ELSEVIER

Journal of Chromatography A, 897 (2000) 259–268

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Supercritical fluid extraction of hydrocarbons and 2-alkylcyclobutanones for the detection of irradiated foodstuffs

P. Horvatovich[a,b], M. Miesch[c], C. Hasselmann[a], E. Marchioni[a,*]

[a]*Département des Sciences de l'Aliment, Faculté de Pharmacie, 74 Route du Rhin, 67400 Illkirch, France*

[b]*Institute of Preservation and Livestock Products Technology, University of Horticulture and Food Industry, Ménesi út 45, 1118 Budapest, Hungary*

[c]*Laboratoire de Chimie Organique Synthétique (UMR 7509), Institut de Chimie, 1 Rue Blaise Pascal, 67000 Strasbourg, France*

Received 14 February 2000; received in revised form 6 July 2000; accepted 28 July 2000

Abstract

Supercritical carbon dioxide can be used to carry out a selective and fast extraction (30 min) of volatile hydrocarbons and 2-alkylcyclobutanones contained in irradiated foods. After elimination of the traces of triglycerides still contained in the extracts on a silica column, the compounds were analysed by gas chromatography–mass spectroscopy (2-alkylcyclobutanones) and gas chromatography-flame ionization detection (volatile hydrocarbons). The present method was applied successfully to freeze-dried samples (1 g or less) of cheese, chicken, avocados and to various ingredients (chocolate, liquid whole eggs) included in non-irradiated cookies. It was faster (4–5 h) than the reference methods EN 1784 (volatile hydrocarbons) and EN 1785 (2-alkylcyclobutanones), which take 1.5 days each. The minimal dose detectable by this method is, in addition, slightly lower than those of the reference methods.

Keywords: Supercritical fluid extraction; Hydrocarbons; 2-Alkylcyclobutanones

1. Introduction

The radiolysis of a fatty acid $C_{m:n}$ (m=number of carbon atoms; n=number of double bonds) leads mainly to the formation of volatile hydrocarbons of formulas $C_{m-1:n}$ and $C_{m-2:n+1}$ by rupture of the side chain in the α and β positions with respect to the carbonyl group [1], and 2-alkylcyclobutanones with a $C_{m-4:n}$ alkyl chain [2].

The presence of volatile hydrocarbons in a foodstuff is not specific to ionizing treatment, but the appearance of a hydrocarbon couple $C_{m-1:n}$/$C_{m-2:n+1}$ for each fatty acid $C_{m:n}$ present indicates unambiguously that such a treatment has been performed [3–6]. Since 2-alkylcyclobutanones are assumed to be formed specifically by irradiation until proved otherwise [7–9], their presence thus also appears to prove the irradiation of this foodstuff.

Analytical methods for these chemical compounds, developed by the European Standardization Committee [10,11] thus allow the detection of irradiated foodstuffs at doses equal to or higher than 0.5 kGy (hence for the purpose of pasteurization) when their lipid contents exceed 1% [9]. The protocols proposed (EN 1784 and EN 1785) have, how-

*Corresponding author. Tel.: +33-3-8867-6944; fax: +33-3-8866-0190.

E-mail address: eric.marchioni@pharma.u-strasbg.fr (E. Marchioni).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00810-4

ever, the 2-fold disadvantage of being time-consuming (1.5–2 days for each protocol), partly owing to the use of a Soxhlet extraction (of 6 h duration), and very expensive on account of the need for a large quantity of Florisil in the solid-phase extraction designed to purify the extracts obtained (retention of the lipids). The protocols are, in fact, very similar (Soxhlet extraction, purification of the extracts on a column of Florisil and separation by gas chromatography, with mass detection in the case of the analysis of the 2-alkylcyclobutanones and flame ionization for the analysis of the volatile hydrocarbons), but they are nonetheless sufficiently different in detail to prohibit both chromatographic analyses being made on the same extract. This obliges the operator wishing to confirm or refute the use of ionizing radiation treatment of a foodstuff by the dual investigation of the volatile hydrocarbons and 2-alkylcyclobutanones to perform two long and complex procedures successively.

The replacement of the Soxhlet extraction by a supercritical fluid extraction might permit both a considerable reduction in the time required for the analyses and a simultaneous extraction of the 2-alyklcyclobutanones and volatile hydrocarbons contained in the samples. Moreover, it is possible to envisage a more selective extraction of these chemical compounds and, consequently, eliminate the need for solid-phase extraction on Florisil specified in the reference methods EN 1784 and EN 1785 or, failing that, if small quantities of lipids are still present in the extracts obtained, to replace it by a purification device simpler to implement and less expensive.

Carbon dioxide in the supercritical phase has already been used for the extraction of the volatile hydrocarbons [12,13] and the 2-alkylcyclobutanones [14] in irradiated foodstuffs. Lembke et al. [12], in fact, subjected to extraction by supercritical carbon dioxide, not the foodstuff sample itself, but its fatty matter pre-extracted with an organic solvent so that the extracts analyzed by gas chromatography not only contained higher concentrations of volatile hydrocarbons but were cleaner. They were apparently able to carry out a selective extraction of these chemical compounds and thus avoid recourse to purification using Florisil. Hampson et al. [13] and Tewfik et al. [14], who performed the supercritical phase extraction directly on the foodstuff sample, did not investigate selective extraction conditions (temperature, pressure) and placed a layer of Florisil on the weighed sample in order to avoid the presence of too large amount of lipids in the extracts obtained. This solution is acceptable for the analysis of the volatile hydrocarbons since these apolar compounds ought not to be retained by Florisil but cannot be recommended for the analysis of the 2-alkylcyclobutanones. These compounds, more polar than the volatile hydrocarbons, will in fact certainly be retained, at least partially, in the Florisil layer if this latter is not saturated with lipids.

The objective of the present work was to suggest a protocol using the extraction by supercritical carbon dioxide for the simultaneous extraction of the volatile hydrocarbons and the 2-alkylcyclobutanones in the foodstuff samples. This protocol ought to be at least as satisfactory as the reference protocols EN 1784 and EN 1785 as regards the minimal detectable irradiation dose and the field of application, while making it possible to perform this dual analysis in a much shorter time and without the use of Florisil, hence more cheaply.

2. Materials and methods

2.1. Chemicals

The 2-alkylcyclobutanone standards [2-hexyl (2-HCB), 2-octyl (2-OCB), 2-decyl (2-DCB), 2-undecyl (2-uDCB), 2-dodecyl (2-DdCB) and 2-tetradecylcyclobutanone (2-TCB)] were synthesized according to the method of Miesch et al. [15]. The volatile hydrocarbon standards [1-tridecene ($C_{13:1}$), 1-tetradecene ($C_{14:1}$), *n*-pentadecane ($C_{15:0}$), 1-hexadecene ($C_{16:1}$) and *n*-heptadecane ($C_{17:0}$)] were Sigma products (St. Louis, MO, USA). The 8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) standards were TeLA products (Berlin, Germany). The carbon dioxide, of 99.9999% purity with a 142-bar helium headspace, was purchased from Air Product and Chemicals (Allentown, PA, USA). *tert.*-Butyl methyl ether (TBME) was from Merck (Darmstadt, Germany). *n*-Hexane, of technical quality, was distilled from calcium hydride. Silica gel 70–230 mesh (Merck) was heated at 100°C overnight and deactivated by addition of distilled water in the proportion

of 4.13 ml of water (Milli-Q+, Millipore, Bedford, MA, USA) to 100 g of silica gel. Florisil, 60–100 mesh (Aldrich, Saint-Quentin, France) was heated at 650°C (5 h) and deactivated by the addition of water in the proportion of 20 ml of water to 100 g of Florisil. Sodium sulfate was purchased from SDS (Peypin, France), heated at 550°C (5 h) and allowed to cool in a dessicator before use. The hydromatrix (Varian, Palo Alto, CA, USA) was washed (6 h soxhlet extraction with *n*-hexane) prior to use.

2.2. Preparation of the foodstuff samples

Sheep's cheese, chocolate and liquid whole eggs were supplied by a French food company. The avocados and chicken were purchased in a local supermarket. The foodstuffs were packaged (5 mm thick) in the presence of air in plastic bags, thermosealed, stored at −20°C and thawed immediately prior to irradiation. The ionizing radiation treatments (3.0 and 100 kGy for the cheese, 0.5 kGy for the avocado, 4.0 kGy for the liquid whole eggs, 3.0 kGy for the chocolate and chicken) were performed at a temperature of 6–8°C. The irradiated liquid whole eggs and chocolate were then stored at −20°C until they were used for the preparation of cookies (2 g% (m/m) of liquid whole eggs and 4 g% (m/m) of chocolate) made by the Lycée Technique d'Hôtellerie et de Tourisme (Strasbourg, France). These cookies were then stored at −20°C.

Just before their analysis, the samples were thawed, homogenized, cooled to −80°C and lyophilized overnight (100 mbar) by means of a Virtis lyophilizer (New York, NY, USA), equipped with an Alcatel rotary vane vacuum pump (Maurepas, France) and a cryogenic trap (−60°C).

2.3. 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 AERIAL (Schiltigheim, France) was used for the irradiation treatments. Irradiation doses were verified with FWT 60.00 radiachromic dosimeters (Far West Technology, Goleta, CA, USA), 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 [16].

2.4. Extraction by means of supercritical carbon dioxide and purification on a silica trap

The lyophilized food sample (1 g of cheese, chicken meat or cookies, 0.5 g of avocado pulp) was finely ground and placed in a stainless steel cylindrical extraction cell of 3 ml internal volume (I.D. 10 mm, length 40 mm). The remaining empty volume of the extraction cell was filled with hydromatrix. Two hundred μl of a *n*-hexane solution of 2-undecylcyclobutanone (1 mg ml^{-1}) and 200 μl of a *n*-hexane solution of 1-tridecene (5 mg ml^{-1}) (internal standards) were added to the sample. The Suprex supercritical extractor used, Prepmaster type (Pittsburg, PA, USA), was equipped with an Accutrap type collector constituted of an automated flow control heated restrictor connected to a stainless steel cylindrical solid trap (maintained at room temperature) of 5 ml internal volume (I.D. 10 mm, length 65 mm) containing 3 g of deactivated silica (rinsed beforehand with 15 ml of *n*-hexane and dried immediately prior to extraction with a stream of nitrogen (99.995% purity, Air Liquide, Paris, France) for 2 s and at 71 bar pressure), the trap itself being connected to a test tube (liquid trap) containing 5 ml of *n*-hexane. The following extraction conditions were selected: pressure, 152 bar; temperature, 80°C; duration, 30 min. The extraction cell was placed vertically in the oven and carbon dioxide is passed through it (from bottom to top) at a flow-rate of 2 ml min^{-1}. The Accutrap collector was equipped with a high pressure pump making it possible to elute (at room temperature) the chemical compounds retained on the solid trap with 15 ml of *n*-hexane then, after removal of the liquid trap, with 35 ml of a mixture of *n*-hexane and TBME (99:1, v/v), only the last 20 ml of which were preserved.

2.5. Gas chromatography

The two fractions obtained were concentrated at 40°C under a stream of nitrogen to a final volume of about 300 μl and analyzed, the one (hexane fraction) by GC–FID (analysis of volatile hydrocarbons) and

the other (*n*-hexane/TBME fraction) by GC–MS (analysis of 2-alkylcyclobutanones). In the first case, a Varian chromatograph was used (type 3300) equipped with a flame ionization detector (300°C) and a split–splitless injector (220°C). It was fitted with a DB5 capillary column (J&W Scientific, Folsom, CA, USA), 30 m×0.25 mm I.D. with a 0.25 μm stationary phase (5% diphenyl–95% dimethylpolysiloxane). The initial column temperature was 80°C (held for 2 min) and was followed successively by a 7°C min^{-1} increase to 155°C (held for 2 min), a 2°C min^{-1} increase to 170°C, and a 10°C min^{-1} increase to 300°C, final temperature held for 5 min. The injection volume was 1 μl. The injection mode was splitless. The carrier (1 ml min^{-1} flow) was nitrogen (99.999% purity, Air Liquide). In the second case, the Varian chromatograph (type 3400) used was equipped with an SPI cooled injector and a mass sensitive detector (Saturn 2000, Varian) operating in the electron impact mode. The mass spectra were recorded between 50 and 300 *m*/*z*. The gas chromatograph was fitted with a ZB-5-MS capillary column (Zebron, Torrance, CA, USA), 30 m×0.25 mm I.D. with a 0.10 μm stationary phase (5% diphenyl–95% dimethylpolysiloxane). The initial injector temperature was 50°C (held for 0.1 min) and was followed by a 230°C min^{-1} increase to 240°C, final temperature held till the end of the column temperature programme. This programme was as follows: 60°C (held for 2 min) followed by an 8°C min^{-1} increase to 300°C, final temperature held for 15 min. The injection volume was 1 μl. The carrier (1 ml min^{-1} flow) was helium (99.9995% purity, Air Liquide).

2.6. Fat analysis

The lipids extracted by the supercritical carbon dioxide were collected directly at the restrictor outlet (without the introduction of the silica trap) in a test tube containing 10 ml of *n*-hexane. After evaporation of the *n*-hexane, the amount of lipids extracted was determined by gravimetry.

3. Results and discussion

3.1. Pressure and temperature of the supercritical fluid

All the food samples analysed during this work were first lyophilized in order to remove their water contents. This water may, in fact, modify the polarity of the supercritical fluid and thus facilitate the undesired extraction of polar compounds. Moreover, it may freeze when the pressure of the supercritical fluid is reduced and clog or partially block the restrictor.

The food sample chosen for the optimization of the conditions of extraction by means of supercritical carbon dioxide was the sheep's cheese. This foodstuff has a high lipid (mainly triglyceride) content (39%) and contains numerous fatty acids in appreciable concentrations. Irradiation was therefore likely to produce detectable quantities of various 2-alkylcyclobutanones and volatile hydrocarbons.

The quantity of lipids extracted after 5 min by supercritical carbon dioxide diminished when the pressure of the supercritical fluid fell and when its temperature rose, at least to the pressure of 152 bar (at higher pressures a modification of the temperature had practically no effect) (Table 1). In order to extract as little lipids as possible, it seemed therefore judicious to perform the extraction at the lowest pressure and the highest temperature available. However, an increase of the temperature above 80°C or a

Table 1
Quantity of lipids (in mg) extracted from a cheese sample by supercritical carbon dioxide (sample weight, 1 g; extraction duration, 5 min; flow-rate, 2 ml min^{-1}) as a function of the pressure and temperature of extraction (standard deviation in parentheses)

Pressure (bar)	Temperature (°C)											
	35		50		60		70		80		100	
125	15	(2)	5.7	(0.5)	2.5	(0.4)	1.9	(0.5)	1.0	(0.3)	0.6	(0.1)
152	18	(2)	9	(1)	5.4	(0.8)	1.7	(0.5)	1.3	(0.7)	0.6	(0.6)
304	108	(15)	112	(15)	93	(8)	96	(16)	81	(18)	82	(23)
456	199	(11)	213	(15)	222	(25)	216	(13)	209	(2)	243	(5)

decrease of the pressure below 152 bar (in other respects difficult to perform because of the 142-bar helium headspace in the carbon dioxide tank) never led to a significant reduction of the quantity of lipids in the extracts. Moreover, such alterations of pressure and temperature will certainly involve a considerable increase in the extraction duration of the volatile hydrocarbons and the 2-alkylcyclobutanones (see below). For these reasons, the extraction parameters finally retained were a pressure of 152 bar and a temperature of 80°C. Under such conditions, and after 30 min of extraction, the quantity of lipids collected was always very low (less than 5 mg) as far as the sample weight was limited to 1.5 g. Above 2.0 g, the quantity of lipids in the extract dramatically increased, probably as a result of an overloading of fat in the extraction cell.

3.2. Purification of the extracts

When extracts (152 bar, 80°C, 30 min, 2 ml min^{-1}) obtained from samples of cheese irradiated at 3.0 kGy were analyzed, the weak quantity of triglycerides entrained appeared nonetheless to be still too large to allow satisfactory isolation of the radio-induced volatile hydrocarbons (data not shown). By using very similar extraction conditions (150 bar, 78°C) and not performing any subsequent purification of the extracts, Lembke et al. [12] succeeded, on the other hand, in identifying the volatile hydrocarbons present in various foodstuffs irradiated at doses ranging from 2.5 to 5.0 kGy, probably because of the use of a preliminary Soxhlet extraction of the fatty matter contained in the food sample, and of a mass detector, which is more selective than the flame ionization detector. The interpretation of the chromatograms obtained by these authors indicates nonetheless the presence of triglycerides in the extracts analyzed, which presents the risk of rapid pollution of the mass detector filters used for the analysis of the 2-alkylcyclobutanones. It thus appeared essential to purify the extracts obtained before analyzing them by chromatography, irrespective of the operating conditions selected.

A solid trap, constituted of deactivated silica (3 g), was thus placed in the extraction device, immediately after the variable restrictor. A first washing of this solid-phase with *n*-hexane (15 ml) enabled the apolar fraction of the extract containing the volatile hydrocarbons to be recovered. A slightly more polar mobile phase (35 ml of *n*-hexane containing 1% of TBME) was necessary to elute the 2-alkylcyclobutanones. The fraction containing these compounds consisted uniquely of the last 20 ml of this eluate.

The gas chromatographic analysis of these two fractions showed that the elimination of the triglyceride impurities had been completely achieved. As regards the analysis of the volatile hydrocarbons, three pairs of these compounds radio-induced from oleic, stearic and palmitic acids could be detected (Fig. 1a). This analytical protocol, on the other hand, did not allow the volatile hydrocarbons formed from other fatty acids to be detected, probably owing to their too high volatility. The five 2-alkylcyclobutanones formed by radiolysis of the saturated fatty acids (C_{10} to C_{18}) could be detected by using a mass detector (Fig. 1b). In fact, the selectivity of the supercritical extraction and the efficiency of the solid-phase extraction on silica were such that it was even possible to detect these compounds by flame ionization (Fig. 1c).

The quantity of silica used for this purification (3 g) proved to be amply sufficient since chromatographic interferences, related to saturation of the silica trap and to the presence of triglycerides in the extracts analyzed, were only observed when the quantity of triglycerides extracted by the supercritical carbon dioxide reached 150 mg, quantity largely higher than that of triglycerides found in the extracts (less than 5 mg, see above). The replacement of the silica by an equivalent amount of Florisil yielded less satisfactory results. Unfortunately silanols groups of the silica degrades when heated above 150°C [17] (whereas Florisil tolerates a temperature of 500°C), which does not guarantee total absence of contamination by volatile hydrocarbons. A preliminary washing of this adsorbent with *n*-hexane, however, rectified this disadvantage.

The kinetic study of an extraction by supercritical carbon dioxide performed on a sample of cheese irradiated at a very high dose (100 kGy), in order to obtain high concentrations of volatile hydrocarbons and 2-alkylcyclobutanones in the foodstuff, has shown that the extraction duration under the conditions recommended (80°C, 152 bar, flow-rate 2 ml min^{-1}) ought to be about 30 min in order to attain maximal concentrations of volatile hydrocarbons and

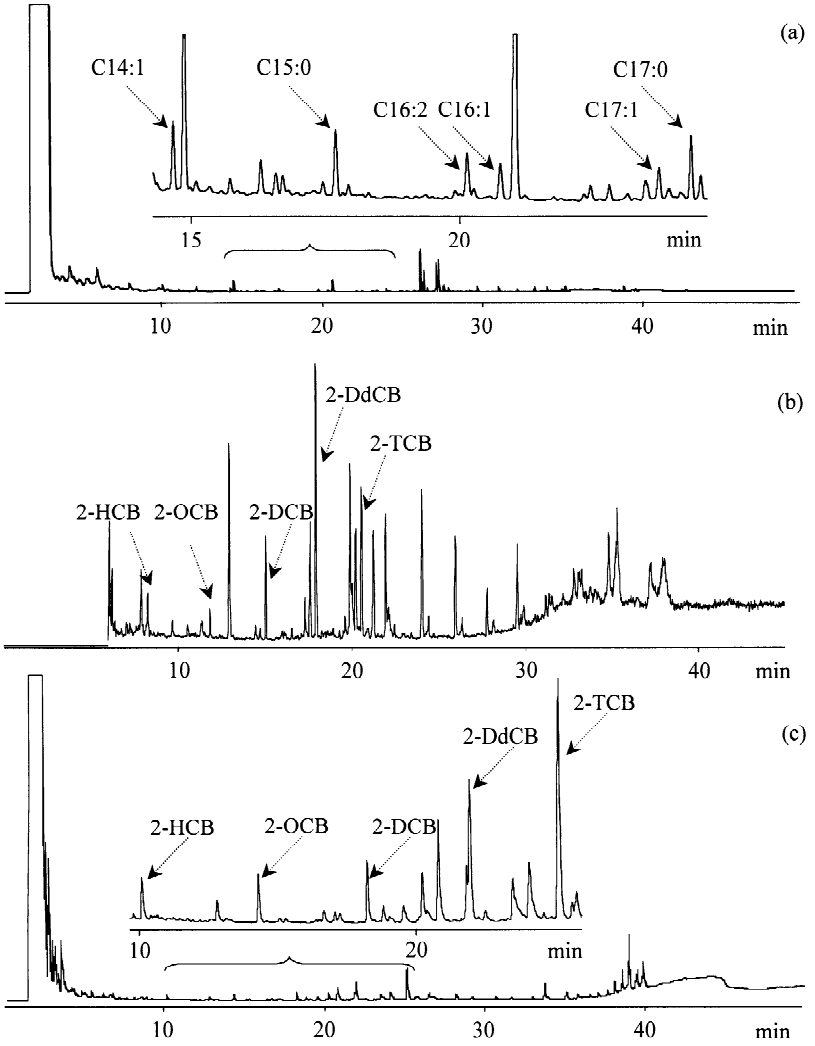

Fig. 1. GC analysis of volatile hydrocarbons (with flame ionization detection (a)) and 2-alkylcyclobutanones (with ion m/z 98 mass sensitive detection (b) and flame ionization detection (c)) extracted from a sample of cheese irradiated at 3.0 kGy by supercritical carbon dioxide (152 bar; 80°C; 30 min; flow-rate 2 ml min^{-1}).

2-alkylcyclobutanones in the extracts obtained (Fig. 2). An increase of the pressure or a lowering of the temperature of the supercritical carbon dioxide has made possible a much more rapid extraction of the volatile hydrocarbons and the 2-alkylcyclobutanones. But, in parallel, it has led to a considerable increase in the quantity of triglycerides present in the extracts (see Table 1), thus causing the extraction performed to lose all its selectivity and as a result necessitating recourse to a much more efficient lipid retention device more complex to implement.

The recovery yields of the suggested protocol were satisfactory for the determination of both the volatile hydrocarbons and the 2-alkylcyclobutanones of highest molecular weight (Table 2). As regards the volatile hydrocarbons, it was nonetheless necessary to place a liquid trap (*n*-hexane) after the silica trap in order to recover by dissolution a part of these compounds (about 20%) extracted by the carbon dioxide in the supercritical phase but not retained by the silica. Under these conditions, the recovery yields of the volatile hydrocarbons were higher (except for

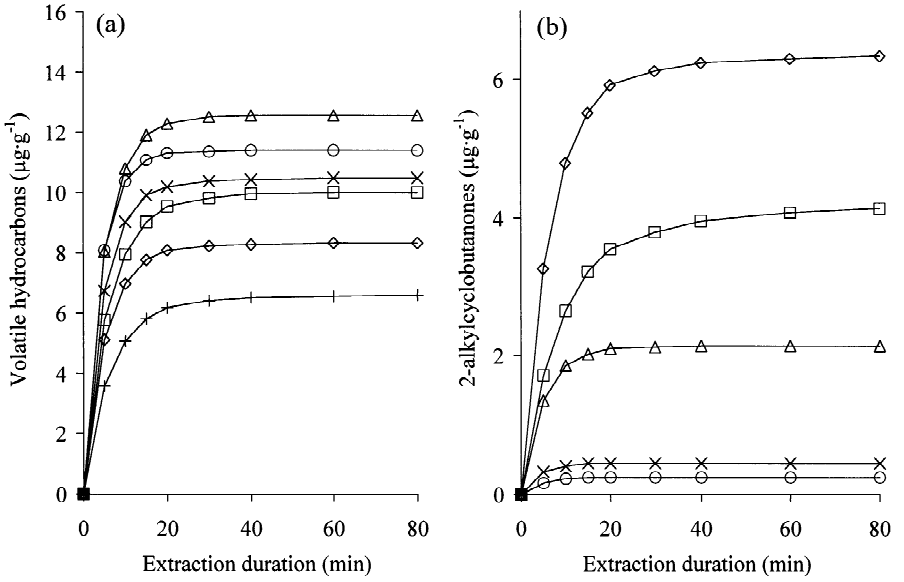

Fig. 2. Kinetics of extraction by supercritical carbon dioxide (152 bar; 80°C; flow-rate 2 ml min^{-1}) of the volatile hydrocarbons (a) and the 2-alkylcyclobutanones (b) contained in a sample of cheese irradiated at 100 kGy. (a) +, 1-heptadecane; ◊, 1-hexadecene; □, 8-heptadecene; △, 1,7-hexadecadiene; ×, *n*-pentadecane; and ○, 1-tetradecene. (b) ○, 2-hexylcyclobutanone; ×, 2-octylcyclobutanone; △, 2-decylcyclobutanone; ◊, 2-dodecylcyclobutanone; and □, 2-tetradecylcyclobutanone.

the 1-tetradecene, the shorter volatile hydrocarbon) than those obtained by application of the reference method EN 1784 [5]. In the case of the analysis of the 2-alkylcyclobutanones, they were slightly lower than those obtained by application of the reference method EN 1785 [9], most probably as a result of the lyophilization step. These recovery yields diminished with shortening of the alkyl chain and thus with the increase of the volatility of these compounds, as had already been observed on the application of the protocol EN 1785 [9].

3.3. Food analysis

The extraction protocol developed was applied to the analysis of samples of chicken irradiated at 3.0 kGy, the lipid (mainly triglyceride) content of which was 16%. The chromatograms obtained indicate excellent resolution of the peaks of the 2-dodecyl and 2-tetradecylcyclobutanones formed from palmitic and stearic acids, the most abundant saturated fatty acids (Fig. 3a) and of the peaks corresponding to the three couples of volatile hydrocarbons (alkanes

Table 2
Recovery yields (in %) of the volatile hydrocarbons and the 2-alkylcyclobutanones added to non-irradiated cheese according to the proposed method[a]

Volatile hydrocarbons	This study	Bibliographic data [5]	2-Alkyl-cyclobutanones	This study	Bibliographic data [9]
1-Tetradecene	49 (2)	68 (5)	2-decyl-	60 (7)	91 (1)
n-Pentadecane	69 (2)	64 (4)	2-dodecyl-	75 (8)	96 (1)
1-Hexadecene	76 (1)	62 (6)	2-tetradecyl-	87 (8)	98 (1)
n-Heptadecane	77 (1)	67 (5)			
1,7-Hexadecadiene	75 (1)				
8-Heptadecene	78 (1)				

[a] Mean of three measurements (standard deviation in parentheses).

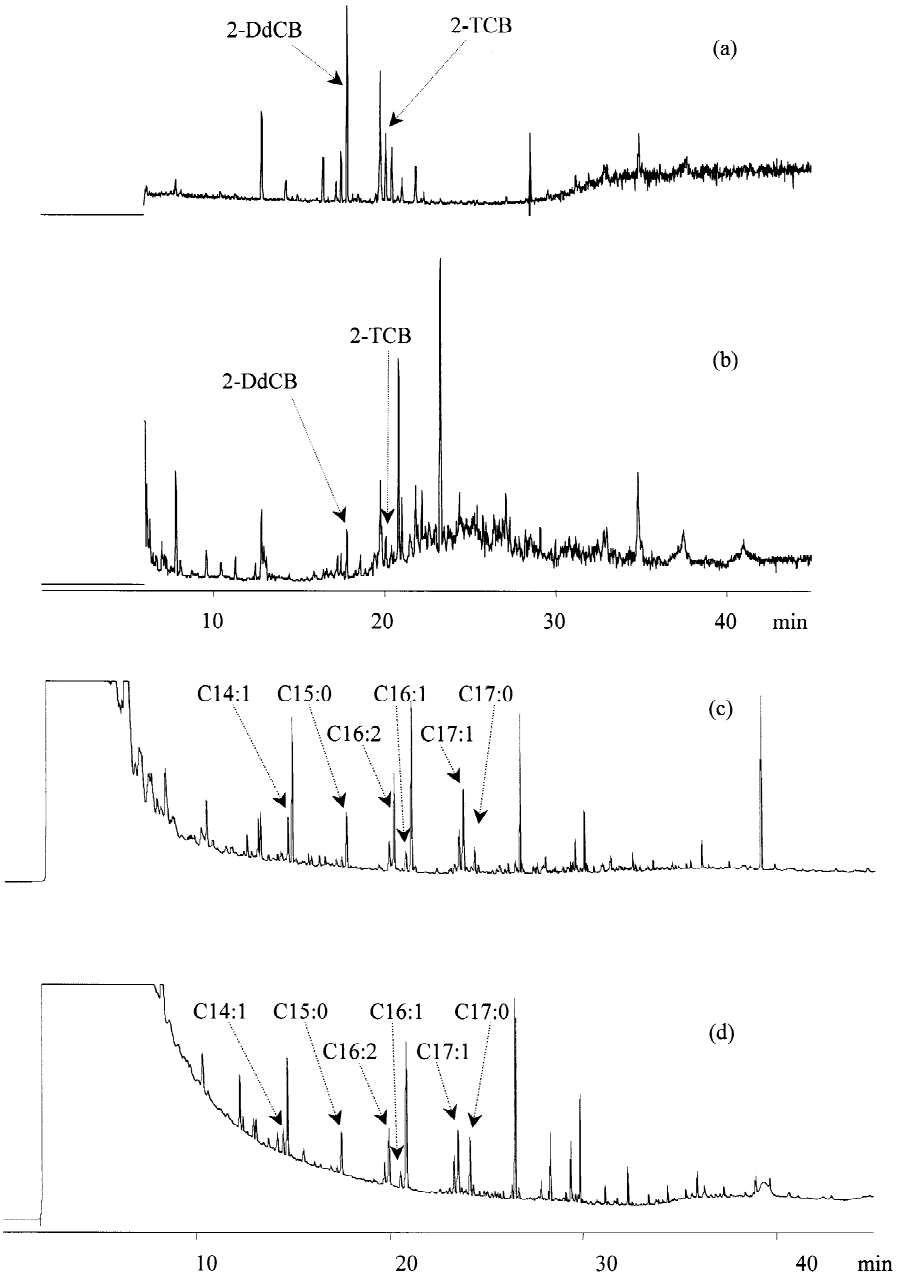

Fig. 3. Chromatograms obtained for the detection of 2-alkylcyclobutanones (ion m/z 98 mass sensitive detection) (a,b) and volatile hydrocarbons (flame ionization detection) (c,d) in a sample of chicken irradiated at 3.0 kGy. Extraction protocols: supercritical carbon dioxide extraction (152 bar; 80°C; 30 min; flow-rate 2 ml min^{-1}) and solid-phase extraction on silica (3 g) (a,c), protocol EN 1784 (d) and EN 1785 (b).

$C_{m-1:n}$ and 1-alkenes $C_{m-2:n+1}$) formed from the two previously mentioned fatty acids and oleic acid, the major fatty acid in chicken meat (Fig. 3c). This resolution is higher than that which can be attained by the methods EN 1785 (Fig. 3b) or EN 1784 (Fig. 3d) (better base line and fewer peaks of impurities),

which indicates that the suggested extraction protocol has a better selectivity than the protocols used in the reference methods.

The minimal irradiation dose (in kGy) detectable by the suggested protocol for the analysis of both the 2-alkylcyclobutanones and the volatile hydrocarbons is given by the formula:

$$D_{\min} = \frac{30 \cdot (1 - w)}{y \cdot [\mathrm{F}]} \cdot \frac{10^3}{[\mathrm{FA}]} \cdot \frac{q_{\lim}}{\rho} \quad (1)$$

in which y is the sample weight (in g) of lyophilized foodstuff, w the water content (in g g^{-1}) of non-lyophilized foodstuff (w is generally included between 0.5 and 0.8), [F] the lipid content of this non-lyophilized foodstuff (in %), $q_{\lim}$ the quantification limit of the compound analysed for the detector used (0.2×10^{-3} nmol for the 2-alkylcyclobutanones and 1×10^{-3} nmol for the volatile hydrocarbons), [FA] the content of the precursor fatty acid (in mmol g^{-1} of triglycerides) and ρ the yield of formation of the compound (1.3 or 2.0 nmol $mmol^{-1}$ of fatty acid precursor kGy^{-1}, respectively, for the 2-alkylcyclobutanones and for the volatile hydrocarbons) [5,9].

In a foodstuff in which lipid content is higher than 5%, the minimal detectable irradiation dose (in kGy) by application of the method EN 1785 and 1784 is given by the formula:

$$D_{\min} = \frac{10^3}{[\mathrm{FA}]} \cdot \frac{q_{\lim}}{\rho} \quad (2)$$

The comparison of formulas (1) and (2) shows that the method developed makes it possible to obtain a lower minimal detectable irradiation dose than those obtained by the reference methods if, for a sample weight of only 1.5 g of lyophilized foodstuff, the triglyceride content of the irradiated foodstuff is greater than or equal to 7%.

On analysis of samples (0.5 g) of avocado irradiated at 0.5 kGy ([F] = 23.5% and $w = 0.7$), the 2-dodecylcyclobutanone and the volatile hydrocarbon couple 8-heptadecene/1,7-hexadecadiene could in fact be easily isolated (Fig. 4). The many peaks of impurities present on the chromatogram obtained on analysis of the volatile hydrocarbons (Fig. 4a), also found on the chromatogram obtained by application of the reference protocol EN 1784 (data not shown), have however not made it possible to detect other hydrocarbon couples, in particular *n*-pentadecane and 1-tetradecene, produced by radiolysis of palmitic

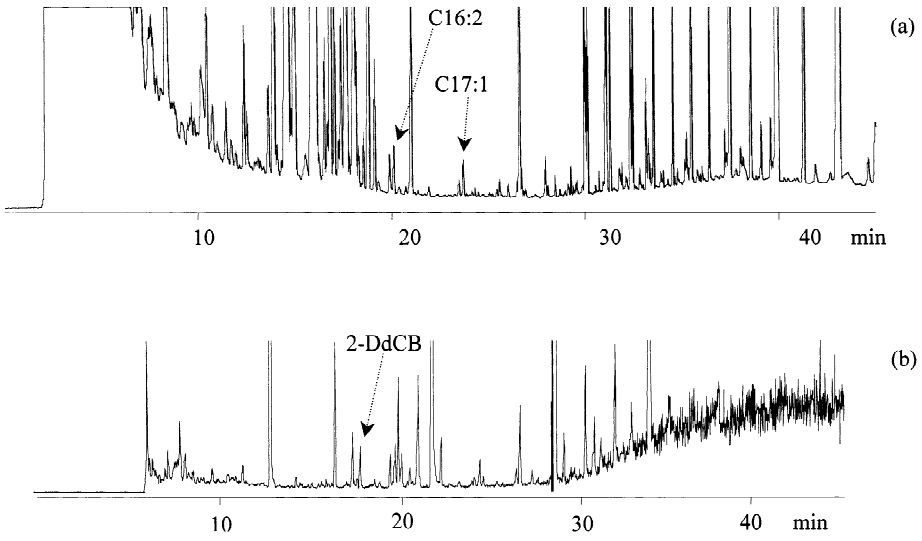

Fig. 4. Chromatograms obtained for the detection of volatile hydrocarbons (flame ionization detection) (a) and 2-alkylcyclobutanones (ion m/z 98 mass sensitive detection) (b) in a sample of avocado irradiated at 0.5 kGy. Extraction protocol: supercritical carbon dioxide extraction (152 bar; 80°C; 30 min; flow-rate 2 ml min^{-1}) and solid-phase extraction on silica (3 g).

acid. Their abundance cannot be attributed to the lack of specificity of the flame ionization detector (chromatograms quite similar were obtained by using a mass detector) but is very certainly explained by the nature of the foodstuff matrix analyzed, and in particular by the presence in this matrix of terpenes [18] extracted by the supercritical carbon dioxide and not retained in the silica trap, contrary to the triglycerides.

The application of the reference method EN 1784 has not always enabled an ionizing radiation treatment to be detected. Thus, on analysis by this method of samples of cookies, only three volatile hydrocarbons but no ($C_{m-1:n}$, $C_{m-2:n+1}$) couple could be detected. A completely identical result was obtained by application of the proposed protocol. The presence of 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone, in larger amount than 2-dodecylcyclobutanone, has made it possible to confirm that lipid ingredients of the cookies had been irradiated. Indeed, the same results can be obtained by the application of the reference protocol EN 1785, but in the case where uninterpretable results are produced by one of the two reference methods EN 1784 and 1785, their successive application will require at least 3 days of work. The proposed protocol, on the other hand, leads very rapidly (4–5 h, care having been taken to perform the lyophilization overnight before the analysis) and almost simultaneously to the results provided by the two methods of detection.

4. Conclusion

The application of the present protocol for the detection of food irradiation, as compared with the application of the reference (CEN) protocols, led to a large saving of time and a considerable reduction of the cost of the analysis. The minimal dose detectable by this protocol is, in addition, slightly lower than those of the reference protocols. Moreover, it allows a simultaneous extraction of both the 2-alkylcyclobutanones and the volatile hydrocarbons, thus giving an immediate confirmation of the result of the analysis. Considering the protection of the environment and the health of the analyst, the important reduction of the quantity of organic solvent used in this protocol is also favourable.

Acknowledgements

The authors wish to thank the French Ministry of Agriculture and Fisheries for financial support (Grants No. R95/09 and R96/25).

References

[1] J.R. Champagne, W.W. Nawar, J. Food Sci. 34 (1969) 335.
[2] P.R. Le Tellier, W.W. Nawar, Lipids 7 (1972) 75.
[3] W.W. Nawar, J.J. Balboni, J. Assoc. Off. Anal. Chem. 53 (1970) 726.
[4] C. Merritt, P. Angelini, E. Wierbicki, G.W. Shults, J. Agric. Food Chem. 23 (1975) 1037.
[5] M. Bergaentzlé, F. Sanquer, C. Hasselmann, E. Marchioni, Food Chem. 51 (1994) 177.
[6] W.W. Nawar, Z. Zhu, H. Wan, E. DeGroote, Y. Chen, T. Aciukewicz, in: C.H. McMurray, E.M. Stewart, R. Gray, J. Pearce (Eds.), Detection Methods for Irradiated Foods. Current Status, The Royal Society of Chemistry, Cambridge, 1996, p. 241.
[7] M.H. Stevenson, A.V.J. Crone, J.T.G. Hamilton, Nature 344 (1990) 202.
[8] M.H. Stevenson, A.V.J. Crone, Food Technol. 48 (1994) 141.
[9] B. Ndiaye, G. Jamet, M. Miesch, C. Hasselmann, E. Marchioni, Radiat. Phys. Chem. 55 (1999) 437.
[10] Anonymous, European Committee for Standardization, Brussels, 1996, EN 1784.
[11] Anonymous, European Committee for Standardization, Brussels, 1996, EN 1785.
[12] P. Lembke, J. Börnert, H. Engelhardt, J. Agric. Food Chem. 43 (1995) 38.
[13] J.W. Hampson, K.C. Jones, T.A. Foglia, K.M. Kohout, J. Am. Oil Chem. Soc. 73 (1996) 717.
[14] I.H. Tewfik, H.M. Ismail, S. Sumar, Lebensm. Wiss. Technol. 31 (1998) 366.
[15] M. Miesch, B. Ndiaye, C. Hasselmann, E. Marchioni, Radiat. Phys. Chem. 55 (1999) 337.
[16] F. Kuntz, E. Marchioni, A. Strasser, The International Atomic Energy Agency, in: Proceedings of an International Symposium on High Dose Dosimetry for Radiation Processing, Vienna (Austria), 1990, SM 314/30, 1991, p. 479.
[17] J.F. Gardais, Ph. Gorin, A. Prevôt, J. Serpinet, J. Tranchant, G. Untz, in: J. Tranchant (Ed.), Manuel Pratique de Chromatographie en Phase Gazeuse, Masson, Paris, 1982, p. 504.
[18] G. Schultzki, A. Spiegelberg, K.W. Bögl, G.A. Schreiber, BgVV Hefte 6 (1997).