

CHAPTER I

INTRODUCTION

1. Problem definition

Canned food is common food packaging method. Canned food has many advantages such as long time storage, retain freshness, easy to transport, etc. In the past, cans were made from steel but since most of food to be canned was acidic which result in can corrosion. Tinsplate steel was used instead but heavy metals can migrate into food causing food poisoning. In addition, high protein foods such as meat can stain the tinsplated surface. Nowadays, polymer is applied to the top layer of the internal can surface. Polymer helps preventing direct contact between food and the metal surface. However, choices of polymer coating must be suitable to types of food and the manufacturing processes. For example, acidic foods require high acid resistance polymer, cans with easy-open lids require high flexibility polymer, etc.

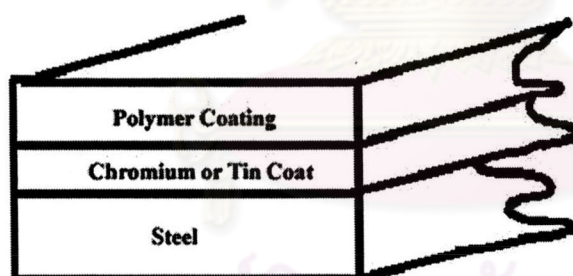


Figure 1-1. Structure of tinsplate.

Types of polymer coatings can be separated into 5 categories. [1, 2]

1. Oleoresins

They are natural polymers produced from oils such as tung oil or linseed oil and natural gums (rosin). These resins are poor acid and chemical resistance, poor color characteristic and have fair flexibility. However, these resins are suitable for wide range of vegetable canning.

2. Acrylic resins

Most common usage of acrylic resins are for pigmented and clear decorative coatings. But acrylic resins can be used for internal coating with good heat resistance. These resins are produced from the polymerization of acids which active double bonds such as acrylic acid ($\text{CH}_2=\text{CH}-\text{COOH}$) or methacrylic acid ($\text{CH}_2=\text{C}(\text{CH}_3)-\text{COOH}$).



Where: R_1 = H, acrylic acid.

= CH_3 , methacrylic acid.

R_2 = substitution group.

Figure 1-2. Synthesis reaction of acrylic polymer.

3. Phenolic resins

Phenolic resins are synthesized by the reaction of phenol with formaldehyde. Under alkali condition and excess formaldehyde, formaldehyde substitutes at the *ortho*- and *para*- positions of the aromatic ring of phenolic. The polymerization of phenolic is formed via methyl ($-\text{CH}_2-$) and ether ($\text{C}-\text{O}-\text{C}$) groups after heating. The production of phenolic resins is shown in Figure 1-3. Phenolic resins found wide usage because of their good chemical resistance, and prevention of sulfur staining. The poor flexibility of resins limit their use to only the three-piece cans and ends.

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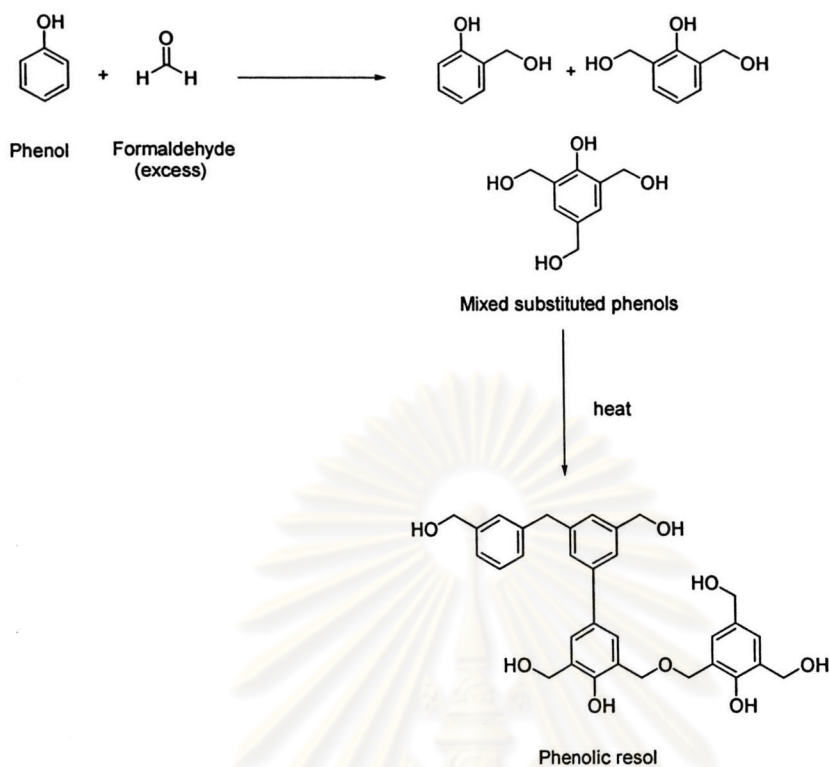


Figure 1-3. The production of phenolic resins.

4. Epoxy resins

Epoxy resins are produced from the condensation reaction of epichlorohydrin and bisphenol A under alkali condition.

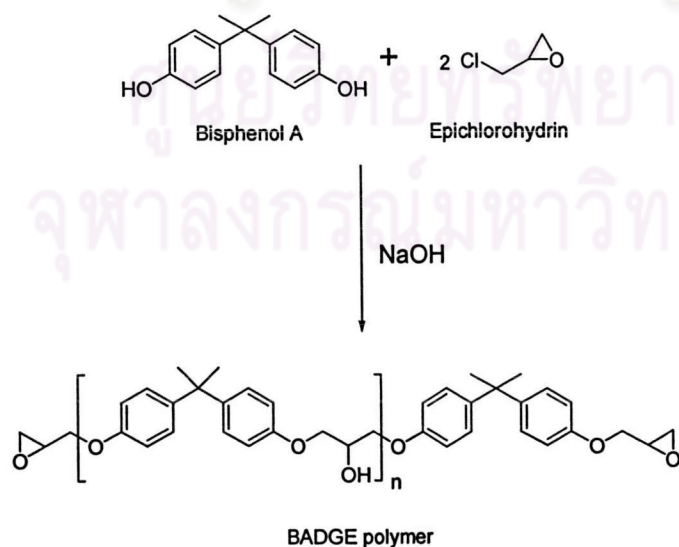


Figure 1-4. The production of epoxy resins.

The resins are normally used in conjunction with other resin materials such as phenolic, polyester, polyamide, amino (urea or melamine) to improve some properties of the resins.

5. Vinyl resins or organosol resins

Organosol resins are solutions of co-polymer resins such as vinyl chloride and vinyl acetate. The production of an organosol resin is shown in figure 1-5.

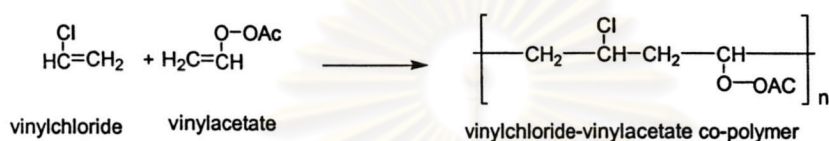


Figure 1-5. The production of vinylchloride-vinylacetate co-polymer.

These resins are highly flexible, good adhesion and tasteless. They are suitable for beers, soft drinks and easy-open cans. In general organosol polymers use other compounds that add into the polymers as additive during curing. Because thermal degradation of organosols normally occurs during the coating process at high temperature where HCl is released and damages the polymer. HCl can be trapped by additives such as BADGE, phenolics, alkyds, etc. Additives such as BADGE, can potentially migrate into food, because they are not bonded to the polymer chain and can be readily released.

In the present day, epoxy and organosol are commonly used for surface coating. Epoxy polymers contain two epoxy groups per molecule. These epoxy groups can react with other compounds (such as phenolic) result in excellent properties polymer. Epoxy polymer technology was described in 1970s in patents [3, 4] on preparations of epoxy polymers based on glycidyl ether included 2, 2-bis[4-(2, 3-epoxypropoxy) phenyl] propane or bisphenol-A-diglycidyl ether (BADGE). The structure of BADGE monomer and BADGE polymer are shown in Figure 1-6. Approximately 75 percent of polymers currently used are derived from BADGE.

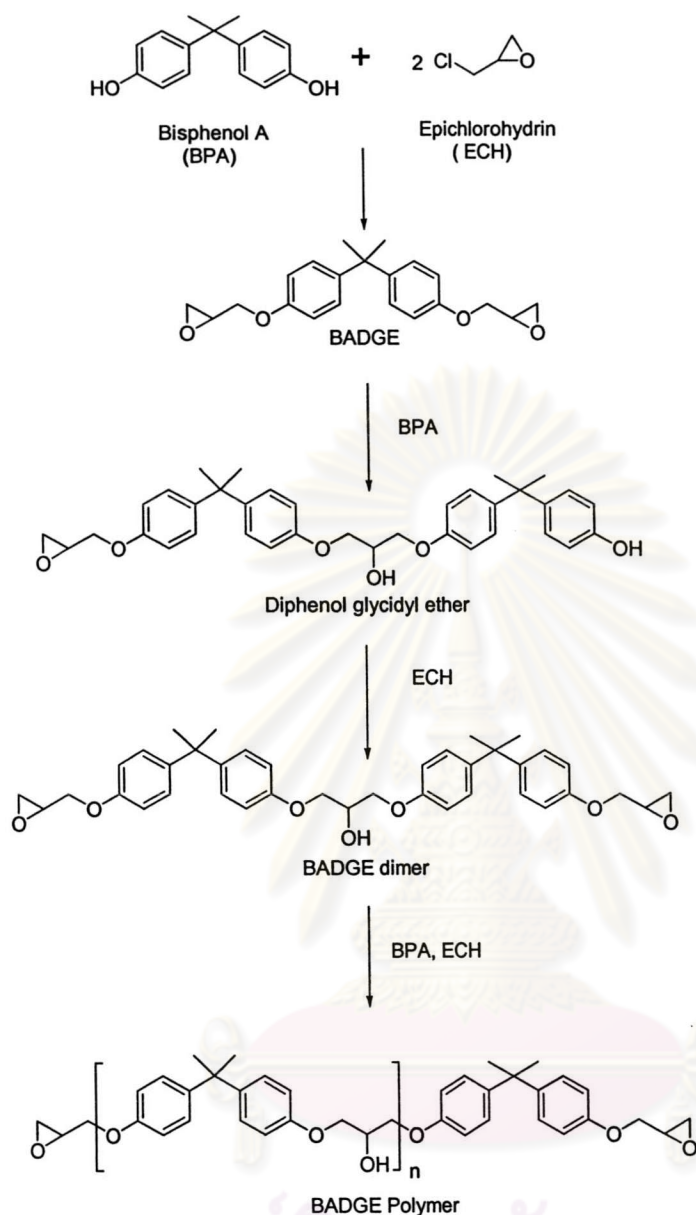


Figure 1-7. The scheme of BADGE polymerization.

Although, can coatings are useful but one important problem is the migration of the monomer and additives into the food content. BADGE was detected in olive oil for the first time in 1996 by M. Biedermann et al. [6, 7]. It was discovered that edible oil can extract BADGE and protects it from further hydrolysis. Therefore, BADGE and its monomeric-chlorohydroxy derivative (BADGE.HCl) were main contaminants in oil-based canned foods. Later, during June 1996 – February 1997, the survey of 242 canned food samples in the Swiss market only detected one sample that was

contaminated more than 1000 $\mu\text{g}/\text{kg}$. Comparing to the result of previous study in 1996 where 9 out of 74 samples were contaminated than 1000 $\mu\text{g}/\text{kg}$, it was obvious that can manufacturers were more careful and that BADGE contamination could be controlled. Up to now, the toxicological data still cannot provide any conclusive answer to the potential danger of these compounds [6, 7]. In 1997, the European Union (EU) set the limit on the sum of BADGE and its derivatives in foodstuff at 1 mg/kg [8]. When BADGE was banned, some can manufacturers shifted to bisphenol-F-diglycidyl ether (BFDGE) as a replacement of BADGE.

Bisphenol-F-diglycidyl ether (BFDGE) is an analogue of BADGE. The structures of BADGE and BFDGE are illustrated in Figure 1-8.

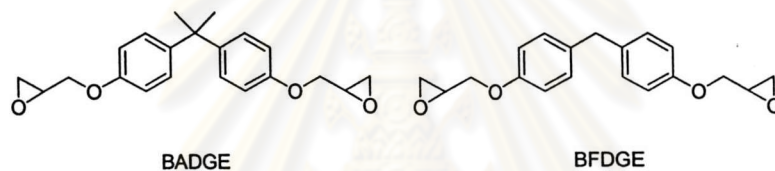


Figure 1-8. The structures of BADGE and BFDGE.

BFDGE is produced from the reaction between bisphenol F and epichlorohydrin. The structures of bisphenol A and bisphenol F are show in Figure 1-9 and bisphenol F reaction scheme in Figure 1-10.



Figure 1-9. The structures of bisphenol A (BPA) and bisphenol F (BPF).

As a result when bisphenol F reacts with epichlorohydrin, 3 isomers of BFDGE are present.

When BFDGE was used as additive in organosols, it can migrate into canned food in the same way as BADGE. The contamination of BFDGE is much more complicated than the situation of BADGE because BFDGE and derivatives comprise of several isomers. In 2001 the European Union (EU) set the limit of BADGE and BFDGE and their derivatives that can migrate into foodstuff at 1 mg/kg or 1 mg/6 dm² (see details in appendix A and B) [10]. However, this regulation is set to be while used for 3 years while toxicological data are assessed.

Contamination Pathways:

There are two possible sources of contamination

1. Starting material.

When Polymerization and curing process are incompleting. BADGE, starting material can migrate into food. In oil-based canned food, the contamination is the largest because edible oil extracts the monomers and protects them from hydrolysis.

2. Additive.

When BADGE or BFDGE are used as additives. They can migrate into food easier than when they are the starting material, because they did not form bond with the polymer chains. However, BADGE or BFDGE additives frequently present as chlorohydroxy derivatives. Chlorohydroxy derivatives are produced from reactions between BADGE or BFDGE and HCl that occurred during thermal degradation of polyvinyl chloride (PVC, organosol). These derivatives can easily migrate into aqueous portion of foods and can be hydrolyzed to hydroxy derivatives. These cause the analysis to be complicated, because the complication depend on types of canned food. The situation gets worst when these derivatives reacted with many types of food matrixes creating many different contamination patterns possible.

In general, canned foods can be separated into 3 categories.

1. Oil-based foods Examples of this type of food are tuna in vegetable oil, fried fish, etc. Because edible oil protects BADGE and BFDGE from hydrolysis, contaminations occur only as BADGE and BFDGE parent forms and their chlorohydroxy derivatives.

2. Aqueous-based foods e.g. tuna in brine, sardines in tomato sauce, etc. In food matrixes rich in water, BADGE and BFDGE are hydrolyzed to corresponded hydroxy derivatives. BADGE and BFDGE parent forms are only present and most contaminants are the hydroxy derivatives.

3. Oil in aqueous-based foods This type of food includes Thai curry, soup, coconut milk, etc. As the name implied, the matrix of oil-in-water foods consists of oil phase and aqueous phase that BADGE and BFDGE can dispersed. BADGE or BFDGE are protected from hydrolysis in the oil phase but they are hydrolyzed to their hydroxy derivatives in aqueous phase. Thus, contaminants could be in all forms.

Migrations of BADGE and BFDGE and their derivatives into food have several forms and can be categorized in to 5 types:

1. BADGE or BFDGE monomers

If BADGE and BFDGE are present in polymer coatings, they can migrate into food. Normally they are protected by the oil phase and do not react with the other food components. The structures BADGE and BFDGE are shown in Figure 1-8.

2. Chlorohydroxy derivatives

In the coating process of organosols at high temperature (270 °C), thermal degradation of polymer occurs releasing HCl. HCl is trapped by BADGE or BFDGE additives forming chlorohydroxy derivatives. The structures of these derivatives are shown in Figure 1-11 and the reaction mechanism is shown in Figure 1-12.

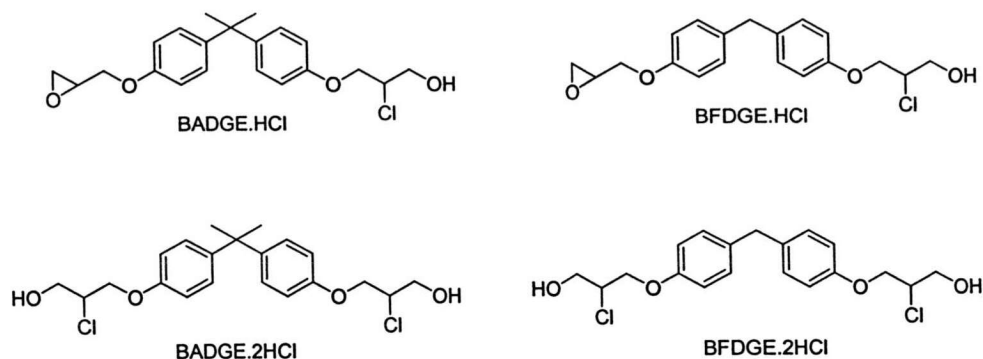


Figure 1-11. Structures of BADGE and BFDGE their chlorohydroxy derivatives.

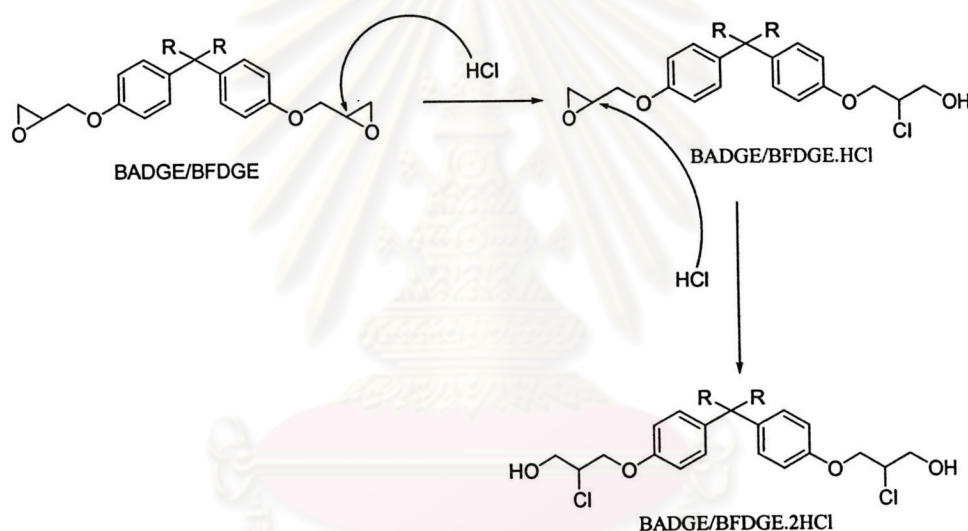


Figure 1-12. Reaction mechanism of chlorohydroxy reaction.

Where R = CH₃ for BADGE compound.

R = H for BFDGE compound.

3. Hydrolysis derivatives

In aqueous and aqueous-based foods, migrated BADGE or BFDGE are readily hydrolyzed at both epoxide groups. In addition, mono-chlorohydroxy derivatives (BADGE.HCl and BFDGE.HCl) extracted into the oil phase can migrate into the aqueous phase and are hydrolyzed as well. The structures show in Figure 1-13 and reaction scheme show in Figure 1-14.

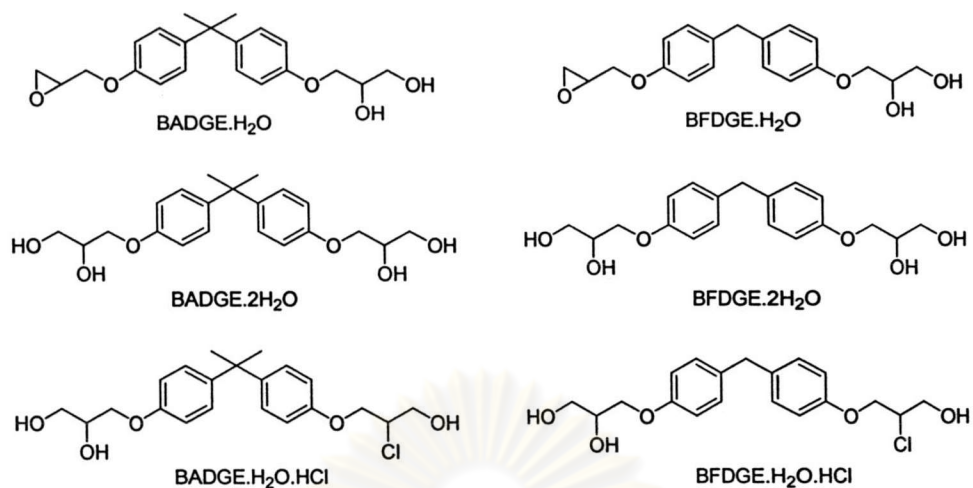


Figure 1-13. Structures of BADGE and BFDGE hydrolysis derivatives.

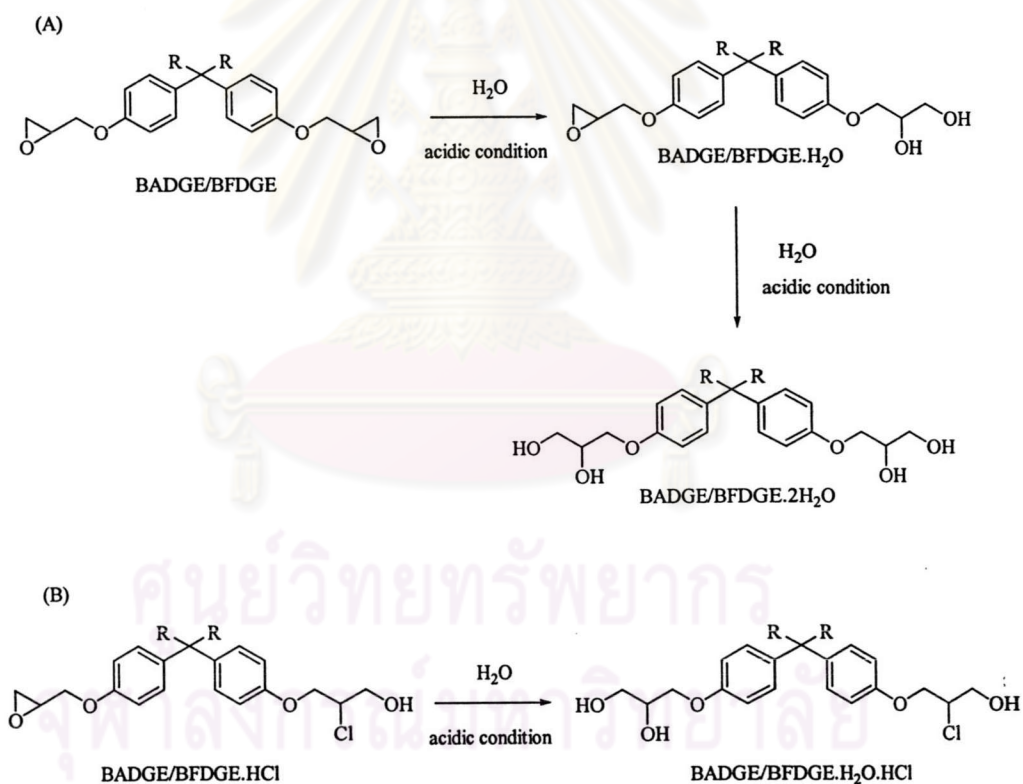


Figure 1-14. Reaction scheme of hydrolysis derivatives.

(A) BADGE or BFDGE reaction scheme.

(B) BADGE.HCl or BFDGE.HCl reaction scheme.

Where R = CH₃ for BADGE compound.

R = H for BFDGE compound.

4. BADGE oligomers

In polymerization process, BADGE monomer condenses with another BADGE monomer results in BADGE dimer. The dimer reacts with other monomer or dimer again results in BADGE oligomers (trimer, tetramer, etc.). Incomplete polymerization can be sources of BADGE oligomers that can migrate into foods and cause contamination in the same way as BADGE monomers.

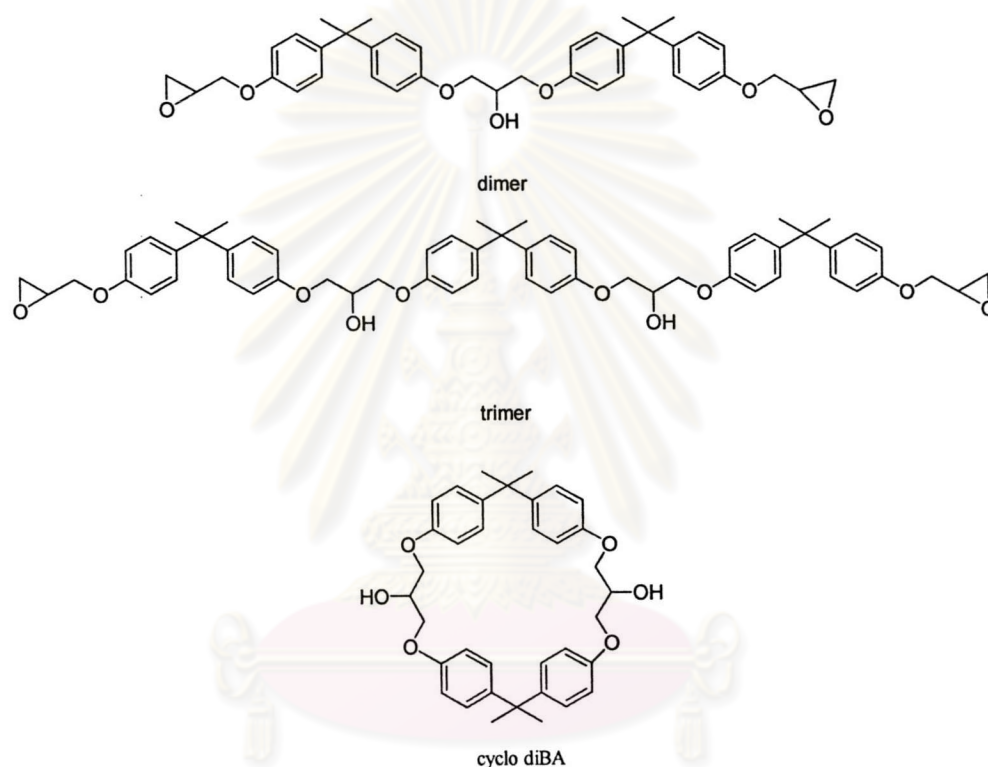


Figure 1-15. Structures of some oligomers of BADGE.

5. Side reaction products

The epoxide groups of BADGE and BFDGE are highly reactive. They can react with solvents with active hydrogen atom such as (phenol, alcohol, etc). The side reactions can be separated into 2 types: normal reaction products and abnormal reaction products. For normal reaction products the reactants attack at position with less steric (higher stability) whereas abnormal reaction products occur when reactants attack at high steric position. Therefore, the yield of abnormal reaction products are very low. The structures of some reaction products are illustrated in Figure 1-16.

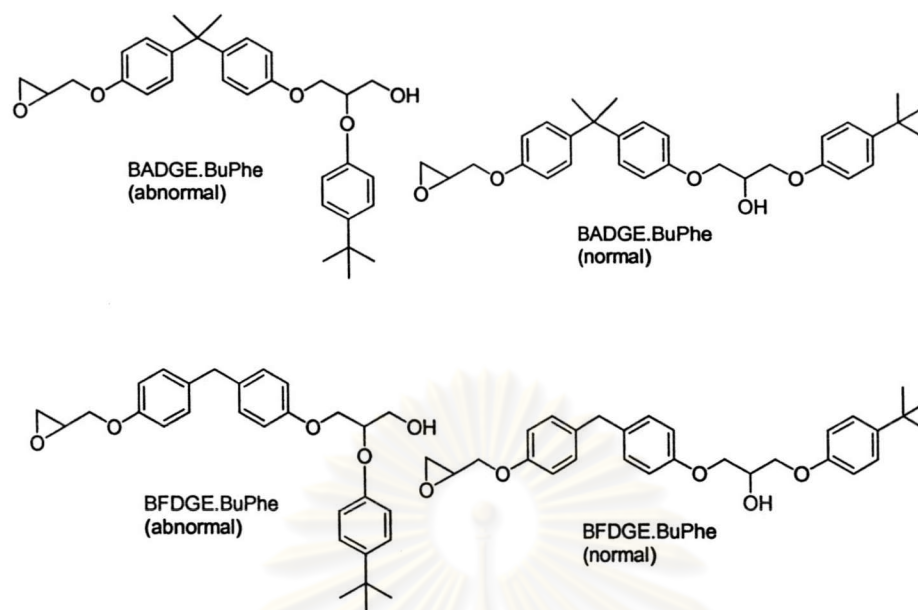


Figure 1-16. Reaction products of BADGE and BFDGE with solvent (phenol).

2. Literature review

Canned foods can contain various types of media such as brine, acid, oil, etc. BADGE or BFDGE can also react with these components. P. P. Losada et al. [11-15] determined BADGE, BFDGE and their hydrolysis products in 3 aqueous simulant media: 3% (w/v) acetic acid, water, and 15% ethanol and varied temperature for kinetic studies. The quantitative of BADGE, BFDGE and their hydrolysis derivatives in kinetic studies were performed on RPLC and MS. In all three media, the rate of hydrolysis was acetic acid > H₂O > ethanol, respectively. Half-life of BADGE and BFDGE were the shortest in acidic media and the longest in ethanol. In addition, the half-life of BADGE and BFDGE decreased when the temperature increased. The team only quantified BADGE, BFDGE and their derivatives in aqueous-based foods as well as characterized their structures.

The canning process parameters such as storage temperature or processing temperature can affect the stability of BADGE. C. Simoneau et al. [16] studied kinetics of BADGE from processed and non-processed can models. The migration was a function of the processing treatment and storage temperature. The processing temperature had the largest effect on the migration of BADGE. High storage temperature significantly influenced migration. They also determined the level of

BFDGE that migrated into oily canned food. From the result of 382 samples, only 3% had contamination level of BFDGE exceeded 1 mg/kg.

L. Hammarling et al. [17] quantitated BADGE and its chlorohydroxy compounds (BADGE.HCl and BADGE.2HCl) by HPLC with fluorescence detector. BADGE level was found up to 5.1 mg/kg in foodstuff and oil phase. The amount of BADGE was the highest in oil-based canned foods. In aqueous and acidic media, BADGE was hydrolyzed to mono- and di-hydrolysis products (BADGE.H₂O and BADGE.2H₂O). In this study, only BADGE.2H₂O was found up to 2.6 mg/kg.

In 1996, M. Biedermann et al. [6, 7] detected BADGE in fat-content canned foods by using normal phase LC-LC with switching valve and fluorescence detector at 225/295 nm. The first column in LC-LC system was cyano bonded phase and the second column was silica. Cyano column separated BADGE from the interferences and transfer BADGE into a switching loop. BADGE was later passed to a silica column to reconfirm with the same fluorescence detector. The analysis method of normal phase chromatography was simple and straight forward for oily samples because sample preparation was unnecessary. The analysis results of 142 samples from the Swiss market found high contamination at more than 10 mg/kg in 10 samples (7%). Fifty cans (35%) are contaminated at 0.2-1.0 ppm and eighty-two cans (58%) are nondetectable. They also found that types of can were related to BADGE contamination level. In cans with easy-open lids (both 2-piece and 3-piece), BADGE contamination was higher than in cans with normal lids. Because the easy open lid required high flexibility, organosol was the polymer of choice and therefore BADGE additive is the potential migrants.

However, the survey result in 1997 (June 1996- February 1997) indicated lower BADGE contamination than the previous study (1996). This is because the manufacturers are more careful and allowed the curing process of coated polymer to completion. However, further results indicated the use of BFDGE and its derivatives in oil-based food from GC-MS confirmation. In the same way as BADGE, the chlorohydroxy derivatives of BFDGE could form by during HCl elimination at high temperature (270 °C).

The epoxide groups of BADGE could react with other components such as alcohol or other solvents. M. Biedermann [19-21] identified BADGE reaction products using GC-MS. These reaction products received attention because most of

the solvents used in coating process containing hydroxide group that could react with BADGE. These new compounds could migrate into food in the same way as BADGE derivatives.

In 1999, M. Biedermann [22-25] determined BADGE, BFDGE and its derivatives in canned food such as vegetables, fruits salad, tomato and other food preparations. In their work, reversed-phase and normal-phase HPLC techniques were used. Reversed-phase HPLC was used to analyze the interest compounds extracted from food because all interested compounds dissolve in polar organic solvent therefore RPLC is suitable to analyze all at the same time. Their method using acetonitrile or methanol as mobile phase did not separate BADGE.H₂O and BADGE.HCl.H₂O apart and normal phase HPLC was used to reconfirm the RPLC result. However, normal phase HPLC required acetylation reaction to improve the separations of all BADGE derivatives. The detector was employed fluorescence detector that is very selective and has higher sensitivity than UV-visible detector. Therefore, it is suitable for detection of BADGE and BFDGE at low concentrations. Because the level of contamination depends on the sterilization time, the data of food preparations showed the highest BADGE contamination level because they required long sterilization. In non-sterilization foods such as tomato the BADGE concentration was low. Fruit in can was badly contaminated by BADGE.HCl (25 µg/kg). Types of lacquers were screened by Bielstein's test that identified organosol coating. IR spectroscopy was used to characterize BADGE functional group in lacquers. If BADGE was an additive in organosol, C-Cl band in IR spectrum was detected. For BADGE starting material, IR spectrum showed OH band and C-O band without C-Cl band.

Recently, J. E. Bile et al. [26] analyzed BADGE and its derivatives in canned food and ready-to-drink beverages in the US Market. Structures of BADGE derivatives were confirmed by GC-MS and LC-MS. IR spectroscopy was used to indicate types of polymer coatings.

J. Lintschinger and W. Rauter [27] synthesized unavailable standards such as BADGE.HCl, BADGE.H₂O.HCl and confirmed their structure by using GC-MS. They reported the BADGE derivatives synthesis methods and developed the method for separating all BADGE derivatives. However, BADGE.H₂O and BADGE.H₂O.HCl which were the critical compounds in gradient RPLC and could be separated by

isocratic RPLC (56: 44, methanol: water). The response factors of unavailable standards were used for quantitative analysis.

In Japan, Y. Uematsu et al. and T. Yoshida et al. [28, 29] determined BADGE.2HCl and BFDGE.2HCl in ready-to-drink canned coffee and canned vegetables from Japanese market. HPLC was the principal analytical method and GC-MS was used for confirmation of the relevant LC fraction. They reported the observation of BFDGE contamination above 1.5 mg/kg. BADGE.2HCl or BFDGE.2HCl were detected in all samples. Beilstein's test was used to confirm the use of organosols.

3. Significant of The Problem

In 2001, EU's set a regulation about BADGE or BFDGE and their derivatives in canned foods at the level of 1 mg/kg or 1 mg/6 dm². If the contamination was found to be above the regulation, the food was rejected for import. Since, canned food is an important export of Thailand. In 2002, Thailand exported fish-in-can worth 86,493.97 million-Baht [30]. The dilemma is that Thailand as the world number one manufacturer and exporter of canned tuna has no control of coating contaminations and thus subjected to international trade barriers and regulations. Therefore, it is crucial that Thai standard methods for the analysis of BADGE, BFDGE and other derivatives in canned food should be developed and validated to international standards.

4. Scope of this research.

This research proposed to develop and validate suitable sample preparation procedure and optimum HPLC method for the analysis of contamination of BADGE, BFDGE, and their respective derivatives in oil-based canned foods which are very important economically to Thailand. Validation scope will strictly comply with the current regulation posed by the European Commission. A preliminary survey of food sampling from the local market using the developed method were conducted to

determined the contamination level in Thailand. The data will be for local consumer protection as well as for the canned food industry. Standard operation procedure (SOP) for sample preparation and HPLC analysis for routine analysis were proposed.

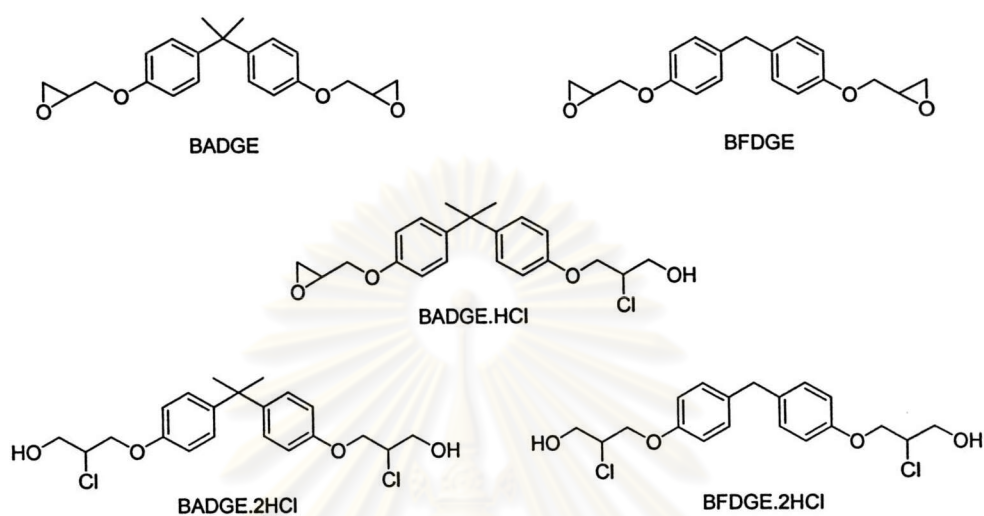


Figure 1-17. The structures of target compounds.

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