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Pesticide and Plasticizer Residues in Citrus Essential Oils from Different Countries

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Residue analyses are very important in the quality control of citrus essential oils. Organophosphorus and organochlorine pesticides, phosphorated plasticizers and chloroparaffins contamination were investigated by HRGC with FPD and ECD detectors in 120 citrus essential oils produced in Italy and in 70 from other countries in the crop year 2006-2007. Results showed that the largest pesticide quantities were found in oils from Brazil and Spain. The presence of such residues might be the result of an improper use of pesticide in citrus growing or of previous contamination of the extractors. However, the pesticide levels showed a measurable decrease in relation to past production years. The absence of phosporated plasticizers and chloroparaffins can be the result of either improvement of the procedures used during the production cycle or in the storage of the essential oils compared with previous years.

Keywords: pesticides, plasticizers, citrus essential oils, countries.

Essential oils are widely used in the alimentary, beverage, cosmetic and pharmaceutical sectors. Regulations are increasingly more strict in terms of residue levels of chemicals used for treatment because of their impact on public health and on the environment. For this reason it is necessary to guarantee genuineness and the quality of essential oils and to determine all organic contaminants, even if a maximum limit of residues has not been established by legislation for this product type. Several methods have been developed to control products on the market. Referring to previous studies [1a-1i], the aim of this research was to determine, both qualitatively and quantitatively, the organophosphorus and organochlorine pesticides, phosphorated plasticizer and chloroparaffin residues in various citrus essential oils from different countries (Table 1), and to evaluate their actual state of contamination.

Organophosphorus pesticide (Table 2) and phosphorated plasticizer residues were determined by HRGC-FPD, and organochlorine pesticide (Table 3) and chloroparaffin residues by HRGC-ECD. Tables 4 and 5 report the mean concentration of contaminated samples (from Italy, Spain, Argentine, Brazil and South Africa) by organophosphorous and organochlorine pesticide residues and the percentage of contaminated samples. Most Italian lemon oils were contaminated by organophosphorus pesticides: in particular, 80% of

Table 1: Analyzed samples of citrus essential oils from different countries.

Samples	Italy	Spain	Brazil	Argentine	South Africa
Lemon	30	-	10	10	10
Orange	30	-	10	-	-
Green mandarin	15	-	10	-	-
Yellow mandarin	15	-	10	-	-
Clementine	-	10	-	-	-
Bergamot	30	-	-	-	-

Table 2: Detection limits (pg) for the analyzed organophosphorus pesticides.

Pesticides		Pesticides	
Acephate	15	Malathion	5
Aldicarb	10	Methamidophos	15
Ethyl azinphos	7	Methidathion	5
Methyl azinphos	16	Ethyl parathion	5
Carbaryl	5	Methyl parathion	5
Ethyl chlorpyriphos	5	Phenthoate	5
Methyl chlorpyriphos	6	Phorate	10
Diazinon	4	Phosalone	10
Dimethoate	22	Phosmet	5
Ethion	1	Methyl pirimiphos	5
Fenamiphos	10	Pyridafenthion	7
Fenitrothion	5	Quinalphos	5
Fenthion	5	Triazophos	15

Table 3: Detection limits (μg/L) for the analyzed organochlorine pesticides.

Pesticides		Pesticides	
Aldrin	30	Dicofol (op'+ pp')	40
α- β- and δ- BHC	55	Dieldrin	20
γ- BHC (Lindane)	20	trans - Difenoconazol	60
Bromopropylate	50	Endosulfan	40
Bromophos-methyl		Endrin	30
Captafol	40	Folpet	60
Chlordane	25	Hexachlorobenzene	45
Chlorfenvinphos	35	Heptachlor + Heptachlor epoxid	50
Clozolinate	45	Methoxychlor	20
o,p'- DDD	30	Penconazol	40
p,p' - DDD	50	Procymidone	50
p,p' - DDE	30	Quintozene	30
o,p'- DDT	70	Tetradifon	20
p,p' - DDT	70	Vinclozolin	35

Table 4: Mean value of contaminated Italian citrus oils by organophosphorus and organoclorine pesticide residues (mg/L ± standard deviation) and percentage of contaminated samples.

		Mean value ± s.d.	Percentage of contaminated samples
Lemon oils			
	Methyl azinphos	0.55±0.10	10.0
	Ethyl chlorpyriphos	0.20±0.06	83.3
	Methyl chlorpyriphos	0.20±0.06	80.0
	Methidathion	0.55±0.10	80.0
	Pyridafenthion	0.35±0.09	13.3
	Dicofol	0.41±0.10	50.0
Orange oils			
	Methyl azinphos	0.24±0.15	86.7
	Ethyl chlorpyriphos	1.56±0.84	80.0
	Methyl chlorpyriphos	1.44±0.73	30.0
	Fenthion	0.64±0.30	30.0
	Malathion	0.82±0.41	26.7
	Pyridafenthion	1.97±0.90	66.7
Yellow mandarin oils			
	Ethyl chlorpyriphos	2.19±0.58	20.0
	Methyl chlorpyriphos	0.94±0.23	13.3
	Phentoate	0.20±0.06	6.7
	Pyridafenthion	0.15±0.05	33.3
	Dicofol	0.61±0.10	33.3

these oils presented a methidathion mean content of 0.55 mg/L and methyl and ethyl chlorpyriphos means of 0.20 mg/L, while methyl azinphos and pyridafenthion residues were present in about 10% of citrus oils. Dicofol was the only recovered organochlorine pesticide at a concentration of 0.41 mg/L in 50% of the samples. In lemon oils from Brazil and Argentina, only ethyl chlorpyriphos and dicofol were present, respectively, in about 2 mg/L and 0.70 mg/L. Methidathion was also determined in 30% of samples from South Africa; this last also showed the highest dicofol concentration (2.50 mg/L).

In orange oils from Italy, ethyl and methyl chlorpyriphos were in high concentrations (1.44 and 1.56 mg/L respectively). Methyl azinphos was present at a smaller level (0.24 mg/L), but in a great number of samples. Residues of organochlorine pesticides were not detected. In Brazilian oils, among the organophosphorus pesticides, methidathion was higher in content (mean value 4.59 mg/L) in many samples, whereas among the organochlorine pesticides, dicofol (3.88 mg/L) was found in 50% of oils.

In Italian yellow mandarin oils, ethyl chlorpyriphos was revealed in 20% of samples with a concentration of 2.19 mg/L; dicofol was the only organochlorine pesticide determined in 33% of samples (mean value 0.61 mg/L); while, in oils from Brazil, only ethyl chlorpyriphos (2.63 mg/L) and malathion (4.80 mg/L) were detected in 70-80% of samples. No residues of any type of pesticide were found in green mandarin oils from Italy whereas, on the contrary, in the Brazilian samples, methidathion was in the highest concentration (mean value 0.34 mg/L) in 40% of oils, while methyl azinphos (0.22 mg/L) and ethyl chorpyriphos (0.08 mg/L) were present in 60% of oils.

Italian bergamot oils showed no organophosphorus and organochlorine contamination: these results confirmed the trend already observed in a previous study [1e] and should probably be connected to the strong resistance of fruits to insect attack and the reduced use of pesticides compared with their use on other citrus species. Clementine oils from Spain had the highest levels of contamination, ranging from 2.00 to 8.50 mg/L. Eighty percent of oils were contaminated by ethyl chorpyriphos (mean content 8.50 mg/L) and about 60% by organochlorine pesticides, such as bromopropilate (3.50 mg/L) and dicofol (2.00 mg/L).

Regarding Italian citrus oils, the pesticide concentrations found in this research were smaller than those determined in samples produced between 1983 and 1991: in fact, in lemon oils methidathion was in the range 0-201 mg/L and dicofol in the range 0-4.8 mg/L. This trend can also be observed for orange oils, which showed not only malathion (range 0-1.17 mg/L) and methyl azinphos (0-5.95 mg/L), but also dicofol residues (0-5.02 mg/L) [1b]. Mandarin oils produced in 1983-1991 were more contaminated compared with the 2006-2007 oils; in fact, they had phentoate in the range 0-1.26 mg/L and dicofol in the range 0-5.83 mg/L [1b].

In all Italian and other essential oil samples, phosphorated plasticizer and chloroparaffin residues were lower than the detection limit. Only in one sample of bergamot oil was a level of 5 mg/L of chloroparaffins determined. On the contrary, as evidenced in previous surveys, some Italian lemon, mandarin and bergamot oils were contaminated by triarylphosphate (range 0.12-2.95 mg/L) and chloroparaffin residues (range mean value 2.5-7.1 mg/L) [1c,1d]. Results from oils produced

Table 5: Mean value of contaminated citrus oils from various countries by organophosphorus and organochlorine pesticide residues (mg/L ± standard deviation) and percentage of contaminated samples (n.d.: not detectable).

		Brazil	%	Argentine	%	South Africa	%	Spain	%
Lemon oils									
	Ethyl chlorpyriphos	2.22±0.18	70.0	1.99±0.15	80.0	0.28±0.05	50.0		
	Methidathion	n.d.	-	n.d.	-	1.50±0.14	30.0		
	Dicofol	0.60±0.09	40.0	0.80±0.10	60.0	2.50±0.20	50.0		
Orange oils									
	Ethyl chlorpyriphos	0.60±0.15	60.0						
	Malathion	0.15±0.08	30.0						
	Methidathion	4.59±1.30	80.0						
	Bromopropilate	0.39±0.10	20.0						
	Dicofol	3.88±0.80	50.0						
Yellow mandarin oils									
	Ethyl chlorpyriphos	2.63±0.90	80.0						
	Malathion	4.80±1.60	70.0						
Green mandarin oils									
	Methyl azinphos	0.22±0.05	60.0						
	Ethyl chlorpyriphos	0.08±0.01	60.0						
	Methidathion	0.34±0.08	40.0						
	Pyridafenthion	0.19±0.05	30.0						
Clementine oils									
	Ethyl chlorpyriphos							8.50±2.30	80.0
	Fenthion							3.96±1.20	70.0
	Fenitrothion							2.00±0.94	30.0
	Methidathion							4.81±1.26	40.0
	Bromopropilate							3.50±1.18	50.0
	Dicofol							2.00±0.55	70.0

elsewhere are lacking, thus making any comparison impossible.

From these results, the following observations can be made:

- organophosphorus pesticides were commonly used; among which ethyl chlorpyriphos was detected in contaminated citrus oils;
- among the organochlorine pesticides, dicofol was recovered from many contaminated samples;
- in general, the largest quantities of pesticides were found in oils from Brazil and Spain;
- the presence of organophosphorus and organochlorine pesticide residues might be the result of improper usage of pesticide in citrus growing or of previous contamination of the extractors;
- pesticide residue levels in Italian citrus oils showed a measurable decrease for organophosphorus and organochlorine pesticides compared with past production years; which suggests that there is a trend towards reduction rational usage of pesticide treatments;
- the absence of phosporated plasticizers and chloroparaffins can be the result of an improvement of the procedures used during the production cycle or in the storage of the essential oils in respect to the previous crop years.

Experimental

Sampling*:* Samples (120) of cold-pressed citrus oils obtained from Italy and 70 from Spain, Argentina, Brazil and South Africa from the production season 2006/2007 were analyzed (Table 1). All samples were kept in small glass bottles at 4°C under nitrogen until analysis.

Chemicals: Twenty-six organophosphorus and 28 organochlorine pesticide standards (99,9%) were purchased from Chebios s.r.l. (Rome, Italy) (Tables 2 and 3). Phosphorated plasticizer standards (99,9%) were obtained from Carlo Erba (Milan, Italy): the solution was composed of 7 different classes of triarylphosphates. Chloroparaffin standards from C_6 to C_{23} (99,9%) were purchased from Witco Corporation (Houston, TX USA). Standard solutions were prepared in *n*-hexane and stored at 5°C in a refrigerator. Bromophos-methyl was used as the internal standard at a concentration of 1 mg/L for quantification by HRGC-FPD and HRGC-ECD. Silica gel (60-220 mesh), dichloromethane (>99,99%) and *n*-hexane (>99,99%) were obtained from J.T.Baker (Deventer, Holland).

Sample preparation for the determination of organophosphorus pesticide and phosphorated plasticizer residues: The citrus essential oil (0.2 mL) was added to 0.2 mL of the internal standard solution and was directly analyzed, without any clean-up procedures [1a-1c,1f-1h], using a FPD-Perkin Elmer

(Waltham, Massachusetts, USA) Clarus 500 gas chromatograph, equipped with a Restek (Bellefonte, PA, USA) RTX5-MS capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness). The injector and the detector were maintained at 260°C. The oven temperature was programmed from an initial value of 100°C to 170°C (10 min hold) at a rate of 25°C/min, then to 195°C at 10°C/min (0 min hold), and finally to 280°C at 7°C/min (5 min hold). Helium was used as the carrier gas (purity 99,9995 %) at a flow rate of 1.2 mL/min; inlet pressure 40 KPa. The detection limits of the analyzed pesticides, calculated according to the expression established [2], are reported in Table 2. Since the quantification of organophosphorus pesticides and phosphorated plasticizers was carried out at the same time, the quantitative analysis was executed by the internal standard method using a 1 mg/L bromophos-methyl standard solution. Under these conditions, a detection limit of 0.01 mg/L of total triarylphosphates was achieved [1c,1f-1h].

Sample preparation for the determination of organochlorine pesticide and chloroparaffin residues: For the clean-up procedures a 10 mm i.d. x 500 mm length glass column with a teflon stopcock was used. The column was packed with 8 g silica gel, previously activated at 550°C for 3 h, in dichloromethane. A 0.2 mL sample of essential oil was introduced into the column and eluted with 30 mL dichloromethane: the first fraction (0-12 mL) was discharged, the second one was collected (12-30 mL) and the solvent was evaporated under a current of nitrogen; 0.2 mL of a solution of the internal standard was added to the sample and was directly analyzed by GC/ECD. Organochlorine pesticide and chloroparaffin residues were determined with a ECD-Perkin Elmer Clarus 500 gas chromatograph, equipped with a Restek RTX5-MS capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness), operating in splitless mode (2 min), then split (1:10). Helium was used as the carrier gas at a flow rate of 1.5 mL/min; inlet pressure 128 KPa. The injector and detector were maintained at 350°C. The column temperature was programmed from an initial value of 150°C to 300°C (10 min hold) at a rate of 4°C/min.

Table 3 reports the detection limits of the analyzed pesticides, calculated according to [2]. A distilled lemon essential oil was used to calculate the recoveries by adding a solution of the organochlorine pesticides to reach pesticide concentrations of 1 mg/L. After the cleanup procedure, the internal standard was added and the solution was then analyzed by GC-ECD. This procedure was repeated 6 times; recovery percentage was $\geq$94.0 %. The quantitative analysis of the total chloroparaffins was conducted by direct calibration, using the above-mentioned standard mixture.

The peak height following the highest part of the chromatogram was taken into account. Because of the large number of isomers, which give the chromatogram a characteristic appearance, the detection limit was estimated to be 1 mg/L based on the sum of all the isomers present [1d,1f-1h]. To calculate chloroparaffin recoveries, a standard mixture solution was added to a distilled lemon essential oil up to a concentration of 5 mg/L. After the cleanup procedure, the solution was analyzed by GC-ECD. After 6 trials, the average recovery was 99.7 ± 1.9%.

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References

[1] (a) Leoni V, D'Alessandro De Luca E. (**1978**) An important aspect of the health problem caused by pesticides: the presence of organophosphorus insecticide residues in essential oils. *Essenze Derivati Agrumari*, ***48***, 39-50; (b) Dugo G, Saitta M, Di Bella G, Dugo P. (**1997**) Organophosphorus and organochlorine pesticide residues in Italian citrus oils. *Perfumer & Flavorist*, ***22***, 33-44; (c) Saitta M, Di Bella G, Bonaccorsi I, Dugo G, Della Cassa E. (**1997**) Contamination of citrus essential oils: the presence of phosphorated plasticizers. *Journal of Essential Oil Research*, ***9***, 613-618; (d) Di Bella G, Saitta M, Lo Curto S, Lo Turco V, Visco A, Dugo G. (**2000**) Contamination of Italian citrus essential oils: presence of chloroparaffin. *Journal of Agricultural and Food Chemistry*, ***48***, 4460-4462; (e) Saitta M, Di Bella G, Salvo F, Lo Curto S, Dugo G. (**2000**) Organochlorine pesticide residues in Italian citrus essential oils, 1991-1996. *Journal of Agricultural and Food Chemistry*, ***48***, 797-801; (f) Di Bella G, Saitta M, Lo Curto S, Salvo F, Licandro G, Dugo G. (**2001**) Production process contamination of citrus essential oils by plastic materials, *Journal of Agricultural and Food Chemistry*, ***49***, 3705-3708; (g) Di Bella G, Saitta M, La Pera L, Alfa M, Dugo G. (**2004**) Pesticide and plasticizers residues in bergamot essential oils from Calabria (Italy). *Chemosphere*, ***56***, 777-782; (h) Di Bella G, Serrao L, Salvo F, Lo Turco V, Croce M, Dugo G. (**2006**) Pesticide and plasticizers residues in biological citrus essential oils from 2003-2004. *Flavour Fragrance Journal*, ***21***, 497-501; (i) Torres CM, Picó Y, Redondo MJ, Mañes J. (**1996**) Matrix solid-phase dispersion extraction procedure for multiresidue pesticide analysis in oranges. *Journal of Chromatography A*, ***719***, 95-103.

[2] Pharmeuropa. The European Pharmacopoeia Forum. (**1999**) *Technical guide for the elaboration of monograph*. 3rd edition, 66.