Contamination of Italian Citrus Essential Oils: Presence of Chloroparaffin

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In this study, the contamination by chloroparaffin of Sicilian and Calabrian citrus essential oils, produced in the crop years 1994−1996, was investigated. The analyses were carried out on 102 lemon oils, 98 orange oils, and 96 mandarin oils, using a dual-channel GC−ECD. It was found that 53% of lemon oil, 33% of orange oil, and 38% of mandarin oil samples were contaminated. The mean contamination levels were 7.1 ppm (lemon), 2.5 ppm (orange), and 5.3 ppm (mandarin). The highest concentration of chloroparaffin found was 60 ppm in a lemon oil sample.

Keywords: *Citrus oils; chloroparaffin; dual-channel GC−ECD*

INTRODUCTION

In previous studies we have used gas chromatography with nitrogen phosphorus (NPD), flame photometric (FPD), and electron-capture (ECD) detectors to determine the levels of organophosphorus and organochlorine pesticide residues in citrus essential oils (Dugo et al., 1987; Dugo et al., 1990; Di Bella et al., 1991; Dugo et al., 1994; Della Cassa et al., 1995; Dugo et al., 1997). More recently we have investigated the contamination of citrus oils by phosphorated plasticizers using GC−FPD and by phthalate esters using GC−MS (Di Bella et al., 1996; Dugo et al., 1997; Saitta et al., 1997; Di Bella et al., 1999). In this study we have investigated the contamination of Italian citrus essential oils by another class of plasticizers, namely chloroparaffins. Short-chain chlorinated paraffins are classified as probably carcinogenic to humans, medium-chain chlorinated paraffins are considered to be unclassifiable with respect to carcinogenesis in humans, and long-chain chlorinated paraffins are classified as possibly carcinogenic to humans (Chan et al., 1994). Chloroparaffins are toxic by ingestion and subcutaneous routes (Sax et al., 1989).

No recent studies on chloroparaffin residues in citrus essential oils are to be found in the literature. Thus, it seemed timely to investigate the presence of these contaminants in Italian citrus essential oils by dual-channel GC−ECD.

MATERIALS AND METHODS

Sampling. 102 lemon oils, 98 orange oils, and 96 mandarin oils were analyzed. All were produced in the crop years 1994−1996 and were commercially available on the Sicilian and Calabrian markets. All samples were collected in small glass bottles and stored at 4 °C under nitrogen atmosphere until analysis.

Standards. Chloroparaffins mixture standard from C6 to C18 was purchased from Witco Corporation (Houston, TX). A 5-ppm standard solution was prepared in *n*-hexane. Silica gel (70−230 mesh) was obtained from Merck (Darmstadt) and dichloromethane from Baker (Deventer, Holland).

Cleanup Procedure. The procedure followed was the same as that previously developed for the determination of organochlorine pesticide residues in citrus essential oils (Saitta et al., 1995; Dugo et al., 1997). A 10-mm i.d. × 500-mm length glass column with a Teflon stopcock was used. Silica gel was activated at 550 °C for 3 h. The chromatographic column was packed with a slurry of silica gel (8 g) in dichloromethane and the excess solvent was then drained off. A 0.2-mL aliquot of essential oil was introduced into the column and the elution was performed with 30 mL of dichloromethane: the first fraction (0−12 mL) was discarded and the second one (12−30 mL) was collected. This sample was then concentrated to 0.5 mL under a gentle stream of nitrogen and directly analyzed by gas chromatography.

Gas Chromatography. The levels of chlorinated paraffins were determined with a dual-channel Shimadzu GC-17A gas chromatograph fitted with Restek RTX-5 (30 m × 0.32 mm, 0.25 μm film thickness) and Restek 1701 (30 m × 0.32 mm, 0.25 μm film thickness) columns operating in the splitless mode. Helium was used as carrier gas at a constant flow rate of 36 cm/sec. The injector temperature was maintained at 230 °C. The column oven was temperature-programmed from an initial value of 50 °C (2 min hold) to 150 °C at a rate of 25 °C/min, then to 270 °C at 4 °C/min (20 min hold).

Chloroparaffin Recovery Assay. A chloroparaffins standard mixture solution was added to a distilled lemon essential oil up to a concentration of 5 ppm. After the cleanup procedure the solution was analyzed by GC−ECD. After six runs the average recovery was 99.4 ± 1.8%.

RESULTS AND DISCUSSION

The chromatogram of the standard solution is shown in Figure 1. Because of the high number of isomers the chromatogram is quite complex and shows a characteristic pattern with numerous peaks, some of which are broad. The detection limit was determined to be 1 ppm, based on the sum of all the isomers present.

Quantitative analysis of the chloroparaffin residues was carried out by the direct calibration method. A standard solution of known concentration was injected before and after every essential oil sample. Determination was based on the height of the highest peak, because this occurred in an area of the chromatogram which was free from interference by organochlorine pesticides (Figure 2).

Lemon Essential Oils. The ECD chromatogram of a lemon oil sample is shown in Figure 2. The levels of

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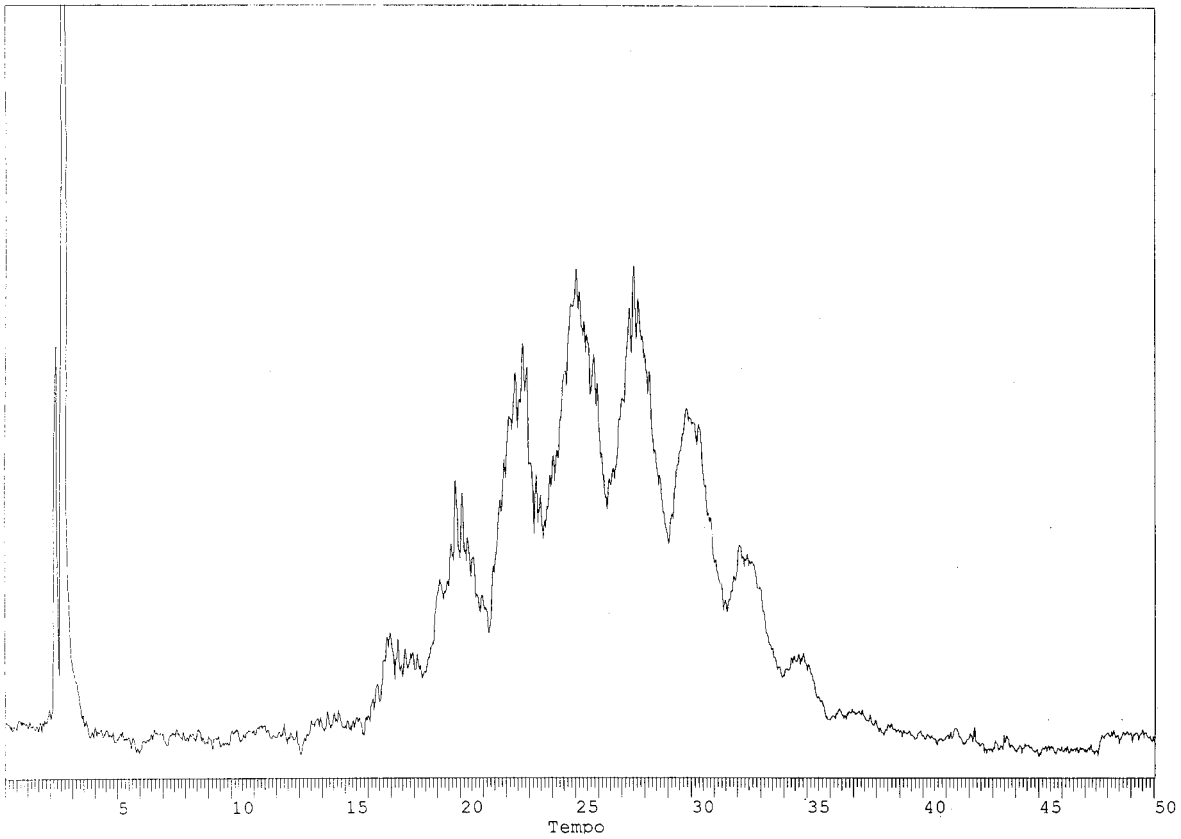

Figure 1. GC−ECD chromatogram of chloroparaffin standard.

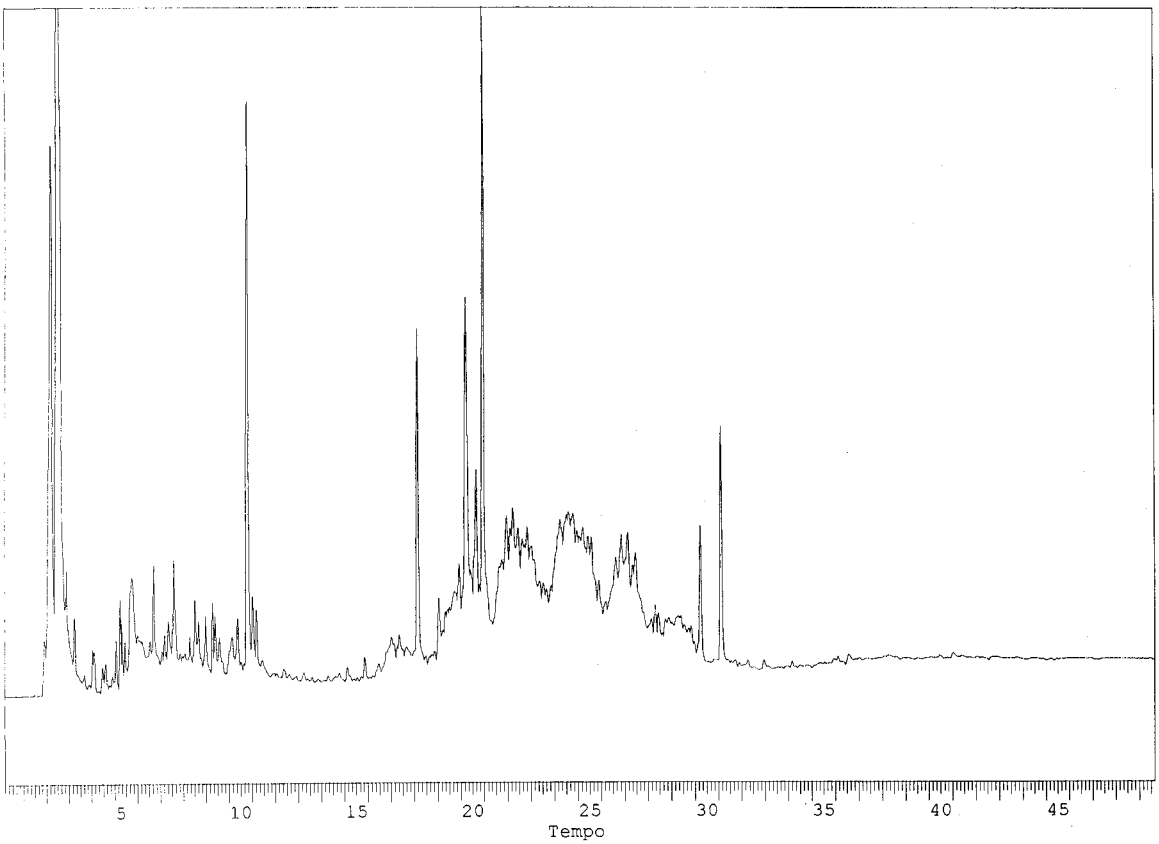

Figure 2. GC−ECD chromatogram of lemon essential oil sample.

chloroparaffin present in the lemon oil samples are reported in Table 1. The highest concentration found was 60 ppm, whereas the average was 7.1 ppm. In Figure 3 the frequencies of the levels of chloroparaffin

Table 1. Chloroparaffin Residues in Citrus Essential Oils Produced in the Crop Years 1994−1996

citrus essential oils (number of samples)	chloroparaffin mean value (mg/Kg)	concentration range[a] (mg/Kg) (min−max)	number of contaminated samples
lemon (102)	7.1	n.d. − 60	54 (53%)
orange (98)	2.5	n.d. − 25.6	33 (33%)
mandarin (96)	5.3	n.d. − 25	41 (38%)

[a] n.d. = not detectable (<1 ppm).

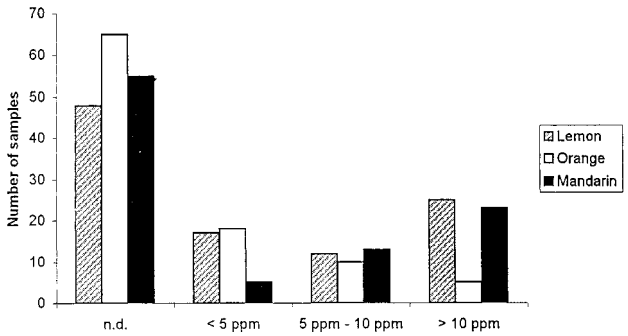

Figure 3. Citrus essential oils: frequencies of the chloroparaffin levels.

are reported: the concentration of chloroparaffin was above the detection limit in 53% of samples and it was higher than 10 ppm in 25% of them.

Orange Essential Oils. The chloroparaffin residues were above the detection limit in 33% of the orange oil samples analyzed (Table 1 and Figure 3). The highest level of contamination in these samples was 25.6 ppm.

Mandarin Essential Oils. Mandarin essential oils showed levels of contamination similar to those of orange essential oils: the chloroparaffin residues were above the detection limit in 38% of the samples (Table 1 and Figure 3). The highest concentration found was 25 ppm and the average concentration was 5.3 ppm.

CONCLUSIONS

Of the citrus essential oils analyzed, the lemon oils were the most contaminated, with a higher percentage of contaminated samples and, within these, a higher average chloroparaffin concentration. Earlier studies have shown that mandarin essential oil samples contained the highest phthalate ester residues, whereas lime oil samples showed the highest levels of contamination by phosphorated plasticizers **(**Di Bella et al., 1996; Dugo et al., 1997; Saitta et al., 1997; Di Bella et al., 1999). Taken together, these results suggest that the contamination of citrus essential oils by plasticizers does not depend on the nature of the oil, but probably correlates to some procedure used during the production cycle or in the storage of the essential oils. Future studies will be devoted to establishing the causes of plasticizer contamination, and more specifically to an examination of the role played by the plastic materials with which the essential oils come into contact.

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