

Determination of 2-Alkylcyclobutanones with Electronic Impact and Chemical Ionization Gas Chromatography/Mass Spectrometry (GC/MS) in Irradiated Foods

PETER HORVATOVICH,[†] DALAL WERNER,[‡] STEPHANIE JUNG,[†] MICHEL MIESCH,[§]
HENRY DELINCEE,^{||} CLAUDE HASSELMANN,[†] AND ERIC MARCHIONI^{*,†}

Laboratoire de Chimie Analytique et Sciences de l'Aliment (LC4, UMR 7178), Faculté de Pharmacie, Université Louis Pasteur, 74, route du Rhin, 67400 Illkirch, France, Aérial, rue Laurent Fries—Parc d'Innovation, 67412 Illkirch, France, Laboratoire de Chimie Organique Synthétique (LC3, UMR 7177), Faculté de Chimie, Université Louis Pasteur, 1, rue Blaise Pascal, 67000 Strasbourg, France, and Institute of Nutritional Physiology, Federal Research Centre for Nutrition and Food, Haid-und-Neu-Strasse 9, D-76131 Karlsruhe, Germany

The use of a column containing 60 g of silica gel for cleanup and the use of isobutane as a reactant reagent for chemical ionization—mass spectrometric analysis of the saturated and monounsaturated alkyl side-chain 2-alkylcyclobutanones (2-ACBs; specifically induced by irradiation from fat in foods until the proof of contrary) has improved both the sensibility and the selectivity of the method when applied for the detection of irradiated foods. The quality of the chromatograms obtained was improved, allowing the detection of food samples (avocados) irradiated at low doses (0.1 kGy) or irradiated ingredients included in low proportions (less than 5%, wt/wt) in nonirradiated culinary foods. These analytical modifications for the detection of 2-ACBs on the official EN 1785 method enable an extension of its current field of application using common equipments of food quality control laboratories.

KEYWORDS: Irradiated food; 2-alkylcyclobutanone; mass spectrometry; electronic impact ionization; chemical ionization

INTRODUCTION

Food irradiation is used to reduce bacterial contamination (1–10 kGy), to disinfest food products (0.15–0.5 kGy), to slow the ripening of fruits and vegetables (0.5–1.0 kGy), and to inhibit the sprouting of bulbs and tubers (0.05–0.15 kGy) (1). This treatment is wholesome (1–3) and improves the hygienic quality of the foodstuffs. Since 1996, the European Committee for Standardization (CEN) has published 10 official protocols for the detection of irradiated foods (4–13), thus enabling food quality control laboratories to distinguish between an irradiated and nonirradiated foodstuff. These tests now allow for a control of the international trade with respect to the appropriate legal labeling, thus giving consumers a guarantee of freedom of choice.

The 2-alkylcyclobutanones (2-ACBs) are formed by radiolysis of triglycerides in foods treated by accelerated electron beams, X-rays, or γ radiation (⁶⁰Co or ¹³⁷Cs). These cyclic compounds with a ring of four carbon atoms have the same carbon number

as the precursor fatty acid. They have a keto group at ring position 1 and an alkyl chain located in ring position 2. The primary fatty acids in food (capric, lauric, myristic, palmitic, palmitoleic, stearic, and oleic acids) give rise to the production of 2-hexyl-, 2-octyl-, 2-decyl-, 2-dodecyl-, *cis*-2-dodec-5'-enyl-, 2-tetradecyl-, and *cis*-2-tetradec-5'-enyl-cyclobutanones (2-HCB, 2-OCB, 2-DCB, 2-dDCB, *cis*-2-dDeCB, 2-tDCB, and *cis*-2-tDeCB, respectively). Up to now, these compounds were found exclusively in irradiated foods and have until now 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 (14–16).

The EN 1785 method was validated for the analysis of the saturated alkyl side chain 2-ACBs (s-2-ACBs). This method comprises three main steps of analysis [fat extraction with *n*-hexane using a Soxhlet apparatus, isolation of 2-ACBs from approximately 200 mg of fat by adsorption chromatography on 30 g of Florisil, and chromatographic separation and detection by gas chromatography—mass spectrometry functioning in electronic impact ionization mode (GC—EI—MS)] (8). This method can be used for the detection of foods irradiated at doses above 0.5 kGy (pasteurization) and containing at least 1 g of fat/100 g of food (1 g %, wt/wt) (14).

However, the official protocol cannot be used to detect foods

* To whom correspondence should be addressed. Telephone: 33-(0)3-90-24-43-26. Fax: 33-(0)3-90-24-43-25. E-mail: eric.marchioni@pharma.u-strasbg.fr.

[†] Laboratoire de Chimie Analytique et Sciences de l'Aliment, Université Louis Pasteur.

[‡] Aérial.

[§] Laboratoire de Chimie Organique Synthétique, Université Louis Pasteur.

^{||} Federal Research Centre for Nutrition and Food.

irradiated at lower doses or nonirradiated foods containing a low quantity of irradiated ingredients. An improvement of this method was attempted using supercritical fluid extraction with carbon dioxide coupled on-line to a small column containing 3 g of silica gel to simplify and decrease the cost and duration of the sample preparation (17). The use of the supercritical fluid extraction resulted in a cleaner extract and had a slightly lower detection limit, thus offering approximately the same application field as the official EN 1785 method but saving time and material costs.

Ndiaye et al. (18) accumulated up to four extracts obtained by the EN 1785 method before the final chromatographic analysis on a silver ion impregnated cation exchanger SPE column. The accumulated sample was then fractionated and analyzed by GC-MS. Using this method, these authors were able to successfully detect the irradiated ingredients in 100 g of chicken quenelles containing 2 g of mechanically recovered meat (2 g %, wt/wt, MRM) irradiated at 5 kGy or in 100 g of cookies containing 3 g of liquid whole eggs (3 g %, wt/wt) irradiated at 4 kGy. As a single food item irradiated at low dose, unpolished rice treated at 0.1 kGy could also be identified.

The direct application of the supercritical fluid extraction on 2 g of fat previously extracted by Soxhlet apparatus allowed for the successful analysis of a 10 times higher amount of fat than described in the EN 1785 method (19, 20). By these means, even 0.5 g % (wt/wt) MRM irradiated at 5 kGy could be detected in otherwise nonirradiated chicken quenelles (20). Using a longer GC column (60 m) for better removal of impurities in the extract, the detection limit of 2-dDCB in cowpeas and rice could be improved to 50 Gy (19). Both methods reduced the detection limit by analyzing higher amounts of sample, requiring a longer analysis time, and using more toxic solvent. In addition, they required the use of expensive laboratory material like a supercritical fluid extractor, which is not common equipment of the most food control laboratories.

Recently, Horvatovich et al. (21) showed that the EN 1785 or the supercritical fluid extraction method can also be used for the detection of monounsaturated alkyl side chain 2-ACBs (μ -2-ACBs) by the use of a slightly more polar (OV-20-MS) and longer (60 m) GC column allowing for the analysis of the most abundant 2-ACBs in irradiated foods. The most abundant fatty acid in foods is frequently oleic acid, which is the precursor of *cis*-2-tDeCB. On the other hand, *cis*-2-tDeCB is fragmented to a higher degree and has a 3 times higher detection limit than the most abundant *s*-2-ACB, the 2-dDCB in electron impact ionization mode, monitoring the sum of ions of mass/charge ratio (m/z) 95 and 98 for the monounsaturated and monitoring the ion m/z 98 for the saturated compound. For this reason, *cis*-2-tDeCB can only be used as the preferential marker for the detection of irradiated foods in the rare case when the ratio of the oleic acid over palmitic acid is greater than 3.

The primary objective of this study is to find a suitable alternative to the uncommon supercritical fluid extraction but still be able to analyze a larger amount of fat (2 g of fat) than in the EN 1785 method. This was attempted by using a larger silica column. The second objective is to lower the detection limit and enhance the specificity by using isobutane as a reactant reagent for chemical ionization mass spectrometry (CI-MS) (22). These attempts could enhance the field of application, but nevertheless, endeavoring us to perform the analysis within a relatively short time and using common equipment available in food control laboratories. Furthermore, the main mass spectroscopic fragmentation pattern of μ -2-ACBs and *s*-2-

ACBs in both EI- and CI-MS will be proposed, and the detection limit of both methods will be compared.

MATERIALS AND METHODS

Chemicals. The 2-ACB standards (2-HCB, 2-OCB, 2-DCB, 2-uDCB, 2-dDeCB, 2-dDCB, 2-tDeCB, and 2-tDCB) were synthesized as described by Miesch et al. (23). The standards of μ -2-ACBs were mixtures of 75% *cis* and 25% *trans* isomers (in the real sample, however, only the *cis* form of μ -2-ACBs was detected). Dilutions (5,000, 2,500, 1,250, 0,500, 0,250, 0,125, 0,05, 0,025, 0,010, and 0,006 μ g mL⁻¹) were prepared in isooctane (Merck, Darmstadt, Germany) for the determination of the limit of detection. A total of 200 μ L of standard mixture of 2-ACBs (2-HCB, 2-OCB, 2-DCB, 2-dDCB, 2-dDeCB, and 2-tDeCB) was used in the dilution of 5 μ g mL⁻¹ in isooctane to determine the optimal elution condition of 2-ACBs on the silica column. *n*-Hexane of technical quality was distilled over calcium hydride (Lancaster Synthesis, Morecambe, U.K.) before use, and its purity was verified by GC. The silica gel (63–200 μ m, 70–230 mesh, Merck, Darmstadt, Germany) was activated prior to use by heating at 100 °C overnight and then deactivated by the addition of 4 mL of ultrapure water (Milli Q+, Millipore, Belford, MA) for 100 g of silica gel. The homogenization was carried out by 5 min of hand shaking (ensuring that the deactivated silica contains no lumps and that the powder flows freely) of a batch of 500 g of silica. The deactivated silica gel was left to equilibrate overnight in a closed glass flask placed in a desiccator. The silica gel under this condition may be used within 1 week.

Food Samples. Avocado, sheep's cheese, and salmon were purchased in a local supermarket. Foodstuffs to be irradiated were sliced (5 mm thick layer), packaged in the presence of air in plastic bags (multilayer ACX, AFP CENPA), thermosealed, stored at -20 °C, and thawed immediately prior to irradiation. The chicken quenelle samples were prepared in a French food company, with a 4% (m/m) inclusion of MRM irradiated at 5.0 kGy starting from the industrial base of quenelles and according to the usual receipt of the factory, namely, before poaching (20 min, 80 °C). The fish quenelle samples [containing a 10% (m/m) inclusion of 5 kGy irradiated salmon] were prepared at the Lycée Technique d'Hôtellerie et de Tourisme (Illkirch, France). Both culinary foods were packaged in the plastic bags after preparation and inclusion of the irradiated ingredients and stored at -20 °C.

Irradiation Treatment and Dosimetry. The MRM was irradiated at the industrial plant in the frozen state with a dose of 5 kGy. A van de Graaff electron beam accelerator, 2.2 MeV, 75 μ A (Vivirad High Voltage, Handschuheim, France) located in the Regional Centre of Innovation and Technology Transfer AERIAL (Schiltigheim, France) was used for the other irradiation treatments. The ionizing radiation treatments (0.1 kGy for avocado and 5 kGy for salmon) were performed at 6–8 °C. Irradiation doses were verified with FWT 60.00 radiachromatic dosimeters (Far West Technology, Goleta, CA), previously calibrated with an alanine dosimeter (Laboratoire National Henri Becquerel, Gif-sur-Yvette, France). Dose uniformity of about \pm 10% within the sample was achieved by the use of a 100 μ m thick copper scattering foil (24). All foodstuffs were placed in plastic bags and stored at -20 °C after the radiation treatment until the analysis.

Fat Extraction and Purification of Extracts. Before extraction, the foods were defrosted, homogenized, frozen at -80 °C, and lyophilized (overnight) with a lyophilizer (Virtis, New York), equipped with a rotary vacuum pump (Alcatel, Maurepas, France), and cryogenic trap cooled to -50 °C. During the lyophilisation process, no loss of 2-ACBs were observed (data not shown).

A total of 20 g of lyophilized food sample was subjected to a *n*-hexane Soxhlet extraction in accordance to the EN 1785 standard. A total of 2 g of the lipid fraction of each food sample and 200 μ L of 2-uDCB (used as an internal standard) of 1 μ g mL⁻¹ were purified by adsorption chromatography on a silica gel column [60 g, 45 cm length, 2.5 cm internal diameter]. The mobile-phase flow was 1 mL min⁻¹. The first 300 mL of *n*-hexane elution containing apolar impurities was discarded. The 2-ACBs (more polar) were collected during the second elution (950 mL of a 1% TBME *n*-hexane solution), of which only the last 450 mL was preserved. This fraction was concentrated with a rotary

Table 1. Values of Emission Current, Target, and Maximum Reaction Time (CI) for Different 2-ACBs

2-ACB	retention time (min)	emission current (μA)	target (ions)	maximum reaction time (ms)
2-HCB	10.00–17.00	60	4000	100
2-OCB, 2-DCB, <i>cis</i> -2-dDeCB and 2-dDCB	17.00–36.50	5	2000	100
<i>cis</i> -2-tDeCB	36.50–39.35	5	2000	60
2-tDCB	39.35–93.00	5	2000	100

evaporator under reduced pressure (200 mbar) at 40 °C to approximately 5 mL. The *n*-hexane evaporation was pursued up to 200 μL under a gentle nitrogen stream. The internal standard 2-uDCB was used for the quantification of both *s*-2-ACBs and μ -2-ACBs.

Instrumental. A Varian Saturn 2000 ion-trap mass spectrometer (Varian, Palo Alto, CA) coupled to a Varian 3400 gas chromatograph equipped with a Varian 8200 autosampler was used for all of the analyses. Ion-trap parameters included a trap temperature of 150 °C, a manifold temperature of 45 °C, a transfer-line temperature of 170 °C, an axial modulation amplitude of 4.0 V, and a scan rate of 1 s. The data were acquired using the automatic reaction control (ARC) feature of the Saturn 2000 mass detector.

The GC was employed with a septum-equipped temperature-programmable injector (SPI), which was initially held at 50 °C before being ramped to 240 °C at a rate of 230 °C min^{-1} . The final temperature was held for 92 min. The SPI injector was cooled between runs by CO_2 (quality 99.9%, Air Liquide, Paris, France). The capillary column was a 60 m OV-20-MS (Ohio Valley, Marietta, OH) with a 0.25 mm i.d. The thickness of the stationary phase (20% diphenyl-/80% dimethylpolysiloxane) was 0.10 μm . The GC oven temperature was initially held at 60 °C for 2 min, ramped at 4 °C min^{-1} to 300 °C, and held for 30 min. The injection volume was 1 μL , and the carrier gas flow (helium, 99.9995% of purity, Air Liquide) was 1 mL min^{-1} .

Electronic Impact (EI) Detection Conditions. The target was fixed at 20 000; the maximum ionization time was at 25 μs ; and the background mass was m/z 45. The ionization filament current was fixed at 10 μA . The acquired mass range was between m/z 50 and 300.

Chemical Ionization (CI) Detection Conditions. Isobutane (quality of 99.95%, Messer, Krefeld, Germany) was used as a CI reagent. The pressure of this gas in the mass spectrometer was adjusted to obtain a 1:1 ratio of the $(\text{CH}_3)_3\text{C}^+$ (m/z 57) and $(\text{CH}_3)_2\text{CH}^+$ (m/z 43) ion abundance. The reaction time was 60 ms; the rf (radio frequency) storage level was m/z 19; the ejection amplitude was set to 15 V; and the maximum ionization time was 2000 μs . The background mass was

m/z 65. Emission current, target, and maximum reaction time were optimized for each 2-ACB (Table 1). The optimization was conducted to maximize the signal/background noise for each 2-ACB. The optimized method is described in Table 1. The acquired mass range was between m/z 50 and 300.

RESULTS AND DISCUSSION

Silica Column Purification. Horvatovich et al. (17) reported that 3 g of silica gel was enough for the retention of about 100 mg of fat. This capacity (33 mg g^{-1} of fat for silica gel) is 5 times higher than that of the Florisil column (200 mg of fat on 30 g of adsorbent) proposed in the EN 1785 standard. A glass column containing 60 g of silica gel (prepared as described in the Materials and Methods) was then used for 2 g of fat for the cleanup of 2-ACBs. The optimization of the elution pattern was performed with 2 g of nonirradiated sheep's cheese fat spiked with the standard mixture of μ -2-ACBs and *s*-2-ACBs. The first elution (300 mL of *n*-hexane) containing the apolar fraction of the fat was discarded. The second elution (950 mL of a 1% TBME *n*-hexane), of which only the last 450 mL was retained, contained the 2-ACBs. Under these conditions, the extract was free of triglycerides and contained mainly components of lipids having oxy groups (aldehydes and ketones). The recovery rate of different 2-ACBs was between 57 and 68%. This recovery rate is lower than the values obtained by the official method (8) (91–98%), the method using supercritical fluid extraction (17) (60–87%), or SFE method on 2 g of Soxhlet extracted fat (19) (90 \pm 3% for 2-dDeCB). This is certainly due to the use of a high solvent volume and for the high mass of the test samples. The collected fraction was concentrated under reduced pressure, down to approximately 200 μL , and was injected in the GC–MS. The analysis of 2 g of fat results in a higher sensitivity versus the EN 1785 standard, in which only 0.2 g of fat is applied.

2-ACB Fragmentation Patterns. The mass spectra of the 2-tDCB and *cis*-2-tDeCB obtained by EI–MS analysis are presented in parts a and b of Figure 1. For μ -2-ACBs, the ions m/z 98 and 95 are of equivalent intensities. The ion m/z 98 was predominant for *s*-2-ACBs. The fragmentation pattern of μ -2-ACB is the mixture of the mass fragments of both the *s*-2-ACB and monounsaturated aliphatic hydrocarbons (21). The spectrum presented fragments in a low-range mass (e.g., 67, 81, 95, 109, 123, 137, 151, 165, 179, 193, 207, and 221) with a difference of CH_2 (14 m/z), frequent for aliphatic monoun-

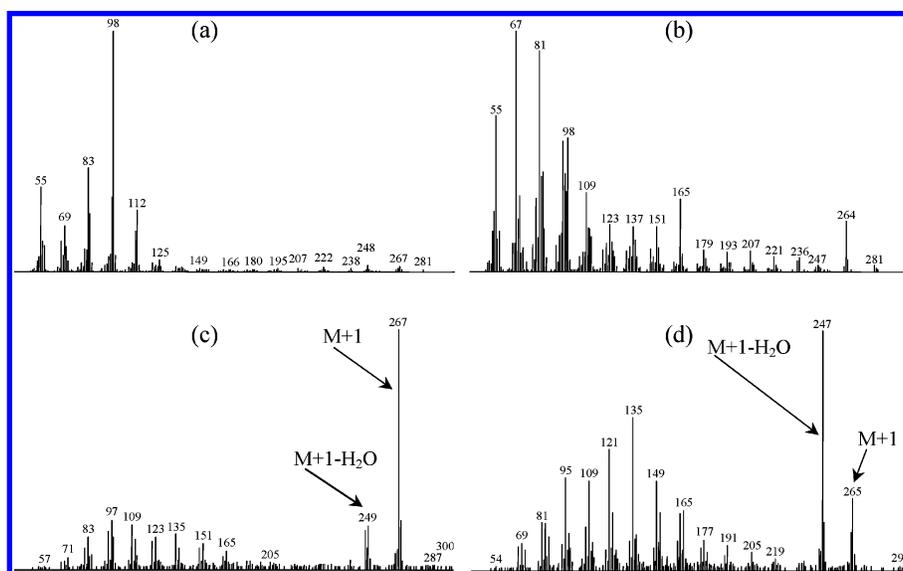


Figure 1. EI (a and b) and isobutane CI (c and d) mass spectra of 2-tDCB (a and c) and *cis*-2-tDeCB (b and d).

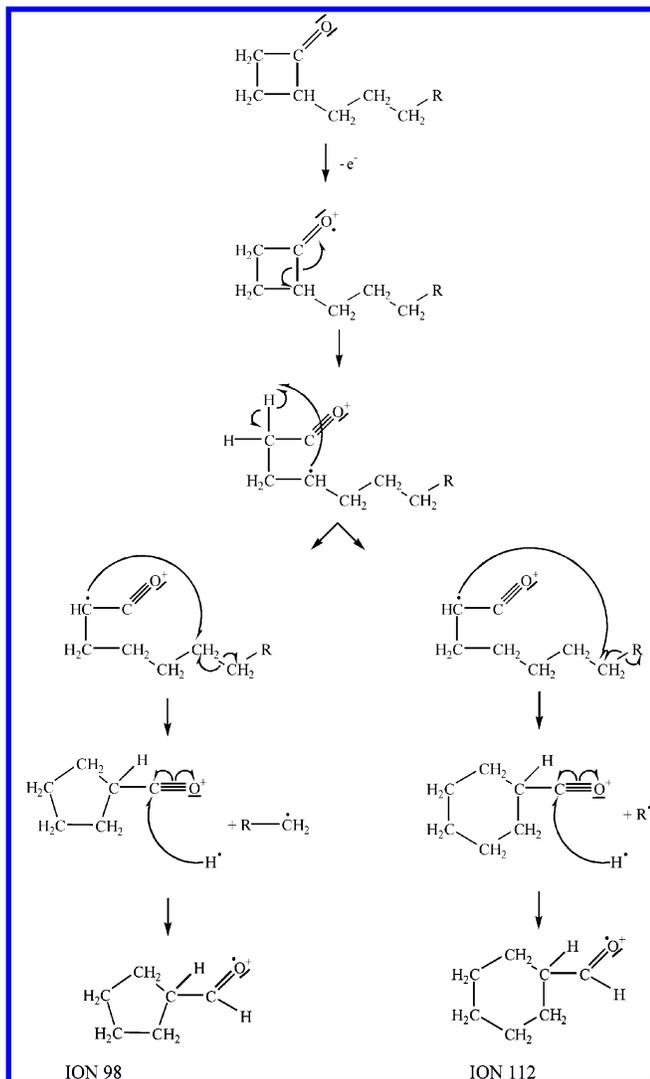


Figure 2. Possible EI ionization fragmentation pattern of s-2-ACBs.

saturated hydrocarbons. A second important and selective fragment with a higher mass was the ion m/z 112. The ratio between these two ions (98/112) was 4:1 for 2-tDCB and 2-dDCB, as previously described in EN 1785 (8). This 98/112 ratio was also 4:1 for 2-uDCB and 2-DCB but increased to 6:1 for 2-OCB and to 56:1 for the 2-HCB (14). As for cyclic ketones, the fragmentation peaks of s-2-ACBs could result from cleavage at the C–C bonds adjacent to the oxygen, with the charge remaining at the oxygenated functional group (Figure 2). Then, hydrogen could shift to convert near the carbon of oxygen, and thereafter, different cleavages of the C–C bond in the alkyl side chain could lead to the formation of the cyclic fragments m/z 98 or 112 (parts a and b of Figure 1).

The CI mode is a soft ionization method contrary to the high-energy electron impact. Emission current, target, and maximum reaction time were optimized for each 2-ACB. The optimization was conducted to maximize the signal/background noise for each 2-ACB. The optimized method for isobutane CI is described in Table 1. With isobutane as an ionization reagent, two predominant ions could be detected (Figure 3): the pseudomolecular ion $(M + H)^+$ [M represent the molecular mass of the neutral compounds] and this pseudomolecular ion minus 18 $(M + H - H_2O)^+$ for both the s-2-ACB and mu-2-ACB. These two fragments probably resulted from the protonation of the oxygen, followed by a rearrangement, which led to the formation of the

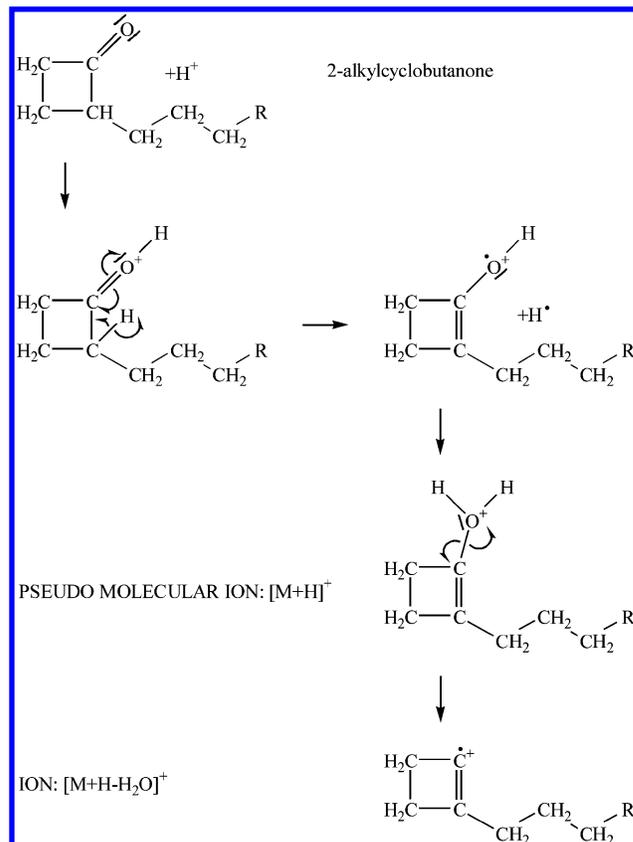


Figure 3. Possible CI fragmentation pattern of s-2-ACBs and mu-2-ACBs.

pseudomolecular ion. Concerning the fragment $(M + H - H_2O)^+$, the cleavage could occur at the oxygen bond (Figure 3).

Indeed, the s-2-ACBs produced a strong pseudomolecular ion (Figure 1c). However, in the case of mu-2-ACBs, the $(M + H - H_2O)^+$ was predominant (Figure 1d) whatever the length of the alkyl chain. On the other hand, this observation indicated that in both CI and EI ionization, the s-2-ACBs underwent less fragmentation than their monounsaturated analogues.

These proposed fragmentation schemes induced by both EI and CI modes were consistent with all observations made with the other 2-ACBs.

Detection Limit and Sensibility of the EI- and CI-MS.

The detection limit (signal/background noise ratio above 3) was determined with regard to a synthesized mixture of s-2-ACB and mu-2-ACB standards, analyzed with both CI and EI ionization (Table 2). When EI ionization was used and the ion m/z 98 was monitored, the lowest detectable amount for s-2-ACBs was 50 pg (i.e., 0.32 pmol of 2-HCB and 0.21 pmol of 2-dDCB, which values are consistent with previous findings) (14). For mu-2-ACBs, the limit of detection was about 250 pg (0.95 pmol of *cis*-2-tDeCB), ~5 times greater than for the saturated compounds. To lower the limit of detection for the detection of mu-2-ACBs, the sum of ions m/z 95 and 98 was considered, which leads, however, to somewhat less selectivity. In this case, the limit of detection was about 150 pg (i.e., 0.57 pmole of *cis*-2-tDeCB), ~3 times greater than for the 2-dDCB considering only the ion 98. These results are in accordance with those reported by Meier et al. (25), Ndiaye et al. (14), and Horvatovich et al. (21).

The use of an ion-trap mass analyzer with automatic gain control allows for a high linear range for both CI and EI ionization modes (Figure 4).

The analysis by CI-MS allowed for a reduction of the limit

Table 2. Limit of Detection of Different 2-ACB Using EI or CI

2-ACB	precursor fatty acid	detection limit (pmol)				sensitivity ratio (EI/CI)
		EI	monitored ion in EI	CI	monitored ion in CI	
2-HCB	capric	0.32	98	0.01	155	32.0
2-OCB	lauric	0.27	98	0.07	183	3.9
2-DCB	myristic	0.24	98	0.06	211	4.0
<i>cis</i> -2-dDeCB	palmitoleic	0.62	95 + 98	0.16	219	3.8
2-dDCB	palmitic	0.21	98	0.07	239	3.0
<i>cis</i> -2-tDeCB	oleic	0.57	95 + 98	0.57	247	1.0
2-tDCB	stearic	0.19	98	0.12	267	1.6

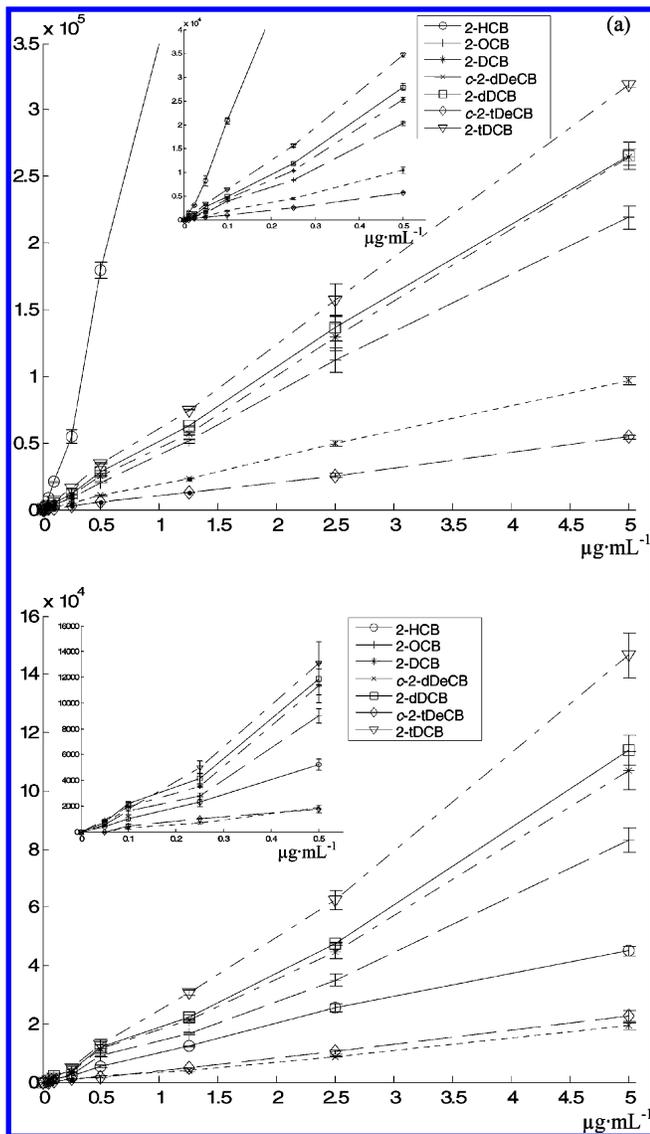
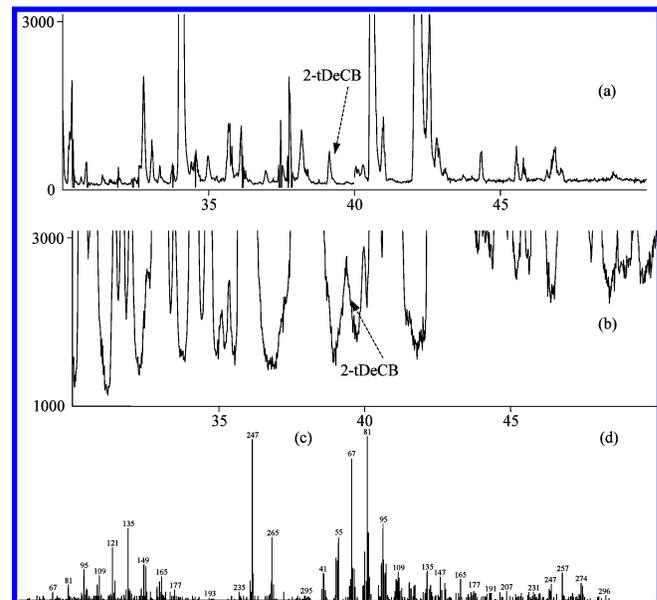


Figure 4. Concentrations [from 5 to 0.0006 $\mu\text{g mL}^{-1}$ (CI) and from 5 to 0.05 $\mu\text{g mL}^{-1}$ (EI)] of 2-ACB versus the signal detector using EI ionization and CI modes. Zoom between 0.5 and 0.0006 $\mu\text{g mL}^{-1}$ for CI and 5–0.05 $\mu\text{g mL}^{-1}$ for EI.

of detection depending upon the emission current and the maximum reaction time used (Table 2). This sensitivity enhancement for the most abundant s-2-ACB in irradiated foods (2-dDCB) is about ~ 3 but remains not significant for the most abundant μ -2-ACB in irradiated food (*cis*-2-tDeCB). Nevertheless, CI has the advantage to allow for a more selective detection, detecting in a higher mass region for the single-ion monitoring (m/z 247 for the 2-tDeCB and 239 for the 2-dDCB).



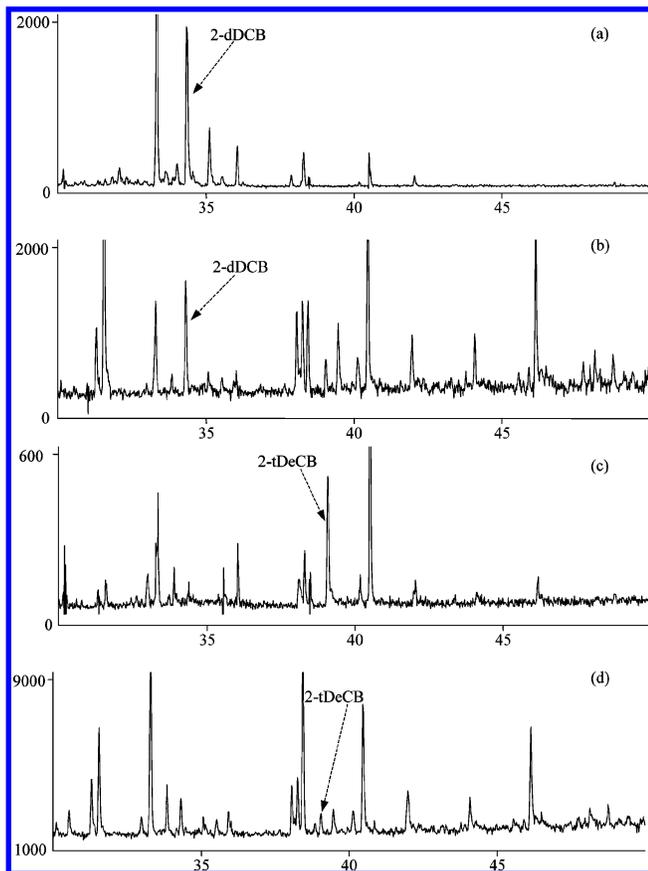


Figure 6. Chromatograms of 2-ACBs extracted from nonirradiated poultry quenelles containing 4% (wt/wt) of mechanically recovered poultry meat irradiated at 5 kGy. Isobutane CI (a, ion m/z 239; c, ion m/z 247) and EI ionization (b, ion m/z 98; d, sum of ions m/z 95 and 98).

number of interfering impurities is greatly increased. The detection of the irradiated ingredients is then much more difficult. The use of the proposed protocol for the analysis of poultry quenelles containing 4% (m/m) of 5 kGy irradiated mechanically recovered meat allowed for the detection of the 2-dDCB (parts **a** and **b** of **Figure 6**) with a signal/noise ratio of 80 (CI mode), whereas it was only 24 when using the EI mode (lower signal and higher noise). The same observation was done concerning the detection of the 2-tDeCB (parts **c** and **d** of **Figure 6**). The signal/noise ratio was 40 when detecting with the CI mode, whereas it was 10 times less when using the EI mode because of the higher background noise.

In the case of fish quenelles containing 10% of 5 kGy irradiated salmon, the 2-dDCB was also detected in the chromatograms obtained with both EI ionization and isobutane CI-MS (parts **a** and **b** of **Figure 7**). As in the case of poultry quenelles, the better specificity of the isobutane CI-MS led to a significant improvement of the signal/background noise ratio (from 8 to 27) for the detection of the 2-dDCB. In addition, *cis*-2-tDeCB was also detected in the fish quenelles when using the CI mode (**Figure 7c**), whereas it was not detected in the EI mode (**Figure 7d**). This observation further underscores the potential advantage of the isobutane CI-MS for the detection of irradiated foods or ingredients containing high amounts of oleic acid.

The replacement of the EI ionization with an isobutane CI resulted in a more sensitive and specific detection of the most abundant 2-ACBs in irradiated foods. The optimized CI parameters enhanced the sensitivity of the detection method 3–4-fold. When this detection mode is coupled with an

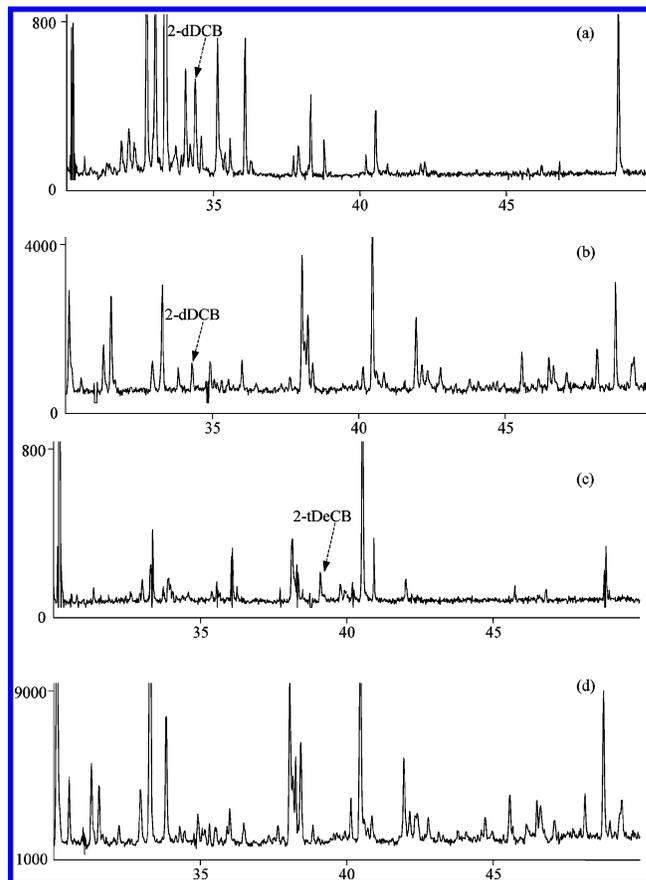


Figure 7. Chromatograms of 2-ACBs extracted from nonirradiated fish quenelles containing 10% (wt/wt) of salmon irradiated at 5 kGy. Isobutane CI (a, ion m/z 239; c, ion m/z 247) and EI ionization (b, ion m/z 98; d, sum of ions m/z 95 and 98).

increased amount of sample (2 g of fat), a very specific and sensitive method for the detection of 2-ACBs was obtained. This was demonstrated for the detection of avocados irradiated at 0.1 kGy and of nonirradiated foods containing low inclusions of irradiated ingredients, enabling us now to perform the routine analysis of practically all irradiated foodstuffs with conventional laboratory equipment in food quality control laboratories.

LITERATURE CITED

- (1) Anonymous. *Safety and Nutritional Adequacy of Irradiated Food*. World Health Organization: Geneva, Switzerland, 1994.
- (2) Anonymous. *Wholesomeness of Irradiated Food*. Report of a joint FAO/IAEA/WHO Expert Committee; World Health Organization: Geneva, Switzerland, *Technical Report Series 659*, 1981.
- (3) Anonymous. *High-Dose Irradiation: Wholesomeness of Food Irradiated with Doses above 10 kGy*. Report of a Joint FAO/IAEA/WHO Study Group; World Health Organisation: Geneva, Switzerland, *Technical Report Series 890*, 1999.
- (4) Anonymous. Foodstuffs. Detection of irradiated food containing bone. Method by ESR spectroscopy. European Committee for Standardization: Brussels, Belgium, *EN 1786*, 1996.
- (5) Anonymous. Foodstuffs. Detection of irradiated food containing cellulose. Method by ESR spectroscopy. European Committee for Standardization: Brussels, Belgium, *EN 1787*, 1996, revised 2000.
- (6) Anonymous. Foodstuffs. Detection of irradiated food from which silicate minerals can be isolated. Method by thermoluminescence. European Committee for Standardization: Brussels, Belgium, *EN 1788*, 1996, revised 2001.

- (7) Anonymous. Foodstuffs. Detection of irradiated food containing fat. Gas chromatographic analysis of hydrocarbons. European Committee for Standardization: Brussels, Belgium, *EN 1784*, 1996, revised 2003.
- (8) Anonymous. Foodstuffs. Detection of irradiated food containing fat. Gas chromatographic/mass spectrometric analysis of 2-alkylcyclobutanones. European Committee for Standardization: Brussels, Belgium, *EN 1785*, 1996, revised 2003.
- (9) Anonymous. Foodstuffs. Detection of irradiated food containing crystalline sugar by ESR spectroscopy. European Committee for Standardization: Brussels, Belgium, *EN 13708*, 2001.
- (10) Anonymous. Foodstuffs. Detection of irradiated food using photostimulated luminescence. European Committee for Standardization: Brussels, Belgium, *EN 13751*, 2002.
- (11) Anonymous. Foodstuffs. DNA comet assay for the detection of irradiated foodstuffs—Screening method. European Committee for Standardization: Brussels, Belgium, *EN 13784*, 2001.
- (12) Anonymous. Foodstuffs. Detection of irradiated food using direct epifluorescence filter technique/aerobic plate count (DEFT/APC)—Screening method. European Committee for Standardization: Brussels, Belgium, *EN 13783*, 2001.
- (13) Anonymous. Foodstuffs. Microbiological screening method for irradiated food using LAL/GNB procedures. European Committee for Standardization: Brussels, Belgium, *EN 14569*, 2004.
- (14) Ndiaye, B.; Jamet, G.; Miesch, M.; Hasselmann, C.; Marchioni, E. 2-Alkylcyclobutanones as markers for irradiated foodstuffs. II. The CEN (European Committee for Standardization) method: Field of application and limit of utilization. *Radiat. Phys. Chem.* **1999**, *55*, 437–445.
- (15) Crone, A. V. J.; Hamilton, J. T. G.; Stevenson, M. H. Effect of storage and cooking on the dose response of 2-dodecylcyclobutanone, a potential marker for irradiated chicken. *J. Sci. Food Agr.* **1992**, *58*, 249–252.
- (16) Crone, A. V. J.; Hand, M. V.; Hamilton, J. T. G.; Sharma, N. D.; Boyd, D. R.; Stevenson, M. H. Synthesis, characterization and use of 2-tetradecylcyclobutanone together with other cyclobutanones as markers of irradiated liquid whole egg. *J. Sci. Food Agr.* **1993**, *62*, 361–367.
- (17) Horvatovich, P.; Miesch, M.; Hasselmann, C.; Marchioni, E. Supercritical fluid extraction of hydrocarbons and 2-alkylcyclobutanones for the detection of irradiated foodstuffs. *J. Chromatogr., A* **2000**, *897*, 259–268.
- (18) Ndiaye, B.; Horvatovich, P.; Miesch, M.; Hasselmann, C.; Marchioni, E. 2-Alkylcyclobutanones as markers for irradiated foodstuffs III. Improvement of the field of application on the EN 1785 method by using silver ion chromatography. *J. Chromatogr., A* **1999**, *858*, 109–115.
- (19) Horvatovich, P.; Miesch, M.; Hasselmann, C.; Marchioni, E. Supercritical fluid extraction for the detection of 2-dodecylcyclobutanone in low dose irradiated plant foods, *J. Chromatogr., A* **2002**, *968*, 251–255.
- (20) Marchioni, E.; Horvatovich, P.; Ndiaye, B.; Miesch, M.; Hasselmann, C. Detection of low amount of irradiated ingredients in non-irradiated precooked meals. *Radiat. Phys. Chem.* **2002**, *63*, 447–450.
- (21) Horvatovich, P.; Miesch, M.; Delincée, H.; Hasselmann, C.; Marchioni, E. Determination of mono-unsaturated alkyl side-chain 2-alkylcyclobutanones in irradiated foods. *J. Agric. Food Chem.* **2005**, *53*, 5836–5841.
- (22) Girod, C.; Staub, C. Analysis of drugs of abuse in hair by automated solid-phase extraction, GC/EI/MS and GC ion trap/CI/MS. *Forensic Sci. Int.* **2000**, *107*, 261–271.
- (23) Miesch, M.; Ndiaye, B.; Hasselmann, C.; Marchioni, E. 2-Alkylcyclobutanones as markers for irradiated foodstuffs. I. Synthesis of saturated and unsaturated standards. *Radiat. Phys. Chem.* **1999**, *55*, 337–344.
- (24) Kuntz, F.; Marchioni, E.; Strasser, A. Homogénéisation de la dose déposée dans un produit ionisé aux électrons accélérés. In *Proceedings of an International Symposium on High Dose Dosimetry for Radiation Processing*, 5–9 November 1990; The International Atomic Energy Agency: Vienna, Austria, 1991; IAEA Report SM 314/30, pp 479–488.
- (25) Meier, W.; Artho, A.; Nägeli, P. Detection of irradiation of fat-containing foods by on-line LC–GC–MS of alkylcyclobutanones. *Mitt. Geb. Lebensm. Unters. Hyg.* **1996**, *87*, 118–122.
- (26) Souci, S. W.; Fachmann, W.; Kraut, H. Avocado. In *Food Composition and Nutrition Tables*, 6th ed.; Medpharm Scientific Publishers: Stuttgart, Germany, 2000; pp 949–950.

Received for review October 3, 2005. Revised manuscript received January 23, 2006. Accepted January 24, 2006.

JF0524426