Korean J. Food Sci. Ani. Resour.
Vol. 31, No. 6, pp. 858~864(2011)
DOI http://dx.do.org/10.5851/kosfa.2011.31.6.858

ARTICLE

Effect of Fat Substitute and Plum Extract on Radiation-induced Hydrocarbons and 2-Alkylcyclobutanones in Freeze-dried Beef Patties

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Abstract

The effect of adding 10% fat substitute (10%F) or 2% plum extract (2%P) on the formation of hydrocarbons and 2-alkylcyclobutanones (2-ACBs) in freeze-dried beef patties, irradiated (IR) at 44 kGy, and freeze-dried irradiated cooked beef patties was investigated. Hydrocarbons, such as $C_{16:3}$, $C_{16:2}$, $C_{17:2}$ and $C_{17:1,}$ were detected only in irradiated samples and their concentrations were high in the order of 2%P+IR, IR and 10%F+IR. Only irradiated beef samples produced 2-ACBs (2-DCB, 2-TCB, 2-TeCB), and their amounts were high in reverse order. The addition of fat substitute or plum extract did not help in reducing hydrocarbons and 2-ACBs in the freeze-dried irradiated cooked beef. However, the amounts of radiation-induced hydrocarbons and 2-ACBs in all irradiated beef patties even at 44 kGy were too small to be of concern for human consumption.

Key words: freeze-dried beef, irradiation, hydrocarbons, 2-alkylcyclobutanones, additives

Introduction

Space foods have been developed since astronaut John Glenn, the first American to orbit the Earth, has taken foods to the weightless conditions of the earth orbit. Dehydrated, retorted, and irradiated foods are consumed for space missions, and the critical factors for designing space foods include light weight, high quality, and long shelf-life. International Space Station (ISS) and planetary outpost missions have 9 mon, 1 year, and 3-5 years of shelf-life requirements for the foods in shuttle, respectively. Another important requirement for space foods is improved mouth feel and taste (Kloeris, 2001).

Reduction of fat content in hamburgers without compromising desirable quality characteristics is important to ensure that the products are acceptable to NASA astronauts as well as other consumers. The drastic reduction of fat content from beef, however, can result in a product with an unpalatable mouth-feel. Fat substitute (Fantesk^{TM}, Heritage Fare Technology, USA), a uniformly-dispersed oil phase (10 to 50 μ droplets) within a carbohydrate matrix (Garzon *et al.,* 2003), and plum extract puree added in meat products can eliminate this quality defect. Fat substitute, Fantesk^{TM}, is reported to bind moisture and maintain the desirable texture and mouth-feel that consumers expect in a juicy hamburger. Also, addition of fat substitute to ground beef can reduce fat content in hamburger patties without compromising mouth-feel (Garzon *et al.,* 2003). This reduction in fat content may also decrease the extent of off-odors generated from lipid oxidation during irradiation and storage. Plum extract contains humectants such as sorbitol, which binds moisture and thus has a potential to alleviate the dry mouth-feel in low-fat meat products (Anon, 1998). The addition of plum extract to low-fat ground beef has enabled the production of hamburgers with similar mouth-feel and texture to those with high-fat beef patties (Anon, 1998). Keeton *et al.* (2001) reported that moisture retention of hamburgers added with plum puree was improved by 15.8% in precooked patties when reheated to 102°C and held warm for up to 4 h. More importantly, plum extract

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contains antioxidants (Nuñez de Gonzalez *et al.,* 2008). Therefore, it can reduce the development of off-odors due to lipid oxidation in meat products that form during irradiation and storage.

Irradiation produces a characteristic odor, which negatively influences the consumer acceptance (Lee *et al.,* 2003). The principle of electron beam irradiation is that a stream of high-energy electrons propelled from an electron gun is absorbed by materials in which the radiation energy converts water molecules to reactive ions or produces free radicals (Woods and Pikaev, 1994; Josephson and Peterson, 2000). Hydroxyl radicals from ionizing irradiation can increase lipid oxidation (O'Connell and Garner, 1983; Thakur and Singh, 1994). Fat and myoglobin that is oxidized by the free radicals in irradiated meat can cause color changes, rancidity, and off-odor in meat (Murano, 1995).

The radiation processing of food is accepted in over 50 countries and commercially implemented in about 40 countries as a mean of enhancing hygienic quality, extending shelf-life and reducing incidence of food-borne diseases (IAEA, 2008). At the same time, demands for reliable detection methods for irradiated foods are growing to help educate consumers and promote international trade of irradiated foods (Delincée, 2002). Hydrocarbons and 2-alkylcyclobutanones (2-ACBs), which can be formed from fat-containing foods when exposed to ionizing radiations, were successfully determined as irradiation markers for many foods (Morehouse and Ku, 1993; Lee *et al.,* 2000; Hwang *et al.,* 2001; Chung *et al.,* 2002; Kwon *et al.,* 2003). Food irradiation is acknowledged as a safe process to improve food quality by reducing microbial contamination. However, the toxicological potential of 2-alkylcyclobutanones (2-ACBs), radiolytic derivatives of triglycerides found exclusively in irradiated foods, is a concern for some consumers (Thakur and Singh, 1994).

The objective of this study was to determine the effect of the fat substitute Fantesk^{TM} and plum extract on the formation of radiation-induced hydrocarbons and 2-ACBs in freeze-dried, irradiated (44 kGy), or freeze-dried and irradiated cooked beef patties typically used by NASA astronauts.

Materials and Methods

Reagents

Hydrocarbon and 2-alkylcyclobutanone standards were purchased from Fluka (Sigma-Aldrich, Switzerland). n-Hexane (HPLC-grade) and florisil (60-100 mesh) were purchased from Fisher Scientific (USA). Florisil was heated at 550°C overnight to remove contaminants, cooled in a desiccator, and deactivated by adding 3% water prior to use.

Sample preparation

Eight raw beef top rounds were obtained from the Meat Laboratory at Iowa State University 6 d post-slaughter. Two top rounds were pooled and treated as a replication. Each round was trimmed off any visible fat and connective tissues, and each replication was ground separately through a 3-mm plate twice. Fat substitute (Fantesk^{TM}, Heritage Fare, Ltd, USA) was obtained from the Heritage Fare Ltd. (USA) and plum extract puree was obtained from the California Plum Board (Sunsweet Growers Inc., USA). Plum extract was dissolved in distilled water prior to use. Fresh ground beef (80% lean) was used to prepare hamburger patties for control (no fat substitute or plum extract added) and 90% lean meat was used for 10% fat substitute or 2% plum extract treatments. For 10% fat substitute treatment, fresh raw beef and the fat substitute were ground through a 3-mm plate separately, and then the ground beef and fat substitute (10% of meat) were mixed for 3 min in a bowl mixer (Kitchen Aid, Inc., USA). For the plum extract treatment, plum extract (2% of meat weight) was dissolved in 4 vol. of distilled water, added to ground beef (90% lean), and mixed for 3 min in a bowl mixer (Kitchen Aid, Inc.) to ensure uniform distribution of plum extract. The mixtures were chilled and patties (110 g) were prepared using an automatic patty machine.

Patties were cooked in an electric oven at 175°C to an internal temperature of 75°C and cooking yield was determined. Internal temperatures of meat during cooking were monitored with thermocouples connected to digital readout devices. All the cooked meat patties were vacuum-packaged in high-oxygen-barrier bags (nylon/polyethylene, 9.3 mL $O_2/m_2/24$ h at 0°C; Koch, USA) immediately after cooking to minimize oxidative changes during handling and storage. The cooked beef patties were frozen, freeze-dried (FD), irradiated (IR), or freeze-dried and irradiated (FD+IR). Freeze-drying of patties was done using a Virtis freeze-dryer (Ultra-35, 8 shelf unit, Virtis Inc., USA). Samples for freeze-drying were held at -20°C in a walk-in freezer prior to loading. Temperature of the freeze-dryer shelves was held initially at < 0°C until a vacuum reading of < 100 millitorr was achieved (approximately 1 h after loading) and then raised to 26°C for the duration of the run. After freeze-drying, patties were individually vacuum-packaged in high-oxygen-barrier bags. Frozen samples were individually vacuum-packaged in high-oxygen-barrier bags and then stored in a -20°C freezer. Irradiation of frozen and freeze-dried samples was done using a Linear Accelerator (Surebeam, USA) at an average dose of 44 kGy as recommended by the NASA. The energy and power level used were 10 MeV and 10 kW, respectively, and the conveyer speed was 1 ft/min. To confirm the target dose, 2 alanine dosimeters per cart were attached to the top and bottom surfaces of a sample. The alanine dosimeter was read using a 104 Electron Paramagnetic Resonance Instrument (Bruker Instruments Inc., USA). The max/min ratio was approximately 1.18 (avg.). After irradiation, samples for the frozen treatment were stored in a freezer at -20°C, whereas FD, IR and FD+IR samples were stored at room temperature (22°C).

Fat extraction

Fat was extracted using the method described by Schreiber *et al.* (1994). Hexane and *n*-pentane/isopropanol (3:2, v/v) were used to extract hydrocarbons and 2-alkylcyclobutanones, respectively. Fifty grams of sample were homogenized with 200 mL *n*-hexane and the homogenate was then centrifuged (Vision, VS-6000 CFN, Korea) at 2,500 g for 20 min. The supernatant was collected in a round bottom flask. The solvent was evaporated using a rotary vacuum evaporator (Heidolph WB-2001, Germany) at 35°C. The extracted fat was flushed with nitrogen and stored at 4°C until separation using florisil column chromatography.

Separation of hydrocarbons

Deactivated florisil (25 g) was packed into a 200×20 mm glass column. Anhydrous sodium sulfate was added on the top of the florisil column (1-cm layer). One gram of extracted fat was mixed with an internal standard (n-eicosane, 4 ppm), applied to the florisil column, and eluted with 60 mL hexane at a flow rate of 3 mL/min. The eluted hexane was concentrated to 2 mL using a rotary vacuum evaporator and further concentrated to 0.5 mL using nitrogen gas (EN 1784, 2003).

Separation of 2-alkylcyclobutanones (2-ACBs)

Fat (0.2 g) was mixed with an internal standard (2-cyclohexylcyclohexanone, 1 ppm), applied to a florisil column, and eluted with 150 mL hexane followed by 120 mL of diethyl ether/hexane (2:98) at a flow rate of 3 mL/min. The eluent of diethyl ether/hexane fraction was concentrated to 2 mL using a rotary vacuum evaporator and further concentrated to 0.2 mL using nitrogen gas (EN 1785, 2003).

GC/MS analysis

GC/MS analysis was carried out with a gas chromatography (Hewlett-Packard 6890 Series, HP Co., USA) equipped with a Mass Spectrometer (HP 5973, Hewlett-Packard). The column used was HP-5 (30 m, 0.32 mm i.d., 0.25 μm film thickness, J & W Scientific, USA). The oven temperature programs used are as follow: for hydrocarbons, initial temperature (60°C) was increased to 170°C at 25°C/min, to 205°C at 2°C/min, and then to 270°C at 10°C/min. For 2-alkylcyclobutanones, the initial temperature (120°C) was increased to 160°C at 15°C/min after holding at 120°C for 1 min, increased to 175°C at 0.5°C/min, and then increased to 290°C at 30°C/min (10 min). The injector and detector temperatures were kept at 250 and 300°C, respectively. The carrier gas was helium at a flow rate of 1.0 mL/min. To analyze hydrocarbons, 2 μL of sample was injected in splitless mode for 2 min and then the inlet was changed to split mode (20:1). To analyze 2-alkylcyclobutanones, 2 μL of sample was injected in splitless mode for 1 min and then changed to split mode (20:1). Hydrocarbons were identified by comparing retention time and mass spectrum of peaks authentic hydrocarbon standards. The concentration of each hydrocarbon in fat was determined using an internal standard (2-cyclohexylcyclohexanone, 1 ppm). 2-Alkylcyclobutanones were analyzed using a GC/MS with selective ion monitoring (SIM) mode. The ion m/z 98 was set for 2-dodecylcyclobutanone (2-DCB), m/z 112 for 2-tetradecylcyclobutanone (2-TCB), m/z 67 and 81 for 2-(5'-tetradecenyl)cyclobutanone (2-TeCB), and m/z 98, 70 and 83 for internal standard 2-cyclohexylcyclohexanone. Mass spectra of 2-alkylcyclobutanones (2-ACBS) were confirmed by a GC/MS with full scan mode (EN 1784, 2003; EN 1785, 2003).

Statistical analysis

Analysis of variance was performed using SAS software (SAS, 2001) and the Student-Newman-Keul's multiple range test was used to compare difference among mean values. Mean scores and standard error of the mean (SEM) were reported.

Results and Discussion

Proximate analyses of cooked beef patties at day 0 when 80% lean as control, 10% fat substitute added, and 2% plum extract added, respectively, are given in Table 1.

Table 1. Proximate analysis of cooked beef patties when added with fat substitute or plum extract

Addition	Protein	Moisture	Ash	Lipid	Carbohydrate	Cooking yield
	(%)					
Control	25.43	58.4	0.96	14.55	0.66	70.99
10% Fat substitute	26.17	63.43	1.03	8.58	0.8	73.75
2% Plum extract	24.58	64.82	1.04	7.71	1.85	72.94

For proximate analyses (n = 4), for cook yield (n = 16)
Control was 80% lean, whereas amended patties were with 90% lean beef.

Radiation-induced hydrocarbons

Figs. 1 show gas chromatograms of hydrocarbons in non-irradiated control and irradiated freeze-dried beef samples. Eight hydrocarbons were detected for the non-irradiated and irradiated samples (Table 2, Fig. 1). Hydrocarbons $C_{14:1}$, $C_{15:0}$, $C_{16:1}$, and $C_{17:0}$ were detected in non-irradiated and irradiated samples, but $C_{16:3}$, $C_{16:2}$, $C_{17:2}$, and $C_{17:1}$ were detected only in irradiated samples (Fig. 1). Concentrations of total hydrocarbons in three non-irradiated samples were not significantly different among treatments ($p > 0.05$), while their amounts increased upon irradiation with highest concentration in 2% plum-added beef sample, followed by control and 10% fat-added samples ($p < 0.05$). In case of irradiated groups, radiation-induced hydrocarbons, such as $C_{16:3}$, $C_{16:2}$, $C_{17:2}$ and $C_{17:1}$, significantly increased in the order of 2%P+IR, IR and 10%F+IR groups.

Beef has high concentrations of oleic (37.07±0.36%), palmitic (25.34±0.09%), and linoleic acids (23.09±0.56%), but a low concentration of stearic acid (7.28±0.10%) (Brito *et al.,* 2002). Two types of hydrocarbons were predominantly produced from fatty acids by irradiation: One is the hydrocarbon with one carbon less than the parent fatty acids (Cn-1) and the other is the one with two carbons less and an additional double bond at position 1 (Cn-2, 1-ene). Nawar (1986) confirmed the production of pentadecane ($C_{15:0}$) and 1-tetradecene ($C_{14:1}$) from palmi-

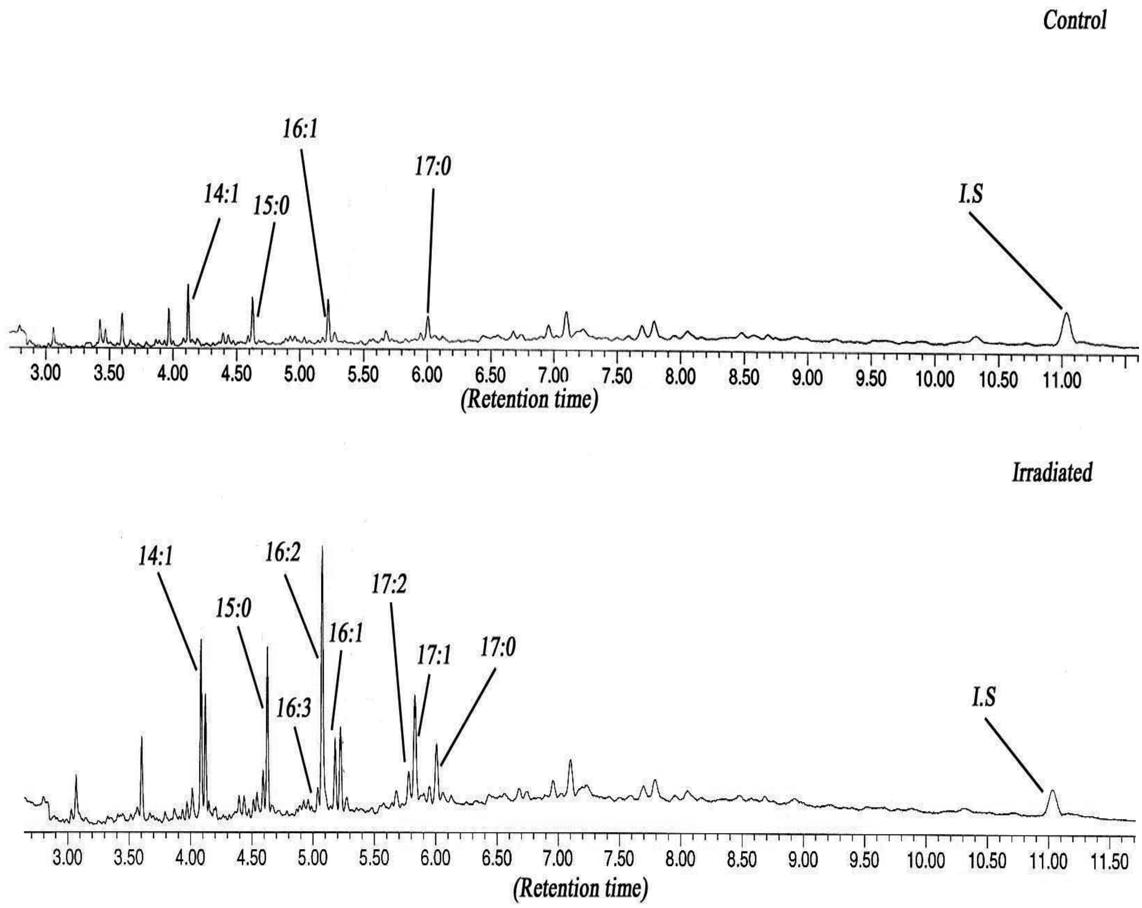

Fig. 1. GC/MS chromatogram of radiation-induced hydrocarbons of nonirradiated and irradiated freeze-dried beef.

Table 2. Concentration of radiation-induced hydrocarbons in freeze-dried beef when added with fat substitute or plum extract (unit: mg/g fat)

Treatment	Hydrocarbon								Total HC
	C14:1	C15:0	C16:3	C16:2	C16:1	C17:2	C17:1	C17:0	
Control	0.31^{c}	1.62^{c}	0^{d}	0^{d}	1.36^{bc}	0^{d}	0^{d}	1.48^{d}	4.77^{c}
IR	5.35^{b}	4.94^{b}	1.14^{b}	10.36^{b}	2.96^{b}	2.71^{b}	6.48^{b}	4.31^{b}	38.25^{b}
2%P	3.61^{b}	3.93^{b}	0^{d}	0^{d}	0.73^{bc}	0^{d}	0^{d}	3.78^{b}	12.05^{c}
2%P+IR	10.21^{a}	7.23^{a}	2.07^{a}	21.56^{a}	5.80^{a}	6.69^{a}	14.93^{a}	5.91^{a}	74.40^{a}
10%F	1.32^{c}	1.64^{c}	0^{d}	0^{d}	0.21^{c}	0^{d}	0^{d}	2.60^{c}	5.77^{c}
10%F+IR	3.38^{b}	3.52^{b}	0.57^{c}	6.34^{c}	1.33^{bc}	1.40^{c}	3.82^{c}	5.53^{a}	25.89^{b}
SEM	0.98	0.60	0.23	2.39	0.58	0.73	1.57	0.48	7.35

$^{a\text{-}d}$Means with different superscripts within a column are significantly different ($p < 0.05$)
Abbreviations: IR, irradiated (44 kGy); P, plum extract added; F, fat substitute added; HC, hydrocarbons; SEM, standard error of the means (n = 4).

tic acid, heptadecane ($C_{17:0}$) and 1-hexadecence ($C_{16:1}$) from stearic acid, 8-heptadecene ($C_{17:1}$) and 1,7-hexadecadiene ($C_{16:2}$) from oleic acid due to irradiation.

According to previous researches, hydrocarbons, particularly saturated hydrocarbons, are frequently produced from packaging materials (Schreiber *et al.,* 1994; Biedermann *et al.,* 1992; Morehouse *et al.,* 1991). Unsaturated hydrocarbons have been observed in non-irradiated foods, such as fish (Schulzki *et al.,* 1993) and beef (Hartmann *et al.,* 1995). It should be noted that the formation of hydrocarbons in foods is not specific for irradiation. Many hydrocarbons are also formed after heating or oxidation. For example, in vegetable oils, long-chain hydrocarbons are found after heating or frying (Nawar, 1983; Nawar, 1988, Lesgards *et al.,* 1993). In roasted pistachio nuts, many hydrocarbons have also been observed (Lembke *et al.,* 1995). In addition, long-chain hydrocarbons were found in animal products such as roasted chicken (Noleau and Toulemonde, 1987). However, production of C_{n-1} and $C_{n-2:1}$ hydrocarbons is the typical patterns resulting from irradiation (Nawar, 1988). Hwang (1999) found $C_{15:0}$, $C_{14:1}$, $C_{17:0}$, $C_{16:1,}$ and $C_{17:1}$ hydrocarbons in non-irradiated pork. Park *et al.* (2001) detected trace amounts of pentadecane and heptadecane in non-irradiated and irradiated pork at 0.1 kGy. Schreiber *et al.* (1994) also reported that radiation-induced hydrocarbons were found in non-irradiated chicken, pork, and beef at low concentrations. Therefore, $C_{16:3}$, $C_{16:2}$, $C_{17:2}$, and $C_{17:1}$ were confirmed to be used as markers for identifying post-irradiation of freeze-dried beef since they were not detected in non-irradiated samples.

Radiation-induced 2-alkylcyclobutanones

Three different 2-alkylcyclobutanones (2-ACBs) were detected only in irradiated samples (Table 3). The concentrations of 2-ACBs were the highest in freeze-dried

Table 3. Concentration of radiation-induced 2-alkylcylclobutanones in freeze-dried beef when added with fat substitute or plum extract (unit: mg/g fat)

Sample	2-Alkylcyclobutanone			Total
	2-DCB	2-TeCB	2-TCB	
Control	0^{d}	0^{b}	0^{c}	0^{d}
IR	5.02^{b}	0.20^{ab}	0.56^{a}	5.78^{b}
2%P	0^{d}	0^{b}	0^{c}	0^{d}
2%P+IR	2.97^{c}	0.45^{a}	0.26^{b}	3.68^{c}
10%F	0^{d}	0^{b}	0^{c}	0^{d}
10%F+IR	7.59^{a}	0.45^{a}	0.43^{a}	8.47^{a}
SEM	0.89	0.06	0.07	1.01

$^{a\text{-}d}$Means with different superscripts within a column are significantly different ($p < 0.05$).
Abbreviations: IR, irradiated (44 kGy); P, plum extract added; F, fat substitute added; 2-DCB, 2-dodecylcobutanone; 2-TeCB, 2-(5'-tetradecenyl) cyclobutanone; 2-TCB, 2-tetradecylcyclobutanone; SEM, standard error of the means (n = 4).

beef with 10%Fat+IR, followed by IR, and 2%P+IR groups ($p < 0.05$). The concentration of 2-DCB was higher than that of 2-TCB because the content of palmitic acid in beef is greater than that of stearic acid (Brito *et al.,* 2002). Similar results were found in irradiated liquid whole egg (Stevenson *et al.,* 1993), beef (Crone, 1992), mango, papaya, salmon meat, Camembert cheese (Stewart *et al.,* 2000), and prawn meat (McMurray *et al.,* 1995). 2-TeCB has also been found in irradiated chicken meat, papaya, and mango (Hamilton *et al.,* 1995; Stewart *et al.,* 2000). Some of 2-ACBs are reported as carcinogenic properties (Delincée and Pool-Zobel, 1998) and hydrocarbons influence the flavor of meat products (Champaign and Nawar, 1969). Raul *et al.* (2002) reported that Wistar rats received 3.2 mg/kg body weight of 2-tetradecylcyclobutanone (2-TCB) or 2-(5'-tetradecenyl) cyclobutanone (2-TeCB) daily for 6 months developed tumors in the colon. However, the amounts of 2-DCB, and 2-TeCB used in this study are far greater than the amounts that humans can be exposed through consuming irradiated foods, which is estimated to be < 5-10 mg/kg body weight (Raul *et al.,* 2002).

Electron beam irradiation of freeze-dried cooked beef patties at 44 kGy resulted in the formation of radiation-induced hydrocarbons ($C_{16:3}$, $C_{16:2}$, $C_{17:2,}$ $C_{17:1}$) and 2-alkylcyclobutanones (2-DCB, 2-TCB, 2-TeCB), which can be used as identification markers for irradiated freeze-dried cooked beef patties. Addition of fat substitute did not change the amounts of radiation-induced hydrocarbons and 2-ACBs in freeze-dried irradiated beef, but addition of plum extract significantly increased them. This indicated that addition of fat substitute or plum extract did not help in reducing hydrocarbons and 2-alkylcyclobutanones in freeze-dried cooked irradiated beef. Although high-dose irradiation (44 kGy) induced the production of hydrocarbons and 2-ACBs in freeze-dried cooked beef patties, the amounts were too small to be of concerns for human.

Acknowledgements

This research was supported by Kyungpook National University Research Fund (2009), Iowa State University, and WCU (World Class University) program (R31-10056) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology.

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(Received 2011.11.14/Accepted 2011.11.21)