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Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography

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A high performance liquid chromatography (HPLC) method was developed for the determination of bisphenol A (BPA) that had migrated into canned fruit and vegetables. BPA was extracted with acetonitrile from the solid portion of canned food, and with an OASIS HLB cartridge from the aqueous portion, respectively. Both extracts were cleaned up on a Florisil cartridge. The HPLC separation was carried out on a Wakosil II 3C18 RS column (4.6 × 150 mm) with acetonitrile-water (40:60, v/v) as a mobile phase with a flow rate of 0.8 ml/min. BPA was detectable by UV detector at 228 nm and determined with the similarity of chromatographic peak spectrum by multiwavelength detector (similarity index was 0.99 or above). The quantification limits were 10 ng/g for the solid portion and 5 ng/ml for the aqueous portion, respectively. BPA was mainly detected in the solid portion of canned food and found at the maximum level of 11 µg per can. To verify migration into the solid portion of canned food, a partitioning experiment was carried out.

Introduction

Bisphenol A (4,4′-isopropylidenediphenol, CAS Registry No.80-05-7, known as BPA) is used as a principal material in the preparation of plastics such as polycarbonate and epoxy resins. The production and use of BPA are increasing as the use of such resins increases. Many food and beverage cans are coated with epoxy resins to protect foodstuffs from a variety of substrates, especially metals, in the inner surfaces of cans. However, Gandara *et al.* (1993) reported that polymerization of epoxy resin reactions might not be fully complete. Some chemicals such as BPA may migrate into foodstuffs from epoxy-coated can surfaces. The Directive of the European Union established specific migration limits in food or in food stimulants: 3 mg/kg for BPA (European Commission 1990) and 0.02 mg/kg for bisphenol A diglycidyl ether, BADGE. Moreover, the EU Commission and the Scientific Committee for Food proposed to increase the specific migration limit for BADGE compounds to 1 mg/kg (SCF 1996). BPA was considered of lesser importance than BADGE at that time, but concerns about the estrogenic activity of BPA were heightened recently. BPA was reported to have estrogen-like behavior and activity in *in vitro* cell culture studies (Krishnan *et al.* 1993, Kuiper *et al.* 1997, 1998).

Furthermore it has been shown that BPA leached from epoxy can coatings to foodstuffs. Biles *et al.* (1997) showed that BPA migrated into infant formula liquid concentrates from epoxy can coatings at the level of about 13 ng/ml. Horie *et al.* (1999) reported that BPA concentration in canned drinks was found at the maximum level of 212 ng/ml. In canned food, Brotons *et al.* (1995) reported that about 23 µg/can of BPA migrated into the aqueous portion in vegetable cans. But the total residue of BPA in canned food was not measured. Therefore, it is necessary to develop analytical methods for the determination of BPA in canned food. The present paper reports the determination of BPA in the solid and aqueous portions of canned vegetables and fruit by using HPLC.

Experimental procedures

Materials and reagents

BPA was purchased from Kanto Chemical Co. (Tokyo, Japan). Distilled water was used, which was

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concentrated 100-fold by using an OASIS HLB (60 mg) solid phase extraction (SPE) cartridge, and the concentrate was detected to have no peaks of BPA (< 0.2 ng/g, 50 µl injection). Sep-Pak plus Florisil cartridges and OASIS HLB (60 mg) extraction cartridges were purchased from Waters (Massachusetts, USA). Before the use of these cartridges, the Sep-Pak plus Florisil cartridges were conditioned by 20 ml of acetone–heptane (20:80,v/v) and 10 ml of acetone–heptane (5:95,v/v), and the OASIS HLB (60 mg) extraction cartridges by 20 ml of methanol, followed by 10 ml of distilled water. Other chemicals were of analytical-reagent or HPLC grade.

Preparation of standard solution

A stock standard solution of BPA was prepared by dissolving 100 mg of BPA in 100 ml of methanol. The standard solutions were reserved at −20°C in amber glass vessels. Working standard solutions were prepared by diluting the stock solution with methanol or acetonitrile-water (40:60, v/v). The working standard solutions were stored at 4°C in the refrigerator.

Apparatus

The analysis of standards and sample extracts was conducted by a JASCO Co. (Tokyo, Japan) HPLC Gulliver system equipped with a pump (PU-980), a degasser (DG-980), an auto sampler (AS1555), a column oven (860-CO), and a multiwavelength detector (MD-1510). The analytical column was Wakosil II 3C18 RS (3 µm,150 × 4.6 mm I.D) purchased from Wako Pure Chemical Industries Co. (Osaka, Japan) and was heated during the analyses at 40°C. The mobile phase was acetonitrile-water (40:60,v/v) with a flow rate of 0.8 ml/min under isocratic conditions. The injection volume was 20 µl and the UV detector wavelength was 228 nm. The final concentration of BPA in the extract was determined by multiwavelength detector. Figure 1 shows a typical chromatogram and a peak spectrum obtained from standard solution of BPA.

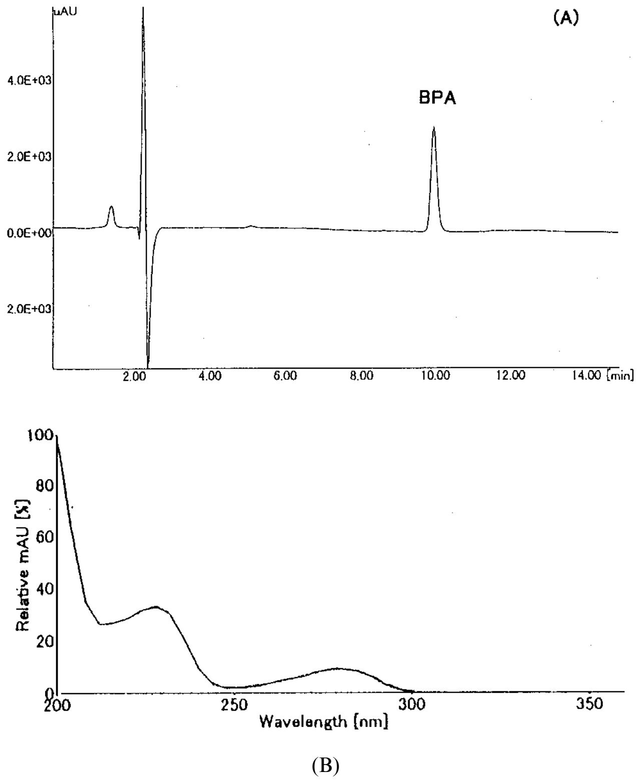


Figure 1. Typical chromatogram (A) and spectrum (B) of standard solution of BPA (0.5 mg/kg).

Sample preparation for the solid portion in cans of vegetables and fruit

Five grams of sample were homogenized for 2 min with 50 ml of acetonitrile and 15 g of sodium sulfate. The homogenate was filtered and the residue was washed by 30 ml of acetonitrile. The filtrate was shaken vigorously for 5 min with 50 ml of hexane saturated with acetonitrile. The acetonitrile layer was transferred to a flask. The hexane layer was shaken with 50 ml of acetonitrile again and the acetonitrile layer was added into the flask. After adding 10 ml of 2-propanol to the acetonitrile layer, the extractant was evaporated to dryness under reduced pressure at 40°C. The residue was dissolved in 10 ml of acetone–heptane (2.5:97.5) and then applied to a Sep-Pak Florisil cartridge. After washing with 10 ml of acetone–heptane (5:95), BPA was eluted from the cartridge with 10 ml of acetone–heptane (20:80). The eluate was evaporated to dryness under reduced pressure at 40°C and the residue was dissolved in

1 ml of mobile phase, then 20 µl of the sample solution was injected into the HPLC system.

Sample preparation for the aqueous portion in cans of vegetables and fruit

A 10 ml sample of the aqueous portion of canned food was added to about 10 ml of distilled water as required. The aqueous portion was applied to an OASIS HLB extraction cartridge. Then after washing with 10 ml of distilled water, BPA was eluted from the cartridge with 10 ml of methanol and the eluate was evaporated to dryness under reduced pressure at 40°C. The residue was applied to a Sep-Pak Florisil cartridge and injected into the HPLC system.

Calibration graphs

Standard solutions (20 µl) at concentrations of 0.05, 0.1, 0.5 and 1.0 µg/ml of BPA were injected into the HPLC system. A linear calibration graph ($r = 0.997$) was obtained by measurement of peak areas at 228 nm.

Results and discussion

Preparation for the solid portion of canned food

Acetonitrile was chosen as an extraction solvent. The reasons for this choice were its high extraction efficiency and the simple procedure for preparation. BPA was recovered in a good yield using acetonitrile, and this permitted direct liquid–liquid extraction with hexane to remove the impurities such as fat from samples. For sample clean-up, a Florisil cartridge was employed, because it is used routinely for analysis of pesticides in vegetables and fruit. When 10 ml of acetone–heptane (5:95) was used as a washing solvent and 10 ml of acetone–heptane (20:80) for elution, good recoveries and clean chromatograms were obtained. BPA was detectable at 228 nm and determined with the similarity of chromatographic peak by multi-wavelength detector at the lowest level of 10 ng/g (similarity index = 0.99).

Table 1. Influence of extraction cartridges on the recovery of BPA (1 µg) from distilled water.

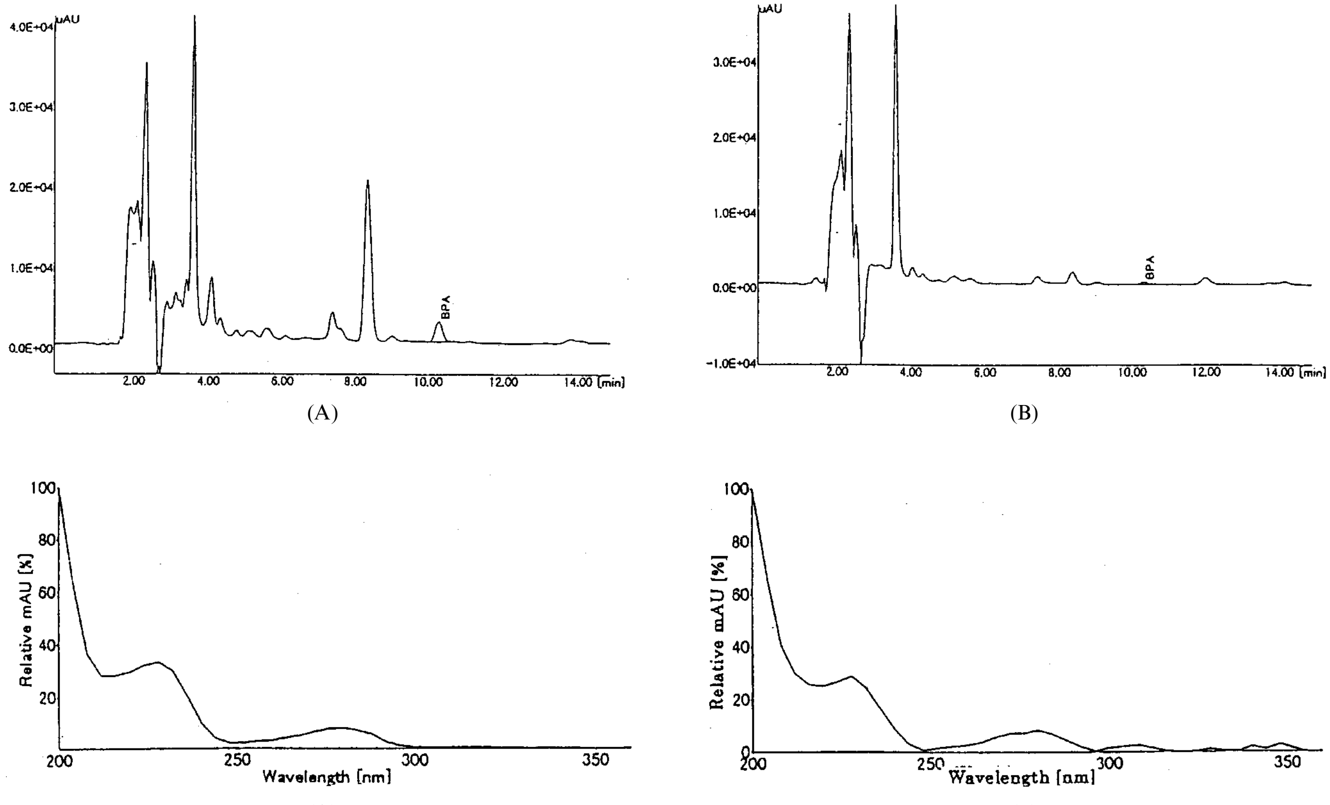
Cartridge		Recovery (%)
C18 Bond Elut	(500 mg)	91.8
PH Bond Elut	(500 mg)	92.5
HLB OASIS	(60 mg)	98.9

Preparation for the aqueous portion of canned food

Biles *et al.* (1997) reported that BPA in infant formula could be concentrated using an SPE cartridge. We also examined the effects of the use of C18 cartridges, PH cartridges, and OASIS HLB cartridges for the aqueous portion of canned food. Each cartridge had been previously washed with 20 ml of methanol and 10 ml of distilled water. After 20 ml of distilled water, which was spiked with 1 µg of BPA, was applied to each cartridge, each cartridge was washed with 10 ml of distilled water and then BPA was eluted by 5 ml of methanol. The OASIS HLB cartridge was found to give the best recovery of BPA (table 1). The OASIS HLB cartridge has a unique copolymer consisting of divinylbenzene (lipophilic monomer) and N-vinylpyrolidone (hydrophilic polymer). However, in some samples, the impurities could not be sufficiently removed. Therefore, after the solid phase extraction, an additional clean-up procedure was performed using a Florisil cartridge. Using two SPE cartridges, the impurities could be removed sufficiently and BPA was determined at the level of 5 ng/ml. Figure 2 shows typical chromatograms and peak spectra obtained from the solid and aqueous portions of canned corn.

Recovery tests of BPA

Uncooked ears of corn grown in Japan were purchased at a supermarket. The ears were cooked in boiling distilled water for about 10 min and the kernels of corn were used to carry out recovery tests of BPA. A 5 g portion of the kernels of corn spiked with 0.25 µg or 1 µg of BPA was analysed with the preparation method for solid portions as described above. The mean recoveries of BPA from the spiked



(A) (B)

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Figure 2. Typical chromatograms and peak spectra obtained from the solid and aqueous portions of canned corn. (A) Solid portion of canned corn (similarity index = 0.993), (B) Aqueous portion of canned corn (similarity index = 0.978).

boiled kernels of corn were 87.1% and 90.1%, and the RSD were 4.2% and 3.4% ($n = 3$), respectively.

Recovery tests for the aqueous portion were carried out as follows:

(1) 10 ml of 1.0% sodium chloride solution at pH 6.0 (nearly the same concentration and pH as the aqueous portion of canned corn) spiked with 0.5 µg or 1 µg of BPA, was respectively analysed with the preparation method for the aqueous portion. The mean recoveries were 85.7% and 89.3%, and RSD were 3.9% and 2.9% ($n = 3$),

(2) 10 ml of the aqueous portion of canned pineapple (pH 3.8), in which BPA was not detectable, spiked with 0.5 µg or 1 µg of BPA was also analysed. The mean recoveries were 84.5% and 86.5%, and RSD were 3.9% and 3.5% ($n = 3$), respectively.

Concentration of BPA in food cans

Fourteen samples of canned foods (corn, mushrooms, asparagus, oranges, peaches, pears, and pineapples) were purchased at supermarkets in Japan. Most cans were products of Japan, China, and the United States.

The residual amounts of BPA found in the solid portion of canned food ranged from < 10 ng/g to 95.3 ng/g, while the amounts of BPA from the aqueous portion of canned food were less than 5 ng/ ml. The total amount of BPA in food cans was a maximum of 11.1 µg (5.8 µg/dm^2) per can as shown in table 2.

In spite of BPA determined in both solid and aqueous portions of canned food, the highest amount of BPA in this study was less than the highest level of BPA reported in the study of Brotons *et al.* (1995). No samples contained levels exceeding the limits estab-

Table 2. Residual Bisphenol A in canned food.

No	Sample	Brand*1	Contents (g)	Amount of solid (g)	Area of inner surfaces (dm^2)	Solid portion BPA (ng/g)	Aqueous portion BPA (ng/mL)	BPA		
								(µg/can)	(g/dm^2)	Origin
1	corn	A1	190	130	2.0	52.5	< 5	6.8	3.4	Unknown (Japan or USA)
2	corn	A2	190	130	2.0	57.4	< 5	7.5	3.8	Unknown (Japan or USA)
3	corn	A3	190	130	2.0	56.4	< 5	7.3	3.7	Unknown (Japan or USA)
4	corn	B	130	116	1.9	95.3	(tr. 4.0)	11.1	5.8	Japan
5	corn	C	226	142	2.2	18.4	< 5	2.6	1.2	USA
6	corn	D	230	145	2.2	28.0	< 5	4.1	1.8	Japan
7	mushroom	E	130	75	1.5	< 10	< 5	—	—	Unknown
8	mushroom	F	190	113	1.8	39.5	< 5	4.5	2.5	Indonesia
9	asparagus	G	250	160	2.1	29.8	< 5	4.8	2.3	China
10	orange	C	435	250	3.2	< 10	< 5	—	—	Unknown
11	orange	H	425	234	3.2	< 10	< 5	—	—	China
12	pear	A	425	240	3.2	< 10	< 5	—	—	Australia
13	peach	G	425	250	3.2	< 10	< 5	—	—	China
14	pineapple	I	227	140	1.9	< 10	< 5	—	—	Thailand

*[1] A1–A3 represent the same lot of the same brand.

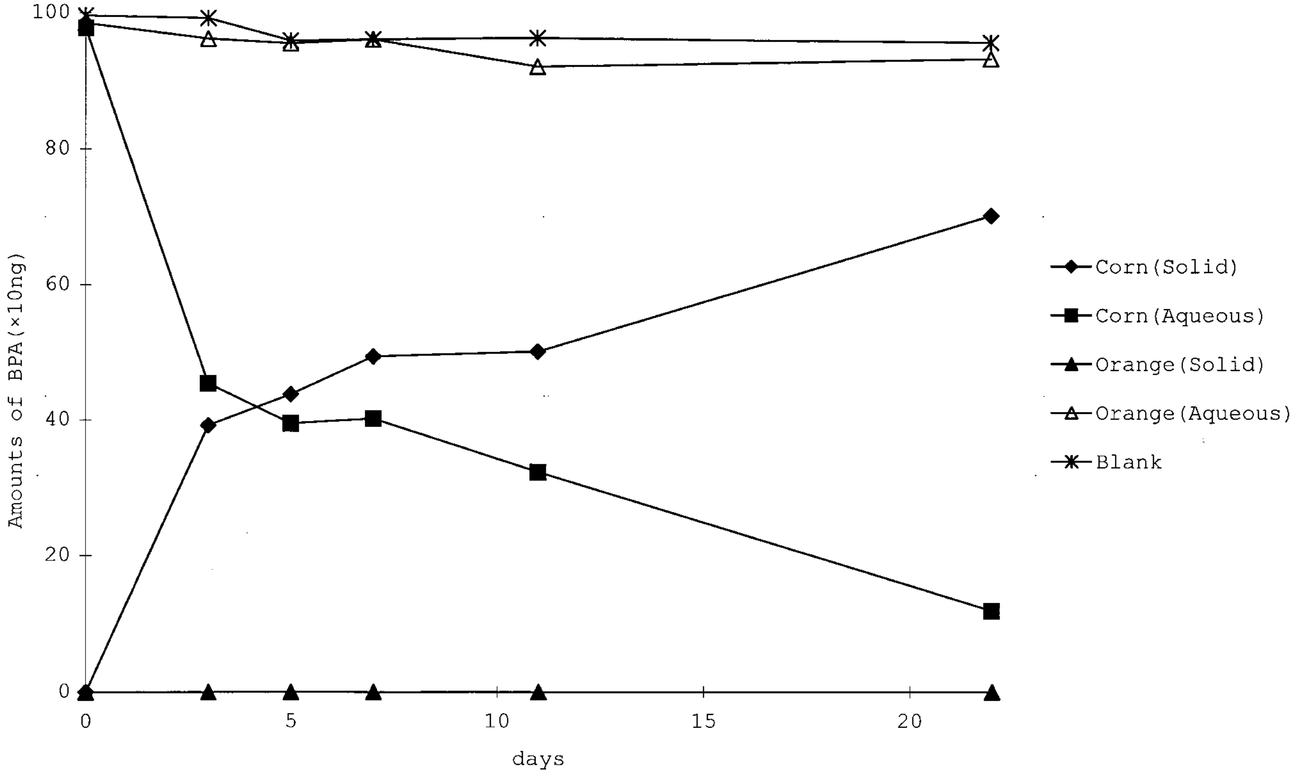

Figure 3. Changes in the amounts of BPA in the solid and aqueous portion of each packed corn and orange.

lished by the EU. There was no relationship between residue levels and area of epoxy coated can surface. Furthermore, no significant relationship was observed between the product's expiration date and the level of BPA. Nevertheless, samples from the same lot of canned corn (sample No.1 to 3) were found to have nearly the same residue level of BPA.

From these results, the wide range of residue levels might be due in part to manufacturing process, sterilization procedure, and type of food contact surfaces, type of food contained and/or storage conditions.

Procedure for estimation of partitioning of BPA in packed corn

Most food cans had no BPA in the aqueous portion, but in some of the corn cans, about 4 ng/ml (under the quantification limit and similarity index = 0.98) of BPA was found in the aqueous portion. In the case of sample No.4, the proportion of the amount of BPA in the solid portion to that in the aqueous portion was about 95 to 4. We were concerned with these results from canned corn analysis. To confirm these results, the experiment below was carried out.

Glass vials with a screw cap, in which a 5 g of boiled corn was packed, were filled with 50 ml of 1.0% sodium chloride solution at pH 6.0 (nearly the same as the concentration and pH of the aqueous portion of canned corn) containing 1 μg of BPA. The vials were shaken once by hand and allowed to stand at 4°C. Then each portion of packed corn was analysed for BPA after 0, 3, 5, 7, 11 and 22 days. For comparison, peeled oranges were also packed and analysed in 10% sucrose solution at pH 5.8 (figure 3).

As a function of time, the amount of BPA that migrated into the corn increased, while in peeled oranges BPA was not detectable. During this partitioning test, no loss of BPA in the control occurred. After 7 days, about 50% (equivalent to 100 ng/g) of BPA in the aqueous portion migrated into packed corn, and 22 days later, about 70% (equivalent to 140 ng/g) migrated. During this test, the amount of BPA that migrated into corn exceeded the levels found in canned corn listed in table 2. It was expected that as time increased, the migrant would increase to a saturation point.

These results suggested that, during the canning process of corn, BPA first leached from the food contact surfaces into the aqueous portion, and then partitioned from the aqueous portion to the solid portion during storage of the canned corn. Consequently, much less BPA was detectable in the aqueous portion than in the solid portion. However, it was not clearly found in this study whether BPA was adsorbed in fibers and/or fat of corn, or other mechanisms may have occurred.

Conclusions

This investigation shows that the real residue of BPA can be quantified in cans of vegetables and fruit. In canned fruit, no BPA was determined (< 10 ng/g), while in canned vegetables, BPA was detectable in the solid portion at a range from < 10 ng/g to 95 ng/g, and in most of the canned vegetables BPA concentrations in the solid portion were higher than those in the aqueous portion. Results from BPA analysis of canned food and the partitioning experiment suggested that BPA leached out of can surfaces might migrate from the aqueous portion to the solid portion during storage in some types of foodstuffs such as canned corn. However, further investigation is necessary to clarify the mechanisms of migration of BPA into canned foods.

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