Specific migration of Bisphenol-A Diglycidyl Ether (BADGE) and its derivatives in four different temperatures in epoxy lacquer

Soheyl Eskandari*[,1], Samira Siami[2], Morteza Shahrestani[1], Abdulrasoul Oromiehie[3], Maryam Mizani[2]

[1] Food and Drug Laboratory Research Center (FDLRC), Food and Drug Control Laboratories (FDCLs), Food and Drug Organization (FDO), Ministry of Health and Medical Education, Tehran, Iran
[2]Science and Research Unit, Azad University, Tehran, Iran
[3]Iran Polymer and Petrochemical Institute, Tehran, Iran

*Corresponding Author: email address: s.eskandari@fdo.gov.ir (S. Eskandari)

ABSTRACT

Migration of compounds from packaging materials is one of the most important aspects of food safety. Epoxy resins have been in inner coatings of food cans since the 1960s. These resins can be produced from Bisphenol-A Diglycidyl Ether (BADGE) that is also utilized as a starter. Migration of potentially toxic compounds in epoxy resins used for commercial cans is a very important food safety issue. Residual BADGE from epoxy coating can be hydrolyzed and chlorohydrolysed into two degradation products, which correspond to its first and second hydrolysis and chlorohydrolyse products. Specific migration of these compounds was evaluated in two water-based food stimulants: %3 acetic acid and %15 ethanol at various temperatures (-6, 5, 25 and 40 ° C) during 10 days. Solid Phase Extraction (SPE) was used to fortify analysts. A flourimetric-detection RP-HPLC was applied to separation and quantification of BADGE, its hydrolysis and chlorohydroxy derivatives. The EU has adjusted the specific migration limit of these compounds in food due to migration from can coatings. Higher levels of migration were found in 15% ethanol than 3% acetic acid. The results illustrated that decreasing of temperature up to –6° C was increased migration. The highest concentration was observed in BADGE.H_2O up to 0.9 mg/Kg. Migration of these compounds takes place in food stimulants; the amounts were lower than exceeding EU limits.

Key words: Migration; Epoxy resins; BADGE, Bisphenol-A diglycidyl ether; RP-HPLC.

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 [1]. 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 stabilizing components for epoxy resins [2]. Epoxy resins are the most used resins in an inner lacquer, because of high chemical resistance and not make second flavor in yield [3]. Bisphenol A diglycidyl ether (BADGE) was obtained commercially by condensation of epichlorohydrin and bisphenol A, that epichlorohydrins were made glycidyl groups. Acidhydrochloroxy presence in organosel lacquer based on PVC makes chlorohydroxy forms of BADGE. Residues BADGE, that do not react during the cure reaction and remain free in the cross-linked network [4, 5]. In acidic and aqueous food simulants is hydrolysed to BADGE.H_2O and BADGE.$2H_2O$. Like BADGE, Derivation of BADGE that are more polar than BADGE with molecular mass M<1000 Da have migration potential into foods [3].

BADGE LD_{50} is 19.6ml/Kg body weight for rats. Observations in man indicate that, BADGE has been shown to cause allergenic contact dermatitis in humans. Workers exposed to epoxy resins in different factories all gave positive reactions in a patch test. And there were no data available to evaluate the carcinogenicity of BADGE in humans [6]. According to 90/128/EEC, BADGE was categorized in toxic potential material. The total migration of BADGE and its derivatives BADGE, BADGE.H_2O and BADGE.2H_2O in contact with food shall not exceed a limit of 9mg/Kg, and for BADGE.HCl, BADGE.2HCl and BADGE.H_2O.HCl must not exceed a limit of 1mg/Kg [7]. However, identification of materials with M<400-500 Da carry out by GC but, BADGE derivatives have M<1000 Da and it is possible to analyses BADGE by LC and also by GC-MS at the upper temperature range [8]. The conjugated nature of the substance gives it good fluorescent properties to use FLD detector [9].

The findings of Sueiro et al. (2001) suggest that BADGE and, to a much lesser extent, the diol epoxide of BADGE may constitute a genotoxic hazard, but not the bis-diol or bis-chlorohydrin of BADGE [10]. In another related study, in acidic, aqueous and oily canned food samples used SPE cartridges to increase recoveries of analytes [11].

It is extremely important to study BADGE and its derivatives migration in epoxy resins that are used in large scales. The purpose of this study was to determine the effect of various storage temperatures on the migration of BADGE and its derivatives used in three-piece cans coat with epoxy resins.

MATERIALS AND METHODS

Materials: Standards and reagents:

All standards of BADGE and its derivatives: Bisphenol A diglycidyl ether (BADGE), bisphenol A-bis(2,3-dihydroxypropyl)-ether (BADGE.2H_2O), bisphenol A-(3-chlor-2-hydroxypropyl)- (2,3-dihydroxypropyl)-ether (BADGE.H_2O.HCl), bisphenol A-glycidyl-(3-chlor-2-hydroxypropyl)-ether (BADGE.HCl), bisphenol A-glycidyl- (2,3-dihydroxypropyl)-ether (BADGE.H_2O), bisphenol A bis-(3-chlor-2-hydroxypropyl)-ether (BADGE.2HCl) (purity %99) were purchased from Fluka Chemicals Co., (Buchs, Switzerland). HPLC-gradient grade water, Methanol and acetic acid from HPLC grade were purchased from Merck Co., (Germany). Ethanol was purchased from Bidestan (Tehran, Iran). SPE cartridges (200mg, 3^{cc}) were purchased from Macherey-Nagel (Düren, Germany) for Solid Phase Extraction.

Experimental

Stock solutions of BADGE and its derivatives were made individually in methanol at a concentration of 50μg/ml and were stored at 4ºC for not more than 3 months. Intermediate solutions of BADGE and its derivatives were prepared at a concentration of 10μg/ml in methanol. Standard curves for all standards were plotted by injection of six duplicate concentration of standard and peak area responses are obtained. A standard graph was prepared by plotting concentration versus area.

The solutions of food simulants (3% acetic acid and 15% ethanol) were prepared and stored in double-lacquer cans coat with epoxy resins. Migration of BADGE and its derivatives were tested during 10 days at four different temperature conditions: 40, 25, 5 and -6 ºC. They were then loaded onto SPE cartridges that were conditioned with 10ml methanol and 10ml water. The analytes were eluted with 5ml methanol into vials [12].

Apparatus and conditions

The analytical determination and quantification of BADGE and its derivatives 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 Agilent Eclipse XEB C18 column (150mm×4.5 mm, 5µm 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: H_2O / Acetonitrile (55:45v/v) to H_2O / Acetonitrile (35:65 v/v) and Flow Rate: 1-1.5 ml/min with run length of 12min were established. The volume of injection was 5 µl [1].

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

Calibration curves were made by plotting the concentration of six duplicate standard solutions. Retention time, standard line regression and correlation coefficient of each standard are shown in Table 1.

Table 1. Retention time, standard line regression and correlation coefficient of BADGE and its derivation

	RT[1] (min)	Standard line regression	(r) [2]	P-Value
BADGE. 2H_2O	1.785	$Y=2217x+5190$	0.999385	<0.000
BADGE. H_2O.HCl	4.089	$Y=1923x+3095$	0.999524	<0.000
BADGE. H_2O	4.361	$Y=1873x+3315$	0.999521	<0.000
BADGE. 2 HCl	9.371	$Y=2617x+4870$	0.999258	<0.000
BADGE. HCl	10.017	$Y=1137x+2085$	0.999851	<0.000
BADGE.	10.723	$Y=3026x+8726$	0.999135	<0.000

1- Retention Time
2- Correlation Coefficient

The effect of various storage temperatures on the migration of BADGE and its derivatives used in three-piece cans coating with epoxy resins in two different simulants were evaluated.
The amount of BADGE and other derivatives individually migrated from can coating into %15 ethanol at four different temperatures during 10 days are shown in Figure 1. In Figure 2, the amount of BADGE and other derivatives individually migrated from can coating into %3 acetic acid at four different temperatures during 10 days are shown.

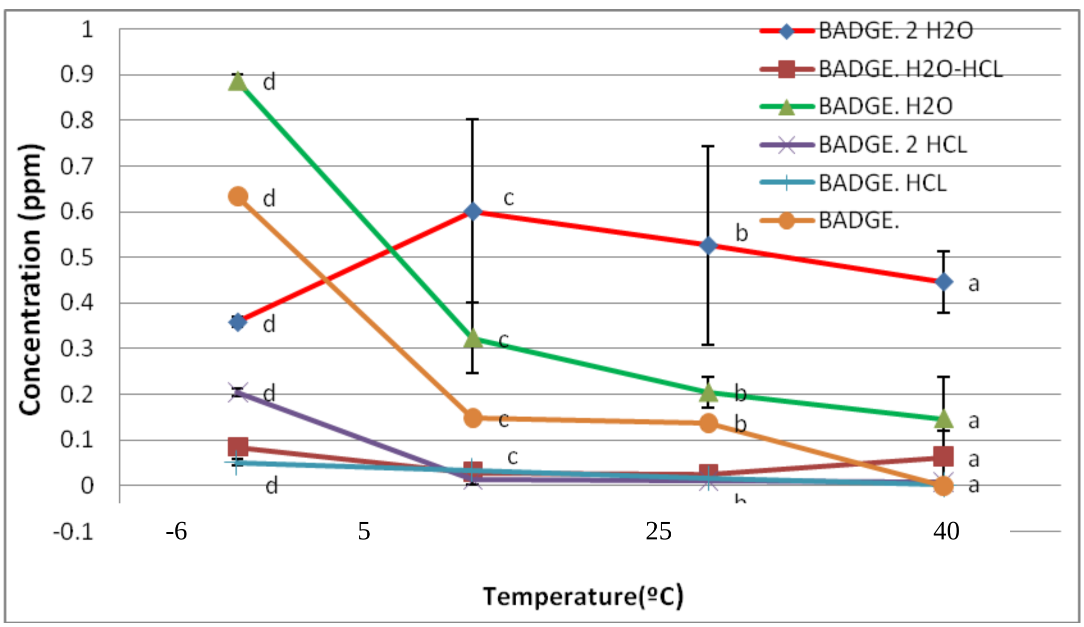

Figure 1. Migration of BADGE and other derivatives from can coat at four different temperatures in 15% ethanol during 10 days

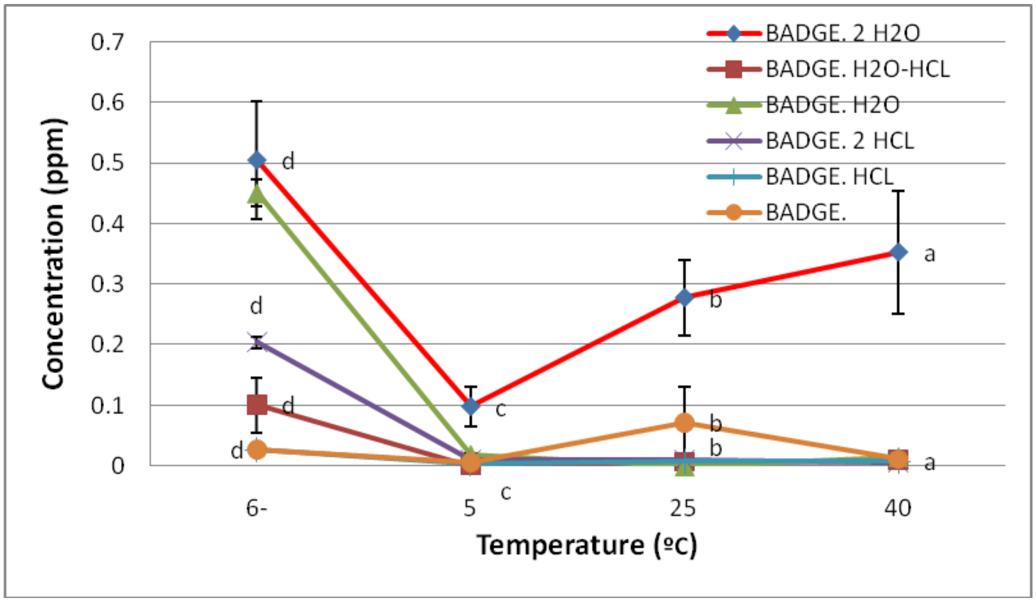

Figure 2. Migration of BADGE and other derivatives from can coat at four different temperatures in 3% acetic acid during 10 days

The limit of migration of BADGE and other derivatives in two food simulants (15% ethanol and 3% acetic acid) were evaluated.

Limit of migration of BADGE.2H_2O in two simulants %15 ethanol and 3% acetic acid is illustrated in Figure 3. In Figure 4, limit of migration of BADGE.H_2O.HCl in the two stimulants has shown. Limit of migration BADGE.H_2O, BADGE.2HCl, BADGE.HCl and BADGE in the two food simulants were shown in Figure 5-8, respectively.

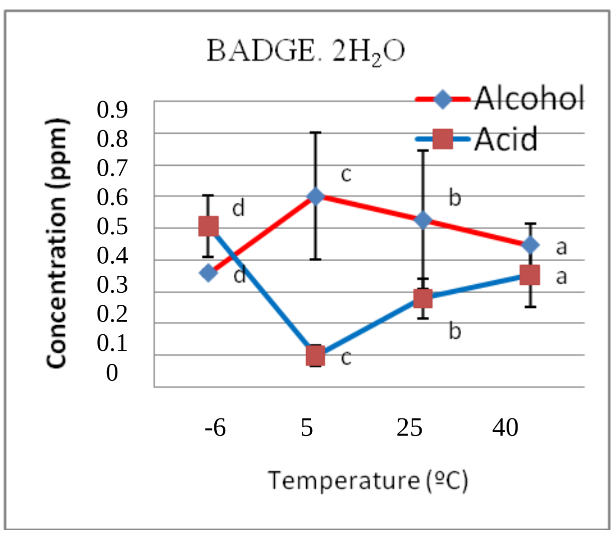

Figure 3. Limit of migration of BADGE.2H_2O in food simulants: %15 ethanol and %3 acetic acid

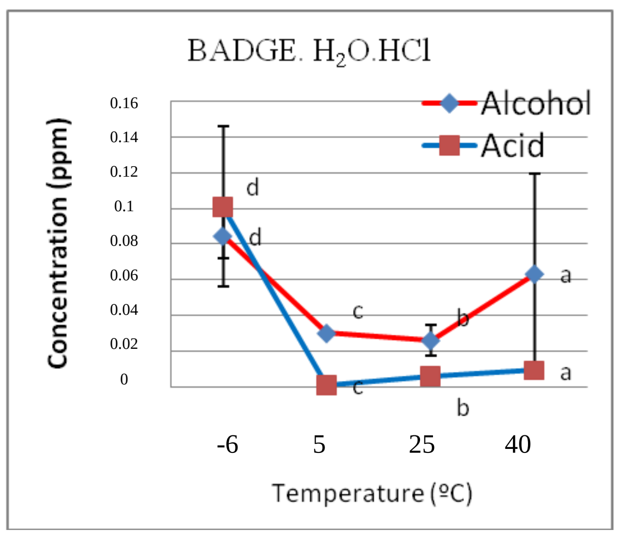

Figure 4. Limit of migration of BADGE.H_2O.HCL, in food simulants: %15 ethanol and %3 acetic acid

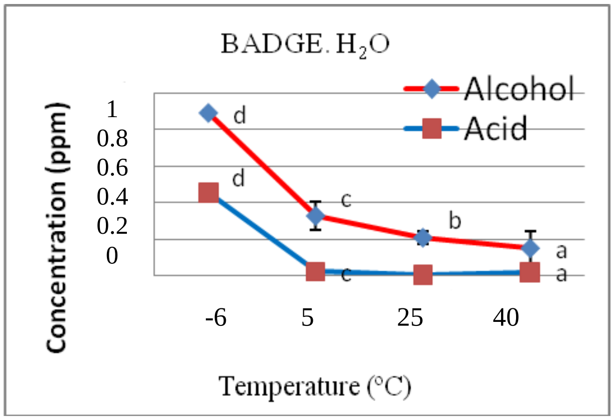

Figure 5. Limit of migration of BADGE. H_2O in food simulants: %15 ethanol and %3 acetic acid

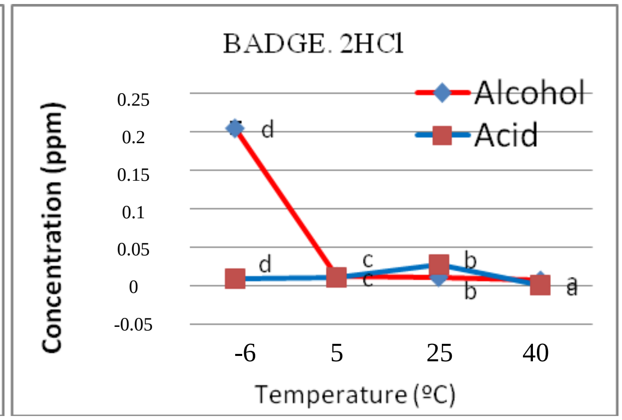

Figure 6. Limit of migration of 2HCl in food simulants: %15 ethanol and %3 acetic acid

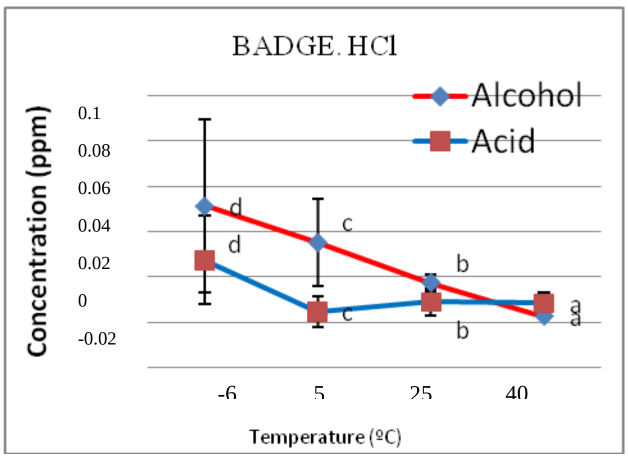

Figure 7. Limit of migration of BADGE.HCl in food simulants: %15 ethanol and %3 acetic acid

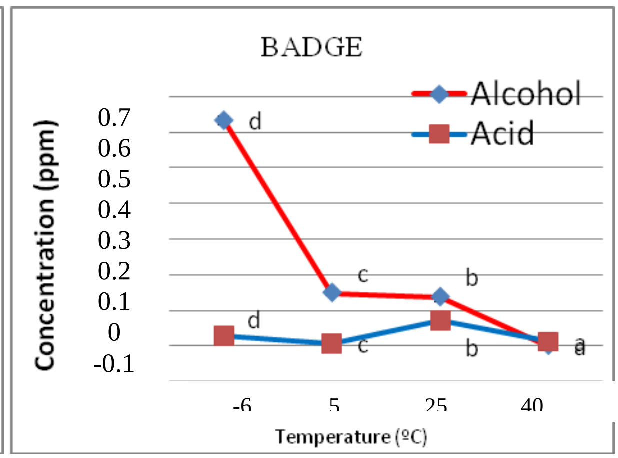

Figure 8. Limit of migration of BADGE in food simulants: %15 ethanol and %3 acetic acid

Results obtained after analysis of food simulants in canned food showed a higher level of migration in %15 ethanol and the highest level of BADGE.H_2O (up to 0.9mg/Kg) were found in %15 ethanol at -6°C. Beside %15 ethanol, BADGE.H_2O was also found in %3 acetic acid in level up to 0.45mg/Kg at -6◦C. BADGE.H_2O was only found in -6°C in %3 acetic acid. It means that migration at 40°C to 5°C was not detected. The same results were found in BADGE.2H_2O and BADGE.H_2O.HCl migration. With decreasing the temperature, a high level of migration was seen. In general, the hydrolysis products were detected in food simulants.

BADGE was found in 15% ethanol at -6 °C up to 0.6mg/Kg where, in 3% acetic acid was not detected in any of the holding temperatures. Detectable levels of BADGE.2HCl and BADGE.HCl were in low concentration in two food simulants in different temperature. But one of the samples, BADGE.2HCl was present in high concentration than the others (up to 0.2mg/Kg) at -6°C. Higher levels of migration were found in 15% ethanol than acetic acid ($p<0.05$). This result is in accordance with other recent investigations. The half-life of BADGE was longer in ethanol than acetic acid. Due to active hydrogen in acetic acid the rings opening happened [13].

The results illustrated that decreasing of temperature up to -6 °C was increased migration; whereas BADGE, BADGE.2HCl and BADGE.HCl were not detected at 40 °C. Amount of BADGE.H_2O and BADGE.2H_2O in low temperature was more than other derivatives ($p<0.05$). The highest concentration was observed in BADGE.H_2O up to 0.9mg/Kg. This may be explained by ascription to fragility of thermoset epoxy resins in low temperature the cross-linked

networks are broken down, so residual BADGE was gone out from matrix. BADGE.HCl was negligible compared with the level of BADGE.H_2O.HCl in food simulants. That is to say, BADGE.HCl in more polarity is hydrolysed in epoxy groups [14].
However, the BADGE amount was low in most of food simulants, presence of other derivatives means that BADGE was there and converted to other derivatives. BADGE hydrolysis exists in aqueous produce toxic components but, the limit is lower than BADGE itself. BADGE.2H_2O toxic according to not having epoxy free groups is less than BADGE.H_2O. Toxicity of BADGE.H_2O.HCL is unknown. The level of BADGE and its derivatives in this study was less than limit of restriction. The FDA has found no evidence or data to indicate regulatory limits or restrictions on bisphenol A are needed. Up to the present there is no replacement for epoxy resins, and its rules only have changed and restricted. There is belief that researches in this field need to continue [15].

CONCLUSION

In this study HPLC method with Fluorescence detector was used for determination of migration of BADGE and derivatives from can containers into food simulants. In conclusion, the level of BADGE and its derivatives in this study were lower than exceeding EU limits.

ACKNOWLEDGEMENTS

The authors would like to thank Food and Drug Control Laboratories (FDCLs) and Food and Drug Laboratory Research Center (FDLRC), Ministry of Health and Medical Education, Tehran – Iran for technical support of conducting this research.

REFERENCES

1.Sajadian SS, Shoeibi Sh, Eshaghi MR, Shahrestani M, Mousavi Khaneghah A. Study of migration of Bisphenol A diglycidyl ether (BADGE) and its derivatives from canned tuna fish in Iran's market. Adv Environ Biol. 2012; 6(2): 768-773.
2.Susanna S, Rosa Ana S, Joaqu´ın G. Genotoxicity of the coating lacquer on food cans, bisphenol A diglycidyl ether (BADGE), its hydrolysis products and a chlorohydrin of BADGE. Mutat Res, 2000; 470: 221-228.
3.Schaefer A. Identification and quantification of migrants from can coatings: An approach to elucidate the total migrate below 1000 Da. Buchbinderei Sanders OHG. 2004; 13-44
4.Simal-Gandara J, Paz-Abuin S, Ahrne L. A critical review of the quality and safety of BADGE-Based epoxy coatings for cans: Implication for legislation on epoxy coating for food contact. Critical reviews in food science and nutrition. 1998; 38 (8): 675-688.
5.Walker FH, Dikenson JB, Hegedus CR, Pepe FR, Keller R. A new polymeric polyol for thermoset coatings: superacid-catalyzed copolymerization of water and epoxy resins. Air products and chemicals Inc. J coating Technol. 2002; 74: 928
6.Sheftel VO. Indirect food additives and polymers: Migration and Toxicology. CRC press LLC. 2000; 41-46, 1070-1072
7.European Commission. Commission Directive 90/128/EEC relating to plastics materials and articles intended to come into contact with foodstuffs. O J L. 1990; 394: 26-47
8.Katan LL. Migration from food contact materials. Chapman & Hall. 1996; 226-239
9.Lintschinger J, Rauter W. Simultaneous determination of bisphenol A-diglycidyl ether and bisphenol F-diglycidyl ether and their hydrolysis and chlorohydroxy derivatives in canned foods. Eur Food Res Technol. 2000; 211:211-217
10.Sueiro RA, Suarez S, M.Araujo, Garrido MJ. Mutagenic potential of bisphenol A diglycidyl ether and its hydrolysis-derived products in the Ames Salmonella assay. Mutagensis. 2001; 16, (4) 303-307.
11.Sun C, Leong L P, Barlow PJ, Chan SH, Bloodworth BC. Single laboratory validation of a method for the determination of bisphenol A, bisphenol A diglycidyl ether and its derivatives in canned foods by reversed-phase liquid chromatography. J Chromatogr A. 2006; 1129:145-148
12.European Commission. Commission Directive 85/572/EEC laying down the list of simulants to

be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. 1985; O J L 372.

13.Losada PP, Simal Lozano J, Paz Abuin S, Lopez Mahia P, Simal Gandara J. Kinetics of the hydrolysis of bisphenol A diglycidyl ether (BADGE) in water-based food stimulants: Implications for legislation on the migration of BADGE-Type epoxy resins into foodstuffs. Fresenius J Anal Chem. 1993; 345: 527-532.

14.Hammarling L, Gustavsson H, Svensson K. Migration of bisphenol A diglycidyl ether and its reaction products in canned foods. Food Addit Contam. 2000; 17(11): 937-943.

15.Poustkova I, Dobias J, Steiner I, Poustka J, Voldrich M. Stability of bisphenol A diglycidyl ether and bisphenol F diglycidyl ether in water-based food stimulants. Eur Food Res Technol. 2004; 219:534-539