DETERMINATION OF SATURATED-HYDROCARBON CONTAMINATION IN BABY FOODS BY USING ON-LINE LIQUID-GAS CHROMATOGRAPHY AND OFF-LINE LIQUID-COMPREHENSIVE GAS CHROMATOGRAPHY COMBINED WITH MASS SPECTROMETRY

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INTRODUCTION

The contamination of foods with mineral oil satured hydrocarbons (MOSH) is a common occurrence. MOSH, as well as mineral oil aromatic hydrocarbons (MOAH), derive from crude oil, with the former fraction containing linear and branched alkanes, as well as cyclic ones (naphthenes). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) published a list of admissible daily intake (ADI) values for white mineral oils: the lowest value was 0.01 mg/kg for a low viscosity product, while 20 mg/kg was the highest ADI value, for a high viscosity one. In 2009, ESFA (European Food Safety Authority) published an opinion on the use of high viscosity white mineral oils, establishing an ADI value of 12 mg/kg bw/day. A limit of 0.6 mg/kg for MOSH (from C10 to C25) contamination in foods, has been recently proposed by the German Federal Ministry of Food, Agriculture and Consumer protection (BMELV).

Heart-cutting LC-GC, with a flame ionization detector (FID), is a prime choice for the quantification of MOSH in vegetable oils. A previously-reported rapid LC-GC-FID method was used in the present work, to analyze homogenized solid baby foods (meat, fish, and fruit). Various degrees of contamination were found in all samples, and the LC fractions, relative to the most contaminated samples, were collected off-line and then analyzed by using comprehensive two-dimensional GC-quadrupole mass spectrometry (LC-GC×GC-qMS). The results herein reported are important; moreover, the use of LC-GC×GC-qMS appears to be an interesting option to "see and identify" what is beneath anonymous LC-GC-FID hydrocarbon humps.

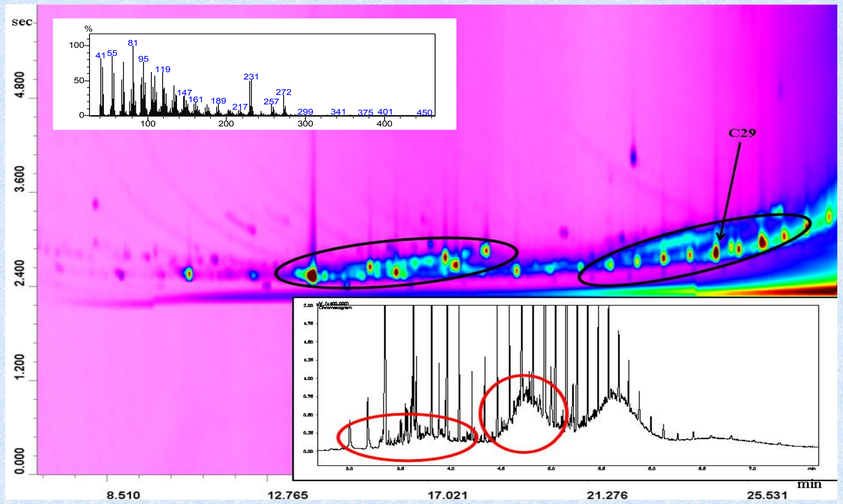

Figure 1. *LC-GC×GC-qMS and LC-GC-FID chromatograms relative to sample Salmon I. A spectrum of a cyclic alkane is reported in the left-hand upper part of the 2D chromatogram.*

Baby food	MOSH (mg/Kg)	≤ C25 alkanes (mg/Kg)
Salmon I	13.8	2.0
Plaice	3.5	0.9
Chicken	3.0	1.4
Beef I	1.9	0.8
Beef II	5.6	2.2
Beef-Ham	4.9	2.0
Turkey	3.2	1.1
Sea bass	1.4	0.9
Calf	2.5	0.7
Rabbit	3.5	1.0
Ostrich	1.6	-
Salmon II	1.1	-
Prune I	0.6	-
Pear	3.6	0.6
Fruit mix	0.3	-
Prune II	10.5	1.8

Table 1. *Samples analysed, total levels of MOSH contamination, and contamination considering an upper boundary defined by C25.*

EXPERIMENTAL

Samples and sample preparation

All the sixteen baby-food samples were purchased from supermarkets located in Messina (Italy). All the samples (500 mg) were extracted for three times with hexane (1 mL each, for 15 min), and then each time filtered. After, the filtrates were added together and dried under a gentle nitrogen stream. The extract was weighed (to derive the % of extract) and then diluted to a final concentration of 25% *v/v* in hexane. Apples, sugar and corn starch were purchased from a supermarket located in Messina. Quantification was achieved through a calibration curve, constructed through external standardization.

LC-GC-FID analyses

All samples, reported in Table 1, were analyzed, by using an LC×GC system (Shimadzu, Kyoto, Japan).

LC conditions: a 100 × 3 mm ID × 5 µm d_p silica column (SUPELCOSIL LC-Si, Supelco, Milan, Italy) was operated under isocratic conditions, using hexane as mobile phase (0.35 mL/min). Injection volume: 20 µL. At the end of the heart cut, the column was backflushed using CH_2Cl_2. Data were acquired by the LCsolution software (Shimadzu).

A Shimadzu AOC-5000 autoinjector, equipped with a dedicated dual side-port syringe, was employed as transfer device.

A Shimadzu GC2010 Plus gas chromatograph was equipped with an Optic 3 PTV injector (ATAS GC International, Eindhoven, The Netherlands). Optic 3 conditions: from 75°C (1 min) to 360°C at 250°C/min. Injection mode: split, at a ratio of 200:1 for 1 min during sample introduction and solvent vent, then splitless for 1 min, then 50:1 for the remaining analysis time. Data were acquired by the GCsolution software (Shimadzu).

GC conditions: an SLB-5ms [silphenylene polymer, virtually equivalent in polarity to poly(5% diphenyl/95% methylsiloxane)] 15 m × 0.10 mm ID × 0.10 µm d_f column (Supelco) was heated from 50°C (1 min) to 360°C (4 min) at 70°C/min. Carrier gas: hydrogen, pressure 529 kPa (constant linear velocity: 100 cm/sec). FID (360°C) sampling frequency was 50 Hz. The LC-GC software (Shimadzu, Duisburg, Germany) enabled the control of each instrument through the respective native software.

GC×GC-MS analyses

All GC×GC applications were carried out on a Shimadzu GC×GC-MS system.

The primary column, an SLB-5ms 30 m × 0.25 mm ID × 0.25 µm d_f column, was connected to an uncoated capillary segment (1.0 m × 0.25 mm ID, used to create a double-loop). The uncoated capillary was then connected to a segment of BPX50 (50% phenyl polysilphenylene-siloxane) 1.5 m × 0.10 mm ID × 0.10 µm d_f column (SGE). Loop-type modulation was carried out every 6 sec. MS parameters: the sample was analyzed in the full scan mode, using a mass range of 40-460 *m/z*; spectra generation frequency: 25 Hz; GC oven temperature program: 80°C to 360°C at 10°C/min. Carrier gas, helium, was supplied at an initial pressure of 250 kPa (constant linear velocity). Injection temperature: 350°C. Injection mode and volume: split (1:10), 4 µL. Data were collected by the GCMS Solution software (Shimadzu); bidimensional visualization was carried out by using the ChromSquare v.1.5 software (Shimadzu Europe, Duisburg, Germany).

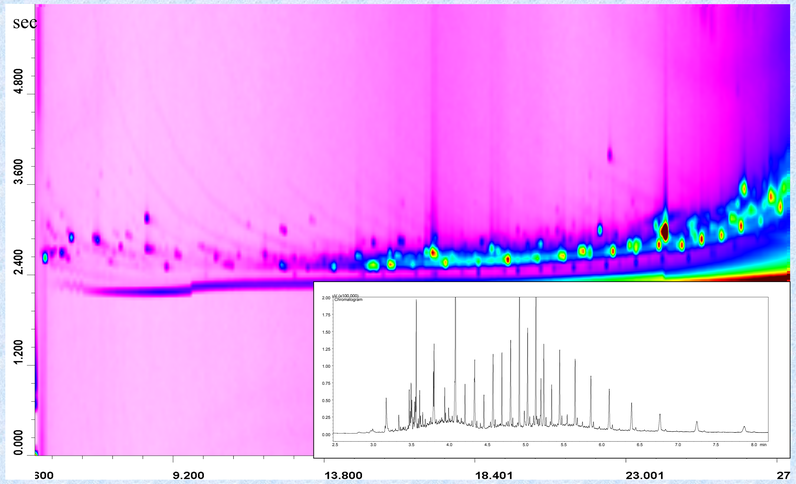

Figure 2. *LC-GC×GC-qMS and LC-GC-FID chromatograms of sample Beef II.*

RESULTS AND DISCUSSION

Twelve homogenized solid baby foods, containing either meat or fish (and vegetable oil), were subjected to analysis (Table 1). The vegetable oil used as ingredient was, in all twelve cases, sunflower oil. As can be observed in Table 1, the meat and fish baby foods were all contaminated. Also reported in the table are MOSH concentrations considering the upper alkane boundary (MOSH up to C25). After quantification, the MOSH "humps" were subjected to a qualitative investigation. A four-dimension off-line LC-GC×GC-qMS experiment was carried out as follows: the 175-µL MOSH fraction was collected, dried, and the residue was solubilised in 10 µL of hexane. The LC-apolarGC×polarGC-qMS results are shown in Figure 1 and 2 (most contaminated samples). The zones defined by the ellipses in Figure 1, show the compounds contained in the first and second hump, namely *n*-alkanes, branched alkanes (continuous line between the *n*-alkanes) and cyclic alkanes (continuous line above the linear and branched alkanes). A mass spectrum derived from the first "cycloalkane zone" is shown in Figure 1. The "cyclics" were identified on the basis of I) bidimensional chromatogram location, and II) information reported in the literature.

At this point, it was decided to extend the study to fruit-based baby foods. Four samples were subjected to analysis, and found to contain MOSH concentrations in the range 0.3-10.5 mg/kg (Table 1). As a consequence of such a finding, an apple baby food was lab-produced using the ingredients reported on the jar label, and then analyzed; the LC-GC result indicated the presence of mineral oil saturated hydrocarbons. The necessity arose, at this point, to analyze each ingredient (apples, sugar and starch): a MOSH contamination was pinpointed in the commercial corn starch (7.3 mg/kg) and sugar (4 mg/kg). All the baby foods analyzed in the present investigation were found to be MOSH contaminated, with the levels observed not to be considered as low (average value: 3.8 mg/kg). It is clear that the origin of the MOSH contamination observed, in all samples, cannot be exactly pinpointed, though the vegetable oil probably plays a role. The hydrocarbon molecular-weight range also varied, meaning that the contamination most certainly came from different sources.

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