Characterization of Irradiated Food by SFE and GC–MSD

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Supercritical fluid extraction (SFE) is a good alternative to the classical organic solvent sample preparation for analysis of irradiated fat-containing food. This technique showed a high extraction selectivity, which made it possible to determine both the characteristic hydrocarbon pattern and the presence of alkylcyclobutanones (irradiation markers) simultaneously in a single run. Sample preparation time and solvent consumption are considerably reduced when compared to conventional techniques.

Keywords: *Irradiated food; supercritical fluid extraction; gas chromatography; volatile hydrocarbons; 2-alkylcyclobutanones*

INTRODUCTION

Irradiation of food for the control and reduction of microorganisms and the extension of product shelf life has become a potential preservation technique in many countries. A Joint Expert Committee on the "Wholesomeness of Irradiated Food" convened by FAO, IAEA, and WHO, in 1980, stated that "The irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard ..." and "... introduces no special nutritional or microbiological problems..." (WHO, 1981). However, many consumers still have doubts about this technique. Therefore, the consumer must be able to chose between irradiated and nonirradiated food. This can only be ensured by the correct labeling of the products and effective control by the regulating agencies. This requires a rapid and reliable routine analysis method.

So far, there are three main methods used for the characterization of irradiated food (Schreiber et al., 1993; *Analytical Detection Methods for Irradiated Foods*, 1991): With **thermoluminescence (TL)**, the adherent mineral residues (e.g. sand) of the particular food (vegetables, spices, herbs, tea, etc.) can be analyzed. **Electron spin resonance (ESR)** can be used to determine radicals formed by irradiation in food of low water content (bones, seeds, etc.). In addition to these sophisticated instrumental techniques, the **analysis by gas chromatography (GC) of hydrocarbons** formed during irradiation of fat-containing foods can be used. This is probably the method of choice for routine analysis in control laboratories with respect to cost and analytical know-how.

The irradiation of fat results in the formation of a characteristic pattern (depending on the individual fatty acid composition of the fat) of saturated and olefinic hydrocarbons, aldehydes, methyl and ethyl esters, and cyclic products (e.g. 2-alkylcyclobutanones) (Nawar, 1978; Delincee, 1983). Two groups of hydrocarbons are formed preferably: one with one fewer carbon atoms (C_{n-1}) than the original fatty acid, and another that has two fewer carbon atoms (C_{n-2}) and an additional double bond in position 1 (first carbon atom). Their formation

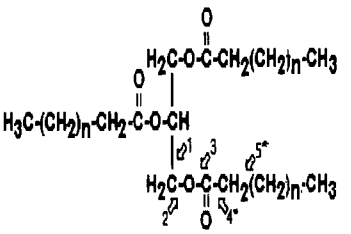

Figure 1. Formation of various irradiation products from fat (triglycerides). The arrow indicates the position of breakage. (1) Fatty acid methyl esters; (2) free fatty acid; (3) aldehyde or 2-alkylcyclobutanone; (4) alkanes/alkenes (n − 1); (5) alkenes (n − 2). (*) Main irradiation products.

can be deduced from the scission pattern shown in Figure 1, in which the arrows indicate the possible splitting position during irradiation.

These volatile compounds derived from lipids must be isolated from the fat fraction prior to GC analysis. For this purpose, open column liquid chromatography on Florisil columns is generally applied (Morehouse and Ku, 1990, 1992; Morehouse et al., 1991; Sjörberg et al., 1992; Ammon et al., 1992). Compared to other applied isolation techniques [e.g. "cold finger distillation" (Balboni and Nawar, 1970)], the Florisil cleanup has a higher sample capacity but is rather time-consuming and requires large amounts of organic solvents. Furthermore, simultaneous cleanup of the characteristic hydrocarbons (C_{n-1} and C_{n-2}) and 2-alkylcyclobutanones on the Florisil column is not possible due to polarity differences of the two compounds. Therefore, coupled techniques such as LC–GC (Biedermann et al., 1989) or LC–LC–GC (Biedermann et al., 1992) have been applied. These show several advantages compared to the classical open column Florisil technique, e.g. reduction of solvent consumption and the risk of contamination during sample preparation. Nevertheless, a complex HPLC–GC system is necessary, and the simultaneous detection of the volatile hydrocarbons and the characteristic 2-alkylcyclobutanones is not possible either. Hence, the potential of supercritical fluid extraction (SFE) as an alternative sample preparation technique has been studied.

EXPERIMENTAL CONDITIONS

Samples and Sample Cleanup Procedures. All of the samples were bought in a local supermarket and stored in a deep freezer until they were divided into two portions. One

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0021-8561/95/1443-0038$09.00/0

Table 1. Fatty Acid Ethyl Ester Composition of the Investigated Food Samples in Weight Percent

FAEE	pork meat	duck breast	German caviar	peanuts	pistachio nuts	instant soup powder
14:0	2	0.4	2			
16:0	27	20.1	3.5	10	10.6	9.2
16:1	4	2.3	21.6		0.8	
18:0	14	7.8	6.3	3.5	1	18.5
18:1	43	56.8	8.3	59	50	54.5
18:2	9	11.3	26.5	20	26	10
18:3	1			0.5	1.6	4
18:4						
20:0	<1	0.6		1.5		
20:4			<0.5			
20:5			12.3			
22:0			3	2.5		
22:6			15.1			

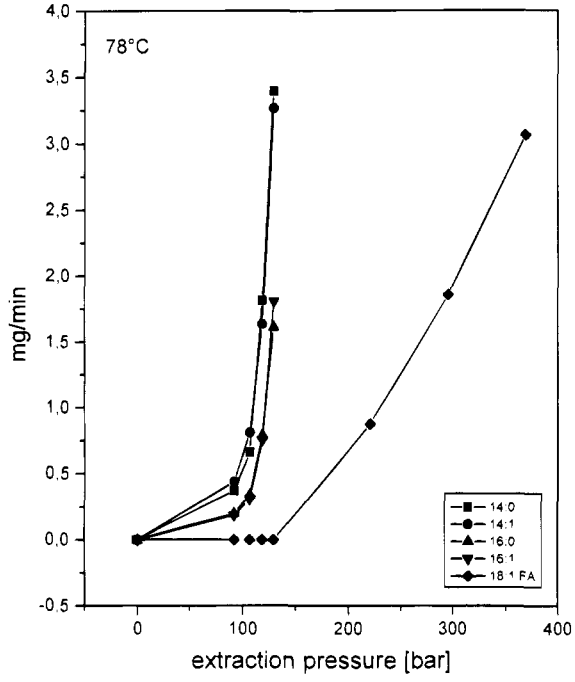

Figure 2. Extraction pressure for hydrocarbons and oleic acid from peanut oil on glass beads. One milliliter of peanut oil coated on 10 g of glass beads (dp ~ 25 μm); extraction time; 4 min; temperature, 78 °C.

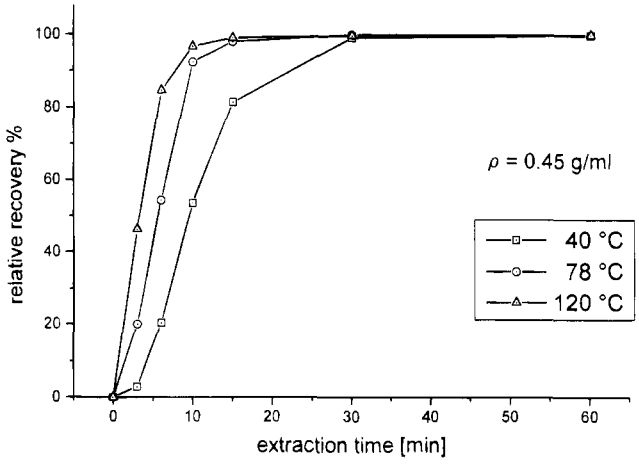

Figure 3. Influence of temperature on the extraction rates for hexadecane. Density: constant at 0.45 g cm^{-3}. Sample was coated on glass beads.

part was treated with a defined dosage (see Table 2) of ionizing radiation, at the German Federal Research Centre for Nutrition (Dr. Delincee) in Karlsruhe/Germany. The dose of

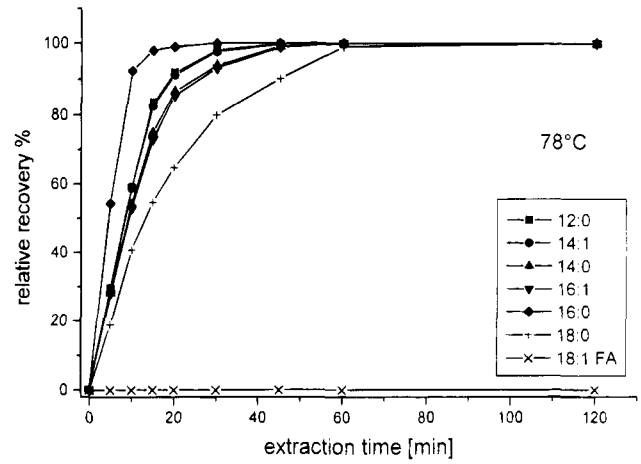

Figure 4. Extraction rates of hydrocarbons. Oleic acid is not extracted under these conditions. Conditions: 78 °C, 152 bar, density 0.45 g cm^{-3}; fluid flow 1.0 mL/min.

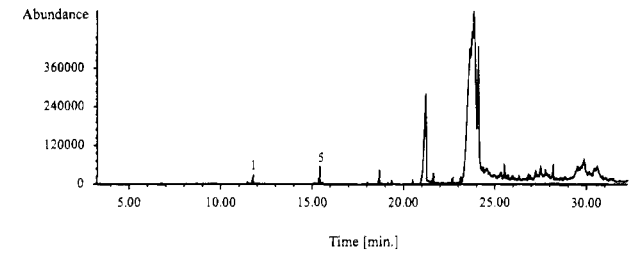

Figure 5. GC analysis of irradiated instant soup powder. Fat extract (liquid extraction) was directly analyzed by GC. GC conditions: see Experimental Conditions.

radiation corresponded to that commonly applied to the individual food samples.

Because literature data on the fatty acid composition of the various exposed samples are quite different, it was necessary to determine the fatty acid composition of the various samples. This, in turn, was necessary to correlate the found hydrocarbon pattern with the fatty acid composition of the sample (see Figure1). For the determination of the fatty acid composition, the fat in the food samples was extracted by classical solvent extraction and GC analysis was carried out after transesterification. The hydrocarbons were selectively extracted by SFE, either from the organic solvent fat extract or directly from the dried sample.

Sample pretreatment was identical for both the irradiated sample and the nonirradiated portion of the following samples:

Peanuts. Approximately 10 g of crushed peanuts was treated with 100 mL of methylene chloride overnight (for 12 h). After filtration over anhydrous Na_2SO_4 and removal of the organic solvent by rotary evaporation, the peanut oil was stored in a brown glass bottle at 4 °C.

Instant Soup Mix Powder. The soup mix powder ("Grünkernsuppe", Knorr, Germany) was treated with 200 mL of methylene chloride for 0.5 h in an ultrasonic bath. After filtration over anhydrous Na_2SO_4, the organic solvent was removed by rotary evaporation and the extracted fat stored in a brown glass bottle at 4 °C.

Roasted Pistachio Nuts. Approximately 10 g of pistachio nuts was crushed and treated with 200 mL of methylene chloride/acetone (2:1 v/v). After filtration over anhydrous Na_2SO_4, the organic solvent was removed by rotary evaporation and the extracted fat stored in a brown glass bottle at 4 °C.

Duck Breast, Pork Meat. Approximately 30–50 g of tissue was homogenized in an electric mixer and treated with 80 mL of extraction solvent (heptane/2-propanol, 3:2 v/v) for 0.5 h in an ultrasonic bath. After centrifugation (10 min at 3000 rpm), the organic phase was removed with a Pasteur pipe. This organic phase was filtered over anhydrous Na_2SO_4, the organic solvent was removed by rotary evaporation, and the remaining fat extract was stored in a brown glass bottle at 4 °C.

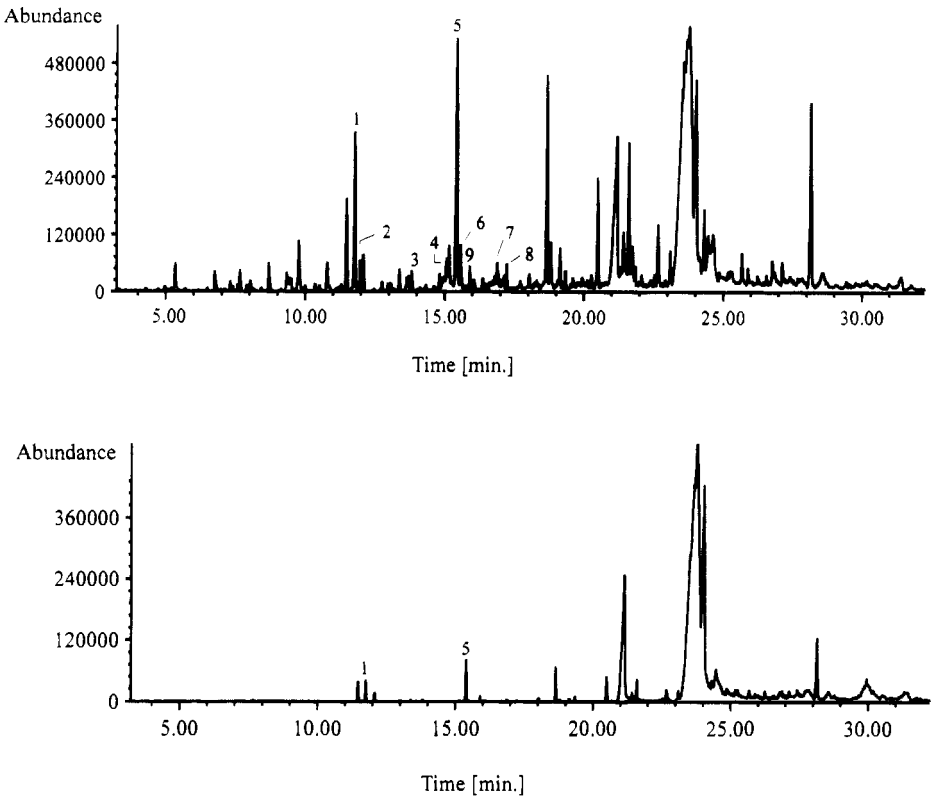

Figure 6. GC analysis of irradiated (A, top) and nonirradiated (B, bottom) instant soup powder after SFE extraction. SFE conditions were as in Figure 4; extraction time, 40 min. Hydrocarbons: (1) 14:1, (2) 14:2, (3) 15:0, (4) 2-alkylcyclobutanone, (5) 16:2, (6) 16:1, (7) 17:1, (8) 17:0, (9) 16:0.

German Caviar. Approximately the same amounts (each 50 g) of anhydrous $CaCl_2$ and caviar sample were carefully ground in a mortar (to bind the water). Then 60 mL of extraction solvent (heptane/2-propanol, 3:2 v/v) was added. This suspension was then exposed three times to ultrasonication (each time for 30 min) and left overnight (approximately 12 h) in the extraction solvent. After filtration over anhydrous Na_2SO_4, the filtrate was cleared by centrifugation (10 min at 3000 rpm). The organic solvent layer was then carefully removed by rotary evaporation, and the fat was stored in a brown glass bottle at 4 °C.

Later experiments showed that a direct and sufficiently selective extraction of the, through radiation induced, hydrocarbons was possible by means of SFE, without any previous organic solvent extraction. In this case the homogenized and dried sample is directly placed into the SFE extraction chamber. Hence, the sample pretreatment procedure and thus the total analysis time are reduced considerably.

Transesterification of Fats and GC Analysis. The transesterification of fat into fatty acid esters (preferably methyl esters) for GC analysis is well-known and described in the literature (Stoffel et al., 1959). The following slightly modified method has been used.

Twenty milliliters of an ethanolic sulfuric acid (10 mL of concentrated H_2SO_4 in 200 mL of ethanol) was added to approximately 100 mg of fat in a round flask. This solution was boiled under reflux for 1 h. After the addition of 20 mL of hexane, the fatty acid ethyl esters (FAEE) were transferred into the organic phase by a separation funnel. The organic phase was then filtered through a filter containing anhydrous Na_2SO_4 and then evaporated to dryness with a rotary evaporator. The residue was redissolved in 1 mL of methylene chloride in preparation for GC analysis.

The GC analysis was carried out on a Carlo Erba (HRGC 5300 Mega Series, Italy) GC, using a DB-5, 15 m × 0.25 mm × 0.25 μm (J&W Scientific, Fisons) capillary column. A flame ionization detector (FID) was used for detection. The following temperature program was utilized: initial hold for 2 min at 150 °C, 3 °C/min to 300 °C, isothermal hold at 300 °C for 5 min, and then return to 150 °C. Splitless injection of 0.5 μL sample solution was utilized.

The fatty acid composition of the fats analyzed are summarized in Table 1.

Supercritical Fluid Extraction (SFE). All SFE experiments were carried out using a Hewlett-Packard 7680T SFE module (Hewlett-Packard, Germany). This SFE module utilized a solid phase trap filled with stainless steel beads, which was thermostated at 15 °C. For sample collection the trapped analytes were washed off the trap with 1.0 mL of *n*-heptane (0.7 mL/min) and collected in glass vials.

For SFE method development, 30 μL of the individual hydrocarbon and fatty acids standards were spiked onto 10 g of glass beads [particle diameter (dp) = 25–50 μm]. To simulate real extraction conditions, 1 g of nonirradiated peanut oil was then added to the glass beads and, after thorough mixing (homogenization), filled into the extraction chamber.

For extraction of the various fat extracts, 1 g of the isolated fat extracts was homogenized in a mortar with approximately 10 g of nonporous glass beads (dp = 25–50 μm). The glass beads were then quantitatively transferred into the extraction chamber. Each extraction was carried out at 78 °C, 150 bar (r = 0.45 g/mL), for 40 min at a flow rate of 1.0 mL/min (liquid carbon dioxide at 4 °C, r = 0.92 g/mL) measured before the extraction chamber (at the pump head).

For quantitative analysis of the extracted samples, the collection solvent *n*-heptane was quantitatively removed from the sample vials by a gentle nitrogen stream, and the resultant residues were dissolved in methylene chloride containing *n*-eicosane (C20:0) as an internal standard. This solution was used for quantification by gas chromatography. The carbon dioxide used for the SFE had a purity of 99.995% and was purchased from AGA-Gas (Dillingen, Germany).

GC–MSD Analysis of SFE Extracts. The characterization and identification of the hydrocarbons and 2-alkylcyclobutanones from the irradiated fats were achieved using a Hewlett-Packard HP 5890 Series II GC, coupled to a mass selective detector (MSD) (HP 5971, EI^+). The capillary column used was a HP-1 (methylpolysiloxane phase), 12 m × 0.22 mm i.d. × 0.33 μm stationary phase thickness. Injection volumes varied between 0.5 and 1.5 μL, depending on the concentration of the irradiation products in the particular sample extract.

Table 2. Irradiated Food Samples: Correlation between Fat Composition and Hydrocarbon Decomposition Pattern

			theor HC pattern		obsd HC pattern		
irradiated sample (dose)	FAEE composition	wt % FAEE	$n-1$	$n-2$	$n-1$	$n-2$	cyclobutanone?
pork meat (2.5 kGy)	18:1	43.0	17:1	16:2	17:1	16:2[a]	no
	16:0	27.0	15:0	14:1	15:0[a]	14:1	
	18:2	9.0	17:2	16:3			
	16:1	4.0	15:1	14:2			
	14:0	2.0	13:0	12:1			
duck breast (2.5 kGy)	18:1	56.8	17:1	16:2	17:1	16:2[a]	yes
	16:0	20.1	15:0	14:1	15:0[a]	14:1	
	18:2	11.3	17:2	16:3	17:2[a]	16:3	
	18:0	7.8	17:0	16:1	17:0	16:1[a]	
	16:1	2.3	15:1	14:2	15:1[a]		
peanuts (5.0 kGy)	18:1	59.0	17:1	16:2	17:1	16:2[a]	yes
	18:2	20.0	17:2	16:3	17:2[a]	16:3	
	16:0	10.0	15:0	14:1	15:0	14:1[a]	
	18:0	3.5	17:0	16:1	17:0	16:1[a]	
	22:0	2.5	21:0	20:1			
	20:0	1.5	19:0	18:1			
pistachio nuts (5.0 kGy)	18:1	50.0	17:1	16:2	17:1	16:2[a]	yes
	18:2	26.0	17:2	16:3	17:2[a]	cb[b]	
	16:0	10.6	15:0	14:1	15:0	14:1[a]	
	18:3	1.6	17:3	16:4			
	18:0	1.0	17:0	16:1	17:0	16:1[a]	
	16:1	0.8	15:1	14:2			
soup (2.5 kGy)	18:1	54.5	17:1	16:2	17:1	16:2[a]	yes
	18:0	18.5	17:0	16:1	17:0	16:1[a]	
	18:2	10.0	17:2	16:3			
	16:0	9.2	15:0	14:1	15:0	14:1[a]	
	18:3	4.0	17:3	16:4			
	20:0	3.0	19:0	18:1			

[a] Main irridiation product. [b] cb, alkylcyclobutanone.

Where possible, the identification of the individual hydrocarbon peaks was done with reference samples and/or with the mass selective detector. A successful separation of the irradiation products was possible using the following temperature program: initial hold for 3 min at 70 °C, 7 °C/min to 240 °C, isothermal hold at 240 °C for 5 min, then 10 °C/min to 300 °C, with a final isothermal hold at 300 °C for 5 min.

RESULTS AND DISCUSSION

Optimization of SFE. The solvating and extraction properties of supercritical carbon dioxide can easily be adjusted by varying its density and adjusting the extraction pressure or temperature. For the extraction of hydrocarbons out of a fatty matrix, the SFE showed good extraction selectivity. As can be seen in Figure 2, the hydrocarbons of interest are already extracted at 78 °C with a pressure of approximately 100 bar (density ca. 0.25 g/mL), whereas oleic acid (C18:1) has a threshold pressure of approximately 150 bar (density ca. 0.36 g/mL). It is therefore possible to extract the hydrocarbons selectively and to keep the fatty acid concentration in the gained extract very low. Triglycerides generally show even higher threshold densities, so that they are not extracted under these conditions. This high selectivity cannot be achieved by classical solvent extraction techniques and shows comparable, or even better, results than a Florisil column cleanup procedure.

Figure 3 shows the influence of increasing temperature, at constant density, on the extraction kinetics of

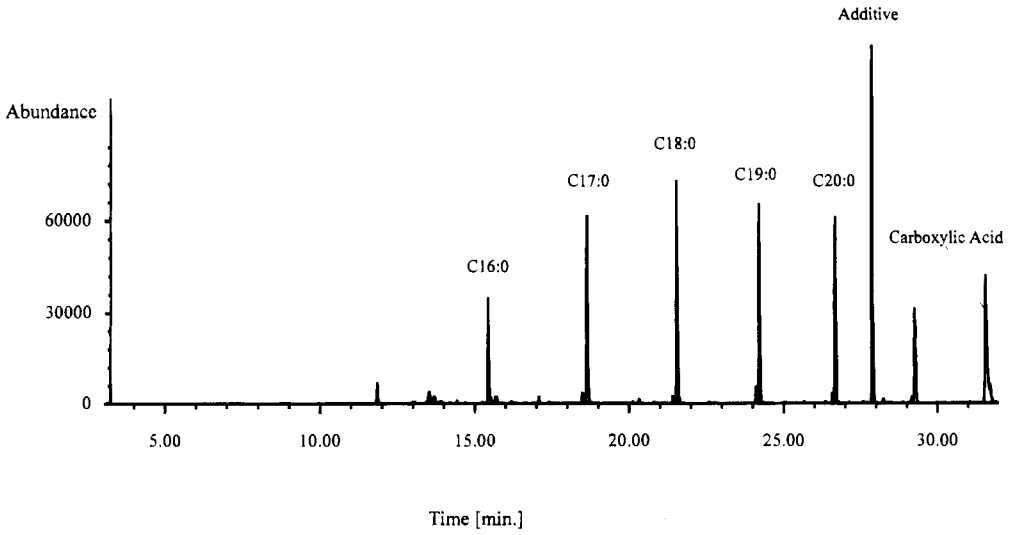

Figure 7. Hydrocarbons extracted (heptane extract!) from deep-freezer plastic bags. GC conditions: see Experimental Conditions.

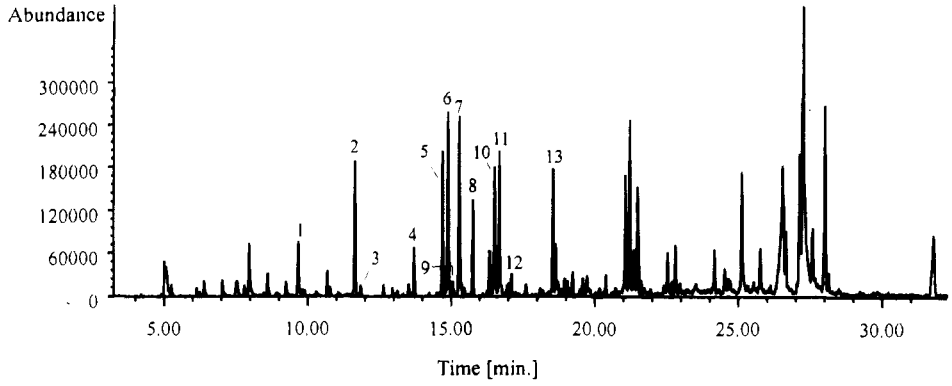

Figure 8. GC analysis of irradiated peanuts: Solvent extraction followed by SFE enrichment. SFE and GC conditions were as in Figures 4–6. Hydrocarbons: (1) unsaturated aldehydes, (2) 14:1, (3) 14:0, (4) 15:0, (5) 2-alkylcyclobutanone, (6) 16:2, (7) 16:1, (8) phosphoric acid ester, (9) 16:0, (10) 17:2, (11) 17:1, (12) presumably heptadecanol.

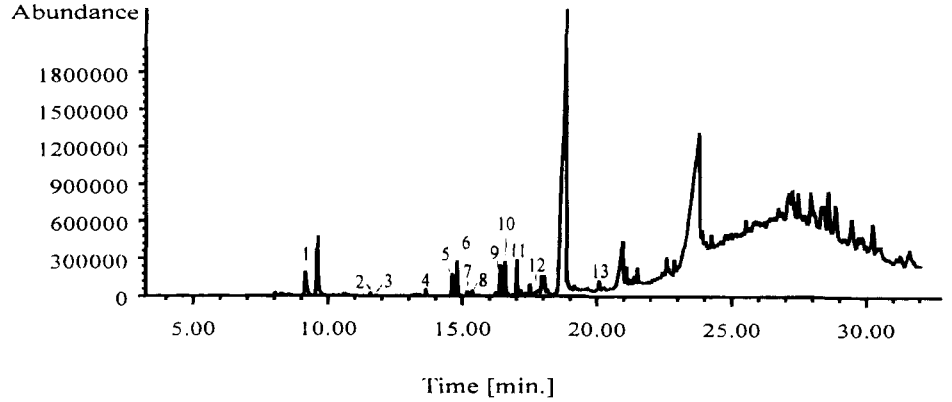

Figure 9. GC analysis of irradiated peanuts after just SFE. SFE and GC conditions as in Figures 4–6. Hydrocarbons: (1) unsaturated aldehydes, (2) 14:1, (3) 14:0, (4) 15:0, (5) 2-alkylcyclobutanone, (6) 16:2, (7) 16:1, (8) 16:0, (9) 17:2, (10) 17:1, (11) 17:0, (12) 18:0, (13) 19:0.

a characteristic hydrocarbon (C16:0) found in irradiated food. With increasing temperature at constant density, the volatility of the hydrocarbon increases also. Consequently, the required extraction time is reduced. However, at 120 °C the selectivity is lost, because under these conditions the fatty acids (e.g. oleic acid) are also extracted to a significant amount. Therefore, to keep extraction time as short as possible and maintain the high selectivity, all samples were extracted at 78 °C and 150 bar.

Using these conditions, as can be seen in Figure 4, a total extraction of the hydrocarbons requires approximately 60 min. Nevertheless, extracting the sample for between 30 and 40 min is sufficient, as more than 90% of the important marker hydrocarbons are extracted within this time. It is important to note that the extraction of the hydrocarbons from the fat sample does not have to be quantitative. This is due to the fact that generally the question is not with *what* dose a particular sample was irradiated, but *whether* it was irradiated at all. If a quantitative statement has to be made concerning the dose of irradiation, then obviously a quantitative extraction of the hydrocarbons has to be ensured. This is because the hydrocarbon formation rate is directly proportional to the irradiation dose (*Analytical Detection Methods for Irradiated Foods*, 1991).

From Figures 2 and 4 it can be deduced that the introduction of an ethyl group into the hydrocarbon molecule changes the extraction behavior of the particular hydrocarbon. With increasing chain length of the hydrocarbon the extraction time increases too, due to a decreasing volatility of the molecule. On the other hand, the introduction of a double bond into the hydrocarbon chain shows no significant effect on its volatility and therefore extraction behavior in supercritical carbon dioxide.

Characterization of Irradiated Foods. The advantage of the SFE extraction can be seen by comparing Figures 5 and 6 for the analysis of a soup mix powder (Grünkernsuppe). In the fat extract gained by liquid extraction, the hydrocarbons can hardly be characterized, whereas after SFE extraction and enrichment, the characterization of the various extracted hydrocarbons and their characteristic pattern (depending on the initial fatty acid composition of the sample) presents no major problem. After the SFE extraction, it is also possible to detect the characteristic irradiation marker 2-alkylcyclobutanone (peak 4 in Figure 6A). This group of substances has so far not been detected in nonirradiated food samples (J. Ammon, personal communication, 1993). The 2-alkylcyclobutanones are formed from fatty acids. Hence, the corresponding 2-alkylcyclobutanone that will be generated depends on the predominant fatty acids in the sample. Unfortunately, the absolute amount of 2-alkylcyclobutanone formed was so small that a reliable identification of the alkyl chain length was not possible. Nevertheless, a reliable qualitative identification as a 2-alkylcyclobutanone was possible with the utilized GC–MSD spectral library.

The observed hydrocarbon patterns corresponded very well to that predicted from the degradation scheme described above in Figure 1 and the fatty acid composition summarized in Table 1. The generated hydrocarbons derived from the fatty acids and their relative abundances are summarized in Table 2. It would be superfluous to mention that in nonirradiated products

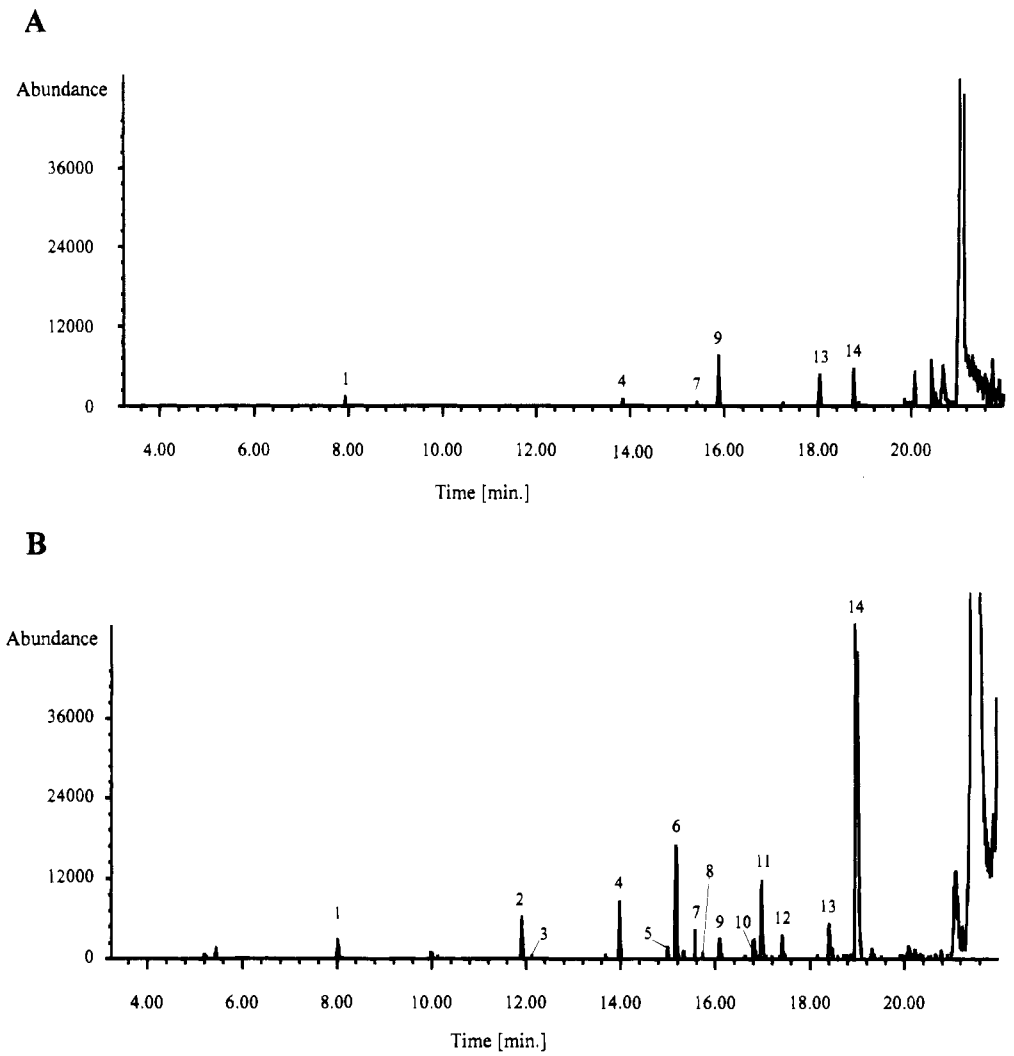

Figure 10. GC analysis of nonirradiated (A) and irradiated (B) fat from duck breasts. SFE and GC conditions were as in Figures 4–6. Hydrocarbons: (1) 12:0, (2) 14:1, (3) 14:0, (4) 15:0, (5) 2-alkylcyclobutanone, (6) 16:2, (7) 16:1, (8) 16:0, (9) phosphoric ester, (10) 17:2, (11) 17:1, (12) 17:0, (13) carboxylic acid or ester, (14) presumably octadecanal.

the characteristic generated hydrocarbon pattern is not present. However, one or the other hydrocarbon can be found in nonirradiated food samples. This is demonstrated in Figure 11A, in which the analysis of nonirradiated, roasted pistachio nuts is shown under the same conditions as in Figure 4.

It is important to note that with the SFE technique it is, for the first time, possible to isolate the marker 2-alkylcyclobutanone along with the hydrocarbons, in a single sample preparation step. With the classical Florisil column sample cleanup technique, two separate procedures have to be carried out, using Florisil packings differing in their activity (water content) (J. Ammon, personal communication, 1993). For the hydrocarbons a Florisil packing activated with 3% water has to be used, whereas for the elution of the marker 2-alkylcyclobutanone, the Florisil has to be activated with a higher water content.

Unfortunately, the samples were stored in plastic deep-freezer bags. Small amounts of hydrocarbons migrated from the plastic bag into the fat of the stored food samples and therefore caused problems during the investigation of the samples. Figure 7 shows a heptane extract of one of the utilized deep-freezer plastic bags. The chromatogram in Figure 7 confirms the observation from Biedermann et al. (1992), who also suggest not storing the particular food samples in plastic bags, plastic containers, or wax paper.

It is very likely that the hydrocarbons originating from the utilized plastic bags migrate into the lipophilic region of the food samples and are coextracted by the supercritical carbon dioxide. Hence, even a nonirradiated food sample can show a number of hydrocarbons and therefore may cause a false conclusion concerning the previous food treatment. However, a reliable determination of an irradiated food sample is still possible, with the help of the characteristic hydrocarbon pattern (resulting from the individual fatty acid composition of the sample) and the presence of the marker 2-alkylcyclobutanone.

The organic solvent extraction of the fats prior to the SFE isolation of the irradiation products is not always necessary. This can be seen by comparing Figures 8 and 9 for the analysis of irradiated peanuts. Both chromatograms show the expected hydrocarbon pattern, including the 2-alkylcyclobutanone peak.

Generally, it is possible to extract any naturally dry (or dried) sample directly with supercritical carbon dioxide, without any need of a solvent extraction step. This reduces the analysis time and consumption of organic solvent significantly and is a great advantage of this method. However, as Figures 8 and 9 indicate, a solvent extraction of the sample fat, prior to the supercritical fluid extraction of the hydrocarbons, has the advantage that much cleaner and more concentrated extracts for GC analysis are obtained.

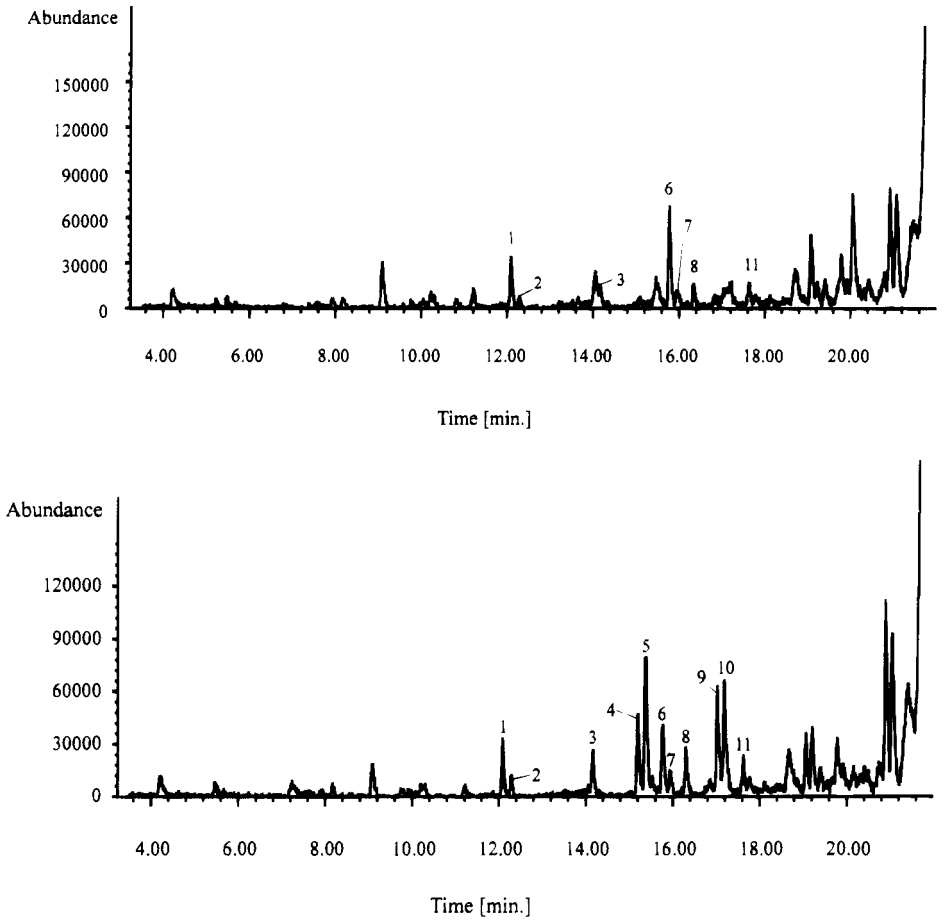

Figure 11. GC fat analysis of (A, top) nonirradiated and (B, bottom) irradiated roasted pistachio nuts after organic solvent extraction followed by SFE enrichment of hydrocarbons. SFE and GC conditions as in Figures 4–6. Hydrocarbons: (1) 14:1, (2) 14:0, (3) 15:0, (4) 2-alkylcyclobutanone, (5) 16:2, (6) 16:1, (7) 16:0, (8) phosphoric acid ester, (9) 17:2, (10) 17:1, (11) 17:0.

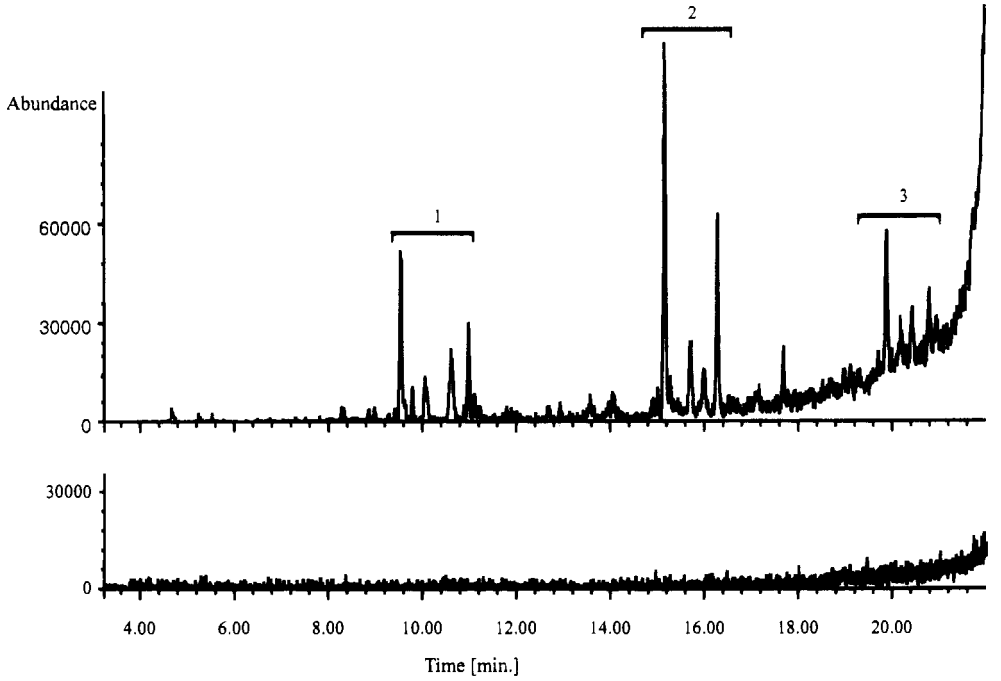

Figure 12. GC analysis of fat extract from German caviar after organic solvent extraction followed by SFE enrichment of hydrocarbons. The regions where unsaturated hydrocarbons elute are marked 1–3. For an explanation, see the text. SFE and GC conditions were as in Figures 4–6.

Further applications of the SFE method are demonstrated in Figure 10 for a meat product (duck breast) and for pistachio nuts in Figure 11. The chromatograms in these figures were only recorded up to 22 min, as only in this region do the hydrocarbons of interest elute. The reason the nonirradiated pistachio nuts (Figure 11A) show numerous hydrocarbons is due to the fact that they were roasted. The roasting process (or any other

heating process as well) induces a hydrocarbon formation similar to irradiation. Nevertheless, this hydrocarbon formation shows no characteristic pattern (*Analytical Detection Methods for Irradiated Food*, 1991). Therefore, irradiation of a food can still be identified by the presence of the characteristic hydrocarbon pattern and the irradiation marker, 2-alkylcyclobutanone.

All of the investigated irradiated food samples showed a hydrocarbon pattern as expected, or very close to the expected one, except the caviar sample (Figure 12). In this case, several groups of unsaturated (one to three double bonds) hydrocarbons were found but no characteristic pattern which could be derived from the fatty acid composition of the sample. The characteristic irradiation marker, 2-alkylcyclobutanone, was not found either. Similar observations were made by Ammon (personal communication, 1993) with other fish products. An explanation for this behavior could be that the highly unsaturated fatty acids of the caviar sample (and other fish samples) are more randomly split by the ionizing radiation, generating numerous shorter hydrocarbons with two and three double bonds.

The fact that no or only very little 2-alkylcyclobutanone is formed during the irradiation can also be explained by the large amount of the highly unsaturated fatty acids in the sample. These do not permit the formation of the particular cyclic products.

In Table 2 the theoretically predicted hydrocarbon pattern of the various investigated food samples is compared with the one actually found. All of the investigated samples show good agreement between the predicted and the found patterns. This demonstrates the potential of the SFE technique for a reliable identification of fat-containing irradiated foods.

Conclusion. This paper shows the potential of supercritical fluid extraction (SFE) for the highly selective, efficient, and fast extraction of hydrocarbons from complex matrices, such as irradiated foods. The technique is easy to perform and reduces the consumption of organic solvents and analysis time. Further, a direct on-line coupling SFE–GC/FID (MS) or SFE–SFC/FID (MS) is possible.

A unique advantage of the SFE sample preparation technique is that hydrocarbons and the irradiation marker, 2-alkylcyclobutanone, can simultaneously be extracted in one run, with a sufficiently high selectivity for the following GC–MS analysis. This provides a unique diagnostic aid for the rapid detection of an irradiated food sample.

A further advantage of the SFE method is that the determination of the total or free fat content of the investigated sample (usually a routine analysis) can be run directly after the selective extraction of the volatile irradiation products, simply by altering the extraction conditions (Lembke and Engelhardt, 1993).

ACKNOWLEDGMENT

We thank Dr. H. Delincee (Federal Research Centre for Nutrition, Karlsruhe/Germany) for irradiating our samples and for the fruitful discussions concerning this work.

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Received for review February 23, 1994. Revised manuscript received August 9, 1994. Accepted October 6, 1994.[⊗]

JF940094U

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1994.