Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 110 (2008) 531–537
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Analytical Methods

Solid phase microextraction as a methodology in the detection of irradiation markers in ground beef

M.M. Caja, M.L. Ruiz del Castillo *, G.P. Blanch

Instituto de Fermentaciones Industriales. Consejo Superior de Investigaciones Científicas (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

Received 14 September 2007; received in revised form 31 January 2008; accepted 10 February 2008

Abstract

The usefulness of solid phase microextraction (SPME) to detect the occurrence of the irradiation markers 2-dodecylcyclobutanone (2-DCB) and 1,3-bis(1,1-dimethylethyl)benzene in irradiated ground beef was evaluated. To that aim, beef samples were irradiated with different irradiation doses and subsequently examined together with non-irradiated beef samples used as control samples. The SPME conditions applied were selected as a result of performing an optimization process including different fibers (PDMS, DVB/CAR/PDMS, polyacrylate and PDMS/DVB), as well as extraction times (10, 25 and 40 min) and temperatures (40 and 60 °C). For comparison, 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene were additionally identified in some of the samples by steam distillation–solvent extraction (SDE). Although this study is a preliminary work, from the results obtained SPME seemed to be a rapid and valuable technique to determine 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene in ground beef subjected to irradiation, offering advantages over other methods reported in the literature. In addition, SPME allowed to confirm the validity of 2-DCB as an useful marker to distinguish non-irradiated from irradiated ground beef. On the contrary, the occurrence of 1,3-bis(1,1-dimethylethyl)benzene was however established in both types of samples by SPME and SDE.

Keywords: SPME; Irradiation; 2-Dodecylcyclobutanone; 1,3-Bis(1,1-dimethylethyl)benzene; Ground beef

1. Introduction

Over the last few years there has been an increasing demand for new techniques for food preservation replacing the use of hazardous chemicals (Bhattacharjee, Singhal, Gholap, Variyar, & Bongirwar, 2003). Among them, food irradiation has been demonstrated to be particularly effective in inactivating pathogens, decreasing microbial load and extending shelf life without appreciable alteration in food quality (Giroux & Lacroix, 1998; Thomas, 1986; Urbain, 1986). However, despite repeated assurances that irradiation is one of the safest methods to preserve foodstuffs, nowadays consumers still demand labeled foods by legislation to avoid unknown risks. For that reason, new methods capable of differentiating between irradiated and non-irradiated foods are currently sought.

A number of biological, physical and chemical methods have been developed for detecting irradiated foods. In this regard, although the biological (Nation, Smittle, & Milne, 1995; Scotter, Beardwood, & Wood, 1995; Wirtanen, Salo, Karwoski, & Sjöberg, 1995) and physical (Desrosiers, 1996; Dodd, 1995; Rahman, Haque, & Sumar, 1995) methods have widely demonstrated their usefulness in the detection of irradiated foods, the chemical methods are the most commonly used. As chemical methods, it can be emphasized those based on the detection of marker compounds of irradiation, such as 2-alkylcyclobutanones (Boyd et al., 1991; Crone, Hamilton, & Stevenson, 1992) and radiation-induced lipid-derived long-chain volatile hydrocarbons (Bergaentzle, Sanquer, Hasselman, & Marchioni, 1994; Morehouse, Kiesel, & Ku, 1993). The usefulness of the former markers is known long ago and the relation

* Corresponding author. Tel.: +34 91 5622900; fax: +34 91 5644853.
E-mail address: ifir312@ifi.csic.es (M.L. Ruiz del Castillo).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.02.024

between its occurrence and the employment of an ionizing radiation is at present doubtless (Stevenson, 1994; Stewart, Moore, Graham, McRoberts, & Hamilton, 2000). Regarding long-chain volatile hydrocarbons, some authors have recently proposed 1,3-bis(1,1-dimethylethyl)benzene as an indicator in the identification of irradiated beef extract powders (Kim, Cho, Ahn, Cho, & Cha, 2005). However, in contrast to 2-alkylcyclobutanones, the validity of this compound as an irradiation marker has only been determined usable in this specific study and more studies would be needed to extend the application field of this indicator to irradiated samples other than beef extract powders.

The European standard (EN1785) method for the identification of irradiated lipid-containing foods is based on the detection of 2-alkylcyclobutanones by means of solvent extraction followed by Florisil chromatography. This method was validated in 1996 by Ministry of Agriculture, Fisheries and Food validated method (MAFF V37) (MAFF, 1996) and implies extremely long overall analysis time (72–84 h), large organic solvent volumes, high economic cost and relatively high explosion risk (McMurray, Brannigen, Hamilton, Boyd, & Stevenson, 1999; Rahman, Haque, & Sumar, 1996). These limitations have been overcome by using a supercritical fluid extraction (SFE) method to also detect 2-alkylcyclobutanones, which has more recently been proposed as an alternative to the official method (Gadgil, Hachmeister, & Smith, 2002; Rahman, Matabudall, Haque, & Sumar, 1995; Tewfik, Ismail, & Sumar, 1998). Advantages of the SFE approach are the remarkable reduction in the extraction time (from 6–18 h to 30 min), the larger amount of sample that can be used and the higher efficiency in extracting low levels of 2-alkylcyclobutanones. Nonetheless, SFE is not a technology easily accessible to all laboratories owing mainly to the high initial economic cost. Accordingly, the search for additional methods to identify irradiated foods would be valuable in routine analysis of irradiated foods.

In this regard, solid phase microextraction (SPME) has demonstrated to be an interesting methodology to isolate minor compounds from complex matrices (Arthur & Pawliszyn, 1998; Pawliszyn, 1995; Zhang & Pawliszyn, 1993; Zhang, Yang, & Pawliszyn, 1994). It is rapid, accessible, inexpensive and simple handling. The usefulness of SPME in studying volatile compounds obtained as a consequence of the application of irradiation to foodstuffs has occasionally been reported. However, most of these works are not aimed to identify irradiation indicators but volatile sulfur compounds, which have been described to give off a pungent odor at low concentrations (Fan & Sokorai, 2002; Fan, Sommers, Thayer, & Lehotay, 2002). In fact, reports on the application of SPME to the determination of irradiation markers are extremely scarce. Specifically, Kim et al. (2005) identified irradiation volatile markers in beef extract powder by SPME while Thomazini, Contreras, and Miyagusku (2006) applied this sample preparation technique to the detection of markers in irradiated chicken thigh.

The goal of this investigation was to study the usefulness of SPME to distinguish irradiated from non-irradiated ground beef through the occurrence of 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene.

2. Materials and methods

2.1. Samples and materials

2-DCB and 1,3-bis(1,1-dimethylethyl)benzene standards used in the identification of the target compounds were obtained from Sigma–Aldrich (Madrid, Spain). Dichloromethane and Milli-Q water employed in the preparation of the standard solution as well as in the SPME and SDE extractions were obtained from SDS (Peypin, France) and from a Milli-Q water purification system (Millipore, Milford, MA), respectively. A standard solution containing 10 mg of each compound in 10 ml of dichloromethane was used to optimize the chromatographic separation and the extraction conditions used in SPME.

Six beef samples (fat content around 30%) used for human consumption, which had not been submitted to any treatment, were purchased from the local market. Five of them were not irradiated to be used as control samples (Samples 1–5). On the contrary, Sample 6 was vacuum-packed in nylon/polyethylene bags (100 g each) to be subjected to irradiation. The bags were irradiated using electron beam at targeted absorbed doses of 2.0 (Sample 7), 4.0 (Sample 8) and 8.0 kGy (Sample 9) (IONMED Esterilización, S.A., Cuenca, Spain). The irradiation dose applied in each case was monitored by using a radiochromic film dosimeter. The rest of Sample 6 was also used as a control sample. Both non-irradiated (Samples 1–6) and irradiated samples (Samples 7–9) were finally frozen at −18 °C until their analysis.

2.2. Solid phase microextraction (SPME)

The isolation of 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene was carried out for all samples by solid phase microextraction (SPME). A fused silica fiber coated with a 100 μm layer of polydimethylsiloxane installed in a holder for manual use (Supelco, Madrid, Spain) was utilized. Before using the SPME fiber, it was conditioned in the injector of the gas chromatograph at 250 °C for 30 min. A 2.0 g weight of the frozen ground beef plus 2.0 ml of Milli-Q water were placed into a 10.0 ml vial, which was sealed with plastic film suitable for the SPME extraction (i.e., low water permeability, insensitivity to moisture vapor and the commonest reagents). The simple addition of water enabled the sample to be thawed. Experimentation was performed by exposing the fiber to the headspace of the sample for 10 min at 40 °C. Prior to the extraction, an incubation time of 10 min was applied to enrich the headspace in the target compounds. As later explained in results and discussion, the extraction conditions (fiber type, extraction temperature and exposure time) were selected as

a result of an optimization process. This optimization was accomplished by applying the same extraction time and temperature as above-described starting from a 0.2 μl volume of the standard solution in 2.0 ml of Milli-Q water. The release of 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene into the headspace and their transfer to the fiber was promoted by applying constant sample stirring throughout the experimentation. Upon completion of the extraction step, the target compounds were thermally desorbed into the GC injector and finally analyzed by gas chromatography–mass spectrometry (GC–MS), as detailed below.

2.3. Steam distillation–solvent extraction (SDE)

For Samples 6–9, the extraction of the interesting compounds was in addition performed by using the high-density solvent configuration of the commercial version (Chrompack, Middelburg, The Netherlands) of the micro-distillation–extraction device, as proposed in the past (Godefroot, Sandra, & Verzele, 1981). A 50 g weight of frozen ground beef was allowed to thaw at room temperature during approximately 15 min before use. Then, 100 ml of Milli-Q water was added to the defrosted sample and the mixture was properly homogenized with an Ultra Turrax homogenizer (Ika-Werk, Germany). The resulting sample was used to obtain the SDE extracts. A 2 ml volume of distilled dichloromethane was employed as the extraction solvent. The sample was heated in a silicone bath at 120 °C whereas dichloromethane was distilled by heating with a water bath at 60 °C. During the extraction process the vapors of both sample and solvent condense in such a way that the continuous reflux was maintained over the extraction time (2 h), the distillable material being finally collected in the dichloromethane. Between consecutive runs the SDE apparatus was carefully cleaned with acetone and Milli-Q water. Once the extraction was finished, a further concentration step (up to a final volume of approximately 0.2 ml) under a nitrogen stream was required to achieve the sensitivity demanded for identification purposes. The SDE-extracts were finally analyzed by using GC–MS as detailed below.

2.4. Gas chromatography/mass spectrometry (GC/MS)

The extracts obtained by both SPME and SDE were analyzed by using a Hewlett–Packard Model 6890 gas chromatograph fitted with a split/splitless injector and mass spectrometry (MS) Model HP5973 detector. For SPME, the fiber desorption was carried out at 250 °C for 10 min whereas a 0.4 μl volume of the SDE-extracts were sampled into the GC injector, which was kept at 250 °C at all times. Splitless mode was used in all instances. GC separations were performed on a 30 m × 0.25 mm I.D. fused silica column coated with a 0.25 μm layer of polyethyleneglycol (BTR-Carbowax, Quadrex, Woodbridge, CT, USA). After holding the initial temperature at 60 °C for 10 min, the column was temperature-programmed at 5 °C/min to 230 °C and kept at this temperature for 5 min. Helium was used as the carrier gas at an initial flow rate of 1 ml/min. The source and the quadrupole temperatures were set at 230 and 100 °C, respectively. With the aim of attaining a higher sensitivity, the SIM mode was mostly used. The ion m/z 175 was monitored for 1,3-bis(1,1-dimethylethyl)benzene whereas the ions m/z 95, 98 and 112 were monitored for 2-DCB. Data acquisition from the MS was accomplished with the HP-ChemStation system. In all instances 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene were identified by matching the mass spectrum and retention time provided by the standards run under the same experimental conditions. The extraction (by both SPME and SDE) and GC-analysis of all samples were carried out at least in triplicate.

3. Results and discussion

The experimental conditions (fiber type, extraction temperature and time) applied during the extraction process were optimized as explained below. The selection of the optimum values were based on those conditions that provided the highest peak areas of the target compounds from the standard solution. First, the extraction temperature and time were fixed at 40 °C and 10 min, respectively, to select the most adequate fiber. We considered in our study: polydimethylsiloxane (PDMS, film thickness 100 μm), divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS, film thickness 50/30 μm), polyacrylate (film thickness 85 μm) and polydimethylsiloxane/divinylbenzene (PDMS/DVB, film thickness 65 μm). Fig. 1 depicts the peak areas obtained for 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene from the four mentioned fibers. As seen, although the best results for 1,3-bis(1,1-dimethylethyl)benzene were provided by DVB/CAR/PDMS, 2-DCB was practically not retained. Nevertheless, the employment of PDMS resulted in satisfactory areas for both analytes. For that reason, we eventually chose PDMS to perform the extractions.

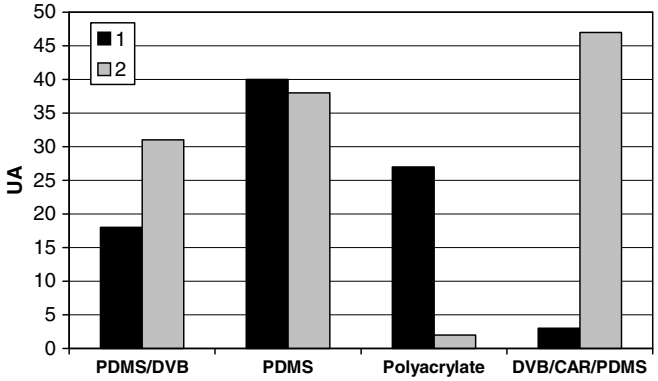

Fig. 1. Peak areas of 2-DCB (compound 1) and 1,3-bis(1,1-dimethylethyl)benzene (compound 2) with the different SPME-fibers utilized during the optimization process. See text for more details.

Subsequently, the exposure time was optimized by setting the SPME fiber (PDMS) and temperature (40 °C) while testing 10, 25 and 40 min. From this experiment, significant differences in the peak areas were not found with any of the values tested. This implies that distribution equilibrium is most likely reached at 10 min in such a way that the extracted amount at longer times remains constant. Taking this into account, we selected 10 min as the extraction time because of the shorter analysis time obtained. Finally, by using PDMS and fixing the exposure time at 10 min, we tried 60 °C as the extraction temperature and compared the areas obtained to those provided by 40 °C. The latter provided higher peak areas and, consequently, a temperature of 40 °C seemed to be more recommendable.

The repeatability of the proposed method was estimated by calculating the relative standard deviation (RSD, $n = 3$) from both the standard solution and real-life sample (ground beef irradiated with 8.0 kGy) under the optimum extraction conditions. The values obtained from the standard solution were 22.5% and 8.5% for 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene, respectively, whereas 17.9% and 5.6% were the values achieved from the beef sample. Likewise, the detection limit was calculated by considering a signal/noise ratio of three from the standard solution under the optimum experimental conditions. The resulting values were 0.35 and 0.14 ppm for 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene, respectively.

Table 1
Identification of irradiated ground beef through the determination of 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene by SPME-GC–MS

Samples	Irradiation dose (kGy)	2-DCB	1,3-Bis(1,1-dimethylethyl)benzene
1	0	Not detected	Detected[b]
2	0	Not detected	Detected
3	0	Not detected	Detected
4	0	Not detected	Detected
5	0	Not detected	Detected
6	0	Not detected	Detected
7	2	Uncertain[a]	Detected
8	4	Detected	Detected
9	8	Detected	Detected

[a] Signal approximately twice the background noise.
[b] Signal at least three times the background noise.

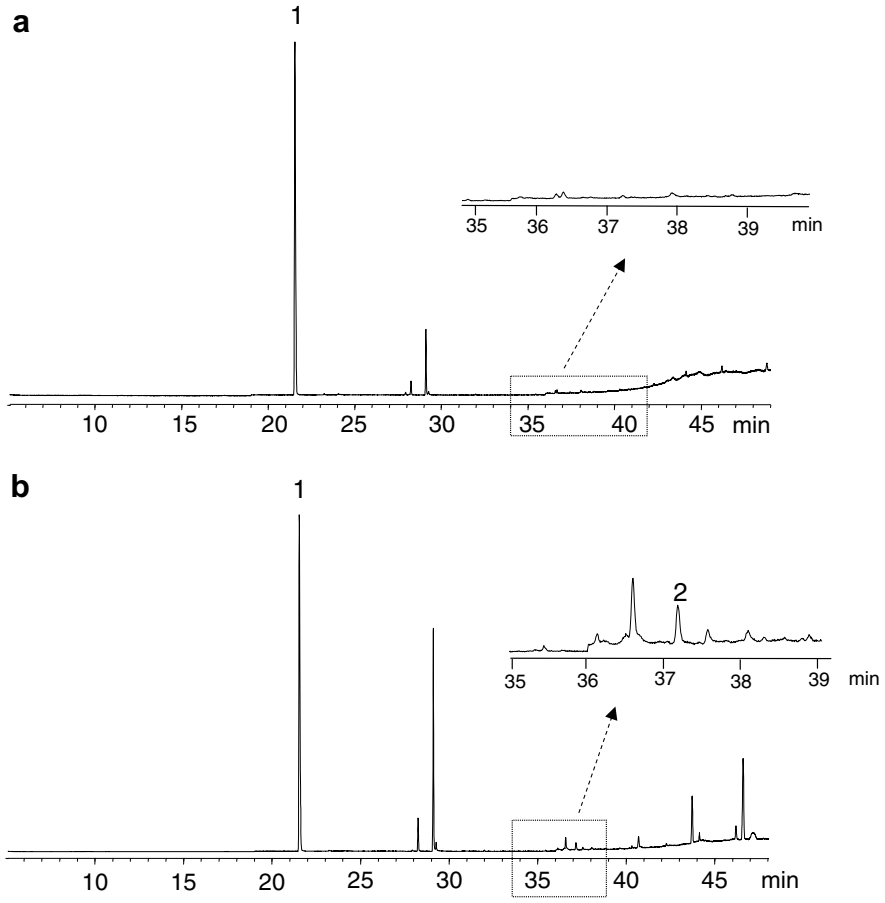

Fig. 2. Chromatograms resulting from the SPME-GC–MS analysis of (a) non-irradiated and (b) irradiated ground beef (Samples 6 and 9 in Table 1, respectively). Peak identification: (1) 1,3-bis(1,1-dimethylethyl)benzene, (2) 2-DCB.

Table 1 illustrates the results obtained from the SPME-GC–MS analysis in the SIM mode for 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene in non-irradiated and irradiated ground beef samples as well as the different irradiation doses utilized. As can be observed, 2-DCB could not be detected in any of the non-irradiated samples used as control samples (Samples 1–6 in Table 1) whereas its occurrence in the irradiated beef (Samples 7–9 in Table 1) was clear when irradiation doses of 4 and 8 kGy were applied. The chromatographic signal corresponding to this marker was, however, uncertain when a 2.0 kGy dose was used to irradiate the sample. Specifically, the obtained signal was equal to two times the background noise in such a way that its detection could not be unambiguously accomplished and, in short, its presence could not be established. Other authors have reported the detection of 2-DCB in ground beef patties irradiated with doses as low as 1.0 kGy by a SFE procedure (Gadgil et al., 2002). Considering, however, the minor concentration at which 2-DCB occurred in the mentioned study, the reason why its presence could not be established in ground beef irradiated with 2.0 kGy in the present study might be the lower content of 2-DCB, as a consequence of the lower fat content, with respect to that in the samples studied by Gadgil et al. (2002).

Considering, thus, not only the viability but also the advantages of using SPME to detect 2-DCB, our aim now is to improve the sensitivity of the method proposed to study samples irradiated at doses lower than 1.0 kGy. As also can be seen in Table 1. 1,3-bis(1,1-dimethylethyl)benzene was identified for certain by mass spectrometry in all samples, i.e., both non-irradiated and irradiated beef. This means that it cannot be used as an irradiation marker in ground beef samples. Consequently, although, according to the literature (Kim et al., 2005), this compound appears to be an useful marker to distinguish non-irradiated from irradiated beef extract powders, its extension to irradiated beef samples which have not been submitted to any treatment did not seem in principle feasible.

As an example Fig. 2 shows the chromatograms obtained from the SPME-GC–MS analysis of (a) non-irradiated (Sample 6 in Table 1) and (b) ground beef irradiated with a 8.0 kGy dose (Sample 9 in Table 1). As can be seen, whereas 2-DCB occurred exclusively in the sample subjected to irradiation (Fig. 2b), the presence of 1,3-bis(1,1-dimethylethyl)benzene was apparent in both samples.

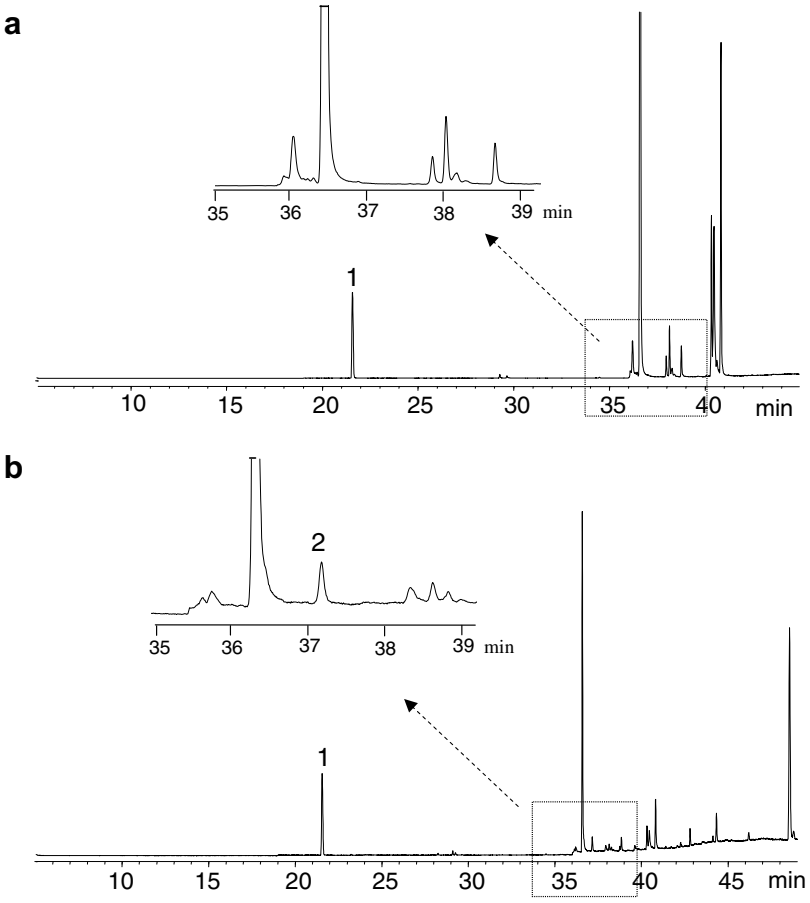

Fig. 3. Chromatograms resulting from the SDE-GC–MS analysis of (a) non-irradiated and (b) irradiated ground beef (Samples 6 and 9 in Table 1, respectively). Peak identification: (1) 1,3-bis(1,1-dimethylethyl)benzene, (2) 2-DCB.

Moreover, to verify the validity of SPME in the detection of 2-DCB and 1,3-bis(1,1-dimethylethyl)benzene as well as the use of 1,3-bis(1,1-dimethylethyl)benzene as an irradiation indicator in ground beef samples, a steam distillation–solvent extraction (SDE) approach was also applied to Samples 6–9. The reason why we elected this extraction method for confirmation purposes was its similarity to the official method for the identification of 2-DCB in irradiated foods (MAFF, 1996). As a result, 2-DCB was not detected in the control sample (Sample 6) and in the sample irradiated with a 2.0 kGy dose (Sample 7) while its presence in Samples 8 and 9 was confirmed. With regard to 1,3-bis(1,1-dimethylethyl)benzene, it was once more detected in both irradiated and non-irradiated samples.

Fig. 3 depicts the chromatograms corresponding to the SDE-GC–MS analysis of (a) non-irradiated (Sample 6 in Table 1) and (b) ground beef irradiated with 8.0 kGy (Sample 9 in Table 1). By comparison of Fig. 3a and b, it is obvious that whereas the usefulness of 2-DCB as an irradiation marker was also confirmed by SDE, the occurrence of 1,3-bis(1,1-dimethylethyl)benzene cannot be directly associated with the application of the ionizing radiation.

In summary, SPME may be an interesting alternative to the official method based on solvent extraction to detect 2-DCB in irradiated ground beef. Advantages of SPME are its rapidness (overall extraction time, 50 min), simplicity, low economic cost, accessibility and no need for the use of large amount of organic solvents. On the other hand, 1,3-bis(1,1-dimethylethyl)benzene cannot be used as an irradiation marker in ground beef since its occurrence in both irradiated and non-irradiated samples can be positively established by SPME-GC–MS and SDE-GC–MS under the experimental conditions described in this work.

In short, we consider that, although this is a preliminary study, the results found in this work are promising. The intention is now to extend the application of SPME to the analysis of a larger number of samples as well as to the search for new irradiation markers with a view to proposing this approach as an alternative to the official method to identify irradiated ground beef.

Acknowledgments

The authors thank financial assistance from Comunidad de Madrid (Project CAM S-0505/AGR/0314) and Débora García for her help in performing some of the experimental work.

References

Arthur, C. L., & Pawliszyn, J. (1998). Solid-phase microextraction with thermal desorption using fused silica optical fibers. *Analytical Chemistry, 62*, 2145–2148.

Bergaentzle, M., Sanquer, F., Hasselman, C., & Marchioni, E. (1994). Detection of gamma irradiated raw milk Camembert cheese by capillary gas chromatographic analysis of volatile hydrocarbons. *Food Chemistry, 51*, 177–182.

Bhattacharjee, P., Singhal, R. S., Gholap, A. S., Variyar, P. S., & Bongirwar, D. R. (2003). Hydrocarbons as marker compounds for irradiated cashew nuts. *Food Chemistry, 80*, 151–157.

Boyd, D. R., Crone, A. V. J., Hamilton, J. T. G., Hand, M. V., Stevenson, M. H., & Stevenson, P. J. (1991). Synthesis, characterization and possible use of 2-dodecylcyclobutanone as a marker for irradiated chicken. *Journal of Agricultural and Food Chemistry, 39*, 789–792.

Crone, A. V. J., Hamilton, J. T. G., & Stevenson, M. H. (1992). Effect of storage and cooking on the dose response of 2-dodecylcyclobutanone, a potential marker for irradiated chicken. *Journal of the Science of Food and Agriculture, 58*, 249–252.

Desrosiers, M. F. (1996). Current status of the EPR method to detect irradiated food. *Applied Magnetic Resonance, 47*, 1621–1628.

Dodd, N. J. F. (1995). Free radicals and food irradiation. In C. A. Rice-Evans (Ed.), *Free radicals and oxidative stress* (pp. 247–258). London, UK: Biochemical Society.

Fan, X., & Sokorai, K. J. B. (2002). Changes in volatile compounds of c-irradiated fresh cilantro leaves during cold storage. *Journal of Agricultural and Food Chemistry, 50*, 7622–7626.

Fan, X., Sommers, C. H., Thayer, D. W., & Lehotay, S. J. (2002). Volatile sulfur compounds in irradiated precooked turkey breast analyzed with pulsed flame photometric detection. *Journal of Agricultural and Food Chemistry, 50*, 4257–4261.

Gadgil, P., Hachmeister, K. A., & Smith, J. S. (2002). 2-Alkylcyclobutanones as irradiation dose indicators in irradiated ground beef patties. *Journal of Agricultural and Food Chemistry, 50*, 5746–5750.

Giroux, M., & Lacroix, M. (1998). Nutritional adequacy of irradiated meat: A review. *Food Research International, 31*, 257–264.

Godefroot, M., Sandra, P., & Verzele, M. (1981). New method for quantitative essential oil analysis. *Journal of Chromatography, 203*, 325–335.

Kim, H., Cho, W.-J., Ahn, J.-S., Cho, D.-H., & Cha, Y.-J. (2005). Identification of radiolytic marker compounds in the irradiated beef extract powder by volatile analysis. *Microchemical Journal, 80*, 127–137.

MAFF validated method V37 (1996). Method for the detection of irradiated food containing fat. *Journal of the Association for Public Analysis, 32*, 39–52.

McMurray, B. T., Brannigen, I. O., Hamilton, J. T. G., Boyd, D. R., & Stevenson, H. (1999). Detection of irradiated foods using cyclobutanones. *Food of the Science and Technology Today, 8*, 99–100.

Morehouse, K., Kiesel, M., & Ku, Y. (1993). Identification of meat treated with ionising radiation by capillary gas chromatographic determination of radiolytically produced hydrocarbons. *Journal of Agricultural and Food Chemistry, 41*, 758–763.

Nation, J. L., Smittle, B. J., & Milne, K. (1995). Radiation induced changes in melanization and phenoloxidase in caribbean fruit fly Larvae (Diptera: Tephritidae) as the basis for a simple test of irradiation. *Annual Entomological Society of America, 88*, 201–205.

Pawliszyn, J. (1995). New directions in sample preparation for analysis of organic compounds. *Trends in Analytical Chemistry, 14*, 113–122.

Rahman, R., Haque, A. K., & Sumar, S. (1995). Physical methods for the identification of irradiated foodstuffs. *Nutrition and Food Science, 2*, 36–41.

Rahman, R., Haque, A. K., & Sumar, S. (1996). Cyclobutanones as markers for irradiated samples of Brie and Camember cheese. *Nahrung, 40*, 55–59.

Rahman, R., Matabudall, D., Haque, A. K., & Sumar, S. A. (1995). A rapid method (SFE-TLC) for the identification of irradiated chicken. *Food Research International, 29*, 301–307.

Scotter, S., Beardwood, K., & Wood, R. (1995). Detection of irradiation treatment of poultry meat using the Limulus Amoebocyte Lysate test in conjunction with a gram negative bacterial count. Collaborative trial. *Journal of the Association for Public Analysis, 31*, 163–178.

Stevenson, M. H. (1994). Identification of irradiated foods. *Food Technology, 48*, 141–144.

Stewart, E. M., Moore, S., Graham, W. D., McRoberts, W. C., & Hamilton, J. T. G. (2000). 2-Alkylcyclobutanones as markers for the detection of irradiated mango, papaya, Camembert cheese and salmon meat. *Journal of the Science of Food and Agriculture, 80*, 121–130.

Tewfik, I. H., Ismail, H. M., & Sumar, S. A. (1998). A rapid supercritical fluid extraction method for the detection of 2-alkylcyclobutanones in gamma irradiated beef and chicken. *Lebensmittel-Wissenschaft und Technologie, 31*, 366–370.

Thomas, A. C. (1986). Radiation preservation of foods of plant origin III. Tropical fruits: Bananas, mangoes and papayas. *Critical Review in Food Science and Nutrition, 23*, 147–206.

Thomazini, M., Contreras, C. C. J., & Miyagusku, L. (2006). Solid phase microextraction for the analysis of irradiated raw chicken thigh. *Italian Food Science, 18*, 329–335.

Urbain, W. M. (1986). *Food irradiation*. Orlando, FL: Academic Press.

Wirtanen, G., Salo, S., Karwoski, M., & Sjöberg, A.-M. (1995). A microbiological screening method for the indication of irradiation of frozen poultry meat. *Zeitschrift Lebensmittel Untersuchung und Forschung, 200*, 194–197.

Zhang, Z., & Pawliszyn, J. (1993). Headspace solid-phase microextraction. *Analytical Chemistry, 65*, 1843–1852.

Zhang, Z., Yang, M., & Pawliszyn, J. (1994). Solid-phase microextraction, a solvent free alternative for sample preparation. *Analytical Chemistry, 66*, 844A–854A.