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Natural Existence of 2-Alkylcyclobutanones

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Increased outbreaks of food-borne illnesses, including deaths in recent years, attributed in large part to consumption of food contaminated with *Escherichia coli* O157:H7, have become a worldwide concern. Food-borne illnesses constitute an unacceptable health risk demanding stringent food safety controls. Despite food irradiation being a known effective method to eliminate pathogens that are difficult to eradicate by conventional methods, consumers and industry at large have been reluctant to adopt it. This is mainly attributed to some apprehensions regarding the safety of irradiated food. One such apprehension relates to 2-alkylcylcyclobutanones, unique radiolytic products thought to be formed in minute quantities in food during radiation processing. We demonstrate here for the first time the natural occurrence of 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone in commercial nonirradiated as well as fresh cashew nut samples and 2-decylcyclobutanone as well as 2-dodecylcyclobutanone in nonirradiated nutmeg samples. The presence of 2-tetradecenylcyclobutanone was also observed in both commercial and irradiated cashew nuts. The present study will provide greater impetus for wider adoption of radiation technology for elimination of food-borne pathogenic bacteria without apprehensions about the technology that can help ensure food safety.

KEYWORDS: ***Anacardium occidentale*; *Myristica fragrans*; 2-alkylcylcyclobutanones (2-ACBs); food irradiation; food safety**

INTRODUCTION

Recent food poisoning outbreaks and product recalls have highlighted the need for the development of effective preventive and therapeutic control strategies for emerging highly virulent races of food-borne pathogens like *Escherichia coli* O157:H7 (*1, 2*). Food-borne illnesses are necessarily not new but have recently originated from relatively less common sources of food poisoning, particularly fresh produce such as raw vegetables and fruits, contributing to a widespread food safety scare. No specific therapeutic regimens are available so far to control *E.coli* O157:H7 infection as antibiotic usage has been contraindicated for this pathogen (*2*). Manning et al. (*3*) emphasized the need for safe agricultural practices in areas where livestock farms and crop fields coexist. Besides, better postharvest management may also play a crucial role in the prevention of food-borne illnesses. The food industry as well as food safety regulators thus need to review and reformulate their food safety strategies. One of the postharvest treatment options available to the food industry today is ionizing radiation technology. It is an effective tool in eliminating pathogens and ensuring food safety without significantly altering quality (*4*). The myths and fallacies surrounding this technology have been effectively addressed over the years with sustained scientific inputs (*4*−*6*). Although there has been increased acceptance and commercial adoption of the technology worldwide, still there are reservations in certain quarters over its wider deployment. One of the recently raised concerns relates to the formation of 2-alkylcyclobutanones (2-ACBs), unique radiolytic products (URPs) of fatty acids, thought to be produced only in irradiated foods. These compounds have so far not been known to occur naturally in food, nor have they been reported to be formed by other food processing techniques (*7*). While several earlier studies have demonstrated their toxic, genotoxic, and tumor promoting potential, recent investigations have revealed their nontoxic nature (*8*). Nevertheless, there is a debate on the health risks related to the consumption of irradiated foods containing 2-ACBs (*9*).

2-ACBs, regarded as URPs, are cyclic compounds formed by the loss of an electron from the oxygen on the carbonyl of a fatty acid or a triglyceride, followed by a rearrangement (*10*). Irradiation of the four major fatty acids found in foods, palmitic, stearic, oleic, and linoleic, produces their corresponding cyclobutanone, namely, 2-dodecyl-, 2-tetradecyl-, 2-tetradec-5′-enyl-, and 2-tetradeca-5′-8′-dienyl cyclobutanone (*10*). The 2-ACB thus formed is specific to the parent fatty acid. They are currently used as routine markers of radiation processing of foods containing a high content of lipids (*11*). Thus, while considering irradiation as a technology option to overcome insect

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infestation of stored cashew nut, reported to contain 45−50% lipid (*12*), our initial aim was to confirm the presence of 2-ACBs in irradiated samples and estimate their content. Because this food commodity has a high lipid content (mainly triglycerides), irradiation is expected to produce detectable quantities of 2-ACBs. The occurrence of 2-ACBs in peanuts and pistachio nuts irradiated at 5 kGy has been reported earlier (*13*). No report, however, exists on the formation of these URPs in irradiated cashew nuts exposed to lower doses (<1 kGy) generally employed for insect disinfestation. Because palmitic acid is one of the major fatty acids of cashew nut (*14*), the formation of 2-dodecylcyclobutanones (2-dDCBs) is expected. 2-dDCBs are also the most abundant saturated 2-ACBs formed and considered the most important markers for the detection of irradiated food (*15*). Therefore, the presence of these compounds was monitored in samples of cashew nut and nutmeg, both nonirradiated and irradiated, using different purification protocols with an aim to unequivocally confirm if these compounds were naturally present or produced only by radiation processing. Here, for the first time, we present evidence of the natural existence of 2-dDCB, 2-tetradecenylcyclobutanone (2-tDeCB), and 2-tetradecylcyclobutanone (2-tDCB) in cashew nut (*Anacardium occidentale*) and 2-decylcyclobutanone (2-DCB) as well as 2-dDCB in nutmeg (*Myristica fragrans*), thus disproving the hypothesis that 2-ACBs are URPs.

MATERIALS AND METHODS

Cashew nuts and nutmeg were procured from a local market, packed (30 g) in sealed polythene bags, and irradiated to an average absorbed dose of 1 kGy of γ-rays using a cobalt-60 source irradiator (GC-5000, BRIT, Mumbai) at a dose rate of 7.07 kGy/h. Standard 2-dDCB was procured from Sigma Chemical Co. (United States) Ltd. 2-tDCB and 2-tDeCB were gifted by Dr. E. Marchioni (Laboratoire de Chimie Analytique et Sciences de l'Aliment, IPHC, UMR 7178, Strasbourg, France).

Extraction and Purification of Lipids. *Soxhlet Extraction−Column Chromatography.* The isolation and subsequent fractionation of lipids to obtain 2-ACBs were performed according to the method adopted by Horvatovich and others (*15*). Ground cashew nuts (30 g) were subjected to Soxhlet extraction using *n*-hexane to obtain a lipid fraction. The lipid fraction (2 g) was purified by adsorption chromatography on a silica gel column (60 g, 45 cm length, 2.5 cm id). The mobile phase flow rate was 1 mL/min. The first 300 mL of *n*-hexane elution containing apolar impurities was discarded. The fractions eluting with 1% diethylether in *n*-hexane (900 mL) were collected and evaporated to dryness to obtain 2-ACBs. The residue was made to a known volume and further analyzed on gas chromatography−mass spectrometry (GC-MS).

Supercritical CO_2 Extraction−Thin-Layer Chromatography (TLC). The conditions used by Lembke et al. (*13*) were adapted for the present work. Ground cashew nuts (30 g) were subjected to SC-CO_2 extraction using laboratory-scale supercritical equipment (SPEED-SFE, of Applied Separations, United States). A plug of glass wool was pushed to the closed end of a SS 316 high-pressure extraction vessel fitted with a filter-containing metal frit and tamped tightly in place. The weighed sample was placed in the vessel (21 cm length, 2.2 cm id), and a second plug of glass wool was placed above the sample matrix. SPEED matrix (a hydromatrix and dispersing agent) was added to eliminate the dead volume of the extraction vessel. A wad of glass wool was put in place just before fastening the vessel with a filter-containing metal frit. The vessel was packed firmly to ensure that CO_2 diffused from bottom to top uniformly through the sample matrix. The vessel was placed in the oven module, and a thermocouple was connected to the vessel body. A pressure tight collection vial of Borosil glass with Teflon caps and two septa of 100 mL capacity were used to collect the extract. A glass flow meter or rotameter (LPM CO_2 black glass float) with a working range of 0.2−2.2 L/min, provided at the collection end, was used to measure the flow rate of CO_2. Pure CO_2 (>99% purity) at a flow rate of 1 mL/min was used for the extraction. The extraction was carried out for 40 min (dynamic) at 80 °C and a pressure of 146−148 bar. A static time of 5 min was maintained for all of the trials undertaken. Blank runs were carried out between each extraction exactly in the same manner as above but without sample.

The supercritical fluid extraction (SFE) extract was subjected to analytical (0.25 mm thickness) and preparative (0.5 mm thickness) silica gel G 60 (E Merck, Germany) TLC using 95:5 *n*-hexane:diethylether as the developing solvent system. The band at R_f values corresponding to 2-ACBs (identified by comparing the R_f with standard spotted on the same plate (visualized by exposure to I_2 vapor) on the preparatory plate was scraped and analyzed by GC-MS.

GC-MS Analysis. GC-MS analysis was carried out on a Shimadzu GC/MS QP5050 model equipped with a GC-16 gas chromatograph and a DB5 (5% diphenyl−95% dimethylpolysiloxane) capillary column (length, 30 m; id, 0.25 mm; film thickness, 0.25 μm). Operating conditions were as follows: The carrier gas was helium with a flow rate of 0.9 mL/min; the column temperature was programmed from 60 to 200 °C at the rate of 4 °C/min thereafter at 280 °C at the rate 10 °C/min held for 5 min at initial temperature and the final temperature. Injector and interface temperatures were 210 and 280 °C, respectively, with a splitless injection mode and an injection volume of 1.5 μL. Diethylether and hexane were injected between two injections. The MS when operated in the single ion mode (SIM) measured ion currents at *m/z* 98, 112, and 238 for the 2-ACBs. The compounds were identified by reference to the retention times and ion ratios of 98:112 of authentic standards analyzed at the same time. All figures provided have retention time (min) on the *X*-axis and total ion current on the *Y*-axis.

Quantitative Determination. A calibration curve of area vs amount of 2-dDCB in ng (R^2, 0.94) was obtained by injecting different volumes (0.1−8 μL) of a 5 μg/mL standard solution into the GC-MS. The amount of 2-dDCB in the unknown samples was determined by interpolating the calibration curve. The sensitivity of the developed method was checked in terms of minimum limit of detection. This was defined as the compound concentration (area) in the GLC chromatogram that produced a signal-to-noise ratio above 3. The ions at masses 98 and 112 were used for quantitative assessment of peak areas.

Recovery. The SFE crude extract was spiked with known amount of 2-tDeCB and analyzed by GC-MS. This mixture was then spotted on a preparative TLC silica gel plate and run in a solvent mixture of 95:5 *n*-hexane:diethyl ether. The band corresponding to that of the standard was scraped and injected into GC-MS. The areas correlating with the two peaks of 2-tDeCB, before and after TLC, were used for the calculation of recovery of the material.

Data Analysis. Data presented are means of values obtained from three independent samples each analyzed in duplicate for every single set of complete analysis. Thus, a total of six replications were carried out for each complete set of analysis, and the values were reported as means ± standard deviations (SDs), $n = 6$. All statistical calculations were performed using Origin software. One-way analysis of variance (ANOVA) was carried out, and multiple comparison of means were done by Tukey's test. The significance level was 0.05.

RESULTS AND DISCUSSION

Protocol I: Soxhlet Extraction−Column Purification. In the first protocol, the traditional solvent extraction using Soxhlet apparatus followed by column chromatography was attempted. Both florisil and silica gel have been routinely used as column materials for the isolation of 2-ACBs (*15*). In our studies, 2-ACBs were not detected in fractions (1% diethylether in *n*-hexane) when florisil was used. Horvatovich et al. (*15*) have earlier reported a five times higher capacity for silica gel (100 mg of lipid on 3 g of adsorbent) to retain lipids when compared to florisil (15 g of adsorbent for 100 mg of lipid). Cleaner extracts from silica gel with lower contents of triacylglycerols in the eluates containing 2-ACBs resulted in a higher sensitivity for detection of these compounds. A higher sensitivity for detection of 2-ACBs was also reported by Horvatovich et al. (*15*) while using silica gel column for purification of these

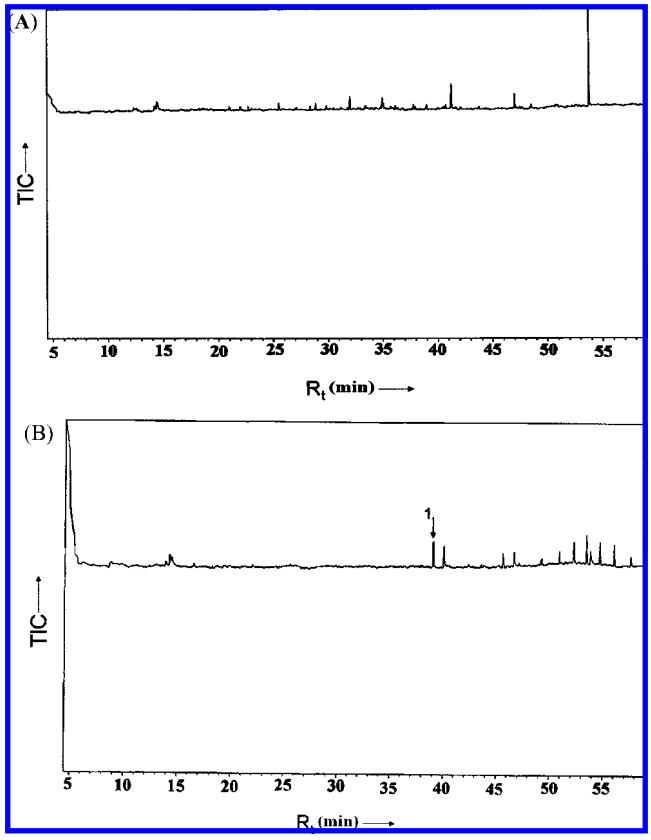

Figure 1. GLC chromatogram of lipid isolates obtained using Soxhlet extraction and subsequent purification by silica gel chromatography. The peak marked 1 corresponds to 2-dDCB identified by comparison of retention time and mass fragmentation with synthetic commercial standard. (**A**) Nonirradiated sample and (**B**) irradiated sample.

compounds. Thus, silica gel instead of florisil was employed as a column material in all subsequent procedures for the isolation of 2-ACBs from the lipid extracts.

The fractions eluting with *n*-hexane:diethylether (99:1) when analyzed by GC-MS in the single ion monitoring mode (SIM) showed a peak at R_t 39.039 (**Figure 1**). This peak corresponding to R_t of standard 2-dDCB suggested the presence of 2-ACBs in the irradiated sample. The presence of this compound in the extract was confirmed from the ratio of the relative intensities of the two important and selective fragments at masses 98 and 112. The ratio between the two ions (98/112), generally employed to confirm the presence of 2-dDCB (*15*), was found to be 4:1, which was comparable with that of the standard. The predominance of ion *m*/*z* 98 also confirmed the absence of unsaturation in the side chain (*15*). The identity of the peak was further confirmed by comparing the retention time (**Figure 2**) as well as the mass fragmentation pattern in scan mode with that of the synthetic commercial standard (**Figure 3**). The protocol was repeated with nonirradiated control samples. No peak corresponding to 2-dDCB was detected supporting the current hypothesis that 2-ACBs are URPs.

Protocol II: Super Critical Fluid Extraction—TLC Purification. Isolation and identification of 2-ACBs by the traditional chromatographic methods involve a complex procedure and is thus time-consuming. Carbon dioxide SFE is currently used as a rapid and effective extraction procedure (*11*). The extraction can be tailored for selective isolation of 2-ACBs, thus obviating the need for further purification on columns such as florisil or silica gel. A higher sensitivity in identification of these compounds using this method has been reported (*11*). In the

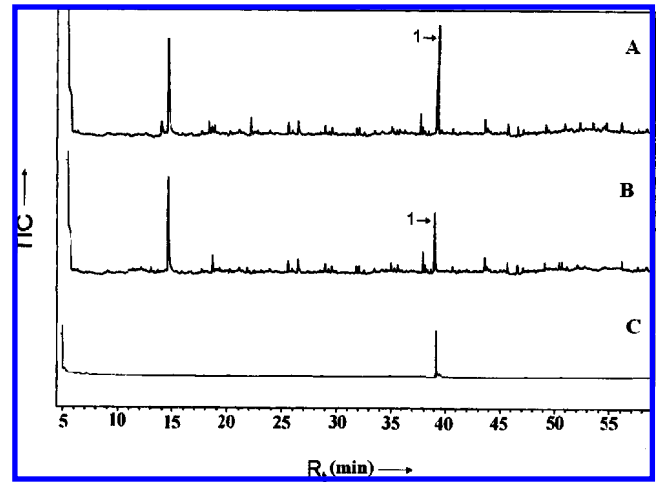

Figure 2. GLC chromatogram of the band (R_f 0.46) obtained from preparative TLC of SFE extract of nonirradiated commercial cashew nut spiked with standard 2-dDCB. (**A**) Spiked sample, (**B**) nonspiked sample, and (**C**) standard 2-dDCB.

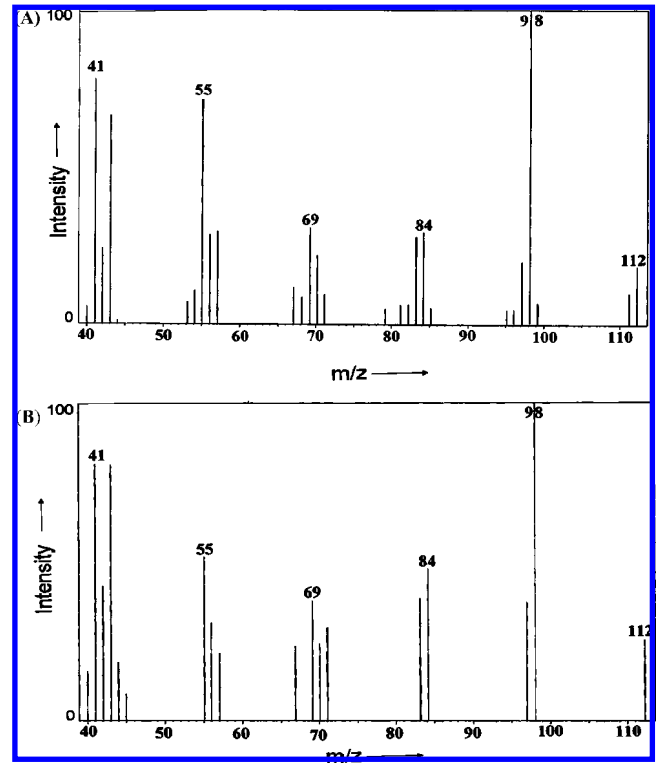

Figure 3. Comparison of mass spectrum of standard 2-dDCB with that of the peak at R_t 39.039 in the sample. (**A**) Standard 2-dDCB and (**B**) nonirradiated cashews.

second protocol, we therefore employed SFE. 2-dDCB was readily identified in the extracts of radiation-processed samples but not in the nonirradiated samples (**Figure 4**). It has been earlier reported (*11*) that overloading of the extraction cell with greater than 2 g of lipid resulted in a high content of triglyceride in the extract. Further purification of the extract was therefore carried out on a solid trap constituted of deactivated silica placed in the extraction device immediately after the variable restrictor. This resulted in elimination of triglyceride impurities completely (*11*). Use of silica column, however, reduces some of the advantages of using SFE. TLC is a simple, rapid, and cost-effective technique as compared to column chromatography. Under the conditions currently used for SFE extraction, the yield of extract obtained was found to be 83 mg per 100 g of cashew

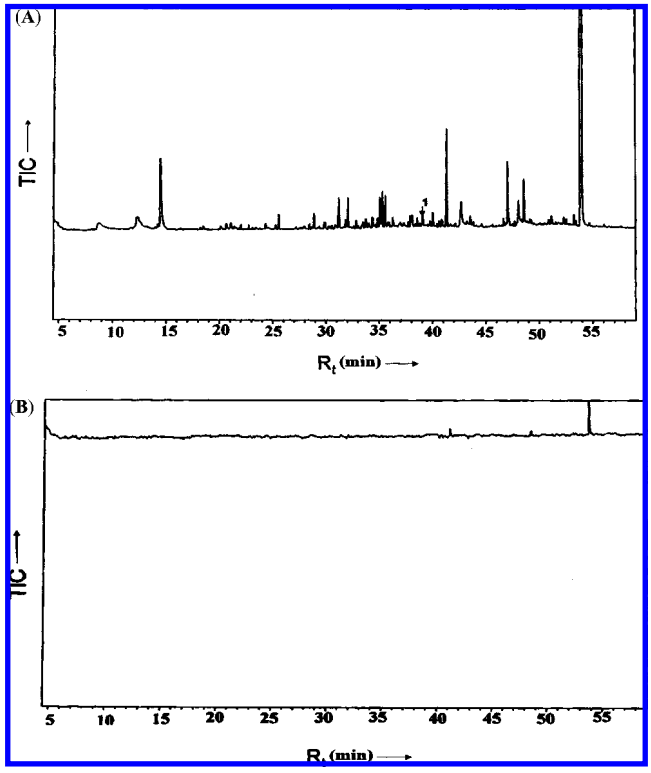

Figure 4. GLC profile of SFE extract of cashew nuts. The peak marked 1 (R_t 39.039) corresponds to 2-dDCB. (**A**) Irradiated sample and (**B**) nonirradiated sample.

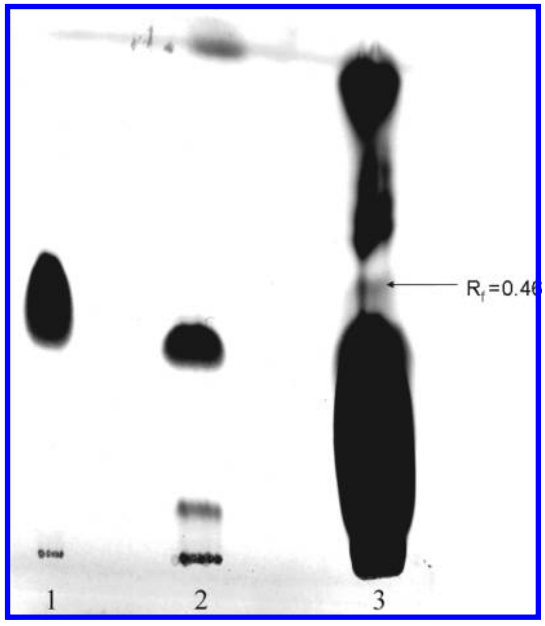

Figure 5. Analytical TLC of SFE extract of irradiated cashew nuts as well as the band (R_f 0.46) corresponding to 2-ACBs in the same sample scraped from preparative TLC. Key: 1, standard 2-dDCB; 2, scraped band; and 3, SFE extract.

nut. Triglycerides are not reported to be extracted under the SFE conditions used (*13*). The ability of the SFE method to selectively extract 2-ACBs was further confirmed by monitoring the chromatographic nature of the isolate on an analytical silica gel TLC plate. A high content of triacylglycerol in the extract was noticed, suggesting a need for prepurification using a preparative silica gel TLC to obtain a fraction rich in 2-ACBs. The band at R_f 0.46 corresponding to the standard 2-dDCB was isolated from the plate (**Figure 5**) and then subjected to GC-

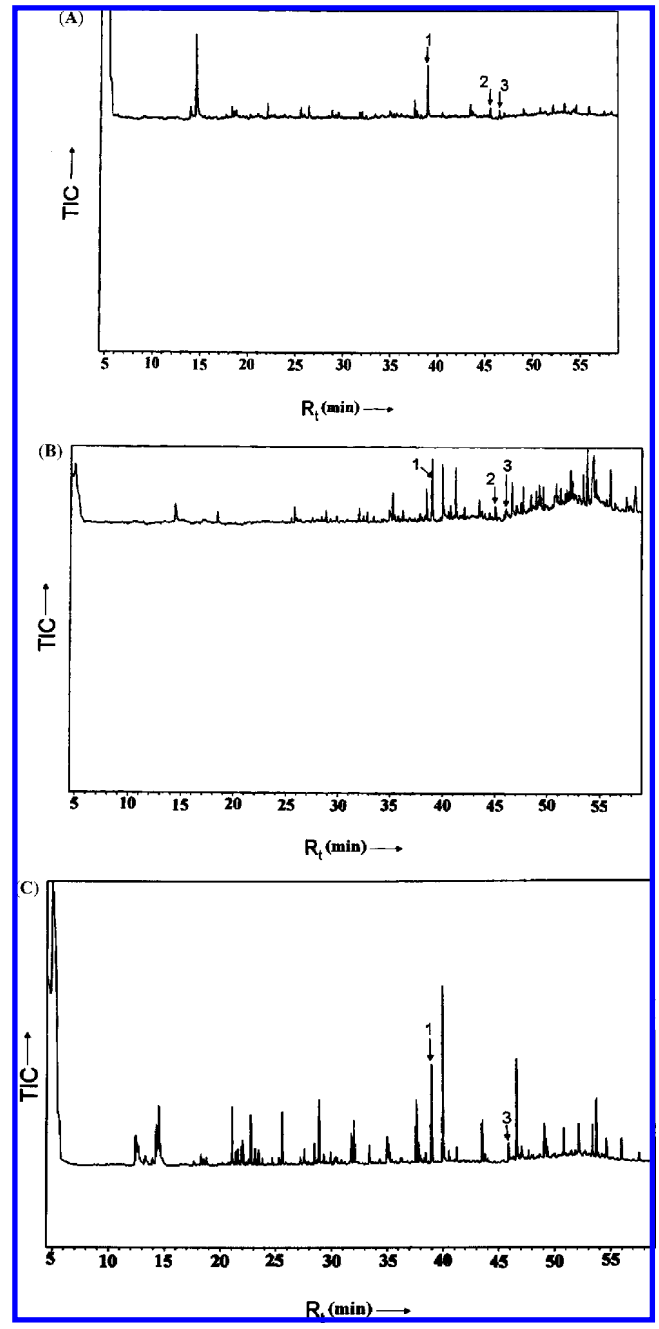

Figure 6. GLC chromatogram of the band (R_f, 0.46) obtained from preparative TLC of SFE extract of irradiated and nonirradiated, commercial cashew nuts and farm fresh cashew nuts. Peaks marked 1, 2, and 3 correspond to 2-dodecyl cyclobutanone, 2-tetradecenyl cyclobutanone, and 2-tetradecyl cyclobutanone at R_t 39.03, 45.01, and 46.07, respectively. (**A**) Irradiated, (**B**) nonirradiated, and (**C**) farm fresh cashews.

MS analysis. The peak at R_t 39.039 readily identified in the chromatograms (**Figure 6**) was confirmed as 2-dDCB from the ratio of the intensities of the fragments with masses 98 and 112 as well as from the mass fragmentation pattern. The combination of SFE with TLC provided higher recovery (96%) of 2-ACBs as compared to extraction using SFE alone (63%). The recovery rate of 2-ACBs using the SFE-TLC method was far higher than that reported earlier (*15*) using SFE directly on food products (60−80%) as well as SFE on Soxhlet extracted fat (90%). The method was, however, comparable to the values (91−98%) obtained by the European committee for standardization officially recommended EN 1785 method for detection of irradiated foods (*16*). A considerable enrichment of 2-ACBs could

Table 1. Amount of 2-ACBs Estimated in Cashew Nut Samples Extracted Using Different Techniques[a]

cashew nut sample	amount of 2-dDCB (μg/g)	amount of 2-tDCB (μg/g)	amount of 2-tDeCB (μg/g)
nonirradiated, Soxhlet extraction + silica gel chromatography	ND	ND	ND
irradiated (1 kGy), Soxhlet extraction + silica gel chromatography	0.95 ± 0.4	ND	ND
nonirradiated, SFE extraction	ND	ND	ND
irradiated (1 kGy), SFE extraction	0.30 ± 0.1	0.13 ± 0.06	ND
nonirradiated, SFE extraction + TLC	2.70 ± 1.71	1.0 ± 0.08	0.52 ± 0.01
irradiated (1 kGy), SFE extraction + TLC	6.12 ± 0.82	2.06 ± 0.4	0.8 ± 0.1
fresh from farm shelled (nonirradiated), SFE extraction + TLC	1.67 ± 0.62	0.9 ± 0.12	ND

[a] Values are means ± SD; $n = 6$ independent replications; ND, not detected.

be attained using the SFE method compensating for its lower sensitivity and resolving power; the minimum limit of detection of 2-dDCB in the sample by gas liquid chromatography was found to be 6.09 pmol. Horvatovich et al. (*15*) using EI ionization and monitoring the ion at *m*/*z* 98 have reported a lowest detectable amount of 0.21 pmol for 2-dDCB.

2-ACBs are known to date to be formed specifically in irradiated foods. Therefore, we employed a combination of SFE with TLC to further confirm the absence/presence of 2-ACBs in the nonirradiated samples. To our surprise, a peak at R_t 39.039 could be readily identified in the chromatogram of both the irradiated and the nonirradiated samples (**Figure 6**). That the peaks were indeed those of 2-dDCB was confirmed by the mass fragmentation pattern as above. The finding thus contradicted the current belief that 2-ACBs are URPs. **Table 1** lists the 2-dDCB content in the extracts of various samples using different methods. The content of 2-dDCB detected in the irradiated sample was found to be approximately 20 times higher (6.12 μg/g) using SFE-TLC as compared to that using SFE alone (0.3 μg/g), suggesting a higher efficiency of the SFE-TLC method in recovering 2-ACBs. This presumably led to the detection of 2-ACBs in the nonirradiated samples. Besides 2-dDCB, 2-tDCB (R_t, 46.07) and 2-tDeCB (R_t 45.01) were also detected in both nonirradiated and irradiated samples (**Figure 6**). The content of 2-ACBs in irradiated sample (**Table 1**) is comparable to the literature values (*17*). The limit of detection of monounsaturated -2-ACBs (μ-2-ACBs) is reported to be far higher than that of saturated ACBs (s-2-ACBs). Monounsaturated -2-ACBs such as 2-tDeCB are therefore generally used as preferential markers of irradiated foods only in rare cases where the ratio of oleic acid over palmitic acid is >3 (*15*). The existence of this compound is thus used as a confirmation of the presence of saturated 2-ACBs (s-2-ACBs). Several analytical difficulties have been encountered by various laboratories in the detection of 2-tDeCB. One such difficulty is related to the coelution of impurities having identical retention times, resulting in poor resolution of the peak corresponding to 2-tDeCB. Aldehydes such as *n*-octadecanal and *n*-hexadecanal have been identified as the major impurities interfering with the peak of 2-tDeCB. Thus, while some researchers were unable to detect this compound in irradiated foods, no clear identification could be made by others when detected in the EI mode. A combination of SFE and TLC as employed in this study aided in a better resolution from interfering impurities such as *n*-octadecanal (R_t 25.892), resulting in a clear identification of this compound. This is also supported by the detection of 2-tDeCB only by using the currently developed method (**Table 1**). The efficacy of the method to detect other 2-ACBs as well was thus clearly demonstrated.

Identification of 2-ACBs in Fresh Cashew Nuts. To further authenticate the above results on the natural occurrence of 2-ACBs, freshly picked, shelled, and dried cashew nuts were subjected to the combined SFE and TLC as above. 2-dDCB could be readily identified by GC-MS in these samples (**Figure 6**). The presence of this compound in fresh nut samples unambiguously demonstrated the natural origin of 2-dDCB (**Table 1**). One-way ANOVA analysis revealed that the values of 2-dDCBs obtained by SFE-TLC method for farm fresh, nonirradiated, and irradiated cashew nuts were significantly different ($p < 0.05$) from each other. A similar trend was also observed in the case of 2tDeCB. However, in the case of 2tDCB, the nonirradiated and farm fresh samples had a similar content of this compound, although their contents were lower than the irradiated samples. The values of 2-ACBs within each treatment were, however, not significantly different. Horvatovich et al. (*17*) in their report on the stability of 2-ACBs during storage have reported losses in these compounds ranging from 40 to 60% in lyophilized poultry meat when stored up to 28 days. Reduction ranging from 21 to 78% has also been reported in poultry meat, cheese, and sardines irradiated at 3 kGy and stored at 4 °C (*7*). Similar reduction has also been reported in mango (2 kGy, 20 °C stored), while higher losses (72%, 2-tDeCB; and 94%, 2-dDCB) have been observed in papaya stored for 2 weeks at 10 °C (*7, 18*). Successful identification of irradiated foods based on 2-ACBs can be significantly affected during postirradiation storage of food. Stewart et al. (*18*) therefore recommended analysis of 2-dDCB in papaya as soon as possible after irradiation when in its freshest state. Loss of 2-dDCB from irradiated liquid whole egg upon heating in oven (64.4 °C, 2.5 min) is also reported (*19*). Hamilton et al. (*20*) have proposed a mechanism of degradation of 2-ACBs via an oxidative conversion of cyclobutanone ring to 4-alkyl-γ-butyrolactone during storage. Thus, degradation of natural 2-ACBs in nonirradiated food during storage may result in their reduced content often below the detection limit of the current methods and the belief of their absence in nonirradiated commodities.

The natural occurrence of cyclobutanones has been reported in the literature (*21*). In studies on the biosynthesis of moniliformin, a fungal toxin possessing a cyclobutanone structure, Franck and Breipohl have reported (*22*) its formation via cyclization of polyketides. Although no naturally occurring 2ACBs have been identified to date, the formation of these compounds either by a similar pathway or via a mechanism of cyclization of lipids as reported during radiation processing could be a distinct possibility. This, however, needs further investigation.

Identification of 2ACBs in Other Nonirradiated Foods. Nutmeg is an important spice of commerce reported to contain 30−40% lipids (*23*). Despite a high content of lipid, the existence of 2ACBs in the irradiated nutmeg and their possible use as markers of radiation processing has not been reported. We, therefore, selected this spice to confirm the natural occurrence of 2-ACBs. Commercial nutmeg samples obtained from a local market were ascertained to be nonirradiated based on the detection of radiation specific hydrocarbon as per the protocol followed by Bhattacharjee et al. (*14*). A radiation dose

Table 2. Amount of 2-ACBs Estimated in Nutmeg Samples[a]

nutmeg samples	amount of 2-DCB (μg/g)	amount of 2-dDCB (μg/g)
nonirradiated, SFE extraction + TLC	2.67 ± 0.21	0.58 ± 0.19
irradiated (5 kGy), SFE extraction + TLC	6.79 ± 0.32	1.74 ± 0.23

[a] Values are means ± SD, $n = 6$ independent replications.

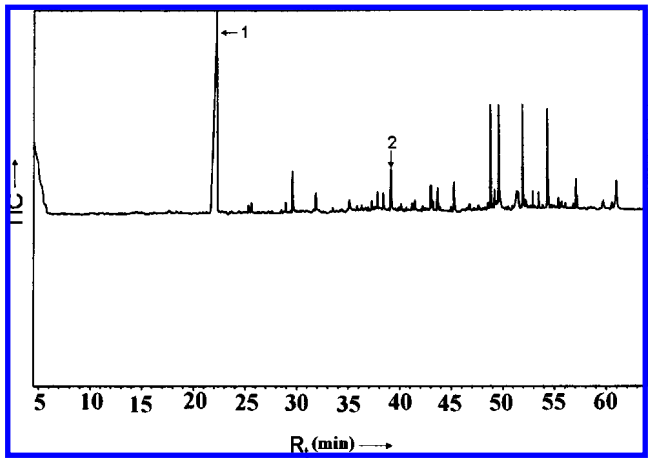

Figure 7. GLC chromatogram of the band (R_f 0.46) obtained from preparative TLC of SFE extract of nonirradiated commercial nutmeg. Peaks marked 1 and 2 correspond to 2-DCB (R_t 22.3) and 2-dodecyl cyclobutanone (R_t 39.03), respectively.

of 10 kGy is normally employed for microbial decontamination of spices (*4*). Our earlier studies have, however, demonstrated the formation of rancid odor in nutmeg beyond a dose of 5 kGy (*24*). Thus, the spice samples (30 g) were exposed to a radiation dose of 5 kGy in the present study. Because myristic and palmitic acid are reported to be the major fatty acids of nutmeg, the formation of 2-DCB and 2- dDCB was expected. The newly developed SFE-TLC protocol was employed for the isolation of 2-ACB from the spice. As in cashew nut, 2-ACBs (2-DCBs and 2-dDCB) were also detected in both the nonirradiated and the irradiated nutmeg. As expected, 2-DCB (R_t 22.3) was found to occur at a much higher concentration than 2-dDCB (**Table 2**). The values of 2-DCB and 2-dDCB in both the nonirradiated and the irradiated samples showed significant differences, although the contents were not significantly different within each treatment. This further confirmed the natural occurrence of 2-ACBs (**Figure 7**). Despite the larger detection limit, larger sample size, and higher enrichment of 2-ACBs, using the SFE-TLC combination may possibly account for the detection of these constituents in this study where others failed.

The present findings on the occurrence of 2-ACBs in nonirradiated food commodities thus contradicted the prevailing belief that these compounds are unique to irradiated foods. This once again strengthens the case for irradiation as a global food safety tool. Wider use of this technology for elimination of food-borne pathogens such as *E. coli* O157:H7 can go a long way in preventing serious outbreaks of food-borne diseases in future.

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