Use of Radiation-Induced Alkanes and Alkenes to Detect Irradiated Food Containing Lipids

Guy Lesgards[a,*], Jacques Raffi[b], Isabelle Pouliquen[a], Abdel-Aziz Chaouch[a], Philippe Giamarchi[a] and Michel Prost[c]

[a]Laboratoire de Chimie des Produits Naturels, Faculté des Sciences et Techniques de Saint-Jérôme, 13397 Marseille Cedex 13, [b]DPVE/SIV, CEN Cadarache, 13108 Saint-Paul and [c]Centre de Formation et d'Investigation Biologique, CEFIBRA, 21560 Couternon, France

To detect irradiated foodstuffs, we used the Nawar relation between lipid structure and radiolysis compounds, such as alkanes and alkenes. We first applied this method to sunflower, olive and peanut oils. Alkanes and alkenes were analyzed by gas chromatography with a head-space system for desorption and concentration of the volatile compounds. The detection limit, obtained both by estimation of the chromatogram area and by a blind trial, is better than 0.15 kGy. The continuity of detection with storage time was also studied. We have compared these results with those obtained by thermolysis: the same method can be used to detect ionized vegetable oils, even if they have been heated. In a second step, we studied three possible commercial situations—the irradiation of avocado-pears, fresh pilchards and poultry meat. Although we can use this lipid method to identify irradiated avocado-pears (for doses above 0.5 kGy) and poultry meat, it is impossible to apply it to fresh pilchards because numerous volatile compounds are already present before irradiation.

KEY WORDS: Alkane, alkene, detection, food, irradiation, lipid.

The use of X- and gamma-rays or electron beams to reduce food losses and to improve the hygienic quality of foodstuffs has been introduced recently. In 1980, a Joint FAO (Food & Agriculture Organization), WHO (World Health Organization) and IAEA (International Atomic Energy Agency) Committee concluded, on the basis of scientific studies, that ". . . the irradiation of food up to an overall average dose of 10 kilogray . . ." (7) (a dose of 1 Gy means the absorption of an energy of 1 joule per kilogram of food) presents no toxic hazard and introduced no special nutritional or microbiological problems (1). Radiation treatment of different foodstuffs is now legal in many countries, such as in the United States and France, although in many others it is still prohibited. Thus, it is important to make methods available to determine if foodstuffs or food ingredients have been treated by irradiation.

To this date, no "unique radiolytic products" (*i.e.*, products that are not also present in regular foods) have been found in irradiated foods, but there are some chemical or physical indicators that seem to exist for the identification of irradiated foods. These indicators are being studied (2) and have led to the first co-trials on identification of irradiated food (3–6).

From the mechanism of lipid radiolysis, a relation between the lipid structure and radiolytic compounds, such as alkanes and alkenes, has been established by Nawar *et al.* (7–10). They proved that a "CN:M" fatty acid, *i.e.*, a fatty acid with a chain of N carbons and M double bonds, leads to the formation of two main radiation-induced alkenes, CN-1:M and CN-2:M+1. Since the first studies, in which a high-vacuum cold-finger distillation was used (7), other techniques have been developed, such as head-space/desorption concentration injection (DCI) chromatography (11). We present here work on different vegetable oils, used as models, and then show how this method can be extrapolated to other foodstuffs.

MATERIALS AND METHODS

Sample irradiation. Samples were irradiated by gamma rays from cesium 137 (source of 18,000 Ci, and approximately 60 Gy/min) at room temperature and generally were kept in a domestic freezer at −20°C.

Triglycerides. They were analyzed with a DI200 chromatograph from Delsi (Argenteuil, France), with helium carrier gas, a split injector and a flame ionization detector (11). The following conditions were used: inlet pressure 0.4 bars, injector and detector temperature 350°C, and fused silica column (Spiral, France), length 5 m, diameter 0.5 mm, stationary phase OV 17; temperature programmed from 280°C to 330°C, at 5°C/min until 300°C and only 2°C/min thereafter.

Volatile compounds. The same chromatograph and integrator systems were used with a head-space system for desorption and concentration of the volatile compounds (Fig. 1). A small oven containing the sample was heated under carrier gas flow to evaporate the volatile compounds. These compounds were then cryo-concentrated on a tenax trap (tenax is a porous polymer of 2,6-diphenyl-P-phenylene oxide) for a few minutes. Afterwards, they were injected in the gas chromatograph by flash heating of the trap.

The hydrocarbons were separated by chromatography and identified by comparison with the retention times of commercial standard hydrocarbons. Quantitative measurements were accomplished by comparison of relative peak areas with those of internal references, such as C18:0 (Alltech, Eke, France).

Extraction of avocado-pear and fresh pilchard lipids. Eight grams of avocado-pear pulp or pilchard fillet were crushed with the same quantity of extra pure sea sand and anhydrous sodium sulfate to obtain a fine powder. The mixture was put in extraction thimbles and extracted by 100 mL of diethyl oxyde in a Soxhlet extractor for 4 h. The solvent from the obtained extract was then evaporated in a vacuum evaporator at room temperature to obtain pure avocado-pear lipids.

Avocado-pear. Different treatment methods were used initially (Fig. 2) to find the best analysis conditions. These methods included: (i) irradiation of avocado-pear oil, which was extracted as above, in small 2-mL glass bottles. This fraction is called "irradiated avocado-pear oil," (ii) irradiation of small avocado-pear pieces (8 pieces per avocado-pear) in small hermetically sealed polyethylene bags. After treatment, the avocado-pear pulp can be analyzed directly or extracted into oil and then analyzed. This last fraction is called "oil from irradiated avocado-pear."

*To whom correspondence should be addressed.

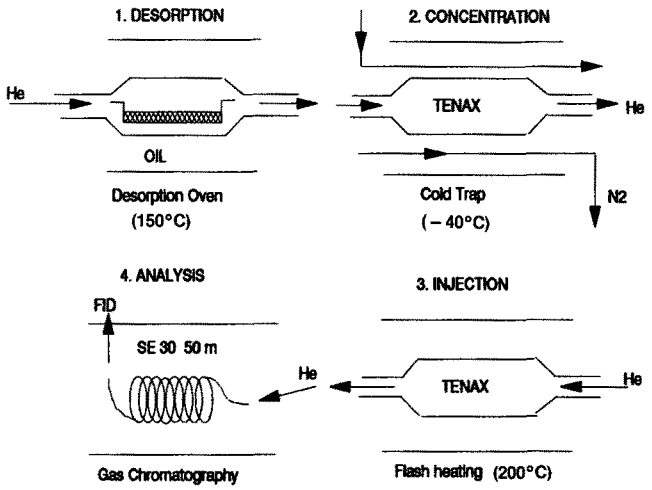

FIG. 1. Desorption concentration injection system. The hydrocarbons included in the oil are desorbed under helium (1) and then trapped on tenax cooled at −40°C (2). Flash heating of the trap (3) leads to desorption of the compounds concentrated on the trap, which are injected and analyzed by gas chromatography (4).

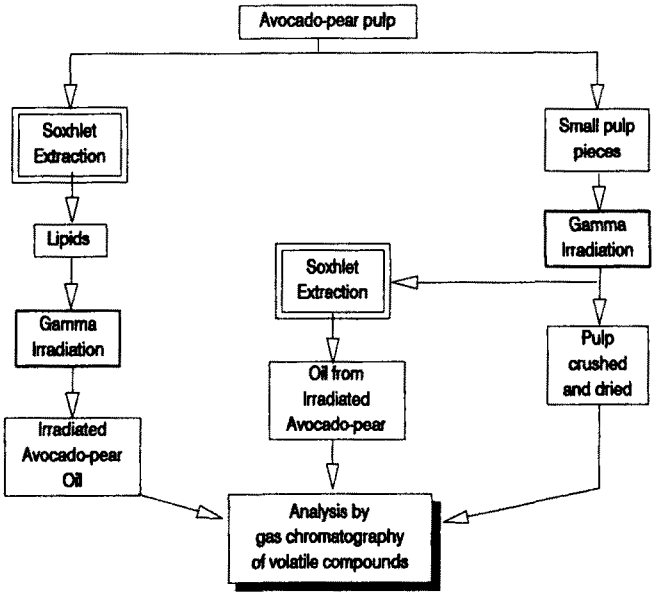

FIG. 2. Different schemes of analysis for avocado-pear.

Pilchards. The oil composition may vary with the fish age, the season and even with different parts of the fish body. Thus, the pilchards were cut in two filets; the first filet of each fish was irradiated, and the second one was kept as a reference sample.

Poultry meat. The meat was crushed in the presence of pentane and isopropanol, after addition of the internal standard. Then the mixture was centrifuged (2000 rpm) and the solution was completely evaporated under vacuum. The remaining lipids were then analyzed in the DCI system.

TABLE 1

Fatty Acid Composition[a] of Oils from Avocado-Pear, Pilchard and Poultry, Determined on Unirradiated[b] and Irradiated[c] samples

	Avocado-pear		Pilchard		Poultry	
Fatty acids	Ref.[b]	10 kGy[c]	Ref.[b]	50 kGy[c]	Ref.[b]	10 kGy[c]
C14:0	0.1	0.1	8.7	8.7	0.8	0.8
C15:0	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.2	0.2
C16:0	18.5	17.0	20.2	20.5	19.5	20.0
C16:1ω7	9.6	10.2	12.5	12.5	2.9	2.8
C17:0	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.3	0.3
C18:0	0.4	0.4	4.4	4.5	8.0	8.2
C18:1ω9	44.5	46.1	20.5[e]	20.8[e]	37.4	37.3
C18:1ω7	6.9	7.1	0.0	0.0[d]	2.2	2.3
C18:2ω6	18.6	17.5	0.5	0.5	24.3	24.1
C18:3ω3	1.1	1.3	0.2	0.2	0.0[d]	0.0[d]
C20:0	0.0[d]	0.0[d]	0.3	0.3	0.1	0.1
C20:1ω9	0.0[d]	0.0[d]	1.6	1.7	0.0[d]	0.0[d]
C21:0	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.5	0.4
C22:0	0.0[d]	0.0[d]	0.1	0.1	0.0[d]	0.0[d]
C22:1	0.0[d]	0.0[d]	1.0	1.1	0.0[d]	0.0[d]
C24:0	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.0[d]

[a]The results, presented here in area percentages of the chromatograms (100% = total of determined fatty acids), are calculated from the average of five determinations, which leads to an accuracy of ±1.5%.
[b]Ref.: unirradiated sample (first column).
[c]Irradiated sample (second column).
[d]0.0: to low to be determined (<0.05).
[e]For pilchard oil, the C18:1ω9 value represents the sum of monounsaturated hydrocarbons C18:0 in ω9 and ω7.

EXPERIMENTAL RESULTS ON PURE OILS

Irradiated vegetable oils. There is no significant difference between the lipid composition of irradiated and unirradiated oils (Table 1). In sunflower, olive (two varieties) and peanut oils, we have shown the appearance of volatile radiation-induced hydrocarbons with 14 to 17 carbons and several degrees of unsaturation (Tables 2 and 3). These hydrocarbons were identified by chromatography (Fig. 3) and measured by comparing peak areas with internal references, leading to histograms, such as in Figure 4. The percentages of main fatty acids CN:M in the oil sample are the same as the percentages of the volatile compounds with one less carbon (CN−1:M) and of those with two less carbons and one more unsaturation (CN−2:M+1) (Table 2). All our experimental results are in good agreement with the proposals of Nawar (7-10). Table 2 shows examples of results obtained on several different oils and the comparison between their fatty acid compositions and the average radiation-induced volatile hydrocarbon compositions. Notice that the shape of the chromatograph curve for the unirradiated oil is linked to the purity of that oil; *i.e.,* olive and extra virgin olive (Fig. 3) oils do not show the same peak intensities. It is thus always necessary to compare the lipid composition with the hydrocarbon composition, instead of only the hydrocarbon chromatograph, to avoid mistakes by considering only the fatty acid compositions described in the literature.

The amount of these volatile radiation-induced hydrocarbons (Table 3) increases linearly with the irradiation dose (11). In sunflower oil we have obtained formation of 1.71 ± 0.05 μg of C 16:3 alkene per kGy per gram of oil. In peanut oil we have obtained 1.27 ± 0.05 μg of

TABLE 2

Relation Between Fatty Acid Compositions of Peanut, Sunflower and Olive Oils and Composition of Radiation-Induced Hydrocarbons[a]

		Hydrocarbons (%)				
Fatty acids	(%)	C15:0 + C14:1	C15:1 + C14:2	C17:0 + C16:1	C17:1 + C16:2	C17:2 + C16:3
Peanut oil						
C16:0	10.5	14.0				
C16:1	0.1		1.5			
C18:0	4.0			5.5		
C18:1	57.0				57.0	
C18:2	21.0					22.0
Sunflower oil						
C16:0	6.2	9.0				
C16:1	0.1		0.5			
C18:0	4.3			5.0		
C18:1	28.0				28.5	
C18:2	68.0					57.0
Extra virgin olive oil						
C16:0	11.0	11.2				
C18:1	83.6				83.0	
C18:2	5.4					5.9

[a]The percentages are average values calculated from the two relative hydrocarbons—accuracy of ±1.5% for fatty acids and ±2.5% for hydrocarbons.

TABLE 3

Structure and Quantity of the Main Radiation-Induced Volatile Hydrocarbons (C-13 to C-18) in Oils[a]

	Quantity of hydrocarbons in μg/100 g of oil															
Sample	C13:2	C13:1	C14:2	C14:1	C14:0	C15:1	C15:0	C16:3	C16:2	C16:1	C16:0	C17:3	C17:2	C17:1	C17:0	C18:1
Peanut oil																
Unirradiated	—	634	—	—	—	—	130	—	—	—	—	—	20	40	—	—
Irradiated 10 kGy	22	531	21	217	14	44	340	351	1267	110	18	58	509	1049	75	—
Heated oxygen	190	31014	—	—	97	—	336	—	—	—	—	—	755	1538	58	—
Heated vacuum	—	4712	3243	—	133	—	87	—	—	—	—	—	131	279	—	—
Sunflower oil																
Unirradiated	—	1120	—	—	—	—	79	102	57	37	—	—	507	271	67	
Irradiated 10 kGy	—	1028	—	230	—	—	230	1697	1070	168	—	258	2063	1059	164	—
Heated oxygen	—	66263	—	296	—	—	144	—	109	—	—	—	1166	843	140	—
Heated vacuum	—	23327	—	—	—	—	197	—	—	—	—	—	500	318	—	—
Extra virgin olive oil																
Uniradiated	—	3619	313	—	—	291	2469	—	—	—	—	—	179	248	—	83
Irradiated 10 kGy	60	3256	247	559	68	295	3076	246	3454	254	54	40	644	2435	112	80
Heated oxygen	633	10740	449	—	—	171	1349	—	—	—	—	—	1176	4549	275	—
Heated vacuum	739	4285	—	—	202	—	246	—	—	—	—	—	603	1905	107	—

[a]Accuracy, ±2.5%.

C16:2 alkene/kGy/g of oil. We have also analyzed irradiated sunflower oils (at 3, 7 and 10 kGy) kept at room temperature after irradiation. The amount of radiation-induced hydrocarbons seems to be unmodified even after 7 mon.

The addition of butylated hydroxytoluene (BHT) antioxidant to the oil in the amount of 200 mg/L seems to have no influence upon the quantities of radiation-induced hydrocarbons. For instance, at 10 kGy, the lipid 18:1 leads to 15.9 ± 0.4 μg/g of 16:3 and 16.8 ± 0.4 μg/g of 17:2 without BHT, and, respectively, to 16.2 ± 0.4 and 17.0 ± 0.4 with BHT. Moreover, these quantities are quite constant with storage time.

A foodstuff irradiation control test. With regard to the products having a high "N"-carbon fatty acid content, the appearance of "N-2"-carbon alkenes on the chromatogram of volatile oil compounds could be used as an irradiation control test by food inspection authorities.

In the case of oils, this method has a detection limit that is less than 0.15 kGy. A trial with unknown samples showed that 0.1-kGy samples are correctly identified, even if the estimated dose is overestimated (Table 4). The absence of interfering compounds in the initial nonirradiated product may allow this method to prove that samples irradiated above 0.15 kGy are detected without any ambiguity; and to be semi-quantitative, at least at doses above 0.3 kGy, assuming that the irradiation was always carried out at the same temperature.

Heated vegetable oils. Unirradiated sunflower, olive and peanut oils have been analyzed after heating for 18 or 40 h in an oven at 180°C. We can observe a decrease in the longest and most unsaturated triglycerides. For example,

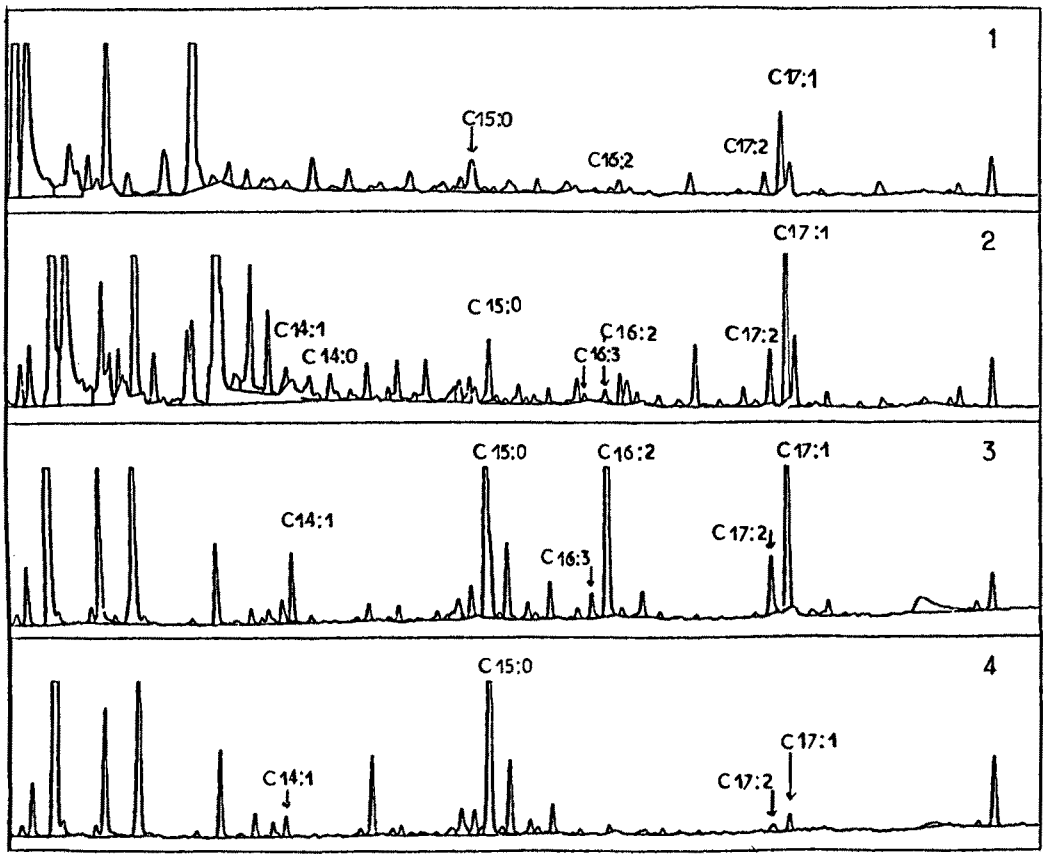

FIG. 3. Chromatograms of extra virgin olive oil heated for 18 h at 180°C without (1) and with (2) oxygen, irradiated at 10 kGy (3) and untreated (4).

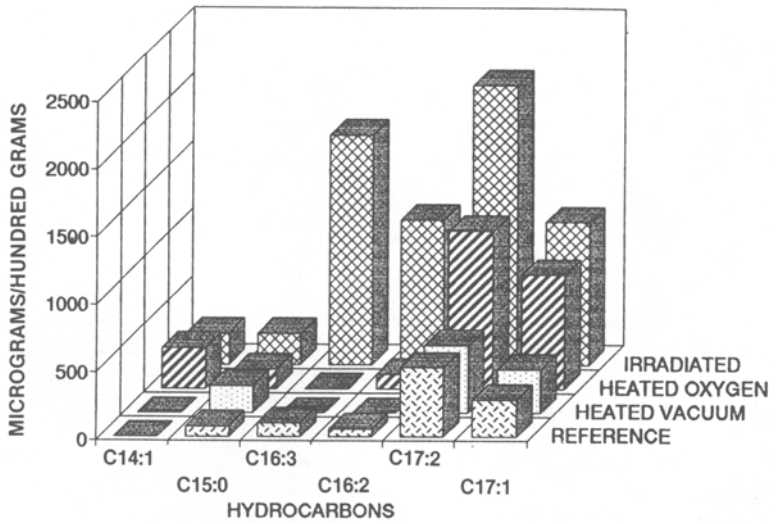

FIG. 4. Sunflower oil irradiated at 10 kGy; amount of induced hydrocarbon as a function of treatment. The hydrocarbon C13:1 is not represented on this histogram because its amount (31014) is too intense with regard to the other compounds. The reference is the unirradiated and unheated sample.

TABLE 4

Results of Blind Test (sunflower oil)

Samples	Studied hydrocarbons C16:3 (μg/g)	Studied hydrocarbons C17:2 (μg/g)	Radiation dose Estimated	Radiation dose Delivered
Known reference[a]	0.00	0.00	0	0
Known "10 kGy"	13.3[b]	14.3	10	10
1	0.31	0.52	0.24	0.11
2	0.29	0.38	0.22	0.27
3	0.03	0.13	0.02[c]	0
4	9.32	9.28	7.03	7
5	3.33	3.03	2.51	3
6	4.12	3.15	3.11	3
7	0.07	0.20	0.06[c]	0
8	9.04	9.02	6.82	7
9	0.35	0.11	0.26	0.26
10	0.23	0.37	0.18	0.11

[a]Unirradiated sample.
[b]Each number (accuracy ± 2.5%) is the average of five measurements. The oil is different from the one used in Table 3, which leads to differences in the radiation-induced quantities of hydrocarbons.
[c]Considered as unirradiated oil samples.

sunflower oil naturally contains 26 ± 3% trilinolein, but only 16 ± 2% after 40 h of heating at 180°C.

In general, analysis of the results shows that heating, as well as irradiation, induces the formation of hydrocarbons, but not in the same relative amounts. The chromatograms (Fig. 3) show that some of them are induced in greater amounts during irradiation and some others during heating (Fig. 4). To analyze the results further, we can separate the detected compounds into three classes: the first contains those compounds that are quite characteristic of irradiation, the second incorporates those that come from heating and the last class contains all other compounds.

For peanut oil (Table 2), in the first class we find the following compounds: C14:1, C15:1, C16:0, C16:1, C16:2, C16:3 and C17:3, which are "characteristic" of irradiation. C13:1 and C14:0 are characteristic of heating, as well as C14:2, which is only induced by heating in the presence of oxygen. The other compounds present on the chromatogram (C15:0, C17:1 and C17:2) are not interesting because they were detected in all cases (*i.e.,* after heating, irradiation or without treatment). The C17:0 hydrocarbon is not present in the reference oil, but cannot be used because it is induced either by heating or irradiation.

For sunflower oil (Table 3), C17:3 is the only hydrocarbon that seems to be strictly characteristic of irradiation. However, the compounds C16:1, C16:2 and C16:3 also can be considered as indicators of irradiation, even though they are detected in the reference oil, because of their large concentration increase upon irradiation. We cannot find any compound characteristic of heating in this oil. The hydrocarbons C15:0, C17:0 and C17:1 cannot be used because they are always present, and that is also true for C14:1 and C17:2, which are both induced by heating and irradiation.

Pure extra virgin oil (Table 3) is similar to sunflower. Only C16:2 is a good irradiation indicator; but we can also use C14:1, C15:0, C16:1 and C16:3. We did detect a compound characteristic of heating, C17:0, which only appears after vacuum heating (Table 3). The compounds C14:0, C14:2, C15:1, C16:0, C17:1 and C17:2 cannot be used because they are all present in reference, heated and irradiated samples.

To summarize, this study shows that the presence of significant amounts of C16 hydrocarbons is characteristic of irradiation. C16:1, C16:2 and C16:3 are all radio-induced in each oil. A large amount of C17:2 can also be considered as characteristic of irradiation, even though it is detected in low amounts in less pure commercial olive oil. On the other hand, we cannot find any compound that is always characteristic of heating for all oils. Moreover, heating under vacuum seems to induce no characteristic product. The difference in the thermolytic and radiolytic reactions is probably due to the mechanisms involved. It would be necessary to carry out further experiments to understand the nature of these different mechanisms. However, it is possible to distinguish irradiated and heated samples from the unirradiated ones, especially by the analysis of 16-carbon volatile hydrocarbons, which are not present or not significantly induced under heating (with or without oxygen), and of other series (C14, C15, C16), which are only present in relatively important amounts in irradiated samples.

EXPERIMENTAL RESULTS ON AVOCADO-PEARS

Triglycerides and fatty acids analysis. We have carried out experiments on avocado oils extracted before or after irradiation (Fig. 2), *i.e.,* "irradiated avocado-pear oil" and "oil from irradiated avocado-pear." No significant differences appear between the two oil samples or with the unirradiated sample, even at the high irradiation dose of 50 kGy.

Volatile compounds in irradiated avocado-pear oil. We have obtained the same results for the "irradiated avocado-pear oil" and the "oil of unirradiated avocado-pear," *i.e.,* the formation of volatile hydrocarbon compounds with sixteen carbons (Fig. 5). However, there is a small amount of hydrocarbons with seventeen carbons in the unirradiated samples, and some interfering compounds are sometimes present in this area with approximately the same retention times.

For the direct avocado-pear pulp analysis we obtained the same results as above, but the quantity of radiation-induced volatile compounds is less important (0.5 μg/kGy/g).

In the avocado-pear oil, the small amount of 17-carbon alkanes and alkenes present naturally in the fruit, as well as some interfering compounds, makes the quantitative analysis more difficult. This phenomenon comes from the fact that avocado-pear oil is a raw oil and not refined, such as sunflower or peanut oil. At present, the interfering peaks in the avocado-pear oil limit the test sensitivity to a 0.5-kGy dose, but there is always a good correlation between the lipid and hydrocarbon composition (Table 1 and Fig. 6).

APPLICATION TO OTHER PRODUCTS

Although this method can be applied without any problem to pure oils, for other products the two main problems are the extraction of radiation-induced volatile hydrocarbons and the presence of "interference" peaks in the chromatograms. For example, for avocado the mini-

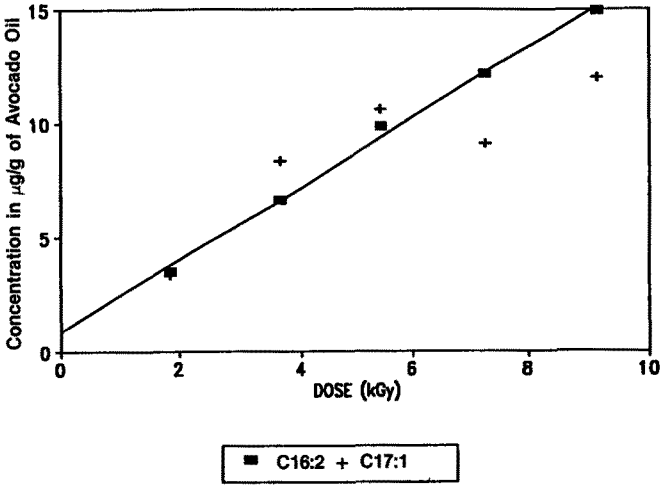

FIG. 5. Correlation between the induced quantity of hydrocarbons in avocado-pear with the irradiation dose.

mum limit of detection is presently 0.5 kGy, which is too high with regard to the possible commercial range values. We have to lower this limit of detection by improving the method (chromatographic conditions, purity of columns and solvents, DCI trap, etc.).

The same is true for the other foodstuff, pilchard, which was studied with the same method. Here, the fish oil has a complex fatty acid composition, more than 40 fatty acids were detectable on the fatty acid chromatogram, of which 13 were identified (Table 1). Consequently, the volatile compound chromatograms are much more complicated; if there are differences between the composition of volatile compounds of irradiated and nonirradiated samples, it is obviously impossible to detect any "new peak" induced by irradiation (Fig. 7).

Consequently, because we never get the reference sample in commercial conditions, we probably cannot conclude whether or not the pilchard has been irradiated. The only remaining way is to compare the peak intensities and to try to find a ratio linked to the irradiation dose, but this requires further systematic quantitative experiments that are beyond the scope of this paper.

The only remaining question is whether this negative result is only due to the complexity of the fatty acid composition (*i.e.*, a limitation due to the nature of the fish) or also to the present analysis conditions (*i.e.*, chromatographic conditions discussed above).

For poultry meat (Table 1) there are really no problems (except the usual ones) linked to the volatile extraction. It was shown by our laboratory, as by all those involved in a preliminary intercomparison (3), that the distinction between untreated and irradiated poultry meat is possible. This is why this product was chosen for the intercomparison sponsored in 1983 by the Community Bureau of Reference (Commission of the European Communities).

The main advantage of this technique is its speed. After lipid extraction, the samples can be directly injected without other pre-treatment (no chromatography needed on Flurosil column, for instance) and, moreover, it is often possible to directly inject the sample without any lipid extraction. However, it is necessary to find the right quan-

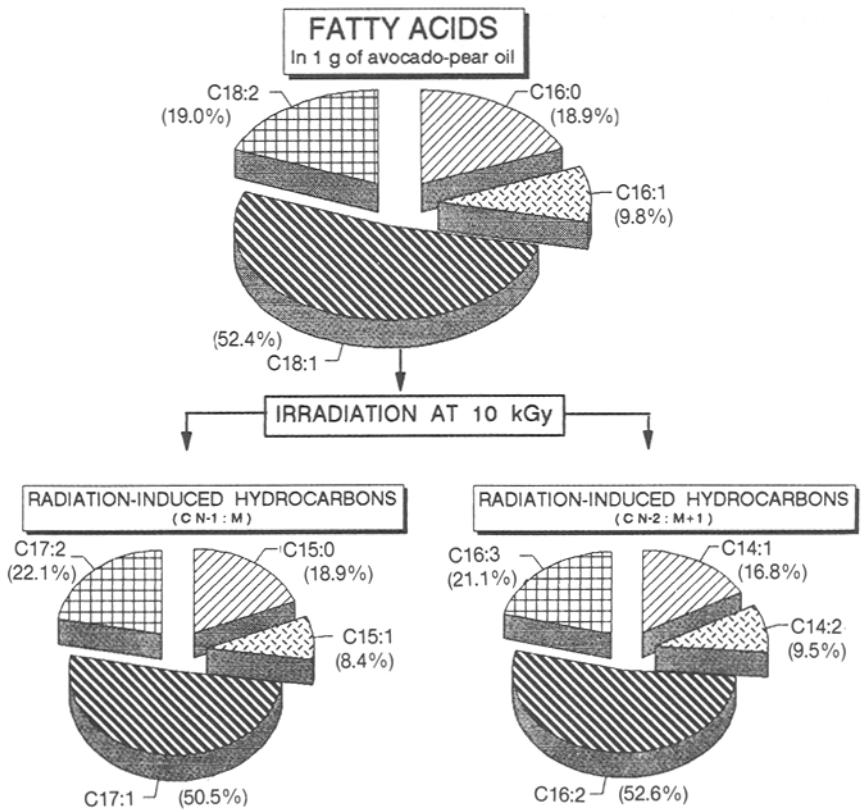

FIG. 6. Hydrocarbon composition of irradiated avocado-pears (10 kGy).

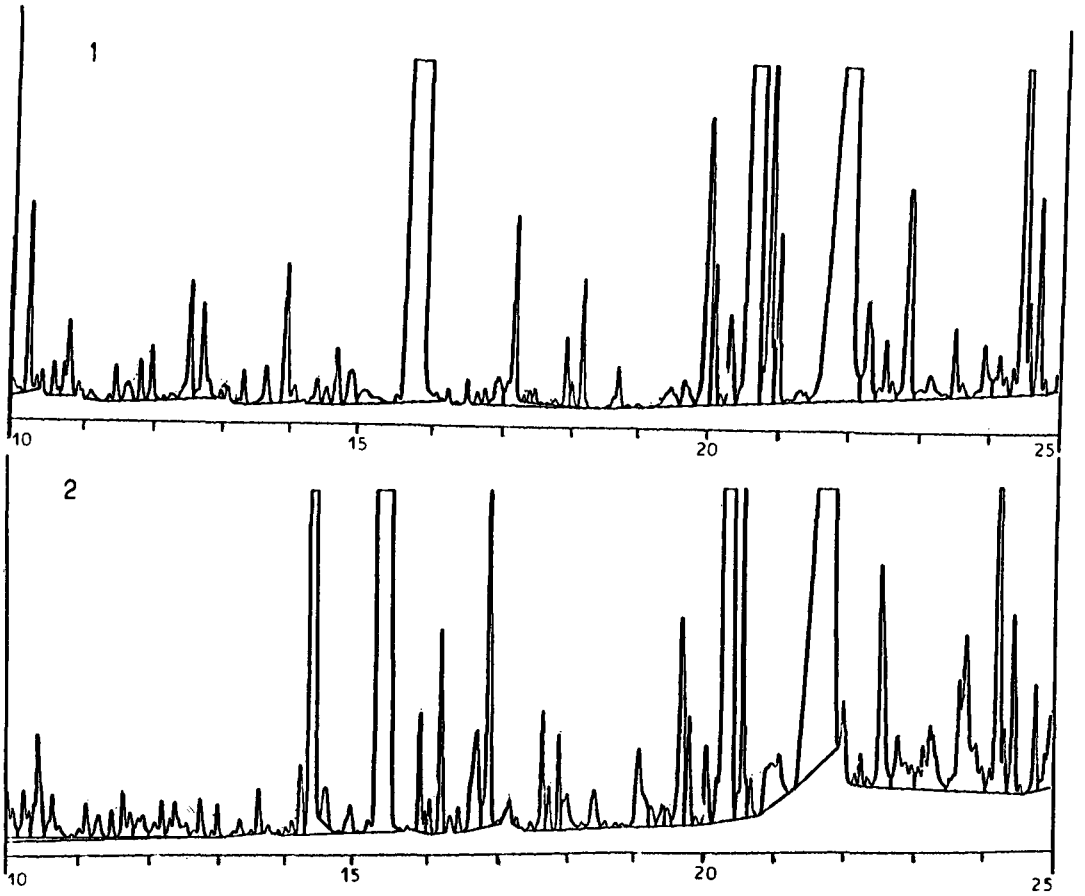

FIG. 7. Chromatograms of pilchard oil irradiated at 10 kGy (1) and untreated (2).

tity of food to take advantage of both sensitivity and speed of the analysis.

Finally, if the specificity of this method, with regard to the irradiation treatment, is corroborated once more, the complexity of the chromatograms for a lot of foodstuffs requires further studies to improve the choice of analysis parameters.

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