Suitability of microwave-assisted extraction coupled with solid-phase extraction for organophosphorus pesticide determination in olive oil

Edwar Fuentes*, María E. Báez, Adalí Quiñones

Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, Santiago, Chile

Keywords:
Organophosphorus pesticides
Olive oil
Microwave-assisted extraction
Chemometric approach

A B S T R A C T

A systematic study of the microwave-assisted extraction coupled to solid-phase extraction of nine organophosphorus pesticides (dimethoate, diazinon, pirimiphos methyl, parathion methyl, malathion, fenthion, chlorpyriphos, methidathion and azinphos methyl) from olive oil is described. The method is based on microwave-assisted liquid–liquid extraction with partition of organophosphorus pesticides between an acetonitrile–dichloromethane mixture and oil. Cleanup of extracts was performed with ENVI-Carb solid-phase extraction cartridge using dichloromethane as the elution solvent. The determination of pesticides in the final extracts was carried out by gas chromatography–flame photometric detection and gas chromatography–tandem mass spectrometry, using a triple quadrupole mass analyzer, for confirmative purposes. The study and optimization of the method was achieved through experimental design where recovery of compounds using acetonitrile for partition ranged from 62 to 99%. By adding dichloromethane to the extracting solution, the recoveries of more hydrophobic compounds were significantly increased. Under optimized conditions recoveries of pesticides from oil were equal to or higher than 73%, except for fenthion and chlorpyriphos at concentrations higher than 0.06 $\mu g\,g^{-1}$ and diazinon at 0.03 $\mu g\,g^{-1}$, with RSDs equal to or lower than 11% and quantification limits ranging from 0.007 to 0.020 $\mu g\,g^{-1}$. The proposed method was applied to residue determination of the selected pesticides in commercial olive and avocado oil produced in Chile.

1. Introduction

Olive oil has been produced for thousands of years in the countries surrounding the Mediterranean Sea and is considered an essential foodstuff in these countries due to its nutritional properties and healthy effects resulting from its high antioxidant and monounsaturated fatty-acid contents. All these positive characteristics have increased the demand for this commodity throughout the world. In order to satisfy the increasing demand and provide new alternatives to consumers, other countries such as Chile, with a Mediterranean climate, are producing olive oil [1]. Olive trees can be attacked by several pests; mainly the olive fruit fly Bactocera (Dacus) Oleae, and require the application of pesticides to control them. The most commonly used ones are the organophosphorus insecticides (OPPs), which provide well-characterized and cost-effective treatments. Most of these compounds are lipophilic and can be concentrated and stabilized for long periods in olive oil [2], constituting a human health hazard and an important parameter of oil quality. Thus, maximum pesticide residue levels have been set by the European Union and the Codex Alimentarius Commission of the Agriculture Organization of the United nations (FAO) for olive and olive oil [3]. Therefore, it is necessary to monitor their residues regularly through multi-residue analytical methods which combine short analysis time, sufficient selectivity and sensitivity. However, class diversity and physicochemical properties make it difficult to develop methodologies that cover all the analytes under study.

The preferred method for the determination of volatile pesticides in oils is capillary gas chromatography (GC) due to its high separation efficiency and variety of selective detection methods than can be used. In this way, GC coupled to tandem mass spectrometry (MS/MS) is particularly useful for qualitative and quantitative purposes, being mandatory to obtain unambiguous identification [4]. The ion-trap mass analyzer has been used for the determination of organophosphorus [5] and pyrethroid pesticides [6] in olive oil. More recently, the use of a triple quadrupole (QqQ) analyzer has been reported to determine multiclass pesticide residues in olive oil, its high acquisition speed, selectivity and detectability being outlined [7]. Although these GC techniques are widely

* Corresponding author. Tel.: +56 2 9782833.
E-mail address: edfuentes@ciq.uchile.cl (E. Fuentes).

Table 1
Experimental factors, levels, matrix, overall desirability (D) and analysis of variance of the Plackett–Burman design

Code	Factor	Lower level (−1)	Upper level (1)
P	Power (W)	300	700
T	Time (min)	5	15
Mo	Mass of oil (g)	3	5
Hd	Dilution with hexane (1:1)	No	Yes
Exs	Extracting solvent	Methanol	Acetonitrile
VExs	Volume of extracting solvent (mL)	2	5
df	Dummy	–	–

Matrix

Run	P	T	Mo	Hd	Exs	VExs	df1	df2	df3	df4	df5	D
1	1	−1	1	−1	−1	−1	1	1	1	−1	1	0.047
2	1	1	−1	1	−1	−1	−1	1	1	1	−1	0.275
3	−1	1	1	−1	1	−1	−1	−1	1	1	1	0.046
4	1	−1	1	1	−1	1	−1	−1	−1	1	1	0.677
5	1	1	−1	1	1	−1	1	−1	−1	−1	1	0.273
6	1	1	1	−1	1	1	−1	1	−1	−1	−1	0.984
7	−1	1	1	1	−1	1	1	−1	1	−1	−1	0.284
8	−1	−1	1	1	1	−1	1	1	−1	1	−1	0.015
9	−1	−1	−1	1	1	1	−1	1	1	−1	1	0.103
10	1	−1	−1	−1	1	1	1	−1	1	1	−1	0.543
11	−1	1	−1	−1	−1	1	1	1	−1	1	1	0.131
12	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	0.014

Analysis of variance

	P	T	Mo	Hd	Exs	VExs	df1	df2	df3	df4	df5
Effect	0.368	0.100	0.119	−0.023	0.089	0.342	−0.134	−0.047	−0.133	−0.003	−0.120
F-ratio[a]	38.8	2.83	4.09	0.15	2.27	33.7	–	–	–	–	–
P-value	**0.000**[b]	**0.093**	**0.043**	0.695	0.132	**0.000**	–	–	–	–	–

[a] F-ratio was obtained from experimental error (0.1022) calculated with the effect of dummy factors.
[b] Bold values indicate significant effect at 90% of confidence level.

used for the analysis of pesticide residues in olive oil, the selective detection systems continue being used to evaluate the performance of new method, including flame photometric (FPD) [8,9], nitrogen–phosphorus (NPD) [10–13] and electron-capture (ECD) [11–13] detection.

Independently of the detection system, an adequate extraction method and an effective cleanup process to remove totally or partially the lipidic components co-extracted with the target compound are mandatory so as to eliminate or diminish interferences and keep the chromatographic system in good working order. Thus, sample preparation is a crucial step, usually time-consuming because of the oil matrix complexity, mainly comprising triglycerides. In a recent review, García Reyes et al. provide a panorama of the most significant methods for determining pesticides in olive oil, paying special attention to the cleanup step [4]. The most widespread extraction technique used is liquid–liquid extraction followed by solid-phase extraction (SPE) using different sorbents as cleanup [6,10,12,14,15]. However, gel permeation chromatography is the most extensively used technique in routine laboratories for the determination of pesticides in olive oil, generally after a liquid–liquid partitioning with acetonitrile [5,7,16]. Solid-phase microextraction [17], matrix solid-phase dispersion [18], freezing [14,19,20] and size-exclusion chromatography [21] have also been proposed as extraction and/or cleanup step. Other authors have proposed a very simple method based only on hexane-acetonitrile partitioning [22] or oil-acetonitrile partitioning [8]. In this case, recoveries were higher than 74%, some chromatographic interferences were observed and the lifetime of the chromatographic system could be shortened.

Table 2
Matrix, experimental factors and overall desirability (D) of the Dohelert matrix designs for the optimization of microwave-assisted liquid–liquid extraction and cleanup steps (code for the cleanup factors: VEls = volume of elution solvent, DCM = dichlomethane content in the elution solvent, Vext = volume of extract)

Run	Coded values			Real values MAE			Real values cleanup			D	
	X_1	X_2	X_3	P (W)	VExs (mL)	T (min)	VEls (mL)	DCM (%, v/v)	Vext (mL)	MAE	Cleanup
1	1	0	0	850	4.5	7.5	5	50	4	0.573	0.168
2	0.5	0.866	0	780	7	7.5	4.3	100	4	0.714	0.463
3	0.5	0.289	0.817	780	5.3	10	4.3	67	6	0.469	0.437
4	−1	0	0	550	4.5	7.5	2	50	4	0.555	0.014
5	−0.5	−0.866	0	620	2	7.5	2.8	0	4	0.035	0.0
6	−0.5	−0.289	−0.817	620	3.7	5	2.8	33	2	0.308	0.0
7	0.5	−0.866	0	780	2	7.5	4.3	0	4	0.071	0.0
8	0.5	−0.289	−0.817	780	3.7	5	4.3	33	2	0.0	0.179
9	−0.5	0.866	0	620	7	7.5	2.8	100	4	0.599	0.614
10	0	0.577	−0.817	700	6.2	5	3.5	83	2	0.432	0.080
11	−0.5	0.289	0.817	620	5.3	10	2.8	67	6	0.448	0.298
12	0	−0.577	0.817	700	2.8	10	3.5	17	6	0.526	0.0
13	0	0	0	700	4.5	7.5	3.5	50	4	0.849	0.103
14	0	0	0	700	4.5	7.5	3.5	50	4	0.817	0.138

Table 3
GC–FPD relative response to standard in acetone for pesticides in olive oil extract with and without cleanup step

Pesticide	MAE olive oil extract	MAE–SPE olive oil extract
Dimethoate[a]	1.33	1.06
Diazinon[b]	1.15	1.06
Parathion methyl[b]	1.27	1.10
Pirimiphos methyl[b]	1.20	1.03
Malathion[a]	1.43	1.12
Fenthion[b]	1.27	1.10
Chlorpyriphos[b]	1.23	1.12
Methidathion[a]	1.44	1.13
Azinphos methyl[a]	1.53	1.01

[a] 0.2 μg mL^{-1}.
[b] 0.1 μg mL^{-1}.

In all these reported methods, liquid–liquid extraction has been performed by a mechanical or manual shaker, which can produce low throughput of samples. Microwave-assisted extraction (MAE) is well suited for routine analysis and offers great reduction in time and solvent consumption, and high throughput of samples. Nevertheless, MAE has not been proposed to assist the extraction of organophosphorus pesticides from olive oil. In this study we propose MAE to assist liquid–liquid extraction with partition of nine organophosphorus pesticides commonly used in olive trees [23] between a solvent and oil, followed by solid-phase extraction using ENVI-Carb cartridge. Optimization of both steps was performed through experimental design and by using a desirability function to study the multiple response generated by the target compounds. The proposed method is simple, low solvent-consuming and has good throughput of samples (ten samples can be analyzed in 4 h). Finally, the method was applied to residue determination of the selected pesticides in commercial olive and avocado oil produced in Chile.

2. Experimental

2.1. Chemicals and reagents

The pesticides used (dimethoate, diazinon, parathion methyl, pirimiphos methyl, malathion, fenthion, chlopyriphos, methidathion and azinphos methyl) had a purity of ≥98% (Supelco, ChemService, Bellefonte, PA, USA). All the solvents used were residue analysis grade (Fisher, Pittsburgh, PA, USA). Triphenylphosphate (TPP) (Aldrich, Milwaukee, WI, USA) was used as an internal standard for GC–FPD determinations. Stock solutions were prepared in *n*-hexane at 1 g L^{-1} and maintained at 4 °C. Working standard solutions were prepared by dilution with acetone and with hexane for spiking purposes.

2.2. Chromatographic analysis

2.2.1. GC–FPD

A Hewlett-Packard (Agilent, Little Falls, DE, USA) Model 5890 Series II gas chromatograph was employed, equipped with split/splitless injector and a flame photometric detector. An HP 5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. Helium and nitrogen (99.995%) were selected as carrier and auxiliary gas, respectively. Pesticides were separated and determined under the following conditions: injector temperature, 250 °C; detector temperature, 280 °C; column temperature program: 70 °C, held for 2 min; increased at a rate of 30°/min up to 180 °C; held for 18 min; increased at the rate of 25 °C/min up to 280 °C; held for 4 min. A 1-μL volume of the extract was injected in the splitless mode (2 min purge). Carrier gas flow in the column

Table 4
Analysis of variance for the second-order models obtained according to the Doehlert design for the optimization of microwave-assisted liquid–liquid extraction and cleanup steps (d.f. = degree of freedom, SS = sum of squares, MS = mean square)

Model	Source[a]	d.f.	Coefficient	SS	MS	*F*-ratio	*P*-value
	Constant	1	0.832				
	P	1	−0.0108	0.00046	0.00046	0.811	0.533
	VExs	1	0.245	0.240	0.240	419	0.031
	T	1	0.129	0.0667	0.0667	117	0.059
	P^2	1	−0.350	0.123	0.123	215	0.043
	P × VExs	1	0.0392	0.00115	0.00115	2.02	0.391
MAE,	*P* × *T*	1	0.158	0.0151	0.0151	26.4	0.123
R^2	$VExs^2$	1	−0.589	0.347	0.347	607	0.026
0.9397	VExs × *T*	1	−0.315	0.0594	0.0594	104	0.062
	T^2	1	−0.538	0.317	0.317	555	0.027
	Regression	9	–	0.8287	0.0921	6.92	0.039
	Residual error	4	–	0.0533	0.0133		
	Lack of fit	3	–	0.0527	0.0176	29.3	0.135
	Pure error	1	–	0.00057	0.00057		
	Total	13	–	0.8820			
	Constant	1	0.121				
	VEls	1	0.0591	0.0140	0.0140	22.4	0.133
	DCM	1	0.285	0.324	0.324	519	0.028
	Vext	1	0.0970	0.0377	0.0377	60.3	0.082
	$VEls^2$	1	−0.0297	0.00088	0.00088	1.41	0.445
	VEls × DCM	1	−0.0872	0.00570	0.00570	9.13	0.204
Cleanup,	VEls × Vext	1	0.00613	0.00002	0.00002	0.04	0.880
R^2	DCM^2	1	0.208	0.0432	0.0432	69.2	0.076
0.9284	DCM × Vext	1	0.350	0.0736	0.0736	118	0.059
	$Vext^2$	1	0.0226	0.00056	0.00056	0.90	0.517
	Regression	9	–	0.4851	0.0539	5.73	0.054
	Residual error	4	–	0.0374	0.0094		
	Lack of fit	3	–	0.0368	0.0123	20.5	0.161
	Pure error	1	–	0.00062	0.00062		
	Total	13	–	0.5225			

[a] See Tables 1 and 2 for the code of source.

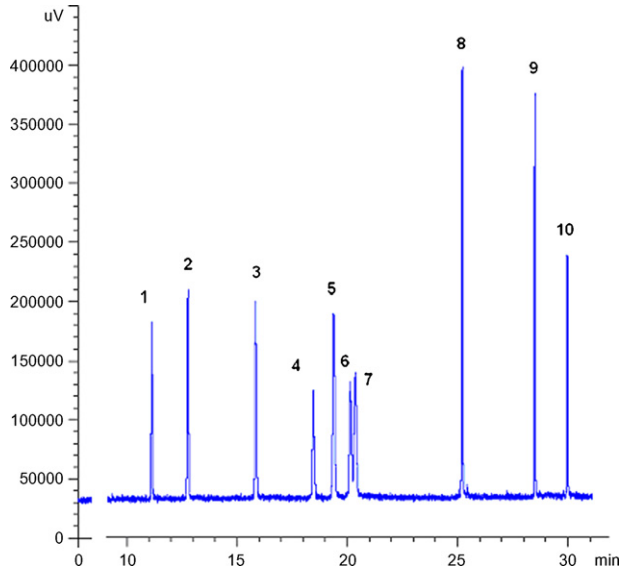

Fig. 1. GC–FPD chromatogram obtained for a spiked to a residue-free oil extract at 0.1 μg mL^{-1} for dimetoathe, malathion, methidathion and azinphos methyl and 0.2 μg mL^{-1} for diazinon, parathion methyl, pirimiphos methyl, fenthion and chlorpyriphos. Pesticides: 1 = dimethoathe; 2 = diazinon; 3 = parathion methyl; 4 = pirimiphos methyl; 5 = malathion; 6 = fenthion; 7 = chlorpyriphos; 8 = methidathion; 9 = TPP (internal standard at 0.1 μg mL^{-1}) and 10 = azinphos methyl.

was 0.95 mL/min. H_2, 75 mL/min and air, 100 mL/min were used as combustion gases. Under these conditions, the mixture of pesticides and internal standard (TPP) was well resolved in a run time of 31.6 min.

2.2.2. GC–QqQ–MS/MS

Analyses were performed with a CP-3800 gas chromatograph from Varian (Walnut Creek, CA, USA) equipped with electronic flow control. Samples were injected with a CP-8400 autosampler into a split/splitless temperature-programmable injector port operated in the split mode. A split gooseneck liner was used. The gas chromatograph was interfaced to a Varian 300 MS triple quadrupole mass analyzer using an electron ionization (EI) source. Argon (99.999%) was used as collision gas at 2.0 mTorr (1 Torr = 133.322 Pa). Aliquots of 2 μL of sample were injected into the gas chromatograph with a split/splitless programmed-temperature injection (PTV). The initial injector temperature was set at 280 °C, held for 1.0 min, and then the temperature was increased at a rate of 200 °C/min^{-1} to 350 °C and then held for 15.0 min. Gas chromatography was performed under the same conditions as used in GC–FPD. The mass spectrometer was operated in EI generating electrons with a kinetic energy of 70 eV and selection reaction monitoring (SRM) acquisition mode. The temperatures of the transfer line, ionization source, and manifold were set at 280, 280, and 40 °C, respectively. Scan time was set at 0.25 s. All compounds were monitored in full scan mode in the range m/z 50–550, using EI mode. Then, the precursor ion was selected with the aim of achieving a compromise between both selectivity (the highest m/z ion is preferred) and sensitivity (the highest abundance ion). Next, the selected precursor ion was submitted to collision-induced dissociation with argon gas at collision energies ranging from 10 to 40 V. A minimum of two MS/MS transitions were selected for each compound.

2.3. Olive oil samples and spiking procedure

In order to ensure the purity of oil as concern pesticide residues, recovery and optimization studies were carried out using a commercial organic extra virgin olive oil purchased at a local supermarket. Another eight samples of Chilean extra virgin olive oil and two samples of Chilean avocado oil purchased between November 2007 and March 2008 were also analyzed in this study. To obtain spiked organic olive oil, 3 mL of standard working solution at a proper concentration in *n*-hexane was added to 60 g of oil in a 100 mL separatory funnel to obtain the desired spiking level. After agitation, the sample was stored for 24 h in the dark at room temperature before extraction assays.

2.4. Microwave-assisted liquid–liquid extraction and SPE cleanup

2.4.1. MAE

For microwave-assisted extraction, a Milestone (Sorisole, Bergamo, Italy) MLS 1200 MEGA high-pressure microwave oven extraction system equipped with an exhaust module EM-45/A was used. An aliquot of 5 ± 0.01 g olive oil was accurately weighed into the microwave extraction vessels. Then, 5 mL of the extracting solution (acetonitrile–dichloromethane 90:10, v/v) was added, the vessels were covered with pressure-resistant holders, and preheated for 2 min at 250 W and then for 8 min at 700 W, using the microwave oven system. After microwave irradiation, the vessels were water-cooled, opened, and their content transferred into a test tube, rinsing the inner wall with 2 mL of acetonitrile. The extract layer was carefully transferred with a Pasteur pipette to a test tube.

2.4.2. Cleanup

A 6-mL internal volume ENVI-Carb cartridge (Supelco, Bellefonte, PA, USA), containing 500 mg of grafitized carbon was conditioned with 4 ml acetonitrile. Then the extract was passed through the cartridge at about two drops per second, preventing the dry column from drying. Elution was performed with 3 ml dichloromethane. The eluents were evaporated to dryness using a vacuum rotary evaporator equipped with a 50 °C water bath; the residue was weighed in order to determine the oil residue passing through the cartridge and subsequently re-dissolved with 2 mL acetone. Internal standard (20 μL of TPP at 5 mg L^{-1}) was added for GC–FPD analysis.

2.5. Optimization approach

2.5.1. MAE

A Plackett–Burman design was developed to assess the effect of six experimental factors (power and time of extraction, type and volume of extracting solvent, mass of olive oil and dilution of oil with *n*-hexane) on extraction efficiency, with two levels for each factor. Five dummy factors were also included to calculate experimental error (Table 1). Olive oil spiked at 0.2 μg g^{-1} for diazinon, parathion methyl, pirimiphos methyl, fenthion and chlopyriphos; and 0.4 μg g^{-1} for dimethoate, malathion, methidathion and azinphos methyl was used in this experiment. Subsequently, a Dohelert design was set up to optimize extraction through evaluation of the response surface (Table 2). In this case, olive oil spiked with the OPPs at 0.05 and 0.1 μg g^{-1} was used.

2.5.2. Cleanup

5 g olive oil was extracted according to the MAE procedure under nonoptimized conditions. The extracts obtained were spiked with the OPPs at 0.1 and 0.2 μg g^{-1}. A Dohelert design was built to optimize the volume of extract, volume of elution solvent (acetonitrile) and its content of dichloromethane (%, v/v); with three, five and seven levels, respectively (Table 2).

Due to the variability of pesticide properties and in order to assess the above-described experiments, we used Derringer desirability function to simplify the OPPs response matrices. With this

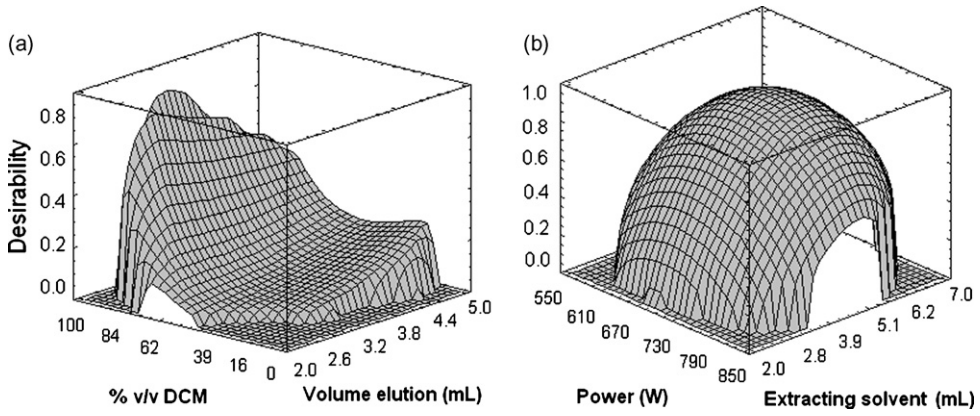

Fig. 2. Response surfaces obtained for the optimization of (a) the SPE cleanup method using ENVI-Carb cartridges at 5 mL volume of extract, and (b) the microwave-assisted extraction method at 8 min of nine OPPs from olive oil.

previous analysis, each individual response "*i*" is associated with its own partial desirability function (d_i). This varies from 0 to 1 according to the closeness of the response to its target value. The *n* individual desirability functions, calculated at all points in the domain, are then combined as the geometric means to obtain the overall desirability function ($D = (d_1, d_2, d_3, \ldots, d_n)^{1/n}$) for the system, whose values can be utilised for screening or optimization purposes into the domain. Statistical software (Statgraphics Centurion XV for Windows, Rockville, MD, USA) was used to build the experimental design and to analyze data from experimental values. The effect of dichloromethane (DCM) concentration on extraction efficiency was studied independently using olive oil spiked with the OPPs at 0.05 and 0.1 $\mu g\,g^{-1}$.

3. Results and discussion

3.1. Matrix effect and optimization of SPE cleanup

At first we evaluated the matrix effect on the chromatographic response. Microwave-assisted extraction was applied under nonoptimized conditions to obtain extracts from an organic olive oil. The mean value of the co-extracted oil, expressed as milligrams per gram of olive oil extracted, was $3.10 \pm 0.23\ mg\,g^{-1}$ (approximately $7.8\ mg\,mL^{-1}$ in the extract). These extracts ($n = 3$) were evaporated, redissolved in acetone, fortified and the solutions were injected in the gas chromatograph. Table 3 shows the relative response to standard in acetone and Fig. 1, a typical chromatogram in matrix. Pesticides in the oil matrix showed higher response that those in acetone, with values ranging from 1.15 to 1.53. Dimetoathe, malathion, methidathion and azinphos methyl showed the higher values. This effect has been called "matrix-induced chromatographic response enhanced" [24] and is caused when matrix components are present to fill active sites in the inlet system, thus reducing analyte interactions and increasing their transference to the chromatographic column. In this case, the effect is probably caused by lipid, pigments and other higher molecular mass components contained in the sample as co-extracts.

To eliminate or diminish the co-extracts and their effects, SPE cleanup procedure using ENVI-Carb cartridges was optimized. Ten responses were included in the overall desirability: the partial desirability of the co-extracted oil obtained by its minimization (unilateral; weight factor, $s = 2$; impact factor, $I = 2$), and the partial desirability for each pesticide obtained by "target is best" fixed at 100% recovery (bilateral; upper limit 110%; weight factors, $s = t = 2$; impact factor, $I = 3$). The overall desirability is shown in Table 2. The analysis of variance (ANOVA) and the estimated coefficients for the second-order model are given in Table 4. The model as fitted represent the data adequately, since lack of fit was not significant ($P > 0.05$). The regression was significant at the 90% confidence level and the coefficient of determination was 0.9284. The ANOVA showed that only the DCM content in the elution solvent had a positive significant effect on the global desirability ($P < 0.05$). In fact, the quantitative recovery of dimethoate, diazinon, parathion methyl, pirimiphos methyl, malathion and methidathion can be achieved in two ways, with major volumes of minor solvent forces (less hydrophobic) or minor volumes, but of major force (major DCM concentration). On the other hand, the quantitative recovery of chlorpyriphos, fenthion and azinphos methyl is achieved only

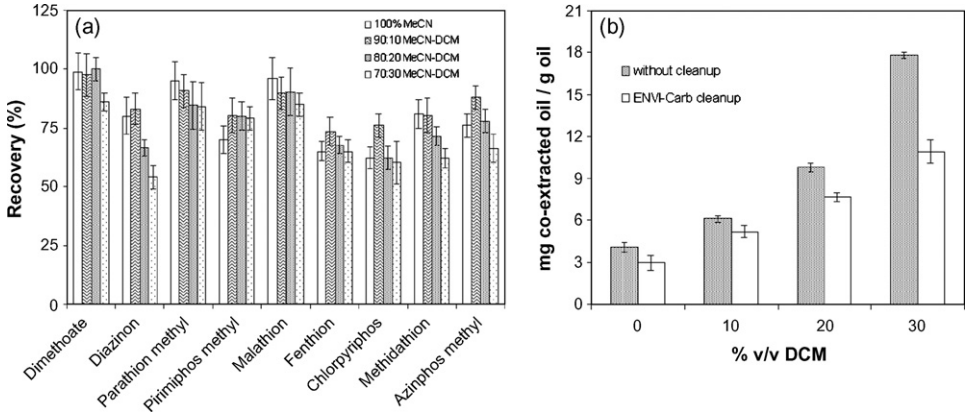

Fig. 3. Effect of dichloromethane concentration in the extracting solution on (a) the extraction efficiency of 9 OPPs; and (b) the co-extracted oil.

Table 5
Analytical characteristics of the MAE–SPE and GC–FPD method for the nine pesticides studied

Pesticide	Linear range ($\mu g\,g^{-1}$)	R	S_b/b (%)	$S_{y/x}/b$ ($\mu g\,g^{-1}$)	mLOQ ($\mu g\,g^{-1}$)	rLOQ[a] ($\mu g\,g^{-1}$)	RSD[b] (%)
Dimethoate	0.024–0.16	0.994	5.1	0.001	0.013	0.010	10.5
Diazinon	0.012–0.08	0.994	5.6	0.001	0.007	0.006	6.9
Parathion methyl	0.012–0.08	0.999	2.1	0.001	0.009	0.003	7.0
Pirimiphos methyl	0.012–0.08	0.983	10.0	0.001	0.015	0.010	7.3
Malathion	0.024–0.16	0.989	7.4	0.002	0.012	0.014	6.7
Fenthion	0.012–0.08	0.991	7.9	0.001	0.013	0.007	9.8
Chlorpyriphos	0.012–0.08	0.985	10.0	0.001	0.013	0.007	7.5
Methidathion	0.024–0.16	0.989	6.8	0.002	0.010	0.014	7.3
Azinphos methyl	0.024–0.16	0.995	5.6	0.001	0.020	0.010	6.1

[a] Limit of quantification from regression model = $10(S_{y/x}/b)[(n-2)/(n-1)]^{1/2}$.
[b] Relative standard deviation ($n = 5$) for 0.05 $\mu g\,g^{-1}$.

by high DCM concentration in the elution solvent. However, as the DCM and volume of extract increase, the elution of the co-extracted oil also increases. Thus, a consensus through global desirability was necessary. The response surface of global desirability is shown in Fig. 2A. The D value increased if the DCM concentration increased and reached a maximum at high DCM content and medium volume of elution. Optimization led to the following experimental conditions: volume of extract, 5 mL; volume of elution solvent, 3 mL of 100% DCM. Applying the optimized conditions, recovery and reproducibility were assessed by means of four extractions. The results obtained as averages were 90–112% and SD 8–12%. Cleanup through ENVI-Carb cartridge gave less olive oil residue in the final extracts (mean $2.05 \pm 0.18\,mg\,g^{-1}$) than those without the SPE cleanup ($3.10 \pm 0.23\,mg\,g^{-1}$). However, excepting for azinphos methyl, the matrix effect was still present for all compounds (Table 3). Thus, spiked extracts of a free residue olive oil (ideally organic oil) or standard additions should be used for calibration purposes in order to avoid quantitative errors [25].

3.2. Optimization of microwave-assisted extraction

In MAE, five factors were defined to evaluate their contribution to extraction efficiency. A Plackett–Burmann design was used to evaluate the effects of single factors, with the individual desirability for the 9 OPPs obtained by maximization (unilateral, $s = 1$, $I = 3$). The ANOVA of regression of experimental factors in global desirability showed that, in decreasing order, power, volume of extracting solvent, mass of oil and time had a significant positive effect on MAE efficiency ($P < 0.10$). Type of extracting solvent (acetonitrile or methanol) and dilution of oil in hexane had no effect on extraction efficiency (Table 1).

Power, volume of extracting solvent (acetonitrile) and time of extraction were simultaneously optimized through a Dohelert design with five, seven and three levels, respectively. Mass of oil was fixed at 5 g without dilution in hexane and the partial desirability for each pesticide was obtained by maximization (unilateral, $s = 1$, $I = 3$). The overall desirability is shown in Table 2. The ANOVA and the estimated coefficients for the second-order model are given in Table 4. The lack of fit was not significant ($P > 0.05$), the regression was significant at the 95% confidence level and the coefficient of determination was 0.9397. In accordance with the results of the screening, the volume of extracting solvent, its quadratic term, and the quadratic term of time and power had significant effect on MAE ($P < 0.05$). In this manner, the volume of extracting solvent, time and power act as limiting factors diminishing the extraction efficiency beyond an optimal value. The response surface showed that optimum conditions are next to the central values of the factors under study (Fig. 2B). Thus, the optimal extraction conditions retained were 8 min at 700 W using 5 mL of acetonitrile as extracting solvent. Under these conditions the recoveries for dimethoate, diazinon, parathion methyl, malathion and methidathion ranged from 80 to 99%. However, for pirimiphos methyl, fenthion, chlorpyriphos and azinphos methyl, recoveries were between 62 and 76%. These low recoveries are related to the more lipophilic character of these compounds, which makes extraction from oil matrix difficult. On the other hand, under these extraction conditions, the co-extracted oil after SPE cleanup was increased from 2.05 ± 0.18 to $2.97 \pm 0.44\,mg\,g^{-1}$.

Table 6
Percent recoveries ± standard deviation ($n = 3$) of pesticides spiked to olive oil at different concentrations

Pesticide	$\mu g\,g^{-1}$		
	0.03	0.06	0.12
Dimethoate	95 ± 2	95 ± 8	104 ± 5
Diazinon	70 ± 1	85 ± 8	99 ± 2
Parathion methyl	93 ± 10	83 ± 8	81 ± 2
Pirimiphos methyl	76 ± 2	77 ± 7	94 ± 3
Malathion	100 ± 10	85 ± 8	85 ± 4
Fenthion	73 ± 7	64 ± 5	60 ± 4
Chlorpyriphos	77 ± 2	64 ± 5	60 ± 2
Methidathion	80 ± 3	81 ± 7	82 ± 2
Azinphos methyl	93 ± 7	95 ± 9	84 ± 6

3.3. Effect of dichloromethane on extraction efficiency of MAE

Authors have observed that a mixture of acetonitrile–dichloromethane increases the recovery of OPPs from sunflower oil [5]. To evaluate the effect of DCM content in the extracting solvent on MAE efficiency, 5 g olive oil was extracted with a mixture of acetonitrile-dichloromethane at 10, 20 and 30% (v/v). The co-extracted oil was also determined with and without the SPE cleanup step. Results are shown in Fig. 3. A significant increase in the recovery was observed for the more hydrophobic compounds (pirimiphos methyl, fenthion, chlorpyriphos and azinphos methyl). With 10% DCM, recoveries were equal or higher than 73% (Fig. 3A). However, with a higher content in DCM the efficiency of extraction was diminished. This effect can be due to co-extracted oil, which increased exponentially with the DCM content (Fig. 3B), diminishing the efficiency of the solvent in this way. Hence, 10% (v/v) was selected as optimum to extract the OPPs compounds from olive oil. Cleanup through ENVI-Carb cartridge gave less olive oil residue in the final extracts (Fig. 3B).

3.4. Analytical performance of the method

Table 5 summarizes the analytical characteristics of the method for the determination of 9 OPPs by GC–FPD. Linearity was studied in the range 0.03–0.2 $\mu g\,mL^{-1}$ (0.012–0.08 $\mu g\,g^{-1}$) for diazinon, parathion methyl, pirimiphos methyl, fenthion and chlopy-

Table 7
Concentrations of organophosphorus pesticides, maximum residue limits (MRL), free acidity, peroxide value and K270 in eight commercially packed extra virgin olive and two commercially packed avocado oil samples from Chile

	Range ($\mu g\,g^{-1}$)	Number of positive samples	MRL ($\mu g\,g^{-1}$)[a] virgin olive oil	MRL ($\mu g\,g^{-1}$)[a] refined olive oil
Compound:				
Dimethoate	nd	–	1	0.05
Diazinon	0.046–0.146	3	nr	nr
Parathion methyl	nd	–	nr	nr
Pirimiphos methyl	nd	–	5	nr
Malathion	nd	–	nr	nr
Fenthion	nd	–	1	1
Chlorpyriphos	0.014–0.021	3	nr	nr
Methidathion	0.010	1	1	2
Azinphos methyl	0.028	1	nr	nr
Quality evaluation:				
Free acidity (% oleic acid)	0.16–0.64			
Peroxide value (meq O_2/kg)	3.70–16.00			
K270 (% λ)	0.07–0.12			

nd: non detected. nr: non reported.
[a] Ref. [3].

riphos; and 0.06–0.4 $\mu g\,mL^{-1}$ (0.024–0.16 $\mu g\,g^{-1}$) for dimethoate, malathion, methidathion and azinphos methyl, measuring in triplicate a matrix-matched standard calibration curve prepared in extracts of blank matrix (organic olive oil free of pesticide residues). Linear calibration graphs were constructed by least-square regression of concentration versus peak area ratio (analyte/IS) of the calibration standards. The responses of all compounds were linear in the range under study, with r values of 0.983–0.999. Moreover, the relative standard deviation of the slope (s_b/b; where s_b is the standard deviation of the slope and b is the slope), which gives a better representation of analytical data, was equal or less than 10%. Analytical sensitivity ($S_{y/x}/b$; where $S_{y/x}$ is the standard deviation of the regression), which indicates the minimal difference in concentration detected by the method, was between 0.001 and 0.002 $\mu g\,g^{-1}$. The limit of quantification of the method (mLOQ) was obtained by spiking an organic olive oil at low concentrations and subjecting it to the sample preparation method (ten times the standard error of the signal obtained for six extracts). For the nine compounds mLOQ values ranged from 0.007 to 0.020 $\mu g\,g^{-1}$. Thus, MRLs required by European and international regulations for the selected compounds can be verified without difficulty [3]. Additionally, the limit of quantification from the regression model (rLOQ) was also obtained, as $10(S_{y/x}/b)[(n-2)/(n-1)]^{1/2}$; where n is the number of pairs of points. The precision of the method, expressed as repeatability (RSD, $n = 5$), was determined on olive oil fortified at 0.05 $\mu g\,g^{-1}$; and the values were equal or lower than 10.5%. Recovery studies are shown in Table 6. The mean recovery of three determinations of three concentrations levels of OPPs ranged from 60 to 104%, with RSD equal or lower than 11%. The lower recoveries were obtained for fenthion and chlorpyriphos at 0.06 and 0.12 $\mu g\,g^{-1}$.

3.5. Real sample analysis

The method developed in this study was applied to analyze OPPs residues in eight commercially packed extra virgin olive oils and two commercially packed avocado oils produced in Chile. A matrix standard prepared by spiking an extract of organic olive oil was analyzed twice with the set of samples in order to achieve accurate quantification. Some parameters of oil quality were also determined in these samples. Results are summarized in Table 7. Two samples of olive oil and the samples of avocado oil contained no detectable residues. Three samples contained diazinon residues at 0.046; 0.110 and 0.146 $\mu g\,g^{-1}$. Chlorpyriphos residues were detected in three samples at 0.014; 0.020 and 0.021 $\mu g\,g^{-1}$. Azinphos methyl residues were detected in one sample at 0.028 $\mu g\,g^{-1}$ and methidathion residues in one of them at 0.010 $\mu g\,g^{-1}$. However, none of the olive oil samples contained residues higher than the permitted MRL recorded by European legislation [3]. The identity of residues in positive samples was confirmed through GC–MS/MS analysis with the experimental conditions detailed in Section 2.2. Free acidity, peroxide value and absorption at 270 nm were lower than maximum levels established for extra virgin olive oil [26].

4. Conclusions

The MAE–SPE and GC–FPD method is simple, selective, and sensitive to determine organophosphorus pesticides at sub-$\mu g\,g^{-1}$ level in olive and avocado oil. Due to the "matrix-induced chromatographic response enhancement effect" caused by lipid, pigments and other components contained in the sample as co-extracts, matched standard in free residue olive oil extracts (ideally organic oil) or standard additions method should be used for calibration purposes to obtain accurate results. The use of acetonitrile–dichloromethane extraction mixture has permitted to increase MAE efficiency to extract the more hydrophobic OPPs from oil. The study and optimization approach through desirability function and experimental design has permitted to assess the variability of the pesticides and the co-extracted matrix. By applying the method to olive and avocado oil samples produced in Chile, diazinon, chlorpyriphos, methidathion and azinphos methyl were detected in six samples of olive oil at concentrations lower than the permitted MRL.

Acknowledgment

The authors thank FONDECYT for financial support through Project 11060461.

References

[1] Agricultura y Mercados, Mercado Del Aceite de Oliva, Oficina de Estudios y Políticas Agrarias (ODEPA), Ministerio de Agricultura de Chile, Santiago, March, 2008.
[2] P. Cabras, A. Angioni, V.L. Garau, M. Melis, F.M. Pirisi, M. Karim, E.V. Minewlli, J. Agric. Food Chem. 45 (1997) 2244.
[3] Codex Alimentarius Commission, Codex Alimentarius Pesticide Residues in Food-Maximum Residues Limits, vol. 2B, second ed., FHO/WHO, Rome, 1996.
[4] J.F. García Reyes, C. Ferrer, M.J. Gómez Ramos, A. Molina Díaz, A.R. Fernández Alba, Trends Anal. Chem. 26 (2007) 239.
[5] M. Guardia Rubio, M.L. Fernández De Córdova, M.J. Ayora Cañada, A. Ruiz Medina, J. Chromatogr. A 1108 (2006) 231.

[6] F.A. Esteve Turrillas, A. Pastor, Miguel de la Guardia, Anal. Chim. Acta 553 (2005) 50.
[7] A. Garrido Frenich, J.L. Fernández Moreno, J.L. Martínez Vidal, F.J. Arrebola Liébanas, J. Agric. Food Chem. 55 (2007) 8346.
[8] G. Dugo, G. Di Bella, L. La Torre, M. Saitta, Food Control 16 (2005) 435.
[9] L.L. Zhiqiang Zhou, C. Pan, C. Qian, S. Jiang, F. Liu, Chromatographia 66 (2007) 625.
[10] E.G. Amvrazi, T.A. Albanis, J. Agric. Food Chem. 54 (2006) 9642.
[11] C. Yagüe, S. Bayarri, P. Conchello, R. Lázaro, C. Pérez-Arquillué, A. Herrera, A. Ariño, J. Agric. Food Chem. 53 (2005) 5105.
[12] R. Raoux, J.L. Coustille, S. Rambaud, Oleagineaux, Corps Gras, Lipides 4 (1997) 71.
[13] E.M. Díaz Plaza, J.M. Cortés, A. Vázquez, J. Villén, J. Chromatogr. A 1174 (2007) 145.
[14] Ch. Lentza Rizos, E.J. Avramides, E. Visi, J. Chromatogr. A 912 (2001) 297.
[15] A.M. Tsatsakis, I.N. Tsakiris, M.N. Tzatzarakis, Z.B. Agourakis, M. Tutudaki, A.K. Alegakis, Food Addit. Contam. 20 (2003) 553.
[16] K. Patel, R.J. Fussell, M. Hetmanski, D.M. Goodall, B.J. Keely, J. Chromatogr. A 1068 (2005) 289.
[17] C. Tsoutsi, I. Konstantinou, D. Hela, T. Albanis, Anal. Chim. Acta 573–574 (2006) 216.
[18] C. Ferrer, M. Jose Gómez, F. García Reyes, I. Ferrer, E.M. Thurman, A.R. Fernández Alba, J. Chromatogr. A 1069 (2005) 183.
[19] Ch. Lentza Rizos, E.J. Avramides, F. Cherasco, J. Chromatogr. A 912 (2001) 135.
[20] M. Garcés García, E.M. Brun, R. Puchades, A. Maquieira, Anal. Chim. Acta 556 (2006) 347.
[21] S. Barrek, O. Paisse, M.F. Grenier Lustalot, Anal. Bioanal. Chem. 376 (2003) 355.
[22] P. Cabras, A. Angioni, M. melis, E.V. Minelli, F.M. Pirisi, J. Chromatogr. A 761 (1997) 327.
[23] L. Rastrelli, K. Totaro, F. De Simeone, Food Chem. 79 (2002) 303.
[24] D.R. Erney, G.R. Gillespie, D.M. Gilvydis, C.F. Poole, J. Chromatogr. A 638 (1993) 57.
[25] C.F. Poole, J. Chromatogr. A 1158 (2007) 241.
[26] D. Boskou, in: D. Boskou (Ed.), Olive Oil: Chemistry and Technology, second ed., American Oil Chemists Society Press, 2006, pp. 113–172 (Chapter 7).