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Hydrocarbons detected in irradiated pork, bacon and ham

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Abstract

Hydrocarbons produced by γ-radiation of pork, bacon and ham were analyzed to determine how irradiation affects the production of the hydrocarbons and whether the hydrocarbons can be used for identifying post-irradiation of pork, bacon and ham. Hydrocarbons were determined by a sequential procedure of lipid extraction by hexane, Florisil column chromatography and gas chromatography. Hydrocarbons C17:1, C16:2, C17:2 and C16:3 were detected in pork, bacon and ham irradiated at 0.5 kGy or higher, but not in non-irradiated ones except C17:1. The detection levels in all the irradiated samples were in the order of C16:2, C17:1, C17:2 and C16:3 from the highest to the lowest.

Keywords: Bacon; Ham; Hydrocarbon; Irradiation detection; Pork

1. Introduction

Pork and its products may be contaminated with microorganisms and/or parasites. Irradiation is one of the most effective and safest methods to control them. The USA, China, Croatia and Mexico permit pork to be irradiated up to 1 kGy for parasite control (IAEA, 1995). In Russia and Ukraine irradiation of pork is permitted up to 8 kGy for shelf-life extension (IAEA, 1995). In spite of the benefits from food irradiation, consumers are concerned about how their foods were previously treated. Foods produced domestically can be controlled easily, but it is hard to determine if imported foods are irradiated.

One of the promising methods to detect the post-irradiation of lipid-containing foods is the analysis of hydrocarbons, which are produced from lipids by irradiation (Champagne and Nawar, 1969; Dubravcic and Nawar, 1969; Kavalam and Nawar, 1969). Two types of hydrocarbons are predominantly produced by irradiation of a fatty acid: one is the hydrocarbon which has one carbon less than the parent fatty acid (Cn-1) and the other is the one that has two carbons less and an additional double bond at position 1 (Cn-2, 1-ene) (Spiegelberg et al., 1994). Oleic acid is the most abundant of the lipids found in pork consisting of 42%, followed by palmitic acid (22%), stearic acid (12%) and linoleic acid (9%) (Hands, 1996). Therefore, 8-heptadecene (C17:1) and 1,7-hexadecadiene (C16:2) from oleic acid, *n*-pentadecane (C15:0) and 1-tetradecene (C14:1) from palmitic acid, *n*-heptadecane (C17:0) and 1-hexadecene (C16:1) from stearic acid, and 6,9-heptadecadiene (C17:2) and 1,7,10-hexadecatriene (C16:3) from linoleic acid are expected to be detected in irradiated pork.

Separation of hydrocarbons from lipids has been considered to be one of the most critical steps in the methodology to detect hydrocarbons. Hydrocarbons have been separated from lipid fractions by cold finger distillation (Nawar et al., 1990), column chromatography (Sjöberg et al., 1992; Schreiber et al., 1994; Spiegelberg et al., 1994), or high performance liquid chromatography (Biederman et al., 1992; Schulzki et al., 1995a,b). Florisil column chromatography has been considered to be one of the most practicable methods for the routine analysis of meats (Spiegelberg et al., 1994). Detection of hydrocarbons has been studied in pork, beef or chicken (Champagne and Nawar, 1969; Vajdi et al., 1979; Sjöberg et al., 1992; Schreiber et al., 1994; Spiegelberg et al., 1994), but not on their processed products.

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In the present study a sequential procedure of lipid extraction by hexane, Florisil column chromatography, and gas chromatography (GC) could be used to detect hydrocarbons not only in irradiated pork but also bacon and ham. The method may also be useful to determine how irradiation affects production of hydrocarbons in pork, bacon and ham.

2. Materials and methods

2.1. Materials and reagents

Pork (shoulder part) was purchased from a local market in Chonju, Korea. Bacon and ham were purchased from a local producer (M. Co., Korea). Sodium sulfate was analytical grade (Pure Chemicals Co., Ltd., Japan). *n*-Hexane and *iso*-octane were from Fisher Scientific (USA). The hydrocarbon standards were purchased from Sigma Chemical Co. (USA) or obtained from the German Federal Institute for Health Protection of Consumers and Veterinary Medicine (Germany). Linoleic acid was purchased from Nu Chek Prep, Inc. (USA). Since 6,9-heptadecadiene (C17:2) and 1,7,10-hexadecatriene (C16:3) standards were not commercially obtainable, linoleic acid was irradiated at 10 kGy, and the two largest GC peaks were identified as the two hydrocarbons by GC/MS.

2.2. Irradiation

Samples (100 g each in bulk) were irradiated at 0.5, 1, 3 and 6 kGy using a ^{60}Co γ-radiation source (100,000 Ci, 71.5 Gy min^{-1}) at the Korea Atomic Energy Research Institute (Korea).

2.3. Fat extraction

Fat extraction, separation of hydrocarbons, and GC analysis followed the previously reported methods (Spiegelberg et al., 1994; Choi and Hwang, 1997) with minor modifications. Thirty grams of meat sample was ground for 3 min in a mixer (FM680T; Hanil Electronic, Korea) with 50 g anhydrous sodium sulfate (previously heated to 650°C for 5 h). One hundred and fifty milliliters *n*-hexane was added and the content was homogenized thoroughly with a homogenizer (M133/1280-0; Biospec Products, Inc., USA) at full speed for 3 min. The mixture was transferred to Teflon centrifuge tubes (Nalge Co., USA) and centrifuged at 2240×*g* for 20 min using a centrifuge (Union 55R; Hanil Co., Korea). The supernatant was collected in a round-bottomed flask. The solvent was evaporated using an Eyela rotary vacuum evaporator (N-N; Tokyo Rikakikai Co., Ltd., Japan) at 35°C, connected to an Eyela aspirator (A-3S; Tokyo Rikakikai Co., Ltd., Japan). The extracted fat was flushed with nitrogen and stored at 4°C until separated by Florisil column chromatography.

2.4. Separation of hydrocarbons by Florisil column chromatography

Florisil (60–100 mesh, F100-3; Fisher Scientific) was heated at 550°C overnight. Just prior to packing the column, it was heated again to 130°C for 5 h and cooled down to room temperature. It was then deactivated by the addition of 3% water. The glass column (2.3 cm i.d.) with a Teflon stopcock was rinsed with hexane and filled with 20 g Florisil. One g of fat sample mixed with 1 ml of hexane containing 8 μg ml^{-1} *n*-eicosane as an internal standard was applied to the column, followed by 60 ml hexane and eluted at 3 ml min^{-1}. Half a Milliliter (0.5 ml) *iso*-octane was added to the eluate, and the eluate was concentrated to a volume of about 4 ml under a nitrogen stream. The concentrated sample was filtered through a Nylon membrane (13 mm diameter, 0.2 μm pore; Whatman International Ltd., UK) contained in a 13 mm syringe holder (Nucleopore Corp., USA), which was connected to a 10 ml Luer-lock syringe (Popper and Sons, Inc., USA). The filtrate was concentrated to 2 ml under nitrogen and transferred into a GC vial. Hydrocarbons from linoleic acid, non-irradiated or irradiated, were separated in the same way.

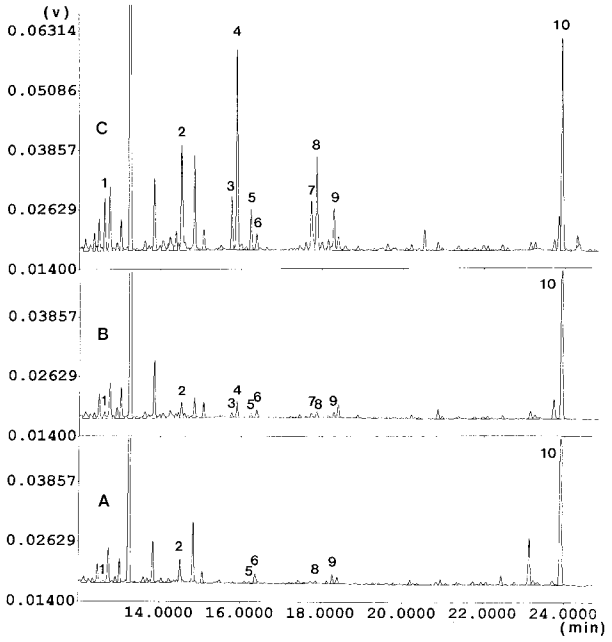

Fig. 1. GC chromatograms of the hydrocarbons in irradiated ham. (A) non-irradiated; (B) irradiated at 1 kGy; and (C) irradiated at 6 kGy.(1) 14:1; (2) 15:0; (3) 16:3; (4) 16:2; (5) 16:1; (6) 16:0; (7) 17:2; (8) 17:1; (9) 17:0; and (10) 20:0 (internal standard). Column: HP-5 (5%-diphenyl–95%-dimethylsiloxane copolymer); 0.32 mm i.d.×30 m, 0.25 μm. Oven: 50°C for 2 min; 50–130°C at 10°C/min; 130–200°C at 5°C/min; 200°C for 2 min; 200–250°C at 25°C/min, and 250°C for 5 min. Injector: splitless, 200°C. Detector: FID, 250°C.

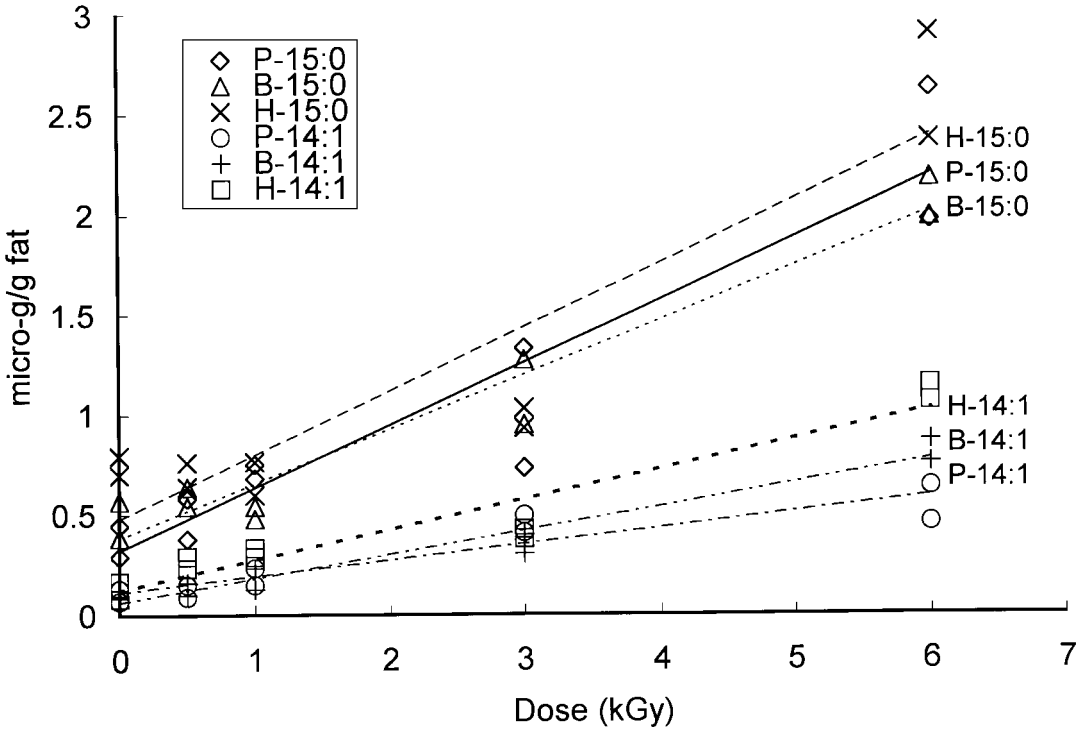

Fig. 2. Hydrocarbons 15:0 and 14:1 detected in irradiated pork, bacon and ham. P: pork; B: bacon; H: ham; spots: single measurements; lines: linear regressions.

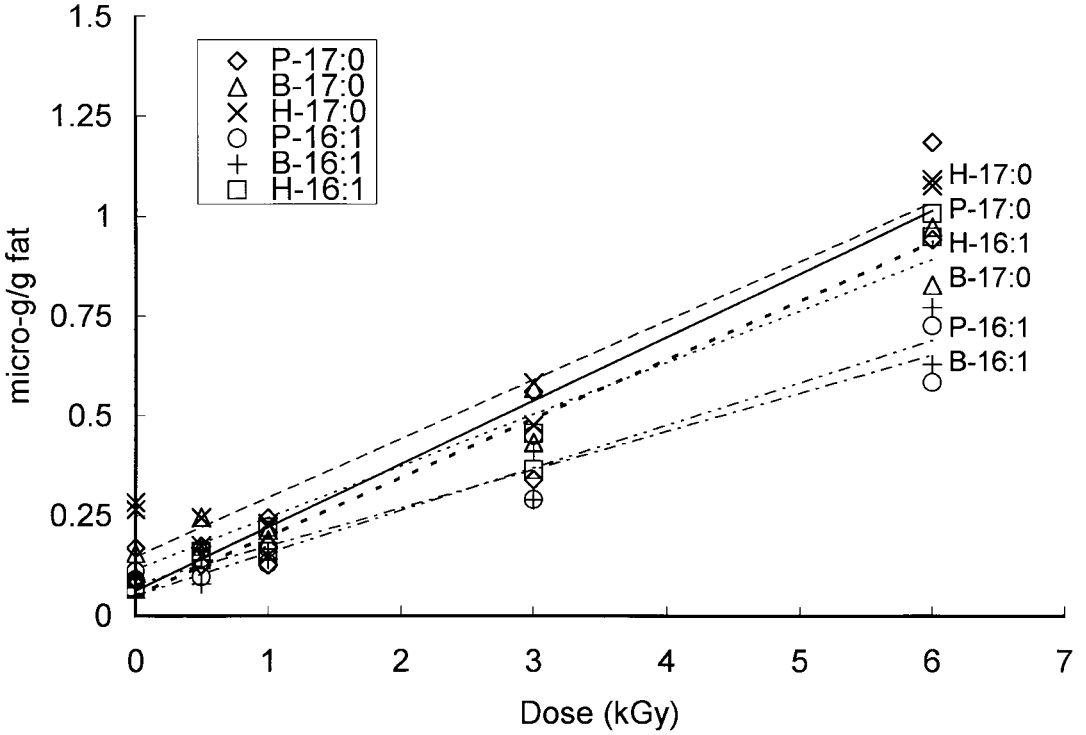

Fig. 3. Hydrocarbons 17:0 and 16:1 detected in irradiated pork, bacon and ham. P: pork; B: bacon; H: ham; spots: single measurements; lines: linear regressions.

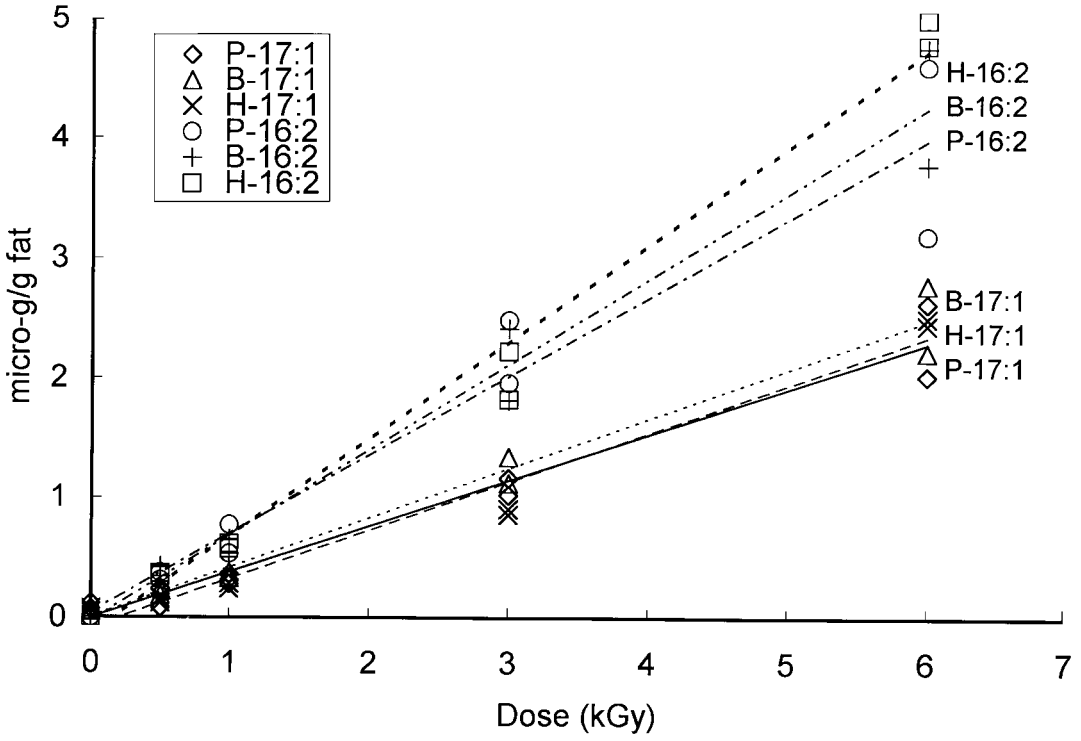

Fig. 4. Hydrocarbons 17:1 and 16:2 detected in irradiated pork, bacon and ham. P: pork; B: bacon; H: ham; spots: single measurements; lines: linear regressions.

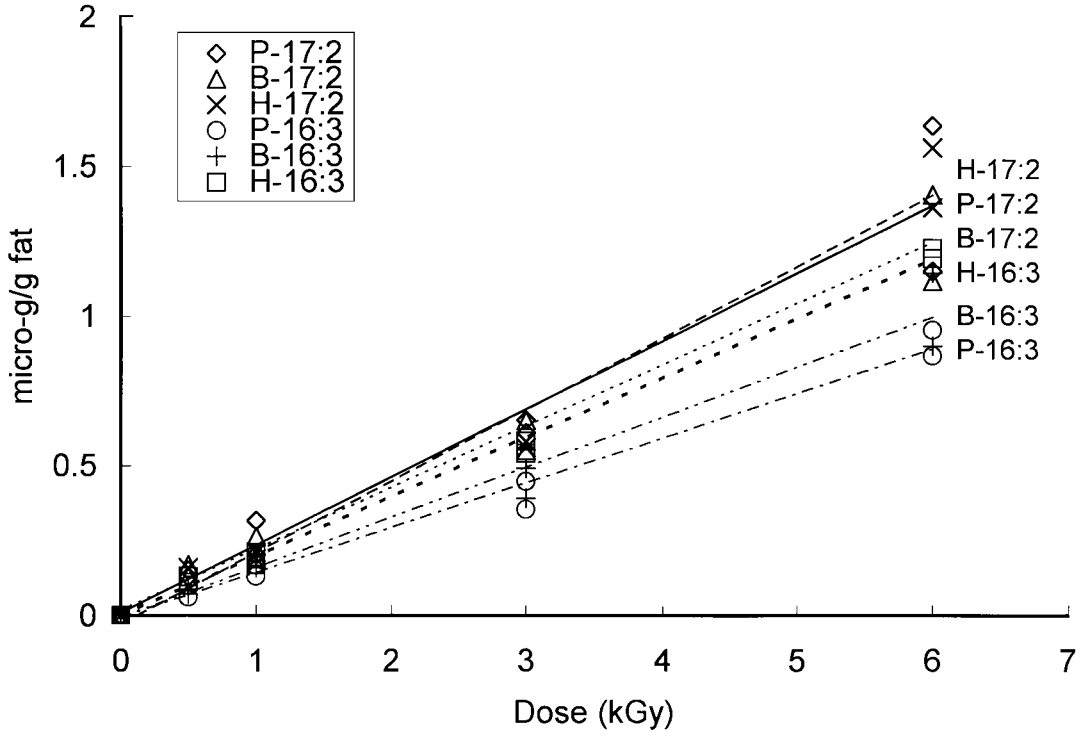

Fig. 5. Hydrocarbons 17:2 and 16:3 detected in irradiated pork, bacon and ham. P: pork; B: bacon; H: ham; spots: single measurements; lines: linear regressions.

2.5. GC analysis of hydrocarbons

The isolated hydrocarbons were analyzed on a Hewlett Packard 6980 Series Gas Chromatograph (Hewlett Packard Co., USA) equipped with a flame ionization detector (FID) and a split/splitless injector. Helium was used as the carrier gas at the flow rate of 2.6 ml min^{-1}. The column was an HP-5 (5%-diphenyl–95%-dimethylsiloxane copolymer) 0.32 mm i.d.×30 m column with 0.25 μm film thickness (Hewlett Packard Co. USA). The initial column temperature was 50°C for 2 min, then programmed at 10°C min^{-1} to 130°C and 5°C min^{-1} to 200°C where it was held for 2 min, then 25°C min^{-1} to 250°C with a final hold of 5 min. The injector and detector temperatures were 200 and 250°C, respectively. The injector was initially set in splitless pulsed for 1 min and purged at 0.6 min. One μl of the sample was injected. Initially unidentified peaks were identified by a GC/MS. All the experiments were in duplicate. The data were analyzed with Microsoft Excel 97 (Microsoft Corp., USA): one-tailed *t*-test ($\alpha = 0.05$) of hydrocarbons between unirradiated and irradiated samples; repeated measures two-way ANOVA among pork, bacon and ham for each hydrocarbon; and linear regressions of hydrocarbons against dose.

3. Results and discussion

The hydrocarbons expected from pork lipids were clearly detected on GC. GC chromatograms of hydrocarbons only for ham are introduced here (Fig. 1), since those for pork and bacon appeared almost in the same manner as for ham. There were some small peaks around those of the hydrocarbons we are interested in. However, the hydrocarbons had relative retention times clearly different from the other peaks.

The hydrocarbons determined in irradiated pork, bacon and ham increased with dose (Figs. 2–5) and showed strong correlations between dose and hydrocarbon production with *r* values higher than 0.9. Hydrocarbons C15:0, C14:1, C17:0, C16:1 and C17:1 were detected in non-irradiated samples. The dose at which each hydrocarbon in each sample was detected with significance is compared with non-irradiated sample (Table 1). C15:0 and C17:0 were detected in fairly high levels in non-irradiated samples and did not show significant increases in the samples irradiated even at 1 or 3 kGy. For C14:1 and C16:1, the differences were seen in the samples which had been irradiated at fairly low doses.

As expected, the detected amount of C17:1 and C16:2 induced from oleic acid was higher than any other pair of hydrocarbons from a fatty acid in all the samples (Figs. 2–5). C16:2 was the largest in the irradiated samples, followed by C17:1. C17:1 was detected in non-irradiated sample, but it was in a fairly low level and increased significantly at lower doses (Table 1). C17:2 and C16:3 derived from linoleic acid were detected at fairly low levels in irradiated samples, but not detected in unirradiated ones. C17:1, C16:2, C17:2 and C16:3 could, therefore, be used as markers for identifying post-irradiation of pork since they were not detected or were detected at a very low level in non-irradiated samples. For all of the irradiation doses, detection levels of the four hydrocarbons were in the order of C16:2, C17:1, C17:2 and C16:3 from the highest to the lowest. Only the detection of some hydrocarbons may be misinterpreted as an irradiated sample, since the sample could be contaminated or during preparation for GC analysis some peaks may appear at the same retention times as for the expected hydrocarbons. Therefore, the pattern of the detected amounts of the four hydrocarbons could be used for correct identification of post-irradiation of pork and its products besides the four hydrocarbons themselves.

The types and pattern of the detected hydrocarbons in non- irradiated and irradiated bacon and ham was

Table 1
The lowest irradiation dose increasing hydrocarbons in significant levels compared with unirradiated samples[a]

	Hydrocarbons (kGy)				
	15:0	14:1	17:0	16:1	17:1
Pork	6	3	6	3	1
Bacon	3	3	3	1	0.5
Ham	3	1	3	0.5	0.5

[a] One-tailed *t*-test ($\alpha = 0.05$) assuming variances are equal ($n = 2$).

Table 2
Differences in detected hydrocarbons among pork, bacon and ham

	ANOVA results[a]	
	Among three samples	Between two samples
15:0	NS[b] ($p = 0.12$)	
14:1	*** ($p = 0.00008$)	Pork-bacon: NS ($p = 0.32$) Pork-ham: *** ($p = 0.0004$) Bacon-ham: *** ($p = 0.0004$)
17:0	NS ($p = 0.15$)	
16:1	*** ($p = 0.009$)	Pork-bacon: NS ($p = 0.86$) Pork-ham: *** ($p = 0.01$) Bacon-ham: *** ($p = 0.005$)
17:1	NS ($p = 0.37$)	
16:2	NS ($p = 0.65$)	
17:2	NS ($p = 0.77$)	
16:3	*** ($p = 0.002$)	Pork-bacon: NS ($p = 0.26$) Pork-ham: *** ($p = 0.00004$) Bacon-ham: *** ($p = 0.027$)

[a] Repeated measures two-way ANOVA ($n = 2$ in each of five doses).
[b] NS: not significant; ***: highly significant. **: significant.

similar to those in pork (Figs. 2–5), although the detection levels showed a little differences, especially in ham from pork and bacon. Two-way ANOVA (dose and kind of sample) showed that there were significant differences among the three samples for C14:1, C16:1 and C16:3, and only ham showed significant differences from the other two samples for those hydrocarbons (Table 2). (For all the hydrocarbons, dose significantly affected production of hydrocarbons in all the samples as noted in Table 1). The higher hydrocarbon levels in ham might result from the change of the medium during the pre-treatments where lipids could be more susceptible to irradiation. However, further studies should be conducted to see whether the ham processing affects the hydrocarbon production by comparing pork and ham made from the same portion of the pork.

The irradiation of pork and its products from 1 to 10 kGy would have certain benefits such as parasite or microbial control. If pork, bacon and ham are irradiated at 1 kGy or higher, C17:1, C16:2, C17:2 and C16:3 could be used for determining whether bacon and ham are post-irradiated as well as pork.

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