Dynamic changes of selected microbiological and physical parameters in fresh jointed beef

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Abstract

During the performance of microbiological analyses, important groups of microorganisms whose action may have a significant influence on the quality of chilled beef meat stored aerobically and vacuum-packed were monitored in samples of beef on days 1, 3, 7 and 14. Samples of beef rump *(musculus semimembranosus)* and sirloin *(musculus longissimus thoracis et lumborum*) from young animals (n = 9) were used. The initial concentration of 10^4 CFU.cm^{-2} increased under aerobic conditions during the seven days of storage to a total of 10^7 CFU.cm^{-2}. Higher total counts of microorganisms were detected in rump than in sirloin at the beginning of storage. A lower count of microorganisms was found in vacuum-packed meat after 14 days of storage than in unpacked meat on day 7. A similar trend was found for the numbers of psychrotrophic microorganisms. Even on day 14 of storage, coliform bacteria counts in vacuum-packed beef were as much as two orders of magnitude lower than in beef stored aerobically. No visible growth of mould was seen in the samples of meat stored. The number of yeast was relatively low at the beginning of storage (of the order of 10^2 CFU.cm^{-2}), though their number then increased gradually up to 10^4 CFU.cm^{-2} in unpacked meat. Lower counts (10^3 CFU.cm^{-2}) were found in vacuum-packed meat. The colour difference ΔE^*_{ab} on day seven of storage of the meat was higher in rump meat (5.2) than in sirloin (4.7). Discoloration of the meat (ΔE^*_{ab} = 5.19) and an increase in pH (6.10) were clearly evident when surface slime, which was evident at microorganism counts of the order of 10^8 CFU.cm^{-2}, appeared.

Shelf life of meat, lightness, CIELAB, pH, discolouration

Introduction

Meat accounts for a significant proportion of man's diet. Meat quality is, however, variable and properties such as taste, structure, colour and aroma vary according to the type of animal, method of rearing and feed, and are influenced by many factors. Meat has a complex and extremely diverse histological structure and variable chemical composition and technical and sensory properties. Its structure and composition depend on the animal's way of life, the functioning of the individual parts of the body, a number of intravital factors (species of animal, breed, sex, age, nutrition, health status, etc.), the course of post-mortal changes and the method of cooking (Pipek 2009). Average values for the composition of beef rump meat, as given by Staruch (2011), are as follows: water 74%, total proteins 21%, connective tissue protein 4.69%, total lipids 2.32%, minerals 1.06%, total amino acids 19.62% (essential 9.2%, semi-essential 1.58%, non-essential 8.84%). Steinhauserová and Steinhauser (2000) give basic compositions for important cuts of beef, e.g. rump beef (73% water, 20.2% proteins and 5.0% fat) and bottom sirloin (57% water, 16.7% proteins, 25% fat).

Meat is a complex and dynamic biological system in which a large number of post-mortal biochemical processes occur. Taken together, these are known as maturing, during which the meat obtains the desired sensory, technical and culinary properties. Thanks to its chemical composition, physical properties and high water content, meat represents an ideal breeding ground for microorganisms (Görner and Valík 2004). The microflora occurring on the surface of meat is extremely diverse. The bacteria occurring, as stated by Jay et al.

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(2005), are primarily comprised of representatives of the genera *Acinetobacter*, *Aeromonas*, *Enterococcus*, *Moraxella*, *Pseudomonas*, *Psychrobacter*, *Alcaligenes, Flavobacterium* and others, microscopic fungi occurring may frequently include moulds of the genera *Cladosporium, Geotrichum, Rhizopus, Sporotrichum, Thamnidium, Aspergillus* and *Mucor*, while the principal yeasts occurring include *Candida, Rhodotorula, Cryptococcus, Saccharomyces*, etc. The occurrence of important pathogenic microorganisms, most notably *E. coli* O157:H7*, Salmonella* spp., *Campylobacter* spp., *Bacillus cereus, Clostridium perfringens, C. botulinum, Listeria monocytogenes, Staphylococcus aureus* and *Yersinia enterocolitica*, whose pathogenic serotypes 0:3 and 0:9 have been isolated from cattle by Simonová et al. (2007), must also be anticipated.

As noted by Adams et Moss (2008), the tissues of healthy animals are protected against infection by a combination of physical barriers and the activity of the immune system, as a result of which the internal organs and muscles of freshly slaughtered animals should be relatively free of microorganisms – the numbers of microorganisms in samples of tissue taken in an aseptic manner are generally lower than 10 $CFU.kg^{-1}$, though they may increase as the result of stress and will, of course, be higher if the animal is suffering from an infection. Muscle tissue and organs are, however, contaminated by technological operations involved in animal slaughtering. The parts of the animal most heavily populated by microbes that may contaminate the meat are the skin and the gastrointestinal tract (Adams et Moss 2008). Evisceration is the riskiest technical operation in the clean slaughter of animals – the content of the guts, as noted by Steinhauserová (2000), contains a large quantity of bacteria (10^8 – 10^{10} bacteria to 1 g of intestinal content) whose metabolic activity may, if they get onto the surface of the muscle tissue, both considerably reduce the shelf life of the meat and represent a health risk. The hands of workers handling meat and tools and equipment used may also be a significant source of contamination if not cleaned regularly and properly (Steinhauserová 2000). Contamination of the meat of slaughtered animals by processing equipment, knives and workers is, however, less important than contamination from the animals themselves according to Adams et Moss (2008).

Meat must be chilled immediately after dressing in order to prevent spoilage. The EU requires meat to be chilled to a temperature below 7 °C, though for a longer shelf life it must be stored at temperatures of around 0 °C (Pipek 2009). According to Görner and Valík (2004), beef halves and quarters attain this temperature after between 15 and 24 hours of intense refrigeration. Meat refrigeration significantly restricts the growth of the mesophilic microorganisms that make up the principal part of the original microflora. The temperature of refrigerated meat allows the development of psychrotrophic microorganisms that come to dominate over the course of time (Jackson et al. 2001). Meat stored in an ordinary atmosphere has a relatively short shelf life that depends, first and foremost, on the quantitative and qualitative composition of primary microbial contamination, the storage temperature, water activity and the availability of oxygen (Steinhauserová 2000). The growth of aerobic microflora is suppressed in meat packed in a modified atmosphere or in a vacuum. Lactic acid bacteria and *Brochothrix thermosphacta* dominate in these conditions (Jackson et al. 2001; Pennacchia et al. 2011). *Shewanella putrefaciens*, psychrotrophic *Enterobacteriaceae*, psychrotrophic clostridia and bacilli also play a part in spoilage, as is stated by Fernandes (2009). The speed and extent of decomposition depends on the temperature and other conditions of storage, for which reason it is essential, as noted by Pipek (2011) and Loretz et al. (2011), to achieve the necessary low temperatures as soon as possible, or to take supplementary preservative and antimicrobial action, such as reducing the pH (spraying with a solution of organic acids, particularly lactic acid), reducing the water activity, using appropriate packaging or modifying the atmosphere in the packaging or storage area.

Colour is an extremely conspicuous characteristic and one of the factors used by the consumer to assess the quality of meat and meat products. It is also associated with many quality characteristics (Pipek and Pour 1998). Colour, as perceived by the consumer, is the physical interaction of light with meat as observed by the human eye and interpreted by the brain. Colours are, therefore, perceived individually. Visual assessment cannot provide a precise evaluation of colours, as man has a poor memory for colour (Musilová et al. 2001). Beef meat from young animals is a bright pale red colour. Bulls have meat of a dark red colour, often with a tinge of copper. In large muscular parts of the body, in places at which fasciae (the fibrous envelopes of muscles) are found, the meat may even have a bluish shimmer. Meat takes on unusually dark brown-red tones when the surface becomes dry. Beef bullocks give meat of a vivid brown-red colour, initially bright, before turning to a dark brick-red colour after storage (Šimek and Steinhauser 2001).

There are, in principle, two ways of assessing meat colour: subjective assessment (visual colour perception) and objective assessment using instruments based on the measurement of absorbance and reflectance. Image analysis is currently a rapidly developing method for assessing meat colour (Mancini 2005). Colour is most frequently expressed objectively by means of the *L**, *a**, *b** colorimetric system developed by the Commission Internationale de l'Eclairage (CIE). Spectrophotometers working in the visible region are used for the objective assessment of colour. Reflex spectrophotometry provides results close to visual perception. During reflex measurement, the proportion of reflected light to incident light is determined in dependence on the wavelength throughout the entire range of visible light, i.e. from 400 to 760 nm (a number of spectrometers even measure from 360 nm). CIE system *L* a* b** quantities are calculated from values for the entire spectrum by means of complex relations (Zhang et al. 2009).

The aim of this experiment was to describe the dynamics of changes playing a significant part in subsequent quality in dependence on various kinds of consumer behaviour.

Materials and Methods

Muscles from cattle carcasses were used for the experiment: rump – *musculus semimembranosus* (MS) and bottom sirloin – *musculus longissimus thoracis et lumborum* (MLTL) taken from young animals (Table 1). This meat was stored under aerobic conditions (AER) and vacuum-packed (VAC) at a temperature of 6 °C.

Table 1. Basic characteristics of slaughtered animals

No of animal in series 1	1	2	3
category	bull	young bull	young bull
breed	Aberdeen Angus	Aberdeen Angus	Charolais
age of animal	20 months	20 months	20 months
weight of animal	521 kg	478 kg	530 kg
No of animal in series 2	4	5	6
category	cow	young bull	young bull
breed	Bohemian Spotted Cattle	Bohemian Spotted Cattle	Bohemian Spotted Cattle
age of animal	24 months	24 months	24 months
weight of animal	542 kg	738 kg	765 kg
No of animal in series 3	7	8	9
category	young bull	young bull	young bull
breed	Bohemian Spotted Cattle	Bohemian Spotted Cattle	Bohemian Spotted Cattle
age of animal	23 months	23 months	23 months
weight of animal	613 kg	628 kg	619 kg

Samples of meat were monitored over the course of 7 or 14 days for numbers of selected groups of microorganisms, changes in colour and (initially) pH. A swab method was used to determine significant groups of microorganisms. Swabs were taken by wiping an area of 5 x 5 cm of the surface of meat samples.

A ten-fold dilution series was prepared after shaking the swab in a sterile physiological solution. One ml of the original solution or a dilution of it was inoculated in a Petri dish and suffused with an appropriate substrate. The Petri dishes were incubated at the pertinent temperatures and, after the cultivation time had elapsed, the numbers of characteristic colonies were read and the results displayed in $CFU.cm^{-2}$ following conversion. The following groups of microorganisms were determined during microbiological analysis: total count of microorganisms (ČSN ISO 2293) on PCA (Plate Count Agar) at 30 °C after 72 hours, psychrotrophic microorganisms (ČSN ISO 6730) on PCA at 6.5 °C after 10 days, coliform bacteria (ČSN ISO 4832) on VRBL (Violet Red Bile Lactose) Agar at 37 °C after 24 hours, heat-resistant and spore-forming bacteria after heating at 85 °C for 10 minutes under aerobic and anaerobic conditions on PCA at 30 °C after 48 hours, and yeasts and moulds (ČSN ISO 13681) on Chloramphenicol Glucose Agar at 25 °C after 120 hours. All substrates used came from Biokar Diagnostics (France).

The reference method for the determination of pH_{ult} is performed in accordance with the standard ČSN ISO 2917. A PORTAMESS 911 Ph KNICK meter with buffers used for calibration at the pH range of 4 to 7 was used for measurement. Two punctures were performed in all cases and their values averaged.

Colour was measured on a Konica Minolta CM-3500d spectrophotometer with CMs-100w Spectramagic NX evaluation software. An aperture of 30 mm, SCE and D65 mode were used for the purposes of measurement. Colour is defined by CIE L^*, a^*, b^* values. The results were processed statistically in the program UNISTAT 5.1 by means of analysis of variance with subsequent multiple factor comparison and statistical differences assessed by a Tukey's test at the level of significance ($P < 0.05$).

Results and Discussion

Important groups of microorganisms whose action may have a significant influence on the quality of chilled meat were monitored in samples of beef during microbiological analyses. The surface of the muscle becomes contaminated during slaughtering in dependence on the hygiene conditions – in first-rate slaughterhouses to a level of 10^2 to 10^3 microorganisms per cm^2, in slaughterhouses with inadequate hygiene to a level of 10^6 to 10^7 microorganisms per cm^2 (Steinhauserová 2000). The best indication of the microbial contamination of meat is provided by the total count of microorganisms (Fig. 1). Under aerobic conditions, the initial concentration of 10^4 $CFU.cm^{-2}$ increased to 10^7 $CFU.cm^{-2}$ over the course of seven days of storage. Higher total plate counts were detected at the beginning of storage in rump (MS) than in sirloin (MLTL). Similar values were recorded in rump after skinning by Gill et al. (1996), though their number fell after subsequent processing. A lower total plate count was found in vacuum-packed meat (VAC) after 14 days of storage than in unpacked meat (AER) on day 7. Storing meat in a vacuum influences primarily the numbers of microorganisms, and not necessarily on their species diversity, as noted by Pennacchia et al. (2011). In ostrich meat, Jevinová et al. (2012) reported 3.0 x 10^7 $CFU.cm^{-2}$ for aerobic storage and 2.0 x 10^5 $CFU.cm^{-2}$ for vacuum-packed meat after 14 days of storage.

A similar trend was also found for psychrotrophic microorganisms (Fig. 2). According to Jay et al. (2005), these microorganisms include *Pseudomonas, Alcaligenes, Moraxella, Shewanella, Brochothrix, Corynebacterium, Flavobacterium* and *Micrococcus*. Bacteria of the genus *Pseudomonas* make up more than 50%, and sometimes as much as 90%, of the microflora causing meat spoilage (Ducková and Čanigová 2005). Pseudomonades have strong oxidative capacities, and use low-molecular nitrogenous substances as a source of energy (Jackson et al. 2001). *Pseudomonas fragi* is the pseudomonas most frequently isolated on spoilt meat, its occurrence ranging from 56.6 to 79%, followed by *Ps. lundensis* and *Ps. fluorescens* (Ducková and Čanigová 2005). The numbers of psychrotrophic microorganisms in vacuum-packed meat reached almost 10^6 $CFU.cm^{-2}$ after 14 days of storage, i.e. approximately one order of magnitude less than in unpacked meat. These are, nevertheless, extremely large numbers in such a short period of time in comparison with the data determined by Rodas-González et al. (2011), who found similar numbers in vacuum-packed meat after more than 10 weeks of storage in a number of cases.

Coliform bacteria are another significant group of microorganisms contaminating meat surfaces. Bacteria of the genera *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella* are known as coliform bacteria. Görner and Valík (2004) state that the original site of coliform bacteria is the digestive tract of humans and animals. These bacteria include not only species that contribute to meat spoilage, but also significant pathogens such as *E. coli* O157:H7. The numbers of coliform bacteria (Fig. 3) were extremely low at the beginning of the storage period. Their numbers were comparable (almost 10^5 CFU.cm^{-2}) at the end of storage in both types of unpacked meat. The numbers of coliform bacteria in vacuum-packed meat were as much as two orders of magnitude lower on day 14 of storage.

Numbers of heat-resistant and spore-forming microorganisms were monitored during anaerobic and aerobic cultivation.

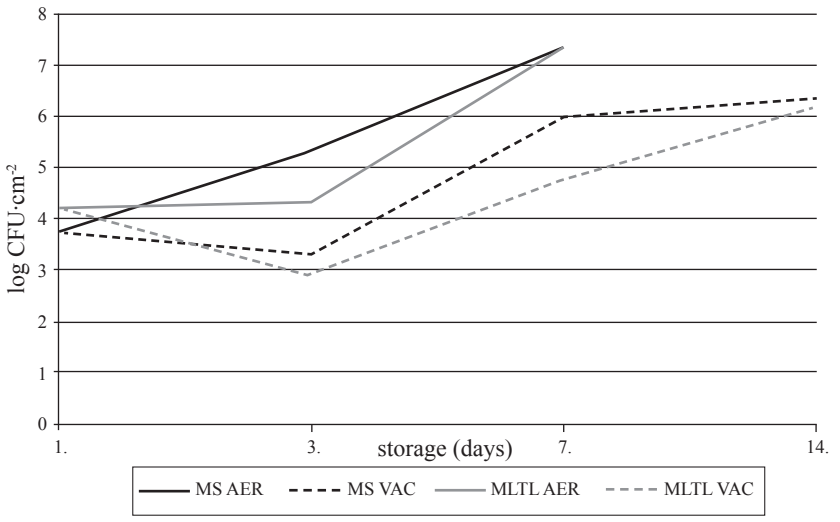

Fig. 1. Average values of total counts of microorganisms

The initial values were low in both cases and their numbers increased only extremely slowly. The dominant component of this group of microorganisms are anaerobic and facultative anaerobic heat-resistant and spore-forming bacteria, first and foremost representatives of the genera *Bacillus* and *Clostridium*. Psychrotrophic clostridia such as *C. putrefaciens*, *C. gasigenes* and *C. algidicarnis* may play a part in the spoilage of vacuum-packed beef, particularly during long periods of storage, as stated by Silva et al. (2012).

No visible growth of mould was discovered on the samples of meat stored. Any spores present were in an inactive state. Extremely low numbers of moulds were discovered following cultivation (no units, single units or tens of units per cm^2) throughout the storage period. The development of moulds was inhibited by competing microflora and, for the vacuum-packed meat, a shortage of oxygen. The numbers of yeasts were also relatively low at the beginning of storage (of the order of 10^2 CFU.cm^{-2}), though their number gradually increased to as much as 10^4 CFU.cm^{-2} in unpacked meat. Their numbers were lower in vacuum-packed meat (10^3 CFU.cm^{-2}). More rapid development of yeasts in an aerobic environment tends to point to the presence of yeasts with a predominately respiratory

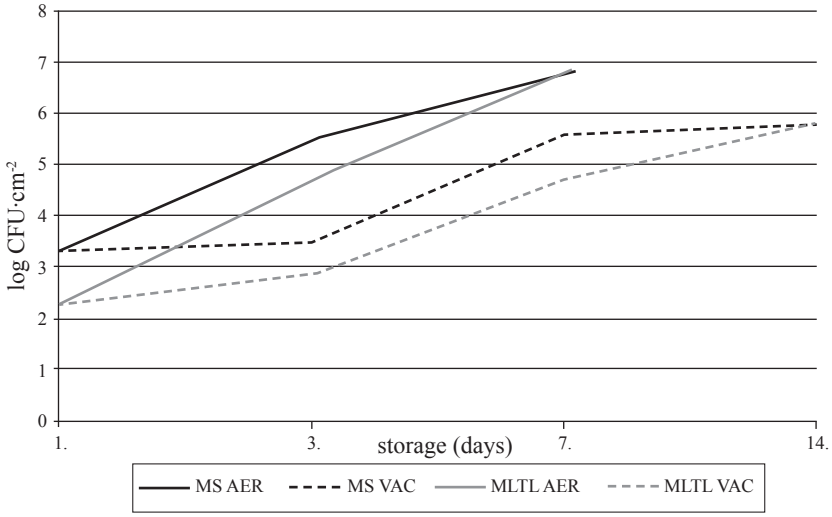

Fig. 2. Average values of psychrotrophic microorganisms

metabolism. In spite of relatively low numbers, the properties of yeasts allow them to play a significant part in meat spoilage, even in vacuum-packed meat.

The development of contaminating microflora was found to be markedly more rapid in unpacked meat stored under aerobic conditions. The total plate counts were as much as 10^7 CFU.cm^{-2} on day 7 of storage. Such numbers of microorganisms can be a cause of the characteristic features of meat spoilage, as pointed out by Adams and Moss (2008), such as off odours caused by the decomposition products of proteins and fats that

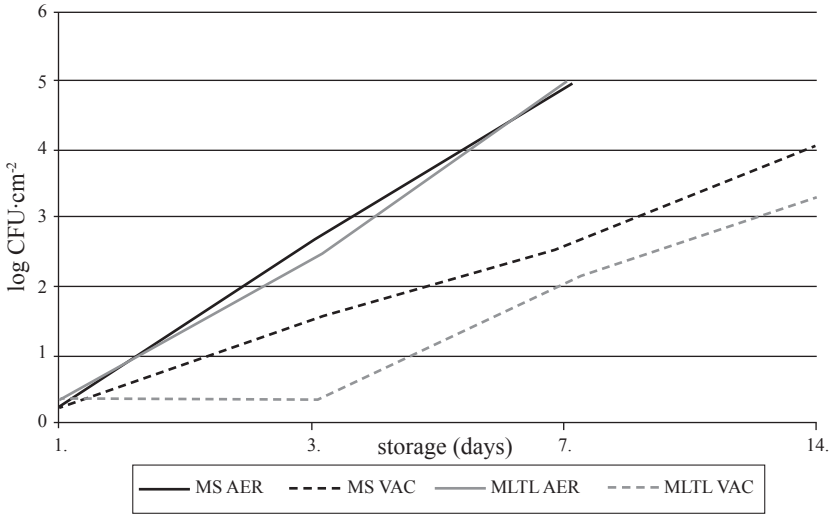

Fig. 3. Average coliform bacteria counts

Table 2. Values of pH and CIE L*a*b* in dependence on period of storage and meat cut ($\bar{x} \pm s_e$)

Cut	Rump (MS)		Sirloin (MLTL)	
Storage	AER	VAC	AER	VAC
pH_{ult}	5.61 ± 0.05		5.59 ± 0.05	
pH – day 7	6.10 ± 0.15[a]	5.75 ± 0.08[b]	6.03 ± 0.13[a]	5.72 ± 0.05[b]
L* – day 1	41.02 ± 0.42[a]		36.68 ± 0.42b	
L* – day 3	43.36 ± 0.44[a]	41.60 ± 0.62[a]	36.27 ± 0.74[b]	36.62 ± 0.45[b]
L* – day 7	40.56 ± 0.57[a]	39.98 ± 0.54[a]	34.91 ± 0.63[b]	34.83 ± 0.29[b]
L* – day 14	-	43.13 ± 0.55[a]	-	39.60 ± 0.52[b]
a* – day 1	14.21 ± 0.38		14.26 ± 0.42	
a* – day 3	11.93 ± 0.29[a]	12.61 ± 0.39[b]	11.21 ± 0.38[a]	12.93 ± 0.40[b]
a* – day 7	10.19 ± 0.26[a]	12.81 ± 2.07[c]	11.04 ± 0.34[b]	12.26 ± 0.41[c]
a* – day 14	-	12.57 ± 0.53	-	13.10 ± 0.37
b* – day 1	15.01 ± 0.33[a]		12.58 ± 0.34[b]	
b* – day 3	14.80 ± 0.37[a]	13.62 ± 0.43[b]	11.54 ± 0.41[c]	11.47 ± 0.35[c]
b* – day 7	11.75 ± 0.35[a]	12.95 ± 0.40[b]	9.61 ± 0.34[c]	10.00 ± 0.33[c]
b* – day 14	-	14.21 ± 0.33[a]	-	12.55 ± 0.26[b]

The index [a, b, c] on lines indicates differences between groups ($P < 0.05$)

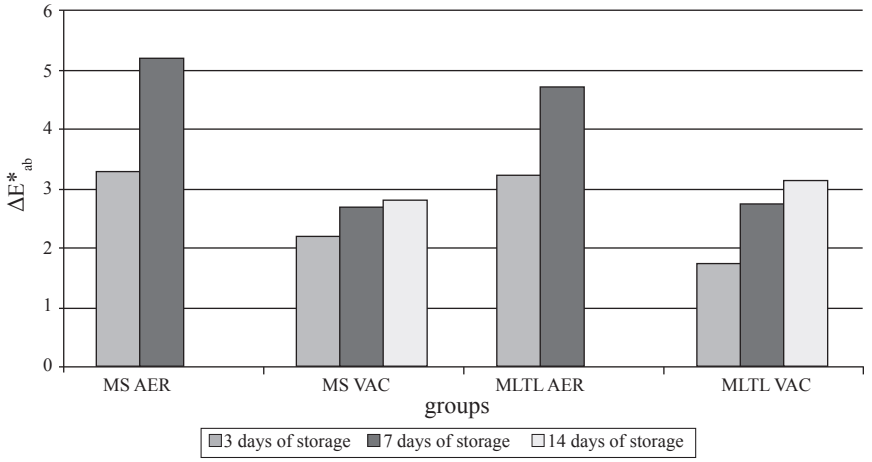

Fig. 4. Changes in colour ΔE^*_{ab} in beef during storage period for rump (MS) and sirloin (MLTL)

appear when these microorganisms reach numbers of around 10^6 CFU.cm^{-2}, surface slime evident at numbers of microorganisms of around 10^8 CFU.cm^{-2}, changes to meat colour ($\Delta E^*_{ab} = 5.19$) and an increase in pH (Table 2).

As a result of the limited supply of air and the protective action of the packaging material, the numbers of microorganisms in vacuum-packed meat increased considerably more slowly – the meat was of acceptable sensory quality after 14 days of storage. Similar conclusions were reached in the study by Pennacchia et al. (2011), who found that lower numbers of microorganisms were recorded in vacuum-packed beef steaks than in steaks

stored under aerobic conditions after 20 days of storage. They also state, in accordance with our findings, that vacuum-packed steaks were acceptable in sensory terms after 20 days of storage.

In the final phase of monitoring numbers of microorganisms, i.e. day 7 for unpacked meat and day 14 for vacuum-packed meat, extremely similar numbers of all groups of microorganisms were found in both rump and sirloin. The greatest differences in meat colour during storage were seen, of course, in meat stored aerobically (Table 2); the deviation of ΔE^*_{ab} on day 7 of meat storage was higher for rump (MS - 5.2) than for sirloin (MLTL - 4.7). After 14 days of vacuum-packed storage, the ΔE^*_{ab} attained lower values ($\Delta E^*_{ab\ MS\ VAC} = 2.79$, $\Delta E^*_{ab\ MLTL} = 3.15$) and the change in colour therefore was not very pronounced.

Conclusions

Markedly more rapid development of contaminating microflora was found in unpacked meat stored under aerobic conditions. In the final phase of monitoring the numbers of microorganisms, i.e. on day 7 for unpacked meat and on day 14 for vacuum-packed meat, extremely similar numbers of all groups of microorganisms were found in both rump and sirloin. Restricting the supply of air and the protective action of the packaging material led to numbers of microorganisms growing considerably more slowly in vacuum-packed meat – the meat continued to be of acceptable sensory quality even after 14 days of storage. These results will be used to monitor quality in a meat-processing plant and for the purposes of an experiment simulating various forms of consumer behaviour.

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