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The Investigation of Bisphenol a Presence in Canned Tuna Fish using High-Performance Liquid Chromatography Method

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Abstract: In this study, total number of 160 canned tuna fish samples were investigated the presence and levels of Bisphenol A (BPA) in four brands (A, B, C, D) of canned tuna fish samples which were collected from Ankara region, Turkey. Additionally, the salt amount, pH value and drain weight level for each sample were determined. Quantitative analysis of BPA in samples was carried out by solid phase extraction method followed by High Performance Liquid Chromatography (HPLC) with fluorescence detection. The mean levels of BPA in the analysed canned fish samples were determined as 0.45±0.09, 0.31±0.09, 0.45±0.09 and 2.33±0.09 mg kg^{-1} for A, B, C, D brands, respectively. According to the findings of this study, it was determined that the mean BPA values of the samples except for the values obtained from D brand were below the Turkish Food Codex (TFC) values (0.6 mg kg^{-1}). Furthermore, the pH values and salt amounts were in accordance with Turkish Standart Instute (TSI) values for all brands. However, mean of drain weight found in all brands were lower than the TSI value. In conclusion, some samples obtained during the period of the study are determined to have high levels of BPA. Therefore, the steps in all food processing should be monitored for preventing the BPA contamination.

Key words: Bisphenol A, HPLC, solid phase extraction, tuna, Turkey

INTRODUCTION

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane-BPA) is a main component of epoxy resins and a monomer that is used in the production of epoxy and polycarbonate plastics and flame retardants (Izzotti *et al*., 2009). These resins are widely used as internal surface coating material in food cans for preventing metal corrosion and migration from the can into the can contents (Garcia-Prieto *et al*., 2008). Epoxy resins are used to make the coating cans of sea products, vegetables, beer, soft drinks, powder milk, wine and water and various types of food containers (Garcia and Losada, 2004). Thus, BPA products are in direct contact with solid and liquid food in the market (Morck *et al*., 2010).

As a result of using high temperatures for sterilisation and microwave heating, the resins can decompose and the migration of bisphenols from packaging to food can be more intensive and rapid (Jordakova *et al*., 2003).

The Tolerable Daily Intake (TDI) for BPA was reviewed by the Scientific Committee on Food in 2002 and was reduced to a temporary value of 0.01 mg kg^{-1} body weight per day (EC, 2002). The U.S. Environmental Protection Agency (EPA) established the Reference Dose (RfD) of 0.05 mg kg^{-1} body weight/day (EPA, 1993). BPA is believed to have estrojen like activity. The levels of BPA in the blood of men and women are associated with some diseases such as reproduction dysfunctions, endometrial hyperplasia, recurrent miscarriages, abnormal karyotypes and polycystic ovarian syndrome (Grumetto *et al*., 2008).

In addition, BPA has an estrogen like effect on MCF-7 cells (human breast cancer cell line) inducing cell proliferation and progesterone receptors *in vitro* (Krishnan *et al*., 1993).

Although, human may be exposed to BPA in the enviroment, the primary route of exposure to BPA is food. In most cases, contamination occurs due to migration of BPA from food containers made with BPA (Kang *et al*., 2006). European Union (EU) legislation was introduced, setting a Specific Migration Limit (SML) for BPA of 3 mg kg^{-1} food but was changed to 0.6 mg kg^{-1} food in 2002 (EC, 1990, 2002). However, SML for BPA issued in Japan is 2.5 mg kg^{-1} (Masuyama, 1994).

In Turkey, the regulations regarding BPA are concerted with European Union directives. In 2005, the SML of BPA in Turkish Food Codex (TFC) was announced as 0.6 mg kg^{-1}. In 2008, this notification was rewritten but the SML value of BPA was not changed.

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The aim was to determine presence and levels of BPA in four brands of canned tuna fish collected from the Ankara region, Turkey. In addition, salt amount, pH value and drain weight level for each sample were determined.

MATERIALS AND METHODS

In this study, a 160 canned tuna fish samples obtained from 4 different brands (A, B, C, D) were used. Canned fish samples collected from brands A, B, C and D in Ankara local market were packed in oil and water. The canned fish samples with different serial numbers and different production dates were used. The sealed cans were stored at room temperature. Upon opening, the total contents of each can were homogenized and an aliquot was taken for extraction.

Drain weight level, pH value and salt content was determined before the extraction in samples. Salt content for samples was determined by using Mohr method (Nielsen, 2003). The pH values were determined by pH meter (TSI, 2002). The drain weight level was determined with TSI method (TSI, 2007, 2010).

The BPA levels in the fish samples were determined by the method of Kang and Kondo (2002). BPA standard (99%) was purchased from Sigma-Aldrich, USA. Solid-phase extraction cartridges (florisil light) were obtained from waters (Milford, MA, USA). All reagents were of HPLC or analytical grade (Merck, Germany).

Extraction of BPA: Briefly, a 5 g sample (fish meat) was extracted with 50 mL acetonitrile and 10 g anhydrous sodium sulfate using a high speed homogenizer. The homogenate was filtered and the residue was washed with 30 mL acetonitrile. The combined filtrate was shaken for 5 min with 30 mL hexane saturated with acetonitrile and the combined solution was allowed to separate by standing for about 15 min. The acetonitrile layer was transferred to a flask. The hexane layer was shaken with 50 mL acetonitrile again and the acetonitrile layer was combined with the first extract in the flask. The acetonitrile solution was evaporated to dryness using nitrogen at 40°C. The residue was dissolved in 20 mL acetone-n-heptane (3:97, v/v) and applied to a Sep-Pak florisil cartridge that was preconditioned with 5 mL acetone-n-heptane (3:97, v/v). Afterwards, cartridges were washed with 10 mL acetone-n-heptane (5:95, v/v) and dried. BPA was eluted from the cartridges with 10 mL acetone-n-heptane (20:80, v/v). The extract was evaporated to dryness under nitrogen at 40°C and dissolved in 1 mL acetonitrile-water (40:60, v/v) (Kang and Kondo, 2002).

HPLC analysis of BPA: BPA extracts were analysed with High Performance Liquid Chromatography (HPLC) (Varian Prostar System) using fluorescence detection (Varian Prostar 363). The column temperature of a column heater Varian ProStar 510 was set at 40°C. The mobile phase was 40% acetonitrile and 60% water with a flow rate of 1 mL min^{-1} under isocratic conditions. The stationary phase was Reverse Phase Column 18 (RPC18) (150×4, 6 mm inner diameter-i.d., 5 μm). Excitation wavelength of the fluorescence detector was 275 nm and the emission wavelength was 300 nm (Kang and Kondo, 2002). The BPA calibration curve was constructed by using a series of dilutions containing different levels of BPA. The coefficient of determination of this calibration curve was $R^2 = 0.9975$. Sample volumes of 50 μL were injected. The BPA levels of the samples were found as μg mL^{-1} from calibration curve and than these levels calculated as mg kg^{-1}. The retention times varied as mean of 7.035±0.011 min when the BPA standard solution (0.1 μg mL^{-1}) was measured six times (Kang and Kondo, 2002).

Statistical analysis: Descriptive statistics and one sample t-test were conducted for statistical evaluation (Daniel, 1991).

RESULTS AND DISCUSSION

In this method, the mean recovery was found 95.64%. The Limit Of Detection (LOD) was determined as 1.96×10^{-3} μg mL^{-1} (Skoog and Leary, 1992; Kang and Kondo, 2002). The technique was found to be reproducible. Percentage Relative Standard Deviation (RSD%) of peak area was found 2.54% for the inter day precision test and 0.68% for the intra day precision test (Snyder *et al*., 1997; Grumetto *et al*., 2008).

The BPA was found for all of the samples and Table 1 shows the levels of BPA in canned tuna fish samples of different brands. In addition, the salt amount, pH value and drain weight for each sample were determined. The results of the BPA analysis were evaluated within the Turkish Food Codex (TFC) value. The mean values of salt, pH and drain weight of samples were evaluated according to Turkish Standart Institute (TSI) values.

BPA mean levels in the analysed fish samples were determined as 0.45±0.09, 0.31±0.09, 0.45±0.09 and 2.33±0.09 mg kg^{-1} for A, B, C, D brands, respectively.

It was determined that the mean BPA values of the samples except for the values obtained from D brand were below the TFC values (0.6 mg kg^{-1}). Mean values of pH

Table 1: Levels of BPA in canned tuna fish samples of different brands

Brands of sample analysed	Number of sample	<0.2 mg kg^{-1}	0.2-0.39 mg kg^{-1}	0.4-0.59 mg kg^{-1}	>0.6 mg kg^{-1} *	Percent
A	40	4	12	13	11	27.5*
B	40	8	25	5	2	5.0*
C	40	-	17	17	6	15.0*
D	40	12	8	-	20	50.0*

*Exceeding TFC and EU standard value (0.6 mg kg^{-1})

of the samples for A, B, C and D brands were determined as 6.04±0.02, 6.00±0.02, 5.99±0.02 and 5.98±0.02, respectively. The pH values of samples were within the TSI values (4.0-6.9). On the other hand, the salt content of the fish samples for brands A, B, C and D were 1.12±0.03, 1.09±0.03, 1.25±0.03 and 1.11±0.03, respectively and were below the TSI value of 2.5%.

The mean of drain weight found in all brands were lower than the TSI value. According to TSI, level of drain weight have to at least 65% (w/w) (TSI, 2010).

The data revealed that the BPA levels found in 24.8% of samples were higher than TFC and EU directive (0.6 mg kg^{-1}). Up to the knowledge, this is the first report on BPA levels in canned tuna fish obtained in Turkey. However, previous study reports the presence of a chemical derivative of BPA-Bisphenol A Diglysidil Ether (BADGE) in canned fish. Erkan *et al.* (2006) reported migration of BADGE in different brands of canned fishes in oil (sardine, thun, sardelle, mackerel, pelamide and trout) obtained in the Turkish market, obtaining highest values of BADGE in canned sardine/sardelle.

In several countries, similar studies were conducted on BPA levels in canned fish. Podlipna and Markl (2007) reported BPA levels in conserved tuna fish in oil and water using 12 samples and found BPA contamination rates to be 12-59 ng g^{-1}. Munguia-Lopez and Soto-Valdez (2001) investigated BPA in aqueus of tuna fish and Jalapeno pepper and determined BPA and found contamination rates 0.6-83.4 μg kg^{-1}, respectively. FSA (2001) reported BPA levels as higher than 70 μg kg^{-1} in 36 samples from a total of 62 food samples. Goodson *et al.* (2002) identified BPA contamination in 62 concerved samples and in 37 samples, 7-380 μg kg^{-1} levels were reported. Sajiki *et al.* (2007) found BPA contamination levels to be as 0-842 ng g^{-1} in 48 samples in Japan. These results are lower than those reports in the present study.

These reports show that the levels of BPA vary in canned fish and other canned foods. The discrepancies in these data could have originated from food canning technology, resin based food cans and food type. In addition, BPA could be present initially in the raw food itself.

CONCLUSION

The present study investigated the presence of BPA in the 160 canned fish samples and was found in all of the samples. According to the findings of the analysis of samples obtained at different times, variability is thought to be observed. Performing assessment of BPA migration-related conditions and contamination risk is important. A long term and excessive consumption of foods containing BPA above the tolerance levels, may be a hazardous impact on human health. For this reason, the processing of canned fish, other foods and drinks should be monitored for preventing the contamination by BPA.

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