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ORIGINAL PAPER

Detection of Hydrocarbons in Irradiated Chilled Beef by HS-SPME–GC–MS and Optimization of the Method

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Abstract The hydrocarbons 1,7-hexadecadiene (1,7-C16:2) and 8-heptadecene (8-C17:1), which are used as markers for identifying irradiated chilled beef, were easily detectable in chilled beef irradiated at 0.5 kGy or higher by using a new method of headspace solid-phase microextraction, gas chromatography and mass spectrometry (HS-SPME–GC–MS). The conditions for using SPME were optimized in this study for qualitative and quantitative determination of 1,7-C16:2 and 8-C17:1 produced by γ-radiation in chilled beef. The relationship between irradiation dose and production of hydrocarbons was also studied, which showed good linear correlation with the coefficients of 0.9942 and 0.9943 for 1,7-C16:2 and 8-C17:1, respectively. HS-SPME–GC–MS is a simple and sensitive method for the analysis of hydrocarbons in irradiated chilled beef.

Keywords SPME · Chilled beef · Hydrocarbons · Gas chromatography

Introduction

Parasites and pathogenic bacteria in chilled beef can be controlled by irradiation. Studies by Chiasson [1] and Turgis [2] showed that *Salmonella typhi* and *Escherichia coli* in ground beef were sensitive to radiation. Countries like China, America, Russia, and South Africa permit chilled beef irradiation for microbial control [3, 4]. However, food irradiation polices, such as those governing irradiation dose, product type, and labeling requirements, vary from country to country [5]. These differences may result in chaos in the international trade of irradiated food, thereby affecting food exports. Thus, a reliable method is needed to detect irradiated food and the allowed absorbed dose of radiation.

According to Nawar [6], two types of hydrocarbons were predominantly produced by irradiation of fatty acids (Cn) in food: Cn-1 (with one carbon fewer than the parent fatty acid) and Cn-2:1 (with two carbons fewer than the parent fatty acid and with an additional double bond). Thus, it is possible to verify whether food containing fat is irradiated or not by analysis of the two types of hydrocarbons. Vajdi [7] and Miyahara [8] studied the detection of irradiated beef by the GC method and found the same results: that 1,7-C16:2 and 8-C17:1 were the main hydrocarbons produced by γ-radiation of beef. Both of their detection methods were similar to EN 1784:2003 [9], which uses organic solvent extraction and separation by a Florisil column for sample pretreatment. Those methods involve many organic solvents and complex procedures.

SPME can be used either for a rapid analysis of volatile compounds for qualitative purposes or for quantitative analysis of particular compounds [10]. It is an analytical technique for the extraction and identification of volatile compounds of foods, because it integrates sampling, extraction, concentration, and sample introduction to GC [11]. The SPME–GC analysis was successfully used to detect the headspace volatile compounds of soybean and corn oils [12], and new fibers like carboxen-polydimethylsiloxane (PDMS) have been shown to detect parts

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per billion levels of chemicals produced in light-irradiated milk [13]. Kim [14] used the SPME method successfully to enrich the volatile compounds, including hydrocarbons in irradiated beef powder. However, the use of SPME for work with hydrocarbons in irradiated chilled beef has not been previously reported.

The objectives of the present study were to optimize the conditions of SPME for the analysis of 1,7-C16:2 and 8-C17:1 produced in irradiated chilled beef, and to determine how irradiation affects production of these two hydrocarbons in chilled beef. A quick and sample method could thereby be developed for verification of irradiated chilled beef and the estimation of irradiation dose.

Experimental Procedures

Materials and Reagents

Fresh chilled beef was purchased from a local supermarket in Beijing, China. The hydrocarbon standards 1,7-C16:2, 8-C17:1 and C20:0 (*n*-eicosane) were purchased from Sigma Chemical Co. (St. Louis MO, USA). Hexane was purchased from Fisher Scientific Co. (USA).

Irradiation

Chilled beef was irradiated at 0.1, 0.5, 1, 2, 4, and 8 kGy using a ^{60}Co radiation source at room temperature in the radiation center of the Chemistry Institute, Peking University. All the samples were stored at 4 °C right after irradiation treatment.

Sample Preparation

Non-irradiated or irradiated chilled beef samples were well homogenized, and 2 g of homogenate were weighed into a headspace bottle.

SPME

The SPME fiber was DVB-PDMS, which was 75 μm and double-polar [15]. The fiber was activated at 250 °C for 10 min before use [16]. It was inserted into a headspace bottle containing 2 g of chilled beef. The hydrocarbon standards were added in the non-irradiated sample to optimize the SPME temperature, time, and desorption time [17].

GC–MS Analysis of Hydrocarbons

The fiber was inserted into a GC-2010 instrument (Shimadzu, Japan) after extraction, with a desorption temperature of 250 °C. The GC machine was equipped with an MS detector in EI mode. Helium was used as the carrier gas. The DB-5 column was 0.25 mm i.d. × 30 m column with 0.25 μm film thickness. The initial column temperature was held at 50 °C for 2 min, and then programmed at 10 °C/min up to 130 °C, then 5 °C/min to up to 200 °C for 2 min, then 25 °C/min up to 250 °C, with a final hold of 5 min [18–21]. The interface temperature was 200 °C. The ion source temperature was kept at 200 °C. The ionization energy was 70 eV. The scanning range was 30–400 *m*/*z* in SIM mode, as the hydrocarbons to be detected were known already [22, 23].

Qualitative and Quantitative Determination Method

1,7-C16:2, 8-C17:1, and C20:0 (internal standard) were qualitatively determined by chromatographic retention time and characteristic ions through GC–MS analysis of hydrocarbon standards. Hydrocarbon standards were added to the samples, which were not irradiated for the internal standard curve. The concentrations were 0.0025, 0.005, 0.025, 0.05, 0.25, and 0.5 μg/g beef. C20:0 was used as the internal standard with a concentration of 0.25 μg/g beef. All experiments were in duplicate unless otherwise stated.

Results and Discussion

Qualitative Analysis Results

As shown in Fig. 1, all the peaks were of good shape and interferants were not apparent. The retention time of 1,7-C16:2, 8-C17:1, and C20:0 were at 16.8, 18.9, 25.3 min, respectively. Abundant ions with preferred characteristics can remove interferents and enhance the detection sensitivity. Figure 2 shows the fragment peaks. For 1,7-C16:2, ions with preferred characteristics were observed at *m*/*z* 67, *m*/*z* 82 and *m*/*z* 96. For 8-C17:1, such ions were observed at *m*/*z* 83, *m*/*z* 97, and *m*/*z* 111. For C20:0, such ions were observed at *m*/*z* 57, *m*/*z* 71, and *m*/*z* 85.

Optimization of the Extraction Temperature

Temperature is an important factor for SPME. Temperature increases cause an increase in volatiles, but a high temperature may enhance the desorption of volatiles absorbed onto the fiber [15]. The effect of temperature on extraction efficiency was studied at 60, 70, 80, 90, and 100 °C. As shown in Fig. 3, SPME temperature had a significant effect on the concentrations of 1,7-C16:2 and 8-C17:1. More and more hydrocarbons evaporated from the sample as the temperature of the water bath increased. However, the contents of these two hydrocarbons decreased when the temperature reached 100 °C. The main reason might be that lots of water

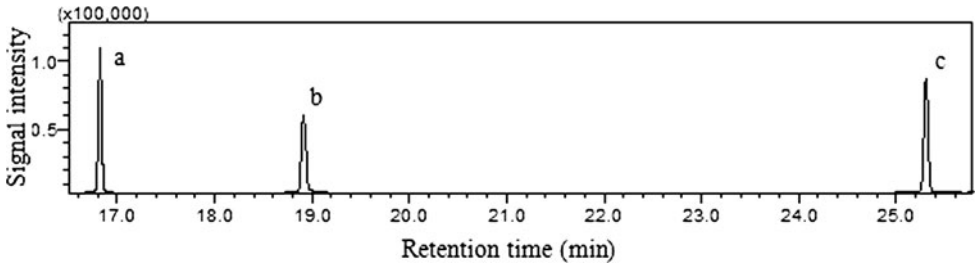

Fig. 1 GC–MS Chromatograms of hydrocarbon standards. **a** 1,7-C16:2, **b** 8-C17:1, **c** C20:0

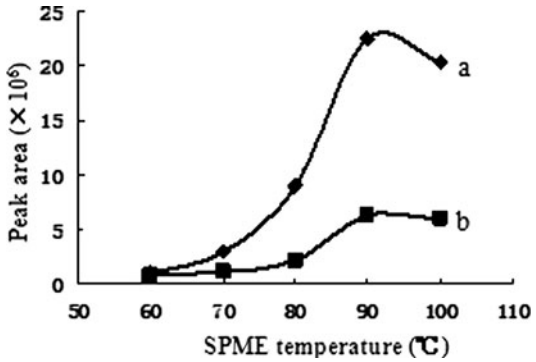

Fig. 2 Effect of SPME temperature on hydrocarbons. **a** 1,7-C16:2, **b** 8-C17:1

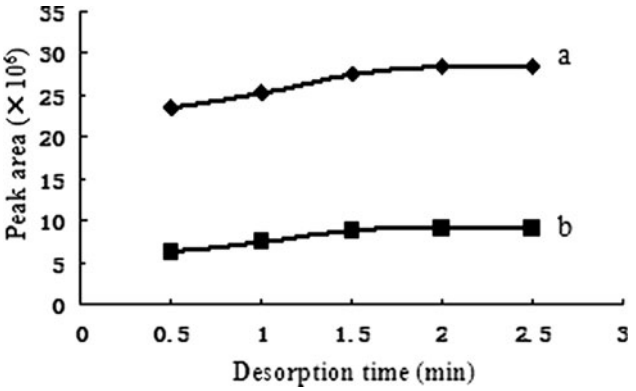

Fig. 4 Effect of desorption time on hydrocarbons. **a** 1,7-C16:2, **b** 8-C17:1

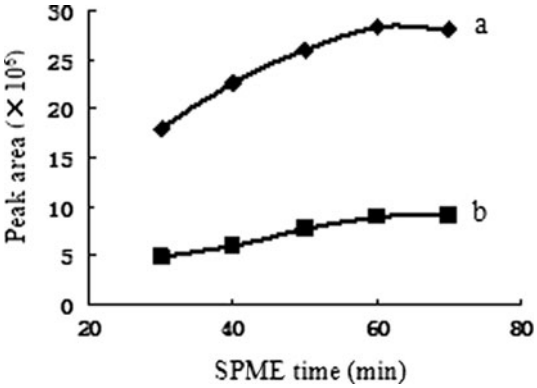

Fig. 3 Effect of SPME time on hydrocarbons. **a** 1,7-C16:2, **b** 8-C17:1

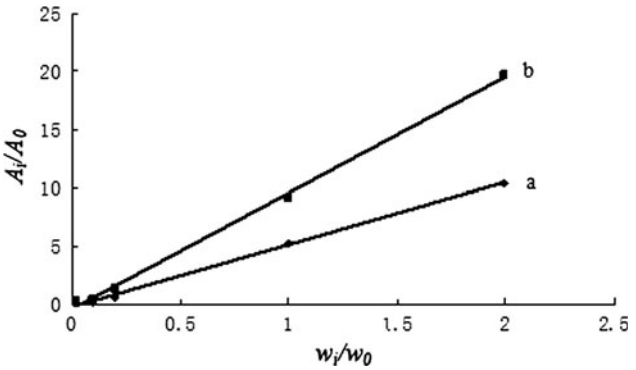

Fig. 5 The standard curves of the internal standard method. **a** 1,7-C16:2, **b** 8-C17:1; (A_i/A_0) peak area ratio of standard to internal standard, (w_i/w_0) concentration ratio of standard to internal standard

evaporated, which affected the adsorption. And the desorption of hydrocarbons was enhanced by the high temperature. Thus, 90 °C would have been the best choice.

Optimization of the Extraction Time

The effect of time on the extraction efficiency was studied at 30, 40, 50, 60, and 70 min. As shown in Fig. 4, the peak areas of 1,7-C16:2 and 8-C17:1 increased with time. However, the trend proceeded slowly when 60 min had been reached. The fiber adsorbed more and more volatiles with time, and became stable so that hydrocarbons could not be adsorbed. If the fiber was still working, the amount of target compounds might have decreased as other volatiles increased. Figure 4 shows that extraction equilibrium was achieved in 60 min.

Optimization of the Desorption Time

As described above, the fiber was immediately transferred to the injection port of a GC instument after extraction. For optimization of desorption time, injections were carried out at 0.5, 1, 1.5, 2, and 2.5 min. As shown in Fig. 5, the desorption efficiency became stable at 2 min, and the peak areas of these two hydrocarbons did not increase.

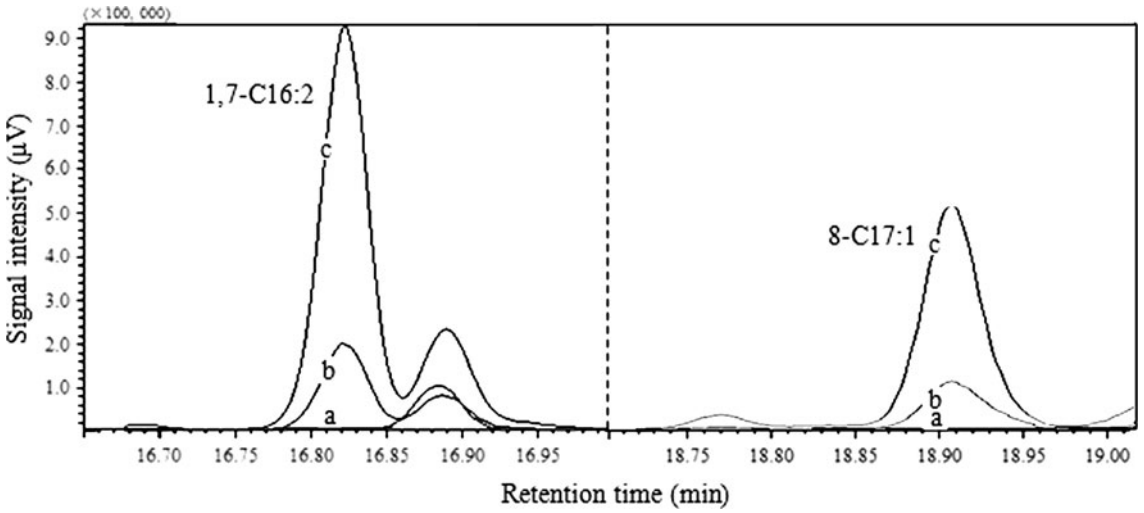

Fig. 6 GC–MS Chromatograms of 1,7-C16:2 and 8-C17:1 in irradiated chilled beef with different doses. **a** 0 kGy, **b** 2 kGy, **c** 8 kGy

Table 1 The linearity range, relative coefficient, detection limit, and RSD of this method

Hydrocarbons	Linear range (μg/g beef)	Linear correlation coefficient	Detection limit (μg/g beef)	RSD/% ($n = 3$)
1,7-C16:2	0.005–0.500	0.9991	2.299×10^{-3}	6.5
8-C17:1	0.005–0.500	0.9994	1.125×10^{-4}	8.7

Therefore, the final choice as the optimum desorption time was 2 min.

Quantitative Characteristics of the Proposed Method

Quantitative characteristics of the proposed method were studied after optimization of the conditions involved. The concentration ranges from 0.005 to 0.5 μg/g of 1,7-C16:2 and 8-C17:1 were studied. The peak areas for the two hydrocarbons were not stable at the concentration of 0.0025 μg/g, so standard curves were drawn during the linear range, as shown in Fig. 6. C20:0 was used as an internal standard. The linear ranges, detection limits and precision levels of these two hydrocarbons were measured as shown in Table 1.

Analysis of Hydrocarbons in Irradiated Chilled Beef

Chilled beef samples irradiated with different doses were analyzed by using the optimized SPME method described above. As shown in Fig. 6, hydrocarbons 1,7-C16:2 and 8-C17:1 did not exist in non-irradiated chilled beef. However, they were both detected in the sample irradiated with the doses of 0.1–8 kGy. 1,7-C16:2 and 8-C17:1 were so sensitive to irradiation that they were suitable to be used as markers to identify whether the chilled beef was irradiated or not. They were also easily produced, even with low doses of radiation. As shown in Table 2, with the dose of 0.1 kGy, the concentration of 1,7-C16:2 and 8-C17:1 in chilled beef measured 0.021 and 0.019 μg/g, respectively. This also means the HS-SPME–GC–MS method can be used to identify low-dose irradiated chilled beef.

Relationship between Radiation Dose and Concentrations of Hydrocarbons

Radiation dose has a significant impact on the radiolysis products. The absorbed dose of samples increased with the increase in radiation dose. Consequently, the yields of radiolytic products increased, too. As shown in Table 2, the greater the radiation dose, the greater the concentrations of hydrocarbons. In the range of 0.1–8 kGy, 1,7-C16:2 and 8-C17:1 had a good linear relationship with the radiation doses (Table 2). According to this linear relationship and under certain conditions, the radiation dose can be determined by the concentration of hydrocarbons. However, basing sample analyses on this linear relationship, can only provide rough estimates of the radiation doses. To make an accurate determination, the factors of storage time and storage temperature should be considered. Hwang [19] reported the hydrocarbons detected in eggs in their shells stored at 30 °C for 10 days or at 5 °C for 8 weeks were a little different from those detected on day 0. Therefore, the change of hydrocarbons during storage remains to be

Table 2 The concentration of 1,7-C16:2 and 8-C17:1 in irradiated chilled beef with different doses

Dose (kGy)	Hydrocarbons (μg/g beef)[a]	
	1,7-C16:2	8-C17:1
0.1	0.021 ± 0.002	0.019 ± 0.003
0.5	0.035 ± 0.001	0.026 ± 0.002
1.0	0.039 ± 0.002	0.029 ± 0.001
2.0	0.069 ± 0.005	0.036 ± 0.002
4.0	0.148 ± 0.01	0.064 ± 0.004
8.0	0.301 ± 0.06	0.116 ± 0.009
Linear equation	$y = 0.0358x + 0.0089$	$y = 0.0122x + 0.0166$
Linear correlation coefficient	0.9942	0.9943

[a] Means ± standard deviations ($n = 3$)

studied further. The temperature of irradiation and oxygen level in the packaging might be two more factors that effect the formation of hydrocarbons. Miyahara [24] reported that the yields of hydrocarbons in fatty acid methyl esters were higher as the temperature of irradiation rose and a significant reduction of hydrocarbon yields was observed in the packaging that contained oxygen absorbers.

Generation Mechanism of Hydrocarbons

Dubravcic's studies have shown that when the fatty acid was irradiated by gamma rays, there were at least four radiolytic hydrocarbons produced because of the break of C–C bonds at the position of α-carbonyl and β-carbonyl [6]. Two of them were typical and had a relatively higher concentration. One is produced by breaking the α-carbonyl C–C bond, resulting in a hydrocarbon that has one carbon fewer than the parent fatty acid; the other is produced by breaking the β-carbonyl C–C bond, resulting in a hydrocarbon that has two carbons fewer than the parent fatty acid, and an additional double bond. According to this theory, 1,7-C16:2 and 8-C17:1 were produced by oleic acid (C18:1) in the carbonylation of the α-C–C and β-C–C bonds of the decomposition products, respectively. In addition, the oleic acid in beef has a high level (26–50%) of total fat, which helps explain why the concentrations of 1,7-C16:2 and 8-C17:1 in irradiated chilled beef are relatively high.

In conclusion, detection of the prominent radiolytic hydrocarbons 1,7-C16:2 and 8-C17:1 in irradiated chilled beef by the HS-SPME–GC–MS method may make it possible to identify whether chilled beef has been previously irradiated at 0.1 kGy or higher. Therefore, detection of hydrocarbons by HS-SPME–GC–MS can be applicable to identifying the post-irradiation of chilled beef. The dose versus quantity of marker response was indicative of the irradiation dose, probably because of the quickness and sample procedure during analysis. Thus, this methodology could be used for detecting of irradiation dose.

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