Determination of Irradiation Induced Hydrocarbons in Beef Jerky by GC-MS

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Summary: This study was aimed to analyze the effects of ionizing radiations on beef jerky through determination of induced hydrocarbons. The samples were irradiated with gamma and electron-beam at 0, 1, 3, 5 and 7 kGy doses. The induced hydrocarbons were extracted by solid phase extraction (SPE) and identified by gas chromatography-mass spectrometry (GC-MS). The application of analytical technique was validated by linearity, limits of detection, correlation variance and spiking recovery experiments. The main hydrocarbons induced by irradiations were from palmitic acid (1-tetradecene and pentadecane), stearic acid (heptadecane and 1-hexadecene) and oleic acid (8-heptadecene and 1,7-hexadecadiene). Stearic acid and oleic acid showed high degradation rate at α-carbon position, while C_{n-2} hydrocarbons were higher than C_{n-1}. The formation pattern of induced hydrocarbons was similar in both gamma-ray and e-beam but their concentrations were variable. The hydrocarbons induced by irradiations were detected in all irradiated samples and remained absent in the non-irradiated controls. Conclusively the detected hydrocarbons could be good marker compounds to determine the irradiated samples of beef jerky.

Keywords: Beef jerky, gamma-ray, e-beam, hydrocarbons, solid phase extraction (SPE), gas chromatography mass spectrometry (GC-MS)

Introduction

Among livestock processed foods, beef Jerky is the most widely used delicatessen product from snack to special purpose foods [1]. In South Korea, it has been used as traditional food; pyebaek or a side dish for drinks. Around the world, beef jerky has been used with proper name according to processed form and storage condition as in China, Japan, USA, South America, and Africa [2]. Like other livestock products beef jerky are well known for microbial growth, and easily suffer from food poisoning and deterioration [3]. Thus proper storage and processing techniques are always needed for consuming of meat products safely and to increase shelf life.

WHO/IAEA/FAO has approved the irradiation processing as non-heat treatment technology which has high usefulness and safety for controlling microorganism deterioration of foods [4]. In developed world such as USA and EU, food irradiation technology has been commercialized for processing foods [5]. Gamma ray (radio isotope (Co^{60}, Cs^{136}), electron beam (high voltage electrons) and x-rays are used for food irradiation around the world. In case of Korea, gamma ray was accepted restrictively for irradiation of about 26 items and since 2012, electron beam has also been using for a food irradiation [6]. The policies of food irradiation in terms of doses, product types, and marking, vary from one country to another around the world [7]. These variances often result in confusions in the worldwide trade of irradiated foods and hence a reliable and authentic method to identify irradiated products is required all the times [8, 9].

Several techniques are applied to identify the irradiated foods including physical, chemical or biological methods. Determination of irradiation induced hydrocarbons is a chemical method applied for identification of irradiated fat-containing foods. In literature several research studies in the recent past have determined hydrocarbons as markers of various irradiated fat-containing foods [10-14]. Studies on irradiation of pork jerky patties [15], beef patties [3], and pork jerky [16], have been successfully applied for identification of irradiated foods. Also irradiation of beef jerky by electron beam has been done for qualitative evaluation and storage effects [17], but the chemical impacts of irradiation have so far not been examined. Therefore this study was designed to evaluate the impact of ionizing radiations, including gamma (γ) and electron (e) beam, on beef jerky through detection of induced hydrocarbons and develop any possible irradiation markers for determination of irradiated food samples and consequently to enhance their global trade.

Experimental

Reagents

The standard hydrocarbons used were purchased from TeLA Co. (Berlin, Germany). HPLC grade solvents (n-pentane, n-hexane, and isopropanol) were purchased from Fisher Scientific (Pittsburg, USA), and further distilled using spiral packed double distilling apparatus (Normschliff Geratebau, Germany) prior to use. Florisil (60-100 mesh, Fisher Scientific, Pittsburg, PA, USA) was heated for 24 hrs at 550 ºC, to remove possible contaminants. Prior to use, the florisil was heated for at least 5 hours at 130 ºC in a drying oven (HB-502M Hanbaek Co, Korea), and cooled in a desiccator. Then deionized water (3%) was added to separate any hydrocarbons by shacking for 20 minutes. The florisil so prepared was stored in the desiccator for 10-12 hours at room temperature before use [18, 19].

Samples Collection and Irradiation

Ten samples of beef jerky were purchased from local supermarkets in Gwangju, South Korea, during July-August, 2012. All samples were belonged to the same brand but different packaging. These were studied in triplicate, thus making a total of 30 samples analyzed.

All samples were irradiated at, 0, 1, 3, 5, and 7 kGy doses, at 12±1°C using a Cobalt-60 γ-irradiator (Sailor 100,000 Ci, IR-79, Nordion International Ltd., Ontario, Canada, 100 kCi) at Korea Atomic Energy Research Institute, Daejeon. The dose rate was 2.5 kGy/h, with an error rate of ±0.02 kGy. Absorbed doses were monitored with either a radical or a ceric-cerous dosimeter. The irradiated samples and non-irradiated control were stored at -18℃ until required for analysis [18, 19].

Electron beam irradiation of the samples was done by electron-beam accelerator (model ELV 4, 2.5 MeV, EB-Tech., Ltd., Korea). The electron beam velocity of 5-10 m/min was having the same absorbed dose as by gamma irradiation. The samples were irradiated at doses of 0, 1, 3, 5 and 7 kGy. The absorbed doses were investigated with cellulose triacetate (CTA) dosimeter. The samples were then stored at -18℃ before analysis [18, 19]

Extraction of fats and determination of fatty acids

Extraction of fats was done by mixing 100 grams from each crushed sample with 500 mL n-hexane and centrifuged at 3400 rpm for 15 minutes. After centrifugation, the supernatant layer was separated, and then using 10 g anhydrous Na_2SO_4 the water content was removed. The extract was concentrated by removing the extraction solvent, first by rotary vacuum evaporator (Büchi, Switzerland) to 3 mL, and then to 0.5 mL using N_2 gas. The concentrated extract samples were kept frozen, before analysis. The fatty acid composition was analyzed by the method of Korean Food Code [6].

Separation of Hydrocarbons

The fats extracted (1 g) from irradiated samples and control were dissolved in 1 mL of n-eicosane (4 μg/mL n-hexane). The solid phase florisil Packed LC Column was conditioned with 30 mL of n-hexane, and then the sample solution was loaded onto the column. After loading, 50 mL of n-hexane was used as an eluent with a flow rate of 1 mL/min. The eluates were collected in glass vials, concentrated to 2 mL using a rotary vacuum evaporator, and further concentrated to 0.5 mL using nitrogen gas. It was kept in a refrigerator at -18 ºC, before GC-MS analysis [20].

GC-MS analysis of hydrocarbons

The GC-MS analyses were carried out on a Shimadzu GC-MS QP-5050A spectrometer (Kyoto, Japan), in EI mode, employing a DB-5 column (30 m × 0.32 mm i.d., 0.25 μm film thicknesses, J & W Scientific, Folsom, CA, USA). The ionization voltage was set at 70 eV, and the injector and ion source temperatures were kept at 250℃. The oven temperature was programmed as follows: 60℃ to 170℃ at 25℃/min, to 205℃ at 2℃/min and finally to 270℃ at 10℃/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The 1 μL of sample was injected in the split mode (20:1) and the hydrocarbons were identified by comparing the retention time and mass spectrum of peaks, as shown in the total ion chromatogram, with that of an authentic standards including 1-tetradecene ($C_{14:1}$), pentadecane ($C_{15:0}$), 1 hexadecene ($C_{16:1}$), 1, 7-hexadecadiene ($C_{16:2}$), heptadecane ($C_{17:0}$), 8-heptadecene ($C_{17:1}$), and eicosane ($C_{20:0}$). The concentration of each hydrocarbon in the fat was determined using n-eicosane (4 μg/mL) as an internal standard [20, 21].

Method Validation

The analytical method followed was validated by measuring several parameters including detection limits, linearity, precision and accuracy.

The limits of detection (LOD) and limits of quantification (LOQ), were calculated with three and

ten times the standard deviation of the blank divided by the slope of the analytical curve respectively [22].

Linearity was established by preparing the calibration curves of the authentic hydrocarbon standards using a non-weighted least-squares linear regression analysis method. All calibration curves were prepared with ten standard solutions, including the blank standard solution. All analyte hydrocarbon concentrations in the samples were within linear range of calibration curve and above the established lower linearity limit [23].

Precision is the degree of variability given by the expression of results, not taking into account sample variability. The percent coefficient of variation (CV%) was obtained by using relative standard deviation of 10 repeated determinations of one sample. The analytical quality control was verified by determining the recovery test. This was done by spiking the samples with the authentic hydrocarbon standards. The recoveries for all spiked hydrocarbons were determined with relative standard deviations (n = 3) [22].

Statistical Analysis

Data were reported as mean ± standard deviation of triplicate measurements. Significant differences ($p < 0.05$) within means were analyzed by analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test in the SPSS Statistics Software Version 18 (IBM, NY, USA).

Results and Discussion

Table-1; enlist method validation parameters for application of the analytical technique in beef jerky. For calibration curves, the correlation coefficient (R^2) values were at least 0.9991. The correlation variance (CV^2) values were lower than 3% in all cases. The percentage recoveries of spiking were from 94.2 to 97.0%. These results for the quality parameters clearly justified the applied analytical technique for analysis of hydrocarbons in beef jerky (22, 23).

Table-1: Correlation coefficient, LOD, LOQ, precision and accuracy values for method validation.

Hydrocarbons	Correlation coefficient (R^2)	LOD (µg/g)	LOD (µg/g)	Precision CV (%)	Accuracy (%)
$C_{14:1}$	0.9998	0.02	0.06	0.73	94.2
$C_{15:0}$	0.9997	0.01	0.03	0.58	96.2
$C_{16:1}$	0.9997	0.03	0.09	0.77	95.2
$C_{16:2}$	0.9995	0.01	0.03	0.62	95.7
$C_{17:0}$	0.9994	0.01	0.03	0.66	97.0
$C_{17:1}$	0.9992	0.01	0.03	0.73	95.9

Lipid profile analysis of the beef jerky samples indicated three fatty acids as the major fat components by contributing 86.83% of the total content (Table-2). These included oleic acid (43.9%), palmitic acid (27.6%) and stearic acid (15.4%). Due to their higher percentage composition, the possibility of hydrocarbons to be induced by gamma rays and electron beam irradiations can be expected from these fatty acids. Palmitoleic acid (4.54%), linoleic acid (3.67%), myristic acid (3.07%) and eicosenoic acid (1.89%) were the other minor fatty acids reported. The fatty acids contents for beef jerky in present research was in conformity to the food composition and nutrition standard tables developed in Japan [24].

Table-2: Fatty acids composition in beef jerky

Fatty acid	g/100g	%
Oleic acid	0.744	43.9
Palmitic acid	0.467	27.6
Stearic acid	0.260	15.4
Palmitoleic acid	0.077	4.54
Linoleic acid	0.062	3.67
Myristic acid	0.052	3.07
Eicosenoic acid	0.032	1.89
Total	1.694	100

From Fig. 1 and Table-3, it can be seen that hydrocarbons were reported for all gamma ray irradiated beef jerky samples at doses of 1, 3, 5, and 7 kGy. The concentration of hydrocarbons induced was found to increase in accordance to irradiation dose. However these appeared quantitatively different for the same dose, due to the difference in fatty acid contents of the subject beef jerky samples.

Among the induced hydrocarbons in beef jerky by gamma rays, pentadecane ($C_{15:0}$), 1-tetradecene ($C_{14:1}$), 8-heptadecene ($C_{17:1}$), 1,7-hexadecadiene ($C_{16:2}$), heptadecane ($C_{17:0}$) and 1-hexadecene ($C_{16:1}$) were confirmed present in all of the samples examined. These findings were in accordance to the published literature of radiolytic products of lipid when legumes were irradiated by gamma rays [25].

8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) were derived from the parent oleic acid of beef jerky by irradiation, with C_{n-2} hydrocarbon ($C_{16:2}$) having higher amount than C_{n-1} ($C_{17:1}$). From stearic acid,1-hexadecene ($C_{16:1}$) and heptadecane ($C_{17:0}$) were derived and the high production rate of C_{n-2} hydrocarbons was again confirmed. However pentadecane ($C_{15:0}$) and 1-tetradecene ($C_{14:1}$) were derived from palmitic acid, with C_{n-1} hydrocarbons ($C_{15:0}$) having higher amounts than C_{n-2} ($C_{14:1}$) (Table-3). The reason for this high content of C_{n-1} hydrocarbons than $C_{n-2,}$ could be because of the hexane solvent interference as also determined the same for irradiation of meat and fish by Hwang et al 1997 [26].

Table-3: Concentrations (µg/g fat) of radiation-induced hydrocarbons in beef jerky by G-ray.

Irradiation dose	Palmitic acid ($C_{16:0}$)		Stearic acid ($C_{18:0}$)		Oleic acid ($C_{18:1}$)	
kGy	$C_{14:1}$	$C_{15:0}$	$C_{17:0}$	$C_{16:1}$	$C_{17:1}$	$C_{16:2}$
0	$0^{a\ 1)}$	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
1	$1.05^{b} \pm 0.14^{2)}$	$1.33^{b} \pm 0.11$	$0.15^{a} \pm 0.08$	$0.51^{b} \pm 0.1$	$1.01^{b} \pm 0.12$	$1.12^{b} \pm 0.12$
3	$1.71^{c} \pm 0.19$	$2.59^{c} \pm 0.22$	$0.44^{a} \pm 0.23$	$1.05^{c} \pm 0.17$	$1.52^{c} \pm 0.13$	$2.32^{c} \pm 0.22$
5	$3.02^{d} \pm 0.22$	$4.09^{d} \pm 0.23$	$1.60^{b} \pm 0.28$	$1.81^{d} \pm 0.24$	$2.85^{d} \pm 0.18$	$3.53^{d} \pm 0.24$
7	$3.62^{d} \pm 0.28$	$4.48^{d} \pm 0.29$	$2.55^{c} \pm 0.31$	$2.29^{e} \pm 0.27$	$4.44^{e} \pm 0.25$	$5.00^{e} \pm 0.32$

1) a-e Values with different superscript letters within a column differ significantly ($P < 0.05$).
2) Mean±Standard deviation (n=3)

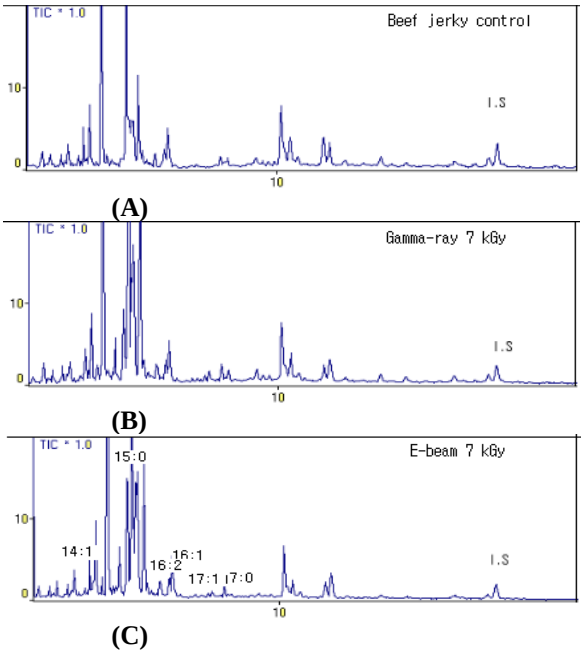
Table-4: Concentrations (µg/g fat) of hydrocarbons induced by e-beam irradiation of jerky.

Irradiation dose	Palmitic acid ($C_{16:0}$)		Stearic acid ($C_{18:0}$)		Oleic acid ($C_{18:1}$)	
kGy	$C_{14:1}$	$C_{15:0}$	$C_{17:0}$	$C_{16:1}$	$C_{17:1}$	$C_{16:2}$
0	$0^{a\ 1)}$	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
1	$0.43^{b} \pm 0.12^{2)}$	$0.63^{b} \pm 0.12$	$0.69^{b} \pm 0.17$	$0.48^{b} \pm 0.12$	$1.08^{b} \pm 0.14$	$1.27^{b} \pm 0.12$
3	$1.06^{c} \pm 0.18$	$2.00^{c} \pm 0.29$	$0.99^{b} \pm 0.22$	$0.54^{b} \pm 0.14$	$1.49^{c} \pm 0.17$	$2.47^{c} \pm 0.15$
5	$1.46^{d} \pm 0.26$	$2.72^{d} \pm 0.27$	$1.60^{c} \pm 0.28$	$1.20^{c} \pm 0.24$	$2.84^{d} \pm 0.18$	$4.00^{d} \pm 0.24$
7	$2.04^{e} \pm 0.28$	$3.12^{d} \pm 0.23$	$2.39^{d} \pm 0.25$	$2.06^{d} \pm 0.28$	$4.22^{e} \pm 0.26$	$4.35^{d} \pm 0.26$

1) a-e Values with different superscript letters within a column differ significantly ($P < 0.05$).
2) Mean±Standard deviation (n=3)

As oleic acid occupied the highest content of fatty acid composition of beef jerky, 1,7-hexadecadiene ($C_{16:2}$) induced by gamma irradiation was identified in highest concentrations than all other hydrocarbons in accordance published studies [27]. 1-hexadecene ($C_{16:1}$) and heptadecane ($C_{17:0}$) were C_{n-2} and C_{n-1} radiolytic products of stearic acid respectively. The content of heptadecane ($C_{17:0}$) has already known to be affected by solvent, and thus 1-hexadecene ($C_{16:1}$) may be only considered for any application of radiolytic studies of stearic acid. In the same way pentadecane ($C_{15:0}$) is obtained in high amount as radiation induced product of palmitic acid but due to the solvent interference as explained for stearic acid it cannot be used for radiolytic application studies. Consequently1,7-hexadecadiene ($C_{16:2}$) derived from oleic acid and 1-hexadecene ($C_{16:1}$) derived from stearic acid were obtained in high quantities in all irradiated samples at all doses and can thus be determined as identification marker compounds of gamma irradiation.

Chromatograms of 0 kGy control and 7 kGy irradiated samples by electron beam are given in Fig. 1. Like for gamma rays discussed above, the hydrocarbons from beef jerky by electron beam also showed increase according to radiation dose, with slightly high concentration in comparison. The amount of induced hydrocarbons was found dependent upon the fatty acid contents of the beef jerky samples irradiated. The identified hydrocarbons in all electron beam irradiated beef jerky samples at all doses included pentadecane ($C_{15:0}$), 1-tetradecene ($C_{14:1}$), 1,7-hexadecadiene ($C_{16:2}$), 1-hexadecene ($C_{16:1}$), 8-heptadecene ($C_{17:1}$) and heptadecane ($C_{17:0}$) (Table-4). Hydrocarbons induced from oleic acid were 8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) with content of C_{n-2} hydrocarbon higher than C_{n-1}. This pattern of hydrocarbon induction by irradiation is the same as explained for ground beef by Hwang (2013) [28]. Stearic acid gave 1-hexadecene ($C_{16:1}$) and heptadecane ($C_{17:0}$) hydrocarbons by irradiation, with high synthetic rate for C_{n-1} than C_{n-2} hydrocarbon product. The third fatty acid which gave significant amount hydrocarbons by electron beam irradiation was palmitic acid with pentadecane ($C_{15:0}$) and 1-tetradecene ($C_{14:1}$) as radiolytic products showing high content in case of C_{n-2} than C_{n-1}.



A.beef jerky control, B. beef jerky gamma-ray 7 kGy, C. beef jerky e-beam 7 kGy

Fig. 1: GC/MS chromatograms of hydrocarbons induced in non-irradiated control and 7 kGy irradiated beef jerky of gamma-ray and e-beam.

The induced hydrocarbons showed increase along irradiation doses, from 1 to 7 kGy. In case of pentadecane ($C_{15:0}$) from palmitic acid, although the concentration is high but the influence of solvent cannot be neglected and therefore cannot be considered for any application of irradiation studies. The other major hydrocarbons derived by e-beam irradiation of beef jerky were 8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) from stearic acid and oleic acid respectively. These can be used as markers of irradiation, as their concentration varied with irradiation doses and were not found in non-irradiated ones. Thus electron beam irradiation of beef jerky and later on GC/MS analysis of induced hydrocarbons was found to be a possible chemical discrimination method for irradiated and non-irradiated samples.

The overall generation flow of induced hydrocarbons by γ-ray and e-beam irradiation was the same for all doses. Oleic acid derived hydrocarbon, 1,7-hexadecadiene ($C_{16:2}$) was high for gamma ray than electron beam at all irradiation doses except 7 kGy. The 8-heptadecene ($C_{17:1}$) from oleic acid and 1-tetradecene ($C_{14:1}$) from Palmitic acid were high for gamma rays only at 1 kGy dose and their electron beam concentrations were comparatively high from 2 to 7 kGy. These research findings were in accordance to the published literature about the gamma and electron beam irradiation study of deboned chicken meat [29].

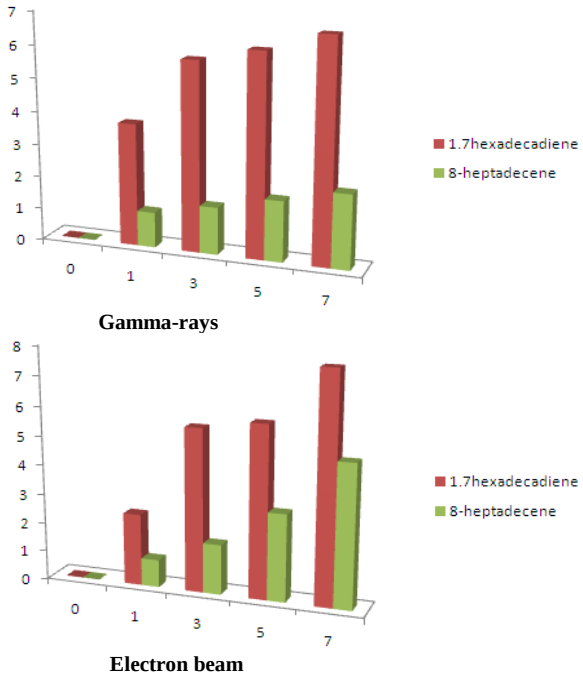


Fig. 2: Concentration variation of 1,7-hexadecadiene and 8-heptadecene, with irradiation doses in Beef jerky.

Stearic acid derived hydrocarbons, 1-hexadecene ($C_{16:1}$) and heptadecane ($C_{17:0}$) were higher for all e-beam irradiation doses than gamma ray. The two major hydrocarbons induced by both gamma ray and electron beam irradiation of beef jerky samples were 8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) from stearic acid and oleic acid respectively (Fig. 2). Their concentrations showed increase along irradiation doses, from 1 to 7 kGy and were not detected in non-irradiated beef jerky samples. Therefore 8-heptadecene and 1,7-hexadecadiene were thus declared as irradiation markers for both electron beam and gamma irradiation, to identify the irradiated samples beef jerky and non-irradiated ones.

Conclusions

Hydrocarbons induced by both gamma rays and electron beam irradiations in beef jerky were dependent upon the parent fatty acids and dose of irradiations. Both gamma-ray and e-beam irradiations induced hydrocarbons with the same general pattern but their concentrations were variable. The main hydrocarbons detected were 1,7-hexadecadiene ($C_{16:2}$) and 8-heptadecene ($C_{17:1}$) from oleic acid. The hydrocarbons induced were detected in all irradiated samples and not in the non-irradiated controls. Therefore it was concluded that the detected two major hydrocarbons of 1,7-hexadecadiene and 8-heptadecene, could be good marker compounds to distinguish the irradiated samples of beef jerky from non-irradiated ones.

Acknowledgments

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