A Rapid Method of Analysis of Organophosphate Pesticides in Mandarin Essential Oils

Detection and hazard assessment of organophosphate pesticides in industrially distilled and cold-pressed mandarin oils

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Pesticides belong to the family of phytosanitary drugs (plant drugs, from the Greek *phyton*). They are largely used in agriculture against animal and vegetable parasites to ensure the quality and productivity of harvest. Despite the health risks and hazards of pesticides for the environment due to their toxicity and persistence, their use is considered essential in modern intensive agriculture so long as they are subject to careful and rigorous controls in terms of application method, frequency, quantity of use and picking times after the last treatment.

Depending on the type of organisms they are used against, pesticides are classified as follows:

- Fungicidals are used to fight cryptogams such as downy mildew, powdery mildew, anthracnose, rust and anthrax.
- Insecticides are aimed at controlling aphids, thrips, aleuroids, red spiders, their grubs and eggs, which are responsible for diseases of apples, pears, grapevines, citrus fruits, etc. It is common opinion that insecticides are toxic to humans, although they are well tolerated by plants. Damages can be due to a toxic action that is either immediate or the result of accumulation; insecticides therefore need to be handled with extreme caution given that they can easily penetrate the skin through contact. For example, organophosphates have an immediate toxic action, blocking the activity of some enzymes, particularly those responsible for nervous system functions.
- Weed killers, or herbicides, present a labor-efficient and economical solution for the control of weeds. Herbicides act on the weeds by direct contact, or systematically through a radical absorption or by blocking vital processes. Other hormonal compounds provoke irregular growths.
- Rat poisons are used for the control of rats and mice.
- Fumigants are used to control insects, rodents and soil nematodes. All are in gas form and therefore can be used in places that are difficult to access—places where it would be impossible to use other types of control processes, such as storehouses and grain silos, rodents' nests, etc.

In terms of their chemical composition, pesticides are classified as follows: organophosphates, organochlorine compounds, carbamates, arsenic and its derivatives, polysulfides, organic tin compounds, coumarin derivatives, organo-nitro compounds, carbon tetrachloride, and carbon disulphide.

Organophosphate Pesticides

Organophosphate pesticides are esters of dialkylthiophosphoric and dialkyldithiophosphoric acid. (**See F-1 and F-2.**) They count among the hundreds of phosphorated compounds with diversified chemical, physical and biological properties and variable behavior. They can be used as fungicidals, weed killers and chemical sterilizers against insects.

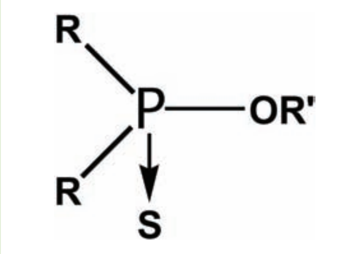
The dangerous effects of organophosphate pesticides are mitigated by their limited stability due to photodegradability and hydrolyzability. As a result, poisoning caused by ingestion of food rich in organophosphate residues is rare. Only an accumulation due to continuous ingestion of treated food can cause phenomena of chronic toxicity. For some time now researchers have been trying to

At a Glance

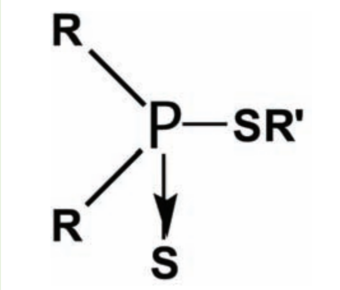
In order to quantify some organophosphate pesticides in industrial cold-pressed mandarin essential oils obtained from Calabrian fruits (Italy), a rapid and simple gas-chromatographic method using capillary columns and a flame photometric detector (FPD) was conducted. The results show that in cold-pressed oils from the 2005–2007 seasons, the following common citrus pesticides were found to be present in decreasing order of concentration: tetrachlorvinphos, methyl parathion, ethyl chlorpyrifos, Malathion, ethyl parathion, chlorfenvinphos and prothiofos. In essential oils obtained through distillation, Malathion and alkyl parathions were found within the sensitivity limits of the method.

convince technicians and farmers to choose phytosanitary drugs more carefully, selecting those combining efficacy and reduced persistence in order to minimize such effects.[1,2] Symptoms of poisoning induced by organophosphates include: vomiting, diarrhea, dyspnoea, lacrimation and convulsions. Treatment is based on atropine and repeated cleansing with water and soap, and with bicarbonate solution to mitigate contact contamination.

Esters of dialkylthiophosphoric acid **F-1**



Esters of dialkyldithiophosphoric acid **F-2**



In previous articles, organochlorines, organophosphates and/or carbamate pesticides have been identified in essential oils from several species of citrus fruits: Navelina orange, bergamot, citron, clementine, mandarin and grapefuit.[3-6] Among insecticides, the following organophosphates are currently the focus of attention: methyl azinphos, chlorfenvinphos, ethyl chlorpyrifos, methyl chlorpiriphos, diazinon, dichlofenthion, disulfoton, ethion, fenchlorphos, Imidan, Malathion, methyl parathion, prothiofos, terbufos, tetrachlorvinphos, Tokuthion and Trichloroate. This article focuses on a gas chromatographic (GC) method with programmed temperature and a specific flame photometric detector (FPD) for a rapid analysis of organophosphoric pesticides in cold-pressed mandarin essential oils.

Experimental Part

Forty-four samples of industrial mandarin essential oil from Calabrian fruits (Italy) obtained by cold-press technology during the 2005–2006 season (25 samples) and 2006–2007 season (19 samples) were analyzed. Each sample was representative of a batch of 200 kg of mandarin oil originating from processing 40,000 kg of fruit. In the cold-pressing process, the mandarin fruits were cleansed in a tub, rinsed with water jets under pressure, brushed and then passed to a screw press. The liquid derived from the screw press underwent two subsequent centrifugations to completely separate the oil from the juice.

For comparison purposes, five samples of oil distilled with the "Peratoner" method were analyzed; this method consisted of recovering the oils from solid residues derived from centrifugation of a water-essential oil emulsion through vapor current distillation under ordinary or reduced pressure.[7] These five samples were derived from all processing waste of the 19 cold-pressed samples in the 2006–2007 season. The presence of organophosphate pesticides was determined through a GC with capillary columns and with a specific FPD. Thanks to this technique, which is very stable and highly selective, it was possible to directly inject oil samples into the GC without any preliminary treatment.

The instruments and experimental conditions adopted in the analysis were as follows: Thermoquest GC 2000 GC; Restek DB-5 column (30 m x 0.25 mm, film thickness 0,25 µm); split injector 1:10 at 250°C; injected volume, 2 µL of oil loaded with 1 mg/L of the internal standard (methyl bromophos); FPD at 250°C, equipped with interferential filter (λ= 254 nm); helium (He) as carrier gas, kept at a constant flux of 1.5 mL/min. The oven temperature was programmed as follows: 80°C for 1 min with a 25°C/min increment up to 173°C; after 2 min at 173°C, a 2°C/min increment up to 195°C; then a 25°C/min increment up to 270°C. This temperature was kept constant for 5 min. The data collection system consisted of a Compaq computer with Chromquest software, version 2.53. Identification of peaks was carried out through a comparison with the retention times relating to the internal standard, methyl bromophos, commercially available as a solution with a standard composition. The quantitative analysis was carried out through a calculation of the relation between the areas of the unknown peaks and the areas of peaks of standard mixtures, taking into account the reaction factors in relation to the internal standard.

The minimum concentration of detectable analyte (LOD) was 1 µg/kg, while the quantification limit (LOQ), calculated as 10 times the standard deviation of the background noise, corresponded to 4 µg/kg. **T-1** shows the absolute time of retention, the relative time of retention and the reaction factor in relation to the internal standard (methyl bromophos) for each pesticide.

F-3 shows FPD GC of one of the analyzed samples of cold-pressed mandarin oil.

Results and Discussion

T-2 shows the results relating to the analyzed samples. The results show that all mandarin oils contained organophosphate pesticides.

As far as the total average content is concerned, there are few differences among the oils produced in the 2005–2006 and 2006–2007 seasons. There are considerable differences, however, in the distribution of single pesticides between the two periods. The active principles constantly found in the two seasons (2005–2006 and 2006–2007) were: Malathion, present in 39 samples, corresponding to 89% of the samples; ethyl chlorpiriphos, present in 36 samples, corresponding to 82% of the samples. Both percentages were similarly distributed over the two seasons. The high frequency of these two active principles was due to the fact that they are part of broad-spectrum insecticide formulation (aphids, cochineals, etc.); for this reason they are largely used in citriculture. Methyl parathion and chlorfenvinphos were also quite common, present in 28 and 26 samples of the two seasons, respectively, with a frequency corresponding to 63% and 60% of the samples.

Retention times and reaction factors of active principles **T-1**

Active principle	Absolute time (min)	Relative time (min)	Reaction factor
Dylox + dichlorvos	4.596	0.161	4.982
Phosdrin	6.675	0.235	3.943
Prophos	11.606	0.408	0.633
O, O, O′, O′ tetraethyldithiopyrophosphate	13.461	0.473	0.295
Phorate	14.021	0.493	0.688
Demeton S	15.477	0.544	0.575
Dimethoate	16.314	0.573	3.079
Dioxathion	17.420	0.612	3.042
Terbuphos	17.626	0.619	0.780
Diazinon	18.750	0.659	0.754
Disulfoton	19.254	0.676	1.324
Dichlofenthion	22.446	0.789	1.383
Methyl clorpyriphos	23.443	0.824	1.735
Methyl parathion	23.737	0.834	1.004
Fenchlorphos	24.052	0.845	1.018
Fenthion	24.524	0.862	3.180
Malathion	26.384	0.927	1.072
Ethyl chlorpyriphos	27.446	0.964	0.446
Ethyl parathion	27.700	0.973	0.869
Trichloroate	28.229	0.992	1.791
Methyl bromophos (internal standard)	**28.462**	**1.000**	**1.000**
Fentoate	29.761	1.046	0.651
Chlorfenvinphos	29.391	1.033	2.109
Protiophos	29.879	1.050	3.555
Tetrachlorvinphos	30.643	1.077	3.870
Tokuthion	31.277	1.099	0.613
S,S,S, tributylphophorotrithioate	31.448	1.105	1.141
Fensulfothion	33.408	1.174	2.134
Ethion	33.209	1.167	1.285
Sulfoprophos	33.785	1.187	1.189
Famphur	34.522	1.213	3.362
Imidan (Phosmet)	37.403	1.314	4.224
EPN	37.309	1.311	1.747
Leptophos	38.946	1.368	4.514
Guthion	39.918	1.403	4.327
Ethyl guthion	42.359	1.488	3.568
Coumaphos	46.864	1.647	4.266

The most commonly found organophosphates in the oils produced in the 2006–2007 citrus season in decreasing order of concentration were: tetrachlorvinphos, methyl parathion, ethyl chlorpyrifos, Malathion, ethyl parathion, chlorfenvinphos and prothiofos. In the oils distilled using the "Peratoner" method from processing waste of the same fruits from which the cold-pressed oils were produced in the 2006–2007 season, residues of pesticides were found in a reduced quantity in two cases only: Malathion (0.1 and 0.05 mg/kg, respectively) and methyl parathion (0.04 and 0.02 mg/kg, respectively).

Organophosphate pesticides are now consistently found in industrially distilled and cold-pressed mandarin oils. Nevertheless, given that the oils are present in food and pharmaceutical products in a reduced quantity, the low level of pesticides found does not constitute a health hazard upon consumption. The lack or occasional low quantity of residues of phytosanitary products in essential oils, obtained through distillation ("Peratoner" method), confirms that pesticides, due to their liposolubility, are probably solubilized and fixed in the waxes and fats present in the non-volatile parts of the mandarin oil.

References

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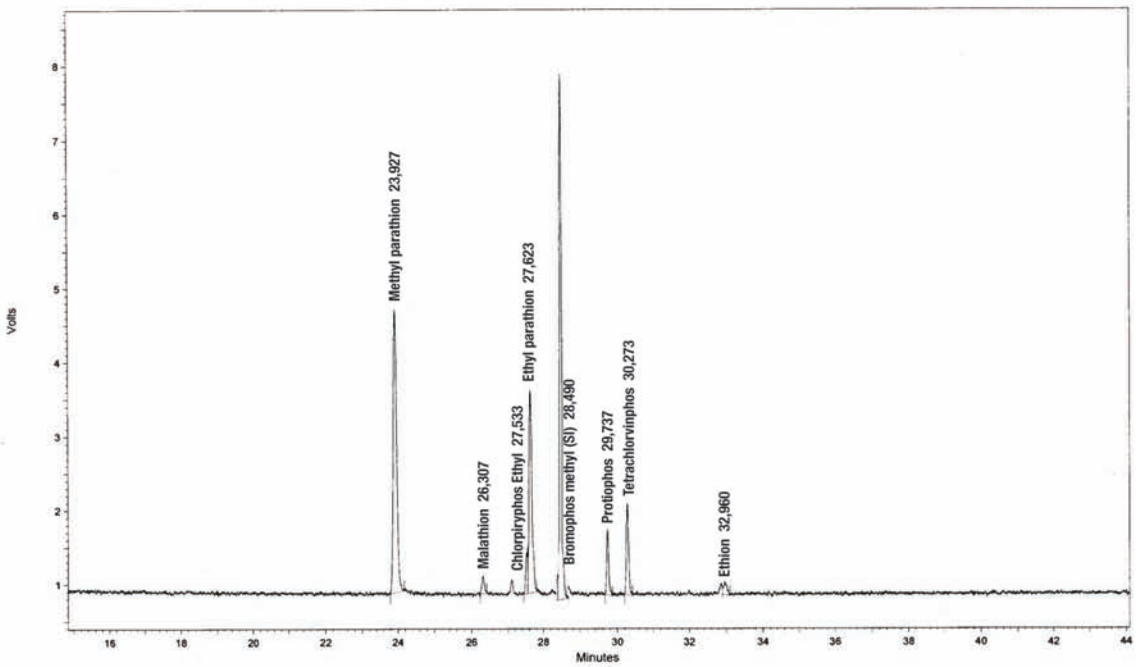
FPD gas chromatogram of a sample of cold-pressed mandarin oil F-3



Pesticides in mandarin essences* T-2

	2005–2006 season				2006–2007 season			
	Minimum	Maximum	Average	Frequency	Minimum	Maximum	Average	Frequency
Methyl azinphos	0.11	1.31	0.73	7				
Chlorfenvinphos	0.01	2.61	0.56	18	0.12	0.32	0.17	8
Ethyl chlorpirifos	0.01	0.31	0.11	18	0.53	1.61	0.98	18
Methyl chloropirifos	0.18	4.63	1.61	23				
Diazinon	0.00	0.16	0.03	7				
Dichlofenthion	0.01	0.01	0.01	1				
Disulfoton	0.01	1.40	0.70	14				
Ethion	0.01	0.01	0.01	1				
Fenchlorfos	0.02	0.02	0.02	1				
Imidan	0.12	0.92	0.45	7				
Malathion	0.01	0.25	0.11	22	0.05	1.04	0.36	17
Ethyl parathion					0.50	0.50	0.50	1
Methyl parathion	0.06	3.69	1.13	9	0.03	1.94	0.68	19
Prothiophos					0.02	0.02	0.02	1
Terbufos	0.01	0.04	0.01	20				
Tetrachlovinphos					0.10	12.36	3.09	16
Tokuthion	0.01	0.44	0.07	20				
Trichloroate	0.01	0.10	0.04	3				
Total content of pesticides	**0.82**	**8.58**	**3.41**	**24**	**1.23**	**13.61**	**4.79**	**19**

*mg/kg

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