Formation of Hydrocarbons in Irradiated Brazilian Beans: Gas Chromatographic Analysis To Detect Radiation Processing

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Radiation processing of beans, which are a major source of dietary protein in Brazil, is a valuable alternative to chemical fumigation to combat postharvest losses due to insect infestation. To ensure free consumer choice, irradiated food will be labeled as such, and to enforce labeling, analytical methods to detect the irradiation treatment in the food product itself are desirable. In two varieties of Brazilian beans, Carioca and Macaçar beans, the radiolytic formation of hydrocarbons formed after α and β cleavage, with regard to the carbonyl group in triglycerides, have been studied. Using gas chromatographic analysis of these radiolytic hydrocarbons, different yields per precursor fatty acid are observed for the two types of beans. However, the typical degradation pattern allows the identification of the irradiation treatment in both bean varieties, even after 6 months of storage.

Keywords: *Food irradiation; irradiation identification; beans; Phaseolus vulgaris L.; Vigna unguiculata L. Walp; gas chromatography; hydrocarbons*

INTRODUCTION

Beans are a major source of dietary protein in Brazil. However, high losses due to insect infestation occur after each harvest. For insect disinfestation, treatment with ionizing radiation offers an attractive alternative to chemical fumigation. Food irradiation is now recognized as a safe and effective process for a range of specific applications, among them the disinfestation of various food products such as cereal grains, legumes, fresh and dried fruit, nuts, dried vegetables, dried fish, and meat (WHO, 1994; Diehl, 1995; Moy, 1985; IAEA, 1991). Irradiated food lasts longer and can be more accessible to a greater number of people at a lower cost. The process of food irradiation is at present approved in about 40 countries worldwide, and increasing amounts of food are circulating in the international trade market (Loaharanu, 1995). Although administrative control of facilities licensed for food irradiation and compulsory certification of irradiated food should provide a reliable control, it is desirable to apply analytical methods to detect the irradiation treatment directly in the food product itself (Delincée, 1991; Raffi et al., 1994; McMurray et al., 1996). International cooperation has led to a large development of detection methods, and five of these have in the meantime been adopted as European Standards by the European Committee of Standardization (CEN). These five standards are electron spin resonance measurements for irradiated food containing bones (e.g., meat, fish, and frog legs) or cellulose (e.g., nut shells and paprika powder), thermoluminescence measurements of irradiated food from which particles of silicate minerals can be isolated (e.g., herbs and spices and shrimps), and gas chromatographic analysis of fat-containing foods (e.g., meat) measuring 2-alkylcyclobutanones or hydrocarbons (Delincée, 1996). This paper describes the gas chromatographic analysis of hydrocarbons from irradiated Brazilian beans.

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MATERIALS AND METHODS

Sample Material. Two kinds of Brazilian beans, Carioca (*Phaseolus vulgaris* L.) and Macaçar beans (*Vigna unguiculata* L. *Walp*), were purchased from a local market, São Paulo.

These beans in polyethylene bags were irradiated in a ^{60}Co source (IPEN, São Paulo, Gammacell 220, AECL) with doses varying between 0, 0.5, 1.0, 2.5, 5.0, and 10.0 kGy (dose rate, 0.44 kGy/h). Following irradiation, beans were stored at room temperature for 3 months in Brazil and then shipped to Karlsruhe, Germany, where storage was continued for another 3 months.

Principle of the Hydrocarbon Detection Method (DIN-EN 1784). During irradiation, chemical bonds are broken in triglycerides mainly in the α and β positions to the carbonyl groups resulting in the C_{n-1} and $C_{n-2:1}$ hydrocarbons. The C_{n-1} hydrocarbon has one carbon atom less than the parent fatty acid, and the $C_{n-2:1}$ hydrocarbon has two carbon atoms less than the parent fatty acid and an additional double bond in position 1. To predict these chief radiolytic products, the fatty acid composition of the samples has to be known (Nawar, 1977, 1978, 1983a,b, 1986, 1988; Nawar et al., 1990, 1996; Merritt et al., 1978, 1985; Delincée, 1983).

For detection of hydrocarbons, the fat is isolated from the sample by solvent extraction. The hydrocarbon fraction is obtained by Florisil chromatography and subsequently separated using gas chromatography and detection with a flame ionisation detector (FID) or a mass spectrometer (MS).

Reagents. All reagents (e.g., solvents, Florisil, sodium sulfate) and materials (e.g., extraction thimbles, tubes) have to be checked for possible contamination with relevant hydrocarbons. Do not use plastic materials for the analysis (Hartmann et al., 1995).

Fat Extraction (Hartmann et al., 1996). Homogenized beans (25 g) were mixed with 25 g of anhydrous sodium sulfate, filled into glass fiber thimbles, and covered with a plug of glass

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wool. Extraction was carried out in a Soxhlet apparatus for 5 h with *n*-hexane under reflux. The solvent is removed by rotary evaporation.

Fatty Acid Composition. Fatty acid analysis was carried out by preparing fatty acid methylesters using trimethylsulfoniumhydroxid (DGF-method C-VI 11e), followed by quantitation employing gas chromatography (DGF-method C-VI 10a) (Deutsche Gesellschaft für Fettwissenschaft e.V. Münster, 1987).

Isolation of the Hydrocarbon Fraction (Hartmann et al., 1996). Fat (150 mg) was mixed with 0.5 mL of *n*-hexane containing 0.8 μg/mL of each *n*-hexadecane (C16:0) and *n*-eicosane (C20:0), applied to a solid phase extraction (SPE) column containing 2 g of activated Florisil. Hydrocarbons are eluted with 4 mL of *n*-hexane at a flow rate of 0.5−1 mL/min. To avoid inadvertent evaporation to dryness, 0.5 mL of *iso*-octane is added to the eluate, which is then concentrated on the rotary evaporator to about 0.5 mL (test solution).

Separation and Identification of Hydrocarbons. The hydrocarbons were separated on a J&W Scientific DB-5 column, 30 m × 0.32 mm (i.d.) with a 0.25 μm stationary phase (5% diphenyl and 95% polysiloxane), coupled with a retention gap, 2 m × 0.53 mm (i.d.), phenyl-desactivated (Macherey & Nagel) on a Carlo-Erba Type MEGA 5300 gas chromatograph equipped with a flame ionization detector. Conditions were as follows:

injection volume	5 μL
injection mode	cool on column
carrier gas	helium (70 kPa, 2mL/min)
detector temperature	300 °C
initial column temperature	95 °C for 1 min
first ramp	15 °C/min to 140 °C
second ramp	3 °C/min to 200 °C
third ramp	15 °C/min to 300 °C
final temperature	is held for 10 min

The hydrocarbons were identified by comparison of retention times employing a hydrocarbon standard solution with concentrations of about 0.8 μg/mL in iso-octane of 1-tetradecene (1, 14:1), *n*-tetradecane (14:0), *n*-pentadecane (15:0), 1,7-hexadecadiene (1,7, 16:2), 1-hexadecene (1, 16:1), *n*-hexadecane (16:0), 8-heptadecene (8, 17:1), 1-heptadecene (1, 17:1), *n*-heptadecane (17:0) and *n*-eicosane (20:0). Standards were purchased from TeLA Technische Lebensmittel- und Umweltanalytik GmbH, Berlin.

Whenever any ambiguity existed in the recognition of the radiation-induced hydrocarbon pattern, mass spectrometry for identification was employed.

Quantitative measurements were based on the comparison of relative peak areas with those of internal standards such as C16:0 (*n*-hexadecane) and C20:0 (*n*-eicosane), taking recovery rates into account. All analysis were carried out in duplicate.

RESULTS AND DISCUSSION

The studied beans contain large amounts of palmitic, oleic, linoleic, and linolenic acids (Table 1). The expected hydrocarbons after an irradiation treatment are the C_{n-1} and $C_{n-2:1}$ hydrocarbons of the main fatty acids (Table 2).

Figures 1 and 2 show the gas chromatograms of the hydrocarbon fraction of unirradiated and irradiated Carioca and Macaçar beans. In agreement with the lipid degradation patterns during irradiation as proposed by Nawar (1977, 1978, 1983a,b, 1986, 1988, 1990, 1996) a number of radiolytic hydrocarbons can be observed. Obviously, the amount of hydrocarbons increase with radiation dose (Tables 3 and 4).

Only the expected hydrocarbons from palmitic, oleic, and linoleic acid were estimated quantitatively in this work since standard solutions of hydrocarbons derived from linolenic acid, namely 6, 9, 12, 17:3 and 1, 7, 10, 13, 16:4 are not available. Some of the hydrocarbons,

Table 1. Fatty Acid Composition of Brazilian Beans[a]

fatty acid	Carioca (%)	Macaçar (%)
C12:0, lauric acid	0.2	
C14:0, myristic acid	0.1	0.4
C15:0, pentadecylic		0.1
C16:0, palmitic acid	11.3	26.8
C16:1, palmitoleic acid	0.1	0.9
C17:0, margaric acid	0.2	0.2
C18:0, stearic acid	1.9	5.9
C18:1, oleic acid	11.0	28.5
C18:2, linoleic acid	35.5	19.3
C18:3, linolenic acid	36.7	11.3
C20:0, arachidic acid	0.5	1.2
C20:1, gadoleic acid	0.1	0.5
C22−24, higher acids	2.3	4.7
total amount of fat (%)	1.0	0.8

[a] Results are presented in area percentage of the chromatograms (100% = total of determined fatty acids).

Table 2. Radiolytic Hydrocarbons Derived from Fatty Acids

	radiation-induced hydrocarbons	
fatty acid	C_{n-1}	$C_{n-2:1}$
C16:0, palmitic acid	15:0	1, 14:1
C18:1, oleic acid	8, 17:1	1,7, 16:2
C18:2, linoleic acid	6,9, 17:2	1,7,10, 16:3
C18:3, linolenic acid	6,9,12, 17:3	1,7,10,13, 16:4

e.g., pentadecane (C15:0) were present already in the unirradiated samples. This kind of contamination has been previously observed, particularly saturated hydrocarbons occur frequently, e.g., from the packaging material (Schreiber et al., 1994; Biedermann et al., 1992; Grob et al., 1991; Lembke et al., 1995; Schulzki et al., 1996; Morehouse et al., 1991, 1992). Also unsaturated hydrocarbons have been observed in unirradiated foods, e.g., fish (Schulzki et al., 1993) or beef (Hartmann et al., 1995). It again should be noticed that the formation of hydrocarbons is not specific for irradiation. Many hydrocarbons are also formed after heating or after oxidation. For example, in vegetable oils, long-chain hydrocarbons are found after heating or frying (Nawar, 1983b, 1988; Lesgards et al., 1993; Hartmann et al., 1997a); in roasted pistacchio nuts, many hydrocarbons were observed (Lembke et al., 1995). Also in animal products, such as roasted chicken (Noleau and Toulemonde, 1987), long-chain hydrocarbons are found. However, the typical degradation pattern with C_{n-1} and $C_{n-2:1}$ hydrocarbons derived from precursor fatty acids is characteristic for irradiation (Nawar, 1988).

The characteristic formation of hydrocarbons during irradiation is illustrated in Figures 3 and 4. It is interesting to note that, for all three fatty acids, C16:0 palmitic acid, C18:1 oleic acid, and C18:2 linoleic acid, the $C_{n-2:1}$ hydrocarbon occurs in a higher yield than the C_{n-1} hydrocarbon, at least at higher doses where originally contaminating hydrocarbons play a minor role.

When the yield of radiolytic hydrocarbons was plotted versus amounts of parent fatty acids (Figures 5 and 6), the linear response with radiation dose is demonstrated. It is also shown that strongly different yields per precursor fatty acid are obtained for the two types of beans. Whereas in Carioca beans 1,7-hexadiene is formed with the highest yield (∼17.0 μg/g of oleic acid/kGy) formation in Macaçar beans is rather low (∼1.7 μg/g of oleic acid/kGy). In Macaçar beans, the highest yield is found for 1-tetradecene (∼6.5 μg/g of palmitic acid/kGy). Such different yields of hydrocarbons from various substrates have been previously observed, e.g., for beef, pork, and chicken meat (Schreiber et al., 1994),

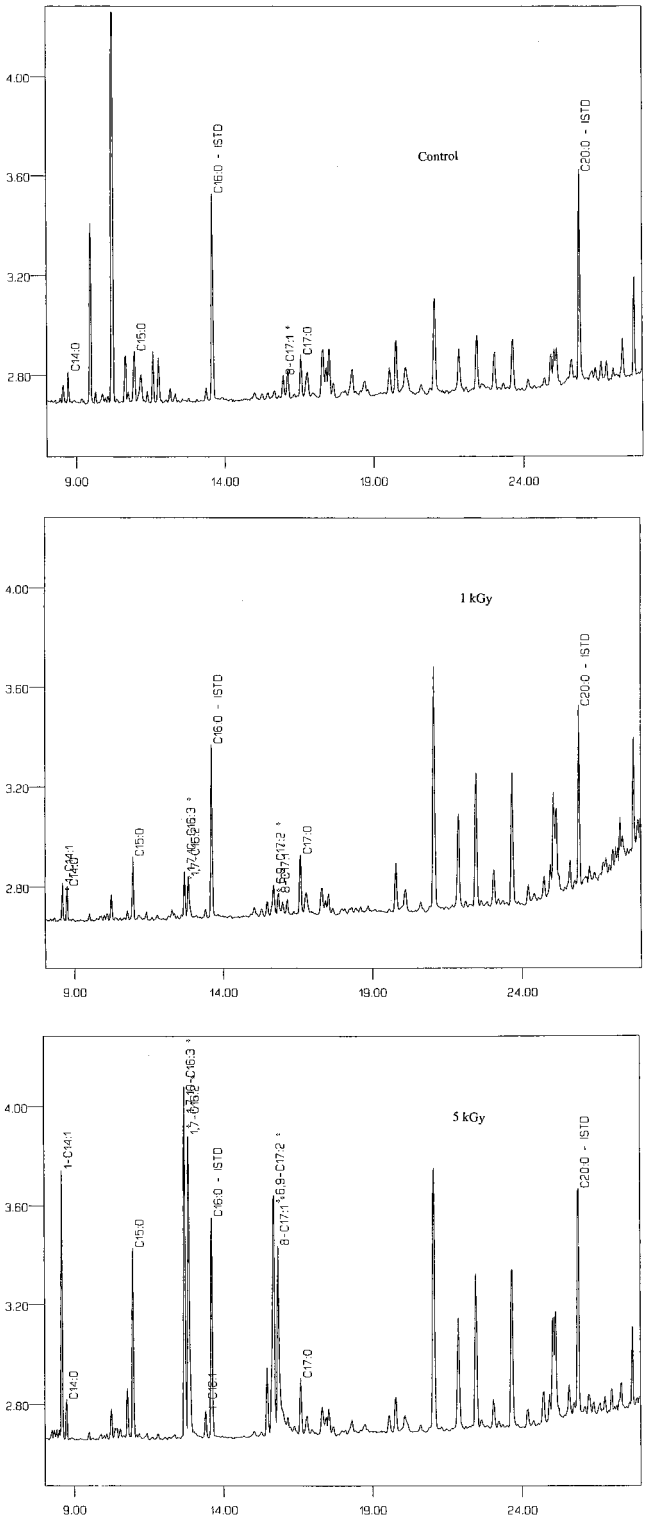

Figure 1. Gas chromatogram (GC/FID) of hydrocarbons from control (0 kGy) and irradiated (1 kGy, 5 kGy) Carioca beans. The *x*-axis in all three panels is the time in minutes. The *y*-axis in all three panels is the signal intensity.

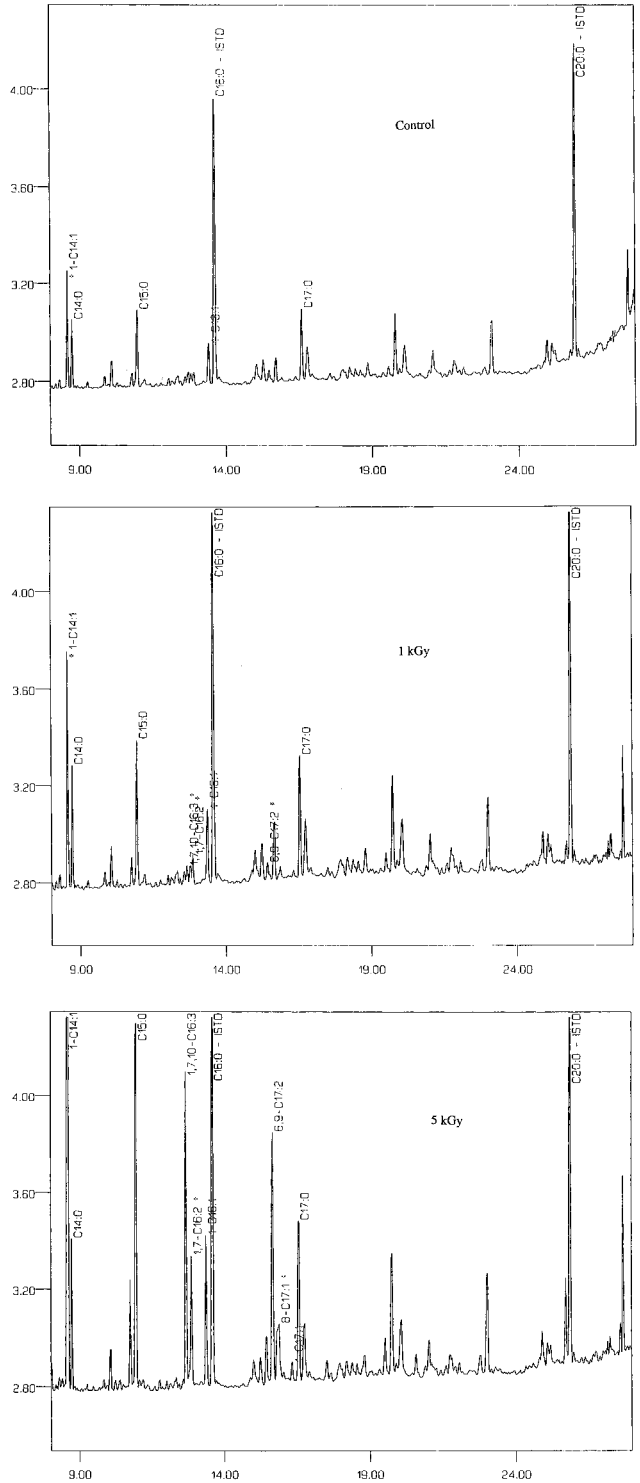

Figure 2. Gas chromatogram (GC/FID) of hydrocarbons from control (0 kGy) and irradiated (1 kGy, 5 kGy) Macaçar beans. The *x*-axis in all three panels is the time in minutes. The *y*-axis in all three panels is the signal intensity.

for vegetable oils (Lesgards et al., 1996), for shrimps and chicken (Morehouse et al., 1993), and various other foods (Nawar et al., 1996).

As already mentioned, β-cleavage leading to $C_{n-2:1}$ hydrocarbons occurs preferential to α-cleavage producing C_{n-1} hydrocarbons in these two types of beans. The ratio of $C_{n-2:1}/C_{n-1}$ hydrocarbons is about 1.1−1.5 for all three fatty acids, C16:0, C18:1, and C18:2, at radiation doses of 5−10 kGy in Carioca beans. In Macaçar beans for C18:2, this ratio is about 1.2, but for C16:0 and C18:1, it has increased twice to 2.1−2.5. Thus, not only the yield but also the ratio of β- and α-cleavage is dependent on the material and the fatty acid, respectively. This has been previously noted for shrimp and chicken (Morehouse et al., 1993), beef, pork, and chicken (Schreiber et al., 1994), vegetable oils (Lesgards et al., 1996), and various other foods (Nawar et al., 1996). It is interesting that also the yields of another lipid derived radiation product, namely the 2-alkylcyclobutanones, do not always reflect the fatty acid composition of the food products. Whereas for

Table 3. Radiation-Induced Hydrocarbons in Carioca Beans

hydrocarbon (μg/g of fat)	radiation dose (kGy)					
	0	0.5	1.0	2.5	5.0	10.0
C15:0	1.47	1.53	1.87	2.07	4.13	6.40
C1, 14:1	<0.13	0.60	0.93	2.13	4.87	8.20
C8, 17:1	0.73	0.47	0.73	2.27	7.33	12.6
C1,7, 16:2		1.33	2.13	5.07	11.4	18.7
C6,9, 17:2		0.89	1.27	2.93	7.53	13.9
C1,7,10, 16:3		0.93	1.53	3.93	9.0	14.9

Table 4. Radiation-Induced Hydrocarbons in Macaçar Beans

hydrocarbon (μg/g of fat)	radiation dose (kGy)					
	0	0.5	1.0	2.5	5.0	10.0
C15:0	1.46	1.47	1.89	2.89	4.60	8.07
C1, 14:1		1.73	2.33	6.27	11.6	17.0
C8, 17:1		<0.13	0.13	0.53	1.0	2.27
C1,7, 16:2		0.13	0.53	1.40	2.33	4.93
C6,9, 17:2		0.53	0.93	2.27	4.13	9.33
C1,7,10, 16:3		<0.13	0.33	2.13	5.27	10.4

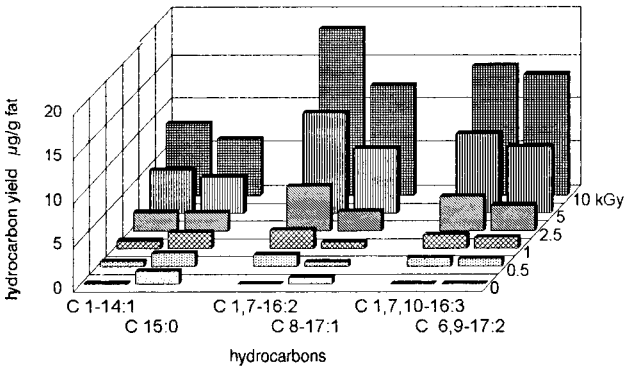

Figure 3. Radiation-induced hydrocarbons in Carioca beans.

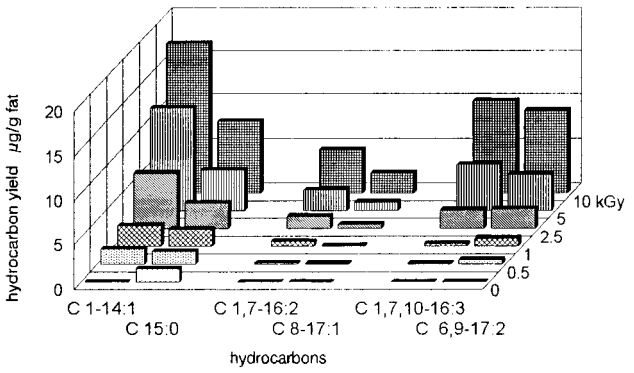

Figure 4. Radiation-induced hydrocarbons in Macaçar beans.

chicken, beef, lamb, and egg, the ratio of palmitic to stearic acid is reflected in the ratio of 2-dodecylcyclobutanone to 2-tetradecylcyclobutanone, this is not the case for pork. This is explained by different positions of the fatty acids on the glycerol backbone (Stevenson, 1994). Certainly the molecular arrangement of the fatty acid will also influence the yield of hydrocarbons during irradiation. It has already been observed that the yield of the C_{n-1} hydrocarbon is much higher for the free fatty acid than for the triglyceride (Dubravcic and Nawar, 1976; Nawar et al., 1996). Thus, the presence of free fatty acids or different types of fat will influence the yields of hydrocarbons during irradiation.

This paper has shown that radiolytic hydrocarbons can be detected in irradiated beans using a relatively simple gas chromatographic analysis. Application of a gas chromatographic analysis of radiolytic hydrocarbons

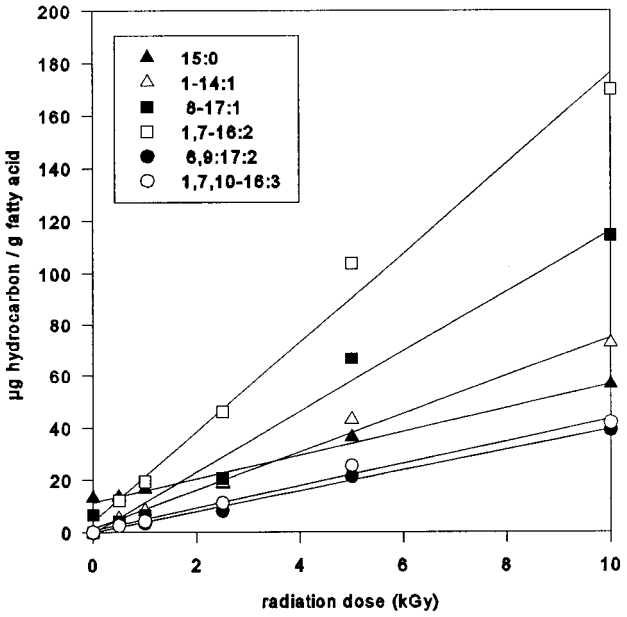

Figure 5. Yield of radiation-induced hydrocarbons per precursor fatty acid in Carioca beans.

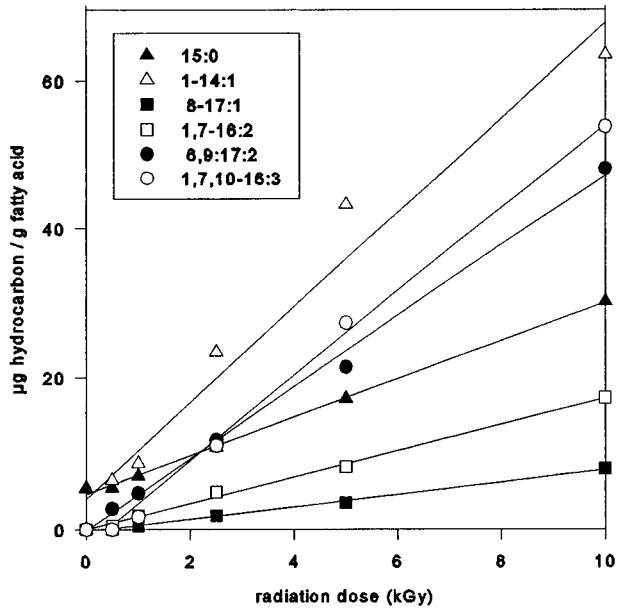

Figure 6. Yield of radiation-induced hydrocarbons per precursor fatty acid in Macaçar beans.

as a tool for detection of an irradiation treatment of food has already been proposed more than 20 years ago (Nawar and Balboni, 1970). Finally, this analysis method has now been standardized on a European level (DIN-EN 1784, 1997). Slight modification of this standard employing a solid-phase extraction (SPE) method saves solvents and chromatographic material like Florisil (Hartmann et al., 1996).

The analysis of the beans after 6 months of storage after the irradiation treatment shows that radiation-induced hydrocarbons exhibit high stability. Since usual storage of beans may last from one harvest to the next, the detection of hydrocarbons offers a suitable identification means of an irradiation treatment under commercial storage conditions. Long-term stability of radiolytic hydrocarbons has already been described for various meat samples (Merritt et al., 1978; Schreiber et al., 1994).

According to the criteria of the European method (DIN-EN 1784, 1997), all the expected hydrocarbons,

C_{n-1} and $C_{n-2:1}$, should be detectable. The sensitivity of the detection method as applied here allows an unequivocal identification of irradiated beans at a radiation dose of 1 kGy and higher. It can be seen on Figures 3 and 4 that 0.5 kGy samples almost certainly also can be identified. Most probably, samples with only low doses of radiation, as for example, used for insect disinfestation, can be identified with higher certainty employing the new procedure of "silver" chromatography (Hartmann et al., 1997b). This procedure enables an enrichment of hydrocarbons with simultaneous separation of the hydrocarbons into several fractions, e.g., alkanes, dienes, and trienes etc., leading to a safer identification. On the other hand, more expensive "sophisticated" techniques like coupled LC-GC, LC-LC-GC (Biedermann et al., 1992; Schulzki et al., 1996), or SFE-GC-MS (Lembke et al., 1995) may be employed to analyze lipid degradation products after irradiation with greater sensitivity.

The development of analytical detection methods for irradiation treatment of food will contribute to an improved food control, thereby enhancing consumer confidence in the proper surveillance of food irradiation. An improved acceptance of this valuable technique of radiation processing will help countries in the fight against food losses (WHO, 1994).

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