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Hydrocarbons as marker compounds for irradiated cashew nuts

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Abstract

Volatile long chain hydrocarbons, such as 1-tetradecene, 1-hexadecene and 8-heptadecene, could serve as marker compounds in cashew nuts irradiated at 0.25–1.00 kGy. Monitoring these markers over a storage period of 6 months under ambient conditions showed them to be persistent. The concentrations of the markers increased linearly with radiation dose at all storage periods of the study. However, their concentration decreased marginally with storage at all the dose levels.

Keywords: Irradiation; Cashew nuts; Hydrocarbons

1. Introduction

Cashew nuts *Anacardium occidentale* Linn, constitute an important item of commerce from India, and contribute to 0.93% of total exports from the country. Being very prone to insect infestation, chemical disinfestation is widely used (Narvaiz, Lescapo, & Kairiyama, 1992). Use of hazardous chemicals has now been restricted, worldwide, on account of the increasing risk they pose to environment and health. One of the most important applications of irradiation is to destroy or reduce ubiquitous pests and pathogens that contaminate raw foods (WHO, 1988). Efforts in the use and acceptance of food irradiation are now being directed towards establishing methodologies capable of detecting ionization in foods, using both qualitative and quantitative techniques (Anon, 1981). Although it has proven difficult to devise a method to detect irradiated food exclusively, considerable progress has been made and a number of approaches based on physical, chemical, microbiological and biological changes have been attempted (Delincee, 1998; Delincee & Ehlermann, 1989; Delincee, Ehlermann, & Boegl, 1988; Grootveld et al., 1989) and reviewed (Glidewell, Deighton, Goodman, & Hillman, 1993; Singhal, Kulkarni, & Rege, 1997).

Irradiation results in the formation of a characteristic pattern of saturated and olefinic hydrocarbons, aldehydes, methyl and ethyl esters, and 2-alkylcyclobutanones, depending on the fatty acid composition of the fat in the concerned food (Nawar, 1978; Nawar & Balboni, 1970). The radiolysis of fatty acids ($C_{m:n}$, m = number of carbon atoms; n = number of double bonds) leads to the formation of two groups of long chain volatile hydrocarbons. The first group comprises hydrocarbons with one carbon less than the original fatty acid ($C_{m-1:\ n}$) the second group has hydrocarbon with two carbon atoms less than the original fatty acid and one additional double bond at position 1 (first carbon atom) ($C_{m-2:\ n+1}$) by rupture of the side chain in the α and β positions with respect to the carbonyl group (Ammon, Mildau, Ruge, & Delincee, 1992; Champagne & Nawar, 1969). The other group is of 2-alkylcyclobutanones with a $C_{m-4:\ n}$ alkyl chain (LeTeller & Nawar, 1972).

Recovery and measurement of hydrocarbons (C_1–C_{22}) from irradiated lipids, using cold finger vacuum distillation and gas chromatography, has been reported (Nawar, Champagne, Dubravcic, & LeTellier, 1969). After several investigations, the most appropriate markers appear to be tetradecene, hexadecadiene and heptadecene, the amounts of which increase with increasing radiation dose (Nawar, Zhu, & Yoo, 1990; Singhal, Kulkarni, & Rege, 1997). The presence of

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volatile hydrocarbons in a foodstuff is not specific to ionizing treatment, but the appearance of a hydrocarbon couple $C_{m-1:\ n}/C_{m-2:\ n+1}$ for each fatty acid $C_{m:\ n}$ present indicates, unambiguously, that such a treatment has been performed (Dubravcic & Nawar, 1968; Nawar & Balboni, 1970; Merritt, Angelini, Wierbicki, & Shults, 1975; Bergaentzlé, Sanquer, Hasselmann, & Marchioni, 1994; Nawar et al., 1996; Horvatovich, Miesch, Hasselmann, & Marchioni, 2000). These have been detected by HPLC-GC-FID in products such as soup mixes, spices, fish and shrimps (Biedermann, Grob, Frohlich, & Meier, 1992; Lembke et al., 1995), in chicken, pork, turkey, duck breast, German Caviar and beef (Horvatovich et al., 2000; Lembke, Börnet, & Engelhardt, 1995; Morehouse, Kiesel, & Yuoh, 1993), in avocado-pear and fresh pilchard lipids (Lesgards, Raffi, Pouliquen, Chaouch, & Giamarchi, 1993), in cheese, chocolate and liquid whole eggs (Bergaentzlé et al., 1994; Horvatovich et al., 2000) and in peanuts and roasted Pistachio nuts (Lembke, Börnet, & Engelhardt, 1995). Pentadecene, tetradecene, heptadecadiene and hexadecatriene, produced from palmitoleic acid and linoleic acid have also been established as markers of irradiation in chicken, since the contents of the two parent fatty acids are high in chicken fat (Nawar, 1990; Morehouse, Kiesel, & Yuoh, 1993; Horvatovich et al., 2000). Similarly nonane and hexadecadiene may be used as markers of irradiated bacon (beef) and pork fats (Champagne & Nawar, 1969; Singh, Kremers, Baressi, George, Delaney, & Marsman, 1989; Singh, Guerrero, & Kremers, 1993; Hampson, Jones, Foglia, & Kohout, 1996). This method has also been well adapted to Camembert cheese (Bergaentzle et al., 1994) and also various irradiated products such as whole liquid egg (Crone, Hand, Hamilton, Sharma, Boyd, & Stevenson, 1993), pork (Stevenson, 1992), fresh and seawater fish as tilapia and mullet (Tewfik, Ismail, & Sumar, 1999) and mango, papaya and salmon meat (Stewart, Moore, Graham, McRoberts, & Hamilton, 2000). These compounds are promising markers, even when irradiated eggs are used as an ingredient in processed foods, such as baked products (Pfordt & Von Grabowski, 1995).

In addition to thermoluminescence and ESR (Electron spin resonance), analysis of these hydrocarbons by gas chromatography (GC) is being used. This is the method of choice for routine analysis in control laboratories with respect to cost and analytical know-how.

The changes induced by irradiation of fatty acids in cashew nut oil into hydrocarbons can be used as the basis for identification of irradiated cashew nuts. No data are available on potential biomarkers of irradiated cashew nuts. The long chain hydrocarbons are difficult to isolate from the lipid matrix by conventional column separation methods. For isolation of these volatile lipids, open column lipid chromatography on Florisil columns has been applied for chicken (Ammon, Mildau, Ruge, & Delincee, 1992; Boyd, Crone, Hamilton, Stevenson, & Stevenson, 1991; Crone, Hamilton, & Stevenson, 1992; Sjöberg, Tuominen, Liutamo, & Luukkonen, 1992), for shrimp and frog legs (Morehouse & Ku, 1992; Morehouse, Ku, Albrech, & Yang, 1991) and for Camembert cheese (Bergaentzlé et al., 1994). Compared to other applied isolation techniques, such as cold finger distillation (Nawar & Balboni, 1970), the Florisil clean up has a higher sample capacity but is rather time consuming and requires large amounts of organic solvents. Hence, supercritical fluid extraction (SCFE) technology, as an alternative sample preparation technique, has been studied and has proved considerably successful in studies with peanuts, instant soup mix powder, roasted Pistachio nuts, duck breast, pork meat and German Caviar (Lembke et al., 1995), beef (Hampson et al., 1996), fresh water fish, tilapia and sea water fish, mullet (Tewfik et al., 1999), and cheese, chocolate and liquid whole eggs (Horvatovich et al., 2000).

In the present work, an attempt has been made to identify hydrocarbon biomarkers of cashew nuts, irradiated at different doses, as a function of storage time. The hydrocarbons were isolated, by both conventional column chromatography and SCFE to check for any merits/demerits of the individual procedures.

2. Materials and methods

2.1. Materials

Glass-distilled *n*-hexane (AR grade) was procured from M/s S.D Fine Chemicals Ltd, Mumbai, Silica-gel plates, 60 F_{254} (TLC aluminium sheets 20 cm×20 cm, particle size 4–6 nm) were purchased from M/s E. Merck India Ltd, Mumbai. Silicic acid (100–200 mesh for lipid chromatography) was obtained from M/s Spectrochem Pvt. Ltd., Mumbai. Whole cashew nuts (commercial grade White Wholes W 320; specification laid down by Government of India under the Export Quality Control and Inspection Act, 1963, implemented by the Cashew Export Promotion Council of India, Cochin) (Woodroof, 1967) were procured from a local market of Mumbai, India.

2.2. Methods

2.2.1. Irradiation process

The cashew nuts, in batches of 100 g each, were packed into 60 gauge thick self-sealable low-density polyethylene (LDPE) bags. All samples were stored under ambient conditions (room temperature, 31–32 °C) during the study. The cashew nuts were irradiated at 0.25, 0.50, 0.75 and 1.00 kGy, using a ^{60}Co gamma source at the Food Technology Division of Bhaba

Atomic Research Center, Mumbai. Fricke's Dosimeter was used for the measurement of the applied irradiation dose. The temperature and the dose rate for all samples were 30 °C and 55 Gy/min respectively.

2.2.2. Oil extraction

The oils from control and irradiated cashew nuts after grinding were extracted by the manual Soxhlet apparatus (Scientific Apparatus Manufacturing Company, Mumbai, India) for 16 h, using distilled AR grade *n*-hexane as the solvent. The oil extraction was carried out prior to column chromatography and/or SCFE at 2-month intervals, i.e. at 0, 2, 4 and 6 months.

2.2.3. Transesterification of cashew nut oil and GC-MS

To know the parent fatty acid composition of cashew nuts, transesterification of the cashew nut oil into fatty acid ethyl esters (FAEE) for GC analysis was done using the method described by Lembke et al. (1995). The FAEE were analyzed using a Shimadzu QP-5050A GC/MS instrument equipped with a GC-17A gas chromatograph (Kyoto, Japan) with a flame ionization detector. The column was a non-polar DB-1 (polymethyl siloxane with 1% phenyl modification) capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm). Helium was used as the carrier gas. Splitless injection of 1.5 μL of sample solution was utilized for analysis. The column was heated from 140 to 210 °C at the rate of 4 °C/min and held at the initial and final temperatures for 5 min. Injector and interface temperatures were maintained at 210 and 230 °C, respectively. Ionization voltage was 70 eV. Electron multiplier voltage was 1 kV. Matching of mass spectral fragmentation pattern with those in the spectral library (WILEY/NIST Library) provided with the instrument identified the compounds of interest.

2.2.4. Isolation of hydrocarbons

2.2.4.1. Column chromatography. Silicic acid (100–200 mesh for lipid chromatography) was washed with methanol and water to remove fines and impurities, and activated at 110–120 °C overnight. Approximately 35–38 g of silicic acid was used for the column of length 100 mm and internal diameter 25 mm. A slurry of silicic acid in *n*- hexane was poured into the column. About 1 g of the extracted cashew nut oil was applied to the column and the hydrocarbons were eluted with *n*-hexane. This was done with the oil from the control sample and that from irradiated samples alike. Hydrocarbons were eluted in the initial fraction itself (180 ml) and concentrated by rotary evaporator (Buchi, Flawil, Switzerland) at 45 °C and 500 mm Hg vacuum and further purified on aluminium TLC plates precoated with silica gel 60 F_{254} and *n*-hexane as developing solvent. This fraction gave two distinct bands after visualising in an iodine chamber. GC-MS analysis of the bands revealed presence of hydrocarbons in the band with R_f value of 0.85–0.9. This band was scraped off and the hydrocarbons were extracted in *n*-hexane. The top band was analyzed for hydrocarbons by GC-MS. The compounds were eluted from the silica gel by repeated washings using *n*-hexane through non-absorbent cotton wool. The residue after concentration was dried with anhydrous sodium sulphate and concentrated to nearly 0.1 ml by slowly purging nitrogen. For all doses of irradiation, the hydrocarbons were extracted in a similar manner.

2.2.4.2. Supercritical fluid extraction (SCFE). For SCFE, a SPEED-SFE model of Applied Separations, Allentown, USA was used. In the preliminary trials, optimization of the SCFE method was done with cashew nuts irradiated at 10 kGy. Non-porous glass beads (average particle diameter, dp = 4.72 mm) were spiked (thorough mixing and homogenization) with 1 g of extracted cashew nut oil and packed in the extraction vessel for SCFE. The parameters of SCFE, such as sample size, temperature, pressure and time of extraction, flow rate of carbon dioxide during collection phase, were optimised to selectively extract only the long-chain hydrocarbons, with minimum triglycerides and fatty acids. The optimized conditions of SCFE that gave the best yield of hydrocarbons with minimum fatty acids were a sample size of 1 g oil at 80 °C and pressure of 150–155 bar for 10 min static and 45 min dynamic and a flow rate of 0.1 l/min of carbon dioxide. The hydrocarbons were collected in ~50 ml *n*-hexane at −2 to −5 °C. These optimised conditions were used in all the subsequent trials with the lower irradiation dosages.

For GC-MS analysis of the extracts, the collection solvent was slowly removed from the sample vials by a gentle nitrogen stream to a volume of 0.1 ml.

2.2.5. GC-MS analysis of the extracts

The extracts were analyzed using the Shimadzu QP-5050A GC/MS instrument as described above. The following temperature programme was used: the column temperature was programmed from 60 to 200 °C at the rate of 4 °C/min, held at the initial temperature and at 200 °C for 5 min and further heated to 280 °C at the rate of 10 °C/min and held at the final temperature for 25 min. Injector and interface temperatures were maintained at 210 and 230 °C, respectively. Ionization voltage was 70 eV. Electron multiplier voltage was 1 kV. Sample solution (1 μl) was used for analysis and the samples were injected in splitless mode. The identification of the hydrocarbons was done with the mass selective detector and the available MS library, as stated above. The relative concentration of the hydrocarbons in the oil is expressed in terms of relative peak area (%) of the same as obtained in the GC chromatogram.

3. Results and discussion

The major fatty acids identified in non-irradiated (control) cashew nuts were 59.9% oleic acid ($C_{18:1}$), 20.9% linoleic acid ($C_{18:2}$), 9.65% palmitic acid ($C_{16:0}$) and 9.45% stearic acid ($C_{18:0}$). This is in accordance with the literature reports (Mahindru, 1977). From the parent fatty acid composition of the cashew nuts, the expected long-chain hydrocarbons would be mainly pentadecane ($C_{15:0}$) and 1-tetradecene ($C_{14:1}$) from palmitic acid ($C_{16:0}$); heptadecene ($C_{17:0}$) and 1-hexadecene ($C_{16:1}$) from stearic acid ($C_{18:0}$); 8-heptadecene ($C_{17:1}$) and 1,8-hexadecadiene ($C_{16:2}$) from oleic acid ($C_{18:1}$) and heptadecadiene ($C_{17:2}$) and hexadecatriene ($C_{16:3}$) from linoleic acid ($C_{18:2}$), according to the theory of Nawar (1986).

Apart from some of the marker hydrocarbons, other hydrocarbons, such as undecane, dodecane and tridecane and parent fatty acids, such as palmitic and stearic, were detected by column chromatography in the cashew nuts irradiated at 1.00 kGy. However, these were not detected in the irradiated samples extracted by the SCFE technique, justifying the use of the high pressure SCFE technique for the extraction of these hydrocarbons over the conventional column chromatography techniques.

The conditions of SCFE that gave the best yield of hydrocarbons have been described above. A pressure more than 155 bar resulted in extraction of the parent oleic, stearic and palmitic acids. At the optimized conditions of SCFE, the selectivity of carbon dioxide for the marker hydrocarbons was the highest. This has been

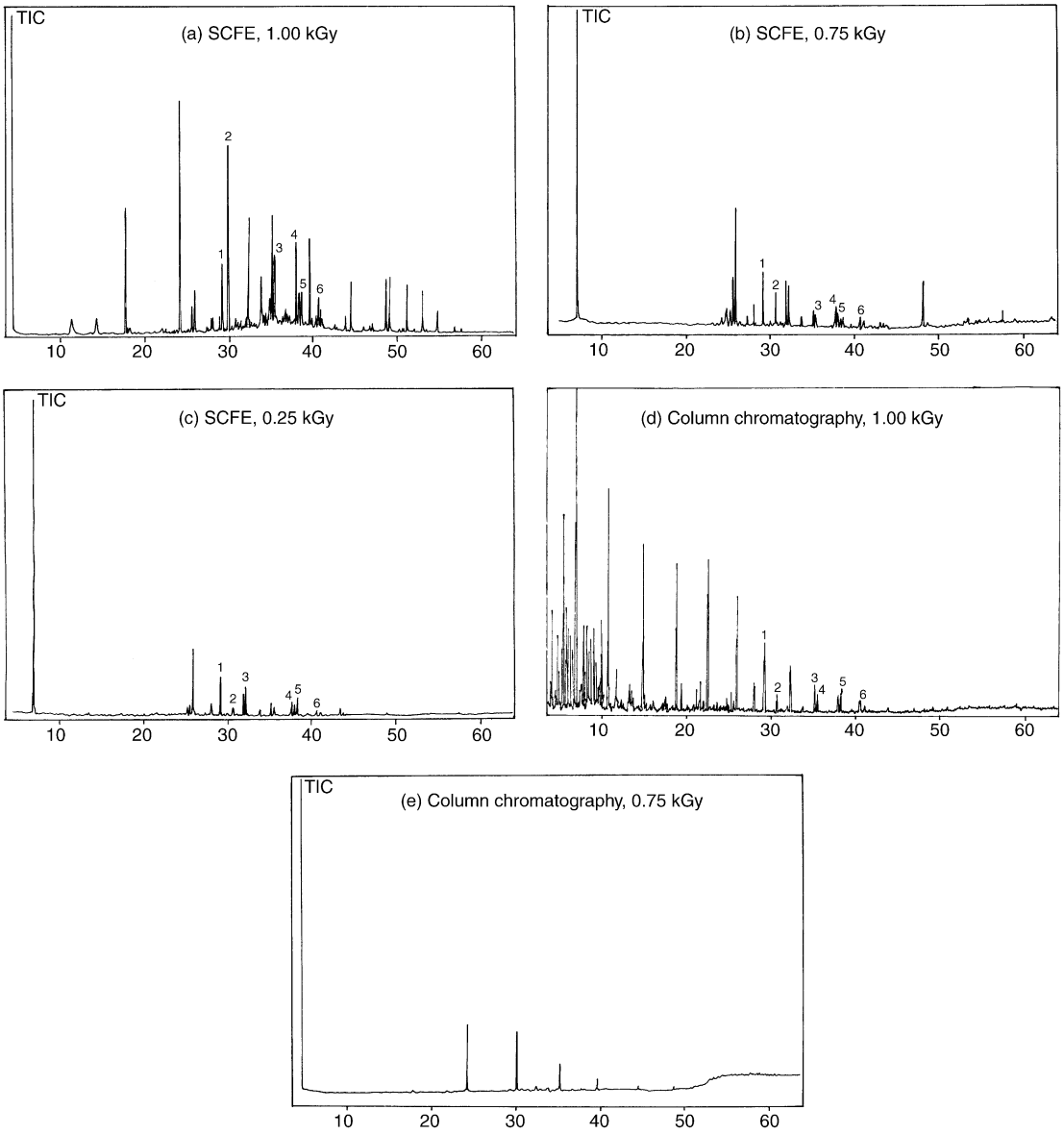

Fig. 1. Comparative profile of marker hydrocarbons detected by SFE and column chromatography for fresh cashew nuts irradiated at different dosages. 1. 14:1 2. 15:0 3. 16:1 4. 17:0 5. 17:1 6. 17:2.

shown to be true for extraction of volatile hydrocarbons from irradiated foods such as peanuts, instant soup mix powder, roasted pistachio nuts, duck breast, pork meat and German caviar (Lembke, Börnet, & Engelhardt, 1995). However, a much lower flow rate of carbon dioxide than that reported for the above could yield an appreciable quantity of the volatile hydrocarbons in the same extraction time.

Fig. 1a–e give a comparative profile of the marker hydrocarbons detected by SCFE and column chromatography for the fresh samples, irradiated at different doses. From the figures it can be clearly seen that, in case of SCFE, it was possible to detect some of these marker hydrocarbons, even at the lowest dose of 0.25 kGy and the extract obtained was much cleaner and free from the non-marker hydrocarbons, whereas it was not possible to detect the marker hydrocarbons at a dosage lower than 1.00 kGy, by column chromatography.

The hydrocarbons detected by SCFE for 0.25 kGy-irradiated cashew nuts, over a 6-month storage period, are tabulated in Table 1. It is seen that the marker hydrocarbons 1-tetradecene (14:1), 1-hexadecene (16:1) and 8-heptadecene (17:1) were persistently present until 180 days of storage. The hydrocarbon pattern, as shown for 1.00 kGy dosage, was qualitatively similar for all the irradiation dosages. These marker hydrocarbons were not detected in the control, non-irradiated sample throughout the storage period. However, two of the hydrocarbons, pentadecane and heptadecane were detected even in the control, non-irradiated cashew nuts as well as in the irradiated ones. Their levels remained fairly constant over the storage period, 2.36–2.02% for pentadecane and 2.11–2.03% for heptadecane from 0–180 days of storage. These hydrocarbons have also been reported for γ-irradiated raw-milk Camembert cheese (Bergaentzle et al., 1994) and for irradiated sesame seeds (Choi & Hwang, 1997). Hence the hydrocarbons that could serve as potential biomarkers of irradiated cashew nuts are 1-tetradecene, 8-heptadecene and 1-hexadecene. These could be detected in substantial quantities in all the irradiation dosages, even after 180 days of storage. In general, the most appropriate markers suggested are tetradecene, hexadecadiene and heptadecene (Nawar, Zhu, & Yoo, 1990; Singhal et al., 1997). Hexadecadiene was not detected in irradiated cashew nuts, possibly because of very low concentration of the same; instead 1-hexadecene proved to be a good alternative.

The relative percentages of the marker hydrocarbons in irradiated cashew nut oil have been listed as a function of storage time and irradiation dose in Table 2. It is seen that the concentrations of the biomarkers increased with irradiation dose for the entire storage period, but their concentrations decreased marginally with storage at all the radiation doses. The correlation of the marker compounds with storage time and irradiation dose are listed in Table 3. A fairly good linear correlation has been obtained for the content of hydrocarbons with both dosage level and storage time.

Table 1
Hydrocarbon composition of cashew nuts irradiated at 0.25 kGy[a]

Storage time (days)	Fatty acid	Theoretical HC pattern		Observed HC pattern		Retention time (min)	
		n-1	*n*-2	*n*-1	*n*-2	*n*-1	*n*-2
0	16:0	15:0	14:1	15:0[b]	14:1	30.79	29.29
	18:0	17:0	16:1	17:0[c]	16:1	38.08	35.57
	18:1	17:1	16:2	17:1		38.41	
	18:2	17:2	16:3	17:2		40.74	
60				15:0	14:1	30.71	29.24
				17:0	16:1	38.42	33.08
				17:1		39.21	
120				15:0	14:1	32.29	29.20
				17:0	16:1	37.99	35.50
				17:1		38.30	
180				15:0	14:1	31.98	29.22
				17:0	16:1	37.99	37.72
				17:1		38.31	

[a] The hydrocarbon pattern was similar for all dosages of 0.25, 0.50, 0.75 and 100 kGy.
[b] No. of carbon atoms.
[c] Compounds present even in non-irradiated cashew nuts.

Table 2
% Relative composition of marker compounds in irradiated cashew nut oil as a function of irradiation dose and storage time

Irradiation dose (kGy)	Storage time (days)	Relative% composition in oil		
		1-Tetradecene (14:1)	1-Hexadecene (16:1)	8-Heptadecene (17:1)
0.25	0	1.30	1.12	0.57
	60	1.28	1.05	0.51
	120	1.23	0.99	0.48
	180	1.21	0.92	0.41
0.50	0	1.47	1.31	0.71
	60	1.44	1.28	0.68
	120	1.39	1.24	0.59
	180	1.35	1.19	0.57
0.75	0	1.63	1.54	0.84
	60	1.60	1.51	0.81
	120	1.58	1.48	0.78
	180	1.52	1.44	0.75
0.75	0	1.75	1.68	1.18
	60	1.71	1.62	1.12
	120	1.66	1.57	1.10
	180	1.61	1.53	0.98

Table 3
Correlation of the marker compounds (Y) with storage time (X) and irradiation dose (X')

Correlation of the marker compound	Marker compound		
	1-Tetradecene	1-Hexadecene	8-Heptadecene
With irradiation dose after storage (days) for			
(a) 0	$Y = 0.604\ X + 1.160$ ($R^2 = 0.99$)	$Y = 0.764\ X + 0.935$ ($R^2 = 0.99$)	$Y = 0.784\ X + 0.335$ ($R^2 = 0.94$)
(b) 60	$Y = 0.580\ X + 1.145$ ($R^2 = 0.99$)	$Y = 0.776\ X + 0.880$ ($R^2 = 0.98$)	$Y = 0.784\ X + 0.290$ ($R^2 = 0.96$)
(c) 120	$Y = 0.592\ X + 1.095$ ($R^2 = 0.98$)	$Y = 0.792\ X + 0.825$ ($R^2 = 0.96$)	$Y = 0.820\ X + 0.225$ ($R^2 = 0.95$)
(d) 180	$Y = 0.548\ X + 1.080$ ($R^2 = 0.99$)	$Y = 0.832\ X + 0.750$ ($R^2 = 0.96$)	$Y = 0.756\ X + 0.205$ ($R^2 = 0.99$)
With storage time at dosage (kGy) of			
(a) 0.25	$Y = -0.0005\ X' + 1.303$ ($R^2 = 0.97$)	$Y = -0.0011X' + 1.119$ ($R^2 = 1.00$)	$Y = -0.0009\ X' + 0.569$ ($R^2 = 0.98$)
(b) 0.50	$Y = -0.0007\ X' + 1.474$ ($R^2 = 0.99$)	$Y = -0.0007\ X' + 1.315$ ($R^2 = 0.99$)	$Y = -0.0008\ X' + 0.714$ ($R^2 = 0.94$)
(c) 0.75	$Y = -0.0006\ X' + 1.635$ ($R^2 = 0.95$)	$Y = -0.0005\ X' + 1.542$ ($R^2 = 0.99$)	$Y = -0.0005\ X' + 0.840$ ($R^2 = 1.00$)
(d) 1.00	$Y = -0.0008\ X' + 1.753$ ($R^2 = 1.00$)	$Y = -0.0008\ X' + 1.675$ ($R^2 = 0.99$)	$Y = -0.001\ X' + 1.188$ ($R^2 = 0.91$)

4. Conclusion

The theoretically predicted hydrocarbon pattern for irradiated cashew nuts compared well with that found experimentally in the case of SCFE extracted samples. This proves the potential of SCFE for a reliable identification of irradiated cashew nuts. 1-Tetradecene, 1-hexadecene and 8-heptadecene could serve as potential biomarkers for irradiated cashew nuts that are also persistent for a 6-month storage period.

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