

E-NOSE MASS SPECTROMETRY METHOD FOR THE EVALUATION OF ARTIFICIALLY CONTAMINATED MEAT

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Abstract.

A new, simple method, derived from mass spectrometry, with the ability to analyze volatile organic compounds (VOC) under atmospheric conditions, is proposed. The method covers a large spectrum of applications, such as detection of off-odors from different sources with high impact in contamination. In particular, this study focused on the analysis of the biogenic VOC respectively, biogenic amines emission during the meat storage and artificially contaminated meat. The samples were fitted to the capillary nose to collect VOC. VOC are transferred in the vacuum chamber, ionized and analyzed by the mass spectrometer. The mass distribution related to the ionic current leads to estimate the composition of the VOC. This method derived from mass spectrometry could be a useful tool in the meat chain control to identify the type of meat and the level of infestation by analysis of the biogenic volatile organic compounds (BVOCs).

Key words: mass spectrometry, biogenic amines, volatile organic compounds, meat analysis

1. Introduction

In the control chain of the food quality required devices for fast screening and detection of odors, relevant for meat products during preservation. The devices should promptly and accurately detect freshness, spoilage, contamination, consistency or inferior products. The odors originate from the food stored in inadequate conditions. Usually, a complex chain of analytical methods are involved to evaluate the food quality. In the initial stage, from farm to fork, the olfactory inspection with human sensory panels are used. When the spoilage is initiated by the bacterial flora existing in the fresh meat or by accidental infestation with different pathogens a qualitative and fast evaluation is necessary down to ppm limits before to use expensive and time-consuming analytical methods. This work proposes a simplified method derived from mass spectrometry: a heated capillary tube connected to differential turbomolecular pumps and a quadrupole mass spectrometer for vapor analysis, i.e. e-nose mass spectrometer

(e-noseMS, a particular class of e-nose). Therefore, the vapors and volatile organic compounds are sampled directly from food under atmospheric conditions. A qualitative analysis can be performed using the relative abundance of the dominant ions from volatile organic compounds (VOCs). During meat spoilage the odors emission takes place due to the microflora activity which release *biogenic amines* (BAs) respectively, *biogenic volatile organic compounds* (BVOCs), (G. Vinci & al. [1]). BAs are organic bases, produced by the decarboxylation of free amino acids or by the amination and transamination of the aldehydes and ketones with aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, β -phenylethylamine) and heterocyclic (histamine, tryptamine) structure (J. Stadnik & al. [2], G. Suzzi & al. [3]). They have an endogenous origin, formed by the breaking down of the proteins in food, by thermal or bacterial enzymatic decarboxylation of free amino acids (C.W. Tabor & al. [4], H.K. Mayer [5]). Tyramine (Tir), putrescine (Put) and cadaverine (Cad) have been reported as indicators for estimating the bacterial meat spoilage (M. Rokka & al. [6]). An increased level of histamine (His) due to free histidine metabolism are reported in fish spoilage. BVOCs originating from bacterial metabolites other than methane, carbon dioxide or monoxide are: Sulphur compounds (e.g. dimethyl sulphide and dimethyl disulphide), isoprenoids (isoprene is one of the most important biogenic hydrocarbons). Each of them is specific for Gram-positive or Gram-negative bacteria activity. The analytical methods for the identification and quantification of BVOCs are usually HPLC (G.L. La Torre & al. [7]) and capillary electrophoresis (M. Křížek & al. [8]), expensive and time-consuming, therefore inappropriate for a fast evaluation. The detection of BAs and BVOCs from meat spoilage *in situ* without any preparative and time-consuming methods is a challenge. There are a few biosensors based on functionalized carbon nanotubes for BAs detection that use electroanalytical methods (amperometric sensors) (J.F. Rochette & al. [9]), radioimmunoassay and enzymatic methods (K. Punakivi & al. [10]) for the detection of by-products such as ammonia (Am) (A. Airoudj & al. [11], S. Carquigny & al. [12]). Electronic noses (e-nose), which mimic the animal olfactory systems, were developed for the BVOCs detection. E-nose are electronic systems based on a sensor array of metal oxide structures (MOX) (Z. Wendong & al. [13]), conducting polymer composites (H. Bai & al. [14]), carbon nanotubes, graphene (Y. Shao & al. [15]) or chemical field effect transistors (ChemFET) (J. Janata [16]). Unfortunately, they are blind sensors, each structure responding to a specific gas or vapour with large interferences (A.C. Romain & al. [17], Y.S. Yang & al. [18]), having the lowest detection limits at around 500 ppm (J. Huang & al. [19]). Recent advances pushed the detection limits down to 100 ppm for NH₃ (P. Bhatia & al. [20], N. Gabouze & al. [21]) and H₂S (M. Singh & al. [22], N. S. Ramgir & al. [23]), ~ 200 ppm for CO (P. Cosoli & al. [24], H. Yamaura & al. [25]) and CO₂ (S.A. Waghuley & al. [26], L.M. Cavanagh & al. [27]). Some sensors use the immobilization of the olfactory receptors on nanostructures in order to extend the range of odors which can be identified (G. Gomila & al. [28]). In spite of the effort to design e-nose based on the array of individual gas sensors, they remain expensive and not suitable to replace analytical methods. In addition, if it is taken into account the low vapor pressure of the biogenic amines and by-products during food spoilage, the identification of the odor emission down to 20 ppm and continuous monitoring with portable bench top MS-analyzer can be achievable due to advances in technology that developed new methods in order to get samples from atmosphere or direct from food packaged in the market or from producer. Based on these assumptions, several years ago, a modular system was developed: a bench top analyzer with the capability to collect off-odors emission of samples under atmospheric conditions and analyzed in the mass range 1-300 Da, which covers a lot of fingerprints of the metabolites released

during infestation or meat spoilage. This concept can be successfully applied in beverage, vintage classification or in plant essential oils analysis. In particular, for the meat spoilage, a series of experiments with fresh meat and controlled infestation with *Pseudomonas aeruginosa* are performed. The meat spoilage is evaluated for volatile organic compounds (VOCs) emission and related with biogenic amines (BAs) as by-products. Note that BAs are considered the index for the food freshness (G. Vinci & al. [1], F. Galgano & al. [29]). *Pseudomonas aeruginosa* is a Gram-negative microorganism with specific BVOCs emissions, i.e: 1) dimethyldisulfide (DMDS), dominant species 2) dimethyltrisulfide (DMTS), 1-undecene (Und) and isoprene in low but still significant concentrations (C. Schöller & al. [30]).

2. Materials and methods

Materials. Commercial slices of fresh meat (pork) were transferred in sterile bags (artificial stomach) and inoculated with *Pseudomonas aeruginosa* ATCC 27853 strain in different concentrations (P₀ - fresh meat blank, P₁-10², P₂-10³, P₃-10⁴, P₄-10⁶ colony - forming units per gram, CFU g⁻¹). The inoculation protocol was performed in agreement with SR EN ISO 13720/2011. Each sample was stored for 24 h and 48 h at 4 °C.

Equipment. E-noseMS derives from a residual gas analyzer, a mass spectrometer of small dimensions, whose function is to analyze the gas inside the chamber, either vacuumed or at normal conditions (M. Bhuyan & al. [31]). The gas molecules in the vacuum chamber are sampled and ionized. The ions are measured according to their molecular mass by a quadrupole mass spectrometer. The capacity to measure up to a 300 Da mass is enough to detect vapors from a large class of complex organic molecules. To bring vapors from atmospheric pressure to operating conditions (~10⁻⁷ mbar) one needs a new experimental set-up, based here on Pfeiffer technology. A gas inlet system consists of a three-way sampling vacuum chamber (Figure 1) with pressure regulated down to 10⁻³ mbar by dry-compressing diaphragm vacuum pump and a Hi Pace turbo pump. The gas inlet is equipped with a capillary (nose) that can be heated up to 350 °C. The capillary is made of stainless steel. The heated capillary prevents vapors condensation during analysis. The third way of the sampling chamber is connected to the PrismaPlus® quadrupole mass spectrometer headed on dry pumping station, composed of a diaphragm vacuum pump MVP and a Hi Pace turbo pump. The Quadera mass spectrometer software enables both qualitative and quantitative analyses to be performed up to 300 Da at a concentration less than 1ppm.

Methods. The meat stored in artificial stomach was transferred into a sterile glass bottle in slices of 5 g. The glass bottle, closed with a rubber stopper, was fitted to the capillary nose with a disposable sterile syringe needle and a sterile HEPA filter with 45-µm pore size to prevent cross-contamination. The vapors were sampled and transferred into mass spectrometer chamber and analyzed the mass distribution related to the ion current for each component. There are three approaches to evaluate the concentration of the vapors in sample. The first approach consists of the evaluation of ionic current of each component to the total ionic current. The second approach evaluates the relative abundance of each vapor related to the main component in agreement with (B.D. Mistry [32]). The third approach takes into account the main molecular fragments resulted from the electronic ionization in the quadrupole mass spectrometer. The relative abundance (Rel abn %) is defined as:

$$\text{Rel abn}(\%) = \frac{\sum \text{Ion Current}_{\text{Compound}}}{\text{Ion Current}_{\text{reference}}} \times 100$$

where the sum is carried over the molecular ion fragment contributions. The ionic current of nitrogen was considered as a reference. This method is a qualitative estimation of the meat spoilage but gives a rapid evaluation

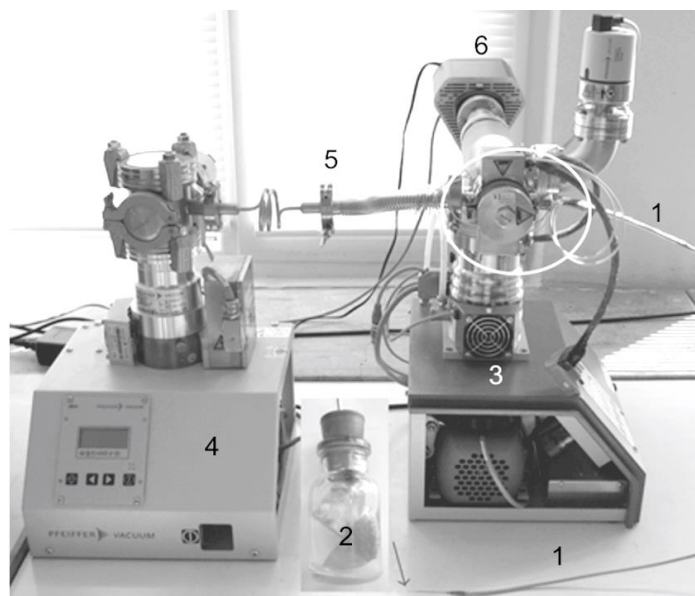


Figure 1. E-nose mass spectrometer, experimental set-up. 1) capillary tube with heating mantle 2) glass bottle for meat storage with connector to the capillary tube 3, 4) turbomolecular pump 5) pressure reduction line 6) MS quadrupole. 3-way vacuum chamber (circle)

3. Results and discussions

BVOCs and BAs analysis at 24 h

In figure 2, BVOCs emission (A) and BAs vapour (B) during spoilage for samples P0-P4 stored 24 h at 4 °C are shown. The relative abundance for BAs is quite very low because they have a very low vapour pressure even at high temperature (for example, His at its boiling point (~ 440 K) has the vapour pressure of about 1 mbar. At room temperature, the vapour pressure is lower than a tenth of microbar). BVOCs are abundant being the by-products from original microflora and from the induced contamination with *Pseudomonas aeruginosa*. In all the samples, ammonia (Am) and hydrogen sulfide (HydS) as well as nitrogen are the dominant components having origin in microflora metabolism (besides CO₂, H₂O₂ and other sulfides (G.J.E. Nychas & al. [33], not shown in figure 2A). Earlier studies with GC-MS up to a 150 Da mass on volatile compounds produced by *Pseudomonas aeruginosa* indexed a large range of significant compounds such as ethyl and methyl esters from C₂-C₈ fatty acids, sulphur-containing compounds, methane and isopropane thiols and their related sulphides and thioesters but not hydrogen sulphide (R.A Edwards & al. [34]). BVOCs, characteristic for *Pseudomonas aeruginosa*, are reported to be: DMDS (dominant component), DMTS, respectively Und. On the other hand, during meat spoilage, the initial microflora releases BVOCs in large range covering C₂-C₈ compounds identified by GC-MS and correlated with the results from a vast literature concerning amoniactal and sulfur compounds. Besides acetone, other compounds like methyl ethyl ketone, dimethyl sulfide and DMDS were established as indexes of microbial spoilage (H.K. Stutz & al. [35], D. Mayr & al. [36]). Therefore, for simplifying the analysis, are taken into account specific features of the BVOCs and biogenic amines vapours indexed

by e-noseMS. All off-odors increase with the level of infestation. The uncontaminated sample P0 shows after 24 h, a very low BVOCs (Figure 2A) concentration respectively BAs (Figure 2B), the Relabn is less than 9×10^{-4} (their presence is explained by decarboxylation reactions induced endogenously, from degradation of amino acids in the fresh meat). Usually, the concentrations of His, Cad, Tir increase with the level of contamination, except spermidine. Spermidine (Spe) is a particular case which decreases when the microbial loading increases (Relabn %: $P0 = 8.8 \times 10^{-4} > P1 = 6.3 \times 10^{-5} > P2 = 5.9 \times 10^{-6} > P3 = 3.4 \times 10^{-8} > P4 = 5.8 \times 10^{-9}$, Figure 2B). The chemical reactions that induce the production of Spe are different compared to other biogenic amines: this compound is found mostly in fresh meat. It is a natural substance that controls the intracellular pH and maintains the membrane potential in the living cell. It decreases during the meat spoilage (P. Paulsen & al. [37]).

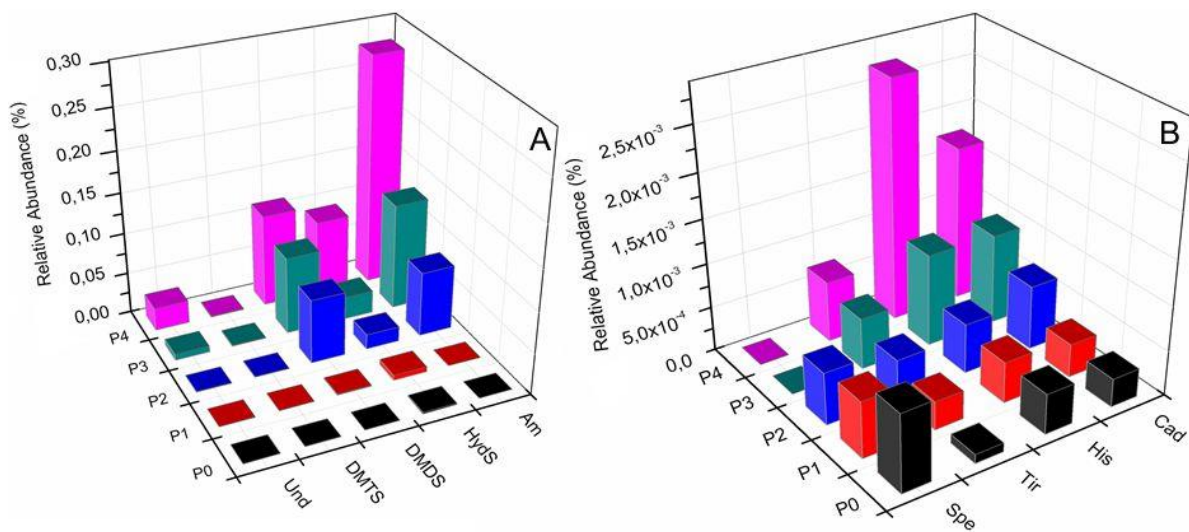


Figure 2. The relative abundance of BVOCs (A) and off-odors emission from BAs (B). The batch of samples P0-P4, stored at 4 °C for 24 h. E-nose capillary heated at 25 °C

The BVOCs concentration increases when the microbial loading increases according to the following relation: $Am > DMDS > HydS > DMTS, Und$. The concentration of BAs vapours increases in a specific order: $His > Cad > Tir$. This is an important conclusion in development of specific chemosensor array for the meat freshness analysis. It is enough to quantify Am, DMDS and His by a chemosensor array in order to establish the level of the meat spoilage.

BVOCs and BAs releasing during storage at 24 h and 48 h

After a longer storage of the inoculated samples, even if they are preserved under proper conditions, the meat spoils fast with the release of a high amount of BA and BVOC. For example, samples P2 and P4 with lower respectively highest level of inoculation after 48 h, approximately doubled and even tripled the quantity of BVOCs. In Table 1, a comparison between two samples, P2 and P4, is shown where the level of inoculation $P4/P2$ is 10^3 times higher. By comparison, for a storage time of 24 h and 48 h, BVOCs increased ~ 1.9 times respectively ~ 2.7 times. The results presented in Figure 3 are in agreement with the growth ratio (GR) for BAs in samples P2 and P4

Table 1. The relative abundance (*Rel. abn.*) for BVOCs in samples P2 (10^3 CFU g^{-1}) and P4 (10^6 CFU g^{-1}) stored 24 h and 48 h

BVOCs	<i>Rel. abn.</i> [%]			<i>Rel. abn.</i> [%]		
	P2-24h	P2-48h	GR	P4-24h	P4-48h	GR
Am	3.438	5.507	1.6	8.572	23.144	2.7
HydS	0.020	0.040	1.6	0.030	0.080	2.7
DMDS	0.147	0.275	1.9	0.933	2.519	2.7
DMTS	0	0	0	0.049	0.132	2.7
Und	0.148	0.275	1.9	0.880	2.377	2.7

GR- growth ratio

The relative abundances for Cad, His, and Tyramine, increase more than two times even though the level of contamination is very low such in the case of P2 (10^3 CFU g^{-1}).

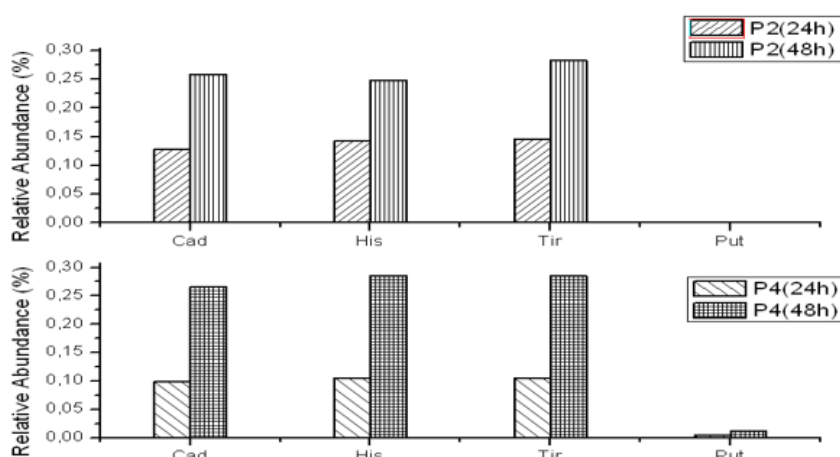


Fig. 3. Relative abundance of BAs in samples P2 and P4 stored at 24 h and 48 h

The putrescine is under detection limit for sample with low inoculation loading, P2 (10^3 CFU g^{-1}) and relative detectable for high loading P4 (10^6 CFU g^{-1}). Taking in account the *Pseudomonas* fingerprints where Und and DMDS are dominant, His, Cad, Tir can be appreciated, at least qualitatively. BVOCs, besides ammonia, found in all types of spoilage, might give a first indicative on the infestation type. This will simplify the analytical methods for a quantitative analysis.

The nose capillary temperature influence on the BVOCs and BAs detection

Another series of experiments were performed related to the influence of temperature on the capillary nose. In Table 2 a new series of samples loaded with the same CFU g^{-1} are recorded BVOCs and BAs for two temperatures of the capillary nose. The heating of the capillary tube increases the sensibility of the detection and prevents the vapour condensation.

For sample P2 (10^3 CFU g^{-1}), putrescine is not detected neither at 25 °C, nor at 75 °C, because the microbial load of this sample was too low and also, the storage temperature between measurement was maintained at 4°C. The difference in values between Table 1 and 2 comes from different sources of the meat but the growth ratio keeps similar values.

Table 2. The relative abundance (*Rel. abn.*) of BVOCs and BAs in samples P2 (10^3 CFU g^{-1}) and P4 (10^6 CFU g^{-1}) at 25 °C and 75 °C (at 24 h)

BVOCs	<i>Rel. abn.</i> [%]		<i>Rel. abn.</i> [%]	
	P2 – 25 °C	P2 – 75 °C	P4 – 25 °C	P4 – 75 °C
Am	3.279	12.420	8.610	32.800
HydS	0.023	0.087	0.038	0.144
DMDS	0.139	0.530	0.898	3.412
DMTS	0	0	0.042	0.160
Und	0.141	0.538	0.873	3.326
BAs				
Put	0	0	0.0063	0.0260
Cad	0.0062	0.0170	0.0071	0.0305
His	0.0078	0.0394	0.0087	0.0426
Tir	0.0048	0.0201	0.0052	0.0283

4. Conclusions

E-nose mass spectrometer can be used in the food chain control at least for a qualitative evaluation. Due to the high level of miniaturization of the e-noseMS, this could be integrated in simple control line either in market or to the producer or retailer where the fresh meat can be evaluated directly by the consumer. This method allows a deep and extensive research of odor and BVOCs emissions in order to identify the type of meat and the level of infestation. Therefore, a comparison with the analytical methods needs to be performed for implementation of this method in the food chain control. BVOCs and BAs vapours are detectable in the ppm range, essential to evaluate the food quality and its preservation under proper conditions. If any infestation will take place at a very low loading level this can be fast detected. The present study on *Pseudomonas aeruginosa* confirms the sensibility of the method. The results obtained in this study using a method of analysis based on the relative abundance correlate with the ones obtained using the microbiological activity via BAs and BVOCs emissions.

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