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Bisphenol-A-Diglycidyl Ether (BADGE) in Edible-Oil-Containing Canned Foods: Determination by LC-LC-Fluorescence Detection

Key words: Bisphenol-A-diglycidyl ether, BADGE, Canned foods, Epoxy coatings

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Introduction

Bisphenol-A-diglycidyl ether (BADGE, fig. 1) is a starting point material for the production of epoxy polymers used, e.g., for coating the internal surfaces of cans used for food preserves. Such lacquers are usually polymerized starting from oligomers obtained by the reaction of bisphenol-A and epichlorohydrin. BADGE is, however, also added to organosol (PVC type) coatings to improve their thermal stability and performance. Organosols are more flexible than epoxy resins and are frequently used for cans with easy-open-lids.

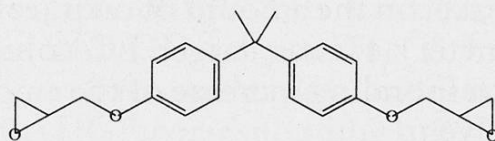


Fig. 1. Structure of BADGE

Swiss legislation requires that packing does not release materials into foodstuffs which are toxic or technically avoidable (legislation before 1995) and that BADGE is undetectable in foods at a detection limit of 0.02 mg/kg (1), for which there is a transition period up to 1998. This value was taken over from the EU, which set the same specific migration limit for foods in contact with plastics, although not (yet) for coatings (2).

Paseiro Losada et al. determined BADGE (3–6) as well as the corresponding bisphenol-F derivative (7) in aqueous media, using reversed phase HPLC (RP-LC) and fluorescence detection or RP-LC coupled to mass spectrometry (MS). The

same group also investigated the kinetics of the epoxide hydrolysis in acidic media (8, 9) and the migration of BADGE into water-based food simulants (10). This work suggests that BADGE is not a problem in water-based foods because it barely survives sterilization of the product and long term storage. *Lopez-Fabal* (11) developed a method for determining BADGE in olive oil. BADGE was isolated from the oil using a Florisil cartridge (SEP-PAK) and analyzed by RP-LC.

Sharman et al. (12) analyzed BADGE in foods, such as pizza, which were in contact with packaging material enhancing browning in microwave ovens (epoxy adhesive was used to attach microwave susceptors to the packaging material). In two of the samples analyzed, high concentrations were found (up to 3000 mg/kg).

We incidentally discovered high concentrations of BADGE in the oil phase of canned fish preserved in oil, such as tuna, sardines, and anchovies, while checking the authenticity of olive oils. In fact, the majority of canned foods in oil was found to be contaminated with BADGE beyond the limit of 0.02 mg/kg. Oil efficiently extracts BADGE from the coating of the can and protects it against hydrolysis, even in presence of acidic aqueous media. We concluded that it is the lipids of canned foods that must be checked.

Method

Principals

The analysis of BADGE by GC or RP-LC presupposes previous removal of the fat or oil. Isolation of BADGE from a fat matrix is difficult, however, because this epoxide may be partly lost. Only normal phase LC enabled the injection of a sufficient amount of oil on to the column to reach the detection limit required (13), i.e. to completely circumvent sample preparation. 2 mm i.d. LC columns were used considering that the sensitivity of the method finally depended on the BADGE concentration in the eluate, i.e. on the amount of oil injected divided by the column flow rate (or column diameter). Hence, larger LC columns merely provided the same sensitivity with a corresponding increase of the amount of sample introduced and consumed far more solvent.

Since neither a silica gel nor a cyano phase reliably separated interfering components from BADGE, the two columns were combined to LC-LC involving heart cutting, i.e. the BADGE peak from the first column was confirmed on the second. The cyano phase was chosen as first column in order to obtain some focusing of the band on the more strongly retaining silica gel column.

The system was designed to record BADGE eluted from the first as well as from the second column by the same detector. This enabled direct control of the fraction transferred as well as a direct comparison of the peak areas of BADGE. During the first separation step, the detector was connected to the first column (fig. 2). From the outlet of the detector, the eluent passed through a transfer loop with an internal volume corresponding to the fraction of BADGE, i.e. 350 μ l. In this way, the

BADGE fraction passed through the detector before the second LC column was connected, which avoided the backpressure of the second column on the detector cell. For the second separation step, pump 2 carried the BADGE fraction from the transfer loop onto the silica gel column. At the same time, the detector was connected to the outlet of column 2. Fluorescence was recorded at 225/295 nm.

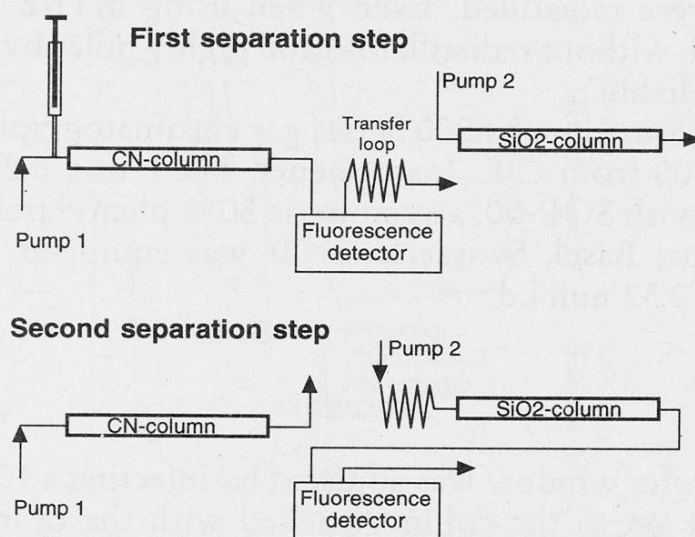


Fig. 2. Principle of LC-LC with heart cutting and recording of BADGE after the first and the second column by the same detector

Instrumentation and materials

The analytical system was constructed on an automated LC-GC system (Dualchrom 3000 from C.E. Instruments, Milan, Italy) equipped with an autosampler, two syringe pumps, a fluorescence detector (Merck F1000), and three switching valves in the LC part. The configuration of the valves is shown in figure 3. Switching of the valves was automated through the Dualchrom software. An LC-GC transfer was simulated in order to obtain the ready signal for the autosampler.

Standard substance: a sample of BADGE was obtained from Ciba. It had the consistency of a glue; pure BADGE is crystalline. A 1:1 solution with n-eicosane injected on-column onto a short apolar GC capillary column and using FID resulted in a peak area of BADGE corresponding to 67% of that of the alkane. The

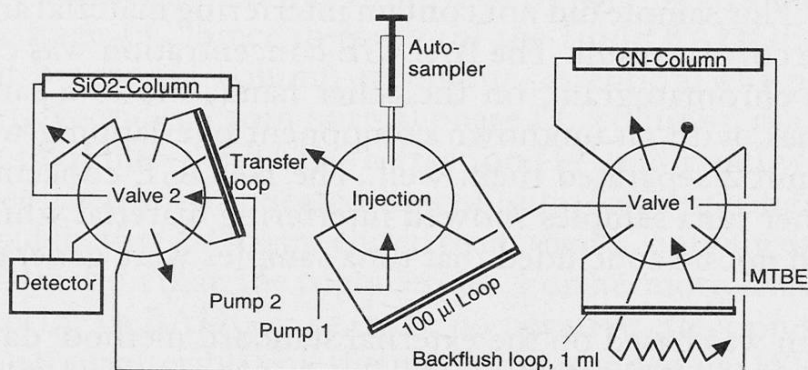


Fig. 3. Configuration of the valve system for LC-LC

FID response to BADGE is reduced owing to the oxygen atoms. Since there is no way to predict the actual FID response, this BADGE was assumed to be 75% pure.

LC was performed on 25 cm x 2 mm i.d. columns packed with silicagel Supelcosil LC-Si 5 μ m and GromsilCN 2 PR 5 μ m (Stagroma, Wallisellen, Switzerland). Pentane and methyl tert. butyl ether (MTBE) of technical grade (Siegfried, Zofingen, Switzerland) were redistilled. Even when using MTBE of high purity, the baseline was too high without redistillation and highly polar by-products rendered retention times unreliable.

GC-MS was performed on a 8000 Series gas chromatograph coupled to a mass spectrometer MD-800 from C.E. Instruments. The 8 m x 0.25 mm i.d. capillary column was coated with SOP-50, a symmetric 50% phenyl polysiloxane obtained from W. Blum (Ciba, Basel, Switzerland). It was equipped with a persilylated precolumn of 3 m x 0.32 mm i.d.

Procedure

The LC-LC-transfer window was adjusted by injecting a 100 ng/ml solution of BADGE in pentane on to the column packed with the cyano phase and using pentane/MTBE 80/20 at 400 μ l/min as the mobile phase (pump 1). Valves 1 and 2 remained in stand-by. The width of the fraction was determined by measuring the base width of the BADGE peak. A transfer loop of corresponding internal volume was installed in valve 2. For the analysis of the samples, valve 2 was switched at the end of the BADGE elution from the first column (end of the corresponding peak, see fig. 4). The mobile phase for the silica gel column consisted of pentane/MTBE 70/30 (400 μ l/min, pump 2). 100 μ l of a pentane solution of edible oil decanted from the can were injected. Samples were analyzed as 10% solutions, but those containing BADGE at more than 1 mg/kg were re-analyzed at a 1% concentration. The detection limit was 5–10 μ g/kg related to the oil.

For the analysis of fish and other foods, an amount of water was added that corresponded to the weight of the sample. After homogenization with a Polytron, 1 g was extracted with 5 ml of hexane and analyzed mostly without further dilution.

The lower chromatogram in figure 4 is from the oil of canned tuna. The first part was recorded at the exit of column 1. The fraction transferred is marked. During the second part of the chromatogram, the detector was connected to the exit of column 2. This sample did not contain interfering material and single column LC provided the correct result. The BADGE concentration was calculated as 0.04 mg/kg. The top chromatogram, on the other hand, shows a sample, oil from a sample of sardines, with an unknown component overlapping with BADGE on column 1. Column 2 separated them well. The BADGE concentration was 0.07 mg/kg. Since other tuna samples showed interfering material while some sardines did not, it should not be concluded that tuna samples were generally more easy to analyze.

Quantification was based on the external standard method; data was related to the oil phase. The standard solution contained 10% rapeseed oil in pentane with

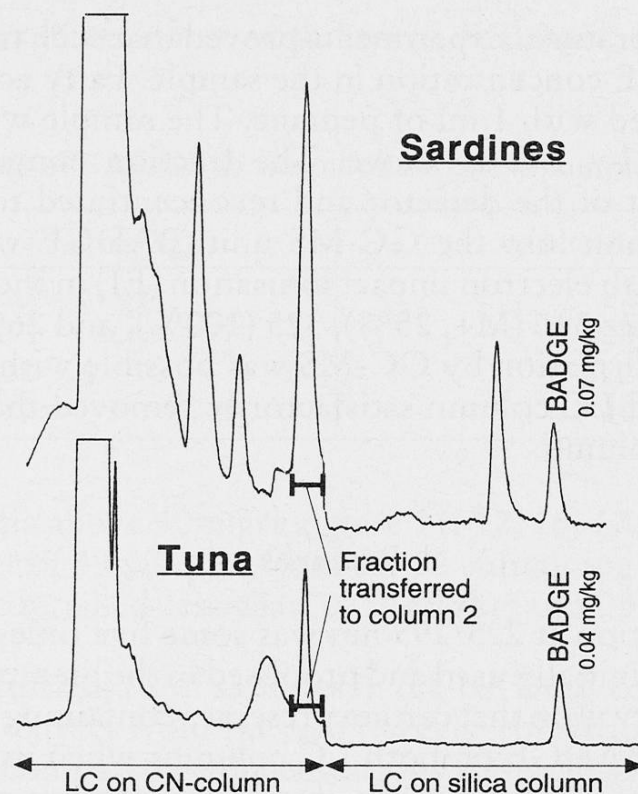


Fig. 4. LC-LC-fluorescence chromatograms of oils without (bottom) and with interfering material (top)

BADGE added to the oil at 0.1, 1, and 10 mg/kg. Linearity of the response was checked repeatedly, since the peak/amount ratio for the smallest concentration was initially some 10% larger than for the highest concentration, but became some 15% smaller after use of the columns for some 500 analyses.

The first LC column could be backflushed with the help of valve 1: 1 ml of MTBE from the backflush loop was sent through the column in reverse direction, followed by 4 ml of eluent. Backflush was necessary after analyzing some samples containing vegetables and spices, but not for oil from canned fish.

GC-MS confirmation

Identity of the BADGE peak was confirmed by GC-MS. Initially, GC-MS was applied to single stage LC. Since injection of the labile BADGE is more reliable when performed by the on-column technique, attention was paid to complete removal of the triglycerides. From normal phase LC columns, the triglycerides are eluted before, but tail into the BADGE fraction (14) and rapidly contaminate the inlet of the GC column when injected on-column. Transesterification solved this problem: fatty acid methyl esters are eluted from the LC column earlier, i.e. tail less into the BADGE fraction than the triglycerides. Furthermore, they are eluted from the GC column prior to BADGE, i.e. have no negative effect on GC of BADGE. 25 mg of oil were transesterified by the method described in (15), i.e. by addition of 0.3 ml MTBE and 0.2 ml of 5% methoxide in methanol, allowing it to react during

5 min at ambient temperature. Experiments proved that such transesterification did not reduce the BADGE concentration in the sample. Fatty acid methyl esters and BADGE were extracted with 1 ml of pentane. The sample was reconcentrated to 250 μ l and analyzed by LC as above. The fraction containing BADGE was recovered at the outlet of the detector and reconcentrated to 30–100 μ l. 5–15 μ l were injected on-column into the GC-MS unit. BADGE was eluted at around 250 °C. MS was run with electron impact ionisation (EI) in the full scan mode. Key ions of BADGE are m/z 340 (M^+ , 25%), 325 (100%), and 269 (15%).

After LC-LC, confirmation by GC-MS was possible without transesterification, because the second LC column satisfactorily removed the triglycerides transferred from the first column.

Remarks

Fluorescence detection at 225/295 nm was some five times more sensitive than that at the 275/320 nm initially used and proposed in the literature. More important, however, was the observation that certain preserves containing vegetables produced a peak coeluted with BADGE on both LC columns when using 275/320 nm, but not at 225/295 nm. At 225/295 nm, no interfering compound has been detected above 20 μ g/kg during the analysis of about 150 samples.

There was partial loss of BADGE over the LC columns. As the LC-LC method detected the peak of BADGE after the first and after the second column, the loss over the second column was directly observed (see also the lower chromatogram in fig. 4). It amounted to about 35% and was practically independent of the concentration, i.e. it did not affect the linearity of the response. That over the first column was around 25%. We have not yet found a way of avoiding these losses. An attempt to deactivate acidic sites within the packing material by dicyclohexylamine added to the mobile phase (up to 0.1%) showed no effect. Introduction into column 2 without passage through the detector did not reduce losses, eliminating the detector as the site of loss.

BADGE was also partially lost during GC-MS analysis, resulting in non-linear response at low concentrations. Performance strongly depended on the activity of the precolumn and the separation column.

Results

BADGE found in samples from the market

Results reported below refer to the oil phase in cans containing foods preserved in oil, i.e. not to concentrations in, e.g., the fish or the whole sample. Some of the concentrations were found to be so high, that rapid analysis was given preference. Data refers to 142 samples from the Swiss market, most of which contained tuna,

sardines or anchovies in oil, some with capers, tomatoes, spices, or other sauces added.

Table 1. Concentrations of BADGE in the oil phase of 142 samples from the Swiss market

Concentration range (mg/kg)	Number of samples
< 0.2	82 (58%)
0.2–1	28 (20%)
1–10	22 (15%)
> 10	10 (7%)

The concentrations above 10 mg/kg were 11, 12, 15, 17, 18, 22, 25, 45, 48, and 57 mg/kg. In a subset of 49 samples from the 82 containing less than 0.2 mg/kg of BADGE in oil, 24 contained less than 0.02 mg/kg and 25 between 0.02 and 0.2 mg/kg.

For 3 samples of tuna and 2 of sardines in oil, BADGE concentrations in the oil phase and in the fat extract from the fish (largely consisting of the oil picked up from outside) were compared. Concentrations differed by at most 20%, with a tendency to be higher in the fish extract, i.e. fish picked up BADGE not only in the fat phase. Since typically 25–30% of the net weight of a sample was oil and the fish contained 9–25% of fat and oil (average of 15%), some 40% of the cans' content consisted of oil. Thus, concentrations given in table 1 divided by 2.5 provide an approximation for the BADGE content over the whole sample. On this basis, 61% of all samples exceeded the 0.02 mg/kg. 12% exceeded it hundred fold at least.

Table 2 shows the concentrations of BADGE in relation to the type of can for 74 samples. Among 13 classical 3-piece cans, none released BADGE to the oil above 1 mg/kg. Products in 3-piece cans with easy-open-lids were somewhat worse: 2 from 9 samples exceeded 1 mg/kg, the highest concentration being 11 mg/kg. All other extremely high concentrations (> 5 mg/kg) were from 2-piece cans with easy-open-lids. More than 30% of this type of cans contaminated the edible oils at concentrations exceeding 1 mg/kg. This might be explained by the type of lacquer used for the lid: to improve protection of the end (lid) from product, particularly in the score and rivet areas, coatings of the organosol/BADGE type are preferred.

Table 2. BADGE concentrations found in the oil phase of cans of different make

Type of can	Number of samples with BADGE at (mg/kg)			
	< 0.1	0.1–1	1–5	> 5
3 piece	11	2	0	0
3 piece, easy open	5	2	1	1
2 piece	5	1	1	0
2 piece, easy open	19	12	8	6

Table 3 shows BADGE concentrations in two lots of cans of the same food product. The lots were produced on the same day, but there was a 6 month gap between the production dates. The mean concentrations differed by a factor 2.5. Variation (as relative standard deviations) between the cans from the same day was 42 and 32%. These results show that only limited conclusions can be reached about the evaluation of a product through the analysis of a single can.

Table 3. BADGE concentrations in the oil phase of ten cans each of two lots of the same product

Lot 1		Lot 2	
Sample	BADGE (mg/kg)	Sample	BADGE (mg/kg)
1	0.24	1	1.88
2	0.54	2	1.52
3	0.67	3	1.02
4	0.46	4	2.2
5	0.55	5	1.28
6	0.94	6	1.32
7	0.48	7	1.16
8	0.67	8	2.12
9	1.12	9	2.62
10	0.41	10	1.36
Mean	0.61		1.65
Rel. stand. dev.	42%		32%

Stability of BADGE in oil in contact with acidic media

Epoxides are readily hydrolyzed in acidic aqueous media. As shown by *Paseiro Losada et al.* (8), the half life time of BADGE in water at 40 °C is 43 h. In 3% acetic acid at 40 °C, it is reduced to 8.1 h. In the light of this it may be surprising that large amounts of BADGE are found in canned foods which have been sterilized at about 125 °C and stored over extended periods of time. Obviously the edible oil phase efficiently protects BADGE from hydrolysis.

Stability of BADGE in presence of acidic aqueous media was of interest in at least two contexts. Firstly, it provided some insight into the range of canned foodstuffs in which BADGE could be present. This range is certainly broader than the fish products primarily analyzed here. Secondly, toxicological assessment of BADGE could be influenced by whether or not BADGE is hydrolyzed in the stomach.

The high concentrations of BADGE found in preserves with oil were a first indication that edible oil protects BADGE. Also the presence of acidic material, such as tomato sauce, did not prevent the presence of large amounts of BADGE.

Stability with acetic acid

The first experiment was performed on the stability of BADGE in the acidic media which may be found in food preserves. To tuna in oil and some 5% of water (typical content of a tuna preserve in oil), 30% of vinegar and 1 mg/kg of BADGE were added. Using a Polytron, this mixture was turned into a milk-like liquid which did not sediment (even over days). Fast hydrolysis could have been expected from the extremely large contact surface between the acidic aqueous phase and the BADGE in oil. However, after 5 days at ambient temperature, the decrease in the BADGE concentration was less than 10%.

Hydrochloric acid, mixing simulating mastication

Hydrolysis of BADGE in edible oil in contact with an aqueous phase is a function of the acidity, temperature, the contact surface, and materials acting as mediators, detergents, or emulgators. For the design of an experiment simulating human intake and digestion, the contact surface was the most difficult parameter to adjust.

In a sample of tuna in oil, the meat was reduced to small pieces with a spatula. Hydrochloric acid of various strengths was added 1:1 and the sample mixed by violent manual shaking. Samples were warmed to 40 °C and analyzed after 1, 2, and 3 h. As part of the material slowly sedimented, the vials were shaken again every about 10 min. After 3 h, the aqueous phase of the sample with 0.1% acid added was at a pH of 5, but those of the samples with the stronger acids remained below pH 1. As shown in table 4, 76% of the BADGE survived 3% hydrochloric acid over 3 h, which suggests that most of the BADGE is likely to pass through the stomach without being hydrolyzed.

Table 4. BADGE hydrolysis in a sample of tuna in contact with hydrochloric acid during 1–3 h at 40 °C. Mixing simulating thorough mastication

HCl concentration	BADGE hydrolysis		
	1 h	2 h	3 h
0.1%	2.5%	6%	10%
1%	8%	16%	18%
3%		11%	24%

Hydrochloric acid, intense mixing

The experiment was repeated with the same sample and the same hydrochloric acids, but using the Polytron to turn the sample into a milk-like homogenate. With 3% acid, degradation after 3 h at 40 °C reached 90% (table 5), which confirms the importance of the contact surface between the aqueous phase and the BADGE in oil, but also shows degradation is incomplete even under these unrealistically drastic conditions.

Table 5. Degradation of BADGE in the presence of hydrochloric acid after intense homogenization by the Polytron (3 h, 40 °C)

HCl concentration	BADGE hydrolysis
0.1%	50%
1%	80%
3%	90%

Conclusion

In the past, contamination of foods by BADGE from packaging materials has not been checked well enough. Aqueous phases used for the previous tests hardly extract BADGE from the coating of the can. Furthermore, BADGE is rapidly hydrolyzed in aqueous media, particularly at low pH. The critical samples are foods containing oil or fat: the edible oil more efficiently extracts BADGE from the coating of the can and protects it from hydrolysis. Laboratory experiments showed that BADGE concentrations in the oil did not noticeably decrease within several days even when the oil was finely dispersed in vinegar.

In future, BADGE will have to be determined working up the whole sample. This will, however, greatly increase the number of possibly interfering compounds in the solution to be analyzed. Furthermore, the range of products analyzed must be widened. Manufacturers of coatings for cans will have to make an effort to solve a problem which has been overlooked for so many years.

Summary

BADGE is a starting point material for the production of epoxy resins and organosol coatings for cans. It is mutagenic in vitro, but in vivo no such activity has been shown. It was analyzed in the oil phase of canned foods, such as tuna, sardines, and anchovies in oil, using normal phase LC-LC with heart cutting and fluorescence detection. Extrapolated to the whole content of the cans, the concentrations found exceeded the Swiss legal limit (non detectable at 0.02 mg/kg detection limit) in more than 60% of the samples by up to a factor of 1000. Treatment with 3% hydrochloric acid over 3 h at 40 °C merely resulted in about 25% hydrolysis, i.e. BADGE in oily foods may pass through the stomach unaffected.

Zusammenfassung

BADGE ist ein Edukt für die Herstellung von Epoxyharzen und Organosolen zur Beschichtung von Dosen. BADGE wirkt mutagen in vitro, doch in vivo wurde bisher keine solche Aktivität nachgewiesen. Es wurde in der Ölphase von Konserven, wie Thon, Sardinen und Sardellen in Öl, mittels Normalphasen-LC-LC und Fluoreszenzdetektion gemessen. Extrapoliert auf den ganzen Doseninhalt überschritten die gefundenen Konzentrationen in

über 60% aller Proben den schweizerischen Grenzwert (nicht nachweisbar bei 0,02 mg/kg Nachweisgrenze) um bis zu einem Faktor von 1000. Bei einer Behandlung mit 3% Salzsäure über 3 h bei 40 °C wurde nur ca. 25% des vorhandenen BADGEs hydrolysiert, was darauf hinweist, dass BADGE in ölhaltigen Lebensmitteln wahrscheinlich weitgehend unverändert den Magen passiert.

Résumé

Le BADGE est une matière première utilisée dans la fabrication de revêtements à base de résines époxydiques et d'organosol pour boîtes. Il est mutagène in vitro, mais une telle activité n'a pas pu être démontrée in vivo. BADGE a été analysé dans la phase huileuse de conserves p. ex. de thon, de sardines ou d'anchois à l'huile, par LC-LC en phase normale avec «heart cutting» et détection fluorimétrique. Extrapolées sur le contenu total de la boîte, les concentrations mesurées dépassaient la limite légale suisse (non-détectable, avec une limite de détection de 0,02 mg/kg) dans plus de 60% des l'échantillons, jusqu'à excéder mille fois cette limite. Le traitement avec une solution à 3% d'acide chlorhydrique pendant 3 h à 40 °C n'hydrolysait qu'environ 25% du BADGE, ce qui semble indiquer que BADGE dans les aliments à base d'huile est peu dégradé lors du passage dans l'estomac.

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