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METHODS FOR ROUTINE CONTROL OF IRRADIATED FOOD: OPTIMIZATION OF A METHOD FOR DETECTION OF RADIATION-INDUCED HYDROCARBONS AND ITS APPLICATION TO VARIOUS FOODS

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Abstract—By comparison of two methods for the isolation of radiation-induced hydrocarbons, high vacuum "cold finger" distillation and Florisil column chromatography, it could be shown that the sensitivity of both was similar whereas the latter seemed to be more practical for routine application. In optimizing studies, the influence of the degree of Florisil activation and the influence of the irradiation temperature on hydrocarbon yields as well as the resolution of hydrocarbons on polar and non-polar gas chromatographic capillary columns have been examined. From the successful application of the method to different fat containing foodstuffs, it is concluded that the Florisil column chromatography is well suited as clean-up procedure for the gas chromatographic/mass spectrometric (GC/MS) detection of irradiated products by routine food control analyses.

INTRODUCTION

The treatment of fatty foods by ionizing radiation induces a series of changes in the fatty fraction. Such changes can not be considered as radiation-specific since they might be caused also by oxidative processes. Pioneering work by Champagne and Nawar (1969), Kavalam and Nawar (1969) and Dubravcic and Nawar (1969) has shown however that subsequent to triglyceride irradiation, two types of volatile hydrocarbons appear in high amounts: hydrocarbons which have one carbon atom (C_{n-1}) less than the quantitatively predominant fatty acids and hydrocarbons which have two carbon atoms less as compared to the most frequently appearing fatty acids and one additional double bond in position 1 ($C_{n-2:1}$). Based on these results, a fission scheme has been developed for irradiated triglycerides that permits a prediction of the main radiolysis products when the proportions of the fatty acids are known (Table 1).

In order to detect the volatile hydrocarbons, it is necessary to isolate them from the fatty matrix. Separation of hydrocarbons and fatty components can be obtained in several ways by taking advantage of differences in volatility or polarity. "Cold finger" distillation (Balboni and Nawar, 1970; Nawar *et al.*, 1990; Spiegelberg *et al.*, 1990, 1991a, b) or Florisil column chromatography (Nawar, unpublished results; Morehouse and Ku, 1990, 1992; Morehouse *et al.*, 1991; Sjöberg *et al.*, 1992; Schulzki *et al.*, 1993) as well as high-performance liquid chromatography (HPLC) (Biedermann *et al.*, 1989; Meier and Biedermann, 1990; Meier *et al.*, 1990) are used for this purpose. Separation of the different hydrocarbons is accomplished by gas chromatography (GC). Flame ionization detectors (FID) and/or mass spectrometers (MS) are used for identification.

These studies have established that the measurement of radiolytic products from food lipids offers the possibility to identify irradiated foods (for review see: Schreiber *et al.*, 1993a). Although the identification of irradiation treatment of meat has been successfully performed using the "cold finger" distillation for isolation of volatiles, an application of this method in routine control seems to be questionable since the capacity for running samples is rather limited. In a previous study Schulzki *et al.* (1993) compared this method with that of Florisil column chromatography and concluded that the latter might be more efficient and practical for routine control purposes. For the preparation of a large-scale German intercomparison between food control laboratories on irradiated meat products (Schreiber *et al.*, 1993b, c), these studies have been extended in order to describe a method optimized for routine control application. Results of these examinations as well as hints important for the exclusion of false positive identifications due to contamination are given.

EXPERIMENTAL

Samples

The comparison of "cold finger" distillation and Florisil chromatography was done on 30 irradiated

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Table 1. Main fatty acids in chicken, pork and beef and their main radiation-induced hydrocarbons

Type of meat	Fatty acid	Approx. fatty acid content [%]	Radiation-induced hydrocarbons C_{n-1}	$C_{n-2:1}$
Chicken	Oleic acid (C 18:1)	32	8 – 17:1	1,7 – 16:2
Chicken	Linoleic acid (C 18:2)	25	6,9 – 17:2	1,7,10 – 16:3
Chicken	Palmitic acid (C 16:0)	21	15:0	1 – 14:1
Chicken	Stearic acid (C 18:0)	6	17:0	1 – 16:1
Pork	Oleic acid (C 18:1)	35	8 – 17:1	1,7 – 16:2
Pork	Palmitic acid (C 16:0)	25	15:0	1 – 14:1
Pork	Stearic acid (C 18:0)	11	17:0	1 – 16:1
Pork	Linoleic acid (C 18:2)	10	6,9 – 17:2	1,7,10 – 16:3
Beef	Oleic acid (C 18:1)	43	8 – 17:1	1,7 – 16:2
Beef	Palmitic acid (C 16:0)	23	15:0	1 – 14:1
Beef	Stearic acid (C 18:0)	10	17:0	1 – 16:1
Beef	Linoleic acid (C 18:2)	2	6,9 – 17:2	1,7,10 – 16:3

(about 5 kGy) and 6 non-irradiated homogenized frozen chicken samples delivered within an intercomparison of the Reference Bureau (BCR) of the European Communities (Meier and Stevenson, 1993). The sample numbers in Table 2 were given by the organizers of this intercomparison. For control experiments and optimization studies of the Florisil method, frozen chicken samples irradiated to about 3 kGy and the analogous controls were used. The method was applied also to pork, beef and (pasteurized) liquid whole egg samples either non-irradiated or irradiated to about 3 kGy.

Lipid Separation from Food

Chicken, pork and beef

Lipid separation by centrifugation after heating to about 50°C. Homogenized meat was heated to about 50°C in a waterbath. The liquefied fat was separated by centrifugation for 10 min at 900 ***g***.

Lipid extraction with a mixture of cold pentane/isopropanol. Fifty grammes of homogenized chicken were mixed with 150 ml pentane/isopropanol (3:2 v/v) in a blender. This mixture was centrifuged for 10 min at 900 ***g***. After collecting the upper pentane layer, the residue was re-extracted with an additional volume of 50 ml solvent mixture. The combined fractions were reduced in a rotary evaporator at 40–45°C to a constant weight.

Lipid extraction with hexane under reflux. Forty grammes of homogenized chicken were mixed with 40 g of anhydrous sodium sulphate and refluxed with 100 ml of hexane for 2 h. The filtered solution was transferred to a 100 ml stoppered cylinder and, after mixing with 5–10 g of anhydrous sodium sulphate, left overnight at room temperature.

Soxhlet extraction of lipids. Forty grammes of homogenized chicken were mixed with 40 g of anhydrous sodium sulphate, filled into an extraction thimble and refluxed with 140 ml hexane for 3 h. The extract was transferred to a 100 ml stoppered cylinder and, after mixing with 5–10 g of anhydrous sodium sulphate, left overnight at room temperature.

Egg

Fifty grammes of liquid whole egg were dried overnight at 100°C. The dried sample was mixed with *ca* 30 g of anhydrous sodium sulphate and refluxed as described above (*Lipid extraction with hexane under reflux*).

Table 2. Yields of hydrocarbons (μg/100 g fat) in homogenized irradiated (about 5 kGy) and non-irradiated chicken samples, isolated (a) by high vacuum "cold finger" distillation (internal standard 13:1) and (b) by Florisil column chromatography (internal standard 20:0)

(a) Isolation by "cold finger" distillation

Sample No.	14:1	15:0	16:2	17:1
78	148	89	127	69
67	142	100	210	113
166	189	94	205	136
165	213	201	439	269
6	216	162	278	177
96	296	229	503	316
146	267	259	543	457
82	195	170	381	237
114	214	123	201	101
27	194	122	308	493
140	180	172	272	170
25	177	106	188	78
40	270	247	622	465
32	193	141	255	151
132	239	239	514	365
Mean value	209	164	336	239
Standard deviation	42	57	148	142
Relative standard deviation (%)	20	35	44	59
Control 25	n.d.	n.d.	n.d.	n.d.
Control 26	n.d.	n.d.	n.d.	n.d.
Control 32	n.d.	n.d.	n.d.	n.d.
Control 33	n.d.	n.d.	n.d.	n.d.

(b) Isolation by Florisil column chromatography

Sample No.	14:1	15:0	16:2	17:1
21	132	162	361	272
94	141	152	337	263
186	163	180	301	291
197	138	144	332	241
48	160	163	362	260
59	131	157	285	284
101	146	150	322	251
181	117	148	327	243
102	144	155	331	271
126	115	149	304	214
167	143	157	288	238
20	157	157	349	246
47	148	169	140	223
113	129	141	202	245
36	168	211	359	274
Mean value	142	160	307	254
Standard deviation	15	17	59	21
Relative standard deviation (%)	11	11	19	8
Control 24	n.d.	n.d.	15*	n.d.
Control 31	n.d.	n.d.	11*	n.d.

n.d. = Not detected.
*Not clearly identified

Lipid Determination of Hexane Extracts

Fifty millilitre flasks were dried overnight at 100°C, cooled, weighed, filled with 5 ml of lipid extract (*Lipid extraction with hexane under reflux* or *Soxhlet extraction of Lipids*) and reduced in a rotary evaporator to dryness. The flasks were dried again overnight at 100°C and reweighed. The volume of extract equivalent to 1 g of lipid was calculated.

Isolation of Hydrocarbons

Isolation of hydrocarbons by high vacuum "cold finger" distillation

Isolation of hydrocarbons by high vacuum "cold finger" distillation was performed as described previously (Spiegelberg *et al.*, 1990, 1991a, b). The hydrocarbons were collected in a "cold finger" apparatus proposed by Balboni and Nawar (1970). Five grammes of lipid dissolved in 1 ml pentane containing 13:1 as internal standard were spread in a thin layer over the bottom of the sample flask and frozen by liquid nitrogen. The flask was connected to the "cold finger" and the system evacuated. The sample was exposed to room temperature for about 10 min. After this thawing period, liquid nitrogen was placed into the reservoir of the "cold finger" and the distillation was carried out at 0.1 Pa for 1 h during which the sample was maintained at 80°C. The condensed hydrocarbons were rinsed from the "cold finger" with 15 ml pentane. This solution was concentrated to 1 ml via a gentle flow of nitrogen gas.

Isolation of hydrocarbons by Florisil column chromatography

Decontamination and activation/deactivation of Florisil. Florisil was heated at 550°C for 5 h to remove volatile contaminants. Prior to use, it was

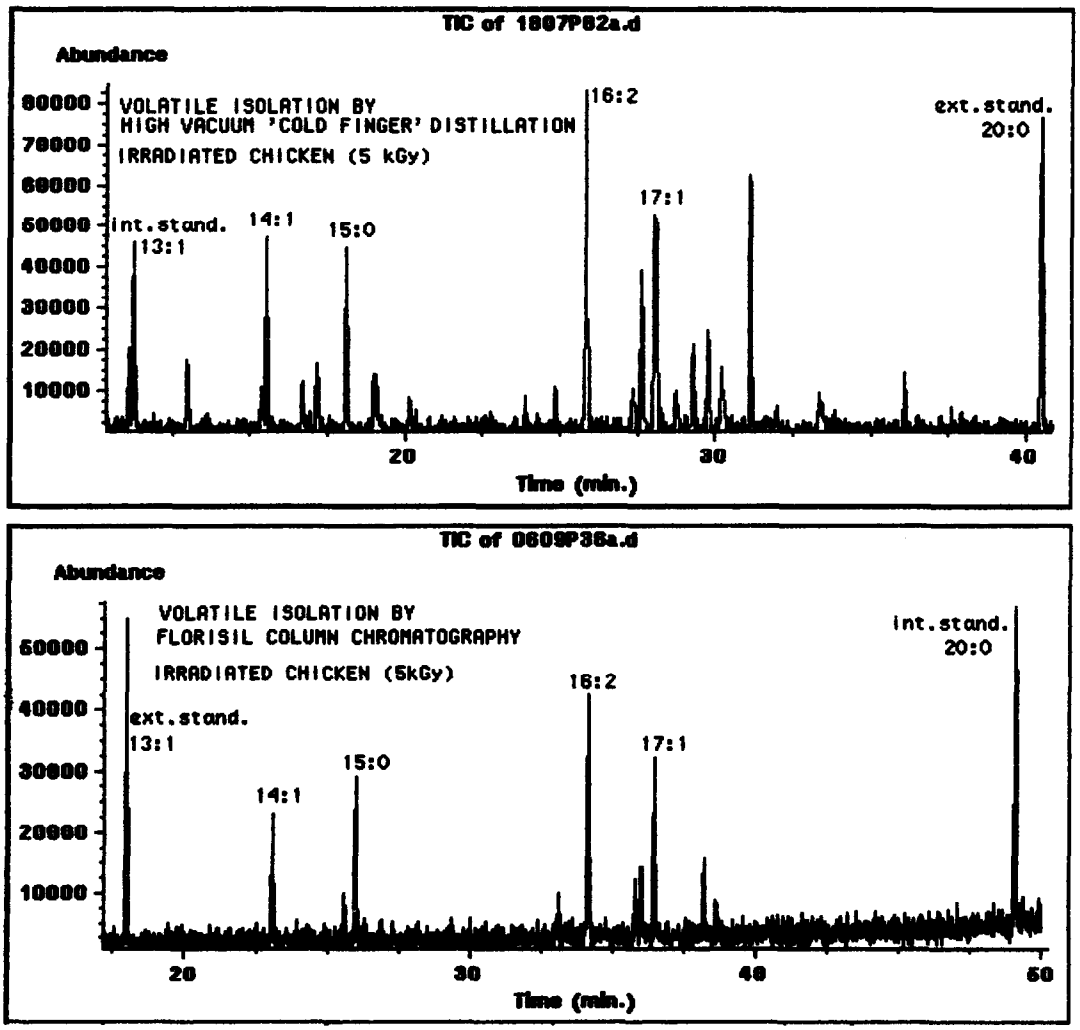

Fig. 1. Gas chromatographic analysis of the volatile hydrocarbons from chicken meat irradiated to 5 kGy. Volatile isolation by high vacuum "cold finger" distillation (top) and by Florisil column chromatography (bottom).

activated at 220°C overnight (approx. 15 h). After cooling to room temperature in a desiccator it was used either freshly activated or after storage in the desiccator for 5 days, or it was used partially deactivated by addition of 3% of distilled water.

Florisil column chromatography. The chromatographic column (20 mm, i.d.) was filled up with about 20 g of activated, stored or partially deactivated Florisil. One gramme of pure lipid or the calculated volume of lipid extract, evaporated to about 5 ml and mixed with 1 ml of the internal standard solution (*n*-eicosane), were applied to the Florisil column. Hydrocarbons were eluted with a volume of 60 ml pentane or hexane at a flow rate of 3–5 ml/min. The eluate was reduced in a rotary evaporator to a volume of approx. 3 ml and finally, to a volume of 1 ml by a gentle stream of nitrogen gas.

Gas Chromatography/Mass Spectrometry (GC/MS)

Apparatus: Hewlett Packard Gas chromatograph/Mass spectrometer (5980/5970B).

Carrier gas: Helium 5.0; sample size: 1 μl; splitless mode.

Columns:

—Polyethylene glycol, Carbowax 20 M (25 m, 0.25 mm i.d., 0.25 μm film thickness) with the following temperature program: 40°C for 2 min, first ramp 2.5°C/min to 170°C, second ramp 5°C/min to 200°C.

—5% diphenyl/95% dimethyl polysiloxane (12 m, 0.2 mm i.d., 0.33 μm film thickness) with the following temperature program: 50°C for 2 min, first ramp 10°C/min to 130°C, second ramp 5°C/min to 200°C.

Calculations

The yields of hydrocarbons were calculated by the following equation:

$$\frac{\text{area hydrocarbon} \times \mu\text{g int. standard} \times 100}{\text{area internal standard} \times \text{g lipid}} = \mu\text{g}/100\,\text{g lipid}$$

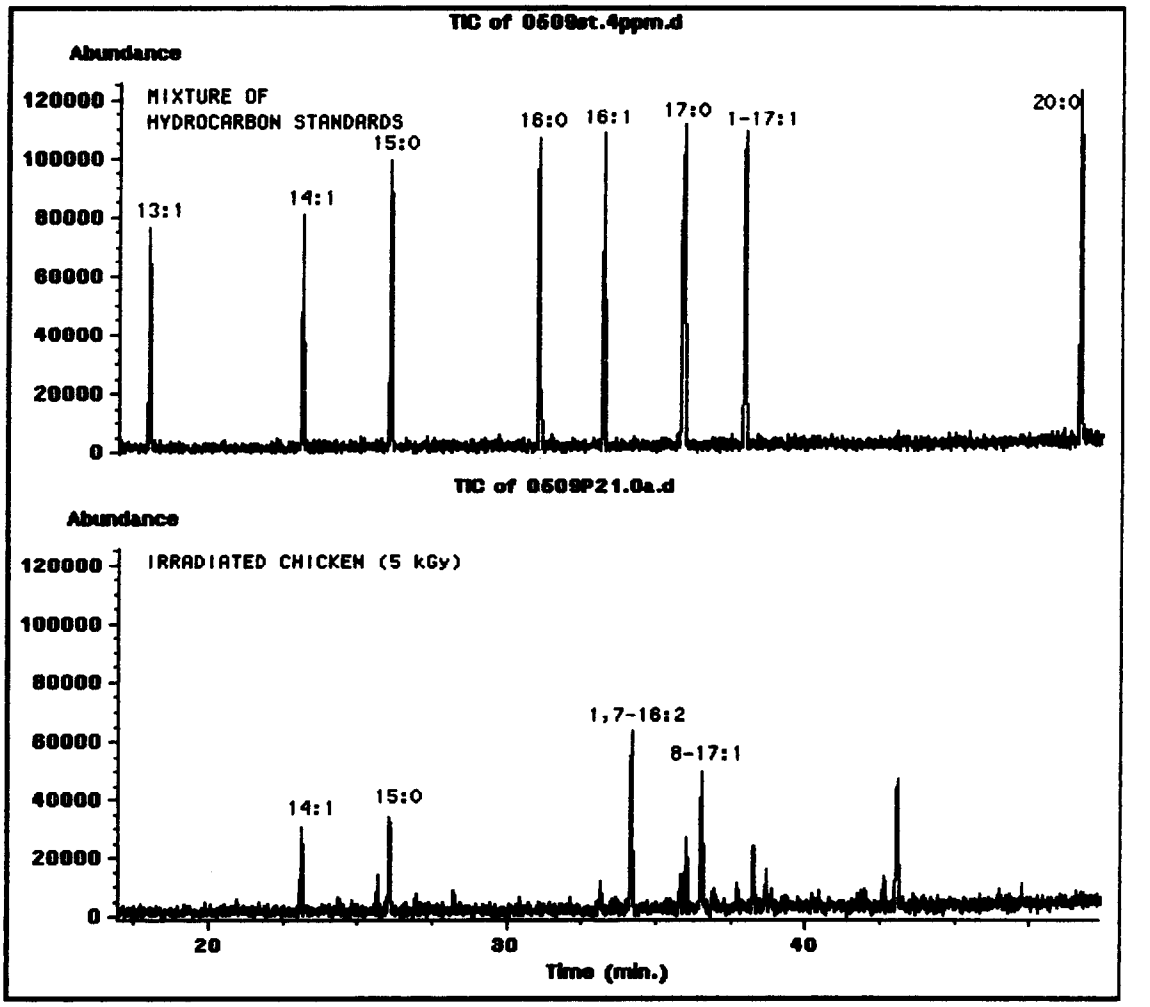

Fig. 2. Gas chromatographic analysis of the volatile hydrocarbons. Top: mixture of hydrocarbon standards. Bottom: hydrocarbons from chicken meat irradiated to 5 kGy.

RESULTS

Comparison of Florisil column chromatography and high vacuum "cold finger" distillation as methods to isolate radiation-induced hydrocarbons

Examination of identical samples. Thirty irradiated (about 5 kGy) and 6 non-irradiated homogenized chicken samples supplied during a BCR intercomparison were subjected to both methods—separation of hydrocarbons by high vacuum "cold finger" distillation as well as Florisil column chromatography using activated Florisil. No qualitative and only minor quantitative differences (Fig. 1 and Table 2) could be found by comparison of both separation methods: whereas the mean values of the quantified hydrocarbons were similar, isolation by Florisil column chromatography resulted in lower standard deviations. By both methods, none of the hydrocarbons 14:1, 15:0 and 17:1 could be detected in non-irradiated chicken. Very small peaks appeared at the retention time of 16:2 in two control samples [Table 2(b)], but due to their small amounts they could not be verified by their mass spectra.

For these analyses, different internal standards had been used for quantification: 13:1 in the case of "cold finger" distillation and 20:0 in the case of Florisil column chromatography. Both hydrocarbons are in general suitable as internal standard, because they are not radiation-induced and not detectable in irradiated samples (Fig. 2). To evaluate the possible influence of different standard substances on the calculated quantities and their variations, 10 lipid extracts derived from irradiated chicken (3 kGy) containing 13:1 as well as 20:0 as internal standards were investigated by column chromatography on activated Florisil. With reference to 13:1, higher amounts of hydrocarbons were calculated than those calculated on the basis of integration areas of 20:0 [Table 3(a and b)]. However, the relative standard deviations were similar for both calculations and the ratios of the areas of both internal standards 20:0/13:1 [Table 3(c)] were similar for all samples. Therefore, higher variations obtained by applying the distillation method could not be related to the use of 13:1 instead of 20:0 as internal standard.

The differences in mean yields of hydrocarbons calculated on the basis of the two standards are due to the dependence of recovery on chain length. Therefore, the calculated amounts have to be corrected for recovery. However, this is only possible for commercially available hydrocarbons.

Time consumption of both isolation methods. For routine analysis, the efficiency of a method is an important aspect. Table 4 demonstrates that the time needed to run one sample using high vacuum "cold finger" distillation is 4 times higher than that needed for running it on a Florisil column.

Lipid sample sizes. Volatile isolation by high vacuum "cold finger" distillation requires a sample amount of at least 3 g, whereas the sample amount for isolation by Florisil column chromatography may be reduced to 0.5 g.

Table 3. Quantitative analysis of hydrocarbons (μg/100 g fat) in irradiated chicken samples (5 kGy) related to two different internal standards (a) 13:1 and (b) 20:0 which had been added to the same samples, simultaneously; (c) Ratios of 13:1 and 20:0 integration areas.

(a) Yields in relation to 13:1

Sample No.	14:1	15:0	16:2	17:1
1	234	307	?	328
2	243	332	?	517
3	194	307	189	473
4	253	327	395	493
5	223	287	418	396
6	226	262	551	484
7	232	287	533	477
8	237	298	359	377
9	220	284	162	437
10	245	305	578	560
Mean value	231	300	398	454
Standard deviation	16	20	148	66
Relative standard deviation (%)	7	7	37	15

(b) Yields in relation to 20:0

Sample No.	14:1	15:0	16:2	17:1
1	94	123	?	132
2	97	133	?	206
3	80	127	78	195
4	101	131	158	197
5	82	105	153	144
6	93	108	227	199
7	95	118	218	196
8	117	147	177	185
9	103	132	75	203
10	96	119	226	219
Mean value	96	124	164	188
Standard deviation	10	12	57	26
Relative standard deviation (%)	10	10	35	14

(c) Ratios of 20:0 and 13:1 integration areas

Sample	1	2	3	4	5	6	7	8	9	10
Ratio	1.8	1.8	1.8	1.8	2.0	1.8	1.8	1.5	1.6	1.9

Improvement of the Florisil column chromatography method for routine use

The comparison has shown that by both methods similar qualitative as well as quantitative results can be obtained. However, the higher efficiency of Florisil column chromatography and the smaller sample sizes needed are important advantages and have led to a more detailed investigation of this method.

Influence of the degree of Florisil activation. In the above described investigations, Florisil was used at

Table 4. Time needed for isolation of hydrocarbons by "cold finger" distillation and Florisil column chromatography

Method	Steps of procedure	Time needed [min]
"Cold finger" distillation	Distillation at room temperature	10
	Distillation at 80°C	60
	Thawing period of "cold finger"	30
	Rinsing and concentration with N_2	20
Total time		about 2 h
Florisil column chromatography	Elution from Florisil column	15
	Rotary evaporation	10
	Rinsing and concentration with N_2	7
Total time		about 30 min

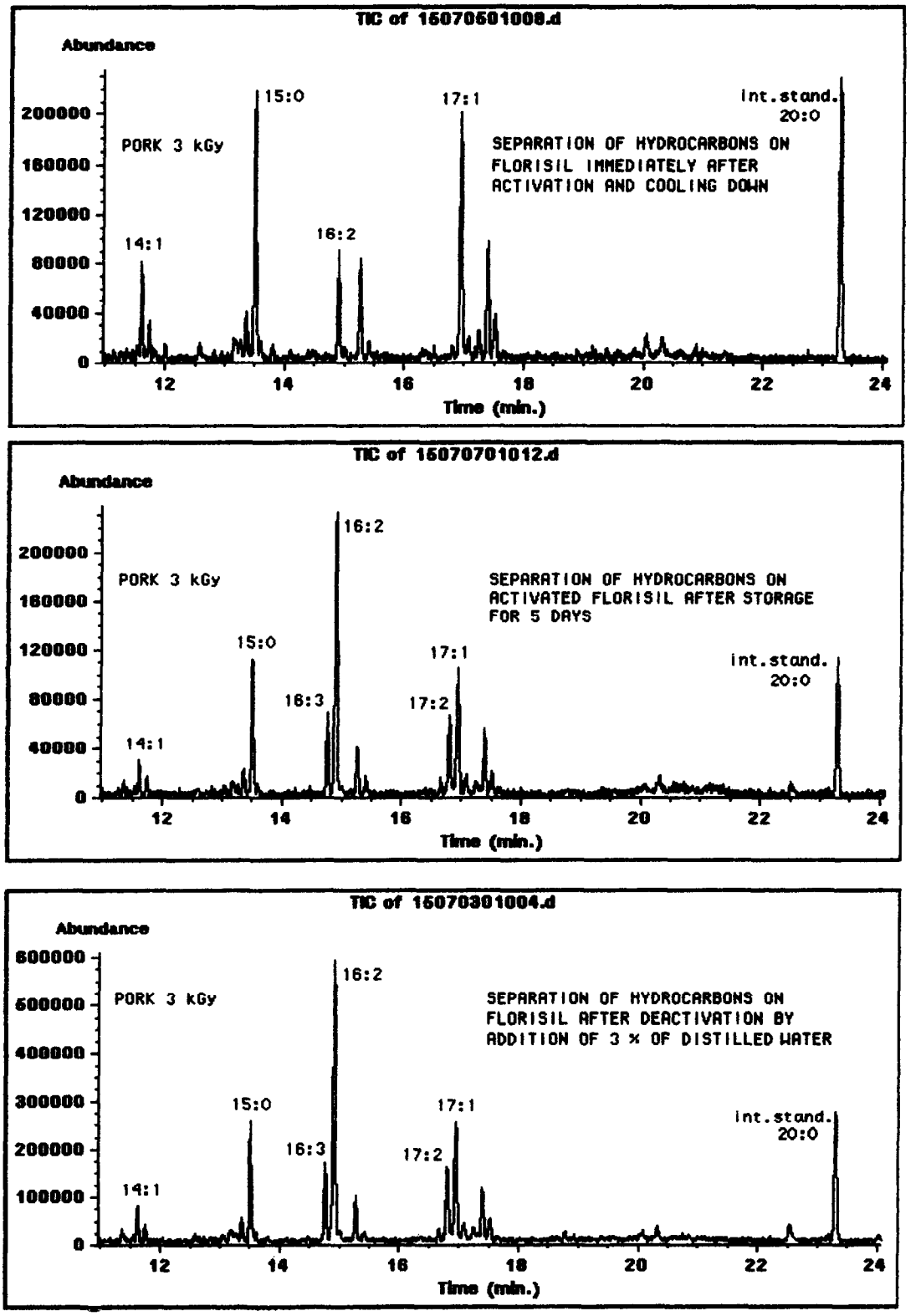

Fig. 3. Influence of the activation state of Florisil on the separation of volatile hydrocarbons from pork meat irradiated to 3 kGy. Top: separation on activated Florisil immediately after cooling down. Centre: separation on activated Florisil after storage for 5 days. Bottom: separation on activated Florisil after partial deactivation by addition of 3% (w/w) of distilled water.

various times after activation. It was noticed that separation of hydrocarbons on freshly activated Florisil resulted in a poor recovery of 16:2. Therefore, the influence of the activation state was examined on an irradiated pork lipid sample (3 kGy) using Florisil

1. immediately after cooling down in a desiccator,
2. after storage in a desiccator for 5 days and
3. after partial deactivation by addition of 3% (w/w) of distilled water.

On freshly activated Florisil, 16:3 and 17:2 were not eluted and the amounts of 16:2 were lower than expected (higher than 17:1), whereas partial deactivation with 3% of distilled water significantly increased recovery of di- and triunsaturated hydrocarbons (Fig. 3). Separation on activated Florisil stored for 5 days resulted in similar amounts of hydrocarbons as did separation with deactivated one.

Possible sources of contamination. Several methods for lipid extraction have been used. If Soxhlet and reflux extractions were used, it was noted that Soxhlet thimbles and filter papers are often contaminated by hydrocarbons: "blank" extractions of a thimble as well as 10 filter papers were carried out with 140 ml of hexane for 2 h in a Soxhlet apparatus. Thirty millilitres of the extracts were evaporated to about 3 ml and concentrated to 1 ml by nitrogen gas. Figure 4(a) and (b) demonstrates contamination of long chain saturated hydrocarbons in thimbles and filter papers. Solvents may also be contaminated with hydrocarbons: in a batch of hexane (concentrated 100:1) 15:0 and 16:0 were detected.

Selection of appropriate capillary columns. Hydrocarbons can be separated on both polar and non-polar capillary columns. Nearly no interaction occurs between hydrocarbons and methyl silicone phases. On these stationary phases, hydrocarbons of interest

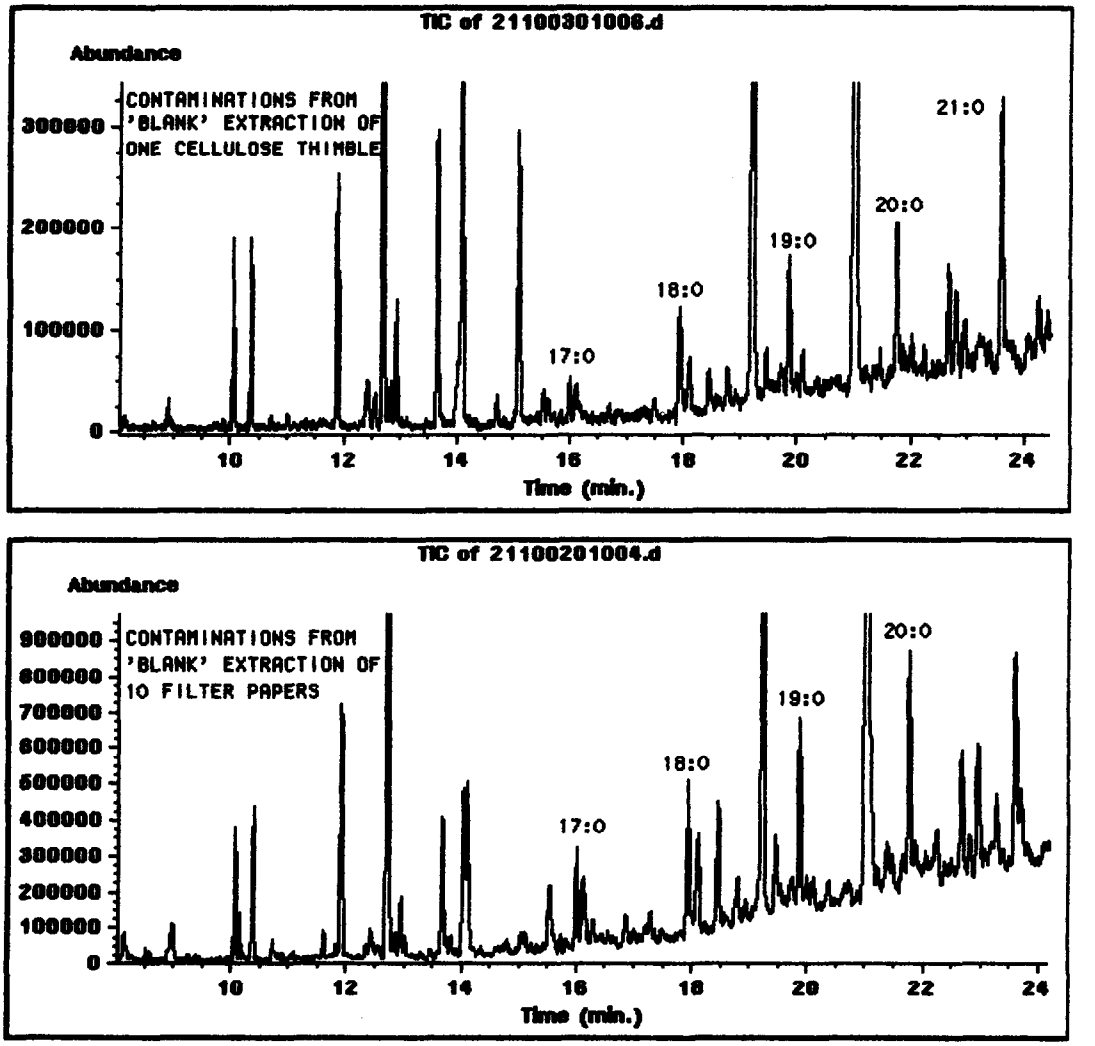

Fig. 4. Contamination with long-chain saturated hydrocarbons. Gas chromatograms from a "blank" extraction of one cellulose thimble (top) and from a "blank" extraction of 10 filter papers (bottom).

elute in the order of their boiling points and are clearly separated [Fig. 5(a)]. In contrast, on polar columns with phases like polyethyleneglycol, retention increases with chain length and number of unsaturated sides [Fig. 5(b)]. On such columns, heptadecene and hexadecatriene tend to overlap.

Influence of irradiation temperature. The yields of hydrocarbons are largely dependent on irradiation temperature (Nawar, 1973). Figure 6 reveals a considerable increase of the amount of hydrocarbons produced by irradiation to about 5 kGy for chicken and beef. This increase is most pronounced for the temperature range of −20 to +20°C which is the commercially used range.

Application of Florisil column chromatography to various foods

The Florisil method was also applied to the detection of hydrocarbons in irradiated pork, beef and egg. Since oleic acid is the most abundant fatty acid in chicken, pork and beef lipids (Table 1), hexadecadiene (1,7 − 16:2) and heptadecene (8 − 17:1) are the main radiolytic hydrocarbons in all these samples (Fig. 7). Pork lipids contain less linoleic acid than chicken lipid. In beef lipids, the amounts of this fatty acid are even smaller (Table 1). Therefore, hydrocarbons 16:3 and 17:2 are induced in smaller amounts in pork lipids than in chicken. In beef, only traces can be detected.

Similar patterns of radiation-induced hydrocarbons are obtained by analysis of lipids derived from irradiated egg and chicken (Fig. 8).

DISCUSSION

The present paper deals with several aspects important for the establishment of a method for the detection of irradiated fatty products in food control

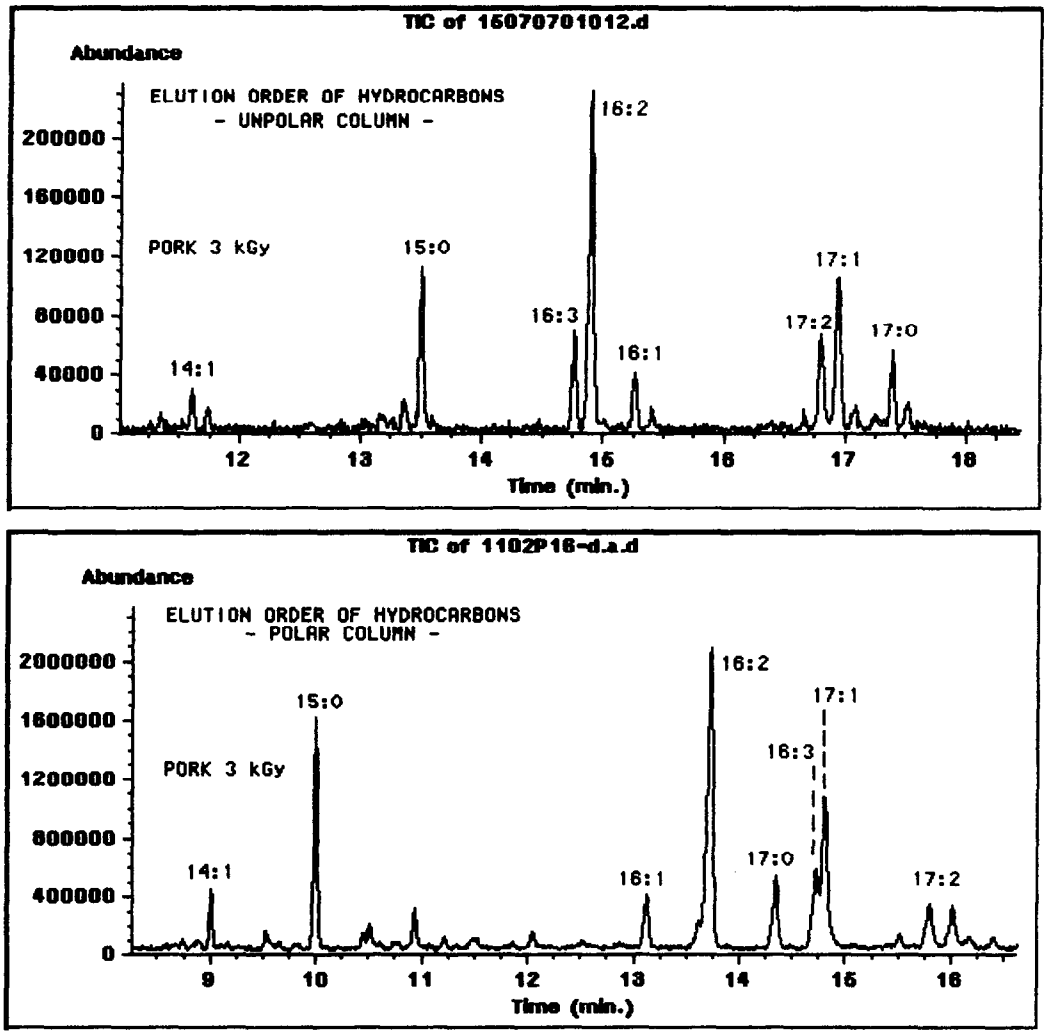

Fig. 5. Influence of different stationary phases on the elution order of volatile hydrocarbons from pork, irradiated to 3 kGy. Top: non-polar column (stationary phase 5% diphenyl/95% dimethyl polysiloxane). Bottom: polar column (stationary phase polyethylene glycol).

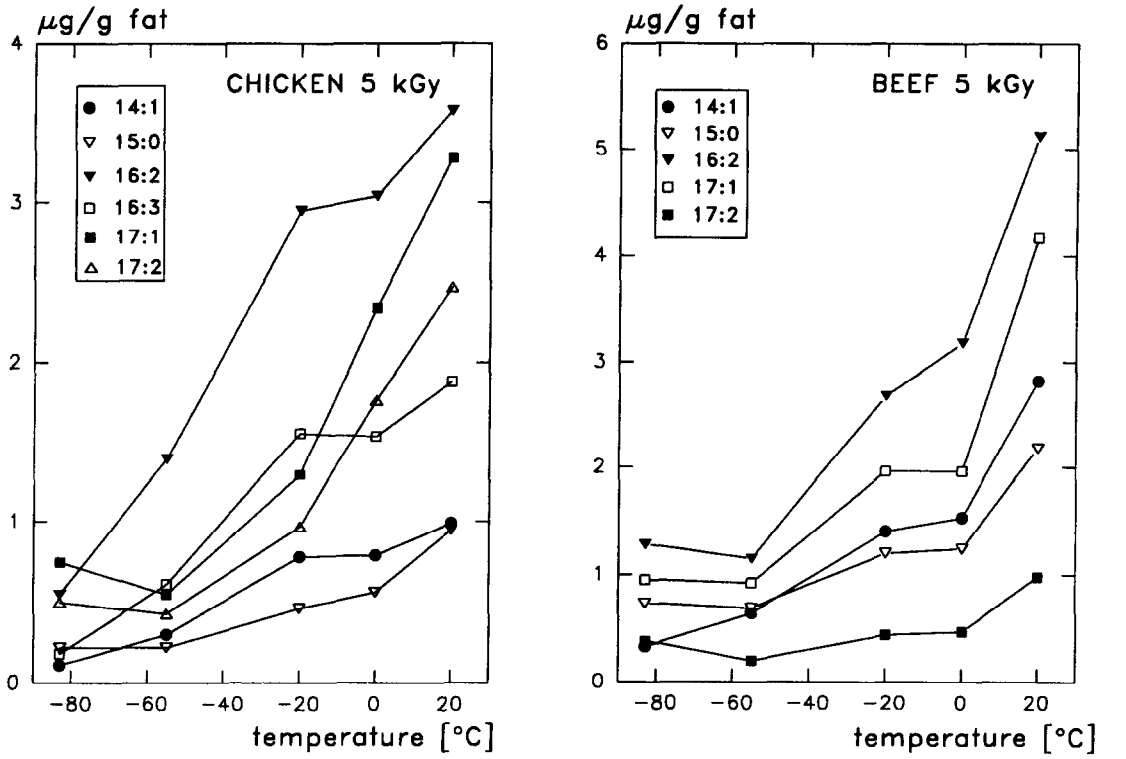

Fig. 6. Effect of irradiation temperature on the yields of radiolytic hydrocarbons in chicken and beef. Per datapoint only one sample had been analysed.

laboratories. These laboratories have to be supplied with detection methods suitable for unequivocal identification of irradiated foods to control the correct labelling of products as well as the adherence to existing national regulations. Labelling and its control are necessary to meet the different regulations in different countries and it ensures the consumer's free choice to buy the irradiated or the non-irradiated product.

The important aspects of routine control are the time consumption and the expenditure needed to run a method and the knowledge about possibilities and limitation to evaluate the possible treatment of a sample qualitatively and quantitatively. Since the separation of hydrocarbons from the fat matrix by "cold finger" distillation as it was originally described by Nawar *et al.* (1969) is not easy to achieve, a comparison of this method with Florisil column chromatography was performed. The mean yields of hydrocarbons obtained with both methods were about the same although the variations were higher if applying the distillation method. However, the time needed for one sample using Florisil chromatography is only a fourth and the sample size needed is much smaller. Being familiar with the method, about 10 Florisil columns may be handled simultaneously whereas a parallel run of several high vacuum distillation apparatus is quite difficult to perform. The higher performance capacity of the Florisil method meets with another important aspect: the Florisil separation technique has already been used in food control laboratories for pesticide residue analysis which makes an establishment of the method for the detection of irradiated food easier to achieve.

A closer examination of the Florisil method revealed not only a higher standard deviation for 16:2 than that achieved for 14:1, 15:0 and 17:1 but in some irradiated samples, 16:2 could not be detected, although it was expected to be present in highest amounts. It could be shown that this was due to the high retention capacities of freshly activated Florisil. A partial deactivation by addition of 3% of water resulted in the expected amounts of hydrocarbons if compared to data published by Nawar *et al.* (1990). Obviously, the retention of hydrocarbons by freshly activated Florisil increases with the number of double bonds.

By an addition of 3% of water, a degree of activation is achieved which remains stable for 3 days whereas the degree of activation decreases constantly after heating if no water is added. This is a further advantage of partial deactivation and in fact also the Florisil used for routine pesticide analysis is deactivated by 3% of water for this reason (Deutsche Forschungsgemeinschaft, 1991).

Prior to deactivation, Florisil has to be heated to 550°C. This is an important step not only for activation but also for decontamination since commercial Florisil may be contaminated with long-chain hydrocarbons. Also high quality solvents might be

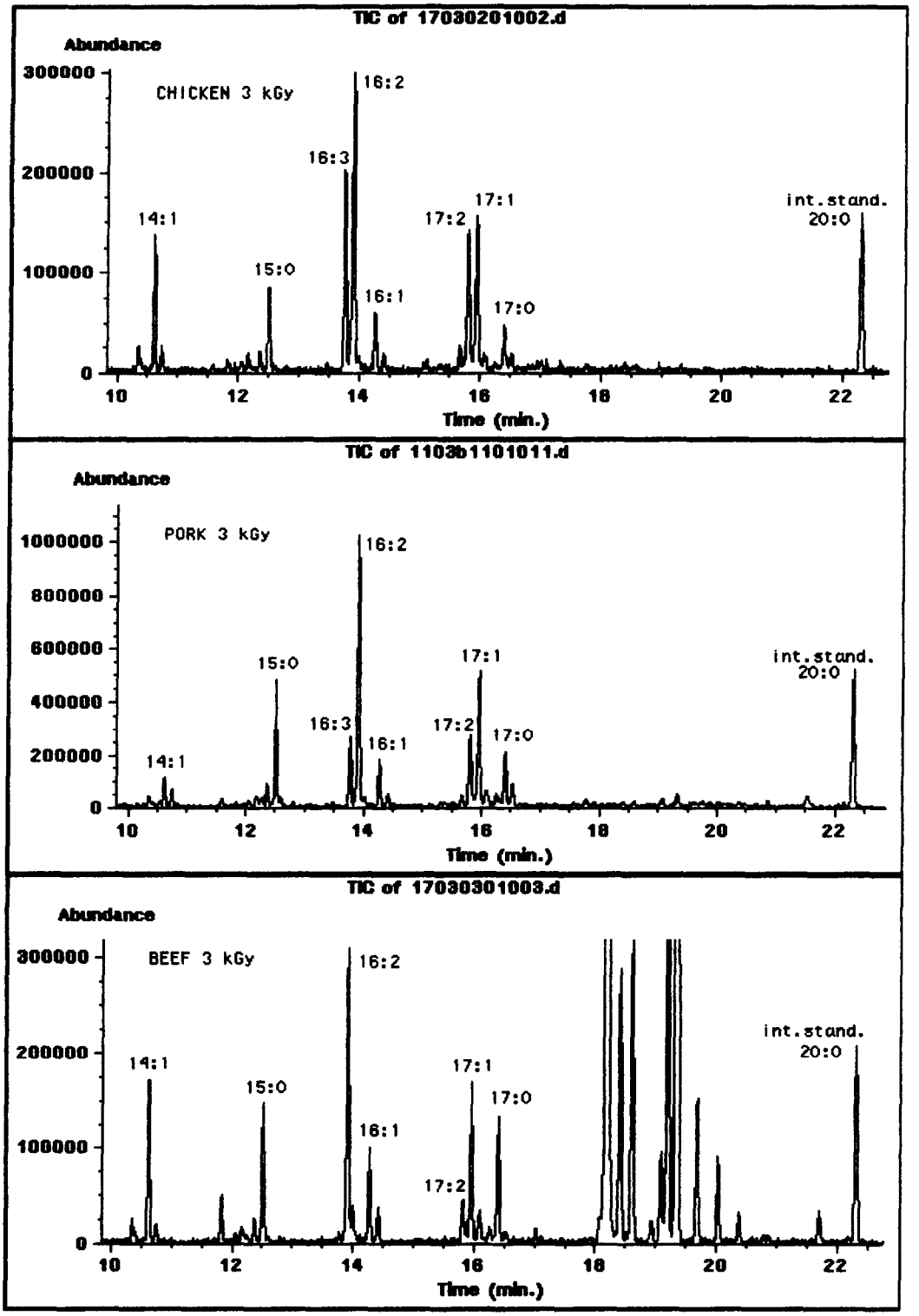

Fig. 7. Gas chromatographic analysis of the volatile hydrocarbons from chicken, pork and beef irradiated to 3 kGy.

contaminated with hydrocarbons of interest as it is normally the case with Soxhlet thimbles and filter papers. Due to this contamination possibility, special care must be taken if fat is recovered by Soxhlet or reflux extraction. Separating the fat by melting gives the lowest raise to contamination and is therefore recommended for samples with a high fat content like fatty pork meat, chicken skin or beef samples

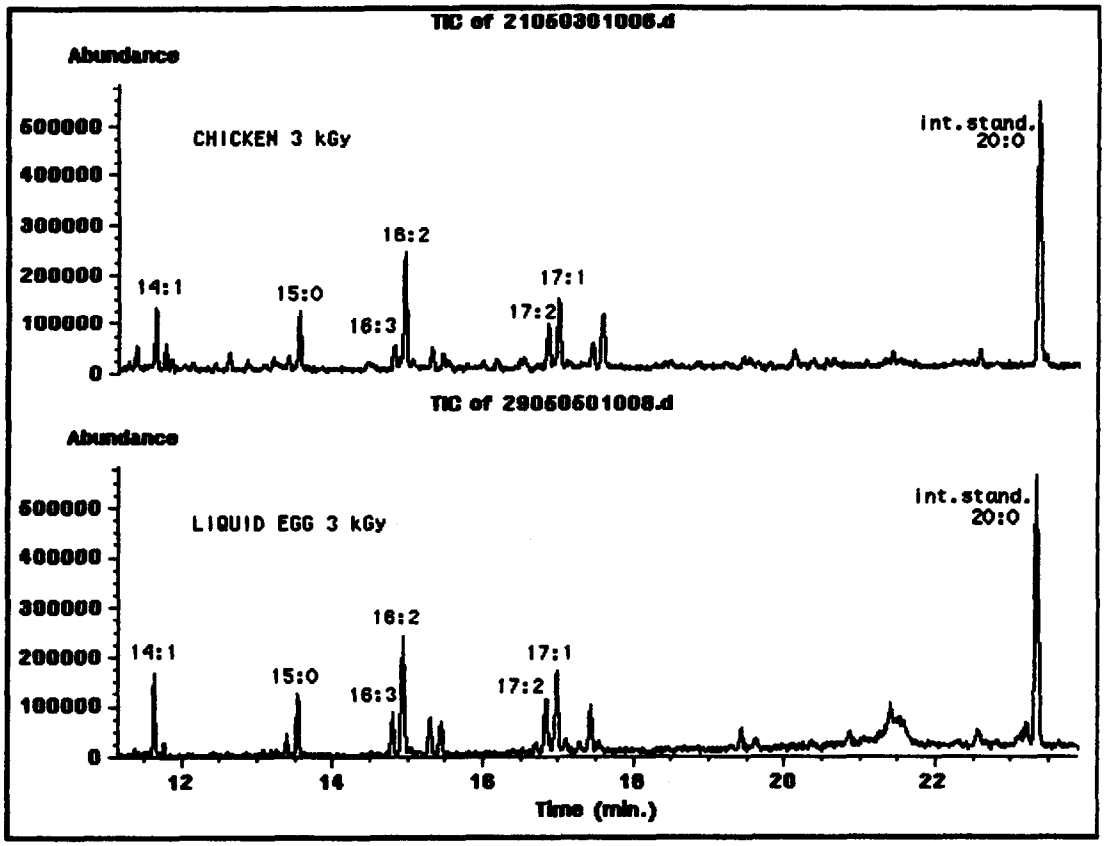

Fig. 8. Gas chromatographic analysis of the volatile hydrocarbons from chicken (top) and liquid egg (bottom) irradiated to 3 kGy.

containing marrow. For samples with low fat content, solvent extraction might be necessary. Among the solvent extraction methods examined, no contamination was observed if cold extraction with pentane/isopropanol had been performed. However, a total evaporation of isopropanol out of the lipid extract is not achievable.

For GC analysis, a non-polar capillary column is preferred because hydrocarbons elute in the order of their boiling points and in groups of the same chain length which facilitates the identification of the compounds, especially the highly unsaturated ones. These are often not commercially available and can only be identified by a comparison of the retention times with expected values and by their mass spectra.

Since the yields of radiation-induced hydrocarbons increase with dose in a linear mode, there should be the possibility of dose quantification. However, as shown by Nawar (1973) and confirmed in this study, the absolute amounts are largely dependent on irradiation temperature, especially in the range of $-20°C$ to $+20°C$. This temperature range is usually applied in commercial practice and as a consequence it will be difficult to estimate the dose of an unknown sample.

Nevertheless, the method is well suited to prove unequivocally an irradiation treatment for doses higher than 0.5 kGy. This is due to the fact that the judgement can rely on a pattern of substances instead of a single marker. A sample can be identified as having been treated by ionizing radiation if all expected hydrocarbons are detectable. This means that the presence of only one or two hydrocarbons is not sufficient if more are expected since they might be contaminants. Especially saturated hydrocarbons often occur as contaminants and should only be used for a positive identification in combination with the presence of unsaturated ones. There is an overall presence of saturated hydrocarbons in nature whereas unsaturated ones with at least one double bond in the middle of the chain (1,7 – 16:2; 6,9 – 17:2; 1,7,10 – 16:3; 8 – 17:1) are not naturally occurring substances.

The suitability of the method described for routine control was recently shown in a German intercomparison between food control laboratories involving chicken, pork and beef samples 3 and 6 months after irradiation. Ninety-eight percent of a total of 864 samples were correctly identified as non-irradiated or irradiated by 17 participating laboratories (Schreiber *et al.*, 1993b, c). The dose levels used were 0.5–0.9, 2.5–3.0 and 6.7–7.5 kGy. Due to this success, many German food control laboratories are beginning now to include this new field in their activities.

The isolation of hydrocarbons by Florisil column chromatography may be applied to various other fat-containing foods such as seafood and certain types of fruit. Examinations were performed on halibut, salmon and herring as well as avocados, mangoes and papayas in our laboratory and will be subject of another paper (Schulzki *et al.*, in preparation). Another method uses the detection of 2-alkyl-cyclobutanones for identification of irradiated fatty foods (Stevenson and Crone, 1990; Stevenson *et al.*, 1993). A combination of the identification of both hydrocarbons and 2-alkyl-cyclobutanones on one Florisil column should facilitate identification in routinely performed analyses; it is therefore under investigation at present.

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