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Characterization of Migrates from Can Coatings by Size Exclusion Chromatography; Total Amounts of Phenolics with Molecular Weight below 1000 D

Key words: Canned foods, Epoxy coatings, Can coatings, Size exclusion chromatography, Bisphenol-A-diglycidyl ether (BADGE)

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Introduction

Internal coatings of food or beverage cans as well as of tubes release complex mixtures of materials to the foods. So far, primarily Bisphenol-A-diglycidyl ether (BADGE) (1–9) and Bisphenol-F-diglycidyl ether (BFDGE) (10, 11) have been analyzed. It is, however, known for a long time that the liquid chromatograms contain many more components observed at the conditions BADGE is detected. Their fairly specific fluorescence suggests that they are closely related to BADGE or BFDGE. Some components have been identified as oligomers of BADGE (12) or as Novolak Glycidyl Ethers (NOGE) with more than two aromatic rings (11). Normal phase HPLC (NPLC) with gradient elution provided a fairly broad overview over the components (13), among which cyclo-di-(Bisphenol-A-mono-glycidyl ether) (cyclo-diBA) was usually the most prominent one.

Total amount of migrants

The work described in this paper was primarily aiming at an estimation of the total amount of components with structures similar to BADGE and BFDGE that could migrate into foods. This data should give a frame to the problem, providing us an idea on the total amount of material to be characterized before decisions can be taken on whether or not the coatings can be considered safe for the consumer.

Using the standardized food simulants, the global migration from coatings usually amounts to 1–5 mg/dm² (the legal limit being set at 10 mg/dm²). Related to the can content, 1 mg/dm² corresponds to a concentration of about 50 mg/kg for

an extremely small (30 g) and of around 6 mg/kg for a large (1 l) can. Hence, concentrations of 250 mg/kg may be reached.

Global migration data must be considered with precautions. On the one hand, coatings commonly contain lubricants (often a mineral oil product (14)) at concentrations of 0.5–2% (15), which may well generate migration of 1–3 mg/dm². Lubricants (as well as catalysts, accelerators, neutralizing agents and other components of non-phenolic structure) were, however, not of interest here. On the other hand, many of the coating components found in edible oils of canned foods (including most of the migrants of interest here) are not extracted by the solvents used as simulants, keeping the data on global migration too low.

Why SEC-FD?

For three reasons, the above goal of determining the total amount of migrants was approached through a Size Exclusion Chromatography (SEC)-fluorescence detection (FD) method:

1. It enabled selective determination of the phenolic components through their fluorescence (leaving, e.g., lubricants out of consideration).
2. The extractable material can be summed up in a few well integratable peaks.
3. The material can be separated into components with a molecular weight below and above 1000 D. Substances of more than 1000 D are generally considered non toxic because of lacking ability to pass through membranes.

The summed amount of fluorescing components with less than 1000 D was the material of interest that should be characterized in the near future. In epoxy coatings, BADGE often not even corresponded to 1% of it, confirming that toxicological assessments of can coatings merely through BADGE may not be meaningful.

Classification by molecular weight through SEC proved, furthermore, to be an important analytical tool, supporting the identification of substances. It may also be useful for cleaning extracts to be analyzed by GC-MS. LC chromatograms could be reduced to the components with molecular weights below 1000 D, i.e. those considered most relevant.

Experimental

Two 250 x 7 mm i.d. SEC columns from Chrompack (Middelburg, The Netherlands) were coupled in series: one packed with Microgel-5 100 Å, the other with Microgel-5 50 Å. A Phoenix SFC syringe pump (CE Instruments, Milan, Italy) delivered the mobile phase, usually tetrahydrofuran (THF, Fluka, for UV spectroscopy) at 400 µl/min. The fluorescence detector (FD) from Merck/Hitachi (F1000) was used at 275/325 nm. Data was collected by a ChromCard system (CE Instruments). Injection volumes were 10 or 100 µl.

HPLC (LC) was performed similar to (13), using a 25 cm x 2 mm i.d. column packed with Gromsil 100 CN 2 PR 5 μm . A normal phase gradient was used with 2% 1-propanol/pentane in pump A and 50% 1-propanol/methyl tert.-butyl ether (MTBE) in pump B, programming as follows: pump A, 7 min, then 1%/min pump B up to 10%, 3%/min pump B up to 22% and 5% pump B up to 60% (3 min). The flow rate was 400 $\mu\text{l}/\text{min}$, the injection volume 80 μl ; fluorescence was detected at 225/295 nm.

Cans were extracted with acetonitrile over 24 h at RT, if possible by opening them only partially and positioning them on the side, such that the solvent was in contact with half of the bottom, side and lid. 10 μl of acetonitrile extracts were directly injected into SEC. For NPLC, usually a partitioning procedure was applied: to 1 ml of extract, 1 ml of 15% dichloromethane/heptane was added, then 20 ml of water. The 15% of dichloromethane were tuned such that materials with molecular weights clearly exceeding 1000 D were strongly discriminated (protection of the LC column); they were near the maximum that avoided broadening of initial bands in LC. In fact, substantial amounts of material precipitated from most coating extracts. Such secondary extracts are, of course, no longer suitable for the determination of total migrates into acetonitrile.

Results

Extraction with acetonitrile

Results shown below are based on acetonitrile extracts from can coatings obtained at 25 °C over 24 h. The question arises as to what extent this simulates the extraction efficiency of edible oils or fats in canned foods over sterilization and storage; extractions are incomplete, in the instance of both oil as well as acetonitrile.

Because of the strong variations between cans of the same lot, experiments were performed with single cans. Used cans were extracted with acetonitrile and the amounts thus obtained compared with those found in the food that has previously been inside the can. The acetonitrile extract shows how much more is extracted than by the oil. Instead of calculating amounts, concentrations were determined in the whole can content as well as in the acetonitrile.

Table 1 shows data for BADGE and cyclo-diBA. In the instances of fish in oil or fatty meat (samples 1–3, 9, 10), BADGE concentrations in the acetonitrile extract were clearly lower than in the oily foods, suggesting that the oil had already extracted most of what can be reached by acetonitrile. Acetonitrile extraction of a new can that has previously been extracted with acetonitrile yielded between 9 and 25% of the BADGE obtained as a sum of the two steps (4 experiments), hence a similar amount as an acetonitrile extraction after the oil. It was concluded that oil extracts BADGE to a similar extent as acetonitrile.

The meat sample nr. 11 extracted a smaller proportion of the BADGE from the can than the samples listed above (73% were in the acetonitrile extract). The product

Table 1. Concentrations ($\mu\text{g/kg}$) of BADGE and cyclo-di-(Bisphenol-A-monoglycidyl ether) (cyclo-diBA) in foods as well as in acetonitrile (AN) extracts from the same cans after emptying and cleaning them; percent related to the sum of the two

Sample	BADGE			cyclo-diBA		
	Food	AN	%	Food	AN	%
1 Sardines in oil	200	75	27			
2 Anchovies in oil	2000	1250	38			
3 Sardines in oil	36	8	18	800	360	31
4 Sardines in oil				2200	500	18
5 Mackerels in oil				1900	200	9
6 Tuna in oil				2000	2900	59
7 Tuna in oil				1400	590	30
8 Tuna in oil				1400	1100	44
9 Meat + fat	150	20	12			
10 Meat + fat	1100	50	5			
11 Meat	13	35	73	280	1350	83
12 Herring in sauce	34	8	19	600	1000	63
13 Sardines in sauce				200	275	58
14 Tuna in tomato sauce				132	2905	96
15 Herring in tomato sauce				414	1589	79

contained a relatively small amount of fat and the latter made poorer contacts to the can wall than, e.g., oil. Other variations, i.e. that between 5–38% of BADGE in oil-containing cans were reached by acetonitrile, are probably related to the coating materials, being differently permeable for fat or oil.

The proportions of cyclo-diBA recovered in the acetonitrile extracts were mostly somewhat higher than those of BADGE (9–59% in samples 3–8), indicating that extraction by oil tends to be weaker. This is related to the poor solubility of cyclo-diBA; in fact, it hardly dissolves in BADGE (it is responsible for the turbidity of BADGE of technical grade). Nevertheless, acetonitrile still seems to be a reasonable simulant also for cyclo-diBA.

Sauces (samples 12–15) showed to be substantially weaker extractants than oil or fat because they were essentially water-based.

The larger molecular weight materials have not been identified so far and probably consist of rather diverse compounds. Components eluted from LC up to the retention time of the tetramer of BADGE were contained in the acetonitrile extract to similar extents as cyclo-diBA, whereas later eluted substances were increasingly extractable by acetonitrile only. Retention times suggest, however, that these materials have molecular weights clearly beyond 1000 D.

For the determination of migrants with molecular weights below 1000 D it is concluded that extraction of can coatings by acetonitrile reasonably simulates that of oily and fatty foods. Acetonitrile extraction should be used together with SEC, specifically determining the small molecular weight material. It is not suitable for

the determination of global migration because high molecular weight components are extracted substantially better than by foods.

SEC Separation

Separations by the two SEC columns of 100 and 50 Å pore size were almost identical. Coupling of the two improved the results compared to the chromatography on a single one of them. No broader comparison with other packings was made.

Using ethyl acetate as a mobile phase, separation was slightly better than with THF. However, large molecular weight material was not sufficiently soluble and the corresponding signals were too small. Acetonitrile was not suitable either, because interactions of the type of reversed phase LC resulted in increased retention times particularly of the higher molecular weight materials.

10 µl of acetonitrile extracts could be injected without noticeably disturbing chromatography. For larger volumes, increase of the retention times primarily of the early eluted material was observed, necessitating an exchange of the solvent.

Analytical SEC was possible for extracts from unused coatings only, because oil and other food components produced interfering peaks. For preparative purposes, however, injection of up to 10 mg of oil did not show an effect on the retention time of BADGE and its oligomers.

Response by fluorescence

Quantitation occurred by fluorescence detection (275/325 nm). Since standards are available of a few components only, no calibration of response factors could be performed for most of the material of interest. It is, however, known from previous work that the responses of Bisphenol A, BFDGE and BADGE, as well as those of the hydrolysis products and the derivatives with hydrochloric acid are almost identical.

The following results confirmed the similarity of the responses. Gravimetrically, acetonitrile extracts from two coatings without lubricant (an epoxy/anhydride and an epoxy/phenolic coating) gave 6.4 and 7.2 mg/dm² of migrants. Quantitating the signals obtained by SEC-FD through calibration with BADGE, the same extracts contained 5.7 and 6.3 mg/dm² of material, respectively. Thus the average response was almost equal to that of BADGE and suggests that this is true also for the individual components. The slightly lower result of SEC-FD might even be explained by the non-fluorescent other material in the coating.

SEC of standards

Figure 1 shows the SEC separations obtained for an advanced BADGE and a NOGE standard. The top SEC-FD chromatogram was obtained from the epoxy resin Araldit GT 7071 from Ciba (Basle, Switzerland). The reverse phase liquid chromatogram (RPLC) and RPLC-MS of this material has been shown in (12). The

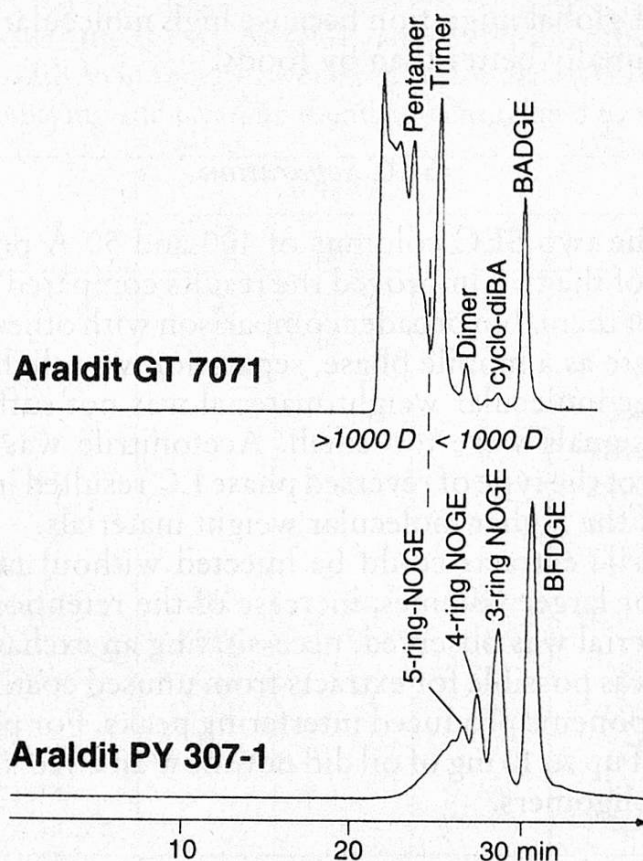


Fig. 1. Standards of an advanced BADGE (with BADGE and its oligomers, top) and of an epoxy-Novolak (NOGE) with components of differing ring numbers (bottom). The cut for distinguishing material of a molecular weight below or above 1000 D is indicated

resin contained about 18% of BADGE. Further, it shows the alternating peak sizes of the glycidoxy-glycidoxy oligomers reaching from the dimer to the pentamer (the tetramer is embedded in the downslope from the pentamer). Since the trimer has a molecular weight of 908 D, the cut distinguishing below and above 1000 D was placed at the onset of the peak of the trimer (shown).

The bottom chromatogram is from the NOGE resin Araldit PY-307-1 from Ciba, the components of which have also been identified by RPLC-MS (11). The predominant peak represents BFDGE and is clearly broadened owing to partial separation of the three isomers. The peaks eluted before correspond to the 3- to 5-ring NOGE. The 4-ring NOGE is eluted slightly later than the dimer of BADGE (molecular weights of 624 and 636, respectively), which is due to the more compact molecular shape.

SEC of extracts from coatings

Figure 2 shows a selection of SEC-FD chromatograms of can extracts with acetonitrile. The extract of can 1, shown at the bottom, primarily shows NOGEs, with clear signals for the BFDGEs and the 3-5-ring NOGEs. The earlier eluted

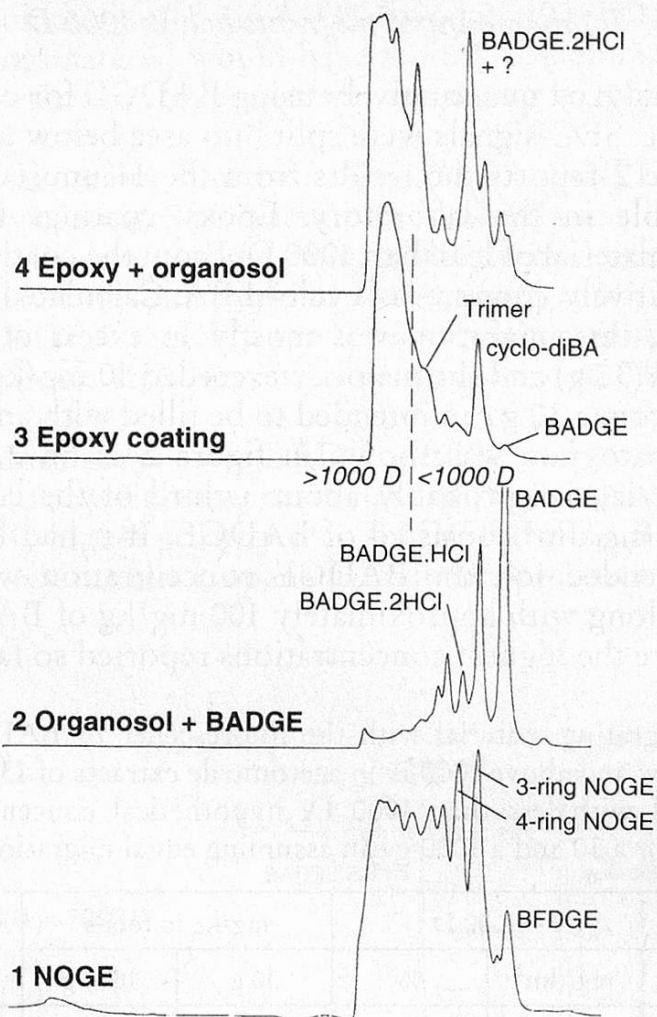


Fig. 2. SEC-FD chromatograms of acetonitrile extracts from empty, unused cans

material is both from higher molecular weight NOGEs and from an epoxy resin of the Bisphenol-A type (determined through characteristic peaks by NPLC).

Chromatogram 2 is typical for the extract from an organosol coating with BADGE added as a stabilizer: about a third of the material detected by fluorescence is BADGE. The large peak eluted just before primarily represents BADGE having reacted with one mole of hydrochloric acid (BADGE.HCl). There are hardly any high molecular weight components, i.e. more than 90% of the material is below 1000 D. Nearly half of the material consists of unidentified components.

Epoxy coatings commonly produce chromatograms like 3. Merely 1.1% of the small molecular weight migrate consisted of BADGE. The cyclo-diBA predominates the small molecular weight material, which has a complex composition. As shown by the broken line, about a third of the extract has a molecular weight below 1000 D.

The top chromatogram is typical for many cans: a mixture of phenolic materials from more than one source. The basic signal resembles that of the epoxy coating of chromatogram 3, but there is also a large BADGE.2HCl, typical of an overheated BADGE-stabilized organosol.

Total amount of migrants below 1000 D

Extracts were analyzed quantitatively using BADGE for calibration and assuming equal response. SEC-signals were split into area below and above 1000 D as shown above. Table 2 reports the results from the 14 unused cans and the tube (sample 15) available in the laboratory. Epoxy coatings from cans released 0.5–5.1 mg/dm² of material of less than 1000 D. From the coatings 1–3, 0.4, 0.3 and 0.25 mg/dm², respectively, consisted of cyclo-diBA. Calculated into concentrations in the can content, this migration was mostly in excess of 100 mg/kg for an extremely small can (30 g) and the majority exceeded 10 mg/kg for a 1 kg can.

Extract 13 was from a 30 g can intended to be filled with anchovies in olive oil. Its SEC-FD chromatogram was shown in figure 2 as nr. 2. It released almost 40 mg/dm² of material, i.e. probably about a third of the coating material was dissolved. Over 12 mg/dm² consisted of BADGE. If it had been filled with the product it was intended for, the BADGE concentration would have reached 600 mg/kg, going along with approximately 100 mg/kg of BADGE.HCl. To our knowledge, these are the highest concentrations reported so far.

Table 2. Total of migrating material with the fluorescence of BADGE and a molecular weight below and above 1000 D in acetonitrile extracts of 13 cans and a tube; % of the material with less than 1000 D; hypothetical concentrations in the foods calculated for a 30 and a 1000 g can assuming equal migration

Sample	> 1000 D	< 1000 D		mg/kg in foods		Remarks
	mg/dm ²	mg/dm ²	%	30 g	1000 g	
1	3.4	1.3	28	65	8	Epoxy/anhydride
2	4.5	2.4	35	120	14	Epoxy/phenol
3	1.4	0.7	33	35	4	Epoxy
4	4.8	2.6	35	130	16	Epoxy
5	7.1	4.9	41	245	29	Epoxy
6	8.8	5.1	37	255	31	Epoxy water-soluble
7	3.1	0.5	14	25	3	Beverage can
8	5.5	2.8	33	140	17	Epoxy
9	2.5	0.9	26	45	5	Epoxy water-soluble
10	3.1	2	39	100	12	Epoxy
11	5.4	3.5	39	175	21	Epoxy
12	5.2	9.4	64	470	56	Organosol/NOGE
13	1.9	38	95	1900	228	Organosol/BADGE
14	1.1	1.3	56	65	8	Epoxy + organosol
15	0.2	0.9	82	45	5	Tube, epoxy

SEC for sample preseparation

Preparative SEC was used for two purposes. Firstly it enabled to remove the high molecular weight material that disturbs LC and GC-MS through interfering

peaks and column contamination. Since GC involved 100–150 µl on-column injection, non-evaporating material would have rapidly contaminated the precolumn. Secondly SEC characterized components by molecular weight and facilitated peak identification.

Figure 3 shows chromatograms from SEC (left) and NPLC of SEC fractions (right) from an epoxy coating. SEC fraction 1 contained a small amount of BADGE (top chromatogram). Fraction 2 was dominated by the cyclo-diBA and the reaction products of BADGE with one mole of tert.-butylphenol (BADGE.BuPhe) or butoxyethanol (BADGE.BuEtOH), the latter two being epoxides on the non-reacted side of the molecule (16). Migration corresponded to 0.08 and 0.06 mg/dm², respectively.

Fraction 3 contained some more BADGE.BuPhe and many unidentified compounds. Since molecular weights were similar, the two non-identified peaks eluted late must be of clearly higher polarity than BADGE.BuPhe. Basically this fraction included the glycidoxy-terminal, open chain dimer (present in a small quantity at most).

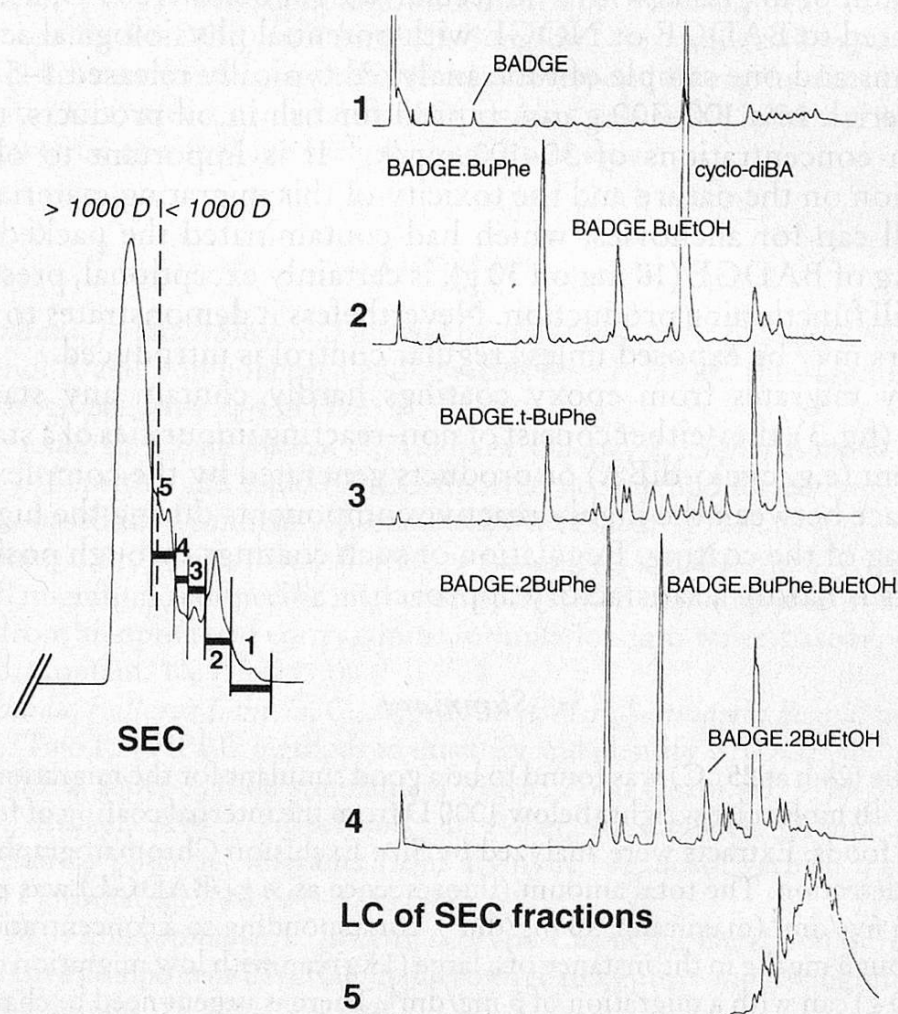


Fig. 3. SEC-FD fractionation of an acetonitrile extract from a typical epoxy coating (left) and LC-FD chromatograms of fractions 1 to 5 (right)

The main components in fraction 4 were BADGE derivatives reacted with two phenols (BADGE.2BuPhe), two glycols (BADGE.2BuEtOH) or one of each (BADGE.BuPhe.BuEtOH). Fraction 5 shows the components with a molecular weight approaching 1000 D and basically included the BADGE trimer (again not positively identified in this extract). None of the components could be identified so far.

Conclusions

The data reported above can be summarized by the following conclusions:

1. Can extracts with acetonitrile provide a good estimate on how much material of a molecular weight below 1000 D will be extracted from a coating by oily foods. Extraction of higher molecular weight components by acetonitrile is clearly more efficient than by oil.
2. SEC pre-separation and detection by fluorescence provides an indication of the total amount of migrants with a molecular weight below 1000 D, i.e. of components related to BADGE or NOGE with potential physiological activity.
3. The 13 cans and one sample of tube analyzed typically released 1–5 mg/dm² of such material. In a 100–300 g can, typical for fish in oil products, this usually results in concentrations of 30–100 mg/kg. It is important to obtain more information on the nature and the toxicity of this migrating material.
4. The small can for anchovies, which had contaminated the packed food with 600 mg/kg of BADGE (18 mg on 30 g), is certainly exceptional, presumably the result of ill functioning production. Nevertheless it demonstrates to what levels consumers may be exposed unless regular control is introduced.
5. Especially migrants from epoxy coatings hardly contain any starting point materials (fig. 3); they either consist of non-reacting impurities of a starting point component (e.g. cyclo-diBA) or products generated by the complex chemistry taking place between the highly reactive components during the high temperature curing of the coating. Regulation of such coatings through positive lists of the educts is hardly a satisfactory approach.

Summary

Acetonitrile (24 h at 25 °C) was found to be a good simulant for the migration of phenolic compounds with molecular weights below 1000 D from the internal coating of food cans into oily and fatty foods. Extracts were analyzed by Size Exclusion Chromatography (SEC) and fluorescence detection. The total amount (fluorescence as, e.g., BADGE) was mostly in the range of 0.5–5 mg/dm² (maximum, 40 mg/dm²), corresponding to a concentration in the can content of about 3 mg/kg in the instance of a large (1 kg) can with low migration or 250 mg/kg for a small (30 g) can with a migration of 5 mg/dm². There is urgent need to characterize this material in order to enable an evaluation of its toxicity.

Zusammenfassung

Acetonitril (24 h bei 25 °C) erwies sich als geeignetes Simulans für die Abschätzung der Migration phenolischer Verbindungen mit einem Molekulargewicht von unter 1000 D aus der Innenlackierung von Konservendosen in öl- und fetthaltige Lebensmittel. Extrakte wurden mittels Size Exclusion Chromatography (SEC) und Fluoreszenzdetektion analysiert. Die totale Menge (Fluoreszenz ähnlich wie z. B. BADGE) lag meistens zwischen 0,5–5 mg/dm² (Maximum: 40 mg/dm²), was in einer grossen Dose mit tiefer Migration einer Konzentration im Füllgut von ca. 3 mg/kg entspricht, in einer kleinen Dose (30 g) mit einer Migration von 5 mg/dm² jedoch 250 mg/kg. Dieses Migrat muss dringend charakterisiert werden, um dessen Toxizität abschätzen zu können.

Résumé

Acétonitrile (24 h à 25 °C) est un paramètre convenable pour l'étude de la migration des composés phénoliques d'un poids moléculaire en dessous de 1000 D, des vernis de boîtes de conserves, dans les aliments gras ou huileux. Les extraits étaient analysés par «Size Exclusion Chromatography (SEC)» avec détection fluorimétrique. Normalement, la quantité totale (fluorescence comme BADGE) était entre 0,5–5 mg/dm² (maximum, 40 mg/dm²). Pour une grande boîte (1 kg) avec une migration basse cela correspond à 3 mg/kg dans l'aliment, pour une petite boîte (30 g) avec une migration de 5 mg/dm² cela arrive à 250 mg/kg. Ce matériel doit être caractérisé en vue d'une évaluation toxicologique.

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