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Evaluation of 2-Dodecylcyclobutanone as an Irradiation Dose Indicator in Fresh Irradiated Ground Beef

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Alkylcyclobutanones (2-ACBs) are radiolytic products formed when fatty acids are irradiated. These cyclobutanones are unique irradiation byproducts and therefore may serve as indicators of irradiation exposure. As only limited information exists about 2-ACB formation in retail meat products, reliable methods that can quantify 2-ACBs and thus estimate irradiation dose in commercial meat products are desired. The cyclobutanone studied in this experiment was 2-dodecylcyclobutanone (2-DCB), which is formed from palmitic acid. The formation of 2-DCB was evaluated in fresh irradiated ground beef patties at two fat levels. Patties containing 15% and 25% fat were irradiated by electron beam at 1.0, 2.0, 3.0, and 4.5 kGy. Commercially available 1-lb irradiated ground beef chubs with different fat levels were analyzed in order to estimate dose absorbed by these samples. The 2-DCB was extracted using supercritical fluid extraction (SFE) and analyzed by gas chromatography−mass spectroscopy (GC−MS) and was detected in all the irradiated samples. The concentration of 2-DCB increased linearly with dose with $R^2 = 0.9646$ for 25% fat samples and $R^2 = 0.9444$ for 15% fat samples. Further, there was no significant difference in 2-DCB concentrations between the two fat levels. The estimated doses applied to the commercial samples ranged between 1.38 and 1.55 kGy, values consistent with doses normally used in the industry (1.0−2.0 kGy). Our results show that 2-DCB can be used to monitor fresh irradiated beef and approximate the absorbed dose.

KEYWORDS: Alkylcyclobutanones; irradiation; 2-dodecylcyclobutanone; supercritical fluid extraction, gas chromatography; mass spectroscopy

INTRODUCTION

Treating food with ionizing radiation improves its safety and helps maintain its quality. The main selling point of irradiated foods is that it is safe from a microbial standpoint. Beginning October 2002, companies could petition the FDA for permission to use terms such as "electronic pasteurization" on the labeling for irradiated foods (*1*). Consumers are already familiar with pasteurization and associate the term with a safe product. Therefore, there must be a protocol in place to test for irradiation in order to check if the product meets with the regulations. Being able to differentiate between irradiated and nonirradiated foods will aid in proving the authenticity and safety of irradiated products and in detecting mislabeled products. In November 2003, Excel Corp. (Dodge City, KS) voluntarily recalled 26 000 pounds of ground beef that was mislabeled as irradiated (*2*). The incident appears to be the first case of its kind, and it emphasizes the need for a method that can reliably distinguish between irradiated and nonirradiated foods.

Ionizing radiation causes changes in the food much like cooking (*3*). Many radiolytic products, such as short chain hydrocarbons, aldehydes, ketones, and flavor volatiles, are formed due to irradiation. Most of these changes are not unique to the process of irradiation, with these compounds being detected in nonirradiated foods as well. At the doses currently approved for food irradiation, the only unique radiolytic products that have been identified are alkylcyclobutanones (*3*). These are cyclic compounds formed by rearrangement of free fatty acids when exposed to irradiation. The resulting compounds have the same number of carbon atoms as the precursor fatty acids, with an alkyl group attached at ring position two (*4*). These compounds have been found in a wide variety of lipid-containing foods and have been universally accepted as indicators of irradiation exposure (*5*−*7*).

Over the years various methods have been evaluated for the detection of alkylcyclobutanones in irradiated lipid-containing foods. These include solvent extraction, high performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and SFE−GC−MS (*8*−*10*). Of these, the solvent extraction method was adopted as a European Standard (EN1785) and Ministry of Agriculture, Fisheries, and Food validated method (Ireland, MAFF V37) in 1996 (*11*). The method involves a long clean up and extraction procedure and uses large volumes of organic solvents. Recent studies using supercritical fluid extraction (SFE) have shown that this procedure offsets the limitations of the solvent extraction method and is better suited

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Table 1. Dosimetry Data Showing the Maximum, Minimum, and Average Absorbed Doses (kGy) for Ground Beef Patties with Two Fat Levels[a]

target dose	maximum dose	minimum dose	average dose
	15% Fat		
1.0	1.15	1.01	1.08
2.0	2.35	2.03	2.19
3.0	3.45	3.07	3.26
4.5	5.15	4.57	4.86
	25% Fat		
1.0	1.14	1.00	1.07
2.0	2.29	2.00	2.15
3.0	3.46	3.01	3.26
4.5	5.09	4.59	4.84

[a] Absorbed dose was measured by alanine dosimeters.

for the extraction of the 2-alkylcyclobutanones (*8*, *12*, *13*). A SFE method previously optimized in this lab was used to extract and detect 2-DCB in γ-irradiated and electron beam irradiated frozen ground beef patties (*14*).

The next step for us was to evaluate 2-DCB formation in fresh irradiated ground beef. We were also interested in investigating the effect of fat level on the formation of 2-DCB. Therefore, the first objective of this experiment was to examine the dose−response relationship of 2-DCB formation in fresh irradiated ground beef patties at two fat levels. The second objective was to quantify 2-DCB in commercially available fresh irradiated ground beef using the data obtained from the first part and estimate the absorbed dose. 2-DCB was selected as it was the most easily detected cyclobutanone according to our previous research (*14*).

MATERIALS AND METHODS

Reagents. Hexane, Florisil (60−100 mesh), anhydrous sodium sulfate, boron trifluoride−methanol (14%) were purchased from Fisher Scientific (Pittsburgh, PA). Wetsupport (diatomaceous earth) and sand were obtained from ISCO, Inc. (Lincoln, NE). The 2-DCB standard was obtained from Acros Organics (Fisher Scientific Co., Pittsburgh, PA).

Ground Beef Patty Preparation and Irradiation. Beef from round muscles and beef trimmings that were 48 h postmortem was coarse ground and packaged in 4.54-kg chubs. Two coarse grind formulations (75/25 or 85/15) were formulated using a commercial processor (Tyson's IBP Fresh Meats, Emporia, KS), transported to Kansas State University (KSU), and stored for 6 days at 1 ± 1 °C. After running the chubs through the grinder (Hobart model number 84145, Hobart Corp., Troy, OH), the fine grind was placed in a lug in a refrigerated unit set at −6.67 °C. Quarter-pound ground beef patties (weight 115 ± 1 g) were made using a patty mold (1.2 cm height × 11 cm diameter) and Plexiglas press. The patties were placed in a single layer on metal trays lined with Butcher paper, crust frozen for 30 min in an open-top case (Model SM0781A11; Star Products Division, Hussmann Corp., Dumas, AR), and individually vacuum-packaged in 3 mil standard barrier (nylon/PE) vacuum pouches (Koch Supplies Inc., Kansas City, MO) using a Multivac A300 Packager (Multivac Packaging Machines, Kansas City, MO). Vacuum was measured using a Kennedy Gauge (Kennedy Enterprises, Lincoln, NE), and the vacuum level was set at 90%. Patties were stored at 0 ± 1.5 °C until shipment to the irradiation facility. Patties were irradiated at the electron beam facility (SureBeam Corp., Sioux City, IA). Temperatures were monitored during storage and throughout transportation using temperature loggers (Omega Engineering, Inc., Stamford, CT). Four patties were irradiated per dose and the dosimetry data is shown in **Table 1**.

Commercial Samples. One-pound chubs of commercially irradiated ground beef chubs were obtained from two sources. Two samples of brand X containing 7% and 20% fat and two samples of brand Y containing 10% and 20% fat were evaluated. These were stored at −80 °C until ready for analysis.

Fatty Acid Profile. A fatty acid profile was obtained to determine the amounts of palmitic acid present in the beef fat. The fat from the ground beef patties was first extracted by blending 5 g of the beef sample with 25 mL of hexane. The extract was passed through Whatman filter paper no. 4 and concentrated to dryness on a Rotavapor (temperature 45 °C). The fatty acid were then converted to their corresponding fatty acid methyl esters (FAME) using boron trifluoride−methanol (14%), according to the procedure described by Ackman (*15*). The extracts were analyzed by GC equipped with a flame ionization detector (FID). The GC−FID conditions were as follows: injector temperature, 250 °C; initial temperature, 60 °C, hold 1 min, 20 °C/min; final temperature, 195 °C, hold 15 min; detector temperature, 280 °C. The instrument used was a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA), with a HP-23 cis/trans FAME column (Agilent Technologies, Palo Alto, CA). The flow rate of carrier gas, helium, was 1 mL/min.

Preparation of Patties for Extraction. All patties and commercial samples were stored at −80 °C prior to analysis. Once ready for analysis, the patties were tempered at room temperature for 20−30 min or until soft enough to cut. The patties were then cut into 1 cm^2 squares and immersed into liquid nitrogen. They were removed after the liquid nitrogen had stopped bubbling (about 30−45 s) and ground in a Waring blender fitted with a stainless steel blending container (Fisher Scientific Co., Pittsburgh, PA). The result was a fine homogeneous powder that was used for the SFE procedure.

SFE Procedure. An ISCO−Suprex prepmaster GA (ISCO, Inc., Lincoln, NE) fat analyzer was used for the SFE procedure. Ground beef was homogenized with Wetsupport in a Waring blender in the ratio of 1 part beef to 2 parts Wetsupport. A 5-mL SFE cartridge (ISCO, Inc., Lincoln, NE) was loaded with sand, Florisil, and about 1.5 g of the beef−Wetsupport mixture prior to being placed in the extractor. The sand protects the seals of the extraction cartridge while the Florisil serves to trap the fat. Three beef patties were extracted for each dose level and two cartridges were prepared per patty. The control and commercial samples were prepared and used in the same way. The entire experiment was performed once.

Extraction was carried out under the following conditions: pressure, 340 atm; temperature, 75 °C; 5 min static and 20 min dynamic with a flow rate of CO_2 of 1 mL/min. These parameters were modified from a previously described procedure (*12*). The cyclobutanones were trapped on glass wool and eluted with about 25 mL of hexane. This extract was concentrated under nitrogen gas to 25 μL for control samples and samples irradiated at doses 1.0 and 2.0 kGy, or to 50 μL for 3.0 and 4.0 kGy and 10 μL for the commercial samples. The extracts (1 FL) were then injected into the GC−MS.

GC−MS Analysis. GC−MS was performed with a Hewlett-Packard 5890 (Palo Alto, CA) fitted with a HP-5 MS column (cross-linked 5% Ph Me siloxane, 30 m × 0.22 mm × 0.25 μm film thickness) and a Hewlett-Packard MSD 5970 detector. The flow rate for the helium carrier gas was 1 mL/min. The GC temperature program was as follows: injector temperature, 250 °C; initial temperature, 55 °C, hold 0.5 min; 20 °C/min; final temperature, 200 °C, hold 1 min; 15 °C/min final temp 270 °C, hold 1 min. The transfer line and ion source were held at 280 °C throughout the runs. The MS was set to selected ion monitoring (SIM) mode and ions *m*/*z* 98 and 112 were monitored for the analysis of 2-DCB. Standard solutions of 1.0, 2.5, 5.0, 7.5, and 10.0 ppm were used to calibrate the standard curve for 2-DCB. The detection limit for 2-DCB was determined on the basis of the signal-to-noise ratio. A signal-to-noise ratio of ≥4 was used for the detection limit and a signal-to-noise ratio of ≥10 was used to set quantitation limit. On the basis of the signal-to-noise ratios, the detection limit for 2-DCB was 0.05 ppm and the quantitation limit was 0.1 ppm. The compounds were identified by comparing retention times and the ion ratios with the standards, and the concentration in the sample was determined from the standard curve.

Statistical Analysis. The experimental design was a completely randomized design with a 2 × 5 factorial structure. Analysis of variance was carried out on the data using the SAS software system release 8.1, (SAS Institute Inc., Cary, NC).

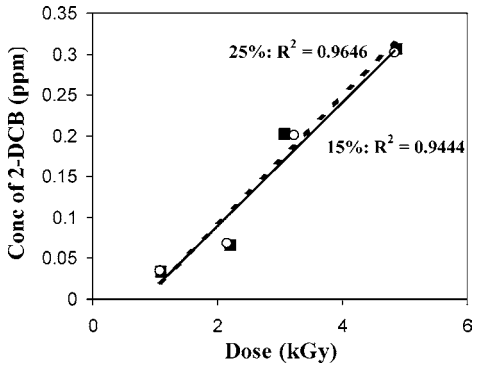

Figure 1. Response of 2-DCB (μg/g of beef) with increasing irradiation dose: (■) 15% fat samples; (○) 25% fat samples.

Table 2. Amount of 2-DCB in Ground Beef Patties Irradiated at Four Dose Levels in Fg/g of Beef (ppm)

targeted dose (kGy)	concn of 2-DCB (ppm)	
	15% fat	25% fat
1.0	0.03 ± 0.002	0.02 ± 0.004
2.0	0.06 ± 0.006	0.04 ± 0.003
3.0	0.20 ± 0.009	0.20 ± 0.008
4.5	0.31 ± 0.008	0.30 ± 0.009

Table 3. Estimated Absorbed Dose for All the Commercial Samples Using Linear Equations Obtained from the 15% and 25% Samples

	using 15% line equation			using 25% line equation	
brand	fat level (%)	dose (kGy)	brand	fat level (%)	dose (kGy)
X	80	1.54	X	80	1.57
	93	1.45		93	1.47
Y	80	1.37	Y	80	1.39
	90	1.53		90	1.56

RESULTS AND DISCUSSION

From fatty acid measurements the amount of palmitic acid in the beef fat was 25.4 ± 1.08%. The 2-DCB was detected in all the irradiated samples and its concentration increased linearly with dose as illustrated in the response curve shown in **Figure 1**. There was no significant difference in the amount formed between the two fat levels. There might be an upper threshold beyond which the amount of fat does not effect 2-DCB formation. This indicates that the amount of fat may not be a factor affecting 2-DCB formation, at least at these fat levels. Thus, the absorbed dose can be estimated for commercial samples with a wide range of fat levels. In a commercial setting, where there is considerable variation in product composition, this would be an advantage. The amount of 2-DCB for the two fat levels is shown in **Table 2**.

The compound was detected in the all commercial samples, and the estimated absorbed doses as calculated from the dose−response curves are shown in **Table 3**. As the fat levels of all the commercial samples did not correspond exactly with the lab samples, line equations obtained both 15% and 25% fat level samples were used to calculate the absorbed dose. Our lab samples were irradiated at a SureBeam facility that irradiated ground beef for retail sale. We expect our samples were processed in much the same way as commercial samples would be and are suitable for estimating applied dose. It should be noted that the absorbed dose values are estimates. There were no true controls for the commercial samples and there was no information about when the samples were irradiated. Therefore, the effect of storage conditions and/or time, if any, was unknown. However, these values are within the range of 1.0−2.0 kGy normally used in the industry (personal communication), indicating that this method was able to approximate the dose applied. The percent recovery for 2-DCB using the SFE−GC−MS was calculated as 99.48% (CV = 18.81%).

Our results are in agreement with previous studies (*6*, *7*, *14*, *16*) and show that the applied dose can be estimated by monitoring 2-DCB formation. The results reiterated the efficacy of the SFE−GC−MS method in evaluating 2-DCB formation and consequently estimating the applied dose. The amount of 2-ACBs formed in irradiated ground beef is very low. Therefore, an extraction method that is efficient is very important. In addition to the high percent recovery, the method is rapid and does not involve excess use of organic solvents. This method has the potential of being applied for routine monitoring of irradiated ground beef with respect to whether it has been irradiated and how much dose was absorbed. More studies are needed to investigate the influence of such variables as storage time and temperature on formation of 2-DCB and other 2-ACBs in ground beef. As further experiments are conducted, it will help establish the SFE−GC−MS method as the method of choice for evaluation 2-ACBs in irradiated ground beef and other lipid−containing foods.

ABBREVIATIONS USED

SFE, supercritical fluid extraction; ACB, alkylcyclobutanone; 2-DCB, 2-dodecylcyclobutanone; GC−MS, gas chromatography−mass spectroscopy; HPLC, high-performance liquid chromatography, ELISA, enzyme-linked immunosorbent assay.

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