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Rapid detection of radiation-induced hydrocarbons in cooked ham

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ABSTRACT

Solid phase microextraction (SPME) coupled with either gas chromatography-ionization flame detector (CG-FID) or multidimensional gas chromatography–mass spectrometry (MDGC–MS) was evaluated for its ability to detect volatile hydrocarbons produced during the irradiation of cooked ham. The chromatogram of an irradiated sample obtained using GC–FID showed a complex pattern of peaks, with several co-eluting peaks superimposed, indicating that the method was unlikely to resolve adequately the volatile hydrocarbons formed during irradiation. Using SPME–MDGC–MS 1-tetradecene ($C_{1-14:1}$), *n*-pentadecane ($C_{15:0}$), 1-hexadecene ($C_{1-16:1}$), *n*-heptadecane ($C_{17:0}$) and 8-heptadecene ($C_{8-17:1}$) were detected in cooked ham irradiated at 0.5, 2, 4 and 8 kGy. This method allows the detection of most n-alkanes and n-alkenes produced during the irradiation of the majority of fatty acids in cooked ham, namely oleic acid, stearic acid and palmitic acid. SPME is rapid and inexpensive and does not require organic solvents. The proposed SPME–MDGC–MS method allows the determination of radiolytic markers in cooked ham in less than 115 min.

1. Introduction

Modern dietary habits have changed; one change being the increase in the consumption of ready-to-eat (RTE) foods of both vegetable and animal origin with very different pack presentation.

The first stages of RTE food production involve a low risk of contamination with pathogenic microorganisms due to the characteristics of the product and to the procedures used. However, RTE food production involves additional manipulations such as cutting, slicing, dicing and packaging. During these subsequent steps, a variety of pathogenic microorganisms can contaminate RTE products from the environment, machinery, tools, and handlers. Several treatments have been developed to avoid post-packaging contamination, such as in-package thermal pasteurization, irradiation and high pressure processing (Zhu et al., 2005).

Contamination with pathogenic organism such as *Listeria monocytogenes* has been reported in RTE products (Cabeza et al., 2007; Sommers & Fan, 2002; Zhu, Du, Cordray, & Ahn, 2005). The use of non-thermal treatments such as irradiation has been proposed to inactivate microorganisms and help maintain the safety of meal products. However, some reports indicate that its application is limited since irradiation can produce changes in lipid oxidation, texture, odor, color and flavor, all of which can affect consumer acceptance (Lee & Ahn, 2005; O'Bryan et al., 2008).

Reliable and routine methods to verify the irradiation history of foods may encourage consumer acceptance of food irradiation and may improve the enforcement of labeling regulation. Available tests rely on a variety of chemical, physical and biological techniques, as reviewed by Chauhan et al. (2009). An important landmark in method standardization came when the European Committee for Standardization adopted two of these methods as European standards for the detection of irradiated food containing fat: one analyzes radiolytic hydrocarbons (BS EN 1784:2003); the other analyzes 2-alkylcyclobutanones (BS EN 1785:2003).

These two methods are complex and involve a long extraction utilizing solvents so are not suited as quick screening tools. To minimize some of these problems, innovative extraction methods, such as solid-phase microextraction (SPME), have been proposed. SPME has been successfully applied to a wide variety of compounds, especially for the extraction of volatile and semi-volatile organic compounds from complex sample matrices (Kataoka et al., 2000; Risticevic et al., 2009). SPME has been used to identify radiolytic markers in meat and meat products (Fan & Sommers, 2006; Kim et al., 2005).

To improve the identification of radiolytic markers, different gas chromatography (GC) methods have been used. However, single-column chromatographic techniques cannot always provide acceptable separations of all components in complex mixtures. Thus, multidimensional gas chromatography (MDGC), which comprises two or more separation steps, has been proposed as a way to enhance peak capacity. For analysis requiring extremely high efficiencies, the "heart-cutting" MDGC technique (Deans, 1981) has proven effective. This approach involves the transfer of one or more unresolved fraction from a first column "precolumn" to a second column, reported as "main column" (Lorenzo et al., 2005; Mondello et al., 1998; Schomburg, 1995).

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The objective of the present study was to analyze radiolytic hydrocarbons formed during the irradiation of cooked ham using SPME to simplify sample preparation, and chromatographic methods GC–FID and MDGC–MS for their ability to identify the greatest number of compounds in the shortest time.

2. Material and methods

2.1. Samples

Cooked ham was acquired in the local market and cut into slices approximately 2 mm thick using an electric slicer. Slices were vacuum-packed in laminated film bags (polyamide and polyethylene, film thickness 90 μm) of low permeability (35 $cm^3/24\ h\ m^2$ bar to oxygen, 150 $cm^3/24\ h\ m^2$ bar to CO_2) and maintained at 4 °C for less than 24 h before irradiation.

The compounds 1-tetradecene ($C_{1-14:1}$), *n*-pentadecane ($C_{15:0}$), 1-hexadecene ($C_{1-16:1}$), *n*-heptadecane ($C_{17:0}$), 8-heptadecene ($C_{8-17:1}$) and 1,7- hexadecadiene ($C_{1,7-16:2}$) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and used as standards. A solution of each compound on its own was prepared at 1 mg/l in *n*-hexane (SDS Purex Analytical Grade, Spain) to determine their retention times under the experimental conditions detailed below for the chromatographic analysis.

2.2. Irradiation treatment

Cooked ham samples were transported under refrigeration (4 °C) to the irradiation plant IONMED Sterilization S.A. (Tarancón, Cuenca, Spain), which houses a linear electron accelerator. Doses of 0.5, 2, 4 and 8 kGy were delivered using a 10-MeV electron beam. The actual dose absorbed was determined by cellulose triacetate dosimeters placed on top of each sample during irradiation treatment. The temperature of samples was controlled at 25 °C, and the increase during irradiation was always lower than 2 °C. After the treatment, the irradiated cooked ham was transported under refrigeration back to the laboratory and frozen at −20 °C, where it was maintained until analysis.

2.3. Solid-phase microextraction

Compounds of interest were retained on a Supelco SPME fiber assembly holder (Bellefonte, PA, USA) provided with an 85-μm carboxen/polydimethylsiloxane (CAR/PDMS) coated fused-silica fiber. Prior to use, the fiber was conditioned for 60 min at 300 °C inside the GC injector port, as recommended by the manufacturer.

Non-irradiated and irradiated cooked ham samples were chopped up to enable volatiles to be released. Aliquots of chopped cooked ham (2 g) were placed into 5 ml vials and sealed with plastic film (parafilm); and the same amount of sample was mixed with 3 ml of 16% (w/v) NaCl, 1/1 (v/v) acetonitrile/water, or pure acetonitrile. In all cases the fiber was exposed to the headspace, and SPME was performed for 30 min at 60 °C by operating the fiber in the headspace mode (i.e., HS–SPME) as previously reported (Barba et al., 2010). The extraction was carried out in a thermostated silicone bath. After the extraction period was over, the target compounds were thermally desorbed into the GC injector (splitless mode) at 250 °C for 2 min and then analyzed by either GC–FID or MDGC–MS as explained below.

2.4. GC–FID analysis of the extracts obtained by HS–SPME

A Varian (Palo Alto, CA, USA) gas chromatograph model CP-3800 was used. Sampling introduction (achieved, as previously mentioned, by thermal desorption of the solutes previously retained onto the SPME fiber) was performed using the PTV injector of the gas chromatograph. The separation was carried out on a 30-m × 0.25-mm i.d. fused-silica capillary column coated with a 0.25-μm layer of ZB-Wax (Micron Analítica, S.A., Madrid, Spain). The oven temperature was initially at 40 °C and it was successively increased up to 95 °C at 4 °C/min, then to 120 °C at 2 °C/min and finally to 230 °C at 4 °C/min. The oven was then maintained at 230 °C for 10 min. The FID detector was operated at 250 °C. Helium was used as carrier gas, and the injector was operated in splitless mode.

2.5. MDGC–MS analysis of extracts obtained by HS–SPME

The MDGC system (See Fig. 1) consisted of two independent Varian (Palo Alto, CA, USA) gas chromatographs (model CP-3800), in which two columns, namely precolumn and main column, were housed. Both columns were serially coupled through a Deans based switching system (Deans, 1981) and a transfer line, which was maintained at 180 °C throughout the experimentation. Preseparation was carried out on a fused-silica capillary column (30 m × 0.25 mm i.d.) coated with a 0.25-μm layer of ZB-Wax (Micron Analítica, S.A., Madrid, Spain), under previously described (point 2.4) chromatographic conditions. The cut between 30 and 43 min, which contained the target compounds, was then transferred to the second dimension (main column) and analyzed on a (25 m × 0.25 mm i.d.) fused-silica capillary column coated with a 0.25-μm film thickness of permethylated Chirasil-β-Dex (Varian, Middelburg); in this case, the oven temperature was initially set at 50 °C (15 min) and then successively raised to 70 °C (1 °C/min), to 140 °C (2 °C/min) and finally it was increased to 200 °C (4 °C/min).

In both dimensions helium served as the carrier gas at an approximate head pressure of 30 psig in the precolumn and 24 psig in the main column. The precolumn was connected to a FID detector, which was always operated at 250 °C, so that separations achieved in the precolumn could be monitored. The main column was connected to a Saturn 2000 ion-trap mass spectrometer (Varian, Palo Alto, CA, USA). Data acquisition was performed using a Star Toolbar system (Varian).

For MS analysis in the MDGC–MS procedure, the electron multiplier was set to 1850 V and ionization was accomplished by electron impact (EI). The temperatures of the transfer line, manifold and trap were fixed, respectively, at 180 °C, 120 °C and 220 °C. The recorded spectra covered the range from 40 to 650 m/z.

The target compounds were identified by matching their GC retention times in both the precolumn and the main column with those obtained from authentic standards analyzed under the same experimental conditions. Mass spectra recorded from the standard compounds were also compared with those provided by the US National Institute of Standards and Technology (NIST) library. Between

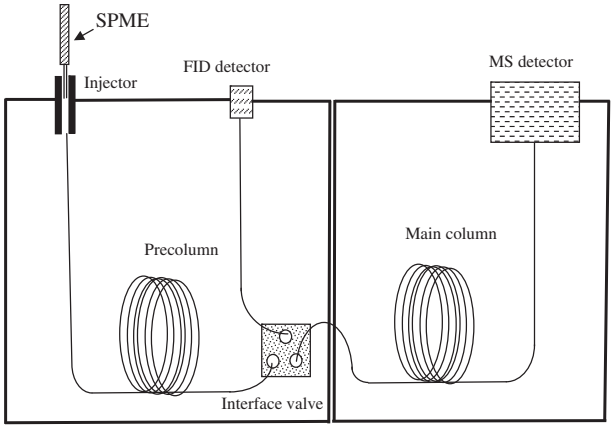

Fig. 1. Schematic diagram of the SPME–MDGC–MS system. The precolumn is connected to the injector and to the transfer valve, which allows communication with the FID detector or with the main column connected to a MS detector.