Detection of 2-Alkylcyclobutanones in Irradiated Meat, Poultry and Egg after Cooking

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2-Alkylcyclobutanones in irradiated and cooked meats, poultry and egg were analyzed to detect the irradiation history of those samples prior to cooking. They were extracted using an accelerated solvent extraction system (ASE), and determined by gas chromatography with mass spectrometry (GC/MS). Irradiated beef, chicken and egg were browned and the irradiated beef and egg were boiled. The irradiated chicken was fried and a pancake was made with the irradiated egg. Radiolytic compounds of 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutane (2-TCB) were detected in all the cooked samples when the raw samples were irradiated with 3–6.5 kGy. Both 2-DCB and 2-TCB were similarly stable in the samples with conventional cooking, which resulted in a temperature less than 100°C inside the samples, whereas they were destroyed when heated to over 200°C. The food samples did not show serious interferences on GC/MS chromatograms due to cooking. The results conclude that the analysis of 2-DCB and 2-TCB in the cooked samples would be a reliable indicator to detect the irradiation history of raw meats, poultry and egg.

Key words —— 2-alkylcyclobutanone, irradiation, food, cooking

INTRODUCTION

The ionizing irradiation of food is used to improve the safety and to maintain the quality of foods by controlling the microorganisms and extending the shelf life.[1,2] The World Health Organization (WHO) concluded, "Irradiation of food up to an overall average dose of 10 kGy presents no toxicological hazard and introduces no special nutritional or microbiological problems."[3] Many countries have allowed the irradiation of foods below the certified dose within the regulations.[4–6] The irradiated foods must be labeled to indicate the irradiation history to consumers in those countries. Some consumers are against the irradiation of food or prefer to buy non-irradiated foods. Such consumers would like to know the irradiation history of pre-cooked foods sold in grocery stores, in which irradiated beef, pork, chicken and egg might have been used as ingredients.

Confirming the irradiation history of foods would promote the consumer's choice and acceptance of the irradiated foods, and may help in the enforcement of labeling regulations. Various methods have been proposed to detect irradiated foodstuffs.[7] Of these methods, The European Union has officially adopted the analysis of 2-alkylcyclobutanones as EN 1785 to detect irradiated foods containing fat, such as chicken, pork and beef.[8] 2-Alkylcyclobutanes are considered to be unique radiolytic products of fatty foods when they are irradiated. 2-Dodecylcyclobutanone (2-DCB) from palmitic acid and 2-tetradecylcyclobutane (2-TCB) from stearic acid have been recommended as markers for the irradiation of lipid-containing foods.[9–12] The EN 1785 method has been confirmed to detect 2-alkylcyclobutane in various irradiated samples; however, the method requires a long sample preparation time and is labor intensive. We have developed to a new analytical method to determine the 2-alkylcyclobutane in fat-containing foods with accelerated solvent extraction (ASE) and mini-column cleanup.[13] The ASE method surpassed the EN 1785 method in operation time and solvent consumption. ASE uses solvents at high pressures and temperatures above boiling point; thus, the solvents solubilize the targeted compounds and penetrate the sample

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matrices better than at atmospheric conditions and room temperature.[14,15] ASE has been effective in PCB analysis[15] and pesticide residue extraction,[16–18] and was comparable with supercritical fluid extraction in residue analysis of pollutants in soils,[19] and also dioxin analysis.[20]

Concerning a toxic aspect of 2-DCB, possibilities of genotoxin and cancer promoter were indicated.[21,22] However, other groups claimed that genotoxic effects of 2-alkylcyclobutanones would be suspicious. A bacterial mutation test with *Escherichia coli*[23] and *Salmonella typhimurium*[24,25] showed that 2-DCB was not mutagenic with and without metabolic activation.

The objective of this study was to confirm that the detection of both 2-DCB and 2-TCB in cooked meat, poultry and egg would be good irradiation markers if they were irradiated while raw. The two compound levels were compared in raw and cooked irradiated samples, and in the overly heated samples. The production of interferences in the cooked samples was also studied. A few studies have been conducted regarding the stability of 2-alkylcyclobutanes with the cooking and processing of irradiated foods.[10,26]

MATERIALS AND METHODS

Reagents —— 2-DCB and 2-TCB were purchased from Fluka (Switzerland). 2-Cyclohexylcyclohexanone, as an internal standard for gas chromatography with mass spectrometry (GC/MS) determination, was purchased from Wako Pure Chemical (Osaka, Japan).

Food Commodities —— Meats, eggs and other foods were purchased at a local market in Osaka, Japan. An approximately 500 g sample was chopped in a conventional food processor (MK-K3, Matsushita, Japan) to obtain the homogenous fat distribution of ground beef. About 40 g of ground beef was put in a ring, which was made of brass with a size of 95 mm in diameter, 6 mm in height, and 2 mm in thickness on the cooking plate, and was spread and filled up the ring to make a patty. The patty in the ring on the plate was almost frozen to make it hard and the patty was removed without disturbing the shape.

Irradiation —— Samples were irradiated with γ-rays from a ^{60}Co source (15 kGy/hr) at the Frontier Science Innovation Center, Osaka Prefecture University. The following doses for irradiation were set up within the maximum levels of European Union[27] or USA.[28] Sliced beef, ground beef and a bite-sized chicken thigh with skin were irradiated at doses of 5.9, 6.5 and 3.0 kGy, respectively, at –19°C, as previously described.[13] Eggs at room temperature were irradiated with γ-rays at 3.1 kGy. Beef patties, which were frozen with powdered dry ice, were irradiated with γ-rays at 4.7 kGy, and they were also irradiated with an electron beam (10 MeV) at 5.3 kGy. The irradiated dose was determined with Radiachromic dye film [FWT-60-00 (1P)], Far West Technology, Inc., CA, U.S.A.) and the irradiated doses were compensated with a calibration curve for frozen temperatures, since the film was less sensitive at cooler temperatures. Irradiated meats were stored at –20°C and the eggs used within a week were refrigerated before cooking. Cooked samples were stored at –20°C until the analysis. Non-irradiated control samples were also cooked and stored under the same conditions.

Cooking —— Irradiated beef slices of 5 mm thickness and 20 g weight were cooked in two ways. The beef was browned on an electric cooking plate (HL-WK8, Mitsubishi, Japan) at 220°C for 30 sec on each side. Gyudon topping, beef bowl topping, was made by combining slices of meat, onion, beef bowl sauce and water, then boiled for 30 min.

Beef patties irradiated from two kinds of sources were heated on an electric cooking plate at 220°C for 3 min and the patty was turned every 30 sec and the inside temperature of the patty was around 78–85°C immediately after heating.

An irradiated bite-sized chicken thigh was cooked in two ways. The chicken thigh was browned on an electric cooking plate at 220°C for 5 min and the inside temperature of the chicken was 55–60°C immediately after heating. The chicken thigh without bone was fried in cooking oil at 180°C for 4 min after sprinkling it with spices and the inside temperature of the chicken was 83–96°C just after heating.

The irradiated eggs were cooked in three ways. An egg was heated in boiling water for 10 min. The yolk and the white were thoroughly mixed for sampling. An egg was agitated on an electric cooking plate at 200°C for 2 min to make a scrambled egg. A whole egg was mixed with 80 g flour, 8 g butter, 30 g sugar and 70 g milk with a food processor to obtain a homogeneous mixture. The mixture was browned on an electric cooking plate at 180°C for 2 min for each of both sides to cook a pancake. The blank pancake without an egg was also cooked.

Oil was not added to the samples cooked without frying.

Heating 2-DCB and 2-TCB ——— An aliquot of 1 ml mixture of 2-DCB and 2-TCB at 1 ppm in *n*-hexane solution was mixed with 1 g of melted beef fat and kept for 30 min at 70°C to remove the *n*-hexane. The mixture was further heated in an electric oven for 1 hr at 100, 150 or 200°C and was dissolved in 20 ml ethyl acetate after being cooled. The remaining 2-DCB and 2-TCB in 10 ml solution were analyzed after cleanup, as described below.

Sample Preparation for Analysis ——— Sample preparation for the analysis of 2-DCB and 2-TCB was performed according to the previously described report.[13] The main procedure involved 10 g each of sample and diatomaceous earth particles (Extrelut for refilling; Merck; Germany) being thoroughly mixed; then the mixture was placed in stainless steel cells for ASE with a Dionex AS 200 (Dionex Corporation, CA, U.S.A.). The ASE conditions were as follows: solvent, ethyl acetate; extraction temperature, 100°C; extraction pressure, 10.3 MPa; twice static extraction period, 5 min; solvent flash, 9.9 ml. The extract was made up to 50 ml with ethyl acetate. An aliquot of 10 ml suspended extract was placed in a 30 ml size test tube with a cap, followed by the addition of 10 ml acetonitrile. For the pancake, a 25 ml extract was taken and 25 ml acetonitrile was added. The mixture was kept at –20°C for 30 min to precipitate the fat. The precipitated fat was removed by immediate filtration with coarse filter paper. Both the flask and filter were washed with 5 ml acetonitrile at –20°C and collected as a filtrate. The filtrate was kept at –20°C for a further 30 min and the appearing fat suspension was removed with filtration. The second filtrate was evaporated almost to dryness and the residue was dissolved in *n*-hexane (about 1 ml) followed by adding to a silica gel cartridge (Mega Bond Elut SI, 1 g, Varian, CA, U.S.A.). Ten ml *n*-hexane was first eluted and discarded. Then, 10 ml of 2% diethyl ether in *n*-hexane was further eluted and collected as a 2-alkylcyclobutanone fraction. The elution was concentrated to 2 ml, after the addition of 2-cyclohexylcyclohexanone to a final concentration of 0.1 μg/ml for compensating the matrix-induced enhancement.[29]

Fat Content ——— An aliquot of 25 ml remaining suspended extract was passed through about 10 g anhydrous Na_2SO_4 on the filter paper and evaporated to almost to dryness. Fat residue was weighed after overnight standing.

GC/MS Determination ——— An Auto Mass 120 (JOEL, Tokyo, Japan) was connected to an HP5890 gas chromatograph (Hewlett-Packard, CA, U.S.A.). The GC conditions were as follows: column, RTX-5ms (Restek Bellefonte, PA, U.S.A.) 30 m × 0.25 mm i.d., 0.25 μm thickness; column temperature program, 60°C (1 min), 60–300°C at 8°C/min, 300°C (5 min); carrier gas, He at 10 psi; injection temperature, 250°C; injection volume, 2 μl with an HP7673 auto sampler (Hewlett-Packard). The MS conditions: ionization mode, electron ionization; ion detection, selected ion monitoring (SIM); ionization voltage, 70 V; ion source temperature 200°C. The monitored ions were *m*/*z* 98, 112, and 98 was selected for quantitation under SIM. The detected 2-DCB and 2-TCB were confirmed under scan mode with *m*/*z* 80–300.

The limits of detection of 2-DCB and 2-TCB were 2.5 ng/ml in the test solution.

Statistics ——— 2-Alkylcyclobutanone residues in the cooked samples and the corresponding raw samples were statistically compared using Student's *t*-test with Microsoft Excel 2004 for Macintosh.

RESULTS AND DISCUSSION

Beef Preparation

2-Alkylcyclobutanone concentrations in the irradiated raw, browned and boiled beef are listed in Table 1, which was expressed with two concentration units, such as the wet weight basis and fat basis. Both 2-DCB and 2-TCB were increased after cooking on a weight basis, whereas the increases after cooking were relatively compensated to near raw levels on a fat basis, since the browned and boiled beef decreased in weight by 14 and 34%, respectively, with cooking (data not shown). The data in Table 1 might indicate the productions of 2-DCB and 2-TCB during cooking on a weight basis concentration; however, it would be recognized as a misunderstanding based on the fat basis concentration. The reason was that the water and fat in the samples might be lost by vaporization or elution during cooking. Thus, water loss could lead to relatively higher concentrations of 2-alkylcyclobutanones on a weight basis when the fat-soluble compounds were not affected. However, fat loss would not affect the concentrations of 2-alkylcyclobutanones on fat basis, so long as exogenous fat was not added during the cooking process. The chromatograms indicated that the peak abundances were increased in boiled beef, whereas the detection of the

Table 1. Effect of Cooking on 2-DCB and 2-TCB in Irradiated Beef Slice

Sample	2-DCB			2-TCB		
	Average[a)]	RSD[b)]	p[c)]	Average[a)]	RSD[b)]	p[c)]
Weight basis	(μg/g weight)	(%)		(μg/g weight)	(%)	
Raw	0.29	14		0.39	12	
Browned	0.36	3	0.02	0.50	2	0.01
Beef bowl	0.38	10	0.06	0.56	13	0.03
Fat basis	(μg/g fat)	(%)		(μg/g fat)	(%)	
Raw	0.76	14		1.00	13	
Browned	0.82	6	0.28	1.12	5	0.13
Beef bowl	0.83	12	0.49	1.22	15	0.19

a) Average of 5 experiments. *b*) Relative standard deviation. *c*) Values with t-test < 0.05 significantly different from the raw sample.

two compounds was not affected in any of the samples. Both 2-DCB and 2-TCB were not detected in the non-irradiated samples (Fig. 1).

Beef patties were irradiated with two sources, such as γ-rays and an electron beam, and cooked under the same conditions. Both 2-DCB and 2-TCB concentrations, which were expressed in weight basis and fat basis units, were not significantly changed after cooking (Table 2). The patties were cooked deeper than the sliced beef, as shown in Table 1. Water and fat were poured from the patties during cooking, which might have caused the unaffected concentrations of 2-DCB and 2-TCB in the browned samples. GC chromatograms of the two compounds in cooked patties with different sources, which looked almost identical, are shown in Fig. 2.

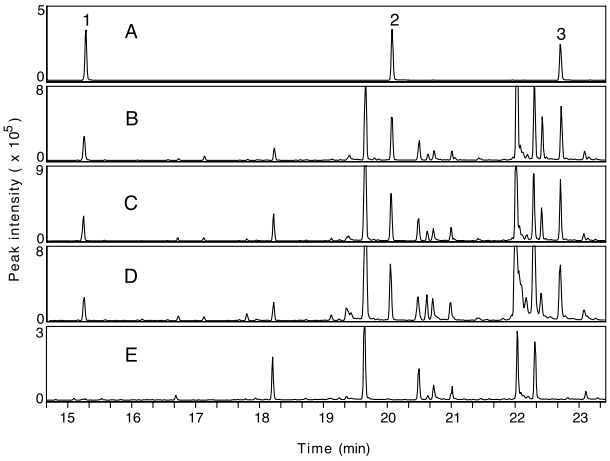

Fig. 1. Typical Chromatograms of 2-Alkylcyclobutanones in Irradiated Beef with Cooking Monitored at *m/z* 98

(A), 2-DCB and 2-TCB at 100 ng/ml; (B), irradiated raw beef; (C), irradiated and browned beef; (D), irradiated and boiled beef; (E), non-irradiated raw beef. Peak labels: 1, 2-cyclohexylcyclohexanone; 2, 2-DCB; 3, 2-TCB.

Chicken Preparation

2-Alkylcyclobutanone concentrations in the irradiated raw, browned and fried chicken are listed in Table 3. Both 2-DCB and 2-TCB concentrations in browned chicken on a weight basis were increased owing to a decrease of water with cooking and their concentrations were similar to the raw levels on a fat basis. The levels in fried chicken were lower than the raw samples, although they were not significant. The exchange of the endogenous fat and water in chicken for cooking oil would take place during frying, such that the weight of chicken was reduced by about 31% and the fat concentration was increased to 16% from 10% after frying. The increase of fat after frying, which did not contain 2-alkylcyclobutanones, was considered to contribute to the 2-DCB and 2-TCB reductions. Large RSD values in the raw and fried samples on weight basis, which might be due to inhomogeneous fat concentrations in each piece, were decreased on a fat basis. Serious interferences were not detected due to cooking (Fig. 2).

Egg Preparation

2-Alkylcyclobutanone concentrations in the irradiated raw egg, boiled egg, scrambled egg and pancake with the irradiated egg are listed in Table 4. 2-Alkylcyclobutanone concentrations on a weight basis were increased in the scrambled egg, possibly due to water loss and were decreased in the pancake due to dilution with other ingredients, such as milk, flour and butter. The concentrations of two compounds in the boiled eggs also showed an increase on a weight basis; however, the reason was not clear. The density of the egg might be increased in the solid state compared with the liquid, since the fat concen-

Table 2. Effect of Cooking on 2-DCB and 2-TCB in Irradiated Beef Patty

Source	Cooking	2-DCB			2-TCB		
		Average[a)]	RSD[b)]	p[c)]	Average[a)]	RSD[b)]	p[c)]
Weight basis		(μg/g weight)	(%)		(μg/g weight)	(%)	
Electron beam	Raw	0.107	15		0.147	12	
	Browned	0.112	5	0.429	0.151	4	0.591
γ-Ray	Raw	0.081	14		0.119	17	
	Browned	0.076	5	0.312	0.107	7	0.322
Fat basis		(μg/g fat)	(%)		(μg/g fat)	(%)	
Electron beam	Raw	0.72	12		1.00	9	
	Browned	0.71	5	0.532	0.95	6	0.073
γ-Ray	Raw	0.46	15		0.68	19	
	Browned	0.47	8	0.867	0.67	9	0.856

a) Average of 5 experiments. *b*) Relative standard deviation. *c*) Values with t-test < 0.05 significantly different from the raw sample.

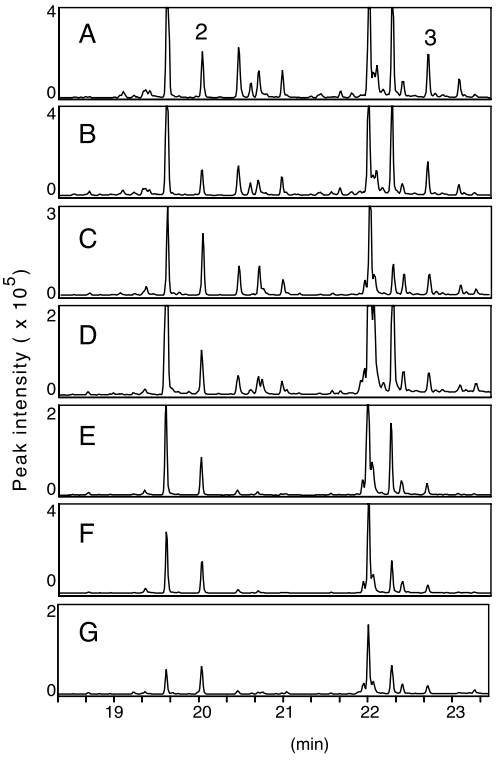

Fig. 2. Typical Chromatograms of 2-Alkylcyclobutanones in the Irradiated Beef Patties, Chicken, and Egg with Cooking Monitored at m/z 98

(A), electron beam irradiated and browned beef patty; (B), γ-ray irradiated and browned beef patty; (C), irradiated and browned chicken; (D), irradiated and fried chicken; (E), irradiated and boiled egg; (F), irradiated and scrambled egg; (G), pancake with irradiated egg. Peak labels: 2, 2-DCB; 3, 2-TCB.

tration was increased in the boiled samples. 2-DCB and 2-TCB concentrations in the boiled and scrambled egg became similar to the raw levels on a fat basis. The two compounds in the pancake were still low on a fat basis. Fat sources, such as butter and milk, were added to the sample to make the pancake. The fat from those ingredients might reduce the concentrations on a fat basis. Thus, the blank pancake without egg was made with the same recipe and the blank fat was extracted as the pancake blank for fat. The blank fat was subtracted from the fat in the pancake with an irradiated egg, and the compensated concentrations of two compounds became similar to the raw levels based on the adjusted fat basis. The results indicated that 2-DCB and 2-TCB in the egg were stable during the cooking process with the methods we tested. Chromatograms of the two compounds in the boiled egg, scrambled egg, and pancake are shown in Fig. 2. The chromatograms indicated that the two compounds could be detected without interference and the cooking did not generate outstanding differences.

Over Heating

Table 5 shows the stability of 2-DCB and 2-TCB in the beef fat, which was stored to protect against the vaporization of the two compounds in the overheated condition. The results indicated that the two compounds were stable below 100°C and they were then reduced by 40% at 150°C and almost broke down after 1 hr at 200°C. The chromatogram indicated that the two compounds were detectable until 150°C, but they were greatly reduced to small peaks, which became indistinguishable from the other peaks at 200°C (Fig. 3). The stability of the two compounds is similar to beef fat, since many unknown peaks, which were broken down products of beef fat, appeared at 200°C, though they were not detected at 150°C or lower.

Table 3. Effect of Cooking on 2-DCB and 2-TCB in Irradiated Chicken

Sample	2-DCB			2-TCB			Weight
	Average[a)]	RSD[b)]	p[c)]	Average[a)]	RSD[b)]	p[c)]	loss
Weight basis	(μg/g weight)	(%)		(μg/g weight)	(%)		(%)
Raw	0.060	46		0.027	39		0
Browned	0.076	11	0.265	0.034	12	0.214	23.6
Fried	0.069	46	0.704	0.031	36	0.653	31.7
Fat basis	(μg/g fat)	(%)		(μg/g fat)	(%)		Fat (%)
Raw	0.59	16		0.27	16		10.3
Browned	0.64	15	0.511	0.29	19	0.748	12.0
Fried	0.41	27	0.099	0.19	23	0.083	16.1

a) Average of 5 experiments. *b*) Relative standard deviation. *c*) Values with t-test < 0.05 significantly different from the raw sample.

Table 4. Effect of Cooking on 2-DCB and 2-TCB in Irradiated Egg

Sample	2-DCB			2-TCB			Fat[a)]
	Average[a)]	RSD[b)]	p[c)]	Average[a)]	RSD[b)]	p[c)]	
Weight basis	(μg/g weight)	(%)		(μg/g weight)	(%)		(%)
Raw	0.053	8		0.031	15		5.3
Boiled	0.076	20	0.027	0.045	25	0.0236	7.3
Scrambled	0.111	16	0.002	0.066	12	0.0005	10.2
Pancake	0.020	8	0.365	0.011	14	0.5935	5.7
Fat basis	(μg/g fat)	(%)		(μg/g fat)	(%)		
Raw	1.01	11		0.59	19		
Boiled	1.08	31	0.600	0.63	35	0.5377	
Scrambled	1.11	22	0.320	0.65	14	0.0926	
Pancake	0.35	16	0.0002	0.20	17	0.0022	
Pancake adjusted[d)]	1.08	41	0.736	0.61	42	0.8350	2.1

a) Average of 5 experiments. *b*) Relative standard deviation. *c*) Values with t-test < 0.05 significantly different from the raw sample. *d*) 2-DCB and 2-TCB concentrations were calculated based on fat only from irradiated egg.

Table 5. Effect of Heating on Stability of 2-DCB and 2-TCB

Temperature (°C)	2-DCB (%)	2-TCB (%)
Room	100	100
100	99	94
150	62	56
200	11	5

Two reports are available on the detection of 2-alkylcyclobutanones in heated foods. Crone *et al*. reported that 2-DCB would be useful for the detection of irradiated cooked chicken products when the γ-ray irradiated chicken thighs were heated at 200°C for 25 min, and the internal temperature was 88°C.[11] It was also pointed out that cooking did not generate 2-DCB. Stewart *et al*. also a made pancake and sponge cake with irradiated liquid whole egg and concluded that 2-DCB could be detected easily,

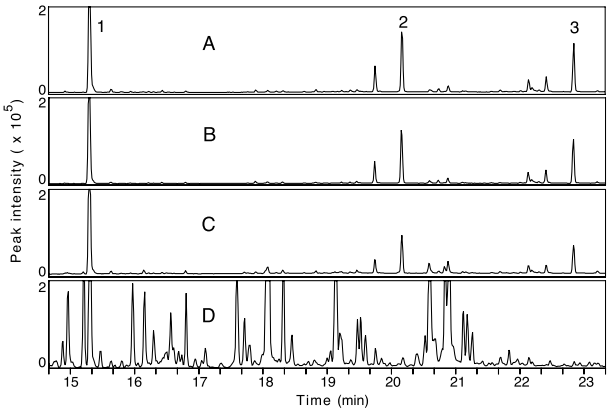

Fig. 3. Chromatograms of 2-Alkylcyclobutanones in Beef Fat Monitored under the Heated Condition at m/z 98

(A), room temperature; (B), heated at 100°C for 1 hr; (C), heated at 150°C for 1 hr; (D), heated at 200°C for 1 hr. Peak labels: 1, 2-cyclohexylcyclohexanone; 2, 2-DCB; 3, 2-TCB. Values with % represent relative concentration to non-heated sample.

whereas 2-TCB could not be unequivocally identified because of the dilution with other fatty ingredients.[26] This study would support these reports with the data of grilled chicken and pancakes and provide additional data for cooked beef.

Conclusion

The results indicated that both 2-DCB and 2-TCB could be detected in irradiated and cooked foods; thus, the analysis of the two compounds in those samples would be an indicator of the samples' irradiation history. The levels of the two compounds were mostly unaffected by cooking on a fat concentration basis. The two compounds are stable provided the inside temperature of sample remains under 100°C during cooking. The concentrations of the two compounds varied because of the loss of water and/or fat during cooking, and the addition of exogenous fat other than the sample. The expression for the fat concentration basis would be suitable to stimulate the level in irradiated raw samples when the samples were cooked without other fat sources. The stability of the two compounds seemed to be similar, since the ratios of 2-DCB/2-TCB were not changed with cooking and both of the compounds were reduced in concentration in the same manner. GC/MS chromatograms indicated that serious interferences were not detected due to cooking, although the surfaces of the samples were heated to around 200°C; thus, the cooking itself was not an obstacle to the determination of the two compounds. The study concludes that the analysis of 2-DCB and 2-TCB in the cooked samples would be a reliable indicator for the irradiation history of raw meats, poultry and egg.

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REFERENCES

1) Diehl, J. F. (2001) Achievements in food irradiation during the 20th century. In *Irradiation for Food Safety and Quality* (Loaharanu, P. and Thomas, P., Eds.), Technomic Publishing Company, Inc., Lancaster, pp. 1–16.
2) Molins, R. A. (ed.) (2001) *Food irradiation: principles and applications*, Wiley-InterScience, New York.
3) WHO (1981) Wholesomeness of irradiated food: report of a joint FAO/IAEA/WHO Expert Committee. *World Health Organ. Tech. Rep. Ser.*, **659**, 1–34.
4) Pauli, G. H. and Tarantino, L. M. (1995) FDA regulatory aspects of food irradiation. *J. Food Prot.*, **58**, 209–212.
5) Roberts, P. B. (2001) Harmonaization of Regulations on food irradiation in accordance with the SPS and TBT agreements. In *Irradiation for Food Safety and Quality* (Loaharanu, P. and Thomas, P., Eds.), Technomic Publishing Company, Inc., Lancaster, pp. 17–25.
6) Vounakis, H. (2001) Food irradiation harmonization in the European Union. In *Irradiation for Food Safety and Quality* (Loaharanu, P. and Thomas, P., Eds.), Technomic Publishing Company, Inc., Lancaster, pp. 26–31.
7) McMurray, C. H., Stewart, E. M., Gray, R. and Pearce, J. (eds.) (1996) *Detection methods for irradiated foods Current status*, The Royal Society of Chemistry, Cambridge, pp. 3–414.
8) Standardization, E. C. F. (2003) *Foodstuffs-Detection of irradiated food containing fat- Gas chromatographic/mass spectrometric analysis of 2-alkylcyclobutanones EN1785*, European Committee for Standardization, Brussels.
9) Stevenson, M. H., Crone, A. V. J. and Hamilton, J. T. G. (1990) Irradiation detection. *Nature* (London), **344**, 202–203.
10) Boyd, D. R., Crone, A. V. J., Hamilton, J. T. G., Hand, M. V., Stevenson, M. H. and Stevenson, P. J. (1991) Synthesis, characterization and potential use of 2-dodecylcyclobutanone as marker for irradiated chicken. *J. Agric. Food Chem.*, **39**, 789–792.
11) Crone, A. V. J., Hamilton, J. T. G. and Stevenson, M. H. (1992) Effect of storage and cooking on the dose response of 2-dodecylcyclobutane,a potential marker for Irradiated chicken. *J. Sci. Food Agric.*, **58**, 249–252.
12) Crone, A. V. J., Hand, M. V., Hamilton, J. T. G., Sharma, N. D., Boyd, D. R. and Stevenson, M. H. (1993) Synthesis, characterization and use of 2-tetradecylcyclobutanone together with other cyclobutanones as markers for irradiated liquid whole egg. *J. Sci. Food Agric.*, **62**, 361–367.
13) Obana, H., Furuta, M. and Tanaka, Y. (2005) Analysis of 2-alkylcyclobutanones with accelerated solvent extraction to detect irradiated meat and fish. *J. Agric. Food Chem.*, **53**, 6603–6608.
14) Richter, B. E., Ezzell, J. L., Felix, D., Roberts, K. A. and Later, D. W. (1996) An accelerated solvent extraction system for the rapid preparation of environmental organic compounds in soil. *Am. Lab.*, **27**, 1033–1039.
15) Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N.

L., Avdalovic, N. and Pohl, C. (1996) Accelerated solvent extraction: A technique for sample preparation. *Anal. Chem.*, **68**, 1033–1039.
16) Obana, H., Kikuchi, K., Okihashi, M. and Hori, S. (1997) Determination of organophosphorus pesticides in foods using an accelerated solvent extraction system. *Analyst* (London), **122**, 217–220.
17) Okihashi, M., Obana, H. and Hori, S. (1998) Determination of N-methylcarbamate pesticides in foods using an accelerated solvent extraction with a mini-column cleanup. *Analyst* (London), **123**, 711–714.
18) Adou, K., Bontoyan, W. R. and Sweeney, P. J. (2001) Multiresidue method for the analysis of pesticide residues in fruits and vegetables by accelerated solvent extraction and capillary gas chromatography. *J. Agric. Food Chem.*, **49**, 4153–4160.
19) Heemken, O. P., Theobald, N. and Wenclawiak, B. W. (1997) Comparison of ASE and SFE with Soxhlet, sonication, and methanolic saponification extractions for the determination of organic micropollutants in marine particulate matter. *Anal. Chem.*, **69**, 2171–2180.
20) Richter, B. E., Ezzell, J. L., Knowles, D. E., Hoefler, F., Mattulat, A. K. R., Scheutwinkel, M., Waddell, D. S., Gobran, T. and Khurana, V. (1997) Extraction of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans from environmental samples using accelerated solvent extraction (ASE). *Chemosphere*, **34**, 975–987.
21) Knoll, N., Weise, A., Claussen, U., Sendt, W., Marian, B., Glei, M. and Pool-Zobel, B. L. (2006) 2-Dodecylcyclobutanone, a radiolytic product of palmitic acid, is genotoxic in primary human colon cells and in cells from preneoplastic lesions. *Mutat. Res*, **594**, 10–19.
22) Raul, F., Gosse, F., Delincee, H., Hartwig, A., Marchioni, E., Miesch, M., Werner, D. and Burnouf, D. (2002) Food-borne radiolytic compounds (2-alkylcyclobutanones) may promote experimental colon carcinogenesis. *Nutr. Cancer*, **44**, 189–191.
23) Sommers, C. H. (2003) 2-dodecylcyclobutanone does not induce mutations in the Escherichia coli tryptophan reverse mutation assay. *J. Agric. Food Chem.*, **51**, 6367–6370.
24) Gadgil, P. and Smith, J. S. (2004) Mutagenicity and acute toxicity evaluation of 2-dodecylcyclobutanone. *J. Food Sci.*, **69**, C713–C716.
25) Sommers, C. H. and Schiestl, R. H. (2004) 2-Dodecylcyclobutanone does not induce mutations in the *Salmonella* mutagenicity test or intrachromosomal recombination in *Saccharomyces* cerevisiae. *J. Food Prot.*, **67**, 1293–1298.
26) Stewart, E. M., McRoberts, W. C., Hamilton, J. T. G. and Graham, W. D. (2001) Isolation of lipid and 2-alkylcyclobutanones from irradiated foods by supercritical fluid extraction. *J. AOAC Int.*, **84**, 976–986.
27) European Commission, Health and consumer protection directrate-general (2000) *List of national authorisations of food and food ingredients which may be treated with ionising radiation (Art 4 (6)), with the exception of products listed in the annex of directive 1999/3/EC*, European Commission, Brussels, Belgium.
28) The U.S. Government Printing Office (2005) Code of Federal Regulations. In *Sec. 179.26 Ionizing radiation for the treatment of food*, Office of the Federal Register National Archives and Records Administration, Washington D. C., pp. 451–453.
29) Erney, D. R., Gillespie, A. M., Gilvydis, D. M. and Poole, C. F. (1993) Explanation of the matrix-induced chromatographic response enhancement of organophophorus pesticides during open tublar column gas chromatogrphy with slpitless or hot on-column injection and flame photometric detection. *J. Chromatogr.*, **638**, 57–63.