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Gas chromatographic evaluation of pesticide residue contents in nectarines after non-toxic washing treatments☆

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Abstract

Washing with aqueous solutions of citric acid, ethanol, glycerol, hydrogen peroxide, potassium permanganate, sodium metabisulfite, sodium laurylsulfate (SLS), sodium hypochlorite, and urea is evaluated for pesticide residue reduction in nectarines and compared with simple tap water washing. Residues of pesticides commonly utilized in nectarines (chlorpyrifos, fenarimol, iprodione, malathion, methidathion, myclobutanil, parathion and pirimicarb) are extracted with ethyl acetate and anhydrous sodium sulfate, extract is concentred and analyzed by GC with nitrogen–phosphorus detection. The formation of possible toxic by-products (chlorpyrifos oxon, malaoxon, methidaoxon and paraoxon methyl) is studied by GC–MS. No toxic by-products are identified in the extracts of the washed samples for the washing-time and concentrations studied, but high levels of sodium hypochlorite, hydrogen peroxide and potassium permanganate form oxons from the organophosphorus pesticides. Ethanol, glycerol and SLS solutions removed near the 50% of the pesticide residues. The other solutions were not more effective than tap water washing. The amount of pesticide removed by washings is related to its water solubility and octanol–water partition coefficient.

Keywords: Pesticide residues; Organophosphorus compounds; Fruits; Nectarines; Oxidative treatments; By-products; GC; Washing

1. Introduction

The pesticides remaining in foods after field treatments generate toxicological, legal and commercial problems. The use of simple and non-toxic washing treatments to reduce such residues in fruit samples [1,2] can facilitate the commercialization and reduce the impact over the consumer health. The solutions for washing fruits must be of low toxicity and easily biodegradable in order to allow their use at home and at processing-food industries. Further economical, sensorial or nutritional aspects should be taken into consideration before use washing treatments for industrial purposes. Products currently used as food additives or for sanitary purposes could be selected for washings. In this way, citric acid, glycerol and sodium metabisulfite are authorized food additives in the EU (E330 antioxidant, E422 emulsifier, stabilizer, thickener and gelling agent, and E223 preservative, respectively). Citric acid is used as an acidulant and synergistic antioxidant in pharmaceutical preparations. Ethanol is itself a component of drinks and used as a bacteriostatic (95% (v/v) in the US Pharmacopoeia) and bactericide (70% (v/v)). Oral solutions of glycerol 50% (v/v) are included in the US Pharmacopoeia, glycerol is also used as humectant in cosmetics and soaps, drug vehicle and solvent for flavours and food colorants. Hydrogen peroxide is a very common disinfectant included in the US Pharmacopoeia (at 3% (v/v)). Potassium permanganate is an antiseptic and disinfectant that can be employed for drinking water treatment at 0.01% (w/v); additionally, it reacts with ethylene delaying the maturation of fruits. Sodium hypochlorite is a very good disinfectant for wa-

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ter (0.5–1.5 mg/l) or for cutaneous applications (0.15–0.5%). Sodium laurylsulfate (SLS) is an excellent dispersing agent for pharmaceuticals included in the US Pharmacopoeia at 20% (w/v) and used as whipping agent in foods in USA. Sodium metabisulfite is an antioxidant for pharmaceuticals and foods. Urea is present in biological fluids and used in pharmaceuticals and cosmetics. In addition, all these compounds are environmental friends because all they are natural substances and/or can be easily degraded in environment.

GC with nitrogen–phosphorous detection (NPD) allows determination of many of the most applied pesticides in nectarines such as chlorpyrifos, fenarimol, iprodione, malathion, methidathion, myclobutanil, parathion methyl and pirimicarb. However, reagents used for washes could produce toxic by-products that can be recognized by GC–MS. In this way, several authors reported that oxidative reagents such as ozone [3], chlorine [4,5] and permanganate [6] form derivative by-products from organophosphorus and urea pesticides.

Multiresidue extraction of pesticides from foods is commonly done with organic solvents [7] such as ethyl acetate [8,9], hexane [10], or acetone followed by dichloromethane re-extraction [6,11] and organic extracts are further analyzed by GC or LC.

In this study, several simple washes (immersion for 3 min followed by 15 s of sprayed tap water) are evaluated on nectarine samples which are previously treated with the doses of pesticides recommended by the producer and doses producing residues near the maximum residue levels (MRL) established in the FAO and EU regulations. The purpose of this study is to know the ability of such simple washes for reducing pesticide residues especially when the MRL are exceeded. In addition, the formation of toxic by-products is investigated by GC–MS.

2. Experimental

2.1. Chemicals

2.1.1. Pesticide standards

Chlorpyrifos (*O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl-phosphorothioate); fenarimol [2,4′-dichloro-α-(pyrimidin-5-yl)benzhydryl alcohol]; iprodione [3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxo-1-imidazolidinecarboxamide]; malathion [*S*-1,2-bis(ethoxycarbonyl)ethyl-*O*,*O*-dimethylphosphorodithioate]; malaoxon [*S*-1,2-bis(ethoxycarbonyl)ethyl-*O*,*O*-dimethylphosphorothioate]; methidathion (*S*-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl-*O*,*O* dimethyl phosphorodithioate); myclobutanil [2-*p*-chlorophenyl-2-(1H-1,2,4-triazol-1-ylmethyl hexanenitrile]; parathion methyl (*O*,*O*-dimethyl-*O*-4-nitrophenylphosphorothioate); paraoxon methyl (*O*,*O*-dimethyl-*O*-4-nitrophenylphosphate) and pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate) with purities up to 98% were obtained from Riedel-de Haen, Seelze, Germany.

2.1.2. Pesticide formulations

Choke containing 48% (w/v) of chlorpyrifos (Afrasa, Paterna, Spain); Rubigan Flow containing 12% (w/v) of fenarimol (Dow AgroSciences Iberica, Madrid, Spain); Rovral containing 50% (w/v) of iprodione (Aventis Crop Science España, Alcácer, Spain); Orothion 50 containing 50% (w/v) of malathion (Químicas Oro, San Antonio de Benageber, Spain); Ultracid 40EC containing 40% (w/v) of methidathion (Syngenta Agro, Madrid, Spain); Systhane Forte containing 24% (w/v) of myclobutanil (Dow AgroSciences); MetilOro containing 20% (w/v) of parathion methyl (Químicas Oro); Aphox containing 50% (w/w) of pirimicarb (Syngenta Agro).

2.1.3. Reagents

Citric acid 1-hydrate, ethanol absolute, ethyl acetate for residue analysis, glycerol, potassium permanganate and sodium metabisulfite were from Merck (Darmstadt, Germany). Hydrogen peroxide (30% (v/v)) was from Peróxidos Farmacéuticos Foret (Barcelona, Spain). Sodium hypochlorite (10% (w/v)), sodium laurylsulfate (SLS), sodium sulfate anhydrous and urea were from Scharlau Chemie (Barcelona, Spain).

2.2. Apparatus

Routine analyses were carried out on a Varian Star 3400CX gas chromatograph (Varian, Walnut Creek, CA, USA) equipped with an 8200CX autosampler, an on-column injector (SPI 1093) containing a glass insert, an NPD system and a Varian Star Chromatography Workstation Version 4.51.

Identification of peaks was performed in a Trace GC gas chromatograph (Thermo Electron Corporation, San José, CA, USA) with a Thermo-Finnigan AS 2000 autosampler, a split/splitless injector, a Trace MS quadrupole mass spectrometer and Xcalibur software.

Compounds were separated on DB-5 ms (J&W Scientific, Folsom, CA USA) fused-silica capillary columns (30 m × 0.25 mm i.d., 0.25 μm film thickness).

2.3. Chromatographic conditions

At the Varian gas chromatograph, the injector and detector were set at 280 °C and 300 °C, respectively. The oven temperature was programmed as follows: initial temperature, 140 °C; held for 1 min, programmed to 280 °C, at 5 °C/min, and held for 11 min. Helium was used as carrier gas at a flow rate of 1.9 ml/min.

At the Trace gas chromatograph injector and transfer line were set at 280 °C, the source temperature at 230 °C. The oven temperature was programmed as follows: the initial temperature (50 °C) was held for 1 min, programmed to 120 °C at 30 °C/min, held for 1 min, then programmed to 275 °C at 5 °C/min, and held for a further 5 min. The mass spectrometer was used with electron impact ionization (−70 eV) in

Table 1
Pesticide concentrations of the aqueous mixtures for immersing nectarine pieces, the remaining residues after 24 h of pesticide application, and before washing and maximum residue levels (MRLs)

Pesticide	Applied pesticide mixture (mg/l)		Remaining pesticide residue after 24 h (mg/kg)		MRL (mg/kg)	
	Level 1	Level 2	Level 1	Level 2	FAO	EU
Chlorpyrifos	720	24	5.91	0.19	0.2	0.2
Fenarimol	24	8.4	1.80	0.59	14	0.5
Iprodione	500	100	14.61	4.81	10	5
Malathion	1000	50	9.52	0.48	6	3
Methidathion	400	20	8.22	0.38	0.2	0.2
Myclobutanil	48	9.6	2.49	0.47	0.5	0.5
Parathion methyl	160	6	5.71	0.22	0.2	0.2
Pirimicarb	500	17	15.01	0.53	0.5	0.5

FAO, Food and Agriculture Organization; EU, European Union.

full scan mode (65–365 u). The carrier gas was helium at 1.5 ml/min. Splitless time was 0.9 min.

2.4. Samples treatment

Nectarine samples were from organic farming without use of pesticides provided by a rural cooperative. The samples utilized for all the assays did not contain residues of pesticides.

Entire nectarines were deepened for 1 min in the aqueous mixtures of pesticide formulations (levels 1 and 2, see Table 1), then wait for 24 h at room temperature in a fume hood. After that, nine pieces were used for investigating the initial residual level of pesticides and other nine pieces were necessary for performing each washing treatment.

The washing treatments consisted of immersing the pieces of the previously pesticide-dipped nectarines in an aqueous washing solution for 3 min, followed by spraying for 15 s with tap water (16–20 °C) with gentle rotation by hand. The assayed washing solutions were the following: (a) 5% (w/v) citric acid; (b) 70% (v/v) ethanol; (c) 15% (v/v) glycerol; (d) 3% (v/v) hydrogen peroxide; (e) 25 mg/l potassium permanganate; (f) 70 mg/l sodium hypochlorite; (g) 5% (w/v) SLS; (h) 5% (w/v) sodium metabisulfite; (i) tap water; (j) 15% (w/v) urea; and (k) a mixture containing 15% (v/v) of glycerol, 70% (v/v) of ethanol and 5% (w/v) of SLS.

2.5. Extraction method

A 50 g portion of sample previously homogenized was weighed in a 500 ml beaker, 100 ml of ethyl acetate and 75 g of anhydrous sodium sulfate were added and the mixture was blended using a home stainless steel-armed blender for 5 min, the resulting mixture was filtered through a thin layer of 20 g of anhydrous sodium sulfate. The solid was washed with 50 ml of ethyl acetate and the organic extract was concentrated to less than 10 ml (dryness was avoided) on a vacuum rotary evaporator using a water bath at 44–46 °C. Finally, the extract was made to 10 ml with ethyl acetate and 2 μl of it was analyzed by GC.

3. Results and discussion

3.1. Validation studies

All the validation studies were performed with previously analyzed pesticide-free nectarines. The linearity in the response was studied by using matrix-matched calibration solutions. Eight point (average of two injections) calibration curves based on peak area data were constructed in the concentration range 0.35–100 μg/ml (equivalent to a pesticide residue of 0.07–20 mg/kg). The resulting correlation coefficients were higher than 0.994 in all cases. The calibration curves were used for quantification purposes. Very high concentrations of pesticides were included within the calibration curves in order to know the actual chromatographic behaviour that reproduces extreme conditions in which the field treatments of fruits should give the highest level of residues that can be found (fruits collected 24 h after the field treatment with commercial pesticides). The injection of such high concentrations of pesticides overloaded the column producing correlation coefficients relatively low (0.994) and increasing the uncertainty.

Recovery assays were done in triplicate by spiking homogenized samples of nectarines at the two levels indicated in the Table 1 as “remaining pesticide residue after 24 h (mg/kg)”. The mean recoveries obtained with spiked samples at the level 2, ranged from 85% to 106% with relative standard deviations (R.S.D.s) below 10%. Nevertheless, variability found when analyzing samples immersed in pesticide mixture formulations was higher (n = 3, R.S.D.s below 15%), this indicate that distribution of the residues of pesticides was not fully homogeneous among all the nectarine pieces. The glass insert was replaced after 20–25 injections to minimize the peak tailing produced by the direct injection of non-purified extracts in the on-column system.

The limits of detection (LODs) (analyte concentration producing a S/N = 3) were verified by analyzing pesticide mixtures at such concentration levels in matrix extracts. LODs ranged from 0.006 mg/kg for parathion methyl to 0.028 mg/kg for pirimicarb, allowing the correct quantification of the selected pesticides at the maximum residue lev-

Table 2
Percent of pesticide residue removed from nectarines by washing treatments (n = 3) and relative standard deviation (R.S.D. (%), in parenthesis)

Pesticide	Water		SLS		H_2O_2		Glycerol		Ethanol	
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
Chlorpyrifos	26 (8)	32 (8)	63 (8)	72 (9)	31 (7)	42 (7)	62 (9)	49 (10)	79 (6)	74 (7)
Fenarimol	30 (10)	17 (9)	46 (11)	34 (10)	44 (9)	35 (10)	64 (15)	33 (15)	51 (8)	51 (9)
Iprodione	10 (8)	15 (9)	61 (14)	39 (15)	31 (9)	24 (8)	59 (10)	48 (10)	60 (11)	55 (9)
Malathion	11 (9)	8 (6)	44 (7)	43 (6)	16 (5)	14 (6)	41 (7)	30 (6)	66 (6)	65 (8)
Methidathion	7 (7)	13 (7)	33 (10)	20 (13)	12 (8)	7 (9)	39 (9)	21 (10)	60 (8)	63 (9)
Myclobutanil	15 (12)	25 (11)	47 (10)	53 (12)	6 (10)	16 (12)	28 (10)	19 (11)	42 (9)	45 (10)
Parathion methyl	15 (5)	10 (6)	45 (6)	44 (7)	22 (8)	7 (8)	38 (9)	25 (9)	66 (5)	63 (8)
Pirimicarb	34 (8)	31 (9)	16 (9)	24 (10)	15 (9)	22 (11)	11 (9)	10 (9)	26 (10)	36 (11)

SLS, sodium laurylsulfate.

els (MRLs) permitted in the FAO and EU regulations (see Table 1).

3.2. Samples treatment

The washing treatments were studied at two concentration levels, the higher one was obtained after application of pesticide formulations in accordance with the label instructions (level 1), the lower one was applied with the intention of produce levels of residues close to the MRL of the EU (level 2). Initially, reagents for washing were chosen in order to solubilize and/or chemically degradate the residues of pesticides. Results of the more effective treatments in decreasing pesticide residues are presented in Table 2.

Fig. 1 illustrates the utility of the ethanol washing for reducing the level of pesticide residues by showing the NPD profiles of a nectarine sample containing the level 1 of pesticide residue before and after the ethanol washing. No peaks from matrix appear at such level according to the high selectivity of the NPD towards the selected pesticides. Ethanol is the most effective removing residues of selected pesticides, near of a 60% of the residues were eliminated from the nectarine, followed by SLS with a 41–44% of residue eliminated and by glycerol with 30–43% of residue eliminated. Simple wash with tap water reduced the residues in a 20% as a whole. Washes with citric acid, hydrogen peroxide, potassium permanganate, sodium hypochlorite, sodium metabisulfite, and urea solutions produced results without significant differences (ν = 23, α = 0.05, t = 2.069) with those obtained with the tap water. No significant differences between the two levels of remaining residues studied were observed and no relationship is found between structure of the pesticide and efficacy of the washing. The uncertainty observed does not allow distinguish small differences within the washing treatments, such occasions are in the practice of little importance. On the contrary, when the differences are of mathematical signification it means that the amount of removed residue is of considerable magnitude and consequently useful for reducing residues.

Effectiveness of a determined washing solution seems to be related with solubility of pesticides in it, ethanol is very effective because all the selected pesticides have high solubilities in it (>30 g/l, see Table 3), whereas pirimicarb that is the most soluble in water (2.7 g/l, see Table 3) is removed in rather the same percent with any wash because all them are water-based solutions. Oxidizing (hydrogen peroxide, potas-

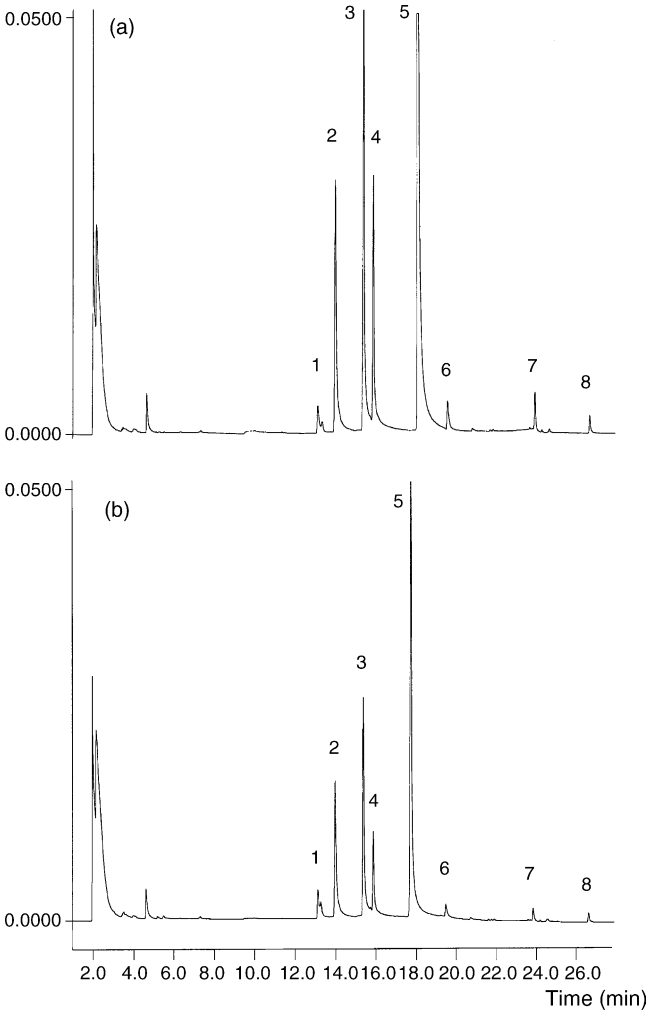

Fig. 1. NPD chromatograms corresponding to a nectarine sample immersed into the level 1 of the pesticide mixture: (a) 24 h after immersing and before washing and (b) after ethanol washing. Identification of peaks: 1, pirimicarb; 2, parathion methyl; 3, malathion; 4, chlorpyrifos; 5, methidathion; 6, myclobutanil; 7, iprodione and 8, fenarimol.

Table 3
Physical and chemical properties of the selected pesticides

Pesticide	Solubility		log *P*	Water half-life (days) (pH 7, 25 °C)
	Water (mg/l) (°C)	Ethanol (g/l)		
Chlorpyrifos	1.4 (25)	630	4.7	72
Fenarimol	13.7 (25)	100	3.69	>28
Iprodione	13 (20)	30	3.1	6.4
Malathion	145 (25)	100	2.89	>12
Methidathion	240 (20)	260	2.52	28.3
Myclobutanil	142 (25)	100	2.94	>28
Parathion methyl	55 (25)	100	2.04	40
Pirimicarb	2700 (25)	250	1.7	>23

P, octanol–water partition coefficient.

sium permanganate and sodium hypochlorite) and reducing reagents (sodium metabisulfite) were not more effective than tap water washing; thus, the main mechanism for removing the residues is dissolution and chemical degradation is unappreciable. On the other hand, SLS forms micelles enabling to involve residues of pesticides such as chlorpyrifos, with high hidrophobicity ($\log P = 4.7$, see Table 3) and low water solubility (1.4 mg/l, see Table 3) such as chlorpyrifos. Results obtained with glycerol are not as good as those of ethanol probably due to the higher viscosity of glycerol solution. The mixture containing 15% (v/v) of glycerol, 70% (v/v) of ethanol and 5% (w/v) of SLS did not show advantages over the single ethanol solution.

In the bibliography, the effectiveness of washing with water is quite irregular. Cabras et al. [10] reported losses of iprodione residues in prunes of near 60% for 5 min water washing, while 25 min for washing did not improve the results. Authors suggested that the pesticide was adsorbed by dust during the field application and washing easily removed both the dust and the adsorbed residue. Krol et al. [1] found that rinsing with tap water for 15–30 s produced significant reductions in residue levels from fruits and vegetables of malathion, iprodione and other pesticides but not of chlorpyrifos. Lentza-Rizos and Chitzanidis [2] reported that immersing peaches for 3 min in tap water reduced residues of iprodione and other pesticides by 40%. Apparent discrepancies are due to many factors such as differences in pesticide application (postharvest, in vitro or in field conditions) and washing mode (immersing, spraying, water temperature, . . .).

Relationship between effectiveness of washing treatments and physical and chemical properties of selected pesticides were studied and results for the level 1 of the remaining pesticide are shown in Table 4. Analogous results were obtained at the level 2 of remaining pesticide (data not-included). The pesticide removed (in %) and its water solubility (as log *S*) are co-related for SLS ($r = 0.8417$) and for glycerol ($r = 0.8379$) washings, that is a higher water solubility produces a lower residue decreasing in SLS and glycerol washings. This rela-

Table 4
Relationship between the effectiveness of the washes (applied pesticide mixture at the level 1) and physical and chemical properties of the selected pesticides

Pesticide studied/washing	Slope (*b*)	Intercept (*a*)	*r*	*n*
PR = $b \times \log S + a$				
All the pesticides/SLS	−13.830	69.031	0.8417	8
All the pesticides/glycerol	−16.899	72.877	0.8379	8
Organophosphorus pesticides/water	−8.005	27.614	0.9759	4
Organophosphorus pesticides/SLS	−11.818	65.242	0.9227	4
Organophosphorus pesticides/H_2O_2	−7.918	32.974	0.9361	4
Organophosphorus pesticides/glycerol	−10.672	62.150	0.8927	4
Organophosphorus pesticides/ethanol	−7.730	80.172	0.9477	4
Pirimicarb, myclobutanil, iprodione, fenarimol/SLS	−15.759	73.364	0.8290	4
Pirimicarb, myclobutanil, iprodione, fenarimol/glycerol	−22.475	84.519	0.9454	4
Pirimicarb, myclobutanil, iprodione, fenarimol/ethanol	−12.840	69.897	0.9397	4
PR = $b \times \log P + a$				
All the pesticides/glycerol	15.732	−3.621	0.6538	8
Organophosphorus pesticides/water	5.686	−2.520	0.6519	4
Organophosphorus pesticides/SLS	8.962	19.028	0.7027	4
Organophosphorus pesticides/glycerol	9.618	15.784	0.9604	4
Organophosphorus pesticides/ethanol	6.105	49.205	0.7829	4
Pirimicarb, myclobutanil, iprodione, fenarimol/SLS	18.320	−9.851	0.6540	4
Pirimicarb, myclobutanil, iprodione, fenarimol/glycerol	27.241	−37.342	0.8106	4
Pirimicarb, myclobutanil, iprodione, fenarimol/ethanol	14.476	3.385	0.6972	4

n, number of points; *P*, octanol water partition coefficient; PR, pesticide removed (in %); *r*, coefficient of correlation (values of $r < 0.650$ are not included); *S*, water solubility (mg/l); SLS, sodium laurylsulfate.

tionship is more evident when all the pesticides with chemical structures that are close (organophosphorus) are either included or excluded in the study, appearing new good correlations for the water, H_2O_2, and ethanol washings and for ethanol washing, respectively. Less intense correlations were found with the pesticide removed (in %) as a linear function of the logarithm of the octanol–water partition coefficient (P). In this last case, correlation is direct, a higher log P value produced a higher residue decreasing. No relationship between the pesticide removed and other properties of the selected pesticides such as ethanol solubility or water half-life were found.

In the literature, Krol et al. [1] indicated that water solubility was not a decisive factor that correlated always with the rinsability of a pesticide, authors explain that some pesticides could translocate into internal plant tissues under field conditions being inaccessible to water rinsing. On the contrary, Nagayama [11] that used boiling water obtained good linear correlations between the log of the leaching ratio and log P for organophosphorus pesticides in brewed tea.

According to the present results and those from the bibliography, it seems that a general relation between solubility and effectiveness of washing with aqueous solutions exists, but some pesticides can have a particular behaviour.

3.3. Identification of by-products

Some of the reagents assayed for washings have redox properties, and thus, can modify the chemical structure of the selected pesticides creating a derived by-product that is more toxic than the parent pesticide. If it is the case, such washing treatment should not be utilized to reduce the pesticide residue levels in fruits. It is well known that some organophosphorus pesticides (actually organothiophosphorus pesticides) such as malathion and parathion methyl react with oxidative reagents producing their respective P=O oxygen analogs (e.g. malaoxon and paraoxon methyl). The assayed washings include the use of hydrogen peroxide, potassium permanganate and sodium hypochlorite that are all them oxidative reagents. The possible formation of toxic by-products was investigated by gas-chromatography mass-spectrometry in SCAN mode on the extracts proceeding from the washings including oxidative reagents but no by-products from the pesticides were found. To gain sensitivity, new runs were performed by monitoring the following characteristic m/z ions: 99, 109, 127 (base peak), 195 and 268 for malaoxon; 96, 109 (base peak) and 247 (molecular weight) for paraoxon methyl; 109, 197, 242, 270 and 298 for chlorpyrifos oxon and 85, 109, 142 and 145 for methidaoxon but traces of such compounds were not detected.

Fig. 2 shows the washing effect of the hydrogen peroxide on the nectarine residues at level 1; under these conditions, the selected pesticides were not chemically modified (no oxons are detected). Nevertheless, a cleaning effect is observed because some peaks (9–16) from endogenous compounds of matrix disappeared from the chromatogram profile.

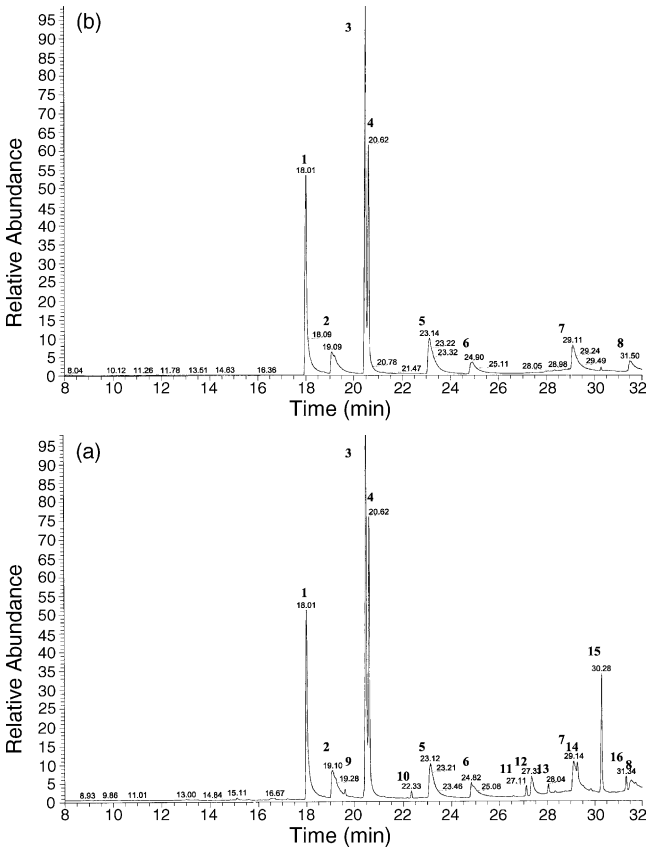

Fig. 2. Total ion chromatogram profile of a nectarine sample immersed into the level 1 of the pesticide mixture: (a) 24 h after immersing and before washing and (b) after hydrogen peroxide washing. Identification of peaks: 1–8, as in Fig. 1; 9–16, from endogenous compounds of the matrix.

To confirm or discard unequivocally the formation of toxic by-products, new assays were done by mixing directly 1 ml of the standard mixture of pesticides (level 1) with 1 ml of the washing oxidative reagent into a 5-ml centrifuge tube, the tube was stoppered and shaken vigorously for 5 min at room temperature in an automatic vibrator, 2 ml of sodium chloride saturated water was added and the mixture was allowed to stand until the two layers had separated, the organic layer was analyzed by GC–MS monitoring the characteristic ions of the oxons. Under such conditions, no traces of oxons were detected.

Finally, other set of assays were performed mixing the standard mixture of pesticides (level 1) with 1 ml of the oxidative reagent at a high concentration such are 30% (v/v) hydrogen peroxide, 10% (w/v) sodium hypochlorite and 0.1 M potassium permanganate. After mixing, the excess of oxidative reagent was removed by rinsing with 1 ml 0.1 M sodium metabisulfite. In such occasion, the organophosphorus pesticides were converted in paraoxon methyl, malaoxon, chlorpyrifos oxon and methidaoxon, whereas the other pesticides were not modified. Hypochlorite was the reagent providing the highest rate of conversion of all the organophosphorus pesticides to the respective oxons, followed by hydrogen per-

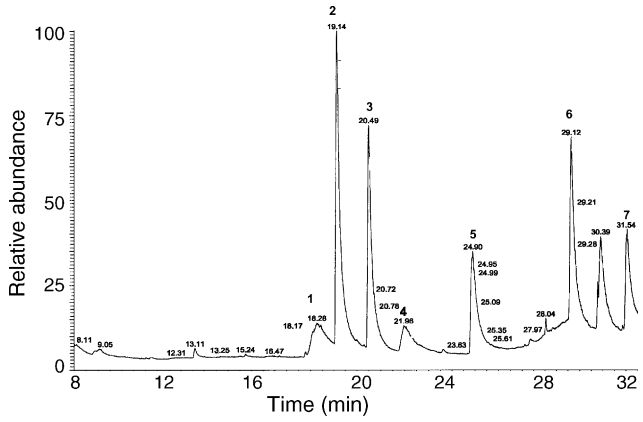

Fig. 3. Total ion chromatogram profile of a mixture of pesticide standard at level 1 in ethyl acetate directly treated with 10% sodium hypochlorite. Identification of peaks: 1, paraoxon methyl; 2, malaoxon; 3, chlorpyrifos oxon; 4, methidaoxon; 5, myclobutanil; 6, iprodione and 7, fenarimol.

oxide and finally by potassium permanganate. The effect of 10% sodium hypochlorite on pesticide standards at level 1 can be seen in Fig. 3. The respective oxons were formed from the organophosphorus pesticides, the peak of pirimicarb disappeared and myclobutanil, iprodione and fenarimol were not modified.

In a published study, in which parathion methyl and vamidothion are included, Cabrera et al. [6] washed pineapples with 100 mg/l sodium hypochlorite for 2 min and formation of paraoxon methyl was not mentioned, but in the same article 0.02% potassium permanganate was utilized to degrade vamidothion and its sulfoxide to vamidothion sulfone. Other authors [5] studied the formation of diazoxon from diazinon in an aqueous buffered solution by reaction with sodium hypochlorite. There are many factors infludings on the activity of the oxidative reagents to form oxons such as solvent, pH, identity of the pesticide, levels of reagents, the reagent it self, reaction time, temperature, endogenous matrix compounds, …. Washings with oxidative reagents to reduce levels of residues or to obtain other technological advantages (e.g. permanganate reacts with ethylene delaying the maturation) must be applied with caution because under certain conditions can produce toxic by-products. Results found in the present study must not be extrapolated to other pesticides, crops or conditions.

4. Conclusions

Simple washing with aqueous solutions help to reduce pesticide residues in nectarines, this phenomenon is related to the water solubility of pesticides. The addition of ethanol to the water washing increases the amount of residue removed from nectarines. Addition of 3% of hydrogen peroxide to the water for washing has cleaning-up effect on chromatograms. If oxidative reagents are utilized for washings, the possible formation of toxic by-products namely oxons from organophosphorus pesticides must be investigated with the particular conditions of washing (level of pesticide residue, concentration of the reagent for washings, time of washing, presence of other matrix compounds, …), because at high levels of oxidative reagents, sodium hypochlorite, hydrogen peroxide and potassium permanganate form toxic oxons from pesticide standards. With the conditions for washings utilized here, no toxic by-products were formed from the selected pesticides.

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