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Importance of control of enzymatic degradation for determination of bisphenol A from fruits and vegetables

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Abstract

The purpose of this study was to develop an analytical method for determination of bisphenol A (BPA) from fruits and vegetables. The present method developed for extraction of BPA from samples was based on solid-phase extraction (SPE) method and solvent extraction. Recovery results in the samples spiked with a 10 ng/ml BPA [no detection (<1 ng/g) to 77%] were lower than those in the samples with a 50 ng/ml BPA (26–96%). The fact that the low recovery results were caused by BPA degradation by enzymes is found. These problems were proved by the pH (pH ≤3) and the heating treatment (at ≥80 °C for 5 min). However, because the heating treatment at temperatures of ≥80 °C for 5 min is more difficult and time-consuming method than the pH control, we suggest that the pH control is useful to prevent BPA degradation. Good recovery results (82–101%) were obtained from all fruit and vegetable samples after pH treatment (pH ≤3). Effective elimination of impurities and a good detection limit (1 ng/g) were obtained with a method involving two SPE cartridges (OASIS HLB and Sep-Pak Florisil cartridge).

Keywords: Bisphenol A; Fruits; Vegetables; Temperature; pH

1. Introduction

Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane; CAS Registry No. 80-05-7) is known as one of endocrine disruptors and has an acute toxicity to aquatic organisms in the range of 1–10 μg/ml for freshwater and marine species [1]. It is made by combining acetone and phenol, and is widely used as a material for the production of epoxy resins, phenol resins, polycarbonates, polyacrylates, polyesters and lacquer coatings on food cans [1]. BPA can be leached from these plastic products and food- and drink-packaging [2–5].

In several extraction methods, liquid–liquid extraction is used as a method for the extraction of BPA from aquatic samples such as river water samples [6]. Recently, solid-phase extraction (SPE) is broadly applied to extract BPA from food [2–5], aquatic [6] and biological samples [6,7] because SPE can reduce the number of steps and total time required for sample clean up and preparation. The SPE method is often used with other extraction methods such as microwave assisted extraction [6,7], solvent extraction [5] or ultrasonic extraction [8].

Moreover, gas chromatography–mass spectroscopy system (GC–MS) [4], high-performance liquid chromatography system (HPLC) equipped with a UV detector [2], a fluorescence detector [3,5] or an electrochemical detector [9] and HPLC–MS [6,7] have been used for the detection of BPA in samples.

Several studies reported analytical methods for determination of BPA in canned fruits and vegetables [2,4,10]. However, there are very few data on the detection of BPA in fruits and vegetables. Moreover, the control of enzymatic degradation may be very important to detect BPA in fruits and vegetables because the possibility of BPA degradation by enzymes in vegetables was suggested [11].

Therefore, the purpose of this study was to develop an analytical method for determination of BPA from fruits and vegetables. The present method developed for extraction of BPA from samples was based on two SPE method and solvent extraction. Our developed method also increased recovery results by the control of enzymatic degradation of BPA. Moreover, HPLC with fluorescence detection was used for determination of BPA in samples.

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2. Experimental

2.1. Reagents and chemicals

Bisphenol A (>99%), polyvinylpyrrolidone and anhydrous sodium sulfate was purchased from Nacalai Tesque (Kyoto, Japan). Solid-phase extraction cartridges, OASIS HLB (60 mg) and Sep-Pak Florisil (900 mg) cartridges, were obtained from Waters (Millipore Co., Milford, MA). Water used in this study was distilled and purified with a Milli-Q water purification system (Nihon Millipore, Yonezawa, Japan). Acetonitrile, acetone, methanol and *n*-heptane were purchased from Kanto Chemical (Tokyo, Japan). Fruits and vegetables were purchased from several stores.

2.2. HPLC apparatus and analytical conditions

BPA in standards and sample extracts was determined using a Waters 600E multisolvent delivery system (Millipore) equipped with a Waters 717plus Autosampler, a Waters Model U6K Universal Liquid Chromatograph Injector (Millipore) and a CR6A Chromatopac integrator (Shimadzu, Kyoto, Japan). The analytical column was a Symmetry Shield RP18 3.5 μm (4.6 mm × 150 mm) with a 3.5-μm guard column (2.1 mm × 10 mm) (Millipore). Fluorescence detector (Model F-1050, Hitachi, Tokyo, Japan) was set with excitation at 275 nm and emission at 300 nm. The mobile phase was acetonitrile–water (40:60, v/v) with a flow rate of 1.2 ml/min under isocratic conditions [5]. The column temperature was 40 °C at a Column heater U-620 Type 30 (Sugaichemi, Osaka, Japan) and the injection volume was 50 μl.

2.3. BPA extraction from sample

The fruit and vegetable samples were cut and homogenized with 0.1 ml of 1N HCl by a mixer for 20 min. Five grams of the homogenized sample was blended with 50 ml of acetone and anhydrous sodium sulfate (30 g), were shaken for 10 min with a mechanical shaker (Model Taiyo SR-IIW, Taiyo, Osaka, Japan) and then were filtered using a paper filter. The filter was washed with 20 ml of acetone. The acetone extract was evaporated with a flow of nitrogen in a 50 °C water bath just until dryness with achieved.

The residue was dissolved with 10 ml of 5% methanol in water and was applied to the OASIS HLB cartridge, which had previously conditioned with 10 ml of methanol and 10 ml of 5% methanol in water. The cartridge was washed with 10 ml of 5% methanol in water and BPA was eluted by 5 ml of methanol. The methanol extract was evaporated to dryness under nitrogen in a 50 °C water bath.

For further removal of the impurities from the residue, a Sep-Pak Florisil cartridge was used. The residue was dissolved in 20 ml of acetone–*n*-heptane (3:97, v/v) and was applied to the Florisil cartridge, which had previously conditioned with 10 ml of acetone–*n*-heptane (20:80, v/v) and 10 ml of acetone–*n*-heptane (3:97, v/v). After washing with 10 ml of acetone–*n*-heptane (5:95, v/v), BPA was eluted from the cartridges with 10 ml of acetone–*n*-heptane (20:80, v/v). The ratio of acetone–*n*-heptane for Sep-Pak Florisil cartridge extraction was based on a previous paper [5]. The eluate was evaporated to dryness, was dissolved with 1 ml mobile phase and then was analyzed by HPLC.

2.4. BPA degradation by crude enzyme

Ten grams of potato sample was homogenized with 10 ml of 0.1 M sodium phosphate buffer (pH 7) and 2 g of polyvinylpyrrolidone to remove phenol compounds from the sample. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C (KUBOTA 6900, Kubota Co., Tokyo, Japan). The supernatant was transferred to Eppendorf tubes and again centrifuged at 15,000 rpm for 15 min. After centrifuging, the supernatant was used as crude enzyme solution. A typical assay solution (pH 7) contained 5 ml of 0.1 M sodium phosphate buffer, BPA (10 and 100 ng/g) in 0.1 M sodium phosphate buffer and 0.5 ml of crude enzyme solution. The control sample with no crude enzyme solution was also prepared. The samples were incubated at 37 °C for 60 min. For the BPA extraction from the mixed assay solution samples, a 1-ml sample was applied to OASIS HLB and Sep-Pak Florisil cartridges and was analyzed as described above.

3. Results and discussion

3.1. Linearity and repeatability

Working standard BPA solutions of 10–1000 ng/ml were prepared by diluting aliquots of the stock solution (1000 mg/ml) in acetonitrile–water (40:60, v/v). A 50-μl volume of the standard BPA solution was injected into the HPLC system. Calibration curves for concentrations (10–1000 ng/ml) versus detector responses (peak heights) in the range of 10–1000 ng/ml were obtained from a linear regression program. The correlation coefficients of peak height to concentration were >0.998.

When BPA was measured consecutively with 10 and 100 ng/ml standard solutions 10 times to evaluate the repeatability of the analytical method, the relative standard deviation was 5.12% and 1.15%, respectively. Moreover, the retention times were 8.17 ± 0.08 min for the standard of 10 ng/ml and 8.16 ± 0.11 min for 50 ng/ml.

3.2. Change of recovery result by pH and temperature

We examined whether pH and temperature could give an effect on the recovery result for BPA or not. The pH and the heating treatment were performed following the homogenization of potato samples. The pH values of samples were adjusted from 2 to 10 by addition of 1N HCl or 1N NaOH. The heating temperature ranged from 20 to 100 °C and the heating time was 5 min. At pH 8, no BPA from samples spiked with both 10 and 50 ng/ml was detected, but recovery results were >80% at pH 10. Recovery results of BPA were >80% from all samples at pH ≤4. Moreover, good recovery results (>about 80%) were obtained at temperatures of ≥80 °C. The pH and the temperature

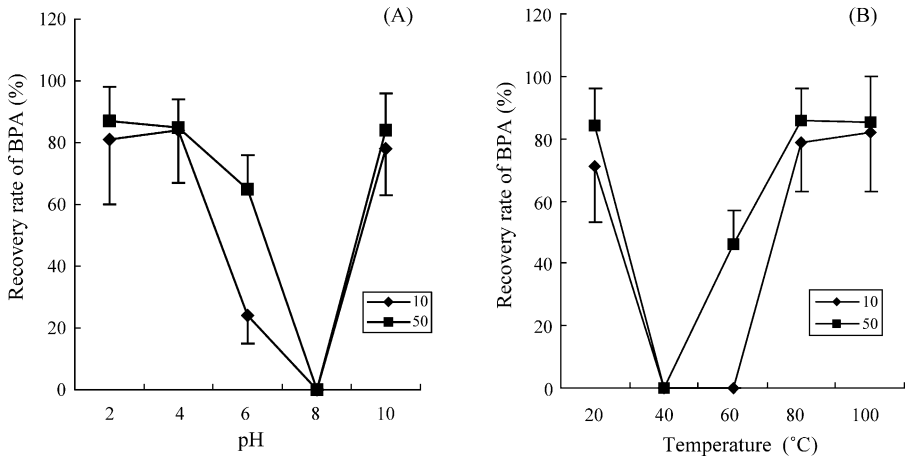

Fig. 1. Change of recovery rates for BPA by (A) pH or (B) heating treatment. The pH values of samples were adjusted from 2 to 10 by addition of 1N HCl or 1N NaOH. The heating temperature ranged from 20 to 100 °C and the heating time was 5 min. Samples spiked with a (♦) 10 ng/ml and (■) 50 ng/ml BPA.

had an important influence on recovery results for BPA in fruit and vegetable samples (Fig. 1).

Generally, the enzyme activity in fruits and vegetables is influenced by pH and temperature. The enzyme activity decreases when pH and temperature are low (generally pH <4 and <10 °C) or high (generally pH >8 and >70 °C) [12,13], respectively. In our study when the pH and the temperature were low (pH ≤4 and 20 °C) or high (pH 10 and ≥80 °C), respectively, recovery results for BPA increased.

However, because some fruits and vegetables such as grape, plum and broad bean pod show enzyme activities at pH 4 [12], we suggest that the optimum pH for the analysis of BPA from fruits and vegetables is pH ≤3.

On the other hand, the heating treatment at temperatures of ≥80 °C for 5 min is more difficult and time-consuming method than the pH control. Therefore, the pH control in fruit and vegetable samples may be useful to protect BPA degradation.

As shown in Table 1, good recovery results (>81%) were obtained from all fruit and vegetable samples after the pH treatment. The addition of 0.1 ml of 1N HCl to samples was enough to adjust to pH <3.

In addition, our results suggest, if BPA is contaminated, that it can continue long in existence in fruit and vegetable foods that are treated with pH <4 or that are heated at high temperature (≥80 °C). For example, BPA contaminations from several canned fruits and vegetables were reported [2–4]. Yoshida et al. reported that BPA migrated from can coatings to fruits and vegetable was not degraded for 20 days [2].

3.3. Extraction of BPA from fruit and vegetable samples

In the present study, the extraction of BPA from samples was based on SPE method with solvent extraction. In our previous study [5], because the recovery result of BPA for OASIS HLB cartridge (99.2%) showed higher than that of other reversed-phase cartridges (the BondElut C_{18}, Sep-Pak tC_{18} and Sep-Pak C_{18}) (92.3–97.6%), the OASIS HLB cartridge was selected for extraction of BPA from fruit and vegetable samples. As shown in Fig. 2, however, several impurities were found by using only OASIS HLB cartridge and the detection limit (signal-to-noise ratio [S/N] = 3) was 3 ng/g. The normal-phase cartridge was used for the further removal of impurities and to obtain a good detection limit. The Sep-Pak Florisil was selected on the basis of a previous study [5]. Effective elimination of impurities and a good detection limit (S/N = 3) (1 ng/g) were obtained from a method involving two SPE.

3.4. Recovery test

For recovery test, fruit and vegetable samples spiked with BPA (10 and 50 ng/g) were extraction and analyzed as described above. The recovery results are presented in Table 1. At no pH treatment, the recoveries for BPA from all samples ranged from ND to 95%. Recovery results were lower in samples spiked with

Table 1
Recovery results of BPA from samples with pH treatment or without pH treatment

Samples[a] (n = 5)	BPA added (ng/ml)	Recovery results (%)[b]	
		No pH treatment	pH treatment[c]
Potato	10	ND[d]	82 ± 18
	50	26 ± 10	88 ± 14
Carrot	10	69 ± 16	97 ± 11
	50	92 ± 7	94 ± 5
Eggplant	10	ND	101 ± 13
	50	68 ± 12	92 ± 8
Apple	10	52 ± 21	96 ± 16
	50	89 ± 5	91 ± 7
Grape	10	71 ± 13	93 ± 7
	50	95 ± 7	92 ± 5
Banana	10	77 ± 14	89 ± 11
	50	96 ± 9	97 ± 4

[a] The samples were extracted using SPE cartridge (OASIS HLB and Sep-Pak Florisil) after solvent extraction with acetone.
[b] Mean ± S.D.
[c] Exactly 0.1 ml of 1N HCl was added into each sample.
[d] ND: no detection (<1 ng/g).

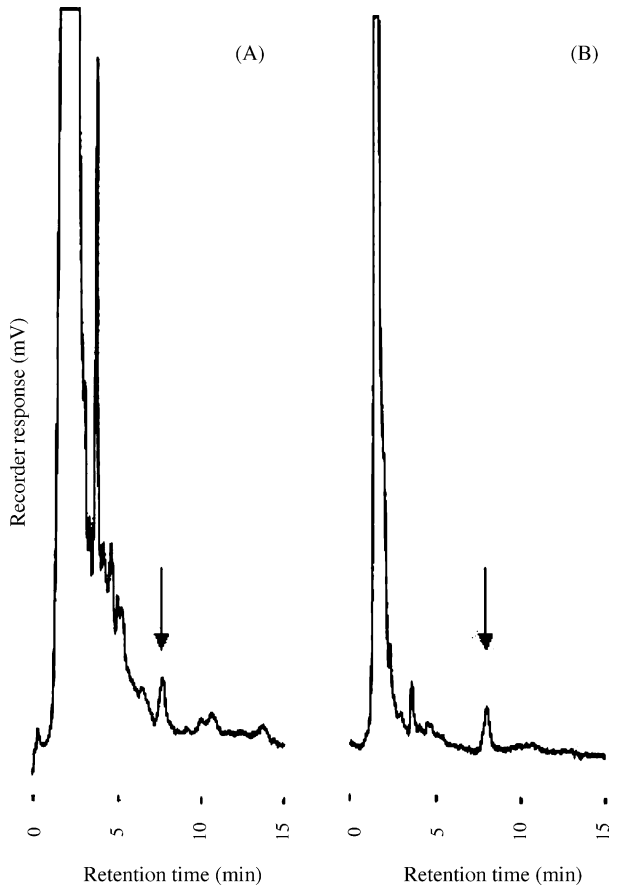

Fig. 2. Chromatograms of potato samples spiked with a 10 ng/ml BPA. After solvent extraction with acetone, BPA was again extracted using (A) the only OASIS HLB cartridge or (B) two SPE cartridges (OASIS HLB and Sep-Pak Florisil cartridge). The BPA peak is indicated by an arrow.

a 10 ng/g BPA compared with samples spiked with a 50 ng/g BPA. These results show that there is potential for BPA degradation in fruit and vegetable samples.

Therefore, we tested whether BPA could be degraded by crude enzyme or not. The potato that had showed the lowest recovery results was used for the test of BPA degradation by crude enzyme. The result of BPA degradation by crude enzyme of potato is presented in Fig. 3. BPA was not found from both samples spiked with a 10 or 50 ng/g BPA in the presence of enzyme solution at 10 min. However, recovery results of BPA from samples in the absence of enzyme solution were >95% and was not changed for 60 min. These results suggest that the low recovery results in fruit and vegetable samples are caused by BPA degradation by enzymes. Similarly, a previous study reported the oxidative degradation of BPA by polyphenol oxidase, one of vegetable enzymes [11].

There are few data from previous studies for determination of BPA in fruits and vegetables with which to compare analytical sensitivity. However, the detection limit of the proposed method (1 ng/g) is lower than that of the method of Goodson and Summerfield using GC/MS (2 ng/g) [4] or that of Yoshida et al. using HPLC/UV (5 ng/g) [2], and comparable with that of the method of Braunrath and Cichna using HPLC/fluorescence

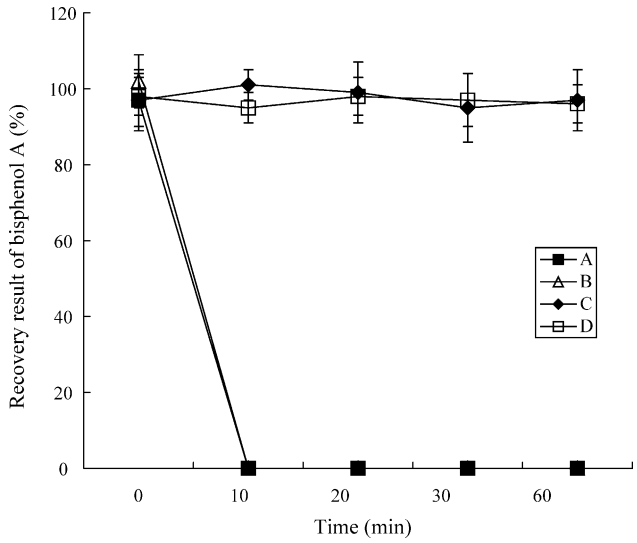

Fig. 3. Recovery results from samples spiked with (A) a 10 ng/ml or (B) 50 ng/ml BPA in the presence of enzyme solution, and samples spiked with (C) a 10 ng/ml or (D) 50 ng/ml BPA in the absence of enzyme solution. The samples were incubated for 60 min at 37 °C.

detector (1.1–1.6 ng/g) [10], compared with detection limits for BPA in canned fruits and vegetables.

For the recoveries of BPA, the proposed method (82–101%) was not widely different from the method (87–91%) by Goodson and Summerfield [4] and Yoshida et al. [2], but better than the method (53–75%) by Braunrath and Cichna [10].

4. Conclusions

To prevent BPA degradation by enzymes is very important to detect BPA from fruit and vegetable samples because the BPA degradation may lead to wrong results. The prevention of BPA degradation by enzymes is simply possible by the pH control. On the other hand, our method based on SPE and solvent extraction is a very selective and sensitive method for the detection of BPA from fruits and vegetables.

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