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Aromatic Hydrocarbons of Mineral Oil Origin in Foods: Method for Determining the Total Concentration and First Results

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An online normal phase high-performance liquid chromatography (HPLC)−gas chromatography (GC)−flame ionization detection (FID) method was developed for the determination of the total concentration of the aromatic hydrocarbons of mineral oil origin with up to at least five rings in edible oils and other foods. For some samples, the olefins in the food matrix were epoxidized to increase their polarity and remove them from the fraction of the aromatic hydrocarbons. This reaction was carefully optimized, because also some aromatics tend to react. To reach a detection limit of around 1 mg kg^{-1} in edible oils, an off-line enrichment was introduced. Some foods contained elevated concentrations of white paraffin oils (free of aromatics), but the majority of the mineral oils detected in foods were of technical grade with 20−30% aromatic hydrocarbons. Many foods contained mineral aromatic hydrocarbons in excess of 1 mg kg^{-1}.

KEYWORDS: Mineral oil aromatic hydrocarbons; food contamination by mineral oil; epoxidation of olefins; online HPLC-GC-FID

INTRODUCTION

Food Contamination by Mineral Oil. Many foods are contaminated with mineral oil products, and there are numerous sources. Mineral batching oil used for spinning jute or sisal fibers contaminated foods packed in corresponding bags (*1*, *2*). Also, other packaging materials release mineral oil products (*3*−*7*). Lubricating oils and release agents used in the food industry often consisted of mineral oil (*8*, *9*). Eggs and meat were contaminated with mineral oil from feeds containing fat from waste collection sites that inadequately separated spent fats and liquid wastes such as used motor oil (*10*). Mineral oil was most probably also added to edible oils as a fraud (*11*). There is an environmental background contamination from soot containing incompletely burned fuel and diesel oil as well as lubricating oil from vehicles without catalysts, primarily diesel engines (*12*, *13*). In numerous other cases, the source of the contamination still awaits investigation (*14*−*16*). The evidence that the hydrocarbons are of mineral origin was discussed in ref *17*, the presence of hopanes, triterpenoid hydrocarbons formed under geological conditions, being a key argument.

The use of certain mineral oil products for food production is tolerated, for example, by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (*18*). Apart from a required high viscosity, principally referring to a high molecular mass, these oils must be "food grade" or "white", which both primarily mean the absence of aromatics.

Human Exposure. The mean dietary exposure to mineral paraffins in the United States was estimated as 0.875 mg kg^{-1} of body weight (bw) (*19*), half of which was from oils used as release agents for baking ware, for dedusting of grain, in confectionaries, and for coatings of fruits and vegetables. In Europe, these practices were never authorized or their use was stopped. Tennant (*20*) estimated the mean exposure in Europe as 0.39 mg kg^{-1} of bw for adults and 0.75 mg kg^{-1} of bw for children. This would correspond to an average mineral oil concentration in foods of around 25 mg kg^{-1}. Unpublished surveillance data by the Official Food Control of Zurich, Switzerland, might have agreed with this in the early 1990s, but since the major uses were stopped, exposure has probably decreased substantially below this level.

Extrapolated from abdominal fat sampled during Caesarean sections of Austrian women, the total amount of mineral hydrocarbons in the human body is on the order of 1 g (*21*). Concentrations in human fat varied between 15 and 360 mg kg^{-1}, with an average of 60.7 mg kg^{-1}. The relative importance of the various potential sources (such as food, cosmetics, pharmaceuticals) is still open. Concentrations in human milk fat were similar to that in the body fat at the beginning of breast feeding.

Mineral oil principally consists of saturated hydrocarbons (paraffins, i.e., straight chain or branched alkanes, and naphthenes, cyclic saturates) and aromatic hydrocarbons. Paraffinic and naphthenic oils are distinguished depending on the predominant class of saturated hydrocarbons (see, e.g., ref *22*). The relevance of this distinction regarding food safety is not clear. For instance, the European Union Scientific Committee for Food (SCF) specified a temporary acceptable daily intake (ADI) for a "white paraffinic mineral oil" without reference to the naphthenics. We use the more general term "mineral oil saturated hydrocarbons" (MOSH) and in this way also distinguish them from the hydrocarbons of plant origin.

The mineral oil aromatic hydrocarbons (MOAH) differ from the widely analyzed polycyclic aromatic hydrocarbons (PAH) formed by pyrolysis at elevated temperature and present in, for example, roasted or smoked food: PAH consist of a limited number of largely nonalkylated ring systems (*23*), whereas the

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MOAH are alkylated to 97−99% and consist of an enormous number of components (*24*).

In the past, mineral oil analysis in foods referred to the MOSH. However, not all mineral oils contaminating foods are white, and the MOAH might well be more of concern than the MOSH. For instance, the batching oil used for jute and sisal fibers is a brownish liquid containing roughly 25% aromatics (*24*). Also, environmental contamination is not limited to MOSH, and the recent contamination of Ukrainian sunflower oil occurred with an oil containing roughly a quarter of aromatic hydrocarbons (*11*). The neglect of the MOAH is primarily due to the lack of a method for their analysis.

Method of Analysis. MOSH are determined by flame ionization detection (FID), as it provides virtually equal response per unit of mass for all hydrocarbons. Gas chromatography (GC) is the separation technique of choice, because it enables the characterization of the MOSH and the separation of these from the hydrocarbons naturally present in foods. However, it is not selective: the MOSH produce a broad hump of unresolved components, and the selectivity to rule out interference by other humps which could be mistaken as MOSH must be from the preseparation.

Online high-performance liquid chromatography (HPLC)−GC provides preseparation at high resolution, enables fully automated analysis of crude food extracts or diluted edible oils, and largely rules out sample contamination during the analytical process (*25*, *26*). Recent progress in MOSH analysis involved activated aluminum oxide for the removal of long-chain *n*-alkanes, in particular those of plant origin often hindering MOSH analysis (*27*). Enrichment on a larger double-bed column containing activated silica gel for retaining lipids and activated aluminum oxide for the retention of long chain *n*-alkanes lowered the detection limit to below 1 mg kg^{-1} (*15*).

PAH can be determined individually, as shown by many methods described in the literature (*23*). This is not possible for the MOAH: they form broad humps of unresolved components and must be analyzed as a sum of all components or of groups, for example, by ring number. Again, there is no selective detector with a predictable response: compounds of differing alkylation do not have the same UV or fluorescence spectra, which means that a group-type calibration in HPLC is impossible. In mass spectrometry (MS), there are no fragments common to groups of components and with an intensity enabling a group-type calibration (*28*). The only system providing virtually the same response for all aromatic hydrocarbons is FID.

Moret et al. (*29*, *30*) developed an online method to measure MOAH in foods and characterize their composition by ring number. The hydrocarbons were isolated from an edible oil or food extract by a large HPLC silica gel column. The eluent of this fraction was evaporated in a miniaturized online solvent evaporator, the MOAH of at least two rings separated by number of rings on an amino HPLC column and analyzed online by GC-FID. This revealed the presence of MOAH on the order of 10 mg kg^{-1} in various foods. For some foods, batching oil on jute bags was the origin, but for others, the sources were not identified.

Scope of this Work. In this paper, a simpler online normal phase HPLC-GC-FID method for determining the total concentration of the MOAH in foods is described, suitable for routine characterization of mineral oil products in foods. Two auxiliary tools were needed for certain samples: epoxidation for the removal of natural olefins when present in disturbing amounts and off-line enrichment to reach a detection limit of about 1 mg kg^{-1} for edible oils or fatty food extracts. Examples illustrate the results. The characterization of the MOAH by ring number can be achieved by comprehensive two-dimensional GC (GC×GC-FID) added to this method (*31*).

MATERIALS AND METHODS

Samples. Food samples were from a local retail market.

Chemicals and Solutions. Hexane from Brenntag (Schweizerhall AG, Basel Switzerland) was redistilled after purification through an aluminum oxide column (2 kg of aluminum oxide for 10 L of solvent). Dichloromethane was from J. T. Baker (Deventer, The Netherlands). Silica gel 60, 0.063−0.200 mm, sodium carbonate, mineral paraffin oil "Paraffin viscous PH Eur, BP, USP", and biphenyl (BP) were from Merck (Darmstadt, Germany). 1,1,2-Trichloroethane, *n*-dodecane (C_{12}), *n*-tetradecane (C_{14}), *n*-hexadecane (C_{16}), hexylbenzene (6B), nonylbenzene (9B), perylene (Per), 1,3,5-tri-*tert*-butylbenzene (TBB), 5-α-cholestane (Cho), 3-chloroperbenzoic acid (mCPBA, ca. 70%), and PS-255 (a dimethylpolysiloxane) were from Fluka/Sigma-Aldrich (Buchs, Switzerland). A sunflower oil containing little mineral oil was selected among edible oils from the market.

The reagent for epoxidation consisted of 10% mCPBA in dichloromethane (slightly turbid; stored at ambient temperature for up to a week). The stock solutions of internal standards contained 100 mg of the components in 10 mL of 1,1,2-trichloroethane: mixture 1, 6B, 9B, and BP; mixture 2, C_{12}, C_{14}, C_{16}; mixture 3, Per and Cho. The internal standard solution contained 100 μL of mixture 1, 300 μL of mixture 2, and 500 μL of mixture 3 in 10 mL of 1,1,2-trichloroethane.

Mixtures of hexylbenzenes and of octadecylbenzenes were obtained by Friedel Kraft reaction of benzene with the corresponding alkyl chlorides: to 5 mmol of benzene dried over sodium sulfate and 0.1 mmol of aluminum chloride cooled in an ice bath was added 5 mmol of 1-chloroalkanes in portions; the onset of the reaction was monitored by hydrogen chloride leaving the condenser. The product was picked up in hexane and washed with water.

Sample Preparation. Of edible oil and fats, 2 g was weighed in and filled to 10 mL with hexane together with 20 μL of internal standard solution. To 20 g of rice, milled noodles, and similar products were added 30 mL of hexane and 20 μL of standard solution, and the mixtures were allowed to stand overnight. Ten milliliters of extract was brought to 1 mL by a rotary evaporator. Turbid solutions were centrifuged. Five grams of chocolate was dissolved in 10 mL of hexane to which 25 μL of standard solution was added.

Epoxidation. In a 15 mL centrifuge tube with a screw cap, 1.5 mL of the above sample solution was brought to dryness by a stream of nitrogen. Of fats and oils, 300 mg was directly weighed in, and 3 μL of internal standard solution was added. For samples containing little fat, 100 mg of sunflower oil containing little mineral oil was admixed before solvent evaporation. Dichloromethane (3 mL) was added and cooled in ice water and then 1 mL of cooled mCPBA solution. The mixture was shaken and allowed to warm to ambient temperature after 5 min. After 15 min, 3 mL of 10% sodium carbonate was added, the solution was intensively shaken for 15 s and then centrifuged. The aqueous supernatant was discarded, and the sample was extracted with 3 mL of water and centrifuged; 1.5 mL of the dichloromethane phase was transferred to an autosampler vial and brought to dryness by a stream of nitrogen. The residue was redissolved in 1 mL of hexane.

Enrichment. Twelve grams of silica gel was packed into a 2 cm i.d. column and conditioned with 40 mL of 20% dichloromethane/hexane. Then 1 g of edible oil or fat in 2 mL of hexane and 10 μL of internal standard solution were added. The first 10 mL of eluate (20% dichloromethane/hexane) was discarded. The subsequent 35 mL was collected in a round flask that contained 40 mg of the clean sunflower oil. The solvent was evaporated using a rotary evaporator and the residue transferred to an autosampler vial with 200 μL of hexane.

If epoxidation was needed, the residue was transferred to a 15 mL centrifuge tube with 1 mL of dichloromethane, and 150 μL of mCPBA solution was added. After the reaction, the solution was washed with 1 mL of 10% sodium carbonate solution and water as described above. The dichloromethane was evaporated and the residue recovered in 200 μL of hexane.

HPLC-GC-FID Analysis. The instrument for automated online HPLC-GC-FID was from Thermo Scientific (Milano, Italy) and consisted of a TriPlus autosampler with a 100 μL syringe, a Phoenix 40 dual syringe pump with three switching valves, a microUVIS 20 UV detector, and a Trace gas chromatograph equipped with an on-column injector, an early vapor exit, and a transfer switching valve.

Of the samples, 5−90 μL was injected onto a 25 cm × 2 mm i.d. HPLC column packed with Lichrospher Si 60, 5 μm (Grom, Rottenburg-Hailfingen, Germany), and chromatographed at 300 μL min^{-1} using a gradient starting with hexane (0.1 min) and reaching 30% dichloromethane after 2 min. The column was backflushed 6 min after the injection with dichloromethane at 500 μL min^{-1} for 9 min and then reconditioned at 500 μL min^{-1} with hexane for 10 min and at 300 μL min^{-1} up to the subsequent injection.

HPLC-GC transfer occurred by the retention gap technique and partially concurrent eluent evaporation (*25*), initially through the on-column and later through the Y-interface (*32*). A 10 m × 0.53 mm i.d. uncoated, deactivated precolumn was followed by a steel T-piece union connecting to the solvent vapor exit and a 15 m × 0.25 mm i.d. separation column coated in the laboratory with a 0.13 μm film of PS-255.

From HPLC, the saturated hydrocarbons were eluted from 2.0 to 3.5 min and transferred to GC at a carrier gas inlet pressure of 60 kPa (helium). The elution window was established by transferring small fractions to GC. The duration of solvent evaporation was determined by the flame method: the effluent from the vapor outlet was lit and the time to the disappearance of the yellow flame measured by means of a stop watch. The solvent vapor exit was switched to a strong restriction 2 s before the end of solvent evaporation, which was around 3.65 min (t = 0 at injection into HPLC). The carrier gas inlet pressure was increased to 150 kPa at the moment of closing of the vapor exit. The oven temperature was programmed at 20 °C min^{-1} from 65 °C (6 min) to 350 °C. The fraction comprising the MOAH ranged from 4.0 to 5.5 min and was transferred at 110 kPa inlet pressure, with an increase to 150 kPa upon closure of the vapor exit (5.7 min). The oven temperature was programmed at 20 °C min^{-1} from 50 °C (8 min) to 350 °C. The FID base block was heated at 370 °C. Sometimes also the intermediate fraction (3.5−4.0 min) was transferred to GC, using the conditions applied to the fraction of the saturated hydrocarbons.

GC×GC for Confirmation. Comprehensive two-dimensional GC (GC×GC) was used for confirming adequate separation of MOSH and MOAH and will be described in ref *31*. Briefly, HPLC fractions were collected from the waste exit of the HPLC-GC transfer valve and reconcentrated to 20 μL. Of this, 10 μL was injected on-column onto a system composed of a 1 m × 0.53 mm i.d. deactivated precolumn followed by a 20 m × 0.25 mm i.d. first-dimension separation column coated with a 0.12 μm film of PS-255 and a 1.5 m × 0.15 mm i.d. second-dimension column coated with a 0.075 μm film of a 50% phenylpolysiloxane. A cryogenic jet modulator was used (Thermo Scientific).

RESULTS AND DISCUSSION

Concept of MOSH/MOAH Separation. During the initial evaluation of potential approaches, comprehensive two-dimensional GC (GC×GC) was considered for the separation of paraffins, naphthenes, and various classes of MOAH (*33*−*38*). This was discarded, because naphthenes, including the steranes and the hopanes, cannot be fully separated from the MOAH by GC×GC (*34*,*39*,*40*) and there would still be the need for isolating the hydrocarbons from the food matrix. Preference was given to a normal phase HPLC-GC method to determine the sum of all aromatics, leaving the option of characterizing the MOAH by GC×GC as an add-on (*31*).

As outlined in the Introduction, MOSH and MOAH consist of extremely complex mixtures. GC-FID is the method of choice but just forms broad humps of unresolved components, reflecting the distillation process in the refinery. Because the MOSH and MOAH are from the same mineral oil fraction, they are of the same volatility range, and the selectivity must be obtained from the preseparation.

Separation of MOSH and MOAH should be complete to the extent that a white mineral oil containing < 1% MOAH can be recognized. Because the possibly small MOAH peak is eluted after the predominating MOSH, a small tailing of the MOSH into the MOAH fraction disturbs the determination. HPLC provides high resolution with minimal tailing. Silica gel was used, because it provides strong retention for aromatic hydrocarbons and a high capacity for retaining triglycerides (*41*). The 25 cm × 2 mm i.d. column used had a capacity to retain 20 mg of fat in such a way that enough packing was left to achieve the MOSH/MOAH separation.

The HPLC separation was optimized and verified by standards marking the edges of the fractions. The start of the MOSH fraction is at breakthrough, whereby the large molecular components are eluted earlier than the smaller ones owing to the size exclusion effect. Naphthenes were eluted last: 5-α-cholestane (Cho) was baseline-separated from *n*-alkanes of similar molecular mass. Cho was used as a marker for the rear edge of the MOSH fraction: it had to be present in the MOSH and absent in the intermediate fraction between the MOSH and the MOAH.

To find the least retained aromatic hydrocarbon, that is, the start of the MOAH fraction, the retentions of trihexylbenzene, dioctadecylbenzene, and 1,3,5-tri-*tert*-butylbenzene (TBB) were compared. TBB, eluted at a similar retention time as the longer chain derivatives, was chosen as the marker owing to its commercial availability: it had to be fully present in the MOAH fraction (area ratio to the internal standard, biphenyl, BP) and absent in the intermediate fraction. Perylene (Per; five fused aromatic rings) was selected as the most strongly retained compound to be included. The correct separation of MOSH and MOAH was checked by GC×GC with FID or MS: HPLC fractions were collected, reconcentrated, and injected on-column into the GC×GC system.

Lichrospher Si 60, a silica gel with strong retention power, provided abundant separation between the MOSH and MOAH, that is, between Cho and TBB. The distance between these was welcome for a robust separation and to minimize the tailing of the MOSH into the MOAH. Strong retention also relies on hexane being free of polar impurities; because it tends to get oxidized during storage (*42*) and these byproducts cannot be removed by distillation, it was purified through activated aluminum oxide prior to distillation.

Strong retention of the MOAH implied a steep gradient to prevent an excessively broad fraction. First experiments with methyl *tert*-butyl ether (MTBE) were not successful: hexane propagated MTBE too slowly through the column, that is, the gradient had a strong delay and did not focus the fraction as efficiently as desirable. Furthermore, it took too much hexane and time for the complete removal of the residual MTBE during reconditioning. Dichloromethane was better suited in both respects. The gradient was started at the moment of the injection to achieve breakthrough of the dichloromethane at a retention time of about 5 min. Backflush with dichloromethane proved to be satisfactory: the silica gel did not lose activity after more than 1000 analyses.

There was a gap of 30 s between the last eluted MOSH and the first MOAH. This intermediate fraction was often analyzed to confirm complete separation of MOSH and MOAH: Cho and TBB as well as humps of mineral oil components had to be absent. Furthermore, the initially used on-column interface struggled with a memory effect of 1.5−4%, and transfer of the intermediate fraction cleaned the transfer system. This was no longer needed with the Y-interface (*32*).

Crude mineral oils hardly contain olefins (*22*), but products may contain them from the raffination process. Furthermore, the food matrix may contain olefins, either of natural origin or as a result of heating or raffination (see below). Monoenes, such as 1-octadecene, calarene, or kaurene, were eluted with the MOSH, sometimes tailing into the intermediate fraction (e.g., kaurene in sunflower oils). Most olefins with more than one double bond (food constituents) were eluted with the MOAH, but usually created no problems, as they form sharp peaks that are ignored

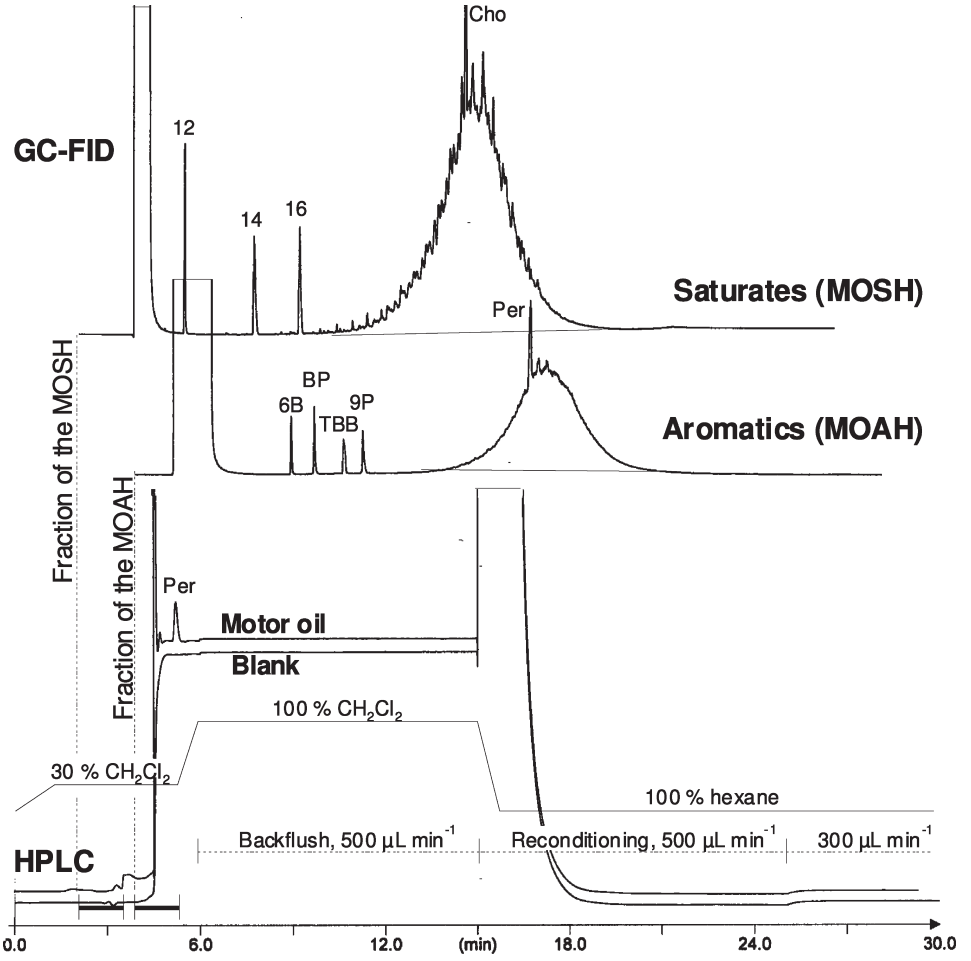

Figure 1. Analytical procedure visualized by the chromatograms of a motor (lubricating) oil. Labeled peaks indicate internal standards for determining concentrations and verification of the performance.

for the determination of the mineral hydrocarbons. Exceptions are those discussed below and sometimes requiring epoxidation.

HPLC-GC Transfer. HPLC-GC transfer involved the retention gap technique to retain rather volatile components by solvent trapping. Partially concurrent eluent evaporation enabled the introduction of the 450 μL fractions into a 10 m $\times$ 0.53 mm i.d. uncoated precolumn (*43*). The on-column interface (transfer line entering the uncoated precolumn through an on-column injector) caused the relatively high memory effect mentioned above, which prompted the development of the Y-interface (*32*).

The transfer of the MOAH fraction had to cope with a rapidly changing eluent composition: the increasing volatility causes the evaporation rate to rise. When evaporation becomes fully concurrent, solvent trapping is lost and the volatile constituents largely leave through the vapor exit. Conditions were adjusted to provide an initially lower evaporation rate than for the transfer of the MOSH.

Verification of Performance; Quantitation. The performance of the method was monitored by verification standards added to the sample together with the internal standards. For the MOSH, the *n*-alkanes C_{14} and C_{16} were used as internal standards. Possible coelution with the same or other components could be detected by one of these standards forming a larger peak (in addition, absolute peak areas were reproducible to within about 10% and an increase was readily detected). The area of *n*-C_{12} was compared to those of *n*-C_{14} and *n*-C_{16} to detect losses of volatile compounds, as they might occur during solvent evaporation in sample preparation or by partially concurrent eluent evaporation under inappropriate conditions. Up to 15% loss of *n*-C_{12} was considered to be acceptable, because compounds of such volatility were not of interest and it confirmed that losses of *n*-C_{14} were negligible. This 15% limit was never exceeded. Full presence of Cho in the MOSH and absence of TBB in the intermediate fraction verified the HPLC separation.

Concentrations of MOAH were calculated using biphenyl (BP) as internal standard. Nonylbenzene (9B) served as a control for coelutions. Hexylbenzene (6B) enabled the detection of loss of volatile components. Adequate cuts of the fraction window were verified by the standards eluted at the edges of the fraction: Cho had to be absent in the intermediate fraction and TBB and Per present in the MOAH. Per was also monitored by UV detection. After epoxidation, Per failed as a verification standard in GC, because it was partly lost.

Peak areas of MOSH and MOAH were determined by integration of the hump of unresolved components above a manually adjusted baseline. Usually the peaks on top of the hump were integrated and subtracted, unless there were mineral *n*-alkanes. Calibration with paraffin oil confirmed the validity of this approach for the MOSH, but due to the lack of a standard, no such control was possible for the MOAH. Alternatively, the concentrations were determined by the use of a paraffin oil as external standard, drawing triangles into the printed chromatograms and using the internal standards for normalizing the absolute areas, as shown in ref *44*.

The detection limit (LOD) and the quantification limit (LOQ) in oils were around 3 and 8 mg kg^{-1}, respectively. The relative

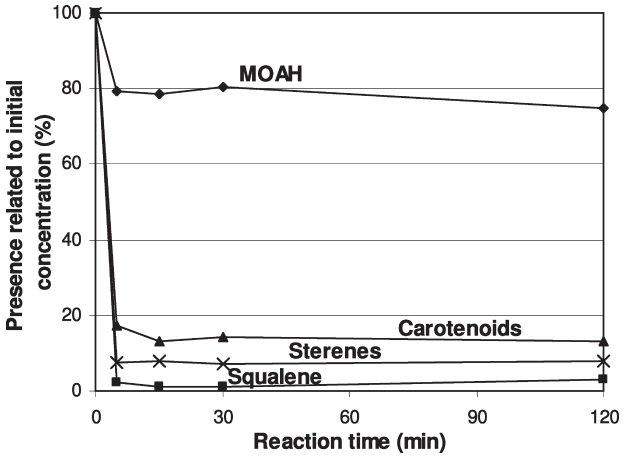

Figure 2. Removal of unsaturated components from a margarine by means of epoxidation at ambient temperature.

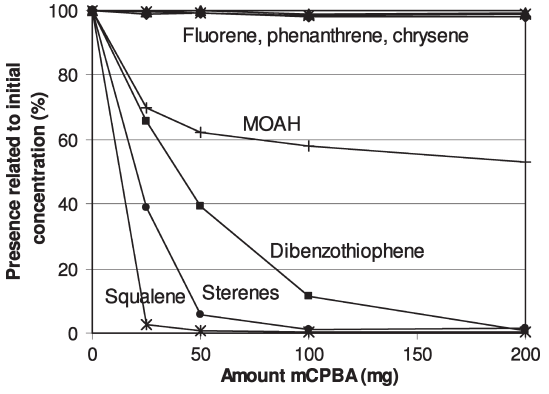

Figure 3. Epoxidation with different amounts of peracid. More peracid better removes squalene and the sterenes, but also the loss of certain aromatics increases.

standard deviations of the peak areas were below 15%, but the measuring uncertainty was usually higher because of the uncertainty about the position of the baseline and possibly the upper contour line of the hump against the natural components on top of the mineral hydrocarbons. Complete recovery of MOSH and MOAH from HPLC-GC-FID was ensured through the recovery of the verification standards at the edges of the fraction.

Method Overview. The method is summarized in **Figure 1**. At the bottom are the HPLC traces recorded by UV detection at 230 nm: a blank and a motor oil (500 μg mL^{-1}, 10 μL injection). They primarily show the eluent composition at the column exit owing to a strong response to dichloromethane. Even though the gradient started at the injection (t = 0), dichloromethane was eluted from the column only after 5 min. Less than half of this delay resulted from the void volume of the tubing, valves, and the column; the main part was from dichloromethane being adsorbed to/retained by the silica gel. The fraction of the MOAH was eluted from within this profile to shortly afterward, as shown by the Per. Backflush with dichloromethane was not recorded, because the eluent left the system through the waste outlet in the backflush switching valve. The strong response at 15 min shows the discharge of the dichloromethane in normal flow direction through the UV detector and the reconditioning of the HPLC column.

The fraction of the MOSH is not visible in HPLC. The GC-FID chromatogram of the saturated hydrocarbons of a motor (lubricating) oil is shown at the top, the fraction of the aromatics below it. Both show humps of unresolved components as they are

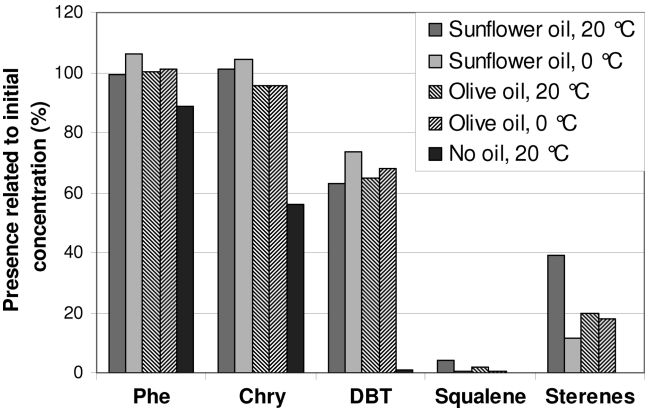

Figure 4. Epoxidation in the presence of edible oil removed little Phe and Chry, but 35% DBT. Without edible oil, the losses were significant for Phe and Chry and virtually complete for DBT. Reaction in the ice bath slightly reduced the losses of MOAH and substantially increased removal of olefins.

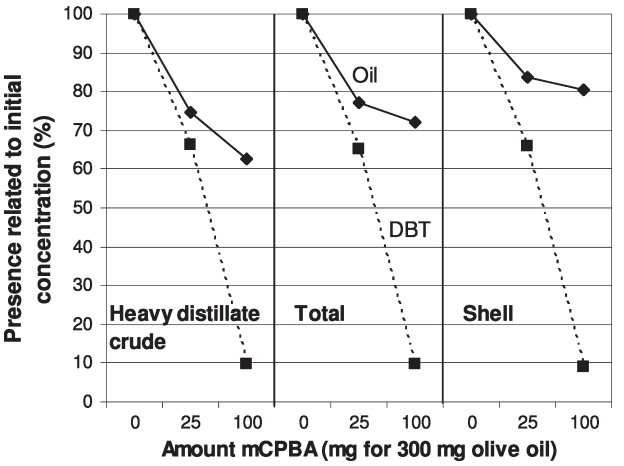

Figure 5. Loss of MOAH by epoxidation in a crude oil distillate and two motor (lubricating) oils of similar molecular mass distribution. Squares with dotted lines: DBT.

typical for mineral oil products of this type (and frequently observed in foods). The *n*-alkanes were removed in the refinery (deparaffination). The absence of signals in the fraction of the aromatics shows the high degree of alkylation and the resulting many isomers. The two humps are almost exactly coeluted; the difference in retention time is largely due to the temperature programs' starting at different levels.

Removal of Olefins by Epoxidation. For some foods, olefins hindered the analysis of the MOAH. In some oils, particularly olive oil, squalene is present at a concentration exceeding the desirable detection limit for MOAH several thousand times. After raffination, part of the squalene is isomerized and forms a hump of unresolved isomers, which is narrower than that of the MOAH but disturbs the quantitative determination of the latter. Raffination of edible oils also forms sterenes, dehydroxylation products of sterols, which are eluted from GC near the center of the most commonly observed MOAH. A hump of unresolved carotenoids is eluted somewhat later. There is no way to separate these polyenes from the MOAH by tuning HPLC selectivity, because the MOAH and the olefins both comprise a rather wide range of polarities.

By derivatization, the polarity of the olefins can be enhanced such that these components are eluted after the MOAH. Bromination was used to remove olefins from the paraffins in a manual

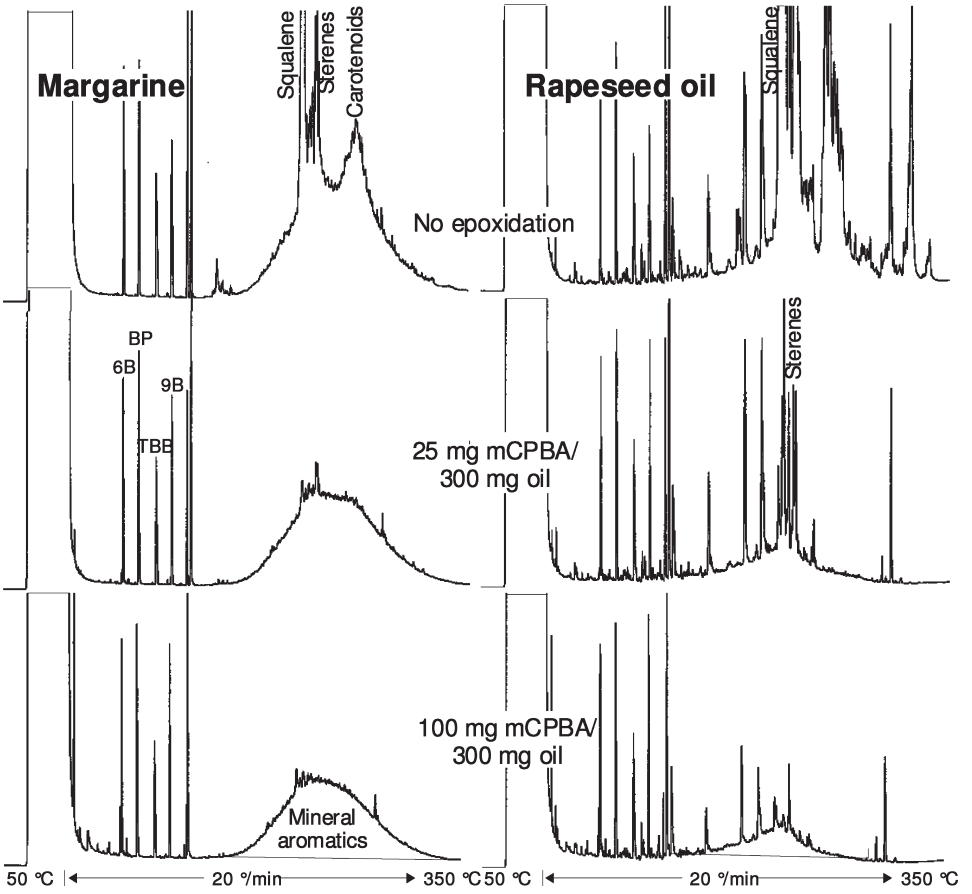

Figure 6. Effect of epoxidation for a margarine and a rapeseed oil: HPLC-GC-FID analysis without epoxidation (top chromatograms) as well as with 25 or 100 mg of mCPBA for 300 mg of fat or oil.

sample preparation approach (*44*). However, under conditions brominating the olefins, also a substantial part of the aromatic compounds was affected. Epoxidation, proposed by Mariani (*45*), was found to be more selective. A single epoxy function increases the retention on silica gel beyond the fraction of MOAH. However, careful optimization of the reaction conditions is required to avoid unacceptable reaction (loss) of aromatic constituents.

The reaction rate of 3-chloroperbenzoic acid (mCPBA) with double bonds was found to decrease from squalene (with six double bonds) to sterenes and fatty acids. Most MOAH tend to react more slowly, but it could not be prevented that also thiophenes and some polycyclic compounds were oxidized. The peracid was added in excess with regard to the olefins, but stoichiometrically corresponding to only a minor fraction of the double bonds in the fatty acids in order to be consumed by the fatty acids before the bulk of the MOAH was attacked. Clean edible oil was added to samples containing little or no fat.

Figure 2 shows the rate by which critical components were removed from the MOAH fraction of a margarine primarily consisting of Ukrainian sunflower oil, which was found to be contaminated by a technical grade mineral oil (*11*). Chromatograms will be shown in **Figure 6**. To 300 mg of this fat was added 30 mg of mCPBA, corresponding to roughly 10% of the amount required for a complete epoxidation of the fatty acids. After 5 min, 98% of the squalene and 83% of the carotenoids were removed. The hump of the MOAH was reduced by 21%. Prolonged reaction had no effect, suggesting that the peracid was depleted in < 5 min.

Figure 3 focuses on the dependence of the removal of olefins and losses of MOAH on the amount of peracid added. Three hundred milligrams of refined sunflower oil was spiked with 10 mg kg^{-1} each of fluorene (Flu), phenanthrene (Phe), chrysene (Chry), dibenzothiophene (DBT), and 500 mg kg^{-1} of a crude heavy mineral oil fraction for manufacturing base oil. This sample was treated with various amounts of peracid during 15 min at room temperature (stopped by the addition of aqueous carbonate). The increase from 25 to 50 mg of mCPBA reduced the residual amount of squalene from 2.6 to 0.8% and that of the sterenes from 39 to 5.7%. With 100 mg of mCPBA, 0.6% of the squalene and 1.3% of the sterenes were left. Flu, Phe, and Chry were not significantly affected, but the loss of DBT (assumed to be one of the most sensitive MOAH) increased from 34 to 61 and 89%. With 200 mg of mCPBA, still less than that stoichiometrically required for a complete epoxidation of the fatty acids, the loss of DBT was almost complete. The MOAH of the crude oil were reduced by 30, 38, and 42% (25, 50, and 100 mg of mCPBA, respectively). The slowed loss at increased amounts of mCPBA suggests that sensitive MOAH constituents reacted readily (e.g., the thiophenes), whereas others were stable. As a conclusion, 100 mg of mCPBA removes the olefins clearly better than 25 mg, but also the losses of MOAH are increased, that is, the larger amount should only be used when really needed.

The experiments reported in **Figure 4** involved 300 mg of refined sunflower or olive oil spiked with 10 mg kg^{-1} each of Phe, Chry, and DBT; 25 mg of mCPBA was added, and the reaction was stopped after 15 min. The reaction occurred either at ambient temperature (with spontaneous warming to about 40 °C) or in an ice bath during the first 5 min and allowed to warm to ambient temperature afterward. The 100% scale refers to the oils without epoxidation. For Phe no loss was observed as long as oil was present. In the absence (dark right bar), the peak was reduced by 11%. This was interpreted by the peracid being more

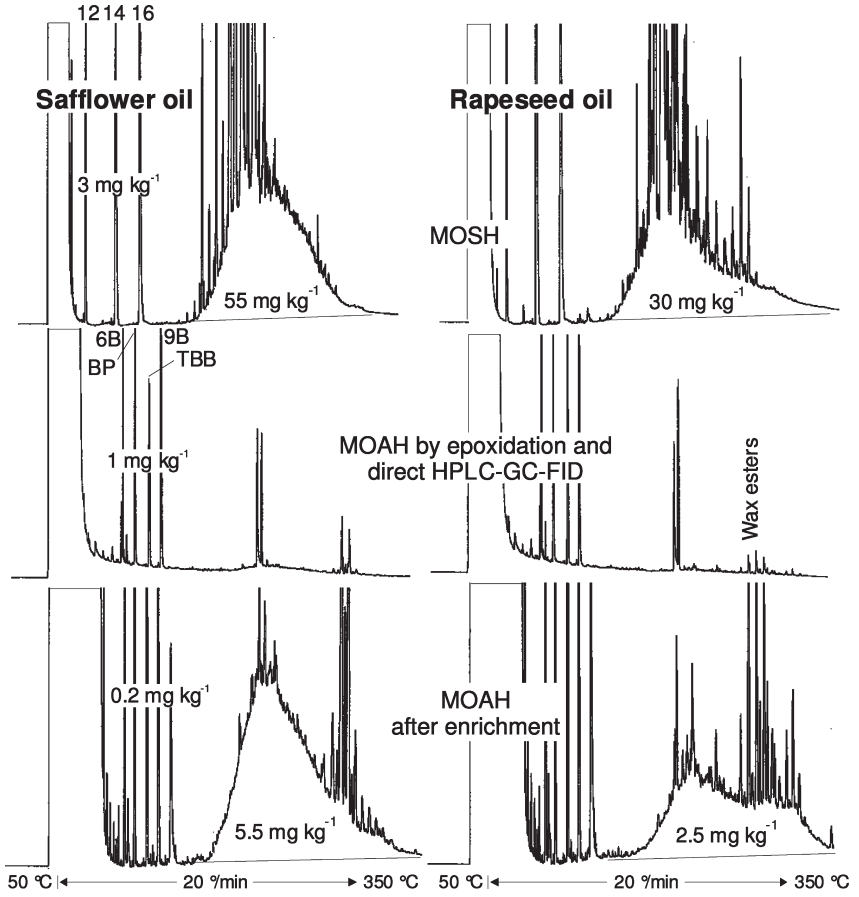

Figure 7. Enrichment for the determination of MOAH in a safflower (left) and a rapeseed oil (right): direct HPLC-GC-FID (center chromatograms) lacks sensitivity, whereas after enrichment (bottom) the limit for quantitation was below 1 mg kg^{-1}.

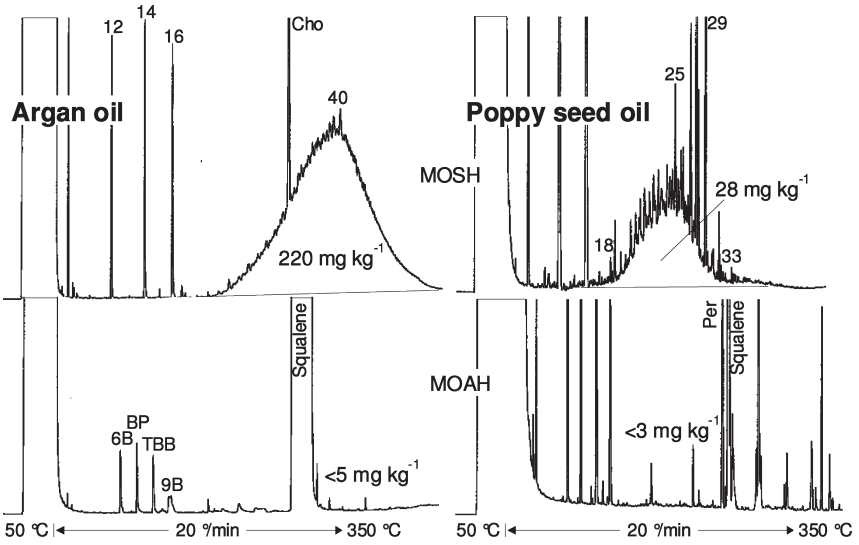

Figure 8. Two edible oils contaminated with white paraffin oils: MOAH analyzed without prior enrichment and epoxidation; same attenuation for MOSH and MOAH.

persistent. For Chry it seems that there was a loss of 5% in olive oil (containing some 45% fewer double bonds than sunflower oil). The loss without oil was increased to 44%. Oxidation of DBT was around 35%, without a difference between the two oils. The loss was slightly lower when the reaction occurred with cooling. Without oil, oxidation of DBT was virtually complete. These results primarily show the effectiveness of unsaturated fatty acids in buffering oxidation.

Epoxidation of squalene was more selective at the lower temperature: in sunflower oil, 96.1% was removed at ambient temperature, but 99.6% was removed in the ice bath (98.1 and 99.7%, respectively, in olive oil). In the instance of the sunflower oil, a substantial difference was also observed for the sterenes. Squalene and the sterenes were absent in the samples without edible oil.

Figure 5 compares the losses for three mineral oil products of similar molecular mass distributions but differing contents of

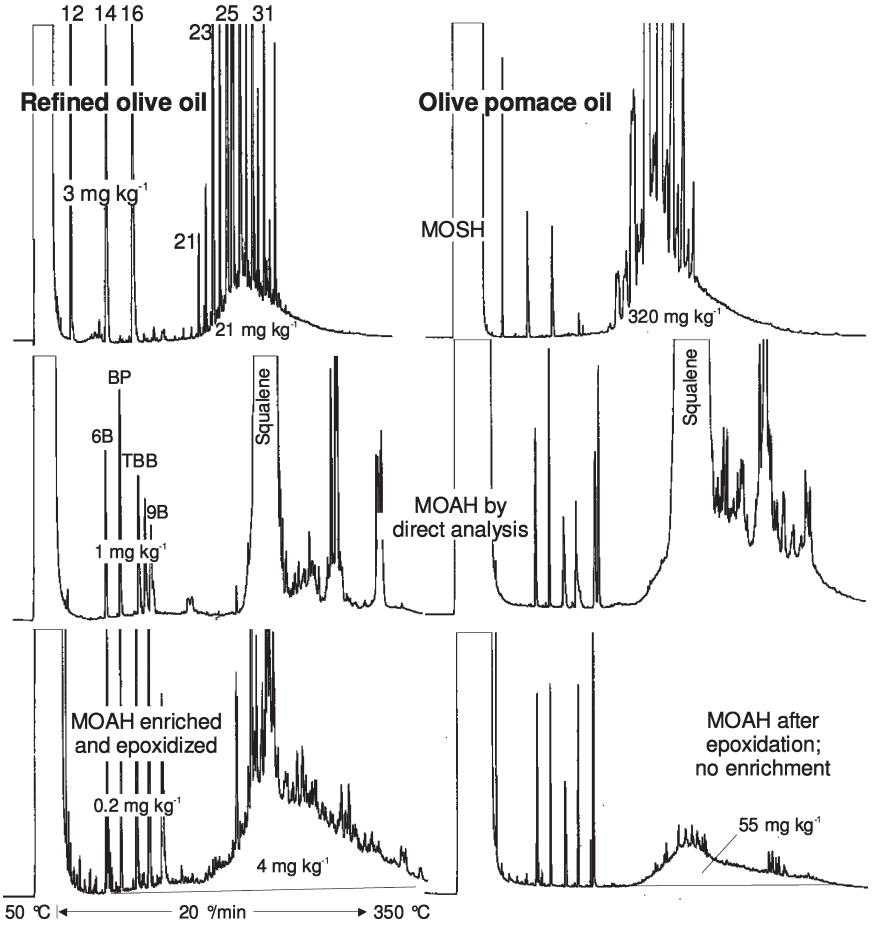

Figure 9. Contamination of a refined olive oil and an olive pomace oil with mineral oil containing about 20% MOAH.

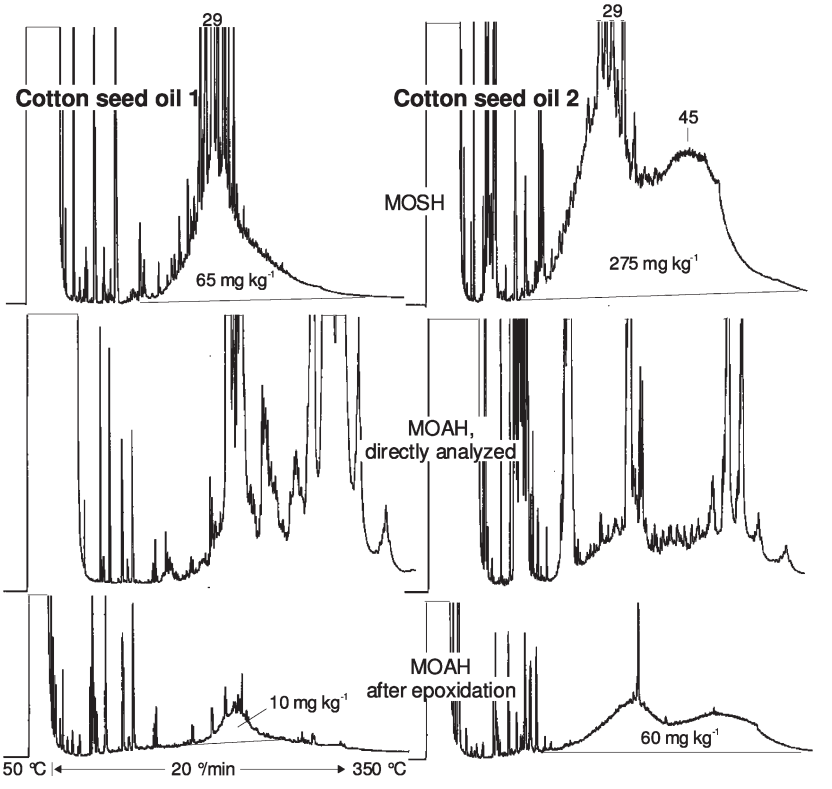

Figure 10. Cotton seed oils for which MOAH analysis presupposes epoxidation, but no enrichment.

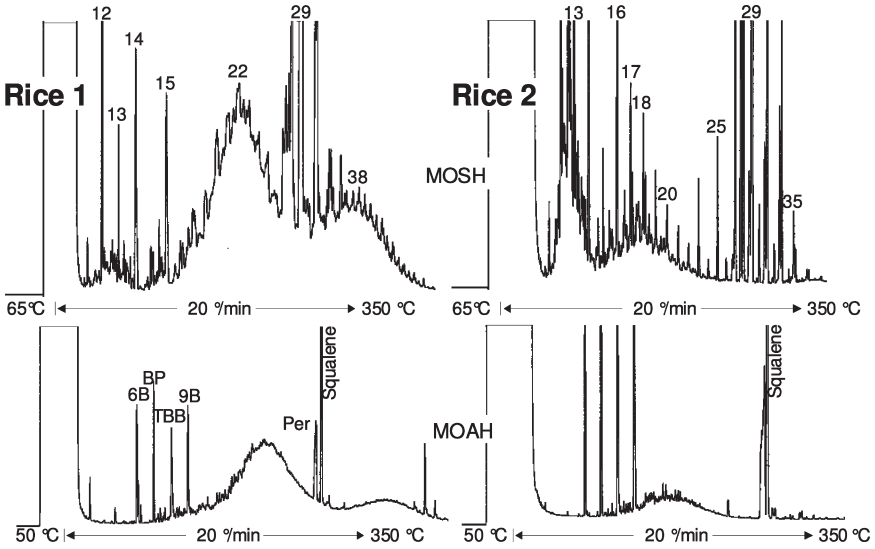

Figure 11. For samples containing little or no fat, low detection limits can be reached by reconcentrating the extracts. Rice 1 contained 10 mg kg^{-1} MOSH and 4.3 mg kg^{-1} MOAH and rice 2, 1.7 and 0.4 mg kg^{-1}, respectively (no epoxidation).

aromatic hydrocarbons. Epoxidation occurred at conditions corresponding to those under Materials and Methods and used in the applications shown below. The mineral oils were added to 300 mg of refined olive oil at 1000 mg kg^{-1} together with 2 mg kg^{-1} DBT and Phe; 25 or 100 mg of mCPBA was added with initial cooling in the ice bath.

The crude heavy mineral oil distillate (left) contained 53% aromatics, of which 25 and 37% were lost with 25 and 100 mg of mCPBA, respectively. The loss of DBT was high (34 and 90%, respectively), whereas no significant loss of Phe was detected (not shown). The Total motor oil contained 26% aromatics, and the losses were lower (23 and 28%). The Shell lubricating oil for cars contained merely 11% aromatic hydrocarbons, and the losses were still lower. The DBT losses were similar, showing the reproducibility of the reaction. These results suggest that the losses depend on the composition of the MOAH. From the refined products, the MOAH are partly extracted, particularly reducing the polyaromatic and sulfur-containing compounds, which are sensitive to oxidation.

The efficacy of epoxidation is illustrated for the margarine shown in **Figure 2** and a refined rapeseed oil. The margarine contained 340 mg kg^{-1} MOSH (not shown). The top chromatogram in **Figure 6** shows the fraction of MOAH without epoxidation. There is a large peak for squalene, probably on a hump of isomerized squalene. It is followed by sterenes and a hump representing carotenoids. The smaller amount (25 mg) of mCPBA was sufficient for a virtually complete removal of these olefins. The use of 100 mg of mCPBA slightly improved the removal of the carotenoids but also increased the losses of MOAH (same attenuation in all three chromatograms). The MOAH in the bottom chromatogram amounted to 100 mg kg^{-1}. In the top chromatogram they would be estimated as 130 mg kg^{-1}; that is, the loss corresponded to 23%. The MOAH chromatogram of the untreated rapeseed oil was severely disturbed. With 25 mg of mCPBA it was improved, but some interference primarily by sterenes remained. The bottom chromatogram clearly revealed 15 mg kg^{-1} MOAH.

Sample Enrichment. Online HPLC-GC-FID of edible oils and fats provided a limit of quantification for MOAH of around 8 mg kg^{-1}, depending on the composition of the MOAH and interferences. This may not be satisfactory. Because the sensitivity of the detection cannot be improved, more sample must be injected, which presupposes a correspondingly larger bed of silica gel to provide the capacity for retaining the triglycerides. As the fraction volume would rapidly become too large for online transfer to GC, an off-line enrichment with a conventional silica gel column was devised, analogous to the method for the MOSH (*15*).

The elution of the MOAH from silica gel requires a modifier in hexane. Because a maximum amount of oil or fatty food extract should be loaded onto the smallest possible bed, this modifier must be selected so that it does not strongly reduce the capacity of the silica gel for retaining fat. A small percentage of MTBE was shown to reduce this capacity by as much as a factor of 10, whereas a larger addition of dichloromethane (e.g., 20%) to achieve the same eluent strength reduces it by hardly a factor of 2 (*41*).

Using 12 g of silica gel and 20% dichloromethane/hexane, safely 1 g of fat or oil could be retained. The fraction was collected into a round flask containing 40 mg of clean edible oil acting as a keeper to prevent evaporation of volatiles in the subsequent solvent evaporation. This oil also buffered the epoxidation when needed and was removed again by the HPLC of the final analysis. The reconcentration to a final 200 μL, of which 90 μL was injected into HPLC-GC-FID, resulted in enrichment by a factor of 50.

Enrichment also increases the concentration of the interferences and renders epoxidation even more important. It was performed after enrichment, because this reduced the amount of mCPBA needed, the impurities of which are also enriched.

Figure 7 illustrates the efficiency of this tool. The top chromatograms show the MOSH in a contaminated safflower and a rapeseed oil from the market. The center chromatograms show that direct HPLC-GC-FID analyses of MOAH (after epoxidation) were insufficient for a quantitative determination. After enrichment, 5.5 and 2.5 mg kg^{-1} MOAH formed large humps and the detection limit was below 1 mg kg^{-1}. The interferences eluted at close to 350 °C consisted of saturated (not epoxidizeable) wax esters.

Illustrative Application to Food Samples. The left chromatograms in **Figure 8** are from argan oil contaminated with 220 mg kg^{-1} MOSH. The analysis of the MOAH was achieved without epoxidation. Squalene seriously overloaded the GC (see internal standards, particularly 9B), but the chromatogram enabled the presence of >5 mg kg^{-1} MOAH to be ruled out, which suggests the at least predominant presence of white paraffin oil. The poppy seed oil at the right contained 28 mg kg^{-1} MOSH. Again, no MOAH were detectable at a detection limit of about 3 mg kg^{-1}.

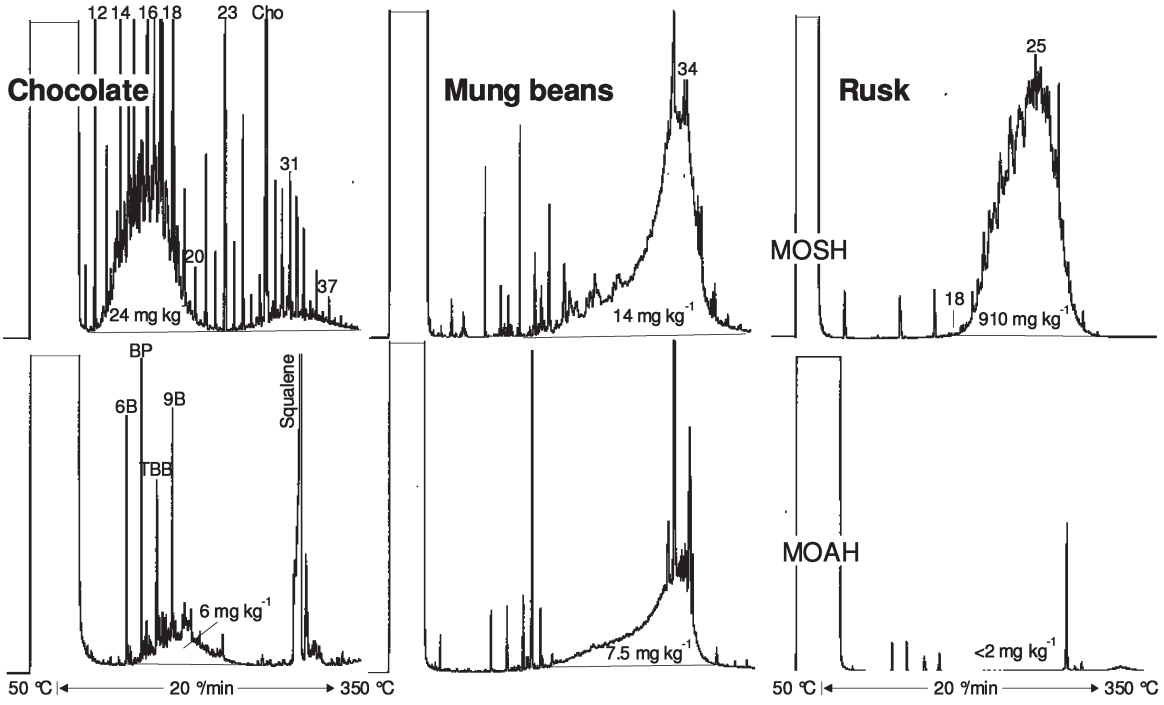

Figure 12. Chocolate and mung beans containing mineral oils of technical quality (concentrations given in the chromatograms) and rusk with a high concentration of a white paraffin oil used as release agent.

Figure 9 shows results for a refined olive oil (left chromatograms) contaminated with 21 mg kg^{-1} MOSH and an olive pomace (sansa) oil with 320 mg kg^{-1} MOSH (determined by preseparation with aluminum oxide to remove the plant *n*-alkanes (*27*)). Epoxidation was a prerequisite (center chromatograms), and for the refined oil also enrichment was needed. The 4 mg kg^{-1} MOAH found after a loss of perhaps 25% during epoxidation suggests that the mineral oil contained about 20% aromatics, which is in the range typical for technical oils. For the pomace oil, no enrichment was needed. Assuming 25% loss during epoxidation, the mineral oil contaminant again contained 20% aromatics.

Also in the two refined cotton seed oils analyzed and shown in **Figure 10**, containing 65 and 275 mg kg^{-1} MOSH, MOAH could not be analyzed without epoxidation (center chromatograms). The samples were not enriched: the 10 mg kg^{-1} MOAH in oil 1 could still be measured. Oil 2 seems to contain two mineral oil products: one with a molecular mass distribution frequently observed for technical oils (similar to that in cotton seed oil 1), the other with an extremely high molecular mass (centered C_{45} and not completely eluted from GC at 350 °C). Both contained MOAH at about 18%.

For samples containing little or no fat, lower detection limits can be reached by reconcentrating the extract. **Figure 11** shows this for two samples of Basmati rice. Rice 1 seems to be contaminated by three mineral oil products. The paraffins centered on *n*-C_{22} could be from jute bags with mineral batching oil. This mineral oil contained about 35% aromatics. The product with the paraffins centered on *n*-C_{38} contained 15% aromatics, whereas none were detected for that with paraffins centered on *n*-C_{13}. The sum of the MOSH amounted to 10 mg kg^{-1} and that of the MOAH to 4.3 mg kg^{-1}. Rice 2 contained 0.9 mg kg^{-1} of a mineral oil product centered at *n*-C_{13} with no detectable MOAH and 0.8 mg kg^{-1} of a product centered on *n*-C_{18} with 35% aromatics, possibly again from jute bags. In samples such as rice, the detection limit for MOAH is around 0.1 mg kg^{-1}.

The chocolate in **Figure 12** contained 30 mg kg^{-1} of a mineral oil centered at *n*-C_{16} with 20% MOAH and a smaller amount of a higher molecular mass mineral oil (no epoxidation). The mung beans contained 22 mg kg^{-1} of a mineral oil with a rather unusual mass distribution. The sample was epoxidized to remove the squalene eluted at the top of the MOAH hump. The MOSH and MOAH profiles well correspond; the MOAH amounted to 35%. The rusk contained 910 mg kg^{-1} of a paraffin oil used as release agent in the bakery with $< 0.2\%$ aromatics (no epoxidation).

Conclusions. A method was developed for determining the sum of the MOAH in foods ranging from highly alkylated benzenes to components with at least five rings. Auxiliary tools were needed for some of the samples: enrichment by the removal of lipids to reach a detection limit of around 1 mg kg^{-1} and the removal of olefins of plant origin with the help of epoxidation. Epoxidation should only be applied when needed, because it causes the loss of roughly 25% MOAH, primarily affecting the thiophenes. This method was investigated in detail and the performance of the critical steps brought under control by verification tools.

Proof of the mineral origin of the aromatic hydrocarbons is primarily indirect: the correspondence of the molecular mass distribution with that of the MOSH for which the mineral origin was demonstrated. For this reason, MOSH and MOAH should be analyzed in parallel.

Presently, a limit of quantitation at 1 mg kg^{-1} seems to be satisfactory, but a toxicological evaluation is needed to determine the concentration (exposure) below which MOAH are no longer of relevant concern. For polycyclic aromatic hydrocarbons, > 100 times lower legal limits are enforced. If the MOAH contained just 1% carcinogenic material, 1 mg kg^{-1} would be of concern.

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