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

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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 analyzers. 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.H2O 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 epichlorohyrdrins 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.H2O and BADGE.2H2O. Like BADGE, Derivation of BADGE that are more polar than BADGE with molecular mass M<1000 Da have migration potential into foods [3]. BADGE LD50 is 19.6ml/Kg body weight for rats. Observations in man indicate that, BADGE has been shown to cause allergic 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.H₂O and BADGE.2H₂O in contact with food shall not exceed a limit of 9mg/Kg, and for BADGE.HCl, BADGE.2HCl and BADGE.H₂O.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.2H₂O), bisphenol A-(3-chlor-2-hydroxypropyl)- (2,3-dihydroxypropyl)-ether (BADGE.H₂O.HCl), bisphenol A-glycidyl-(3-chlor-2-hydroxypropyl)-ether (BADGE.HCl), bisphenol A-glycidyl- (2,3-dihydroxypropyl)-ether (BADGE.H₂O), 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") 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 4°C 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°C. 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: H₂O / Acetonitrile (55:45 v/v) to H₂O / 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 |
|---------------------------------|---------------------------------|-----------------|--------|
| BADGE. 2H₂O                     | 1.785                           | \( Y = 2217x + 5190 \) | 0.999385 |
| BADGE. H₂O.HCl                  | 4.089                           | \( Y = 1923x + 3095 \) | 0.999524 |
| BADGE. H₂O                      | 4.361                           | \( Y = 1873x + 3315 \) | 0.999521 |
| BADGE. 2 HCl                    | 9.371                           | \( Y = 2617x + 4874 \) | 0.999258 |
| BADGE. HCl                      | 10.017                          | \( Y = 1137x + 2068 \) | 0.999851 |
| BADGE                           | 10.723                          | \( Y = 3026x + 8726 \) | 0.999135 |

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](image-url)
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.2H2O in two simulants %15 ethanol and %3 acetic acid is illustrated in Figure 3. In Figure 4, limit of migration of BADGE.H2O.HCl in the two simulants has shown. Limit of migration BADGE.H2O, BADGE.2HCl, BADGE.HCl and BADGE in the two food simulants were shown in Figure 5-8, respectively.

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

Figure 3. Limit of migration of BADGE.2H2O in food simulants: %15 ethanol and %3 acetic acid

Figure 4. Limit of migration of BADGE.H2O.HCl 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.H₂O (up to 0.9mg/Kg) were found in %15 ethanol at -6°C. Beside %15 ethanol, BADGE.H₂O was also found in %3 acetic acid in level up to 0.45mg/Kg at -6°C. BADGE.H₂O 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.2H₂O and BADGE.H₂O.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.H₂O and BADGE.2H₂O in low temperature was more than other derivatives (p<0.05). The highest concentration was observed in BADGE.H₂O 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.H2O.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.2H2O toxic according
to not having epoxy free groups is less than
BADGE.H2O. Toxicity of BADGE.H2O.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.

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