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ORIGINAL PAPER

Study of the migration behavior of acetyl tributyl citrate from PVDC/PVC film into fish fillets as affected by intermediate doses of electron beam radiation

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Abstract Food-grade plasticized polyvinylidene chloride/polyvinyl chloride (PVDC/PVC) film (saran wrap) containing acetyl tributyl citrate (ATBC) plasticizer was used to wrap cod and herring fillets. The ratio of film surface to weight of food was ca. 89:1, in contrast to the generally agreed relationship of 6 dm^2 to 1 kg food (6:1). Wrapped fish samples were subjected to electron beam irradiation at doses equal to 5 and 10 kGy, stored at 4 °C and analyzed for ATBC content at time intervals between 12 and 240 h of contact. Determination of the analyte was performed by ultrasonic-assisted solvent extraction followed by analysis on a gas chromatograph coupled with flame ionization detector. Final ATBC concentrations in cod fillets ranged from 11.1 to 12.8 mg/kg, while the corresponding values for herring samples were between 32.4 and 33.4 mg/kg. Data showed that e-beam radiation at pasteurizing doses did not significantly affect the copolymer's specific migration characteristics. On the contrary, fat content of the packaged fish fillets substantially affected the diffusion coefficient (*D*) values, as well as the extent to which migration of ATBC occurred. No violations of the tolerable daily intake (TDI) of 1.0 mg/kg body weight set by the EU for ATBC were found in the present study. However, food overwrapping or rewrapping with flexible films is often applied in household applications. Since in such cases unrealistically high plasticizer concentrations are determined experimentally, present specific migration limits (SML) should be redefined on a different basis.

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Introduction

Irradiation, as a method of food preservation, has been explored and documented over the past 50 years. Ionizing radiation, including gamma and electron beam radiation, is being used at various dose levels for food preservation, as well as sterilization of packaging materials and medical products. The Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (JECFI) held in Geneva (1980) concluded that irradiation at a dose up to 10 kGy does not impart any toxicological risk to the food and does not affect its nutritional value [1–4].

Fish comprises an ideal substrate for the growth of spoilage microorganisms (e.g., genera including *Pseudomonas* spp., *Bacillus* spp., *Shewanella* spp., *Psychrobacter* spp., *Flavobacterium* spp.), pathogens (including *Vibrio* spp., *Aeromonas hydrophila*, *Clostridium botulinum*, *Salmonella* spp., *Listeria monocytogenes*), and parasites (e.g. *Anisakis* spp., *Paragonimus* spp.). Irradiation of fish and shellfish at low (<1 kGy) and medium (1–10 kGy) doses of ionizing radiation combined with refrigeration and proper packaging, significantly prolongs the shelf life of such products through a drastic reduction in their microbial load. In addition, public health is ensured via elimination of pathogens [2, 5–9].

Currently, more than 20 countries worldwide have legalized irradiation of fish and seafood as a method of preservation. Certain EU member states (Belgium, France, Netherlands, Germany, and Czech Republic) have national permissions for irradiation of such commodities**.** Ionizing

radiation applications in the US include processing of shellfish (fresh or frozen) at doses up to 5.5 kGy [10].

Products such as fish fillets are usually packaged in plastic films before being subjected to irradiation in order to avoid microbial recontamination. Thus, the effect of radiation on plastic packaging materials is of prime importance, since it relates directly to the quality and safety of packaged foodstuffs. Depending on the particular material, nature of the additives used to compound the plastic, processing history, and specific conditions of irradiation (absorbed dose, dose rate, temperature, and atmosphere), changes in the polymer structure may occur affecting, among others, its migration characteristics. According to the literature, irradiation may result in: (a) formation of crosslinks and (b) scission of polymer chains. The first phenomenon is expected to repress migration of additives, while the second one is expected to enhance it [11–18]. Furthermore, irradiation of polymers may induce the formation of low-molecular-mass degradation compounds (radiolysis products) [19].

Among potential migrants, plasticizers have raised considerable concern within the scientific community from the food safety point of view, since they are present in plastics films in significant amounts (up to 30% w/w in PVC). Tri-n-butyl acetyl citrate or acetyl tributyl citrate (ATBC) (at levels up to 4.8% w/w) is the most widely used plasticizer in vinylidene chloride film copolymerized with up to 20% vinyl chloride (PVDC/PVC). This clear, flexible film is currently sold under the trade name of "saran". Saran is used in food applications where low permeability to oxygen, moisture and odors, and high grease resistance are important. Hence, it is being widely used for the wrapping of cheese, frozen meat/poultry, fish, and bakery products at a retail level and is also available as a domestic film wrap [20–22].

Acetyl tributyl citrate was first considered by the Scientific Committee on Food (SCF) in 1998 and classified in SCF List 7, under "*Substances for which some toxicological data exist, but for which an Acceptable Daily Intake (ADI) or a Tolerable Daily Intake (TDI) could not be established*". ATBC was also evaluated by the European Food Safety Authority (EFSA) in 2005. On the basis of lack of genotoxicity, overall low toxicity, lack of carcinogenicity and extensive metabolic transformation into compounds of low toxicity, the substance was classified in SCF List 2 with a TDI of 1.0 mg/kg body weight. The above TDI value was derived by applying the default uncertainty factor of 100 to the No-Observed-Adverse-Effect-Level (NOAEL) of 100 mg/kg bw/day for general toxicity [22]. ATBC was introduced into the EU legislation on food contact plastics by Directive 2007/19/EC with no specific restriction or specification [23, 24].

Numerous studies have been published in the literature with respect to ATBC migration. Several methods, based on either measuring the loss of citrate from the film using infrared spectroscopy (FTIR) or determining (GC/FID-MS) the migrant after proper extraction from the matrix and separation from the co-extracted matter, have been employed to assess migration from saran films into several foodstuffs (e.g., meat, poultry, cheese) especially during microwave cooking [21, 25–29]. However, to the best of our knowledge, there are no published data on the migration of plasticizers from flexible films into fish as affected by irradiation.

Thus, the objectives of the present study were (a) to propose a simple, rapid, and efficient analytical method for the determination of ATBC plasticizer in complex food matrices, (b) to carry out a kinetic study on the migration of ATBC from saran film into marine fish species based on the aforementioned approach, (c) to determine the effect of irradiation with high-energy electrons on the kinetics of ATBC migration, and (d) to check compliance of the specific packaging material with the TDI restriction stemming from the latest EFSA evaluation (2005) under present experimental conditions (irradiation combined with food overwrapping).

Materials and methods

Materials and reagents

Plasticized PVDC/PVC film was commercial saran wrap (12 μm in thickness) supplied by Saropack AG (Rorschach, Switzerland). The content of ATBC plasticizer was disclosed by the manufacturer as being (4.5 ± 0.1) % w/w, which was confirmed by chloroform extraction of the film followed by capillary GC analysis as described below.

Analytical grade ATBC was purchased from Unitex Chemical (Greensboro, NC, USA). Analytical grade octadecane, used as internal standard (IS), was purchased from Fluka (Buchs, Switzerland). Analytical grade chloroform, *n*-hexane, and anhydrous sodium sulfate were purchased from Merck (Darmstadt, Germany).

Fish samples

Fresh cod and herring (*Gadus morhua* and *Argentina silus*) were purchased from retail outlets located in Karlsruhe (Germany), where it had been established that they had not previously been in contact with plasticized film. Blank measurements were taken alongside migration experiments to examine whether fish samples were ATBC contaminated. The specific fish species were selected due to substantial differences in fat content.

Determination of the film's ATBC content

A small piece of saran film (6 × 10 cm) was weighed and extracted in an ultrasonic bath (Bandelin Sonorex RK 100, Germany) for 1 h with 150 mL of chloroform. After solvent evaporation (Rotavapor R-114 flash evaporator, Buchi, Switzerland), the residue was dissolved in 10 mL of *n*-hexane containing octadecane at 1 mg/mL.

For plasticizer determination, a gas chromatograph HP 5890 series II (Hewlett-Packard, Wilmington, DE, USA) with a flame ionization detector (FID) was used, equipped with a 30 m × 0.32 mm fused silica capillary column coated with 0.25 μm film (J & W Scientific, Folsom, USA). The carrier gas was helium at 75 kPa (2 mL/min). The column temperature was held at 200 °C for 1 min, then programmed at 20 °C min^{-1} to 280 °C held for 3 min. Injector was at 250 °C and detector at 300 °C. Injection volume was 1 μL, and detection was performed at a split (2:40) mode.

Lipid and moisture analysis

Fat content of the fish species was determined by the AOAC Soxhlet method [30]. Moisture content was determined gravimetrically; 5 g of minced fish fillet was dried at 105 °C until constant weight.

Irradiation and migration experiments

Cod and herring were manually filleted. Fillets (with skin) of approximately the same weight and surface area were brought into contact (wrapped on both sides) with the saran film. The average weight of fish samples was 45 ± 5 g and the total film/fish contact area was ca. 400 cm^2 for each sample. The ratio of film surface to weight of food was ca. 4 dm^2 to 45 g fish (89:1) in contrast to the generally agreed relationship of 6 dm^2 to 1 kg food (6:1) [31]. The former ratio corresponds to more realistic food packaging applications.

Wrapped fish samples were irradiated using a linear accelerator (LINAC–CIRCE III, Linac Technologies SA; Orsay, France, 10 MeV) at doses equal to 5 and 10 kGy. Absorbed doses were measured using an Alanine-ESR dosimeter, and mean dose rate was 10^7 Gy/s for all samples. Irradiation was carried out at 0 to (4 ± 2) °C, using ice, and samples were subsequently stored at 4 ± 1 °C. Irradiation was conducted at the Federal Research Center for Nutrition and Food (Karlsruhe, Germany).

Sampling for plasticizer determination was carried out at predetermined time intervals; namely 12, 24, 48, 96, 144, 192, and 240 h. By the end of storage period, sensory characteristics of both fish species had begun to deteriorate; especially, those with a higher fat content. Nevertheless, the aforementioned conditions were chosen on a strict experimental basis in order to (a) allow feasibility of a kinetic study and (b) examine the extent to which radiation dose affects the levels of plasticizer migration.

All experiments were carried out in duplicate with three determinations per replicate in order to ensure, as far as possible, reproducible conditions. Identical non-irradiated (control) samples were also analyzed for ATBC content under the same conditions for comparison purposes.

Plasticizer analysis

Overview of the methodology

The quantitative determination of plastics additives in food matrices is associated with two main difficulties; namely, the low detection limit required and the diversity of potential interferences present in foodstuffs.

Alkanes, as well as their mixtures with acetone (usually 1:1 v/v), are the most common solvents reported in the literature for the extraction of adipates, phthalates, and citrates from various food substrates [32–36]. Nerin et al. [34] examined several mixtures of hexane/acetone of different polarity with respect to their efficiency in extracting DEHA from cheese with minimum fat dissolution. Based on the results obtained, the authors concluded that acetone enhances dissolution of the fat matter. The above conclusion, along with the high extraction efficiency of hexane, has also been stressed by other researchers [37]. Thus, *n*-hexane was chosen as the extraction solvent for the present study.

The most common analytical methods with respect to solvent extraction of plasticizers from foodstuffs reported in the literature involve (a) continuous extraction in a Soxhlet apparatus, (b) extraction by sample homogenization, and (c) extraction by means of ultrasounds or microwaves [25, 33–35, 38–42].

Soxhlet extraction needs to be combined with a clean-up step; furthermore, it is laborious, time consuming and cannot be applied to multiple samples simultaneously. On the other hand, direct chromatographic analysis of the organic extract obtained by sample homogenization is unsatisfactory due to significant fat interference.

With respect to the microwave-assisted extraction, it is reported in the literature that microwaves are mainly applied when solvents with polar groups are involved; non-polar solvents, as well as fat, which have a small relative dielectric constant, absorb the energy of microwaves to a lesser extent [43]. Nerin et al. [34] reported that ultrasonics yield maximum recoveries along with minimum fat interference, because the plasticizer is quantitatively extracted without dissolving fat. Hence, sonication was chosen as the extraction method for the present study.

Sample preparation

ATBC plasticizer was determined by the analytical methodology developed by Nerin et al. [34] for the analysis of bis(2-ethylhexyl) adipate in cheese after appropriate adaptation: Whole fish fillet samples (45 ± 5 g) were sonicated (30 °C for 15 min) with 75 mL of *n*-hexane. An ultrasonic thermostated bath (Bandelin Sonorex RK 100, Germany) was used. The organic extract was decanted, and samples were re-extracted (twice) with fresh solvent (150 mL). Following the extraction step, fish samples were washed with ca. 25 mL of solvent. The extracts, including the portion used for washing, were combined and dried with the addition of anhydrous sodium sulfate (ca. 30 g). The mixture was filtered to remove Na_2SO_4, and the filtrate was subsequently evaporated to dryness with the aid of a flash evaporator (Rotavapor R-114, Buchi, Switzerland). The extracted plasticizer was finally collected with 10 mL of *n*-hexane to which one milliliter of an octadecane solution (0.1 mg/mL) had been added. All glassware used were heated at 200 °C for 2 h, cooled and then rinsed with *n*-hexane before use.

Gas chromatography

Gas chromatographic analysis of ATBC was performed on a gas chromatograph model HP 5890 series II with FI detection. A 30 m × 0.32 mm i.d. × 0.25 μm HP-5 column operated at a helium flow rate of 2 mL/min was used. The temperature program of the GC oven was the following: 200 °C (1 min hold); rating 8 °C min^{-1} to 280 °C (3 min hold); rating 20 °C min^{-1} to a final temperature of 300 °C (15 min hold). The injector was at 250 °C, and the detector was set at 320 °C. Sample injections were alternated with pure solvent (*n*-hexane) injections to remove excess of co-extracted lipophilic compounds from the column. Injection volume was 1 μL and detection was performed at a split (2:10) mode.

Analytical curve

One hundred microliters of ATBC were diluted with *n*-hexane to prepare a 1% (10,500 mg/L) stock solution. Appropriate volumes of this solution were added to ca. 45 g fish to cover the range 2.6–78 mg/kg. The extraction procedure was applied and analytical curve equation was obtained by linear regression.

Recovery tests

Fillets (45 ± 5 g) from both fish species, which had not previously been in contact with the plastics film, were spiked with ATBC plasticizer. They were stored for a short period of time (e.g., overnight) before being subjected to the extraction procedure described earlier.

Spiking was carried out at three concentrations (5.2, 13.0, and 35.1 mg/kg) in the proximity of the real sample extracts by addition of appropriate volumes of a 1% ATBC stock solution.

Statistical analysis

Experiments were replicated twice on different occasions. All analyses were run in triplicate for each replicate ($n = 6$). Average values and standard deviations were calculated for all data. Differences between pairs were defined by Student's *t*-test and were considered to be significant at the $p \leq 0.05$ level.

Results and discussion

Optimization experiments

Effect of sample size

The first part of the study involved the evaluation of the effect of the sample's size on the accuracy of the analytical procedure. Two series of experiments were set up as follows: (a) spiked fish fillets of approximate dimensions 6 × 4 × 1.3 cm (45 ± 5 g) were extracted in their entirety and (b) spiked fillets were extracted after being cut into sub-samples (ca. 3 × 2 × 1.3 cm each).

As shown in Table 1, no significant differences were observed in terms of recovery; thus, we chose to apply the extraction procedure on whole fish fillets. Average recoveries for cod samples ranged from 80 to 95% at all spiking levels tested, while for herring samples recoveries were somewhat lower, especially at high plasticizer concentrations (with all individual values still exceeding 70%). Obviously, ATBC did not penetrate far into the fish fillets but remained essentially at the surface; hence, *n*-hexane performed adequately at such sample thickness. Besides, as we have already reported, ATBC penetration into fatty foodstuffs does not exceed 7.5 mm, even in cases of high fat content (30% w/w) and relatively high temperature (25 °C) [40]. The fact that migration into solid foodstuffs occurs only into the topmost few millimeters of contact surface has also been reported by other researchers [37, 44]. The optimized analytical parameters are summarized in Table 2.

Typical chromatograms obtained by both series of experiments and for both fish species are depicted in Fig. 1. The method proved to be selective, since ATBC was effectively determined without significant interference of the sample matrix.

Table 1 Recoveries of ATBC plasticizer from spiked fish fillets after ultrasonic solvent extraction and GC-FID analysis as affected by sample size

Plasticizer	Fish species	Added (mg/kg)	Determined[a] (mg/kg)		Recovery (%)	
			Whole fish fillets[b]	Fish fillets divided in sub-samples[c]	Whole fish fillets[b]	Fish fillets divided in sub-samples[c]
ATBC	Cod	5.2	4.8 ± 0.2	4.9 ± 0.2	92 ± 4	95 ± 3
		13.0	12.4 ± 0.4	11.8 ± 0.4	95 ± 3	91 ± 3
		35.1	28.8 ± 1.4	28.1 ± 1.8	82 ± 4	80 ± 5
	Herring	5.2	4.6 ± 0.2	4.8 ± 0.3	89 ± 3	93 ± 5
		13.0	11.7 ± 0.3	10.7 ± 0.5	90 ± 2	82 ± 4
		35.1	26.7 ± 1.4	27.3 ± 1.8	76 ± 4	78 ± 5

[a] Values represent the mean of two experiments and three injections of each extract

[b] Fish fillets extracted in their entirety (ca. 6 × 4 × 1.3 cm)

[c] Fish fillets cut into four sub-samples (ca. 3 × 2 × 1.3 cm each) prior to solvent extraction

Table 2 Analytical features of the method

Parameter	ATBC
Retention time (min)	7.48 ± 0.05
LOD[a] (mg/kg)	1.6
LOQ[b] (mg/kg)	5.2
RSD[c] (%)	4.8
Regression equation	$E = 44.7 \times 10^{-3}$ C (mg/kg) − 12.5×10^{-2d}
Correlation coefficient (r)	0.9998

[a] Limit of detection, defined as three times the signal-to-noise ratio

[b] Limit of quantitation, defined as ten times the signal-to-noise ratio

[c] Relative standard deviation obtained from two experiments and three injections of each extract ($n = 6$)

[d] Peak area (arbitrary units)

Lipid and moisture analysis

Lipid and moisture content of the muscle of cod (g/100 g muscle) were ca. 0.7% and 82%, respectively. Respective values for herring were 13.2% and 67%. Cod was chosen as a typical non-fatty fish, while herring was chosen as a typical fatty fish.

Migration results

Kinetic study

The mean migration levels of ATBC into both fish species monitored as a function of time are shown in Tables 3 and 4. Equilibrium conditions were reached after ca. 6 days of contact for non-irradiated, as well as irradiated samples of both marine fish species studied. Results indicate that the energy transferred to the polymer by ionizing radiation does not accelerate the attaining of equilibrium state (kinetics).

The final concentrations of ATBC in cod fillets ranged from 11.1 to 12.8 mg/kg (0.11–0.13 mg/dm^2), representing losses from the film between 1.0 and 1.1% of the available plasticizer. Respective values for herring samples were 32.4–33.4 mg/kg (0.32–0.33 mg/dm^2), corresponding to losses of ca. 2.9–3.0% of the available plasticizer. Data showed that irradiation with high-energy electrons at pasteurizing doses did not considerably affect the film's specific migration characteristics. Statistically significant differences ($p < 0.05$) were only observed between non-irradiated and irradiated at 10 kGy cod fillet samples. On the contrary, statistically significant differences in plasticizer migration were found between the two fish species examined. Thus, fat content of the packaged fish fillets substantially affected the extent to which migration of ATBC occurred.

The amount of ATBC that migrated into fish fillets cannot be discussed in relation to a specific migration limit (SML), since to date no such restriction has been established for this additive [24]. Nevertheless, contamination levels reached by the end of the storage period are far below the overall migration limit set by the EU (10 mg/dm^2) [45].

The relatively high migration levels (expressed in mg/kg) observed, especially in herring samples, are attributed to the high ratio of film surface to weight of food (89:1), which is used in the present study and represents realistic use (i.e., food overwrapping or rewrapping). Grob et al. [46] reported that for small packs with a high ratio of contact surface area to volume, present European legislation tolerates extremely high migration levels. The overall migration (OM) or specific migration (SM) of certain additives, e.g., DEHA from PVC cling films into cheese, demonstrate that such high migration values are realistically encountered [21, 36, 47]. Therefore, the authors propose that migration limits should be redefined and

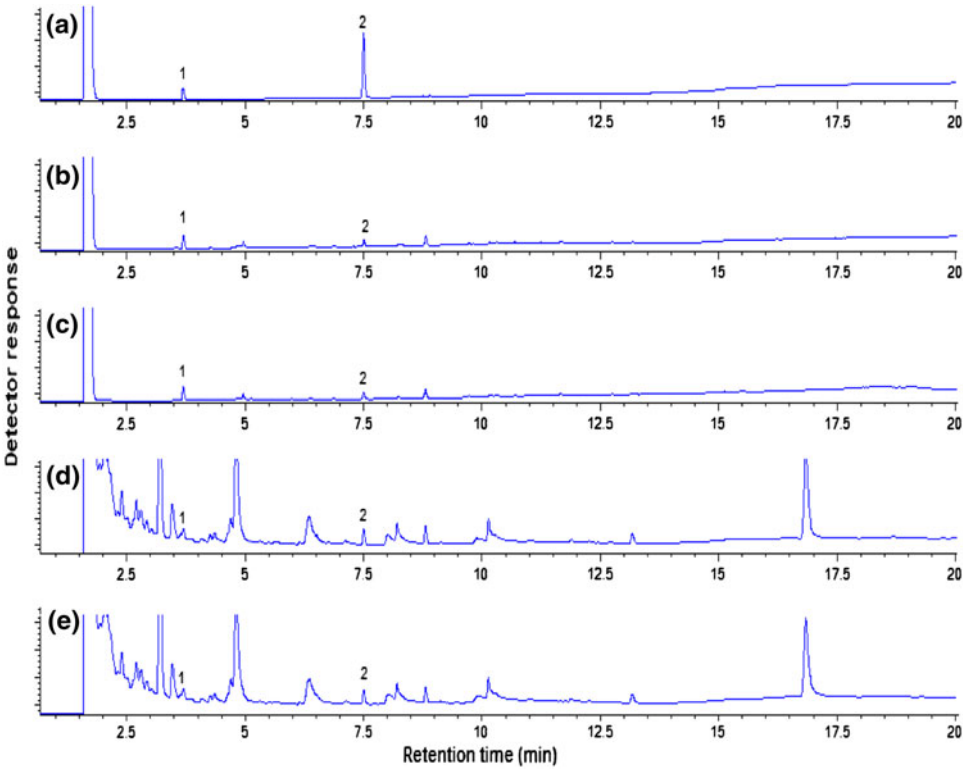

Fig. 1 Chromatograms (GC-FID) **a** ATBC working solution in *n*-hexane at 105 mg/L, **b** spiked (5.2 mg/kg) whole cod fillet sample after ultrasonic solvent extraction, **c** spiked (5.2 mg/kg) cod fillet sample divided in sub-samples prior to ultrasonic solvent extraction, **d** spiked (13.0 mg/kg) whole herring fillet sample after ultrasonic solvent extraction, **e** spiked (13.0 mg/kg) herring fillet sample divided in sub-samples prior to ultrasonic solvent extraction; peaks: [1] $C_{18}H_{38}$ internal standard, t_R 3.68 min and [2] ATBC, t_R 7.48 min). {GC conditions: injector 250 °C; detector 320 °C; column 200 °C (1 min), 8 °C min^{-1} to 280 °C (3 min), 20 °C min^{-1} to 300 °C (15 min); He 75 kPa; split 2:10}

Table 3 Migration values of ATBC plasticizer from control and electron-irradiated saran film into cod fillets at 4 ± 1 °C

Contact time (h)	ATBC migration[a]								
	Non-irradiated			5 kGy			10 kGy		
	(mg/kg)	(mg/dm^2)	% loss	(mg/kg)	(mg/dm^2)	% loss	(mg/kg)	(mg/dm^2)	% loss
12	2.3 ± 0.1	0.02 ± 0.001	0.21 ± 0.01	2.4 ± 0.1	0.02 ± 0.001	0.21 ± 0.01	3.0 ± 0.1	0.03 ± 0.001	0.26 ± 0.01
24	5.6 ± 0.3	0.06 ± 0.003	0.50 ± 0.02	5.8 ± 0.3	0.06 ± 0.003	0.51 ± 0.02	6.7 ± 0.3	0.07 ± 0.002	0.60 ± 0.02
48	7.7 ± 0.4	0.08 ± 0.004	0.69 ± 0.03	8.4 ± 0.4	0.08 ± 0.002	0.75 ± 0.04	9.3 ± 0.4	0.09 ± 0.004	0.83 ± 0.04
96	9.8 ± 0.5	0.10 ± 0.003	0.87 ± 0.04	9.9 ± 0.5	0.10 ± 0.005	0.88 ± 0.03	10.8 ± 0.5	0.11 ± 0.004	0.96 ± 0.05
144	11.1 ± 0.5	0.11 ± 0.005	0.99 ± 0.05	11.2 ± 0.5	0.11 ± 0.003	0.99 ± 0.05	12.0 ± 0.6	0.12 ± 0.006	1.06 ± 0.03
192	11.6 ± 0.6	0.12 ± 0.004	1.03 ± 0.03	12.0 ± 0.6	0.12 ± 0.005	1.06 ± 0.04	12.8 ± 0.6	0.13 ± 0.004	1.14 ± 0.04
240	11.4 ± 0.5	0.11 ± 0.005	1.01 ± 0.03	12.0 ± 0.6	0.12 ± 0.005	1.06 ± 0.04	12.8 ± 0.6	0.13 ± 0.006	1.14 ± 0.04

[a] Values represent the mean of two experiments and three injections of each extract ±SD

expressed as migration amount per contact surface area by taking into account a 20 dm^2/kg surface to weight ratio, rather than the currently used 6 dm^2/kg ratio.

Based on the existing data, a realistic consideration for a significant part of the population would be: For a 60 kg adult, a daily consumption of 150–200 g fish packaged in this type of saran film would result in a maximum daily intake equal to 0.04 or 0.11 mg ATBC/kg bw for cod and herring species, respectively. The above daily intake values are far below the TDI of 1.0 mg/kg bw derived from the latest risk assessments carried out by EFSA with respect to ATBC. The fact, though, that an adult consumes a variety of fatty foodstuffs, on a daily basis, which may have been in contact with PVDC, PVC, or PVDC/PVC polymer blend food films, may pose a safety concern. Currently, high ATBC daily intakes are expected due to an increased usage of VDC copolymers in microwave applications.

Data of the present study are comparable with those reported by other researchers. Till et al. [48] reported a migration value of dioctyl adipate (DOA) plasticizer from PVC film into fish fillets equal to 2.4 mg/dm^2 (1.3% loss from film) after 7 days of contact at 4 °C, while the respective migration value determined in lean beef was 1.4 mg/dm^2 (0.7% loss). This is a contradictory finding,

Table 4 Migration values of ATBC plasticizer from control and electron-irradiated saran film into herring fillets at 4 ± 1 °C

Contact time (h)	ATBC migration[a]								
	Non-irradiated			5 kGy			10 kGy		
	(mg/kg)	(mg/dm^2)	% loss	(mg/kg)	(mg/dm^2)	% loss	(mg/kg)	(mg/dm^2)	% loss
12	6.5 ± 0.3	0.06 ± 0.003	0.57 ± 0.03	6.8 ± 0.3	0.07 ± 0.003	0.60 ± 0.03	7.3 ± 0.4	0.07 ± 0.003	0.65 ± 0.03
24	18.6 ± 0.9	0.19 ± 0.009	1.65 ± 0.08	18.6 ± 0.9	0.19 ± 0.009	1.65 ± 0.08	19.5 ± 0.9	0.20 ± 0.010	1.73 ± 0.08
48	24.4 ± 1.2	0.24 ± 0.012	2.17 ± 0.10	24.1 ± 1.2	0.24 ± 0.012	2.14 ± 0.10	24.9 ± 1.2	0.25 ± 0.012	2.21 ± 0.11
96	28.4 ± 1.4	0.28 ± 0.013	2.52 ± 0.11	28.9 ± 1.4	0.29 ± 0.013	2.57 ± 0.12	29.7 ± 1.4	0.30 ± 0.013	2.64 ± 0.11
144	32.4 ± 1.6	0.32 ± 0.013	2.88 ± 0.12	32.9 ± 1.6	0.33 ± 0.015	2.93 ± 0.14	33.4 ± 1.6	0.33 ± 0.016	2.97 ± 0.13
192	33.3 ± 1.6	0.33 ± 0.015	2.96 ± 0.13	33.0 ± 1.6	0.33 ± 0.015	2.93 ± 0.13	33.4 ± 1.6	0.33 ± 0.015	2.97 ± 0.13
240	32.7 ± 1.6	0.33 ± 0.015	2.91 ± 0.11	32.9 ± 1.6	0.33 ± 0.016	2.92 ± 0.13	33.4 ± 1.6	0.33 ± 0.016	2.97 ± 0.14

[a] Values represent the mean of two experiments and three injections of each extract ±SD

given the fact that fat content of lean beef is significantly higher than that of fish. Such differences may be attributed to the nature of fish lipids; it is well known that fish fat contains a high percentage of polyunsaturated fatty acids and thus it is fluid.

Castle et al. [21] reported migration values of ATBC (4.8% w/w) from saran film into cheese ranging from 1.3 to 7.7 mg/kg after 5 days of contact at room temperature. According to the same authors, migration levels of ATBC from the same film into cheese, cake, and sandwich reached 6% (0.6 mg/dm^2), 2% (0.2 mg/dm^2) and 1% (0.1 mg/dm^2), respectively, after 5 days of contact at 5 °C.

According to Goulas and Kontominas [39], no statistically significant differences were found in DOA (28.3% w/w) migration from PVC film into chicken meat (7% fat) between gamma-irradiated (4 and 9 kGy) and non-irradiated samples after a 10-day contact period at 4–5 °C.

Goulas et al. [47] studied the migration of DEHA plasticizer (28.3% w/w) from PVC film into hard and soft cheeses. The authors reported that after 240 h of contact under refrigeration, the migration of DEHA was approximately 345 mg/kg (18.9 mg/dm^2) for Kefalotyri cheese, 223 mg/kg (12.2 mg/dm^2) for Edam cheese, and 134 mg/kg (7.3 mg/dm^2) for Feta cheese, corresponding to plasticizer losses from the film equal to 37.8, 24.3, and 14.6%, respectively. Migration values exceeded, in all cases, the SML set by the EU for DEHA (18 mg/kg or 3 mg/dm^2) due to the high ratio of film surface to weight of food applied in the study (18:1). According to the authors, the different fat and moisture content of the three types of cheese tested resulted in statistically significant differences in plasticizer migration values.

It should be stressed at this point that migration values (expressed in mg/dm^2) of ATBC plasticizer to both fish species reported in the present study are consistent with those attained for low-fat foods and may be attributed to a variety of factors, including (a) the film's low initial ATBC content, (b) the high compatibility of ATBC with vinyl resins, (c) the small thickness of saran film, (d) the low storage temperature of fish samples, (e) the low fat and high moisture content of fish samples, and (f) the low fat content of fish skin, since fat of the specific marine species is mainly located within the muscular tissue.

Calculation of diffusion coefficients

The diffusion of a substance within a polymer matrix generally obeys *Fick's second law of diffusion* [49]:

$$\frac{\partial C_p}{\partial t} = \frac{\partial}{\partial x}\left(D_p \frac{\partial C_p}{\partial x}\right), \quad (1)$$

where D_p is the constant diffusion coefficient of the migrant within the polymer. Equation (1) can be used in its simplified form (2) for the initial stages of migration assuming long migration times and a D value independent of concentration [49, 50]:

$$M_t \approx 2C_{po}(Dt/\pi)^{1/2}, \quad (2)$$

where M_t (expressed in mg/cm^2) is the amount of substance migrated in time t (expressed in s) and C_{po} (expressed in mg/cm^3) is the initial concentration of the migrant in the polymer.

A plot of M_t/C_{po} against $2(t/\pi)^{1/2}$ is initially linear and has a slope of $D^{1/2}$. The diffusion coefficient D represents the migration rate of a molecule within the polymer matrix and is expressed in cm^2/s.

Table 5 shows diffusion coefficients of ATBC plasticizer for both fish species calculated as mentioned earlier. The average D value of ATBC for film in contact with herring fillets was significantly higher (ca. one order of magnitude) than the corresponding one calculated for cod. Differences observed are probably the result of changes in the diffusivity of the material due to fat absorption. On the other hand, irradiation with high-energy electrons at

Table 5 Diffusion coefficients of ATBC plasticizer into control and irradiated saran film in contact with fish fillets at 4 ± 1 °C

D_{ATBC} (cm^2/s) ($\times 10^{-16}$)			
	0 kGy	5 kGy	10 kGy
Cod	5.8	7.4	8.0
Herring	63.0	58.7	60.6

pasteurizing doses did not produce a considerable increase in the plasticizer's diffusion rate.

Kondyli et al. [41] reported D values of 1.6×10^{-17}, 2.8×10^{-15}, and 6.2×10^{-15} cm^2/s after contact of PVC film with ground beef (3, 12, and 30% fat) for 8 days at 4 °C. The above values are comparable with those of the present study taking into consideration the distinct nature of fish fat. To the best of our knowledge, there are no data in the literature regarding D values of migrating plasticizers from flexible films into fish subjected to irradiation.

Conclusions

An analytical methodology for the extraction of additives of medium polarity, such as plasticizers, from complex food matrices was proposed. An effort was made to eliminate the clean-up step, necessary for the separation of the target compound from the co-extracted fat. The application of such an analytical approach allows for (a) the efficient extraction of ATBC plasticizer at ppm levels and (b) the determination of the analyte by GC-FID with no impairment of the column's separation efficiency due to interference of the food matrix. Moreover, the proposed method is simple, rapid, and accurate and fulfills the requirement of applicability to multiple samples simultaneously.

ATBC migration into fillets of cod and herring fish species yielded final concentrations in the respective ranges 11.1–12.8 mg/kg (0.11–0.13 mg/dm^2) and 32.4–33.4 mg/kg (0.32–0.33 mg/dm^2). An issue to be noted is that of public health in relation to the TDI restriction (1.0 mg/kg body weight) based on the latest EFSA evaluation of ATBC. No violations of such restriction were found in the present study. However, in case of food overwrapping with flexible films, the experimentally determined plasticizer concentration appears unrealistically high for the complete product due to a high contact surface area to weight ratio.

By comparing ATBC migration values between irradiated and non-irradiated fish samples packaged in saran cling film, it becomes apparent that intermediate doses ($\leq$10 kGy) of e-beam radiation do not significantly affect the copolymer in terms of its specific migration behavior. Differences observed, probably are due to changes induced in the polymer matrix as a result of the energy transfer. It appears that the disruption of some polymer–plasticizer and/or polymer–polymer bonds, due to irradiation, corresponds to an increase in matrix free volume and thereby in increased additive migration. On the contrary, fat content of the packaged foodstuff significantly affects the additive diffusion rates (due to fat absorption by the polymer matrix), as well as the total migration levels.

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