Hydrocarbon Productions in Hexane Solutions of Fatty Acid Methyl Esters Irradiated with Gamma Rays

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(Received May 13, 2002; Accepted June 12, 2002; Published online June 12, 2002)

Five fatty acid methyl esters in hexane were irradiated in order to obtain basic data regarding the detection of irradiation of fatty foods. Fifteen hydrocarbons which were formed by radiolysis of fatty acid methyl esters were detected using capillary gas chromatography accompanied by mass spectrometry. Dose response was observed at the range of 0.74 kGy to 10 kGy. The yields of the hydrocarbons increased as the dose increased. Clear dose rate effects were not observed at the range of 10 kGy/hr to 500 Gy/hr. Temperature effects on the formation of the hydrocarbons were observed at the range of -40 to 20 degrees Celsius. Their yields were increased as the temperature rose. The effects of oxygen level in the container were examined. The yields in the containers that contained oxygen absorbers were 5–41% of those under normal pressure. Eminent reduction of hydrocarbon yields were observed in the container oxygen absorbers. Remarkable solvent effects on the formation of the hydrocarbons were observed. The yields of hydrocarbons in benzene solution were reduced to 60–95% of those in hexane. Thus, radiolytic degradation of fatty acid methyl ethers were affected mainly by absorbed dose, irradiation temperature, oxygen pressure, and fatty acid components.

Key words — hydrocarbon detection method, irradiated food detection, MS detection, capillary gas chromatography

INTRODUCTION

Food irradiation is still focused because of its ability to reduce foodborne illness.^{1,2)} Especially, processed ground meats requires good quality of starting material. These meats, however, frequently contaminated with low levels of bacterial populations.³⁾ To ensure material safety, meat needs to be examined regardless of proper irradiation before further processing.

Our program to develop analytical methods for the detection of irradiated foods has been expanded to include the detection of radiolytic products of fatty acids. Establishing a chemical method for detecting irradiated foods is made difficult by the low-level analytes in complex matrix, and by the poor dose response of radiolytic products. Several detection methods have been suggested for the enforcement of labeling requirements.⁴⁾ The ESR detection method for boned meat, the thermoluminescence method for spices, and the half-embryo method for citrus requires bone, inorganic dusts, and seeds in the samples, respectively.⁵⁾ Such methods strongly depend on the presence of specific materials in/on food; therefore, the range of irradiated foods which can be detected by those methods is narrower than that by the hydrocarbon detection method.⁶⁾ Hydrocarbon detection has good potential, and is the most reliable method for the detection of irradiated foods because many foods contain some level of precursors (fatty acid esters) for radiolytic products (hydrocarbons) in their samples.⁷⁾

Product analysis of hydrocarbons in irradiated foods were extensively performed at high-dose ranges (20–100 kGy),⁸⁾ resulting in the detection/ identification of many hydrocarbons and related compounds.⁹⁾

However, hydrocarbons are not "unique radi-

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Fig. 1. Schematic Chart for Radiolytic Reaction of Fatty Acid Esters in Hexane Solution by Gamma-Irradiation of ⁶⁰Co

olytic products" and their amounts can be detected even in some non-irradiated foods. Therefore, it is difficult to estimate the absorption dose based on hydrocarbon levels. A second problem is that the components of fatty acids vary in food samples.¹⁰⁾ As a result, some irradiated foods are not found to be "irradiated" due to insufficient fatty acid components in the sample. Thus, an actual food sample is affected by many factors, such as the sample's individual nature.¹¹⁾ Therefore, study of a model system allows the elucidation of the fundamentals of the hydrocarbon method.

When fatty acid ester (FA) solutions are irradiated by gamma-rays, the FA esters ($C_{n:m}$) are degraded to produce many volatile hydrocarbons HC ($C_{n-1:m}$, $C_{n-1:m+1}$, $C_{n-2:m}$, $C_{n-2:m+1}$, n = 14, 16, and 18, m = 0 or 1) by hydroxyl radicals that are generated by the ionization of trace water in hexane, as shown in Fig. 1. The hydrocarbons we studied are listed in Table 1. This table also shows the relationships between mother fatty acid esters and daughter hydrocarbons.

This paper presents a critical study of the hydrocarbon production in a model system in order to elucidate the behavior of the irradiation. This paper also describes the effects of gamma-irradiation conditions (temperature, oxygen level, *etc.*) on hydrocarbon formation in order to assess the scope and limitation of this method.

MATERIALS AND METHODS

Apparatus —

Irradiation Apparatus: A wet-type ⁶⁰Co Plate source irradiation apparatus at the Takasaki Establishment of Japan Atomic Energy Institute, Takasaki City, Gunma, and a table-type ⁶⁰Co rod source at the Tokyo Metropolitan Institute of Industrial Technology, Setagaya, Tokyo, were used for irradiation.

GC Equipment and Operating Conditions: The gas chromatograph used was a Hewlett-Packard model of 5890 Ser.II equipped with a mass spectrometric detector (a Hewlett-Packard model of 5971). A capillary column [25 m × 0.2 mm i.d., Film thickness, 0.33 μ m; Hewlett-Packard Ultra 1 (Hewlett-Packaged, Co.)] was used. Injector temperature was 200°C. Detector temperature was 280°C. The carrier gas was helium, and the flow rate was 1 ml/min. The injection volume was 2 μ l. The injection mode was splitless. The column oven was controlled as follows: initially, the temperature was maintained at 40°C for 2 min, was raised to 170°C at 2.5°C/ min, then to 200°C at 5°C/min, and finally was maintained at 200°C for 5 min.

Quantitative Determination: The calibration curve was obtained by injecting 0.313, 0.625, 1.25, 2.5, 5, and 10 μ g/ml of standard solutions. The internal standard method was used with the addition of 100 μ g of *n*-eicosan(C₂₀).

Reagents and Other Materials: The hydrocarbon standards used are shown in Table 1 . The purities of the standard materials were over 98%. They were purchased from Tokyo Kasei Chemical Co., Inc., Tokyo, Japan, and Tela Chemicals, Germany.

All reagents for analysis were of the Japanese Industrial Standards (JIS) extra-pure grade, which may be compatible with ACS grade. Hexane was of HPLC grade (Kanto Chemical, Co., Tokyo, Japan). **Standard Solutions** — One hundred milligrams of each hydrocarbon were dissolved in 100 ml of hexane.

Fatty Acid Ester Solutions — Ten grams of each fatty acid methyl ester were dissolved in 100 ml of hexane.

Irradiation Procedure — Five milliliters of fatty acid methyl ester solution was placed in a 10 ml Pyrex tube with a stopper. The sample was irradiated at 6 kGy/hr using a plate-type source for precision irradiation. A sample was routinely irradiated using a rod-type source at an appropriate dose rate. Irradiation was conducted at room temperature, in the vessel with a stopper. After irradiation, the

ester	radiolate	abbreviation	structure
methyl	1-dodecene (a)	1-C _{12:1}	$CH_2 = CH(CH_2)_9 CH_3$
myristate	dodecane (b)	C _{12:0}	$CH_3(CH_2)_{10}CH_3$
	1-tridecene (c)	1-C _{13:1}	$CH_2 = CH(CH_2)_{10}CH_3$
	tridecane (d)	C _{13:0}	CH ₃ (CH ₂) ₁₁ CH ₃
methyl	1-tetradecene (e)	1-C _{14:1}	$CH_2 = CH(CH_2)_{11}CH_3$
palmitate	tetradecane (f)	C _{14:0}	$CH_3(CH_2)_{12}CH_3$
	1-pentadecene (g)	1-C _{15:1}	$CH_2 = CH(CH_2)_{12}CH_3$
	pentadecane (h)	C _{15:0}	CH ₃ (CH ₂) ₁₃ CH ₃
methyl	1-hexadecene (j)	1-C _{16:1}	$CH_2 = CH(CH_2)_{13}CH_3$
stearate	hexadecane (k)	C _{16:0}	$CH_3(CH_2)_{14}CH_3$
	1-heptadecene (n)	1-C _{17:1}	$CH_2 = CH(CH_2)_{14}CH_3$
	heptadecane (o)	C _{17:0}	$CH_3(CH_2)_{15}CH_3$
methyl	1,7-hexadecadiene (i)	1,7-C _{16:2}	$CH_2 = CH(CH_2)_4 CH = CH(CH_2)_7 CH_3$
oleate	7-hexadecene	7-C _{16:1}	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ CH ₃
	1,8-heptadecadiene	1,8-C _{17:2}	CH ₂ =CH(CH ₂) ₅ CH=CH(CH ₂) ₇ CH ₃
	8-heptadecene (m)	8-C _{17:1}	CH ₃ (CH ₂) ₆ CH=CH(CH ₂) ₇ CH ₃
methyl	1,7,10-hexadecatriene	1,7,10-C _{16:3}	CH ₂ =CH(CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₄ CH ₃
linoleate	6,9-hexadecadiene	6,9-C _{16:2}	CH ₃ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₅ CH ₃
	1,8,11-heptadecatriene	1,8,11-C _{17:3}	$CH_2 = CH(CH_2)_5 CH = CHCH_2 CH = CH(CH_2)_4 CH_3$
	6,9-heptadecadiene (l)	6,9-C _{17:2}	CH ₃ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₆ CH ₃

 Table 1. Starting Fatty Acid Esters and Their Radiolates

samples were stored at -20° C.

Irradiation Temperature Control of Sample — When the temperature of a sample needed to be maintained at 0°C, samples were dipped in a bath containing a mixture of water and ice. When the temperature needed to be maintained at –40°C, samples were placed in an air-bath which was cooled by dry ice.

Dosimetry — Absorbed doses were measured using the GammaChrom YG (Hawell, U.K.), while the Radix RN-15 (Radie Kogyou, Japan) was used for calibration.

Caution — The gamma-irradiator should be operated with careful monitoring and supervision by someone experienced in irradiation. Some organic solvents used in this study are suspected carcinogens and should be handle them with care.

RESULTS AND DISCUSSION

Gas Chromatographic and Mass Spectrometric Identification of Hydrocarbons

As shown in Fig. 2-a), all the standard hydrocarbons were separated and identified using an internal standard method under the gas chromatographic conditions. The calibration curve and mass spectroscopic characteristics are also summarized in Table 2. As shown in Fig. 2 b-1, the targeted hydrocarbons [*i.e.* 1-dodecene, $HC(1-C_{12:1})$ (a), dodecene $HC(C_{12:0})$ (b), 1-tridecene $HC(1-C_{13:1})$ (c), and tridecane, $HC(C_{13:0})$ (d)] were below the lower limits of detection in non-irradiated myristic acid methyl ester $FA(C_{14:0})$ solution. As shown in Fig. 2 b-2, 1-dodecene, $HC(1-C_{12:1})$ (a), dodecene $HC(C_{12:0})$ (b), 1-tridecene $HC(1-C_{13:1})$ (c), tridecane, $HC(C_{13:0})$ (d), and tetradecane, $HC(C_{14:0})$ (f) were found in a 10kGy-irradiated $FA(C_{14:0})$ solution. Those compounds were also identified in terms of their retention times and mass spectroscopic characteristics as shown in Table 2. Corresponding results regarding methyl palmitate and methyl stearate were obtained and are shown in Figs. 2 c-1), c-2), d-1), and d-2), respectively. These results show that all the saturated hydrocarbons that were generated by irradiation were confirmed in the model system using standard compounds.

In Fig. 2-e1, background hydrocarbon levels in non-irradiated methyl oleate, $FA(9-C_{18:1})$, and no target hydrocarbons were below the detection limits. As shown in Fig. 2 e-2, 1,7-hexadecadiene HC(1,7-C_{16:2}) (i), 1,8-heptadecadiene HC(1,8-C_{17:2}), 8-heptadecene HC(8-C_{17:1}) (m), and 8-octadecene HC(8-C_{18:1}) were found in 10 kGy irradiated methyl oleate solution in *n*-hexane.

The hydrocarbon standards which were not avail-



Fig. 2. Chromatograms of Hydrocarbons

All the letters at the peaks in the chromatograms correspond to the compounds listed in Table 2. a) is a chromatogram of 15 standard hydrocarbons (2 ng); b-1), c-1), d-1), e-1), and f-1) are those of 10% solutions of non-irradiated methyl myristate, methyl palmitate, methyl stearate, methyl oleate, and methyl linoleate in hexane, respectively, and b-2), c-2), d-2), e-2), and f-2) are those of the 10-kGy irradiated methyl esters in hexane at room temperature.

compound		retention time		mass (m/e)				calibration curve			
								(0.313–10 µg/ml)			
		rt (min)	rrt	M^+	1	2	3	a	b	r	
1-C _{12:1}	(a)	26.29	0.431	168	69	55	83	1.51	-0.0222	0.9997	
C _{12:0}	(b)	26.89	0.441	170	57	71	85	1.13	-0.0002	0.9995	
1-C _{13:1}	(c)	31.64	0.519	182	69	70	55	1.26	-0.0200	0.9995	
C _{13:0}	(d)	32.23	0.529	184	57	71	85	1.38	-0.0130	0.9945	
1-C _{14:1}	(e)	36.80	0.604	196	97	83	69	1.31	-0.0241	0.9995	
C _{14:0}	(f)	37.30	0.612	198	71	57	85	1.23	0.0001	0.9994	
1-C _{15:1}	(g)	41.60	0.682	210	83	69	97	1.23	-0.0276	0.9995	
C _{15:0}	(h)	42.13	0.691	212	57	71	85	1.72	-0.0243	0.9955	
1,7-C _{16:2}	(i)	45.25	0.743	222	82	67	96	1.12	-0.0333	0.9994	
1-C _{16:1}	(j)	46.26	0.759	224	97	83	111	1.46	-0.0156	0.9996	
C _{16:0}	(k)	46.90	0.770	226	71	57	85	1.10	-0.0015	0.9995	
6,9-C _{17:2}	(1)	49.46	0.820	236	82	67	96	0.62	-0.0351	0.9975	
8-C _{17:1}	(m)	49.89	0.827	238	69	83	97	1.27	-0.0193	0.9996	
1-C _{17:1}	(n)	50.57	0.830	238	69	83	97	1.38	-0.0234	0.9997	
C _{17:0}	(o)	51.03	0.838	240	57	73	85	1.07	-0.0096	0.9997	

Table 2. Characteristics of Hydrocarbons

Retention time for IS, 60.9 min; rrt = (retention time)/60.9; M⁺, ion peak; 1,2 and 3: the three most intense peaks; Calibration curve, y = ax+b; r = co-relation coefficient.

Table 3. Hydrocarbons Characterized by GC-Mass Spectrometrv

compound	retention	mass $(m/e)^{c,d)}$				
	rt (min)	rrt	M^+	1	2	3
7-C _{16:1}	46.69	0.774	226	71	85	57
1,8-C _{17:2}	49.45	0.812	236	67	81	96
8-C _{18:1}	53.97	0.886	252	55	69	83
1,7,10-C _{16:3}	44.74	0.734	220	67	79	81
1,8,11-C _{17:3}	49.00	0.805	234	67	81	79
6,9-C _{16:2}	45.13	0.741	222	67	81	79
6,9-C _{18:2}	53.55	0.879	250	67	81	95

a) Retention time for IS, 60.9 min; b) rrt = (relative retention time)/60.9; c) M⁺, ion peak; d)1,2 and 3: the three most intense peaks.

able were identified by mass spectroscopy, the results of which are shown in Table 3. However, 7hexadecene, $HC(7-C_{16:1})$ that may be generated through the scission at the beta-position of the ester (b bond in Fig. 1) was not found in our experiment. Corresponding results regarding methyl linoleate are shown in Figs. 2 f-1) and f-2).

Dose Response of Hydrocarbon Levels

It is very important that candidate hydrocarbons, which can constitute evidences of irradiation show sharp dose-response curves. The five esters were irradiated in a range from 0 to 10 kGy and the generated hydrocarbons were determined. The generated hydrocarbons were classified into 4 compound types as indicated in Fig. 1. As shown in Fig. 3a), the major products of each ester were the $(C_{n-2,m+1})$ -hydrocarbon group (i.e., 1-dodecene, from myristate, 1tetradecene from palmitate, 1-hexadecene from stearate, 1,7-hexadecadiene from oleate, and 1,7,10hexadecatriene from linoleate) generated through the cleavage of the b-bond in ester shown in Fig. 1. The yields increased from $0.5-1.5 \,\mu g/g$ at 1 kGy, to 3 to $8 \,\mu g/g$ at 10 kGy. On the other hand, although a doseresponse could be observed, as to the other hydrocarbons [HC(C_{n-2,m}), HC(C_{n-1,m}), and HC(C_{n-1,m+1})], their amounts were less than $4 \mu g/g$ at 10 kGy. (Figs. 3 b, c, d). The results show that the hydrocarbons ($C_{n-2,m+1}$) provide effective evidence of irradiation. In practical analysis, the amounts of these hydrocarbons depend on the content of mother fatty acids. But in ordinary food, the main fatty acid is oleate; therefore, 1,7-hexadecadiene will be an accurate marker.

Effect of Temperature

The effects of temperature on the formation of hydrocarbons were examined. The samples were irradiated with 10 kGy at -40, 0, 20°C and the results were classified into the 4 radiolytic hydrocarbon types and shown in Fig. 4. Yields of 1,7-hexadecadiene (i), dodecane (b), and 6,9-heptadecadiene



Fig. 3. Relationship of Absorbed Dose to Hydrocarbon Yields The hydrocarbons were classified into four groups; *i.e.*, a) $HC(C_{n-2,m+1})$, b) $HC(C_{n-2,m})$, c) $HC(C_{n-1,m+1})$, and d) $HC(C_{n-1,m})$. The values for compounds with asterisks are the ratios of the peak area of the corresponding compounds to the peak area of the internal standard. Hexane solutions containing 10% fatty acid ester were irradiated at room temperature.

were increased as the temperature rose(from 0.5– 7.4 μ g/g at -40°C to 0.3–11 μ g/g at 20°C), while the other products were left unchanged. In our results, these 3 hydrocarbons showed observable temperature effects, because their intermediates may be stable at lower temperatures. Temperature effects on the formation of *o*-tyrosine have also been reported and the yield increased with the rise in temperature.¹²⁾ In both cases, there may be a suitable temperature for the highest yield of each radiolytic product.

Effect of Fatty Acid Ester Concentration

The effects of fatty acid ester concentration on hydrocarbon yields were examined. Two point five, 5, and 10% solutions of fatty acid esters in hexane were irradiated at room temperature for 10 kGy. The results are shown in Fig. 5. Most hydrocarbon yields were affected slightly by the concentration of mother fatty acid esters. But hydrocarbons in the $C_{n-2:m+1}$ were affected completely.

This illustrates how oxidation by gamma irradiation is affected by components which exist around the fatty acid esters. Therefore, the reproducibility



Fig. 4. Temperature Effect

The hydrocarbons were classified into four groups; *i.e.*, a) $HC(C_{n-2,m+1})$, b) $HC(C_{n-2,m})$, c) $HC(C_{n-1,m+1})$, and d) $HC(C_{n-1,m})$. The values for compounds with asterisks are the ratios of the peak area of the corresponding compounds to the peak area of the internal standard.

of the hydrocarbon yields in the actual complex matrix may be poor. This result is inconsistent with an earlier description of the hydrocarbon method for detecting irradiated foods.⁹⁾

Effect of Dose Rate

The effects of dose rate were examined over a range from 0.75 kGy/hr to 10 kGy/hr at room temperature, although some hydrocarbons were not observed due to their low yields. As shown in Fig. 6, the fatty acid esters in hexane solution gave almost constant yields regardless of dose rate. For example, the yield of 1,7-hexadecadiene was 10 μ g/g at any dose rate. Thus, there was no significant difference in the yield of radiolytic products among the dose rates we examined. These results were in accord with those of *o*-tyrosine.¹²

Effect of Low Oxygen Level

The effects of oxygen level in the samples were examined. Sample solutions in vials were irradiated



Fig. 5. Concentration Effect

The hydrocarbons were classified into four groups; *i.e.*, a) $HC(C_{n-2,m+1})$, b) $HC(C_{n-2,m})$, c) $HC(C_{n-1,m+1})$, and d) $HC(C_{n-1,m})$. The values for compounds with asterisks are the ratios of the peak area of the corresponding compounds to the peak area of the internal standard. Hexane solutions containing 10% fatty acid ester.

under reduced oxygen pressure. Reduction of oxygen in the atmosphere was performed by employing a de-oxygen reagent pack or oxygen-carbon dioxide exchanger. In the atmosphere de-oxygenated [reduced oxygen (RO) in Fig. 7] by de-oxygen reagent pack, the oxygen content was less than 1% and total pressure was about 0.8 atom. By employing an oxygen-carbon dioxide exchanger, the decrease in pressure caused by the reduction of oxygen was compensated for by the generation of carbon dioxide gas in the vials [replaced with carbon dioxide (RWCD) in Fig. 7]; therefore, total pressure in the vial was maintained at about 1 atm. The yields under the two conditions were compared with those in *n*-hexane solution under normal oxygen pressure. The yields were normalized among the class of hydrocarbons, because the reaction paths were the same in each class [for example, the yield of $C_{n-2,m+1}$ was average of the yields of 1-dodecene (a), 1-tetradecene (e), 1-



Fig. 6. Effect of Dose Rate

The hydrocarbons were classified into four groups; *i.e.*, a) $HC(C_{n-2,m+1})$, b) $HC(C_{n-2,m})$, c) $HC(C_{n-1,m+1})$, and d) $HC(C_{n-1,m})$. The values for compounds with asterisks are the ratios of the peak area of the corresponding compounds to the peak area of the internal standard. Hexane solutions containing 10% fatty acid ester were irradiated at room temperature.

hexadecene (j), and 1,7-hexadecadiene (i)]. The results are shown in Fig. 7. The horizontal axis in Fig. 6 indicates the relative average yields under reduced oxygen pressures against those under normal pressure in *n*-hexane. Thus, all hydrocarbon yields under both de-oxygenated and oxygen-carbon dioxideexchanged atmospheres were reduced to 5–41% and 25–60%, relatively of those under normal pressure. Especially, eminent reductions of hydrocarbon yields were observed in the containers that contained oxygen absorbers. These results show this radiolysis reaction needs oxygen and may be controlled by the indirect effects of radiation.

Solvent Effect

The solvent effects on the hydrocarbon yields were examined. Samples in benzene were irradiated at room temperature at 10 kGy. The yields in benzene were compared with those in n-hexane. The



Fig. 7. Effect of Oxygen Level

The hydrocarbons were classified into four groups; *i.e.*, a) $HC(C_{n-2,m+1})$, b) $HC(C_{n-2,m})$, c) $HC(C_{n-1,m+1})$, and d) $HC(C_{n-1,m})$. The average of each group is shown. Fatty acid methyl esters in hexane were irradiated in sealed glass tubes at a dose of 10 kGy at room temperature. The yields under the reduced oxygen pressure (less than 1%) (RO) and under the modified air in which oxygen was removed and was replaced with carbon dioxide (RWCD) relative to those in *n*-hexane under normal oxygen pressure (21%).

yields were normalized among the class of hydrocarbons, i.e., the same treatment as that described in the previous section, because the reaction paths may the same in each class [for example, the yield in benzene of $C_{n-2,m+1}$ was the average of the yields of 1-dodecene (a), 1-tetradecene (e), 1-hexadecene (j), and 1,7-hexadecadiene (i)]. The results are shown in Fig. 8. The horizontal axis indicates the relative yield of the average in benzene against those in *n*hexane. The yields in benzene were reduced to 60– 95% of those in hexane. This illustrates that intermediate radicals were stable enough to be trapped by the solvent.

In conclusion, an analytical procedure was developed to determine hydrocarbon concentration in fatty acid ester solutions which were irradiated with gamma-rays. The radiolytic reaction of fatty acid ester in hexane was affected by several irradiation conditions, including dose, temperature, concentration of fatty acid ester, oxygen level, and the solvent which traps radicals. These factors may affect the results of test for identifying irradiated food. Further study using irradiated food is underway.

Acknowledgements This study was financially supported by the Budget for Nuclear Research of the Ministry of Education, Culture, Sports, Science and Technology, based on the screening and counseling by the Atomic Energy Commission.



Fig. 8. Effect of Solvent

The hydrocarbons were classified into four groups; *i.e.*, a) $HC(C_{n-2,m+1})$, b) $HC(C_{n-2,m})$, c) $HC(C_{n-1,m+1})$, and d) $HC(C_{n-1,m})$. The average of each group is shown. Fatty acid methyl esters in benzene were irradiated at a dose of 10 kGy at room temperature. The yields in benzene were relative to those in *n*-hexane.

We thank to Dr. K. Morehouse and Dr. C. Warner of the US Food and Drug Administration, Washington, DC, and Dr. Eric Marchioni, the University of Louis Pasteur, for useful their advise and discussions.

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