JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometric Analysis of 2-Alkylcyclobutanones in Irradiated Chicken by Precolumn Derivatization with Hydroxylamine

Yuran Ye,[†] Hanxia Liu,[‡] Peter Horvatovich,[§] and Wan Chan*[,†]

[†]Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR

[‡]Chinese Academy of Inspection and Quarantine, Beijing 100123, China

[§]Department of Analytical Biochemistry, Centre for Pharmacy, University of Groningen, Groningen, The Netherlands

ABSTRACT: Food irradiation is a common preservation method that is used in many countries. The ability to identify irradiated food is important for assuring compliance with regulatory policies, such as food labeling requirements, and for informed consumer choice. There is thus a significant demand for analytical methods of high sensitivity and selectivity to identify irradiated food, especially for foods subjected to low-dose irradiation and for processed or composite foods that contain small quantities of irradiated ingredients. 2-Alkylcyclobutanones (2-ACBs) are uniquely formed during food irradiation and have been adopted by the European Committee for Standardization as signature biomarkers for the identification of irradiated foods. We now report the development of a novel assay for quantification of 2-ACBs in γ-irradiated food by liquid extraction of fat content followed by precolumn derivatization and liquid chromatography–tandem mass spectrometric (LC-MS/MS) detection. Precolumn derivatization with hydroxylamine introduced a polar functional group into the otherwise nonpolar 2-ACBs, which greatly enhanced ESI-MS response. The method was validated for extraction efficiency, precision, accuracy, and detection limit. In comparison with the current GC-MS based European official method (EN1785:2003) for 2-ACBs determination, our new LC-MS/MS method offers a more efficient sample processing protocol with reduced solvent consumption. More importantly, the combination of chemical derivatization and LC-MS/MS detection significantly enhanced the analytical sensitivity of the method, which allows confident identification of food irradiated with as little as 10 Gy. To the best of our knowledge, this is the first report of 2-ACB determination by LC-MS/MS and the first analytical method allowing confident identification of irradiated food at dosage of down to 10 Gy.

KEYWORDS: *γ-irradiated food, 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone, LC-MS/MS, precolumn derivatization*

INTRODUCTION

Irradiation is a food preservation method that is used in many countries. Currently, food irradiation has been adopted in at least 39 different countries and on 49 different products to control microbial growth and improve the shelf life.[1] Increasing and widespread application of ionizing radiation in the food industry has led to a growing demand for robust analytical methods to identify irradiated foodstuff for enforcement of labeling policies and informing consumers.[2,3]

Current methods for irradiated food identification include the nontargeted spectroscopic approach and targeted analysis of radiolytic products.[2−4] The goal of these analyses is to quantify markers of radiation exposure, which are largely comprised of oxidized biomolecules that are unique to the exposure. For example, 2-alkylcyclobutanones (2-ACBs) are hydrocarbon chain-containing cyclobutanone derivatives that are formed in abundance when triglycerides are exposed to ionizing radiation, as opposed to other biologically relevant oxidative stresses.[5−7] It has been demonstrated that the level of 2-ACBs is unaffected by cooking methods, including streaming and roasting,[8] or by storage conditions ($t_{1/2}$ = 6 months at −20 °C).[9,10]

Among the 2-ACBs, two predominant members have been adopted by the European Committee for Standardization as signature markers of food irradiation: 2-dodecylcyclobutanone (2-DCB, Figure 1) from radiolysis of palmitic acid and 2-tetradecylcyclobutanone (2-TCB) derived from stearic acid.[11] Currently, the European official method (EN1785:2003) based on gas chromatography–coupled mass spectrometric (GC-MS)

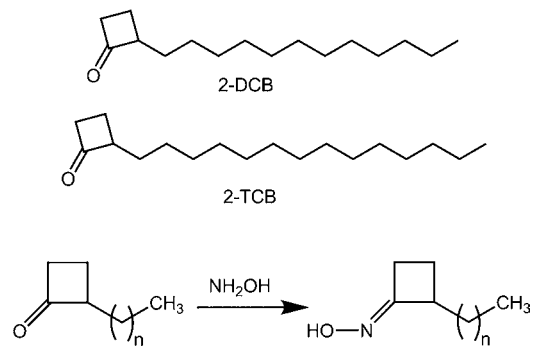

Figure 1. Chemical structures of 2-DCB and 2-TCB as well as their reaction with HA, forming stable ketoximes of enhanced ESI-MS response for LC-ESI-MS/MS analysis.

Received: April 2, 2013
Revised: May 27, 2013
Accepted: May 28, 2013
Published: May 28, 2013

detection of 2-DCB and 2-TCB has been widely adopted for the identification of irradiated food, meat, and meat products in particular.[6,10−14] However, in spite of its validation in interlaboratory tests with different food commodities, the method has met with serious limitations for the growing demands of monitoring irradiated food.[2,3,15] Specifically, the method is insensitive and laborious. To accommodate the evolving technology for food irradiation, there is a need for increased analytical sensitivity for the analysis of food that is exposed to radiation of below 100 Gy and for the analysis of food products containing irradiated ingredients.[3] Further, the Soxhlet extraction and flash column chromatography cleanup required for EN1785:2003 is both time- and solvent-consuming.[3,15−17]

To address these problems, we undertook the development of a method that both simplified sample preparation and increased the sensitivity of detecting 2-ACBs by an order of magnitude. The first goal was to explore the feasibility of simplifying the sample cleanup process in the standard method EN1785, with the aim of substituting the Soxhlet extraction and flash column chromatography with a more environmentally friendly and higher efficiency solvent extraction-coupled solid phase extraction approach. The second goal was to develop a liquid chromatography−coupled tandem mass spectrometry (LC-MS/MS) method with higher sensitivity for quantification of radiation-induced 2-ACBs, with the aim of lowering the detection of irradiation to less than the current limit of 100 Gy. While LC-MS/MS has been widely used for analyzing polar compounds in many applications, such as clinical toxicology and food safety testing,[18−23] the analysis of 2-ACBs by LC-MS/MS has not been reported in the literature, to the best of our knowledge. The low polarity and lack of an easily ionizable functional group have hampered the detection of 2-ACBs by electrospray ionization mass spectrometry (ESI-MS).

We have addressed these issues by an approach that involves derivatization of 2-ACBs as oximes of higher polarity and quantification by LC-ESI-MS/MS. Hydroxylamine (HA, Figure 1) reacts with ketones and aldehydes to form stable oximes. As a result, HA has been applied in derivatizing steroids to enhance detection by LC-MS/MS.[20,22,24] Here we show that HA forms stable oxime derivatives with 2-DCB and 2-TCB, which facilitates their detection by ESI-triple quadrupole mass spectrometry. The approach provides single-digit femtomole sensitivity for identification of 2-DCB and 2-TCB in samples that were exposed to 10 Gy of γ-radiation.

MATERIALS AND METHODS

Chemicals and Reagents. All chemicals and reagents were of the highest purity available and were used without further purification unless noted otherwise. 2-Dodecylcyclobutanone (2-DCB), 2-tetradecylcyclobutanone (2-TCB), 2-(2-ethylhexyl)-cyclohexanone (2-ECH), and hydroxylamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Chicken samples were obtained from a local market and tested to be 2-ACBs free. HPLC-grade acetonitrile was purchased from Honeywell Burdick & Jackson (Muskegon, MI). *n*-Hexane was obtained from Mallinckrodt (St. Louis, ME). Water was produced by a Milli-Q Ultrapure water system with the water outlet operating at 18.2 MΩ (Millipore, Billerica, USA).

Preparation of Standards. Stock solution mixture of 2-DCB and 2-TCB at 0.95 and 1.07 mg/mL, respectively, was prepared in acetonitrile and stored at −80 °C until diluted and derivatized with HA for LC-MS/MS analysis. The HA derivative of the internal standard 2-ECH (2.10 mg/mL) was prepared in overnight reactions with an excess of HA at ambient temperature. Working standard solutions of 2-DCB-HA (1.19−238.4 ng/mL) and 2-TCB-HA (1.33−266.4 ng/mL) were prepared daily by spiking the 2-DCB and 2-TCB mixture to blank nonirradiated sample extracts, derivatized, and LC-MS/MS analyzed in parallel with the sample extracts from irradiated samples. The internal standard, 2-ECH-HA, was spiked to individual standard solutions at final concentration of 0.11 mg/mL prior to LC-MS/MS analysis.

Irradiation Procedure. Gamma irradiation was carried out using a ^{137}Cs irradiator (Gammacell-1000 Elite, MDS Nordion, Canada) at ambient temperature. The dosage rate of the irradiator was calibrated by using a radiochromic film. Chicken samples (n = 5) were placed separately in plastic bags and exposed to γ-radiation over a range of 10−4000 Gy at a dose-rate of 3.3 Gy/min. Nonirradiated chicken was used as the control samples. The samples were stored at −80 °C prior to sample pretreatment.

Sample Preparation. *Liquid Extraction.* The chicken skin of high fat content was used in this study. The chicken samples (∼3 g) were cut into small pieces and homogenized in 3 mL of water. The mixtures after centrifugation at 7000 rpm for 5 min were put at −80 °C freezer for overnight, after which the 2-ACBs-containing semisolid fat content in the frozen sample homogenate was excised. Approximately 1 g of the isolated fat was accurately weighted and extracted three times with 10 mL of acetonitrile. The acetonitrile supernatants were combined, dried with anhydrous magnesium sulfate, and evaporated to dryness under a gentle stream of nitrogen at 35 °C. The residue after evaporation of acetonitrile was dissolved in 1 mL of *n*-hexane and loaded onto a silica SPE column for sample cleanup.

SPE Cleanup. The sample extract was loaded onto a silica Sep-Pak cartridge (690 mg sorbent per cartridge, Waters Corp., Milford, MA) that was conditioned with 5 mL of *n*-hexane/methyl *tert*-butyl ether (95:5 v/v) followed by 10 mL of *n*-hexane. After loading the sample, the column was washed with 5 mL of *n*-hexane before eluting with 7 mL of hexane/methyl *tert*-butyl ether (98:2 v/v). This eluate was collected and evaporated to dryness under a stream of nitrogen at 37 °C. The residue was dissolved in 500 μL of acetonitrile and centrifuged at 13800 rpm for 5 min prior to chemical derivatization.

Derivatization of 2-ACBs with HA. To 50 μL of the reconstituent was added 5 μL of the internal standard (2.10 μg/mL) and 45 μL of HA solution (27.8 mg/mL). The sample mixtures were incubated at room temperature for overnight to allow chemical reaction of 2-ACBs with HA before analyzed by LC-MS/MS.

LC-MS/MS Analysis. Derivatized sample extract (10 μL) was analyzed by LC-MS/MS. Chromatographic separation was performed on an Agilent 1200 series HPLC system (Palo Alto, CA) equipped with an Agilent Eclipse Plus C18 column (50 mm × 3.0 mm, 1.8 μm) heated at 40 °C. The mobile phase consisted of two solvents, with solvent A being water containing 0.4% formic acid, solvent B being acetonitrile. Gradient elution at constant flow rate of 800 μL/min was adopted for the chromatography, with the solvent gradient as follows: 50% B programmed linearly to 100% B in 8 min and held for another 5 min. The first 3 min of the column effluent was diverted away from the MS by a Valco switch valve (VICI, Houston, TX) so as to minimize the ESI-MS source contamination.

The HPLC was coupled to an API4000$^+$ triple quadrupole (QqQ) mass spectrometer (AB Sciex, Foster City, CA) with a turbo V ion source. MS data were acquired in positive electrospray ionization (ESI) mode with the ionization source parameters optimized as follows: ionspray voltage, 5500 V; declustering potential, 70 V; entrance potential, 8; and collision energy, 50. The ion source gas I (GSI), gas II (GSII), curtain gas (CUR), collision gas (CAD), and the temperature of GSII were set at 40, 20, 20, 5, and 400 °C, respectively. The multiple reaction monitoring (MRM) transitions for the 2-DCB-HA, 2-TCB-HA, and the internal standard 2-ECH-HA derivatives were set as follows: 2-DCB-HA: m/z 254→82, 254→96, and 254→110; 2-TCB-HA: m/z 282→82, 282→96, and 282→110. 2-ECH-HA: m/z 226→69. The dwell time for each transition was set at 50 ms.

dx.doi.org/10.1021/jf401446n | J. Agric. Food Chem. 2013, 61, 5758−5763

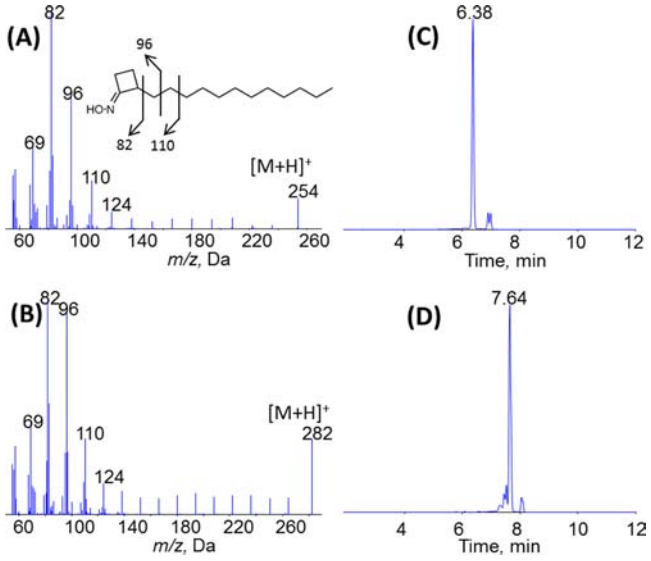

Figure 2. Product ion spectra of the $[M + H]^+$ ion of the 2-DCB-HA (A, m/z 254) and 2-TCB-HA (B, m/z 282), as well as their extracted ion chromatograms at MRM transitions of m/z 254→82 (C) and m/z 278→82 (D), respectively. Shown in the inset of (A) is the cleavage reactions for the formation of major fragment ions in the product ion scan of the 2-DCB-HA derivative.

RESULTS AND DISCUSSION

LC-MS/MS Characterization of the Hydroxylamine Derivatives of 2-ACBs. LC-MS/MS is an inherently sensitive and selective method for analyzing polar compounds.[18,19,21,23,25] However, nonpolar compounds do not ionize well in ESI-MS and are thus difficult to analyze by LC-MS/MS.[20,24,26] To solve this problem for nonpolar 2-ACBs, which are currently limited to GC-MS analysis,[1−3] we adopted a chemical derivatization approach convert the carbonyl functional groups of 2-ACBs to stable and polar oximes for enhanced ESI-MS response (Figure 1).[20,24,26]

Results from LC-MS/MS analysis showed that the 2-ACBs derivatives were stable up to 60 h of storage at room temperature (data not shown). Collision-induced dissociation of the $[M + H]^+$ ion of the 2-DCB-HA oxime derivative at m/z 254 led to the formation of ion peak at m/z 82 as the major fragment (Figure 2A). We also observed cyclobutanone oxime fragment ions at m/z 96, 110, and 124, which contain side chains of different chain lengths at the 2′-C. Similar fragment ions at m/z 82, 96, 110, and 124 were also observed for the 2-TCB-HA oxime derivative (Figure 2B).

The fragment ions of highest abundance were selected for the quantitative analysis of 2-ACBs by multiple reaction monitoring mode (MRM) of triple-quadrupole mass spectrometry. The summation of the chromatographic peaks at MRM transitions of m/z 254→82, 254→96, and 254→110 was used for the quantification of the 2-DCB-HA oxime derivative. Similarly, the summation of the chromatographic peaks at MRM transitions of m/z 282→82, 282→96, and 282→110 was used for the quantification of the 2-TCB-HA oxime derivative. Under the chromatographic conditions described previously, the oxime derivatives of 2-DCB and 2-TCB eluted at retention times of 6.4 min (Figure 2C) and 7.6 min, respectively (Figure 2D).

Optimization of Derivatization Conditions. The derivatization reaction conditions, including the reaction temperature, reaction time, and the ratio of the derivatization agent to analyte, were optimized for the highest reaction yield of the oxime derivatives. The effects of reaction time and temperature on the derivatization yield were studied over 1−25 h and 25−80 °C, respectively. Our results showed that a 10 h of reaction at ambient temperature gave the best MS/MS responses (Figure 3A). In contrast to previous studies showing that elevated temperature favors oxime formation,[15,22,24] we observed that ambient temperature is the optimal condition for maximizing the derivatization yield of the 2-ACB oxime derivatives. The basis for this observed discrepancy includes potential instability of the four-membered ring oxime derivative at the elevated temperature.

The effect of the molar ratio of HA to 2-ACBs on the derivatization efficiency was also investigated. By analyzing reaction mixtures consisting of HA and 2-ACBs in molar ratio from 50:1 to 20000:1, it was found that the highest analytical signal was achieved with molar ratio of HA to 2-ACB above 10000 (Figure 3B). A molar ratio of HA to 2-ACBs of 10000:1 was selected and used in all studies.

Linearity, Recovery, Reproducibility, Accuracy, and Detection Limits. The matrix matched calibration method which corrects for the effect of sample matrix on the chemical derivatization and on LC-MS/MS analysis was adopted. The linearity of the LC-MS/MS assay for 2-DCB and 2-TCB was

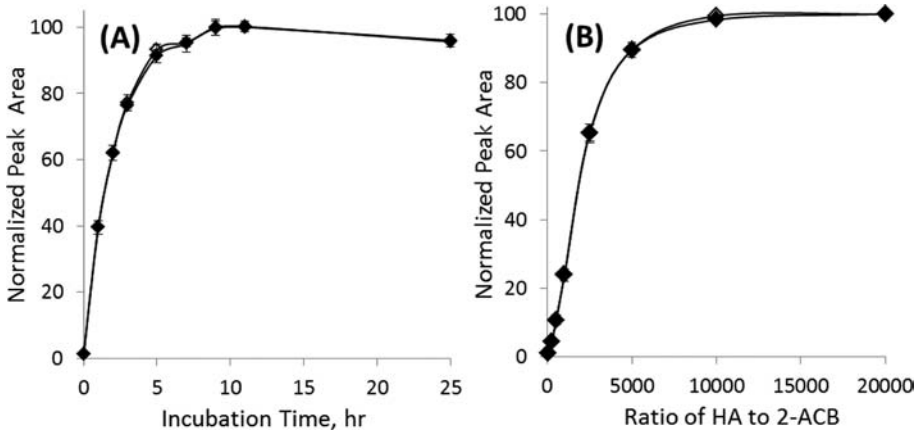

Figure 3. Influence of (A) reaction time and (B) molar ratios of HA over 2-ACBs on the derivatization reaction yield for 2-DCB (◆) and 2-TCB (◇). The data represent mean ± SD for three independent experiments performed at room temperature.

dx.doi.org/10.1021/jf401446n | J. Agric. Food Chem. 2013, 61, 5758−5763

Table 1. Intraday, Interday Precision and Accuracy for the Determination of 2-DCB and 2-TCB in Blank Sample Extracts

		precision		accuracy	
	nominal concentration (nM)	intraday[a] (% RSD)	interday[a] (% RSD)	measured concentration (nM)	error (%)
2-DCB	10	5.14	15.5	8.44 ± 0.43	15.6
	50	2.49	3.76	50.20 ± 1.25	0.45
	500	2.10	1.93	496.71 ± 10.43	0.66
2-TCB	10	11.41	12.6	8.58 ± 0.98	14.2
	50	4.58	7.11	49.06 ± 2.25	1.88
	500	1.23	4.16	518.25 ± 6.39	3.65

[a] $n = 5$.

Table 2. Comparison of the Limit of Detection of the Developed LC-MS/MS Method and Other Methods Reported in the Literature for 2-DCB Detection

analysis	extraction	cleanup	LOD, ng/g fat	minimum dosage detected, kGy	ref
ELISA	Soxhlet	florisil	64	2.5	28
GC-MS[a]	ASE	silica	100	0.5	29
GC-MS[b]	SFE	silica	70	0.1	13
GC-MS[c]	Soxhlet	florisil	10	1	15
LC-MS/MS[d]	direct extraction	silica SPE	0.2 (1.76 pg on-column)	0.01	current
HPLC-FLD[e]			5 ng on-column	NA	31
LC-MS/MS[f]			0.24 ng on-column	NA	current

[a]Electron impact ionization GC-MS. [b]Chemical ionization GC-MS. [c]Electron impact ionization GC-MS with precolumn derivatization with pentafluorophenyl hydrazine. [d]LC-MS/MS method with precolumn derivatization with hydroxylamine. [e]HPLC-fluorescence detection of 2-DCB after reduction to alcohol and labeling with 7-diethylamine 3-carbonylazide. [f]LC-MS/MS method without precolumn derivatization.

found to be linear over the concentration range of 1.19−238.4 ng/mL and 1.33−266.4 ng/mL, respectively. The recovery as determined by spiking 2-DCB (23.84 ng/mL, 119.20 μg/mL, and 238.4 ng/mL) and 2-TCB (26.64, 133.20, and 266.40 ng/mL) into a blank sample matrix, followed by derivatization and analyzsis using the method described above. Recovery was 62.1 ± 6.5% and 68.2 ± 4.3%, respectively. Measurements of 2-DCB and 2-TCB in irradiated samples were thus corrected by a factor of 1.61 and 1.47, respectively, to correct for their respective loss during the sample workup processes.

The instrumental precision was evaluated by analyzing blank sample extracts spiked with 2-ACBs at three different concentrations (2-DCB, 23.84, 119.20, and 238.4 ng/mL; 2-TCB, 26.64, 133.20, and 266.40 ng/mL) on the same day (n = 5) and over five separate days. The intraday precision of the assay at low, medium, and high concentrations was found to occur with a peak area standard deviation of less than 11.4% for both the 2-DCB and 2-TCB (n = 5, Table 1). Over a period of 1 week, the reproducibility of the method for the 2-ACBs determination varied by less than 15.5% (n = 5). The method accuracy, as determined by analyzing spiked samples with three different concentrations of 2-ACBs in sample extracts (n = 5), was found to be less than 15.6% and 14.2% for 2-DCB and 2-TCB, respectively (Table 1). The method parameters for the analysis of 2ACBs by LC-MS/MS are shown in Table 1.

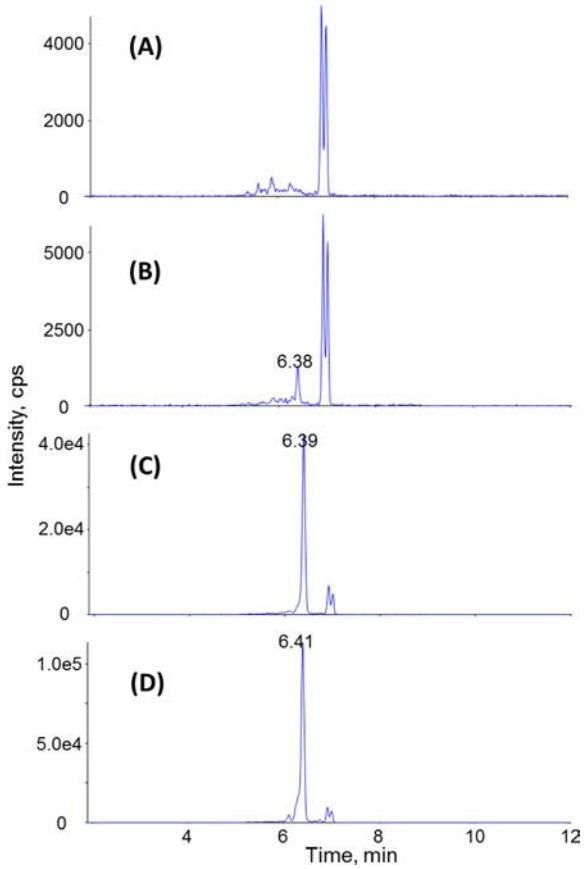

Figure 4. Typical LC-MS/MS chromatograms from multiple reaction monitoring of the m/z 254 → 82 for 2-DCB-HA in (A) nonirradiated chicken sample and in chicken samples exposed to (B)10 Gy, (C) 500 Gy, and (D) 2000 Gy of γ-radiation, respectively. The ketoxime derivatives of 2-DCB was eluted at 6.4 min under the chromatographic condition described under Materials and Methods.

The detection limits for 2-DCB and 2-TCB, at a signal-to-noise ratio of 3,[19,26] were calculated as 1.76 pg/injection (0.18 ng/g fat) and 3.14 pg/injection (0.31 ng/g fat), respectively (Table 2). This is at least 50 times lower than that of existing analytical methods for quantification of 2-ACBs.[6]

Determination of 2-ACBs in γ-Irradiated Chicken Samples. The developed method combining chemical derivatization and LC-MS/MS detection was applied to the determination of 2-ACBs in γ-irradiated chicken samples. As depicted in Figure 4, dose-dependent formation of 2-ACBs was observed in the irradiated chicken samples. Our results showed that γ-irradiation produced 2-DCB and 2-TCB at radiation yield of 104.7 ng per gram of fat per kGy (r^2 = 0.985) and 45.0

dx.doi.org/10.1021/jf401446n | J. Agric. Food Chem. 2013, 61, 5758−5763

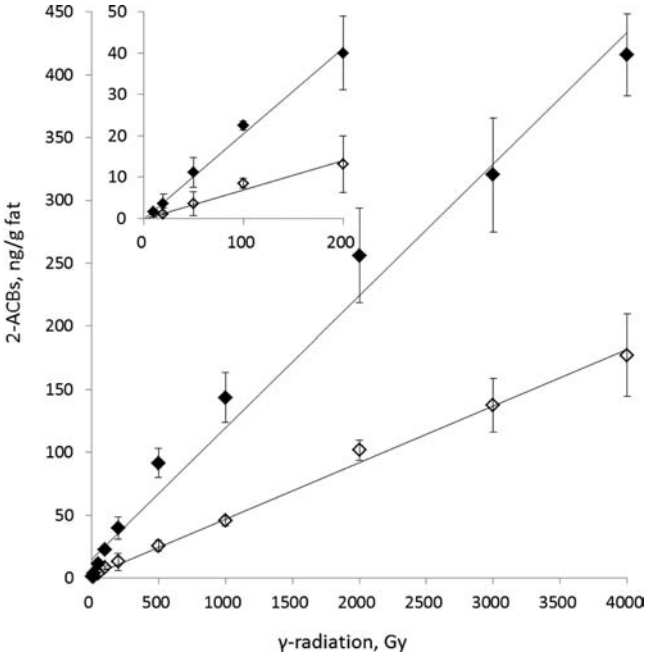

Figure 5. Formation of 2-DCB (◆) and 2-TCB (◇) in γ-irradiated chicken samples. Samples were exposed to γ-radiation from 10 to 4000 Gy at ambient temperature and analyzed by LC-MS/MS analysis as described under Materials and Methods. The data represent means ± SD of five independent experiments. Fitting the data by linear regression yielded lines with the following equations: 2-DCB, $y = 0.105x + 15.07$ ($r^2 = 0.985$); 2-TCB, $y = 0.045x + 2.12$ ($r^2 = 0.996$). Shown in the inset is the dose-dependent formation of 2-ACBs in chicken samples exposed to low dose γ-irradiation between 10 and 200 Gy.

ng per gram of fat per kGy ($r^2 = 0.996$, Figure 5), respectively. These results are comparable to the earlier reported radiation-induced formation of 2-ACBs in γ-irradiated chicken samples.[1,10,27]

Using the GC-MS approach, Tewfik et al. reported dose-dependent formation of 2-DCB in γ-irradiated chicken sample at a formation frequency of 110 ng/g lipid.[27] Our result of 104.7 ng 2-DCB per gram fat per kGy γ-irradiation is in good agreement with the reported frequencies of γ-radiation-induced formation of 2-DCB in chicken samples. The molar ratio of 2-DCB to 2-TCB as determined in this study was 2.3:1, which is consistent with the results obtained by Elliott et al. (2.1:1).[28] The higher radiation yield of 2-DCB than that of 2-TCB is in line with the higher concentration of their corresponding precursor fatty acids (i.e., palmitic acid and stearic acid) in chicken samples.[29,30] These results suggest that our method is suitable for the determination of radiation induced 2-ACBs in irradiated food.

In this study, no 2-ACBs were detected in the nonirradiated chicken samples using the developed LC-MS/MS method (Figure 4A). This observation is consistent with the majority of results reported in previous studies showing that 2-ACBs were only identified in irradiated foodstuff,[3] with an exception based on the recent studies reported by Variyar et al., which revealed the possibility of 2-ACBs in nonirradiated cashew nuts and nutmeg.[12] Our new LC-MS/MS method combining precolumn derivatization with SPE cleanup has the enhanced sensitivity and selectivity necessary to resolve these discrepancies, and it should serve an alternative to the existing GC-MS based European official method (EN1785:2003) for verifying the existence of 2-ACBs in nonirradiated food and in food exposed to low dosage of ionizing radiation.

In conclusion, we have developed and validated a novel LC-MS/MS method for quantification of 2-ACBs as markers of food irradiation. One of the key features of the assay is that derivatization with hydroxylamine dramatically increases the analytical sensitivity for the nonpolar 2-ACBs. The enhanced analytical sensitivity allows confident identification of 2-ACBs in food samples exposed to doses of γ-radiation approaching 10 Gy, which overcomes one of the major limitations of the current GC-MS method for irradiated food identification. The other advantage of our method is that the sample processing time as well as the solvent consumption can be significantly reduced. It is anticipated that the method will facilitate the growing market for food irradiation.

AUTHOR INFORMATION

Corresponding Author

*Phone: 852-2358-7370. Fax: 852-2358-1594. E-mail: chanwan@ust.hk.

Funding

This research was supported by startup funding from The Hong Kong University of Science and Technology and Direct Allocation Grant (DAG12SC02) from the Research Grant Council of Hong Kong.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank AB Sciex for providing the LC-MS/MS system for this research. The food irradiation experiments were performed on a gamma irradiator in the Division of Life Science, HKUST.

REFERENCES

(1) Stewart, E. M.; McRoberts, W. C.; Hamilton, J. T.; Graham, W. D. Isolation of lipid and 2-alkylcyclobutanones from irradiated foods by supercritical fluid extraction. *J. AOAC Int.* **2001**, *84*, 976−986.

(2) Delincee, H. Analytical methods to identify irradiated food—a review. *Radiat. Phys. Chem.* **2002**, *63*, 455−458.

(3) Crews, C.; Driffield, M.; Thomas, C. Analysis of 2-alkylcyclobutanones for detection of food irradiation: current status, needs and prospects. *J. Food Compos. Anal.* **2012**, *26*, 1−11.

(4) Zanardi, E.; Caligiani, A.; Padovani, E.; Mariani, M.; Ghidini, S.; Palla, G.; Ianieri, A. Detection of irradiated beef by nuclear magnetic resonance lipid profiling combined with chemometric techniques. *Meat Sci.* **2013**, *93*, 171−177.

(5) Ndiaye, B.; Horvatovich, P.; Miesch, M.; Hasselmann, C.; Marchioni, E. 2-Alkylcyclobutanones as markers for irradiated foodstuffs. III. Improvement of the field of application on the EN 1785 method by using silver ion chromatography. *J. Chromatogr., A* **1999**, *858*, 109−115.

(6) Sin, D. W.; Wong, Y. C.; Yao, W. Y. Analysis of gamma-irradiated melon, pumpkin, and sunflower seeds by electron paramagnetic resonance spectroscopy and gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **2006**, *54*, 7159−7166.

(7) Gadgil, P.; Hachmeister, K. A.; Smith, J. S.; Kropf, D. H. 2-Alkylcyclobutanones as irradiation dose indicators in irradiated ground beef patties. *J. Agric. Food Chem.* **2002**, *50*, 5746−5750.

(8) Lee, J.; Kausar, T.; Kwon, J. H. Characteristic hydrocarbons and 2-alkylcyclobutanones for detecting gamma-irradiated sesame seeds after steaming, roasting, and oil extraction. *J. Agric. Food Chem.* **2008**, *56*, 10391−10395.

(9) Obana, H.; Furuta, M.; Tanaka, Y. Detection of irradiated meat, fish and their products by measuring 2-alkylcyclobutanones levels after frozen storage. *Shokuhin Eiseigaku Zasshi* **2007**, *48*, 203−206.

(10) Victoria, A.; Crone, J.; Hamilton, J. T. G.; Stevenson, M. H. Detection of 2-dodecylcyclobutanone in radiation-sterilized chicken meat stored for several years. *Int. J. Food Sci. Technol.* **1992**, *27*, 691−696.
(11) *Foodstuffs−Detection of Irradiated Food Containing Fats Gas Chromatographic/Mass Spectrometric Analysis of 2-Alkylcyclobutanones*; EN1785; European Committee for Standardization: Brussels, 2003.
(12) Variyar, P. S.; Chatterjee, S.; Sajilata, M. G.; Singhal, R. S.; Sharma, A. Natural existence of 2-alkylcyclobutanones. *J. Agric. Food Chem.* **2008**, *56*, 11817−11823.
(13) Horvatovich, P.; Werner, D.; Jung, S.; Miesch, M.; Delincee, H.; Hasselmann, C.; Marchioni, E. Determination of 2-alkylcyclobutanones with electronic impact and chemical ionization gas chromatography/mass spectrometry (GC/MS) in irradiated foods. *J. Agric. Food Chem.* **2006**, *54*, 1990−1996.
(14) Zanardi, E.; Battaglia, A.; Ghidini, S.; Conter, M.; Badiani, A.; Ianieri, A. Evaluation of 2-alkylcyclobutanones in irradiated cured pork products during vacuum-packed storage. *J. Agric. Food Chem.* **2007**, *55*, 4264−4270.
(15) Sin, D. W.-m.; Wong, Y.-c.; Yao, M. W.-y. Application of pentafluorophenyl hydrazine on detecting 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone in irradiated chicken, pork and mangoes by gas chromatography−mass spectrometry. *Eur. Food Res. Technol.* **2006**, *222*, 674−680.
(16) Horvatovich, P.; Miesch, M.; Hasselmann, C.; Marchioni, E. Supercritical fluid extraction of hydrocarbons and 2-alkylcyclobutanones for the detection of irradiated foodstuffs. *J. Chromatogr., A* **2000**, *897*, 259−68.
(17) Tewfik, I. H.; Ismail, H. M.; Sumar, S. A rapid supercritical fluid extraction method for the detection of 2-alkylcyclobutanones in gamma-irradiated beef and chicken. *Lebensm.−Wiss. Technol.* **1998**, *31*, 366−370.
(18) Chan, W.; Zheng, Y.; Cai, Z. Liquid chromatography−tandem mass spectrometry analysis of the DNA adducts of aristolochic acids. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 642−650.
(19) Hong, H.; Wang, Y. Derivatization with Girard reagent T combined with LC-MS/MS for the sensitive detection of 5-formyl-2′-deoxyuridine in cellular DNA. *Anal. Chem.* **2007**, *79*, 322−326.
(20) Santa, T.; Al-Dirbashi, O. Y.; Fukushima, T. Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry for biomedical analysis. *Drug Discovery Ther.* **2007**, *1*, 108−118.
(21) Lopez, M. I.; Feldlaufer, M. F.; Williams, A. D.; Chu, P. S. Determination and confirmation of nitrofuran residues in honey using LC-MS/MS. *J. Agric. Food Chem.* **2007**, *55*, 1103−1108.
(22) Lampinen-Salomonsson, M.; Beckman, E.; Bondesson, U.; Hedeland, M. Detection of altrenogest and its metabolites in post administration horse urine using liquid chromatography tandem mass spectrometry—increased sensitivity by chemical derivatisation of the glucuronic acid conjugate. *J. Chromatogr., B* **2006**, *833*, 245−256.
(23) Pang, B.; Zhou, X.; Yu, H.; Dong, M.; Taghizadeh, K.; Wishnok, J. S.; Tannenbaum, S. R.; Dedon, P. C. Lipid peroxidation dominates the chemistry of DNA adduct formation in a mouse model of inflammation. *Carcinogenesis* **2007**, *28*, 1807−1813.
(24) Regal, P.; Nebot, C.; Vazquez, B. I.; Cepeda, A.; Fente, C. A. Determination of the hormonal growth promoter 17alpha-methyltestosterone in food-producing animals: bovine hair analysis by HPLC-MS/MS. *Meat Sci.* **2010**, *84*, 196−201.
(25) Wang, J.; Yuan, B.; Guerrero, C.; Bahde, R.; Gupta, S.; Wang, Y. Quantification of oxidative DNA lesions in tissues of Long−Evans Cinnamon rats by capillary high-performance liquid chromatography−tandem mass spectrometry coupled with stable isotope-dilution method. *Anal. Chem.* **2011**, *83*, 2201−2209.
(26) Sun, C.; Sun, H.; Lai, Y.; Zhang, J.; Cai, Z. Liquid chromatography/mass spectrometry method for determination of perfluorooctane sulfonyl fluoride upon derivatization with benzylamine. *Anal. Chem.* **2011**, *83*, 5822−5826.
(27) Tewfik, I. A rapid direct solvent extraction method for the extraction of cyclobutanones from irradiated chicken and liquid whole egg. *Int. J. Food Sci. Technol.* **2008**, *43*, 108−113.
(28) Elliott, C. T.; Hamilton, L.; Stevenson, M. H.; McCaughey, W. J.; Boyd, D. Detection of irradiated chicken meat by analysis of lipid extracts for 2-substituted cyclobutanones using an enzyme linked immunosorbent assay. *Analyst* **1995**, *120*, 2337−2341.
(29) Obana, H.; Furuta, M.; Tanaka, Y. Analysis of 2-alkylcyclobutanones with accelerated solvent extraction to detect irradiated meat and fish. *J. Agric. Food Chem.* **2005**, *53*, 6603−3308.
(30) Tsutsumi, T.; Todoriki, S.; Nei, D.; Ishii, R.; Watanabe, T.; Matsuda, R. Detection of irradiated food using 2-alkylcyclobutanones as markers: verification of the european committee standardization method EN1785 for the detection of irradiated food containing lipids. *Food Hyg. Saf. Sci.* **2011**, *52*, 321−329.
(31) Morsel, J.-T.; Schmiedl, D. Determination of 2-alkylcyclobutanone using fluorescent labeling. *Fresenius' J. Anal. Chem.* **1994**, *349*, 538−541.