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Determination of Monounsaturated Alkyl Side Chain 2-Alkylcyclobutanones in Irradiated Foods

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The 2-alkylcyclobutanones (2-ACBs) are formed from triglycerides by irradiation treatment and may be used as markers for this type of food processing. This paper describes a detection method for the analysis of monounsaturated alkyl side chain 2-ACBs, which is formed upon irradiation from monounsaturated fatty acids which frequently are the most abundant fatty acids in foods. The estimated radioproduction yields of the *cis*-2-(dodec-5′-enyl)-cyclobutanones (*cis*-2-dDeCB) and the *cis*-2-(tetradec-5′-enyl)-cyclobutanones (*cis*-2-tDeCB) were 1.0 ± 0.5 and 0.9 ± 0.2 nmol·mmol^{-1} precursor fatty acid·kGy^{-1}, respectively, being similar to that of saturated 2-ACBs. The stability study of the s- and mu-2-ACBs in poultry meat samples irradiated at 10 kGy and stored for 3−4 weeks at 4 °C and 25 °C showed that these compounds undergo some transformation, their amounts being reduced by about 50%. These storage losses did not depend on the saturation state of the alkyl side chain. The EI-MS detection limit of 2-tDeCB is 3 times higher (0.6 pmol) than that of 2-dodecylcyclobutanone (0.2 pmol). Consequently, when the oleic acid content of the analyzed food exceeds the content of palmitic acid by a factor of 3, it would be of an advantage to apply 2-tDeCB as a marker for detection of the irradiation treatment.

KEYWORDS: Monounsaturated alkyl side chain 2-alkylcyclobutanone; detection of irradiated foods; supercritical fluid extraction; food preservation

INTRODUCTION

2-Alkylcyclobutanones (2-ACBs) are formed by radiolysis of triglycerides in foods treated by accelerated electron beams, X-rays, or γ radiation of ^{60}Co or ^{137}Cs. These cyclic compounds present an alkyl chain in position 2, this side chain containing four carbon atoms less than its fatty acid precursor. The main fatty acids in food (palmitic, palmitoleic, stearic, and oleic acids) give rise to the production of 2-dodecyl-, *cis*-2-(dodec-5′-enyl)-, 2-tetradecyl-, and *cis*-2-(tetradec-5′-enyl)-cyclobutanones (2-dDCB, *cis*-2-dDeCB, 2-tDCB, and *cis*-2-tDeCB, respectively). Although trace amount of trans fatty acids may be present in processed oil, the monounsaturated fatty acids are naturally occurring in cis form; therefore, it is the cis form of the monounsaturated alkyl side chain cyclobutanones (mu-2-ACBs) which is mainly formed. Up to date, the 2-ACBs has never been detected in nonirradiated foods treated by other food processes such as freezing, heating, microwave heating, UV irradiation, high-pressure processing, or simple preservation treatments (*1*−*3*).

The saturated alkyl side chain 2-ACBs (s-2-ACBs) were largely studied (*1*, *2*, *4*−*12*) for the detection of irradiated food. The European Committee for Standardization (CEN) published as European standard the EN 1785 analytical method for the detection of these saturated compounds (*11*). Recently, it was proposed to use the unsaturated 2-tDeCB, presumably the most abundant 2-ACB (induced from oleic acid, frequently the most abundant fatty acid in food), as an additional irradiation marker (*13*−*16*). Until now, no interlaboratory studies have been organized to validate the use of this mu-2-ACB for food irradiation detection, and only few studies have been designated to the analysis of this compound (*3*, *13*).

Crone et al. (*3*) did not have unsaturated standards at their disposal but detected the presence of 2-tDeCB in triolein irradiated in a vacuum at the very high dose of 52 kGy. On the basis of the spectral and chromatographic characteristics of the isolated compound, they were able to detect the 2-tDeCB in eggs irradiated at 10 kGy (extraction and analyses according to a similar protocol as EN 1785). Formal identification of this

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compound was realized by hydrogenation of extracts under pressure, leading to the formation of the corresponding s-2-ACB and therefore to the increase of 2-tDCB content. This saturated compound was easily identified by comparison of its mass spectra and retention time with those of the available standard.

Hamilton et al. (*17*) have synthesized the pure cis isomer standard of 2-tDeCB and were able, using the EN 1785 method, to detect this compound in poultry meat irradiated at 2.5 kGy and also in papaya pips irradiated at the very low dose of 0.11 kGy for commercial use to prevent ripening of the fruit (*18*). However, the authors monitored the ions 165, 179, 236, and 264 $m \cdot z^{-1}$ only and did not present the mass spectra of the peak attributed to the *cis*-2-tDeCB in the irradiated samples. However, they presented a chromatogram of nonirradiated samples presenting no peak at the retention of the standard. Quantitative results and radioproduction yields were not given. Rahman et al. (*19*) reported the presence of 2-tDeCB traces in Brie and Camembert cheeses irradiated at 3.5 kGy. However, the presented chromatograms did not support the detection of 2-tDeCB. This compound should elute at a shorter retention time than the 2-tDCB on the CPSIL-8 capillary column used in this study (whereas the authors indicated that the peak attributed to the 2-tDeCB is located 2 min after its saturated alkyl chain homologue), and the exact identification of 2-tDeCB by its mass spectra was not presented. Lee et al. (*13*) detected 2-tDeCB in perilla seeds (*Perillae frutescentis*) treated at 0.5 kGy and above, but the presented chromatograms gave evidence of important coelution problems giving rise to a bad resolution of the peak attributed to the 2-tDeCB. Gadgil (*16*) did not detect any 2-tDeCB in beef patties irradiated at doses below 5 kGy despite the substantial amount of precursor oleic acid in beef fat. In addition, the presented chromatogram of the sum of ions 98, 165, 236, and 264 is highly charged at the domain of elution of 2-tDeCB (high baseline level, elution of large peak nearby and an important shoulder at the retention time of 2-tDeCB). These authors reported a 5 times higher detection limit by monitoring the former ions for 2-tDeCB (0.25 ppm) than for 2-dDCB (0.05 ppm). Stewart et al. (*15*) observed that in irradiated mangoes twice as much 2-tDCB as 2-tDeCB was formed which did not reflect the ratio of stearic to oleic acid in the studied foods. In irradiated papayas, relatively more 2-tDeCB than 2-dDCB was formed. In addition, at lower radiation doses, identification of 2-tDeCB seemed only possible using the molecular ion $m \cdot z^{-1}$ 264. Therefore, the authors concluded that relying on only one ion is not good practice. Obviously, considerable analytical difficulties were encountered by laboratories in the detection of 2-tDeCB in irradiated food samples. This is certainly the reason this compound is until now not included in the EN 1785 standard.

The main objective of this work is the development of a selective and accurate detection and quantification method for mu-2-ACBs. The radioproduction yield, the stability during storage, and the detection sensitivity of s-2-ACBs and mu-2-ACBs will be compared to determine the most sensitive marker for the detection of irradiated food.

MATERIALS AND METHODS

Chemicals. The 2-ACB standards [2-(undec-5′-enyl)- (2-uDeCB), 2-undecyl (2-uDCB), 2-(dodec-5′-enyl)- (2-dDeCB), 2-dodecyl- (2-dDCB), 2-(tetradec-5′-enyl)- (2-tDeCB), and 2-tetradecylcyclobutanone (2-tDCB)] were synthesized according to the method of Miesch et al. (*20*). The standard of mu-2-ACBs was a mixture of 75% cis and of 25% trans isomers (in the irradiated food samples, however, only the *cis* form of mu-2-ACBs was detected). The *n*-hexadecanal was synthesized with the following method: the pyridinium chlorochromate (PCC, Sigma Aldrich, Saint Quentin, France) (5.0 g; 23.0 mmol) was added at room temperature to 1-hexadecanol (4.0 g; 16.0 mmol, Sigma Aldrich) in dichloromethane (80 mL) (Carlo Erba, Rodano, Italy). After 3 h of stirring at room temperature, the reaction mixture was diluted with diethyl ether (200 mL) and filtered on Celite (SDS, Peypin, France). After solvent evaporation (25 °C; 15 mmHg), the reaction residue was chromatographed (80 g SiOH 40−63 μm [Merck, Darmstadt, Germany]; *n*-hexane) which allowed the isolation of the *n*-hexadecanal (3.1 g; 12.6 mmol; 78%). The same procedure was used for the synthesis of *n*-octadecanal starting from 1-octadecanol (4.3 g; 16.0 mmol, Sigma Aldrich).

The *tert*-butyl methyl ether (TBME) was a Merck product. Methanol of HPLC grade was supplied by Carlo Erba. *n*-Hexane, of technical quality, was distilled from calcium hydride (Lancaster Synthesis, Morecambe, United Kingdom). Its purity was checked by gas chromatography. The carbon dioxide, of 99.9999% purity with a 142 bar helium headspace and pickup tube, was purchased from Air Product and Chemicals (Allentown, PA). Silica gel of 63−200 μm (i.e., 70−230 mesh) (Merck) was heated at 100 °C overnight and then was allowed to cool to room temperature and was deactivated by addition of ultrapure (Milli Q+, Millipore, Bedford, MA) water in proportion of 4 mL of water to 100 g of silica gel. The activated silica was stored (not more than 1 week) in a stoppered flask in a desiccator at room temperature. Sodium sulfate was purchased from SDS, heated at 550 °C (5 h), and allowed to cool to room temperature in a desiccator before use. The hydromatrix (Varian, Palo Alto, CA) was washed (6 h Soxhlet extraction with *n*-hexane) prior to use.

Preparation of Food Samples. The liquid whole eggs were supplied by a French food company. Sheep's cheese, poultry meat (breast), and avocados were purchased in a local supermarket. The foodstuffs were sliced (5-mm-thick layer) and packaged in the presence of air in plastic bags (multilayer ACX, AFP CENPA), thermosealed, stored at −20 °C, and thawed immediately prior to irradiation.

Irradiation Treatment and Dosimetry. A Van de Graaff electron beam accelerator, 2.2 MeV, 75 μA (Vivirad High Voltage, Handschuheim, France), located in the Regional Centre of Innovation and Technology Transfer Aérial (Illkirch, France) was used for the irradiation treatments. The ionizing radiation treatments (100 kGy for sheep's cheese; 0.5, 1, 3, and 5 kGy for liquid whole eggs; 0.1, 0.5, and 1 kGy for avocados; and 0.5, 1, 3, and 10 kGy for poultry meat) were performed at 6−8 °C. Irradiation doses were monitored with FWT 60.00 radiochromic dosimeters (Far West Technology, Goleta, CA), previously calibrated with an alanine dosimeter (Laboratoire National Henri Becquerel, Gif-sur-Yvette, France). Dose uniformity of about ±10% within the sample was achieved by the use of a 100-μm-thick copper scattering foil (*21*). All the food samples were stored in plastic bags at −20 °C after the radiation treatment until the final analysis.

Sample Preparation for the Stability Study of the 2-ACBs in Irradiated Poultry Meat. A 30-g sample (weighed exactly) was taken out of 400 g of poultry meat irradiated at 10 kGy. The sample was immediately analyzed (in triplicate). The remainder (370 g) was divided into two equal parts. Each part was transferred to a plastic bag which was thermosealed. One bag was stored at 4 °C, and the second was stored at 25 °C. Sampling (30 g) was regularly carried out (every 7 days during 21 and 28 days for temperature storage of 25 and 4 °C, respectively) from these bags (immediately re-thermosealed).

Supercritical Fluid Extraction and Purification on a Silica Trap. Before analysis, the food samples were thawed, homogenized, cooled to −80 °C, and lyophilized overnight (100 mbar) by means of a Virtis lyophilizer (New York, New Jersey), equipped with an Alcatel rotary vane vacuum pump (Maurepas, France) and a cryogenic trap (−60 °C). Two hundred microliters of a *n*-hexane solution of 2-uDCB and 2-uDeCB (1 $\mu g \cdot mL^{-1}$) as internal standards was added to the sample prior to extraction. The extraction procedure was the same as previously proposed by Horvatovich et al. (*12*).

EN 1785 Protocol. The official method is described in the European Standard EN 1785 (*11*).

Gas Chromatography. The fractions containing 2-ACBs were analyzed by Varian chromatograph (type 3400) coupled with an ion trap mass sensitive detector (Saturn 2000, Varian). The gas chromato-

graph was fitted with an OV-20-MS capillary column (Ohio Valley, Marietta, OH), 60 m, 0.25-mm i.d. with a 0.10-μm stationary phase (20% diphenyl-, 80% dimethylpolysiloxane) or with a ZB-5-MS (Zebron, Torrance, CA), 30 m, 0.25-mm i.d. with a 0.10-μm stationary phase (5% diphenyl-, 95% dimethylpolysiloxane). The GC was equipped with a septum-equipped temperature-programmable injector (SPI), which the initial temperature (50 °C, held for 0.1 min) was followed by a 230 °C·min^{-1} increase to 240 °C, and the final temperature was held until the end of the column temperature program. The SPI injector was cooled between runs by CO_2 (quality 99.9%, Air Liquide, Paris, France). The column temperature program was as follows: 60 °C (held for 2 min) followed by an 8 °C·min^{-1} increase to 300 °C, final temperature hold for 15 min. The injection volume was 1 μL. The carrier (1 mL·min^{-1} flow) was helium (99.9995% purity, Air Liquide, Paris, France). The mass spectrometer was operating in electron impact ionization mode (emission current: 10 μA; axial modulation voltage: 4.0 V, AGC: 20 000 ions; trap, manifold, and transfer line temperature, respectively, 150, 40, and 270 °C; electron-multiplier voltage: 1800 V). The mass spectra were recorded in full scan mode between 50 and 300 m·z^{-1}. For the quantitative determination of mu-2-ACBs, the internal standard of 2-uDeCB was used by choosing the ion trace of sum of ions m·z^{-1} 95 and 98. Concerning the s-2-ACBs, the 2-uDCB and the ion m·z^{-1} 98 were used as internal standard and ion trace.

Determination of Fatty Acid Composition. About 6 mg (exactly weighed) tripentadecanoin (internal standard) was added to the lipids to be analyzed (~100 mg, exactly weighed). The various fatty acids obtained by hydrolysis of triglycerides, followed by derivatization to methyl esters (*22*), were separated using the previously reported ZB-5-MS column and were quantified by GC-FID (*23*).

Radioproduction Yield Determination. To estimate the radioproduction yield of *cis*-2-dDeCB, 2-dDCB, *cis*-2-tDeCB, and 2-tDCB, three different irradiated foods (liquid whole eggs, poultry meat, avocados) were studied. The analysis was performed by supercritical fluid extraction of lyophilized samples similarly as described by Horvatovich et al. (*12*) for the analysis of s-2-ACBs using the OV-20-MS capillary GC column. The radioproduction yield of 2-ACBs was calculated as the slope gradient of the molar quantity of 2-ACBs divided by the molar quantity of precursor fatty acids versus the adsorbed doses as described by Ndiaye et al. (*1*). The quantification was corrected by taking into account the recovery rate for each compound, respectively. The statistical evaluations were performed with the Student *t*-test (*t* 95% confidence).

RESULTS AND DISCUSSION

Improved Chromatographic Resolution. The first aim of this study was to increase the chromatographic resolution of GC-MS analysis used in the analytical EN 1785 method to get a better separation of the mu-2-ACBs allowing the unambiguous identification in irradiated foods by comparison of their mass spectra and chromatographic parameters with those of authentic synthesized standards.

The analysis of mu-2-ACBs in food was first performed on liquid whole egg samples treated at 3.0 kGy. The obtained chromatogram using the extraction conditions and chromatographic separation suggested for the analysis of s-2-ACBs (*1*, *11*, *12*) (in particular the use of a 30-m-length GC capillary column of type ZB-5-MS, 5% phenyl, 95% dimethylpolysiloxane generally used for this type of analysis) was not satisfactory (**Figure 1a**). The presence of impurities in the obtained extract, with retention times identical to those of *cis*-2-dDeCB and *cis*-2-tDeCB, troubled the detection. The mass spectrum (**Figure 1c**) of the peak attributed to the *cis*-2-tDeCB, compared against the mass spectrum of the standard (**Figure 1d**), showed the reduction of the intensity of the ions 95 and 98 m·z^{-1}, the increase in the intensity of ion 96 m·z^{-1}, and a decrease in the intensity of ion 165 m·z^{-1} and of the molecular ion (m·z^{-1} 264). The same kind of observation was made with the attributed peak

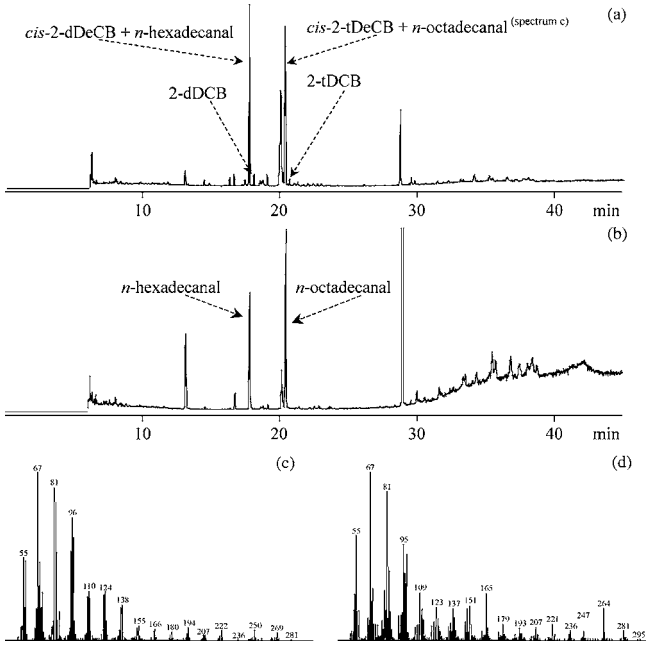

Figure 1. Chromatograms obtained for the detection of mu-2-ACBs (ion trace of sum of ions 95 and 98 m·z^{-1}) in liquid whole egg samples irradiated at 3.0 kGy (**a**) and in nonirradiated samples (**b**) with addition of mass spectra of the peak attributed to the *cis*-2-tDeCB (**c**) and of the pure *cis*-2-tDeCB standard (**d**). The mass spectra of the chromatographic peaks are indicated in the label of the peaks with the corresponding letter. Extraction protocol: supercritical carbon dioxide (152 bar, 80 °C, 30 min, 2 mL·min^{-1}) and solid-phase extraction on silica column (3 g). GC capillary column: ZB-5-MS, 30 m.

of *cis*-2-dDeCB and the pure standard. These results indicate that the chromatographic peak attributed to the mu-2-ACBs corresponded in fact to a superposition of the spectra of the expected 2-ACB and of some impurities which were identified after separation, by the comparison of their spectral and chromatographic data with authentic synthesized reference compounds, as being *n*-octadecanal and *n*-hexadecanal. It is well-known that these aldehydes are intermediates of oxygen-induced radical lipid degradation compounds, thus occurring naturally in all types of foods. It was thus not surprising that these impurities were already present in nonirradiated liquid whole eggs (**Figure 1b**), proving the nonexclusive radiolytic origin of these compounds. These impurities were also found in all other studied food, irrespectively of the use of the European Standard EN 1785 (*11*) method or of the supercritical fluid extraction method presented below. It is therefore highly probable that these aldehydes also interfered with the detection of *cis*-2-tDeCB when other authors analyzed mu-2-ACBs applying the EN 1785 method using a 30-m-long chromatographic column containing 5% diphenyl- and 95% dimethylpolysiloxane as stationary phase (e.g., DB-5 or ZB-5 columns).

To avoid this interference, an improvement of the chromatographic resolution was realized by replacing the GC capillary column usually used for the analysis of 2-ACBs (ZB-5-MS) by a slightly more polar (OV-20-MS, 20% phenyl, 80% dimethylpolysiloxane) and longer (60 m) column. This capillary column resulted in a good separation of the *cis*-2-dDeCB and the *cis*-2-tDeCB from their corresponding impurities (**Figure 2a**), and the identification of these compounds by mass spectra (**Figure 2b**) was now unambiguous. In addition, no peak was observed at the retention time of the mu-2-ACBs in nonirradiated samples. Furthermore, using these chromatographic conditions the detection of s-2-ACB compounds on the same

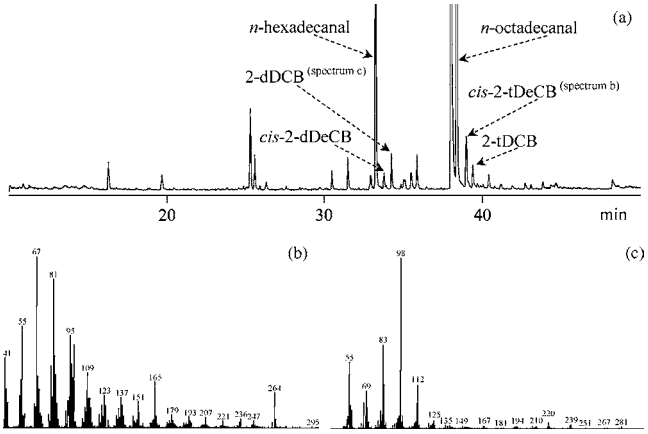

Figure 2. Chromatogram obtained for the detection of mu-2-ACBs (ion trace of sum of ions 95 and 98 m·z^{-1}) in liquid whole egg samples irradiated at 3.0 kGy (**a**) with addition of mass spectra attributed to *cis*-2-tDeCB (**b**) and to 2-dDCB (**c**). The mass spectra of the chromatographic peaks are indicated in the label of the peaks with the corresponding letter. Extraction protocol: supercritical carbon dioxide (152 bar, 80 °C, 30 min, 2 mL·min^{-1}) and solid-phase extraction on silica column (3 g). GC capillary column: OV-20-MS, 60 m.

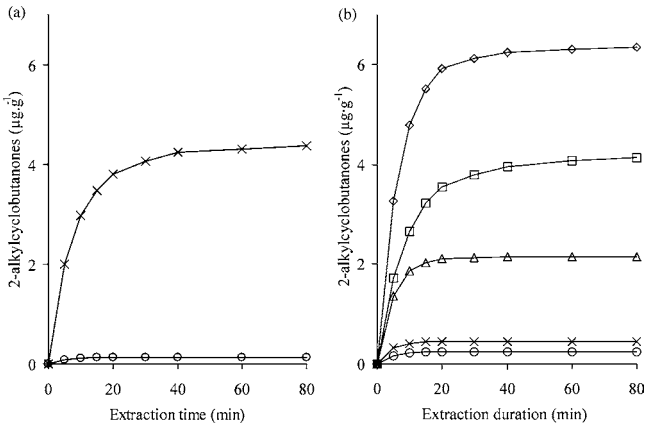

Figure 3. Extraction kinetics of s-2-ACBs and mu-2-ACBs present in 1 g of lyophilized sheep cheese irradiated at 100 kGy using supercritical fluid carbon dioxide (152 bar; 80 °C; 2 mL·min^{-1}): *cis*-2-dDeCB (–○–) and *cis*-2-tDeCB (–×–) (**a**), and 2-HCB (–○–), 2-OCB (–×–), 2-DCB (–△–), 2-DdCB (–◇–), and 2-TCB (–□–) (**b**). **Figure 3b** reprinted with permission from ref *12*. Copyright 2000 Elsevier.

chromatogram was also possible as shown by the mass spectra of the peak attributed to 2-dDCB (**Figure 2c**).

To establish a more selective and rapid analytical method for the analysis of mu-2-ACBs, the supercritical fluid extraction method previously developed (*12*) was used.

Extraction and Purification by SFE and EN1785. It was first necessary to verify if the extraction conditions recommended by Horvatovich et al. (*12*) for the detection and quantification of s-2-ACBs are suitable to the analysis of mu-2-ACBs in food matrix. One gram of lyophilized sheep's cheese sample [39% (wt:wt) lipid content and numerous unsaturated fatty acids in appreciable concentrations] irradiated at high dose (100 kGy) was applied to obtain a high concentration of mu-2-ACBs in the test sample. This high concentration was necessary for adequately following the kinetics of the supercritical fluid extraction. The extracted amount of mu-2-ACBs was quantified at increasing time intervals. The obtained kinetic curves (**Figure 3a**) were of the same shape as those presented previously for the extraction of s-2-ACBs (**Figure 3b**, *12*). This

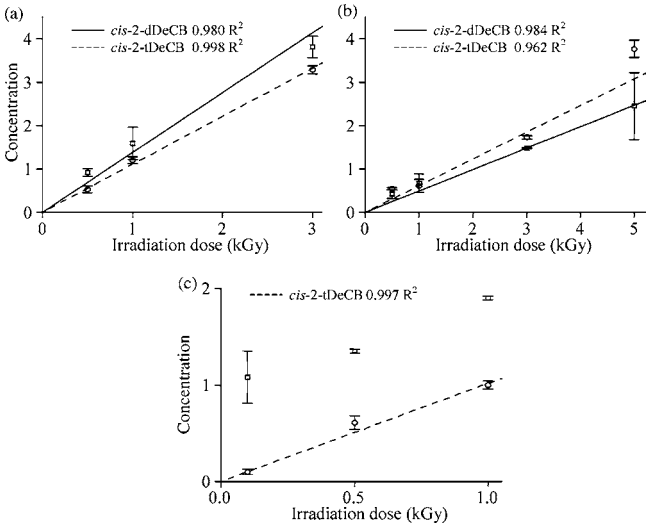

Figure 4. Concentrations of *cis*-2-dDeCB and *cis*-2-tDeCB (in nmol·mmol^{-1} precursor fatty acid) in poultry meat (**a**), liquid whole egg (**b**), and avocado (**c**) samples as a function of the adsorbed doses (in kGy): *cis*-2-dDeCB (–□–) and *cis*-2-tDeCB (–○–). The correlation coefficients (R^2) are presented in the diagrams. The gradient of the slopes as radioproduction yield and the standard deviation are presented in **Table 1**.

Table 1. Radioproduction Yield of mu-2-ACBs and s-2-ACBs (in nmol·mmol^{-1} Precursor Fatty Acid·kGy^{-1}) in Poultry Meat, Liquid Whole Egg, and Avocado Samples ($n = 3$ for Each Dose, SD in Parentheses)

2-ACB	chicken	liquid whole eggs	avocados	average
cis-2-dDeCB	1.3 (0.2)	0.6 (0.1)	[a]	1.0 (0.5)
cis-2-tDeCB	1.0 (0.1)	0.7 (0.1)	1.0 (0.1)	0.9 (0.2)
2-dDCB	1.1 (0.1)	1.2 (0.1)	1.7 (0.2)	1.2 (0.1)
2-tDCB	1.3 (0.2)	1.8 (0.1)	[a]	1.5 (0.4)

[a] Not quantifiable.

proved that the solubility in supercritical carbon dioxide of mu-2-ACBs was near to those of s-2-ACBs for the studied conditions of extraction.

The optimal time required for the complete extraction was about 30−40 min, although the polarities of *cis*-2-dDeCB and *cis*-2-tDeCB are slightly higher than their respective saturated alkyl side chain homologues. In addition, the complete elution of *cis*-2-dDeCB and *cis*-2-tDeCB deposed on silica trap after extraction was achieved in the same fraction as the s-2-ACBs [20 mL of *n*-hexane/TBME 1% (v/v)] (*12*). The recovery rate for *cis*-2-dDeCB and *cis*-2-tDeCB was 80 ± 6% and 82 ± 7%, respectively. These values were consistent with those (89% for *cis*-2-tDeCB) presented by Gadgil (*16*) and those observed using the same method for the analysis of 2-dDCB (75 ± 8%) and 2-tDCB (82 ± 8%) (*12*).

The extracts obtained by the official method EN 1785 (presenting sometimes more charged chromatograms) presented mu-2-ACBs and s-2-ACBs in the same fraction and can be analyzed in one single chromatogram similarly to the extracts obtained by the SFE method. The recovery rate obtained with the EN 1785 method was 92 ± 5% for *cis*-2-dDeCB and 95 ± 3% for *cis*-2-tDeCB, slightly greater than those obtained with the SFE method.

Radioproduction Yield. The concentrations of *cis*-2-dDeCB and *cis*-2-tDeCB, as a function of the adsorbed doses, showed a linear relation (the correlation coefficient R^2 was always between 0.96 and 0.99, **Figure 4**) except for the value obtained for *cis*-2-dDeCB in avocados (**Figure 4c**). This is certainly due

Table 2. Effect of Storage Duration (up to 28 Days at 4 °C and 21 Days at 25 °C) on the Concentration ($\mu g \cdot g^{-1}$ of Lyophilized Poultry Meat) of 2-dDCB, *cis*-2-dDeCB, 2-tDCB, and *cis*-2-tDeCB in 10 kGy Irradiated Poultry Meat[a]

25 °C	0	7	14	21	28	losses
cis-2-dDeCB	0.591 (0.004)	0.360 (0.028)	0.273 (0.061)	0.252 (0.037)	n.p.[c]	57% (6)
2-dDCB	2.211 (0.263)	1.432 (0.078)	1.107 (0.183)	1.044 (0.053)	n.p.	53% (2)
cis-2-tDeCB	2.744 (0.082)	2.315 (0.138)	1.861 (0.459)	1.630 (0.310)	n.p.	41% (11)
2-tDCB	0.740 (0.089)	0.537 (0.035)	0.427 (0.069)	0.348 (0.020)	n.p.	53% (3)
4 °C	0	7	14	21	28	losses
cis-2-dDeCB	0.591 (0.004)	0.339 (0.043)	0.257 (0.005)	0.219 (0.007)	0.207 (0.016)	65% (3)
2-dDCB	2.211 (0.263)	1.594 (0.179)	1.052 (0.021)	1.124 (0.265)	1.159 (0.100)	46% (5)
cis-2-tDeCB	2.744 (0.082)	2.756[b] (0.207)	2.195 (0.064)	2.180 (0.023)	1.882 (0.165)	31% (6)
2-tDCB	0.740 (0.089)	0.683[b] (0.100)	0.450 (0.047)	0.363 (0.101)	0.456 (0.021)	38% (3)

[a] The last column shows the total 2-ACB losses in percentage of the initial value. The standard deviations are in parentheses (triplicate samples). [b] Are not significantly different from the initial value. [c] n.p.: not performed.

to the greater quantification error on the small peaks obtained because of the very low palmitoleic precursor fatty acid concentration in this fruit. The existence of this linear relation was already reported by Crone et al. (*2*, *5*), Stevenson et al. (*8*), and Ndiaye et al. (*1*) for the formation of s-2-ACBs in various foods (e.g., poultry meat, sardine, mangos, beef, etc.). This linear relation allows the calculation of the 2-ACB radioproduction yield in foods which express the molar production of 2-ACB per mole of precursor fatty acid and per kGy of adsorbed doses (*1*).

The average values of the radioproduction yield of the mu-2-ACBs (0.9 ± 0.3 nmol·mmol^{-1} of precursor fatty acid·kGy^{-1}) and the s-2-ACBs (1.4 ± 0.4 nmol·mmol^{-1}·kGy^{-1}) are not statistically different within 5% of the confidence interval (**Table 1**). The average radioproduction values obtained for the *cis*-2-dDeCB, *cis*-2-tDeCB, 2-dDCB, and 2-tDCB were 1.0 ± 0.5, 0.9 ± 0.2, 1.2 ± 0.1, and 1.6 ± 0.4 nmol·mmol^{-1} of precursor fatty acid·kGy^{-1}, respectively, values being not statistically different within the 5% of the confidence interval. These values remained consistent with the one (1.3 ± 0.2 nmol·mmol^{-1}·kGy^{-1}) found by Ndiaye et al. (*1*) for the s-2-ACBs using the EN 1785 method in cheese, sardine, trout, beef, and poultry meat irradiated between 0.1 and 3.1 kGy.

Stability of mu-2-ACBs. The stability study was performed using poultry meat samples irradiated at 10 kGy and stored during 28 and 21 days, respectively, at +4 °C and +25 °C to compare the stability of *cis*-2-tDeCB and *cis*-2-dDeCB against the corresponding s-2-ACBs (2-tDCB and 2-dDCB). The determined quantity of mu-2-ACBs and s-2-ACBs during storage showed that these compounds undergo some transformation, their amounts being reduced by about 50%. Moreover, this degradation did not significantly depend on the saturation state of the alkyl side chain (**Table 2**). Curiously, *cis*-2-tDeCB even appeared to be more stable than 2-tDCB, especially during storage at +4 °C. At room temperature, the eventual bacterial proliferation (contamination of the irradiated sample occurred when the plastic bags were opened for sampling) and the fat rancidation did not induce any additional significant decrease of the 2-ACB content. The degradation of *cis*-2-tDeCB and *cis*-2-dDeCB during the prolonged storage at elevated temperature cannot be ascribed to the peroxidation phenomena of the monounsaturated alkyl side chain as there are no significant degradation differences between the saturated and monounsaturated compounds. This degradation may be explained with the oxidation of the cyclobutanone part, producing the respective 4-alkyl-γ-butyrolactone (*24*). Stewart et al. (*15*) reported incoherent results about the reduction of 2-dDCB, 2-tDCB, and *cis*-2-tDeCB concentration in exotic fruits stored during 2 weeks at 10 °C. Although losses in mangoes (22%, *cis*-2-tDeCB and 4%, 2-tDCB) are consistent with our findings, those in papayas (72%, *cis*-2-tDeCB and 94%, 2-dDCB) are strongly inconsistent from ours. Concerning the s-2-dDCB, our observed losses were consistent with those already reported by Crone et al. (*2*) (−19%) and Boyd et al. (*4*) (−26%) for poultry meat samples irradiated at 5 kGy and preserved at +4−5 °C during 20 days. Ndiaye et al. (*1*) have also reported a reduction of −78 to −21% of the concentrations of 2-hexyl-, 2-octyl-, 2-decyl-, 2-dodecyl-, and 2-tetradecyl-cyclobutanones during 1 month of storage in the presence of air (the longer the alkyl chain, the lower the losses) at 4 °C in poultry meat, sheep's cheese, and sardine irradiated at 3 kGy and at 20 °C for mango irradiated at 2 kGy.

Use of the mu-2-ACBs as Indicators of Irradiated Foods. The most abundant fatty acid in food is mostly oleic acid, which is the precursor of *cis*-2-tDeCB. It is 1.3−1.8 times more abundant in sheep's cheese and poultry meat and up to 4 times more abundant in liquid whole eggs, avocados, and papaya pips than in palmitic acid (precursor of 2-dDCB), which is mainly the most abundant saturated fatty acid. Our results indicate that the radioproduction yield and the stability during storage of both classes of 2-ACBs are similar; thus, the choice between *cis*-2-tDeCB and 2-dDCB, for the most sensitive marker for the detection of irradiated food, will depend on their detection sensitivity. *cis*-2-tDeCB presents a more intensive fragmentation pattern when compared with 2-dDCB (**Figure 2b** and **2c**, respectively) resulting in an important reduction of the 98 m·z^{-1} ion intensity. The limit of detection of the mu-2-ACB was consequently 5 times higher than that for s-2-ACB when monitored with the 98 m·z^{-1}, which is specific and is used for the chromatographic analysis of s-2-ACBs (**Figure 2c**). For this reason, the detection and quantification of the mu-2-ACB was realized using the sum of ions 95 and 98 m·z^{-1}. The detection limit is still 3 times higher for *cis*-2-tDeCB (0.6 pmol) by monitoring this sum of ions than when monitoring only the ion 98 m·z^{-1} for the detection of 2-dDCB (0.2 pmol) using a mass spectrometric detector set in electronic impact ionization mode. Additionally, this setting also resulted in a slight deterioration of the detection selectivity. Thus, the detection of *cis*-2-tDeCB should only be preferred over 2-dDCB when the concentration of its precursor oleic acid is at least 3 times higher than that of palmitic acid (e.g., liquid whole eggs, avocados, papaya seeds). For other foods, more likely the detection of mu-2-ACB may only be used as a confirmation of the detection of the s-2-ACB.

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