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Detection of Radiation-Induced Hydrocarbons in Green, Black and Oolong Teas

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Abstract

Hydrocarbons induced by gamma-irradiation of green, black, and oolong teas were analyzed to determine whether the hydrocarbons can be used as markers for detecting post-irradiation of these teas. The samples were irradiated at 0, 2.5, 5, 7.5, and 10 kGy. Detection was attempted by extracting fat from the teas, separation of hydrocarbons with florisil column chromatography, and identification of hydrocarbons by gas chromatography-mass spectroscopy (GC-MS). Concentration of hydrocarbons increased with the irradiation dose. The major hydrocarbons in irradiated green, black, and oolong teas were 1-tetradecence (14:1), pentadecane (15:0), 1,7-hexadecadiene (16:2), 1-hexadecene (16:1), 8-heptadecene (17:1), and heptadecane (17:0). Radiation-induced hydrocarbons in teas were 1,7- hexadecadiene and 8-heptadecene. These compounds were not detected in non-irradiated samples, so the hydrocarbons (16:2, 17:1) can be used as markers for detecting post-irradiation of the teas. Furthermore, detection of hydrocarbons after 12 months storage at room temperature remains a suitable method for identifying irradiated teas.

Key words: teas, gamma irradiation, hydrocarbons, detection

INTRODUCTION

Irradiation has been permitted for microbial control and insect disinfestation of teas and herbs in Yugoslavia, Brazil, South Africa, Croatia, Ghana, and Mexico (1). Food irradiation is now becoming a reality in many countries, but consumer acceptance is progressing slowly. A mandatory infrastructure for guaranteeing honest and accurate information about irradiated products that considers both the technological and social points of view is needed to enhance the consumer's understanding and to facilitate international trade of irradiated foods. Many methods for the detection of irradiated food have employed. They can be categorized as chemical, physical and microbiological methods. Physical methods use electron spin resonance (ESR) spectroscopy to identify stable free radicals trapped in bone and fiber (2) and thermoluminescence (TL) to measure minerals adhering to the surface of spices and dried vegetables (3). Biological methods include DNA (4) and direct epifluorescent filter technique/aerobic plate count (DEFT/APC) analysis (5). In addition, chemical methods can be used to detect hydrocarbons or 2-alkylcyclobutanones formed from fat-containing irradiated food by GC or GC/MS (6). Of the available chemical methods for detecting irradiated food, one includes the detection of radiation-induced lipid derived volatile hydrocarbons is of interest (6-9). Nawar and Balboni (10) reported that irradiation of fatty acids in foods produces hydrocarbons with one (C_{n-1}) or two (C_{n-2}) fewer carbon atoms than parent fatty acid.

A number of studies on the use of these compounds as detection markers of irradiated foods have been conducted (11-17), but to date no study has evaluated it use in irradiated teas. This study was performed to identify hydrocarbons formed in green, black, and oolong teas that may be useful for detecting whether teas have been irradiated.

MATERIALS AND METHODS

Materials and reagents

Green (Korean), black (Sri Lankan), and oolong (Chinese) teas were purchased from H. Tea Co. in Korea. They were packed in commercial PE film and gamma irradiation was applied in ^{60}Co irradiator at the Korean Atomic Energy Research Institute (KAERI), Daejon. The hydrocarbon standards were purchased from Fluka (Sigma-Aldrich, Steinheim, Switzerland). HPLC grade solvent (n-hexane) was purchased from Fisher Scientific (Korea). Florisil (60~100 mesh) was obtained from Fisher Scientific (New Jersey, USA) and heated at 550°C overnight to remove the contaminants. Before use, the florisil

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was heated for at least 5 hours in a 130°C dry oven, cooled in a desiccator, and deactivated by the addition of 3% water.

Fat extraction

Fat extraction and the separation of hydrocarbons were performed using the method described by Schreiber et al. (6). A powdered sample (300 g) was shaken with 600 mL n-hexane overnight. After filtration, the filtrate was evaporated using a rotary vacuum evaporator in a water bath (35°C). The extracted fat was flushed with nitrogen and stored at 4°C until being separated by florisil column chromatography.

Separation of hydrocarbons

Deactivated florisil (25 g) was packed into a 200× 20 mm glass column. Anhydrous sodium sulfate was added on top of the florisil column in a 1 cm layer. One gram of extracted fat was mixed with an internal standard, 1 mL n-eicosane (4 ppm), applied to the column of florisil, and eluted with 60 mL hexane at a flow rate of 3 mL/min. The eluted hexane was concentrated to a volume of 2 mL using a rotary vacuum evaporator and further concentrated to a volume of 0.5 mL by means of nitrogen gas (8).

GC/MS analysis

GC/MS analysis was performed with a Hewlett-Packard 6890 Series (HP Co., Wilmington, DE, USA). The column used was HP-5 (30 m×0.32 mm i.d., 0.25 m film thickness, J & W Scientific, Folsom, CA, USA). The oven temperature programs for the analysis of hydrocarbons used were 60°C to 170°C at 25°C/min and to 205°C at 2°C/min then to 270°C at 10°C/min. The injector and detector temperatures were kept at 250°C and 300°C, respectively. The carrier gas was helium at a flow rate of 1.0 mL/min. To analyze hydrocarbons, 2 μL of the sample was injected in splitless mode for 2 min and then in split mode (20:1). Hydrocarbons were identified by comparison of retention time and mass spectrum of peaks as shown in the total ion chromatogram with that of authentic hydrocarbon standards (9). The concentration of each hydrocarbon in the fat was determined using an internal standard.

RESULTS AND DISCUSSION

Tea leaves have an oil content of approximately 4% by weight. Tea seed also contains oil which is composed of 14% saturated fatty acid (palmitic acid, stearic acid and myristic acid) and 86% unsaturated fatty acid (oleic acid and linoleic acid) (18). When these fatty acids are irradiated, two primary types of hydrocarbons are formed. One of the hydrocarbons contains one less carbon atom than its parent fatty acid. This hydrocarbon is formed as a result of the loss of the carboxyl group. The other hydrocarbon contains two less carbon atoms than its parent fatty acid. It also forms a double bond at the C_1 position (19). Accordingly, we were able to identify pentadecane ($C_{15:0}$) and 1-tetradecene ($C_{14:1}$) from palmitic acid, heptadecane ($C_{17:0}$) and 1-hexadecence ($C_{16:1}$) from stearic aciid, 8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) from oleic acid in the irradiated teas.

Fig. 1 shows the gas chromatograms of hydrocarbons from non-irradiated and 5 kGy irradiated green tea. In agreement with the lipid degradation patterns during irradiation as proposed by Nawar (19), a number of radiolytic hydrocarbons could be detected. The concen-

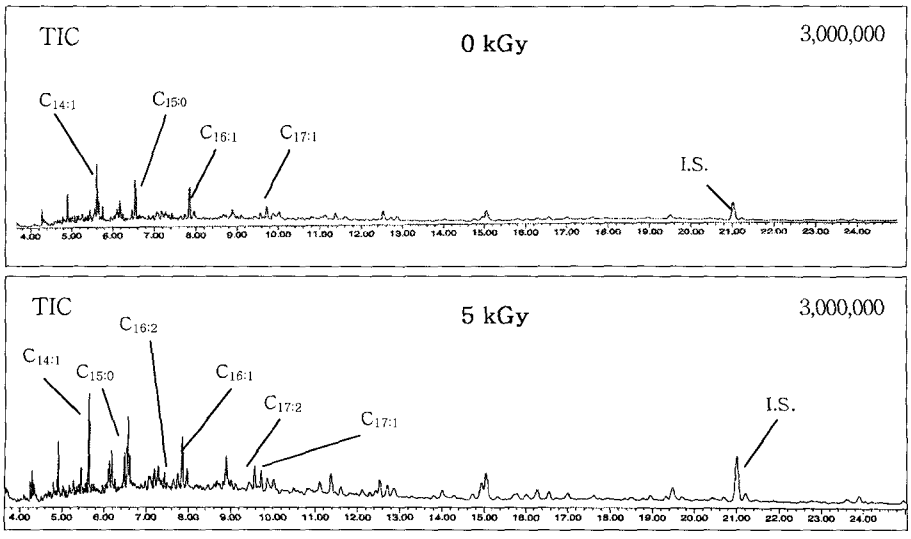

Fig. 1. Gas chromatograms of hydrocarbons of non-irradiated and 5 kGy irradiated green tea.

tration of hydrocarbons increased with the irradiation dose (Table 1~3). Hydrocarbon $C_{15:0}$ and $C_{14:1}$ were detected at fairly high levels in non-irradiated samples. Hydrocarbon $C_{17:0}$ and $C_{16:1}$ were detected in fairly low levels in non-irradiated samples and at remarkably greater levels at higher irradiation doses. $C_{17:1}$ and $C_{16:2}$ derived from stearic acid were detected in irradiated, but not non-irradiated, samples.

Previous studies have demonstrated that hydrocarbons, particularly saturated hydrocarbons, occur frequently e.g. in packaging material (6,20-24). Unsaturated hydrocarbons have also been observed in non-irradiated foods, e.g., fish (23) and beef (25). It should be noted that the formation of hydrocarbons is not specific for irradiation. Many hydrocarbons are also formed after heating or after oxidation. For example, in vegetable oils, long-chain hydrocarbons are found after heating or frying (26-29), and have discovered pitachio nuts after being roasted (22). Additionally, long chain hydrocarbons are found in animal products such as roasted chicken (30). However, the degradation pattern with C_{n-1} and $C_{n-2:1}$ hydrocarbons derived from precursor fatty acids is characteristic for irradiation (27). Hwang (9) reported $C_{15:0}$, $C_{14:1}$, $C_{17:0}$, $C_{16:1}$, and $C_{17:1}$ in non-irradiated pork. Park et al. (15) detected pentadecane and heptadecane in non-irradiated pork in trace amounts and higher quantities in pork irradiated at a dose of 0.1 kGy. However, Schreiber et al. (6) reported that radiation-induced hydrocarbons were found in non-irradiated chicken, pork, and beef at low concentrations. Morehouse and Ku (11) also reported that pentadecane and heptadecane were detected in non-irradiated shrimp mainly due to contamination. Therefore, $C_{17:1}$, and $C_{16:2}$ could be used as markers for identifying post-irradiation of green, black, and oolong teas since

Table 1. Concentrations of hydrocarbons in irradiated green tea (μg/g fat)

Hydrocarbon	Storage period (month)	Irradiation dose (kGy)				
		0	2.5	5	7.5	10
$C_{14:1}$	0	0.56 (±0.128)[1)]	0.73 (±0.038)	2.02 (±0.049)	2.71 (±0.029)	4.00 (±0.149)
	12	0.29 (±0.106)	0.30 (±0.049)	1.18 (±0.064)	1.70 (±0.021)	2.27 (±0.057)
$C_{15:0}$	0	2.40 (±0.073)	2.60 (±0.033)	2.76 (±0.185)	3.26 (±0.065)	3.45 (±0.281)
	12	1.32 (±0.120)	1.48 (±0.170)	1.70 (±0.092)	2.10 (±0.233)	2.49 (±0.057)
$C_{16:1}$	0	1.88 (±0.031)	2.14 (±0.020)	2.36 (±0.286)	2.69 (±0.143)	2.75 (±0.148)
	12	0.75 (±0.191)	1.08 (±0.085)	1.39 (±0.042)	1.71 (±0.021)	1.82 (±0.099)
$C_{17:0}$	0	0.86 (±0.038)	0.95 (±0.054)	1.19 (±0.061)	1.61 (±0.092)	1.81 (±0.078)
	12	0.41 (±0.078)	0.59 (±0.085)	1.05 (±0.085)	1.21 (±0.014)	1.24 (±0.318)
$C_{16:2}$	0	-	0.82 (±0.047)	1.08 (±0.182)	1.19 (±0.281)	1.26 (±0.281)
	12	-	0.39 (±0.042)	0.91 (±0.021)	1.08 (±0.057)	1.24 (±0.304)
$C_{17:1}$	0	-	1.09 (±0.035)	1.59 (±0.074)	1.68 (±0.148)	1.78 (±0.286)
	12	-	1.04 (±0.071)	1.20 (±0.141)	1.26 (±0.078)	1.32 (±0.290)

[1)]Means of duplicate±standard deviation.

Table 2. Concentrations of hydrocarbons in irradiated black tea (μg/g fat)

Hydrocarbon	Storage period (month)	Irradiation dose (kGy)				
		0	2.5	5	7.5	10
$C_{14:1}$	0	0.33 (±0.042)[1)]	0.59 (±0.032)	0.81 (±0.050)	1.07 (±0.052)	1.41 (±0.057)
	12	0.26 (±0.071)	0.39 (±0.035)	0.40 (±0.014)	0.83 (±0.049)	1.13 (±0.141)
$C_{15:0}$	0	1.24 (±0.025)	1.35 (±0.141)	1.93 (±0.052)	3.78 (±0.063)	4.70 (±0.338)
	12	1.11 (±0.141)	1.15 (±0.071)	1.27 (±0.078)	2.67 (±0.156)	3.20 (±0.014)
$C_{16:1}$	0	0.82 (±0.042)	0.87 (±0.108)	1.83 (±0.042)	3.02 (±0.046)	4.45 (±0.306)
	12	0.26 (±0.035)	0.57 (±0.057)	1.41 (±0.141)	2.08 (±0.085)	2.51 (±0.078)
$C_{17:0}$	0	0.60 (±0.026)	0.69 (±0.042)	0.92 (±0.051)	1.84 (±0.043)	2.64 (±0.069)
	12	0.37 (±0.064)	0.41 (±0.057)	0.67 (±0.057)	1.18 (±0.042)	1.07 (±0.035)
$C_{16:2}$	0	-	0.36 (±0.020)	0.97 (±0.324)	1.40 (±0.066)	2.19 (±0.025)
	12	-	0.20 (±0.021)	0.43 (±0.021)	1.20 (±0.120)	1.15 (±0.057)
$C_{17:1}$	0	-	0.39 (±0.024)	0.78 (±0.036)	1.08 (±0.078)	1.61 (±0.032)
	12	-	0.19 (±0.064)	0.31 (±0.092)	1.05 (±0.085)	1.23 (±0.184)

[1)]Means of duplicate±standard deviation.

Table 3. Concentrations of radiation-induced hydrocarbons in irradiated oolong tea (μg/g fat)

Hydrocarbon	Storage period (month)	Irradiation dose (kGy)				
		0	2.5	5	7.5	10
$C_{14:1}$	0	0.32 (±0.061)[1)]	0.58 (±0.059)	0.85 (±0.028)	1.39 (±0.060)	2.43 (±0.038)
	12	0.20 (±0.021)	0.39 (±0.035)	0.58 (±0.021)	0.99 (±0.057)	1.41 (±0.028)
$C_{15:0}$	0	1.29 (±0.138)	2.90 (±0.013)	3.02 (±0.178)	3.95 (±0.111)	6.62 (±1.319)
	12	0.99 (±0.042)	2.22 (±0.148)	2.57 (±0.085)	3.01 (±0.078)	3.59 (±0.042)
$C_{16:1}$	0	1.09 (±0.032)	2.53 (±0.194)	2.96 (±0.097)	3.43 (±0.147)	5.80 (±0.245)
	12	0.98 (±0.028)	1.83 (±0.191)	2.18 (±0.042)	3.00 (±0.085)	3.71 (±0.106)
$C_{17:0}$	0	1.41 (±0.080)	1.64 (±0.049)	1.79 (±0.129)	2.45 (±0.231)	4.87 (±0.067)
	12	1.07 (±0.071)	1.17 (±0.064)	1.33 (±0.431)	1.54 (±0.127)	2.88 (±0.085)
$C_{16:2}$	0	-	1.19 (±0.183)	1.55 (±0.135)	1.73 (±0.034)	1.88 (±0.060)
	12	-	1.05 (±0.092)	1.19 (±0.247)	1.43 (±0.163)	1.60 (±0.042)
$C_{17:1}$	0	-	0.90 (±0.048)	1.14 (±0.040)	1.32 (±0.060)	1.90 (±0.092)
	12	-	0.38 (±0.049)	0.92 (±0.035)	1.16 (±0.071)	1.69 (±0.049)

[1)]Means of duplicate ± standard deviation.

they were not detected in non-irradiated samples.

Hydrocarbons were also analyzed in the teas after storage at room temperature for 12 months. The concentration of hydrocarbons increased with irradiation dose, but their concentration decreased marginally with storage in samples treated with all irradiation doses. Therefore, from these results, it can be demonstrated that, although there was a decrease in the concentration of hydrocarbons, these radiation-induced hydrocarbons exhibit high stability. Long-term stability of radiolytic hydrocarbons has already been described for various meat samples (6,31,32) and Brazilian beans (33).

Finally, it can be concluded that hydrocarbons formed during the irradiation of green, black, and oolong teas increase proportionally with the irradiation dose. Radiation-induced hydrocarbons were 1,7-hexadecadiene and 8-heptadecene, which were detectable even after 12 months of storage at room temperature. Thus, it is possible to use these hydrocarbons as markers to identify irradiated teas.

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