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Short communication

Sensitive and simple determination of bromate in foods disinfected with hypochlorite reagents using high performance liquid chromatography with post-column derivatization

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ABSTRACT

A novel analytical method for the quantification of bromate in fresh foods using high performance liquid chromatography (HPLC) with a post-column reaction has been developed. The fresh food sample solutions were pretreated with homogenization, centrifugal ultrafiltration and subsequent solid phase extraction using a strong anion-exchange resin. After separation on a strong anion-exchange chromatography column using a highly concentrated NaCl solution (0.3 M) as the eluent, the bromate was quantified by detection using a post-column reaction with a non-carcinogenic reagent (tetramethylbenzidine). The developed HPLC technique made it possible to quantify bromate in salt-rich fresh foods. The recoveries from fresh foods spiked with bromate at low levels (2 or 10 ng/g) satisfactorily ranged from 75.3 to 90.7%. The lowest quantification limit in fresh foods was estimated to be 0.6 ng/g as bromic acid. The method should be helpful for the quantification of bromate in fresh foods disinfected with hypochlorite solutions.

1. Introduction

Bromate is classified as possibly carcinogenic to humans (Group 2B) [1]. A decade ago, it was reported that bromate was present in the sodium hypochlorite solutions historically employed as disinfectants for drinking water [2]. Sodium hypochlorite is prepared from the reaction of chlorine and sodium hydroxide, which are produced from the electrolysis of brine which is a solution of sodium chloride. While, bromide ions are found to a very varying extent in the sodium chloride. Accordingly, the bromine in chlorine has been thought to be related to bromate production [2]. Therefore, a provisional guideline value of 10 ng/mL in drinking water is recommended by the WHO [3]. In general, sodium hypochlorite solutions have also been employed for the disinfection of fresh foods, including vegetables, meats and fishes, in many countries. However, residual bromate remaining in fresh foods treated with sodium hypochlorite solution remains obscure.

To date, several analytical methods for bromate detection have been reported [4–8]. Initially US Envirinmental Protection Agency (EPA) 300.1 method was established by using ion chromatography with conductivity detection. As more information concerning bromate toxicity became available, lower regulatory limits were imposed, resulting in demands for lower detection limits. This led to development of post-column derivatization and visible detection method EPA 317. In this method, bromate is specifically reacted with a post-column reagent, o-dianisidine, and detected by visible absorbance detection (450 nm). Separation is basically the same as in EPA 300.0. The ion chromatography method with a post-column reaction was reported for the quantification of residual bromate in bread [9] and announced as the official method by the Japanese Ministry of Health and Welfare (published in 1997). However, the use of o-dianisidine is a safety concern because of its carcinogenicity. In addition, the method requires time-consuming clean-up steps for the bread sample extract, including a C18 cartridge for defatting, a silver cartridge for removal of chloride ions, centrifugal ultrafiltration for deproteinization and a cation-exchange cartridge for removal of silver ions [7]. Furthermore, the method is difficult to apply to fresh foods, because excess amounts of byproducts derived from hypochlorite prevent the quantification of bromate. For the quantification of bromate in fresh foods using HPLC, we considered it necessary to develop a separation method and a detection system, as well as a pretreatment method that is closely linked to the separation and detection systems. In the present study, we report a novel analytical method for the quantification of bromate in fresh foods that is based on HPLC with post-column derivatization using tetramethylbenzidine (TMBz) [10,11].

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Table 1
Recoveries of bromate from fresh foods including cabbage, poultry and horse mackerel.

	Added (ng/g)	Found (ng/g)	Recovery (%)
Cabbage	0.0	N.D.	–
	2.0	1.81 ± 0.02	90.7 ± 1.2
	10.0	8.77 ± 0.29	87.7 ± 2.9
Poultry	0.0	Trace[a]	–
	2.0	1.66 ± 0.07	82.9 ± 3.4
	10.0	7.53 ± 0.50	75.3 ± 5.0
Horse mackerel	0.0	N.D.	–
	2.0	1.77 ± 0.15	88.4 ± 7.6
	10.0	7.89 ± 0.08	78.9 ± 0.8

Each value represents the mean ± SD of three individual analyses.
[a] Bromate peak was detected, but the content was less than the lowest quantification limit (0.6 ng/g as bromic acid).

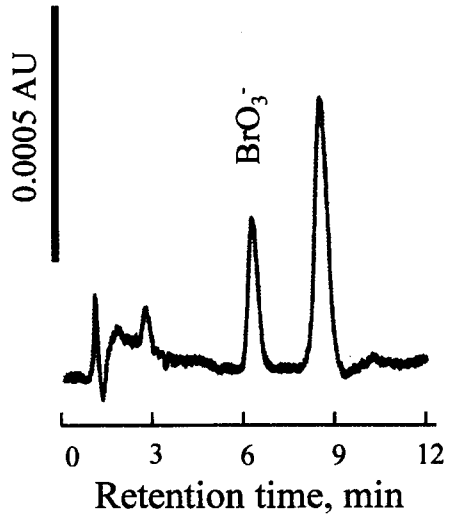

Fig. 4. Chromatogram of drinking water collected at Gunma prefecture in Japan. Fifty millilitres of drinking water were passed through 120 μL of Muromac AG 1X8 ion exchange resin, and the bound bromate on the resin was then eluted by 0.3 M NaCl. Two hundred microlitres of the bromate fraction (volume, 1.0 mL) were subjected to HPLC. The chromatographic conditions are described in Section 2.

with a hypochlorite solution. Sodium hypochlorite solutions have been historically employed as disinfectants for drinking water. In fact, a trace amount of bromate was detected in drinking water, as shown in Fig. 4. Therefore, if bromate penetrates into fresh foods when they are washed with drinking water, the residual bromate can be detected.

4. Conclusion

We established a sensitive, selective and safe HPLC method for the quantification of bromate in fresh foods. The lowest quantitative limit was 0.6 ng/mL (S/N = 5), and the linearity of the correlation between the peak response and bromate concentration was confirmed, even for the lowest quantitative limit ($R^2 = 1.000$). The sample solutions were prepared from fresh foods through minimal steps as follows: (1) homogenization, (2) centrifugation, (3) ultrafiltration and (4) solid-phase extraction. In the present study, recovery tests for cabbage, poultry and horse mackerel were performed on different days, and the recovery of bromate from these fresh foods at 2 ng/g wet weight ranged from 82.9% to 90.7%; there was no statistical significance. It should be noted that bromate appears to be relatively stable in fresh foods. Through these pretreatment steps, the bromate in 1 g of fresh food was transferred into 1 mL of a 0.3-M NaCl solution. Therefore, the lowest quantitative limit of bromate in fresh foods was 0.6 ng/g of wet weight.

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