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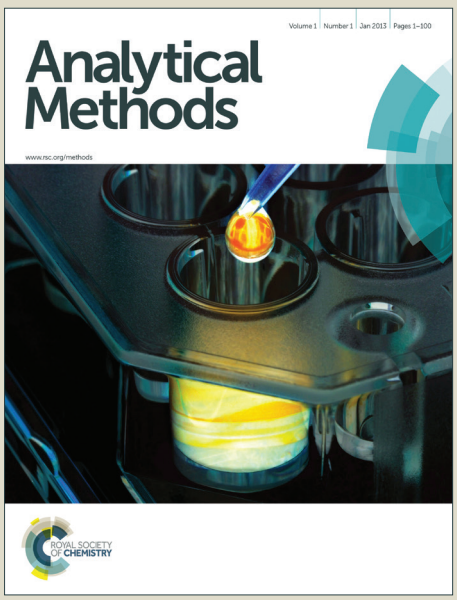

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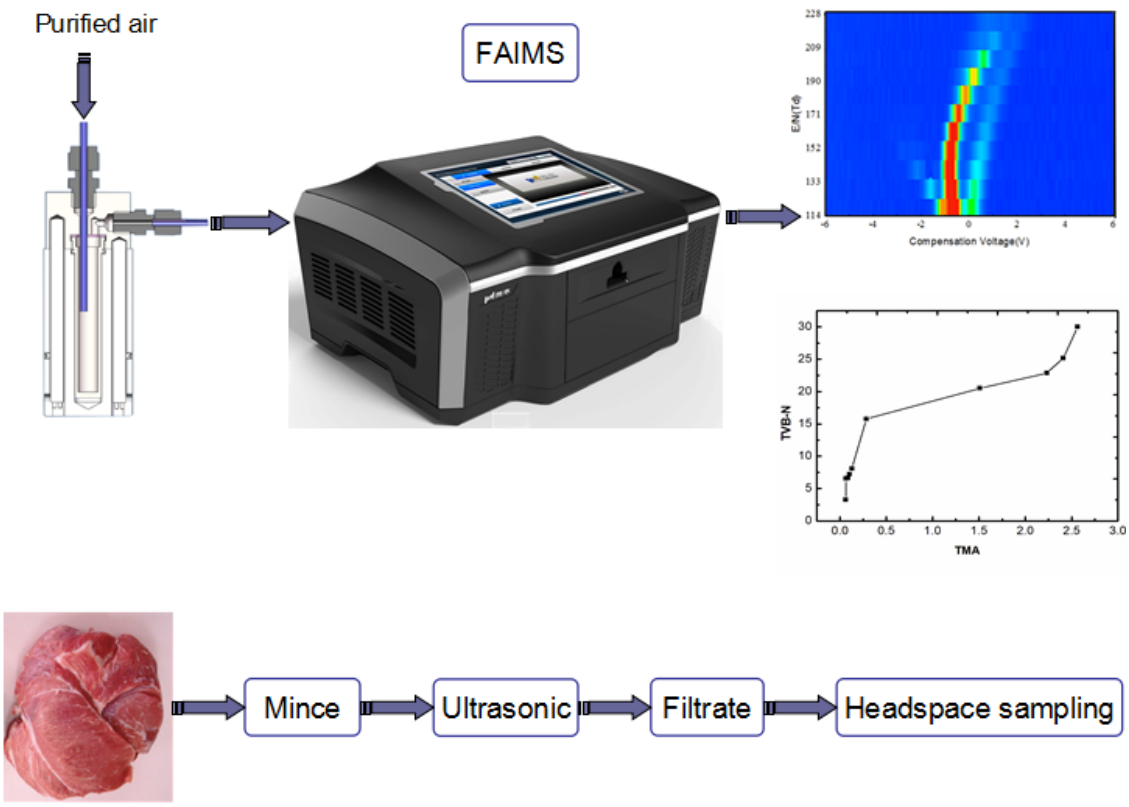

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Rapid Microchip-based FAIMS Determination of Trimethylamine, an Indicator of Pork Deterioration

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ABSTRACT: A fast and quantitative method for detection of biogenic amines based on microchip-based high-field asymmetric waveform ion mobility spectrometry (FAIMS) technique was established for evaluating the degree of spoilage of pork stored at room temperature. Trimethylamine (TMA) was selected as a good indicator for its volatility and high proton affinity. Analysis from standard TMA showed that it exhibited unique characteristic FAIMS spectrum out of that of fresh pork as background. The limit of detection of the system for TMA dissolved in deionized water with adding 100 μL of 8 mol/L NaOH was 3 ng and the measurement was accomplished within 15 s. The relative standard deviation (RSD) was 6.20%. The delivery of sample gas was based on the dynamic headspace method. The effect of storage time on the formation of biogenic amines was examined, and not surprisingly, the content of TMA increases over time. The total volatile basic nitrogen (TVB-N) was measured simultaneously based on the classical semimicro Kjeldahl method. A good correlation coefficient between these two methods was obtained, correlation coefficient was 0.952. Therefore the microchip-based FAIMS technique provides a quantitative classification of the spoilage or freshness of pork, especially when a rapid detection is needed.

KEYWORDS: *High-field asymmetric waveform ion mobility spectrometry (FAIMS); pork spoilage; trimethylamine (TMA); semimicro Kjeldahl method*

INTRODUCTION

In recent years, the topic of monitoring the quality of pork has been paid more attention with food safety accidents happening frequently.[1] During storage, transport and food processing, biogenic amines are formed in the presence of enzymatic decarboxylation of specific amino acids in meat.[2] The content of biogenic amines in meat was used as a freshness indicator or as a spoilage indicator.[3] Spoilage pork doesn't meet the needs of people on taste, and contains ranges of proved chemicals which potentially

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jeopardize to people's health.[4] Therefore, it is very necessary to establish a rapid detection method for the spoilage status of pork during storage.

There are several analytical methods to determine the volatile biogenic amines emanating from spoilage meat, such as gas chromatography-flame ionization detection (GC-FID),[5,6] high performance liquid chromatography (HPLC)[7,8] and gas chromatography-mass spectrometry (GC-MS).[9] Each of chromatographic techniques present good selectivity and sensitivity. However, these chromatography techniques require extra sample preparation, such as extraction, purification and derivatization of the amines to form non-polar volatile compounds. And that several recent publications involved the use of ion mobility spectrometry (IMS), which provides a rapid detection method for biogenic amines. IMS is simple, fast and sensitive to a wide range of compounds making it potential to detect the biogenic amines.[10,11] Zeev Karpas[12] studied the effects of storage time and temperature and the type of meat on the formation of biogenic amines with taking TMA as an indicator thanks to its volatility using the IMS technique. The research work demonstrated that biogenic amines may be readily monitored by IMS. However, the spectrum of TMA from IMS showed a lower resolution which was about 15. This is an obstacle to wide application of IMS technique because of the complexity in classes of species of real samples.

High-field asymmetric waveform ion mobility spectrometry (FAIMS) is an analytical technique for atmospheric pressure separation of gaseous ions, which has broad application in areas such as explosives,[13] hazardous chemicals,[14] chemical warfare agents,[15] proteomics[16,17] and drug analysis.[18] FAIMS technique is developed on the basis of traditional IMS technique. In contrast, the biggest difference is that FAIMS technique distinguished different ions by scanning compensate voltage (CV) and electric field strength,[19] with introducing a new dimension to scan parameters, the spectra of

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FAIMS presented more fingerprint information of sample molecules. And that the home-made microchip-based FAIMS with scanning period of 1 second from CV of -6 V to +6 V under fixed electric field is an ultrafast version compared to the traditional FAIMS technique, which enabled a multi-dispersion field CV scanlines as a spectrum to be presented in a very short period of time which was enough for continuous sample introduction.[20] The FAIMS ion drift chip was fabricated using Micro-electromechanical systems (MEMS) technology with a structure the same as [20]. The field intensity can be as high as 75000 V/cm much higher than the largest separation field intensity of traditional FAIMS which is only 20000-30000 V/cm. Potentially it would lead to higher resolving power for the reason that compensation field (E_c) of many chemicals is proportional to the separation field(E_d). The purpose of current research is to evaluate the performance of an FAIMS device as a potential instrument that could rapidly identify the spoilage status of pork quantitively. One of the unique features of FAIMS is that it is sensitive to compounds with high proton affinity or electron affinity. Among these amines, the proton affinity of TMA is 948.9 kJ/mol (NIST Chemistry WebBook), which is close to the top of the proton affinity scale. So we took TMA as the indicator because of its volatility and high proton affinity. Then the TMA spectra of different storage time of pork spoilage were compared with the concentrations of TVB-N, indicating the spoilage status of pork. Finally we demonstrated that the FAIMS technique could be applied in the identification of different spoilage status of pork successfully in the laboratory.

Experimental

Instrumentation

The instrument used in this study was developed by Suzhou Weimu Intelligent System Co. Ltd based on the FAIMS technology referenced by Shvartsburg, et al.[20] The dispersion field can achieve as high

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as 61000 V cm^{-1} (284 Td at standard atmosphere) with 35 μm gap. The frequency of waveform is 25 MHz. A full compensation voltage scan for both positive ions and negative ions from -6 V to +6 V takes less than 1 second. To fully take advantage of scan speed, a whole dispersion field vs. compensation voltage (CV) spectrum consists of eleven selected CV scans at different dispersion field, which implies an eleven seconds detection for one sample.

GC-MS measurements for TMA hydrochloride standard

TMA hydrochloride (CAS 593-81-7) was purchased from Aladdin Industrial Corporation and used without further purification after its purity was tested with GC-MS (Agilent, 7890a GC with 5975C MS). 27 mg of TMA hydrochloride samples was dissolved in 5 mL of deionized water, another 5 mL of 8 mol/L NaOH was added to enhance the volatilization of the TMA. Sample solution was placed in a vial with a volume of 20 mL hermetically sealed with silica gel cap and stitched with aluminum head. The sample solution was heated for 10 min at 80□ by using water bath heating. Then 0.1 mL of headspace vapor was directly introduced to the injector port of gas chromatograph.Separation was carried out on a DB-1701 chromatograph column (30m × 0.32mm i.d., 0.25 μm film thickness) was used with an oven temperature program of 50 □ (3 min) at 10 □ min^{-1} up to 180 □ (4 min). The carrier gas was high purity helium (He >99.999%) from Jinhong (Suzhou, China) with a flow rate of 1.5 mL min^{-1} and the split ratio of chromatographic injection is 1:20.

FAIMS analytical conditions

Detected ion concentration and spectrum were influenced by headspace sampler temperature, carrier gas flow rate, pressure and humidity. Among all these conditions, headspace sampler temperature and carrier gas flow rate influenced the concentration and transmission of sample molecules respectively;

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the pressure affected the mobility; and humidity mainly affected the species of sample product ions. For this reason, optimal conditions should be selected to ensure that the spectra of target sample have suitable response and resolution. Since the experiment was performed at atmospheric pressure to guarantee the possibility of in-situ applications, we optimized the remaining three factors. Through the optimization experiment, the headspace sampler temperature was set to 90□, the sampling flow was set to 2200 mL min^{-1}, and the absolute humidity of the sample flow is 1.78 $g.m^{-3}$ by adding a humidity generator in the flow path.

Sample preparation and measurements

Standard solutions were made of trimethylamine hydrochloride taken directly from the flask. Concentrations were: 0.5 μg mL^{-1}, 1.0 μg mL^{-1}, 2.0 μg mL^{-1}, 4.0 μg mL^{-1}, 6.0 μg mL^{-1}, 8.0 μg mL^{-1} and 10.0 μg mL^{-1}. A 100 μL of these solutions and 100 μL of 8 mol/L NaOH were added to dynamic headspace sampler (Fig. 1a). The flow path in this study is shown in Fig. 1b, a dry air flow and a moisture air flow were mixed and the proportion could be regulated to control the humidity of the total flow into the instrument.

Pork samples were purchased at supermarket in Suzhou. Ground and put into a clean plastic bag and then placed on a table in the room kept at 30□ constantly. For comparison of the FAIMS measurements with TVB-N method, on a more quantitative basis, classical semimicro Kjeldahl method was implemented. A 10 g sample of pork was ultrasonic vibrated with deionized water for 10 min at room temperature every three hours. The stock solution thus obtained, which is known as “broth”. Then these solutions were placed in freezer for standby.

100 μL of pork broth was delivered to a mental diffusion tube, and 100 μL of 8 mol/L NaOH was

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added for accelerating the volatilization of biogenic amines. The sample vapor was carried into the FAIMS in a flow of pure air. Then the concentration of TMA in pork can be obtained by comparing the height of TMA with standard curve. As a contrast, the concentration of TVB-N in broth was determined by semimicro Kjeldahl method. 5 mL of pork filtrate was mixed with 5 mL of magnesium oxide suspension. The mixture was then transferred into the distillation reaction chamber for 5 minutes. The TVB-N was absorbed by boric acid after vaporization from weak alkaline solution. The content was titrated with standard acid solution to violet.[21] Blank experiment was preceded simultaneously. The criterion of evaluating the pork freshness based on the concentration of TVB-N in pork was summarized to three grades[22] as below: less than 15 mg/100 g for fresh pork; greater than 15 mg/100 g and less than 25 mg/100 g for sub-fresh pork; greater than 25 mg/100 g for corrupted pork.

RESULTS AND DISCUSSION

Purity of TMA

Only one peak with a retention time of 1.309 min existed in the total ion chromatogram (TIC) of mass spectrometer as shown in Fig. 2a. The mass spectrum corresponding to the peak is shown in Fig. 2b was identified as TMA by comparing with the NIST-library mass spectrum of TMA (Fig. 2c). The ions around m/z 59 are attributed to the molecular ion, while the ions around m/z 58 are considered to be the quasi-molecular ion.

Optimization of the FAIMS conditions

Effect of dynamic headspace sampler temperature.

The selection of the temperature is important to the dynamic headspace sample introduction. The higher the temperature, the more sample molecules were dynamically brought into the FAIMS detector,

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which obviously promoted the detecting lower limit. However, an excessive temperature has an unfavorable influence on detecting by promoting different excessive chemicals competing for charges with TMA molecules in the ionization region as well as elevating the thermal effect during the ion drifting which as a result lower the sensitivity, and moreover potentially leads to sample molecules instantaneous diffusion or decomposition.

Fig. 3 depict the effect of temperature on the magnitude of signal of TMA . It can be found that the ion current of TMA increased when the temperature increased from 30 ℃ to 90 ℃. However, the ion current was significantly decreased when the temperature was above 90 ℃. This phenomenon was mainly because the samples exhausted due to the instantaneous diffusion rather than decomposition. We chose the 90 □ as the optimal temperature.

Effect of carrier gas flow rate

The effect of carrier gas flow rate on sensitivity and resolution of TMA was studied over the range of 1.0 to 3.1 L min^{-1}. A smaller flow rate produces poor performance on the sensitivity of detection, while extra flow rate would affect the resolution.[23] As can be seen from Fig. 4a, the ion current of the characteristic peak presented the increasing trend. This is because more sample molecules reached the detector at the same time with the increase of flow rate. However, Fig. 4b shows that the resolution of the characteristic peak showed opposite trend. The main reason for this phenomenon is that the increase of gas flow rate lead to the reduction of the sample migration time, and reduce the deflection cycle number of product ions in the asymmetric strong field as well as reducing the displacement alone the vertical direction of electrode plate, which decreases the chances of product ions collisions on the plate and result in a increase of full width at half maximum and the decline of resolution.[24] As a trade-off between sensitivity and resolution, 2.2 L min^{-1} was chosen as the optimal flow rate.

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Effect of humidity.

The ion reaction mechanism of Ni63 ion source was described in [25]. The reactant ions ($H^+(H_2O)n$ and $O_2^-(H_2O)n$) were generated after a serial reaction excited by the high energy electron beam from Ni63 source. Sample molecules (M) are ionized and generated product ions by the collisions with reactant ions and the water molecules have been replaced from the hydrated proton,[25] In this paper, we analyze the positive ions because TMA ions only exist in this mode.

$$M + H^+(H_2O)n \Longleftrightarrow MH^+(H_2O)n \Longleftrightarrow MH^+(H_2O)n–x + x(H_2O) \quad (1)$$

The reaction is reversible. The concentration of water vapour in the flow path impacted the formation of product ions by providing enough reactant ions as well as competing for hydro-protons in the process of soft ionization, thereby affecting the analyzing performance of FAIMS. Enough reactant ions for product ions were created by plenty of water and excessive water molecules then prevent the formation of product ions. In this study, we controlled the humidity by applying a humidity generator (HG-01, Weimu Intelligent System Co. Ltd) in the diluting flow in the flow path, and regulated the ratio of diluting flow rate to the total flow rate. The effect of humidity on the response of TMA was shown in Fig. 5. The absolute humidity of 1.78 $g.m^{-3}$ corresponding to 200 mL min^{-1} of moist air flow rate was chosen as the optimal humidity.

The FAIMS spectrum of TMA

The FAIMS spectrum of background and TMA with concentration of 5 μg mL^{-1} in positive mode are shown in Fig. 6a and 6b respectively. A single CV scan for both TMA and background at 142 Td was extracted and layed out in Fig. 6c. A unique peak appears at CV= -0.77 V, which is appointed to TMA monomer. The magnitude of reactant ion peak located on the left decreased greatly in the scan for TMA for the reason that TMA with higher proton affinity favored hydro-protons in the process of ionization.

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The formation of dimer[26] was appointed to the peak at CV= 0.45 V. The Standard curve with R^2 of 0.99 was established. The limit of detection was 3 ng and the limit of quantification was found to be 10 ng for TMA dissolved in deionized water.

The spectra of pork at room temperature (average 30 □) from FAIMS.

Pork with different storage periods from 3 hours to 30 hours were analyzed at 142 Td. Shown in Figure 7a, the spectra of filtrate of pork with different storage periods manifested the characteristic peak of TMA. It is indicated that TMA has a higher proton affinity than other volatile compounds of spoilage pork. As the time went on, the response of reactant ion peak decreased gradually while the response of TMA increased. Small changes in the peak magnitude of TMA for the samples stored for several hours were followed by a sharp elevation for the pork sample stored for 21 hours, which might be related to the result of the fulminant growth of microorganisms. Each sample of pork filtrate was repeated for three times. Fig. 7b shows the chronological change of quantities of TMA in pork sample. Table 1 summarized the concentration of TMA in the field of 142 Td and the content of TVB-N in every 100 g pork from semimicro Kjeldahl method over time. The concentration of TVB-N and TMA present similar trends and which supports the validity of the approach of taking TMA as the indicator for pork freshness in this study.

Correlational analysis between content of TMA and that of TVB-N.

The content of TMA and that of TVB-N over time were correlated in Fig. 8. Distinctive positive correlation is achieved. The correlation coefficient between them was calculated to be 0.952. The definition of conversion phase from fresh to spoilage pork can be set to the point at the concentrations of 15 and 25 for TVB-N and the response at 0.35 (Concentration of 0.791 mg/100 g) and 2.41

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(Concentration of 4.95 mg/100g) for TMA respectively. The criterion of fresh pork determined by FAIMS can be expressed as that the response value of TMA is less than 0.35 in a pork sample with the preparation process described as before. When this value is greater than 0.35 and less than 2.41 from FAIMS detection, actions must be taken otherwise the pork is not suitable for food anymore. The sample has to be abandoned if this value is greater than 2.41.

Validation of analytical methods

System suitable

The determination of background was required before each test. In this progress, we would inspect the consistency of compensation voltage value, the ion current and the FWHM in order to ensure the reliability of this analytical method.

Specificity

The spectra of TMA and 24 h pork broth were analyzed at 142 Td, 170 Td and 199 Td, respectively. Fig. 9a, 9b and 9c show that the characteristic peak of 24 h pork broth was proved to be TMA because of the consistency of their compensation voltages under the different field.

Precision and accuracy

Under optimized conditions, the TMA standard solution with concentration of 4 μg/mL was determined for six times. 100 μL of NaOH solution was added to accelerate the volatilization of the TMA. The RSD of ion current was 6.20 %. Tested the same solution at 0 h, 6 h, 12 h, 24 h, 48 h, the result showed that the solution is stable within 48 h. Compared with the predicted concentration value and the real concentration value, the accuracy was 8.13 %.

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CONCLUSIONS

The work demonstrated that trimethylamine in pork can be detected directly and quantitatively evaluated using microchip-based FAIMS technique. TMA was presented unique peak in pork because of its volatility and high proton affinity. The biogenic amines of TMA, was readily identified from their compensate voltage in the drift region. The concentrations of TMA was proportional to the measured peak height, so that the quantity of the TMA could be estimated.

The effect of storage time on the spoilage progress was clearly demonstrated in this work. At room temperature (about 30 □) the slight degradation progress can be observed after 3 hours, and no significant increase until 18 hours. Hereafter, it was reflected in the FAIMS spectra as large TMA peak and means that there were significant changes in the spoilage status of pork. FAIMS technique provide a rapid screening method for meat quality using pork filtrate. A classification of different spoilage grades for pork can be obtain by comparing to the classical semimicro Kjeldahl method. Final results indicated that the response value of 0.35 (concentration of 0.791 mg/100 g) and 2.41 (concentration of 4.95 mg/100 g) were considered to be the borders of three grades of freshness. All these results demonstrated that FAIMS had the advantages of rapid characterization, good repetition and free of multistep sample pretreatment. Based on these results, further efforts is worthy to devoting to develop the online monitoring FAIMS system for meat freshness determination.

ABBREVIATIONS USED

FAIMS, high-field asymmetric waveform ion mobility spectrometry; TMA, trimethylamine; TVB-N, total volatile basic nitrogen; GC-FID, gas chromatograph-flame ionization detection; HPLC, high performance liquid chromatograph; GC-MS, gas chromatograph-mass spectrometry; IMS, ion mobility spectrometry; CV, compensation voltage; FWHM, full width at half maximum; DRIE, deep reactant

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ion etch; TIC, total ion chromatogram.

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