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Migration of Bisphenol A (BPA) from Epoxy Can Coatings to Jalapeño Peppers and an Acid Food Simulant

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Effects of heat processing, storage time, and temperature on migration of bisphenol A (BPA) from an epoxy type can coating to an acid food simulant and jalapeño peppers were determined. Commercial jalapeño pepper cans (8 oz, dimensions 211 × 300) were stored at 25 °C for 40, 70, and 160 days. A solution of 3% acetic acid was canned in 211 × 300 cans from the same batch used for jalapeño peppers. Heat processing was applied to two-thirds of the cans, and the remaining cans were not heat processed. Cans were stored at 25 and 35 °C for 0, 40, 70, and 160 days. Results showed that there is a minimal effect of heat treatment. An effect of storage time on migration of BPA during the first 40 days at 25 °C was observed. An increase on migration of BPA was observed with storage time at 35 °C. The highest level of migration was 15.33 μg/kg of BPA at 160 days at 35 °C. A correction factor of ∼0.4 was calculated for migration under simulating conditions of storage compared to the real ones. The highest level of BPA found in jalapeño peppers cans, surveyed from three supermarkets, was 5.59 ± 2.43 μg/kg. Migration of BPA, performed according to the European and Mercosur conditions, was 65.45 ± 5.29 μg/kg. All the migration values found in this study were below those legislation limits (3 mg/kg).

KEYWORDS: Bisphenol A; epoxy resins; canned jalapeño peppers

INTRODUCTION

Metallic cans are protected against corrosion by the application of inner coatings based on epoxy type resins (*1*). The synthesis of bisphenol A (BPA) type epoxy resins includes the condensation between BPA and epichlorhydrin to yield bisphenol A diglycidyl ether and polymers of different molecular mass (*2*, *3*). BPA may remain unreacted when polymerization conditions are insufficient, having a potential to be transferred to the canned food.

BPA is among estrogenic xenobiotics that may affect the reproductive system of animals and causes proliferation of breast cancer cells in vitro (*4*, *5*). The European Commission is considering extension of the current legislation on plastics for food contact to surface coatings on cans. At the moment, migration limits for BPA is 3 mg/kg of food or food simulant (*6*). Mercosur legislation on food contact materials set a migration limit for BPA (3 mg/kg of food or food simulant) (*7*).

Brotons et al. (*8*) reported estrogenic activity (determined by E-screen bioassay) from canned peas, artichokes, and mixed vegetables. They found that BPA was transferred to water after a second heat process was applied to cans in which the original contents had been removed. Munguia-Lopez and Soto Valdez (*3*) reported migration of 81.8 μg/kg of BPA to an aqueous food simulant from organosol-coated tuna cans. They also found levels as high as 4.2 μg/kg of BPA from the 211 × 300 cans reported in this paper to the same simulant. BPA is also the main reactant in the preparation of polycarbonate (PC), used for the fabrication of baby bottles, training cups, and water carboys. Biles et al. (*9*) reported residual amounts of BPA in the above PC articles from 7 to 58 μg/g. Migration levels to water, ethanol/water, and Miglyol food simulants ranged from 13 to 368% of those residual amounts.

Jalapeño peppers are among the most popular and widely consumed canned foodstuffs in Mexico. They consist of green pickled peppers preserved in a vinegar solution (about 1−2% acetic acid). They are used as a relish or dressing. The aim of this work was to determine and compare the effect of heat processing and storage time on migration of BPA in an acid food simulant and jalapeño peppers canned in 211 × 300 cans. The levels of BPA found in a local survey of commercial jalapeño peppers are also reported.

MATERIALS AND METHODS

Chemical and Reagents. The BPA standard was of analytical grade (Aldrich, Milwaukee, WI). A 3% (w/v) acetic acid solution (JT Baker, Phillipsburg, NJ) was used as the acid food simulant. Acetonitrile (ACN) was HPLC grade (EM Science, Gibbstown, NJ). Methanol was HPLC grade (EM Science). Water for chromatographic analyses was HPLC grade (JT Baker, Xalostoc, Mexico).

Apparatus. A high-pressure liquid chromatographic system (HPLC) was used for quantification of BPA (Varian, Star 9012, pump, Mexico

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DF, Mexico) equipped with a fluorescence detector (Varian 9075) and a Star 5 chromatography workstation (Varian). BPA identity was confirmed by gas chromatography−mass spectrometry (GC−MS) (electron impact ionization mode) using a Varian 3400CX gas chromatograph (Varian) coupled to a Saturn III model mass spectrometer (Varian), with a NIST 92 data system. *Spectra for the Identification of Monomers in Food Packaging* was used for identification (*10*).

Liquid Chromatographic Conditions. The fluorescence detector excitation wavelength was 224 nm, and the emission wavelength was 310 nm. A 15 cm × 5 mm i.d. Micropak C_{18} MCH-5-N-CAP column (Varian), protected by C_{18} guard columns, was used. A Rheodyne 7125 injector with a 10 μL loop was used (*3*). A 1 mL/min flow with the following gradient program was used: 15 min gradient elution from 35:65 to 55:45 (ACN:water), 20 min gradient elution to 100:0, and 10 min isocratic elution for cleaning. The column temperature was 35 °C.

Gas Chromatographic Conditions. The gas chromatograph was fitted with a DB5 fused silica capillary column (30 m × 0.25 mm i.d., film thickness = 0.25 μm) supplied by J&W Scientific (Alltech, Mexico, DF, Mexico). The oven temperature was programmed from 90 to 300 °C at 30 °C/min and held for 10 min. The injector temperature was 150 °C, and that of the transference line was 200 °C. Under these chromatographic conditions, the retention time for BPA was 8.5 min.

Migration tests (Experiment 1). Nine commercial cans no. 211 × 300 (8 oz) were filled with jalapeño peppers (∼200 g), sealed, heat processed at 100 °C for 9 min (HP), and stored for 40, 70, and 160 days at 25 °C. Other 36 cans were filled with the acid food simulant (204 g), sealed, and processed as follow: the same heat processing conditions applied to the foodstuff (HP) were applied to 24 cans (100 °C for 9 min). No heat process (NHP) was applied to the remaining 12 cans. A total of 12 HP cans were stored at 25 °C and the other 12 at 35 °C for 0, 40, 70, and 160 days. NHP cans were stored at 25 °C for 0, 40, 70, and 160 days. NHP cans for 0 days storage were in contact with the resin for ∼4 h before analysis. All cans used in this experiment 1 belong to the same batch number and were provided by a canning factory.

Migration Test According to European and Mercosur Conditions. To verify if the cans comply with the European and Mercosur legislation, a set of 3 cans were filled with the acid food simulant. A heat processing (121 °C/30 min) was applied followed by storage at 40 °C for 10 days, in accordance with the conditions laid down in Directive 82/711/EEC (*11*).

BPA Analysis in Food Simulant. Each can sampled was opened and the simulant evaporated by rotary evaporator at temperature up to 45 °C. The dry residue was redissolved in 5 mL of ACN and filtered through 0.22 μm Durapore membrane filters (Millipore, Cork, Ireland) before injection into the HPLC system. Quantitation was performed by using a calibration curve prepared from 5 to 1200 μg/L of BPA in ACN. Results are presented in μg of BPA/kg of food simulant.

BPA Analysis in Canned Jalapeño Peppers. Each can tested was opened, peppers and liquid were homogenized, and the weight of the total content was recorded. A 120 g aliquot was blended with 150 mL of methanol. The mixture was stirred for 1 h and left to stand overnight. A 20 min stirring period was applied before the sample was filtered through Whatman no. 1 filter paper. The solid residue was stirred with 120 mL of methanol for 1 h and filtered, and both liquids were evaporated to dryness at temperature up to 45 °C. The evaporator flask was rinsed with 5 mL of ACN and filtered through 0.22 μm Durapore membrane filters (Millipore, Cork, Ireland) before injection into the HPLC system. Quantitation was performed as mention above for the food simulant.

Recoveries. Acid food simulant and peppers cans were spiked with a standard solution to get a 18.4 μg/kg concentration of BPA. The analyte was extracted and quantified and recoveries were calculated. Three replicates were determined for the simulant and the foodstuff.

Correction Factor. To compare the performance of the simulating conditions to the real ones, a correction factor was calculated by dividing the BPA levels found in jalapeño peppers (including solids and liquid) by the levels found in the acid food simulant.

Survey (Experiment 2). Eight canned jalapeño peppers samples (3 cans from the same batch number for each sample) were purchased from three local (Hermosillo, Son, Mexico) supermarkets. They belong to 4 brands listed in **Table 2 a**s A−D. BPA was analyzed in each can as stated above.

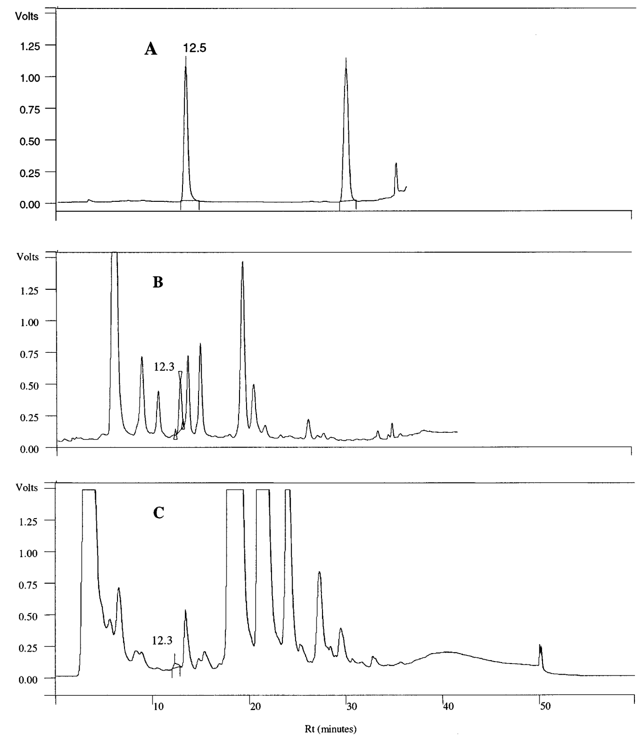

Figure 1. HPLC chromatograms: A = standard solution of 1200 μg/L of BPA in ACN; B = extract from the acid simulant, redisolved in ACN; C = extract from the jalapeño peppers, redisolved in ACN.

RESULTS AND DISCUSSION

Inner coatings of all cans were identified by infrared spectrophotometry as epoxy resins (*3*).

Spectra showed typical aromatic bands at 3040, 1605, 1508, and 826 cm^{-1}. The HPLC chromatogram A in **Figure 1** was obtained from a 1200 μg/L standard solution of BPA. HPLC chromatogram B corresponds to the acid food simulant sample in ACN. HPLC chromatogram C corresponds to the jalapeño peppers sample in ACN. The acid food simulant chromatogram is very similar to the one reported from an aqueous food simulant canned in the same type of cans (*3*). In both cases, the simulants are evaporated to dryness and redisolved in ACN. Therefore, all the signals correspond to compounds present in the resin and they are potential migrants to the can content. Under the analytical conditions of this work, some of these signals were of higher intensity than those of BPA. Identification of these signals was out of the scope of this work. BPA was confirmed in selected samples by GC-MS (**Figure 2**). Electron impact ionization MS spectra showed main ion fragments at *m*/*z* 213, 228, 119, and 91 (*10*). Bisphenol A diglycidyl ether (BADGE), another monomer with potential to migrate from epoxy resins, was not detected in either acid food simulant or peppers. This compound is unstable under acid conditions yielding hydrolysis products identified as BADGE monool epoxide and BADGE diol epoxide (*2*, *12*). BADGE decomposition reaction half-life in 3% acetic acid is reported to be between <1 and 10 h. Moreover, this compound is completely lost at 40 °C for 10 h (*13*). In the present work BADGE was not even detected in the acid simulant after 4 h in contact with the resin. However, migration of 2.7 μg/kg was detected in aqueous food simulant after the heat process was applied (*3*).

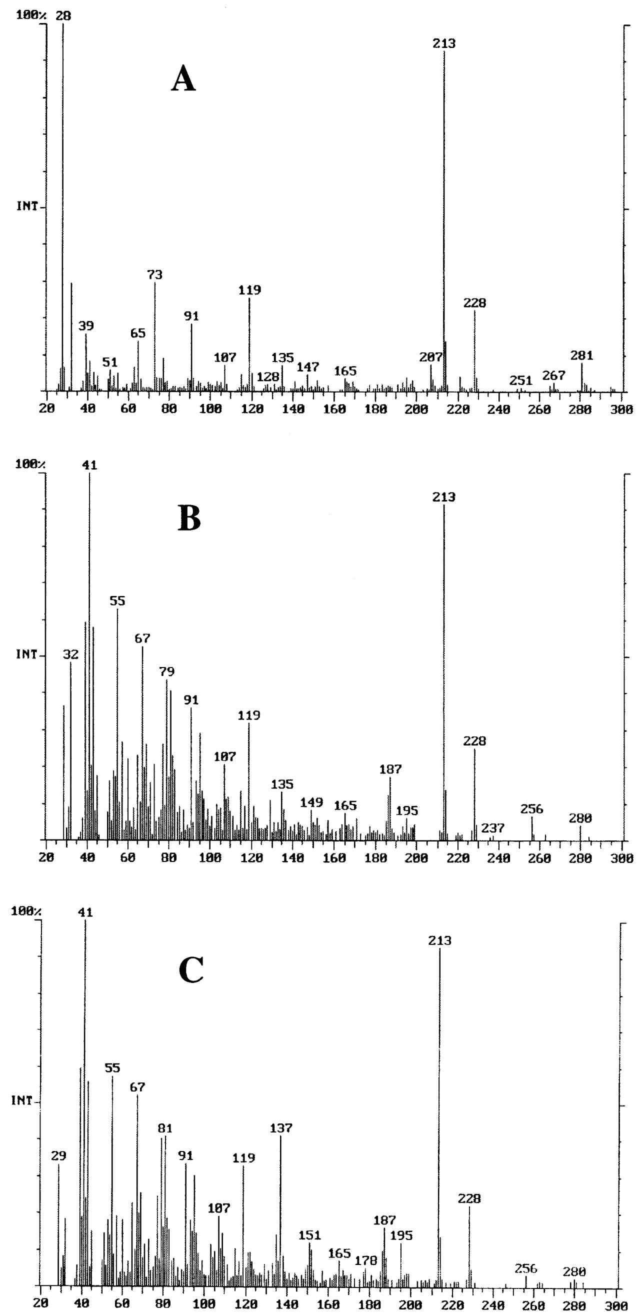

Figure 2. Electron impact ionization mass spectra of BPA: A = standard solution of BPA; B = extract from an acid simulant can; C = extract from a jalapeño peppers can.

Recoveries of BPA in acid food simulant and jalapeño peppers were 89.15 ± 1.07 and 90.61 ± 5.29%, respectively. The regression coefficient for calibration curve was 0.997. The limit of detection (LOD) of the instrument was 2.0 μg/L. The lowest concentration that could be quantified by the instrument was 5.0 μg/L, the first point on the calibration curve. The limits of quantitation (LOQ) of the procedures used in this work were 0.12 and 0.2 μg/kg for BPA in acid food simulant and jalapeño peppers, respectively.

Effect of Heat Processing and Storage Time on Migration of BPA (at 25 °C). Table 1 shows the concentration of BPA found in both acid food simulant and jalapeño peppers during storage. The effect of heat processing on migration was minimal. Migration of BPA to the food simulant increased from <0.12 to 1.73 μg/kg after 40 days of storage for the NHP cans. After that, no significant changes were observed for NHP cans stored at 25 °C. The same behavior was followed by the HP cans stored at 25 °C. The European and Mercosur legislation limit of migration for BPA is 3 mg/kg; the levels found in this study are far lower than those. Similar results were found when migration was determined in the same cans, using an aqueous food simulant (*3*). The highest level reported was 4.2 μg/kg of BPA at 70 days of storage at 25 °C. Thus, migration of BPA from epoxy-coated cans to aqueous food simulants is not affected by acid conditions.

Table 1. Migration of BPA from an Epoxy Type Can Coating to an Acid Food Simulant and to Jalapeño Peppers[a]

	μg/kg of BPA			
	acid food simulant[b]			jalapeño peppers[c]
storage time (days)	NHP, stored at 25 °C	HP, stored at 25 °C	HP, stored at 35 °C	HP, stored at 25 °C
0	<LOQ[a]	<LOQ[a]	<LOQ[a]	ND
40	1.97[b] ± 0.72	4.65[b] ± 2.41	7.67[b] ± 0.81	2.00[a] ± 0.52
70	1.73[b] ± 0.94	3.67[b] ± 2.20	9.52[b] ± 5.43	1.62[a] ± 0.47
160	2.25[b] ± 1.25	3.62[b] ± 1.67	15.33[c] ± 1.12	1.35[a] ± 0.22

[a] Numbers are mean of three replicates ± standard deviation. Different letters indicate significant differences (Tukey's test; $p < 0.05$) within a column. NHP = no heat process applied. HP = heat process applied (100 °C/9 min). LOQ = limit of quantitation of the simulant method: 0.12 μg/kg. ND = not determined. [b] 3% acetic acid. [c] Solids and liquid.

Effect of Storage Time and Temperature on Migration of BPA (at 35 °C). An increase on migration of BPA to the acid food simulant was observed with time at a storage temperature of 35 °C. Levels as high as 15.33 μg/kg of BPA at 160 days were observed (**Table 1**). However, these levels are still far low from legislation limits. This temperature was chosen to simulate conditions in warehouse rooms with no air conditioning in hot weather places. Therefore, although there was no effect of time on migration of BPA at simulated home storage, it can be enhanced at higher temperatures such as those reached on warehouse storage.

Effect of Storage Time on Migration of BPA to Foodstuff (at 25 °C) and Correction Factor. Migration levels of BPA to jalapeño peppers (**Table 1**) were up to 2.00 μg/kg (40 days at 25 °C). There were no significant differences at 70 and 160 days of storage. The correction factor, calculated for acid simulant, was ∼0.4. An overestimation of 2.5 times the migration levels when using the acid simulant was observed. By applying this factor, it is possible to estimate real migration levels of BPA at domestic conditions, when using the acid food simulant.

Migration According to the European and Mercosur Regulations. The migration level according to the European and Mercosur conditions was 65.45 ± 5.29 μg/kg (lower than these regulations limit). This value is ∼4.3 times the level found in cans stored for 160 days at 35 °C. The heat process applied, according to the regulations (121 °C/30 min), increased the migration level of BPA since the real heat process applied was 100 °C/9 min. Jalapeño peppers foodstuff is a high acid food, so it does not need a drastic heat process for commercial sterilization.

Migration of Surveyed Foodstuffs. The highest level of BPA found in jalapeño peppers cans, surveyed from three supermarkets, was 5.59 ± 2.43 μg/kg (**Table 2**). Brotons et al. (*8*) found higher levels of BPA in the liquid phase of canned peas, artichokes, and mixed vegetables (22−76 μg/kg). Biles et al.

Table 2. Levels of BPA Found in Jalapeño Peppers Cans Surveyed in Supermarkets[a]

supermarket	brand	μg/kg of BPA	supermarket	brand	μg/kg of BPA
Soriana	A	<LOQ	VH	C	2.44 ± 0.70
	B	5.59 ± 2.43	Ley	A	2.42 ± 0.74
	C	1.62 ± 0.35		C	2.65 ± 0.65
VH	A	1.58 ± 0.25		D	<LOQ

[a] Numbers are mean of three replicates ± standard deviation. LOQ = limit of quantitation of the jalapeño peppers method: 0.20 μg/kg.

(*14*) reported migration of 0.1−13 μg/kg of BPA from epoxy-coated can surfaces to infant formula concentrates. From the results presented in this work, is not possible to deduce that contamination of jalapeño peppers with BPA could be a serious problem. In fact, these values are far below the European and Mercosur limit of migration for BPA (3 mg/kg).

CONCLUSIONS

Migration levels of BPA from 211 × 300 can coating to an acid food simulant and to the foodstuff were far below the European and Mercosur legal limits. A minimal effect of heat processing on migration of BPA was found. There is an effect of storage time on migration of BPA during the first 40 days at simulated home storage. The effect at longer storage can be enhanced at higher temperatures such as those reached on warehouse storage. There is an overestimation of the levels of migration when using the acid food simulant compared to the real foodstuff.

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