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Study of migration of Bisphenol A diglycidyl ether (BADGE) and its derivatives from canned tuna fish in Iran's market

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S.S. Sajadian, Sh. Shoeibi, M.R. Eshaghi, M. Shahrestani, A. Mousavi Khaneghah; Study of migration of Bisphenol A diglycidyl ether (BADGE) and its derivatives from canned tuna fish in Iran's market

ABSTRACT

Migration of compounds from packaging materials is one of the most important aspects of food safety. The main purpose of this study is determination and quantification of migrated BADGE and its derivatives in canned tuna fishes by a rapid HPLC (High Performance Liquid Chromatography) method with fluorescence detector. Samples have been collected from Iran's market by PMS method (Post Marketing Service) from different regions of Iran. Migrated compounds were extracted with methanol then analyzed by a HPLC system. This method can detect the amount of BADGE and its derivatives in low concentration. Whereas detection limit for BADGE.2H2O was detected in 0.04 ppm. Also the present of recovery for BADE and its derivatives in comparison to previous investigations is suitable. Results showed that the BADGE.HCL, BADGE.H_2O.HCL and BADGE.H_2O were not detected in any samples. However, BADGE, BADGE.2H2O and BADGE.2HCL were detected in samples but the amounts of specific migration of BADGE and its derivatives from metal cans into the samples conform to EU legislation (EC 1895/2005 that determined specific migration limits (SML) for sum of the BADGE and its hydrolyzed derivatives is 9 mg/kg and Sum of chlorinated derivatives is, 1 mg/kg), which confirm the safety of these cans container that were used for packaging of tuna fish in Iran's market.

Key words: BADGE, Chlorinated derivatives, HPLC, Hydrolyzed derivatives, Specific Migration.

Introduction

Food packaging is one of the most important fields in food industry and also has certain effects on food safety issue. Most of cans which use for preserving food are coated by interior lacquer based on epoxy resins to have a barriers role between the food or beverage and the metal surface of the cans for presenting good condition for products.

Bisphenol A diglycidyl ether (BADGE) is the condensation reaction product of one mole of BPA with two moles of epichlorohydrin. BADGE was used as a starting substance or stabilising components for epoxy resins [14].

BADGE hydrolyses occurs in contact with aqueous and acidic food and it may be convert to forms of mono- and dihydrolysed products (BADGE.H2O and BADGE.2H2O) of BADGE or chlorinated products (BADGE. Hcl, BADGE.2Hcl) or BADGE H_2O Hcl, that can be migrate from can lacquers into food stuff (Fig. 1) [4,12,3]. BADGE may break down and stability of BADGE depended on condition and environment of storage, Also the natural of food has effect of this stability [9].

The epoxy resins are alkylating bifunctional agents and they have specific cytotoxic actions in tissues with high rates of cell division vitro assays using different endpoints. In the case of hydrolysis products and BADGE chlorohydrin no data exists about their genotoxicity [14]. BADGE cause disfunctionality of sex (female) hormones and also is of concern due to its teratogenicity and carcinogenicity [10,8,5,11]. With consider to this issue there is a lack of information concerning BADGE and its derivatives migration from can coatings into canned sea food in oil which has a high level of consumption in Iran market.

In 1996 in Switzerland market, BADGE was detected in oil that extracted canned fish. They had a higher level of the maximum permissible amount of Switzerland legislation and removed of the consumption market [13].

Rauter, *et al*. [10] determined bisphenol A diglycidyl ether (BADGE) and its hydrolysis products in canned oily foods from the Austrian

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market by using a new simplified HPLC method. Samples are extracted with pentane, back extracted with methanol, and finally dissolved in the mobile phase (cyclohexane/*tert*–butyl methyl ether). Separation is performed on a normal phase HPLC column using fluorescence detection. Results showed that from 67 analyzed cans, containing various fatty meat or Fish products, 16% were above the maximum quantity of 1 mg/kg tolerated by the European Community, 45% were in the range between 0.1–1 mg/kg, 24% between 0.02 and 0.1 mg/kg, and in 15% the BADGE concentrations were below the detection limit of 0.02 mg/kg. The hydrolysis product BADGE.H2O was not detected in any sample, whereas BADGE.2H2O was found in some samples up to a concentration of 0.5 mg/kg [10]. BADGE has been examined in canned fish in oil. Cans and lids were extracted by acetonitryl separately. BADGE extracted of fish with Hexan and re-extracted with acetonitryl. The method of analysis was by RP-HPLC with fluorescence detector. Finally BADGE was detected in 12% of fishes, 24% of the cans and 18% of the lids [15]. Migration of BADGE and its derivatives in canned foods have been determined by Hammarling, *et al*. in 2000. In this research the greatest amount of BADGE was found in canned fish in oil (5/1 mg/kg). BADGE was also found up to 1/1 mg/kg in the fish in tomato soaus [4] Leepipatpiboon, *et al*. [6] determined bisphenol-A-diglycidyl ether,bisphenol-F-iglycidyl ether, and their derivatives in oil-in-water and aqueous-based simultaneously in canned foods which was applied by high-performance liquid chromatography with fluorescence detection. The method detection limits range 0.72–4.20 ppb and the method quantitation limits range 2.40–14.85 ppb, respectively [6]. Cabado, *et al*. [1] evaluated kinetics migration of BADGE and BFDGE from varnish into canned products (sardines, tuna fish, mackerel, mussels, cod and mackerel eggs) by HPLC in 70 samples after 6, 12 or 18 months of storage. Results of this resaerch showed that there is no migration of BADGE in tuna fish, sardines, mussels or cod. However, migration of BFDGE occurs in all species, in a storage time-dependent way and content of fat, although migration of these compounds is not affected by sterilization conditions [1].

Specific migration limit (SML) for sum of BADGE, BADGE. H_2O and BADGE.$2H_2O$ is set to 9 mg/kg and for sum of BADGE. HCl, BADGE .2HCl and BADGE H_20 HCl is set to 1mg/kg in the legislation [2].

The main purpose of this study is determination of migrated BADGE and its derivatives in canned fishes by HPLC Method which has been collected from Iran's market that it has been done by PMS (Post Marketing Service) in different location of Iran. Also, comparison of final result with European legislation has been done for confirmation safety of used lacquer in can.

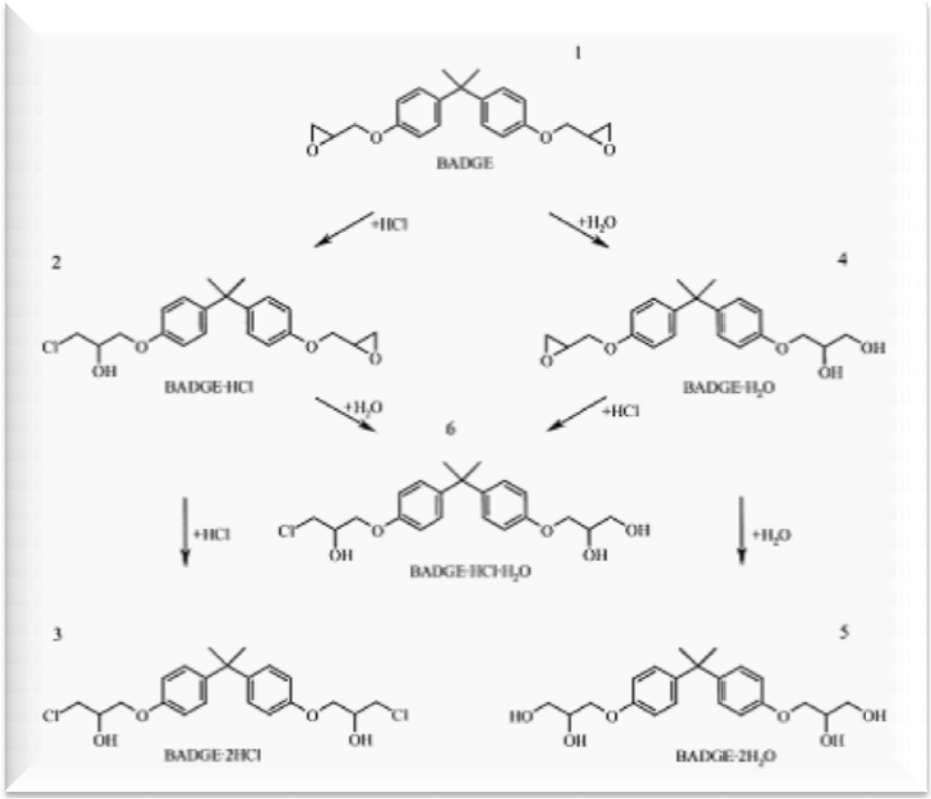


Fig. 1: Bisphenol A diglycidy ether (*BADGE*) and its reaction products [8].

Materials and Methods

Materials: Standards and reagents:

All standards of BADGE and its derivatives (BADGE.H20, BADGE.2H_2O, BADGE.HCL, BADGE.2HCL, and BADGE.H_2OHCL) were purchased from Fluka chemical Co., Swiss. Stock solutions of BADGE and its derivatives were made in methanol at a concentration of 100µg/ml and were stored at 4 °C .Intermediate solutions of BADGE and its derivatives were prepared at a concentration of 10 µg/ml in methanol. HPLC-gradient grade water and methanol (grade) solvents were purchased from Merck Co., Germany.

Samples:

The experiments have performed on two groups of cans, local samples and one of famous brand which contain fish tuna in oil and purchased from markets of five different regions in Iran. These regions consist of West North, North, South, Center, and Capital of Iran. Samples were purchased from Market by PMS method and were analyzed at least 6 months after producing.

Extraction process:

To perform extraction of BADGE and its derivatives, 2.5 g of homogenized sample (net product) were mixed with 5 ml methanol and centrifuged. This operation was repeated two times. The collected methanol fraction was evaporated under nitrogen stream up to approximately 4ml, after adding 1ml distillated water Centrifuged in refrigerate at 3500 rpm in -2 °C for 10 min in order to separation, extracted samples were filtered through a micro filter (whatman, diameter 13 mm, pore size 0.45µm) then analyzed by HPLC with Fluorescence detector for determination of migrants.

Measurements of BADGE and its derivatives by HPLC:

Standards and the content of these monomers in samples were separated and quantified by using a HPLC system (Agilent 1200, Germany) equipped with an Agilent G1311A quaternary pump, an Agilent G1315A Florescence Detector (FLD) and C18 Agilent column (150mm, 5 µm particle diameter, and 4.6mm internal diameter was used). The column temperature was kept at 30°C by using a column oven. The used wavelengths for detection of monomers were 225 nm (excitation wavelength) and 305 nm (emission wavelength). The binary gradient conditions were used: H2O / Acetonitrile (60:40v/v) to H2O / Acetonitrile (40:60 v/v) and Flow Rate: 0.75 to 1.5 ml/min were established. The volume of injection was 5 µl [7].

A mix stock standard solution (10 ppm) were prepared from BADGE and its derivatives in methanol and stored in the dark place at refrigerator temperature. Calibration standards solutions were prepared on the day of use at levels 5, 2, 1, 0.5, 0.25; 0.125and 0.0625µg/ml of stock standards solution and 5 µl of each standard were injected to HPLC**.** Standard curves for all standards were plotted by injection 7 concentration of standard and peak area responses are obtained. A standard graph was prepared by plotting concentration versus area [7].

Validation of method:

Recovery studies were carried out by spiking selected samples of homogenized Tuna Fish with mixed standard solution (mix of BADGE and its derivatives) at four different concentrations (0.25, 0.5, 2 and 4ppm). The spiked samples as well as controls were analyzed in 3 replicate experiments. The recoveries were calculated by using standard calibration curves.

The detection limit (LOD) and quantitation limit (LOQ) are defined as the amount of analyte in standard solutions that yields an instrumental signal significantly different from the blank or background signal which equals to 3 and 9, respectively. Table 1 summarizes LOD and LOQ values of individual compounds and clearly indicates that the analytical method has excellent sensitivity.

Table 1: Results of method validation

Type of Analyte	R^2	*LOD**	*LOQ**	*Recovery***	*CV***
BADGE	0.999	0.083	0.250	74 -114	4.81-17.16
BADGE.H_2O	0.997	0.083	0.250	77 - 98	3.90-10.25
BADGE.2H_2O	0.994	0.040	0.120	83 - 98	5.6 -11.14
BADGE.HCL	0.998	0.160	0.500	89 - 100	0.46- 5.22
BADGE.2HCL	0.996	0.083	0.250	95 - 105	5.86-19.70
BADGE.H_2O.HCL	0.995	0.050	0.150	99 - 116	0.83-7.63

***Per ppm** ****Per percent**

2.6- Statistical Analysis:

Experiments on of selected Cans were performed at three times. Statistical analyses were done with SPSS Ver 16 (SPSS Inc. Chicago, USA).

Results and Discussion

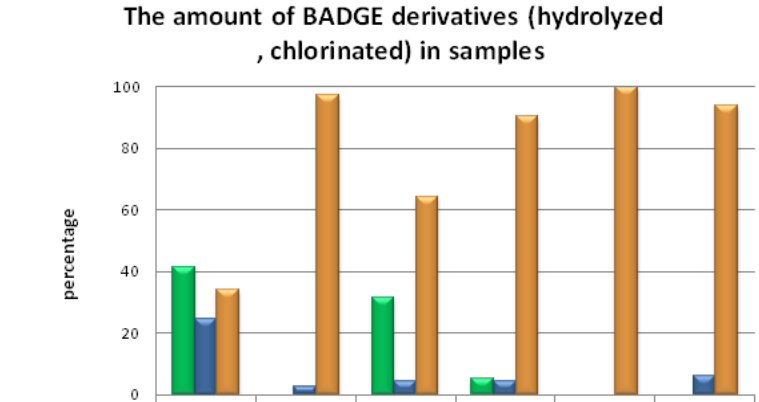
BADGE and its derivatives in samples:

Result shows that BFDGE and its derivatives not detected in any samples. Also BADGE.HCL,

BADGE.H_2O.HCL and BADGE.H_2O were not detected.

From all samples were analyzed 67.5 % of hydrolyzed derivatives and 5.2 % chlorinated derivatives of BADGE were above quantification limit.

Respectively BADGE, 2H_2O with 41.22%, BADGE with 31.57% and BADGE.2HCL with 5.26% were detected in samples (Fig 2).



	B.2H2O	B.H2O	BADGE	B.2HCL	B.HCL	B. H2O HCL
>LOQ	41.22	0	31.57	5.26	0	0
LOD < <LOQ	24.56	2.63	4.38	4.38	0	6.14
ND	34.21	97.36	64.03	90.35	100	93.85

Fig. 2: The amount of BADGE derivatives (hydrolyzed and chlorinated) in samples.

Migration of BADGE in different regions:

Local samples:

Results of ANOVA's hydrolyzed derivatives on local samples show that there are significant differences depending on the region ($p<0.01$) which has confirmed by this order: West north > North >Center > Capital, South. There are no significant differences in west north and north with respect to hydrolyzed derivatives migration as the same capital, center and south. But also there are significant differences in west north and north with capital, center and south .May be kind and curing of lacquers or poor storage conditioning cause these, on the other hands there are no significant differences ($p<0.05$) in local samples with respect to chlorinated derivatives migration in five regions.

Brand samples:

There are no significant differences ($p<0.05$) in brand samples with respect to hydrolyzed derivatives migration in three regions otherwise significant differences ($p<0.05$) according to ANOVA's chlorinated derivatives in brand samples in three regions was obtained which has confirmed by this order: Center> capital and south. Small amounts of BADGE.HCL and BADGE.2HCL may be formed from BADGE when salty foods are processed in coated cans therefore the significant differences occur in migrated chlorinated derivatives in sample of these regions [4].

Comparison Hydrolyzed & Chlorinated Derivatives of BADGE in Brand and Local Samples:

Figure 3 and figure 4 show the amount of hydrolyzed and chlorinated Derivatives of BADGE determined in three and five regions in brand and local samples, respectively. Result shows that chlorinated derivatives of BADGE only were found at center region but migrated compounds of hydrolyzed derivatives were found at three regions in brand samples.

In local samples chlorinated derivatives were found in north, south and capital and hydrolyzed derivatives in north and west north is the most.

The hydrolyzed derivatives that were found in both groups of samples are more than chlorinated derivative. It shows that hydrolyzation of BADGE is more common in these kind of canned food.

Conclusion:

HPLC method with Fluorescence detector was used for determination of migration of BADGE and derivatives from can containers into tuna fish. This study was carried out for investigation of quantify migration of hydrolyzed and chlorinated derivatives of BADGE in canned tuna fish in oil. Extraction with centrifuge under cold condition (-2 $^{\circ}$C) provides a rapid and accurate method for determination of

BADGE and its derivatives by HPLC with fluorescence detection especially decreased run time in comparison with previous investigations that have been performed on canned food [10,4,1].

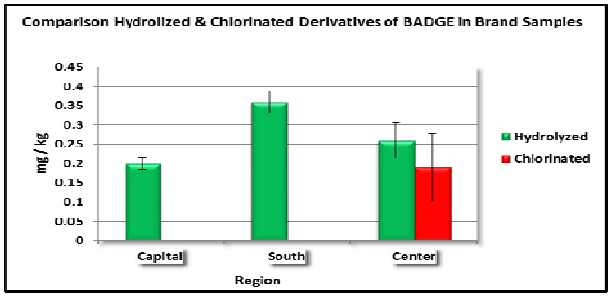


Fig. 3: Comparison Hydrolyzed & Chlorinated Derivatives of BADGE in Brand Samples.

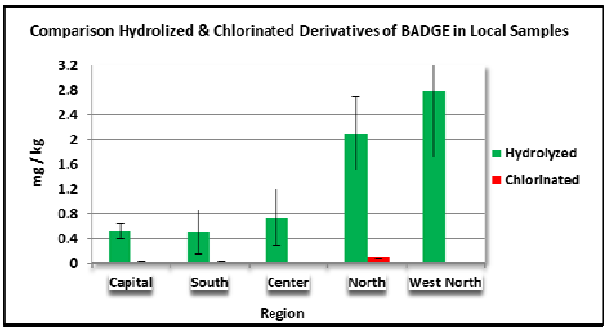


Fig. 4: Comparison Hydrolyzed & Chlorinated Derivatives of BADGE in Local Samples.

More studies should be done on migration of BADGE and its derivatives at different types of canned food. It is necessary to control and improve the distribution chains and the conditions of storage to ensure the safety of products.

This method in this study can detect the amount of BADGE and its derivatives in low concentration. Whereas detection limit for BADGE.2H_2O was detected in 0.04 ppm. Also the limit of recovery for BADE and its derivatives in comparison to previous investigations is suitable [10,4,1].

Results showed that the BADGE.HCL, BADGE.H_2O.HCL and BADGE.H_2O were not detected in any samples. However, BADGE, BADGE.2H2O and BADGE.2HCL were detected in samples but the amounts of specific migration of BADGE and its derivatives from metal cans into the samples conform to EU legislation (EC 1895/2005 that determined specific migration limits (SML) for sum of the BADGE and its hydrolyzed derivatives is 9 mg/kg and Sum of chlorinated derivatives is, 1 mg/kg), which confirm the safety of these cans container that were used for packaging of tuna fish in Iran's market.

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