Bacterial populations and volatile organic compounds associated with meat spoilage

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Abstract. The aim of the study was to detect volatile organic compounds acting as markers of spoilage for raw chilled beef stored under vacuum at 4 °C for 15 days. We also determined the relationship of the volatile compounds with microbial and organoleptic properties associated with shelf life. Volatile organic compounds were analysed by multisensory analysis using an electronic nose sensor. Increasing aldehydes, ketones, and alcohols were measured in beef during the storage period. An increase in volatile chemical compounds during storage was correlated with an increased level of Lactobacillus, the predominant group of microorganisms on the beef at the end of the study. Maximum concentrations of volatile chemical compounds were determined at the end of the shelf life of the stored beef. Lactic acid bacteria were the main microorganisms that caused spoilage and are suitable for predicting the shelf life of raw chilled vacuum packaged beef using the electronic nose device at the threshold of 5.0-6.0 log CFU/g.

1. Introduction
The initial microbial contamination of meat influences the shelf life. The initial microbiota of meat is composed mainly of Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus, Micrococcus, lactic acid bacteria (LAB) and Enterobacteriaceae [1]. The composition of meat, its favourable pH (5.5-6.5) and its high moisture content ensure this food provides good conditions for microbial growth [2]. There are also external factors such as storage temperature and packaging type that influence the growth of microorganisms on meat during its storage [3].

The combination of chill storage with modified atmosphere packaging or vacuum packaging promotes the growth of certain microorganisms: Pseudomonas spp., Enterobacteria, Brochothrix thermosphacta and LAB [4]. Pseudomonas spp. prevailed over other spoilage microorganisms during aerobic storage at the fridge temperature [5]. The dominant factor in ensuring microbial meat quality and safety is the presence and/or growth of the specific microbial community according to the the meat type, meat hygiene and storage conditions [2]. Microbial meat spoilage is caused by the growth of microorganisms that produce metabolites such as aldehydes, ketones, esters, alcohols, organic acids, amines and sulphur compounds.

Currently, numerous studies are aimed at establishing the relationship between organoleptic properties, microbiota and bacterial metabolites (chemical markers) in food products, which can change during storage depending on the type of packaging [6]. Identification of aromatic compounds in meat products that have potential to be used as chemical markers to establish sensory changes during storage was the main aim of these studies [7, 8]. One approach to assessing the shelf life of foods is to evaluate
chemical spoilage indices (CSI). Volatile organic compounds (VOCs), which are metabolites produced by microorganisms, can be used as CSI. The increase of these markers indicates the presence of spoilage microorganisms.

One of the methods used to monitor and measure the gas environment surrounding meat or the gas phase released from the meat on heating is the electronic sensor commonly called the electronic nose [9]. The electronic nose produces a visual imprint of odour resulting from the complex of all substances that form the odour (similar to the human nose).

The main problem in predicting the shelf life of raw meat and meat products is the lack of a clear relationship between the organoleptic and chemical changes that occur during storage. A new model for predicting meat spoilage is needed. A predominant factor for designing the new model is understanding the dynamic changes associated with spoilage caused by microbial communities and their metabolites, and their impacts on sensory qualities of the meat.

2. Materials and Methods

2.1 Sample preparation
Vacuum packaged, chilled, small-sized beef was examined. The beef was stored chilled with high precision temperature control at 4°C±0.2°C and was examined immediately after production and on days 5, 10 and 15 of storage.

2.2 Microbiological analysis
Amounts (10 g) of beef were homogenised in 90 ml of 0.9% saline solution (NaCl) (Vekton, Russia) using a MIX2 homogeniser (AES, France). Then a series of tenfold dilutions were prepared from each initial suspension. Then suitable aliquots were spread on the following growth media: Plate Count Agar (PCA, Merck) incubated at 30 °C for 72 h for total microbial count; Crystal-violet neutral-red bile glucose agar (VRBD, Merck) for Enterobacteriaceae, incubated at 37 °C for 24 h; MRS agar (ФБУН ГНЦ ПМБ, Russia) for LAB, incubated at 30 °C for 72 h; Dichloran Rose-Bengal Chloramphenicol Agar (DRBC, Oxoid) for yeasts incubated at 25 °C for 5 days. Bacterial colony counts were expressed as the means of log_{10} CFU/g for all replicates. Identification of microorganisms was performed using the MALDI Biotyper complex on the basis of a desktop time-of-flight mass spectrometer with matrix laser desorption/ionisation (MALDI-TOF) Microflex (Bruker Daltonik, GmbH).

2.3 pH measurement
The pH of beef samples (10 g each) was measured using a digital pH meter FiveEasyFE20 (Mettler Toledo, Switzerland) after calibration with commercial buffer solutions at pH 7 and 4. Each sample was homogenised with 100 mL distilled water, and the pH was recorded after signal stabilisation.

2.4 Multisensory analysis of volatile organic compounds
From each piece of beef to be analysed, three samples were taken, one each from the superficial, mid and deep layers. To obtain an average level of VOCs in the beef, each sample under study was crushed, and the required amount was placed in a special glass container (vial). The vial was tightly closed and thermostatically maintained at 5 °C for 5 minutes. At the end of the thermostating time, a needle was inserted into the vial to automatically select the gas to be analysed, which was fed to the VOCmeter. The processing of sensor readings (MOS) was performed using the Argus program 2.5.

3. Results and discussion
Initially, specific spoilage microorganisms were present at low levels, but they grew faster than other microorganisms and were present at high levels at the end of beef storage. For example, Lactobacillus at the initial stage (day 0) numbered less than 1 log CFU/g, but by day 15, had increased to 7.4 log CFU/g.
The maximum microbial levels detected reflected the competition between some of the microbial populations (Figure 1). The total microbial count ranged from 3.6 to 6.7 log CFU/g. The number of other microorganisms (yeasts and *Enterobacteriaceae*) did not exceed 3 log CFU/g throughout the study.

**Figure 1.** Microbial populations during anaerobic storage at 4 °C of vacuum packaged beef.

The predominance of *Lactobacillus sakei* among the *Lactobacillus* spp. was established, and *Hafnia alvei* from the *Enterobacteriaceae* family was also identified. On day 15 of storage, an intensive change in organoleptic characteristics of the beef was determined. This was the appearance of an acidic odour and taste, mucus formation and discoloration. In addition, the content of lactic acid was double that of the control beef examined on day 0, which correlated with the proliferation of *Lactobacillus* during storage. The accumulation of acetic acid was not as intense, but was also increased compared with the level measured at the beginning of the study. The pH of the beef decreased during storage in comparison with the background pH (data not shown).

Organoleptic changes were observed when the count of *Lactobacillus* was 7-8 log CFU/g, which correlated with the results of multisensory analysis of the odour of the beef (Figure 2).

**Figure 2.** Accumulation of volatile organic compounds in vacuum packaged beef stored at 4 °C for 15 days.

In multisensory analysis, a gradual increase in the area of visual imprints (sensors M1-M4, sensitive to aldehydes, ketones and alcohols) was measured, corresponding to the length of storage time. This increase in the amount of VOCs in the gas phase released from the beef was likely associated with the proliferation of the various microorganisms.
Despite this, negative organoleptic changes were observed only at the end of the study (day 15), when multisensory analysis registered high concentrations of VOCs, and the levels of two key microbial indicators were high, i.e. total microbial count (5.9 log CFU/g) and *Lactobacillus* (6.6 log CFU/g).

4. Conclusion

The correlation between increases in chemical markers (VOCs) characterising vacuum packaged beef spoilage and increases in the total counts of spoilage microorganisms (including the prevailing microbial spoilage groups for our anaerobic chilled storage conditions) was established. *Lactobacillus* is proposed as the main group of microorganisms causing spoilage, suitable for predicting shelf life at a threshold level of 5-6 log CFU/g when evaluating the gas phase composition of beef packaged under vacuum and chill stored. Multisensory studies have shown that the sensors in the electronic nose device can detect changes in the odour of raw beef during storage, reflecting the qualitative composition and the quantitative content of substances, depending on the number of microorganisms.

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