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RESEARCH NOTE

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The Microwave-Assisted Extraction of Fats from Irradiated Meat Products for the Detection of Radiation-Induced Hydrocarbons

Joong-Ho Kwon*, Tusneem Kausar[1], Jeongeun Lee, Hyun-Ku Kim[2], and Dong U Ahn[3]

Department of Food Science and Technology, Kyungpook National University, Daegu 702-701, Korea

[1]Department of Food Science Technology, University of Sargodha, Pakistan

[2]Korea Food Research Institute, Seongnam, Gyeonggi 463-420, Korea

[3]Department of Animal Science, Iowa State University, Ames, IA 50010-3150, USA

Abstract Hydrocarbons have been successfully used as a chemical marker in order to identify irradiated from non-irradiated foods. The method for determining hydrocarbons consists of extraction of fats, followed by separation of hydrocarbons by florisil column chromatography, and then identification of hydrocarbons by GC/MS. Currently, solvent extraction method for fats has certain limitations with regard to extraction time and solvent consumption. Commercial hams and sausage were irradiated at 0 and 5 kGy, and the efficiency of microwave-assisted extraction (MAE) and conventional solvent extraction (CSE) methods on the extraction of radiation-induced hydrocarbons from the meat products was compared. Significant levels of hydrocarbons, mainly composed of 1,7-hexadecadien, 1,7,10-hexadecatriene, and 6,9-heptadecadiene, were detected in the extracts from irradiated hams and sausages by both CSE and MAE methods. Both methods were acceptable in extracting hydrocarbons from samples, but MAE method required apparently reduced amounts of solvent from 150 (CSE) to 50 mL and reduced extraction time from 23 (CSE) to 5 min.

Keywords: radiation-induced hydrocarbon, microwave extraction, ham, sausage

Introduction

Irradiation processing with gamma rays, electron beam, and X-rays is a well-known and reliable method for destroying pathogenic and spoilage microorganisms in foods without compromising nutritional properties. The commercial use of food irradiation is gradually increasing worldwide (1-3). Recently, detection methods for irradiated foods have been studied not only to increase consumer confidence but also to promote international trade of treated foods.

When foods are irradiated, free fatty acids and triglycerides in foods decomposed into hydrocarbons and 2-alkylcyclobutanones. Nawar and Balboni (4) reported that irradiation of fatty acids in foods produced hydrocarbons with one (C_{n-1}) or two (C_{n-2}) fewer carbon atoms than the parent fatty acids. A number of studies on the use of these compounds as detection markers for irradiated foods have been conducted (5-9).

Because meat products contain fairly high amounts of fat, hydrocarbons can be used as a tool to detect whether meat products have been irradiated. The method consists of extraction of fat, separation of hydrocarbons by florisil column chromatography, and identification of hydrocarbons by gas chromatography/mass spectrometry (GC/MS) method (7). Fat extraction is the basic step in the determination of radiation-induced hydrocarbons in foods. Due to the fact that conventional solvent extraction method uses large amounts of solvents and is time-consuming, promising alternative methods such as microwave-assisted extraction (MAE) or supercritical fluid extraction have been studied extensively (10, 11). Simoneau *et al.* (12) compared the Soxhlet, supercritical fluid extraction (SFE), and MAE for extraction of fat and recommended to use MAE for routine extraction of fat because it took less time and required smaller amount of solvent than other methods tested.

The aim of present study was to compare hydrocarbon profiles of fats extracted from irradiated ham and sausage with different extraction methods, conventional solvent extraction (CSE) and microwave assisted extraction (MAE).

*Corresponding author: Tel: 82-53-950-5775; Fax: 82-53-950-6772
E-mail: jhkwon@knu.ac.kr
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Materials and Methods

Materials and reagents Ham and sausage were purchased from a local market in Daegu, Korea and irradiated at 0 and 5 kGy using a ^{60}Co γ-irradiator (AECL, IR-79, MDS; Nordion International Co., Ltd., Ottawa, ON, Canada) at the Korea Atomic Energy Research Institute, Daejon, Korea. The validation of applied doses was verified by a ceric/cerous dosimeter (±5.6%). The standards of hydrocarbons were purchased from Fluka (Sigma-Aldrich, Steinhein, Switzerland). HPLC-grade hexane was purchased from Fisher Scientific (Pittsburgh, PA, USA). Florisil (60-100 mesh; Fisher Scientific) was pre-treated (heated overnight at 550°C) to remove possible contaminants and then stored. Florisil was deactivated for 5 hr at 130°C in a dry oven and cooled in a desiccator before use. The deactivated florisil was added with 3%(w/w) water, shaken for 20 min, and then stored at room temperature at least for 12 hr before use.

Extraction of fat with MAE and CSE Based upon our preliminary study on MAE, Microdigest 3.6 (Prolabo, France) was operated with focused irradiation under atmospheric pressure conditions of 100 W for 5 min at an

emission frequency of 2,450 MHz (13, 14). It is equipped with a 250 mL quartz vessel, a Graham-type refrigerant column (400 mm in length), and a bent extraction tube. Commercial samples (30 g) were mixed for 3 min in a mixer (FM680T; Hanil Electronic, Korea) with 50 g of anhydrous sodium sulfate. Twenty g of mixture was transferred to a 250-mL vessel and added with 50 mL of *n*-hexane. Upon completion of microwave irradiation, the solvent extract was filtered through a Whatman 41 filter paper under vacuum and collected for evaporation using a rotary vacuum evaporator. The extracted fat was flushed with nitrogen and stored at 4°C until separated by florisil column chromatography. CSE was performed following the methods of Spiegelberg *et al.* (15) and Choi and Hwang (16). Thirty g of sample was mixed for 3 min in a mixer with 50 g of anhydrous sodium sulfate. The mixture was transferred to a 250-mL flask, added with 150 mL of *n*-hexane, and then homogenized for 3 min at full speed (Type PT 10/35; Brinkman Instrument Inc., Westbury, NY, USA). The homogenate was centrifuged at 2,240×g for 20 min (VS-6000 CFN; Vision, Gyeonggi, Korea). The supernatant was filtered through a Whatman No. 41 filter paper and collected in a round-bottom flask. The solvent was evaporated using a rotary vacuum evaporator (Heidolph WB-2001; Kelhein, Germany) at 35°C. The extracted fat was flushed with nitrogen and stored at 4°C until 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 g of dried fat was mixed with an internal standard (1 mL, 4 ppm *n*-eicosane), applied to the Florisil column, and then 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 by means of nitrogen gas (7, 17).

GC/MS analysis Hydrocarbons were analyzed using gas chromatography/mass spectrometry (GC/MS, HP 6890/5973; Hewlett-Packard Co., Wilmington, DE, USA). An HP-5 column (30 m × 0.32 mm i.d., 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA) was used. The amount of sample injected was 2 μL and inlet was splitless mode for 2 min and then changed to split mode (20:1). The oven temperature was programmed to start at 60°C, increased to 170°C at 25°C /min, to 205°C at 2°C/min, and then to 270°C at 10°C/min. The carrier gas was helium at a flow rate of 1.0 mL/min. Hydrocarbons were identified by comparing the retention time and mass spectrum of authentic standards with those of samples. The concentration of each hydrocarbon in the fat was determined by using an internal standard.

Statistical analysis Analysis of variance was performed using the SAS software (18), and the Student-Newman-Keuls' multiple range test was used to compare difference among mean values. Mean scores and standard error of mean (SEM) were reported.

Results and Discussion

Pork is the major ingredient of ham and sausage, and pork fat contains oleic, palmitic, stearic, and linoleic acids (19). When these fatty acids are irradiated, bonds at α and β positions of the carbonyl group in fatty acids are degraded and 2 types of hydrocarbons are subsequently formed. One hydrocarbon contains one less carbon atom than its parent fatty acid. This hydrocarbon is formed as a result of loss of carboxyl group. The other hydrocarbon contains two less carbon atoms than its parent fatty acid. It also forms a new double bond at C_1 position (20). As a result, pentadecane ($C_{15:0}$) and 1-tetradecene ($C_{14:1}$) from paltmitic acid, heptadecane ($C_{17:0}$) and 1-hexadecene ($C_{16:1}$) from stearic acid, 8-heptadecane ($C_{17:1}$) and 1-hexadecene ($C_{16:1}$) from oleic acid, and 6,9-heptadecadine ($C_{17:2}$) and 1,7,10-hexadecatriene ($C_{16:3}$) from linoleic acid can be formed (5). Therefore, these hydrocarbons were monitored for ham and sausage irradiated at 5 kGy. Hydrocarbons, such as $C_{14:1}$, $C_{15:0}$, $C_{16:1}$, $C_{16:0}$, $C_{17:1}$, and $C_{17:0}$, were detected in non-irradiated samples. Nine different hydrocarbons, including $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$, however, were detected in

Table 1. Profiles of irradiation-induced hydrocarbons (μg/g fat) in irradiated ham prepared by conventional solvent extraction (CSE) and microwave-assisted extraction (MAE) of fat extractions[1)]

Hydrocarbon	CSE		MAE		SEM
	Control	5 kGy	Control	5 kGy	
14:1	1.30±0.04[1)a]	1.37±0.02[a]	0.58±0.01[c]	1.11±0.06[b]	0.23
15:0	1.61±0.01[b]	2.09±0.02[a]	0.82±0.11[c]	1.47±0.09[b]	0.35
16:3	0[b]	0.70±0.01[a]	0[b]	0.66±0.04[a]	0.02
16:2	0[b]	2.15±0.05[a]	0[b]	2.23±0.13[a]	0.06
16:1	0.21±0.09[b]	0.53±0.01[a]	0.45±0.01[a]	0.46±0.03[a]	0.10
16:0	1.84±0.12[a]	1.92±0.01[a]	0.47±0.01[c]	0.69±0.05[b]	0.50
17:2	0[b]	0.98±0.02[a]	0[b]	0.97±0.05[a]	0.02
17:1	0.20±0.06[c]	1.66±0.05[a]	0.81±0.12[b]	1.73±0.08[a]	0.48
17:0	1.45±0.33[ab]	1.76±0.02[a]	0.78±0.05[c]	1.13±0.08[bc]	0.28

[1)]Mean of duplicate±SD.
[2)]Means within a row with the same superscript are not significantly different ($p<0.05$).

Table 2. Profiles of radiation-induced hydrocarbons (μg/g fat) in irradiated sausage prepared by conventional solvent extraction (CSE) and microwave-assisted extraction (MAE) of fat extractions[1)]

Hydrocarbon	CSE		MAE		SEM
	Control	5 kGy	Control	5 kGy	
14:1	2.67±0.45[2)a]	3.29±0.08[a]	2.57±0.24[a]	2.99±0.18[a]	0.26
15:0	2.62±0.11[b]	3.74±0.18[a]	2.87±0.12[b]	3.47±0.13[a]	0.35
16:3	0[c]	1.23±0.02[a]	0[c]	1.01±0.03[b]	0.43
16:2	0[c]	3.18±0.04[a]	0[c]	2.14±0.08[b]	1.04
16:1	0.60±0.03[c]	1.16±0.04[a]	0.98±0.02[b]	1.14±0.01[a]	0.17
16:0	2.09±0.08[c]	2.56±0.01[b]	2.66±0.21[b]	3.07±0.14[a]	0.27
17:2	0[c]	1.96±0.04[a]	0[c]	1.68±0.01[b]	0.69
17:1	0.26±0.01[d]	2.53±0.03[a]	0.95±0.395[c]	1.85±0.01[b]	0.66
17:0	2.95±0.12[b]	3.81±0.04[a]	3.48±0.148[a]	3.71±0.07[a]	0.26

[1)]Mean of duplicate±standard deviation.
[2)]Means within a row with the same superscript are not significantly different ($p<0.05$).

irradiated samples. Therefore, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$ can be used as markers for irradiated ham and sausage. These hydrocarbons were detected in fats extracted from irradiated ham and sausage using both CSE and MAE methods. The method of fat extraction did not affect the profile of hydrocarbons in irradiated meat products.

A number of radiolytic hydrocarbons were detected in irradiated samples (data not shown). Hydrocarbons, such as $C_{14:1}$, $C_{15:0}$, $C_{16:0}$, and $C_{17:0}$, were detected in both non-irradiated and irradiated ham with higher concentrations in CSE method than MAE. However, extraction methods had no effect on the extraction of radiation-induced hydrocarbons, such as $C_{16:3}$, $C_{16:2}$, and $C_{17:2}$ (Table 1) from the meat products. Table 2 shows the data of the quantitative analysis for these hydrocarbons. Concentrations of $C_{16:3}$, $C_{16:2}$, $C_{17:2}$, and $C_{17:1}$ were significantly higher in samples prepared with CSE than MAE ($p<0.05$) method for irradiated samples, while the concentrations of other hydrocarbons were not different. Hwang (6) reported that hydrocarbons, such as $C_{17:1}$, $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$, were detected in pork, bacon, and ham irradiated at 0.5 kGy or higher but not in non-irradiated ones except $C_{17:1}$, which is in good agreement with our results. Kwon *et al.* (21) found that the overall profiles of hydrocarbons detected in irradiated sausage were not influenced by fat contents, which supports the possibility of applying MAE to extract fat for determining hydrocarbons in irradiated meat products in reduced amounts of solvent and reduced extraction time (22, 23).

In conclusion, significant amounts of radiation-induced hydrocarbons, such as $C_{16:2}$, $C_{17:2}$, and $C_{16:3}$, were detected in irradiated meat products samples prepared with CSE and MAE methods, but MAE method required apparently reduced amounts of solvent from 150 (CSE) to 50 mL and reduced extraction time from 23 (CSE) to 5 min.

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