JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Analysis of 2-Alkylcyclobutanones in Cashew Nut, Nutmeg, Apricot Kernel, and Pine Nut Samples: Re-evaluating the Uniqueness of 2-Alkylcyclobutanones for Irradiated Food Identification

Elvis M. K. Leung, Phyllis N. Y. Tang, Yuran Ye, and Wan Chan*

Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

ABSTRACT: 2-Alkylcyclobutanones (2-ACBs) have long been considered as unique radiolytic products that can be used as indicators for irradiated food identification. A recent report on the natural existence of 2-ACB in non-irradiated nutmeg and cashew nut samples aroused worldwide concern because it contradicts the general belief that 2-ACBs are specific to irradiated food. The goal of this study is to test the natural existence of 2-ACBs in nut samples using our newly developed liquid chromatography−tandem mass spectrometry (LC−MS/MS) method with enhanced analytical sensitivity and selectivity (Ye, Y.; Liu, H.; Horvatovich, P.; Chan, W. Liquid chromatography−electrospray ionization tandem mass spectrometric analysis of 2-alkylcyclobutanones in irradiated chicken by precolumn derivatization with hydroxylamine. *J. Agric. Food Chem.* **2013**, *61*, 5758−5763). The validated method was applied to identify 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) in nutmeg, cashew nut, pine nut, and apricot kernel samples (n = 22) of different origins. Our study reveals that 2-DCB and 2-TCB either do not exist naturally or exist at concentrations below the detection limit of the existing method. Thus, 2-DCB and 2-TCB are still valid to be used as biomarkers for identifying irradiated food.

KEYWORDS: *2-Alkylcyclobutanone, 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone, LC−MS/MS, irradiated food*

INTRODUCTION

Food irradiation is a commonly used preservation method for limiting microbial growth and extending the shelf life of the food commodity.[1−3] 2-Alkylcyclobutanones (2-ACBs), a mixture of hydrocarbon-chain-containing cyclobutanones, are generally discovered in a wide variety of irradiated food.[4−8] Among the 2-ACBs, 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) from radiolysis of palmitic-acid- and stearic-acid-containing triglycerides, respectively, have been detected as the predominant constituents in irradiated food.[9−11] The uniqueness of 2-ACBs and the predomination of 2-DCB and 2-TCB in irradiated food have rendered them biomarkers for irradiated food identification.[8,11]

Since the 1990s, an analytical method based on mass spectrometric detection of 2-DCB and 2-TCB after gas chromatographic separation (GC−MS) has been adopted by the European Committee for Standardization as one of the standard methods (EN 1785) for identifying food irradiation.[11−13] The method of combining Soxhlet extraction, adsorption chromatography cleanup, and GC−MS analysis was validated in interlaboratory trials and tested on a variety of foodstuffs.[11,13] Currently, EN 1785 is used globally as one of the standard methods for verifying fat-containing food that is preserved by irradiation.

Until recently, there has been no evidence that 2-ACBs can be detected in non-irradiated food or in food processed by preservation techniques other than irradiation. However, a recent controversial report by Variyar et al. presented evidence of the natural existence of 2-DCB and 2-TCB in non-irradiated cashew nuts and 2-decyclobutanone in non-irradiated nutmeg.[14] These findings have aroused worldwide attentions because they contradict the historical belief that 2-ACBs can only be found in irradiated food.[11,15,16] The emerging possibility of the natural existence of 2-ACBs and the challenges in identifying food composites containing irradiated ingredient(s) had highlighted the demand for analytical methods with sufficiently high sensitivity.[11,16]

Recently, we have developed a novel liquid chromatography coupled with electrospray ionization tandem mass spectrometric (LC−MS/MS) method for the analysis of 2-ACBs in γ-irradiated food.[17] The method entails extraction of 2-ACBs with acetonitrile from isolated fat samples and precolumn derivatization with hydroxylamine prior to LC−MS/MS analysis. The developed method offers superior sensitivity to the existing GC−MS-based European official method (EN 1785:2003) for the detection of 2-ACBs. The goal of the present study was to apply the newly developed method with enhanced sensitivity and selectivity for investigating the natural existence of 2-ACBs and, thus, the suitability of using 2-ACBs as indicators for irradiated food identification.

MATERIALS AND METHODS

Chemicals and Reagents. All chemicals and reagents were of the highest purity available and were used without further purification. 2-Dodecylcyclobutanone (2-DCB), 2-tetradecylcyclobutanone (2-TCB), 2-(2-ethylhexyl)-cyclohexanone (2-ECH), formic acid, and hydroxylamine hydrochloride (HA) were purchased from Sigma-Aldrich (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade acetonitrile and n-hexane were purchased from Tedia (Fairfield, OH). Water was produced by a Milli-Q Ultrapure water system with the water outlet operating at 18.2 MΩ (Millipore, Billerica, MA). Cashew

Received: August 6, 2013
Revised: September 25, 2013
Accepted: September 25, 2013
Published: September 25, 2013

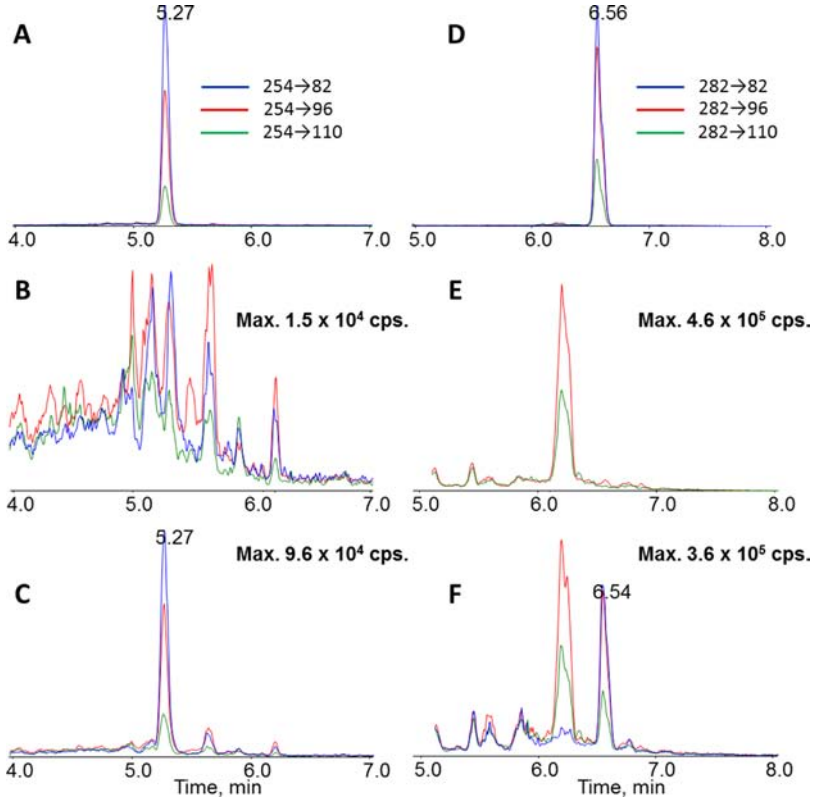

Figure 1. Typical chromatograms from LC−MS/MS analysis of 2-DCB in (A) 2.39 ng/mL 2-DCB standard solution, (B) non-spiked cashew nut sample, and (C) cashew nut sample fortified with 2-DCB at 6.8 ng/g of nut and 2-TCB in (D) 2.67 ng/mL 2-TCB standard solution, (E) non-spiked cashew nut sample, and (F) cashew nut sample fortified with 2-DCB at 7.6 ng/g of nut. The ketoxime derivative of 2-DCB and 2-TCB was eluted at 5.27 and 6.56 min, respectively, under the chromatographic conditions described in the Materials and Methods.

nuts, pine nuts, apricot kernel, and nutmeg (n = 22) were procured from local markets in Hong Kong. The nut samples were pulverized and sealed in polythene bags before use.

Preparation of Standard Solutions. A stock solution mixture of 2-DCB and 2-TCB at 476.8 and 533.0 μg/mL, respectively, was prepared in acetonitrile and kept at −80 °C in a freezer. A working standard solution mixture of 2-DCB and 2-TCB of concentrations ranging from 2.39 to 476.8 ng/mL and from 2.67 to 533.0 ng/mL, respectively, were prepared by serial dilution from the stock solution. The internal standard, the HA derivative of 2-ECH (2-ECH-HA), was prepared in acetonitrile by overnight reaction of 2-ECH (2.1 μg/mL) with 100-fold excess of HA at ambient temperature. The derivatization agent was prepared by dissolving 27.8 mg of solid HA in 1 mL of a 1:1 water/acetonitrile mixture.

Sample Preparation. *Soxhlet Extraction.* The sample preparation was performed essentially as described before,[4,9,13] with modifications. In brief, 7 g of the blended nut samples was mixed with 5 g of anhydrous sodium sulfate and extracted with 100 mL of *n*-hexane for 6 h using a Soxhlet extractor. The hexane extracts were then evaporated to dryness using a rotary evaporator, after which 2-ACBs were extracted from the lipid residuals by direct solvent extraction.

Direct Solvent Extraction. The direct solvent extraction, which had been proven to be efficient in extracting 2-ACBs from the food matrix was adopted in this study to extract the targets from the Soxhlet-isolated lipid.[18] Basically, the lipid residuals from the Soxhlet extraction were serially extracted thrice with 8 mL of acetonitrile each time. The acetonitrile extracts were combined and evaporated to dryness under a gentle stream of nitrogen at 35 °C. The samples were reconstituted in 0.5 mL of acetonitrile, centrifuged at 13 800 rpm for 5 min, and prepared for chemical derivatization.

Derivatization with Hydroxylamine. The derivatization was conducted essentially as described previously.[17] To 100 μL of the sample extract, 90 μL of the derivatization agent and 10 μL of the internal standard were added. The sample mixtures were vortex-mixed and incubated at room temperature overnight before analyzed by the developed LC−MS/MS method.

LC−MS/MS Analysis. LC−MS/MS analysis was performed on a Waters Acquity UPLC (Waters, Milford, MA) coupled with a 6500 QTRAP (AB Sciex, Foster City, CA) LC−MS/MS system. Typically, 10 μL of the derivatized sample mixture was injected into an Eclipse Plus C18 column (50 × 3.0 mm, 1.8 μm; Agilent) heated at 40 °C. The mobile phase system consisted of two components, with component I being 0.4% formic acid in water (A) and component II being acetonitrile (B). The solvent gradient started from 50% B, programmed linearly to 100% B in 8 min, and then held for another 5 min before reconditioning, at a flow rate of 0.8 mL/min. The effluent of the first 2 min from the liquid chromatograph was diverted to waste to protect the system from dirtiness, which might ruin the detection efficiency.

MS data were acquired in positive electrospray ionization (ESI) mode, with the ESI source parameters optimized as follows: ionspray voltage, 5500 V; declustering potential, 90 V; entrance potential, 8; collision energy, 50; gas I (GSI), 50; gas II (GSII), 30; curtain gas (CUR), 30; collision gas (CAD), 6; and temperature of GSII, 550 °C. Samples were analyzed in multiple reaction monitoring (MRM) mode, with the following transitions: m/z 254 → 82, 254 → 96, and 254 → 110 for 2-DCB-HA; m/z 282 → 82, 282 → 96, and 282 → 110 for 2-TCB-HA; and m/z 226 → 69 for the internal standard 2-ECH-HA. The dwell time for each transition was set at 50 ms. While transitions at m/z 254 → 82 and 282 → 82 were used for the quantitative purpose for 2-DCB-HA and 2-TCB-HA, respectively, transitions of 2-DCB-HA at m/z 254 → 96 and 254 → 110 were used for the qualitative confirmation. Similarly, transitions of 2-TCB-HA at m/z 282 → 96 and 282 → 110 served the same purpose as well.

Method Validation. The method performance of the developed method was evaluated by spiking 100 μL of 2 μM 2-DCB (0.48 μg/mL) and 2-TCB (0.53 μg/mL) to 7 g of the blended nut samples. The nut samples fortified with 2-DCB and 2-TCB after standing overnight

dx.doi.org/10.1021/jf403500j | J. Agric. Food Chem. 2013, 61, 9950−9954

Table 1. Summary of Chromatographic Retention Times and the Peak Area Ratios of the Qualitative Transition Ions to the Quantitative Transition Ion Transitions for 2-DCB and 2-TCB from LC−MS/MS Analysis of the Standard Solutions and Spiked Nut Samples

		standard	spiked cashew nut	spiked apricot kernel
2-DCB	retention time (min)	5.27	5.27	5.27
	peak area ratio (m/z 254 → 96/254 → 82)	0.62	0.68	0.74
	peak area ratio (m/z 254 → 110/254 → 82)	0.18	0.19	0.24
2-TCB	retention time (min)	6.56	6.54	6.54
	peak area ratio (m/z 282 → 96/282 → 82)	0.82	0.96	0.93
	peak area ratio (m/z 282 → 110/282 → 82)	0.30	0.38	0.34

at room temperature were mixed with 5 g of anhydrous sodium sulfate, extracted, derivatized, and analyzed using the method described above.

RESULTS AND DISCUSSION

Technological advancement has brought to researchers analytical techniques with improved analytical power. For example, tandem quadrupole MS/MS systems in MRM mode, in which Q1 and Q3 are parked at preselected m/z values of the parent and daughter ions, respectively, are one of the most sensitive and selective analytical platforms for many applications.[19−21] Recently, we have developed a novel LC−MS/MS method for the sensitive detection of 2-ACBs in γ-irradiated food.[17] The detection limit of our assay, which combined chemical derivatization with hydroxylamine and LC−MS/MS analysis, is at least 50 times lower than that of the existing analytical methods for 2-ACB determination. The goal of this study was to apply the newly developed assay in testing the natural existence of 2-ACBs in nut samples.

The Soxhlet extraction method that was proven to be efficient in extracting 2-ACBs from nut samples was used in this study.[4,9,13] The 2-ACBs in the lipid extracts after acetonitrile extraction were derivatized by HA and analyzed by the developed LC−MS/MS method. The performance of the assay for 2-ACB determination was evaluated by analyzing cashew nut and apricot kernel samples fortified with 2-DCB and 2-TCB at fixed concentrations. Depicted in Figure 1 are the chromatograms obtained from LC−MS/MS analysis of a representative cashew nut sample that was spiked with 6.8 and 7.6 ng/g of 2-DCB (Figure 1C) and 2-TCB (Figure 1F), respectively.

In comparison to the concentrations of 2-ACBs in non-irradiated cashew nut samples reported by Variyar et al. (2.70 ± 1.71 μg/g of 2-DCB in cashew nut and 1.0 ± 0.08 μg/g of 2-TCB in cashew nut),[14] our developed assay allowed for the detection of the target analytes at significantly lower concentrations. Therefore, the method is capable of detecting 2-ACBs in the non-irradiated nut samples, if they indeed exist at the reported concentrations.

The existence of 2-ACBs in the tested nut samples was verified by comparing the retention time to the reference standard (panels A and D of Figure 1). Under the optimized chromatographic conditions described in the Materials and Methods, the oxime derivatives of 2-DCB and 2-TCB were eluted at 5.27 min (Figure 1A) and 6.56 min (Figure 1D), respectively, which were used as the first criterion for compound identification.

In addition to the retention times, the 2-ACBs were further confirmed using the relative peak area of the qualitative ion transitions to that of the quantitative ion transition.[22−24] Under the collision-induced dissociation MS/MS conditions as described in the Materials and Methods, the peak area ratios of the two transitions were 0.62 (m/z 254 → 96/254 → 82) and 0.18 (m/z 254 → 110/254 → 82) for 2-DCB (Figure 1A), whereas the ratios for 2-TCB (Figure 1D) were 0.82 (m/z 282 → 96/282 → 82) and 0.30 (m/z 282 → 110/282 → 82) in the standard solution mixture containing 2-DCB and 2-TCB at 2.39 and 2.67 ng/mL, respectively.

The analytes, characterized by the corresponding retention time and the peak area ratios of the qualitative ion to the quantitative ion transitions (Table 1), were tested for their natural existence in nut samples. A pool of 22 nut samples from different origins was purchased from several local markets in Hong Kong (Table 2). The collected samples, including cashew nut, nutmeg, apricot kernel, and pine nut, were processed and analyzed in parallel with the spiked samples. Our results revealed that neither 2-DCB nor 2-TCB was detected in the spike-free samples (Table 2), but distinct peaks at retention times of 5.27 min (2-DCB) and 6.54 min (2-TCB) were observed in the spiked samples.

Table 2. Results of LC−MS/MS Analysis of Nut Samples Collected at the Retailing Stage in Hong Kong

			target analyte	
nut samples analyzed	country of origin	number of samples analyzed	2-DCB	2-TCB
cashew nut	China	2	ND[a]	ND
	India	3	ND	ND
	Vietnam	3	ND	ND
nutmeg	Burma	1	ND	ND
	China	3	ND	ND
apricot kernel	China	8	ND	ND
pine nut	China	2	ND	ND

[a]ND = not detected.

Table 1 summarizes the chromatographic retention time and the peak area ratios of the qualitative transition ions to the quantitative transition ion for 2-DCB and 2-TCB from LC−MS/MS analysis of the standard solutions and the spiked nut samples. Because the retention time of the peaks was within ±0.02 min and the peak area ratios of the MRM transitions were within ±20% of that in the standard solution mixture containing 2-DCB and 2-TCB at 2.39 and 2.67 ng/mL, respectively (panels A and D of Figure 1), the peaks were confidently affirmed as 2-DCB and 2-TCB. Figure 1 shows the chromatograms extracted from LC−MS/MS analysis of the non-spiked (panels B and E of Figure 1) and spiked (panels C and F of Figure 1) cashew samples.

The observation that all of the spiked samples gave positive identification of 2-DCB and 2-TCB and no distinct peaks were recorded in any of the non-spiked samples indicated that 2-DCB and 2-TCB either did not exist naturally in the tested

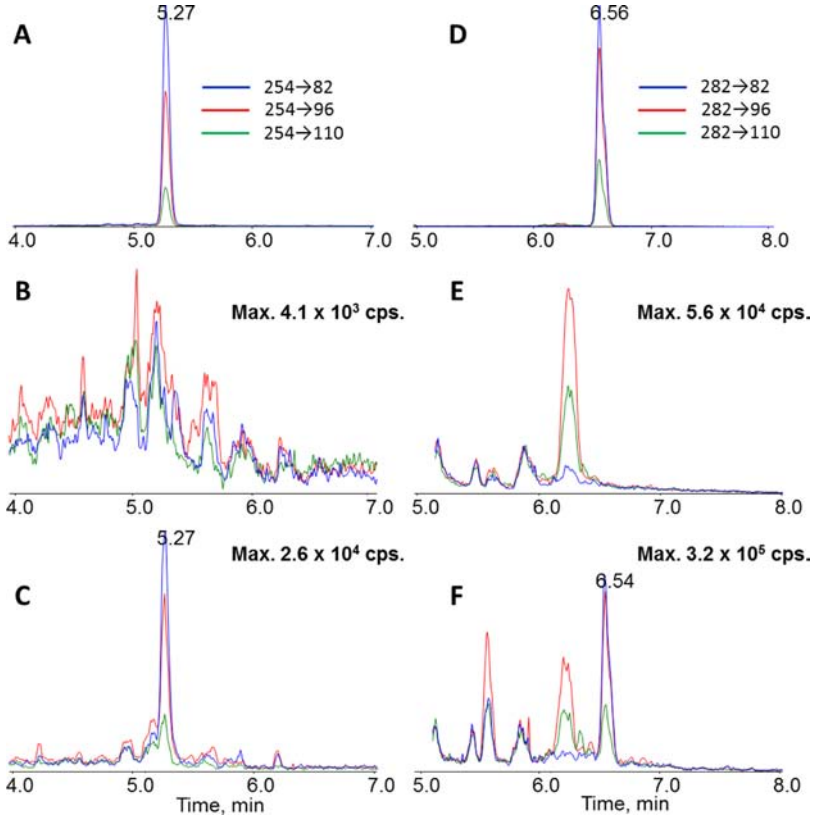

Figure 2. Typical chromatograms from LC−MS/MS analysis of 2-DCB in (A) 2.39 ng/mL 2-DCB standard solution, (B) non-spiked apricot kernel, and (C) apricot kernel fortified with 2-DCB at 6.8 ng/g of nut and 2-TCB in (D) 2.67 ng/mL 2-TCB standard solution, (E) non-spiked apricot kernel, and (F) apricot kernel fortified with 2-DCB at 7.6 ng/g of nut. The ketoxime derivative of 2-DCB and 2-TCB was eluted at 5.27 and 6.56 min, respectively, under the chromatographic conditions described in the Materials and Methods.

cashew nut sample or existed at a concentration lower than the detection limit of the present method (0.2 ng/g of 2-DCB in cashew nut and 0.3 ng/g of 2-TCB in cashew nut).

The controversial publication that 2-ACBs exist naturally in cashew nut and nutmeg by Variyar et al. has attracted widespread attention among food scientists because the argument disproved the prevailing belief about the uniqueness of 2-ACBs in radiolytic food.[11,14] Using our newly developed LC−MS/MS assay,[17] however, neither 2-DCB nor 2-TCB was traced in non-irradiated nutmeg and cashew nut samples (Table 2). Our observation is in line with the results reported by Chen et al. that negative identification of 2-DCB and 2-TCB was confirmed in non-irradiated nutmeg samples.[15]

With the emerging possibility that 2-ACBs occur naturally in nut samples, we have also analyzed apricot kernel and pine nuts (Table2) that are known to contain a high level of palmitic and stearic acids,[25−27] which are precursors for 2-DCB and 2-TCB, respectively. In both samples, neither 2-DCB nor 2-TCB was identified as well. Figure 2 is the chromatograms obtained from LC−MS/MS analysis of representative non-spiked and spiked apricot kernel samples. Similar observations were also reported by Sin et al., who had analyzed non-irradiated melon, pumpkin, and sunflower seed samples.[4] Apart from nut samples, no positive identification of 2-ACBs in non-irradiated foods, such as mangos, beef, chicken, pork, fish, and egg, were reported in the literature.[5,7,13,16]

In summary, our study using a state-of-the-art analytical technique revealed that 2-ACBs either do not exist naturally or may exist at concentrations below the detection limit of our existing analytical technology. Thus, we ensure the belief that 2-DCB and 2-TCB are unique radiolytic products and still valid to use as marker molecules for identifying irradiated foods.

AUTHOR INFORMATION

Corresponding Author

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

Funding

Financial support from the National Natural Science Foundation of China (21305022) is acknowledged.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Reale, A.; Sorrentino, E.; Iacumin, L.; Tremonte, P.; Manzano, M.; Maiuro, L.; Comi, G.; Coppola, R.; Succi, M. Irradiation treatments to improve the shelf life of fresh black truffles (truffles preservation by γ-rays). *J. Food Sci.* **2009**, *74*, M196−M200.

(2) Fan, X.; Sommers, C. H.; Sokorai, K. J. Ionizing radiation and antioxidants affect volatile sulfur compounds, lipid oxidation, and color of ready-to-eat turkey bologna. *J. Agric. Food Chem.* **2004**, *52*, 3509−3515.

(3) Dussault, D.; Caillet, S.; Le Tien, C.; Lacroix, M. Effect of γ-irradiation on membrane fatty acids and peptidoglycan's muropeptides of *Pantoea agglomerans*, a plant pathogen. *J. Appl. Microbiol.* **2009**, *106*, 1033−1040.

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

(5) 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*, 6603−6608.
(6) 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.
(7) 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.
(8) Stevenson, M. H.; Hamilton, J. T. Irradiation detection. *Nature* **1990**, *344*, 202−203.
(9) 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.
(10) 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*, 259−268.
(11) 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.
(12) Delincee, H. Analytical methods to identify irradiated food—A review. *Radiat. Phys. Chem.* **2002**, *63*, 455−458.
(13) European Committee for Standardization (CEN). *EN 1785. Foodstuffs—Detection of Irradiated Food Containing Fat—Gas Chromatographic/Mass Spectrometric Analysis of 2-Alkylcyclobutanones*; CEN: Brussels, Belgium, 2003.
(14) 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.
(15) Chen, S.; Tsutsumi, T.; Takatsuki, S.; Matsuda, R.; Kameya, H.; Nakajima, M.; Furuta, M.; Todoriki, S. Identification of 2-alkylcyclobutanones in nutmeg (*Myristica fragrans*). *Food Chem.* **2012**, *134*, 359−365.
(16) European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF).. Scientific opinion on the chemical safety of irradiation of food. *EFSA J.* **2011**, *9*, 1930.
(17) Ye, Y.; Liu, H.; Horvatovich, P.; Chan, W. Liquid chromatography−electrospray ionization tandem mass spectrometric analysis of 2-alkylcyclobutanones in irradiated chicken by precolumn derivatization with hydroxylamine. *J. Agric. Food Chem.* **2013**, *61*, 5758−5763.
(18) Hijaz, F.; Kumar, A.; Smith, J. S. A rapid direct solvent extraction method for the extraction of 2-dodecylcyclobutanone from irradiated ground beef patties using acetonitrile. *J. Food Sci.* **2010**, *75*, T118−T122.
(19) Chu, P. S.; Lopez, M. I. Determination of nitrofuran residues in milk of dairy cows using liquid chromatography−tandem mass spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 2129−2135.
(20) Guo, L.; Wu, H.; Yue, H.; Lin, S.; Lai, Y.; Cai, Z. A novel and specific method for the determination of aristolochic acid-derived DNA adducts in exfoliated urothelial cells by using ultra performance liquid chromatography−triple quadrupole mass spectrometry. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2011**, *879*, 153−158.
(21) Li, J.; Leung, E. M.; Choi, M. M.; Chan, W. Combination of pentafluorophenylhydrazine derivatization and isotope dilution LC−MS/MS techniques for the quantification of apurinic/apyrimidinic sites in cellular DNA. *Anal. Bioanal. Chem.* **2013**, *405*, 4059−4066.
(22) Kushnir, M. M.; Rockwood, A. L.; Nelson, G. J.; Terry, A. H.; Meikle, A. W. Liquid chromatography−tandem mass spectrometry analysis of urinary free cortisol. *Clin. Chem.* **2003**, *49*, 965−967.
(23) Sphon, J. A. Use of mass spectrometry for confirmation of animal drug residues. *J.—Assoc. Off. Anal. Chem.* **1978**, *61*, 1247−1252.
(24) Li, L. Y.; Campbell, D. A.; Bennett, P. K.; Henion, J. Acceptance criteria for ultratrace HPLC-tandem mass spectrometry: Quantitative and qualitative determination of sulfonylurea herbicides in soil. *Anal. Chem.* **1996**, *68*, 3397−3404.
(25) Femenia, A.; Rossell, C.; Mulet, A.; Caiiellas, J. Chemical composition of bitter and sweet apricot kernels. *J. Agric. Food Chem.* **1995**, *43*, 356−361.
(26) Chung, K. H.; Shin, K. O.; Hwang, H. J.; Choi, K. S. Chemical composition of nuts and seeds sold in Korea. *Nutr. Res. Pract.* **2013**, *7*, 82−88.
(27) Destaillats, F.; Cruz-Hernandez, C.; Giuffrida, F.; Dionisi, F. Identification of the botanical origin of pine nuts found in food products by gas−liquid chromatography analysis of fatty acid profile. *J. Agric. Food Chem.* **2010**, *58*, 2082−2087.