# Rapid Analysis of Nitrite and Nitrate in Meat Products Using Microwave Assisted and Solid Phase Extraction

*Tzu-Yun Chu*

## Abstract

A rapid and simple method was developed for the determination of nitrite and nitrate in meat products. Microwave assisted extraction was employed to greatly reduce the sample preparation time, followed by C18 and IC-Ag solid phase extraction to simultaneously eliminate protein, fat, and chloride anion from salt in sample. The extracted analytes were determined by ion-exchange chromatography with non-suppressed conductivity detector. For the nitrite measurements, the relative standard deviation and the recovery in three level standard mixture added to ham and bacon were 1.8 to 7.5% and 84.0 to 94.5%, respectively. The corresponding results for the nitrate were 1.9 to 7.5% and 95.3 to 100.7%, respectively.

Keywords : Microwave assisted extraction, solid phase extraction, nitrite, nitrate,

Tzu-Yun Chu : Instructor, Department of Chemical Engineering

## Introduction

Nitrite and nitrate are often added as colorants also as preservatives for meat products. Several epidemiological studies have shown an association between brain cancer and nitrite from intake of cured meat.[1] Murphy *et al.* further studied the hypothesis, they have found that the cured meat consumption and cancer trends are not consistent.[2] However, nitrite reacts with secondary and tertiary amines to form carcinogenic nitrosamines.[3] The correlation of high nitrite density in saliva with the cancer incidence rate was reported.[4]

Due to the toxicity and suspected carcinogenicity in humans, the determination of nitrite and nitrate in meat products is important. Many methods have been developed for the measurement of nitrite and nitrate in meat products. Traditional spectrophotometric methods were often time-consuming and lack of sensitivity for complex samples.[5,6] Although a direct potentiometric method have been reported,[7] the need for the use of ion-selective electrodes limited the analysis to a single species at one time. High performance liquid chromatography techniques were much more rapid, sensitive and selective than spectrophotometric methods, which include ion-exchange chromatography,[8] ion-exclusion chromatography,[9] and reverse phase ion-interaction chromatography.[10,11,12] However, most of them required time-consuming sample pretreatment by acid digestion or by adding the Carrez reagents as protein precipitant. Herein, we would like to describe an easy and efficient method for the rapid assessment of nitrite and nitrate in meat products.

In this work, a simple ion-exchange chromatography with non-suppressed conductivity detection was used. Microwave assisted extraction reduced the sample preparation time significantly. Subsequent passing the sample extracted through a C18 SPE cartridge and an IC-Ag cartridge successively removed the interference matrix components concurrently. Thus our method avoided the tedious work for digestion and protein precipitation.

## Experimental

### Reagents

Meat samples of ham and bacon were purchased from a local supermarket. Sodium chloride, potassium nitrate, potassium nitrite and phthalic acid were obtained from Merck (Darmstadt, Germany). Tris(hydroxymethyl)aminomethane was obtained from Ferak (Laborat, Berlin). HPLC grade methanol was obtained from ALPS (Taipei, Taiwan).

### Apparatus

The solid phase extraction was carried out with a Supelco Visiprep DL Disposable Liner SPE Vacuum Manifold. The C18 SPE cartridges were obtained from Chrom Expert and the Maxi-Clean IC-Ag cartridges were obtained from Alltech (Deerfield, USA). A TOA chromatography system consisted of a super intelligent LC pump (ICA-5120), a model 3051 column oven, an injector valve with a fixed 100 μL sample loop, and a conductometric detector (ICA-3030). Chromatographic data were analyzed using a SISC-LAB chromatography data system. Meat samples were homogenized using a Krups MiniPro food processor (Ireland). A domestic microwave oven was purchased from Sunhow (Taiwan).

### Sample Preparation

1. Water Extraction

Meat products were grounded with a food processor to produce homogeneous samples. 5.0 g of the homogenized meat sample was mixed with 15 mL deionized water in a 250 mL beaker. The mixture was placed into a microwave oven with a watch-class on top of the beaker, and irradiated at 60% power (318 W) for 30 seconds. The extracted sample was allowed to cool to room temperature and transferred to a volumetric flask, enough deionized water was added to a final volume of 25 mL. After filtration, 2 mL portion of this filtrate was dilute to the volume of 5 mL with deionized water.

2. SPE Extraction

The C18 SPE cartridge was first conditioned with 4 mL of methanol followed by 3 mL of deionized water. The SPE cartridge was then connected to a syringe

filter, followed by an empty syringe and a Maxi-Clean IC-Ag cartridge which was attached to the SPE vacuum manifold. 3 mL of diluted sample solution was applied to the top of the SPE cartridge and passed successively through the cartridge system dropwise. The first 1 mL of eluate was discarded, the remaining eluate was collected for the liquid chromatography analysis.

**Chromatography**

An anion-exchange quaternary ammonium column TOA PCI-201S (100 mm x 4.6 mm) was used. The mobile phase was 2.0 m*M* phthalic acid – 2.5 m*M* tris(hydroxymethyl)aminomethane buffer solution. 100 μL of the extracted sample was injected, the nitrite and nitrate were separated under 42℃ with a flow rate of 1.2 mL/min. And the analytes were detected using a conductometric detector.

## Results and Discussions

Three different extraction methods were assayed and the results were compared in Table 1. For conventional heating, the homogenized meat samples were placed in a boiling water bath for 1 hour. For ultrasonic method, the homogenized meat samples were sonicated in a Branson ultrasonic cleaner for 10 minutes at room temperature. For microwave-assisted extraction, samples were irradiated in a domestic microwave oven at 60% power (318W) for 30 seconds. There were no significant differences in the peak areas between conventional heating and microwave-assisted methods. However, the microwave-assisted extraction is considerably more convenient and time saving than the conventional one. The chromatogram of chloride, nitrite and nitrate mixed standard is shown in Figure 1. The retention time of the nitrate ion was less than 5 min, which was demonstrated the rapidity and efficiency of the analytical conditions developed by us.

Figure 2 depicts the results of elimination of the interference of chloride anion from meat samples. As shown in (a), the chromatogram of the meat sample extract passed directly through a C18 SPE cartridge and analyzed utilizing the same chromatographic conditions used for the standards. Although the chloride

ion peak was completely separated from the nitrite ion signal in the standards, it totally obscured the nitrite peak which prevented the quantitation of nitrite ion in the meat samples. In order to remove the interfering chloride ion from the meat samples to facilitate the analysis of nitrite ion, an efficient clean-up procedure was required. Thus, 0.4 mL of 0.05 *M* silver acetate was mixed with the eluent from the C18 SPE cartridge to precipitate out the chloride ion in the meat sample as silver chloride. The result displayed in chromatogram (b) clearly revealed that the chloride ion peak was significantly reduced. Unfortunately, the particle size of the precipitate was extremely fine and was not separated from the solution after centrifuged for 10 min with a low speed centrifuge. The resulting supernatant was still cloudy and therefore was filtered through a 0.45 $\mu$m syringe filter in an attempt to remove the precipitate. However, the silver chloride precipitate caused a lot of difficulties during filtration. As a result, an alternative method for removing chloride ion was sought and the use of Maxi-Clean IC-Ag cartridge to reduce the chloride anion content in the meat sample was investigated. The Maxi-Clean IC-Ag cartridge selectively precipitates the $Cl^-$ ion by reacting it with $Ag^+$ ion, hence reduces the concentration of chloride ion in samples. The sample should be passed through the cartridge slowly to allow the chemical interaction to occur. The chromatogram illustrated in (c) evidently showed that the intensity of the chloride anion was greatly reduced which made quantitation of nitrite ion feasible. At the meantime, the size of the injection peak was also diminished due to the removal of chloride ion in the meat sample by the Maxi-Clean IC-Ag cartridge. This result suggests that part of large injection peak in (a) may be arisen from eluent anions, which were displaced from the resin by the chloride anion originally in the meat sample.

The recovery of added analytes were studied at 20, 30, and 40 ppm levels and were analyzed following the same extraction and separation procedures. The results of recovery studies are summarized in Table 2. The average range was between 95.3% and 100.7% for nitrate, 84.0% and 94.5% for nitrite. The reproducibility among individual determinations was expressed as relative standard deviations (RSDs). Table 2 shows that the concentration RSDs of the nitrate and nitrite added to bacon and ham samples were between 1.8% to 7.5% at three spiked levels. Detection limits for nitrite and nitrate were based on three SDs of the

mean assay value of each analyte at the lowest of the three concentration levels, there were 4.2 mg/L and 1.8 mg/L for ham samples, 1.8 mg/L and 4.5 mg/L for bacon samples, respectively.

The regression of the detector response verses the concentration of the standard exhibit linearity, with the correlation coefficients of five replicated determinations for nitrite and nitrate ranging from 0.9866 to 0.9653 (Table 3).

## Conclusion

A practical and rapid analytical method has been established for the determination of nitrite and nitrate in meat samples. Microwave assisted extraction proved to be extremely efficient and much more convenient in operation than the conventional heating method. The extracted samples were cleaned up with C18 solid phase extraction cartridges followed by an IC-Ag cartridge to remove the interfering matrix components such as protein, fat and chloride anion simultaneously. The measurements were carried out with anion exchange liquid chromatography and conductivity detection. The method developed by this study is a new procedure with minimum sample preparation, and is applicable to other complex materials.

**Table 1.** Comparison of extraction method for the determination of nitrite[a]

| | Conventional Heating | Ultrasonic Vibration | Microwave Irradiation |
|---|---|---|---|
| Area | 656427 | 545165 | 642877 |
| RSD, % | 6.6 | 0.3 | 0.2 |

[a]: The amount of nitrite was expressed as peak area (n=3).

**Table 2.** Recovery and RSD data of nitrite and nitrate

| sample | Spike, ppm | Nitrite | | | Nitrate | | |
|---|---|---|---|---|---|---|---|
| | | found[a] | RSD,% | Rec,% | found[a] | RSD,% | Rec,% |
| ham | 20 | 18.2±1.4 | 7.5 | 90.8 | 19.1±0.6 | 3.4 | 95.3 |
| | 30 | 25.2±1.7 | 6.8 | 84.0 | 29.0±2.0 | 6.9 | 96.6 |
| | 40 | 36.7±1.1 | 3.1 | 91.7 | 40.2±0.8 | 1.9 | 100.4 |
| bacon | 20 | 17.2±0.6 | 3.3 | 85.9 | 19.9±1.5 | 7.5 | 99.7 |
| | 30 | 26.5±1.4 | 5.3 | 88.4 | 28.8±1.2 | 4.0 | 96.0 |
| | 40 | 37.8±0.7 | 1.8 | 94.5 | 40.3±0.9 | 2.2 | 100.7 |

[a] : average of five determinations.

**Table 3.** Linearity of response for nitrite and nitrate[a]

| component | slope | intercept | $R^2$ |
|---|---|---|---|
| Nitrite | 9416.9 | 366726 | 0.9866 |
| nitrate | 9333.1 | 372982 | 0.9653 |

[a] : average of five determinations.

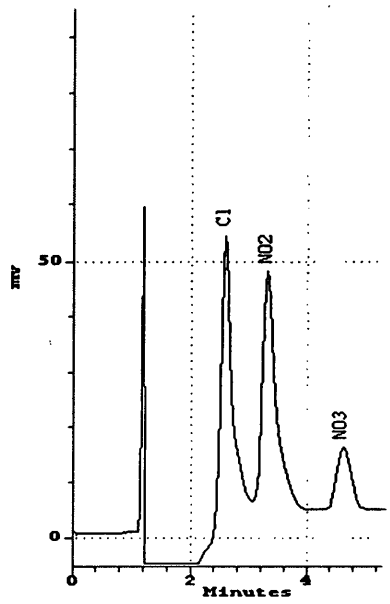


**Fig. 1.** Chromatogram of chloride, nitrite and nitrate mixed standards. Column: TOA PCI-201S. Temperature: 42 °C. Eluent: 2.0 m*M* phthalic acid – 2.5 m*M* tris(hydroxymethyl)aminomethan buffer solution, 1.2 mL/min flow-rate.

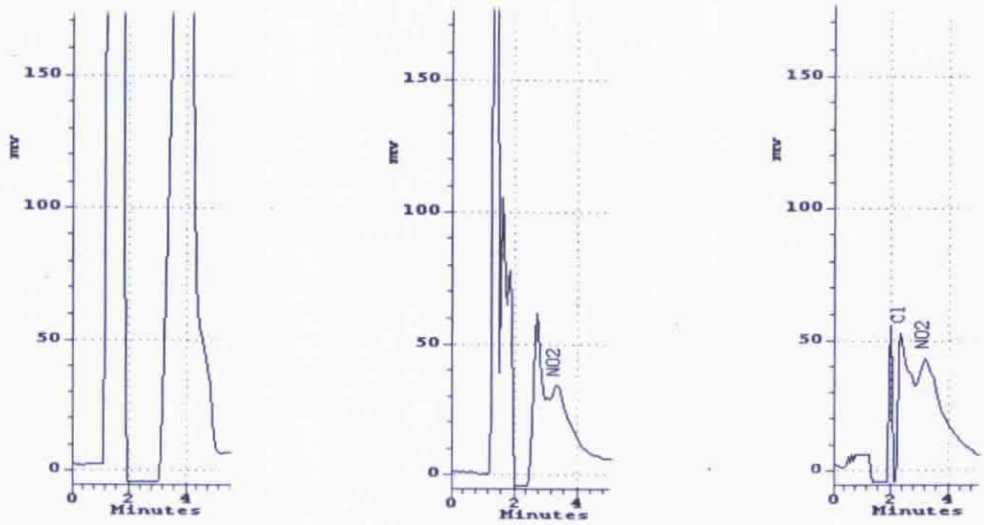


**Fig. 2.** Chromatogram of dilute ham extract. Column: TOA PCI-201S. Temperature: 42 °C. Eluent: 2.0 m*M* phthalic acid – 2.5 m*M* tris-(hydroxymethyl)aminomethan buffer solution, 1.2 mL/min flow-rate. (a) extracted meat sample (b) extracted meat sample treated with silver acetate (c) extracted meat sample passed through IC-Ag cartridge

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# 應用微波技術與固相萃取之肉品中亞硝酸鹽及硝酸鹽的快速檢測方法

朱紫雲

## 摘 要

本研究發展出一對於肉品中亞硝酸鹽及硝酸鹽之快速簡便檢測方法。微波萃取技術之應用大幅減少試樣製備所需時間，而 C18 及 IC-Ag 固相萃取能同時除去樣品中蛋白質、脂肪及食鹽的氯離子。萃取之試樣最後以離子交換色層分析法分離，並以電導偵檢器檢測之。對於亞硝酸鹽之測定，其添加 20，30 及 40 ppm 標準混合溶液至火腿及培根樣品之回收率爲 84.0 至 94.5%，相對標準偏差爲 1.8 至 7.5%。以相同方法對於硝酸鹽之測定其回收率爲 95.3 至 100.7%，相對標準偏差爲 1.9 至 7.5%。

關鍵詞：微波萃取，固相萃取，亞硝酸鹽，硝酸鹽

朱紫雲：化學工程科講師