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Quantitative Analysis of Radiation-induced Hydrocarbons in Irradiated Chicken at Various Dose Levels

Eun-Ah Kim, Hae-Jung Lee, Jae-Seung Yang* and Kyong-Su Kim†

Dept. of Food and Nutrition, Chosun University, Kwangju 501-759, Korea
*Dept. of Food irradiation, Korea Atomic Energy Research Institute, Taejon 305-353, Korea

Abstract

Gamma-irradiated chicken at dose levels of 0.1 to 10 kGy was subjected to detection of radiation-induced hydrocarbons whether irradiated or not. The hydrocarbons extracted from chicken fat were separated by florisil column chromatography and identified with GC-FID and GC/MS methods. Eight kinds of hydrocarbons were identified from irradiated chicken, among which 1,7-hexadecadiene and 8-heptadecene were detected as major compounds. Remarkably radiation-induced hydrocarbons in irradiated chicken were detected at 0.5 kGy and over. The concentration of radiation-induced hydrocarbons was relatively constant during 16 weeks.

Key words: chicken, florisil column chromatography, GC/MS, radiation-induced hydrocarbons

INTRODUCTION

Ionizing radiation of foods extends shelf-life, decreases microbial and insect infestations, prevents sprouting and delays ripening. Interest in the use of irradiation for the treatment and preservation of foods is increasing throughout the world. Thus, much research has been directed to develop the detection method for irradiated food by international organizations such as IAEA, FAO and WHO. The various detection methods based on chemical, physical, microbiological and biological changes occurring in irradiated foods have been studied. Chemical method uses GC or GC/MS analyzer for the detection of hydrocarbons or cyclobutanones formed in irradiated fat-containing food (1-9). Physical method that were used include electron spin resonance (ESR) spectroscopy for the identification of stable free radicals trapped in bone and fiber (10-12) and thermoluminescence (TL) for the measurement of minerals adhering to the surface of spices and dried vegetables (13-15). Biological methods used were DNA (16), limulus amoebocytes lysate (LAL) (17) and direct epifluorescent filter technique/aerobic plate count (DEFT/APC) (18) analyses. However, there is no certain general detection method as of yet. Therefore a reliable method is needed to detect whether foods have been irradiated and which in compliance with the allowable absorbed radiation dose.

Recently, the import of irradiated food has increased in Korea. The use of irradiation of poultry is prohibited in Korea; while in other countries it has been allowed at a dose of 3~10 kGy (19). GC analysis is effective for the detection of hydrocarbons formed in fat-containing food, but so far systematic data is insufficient to apply this method to every sample.

Therefore, this study was conducted to identify hydrocarbons formed in irradiated chicken, thereby providing a quantitative identification basis for the detection of irradiated foods.

†Corresponding author

MATERIALS AND METHODS

Materials

Chickens were irradiated at each of the following doses, 0.1 kGy, 0.5 kGy, 1 kGy, 3 kGy, 5 kGy and 10 kGy using a ^{60}Co γ-irradiator at the Korea Atomic Energy Research Institute. The irradiated chicken and the control were stored at -70°C.

Reagents

The hydrocarbon standards were purchased from TeLA (Germany). HPLC grade solvents (n-pentane, n-hexane and isopropanol) were purchased from Sigma Chemical Co. (USA) and distilled with spiral packed double distilling apparatus prior to use. Florisil (60~100 mesh) was obtained from Fisher Scientific and heated at 550°C overnight to remove the contaminants. Before use, florisil was heated for at least 5 hrs in an 130°C dry oven and cooled in a desiccator. After that 3% water (w/w) was added and shaken for at least 20 min. This mixture was stored for 10~12 hr. Florisil deactivated in this way was used for 3 days. Otherwise, the florisil was reheated at 130°C and deactivated again.

Extraction of fat from chicken

Chicken fat was extracted by a method previously described by Schreiber et al. (20). Chickens were thawed at 4°C. 30 g of ground chicken samples were placed in beakers, and mixed with 30 ml solvent (n-pentane/isopropanol 3 : 2, v/v). The mixture was homogenized for 2 min with a Ultra Turrax (Janke & Kunkel, Germany). The mixture was centrifuged for 20 min at 900×g to obtain the fat. The residues were re-extracted with one third amount of solvent and centrifuged again. The solvent phase was concentrated using a rotary vacuum evaporator and nitrogen gas. The extracted fat was stored at -20°C.

Separation of hydrocarbons from fat by florisil column chromatography

25 g of deactivated florisil was packed into 200×20 mm glass column. Anhydrous sodium sulfate was added on top of a florisil column in a 1 cm layer. 1 g of extracted fat was mixed with an internal standard (4 μg/ml n-eicosane) and put on the column. The hydrocarbons were 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 1 ml by means of nitrogen gas before analysis by GC-FID and GC/MS. The concentration of each radiolytic hydrocarbon in the fat was determined by the use of an internal standard (n-eicosane).

GC-FID and GC/MS analyses of hydrocarbons

A GC analysis was carried out with a Hewlett-Packard (HP) 5890 II Plus gas chromatograph equipped with a flame ionization detector (FID). The column used was DB-5 (J&W Scientific, 30 m×0.32 mm i.d., 0.25 μm film thickness, Folsom, CA). The oven temperature program used was : 60°C to 170°C at 25°C/min and at 2°C/min to 205°C then at 10°C/min to 270°C. 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. 1 μl of sample was injected in splitless mode for 2 min and then in split mode (20 : 1). A GC/MS for hydrocarbons analysis was carried out on a Shimadzu GC/MS QP-5000 spectrometer in the EI mode. The ionization voltage was 70 eV and ion source temperature was set to 230°C. The column was a DB-5 (J&W Scientific, 30 m×0.32 mm i.d., 0.25 μm film thickness, Folsom, CA). The other conditions were the same as described for the GC analysis.

RESULTS AND DISCUSSION

When fats are irradiated, mainly two types of hydrocarbons are formed. One hydrocarbon contains one less carbon atom than its parent fatty acid. This hydrocarbon is formed as a result of the loss of a carboxyl group. The other hydrocarbon which is formed by the loss of CH_3COOH contains two less carbon atoms than its parent fatty acid. It also forms a double bond at the C_1 position (21).

Based on these, radiolytic C_{n-1} and C_{n-2} hydrocarbons for fatty acids are shown in Table 1. Oleic acid comprises 32% of the total fatty acids in chicken (20) and therefore the major hydrocarbons can be expected to be 8-heptadecene and 1,7-hexadecadiene originating from this fatty acid. For quantitative analysis, an internal standard (n-eicosane) was used. The identification of the various peaks in the chromatograms was confirmed by comparison of the mass spectrum (Fig. 1).

The results of quantitative analysis of radiation-induced hydrocarbons in chicken were the same as in Table 2. All the determinations were carried out in triplicate. Fig. 2 shows

Table 1. Radiolytic C_{n-1} and C_{n-2} hydrocarbons from main fatty acids in chicken

Fatty acid	C_{n-1}	C_{n-2}
Palmitic acid ($C_{16:0}$)	Pentadecane ($C_{15:0}$)	1-Tetradecene ($C_{14:1}$)
Stearic acid ($C_{18:0}$)	Heptadecane ($C_{17:0}$)	1-Hexadecene ($C_{16:1}$)
Oleic acid ($C_{18:1}$)	8-Heptadecene ($C_{17:1}$)	1,7-Hexadecadiene ($C_{16:2}$)
Linoleic acid ($C_{18:2}$)	6,9-Heptadecadiene ($C_{17:2}$)	1,7,10-Hexadecatriene ($C_{16:3}$)

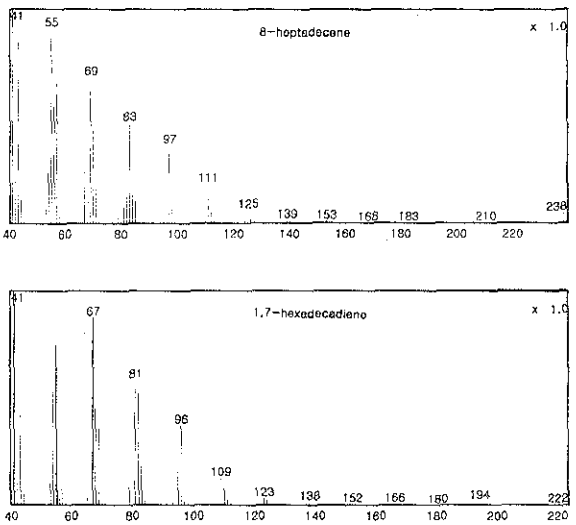

Fig. 1. Mass spectrum of radiation-induced hydrocarbons.

GC chromatogram of radiation-induced hydrocarbons of chicken at various dose levels. Fig. 3 indicates that concentration of hydrocarbons detected increased linearly with the dose levels of irradiation. Pentadecane and 1-tetradecene were the major hydrocarbons from palmitic acid. Pentadecane was detected in a relatively large amount as compared to 1-tetradecene. Heptadecane and 1-hexadecene were formed from stearic acid. The amount of these hydrocarbons was small because of the small amount of stearic acid in chicken. 8-Heptadecene and 1,7-hexadecadiene were the major hydrocarbons from oleic acid. 1,7-Hexadecadiene was the highest amount in the irradiated chickens because of the large amount of oleic acid. 6,9-Heptadecadiene and 1,7,10-hexadecatriene were formed from linoleic acid. Concentration of these hydrocarbons was similar to each other. Morehouse et al. (22) showed that radiolytic hydrocarbons of chicken thigh and breasts, beef, pork and turkey exhibited a linear relationship. They also observed that since the amount of fat present altered the free-radical reaction, the radiolytic hydrocarbons were matrix dependent. Sjöberg et al. (23) investigated the radiation-induced hydrocarbons of chicken and chicken meat ball. The result in the skin of the irradiated chicken was similar to our finding. But in the irradiated chicken meat ball, there was no linear relationship between

Table 2. Concentrations of radiation-induced hydrocarbons in irradiated chicken (μg/g fat)

Dose (kGy)	Palmitic acid		Stearic acid		Oleic acid		Linoleic acid	
	$C_{15:0}$	$C_{14:1}$	$C_{17:0}$	$C_{16:1}$	$C_{17:1}$	$C_{16:2}$	$C_{17:2}$	$C_{16:3}$
0	t[1)]	-	t	-	-	-	-	-
0.1	0.12(±0.02)[2)]	0.06(±0.02)	0.10(±0.04)	0.07(±0.02)	0.10(±0.04)	-	0.08(±0.03)	0.10(±0.05)
0.5	0.14(±0.10)	0.13(±0.05)	0.13(±0.02)	0.11(±0.03)	0.17(±0.04)	0.27(±0.10)	0.17(±0.02)	0.16(±0.03)
1	0.22(±0.09)	0.19(±0.10)	0.15(±0.02)	0.14(±0.03)	0.25(±0.08)	0.38(±0.11)	0.23(±0.05)	0.22(±0.04)
3	0.62(±0.10)	0.88(±0.28)	0.28(±0.06)	0.27(±0.08)	1.02(±0.15)	1.63(±0.21)	0.71(±0.21)	0.69(±0.17)
5	0.97(±0.25)	1.43(±0.14)	0.37(±0.12)	0.38(±0.13)	1.78(±0.22)	2.80(±0.27)	1.13(±0.31)	1.09(±0.28)
10	2.34(±0.27)	2.76(±0.35)	0.66(±0.27)	0.70(±0.37)	3.88(±0.34)	6.16(±0.25)	2.31(±0.38)	2.27(±0.21)

[1)]Trace, [2)]Standard deviation

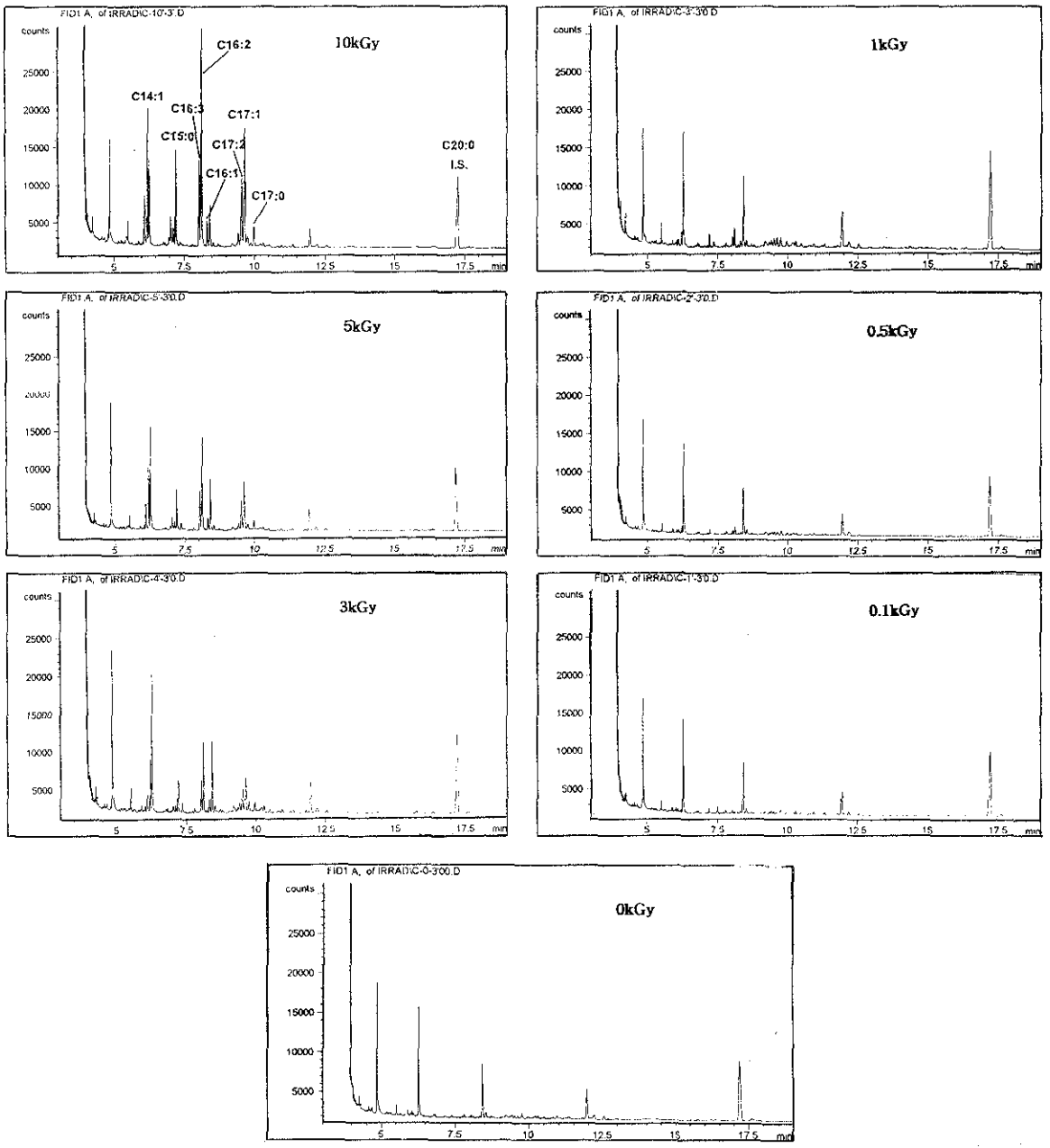

Fig. 2. GC chromatograms of radiation-induced hydrocarbons in chicken at various dose levels.

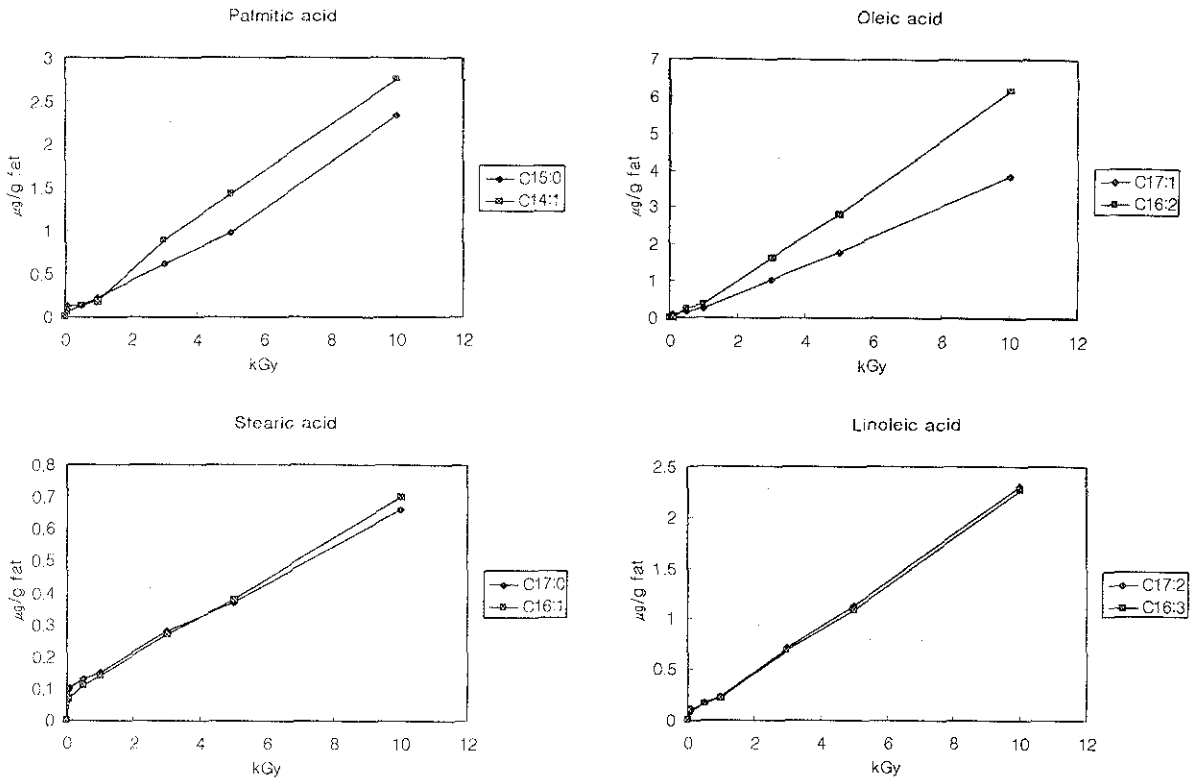

Fig. 3. Effect of radiation dose on radiation-induced hydrocarbons in chicken.

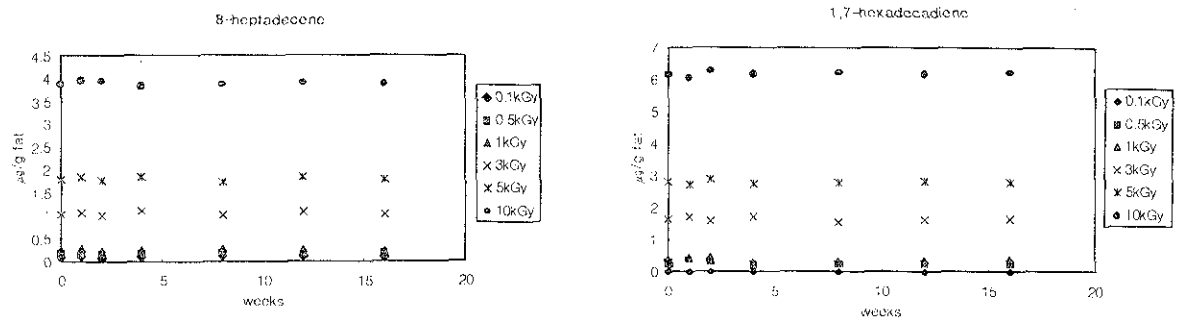

Fig. 4. Changes in concentration of radiation-induced hydrocarbons in chicken during storage.

dose and amount of hydrocarbons formed.

Radiation-induced hydrocarbons except for 1,7-hexadecadiene could be detected in the chicken irradiated with a dose of 0.1 kGy and remarkably these were detected at dose of 0.5 kGy or higher (Fig. 2). Radiation-induced hydrocarbons except for pentadecane and heptadecane were not detected in unirradiated chicken. Pentadecane and heptadecane were found in the unirradiated control chicken in trace. It was proposed that pentadecane and heptadecane detected in irradiated chicken were due to solvent contamination. Schreiber et al. (20) reported that radiation-induced hydrocarbons were found in unirradiated chicken, pork, beef at low concentrations. Morehouse and Ku (24) also indicated that pentadecane and heptadecane were detected in unirradiated shrimp because of contamination. Therefore precaution must be taken to prevent misidentifying the unirradiated food.

Changes in concentration of radiation-induced hydrocarbons during 16 weeks are presented in Fig. 4. Almost the same concentration levels of hydrocarbons were detected before and after storage. Therefore, long term storage of irradiated chicken had no influence on the concentration levels of hydrocarbons. Tuominen et al. (25) reported the identification of irradiated chicken by GC and the concentration levels of hydrocarbons after storage for 5 weeks. Lesgards et al. (26) also studied the analysis of irradiated sunflower oil after 3 weeks. Their results were in agreement with our results.

Finally, hydrocarbons formed in irradiated chicken increased linearly with the dose levels of irradiation. All radiation-induced hydrocarbons could be remarkably detected at doses of 0.5 kGy or higher in chicken and their concentrations were

not affected by storage time. Further works in several different types of foods are currently in progress to obtain more complete quantitative and statistical data.

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