Detection of Radiation-Induced Hydrocarbons in Camembert Irradiated before and after the Maturing Process—Comparison of Florisil Column Chromatography and On-Line Coupled Liquid Chromatography–Gas Chromatography

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The influence of the maturing process on the detection of radiation-induced volatile hydrocarbons in the fat of Camembert has been investigated. Two analytical methods for separation of the hydrocarbon fraction from the lipid were applied: Florisil column chromatography with subsequent gas chromatographic–mass spectrometric (GC–MS) determination as well as on-line coupled liquid chromatography–GC–MS. The maturing process had no influence on the detection of radiation-induced volatiles. Comparable results were achieved with both analytical methods. However, preference is given to the more effective on-line coupled LC–GC method.

Keywords: *Food irradiation; cheese; volatile hydrocarbons; GC; on-line coupled LC–GC*

INTRODUCTION

Cheese produced from raw, unpasteurized milk may be infected with the bacterium *Listeria monocytogenes*. The surface of cream cheese such as Camembert with its fungal growth provides an especially good substratum for multiplication of this bacterium, which may cause the infectious disease listeriosis. In 1993, the application of radiation technology was permitted, in France, to reduce the number of *Listeria* in raw milk. During maturation, irradiation is performed with a dose range of 2.25–3.5 kGy. After irradiation, maturing is continued for some further days.

For the detection of food treated with ionizing radiation, various methods have been developed covering a broad spectrum of food (Schreiber et al., 1993a). One of these methods is based on the identification of hydrocarbons formed in the lipid fraction of food containing fat (Nawar, 1972, 1986). If triglycerides are irradiated, preferential cleavage occurs in the α- or β-position to the carbonyl groups and, as a result, two main hydrocarbons are formed from a certain fatty acid. One hydrocarbon has one carbon atom less than the parent fatty acid (C_{n-1}) and the other hydrocarbon has two carbon atoms less and an additional double bond in position 1 ($C_{n-2:1}$). Interlaboratory trials have established that this procedure can be applied as a routine method for the detection of irradiated chicken, pork, and beef (Meier and Stevenson, 1993; Schreiber et al., 1993b, 1994). It has been successfully applied to other foods such as frog legs (Morehouse et al., 1991), shrimp (Morehouse and Ku, 1992), liquid egg (Spiegelberg et al., 1994), and exotic fruit (Spiegelberg et al., 1993). For the products examined, it could be established that the unsaturated hydrocarbons with internal double bonds cannot be detected in nonirradiated samples and provide a clear proof of irradiation treatment. However, the saturated hydrocarbons may already be present in the raw material. In many cases, the food is contaminated during production and processing or from packaging material. For Camembert it had to be clarified if hydrocarbons typical of radiation treatment might form by the action of microorganisms added for maturation or if changes in the lipids and proteins which occur on a large scale in the course of the maturing process could influence the detectability of hydrocarbons.

In the present study, possible differences in the formation and detection of radiation-induced hydrocarbons in Camembert irradiated before and after maturing at different doses have been examined. Furthermore, two methods for isolation of the hydrocarbons from the lipid matrix were compared. One of them, the isolation by Florisil column chromatography, has been included in the *Collection of Official Methods according to Article 35 of the German Foods Act LMBG* (LMBG Method L 06.00-27, 1994) and described in detail by Spiegelberg et al. (1994). The other method uses the technique of on-line coupled liquid chromatography–gas chromatography (LC–GC) (Biedermann et al., 1989, 1992; Grob, 1991). With the latter technique the lipid extracts are injected into a normal phase LC column to isolate the hydrocarbon fraction. This fraction is transferred directly to the gas chromatograph.

EXPERIMENTAL METHODS

Materials. Thirty freshly produced Camembert cheeses (45% fat in dry matter) of the same production batch of a dairy were divided into two parts; 15 cheeses were sealed singly in sterilized polyethylene bags and transported to the irradiation plant. The samples were irradiated with ^{60}Co γ rays at a dose rate of 17 Gy/min. The applied doses were 0.25, 0.5, 1.0, and 2.0 kGy (three samples per dose). Three samples served as nonirradiated controls. During transport and radiation treatment, the cheese was exposed to an average temperature of 15 °C for about 4 h. After that, the samples were unpacked and left in the maturing chamber together with the nonirradiated ones. The maturing process took 7 days at 18 °C. The second half of the cheese samples was irradiated after the maturing period under the same conditions as described. The samples were kept for 3 days at 6 °C until lipid extraction.

To examine the variety in fatty acid composition, different Camembert products were purchased from local suppliers.

Extraction of Fat from Cheese. Sixty grams of the homogenized sample was mixed thoroughly with 40 g of

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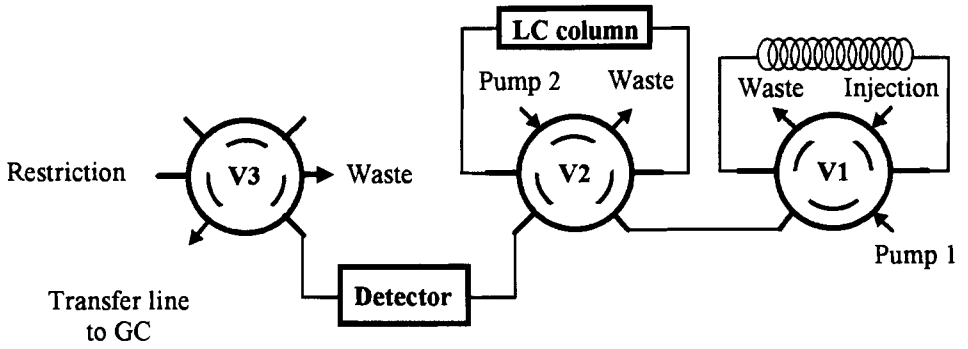

Figure 1. Arrangement of the LC system.

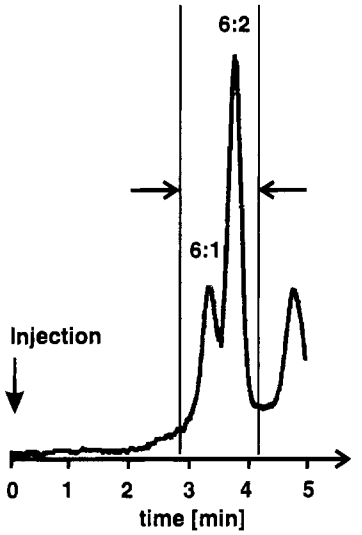

Figure 2. LC–UV chromatogram of a Camembert sample marked by the standards 1-hexene (6:1) and 1,5-hexadiene (6:2). The fraction transferred including alkanes, alkenes, and alkadienes is indicated by the bars.

anhydrous Na_2SO_4 (previously heated at 550 °C for 5 h) in a beaker. The fat was extracted by blending the sample with 100 mL of *n*-hexane for about 2 min. After centrifugation of this mixture, the clear supernatant was collected and the solvent removed by rotary evaporation at 40 °C under vacuum. The fat was stored at 6 °C after complete removal of the solvent by introduction of nitrogen gas.

Fatty Acid Analysis of Cheese. The fatty acid profile was determined according to a modification of DGF Method C-VI-11a (1981). Twenty to thirty milligrams of fat was saponified with 3 mL of 0.5 M methanolic NaOH and methylated with 3 ml of BF_3–methanol under reflux. The fatty acid methyl esters (FAMES) were extracted with 10 mL of *n*-heptane. After mixing with anhydrous Na_2SO_4, the extract was kept at 6 °C until GC analysis.

FAMES were analyzed gas chromatographically using a DB-FFAP column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, CA). The samples were injected in split mode at an injector temperature of 250 °C. The oven was programmed from 140 to 250 °C at a rate of 6 °C/min.

Separation of Hydrocarbons by Florisil Column Chromatography. The separation of hydrocarbons from the fat by Florisil column chromatography was carried out as described elsewhere (Spiegelberg et al., 1994), using 3% deactivated Florisil (Promochem GmbH, Wesel, Germany). The applied sample amount was 1 g of lipid mixed with 1 μg of the internal standard, *n*-eicosane in *n*-hexane.

Separation of Hydrocarbons by On-Line Coupled LC–GC. The principle of coupling LC to GC has been described in detail by Grob (1991). The arrangement of the LC system applied in the present study is demonstrated in Figure 1. It consists of two electrically actuated two-position valves (EC6W, Valco Instruments Co. Inc., Houston, TX) used as injection valve (V1) and as switching valve (V2) for selection of the appropriate eluent mode (normal or backflush through the LC column). A 50 mm × 4 mm i.d. cartridge packed with LiChrospher Si 60, 5 μm (E. Merck, Darmstadt, Germany), is used for separation of the entire hydrocarbon fraction from the lipid. Ten microliters of a 20% (w/v) lipid solution containing 100 μg/mL each 1-hexene and 1,5-hexadiene (Fluka Chemie AG, Buchs, Switzerland) as internal standards for LC and 0.1 μg/mL *n*-eicosane as internal standard for GC is injected into the LC column. The hydrocarbon fraction is eluted with *n*-hexane (GR, E. Merck) at a flow rate of 200 μL/min. The eluents are delivered by the syringe pump system Phoenix 20 (Fisons Scientific, Leicestershire, U.K.). For monitoring the elution of the hydrocarbon fraction marked by the standards 6:1 and 6:2, the UV–Vis spectrophotometric detector LC 55 (Perkin-Elmer & Co. GmbH, Überlingen, Germany) is set to 200 nm. A typical LC chromatogram of a

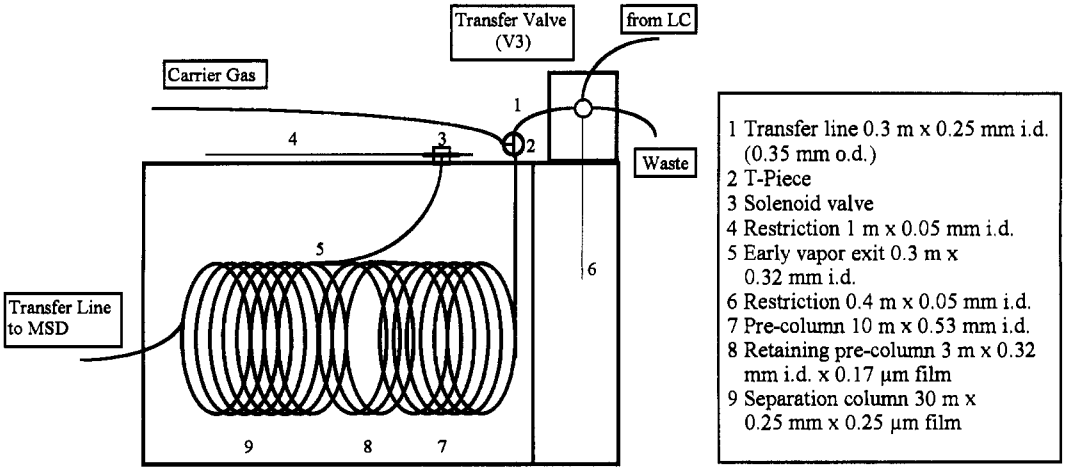

Figure 3. On-line coupling of LC and GC: interface and capillary column system.

Table 1. Timetable of a LC–GC Run

time (min:s)	event	remarks
0:00	start pump A	LC column conditioned with *n*-hexane, V2 in normal flow mode, V1 in load position, V3 in standby mode (see Figure 1)
12:00	V1 inject	sample injection
14:50	V3 transfer	start transfer to GC, start GC run
	solenoid valve ON	vapor exit opened
16:00	start pump B	MTBE flow started
16:10	V3 standby	end transfer to GC, LC column flushed in reversed mode with MTBE
	V2 backflush	
16:50	solenoid valve OFF	vapor exit closed
17:00	stop pump A	hexane flow stopped
35:00	stop pump B	pump and valve programs finished, pumps refilled, valves switched to their initial position
	V1 load	
	V2 normal flow	

Table 2. Main Fatty Acids of the Examined Camembert and Their Radiation-Induced Hydrocarbons

		radiolytic hydrocarbons	
fatty acid	content (%)	C_{n-1}	$C_{n-2:1}$
myristic acid (14:0)	12	13:0	1-12:1
palmitic acid (16:0)	37	15:0	1-14:1
stearic acid (18:0)	13	17:0	1-16:1
oleic acid (18:1 ω9)	23	8-17:1	1,7-16:2

Camembert sample is shown in Figure 2. After leaving the detector, the LC flow passes a third electrically actuated two-position valve (EC6W, Valco). This valve (V3) controls the transfer from the LC system to the GC. When V3 is switched to transfer mode, the eluent flows through a transfer line (30 cm × 0.25 mm i.d. fused silica tube with 0.35 mm o.d.) which is connected "on-column" to a 10 m × 0.53 mm i.d. fused silica precolumn situated in the GC oven. Since the GC was not equipped with an on-column facility, a T-piece (Valco) was installed for introducing the carrier gas into the precolumn (Figure 3).

Within the precolumn, partially concurrent evaporation of the transferred LC fraction takes place. The uncoated precolumn is connected to a retaining precolumn (3 m × 0.32 mm × 0.17 μm film of 95% methyl, 5% phenyl polysiloxane), which is linked by a Y-connector with the early vapor exit and the separation column. GC–MS conditions are described below.

To remove the lipid from the LC column, V2 is switched immediately after the LC–GC transfer has finished and the column is backflushed in reversed mode with *tert*-butyl methyl ether (MTBE, LiChrosolv, E. Merck).

The two-position valves are switched automatically by the programmed serial valve interface (SVI, Valco). The syringe pump system runs separately through programmed cycles. The timetable of the procedure is given in Table 1.

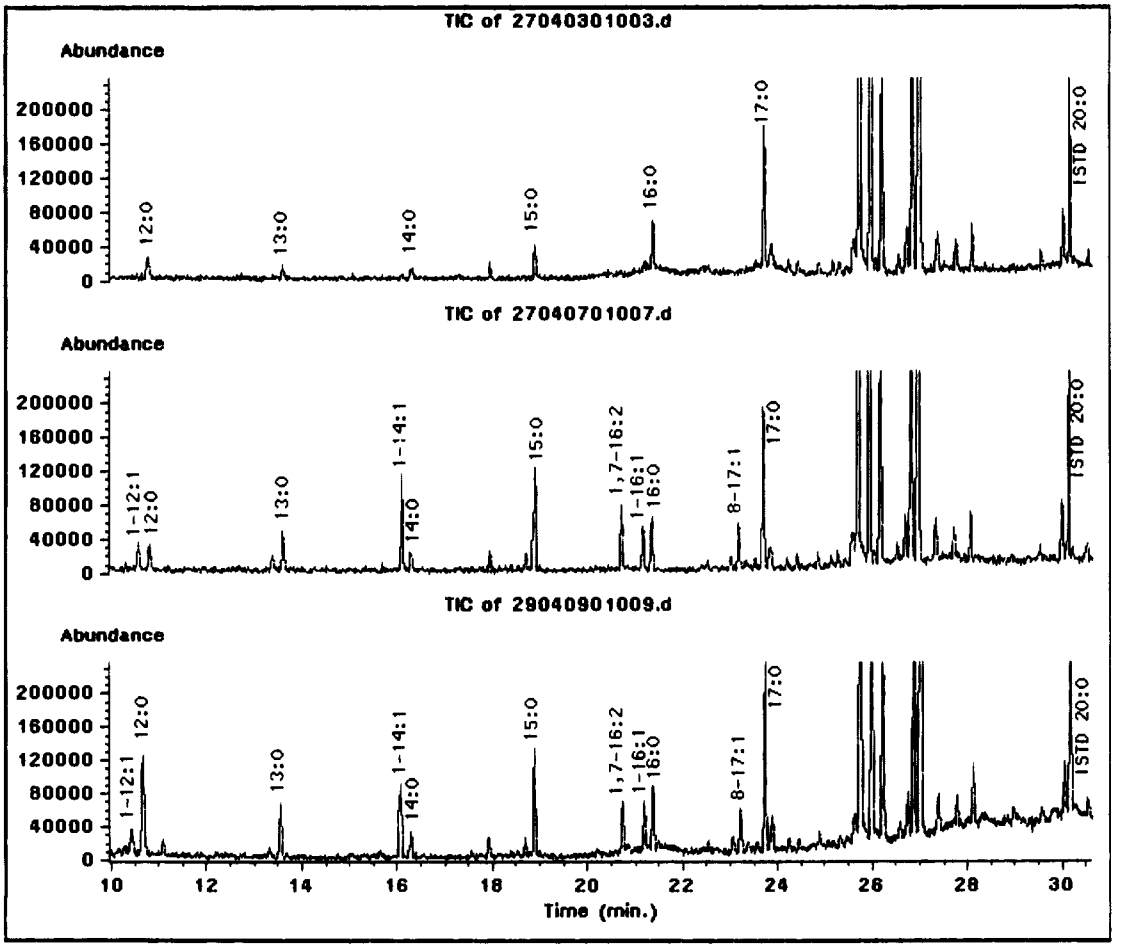

Figure 4. Gas chromatograms of the hydrocarbon fraction of Camembert analyzed by on-line coupled LC–GC–MS. The cheese was treated before (top, center) and after (bottom) maturing. (Top) Control; (center and bottom) irradiated with 500 Gy.

GC–MS of Hydrocarbons. Analyses were performed on a HP GC–MS system 5890-5970 B (HP, Hewlett-Packard Co., Avondale, PA), using helium as carrier gas.

For *off-line determination* of the hydrocarbons after Florisil column chromatography, the autosampler (HP 7673) injects 1 μL of the sample solution. The injector runs in splitless mode at a temperature of 200 °C. The radiolytic hydrocarbons are separated on a 30 m × 0.2 mm i.d. × 0.33 μm fused silica column (Ultra II HP). After an initial period of 2 min at 55 °C, the oven temperature is increased at a rate of 12 °C/min to 155 °C followed by a second ramp at 5 °C/min to 230 °C.

For *on-line coupled LC–GC*, a 30 m × 0.25 mm × 0.25 μm DB 5 capillary column (J&W) is used for separation. When the transfer valve (V3) is switched to transfer mode, the GC run is started and the early vapor exit is opened. Opening and closure time of the solenoid valve of the vapor exit are controlled by the GC (purge on/off time). After a transfer period of 80 s (transfer volume 267 μL) at an evaporation rate of 158 μL/min, the vapor exit is closed after 2 min (20 s is the dead time between transfer valve and vapor exit). The oven temperature during transfer of 78 °C is held for 3 min and increased subsequently to 240 °C, at a rate of 5 °C/min.

The transfer line to the MSD has a temperature of 270 °C. Ion masses are scanned from 50 to 300 amu. Standard substances are available to identify all predicted radiation-induced hydrocarbons. The saturated hydrocarbons and the 1-unsaturated ones were purchased from Sigma Chemical Co., St. Louis, MO. 8-Heptadecene and 1,7-hexadecadiene are available from TeLa (Technische Lebensmittel- und Umweltanalytik GmbH) Berlin, Germany. For quantification of the hydrocarbons, response factors according to the internal standard *n*-eicosane (20:0) are determined separately for both methods.

RESULTS

The fatty acid profiles of different types of Camembert were determined in independent analyses. No significant differences in the amounts of the main fatty acids could be established, and the data were in good correspondence with literature data for milk and Camembert (Souci et al., 1991). Table 2 presents the main fatty acids in the lipid fraction of the Camembert samples examined and their main radiolytic hydrocarbons.

When both analytical methods, Florisil column chromatography as well as on-line LC–GC, were applied, all predicted hydrocarbons were detected in irradiated Camembert samples. Besides other saturated ones, the saturated hydrocarbons tridecane (13:0), pentadecane (15:0), and heptadecane (17:0) were present in small amounts in all control samples (Figure 4). The unsaturated hydrocarbons 1-dodecene (1-12:1), 1-tetradecene (1-14:1), 1-hexadecene (1-16:1), 1,7-hexadecadiene (1,7-16:2), and 8-heptadecene (8-17:1) could only be identified in irradiated cheese. These hydrocarbons were detectable at and above doses of 250 Gy, and their amounts increased linearly with dose in the examined range (0.25–2 kGy, Figure 5). When both analytical methods are compared, a good correspondence of the hydrocarbon amounts can be stated (Figure 5). It should be noted that the response factors used for calculation of the hydrocarbon amounts differed for both methods because a partial loss of the shorter-chained hydrocarbons (C_{12}–C_{15}) during evaporation of the eluent through the vapor exit was observed with the LC–GC method.

No differences in the hydrocarbon pattern could be detected by comparing the chromatograms of samples irradiated before and those irradiated after maturing (Figure 4). The amounts of radiation-induced hydrocarbons per dose were nearly the same (Figure 5).

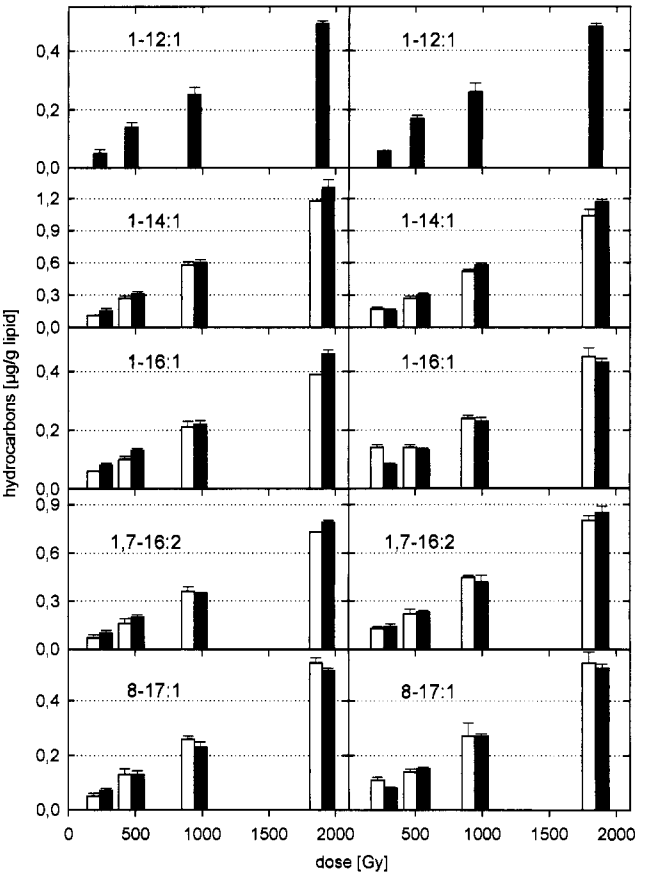

Figure 5. Comparison of hydrocarbon amounts obtained by both analytical methods, Florisil column chromatography and on-line LC–GC, for the two sample sets irradiated before (left) and after (right) maturing at different doses. Because of disturbances in the gas chromatograms of some samples, the 1-12:1 hydrocarbon was not calculated for the Florisil method. None of the unsaturated hydrocarbons was found in nonirradiated samples.

DISCUSSION

In a former study, irradiation treatment of the already matured cheese could be detected successfully (Spiegelberg et al., 1993). However, in practice, irradiation is carried out in the course of the maturing process and maturing is continued subsequently. Therefore, it was necessary to examine possible effects of the maturing process on the detection of the hydrocarbons. This study was performed on Camembert irradiated before and after maturing. According to the results, it can be stated that there is no dependence of hydrocarbon detection and therefore no dependence of hydrocarbon formation on the time of irradiation treatment. Also, it can be concluded that changes in the sample matrix during maturation do not influence the extraction of the hydrocarbons. It can be ruled out that the saturated hydrocarbons found in all controls are a product of the maturing process, for example as a result of microbial decarboxylation of the lipids, resulting in the formation of the C_{n-1} hydrocarbons. If microbial decarboxylation had taken place to a detectable extent, an unsaturated hydrocarbon from oleic acid (8-17:1) should have been found. Other authors who investigated the detection of the hydrocarbons 1-12:1, 13:0, 1-14:1 and 1-16:1 (1,7-16:2 and 8-17:1 were not available as standards) in Camembert over an extended storage period found that these hydrocarbons never appeared in the control samples (Bergaentzle et al., 1994). For the identification of irradiated Camembert cheese, the unsaturated hydrocarbons proved to be reliable markers.

The Florisil method can be used for the detection of irradiated Camembert already at 250 Gy. Thus, the detection limit is far below the permitted dose range of 2.25–3.5 kGy. Using the on-line LC–GC method, it is possible to detect doses even lower than 250 Gy because the lipid amount used for one analysis may be increased by up to 5 times. With both analytical methods, comparable hydrocarbon amounts per dose were found. However, on-line coupled LC–GC is not only equivalent to but in fact superior to the approved Florisil method. There are some advantages that should give preference to this method in the future. If an HPLC autosampler is available, the analysis may be performed in fully automated mode after the lipid extraction. The sample amount and the consumption of chemicals are drastically reduced. In the present study, only 200 mg of lipid was used to prepare a sample solution of 1 mL, which could be injected at least 30 times. For one LC run, approximately 3.5 mL of *n*-hexane and 4 mL of MTBE are consumed, whereas approximately 100 mL of *n*-hexane is needed for one Florisil column. The evaluation of an unknown sample can be carried out rapidly since preparative work such as the heating and deactivation of Florisil is no longer needed.

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