JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Bisphenol A in Canned Foods by Immunoaffinity Chromatography, HPLC, and Fluorescence Detection

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Bisphenol A (BPA) concentrations were determined in canned beverages, fruits, vegetables, and fat-containing foodstuffs bought in Austrian supermarkets. The analysis method consisted of sol−gel immunoaffinity chromatography followed by high-performance liquid chromatography with fluorescence detection. With one exception traces of BPA were detected in all samples. BPA recovery strongly depended on the food matrix, ranging from 27% in goulash to 103% in a lemon soft drink. The results obtained allow a more realistic picture of the BPA exposure caused by cans with an epoxy resin protective coating because—in contrast to several previous studies—only those fractions of the can contents that are actually consumed were analyzed. BPA concentrations ranging from 0.1 ng/mL (lemon soft drink) to 38 ng/g (ready-to eat soup from Thailand) were significantly lower than the European Union migration limit of 0.6 mg of BPA/kg of food.

KEYWORDS: Bisphenol A; migration; canned food; food analysis; immunoaffinity chromatography; sol−gel

INTRODUCTION

In the past decade endocrine disruptors have become a central topic in the international discussion in environmental and food chemistry. Both in vitro (*1*−*3*) and in vivo (*4*, *5*) studies have shown the estrogenic potential of bisphenol A (BPA), which is an anthropogenic substance primarily used as a monomer for producing polycarbonates and epoxy resins. Many household goods are based on polycarbonate, some of them being directly in contact with foodstuff as in one-way beverage bottles and food-storage containers. Epoxy resins are commonly used as protective coatings in metal food and beverage cans.

Recent studies have indicated the migration of BPA traces from both polycarbonate-based packaging (*6*−*8*) and epoxy resins (*9*) into food-simulating liquids, with the amount of BPA leached depending on liquid composition and pH. Toxicological data obtained by in vivo experiments show that the toxic effects observed strongly depend on the animal model used, with some models showing effects at much lower concentrations than for other contaminants not interfering with the endocrine system (*10*−*12*).

To assess potential human health risks caused by BPA exposure, it is therefore essential to start from accurate data on BPA levels in foodstuffs at very low concentrations. Several surveys have already been performed to determine BPA in canned foodstuffs (*13*−*17*), with data available for Japan (*14*), the United Kingdom (*15*), Spain (*16*), and New Zealand (*17*). The data are, however, hardly comparable because they are related to different sample fractions. In some studies sampling was carried out without taking into account which parts of the can content are actually consumed. In their pioneering study Brotons et al., for example, analyzed only the aqueous part of vegetables packaged in cans, but not the vegetables actually eaten (*16*). On the other hand, Goodson et al. (*15*) and Thomson et al. (*17*) determined BPA levels after homogenizing the whole content of food containers, whereas Yoshida et al. (*14*) analyzed both the aqueous and the solid portions of vegetable and fruit cans separately.

To get information on the actual BPA exposure of consumers the present work provides a consistent set of data on BPA levels of those fractions of canned foods that are commonly consumed. Thus, for beverages, fruits, crushed tomatoes, and fat-containing ready-to-eat foodstuffs the total content of the can was used for analysis, whereas for vegetables and fish the liquid fractions were removed and only the solid parts were analyzed.

The cleanup step is very critical in the determination of traces of analytes in complex food samples. Up to now, liquid−liquid extraction and solid-phase extraction (SPE) have been the most common sample preparation steps applied in the analysis of BPA in foodstuffs (*13*−*21*). The data given in the present paper were obtained by applying a recently developed analysis method, including sol−gel immunoaffinity chromatography followed by HPLC with fluorescence detection (*22*). The preparation of immunoaffinity columns by using the sol−gel method offers several advantages compared to covalently binding the antibodies to a solid support material; for example, the synthesis is

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10.1021/jf051525j CCC: $30.25
Published on Web 00/00/0000 **PAGE EST: 6.1**

Table 1. HPLC Methods Using System 1

HPLC method	column	elution mode	elution conditions[a]	flow rate (mL/min)	BPA retention time (min)	LOD (ng/mL) (S/N = 3)	samples analyzed
1	Spherisorb S ODS1, 5 μm, 250 × 4.6 mm i.d. (Knauer)	isocratic	60% A, 40% B	1.0	11.2	0.2	beverages (except cola and energy drink), fruits (except mango), and vegetables (except red kidney beans)
2	Intersil ODS-2, 5 μm, 150 × 2.1 mm i.d.	isocratic	65% A, 35% B	0.3	17.2	0.8	cola, energy drink, mango, red kidney beans, and coconut cream
3	Spherisorb S ODS1, 5 μm, 250 × 4.6 mm i.d. (Knauer)	gradient program 1	0 min: 70% A/30% B 5 min: 70% A/30% B 7 min: 63% A/37% B 17 min: 63% A/37% B	1.0	17.4	0.5	goulash, potato soup, and soup Tom Kha
4	Spherisorb S ODS1, 5 μm, 250 × 4.6 mm i.d. (Knauer)	gradient program 2	0 min: 70% A/30% B 10 min: 70% A/30% B 12 min: 69% A/31% B 27 min: 69% A/31% B 29 min: 28% A/72% B 39 min: 28% A/72% B	1.0	22.3	0.4	fish

[a] Mobile phase A, 50 mM sodium acetate buffer (pH 4.8, adjusted with acetic acid); mobile phase B, ACN.

less labor-intensive, less time-consuming, and less costly. Because the entrapment of antibodies in the pores of the sol−gel glass is carried out under mild conditions, the affinity of the antibody to the antigen is largely retained. Sol−gel immunoaffinity columns have been shown to efficiently remove interfering matrix compounds from food (*22*, *23*) and environmental (*24*−*26*) as well as urine samples (*27*).

MATERIALS AND METHODS

Chemicals and Reagents. Purified polyclonal bisphenol A (BPA) antibodies (5 mg/240 μL of PBS buffer) were a gift from Japan EnviroChemicals, Ltd. (Tokyo, Japan). BPA, sodium acetate, glacial acetic acid, and sodium hydroxide were obtained from Sigma (St. Louis, MO). Acetonitrile (ACN), gradient grade for HPLC, was purchased from Fisher Scientific (Leicestershire, U.K.). Tetramethoxysilane (TMOS) and hexane were from Fluka (Buchs, Switzerland).

A stock solution of BPA was prepared by dissolving 20.0 mg of BPA in 20.0 mL of ACN. This solution was further diluted with mobile phase (see **Table 1**) and stored at 4 °C. Phosphate-buffered saline (PBS), pH 7.6, was prepared by dissolving 12.46 g of $Na_2HPO_4 \times 2H_2O$, 1.56 g of $NaH_2PO_4 \times 2H_2O$, and 8.5 g of NaCl in 1 L of bidistilled water.

Sol−Gel Columns. Immunoaffinity columns were prepared using the protocol described in a previously published paper (*22*). In brief, mixing 1 mL of PBS containing 1 mg of BPA antibody with 1 mL of prehydrolyzed TMOS resulted in the formation of a gel. Aging of the gel was stopped when it had lost 50% of its initial weight. The resulting silica glass was ground in an achate mortar and packed into a 3 mL glass column (Merck, Darmstadt, Germany) equipped with a polytetrafluoroethylene frit (9 mm diameter, Merck). After the immunoaffinity column had been flushed in sequence with 20 mL of PBS, 15 mL of ACN/water (40:60, v/v), and 20 mL of PBS, it was stored in PBS at 4 °C. After usage, the column was regenerated with 20 mL of PBS.

To prevent clogging of the immunoaffinity column, food extracts were filtered through sol−gel precolumns before being applied to the immunoaffinity column. Sol−gel precolumns were prepared as described above but by mixing PBS (without antibodies) with prehydrolyzed TMOS. Half a gram of the resulting pure sol−gel glass was packed into 8 mL glass columns (Merck) equipped with glass microfiber filters GF/F from Whatman (Kent, U.K.). Columns were flushed with ~20 mL of PBS and stored in PBS at 4 °C. Before a precolumn was used, it was conditioned with 10 mL of ACN/bidistilled water (10:90, v/v). After a single use, the precolumns were discarded.

HPLC Separation and Detection. Samples were analyzed using two HPLC analysis instruments composed of different modules, making it possible to make use of the selectivity provided by different chromatographic phase systems, operation modes (isocratic, different gradients), and different detection principles (spectrofluorimetry, multichannel coulometric array detection).

HPLC system 1 consisted of a high-pressure gradient pump (model L-7100, Merck), a column thermostat (model bfo-04 dt, W.O. Electronics, Langenzersdorf, Austria), a six-port injection valve (model 7161, Rheodyne) equipped with a 100 μL injection loop, and a fluorescence detector (model 1080, Merck) set at 275/305 nm. Peaks were integrated using the McDacq software (Bischoff, Leonberg, Germany). Data given in the present work were obtained in a time period of >2 years by applying different HPLC methods. Details of the HPLC methods are summarized in **Table 1**.

To confirm the identity of BPA a second HPLC system (HPLC system 2) was used. It consisted of a high-pressure gradient pump (model L-6200, Merck), an autosampler (model AS-2000 A), and a coulometric electrode array detector (ESA, Chelmsford, MA) equipped with two cell blocks consisting of eight working electrodes. The analytical column was an ACE 3 C18 column, 150 × 3 mm i.d., 3 μm. Elution was carried out at room temperature with 0.01 M sodium acetate buffer, pH 4.8/ACN (66:34, v/v) by applying a flow rate of 0.45 mL/min. The injection volume was 100 μL. Potentials were set at +300, +400, +500, +550, +600, +650, and +700 mV against palladium reference electrodes; the eighth electrode did not work. Peaks were integrated using the CoulArray Win software.

External Calibration. Each HPLC method given in **Table 1** was calibrated by injecting six standard solutions in the concentration range from 0.2 to 50 ng/mL in mobile phase. Analysis functions were obtained by linear regression of peak areas on standard concentrations. Limits of detection (LODs) were calculated for a signal-to-noise ratio of 3 and limits of quantification (LOQs) for a signal-to-noise ratio of 6.

Analysis of Samples. Food cans were bought in supermarkets in Vienna and stored at room temperature for <1 month. The type of the internal coating of the cans was not investigated.

Sample Preparation. *Beverages.* After the beverage had been degassed in an ultrasonic bath, an aliquot of either 15.0 or 20.0 mL was diluted 1:1 with PBS buffer. If necessary, pH values were adjusted to 7.0 with 1 M NaOH. The sample was applied to the immunoaffinity column by using a 16 port Waters Sep-Pak SPE station (Milford, MA). After the column had been washed with 5 mL of ACN/water (10:90, v/v), BPA was eluted with 4 mL of ACN/water (40:60, v/v). The eluate was collected in a 5 mL measuring flask, which was then filled to the ring mark with ACN/water (40:60, v/v). An aliquot of 100 μL was injected into HPLC system 1, applying either method 1 or 2 (see **Table 1**).

Fruits and Vegetables. In the case of fruits the whole content of the can—and in the case of vegetables, only the solid portion—was homogenized with an Ultra-Turrax mixer. One gram of the homogenized sample was mixed with 1 mL of ACN in a 15 mL polypropylene centrifuge tube (Bibby Sterilin Ltd., Staffordshire, U.K.). After 1 min

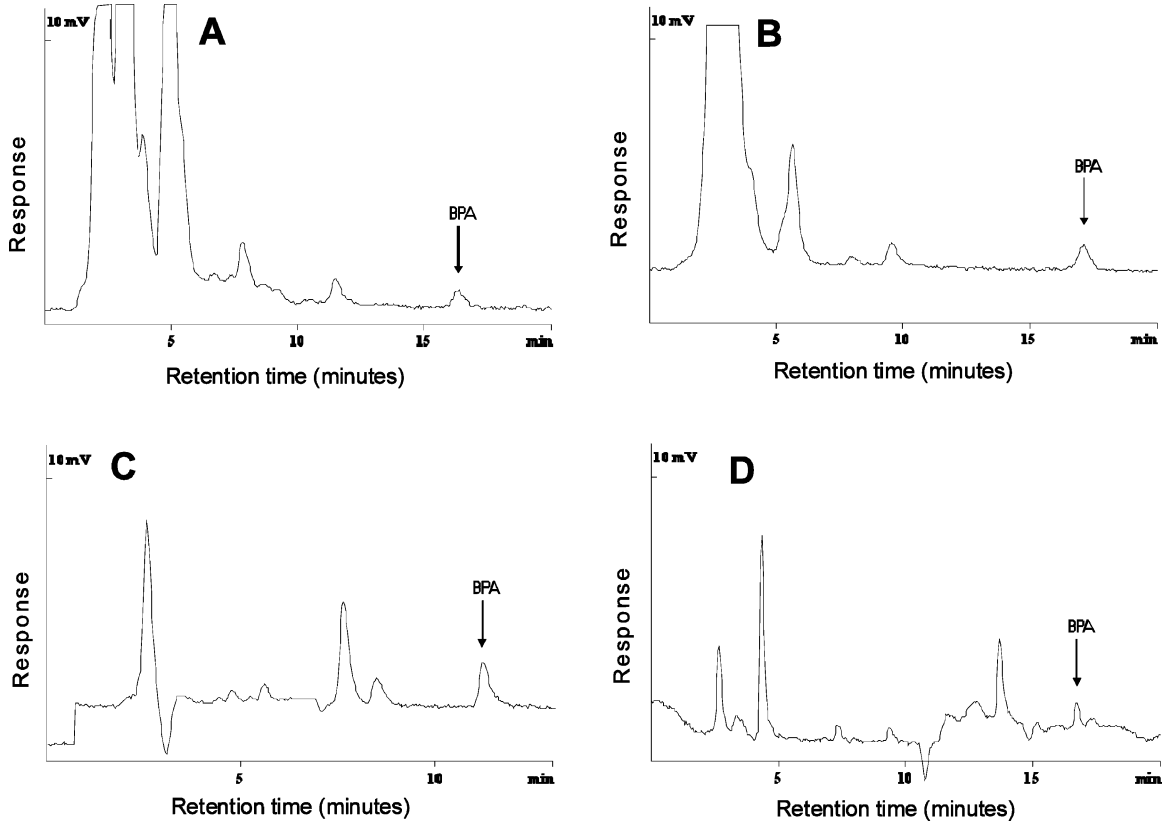

Figure 1. Chromatograms obtained with HPLC system 1 (HPLC–fluorescence detector) by injecting 100 μL aliquots of food samples after purification by immunoaffinity chromatography: (**A**) cola (HPLC method 2); (**B**) energy drink A (HPLC method 2); (**C**) lentil extract (HPLC method 1); (**D**) potato soup with mushrooms (HPLC method 3). HPLC methods are given in **Table 1**.

of shaking, centrifugation was carried out at 2800*g* for 5 min (Sigma centrifuge, model 4 K 10, Vienna, Austria). The supernatant liquid was removed with a Pasteur pipet, and the solid residue was extracted for a second time with 1 mL of ACN. The combined supernatants were filtered through a glass microfiber filter GF/F. After dilution of the filtrate 1:10 with water, a PTFE suction tube was used to apply the sample to a sol−gel precolumn, which was on-line coupled with the immunoaffinity column. When the sample had been loaded to the immunoaffinity column, the precolumn was discarded. After the immunoaffinity column had been washed with 5 mL of ACN/water (10:90, v/v), BPA was eluted with 4 mL of ACN/water (40:60, v/v). The eluate was collected in a 5 mL measuring flask, which was then filled to the ring mark with ACN/water (40:60, v/v). An aliquot of 100 μL was injected into HPLC system 1, applying either method 1 or 2 (see **Table 1**).

Fat-Containing Foodstuffs. For the analysis of goulash, potato soup, and soup Tom Kha, the whole content of the can was homogenized; in the case of tuna and sardines the liquid part was removed by sieving, and only the solid portion was homogenized. A 1 g aliquot of the homogenized sample (fish, 10 g) was filled in a centrifuge tube. One milliliter of ACN and 1 mL of hexane (fish, 10 mL of ACN and 10 mL of hexane) were added, and the mixture was shaken for 1 min. After centrifugation at 2800*g* for 20 min, the ACN phase was transferred into a centrifuge tube. The solid residue and the hexane phase were extracted for a second time by adding a fresh 1 mL (fish, 10 mL) portion of ACN. The combined ACN extracts were filtered through a glass microfiber filter GF/F. The filtrate was evaporated under a constant stream of nitrogen to ~2 mL, diluted 1:10 with water, and purified by immunoaffinity chromatography as described above. An aliquot of 100 μL was injected into HPLC system 1, applying either method 3 or 4 (see **Table 1**).

Standard Addition. For each food matrix one nonspiked and three spiked samples were analyzed as described above. Spiking levels depended on the amount of BPA in the nonspiked samples and were determined in preliminary experiments. In most cases the addition of 10, 20, and 30 ng of BPA (100, 200, and 300 μL of a 100 ng/mL BPA standard solution in ACN) to the sample aliquot used proved to be appropriate. Peak areas were plotted against the amount of analyte added. The recovery was determined by dividing the slope of the linear regression line for the standard addition by the slope of the linear regression line of BPA standard solutions.

RESULTS AND DISCUSSION

Sol−Gel Immunoaffinity Chromatography. Details of the characterization and operation of immunoaffinity columns prepared by entrapping BPA antibodies into sol−gel glass have been published recently (*22*). The investigations indicated that BPA is actually retained in the column due to interactions with the entrapped BPA antibodies and not due to nonspecific adsorption to the sol−gel glass. The binding capacity of a column containing 1 mg of antibodies was shown to be 280 ng of BPA. Loading BPA standard solutions resulted in a mean recovery of 98% with a standard deviation of 3% ($n = 5$). The immunoaffinity columns proved to be very stable and could be used for cleanup of at least 15 food samples. Cross-reactivities of the immunoaffinity column have been determined and were given in the previously published paper (*22*).

Figure 1 shows typical chromatograms obtained by injecting purified extracts of food samples into HPLC system 1. Because the data summarized in the present paper have been generated in a time period of ~2 years, different analytical columns and mobile phases were used (see **Table 1**). The chromatograms demonstrate the high efficiency of the immunoaffinity column in removing interfering matrix compounds. Systematic errors due to the cross-reactivity of the BPA antibodies were eliminated by selecting chromatographic conditions that allow the separa-

Table 2. Analytical Data

product	sample amount	HPLC method	standard addition correlation coefficient *r*	mean recovery ± SD (%)	LOD (S/N = 3)
beverages					
cola drink	20.0 mL	2	0.9947	59 ± 6	0.3 ng/mL
orange soft drink	15.0 mL	1	0.9959	63 ± 4	0.1 ng/mL
lemon soft drink	15.0 mL	1	1.0000	103 ± 1	0.1 ng/mL
beer	15.0 mL	1	0.9879	41 ± 5	0.1 ng/mL
energy drink	10.0 mL	2	0.9905	30 ± 2	0.9 ng/mL
vegetables					
young peas, very fine	1.0 g	1	0.9889	53 ± 9	1.9 ng/g
corn	1.0 g	1	0.9949	35 ± 3	2.9 ng/g
haricot beans	1.0 g	1	0.9644	23 ± 2	4.3 ng/g
red kidney beans	1.0 g	2	0.9911	47 ± 7	7.4 ng/g
lentils	1.0 g	1	0.9941	90 ± 19	1.1 ng/g
crushed tomatoes	1.0 g	1	0.9905	52 ± 5	1.9 ng/g
fruits					
pineapple light	1.0 g	1	0.9957	85 ± 6	1.2 ng/g
peaches	1.0 g	1	0.9991	75 ± 8	1.3 ng/g
lychees	1.0 g	1	0.9885	83 ± 15	1.2 ng/g
mango	1.0 g	2	0.9943	73 ± 3	5.4 ng/g
fat-containing products					
sweetened coconut cream	1.0 g	2	0.9996	31 ± 3	6.4 ng/g
goulash	1.0 g	3	0.9984	27 ± 4	9.3 ng/g
potato soup	1.0 g	3	0.9983	40 ± 7	6.3 ng/g
Tom Kha soup	1.0 g	3	0.9955	39 ± 7	6.4 ng/g
tuna	10.0 g	4	0.9985	45 ± 5	0.4 ng/g
sardines	10.0 g	4	0.9938	97 ± 12	0.2 ng/g

tion of BPA from all substances with a known cross-reactivity of >1%.

Method Performance. For each HPLC method the calibration curve obtained by injecting BPA standard solutions from 0.2 to 50.0 ng/mL showed good linearity ($r > 0.9993$, $n = 6$). The LOD depended on the HPLC method and ranged from 0.2 to 0.8 ng/mL (see **Table 1**); the LOQ ranged from 0.4 to 1.5 ng/mL.

The analytical performance of the whole analysis method was assessed by applying the standard addition method. Correlation coefficients, mean recoveries, standard deviations of the recoveries, and LODs are summarized in **Table 2**. BPA recoveries varied with the sample matrix. This result can be explained by the fact that the efficiency of the liquid−liquid extraction depends on the type and concentration of matrix components. In addition, when the extract is loaded to the immunoaffinity column, coextracted matrix compounds can influence the interactions between BPA and the antibodies immobilized. Relatively high recoveries (>80%) were obtained for lemon soft drink, lentils, pineapples, and lychees, whereas analysis of energy drink, haricot beans, and goulash resulted in recoveries of <30%. LODs, depending on the sample amount and the HPLC method as well as the food matrix, were in the range from 0.1 ng/mL to 9.3 ng/g.

BPA Levels in Canned Foodstuffs. BPA levels found in canned foodstuffs are given in **Tables 3**−**6**. Traces of BPA were detected in all samples except one energy drink.

Very low BPA levels ranging from nondetectable to 3.4 ng/mL were found in beverages. In contrast to previous studies aimed at investigating the migration of BPA into foodstuffs (*15*, *17*), traces of BPA were detected in soft drinks. The analysis methods applied in those investigations suffered from higher LOD values and could therefore not be applied to the determination of BPA concentrations in the low parts per billion range.

To confirm the identity of BPA detected in beverages, samples of purified cola soft drink and energy drink A were injected into HPLC system 2, which was equipped with a coulometric electrode array detector. **Figure 2** shows the chromatograms of (A) a BPA standard solution (10 ng/mL), (B) the cola soft drink, and (C) energy drink A. Due to the lower sensitivity of the coulometric electrode array detector, the cola soft drink had to be concentrated to a factor of ∼4 before it was injected into HPLC system 2. Chromatograms of the BPA standard solution, the cola soft drink, and the energy drink contained a peak with a retention time of 15.4 min, indicating the presence of BPA in both beverages. **Figure 3** demonstrates the similarity of the current−voltage curves of the 15.4 min peaks, adding further evidence for the identity of BPA peaks in the chromatograms of the cola soft drink and the energy drink.

In the case of canned vegetables (except crushed tomato) BPA concentrations were determined in the solid portions, which are the parts of the can content actually consumed. Young peas exhibited significantly lower BPA levels (8.5 ng/g) than the other samples investigated, which gave results ranging from 26 to 35 ng/g. In corn, BPA concentration was found to be 28 ng/g of solid portion, which is in agreement with previously reported data ranging from 18.4 to 95.3 ng/g (*14*). In tomatoes BPA concentration, determined by analyzing the whole content of the can, was 20 ng/g, which is consistent with the amount of BPA measured in ref *17* (<10−21 ng/g).

Table 3. BPA Concentrations in Canned Beverages

product	country of origin	content (mL)	best before date	date of analysis	time left before expiration date (months)	area of inner surface (dm^2)	BPA ± SD (ng/mL)	BPA ± SD (μg/can)	BPA ± SD ($\mu g/dm^2$)
cola drink light	Austria	330	Nov 2003	June 2003	5	2.5	0.7 ± 0.1	0.23 ± 0.03	0.09 ± 0.01
cola drink	Austria	330	Nov 2005	April 2005	7	2.5	0.4 ± 0.0[a]	0.13 ± 0.01	0.05 ± 0.00[a]
orange soft drink	Austria	330	May 2004	June 2003	11	2.5	0.4 ± 0.1	0.13 ± 0.03	0.05 ± 0.01
lemon soft drink	Austria	330	May 2004	June 2003	11	2.5	0.1 ± 0.0[a]	0.03 ± 0.00[a]	0.01 ± 0.00[a]
beer	Austria	500	Jan 2004	July 2003	6	3.5	1.5 ± 0.3	0.75 ± 0.15	0.21 ± 0.04
energy drink A	Austria	250	June 2005	Sept 2003	21	2.4	3.4 ± 0.4	0.85 ± 0.10	0.35 ± 0.04
energy drink B	Austria	250	July 2006	April 2005	15	2.4	nd[b]		

[a] Obtained by rounding standard deviation data calculated from four determinations. [b] Below LOD (0.9 ng/mL).

Table 4. BPA Concentrations in Canned Vegetables

product	country of origin	content (g)	amount of solid (g)	best before date	date of analysis	time left before expiration date (months)	BPA ± SD (ng/g)	BPA ± SD (μg in solid portion)	BPA ± SD (μg in can)
young peas, very fine	Germany	200	140	July 2007	Sept 2003	46	8.5 ± 2.0	1.2 ± 0.3	
corn	Germany	300	285	Sept 2006	Sept 2003	36	28 ± 3.1	8.0 ± 0.9	
haricot beans	Germany	400	250	Nov 2007	Oct 2003	49	35 ± 9.1	8.8 ± 2.3	
red kidney beans	Italy	800	510	Dec 2007	April 2005	32	26 ± 4.8	13.3 ± 2.4	
lentils	Germany	400	265	Nov 2007	Nov 2003	48	26 ± 3.3	6.9 ± 0.9	
crushed tomatoes	Italy	400		Aug 2006	Nov 2003	33	20 ± 2.9		8.0 ± 1.2

Table 5. BPA Concentrations in Canned Fruits

product	country of origin	content (g)	amount of solid (g)	best before date	date of analysis	time left before expiration date (months)	area of inner surface (dm^2)	BPA ± SD (ng/g)	BPA ± SD (μg/can)	BPA ± SD ($\mu g/dm^2$)
pineapple light	Indonesia	565	340	Dec 2004	July 2003	17	3.8	5.0 ± 1.2	2.8 ± 0.7	0.74 ± 0.18
peaches	South Africa	420	250	Dec 2005	Aug 2003	28	3.3	6.4 ± 0.7	2.7 ± 0.3	0.83 ± 0.09
lychees	Thailand	425	200	Dec 2004	Aug 2003	16	3.8	6.8 ± 2.6	2.9 ± 1.1	0.76 ± 0.29
mango	Thailand	425	230	Dec 2006	April 2005	20	3.8	24 ± 4.0	10.2 ± 1.7	6.3 ± 1.05

Table 6. BPA Concentrations in Canned Fat-Containing Products

product	country of origin	content	amount of solid (g)	best before date	date of analysis	time left before expiration date (months)	area of inner surface (dm^2)	BPA ± SD (ng/g)	BPA ± SD (μg/can)	BPA ± SD (μg/solid portion)	BPA ± SD ($\mu g/dm^2$)
sweetened coconut cream	Thailand	400 mL		Nov 2006	May 2005	18	3.3	29.7 ± 1.5	11.9 ± 0.6		3.61 ± 0.18
goulash	Austria	800 g		2007	Dec 2003	48	4.8	22.0 ± 1.5	17.6 ± 1.2		3.64 ± 0.25
goulash	Austria	800 g		2003	Dec 2003	0	4.8	9.6 ± 0.7	7.7 ± 0.6		1.60 ± 0.12
potato soup	Austria	500 g		June 2006	Nov 2003	31	3.8	20.7 ± 1.3	10.4 ± 0.7		2.76 ± 0.17
potato soup	Austria	500 g		April 2003	Nov 2003	–7	3.8	9.6 ± 0.6	4.8 ± 0.3		1.26 ± 0.08
Tom Kha soup	Thailand	410 g		May 2005	Jan 2004	16	3.3	37.6 ± 3.6	15.4 ± 1.5		4.74 ± 0.45
Tom Kha soup	Thailand	410 g		April 2003	Jan 2004	–9	3.3	17.0 ± 1.6	7.0 ± 0.7		2.11 ± 0.20
tuna in oil	Mauritius	195 g	150	April 2007	Feb 2004	38	2.1	43 ± 6.4		6.5 ± 1.0	
sardines in oil	Morocco	125 g	90	Dec 2007	May 2004	43	2.1	2.1 ± 0.3		0.2 ± 0.0[a]	

[a] Obtained by rounding standard deviation data calculated from four determinations.

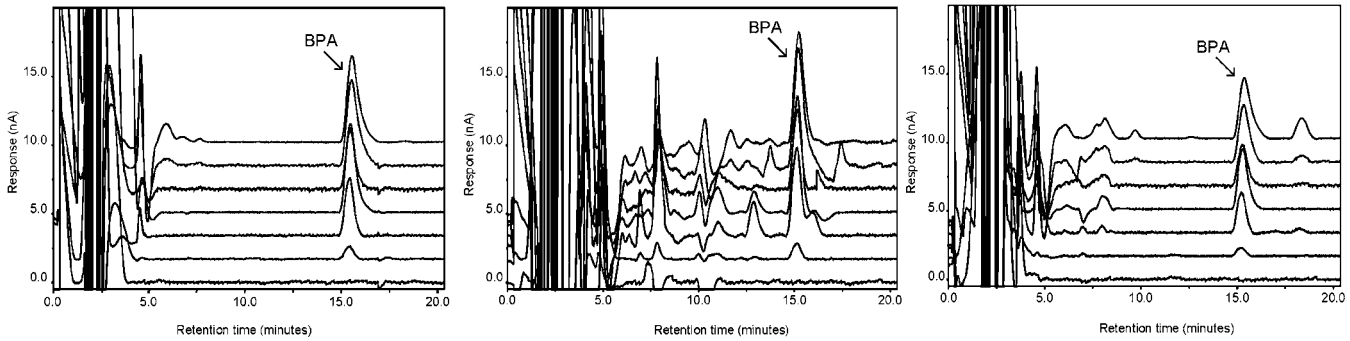

Figure 2. Chromatograms obtained with HPLC system 2 (HPLC–coulometric electrode array detector) by injecting 100 μL aliquots of (**A**, left) a BPA standard solution (10 ng/mL), (**B**, middle) the cola soft drink purified by immunoaffinity chromatography (eluate concentrated to a factor of ~4), and (**C**, right) energy drink A purified by immunoaffinity chromatography.

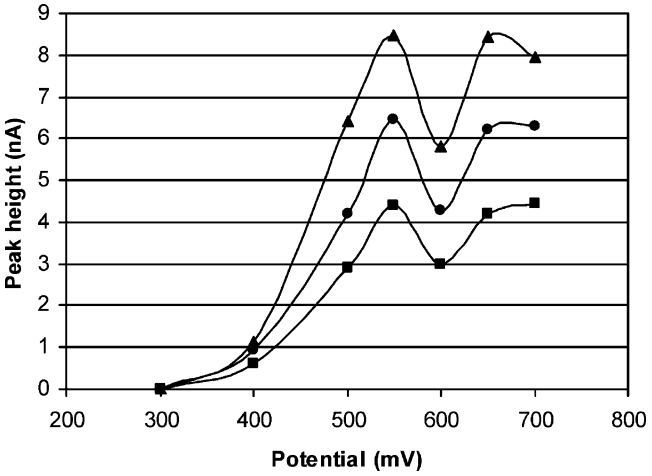

Figure 3. Current-voltage curves of the BPA standard solution (10 ng/mL) (●), the cola soft drink (▲), and energy drink A (■).

Most canned fruits exhibited BPA levels in the range from 5.0 to 6.8 ng/g, which corresponds to the data published by Thomson et al. (BPA levels in peaches and pineapple, <10 ng/g) (*17*). In the present work a significantly higher BPA value of 24 ng/g was found only in mango.

In fat-containing ready-to-eat products BPA levels were in the range from 21 to 38 ng/g. The BPA concentration in the solid portion of a tuna can (43 ng/g) was significantly higher than that in a sardine can (2.1 ng/g). The maximum amount of BPA in food cans was 17.6 μg per can, detected in an 800 g can of goulash.

When BPA levels are related to the epoxy-coated can surface, BPA levels of >1 $\mu g/dm^2$ were found in mangoes as well as in the fat-containing products. In agreement with previously published papers, there was no strong correlation between BPA levels and the area of inner surface.

When nonexpired foodstuffs were analyzed, no relationship was observed between the expiration date of the product and the BPA level found. However, **Table 6** includes data on three ready-to-eat products (potato soup with mushrooms, goulash, and soup Tom Kha), which were already beyond their expiration date of 2003. The three cans were of the same size as the nonexpired cans of the same product. The visual appearance of those foodstuffs differed from that of the "fresh" products—they did not look appetizing. As can be seen in **Figure 4** already expired samples exhibited BPA levels ~55% lower than the nonexpired ones. However, we did not check if this decline is caused by microbial degradation or, as one reviewer remarked, by a reaction of BPA with free radicals.

In 1990 a tolerance limit of 3 mg/kg for BPA migration into food was established by the European Union Commission. In

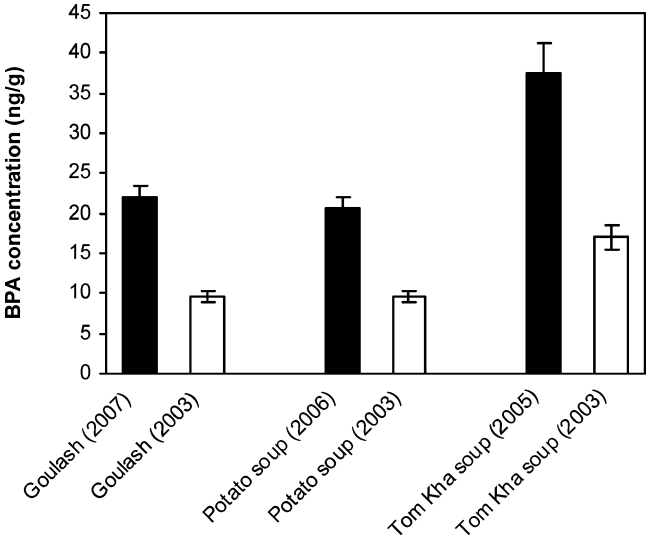

Figure 4. BPA levels of food samples beyond the date of expiration. Expiration dates are given in parentheses.

2004 this limit was reduced to 0.6 mg/kg (*28*). All samples analyzed for the present paper contained BPA levels significantly lower than this limiting value.

ACKNOWLEDGMENT

We thank Japan EnviroChemicals, Tokyo, Japan, for supporting our work by providing BPA antibodies and Heidi Schwartz for carrying out the confirmation studies with the HPLC system equipped with the electrochemical detector.

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Received for review June 27, 2005. Revised manuscript received September 5, 2005. Accepted September 5, 2005.

JF051525J