1. Introduction

Mineral oil products derive from crude petroleum, through distillation processes and various refining steps, and contain proportions of mineral oil saturated hydrocarbons (MOSH, including n-alkanes, isoparaffins and cycloparaffins), and mineral oil aromatic hydrocarbons (MOAH), mainly consisting of alkylated polyaromatic hydrocarbons (PAH). MOSH contamination in foods, deriving from a variety of sources, has been studied for quite a long time[1]. One of the major sources of contamination is paperboard packaging, an issue known since 1997[2], even though it has gained great attention only recently[3]. Such a contamination derives from the printing inks applied directly to the packaging, and/or from the ink used in the newspapers, employed to produce recycled fiber. It has also been demonstrated that mineral oil migrating from paperboard usually contains a large proportion (15-20%) of MOAH[4], which is more of a worry from a toxicological viewpoint. The occurrence and danger of mineral oil products in foods has been discussed widely in recent years[5-10].

The Joint IAGOS/WHO Expert Committee on Food Additives (JECFA), in 2002, reported a list of admissible daily intake (ADI) values for different white mineral oil[11]. Based on such data, an environmental limit of 0.6 mg kg-1 was proposed for MOSH migration (up to C25) in dry foods from paperboard packaging[12]. The European Food Safety Authority (EFSA), published an opinion in June 2012[13], casting doubts on the “JECFA list, due to the lack of sufficient toxicological information and, as a consequence, the JECFA values were recently withdrawn[14]. Furthermore, even though EFSA emphasized the potential carcinogenic risk of MOAH constituents[15] an official approved evaluation of MOSH is still lacking.

2. Experimental

2.1. Samples and chemicals

Chromatographic grade hexane, dichloromethane (50:50 n-hexane/dichloromethane v/v), n-hexane, CH2Cl2, and AgNO3, were purchased from Sigma-Aldrich (Milan, Italy), and distilled before use. The C7-C40 standard mixture, the paraffin oil (code 18512), AgNO3, and AgNO3, were purchased from Supelco and Sigma-Aldrich (Milan, Glass SPE cartridges (6 ml, glass tubes with a frit) were purchased from Macherey-Nagel (Crobomond, Dieren, Germany).

2.2. Samples and preparations

Samples of pasta, rice and icing sugar, were purchased in a supermarket. The ground samples were extracted overnight using n-hexane, and then purified through Ag-SPE. Briefly, a 1 L food solvent ratio was employed to extract MOSH and MOAH from the samples. After, an aliquot of the extract was concentrated prior to SPE clean-up, on a Ag silicon SPE cartridge. Silver silica gel SPE cartridges have been developed for MOSH and MOAH determination[16,17]. With regards to detectors, flame ionization (FID) systems have been widely employed for the reliable quantification of the humps of unresolved complex mixtures (UCM), generated in MOSH/MOAH applications. FIDs are useful because they provide virtually the same response per mass of hydrocarbons, even though the lack of structural information is certainly a major drawback[18]. In the last, the attainment of profound information on the composition of MOSH and MOAH constituents, can provide fundamental information on potential toxicity, and on the contamination source. Such an objective was reached by Biedermann and Grob, who used an MS detector, along with the additional information generated by a comprehensive 2D GC (GCxGC) analysis[19,20]. A pre-separation of the MOSH and MOAH groups was achieved through offline LC, a process necessary to avoid the overlapping of steranes and hopanes (present in the MOSH fraction), with alkylated (non- and tri-ring) aromatics. The GCxGC system was coupled alternately with an MS system, for qualitative purposes, and with an ID system for quantification, and, hence, two applications were required to obtain both information types. A GCxGC-MS method, after an offline LC, pre-separation step, has also been exploited by Mondello and co-workers, to attain a more expanded view on MOSH contamination in homogenized baby food[21].

The present document describes a GCxGC method, characterized by dual MS/FID detection, for the qualitative and quantitative analysis of MOSH and MOAH in various foods. The pre-separation step was performed by using Ag-SPE.

The eluted fractions were concentrated to a final volume of 100 µL to increase sensitivity, since large volume injection (LVI) was not used.

3. Results and Discussion

3.1. GCxGC-MS/FID optimization and validation

GCxGC method optimization was achieved by using offset printing ink, which is formed mainly of MOSH (>90%), and by a minor MOAH fraction. Apart from problems related to co-elution, the offset ink had been injected neat into the GCxGC system, and the MOAH fraction would have overlapped the columns and modulator, while the MOSH constituents would have been barely detected; therefore, a pre-separation on the Ag-SPE cartridge was necessary.
The eluted fractions were concentrated to a final volume of 100 µL to increase sensitivity, since large volume injection (LVI) was not used.

2.3. GC-GC/MS/FID analysis

GC-GC experiments were performed on a system consisting of a GC2010 gas chromatograph, and a QP2010 Ultra quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The primary column, an SLB-5ms 30 m × 0.25 mm ID × 0.25 µm (diluted poly(dimethylsiloxane)) and a 0.25 m × 0.05 mm ID capillary column (Zefen 50, Agilent, Germany), were used to create a double-column method. The second column was connected through a capillary column splitter (50:50) to two uncoated capillaries, with these linked to the FID (0.5 m × 0.1 mm ID) and to the MS (0.25 m × 0.05 mm ID) systems.

2.4. Method parameters

Modulation was performed every 6000 sec, by using a loop-type modulator (under license from Zoex Corporation, Houston, TX, USA). The duration of the hot pulse (350°C) was 375 msec.

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GCxGC method optimization was achieved by using offset printing ink, which is formed mainly of MOSH (>90%), and by a minor MOAH fraction. Apart from problems related to co-elution, if the offset ink had been injected neat then the MOSH group would have overloaded the columns and modulator, while the MOAH constituents would have been barely detected; therefore, a pre-separation on the Ag-SPE cartridge was necessary.
Flow division between the FID and MS units was a compromise among different necessities, the main one being the attainment of a satisfactory sensitivity for quantification purposes. Because the detectors employed operate under different pressure conditions, the employment of too branched with equal IDs proved to be a non-ideal choice, the reason was related to the fact that an excessively long “MS” branch was required to generate an adequate flow resistance, to divert the majority of the effluent to the FID. Such a configuration would have led to substantial differences in the second-dimension elution times, between the qualitative and quantitative experiments. A good compromise was found through the use of an MS-linked 0.25 m x 0.05 mm ID branch, and a 0.5 m x 0.1 mm ID FID one.

Such a splitting configuration produced the following flow conditions: about 84% and 16% of the effluent reaching the FID and MS at the initial analysis temperature, respectively. The split ratio changed slightly during the GC run, with about 87% and 13% of the effluent diverted to the FID and MS, at the end. Since the calibration curve was constructed under the same analytical conditions, the quantitative results were not affected.

The GC×GC dual-detection operational conditions were optimized with the aim of maintaining the same chromatography performance, compared to an MS-only system, as shown in Fig. 1. In the MS-only approach, with the same analytical columns, the head pressure (approx. 150 kPa) was selected to generate about 20 minutes and 210 minutes, in the first and second dimension, respectively. In the dual-detection approach, a 243-kPa pressure produced the same gas velocity in the first dimension (to attain the same elution temperature), and a slightly lower one in the second (180 cm/sec).

Table 1: Quantification values relative to the MOSH and the MOAH fractions, in samples of pasta, icing sugar, and rice, using Ag-SPC-GC×GC-MS/FID.

<table>
<thead>
<tr>
<th>Food</th>
<th>MOSH &lt;C25 (mg/Kg)</th>
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3-2. Food analysis

MOSH and the MOAH fractions, relative to pasta, icing sugar and rice, were quantified up to C25 (as required by the European regulations). For each calibration point, attention was paid, during integration, to eliminate the natural artefacts from the MOSH compounds, and the “unknown” peaks from the MOAH group. Specifically, for GC×GC-FID quantification, the “polymer integration function” was applied, which enabled the definition of a polygonal area in which all the integrated peaks are automatically summed, and the data relative to each peak is saved as well. Thus, the underscored peaks can be easily selected, and subtracted from the total area. Quantification information, relative to the three foods, is listed in Table 1.

MOSH and the MOAH fraction results for the MOAH fraction

The peaks present in the GC×GC-chromatograms, for the three samples, were tentatively-identified on the basis of MS database similarities (≤ 85%) and in accordance with linear retention indices (LRI), contained in the same database. Since a widely-accepted procedure for the calculation of GC×GC LRI values has not been developed, such data were calculated in a one-dimensional mode; furthermore, a rather wide LRI filter window (± 10 units) was applied (to eliminate wrong matches), to compensate for the retention effects of the polar capillary. The tentatively identified compounds, along with experimental and database LRI, are listed in Table 2.

Two compounds were outside the LRI range, specifically, octyltetradecanoate and octyltetradecanoate were characterized by a difference of ±56 and ±57 units, respectively. It noteworthy that, in these cases, the database LRI values, (http://wileybook.nist.gov/chemistry), were attained using a methyl silicone capillary column (Ultra-1 25 m x 0.32 mm x 0.25 μm), while in the present research a 30 m x 0.25 mm ID (0.25 μm) m-silphenylene polymer phase was used. Since the similarity matches were satisfactory, and the analyte locations in the 3D chromatogram gave a further idea on the chemical structure, these solutes were given a name.

Figures 2, 3 and 4 show GC×GC-MS chromatograms for the pasta, icing sugar and rice samples, respectively.
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Fig. 1 Comparison of raw TIC chromatogram expansions (printing ink analyses), obtained using a GC×GC-MS and a GC×GC-MS/FID system.

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Food safety

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MOSH and the MOAH fractions, relative to pasta, icing sugar and rice, were quantified up to C25 (as required by the enrolement limit), using the aforementioned method; attention was paid, during integration, to eliminate the natural alkanes from the MOSH compounds, and the "unknown" peaks from the MOAH group. Specifically, for GC×GC-FID quantification, the "polygonal integration function" was applied, which enabled the definition of a polygonal area in which all the integrated peaks are automatically summed, and the data relative to each peak is saved as well. Thus, the undetected peaks can be easily selected, and subtracted from the total area. Quantification information, relative to the three foods, is listed in Table 1.

Figures 2, 3 and 4 show GC×GC-MS chromatograms for the pasta, icing sugar and rice samples, respectively.

Table 2: Compounds identified in the "MOAH" GC×GC–MS analysis; database-derived (database LRI) and experimental LRI (defined as LRI) values, and spectral similarities (MS%) (×100,000).

3.3. GC×GC-MS results for the MOAH fraction

The peaks present in the GC×GC-chromatograms, for the three samples, were tentatively-identified on the basis of MS database similarities (≥ 80%) and in accordance with linear retention indices (LRI), contained in the same database. Since a widely-accepted procedure for the calculation of GC×GC LRI values has not been developed, such data were calculated in a one-dimensional mode; furthermore, a rather wide LRI filter window (± 25 units) was applied (to eliminate wrong matches), to compensate for the retention effects of the polar capillary. The tentatively identified compounds, along with experimental and database LRI, are listed in Table 2.

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Fig. 2 GC×GC-MS chromatogram, relative to the pasta MOAH fraction. Identification as reported in Table 2.

Fig. 3 GC×GC-MS chromatogram, relative to the icing sugar MOAH fraction. Identification as reported in Table 2.

Fig. 4 GC×GC-MS chromatogram, relative to the rice MOAH fraction. Identification as reported in Table 2.
The identification of the specific aromatic compounds, present in the MOAH "cloud", was outside the scope of the investigation; however, even if desired, the identification of such compounds could not have been performed with satisfactory reliability, because of the low amounts of such compounds. However, it was possible to determine the MOAH quantities (TIC trace), and patterns, which are highly important to define the contamination source.

A series of peaks, present in the MOAH fraction, were identified as esterified fatty acids. However, their presence did not affect reliable quantification because these compounds were subtracted from the total MOAH area. The esterified fatty acids derived from the paperboard packaging. In fact, in a sample of pasta analyzed prior to box packing, no sign of MOAH contamination was observed.

The possibility to use offset printing ink, based on vegetable oils, has been known for more than fifteen years, though its use has become more frequent since contamination from food packaging has become an issue of worry. A series of "unknowns" in the GC×GC-MS chromatograms were labeled as "undefined FA esters", since the relative spectra were clearly that of FA esters, although the database searches gave different possible "homologous" matches with good similarities, but not always with a corresponding LRI value. Hence, it was not possible to identify such compounds with sufficient reliability, even though they were marked in the figures, since their chemical nature was evident. It could also not be excluded that such FA esters were not contained in the MS database. For example, in the pasta sample (Fig. 2), only three out of the four main peaks were identified, namely octyldecanoate, octyltetradecanoate, and octyloctadecanoate. However, it can be deduced from its 2D position that the "undefined FA ester" was most probably octyloctadecanoate, even though such a compound was not present in the MS database used. A good "match" similarly was observed with the spectrum reported in the NIST web site, however no LRI information was found, thus this compound remained unidentified.

It is noteworthy that practically the same compounds were found in all the samples subjected to analyses; however, different quantitative profiles were observed, probably due to a different ink-type and/or to a different contamination source. It can be hypothesized that the vegetable oil offset printing ink was directly used in the pasta packaging (highly contaminated), while it was present, in different amounts, in the recycled fiber used for the packaging of the other two food samples.

Reference