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Short communication

2-Alkylcyclobutanones as markers for irradiated foodstuffs III. Improvement of the field of application on the EN 1785 method by using silver ion chromatography

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Abstract

The inclusion of a purification step by silver ion chromatography in the EN 1785 analytical protocol for 2-alkylcyclobutanones (validated by the European Committee for Standardization for the detection of ionizing radiation treatment) has considerably improved the quality of the chromatograms obtained, allowing the detection of food samples irradiated at very low doses (0.1 kGy) or irradiated ingredients included in low proportions in non irradiated foodstuffs. This analytical modification of the protocol EN 1785 ought thus to permit a very considerable extension of its current field of application.

Keywords: Food analysis; EN 1785; 2-Alkylcyclobutanones

1. Introduction

The method of detection EN 1785 of irradiated foodstuffs based on the detection of 2-dodecyl- and 2-tetradecylcyclobutanones [1], validated by the European Committee for Standardization, was developed from studies conducted by Stevenson and her group between 1990 and 1993 [2–7]. It can be used provided that the foodstuffs have a fat content of at least 0.01 g g^{-1} and are treated at doses higher than 0.5 kGy [8]. Since 1993, other analytical methods for 2-alkylcyclobutanones, very different from the method EN 1785 [by high-performance liquid chromatography (HPLC)–fluorimetry [9], by coupled liquid chromatography–gas chromatography–mass spectrometry (LC–GC–MS) [10], by enzyme-linked immunosorbent assay [11], by supercritical fluid extraction–thin layer chromatography (SFE–TLC) [12] or by SFE–GC–MS [13]], have been published. However, in every case the objective pursued was not to attempt to enlarge the field of application of the EN 1785 method but simply to try to develop analytical protocols easier to use than that

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validated by the European Committee for Standardization.

The objective of this study, on the contrary, was to make possible an extension of the field of application of the protocol EN 1785 by providing a modification entailing the use of solid-phase extraction by a cation exchanger impregnated with silver ions (after passage of the test sample through a column of Florisil) in order to try to improve the quality of the extracts subsequently analyzed by GC–MS.

The purification by silver ion chromatography, recently proposed by Hartmann et al. [14] for fractionation, as a function of the degree of unsaturation, of the volatile hydrocarbons contained in irradiated foods and for the removal of substances interfering with the analyzed extracts, might in fact reduce the amplitude of the background noise of the chromatographic recording (often excessive when the EN 1785 method is used, in particular on analysis of complex foodstuffs). It might thus enable the sample weight of fat taken (limited to 200 mg in the method EN 1785) and, correspondingly, the concentration of the 2-alkylcyclobutanones in the test extract to be considerably increased, while the amounts of impurities present in this extract are maintained at a level which does not disturb the chromatographic isolation of these chemical compounds.

2. Experimental

2.1. Chemicals

The 2-alkylcyclobutanone standards [2-hexyl- (2-HCB), 2-octyl- (2-OCB), 2-decyl- (2-DCB), 2-dodecyl- (2-DdCB), 2-dodecenyl- (2-DdeCB), 2-tetradecyl- (2-TCB) and 2-tetradecenylcyclobutanone (2-TdeCB)] were obtained according to the method of Miesch et al. [15].

Florisil [150 to 250 μm (60–100 mesh)] was supplied by Aldrich (Saint-Quentin, France). It was activated prior to use by heating at 550°C for 5 h, then deactivated by addition of water (20 ml of water per 100 g of Florisil). The cation exchanger SCX (propylbenzene sulfonic acid, 75 μm) was supplied by ICT (Bad-Homburg, Germany). *tert*.-Butyl methyl ether (TBME), acetone and ammonium acetate were Merck products (Darmstadt, Germany). Silver nitrate was supplied by Sigma (Saint-Quentin, France). Methanol of HPLC-grade was supplied by Carlo Erba (Rodano, Italy). The *n*-hexane, of technical quality, was distilled from calcium hydride before use.

The internal standard was 2-cyclohexylcyclohexanone (Fluka, Saint-Quentin, France).

2.2. Test sample preparation

The rice samples were purchased in a supermarket. The liquid whole eggs were supplied by a French agri-foodstuffs company. These foodstuffs were placed in the presence of air in plastic sachets which were then thermosealed. They were irradiated at room temperature at doses of 0.1 kGy (rice) and 4.0 kGy (liquid whole eggs) and stored at −20°C until they were analyzed (rice) or used (liquid whole eggs) for the preparation of a precooked meal [cookies containing irradiated liquid whole eggs (3%, m/m) prepared at the Lycée Technique d'Hôtellerie et de Tourisme, Strasbourg, France].

The chicken quenelle samples, prepared with inclusions (2 and 4%, m/m) of mechanically recovered meat (MRM) irradiated at 5.0 kGy (at the factory) were supplied by a French company specialized in the production of precooked meals.

After being prepared, the precooked meals (cookies and chicken quenelles) were stored at −20°C until they were analyzed.

2.3. Irradiation conditions and dosimetry

A CIRCE III linear electron accelerator, 10 MeV, 0.5 mA (MeV Industrie, Paris, France) was used for the irradiation of the food samples (dose rate 3 kGy s^{-1}). The temperature during the irradiation was 6 to 8°C. Irradiation doses were verified with FWT 60.00 (Far West Technology, Goleta, USA) radiachromic dosimeters, previously calibrated with an alanine dosimeter (Laboratoire de Métrologie des Rayonnements Ionisants, Gif-sur-Yvette, France).

2.4. Preparation and regeneration of the cation exchanger impregnated with silver ions

The cation exchanger was loaded onto the frit of a glass column (80 mm×10 mm I.D.). It was treated

successively with 10 ml of an aqueous ammonium acetate solution (1%, m/v), 10 ml of distilled water, then rinsed with 2 ml of an aqueous silver nitrate solution (1%, m/v) and finally washed successively with 10 ml of methanol, 10 ml of TBME and 10 ml of *n*-hexane. The column was then wrapped in aluminium foil for protection against light.

After use, the cation exchanger phase was successively rinsed with 5 ml of acetone, 2 ml of TBME and 2 ml of *n*-hexane. Such a column can be reused 20 times.

2.5. Extraction of fat from foodstuffs and purification of the extracts obtained

The extraction of the lipids from a foodstuff sample [20 g (cheese, cookies and quenelles), 40 g (rice)] with *n*-hexane (and their quantification) were performed in accordance with the EN 1785 standard. A preliminary filtration step [through a regenerated cellulose membrane (0.45 μm), Sartorius, Goettingen, Germany] was performed on the fat prior to the analysis of the rice extract samples (owing to the presence of starch in suspension).

The quantity of fat analyzed (from 200 mg to 800 mg depending on the sample analyzed) was distributed in one or more 200-mg fractions. Each fraction was then purified by solid-phase extraction on a column of Florisil (protocol EN 1785). The hexane extracts containing the 2-alkylcyclobutanones were pooled and concentrated to a final volume of 3 ml by heating at 40°C under reduced pressure.

This hexane extract was then loaded onto the cation-exchange column impregnated with silver ions. Elution was performed successively with 10 ml of *n*-hexane, 5 ml of *n*-hexane–TBME (95:5, v/v) and 5 ml of TBME–methanol (95:5, v/v). The different eluates thus obtained were evaporated to dryness under a stream of nitrogen, then redissolved in 200 ml of *n*-hexane containing 0.5 mg ml^{-1} of 2-cyclohexylcyclohexanone (internal standard).

2.6. Gas chromatography–mass spectrometry

Separation of 2-alkylcyclobutanones was performed on a 3400 type (Varian, Palo Alto, CA, USA) gas chromatograph directly linked to a Saturn 2000 (Varian) electron impact mass-sensitive detector. The gas chromatograph was fitted with a Varian DB 5MS capillary column, 30 m×0.25 mm I.D. with a 0.25 μm stationary phase (95% dimethylpolysiloxane–5% phenylmethylpolysiloxane). Conditions used were as follows: SPI injector temperature programme: 50°C (1 min), ramp 180°C min^{-1} to 240°C; column temperature programme: 60°C (1 min), ramp 8°C min^{-1} to 300°C, held for 10 min; injection volume 1 μl, injection mode on-column; carrier, helium (5.0 quality), 1 ml min^{-1}. The chromatograms were recorded in the total ion current (TIC) mode. The 2-alkylcyclobutanones were quantified with respect to the ion m/z 98 (2-alkylcyclobutanones with a saturated alkyl chain) and the ion m/z 95 (2-alkylcyclobutanones with a monounsaturated alkyl chain).

3. Results and discussion

The silver ion chromatography proposed by Hartmann et al. (1997) for fractionation as a function of the degree of unsaturation, of the volatile hydrocarbons, was applied with minor modifications of the composition of the mobile phases, to the purification of the fat extracts containing the 2-alkylcyclobutanones with saturated and unsaturated alkyl chains.

The analysis by GC–MS of the different solvent fractions obtained after elution from the silver ion column, showed that none of these 2-alkylcyclobutanones was eluted by *n*-hexane (fraction 1). The 2-alkylcyclobutanones with a saturated alkyl chain, which were found quantitatively in the second fraction (*n*-hexane–TBME, 95:5, v/v), have thus interacted with the stationary phase (probably through the intermediary of their carbonyl group) and required for their elution a slightly less apolar mobile phase than *n*-hexane. The 2-alkylcyclobutanones with a monounsaturated alkyl chain (which have not been investigated in the foodstuffs studied in this work) were eluted only with a mobile phase (TBME–methanol, 95:5, v/v) much more polar than the previous one, which can no doubt be explained by the presence of a double bond in the alkyl chain and therefore by a much stronger interaction with the silver ions than that observed for the 2-alkylcyclobutanones with a saturated alkyl chain.

The results obtained, and especially the demonstration of an interaction between the silver ions and the 2-alkylcyclobutanones with a saturated alkyl chain, have thus suggested the use of this silver ion chromatography as an additional purification step in the case where the strict application of the protocol EN 1785 might not allow the detection of the presence or absence of 2-alkylcyclobutanones in the sample analyzed, owing to the existence of many interfering peaks on the chromatogram.

Ndiaye et al. [8] were able to show, using the protocol EN 1785, that the minimal detectable level of inclusion of mechanically recovered chicken meat irradiated at 5 kGy in non irradiated chicken quenelles, was more than 6%. It was in fact confirmed that the detection of the 2-dodecyl- and 2-tetradecylcyclobutanones was impossible when the level of inclusion was 4% (Fig. 1a). Their detection was made possible, on the other hand, by performing purification of the extracts by silver ion chromatography (Fig. 1b). The presence of 2-dodecylcyclobutanone could be clearly detected even when its

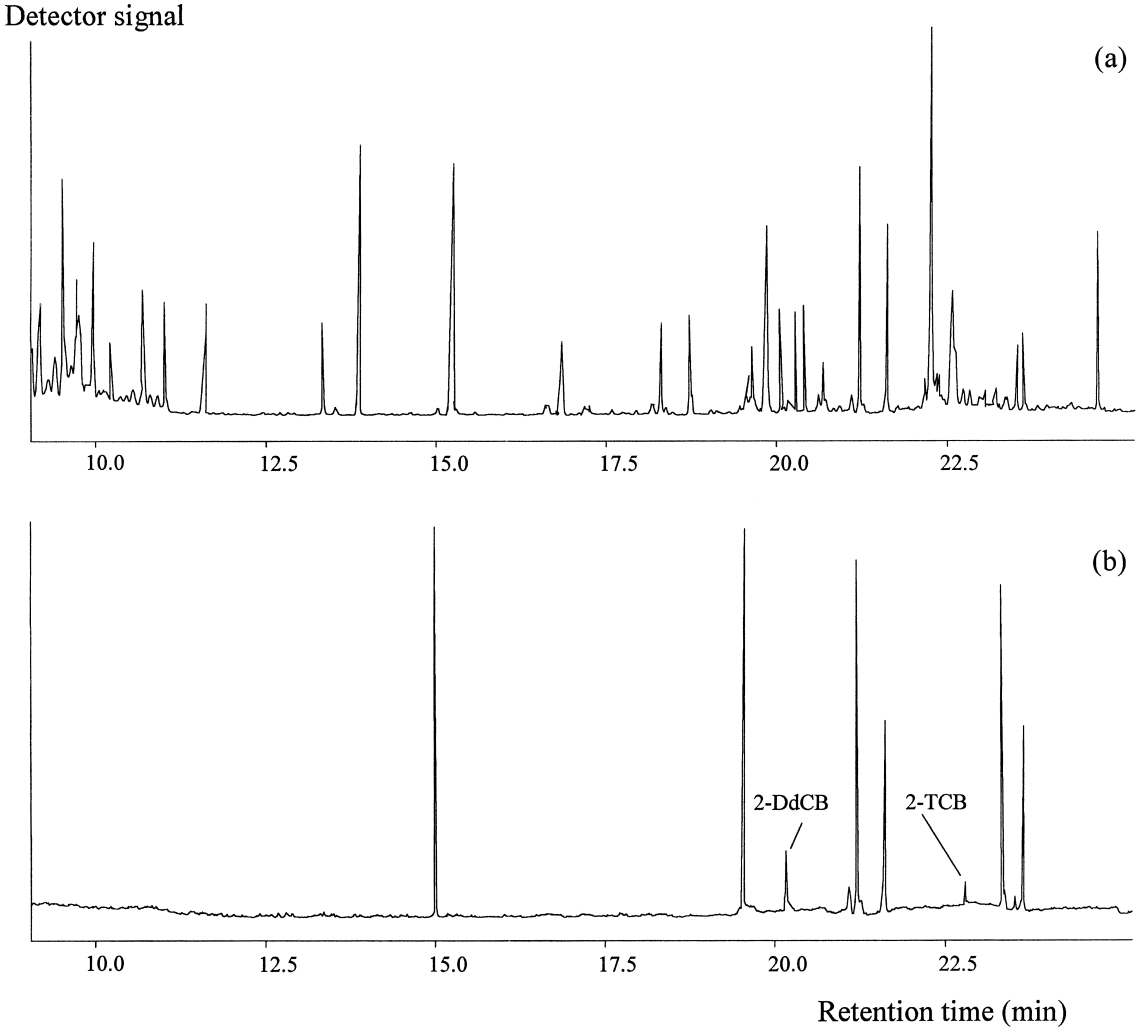

Fig. 1. Chromatograms obtained for the detection of 2-alkylcyclobutanones (selected ion monitoring of the ion m/z 98) in samples of chicken quenelles containing mechanically recovered meat (4%, m/m) irradiated at 5.0 kGy: (a) method EN 1785 and (b) method EN 1785 after additional purification by silver ion chromatography.

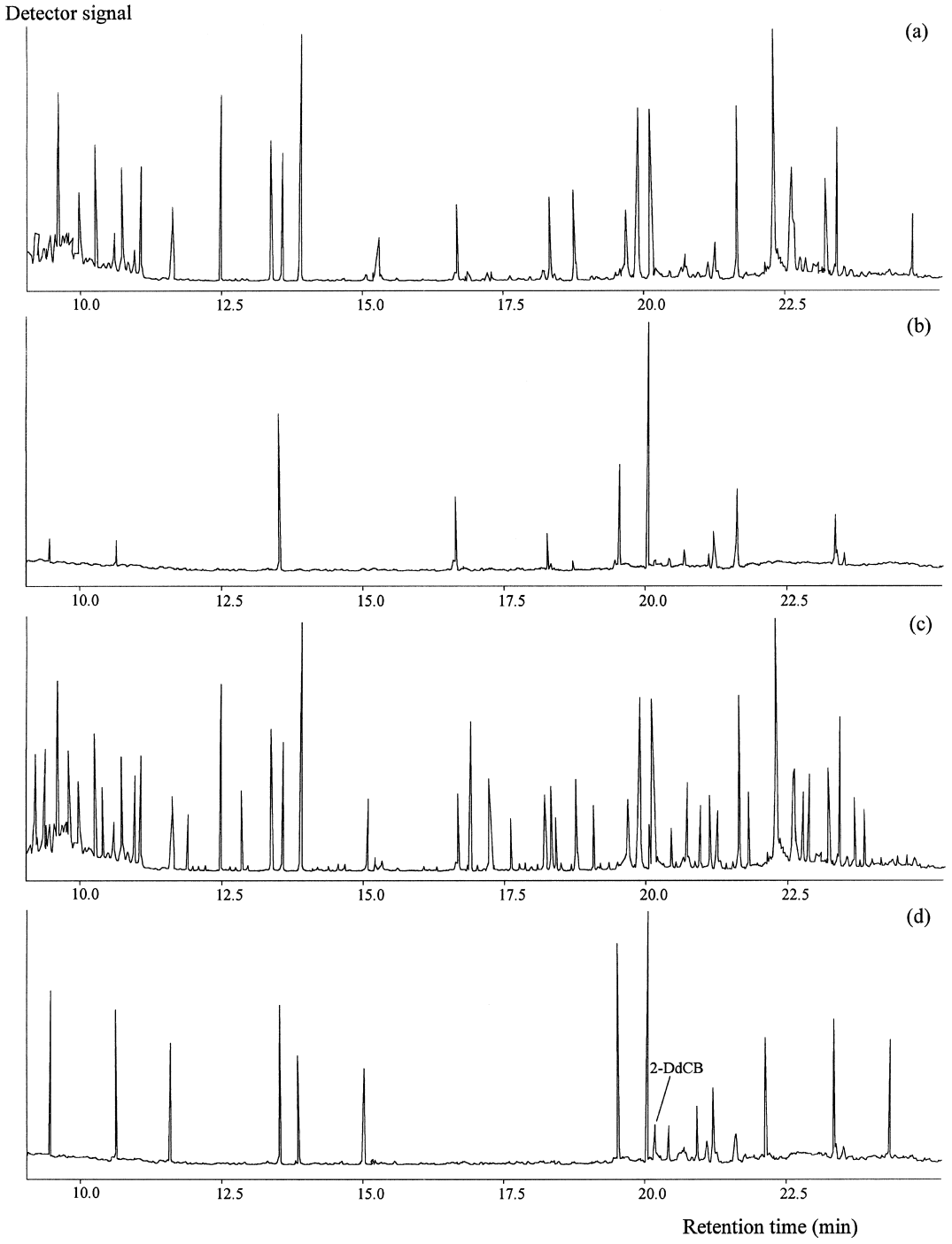

Fig. 2. Chromatograms obtained for the detection of 2-alkylcyclobutanones (selected ion monitoring of the ion m/z 98) in a unpolished rice sample irradiated at 0.1 kGy: (a) method EN 1785, (b) method EN 1785 after additional purification by silver ion chromatography, (c) method EN 1785 with four-fold increase in the amount of fat analyzed and (d) method EN 1785 with four-fold increase in the amount of fat analyzed and additional purification by silver ion chromatography.

level of inclusion was only 2% (chromatogram not shown).

When very low doses of irradiation were used, the sensitivity of the mass detector used in the protocol EN 1785 may not prove to be low enough to detect the quantities of 2-alkylcyclobutanones produced in the foodstuff samples analyzed. In a sample of unpolished rice for example (a cereal in which palmitic acid is the predominant saturated fatty acid), it has never in fact been possible to demonstrate the presence of 2-dodecylcyclobutanone after irradiation at 0.1 kGy (dose used for insect control in this foodstuff) (Fig. 2a). The use of purification by means of silver ion chromatography has certainly led to the disappearance of many interfering peaks from the previous chromatogram (Fig. 2b) but the only useful result it has led to from the point of view of the detection of irradiation treatment was to show unambiguously that the sensitivity of the protocol EN 1785 (nonetheless slightly improved by this additional purification) did not allow the detection of the 2-alkylcyclobutanones. A considerable diminution of the minimal detectable irradiation dose necessarily implies in this case an increase in the concentration of 2-alkylcyclobutanones present in the extract analyzed by GC–MS. This increase could be obtained very simply by analyzing a quantity of fat greater than that recommended in the protocol EN 1785

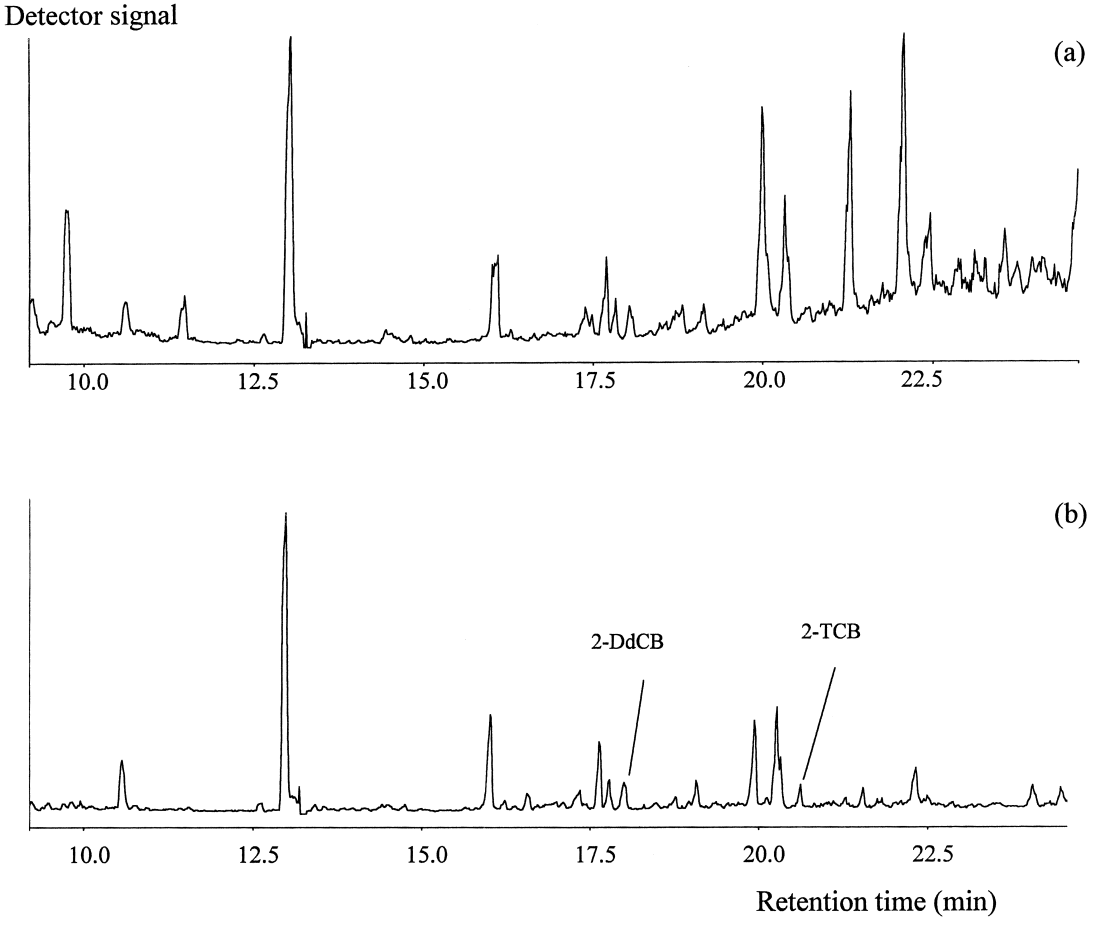

Fig. 3. Chromatograms obtained for the detection of 2-alkylcyclobutanones (selected ion monitoring of the ion m/z 98) in a sample of cookies containing liquid whole egg (3%, m/m) irradiated at 4.0 kGy: (a) method EN 1785 and (b) method EN 1785 with three-fold increase in the amount of fat analyzed and additional purification by silver ion chromatography.

(800 mg instead of 200 mg). This has also had the foreseeable consequence of increasing the amounts of impurities present in the extract analyzed and of making the interpretation of the chromatogram obtained completely impossible (Fig. 2c). The problem of the detection of irradiated rice, on the other hand, could be resolved by combining this increase of the sample taken with purification of the extracts analyzed by the use of silver ion chromatography. With such a protocol, 2-dodecylcyclobutanone could be easily detected (Fig. 2d), thus making it possible to characterize the irradiation of rice at 0.1 kGy.

The application of protocol EN 1785 to the detection of liquid whole egg irradiated at 4.0 kGy included at a level of 3% (m/m) in non irradiated cookies also met with failure (Fig. 3a). This result was hardly surprising since the fat content of the ingredient (0.11 g g^{-1}) was markedly lower than that of the manufactured food (0.20 g g^{-1}) and, consequently, only a very small portion of the fat analyzed belonged to the irradiated ingredient. Just as in the case of rice, the increase in the sample weight of fat analyzed (600 mg instead of 200 mg), combined with purification by silver ion chromatography of the test extract made it possible to detect a 2-tetradecylcyclobutanone peak in the chromatogram obtained (Fig. 3b) and thus to characterize the ionizing radiation treatment of liquid whole egg included in the cookies. It was not possible to detect 2-dodecylcyclobutanone with certainty. The ions m/z 98 and 112 were indeed present in the mass spectrum recorded at the retention time of this compound but the complete spectrum obtained did not correspond to that for this molecule, owing to the coelution of an impurity.

In the light of the results obtained during this investigation, the use of silver ion chromatography as a purification procedure for foodstuff extracts containing 2-alkylcyclobutanones may reveal itself to be an excellent analytical solution when the analysis of these chemical compounds is impossible by the protocol EN 1785, either because of the presence of interfering peaks on the chromatogram or because these compounds are present in the foodstuff in quantities to low to be studied. It ought to lead to a very considerable extension of the field of application of the protocol EN 1785 for the detection of the foodstuffs irradiated at low doses (the case of cereals) or irradiated ingredients included in non irradiated complex matrix.

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