Note

Specific Determination of Bromate in Bread by Ion Chromatography with ICP-MS

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A sensitive method for detecting bromate in bread by ion chromatography with inductively-coupled plasma mass spectrometry (IC/ICP-MS) was developed. Bromate was extracted from bread with water. The clean-up procedure included a 0.2 μ m filter, a C₁₈ cartridge for defatting, a silver cartridge to remove halogen anions, a centrifugal ultrafiltration unit to remove proteins, and a cation-exchange cartridge to remove silver ions. A 500 μ L sample solution was applied to IC/ICP-MS. The detection limit and the quantitation limit of bromate in the solution were 0.3 ng/mL and 1.0 ng/mL, expressed as HBrO₃, respectively, which corresponded to 2 ng/g and 5 ng/g, respectively, in bread. Recovery of bromate was about 90%, and the CV was about 2%. Based on the detection limit in solution and recovery from bread, the detection limit of bromate in bread was estimated to be 2 ng/g.

Key words: bromate; bread; ion chromatography with inductively-coupled plasma mass spectrometry; ICP-MS; silver cartridge

Introduction

Potassium bromate has long been used as a flour improver in the baking industry. Although it is permitted as a food additive in Japan¹⁾, its use is restricted to wheat flour used in baking because of its carcinogenicity^{2), 3)}. Since the amount of bromate added, expressed as HBrO₃, must not exceed 0.030 g/kg of flour and bromate must be decomposed or removed before preparation of the finished food products¹⁾, sensitive methods are needed to detect residual bromate in bread.

Several analytical methods for detection of bromate in bread, including GC/MS⁴⁾ and HPLC with post-column reaction⁵⁾⁻⁷⁾, have been reported, and one of them, the method using HPLC with postcolumn reaction, was published as a notification by the Ministry of Health and Welfare in 1997⁸⁾. Although this method had a very low detection limit for residual bromate and its sensitivity was improved recently⁷⁾, it consumes a large amount of *o*-dianisidine, which is carcinogenic. It should also be noted that oxidants besides bromate are also detected by this method.

Inductively-coupled plasma mass spectrometry (ICP-MS) should be considered as an alternative method, because it can detect bromine specifically. There have been two reports on detection of bromate in bread by ion chromatography (IC) with ICP-MS. Dennis *et al.*⁹⁾ reported a detection limit of 20 ng/g, while Heitkemper *et al.*¹⁰⁾ reported a detection limit of 6 ng/g and the

detection of 10 ng/g bromate in a spike experiment. This study reexamined the use of ICP-MS for detection of bromate in bread. The detection limit and recovery were examined by analyzing bread extracts by IC/ICP-MS, and a detection limit lower than those previously reported was obtained.

Materials and Methods

Samples and reagents

Bread, bun and roll specimens were obtained from local stores. Bun specimens containing bromate was prepared by Yamazaki Baking Co., Ltd. from flour containing potassium bromate (7.5 mg/kg as HBrO₃). Potassium bromate was purchased from Merck, and other chemicals were of reagent or HPLC grade. Ultrapure water (>18 M Ω cm) prepared with a Milli-Q SP Reagent Water System (Millipore) was used throughout the experiments.

Hereafter "bread" represents bread, buns or rolls.

Extraction

Each specimen was cut into small pieces and homogenized in a blender (Oster blender ST-1, Osaka Chemical). The crumbs (10 g) were placed in a 100 mL glass beaker and stirred with 50 mL of water for 15 min, with a magnetic stirrer.

Clean-up

The filters and sample preparation cartridges used to

clean up the bread extract were: filter paper (filter paper 5A, Advantec Toyo); a 0.2 μm cellulose acetate syringe filter (Maishori disc W-25-2, Tosoh); a C_{18} sample preparation cartridge (Sep-Pak Plus tC_{18} Environmental Cartridge, Waters) activated in advance with 10 mL of methanol followed by 10 mL of water; a silver cartridge (OnGuard-Ag cartridge, Dionex) preconditioned with 50 mL of water; a centrifugal ultrafiltration unit (Ultrafree-4 centrifugal filter unit UFV4 BTK, Millipore); and a cation exchange cartridge (TOYOPAK IC-SP M, Tosoh) preconditioned with 20 mL of water.

The bread extract was centrifuged at $8,000 \times g$ for 10 min, and the supernatant was filtered through filter paper. The filtrate was passed through a 0.2 μm cellulose acetate syringe filter, an activated C_{18} cartridge and a silver cartridge, attached in tandem in that order. The first 2 mL of eluate was discarded, and the next 3 mL was placed in the upper cup of the centrifugal ultrafiltration unit and centrifuged till the filtrate in the reservoir exceeded 2 mL. After passing 2 mL of filtrate through a cation-exchange cartridge, the first three drops were discarded, and the remaining filtrate was collected as a sample solution.

IC/ICP-MS

An Agilent 1100 system and an Agilent 7500 omega (Agilent Technologies Company) were used as the IC and ICP-MS systems, respectively, and they were connected by a 500×0.3 mm i.d. ethylene tetrafluoroethylene (ETFE) tube. A GL-IC-A15 anion-exchange column connected to a GL-IC-A15G guard column (Hitachi Chemical) was used as the separation column. The operating conditions of IC and ICP-MS are described in Table 1. The bromate content was calculated from the peak area by an absolute calibration method.

Results and Discussion

Sensitivity of the system

Ammonium carbonate (30 mmol/L) were used as the mobile phase for IC. Ammonium salt was chosen to prevent salt deposition and clogging at the sampling orifice of the ICP-MS system by sodium in the mobile phase¹¹⁾. The injection volume was set at 500 μL to achieve sufficient sensitivity. The retention time of bromate was 5.8 min at a flow rate of 1.0 mL/min. Bromide, which had a retention time of 8.0 min, was separated from bromate.

The sensitivity of our IC/ICP-MS system was assessed. The detection limit and the quantitation limit for ^{79}Br and ^{81}Br were assessed from the baseline noise and the peak height of potassium bromate solution. Baseline noise, N , was defined as a signal response equal to twice the standard deviation of the baseline signal. The detection limit and the quantitation limit were calculated as the concentrations of bromate that would result in a peak height equal to three times and ten times, respectively, the baseline noise. When ultrapure water was analyzed, N was 28 counts at m/z 79, and a detection limit ($S/N=3$) of 0.3 ng/mL as HBrO_3 and a quantita-

Table 1. Operating Conditions of IC and ICP-MS

IC conditions	
Column	GL-IC-A15G/GL-IC-A15
Mobile phase	30 mmol/L $(\text{NH}_4)_2\text{CO}_3$
Flow rate	1.0 mL/min
Column temperature	40°C
Injection volume	500 μL
ICP-MS conditions	
Radio frequency power	1.5 kW
Plasma gas flow	15 L/min
Auxiliary gas flow	1.0 L/min
Carrier gas flow	1.05 L/min
Sampling depth	8.0 mm from load coil
Nebulizer type	concentric nebulizer (quartz)
Interface	Ni sampling cone, Ni skimmer cone
Detection mass	Br: $m/z=79, 81$
Dwell time	0.5 s/amu

tion limit ($S/N=10$) of 1.0 ng/mL were calculated. They correspond to 2 ng/g and 5 ng/g bromate in bread, because a 10 g sample is extracted with 50 mL of water (see below). Similarly, a detection limit of 0.5 ng/mL in solution and 3 ng/g in bread was calculated at m/z 81. Sensitivity was better at m/z 79 than at m/z 81. Aqueous solutions at concentrations of 0.5–5 ng/mL were analyzed, and a calibration curve was plotted according to the peak area of the chromatograms at m/z 79. A good correlation ($R^2=1.000$) was observed. The ionization efficiency of bromine was relatively low. High-resolution ICP-MS yielded better sensitivity in preliminary experiments, but it is elaborate and expensive. The sensitivity of our present system is adequate and practical.

Clean-up

The clean-up method, including a silver cartridge, that was developed in previous studies by Himata *et al.*^{5), 6)} was applied to preparation of sample solutions, with some modifications. A 10 g bread sample was extracted with 50 mL of water, and after filtration through filter paper, a small portion of the extract was passed through a system constructed from a 0.2 μm filter, a C_{18} cartridge for defatting, and a silver cartridge. The silver cartridge, which is a cation-exchange cartridge in silver form, was used to remove anions such as chloride and bromide that may affect the baseline noise of bromine and the resolution of the bromate peak. The centrifugal ultrafiltration unit was then used to remove proteins, and the filtrate in the lower tube was applied to a cation-exchange cartridge to remove silver ions. The volume of the final sample solution was about 1.5 mL. Assuming no loss, 5 ng/g of bromate in a bread sample corresponds to 1 ng/mL in the final solution.

Analysis of bread samples

Sliced bread A, which had been demonstrated in advance to be made without potassium bromate, was used to evaluate the detection limit, the quantitation limit, and recovery. First, a blank bread sample was analyzed

Fig. 1. Chromatograms of spiked and blank samples of sliced bread A
A: aqueous solution of potassium bromate; B and C: spiked sample; D: blank sample
A, B and D: chromatograms at m/z 79; C: chromatogram at m/z 81.

after extraction and the clean-up procedure. No peak was observed in the chromatogram at m/z 79 (Fig. 1D), and the baseline noise (N) of the blank was 30 counts at m/z 79. Based on the N value, a detection limit ($S/N=3$) of 0.3 ng/mL as $HBrO_3$ and a quantitation limit ($S/N=10$) of 1.0 ng/mL were calculated. These values correspond to 2 ng/g and 5 ng/g bromate in bread. They are almost the same as those obtained from ultrapure water. Components from the bread matrices did not affect the baseline noise. Since several peaks were observed up to 20 min when the same sample was analyzed by IC with UV detection or conductivity detection, the interval between the two injections was set at 20 min to allow these components to elute from the column.

A spiked bread sample to which potassium bromate was added at a concentration of 5 ng/g (expressed as $HBrO_3$) was prepared, and the extract was analyzed after the clean-up procedure. A clear bromate peak was detected in the chromatogram at m/z 79 (Fig. 1B) as well as at m/z 81 (Fig. 1C). The retention time of the bromate peak was 5.6 min, 0.2 min shorter than that of the aqueous potassium bromate solution. This difference may be attributable to anionic substances in the

sample.

Recovery and reproducibility were also examined. The reproducibility of the instrument was assessed first. Several preparations were mixed in a tube, and this homogeneous sample was injected 5 times. Average recovery was 88%, and CV was 2.1%. Next, the reproducibility of the entire analysis was examined by analyzing 3 independent preparations. Average recovery was 90%, and CV was 1.9%. The clean-up procedure did not affect reproducibility, because no significant difference was observed between the CV of 3 preparations and of 5 injections of the same preparation. The blank sample solution was analyzed after adding potassium bromate to a final concentration of 1 ng/mL as $HBrO_3$. Recovery was 100%, and the loss of 10% can therefore be attributed to the clean-up procedure alone. Based on the detection limit in solution and recovery of the clean-up procedure, a detection limit as $HBrO_3$ of 0.4 ng/mL in the sample solution and 2 ng/g in bread was estimated. A quantitation limit of 1.2 ng/mL in the sample solution and 6 ng/g in bread was estimated in a similar manner.

Bun specimens containing bromate, made from flour containing potassium bromate (7.5 mg/kg as $HBrO_3$), were analyzed. Bromate was detected at 6.2 ng/mL in the sample solution, which corresponded to 31 ng/g bromate in the bun. No bromide was detected. Three commercial baked goods, sliced bread B, a roll, and a bun, were also analyzed, and no bromate was detected.

In summary, a detection method for bromate in bread by IC/ICP-MS was developed. IC on an anion-exchange column and ammonium carbonate successfully separated bromate and bromide ions. A clean-up method that used a silver cartridge to remove halogen anions was applied. A detection limit of 2 ng/g and a quantitation limit of 5 ng/g as $HBrO_3$ in bread were calculated. Recovery of bromate was about 90%, as estimated on the basis of spike experiments. From the detection limit in solution and recovery from bread, the detection limit was estimated to be 2 ng/g bromate in bread.

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