ELSEVIER

International Journal of Mass Spectrometry 223–224 (2003) 229–235

International Journal of Mass Spectrometry

www.elsevier.com/locate/ijms

Detection of the spoiling of meat using PTR–MS

D. Mayr[a], R. Margesin[b], F. Schinner[b], T.D. Märk[a,c,*]

[a] *Institut für Ionenphysik, Universität Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria*
[b] *Institut für Mikrobiologie, Universität Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria*
[c] *Department of Plasmaphysics, Comenius University, 84248 Bratislava, Slovak Republic*

Received 1 March 2002; accepted 8 April 2002

Abstract

Using the recently developed proton transfer reaction–mass spectrometer (PTR–MS) method, we investigated the spoiling of air- and vacuum-packed meat (beef, pork, and poultry) that was stored at 4 °C for up to 13 days. We measured and identified partly the emitted volatile organic compounds (VOCs) as a function of storage time and found a large increase in these emissions after a few days of storage. Also a large difference in the spoiling behavior between vacuum- and air-packed meat was observed. (Int J Mass Spectrom 223–224 (2003) 229–235)

Keywords: PTR–MS mass spectrometry; Volatile organic compounds; Headspace of meat; Meat storage

1. Introduction

The preservation of appearance and quality is essential during the distribution and merchandising of perishable products like raw meat. The only criterion consumers have at the point of purchase to select meat cuts is visual appearance. Retail appearance is influenced by numerous factors, including species and muscle of origin, duration of aging, display lighting, temperature [1] and numbers of microorganisms [2].

The principal function of preservative packaging is to delay biodeterioration by restricting the growth of spoilage microorganisms, but to be commercially useful abiotic deterioration—like preservation of the meat color—must also be controlled. Delaying the bacterial spoilage of raw meat requires good hygienic condition of the product and low-storage temperatures as well as the use of preservative packaging. Under aerobic conditions, the dominant spoilage microorganisms are strictly aerobic pseudomonads. They produce offensive byproducts that cause putrid odors and flavors. Under vacuum, the anaerobic conditions prevent growth of these pseudomonads, the microflora is composed mainly of lactobacilli. If the initial number of spoilage bacteria is small, meat will be spoiled slowly by the relatively innocuous byproducts of lactic bacteria. Storage at low temperatures results in reduced growth of spoilage bacteria [3]. The method currently used for determining the status of meat, with respect to spoilage, is analysis of the counts of total viable bacteria and/or specific spoilage bacteria. An obvious drawback with a bacteriological method is the incubation period of 1–3 days that is required for colony formation. For enrichment cultures several days are needed.

* Corresponding author. E-mail: tilmann.maerk@uibk.ac.at

1387-3806/02/$ – see front matter
PII S1387-3806(02)00793-5

In the present work, we have measured the concentrations of several "spoiling compounds" in the headspace air of different kinds of meat that was stored at 4 °C. We compared beef, pork, and poultry as well as the effect of different kinds of packaging (normal air vs. vacuum). The goal of these investigations is to replace the time-consuming bacteriological method by fast headspace air measurements to facilitate the investigation of a huge number of meat samples in very short time and to determine the remaining shelf life of meat during storage from the emissions. Such on-line measurements can also help to investigate the growth of different groups of bacteria, their activities and their metabolites produced in different surroundings (e.g., vacuum/air).

Werner Lindinger always wanted to investigate the spoilage of meat by proton transfer reaction–mass spectrometer (PTR–MS) to find a correlation between volatile organic compounds (VOCs) emitted and the bacteriological contamination. He realized that PTR–MS could be a useful tool to determine the status of a meat sample within minutes and make meat controls in series possible. So nearly every student who was under his supervision, the past 5 years started to do some measurements with meat. However, only a small part of these results got published; they are part of a diploma thesis [4]. Only recently, during the last summer, we started with a new and systematic investigation based on collaboration with M-Preis, Innsbruck supplying the necessary meat cuts. In this paper, we present first results from this campaign and we are dedicating these to the memory of Professor Werner Lindinger.

2. Experimental

2.1. Proton transfer reaction–mass spectrometer (PTR–MS)

The measurements were performed using a PTR–MS system that allows an on-line measurement of trace components with concentrations as low as a few pptv. The method is based on reactions of H_3O^+ ions, which perform non-dissociative proton transfer to most of the common VOCs, but do not react with any of the components present in clean air. The generation of the primary H_3O^+ and the chemical ionization of the VOCs are individually controlled and spatially and temporally separated processes. One important consequence is that absolute headspace concentrations can be calculated without calibration or use of standards. Another big advantage of PTR–MS is that the volatile compounds do not need any preparation like pre-concentration. Thus, the measured mass spectral profiles closely reflect genuine headspace distribution. The PTR–MS system and measuring procedure has been described in detail in [5,6].

2.2. Sample preparation and analysis of VOCs

The meat samples were all cut from the same piece of meat in the M-Preis meat-processing factory, and each of them was separately packed and stored at 4 °C in a refrigerator. For measuring the VOCs, a small piece (about 15 g) was cut from the individual meat samples and was placed in a glass flask (300 mL) that was incubated at 25 °C. The headspace air was drawn at 114 mL/min by a vacuum pump, 14 mL/min of which was led through a heated teflon transfer line into the PTR–MS system for on-line analysis. The mass spectrometric data were collected over a mass range of $m/z = 20$–260 amu.

Table 1
The VOCs emitted by different kinds of normal- and vacuum-packed meat samples were measured as a function of a given time (storage time). The total number of the measured samples throughout the storage time is shown, as well as the number of the figure where the results can be seen

Kind of meat	Packaging	Number of samples	Total storage time (days)	Figures
Pork	Normal	22	13	Fig. 1
Beef	Normal	18	10	Fig. 2
Poultry	Normal	18	10	Fig. 3
Beef	Vacuum	16	10	Fig. 4
Poultry	Vacuum	19	10	Fig. 5

Table 2
We used meat that was initially vacuum packed and stored for a certain time (age) at 4 °C which was then unwrapped and exposed to air for 2 or 4 days, respectively, at 25 °C. We performed the measurements immediately after unwrapping (measurement 1) and some hours later (measurements 2–4). The results can be found in the Figs. 6 and 7

Kind of meat	Age (days)	Exposed to air at 25 °C (days)	Hours after exposing to air				Figures
			Measurement 1	Measurement 2	Measurement 3	Measurement 4	
Beef	6.4	2	0	6.5	49.25	–	Fig. 6
Poultry	5.6	4	0	27.5	38.5	94	Fig. 7

.

2.3. Performed measurements

The emissions of different kinds of meat samples packed in normal air and vacuum were measured one by one in the course of a given time (Table 1). An additional experiment was to measure the VOCs of vacuum-packed meat that was exposed to air after unpacking, and incubated for a few days at 25 °C (Table 2). This additional investigation was carried out for vacuum-packed beef and poultry.

3. Results and discussion

3.1. Measured VOCs

The concentration of many VOCs emitted by the investigated meat samples showed a large increase in the course of storage time. Most of them could be identified as known typical spoiling compounds [7–10]. Just a few of them are shown in Figs. 1–7 (one figure for each kind of stored meat): the sum of ethylacetate, methylpropionate, and propylformate (C4-esters detected on mass 89) in graph A as well as ethanol (it is produced under anaerobic conditions) in graph A, acetaldehyde, and 2-butenal in graph B in each figure.

3.2. Growth of bacteria

A typical bacterial growth curve looks like the following: an initial lag phase where the bacteria get used to the medium components is followed by an exponential increase in the bacterial biomass and metabolic activity. After reaching a maximum, the number of bacteria stays constant for a certain time due to balanced biomass production and cell death before it decreases. We noticed a similar trend in the emitted concentrations of many compounds like those shown in Figs. 1–5. The concentrations started

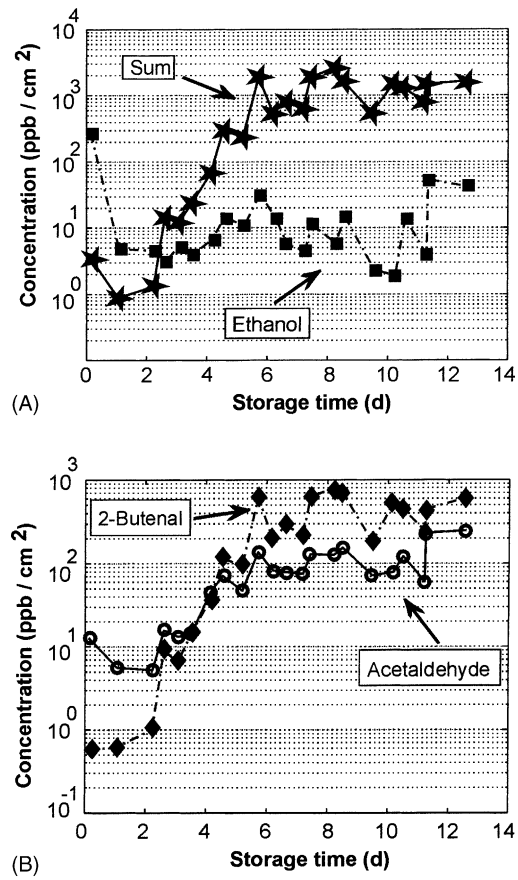

Fig. 1. Concentrations of typical spoiling compounds (✦ : sum of ethyl acetate, methyl propionate, propyl formate; ■: ethanol; ◆: 2-butenal; ○ : acetaldehyde) emitted by normal-packed pork pieces that were stored at 4 °C up to 13 days.

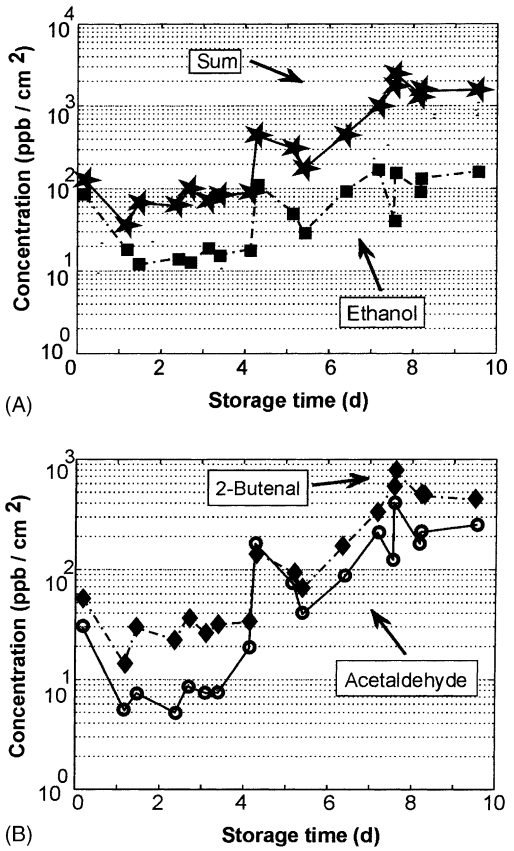

Fig. 2. Concentrations of typical spoiling compounds (✶ : sum of ethyl acetate, methyl propionate, propyl formate; ■: ethanol; ◆: 2-butenal, ○ : acetaldehyde) emitted by normal-packed beef pieces that were stored at 4 °C up to 10 days.

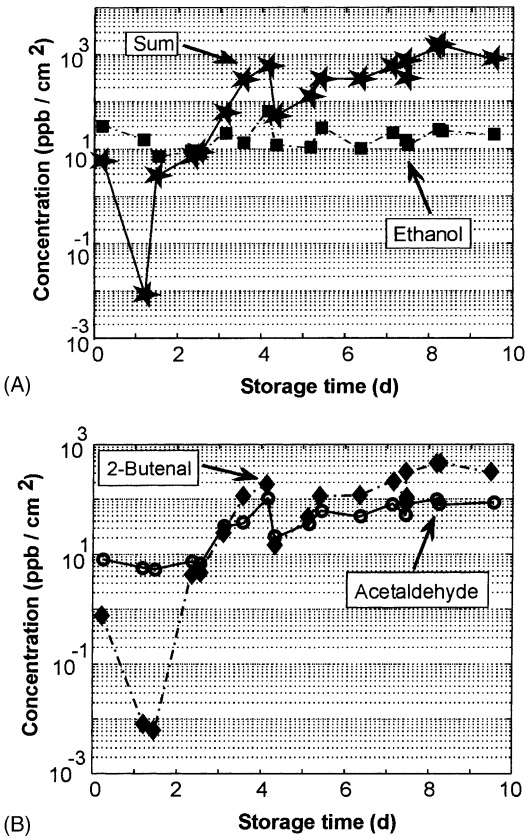

Fig. 3. Concentrations of typical spoiling compounds (✶ : sum of ethyl acetate, methyl propionate, propyl formate; ■: ethanol; ◆: 2-butenal; ○ : acetaldehyde) emitted by normal-packed poultry pieces that were stored at 4 °C up to 10 days.

to exponentially increase after a few days. Then the concentrations remained more or less unchanged. During this stationary phase, the measurements were stopped, so the following decrease was not observed. Because of this pattern similar to a bacterial growth curve, we conclude that bacteria produced these components. This finding holds for all the investigated kinds of meat and packaging.

3.3. Comparison of different kinds of meat

The emissions of the normal-packed pork, beef, and poultry are shown in Figs. 1–3.

Similarities: The emission of the C4-esters, 2-butenal, and acetaldehyde increased in the course of storage time, the concentrations of the C4-esters reached the highest values. The measured concentrations of ethanol against time showed a large variation and no clear trend. The shape of the curves for the C4-esters and 2-butenal were the same for all tested kinds of meat. The VOCs emitted by poultry and pork looked very similar to each other.

Differences: The emissions of beef were larger in the first few days of storage and showed a later and weaker increase than those of pork and poultry.

Conclusion: The C4-esters and 2-butenal were probably produced by the same kind of bacteria at the same time. Acetaldehyde was probably emitted by another kind of bacteria. The spoiling bacteria grew on each

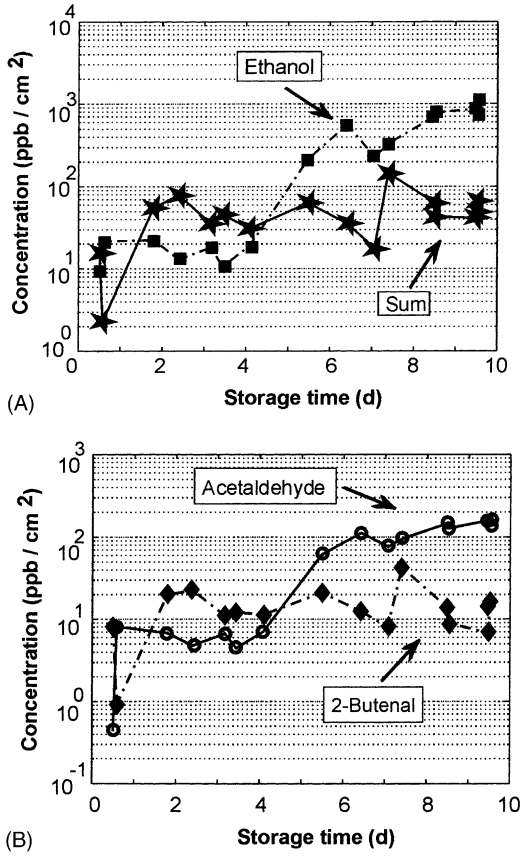

Fig. 4. Concentrations of typical spoiling compounds (✦ : sum of ethyl acetate, methyl propionate, propyl formate; ■: ethanol; ◆: 2-butenal; ○ : acetaldehyde) emitted by vacuum-packed beef pieces that were stored at 4 °C up to 10 days.

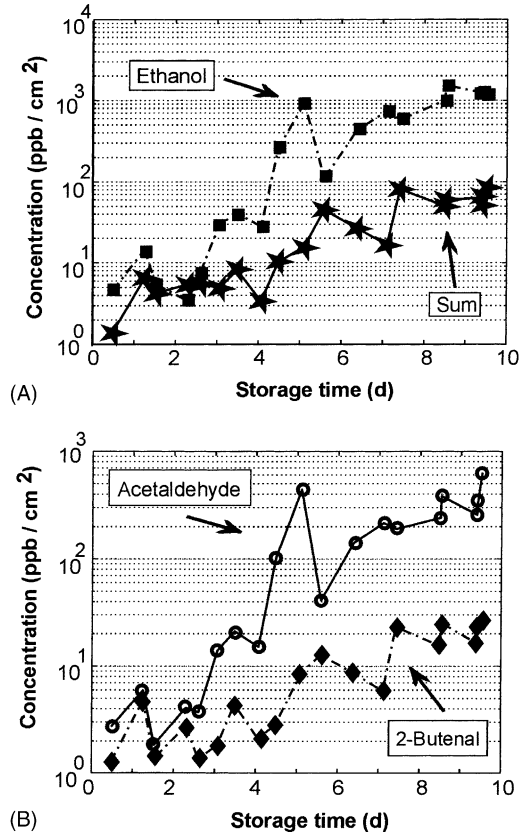

Fig. 5. Concentrations of typical spoiling compounds (✦ : sum of ethyl acetate, methyl propionate, propyl formate; ■: ethanol; ◆: 2-butenal; ○ : acetaldehyde) emitted by vacuum-packed poultry pieces that were stored at 4 °C up to 10 days.

of the three kinds of normal-packed meat at 4 °C and produced no ethanol.

The emissions of the vacuum-packed beef and poultry are shown in Figs. 4–5.

Similarities: The emission of acetaldehyde and ethanol became stronger during storage. Furthermore, the curves for ethanol and acetaldehyde as well as those for 2-butenal and C4-esters had the same shape.

Differences: In the case of vacuum-packed beef the concentrations of the C4-esters and 2-butenal showed a big variation without a clear trend. For vacuum-packed poultry these compounds increased in the course of storage time.

Conclusion: One kind of bacteria—probably heterofermentative lactic bacteria—produced acetaldehyde and ethanol. Under anaerobic conditions, they are metabolically active and in addition to lactic acid also produce ethanol. Another kind of bacteria, which only grew on poultry, emitted 2-butenal and C4-esters.

3.4. Comparison of metabolites produced from normal- and vacuum-packed meat

The concentration of the C4-esters increased strongly under aerobic conditions (normal-packed meat) and stayed nearly constant (beef) or showed just little enhancements (poultry) under anaerobic

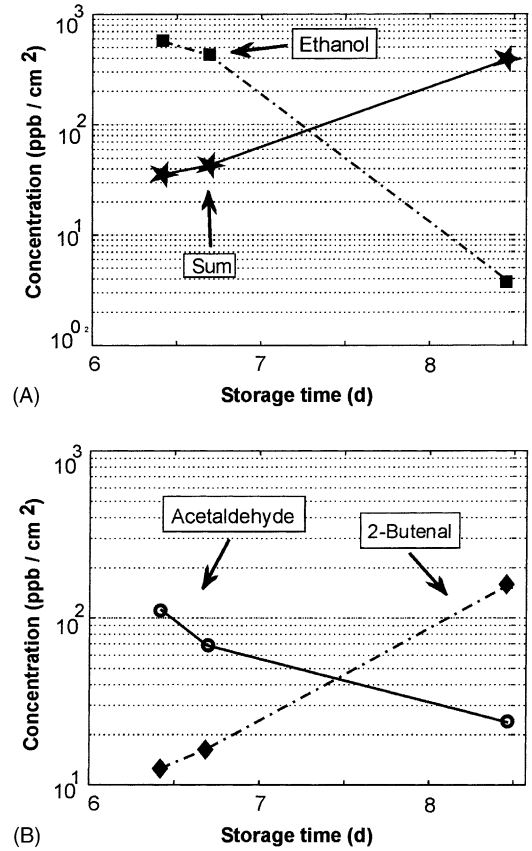

Fig. 6. A vacuum-packed beef sample (previous storage time = 6.4 days at 4 °C) was stored again after a first measurement unwrapped in an open glass flask for 2 days at 25 °C, and emissions (✦ : sum of ethyl acetate, methyl propionate, propyl formate; ■: ethanol; ◆: 2-butenal; O : acetaldehyde) were measured 6.5 and 49.25 h after the first measurement. Ethanol, the typical spoiling compound emitted by vacuum-packed meat, strongly decreased and the C4-esters and 2-butenal increased.

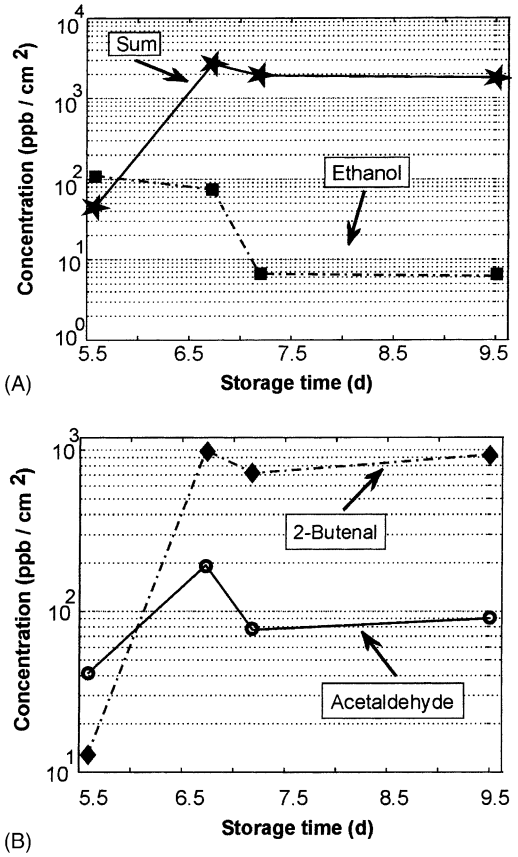

Fig. 7. A vacuum-packed poultry sample (previous storage time = 5.6 days at 4 °C) was stored again after a first measurement unwrapped in an open glass flask for 4 days at 25 °C, and emissions (✦ : sum of ethyl acetate, methyl propionate, propyl formate; ■: ethanol; ◆: 2-butenal; O : acetaldehyde) were measured 27.5, 38.5, and 94 h after the first measurement. Ethanol, the typical spoiling compound emitted by vacuum-packed meat, strongly decreased and the C4-esters and 2-butenal increased.

conditions (vacuum-packed meat). The vacuum-packed meat showed a strong increase of ethanol and just a little (poultry) or none (beef) for normal-packed meat. Acetaldehyde increased much more in the case of vacuum-packed meat than in normal-packed meat. This compound is metabolized under non-strictly anaerobic conditions or formed from ethanol in case of contact with air. The concentration of 2-butenal strongly increased with time in the case of normal-packed meat and weakly (poultry) or not at all (beef) for vacuum-packed meat. The concentration of 2-butenal, possibly an indication of aerobic metabolism, was higher (lower) than that of acetaldehyde (possibly an indication of anaerobic metabolism) for normal-packed (vacuum-packed) meat.

Conclusion: Bacteria producing C4-esters and 2-butenal mainly grew under aerobic conditions (normal-packed meat). Bacteria producing ethanol (like heterofermentative lactic bacteria) and acetaldehyde were typical for vacuum-packed meat.

3.5. Emissions of vacuum-packed meat (beef and poultry) exposed to air

Similarities: The aerobic metabolites, C4-esters, and 2-butenal, strongly increased during the measurement while ethanol, the typical spoiling compound emitted under anaerobic conditions (vacuum-packed), strongly decreased (Figs. 6–7).

Differences: Acetaldehyde decreased in the case of beef and increased in the case of poultry. The increase (decrease) of 2-butenal and the C4-esters (ethanol) was much stronger (weaker) and faster (slower) for poultry than for beef.

Conclusion: Lactic acid bacteria producing ethanol under anaerobic conditions can also grow under aerobic conditions but stop their ethanol production. So either lactic bacteria switched over their metabolism or aerobic bacteria producing C4-esters and 2-butenal replaced the lactic acid bacteria with time.

4. Conclusion

In the present work, we have found that the time dependence of some VOCs emitted by spoiling meat seems to be comparable to a bacterial growth curve. We have observed big differences in the emissions of normal air- and vacuum-packed meat, which result from different bacteria living under aerobic and anaerobic conditions with different metabolic activities.

Encouraged by these results, we will repeat the measurements and additionally, we will carry out a bacteriological examination at the same time. The aim of these experiments is to replace bacteriological examination by fast measurements of the VOC concentrations in the headspace air of the meat sample to facilitate the investigation of a huge number of pieces of meat in very short time and to determine the maximum storage time and storage temperature from the emissions. We will also use this method to investigate the growth of various bacteria, the changes in the microbial composition and the influence of various environmental conditions such as temperature, pH, chemical, and microbial preservation techniques.

Acknowledgements

The authors are grateful to Anton Mölk (head of the M-Preis, Innsbruck) by supporting this study in providing us with the meat samples. This work was supported by the Austrian “Fonds zur Förderung der wissenschaftlichen Forschung” and the “Osterreichische National bank”.

References

[1] L.E. Jeremiah, Food Res. Int. 34 (2001) 749.
[2] M. Al-Bachir, A. Mehio, Food Chem. 75 (2001) 169.
[3] C.O. Gill, Meat Sci. 43 (1996) 99.
[4] H. Judmaier, Master Thesis, Universität Innsbruck, 1997, p. 110.
[5] W. Lindinger, A. Hansel, A. Jordan, Int. J. Mass Spectrom. Ion Processes 173 (1998) 191.
[6] A. Hansel, A. Jordan, R. Holzinger, P. Prazeller, W. Vogel, W. Lindinger, Int. J. Mass Spectrom. Ion Processes 149/150 (1995) 609.
[7] R.A. Edwards, R.H. Dainty, C.M. Hibbard, J. Appl. Bacteriol. 62 (1987) 403.
[8] H.K. Stutz, G.J. Silverman, P. Angleini, R.E. Levin, J. Food Sci. 56 (1991) 1147.
[9] D.U. Ahn, K.C. Nam, M. Du, C. Jo, Meat Sci. 57 (2001) 419.
[10] M.F. King, B.L. Hamilton, M.A. Matthews, D.C. Rule, R.A. Field, J. Agric. Food Chem. 41 (1993) 1974.