ELSEVIER

Journal of Chromatography A, 968 (2002) 251–255

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Supercritical fluid extraction for the detection of 2-dodecylcyclobutanone in low dose irradiated plant foods

Peter Horvatovich[a], Michel Miesch[b], Claude Hasselmann[a], Eric Marchioni[a,*]

[a]*Laboratoire de Chimie Analytique et Sciences de l'Aliment (UMR 7512), Faculté de Pharmacie, Université Louis Pasteur, 74 Route du Rhin, 67400 Illkirch, France*

[b]*Laboratoire de Chimie Organique Synthétique (UMR 7123), Faculté de Chimie, Université Louis Pasteur, 1 Rue Blaise Pascal, 67000 Strasbourg, France*

Received 19 March 2002; received in revised form 30 May 2002; accepted 10 June 2002

Abstract

Supercritical carbon dioxide extraction [152 bar (15 200 kPa), 80 °C, 4 ml min^{-1}, 60 min], performed on lipids (2 g) previously extracted from irradiated plant foods, allowed a selective extraction of 2-dodecylcyclobutanone and its further detection by gas chromatography–mass spectrometry in 50 Gy irradiated cowpeas and 100 Gy irradiated rice. However, because of the higher quantities of lipid impurities in these test samples compared to those present in meat samples, a longer and slightly more polar capillary column than the one proposed in the official standard EN 1785 method should be used to obtain a satisfactory resolution.

Keywords: Food analysis; Supercritical fluid extraction; 2-Dodecylcyclobutanone

1. Introduction

The analytical protocol proposed by Horvatovich et al. [1] for the analysis of the saturated alkyl side chain 2-alkylcyclobutanones for the detection of irradiated food allowed a rapid determination (4 h) of these compounds. The sensitivity of this new method, however, just like that of the standard method [2], is not adequate for the detection of foodstuffs irradiated at low doses for insect disinfestation (cereals and leguminous plants) [3].

A reduction in the detection limit of this method, leading to an increase in its field of application, could be obtained by increasing the quantity of lipids subjected to the supercritical extraction, for example by placing the lipid phase of the sample pre-extracted by a Soxhlet apparatus in the extractor cell, instead of the dry or freeze-dried sample, as previously. Various experiments were performed on two food samples (rice and cowpea) irradiated at low doses (≤100 Gy). Only 2-dodecylcyclobutanone, resulting from the radiolysis of the most abundant saturated fatty acid present in these foods (palmitic acid), was investigated.

*Corresponding author. Tel.: +33-3-9024-4326; fax: +33-3-9024-4159.

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

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00822-1

2. Experimental

2.1. Preparation of food samples

Sheep's cheese, cowpea seeds and unpolished rice were purchased in local stores. The foodstuffs were packaged (5 mm thick) in the presence of air in plastic bags, thermosealed, stored at −20 °C and defrosted immediately prior to irradiation. The ionizing radiation treatments (100 kGy for sheep's cheese; 100 and 50 Gy for rice and cowpea seeds) were performed at 6–8 °C. Irradiation parameters and dosimetry were the same as those proposed previously [1]. All the irradiated food samples were stored in plastic bags at −20 °C.

2.2. Chemicals

The chemicals were the same as those used by Horvatovich et al. [1].

2.3. Soxhlet extraction

The extraction conditions for sheep's cheese samples were the same as those described previously [1]. Cowpea and rice samples (700 g), finely ground, were placed in two cellulose thimbles (57×170 mm, Sartorius, Goettingen, Germany) and extracted 6 h with *n*-hexane in a 1000-ml Soxhlet apparatus (4 cycles h^{-1}) (Verrerie Striegel, Strasbourg, France). The extracts were concentrated to dryness with a rotary evaporator [200 mbar (20 kPa), 35 °C] and stored at −20 °C until they were used.

2.4. Extraction by supercritical carbon dioxide

Two grams of lipids obtained after Soxhlet extraction were placed in a 10-ml internal volume stainless steel cylindrical extraction cell (I.D. 10 mm, length 128 mm) on a ~1-g hydromatrix bed. Two hundred μl of a *n*-hexane solution of 2-undecylcyclobutanone (1 mg ml^{-1}) (internal standard) were added to the sample. The remaining empty volume was filled with ~1.5 g hydromatrix. The cell was placed vertically in the oven of a Suprex supercritical extractor, Prepmaster type (Pittsburg, PA, USA) equipped with a 5-ml internal volume stainless steel cylindrical solid trap (maintained at room temperature) (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 4 bar (400 kPa) pressure]. The carbon dioxide passed through the extraction cell (from bottom to top) at a flow-rate of 4 ml min^{-1} [152 bar (15 200 kPa), 80 °C, 60 min]. The Accutrap collector was equipped with a high pressure pump making it possible to wash (2 ml min^{-1}, 20 °C) the silica trap with *n*-hexane (15 ml), then with a mixture of *n*-hexane and TBME (99:1, v/v, 55 ml). Only the last 40 ml of this mixture were retained, concentrated at 40 °C under a nitrogen stream (99.995% purity, Air Liquide™) to roughly 200 μl and analysed by gas chromatography.

2.5. Gas chromatography

The extracts were analysed following the same chromatographic conditions as proposed previously [1]. The chromatograph was fitted, however, with an OV-20-MS capillary column (Ohio Valley, Marietta, OH, USA), 60 m, 0.25 mm I.D. with a 0.10-μm stationary phase (20% diphenyl-, 80% dimethylpolysiloxane).

3. Results and discussion

The placing in the extraction cell of the lipids contained in the lyophilized (or dry) food sample rather than the lyophilized (or dry) food sample itself in order to increase the quantity of 2-dDCB subjected to supercritical phase extraction has certainly made it necessary to modify the extraction conditions previously recommended by Horvatovich et al. [1].

The kinetic study of an extraction by supercritical carbon dioxide was performed on 2 g of lipids, previously extracted from a sample of cheese irradiated at a very high dose (100 kGy) in order to obtain a high concentration of 2-dodecylcyclobutanone in the foodstuff. The study showed that by being placed under the most selective extraction conditions of temperature and pressure for the 2-alkylcyclobutanones previously established [80 °C, 152 bar (15 200 kPa)] and by using a flow-

rate of 4 ml min^{-1}, the time of extraction needed to attain the maximal concentration of 2-dodecylcyclobutanone in the extract was at least 60 min.

As long as the quantity of lipids (essentially triglycerides) placed in the extraction cell did not exceed 2 g, the quantity of these lipids entrained by the carbon dioxide under the extraction conditions selected [direct collection at the restrictor outlet (without the introduction of the silica trap) in a test tube containing 10 ml of *n*-hexane and determination by gravimetry after evaporation of the solvent] has always been very low (<20 mg). On the other hand, it has been very considerably increased for larger samples (170 and 590 mg, respectively, for samples of 2.5 and 3 g). Hence it seemed clear that the choice of a sample weight higher than 2 g induced a very considerable loss of the selectivity of the extraction protocol proposed and would require the use of an additional purification step of the extract prior to the chromatographic isolation of 2-dDCB. The solid silica trap (3 g) placed at the restrictor outlet during the analysis of the 2-dDCB, did not allow quantities

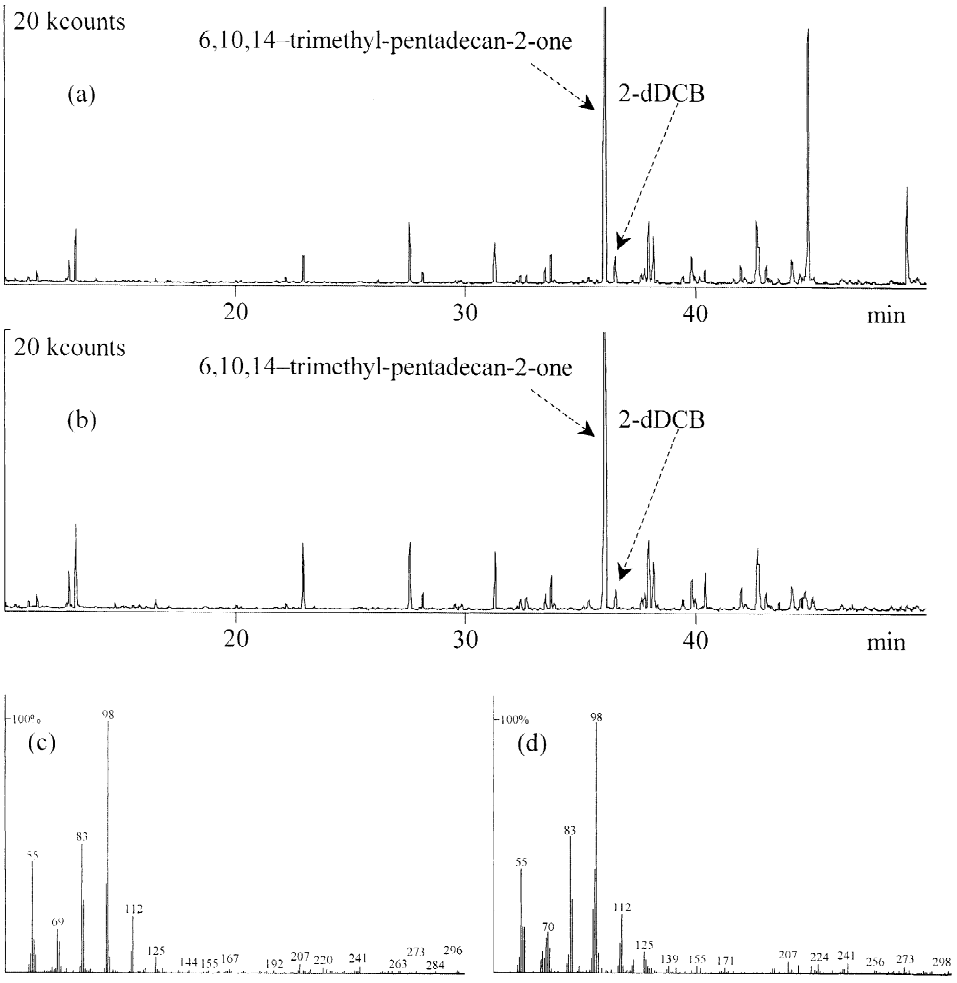

Fig. 1. Chromatograms obtained for the detection of 2-dodecylcyclobutanone (mass spectrometer detector with electron impact ionization, *m*/*z* 98) in irradiated cowpea extracts with addition of the mass spectra of the chromatographic peak attributed to 2-dDCB in samples irradiated at 100 Gy (a,c) and 50 Gy (b,d).

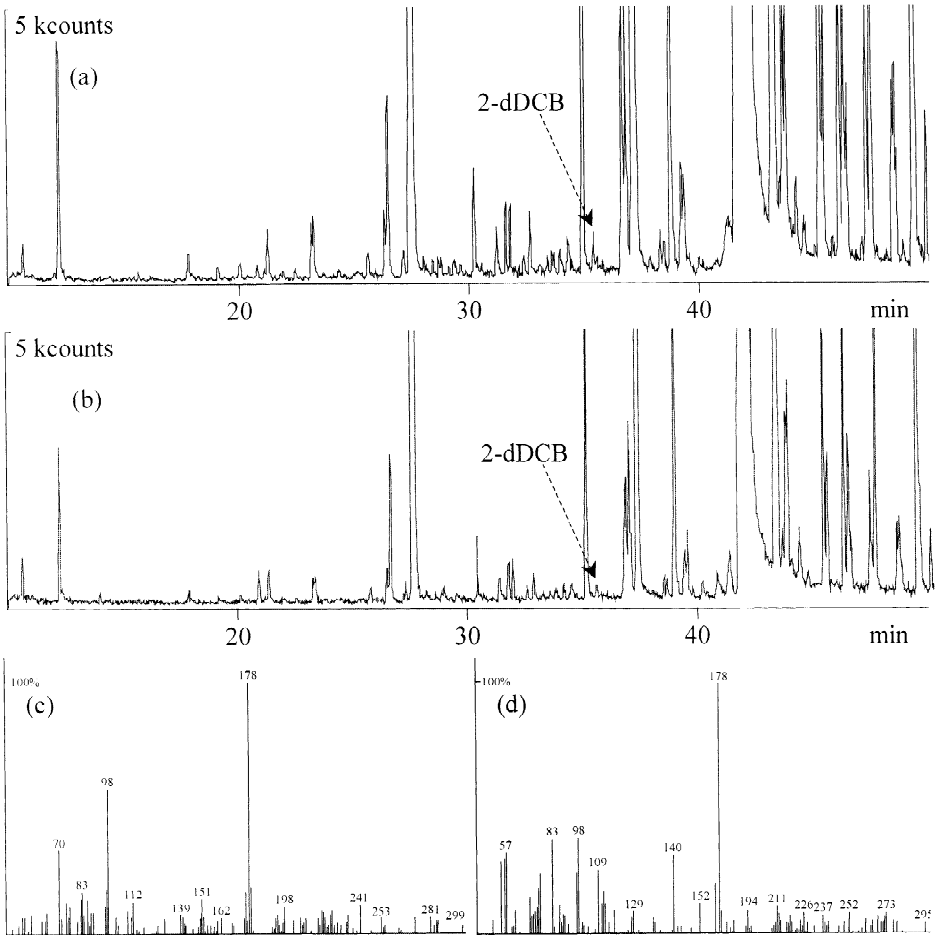

Fig. 2. Chromatograms obtained for the detection of 2-dodecylcyclobutanone (mass spectrometer detector with electron impact ionization, m/z 98) in irradiated rice extracts with addition of the mass spectra of the chromatographic peak attributed to 2-dDCB in samples irradiated at 100 Gy (a,c) and 50 Gy (b,d).

of lipids higher than 100 mg to be retained [1]. Hence it was decided to limit the sample weights to 2 g of lipids. Under these conditions of extraction, the recovery yield of 2-dDCB was (90±3)% (n=3).

Two foodstuffs capable of being irradiated at doses of the order of 100 Gy (for the purposes of disinfestation) were chosen for this study: a cereal (unpolished rice) and a pulse (cowpea seeds). Given the mean contents of water, lipids and palmitic acid of these foodstuffs [4], the quantification limit of 2-dDCB with the detector used (0.2×10^{-3} nmol) and the mean formation yield of this 2-alkylcyclobutanone (1.3 nmol mmol^{-1} of precursor palmitic acid kGy^{-1} [3]), the application of the protocol initially suggested by Horvatovich et al. [1] cannot lead to the detection of whether these foodstuffs have undergone irradiation treatment. The minimal detectable doses of irradiation would in effect be about 1.9 kGy for rice and 3.0 kGy for the cowpea which contain only small amounts of lipids (2.2 and 1.6 g%, respectively) and water (0.13 and 0.10 g g^{-1}, respectively). The analytical modification proposed (replacement of a sample of 1 g of dry or lyophilised food by 2 g of lipids extracted from these foods) will lead to a considerable lowering of the minimal detectable dose of irradiation (factors of 80 for rice

and of 125 for cowpea seeds) and ought to permit the detection in these two foodstuffs of an irradiation treatment at doses higher than 50 Gy.

In a preliminary study it was, however, not possible to demonstrate clearly the presence of 2-dDCB in a sample of cowpeas irradiated at 100 Gy by using the chromatographic column recommended by Horvatovich et al. [1] (stationary-phase 5% diphenyl-, 95% dimethylpolysiloxane, length 30 m). The mass spectrum of the peak attributed to this chemical compound indicated clearly the presence of characteristic ions m/z 98 and 112, but in a ratio of 1.5/1 which is, however, very different from the usual ratio (3.5/1) [2] owing to the co-elution of an impurity in very considerable quantity. The resolution of this co-elution could in fact be obtained by using a longer column (60 m), the stationary phase of which was slightly more polar (20% diphenyl-, 80% dimethylpolysiloxane) (Fig. 1a). The 2-dDCB could then be characterized without ambiguity by its mass spectrum, when the samples of cowpeas were irradiated both at 100 and 50 Gy (Fig. 1). It was then possible to establish the structure of the impurity from its mass spectrum. It was 6,10,14-trimethyl-penta-decan-2-one, a ketone frequently found in the plant products [5–7].

The results obtained concerning the analysis of the irradiated samples of rice were slightly less satisfactory on account of a greater number of impurities in the extracts analysed (presence of the ions m/z 178 in the mass spectra), resulting in an imperfect chromatographic isolation of 2-dDCB (Fig. 2). When the irradiation was performed at 100 Gy (Fig. 2a,c), the analysis of the mass spectra attributed to 2-dodecylcyclobutanone nonetheless indicated in both cases the presence of the characteristic ions m/z 98 and 112 in their usual ratio of 3.5/1 (Fig. 2c), which makes it possible to assert quite unambiguously that 2-dDCB was present in the samples and that in consequence the rice analysed had been previously irradiated. When the dose of irradiation for this foodstuff was only 50 Gy, it becomes more difficult, on the other hand, to distinguish the peaks attributed to 2-dDCB from the baseline fluctuations (Fig. 2b). The detection limit of the method has probably been reached. The presence of the ions m/z 98 and 112 in the mass spectrum recorded (Fig. 2d), admittedly in a ratio which is no longer characteristic, nonetheless very strongly suggests in this case that these foodstuffs have been irradiated.

4. Conclusion

The application of the present protocol to the detection of food irradiation, as compared with the application of the reference (CEN) protocol [2] or the protocol of Horvatovich et al. [1], led to a considerable reduction of the minimal detectable dose, principally for the analysis of foodstuffs with low water and lipid contents (rice and cowpeas). It thus enables foodstuffs irradiated at doses as low as 100 Gy, currently practised for disinfestation treatments, to be detected without ambiguity. The minimal dose detectable by the protocol developed seems to be around 50 Gy.

Acknowledgements

The authors wish to thank the French Ministry of Agriculture and Fisheries for financial support (Grants Nos. R95/09 and R96/25).

References

[1] P. Horvatovich, M. Miesch, C. Hasselmann, E. Marchioni, J. Chromatogr. A 897 (2000) 259.
[2] Anonymous, European Committee for Standardization, EN 1785, Brussels, 1996.
[3] B. Ndiaye, G. Jamet, M. Miesch, C. Hasselmann, E. Marchioni, Radiat. Phys. Chem. 55 (1999) 437.
[4] S.W. Souci, W. Fachmann, H. Kraut, in: Food Composition and Nutrition Tables, 5th edition, MedPharma Scientific Publishers/CRC Press, Stuttgart/Boca Raton, 1994, p. 1090.
[5] S.H. Chen, T.C. Huang, C.T. Ho, P.J. Tsai, J. Agric. Food Chem. 46 (1998) 1101.
[6] E. Bylaité, R. Venskutonis, J.P. Roozen, M.A. Posthumus, J. Agric. Food Chem. 48 (2000) 2409.
[7] N. Aligiannis, E. Kalpoutzakis, I.B. Chinou, S. Mitakou, J. Agric. Food Chem. 49 (2001) 811.