Production of Radiation-Induced Compounds in Irradiated Raw and Cooked Meats

A.S. Leaflet R2388

Joong-Ho Kwon, [P]rofessor; Youngju Kwon, graduate
assistant; Tusneem Kausar, graduate assistant;
Department of Food Science & Technology, Kyungpook
National University;
Ki-Chang Nam, postdoctoral research associate;
Byong Rok Min, graduate assistant;
Eun Joo Lee, assistant scientist;
Dong Ahn, professor, Department of Animal Science

Summary and Implications

The objective of this study was to determine the best irradiation markers in irradiated meat. Raw and cooked beef loins, pork loins and chicken thighs were irradiated at 0 or 5 kGy. The amounts of hydrocarbons, 2-alkylcyclobutanones, and sulfur volatiles were determined after 0 and 6 months of frozen storage. Two hydrocarbons (8-heptadecene ($C_{17:1}$) and 6,9-heptadecadiene ($C_{17:2}$)) and two 2-alkylcyclobutanones (2-dodecylcyclobutanone (DCB) and 2-tetradecylcyclobutanone (TCB)) were detected only in irradiated raw and cooked meats. Although pre-cooked irradiated meats produced more hydrocarbons and 2-alkylcyclobutanones than the irradiated cooked ones, the amounts of individual hydrocarbons and 2-alkylcyclobutanones, such as 8-heptadecene, 6,9-heptadecadiene, DCB, and TCB, were sufficient enough to identify whether the meat was irradiated or not. Dimethyl disulfide and dimethyl trisulfide were detected only in irradiated meats, but dimethyl trisulfide disappeared after 6 months of frozen storage under oxygen permeable packaging conditions. This indicated that only dimethyl disulfide could be used as an irradiation marker for the meat stored in frozen conditions for a long time.

Introduction

Since mid 1980s, extensive research for developing detection methods for irradiated foods has been conducted. Five tests, which include microbiological screening, electron spin resonance (ESR) spectroscopy, thermoluminescence (TL), and monitoring the formation of long-chain hydrocarbons and 2-alkylcyclobutanones (2-ACB), were adopted as standard reference methods for the detection of irradiated food by the European Committee for Normalization (CEN).

Some chemical changes in foods, which can be used as irradiation indicators or markers, occur during irradiation by free radical reactions. 2-Alkylcyclobutanones (2-ACB) such as 2-dodecylcyclobutanone (DCB) and 2-tetradecylcyclobutanone (TCB) are formed in irradiated fat or oil by the loss of an electron from acyl-oxygen bond in fatty acids, followed by a rearrangement process to produce 2-alkylcyclobutanones specific to their parent fatty acids. Because 2-dodecylcyclobutanone (DCB) and 2-tetradecylcyclobutanone (TCB) are not detected in nonirradiated foods, they were used as markers for detecting irradiated foods. These 2-ACBs are extracted using n-hexane or n-pentane along with fat, fractionated using adsorption chromatography prior to separation using gas chromatography (GC) and detection using a mass spectrometer (MS). Other 2-ACBs such as 2-(tetradec-5'-enyl) cyclobutanone derived from oleic acid also have been identified in irradiated foodstuffs. Hydrocarbons (HC) in fat-containing foods were generated by the primary and secondary reactions after the chemical bonds in fatty acids are broken by irradiation. The fatty acid moieties of triglycerides are mainly broken at alpha and beta positions of carbonyl groups and two types of hydrocarbons, which contain one (Cn-1) or two (Cn-2:1) less carbon atoms than its parent fatty acids, are formed.

Ionizing radiation also generates hydroxyl radicals in aqueous or oil emulsion systems. Hydroxyl radical is the most reactive oxygen species and can initiate lipid oxidation by abstracting a hydrogen atom from fatty acyl chain of a polyunsaturated fatty acid (PUFA) and form a lipid radical. In the presence of oxygen, the lipid radical rapidly reacts with oxygen to form a peroxyl radical which, in turn, can extract a hydrogen atom from another fatty acyl chain, yielding a new free radical that can perpetuate the chain reaction, and a lipid hydroperoxide that can be degraded into various volatile compounds including aldehydes, ketones, hydrocarbons, and sulfur compounds, after a series of secondary reactions. Some gases such as carbon monoxide, carbon dioxide and methane are also produced by reactions between meat components and free radicals. However, little information on the chemical changes induced by free radicals in precooked irradiated or irradiated cooked meats is available. The objective of this study was to identify the best irradiation markers that can be used for ground beef, pork, and chicken irradiated before or after cooking.

Materials and Methods

Fresh meats (beef loin, pork loin, and chicken thigh) were purchased from local supermarkets, ground through a 5-mm plate, and vacuum-packaged in an oxygen-impermeable nylon/polyethylene bags within 6 hr of purchase. Five treatments for each meat species were prepared depending on cooking and irradiation conditions; 1) nonirradiated raw meat (uncooked-0 kGy), 2) irradiated raw meat (uncooked-5 kGy), 3) nonirradiated cooked meat (cooked-0 kGy), 4) precooked irradiated meat (cooked-5

kGy), 5) irradiated cooked meat (5 kGy-cooked). For cooked irradiated meat, cooking was done in an 85°C water bath to an internal temperature of 75°C. Cooked and raw meat were repackaged in oxygen permeable nylon bags and irradiated at 5 kGy using a Linear Accelerator. For irradiated cooked meat, raw meats were irradiated first and then cooked immediately after irradiation using the same conditions as above. Samples were analyzed at 0 day and after 6 months of storage at -40°C under oxygen permeable packaging conditions. The fat in raw meats was extracted using Folch's method. Fatty acid composition was analyzed after methylating fats using BF_3-methanol. The fatty acid methyl esters were separated using a gas chromatograph equipped with a flame ionization detector (FID).

Hydrocarbons and 2-alkylcyclobutanones were extracted and analyzed using a gas chromatograph/mass spectrometry (GC/MS). Sulfur volatile compounds were determined using a dynamic headspace GC/MS method. Analysis of variance was done using the generalized linear model procedure of SAS software.

Results and Discussion

Cooking newly produced 1-hexadecene in pork and chicken or significantly increased its concentration in beef. Thus, 1-hexadecene cannot be used as an irradiation marker for meat. However, 8-heptadecene ($C_{17:1}$) and 6,9-heptadecadiene ($C_{17:2}$), derived from oleic acid and linoleic acid, respectively, were found at high concentrations in irradiated meats but not detected in nonirradiated meat. Therefore, 8-heptadecene and 6,9-heptadecadiene can be used as markers for irradiated beef, pork, and chicken. New hydrocarbons can also be formed by heating. Although cooked meats before or after irradiation had a significantly higher number of hydrocarbons than raw meat, 8-heptadecene and 6,9-heptadecadiene were not detected in cooked non-irradiated meats. Although the concentrations of hydrocarbons decreased during storage, the radiation-induced hydrocarbons, 8-heptadecene and 6,9-heptadecadiene, exhibited high stability even after 6 months of frozen storage.

2-Dodecylcyclobutanone (2-DCB) and 2-dodecylcyclobutanone (2-TCB) were detected only in irradiated meats regardless of cooking treatment. Among the 2-ACBs, 2-(5'-teradecenyl) cyclobutanone (2-TeCB) was present at the highest level in all three irradiated meat species, but was also detected in non-irradiated meats. 2-DCB was the best candidate for irradiation marker among the 2-ACB compounds because it was detected only in irradiated meats. During storage, the concentrations of 2-ACBs decreased drastically in all irradiated meats, but detectable level of them were still remained in the meat after 6 months of storage at -40°C.

The amount of dimethyl disulfide was significantly higher in irradiated meats than non-irradiated ones. Dimethyl disulfide and dimethyl trisulfide were not found in non-irradiated meats, but present in irradiated ones. The amounts of these sulfur compounds increased as the irradiation dose increased. Due to low threshold for odor detection, even small amounts of these sulfur compounds are important for irradiation off-odor. The ratios among sulfur volatiles in meat also changed during storage, which should have significant impact on the overall odor characteristics of irradiated meat because each sulfur compound has its own characteristic odor notes. Among the meat species, irradiated pork and chicken produced more sulfur volatiles than irradiated beef. Most of the sulfur-containing compounds disappeared after 6 months of storage at -40°C. Nevertheless, dimethyl disulfide was still detectable in all meats after 6 months of frozen storage under oxygen permeable packaging conditions, indicating that it could be used as an irradiation marker for raw and frozen cooked meat.

Table 1. Concentration of irradiation-induced hydrocarbons in beef, pork, and chicken during storage at -40℃.

Hydrocarbons	Storage time (mo)	Raw meat		Cooked before IR		Cooked after IR
		0 kGy	5 kGy	0 kGy	5 kGy	5 kGy
				---------------------- (µg/g fat) ----------------------		
Beef						
1-Tetradecene($C_{14:1}$)	0	-[1]	9.30 ± 0.21^{bx}	-	12.23 ± 0.92^{ax}	8.06 ± 0.07^{cx}
1-Tetradecene($C_{14:1}$)	6	-	6.37 ± 0.20^{by}	-	7.35 ± 0.03^{ay}	7.58 ± 0.26^{ay}
Pentadecane($C_{15:0}$)	0	1.61 ± 0.51^{cx}	7.12 ± 0.16^{bx}	1.35 ± 0.41^{cx}	14.39 ± 0.96^{ax}	7.86 ± 0.06^{bx}
Pentadecane($C_{15:0}$)	6	1.46 ± 0.03^{dx}	5.10 ± 0.02^{cy}	1.04 ± 0.37^{ex}	9.9 ± 0.23^{ay}	5.78 ± 0.24^{by}
1-Hexadecene($C_{16:1}$)	0	0.86 ± 0.39^{dx}	6.74 ± 0.90^{cx}	1.33 ± 0.73^{dx}	9.91 ± 0.93^{bx}	19.616 ± 0.39^{ax}
1-Hexadecene($C_{16:1}$)	6	-	4.47 ± 0.55^{cy}	0.56 ± 0.08^{dx}	5.97 ± 0.36^{by}	15.80 ± 0.25^{ay}
6,9-Heptadecadiene($C_{17:2}$)	0	-	2.51 ± 0.15^{ax}	-	2.54 ± 0.14^{ax}	1.73 ± 0.715^{bx}
6,9-Heptadecadiene($C_{17:2}$)	6	-	1.82 ± 0.64^{ax}	-	1.98 ± 0.0^{ay}	1.49 ± 0.39^{ax}
8-Heptadecene($C_{17:1}$)	0	-	7.00 ± 0.21^{ax}	-	6.94 ± 0.40^{ax}	4.53 ± 0.75^{bx}
8-Heptadecene($C_{17:1}$)	6	-	5.05 ± 0.25^{by}	-	5.75 ± 0.08^{ay}	3.07 ± 0.12^{cy}
n-Heptadecane($C_{17:0}$)	0	2.88 ± 0.06^{bx}	5.43 ± 0.18^{ax}	3.44 ± 0.19^{bx}	6.18 ± 0.78^{ax}	6.17 ± 0.26^{ax}
n-Heptadecane($C_{17:0}$)	6	0.37 ± 0.03^{cy}	3.83 ± 0.42^{by}	0.38 ± 0.19^{cy}	5.24 ± 0.98^{ax}	5.51 ± 0.53^{ax}
Pork						
1-Tetradecene($C_{14:1}$)	0	0.42 ± 0.00^{bx}	5.63 ± 0.37^{ax}	0.3744 ± 0.03^{bx}	5.50 ± 0.09^{ax}	5.10 ± 0.84^{ax}
1-Tetradecene($C_{14:1}$)	6	-	4.81 ± 0.07^{ay}	-	4.23 ± 0.04^{cy}	4.47 ± 0.35^{bx}
Pentadecane($C_{15:0}$)	0	0.59 ± 0.00^{cx}	6.13 ± 0.69^{ax}	1.41 ± 0.01^{cx}	4.50 ± 0.57^{bx}	5.15 ± 0.69^{bx}
Pentadecane($C_{15:0}$)	6	-	5.35 ± 0.37^{ax}	1.33 ± 0.23^{dx}	2.97 ± 0.04^{cy}	4.03 ± 0.11^{by}
1-Hexadecene($C_{16:1}$)	0	-	3.19 ± 0.42^{ax}	1.93 ± 0.00^{bx}	3.22 ± 0.65^{ax}	3.12 ± 0.29^{ax}
1-Hexadecene($C_{16:1}$)	6	-	2.89 ± 0.90^{ax}	$1.\,80\pm0.21^{bx}$	3.25 ± 0.48^{ax}	1.06 ± 0.00^{by}
6,9-Heptadecadiene($C_{17:2}$)	0	-	3.37 ± 0.18^{ax}	-	3.51 ± 0.47^{ax}	3.09 ± 0.57^{ax}
6,9-Heptadecadiene($C_{17:2}$)	6	-	1.17 ± 0.10^{cy}	-	1.82 ± 0.02^{ay}	1.55 ± 0.37^{by}
8-Heptadecene($C_{17:1}$)	0	-	6.25 ± 0.92^{ax}	-	5.57 ± 0.89^{ax}	6.62 ± 0.56^{ax}
8-Heptadecene($C_{17:1}$)	6	-	4.57 ± 0.29^{ay}	-	4.11 ± 0.51^{ax}	4.20 ± 0.026^{ay}
n-Heptadecane($C_{17:0}$)	0	0.86 ± 0.32^{cx}	4.13 ± 0.78^{ax}	1.34 ± 0.3^{bcx}	1.02 ± 0.41^{cx}	2.16 ± 0.33^{bx}
n-Heptadecane($C_{17:0}$)	6	0.20 ± 0.00^{cy}	4.12 ± 0.43^{ax}	0.37 ± 0.04^{bcy}	0.62 ± 0.00^{bcx}	0.70 ± 0.08^{by}
Chicken						
1-Tetradecene($C_{14:1}$)	0	2.15 ± 0.81^{dx}	8.39 ± 0.52^{bx}	4.04 ± 0.08^{cx}	10.46 ± 0.73^{ax}	1.63 ± 0.69^{dx}
1-Tetradecene($C_{14:1}$)	6	0.51 ± 0.01^{dy}	5.69 ± 0.23^{by}	1.43 ± 0.00^{cy}	8.39 ± 0.35^{ay}	0.73 ± 0.26^{dx}
Pentadecane($C_{15:0}$)	0	0.54 ± 0.02^{ex}	3.92 ± 0.31^{cx}	1.67 ± 0.25^{dx}	7.47 ± 0.30^{ax}	6.43 ± 0.89^{bx}
Pentadecane($C_{15:0}$)	6	-	2.63 ± 0.17^{by}	0.65 ± 0.04^{cy}	4.77 ± 0.67^{ax}	4.02 ± 0.67^{ay}
1-Hexadecene($C_{16:1}$)	0	-	21.63 ± 0.58^{ax}	3.36 ± 0.05^{cx}	6.52 ± 0.72^{bx}	3.22 ± 0.22^{cx}
1-Hexadecene($C_{16:1}$)	6	-	15.76 ± 0.57^{ay}	1.14 ± 0.01^{dy}	5.51 ± 0.17^{bx}	2.5 ± 0.83^{cx}

6,9-Heptadecadiene($C_{17:2}$)	0	-	3.65 ± 0.85^{ax}	-	4.39 ± 0.36^{ax}	3.30 ± 0.47^{ax}
6,9-Heptadecadiene($C_{17:2}$)	6	-	1.77 ± 0.63^{bx}	-	4.35 ± 0.28^{ax}	1.67 ± 0.20^{by}
8-Heptadecene($C_{17:1}$)	0	-	5.22 ± 0.74^{bx}	-	6.99 ± 0.49^{ax}	5.16 ± 0.65^{bx}
8-Heptadecene($C_{17:1}$)	6	-	3.96 ± 0.28^{bx}	-	6.44 ± 0.06^{ax}	4.10 ± 0.29^{bx}
n-Heptadecane($C_{17:0}$)	0	5.03 ± 0.36^{ax}	2.29 ± 0.62^{bx}	2.52 ± 0.80^{bx}	3.66 ± 0.77^{bx}	2.61 ± 0.36^{bx}
n-Heptadecane($C_{17:0}$)	6	4.49 ± 0.06^{ax}	0.60 ± 0.11^{dy}	1.90 ± 0.11^{cx}	3.39 ± 0.20^{bx}	0.47 ± 0.19^{dy}

[a-e]Means with different letters within a column of the same animal species are significantly different ($p < 0.05$), n = 3.
[x,y]Means with different letters within a row are significantly different ($p < 0.05$), n = 3.

Table 2. Concentrations of irradiation-induced 2-alkylcyclobutanones in beef, pork, and chicken during storage at -40℃.

Cooking treatment	IR dose (kGy)	2-DCB		2-TCB		2-TeCB	
		0 month	6 months	0 month	6 months	0 month	6 months
		----- (μg/g fat) -----					
Beef							
Raw meat	0	-	-	-	-	1.28 ± 0.35^{cx}	0.55 ± 0.16^{cy}
Raw meat	5	1.93 ± 0.02^{ax}	0.96 ± 0.04^{by}	0.51 ± 0.01^{ax}	0.14 ± 0.06^{ay}	5.81 ± 0.06^{ax}	2.97 ± 0.02^{ay}
Cooked before IR	0	-	-	-	-	0.67 ± 0.04^{cx}	0.42 ± 0.11^{cy}
Cooked before IR	5	1.63 ± 0.69^{ax}	1.06 ± 0.06^{ax}	0.25 ± 0.18^{bx}	0.05 ± 0.03^{bx}	4.39 ± 1.51^{bx}	2.76 ± 0.78^{ax}
Cooked after IR	5	0.76 ± 0.47^{bx}	0.23 ± 0.06^{cx}	0.13 ± 0.07^{bx}	0.10 ± 0.02^{abx}	2.09 ± 0.53^{cx}	1.77 ± 0.20^{bx}
Pork							
Raw meat	0	-	-	-	-	0.25 ± 0.06^{ax}	0.22 ± 0.02^{cx}
Raw meat	5	0.41 ± 0.09^{ax}	0.08 ± 0.01^{by}	0.44 ± 0.05^{ax}	0.11 ± 0.01^{ay}	0.82 ± 0.20^{ax}	0.40 ± 0.02^{cy}
Cooked before IR	0	-	-	-	-	0.30 ± 0.01^{ax}	0.27 ± 0.22^{cx}
Cooked before IR	5	0.45 ± 0.31^{ax}	0.25 ± 0.16^{ax}	0.65 ± 0.62^{ax}	0.42 ± 0.35^{ax}	0.88 ± 0.36^{ax}	0.68 ± 0.32^{bx}
Cooked after IR	5	0.46 ± 0.13^{ax}	0.23 ± 0.02^{ay}	0.38 ± 0.05^{ax}	0.19 ± 0.01^{ay}	0.54 ± 0.48^{ax}	0.95 ± 0.01^{ax}
Chicken							
Raw meat	0	-	-	-	-	0.30 ± 0.01^{ax}	0.26 ± 0.03^{ay}
Raw meat	5	0.08 ± 0.01^{ax}	0.07 ± 0.01^{ax}	0.13 ± 0.03^{bx}	0.10 ± 0.06^{ax}	0.34 ± 0.15^{ax}	0.35 ± 0.02^{ax}
Cooked before IR	0	-	-	-	-	0.41 ± 0.11^{ax}	0.32 ± 0.06^{ax}
Cooked before IR	5	0.13 ± 0.13^{ax}	0.075 ± 0.02^{ax}	0.18 ± 0.01^{ax}	0.06 ± 0.05^{ay}	0.42 ± 0.01^{ax}	0.23 ± 0.11^{ay}
Cooked after IR	5	0.11 ± 0.01^{ax}	0.06 ± 0.02^{ay}	0.10 ± 0.03^{bx}	0.09 ± 0.01^{ax}	0.24 ± 0.11^{ax}	0.23 ± 0.08^{ax}

[a,b]Means with different letters within a column the same animal species are significantly different ($p < 0.05$), n = 3.
[x,y]Means with different letters within a row are significantly different ($p < 0.05$), n = 3.

Table 3. Effect of irradiating beef, pork, and chicken before or after cooking on sulfur compounds during storage at -40℃.

Cooking treatment	IR dose (kGy)	Dimethyl sulfide 0 month	6 months	Dimethyl disulfide 0 month	6 months	Dimethyl trisulfide 0 month	6 months
		---------- (Total ion counts $\times 10^4$) ----------					
Beef							
Raw meat	0	721	0	0^b	0^c	0^b	0
Raw meat	5	1064	0	2833^{ab}	2054^b	0^b	0
Cooked before IR	0	790	0	0^b	0^c	0^b	0
Cooked before IR	5	0	0	2872^{ab}	2255^b	0^b	0
Cooked after IR	5	662	0	5933^a	3835^a	485^a	0
SEM		537	0	945	424	149	0
Pork							
Raw meat	0	907	0	0^b	0	0^b	0
Raw meat	5	2135	0	3394^{ab}	136	0^b	0
Cooked before IR	0	573	0	0^b	0	0^b	0
Cooked before IR	5	249	0	4317^a	44	489^a	0
Cooked after IR	5	815	0	3405^{ab}	690	535^a	0
SEM		493	0	828	247	59	0
Chicken							
Raw meat	0	0	0	0^c	0^c	0^c	0
Raw meat	5	0	0	0^c	723^c	0^c	0
Cooked before IR	0	0	0	0^c	0^c	0^c	0
Cooked before IR	5	3443	0	12027^a	5579^a	1545^a	0
Cooked after IR	5	1062	0	7515^b	3428^b	673^b	0
SEM		1563	0	830	534	5	0

[a-c]Means with different letters within a column the same animal species are significantly different ($p < 0.05$), n = 3.