Mikołajczak B., Iwańska E., Rzosińska E., Czyżak-Runowska G., 2010. Evaluation of variations in principal indices of the culinary meat quality obtained from young bulls of various breeds. Acta Sci. Pol., Technol. Aliment. 9 (2), 133-149.
- Khalafalla F.A., Nagwa S.S., Ata Mona A.E., Elshabrauy Azza S.M., Abu Elnaga Dorgham S.M., Khairy A.E., 2010. Effect of packing on extension of self life of retail meat. J. Am. Sci. 6 (12), 1049-1058.
- Kołczak T., 2008. Jakość wołowiny [Quality of beef]. Żywn. Nauka Techn. Jakość 1 (56), 5-22 [in Polish].
- Krasnowska G., Sobków B., Dąbrowska M., Pelc M., 2005. Określenie przydatności wybranych preparatów enzymatycznych do poprawy jakości mięsa wołowego [Determination of suitability of selected enzymatic preparations to improve beef quality]. Żywn. Nauka Techn. Jakość 2 (43), 73-83 [in Polish].
- Labadie J., 1999. Consequences of packaging on bacterial growth. Meat is an ecological niche. Meat Sci. 52, 299-305.
- Otles S., Yalcin B., 2008 a. Intelligent food packaging. LogForum 4, 4, 3.
- Otles S., Yalcin B., 2008 b. Smart food packing. LogForum 4, 3, 4.
- Otles S., Yalcin B., 2010. Nano-biosensors as new tool for detection of food quality and safety. LogForum 6, 4, 7.
- Ozimek L., Pospiech E., Narine S., 2010. Nanotechnologies in food and meat processing. Acta Sci. Pol., Technol. Aliment. 9 (4), 401-412.
- PN-A-82055-17:1997. Mięso i przetwory mięsne. Badania mikrobiologiczne. Oznaczanie liczby bakterii kwasu mlekowego [Meat and processed meats. Microbiological analyses. Determination of lactic acid bacteria counts; in Polish].
- PN-A-04023:2001. Mikrobiologia żywności. Wykrywanie i identyfikacja drobnoustrojów z rodziny *Enterobacteriaceae* [Food microbiology. Detection and identification of microorganisms from the family *Enterobacteriaceae*; in Polish].
- PN-A-82055-6:1994. Mięso i przetwory mięsne. Badania mikrobiologiczne. Oznaczanie ogólnej liczby drobnoustrojów [Meat and meat products. Microbiological analyses. Determination of total bacterial counts; in Polish].
- Rosiak E., Kołożyn-Krajewska D., 2005. Modele wzrostu bakterii *Pseudomonas* w produktach gotowych do spożycia [Models of growth for *Pseudomonas* bacteria in ready-to-eat products]. Żywn. Nauka Techn. Jakość 3 (44) Suppl. 191-205 [in Polish].
- Commission Regulation (EC) no. 1441/2007 of 5 December 2007 amending Regulation (EC) no. 2073/2005 on microbiological criteria for foodstuffs.
- Signorini M.L., Ponce-Alquicira E., Gerreiro-Legaretta I., 2006. Effect of lactic acid and lactic acid bacteria on growth of spoilage microorganisms in vacuum-packaged beef. J. Musc. Foods 17 (3), 277-290.
- Watkinson B.M., Kütemeyer C., Reinhold T., Werlein H.-D., 2004. Wild-eine Alternative zu Rindfleisch? Fleischwirtschaft 3, 53-57.

# TRWAŁOŚĆ MIKROBIOLOGICZNA WYBRANYCH ELEMENTÓW MIĘSA WOŁOWEGO PODDANEGO ZABIEGOM TECHNOLOGICZNYM I PRZECHOWYWANEGO W WARUNKACH TLENOWYCH I PRÓŻNIOWYCH W TEMPERATURZE 5°C

**Wstęp.** Uzyskanie mięsa wołowego o odpowiedniej kruchości wymaga dłuższego czasu składowania w warunkach chłodniczych. Czas przechowywania oraz inne czynniki, jak rodzaj mięsa, sposób pakowania czy zastosowane zabiegi technologiczne wpływają na jego stan mikrobiologiczny. Celem pracy było porównanie koncentracji drobnoustrojów (bakterie tlenowe, kwasu mlekowego i z rodzaju *Enterobacteriacea*) w mięśniu najdłuższym lędźwi (*longissimus dorsi* – rostbef) oraz mięśniu półścięgnistym (*semimembranosus dorsi* – ligawa) przechowywanych w temperaturze 5°C w warunkach tlenowych i próżniowych. Aby poprawić kruchość mięsa, wprowadzono do niego solankę zawierającą 1% NaCl oraz 1% NaCl, 0,3% trójfosforanu pięciosodowego E 451i.

**Materiał i metody.** Materiałem do badań było mięso wołowe: rostbef (R) i ligawa (L). Mięso peklowano solanką A, zawierającą 1% NaCl w masie całkowitej lub solanką B, zawierającą 1% NaCl, 0,3% trójfosforanu pięciosodowego E 451i (w tym 56% P<sub>2</sub>O<sub>5</sub>). Próby pakowano próżniowo (P) bądź pozostawiano w otwartych pojemnikach (T) i przechowywano w warunkach chłodniczych (5°C): 1, 3, 7, 10 i 15 dób. Po każdym okresie przechowywania oznaczano: ogólną liczbę bakterii tlenowych mezofilnych, liczbę bakterii kwasu mlekowego, liczbę bakterii z rodziny *Enterobacteriaceae*, w tym bakterie z grupy coli i *E. coli*, wartość pH.

**Wyniki.** Pakowanie próżniowe w porównaniu z przechowywaniem w warunkach tlenowych spowalnia rozwój mikroflory w mięsie wołowym, przy czym największy efekt występuje w przypadku bakterii tlenowych; do 10 doby przechowywania w temp. 5°C w mięsie pakowanym próżniowo stwierdzono większą koncentrację bakterii kwasu mlekowego w porównaniu z mięsem składowanym w warunkach tlenowych. Po dłuższym czasie różnice zależą od rodzaju mięśnia i rodzaju zastosowanej solanki; w mięsie pakowanym próżniowo stwierdzono większą koncentrację bakterii z rodz. *Enterobacteriacea* w ligawie niż w rostbefie.

**Wnioski.** Na zanieczyszczenie mikrobiologiczne mięsa wołowego przechowywanego w temperaturze 5°C statystycznie istotny wpływ ma nie tylko czas przechowywania, ale także rodzaj elementu kulinarnego i zastosowane zabiegi technologiczne.

Slowa kluczowe: mięso wołowe, solenie, pakowanie próżniowe, trwałość mikrobiologiczna

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