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Multiclass Pesticide Determination in Olives and Their Processing Factors in Olive Oil: Comparison of Different Olive Oil Extraction Systems

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The processing factors (pesticide concentration found in olive oil/pesticide concentration found in olives) of azinphos methyl, chlorpyrifos, λ-cyhalothrin, deltamethrin, diazinon, dimethoate, endosulfan, and fenthion were determined in olive oil production process in various laboratory-scale olive oil extractions based on three- or two-phase centrifugation systems in comparison with samples collected during olive oil extractions in conventional olive mills located at different olive oil production areas in Greece. Pesticide analyses were performed using a multiresidue method developed in our laboratory for the determination of different insecticides and herbicides in olive oil by solid-phase extraction techniques coupled to gas chromatography detection (electron capture detection and nitrogen phosphorus detection), optimized, and validated for olive fruits sample preparation. Processing factors were found to vary among the different pesticides studied. Water addition in the oil extraction procedure (as in a three-phase centrifugation system) was found to decrease the processing factors of dimethoate, α-endosulfan, diazinon, and chlorpyrifos, whereas those of fenthion, azinphos methyl, β-endosulfan, λ-cyhalothrin, and deltamethrin residues were not affected. The water content of olives processed was found to proportionally affect pesticide processing factors. Fenthion sulfoxide and endosulfan sulfate were the major metabolites of fenthion and endosulfan, respectively, that were detected in laboratory-produced olive oils, but only the concentration of fenthion sulfoxide was found to increase with the increase of water addition in the olive oil extraction process.

KEYWORDS: Pesticides; processing factors; olives; olive oil; extraction systems; metabolites

INTRODUCTION

The annual cost of olive pest control exceeds 100 million Euros worldwide, 50% of which corresponds to pesticide use, not including the cost of the adverse side effects of pesticide use (*1*). To serve the purpose of the consumers' health protection, the European Union has established maximum residue limits (MRLs) of pesticides in olives as a commodity (*2*), while with Codex Alimentarius, the European Commission has extended this early legislation establishing MRLs for several pesticides in olive oil (*3*). The latest amendment of Codex Alimentarius (*4*) considers olive oil a processed food and proposes the establishment and application of processing factors (*F*) in the established MRLs in the raw commodity, to ensure safety of ready-to-eat foods.

Industrial processing may alter pesticide residues when compared with raw crops via chemical and biochemical reactions (hydrolysis, oxidation, microbial degradation, etc.) and physicochemical processes (volatilization, absorption, etc.). Thus, processing factors of pesticides in certain industrial processes depend on pesticide physicochemical properties as well as the nature and composition of crops under process. Fruit processing (e.g., washing, peeling, and cooking) is known to reduce and/or decompose pesticide residues in final products (*5–10*). In addition, low processing factors of certain pesticides have been reported in juicing and vinification processes as well as in jam preparation processes (*11–15*). However, in some cases, residue levels may increase in the final product as in the production of dry fruit (e.g., resins and prunes) (*16–18*) and unrefined vegetable oil (*19–25*) due to concentration factors of raw commodities in the process of the final product.

The extraction process of unrefined olive oil involves the washing and milling of the fruit, the malaxation of the produced olive paste by slow mixing at a constant temperature (usually bellow 30 °C) for 30−90 min, and the separation of oil by a press or a decanter (centrifugation system). In the past 30 years, olive processing technology has undergone an evolution. Pressure systems have been replaced by three-phase centrifugation systems, which combine reduced manufacturing costs with a higher production capacity that requires a shorter storage time of olives before processing. However, the three-phase centrifugation system presents certain disadvantages: reduction in the phenol content of the oil due to the addition of warm water to dilute the olive paste and an increased amount of wastes due to

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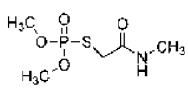
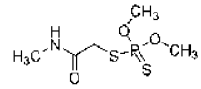
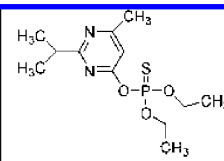
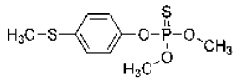
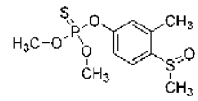
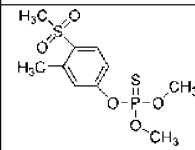
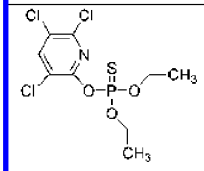
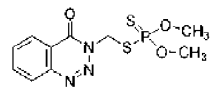
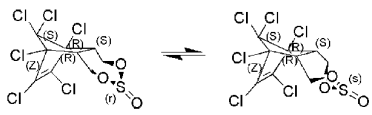
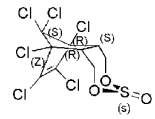
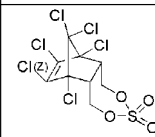
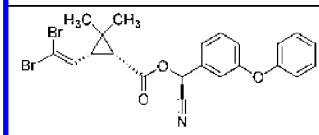
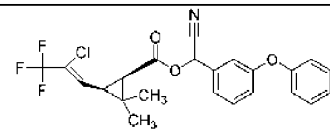
Table 1. Chemical Structures and Main Physicochemical Properties (*30*) of Pesticides Used in This Study[a]

 Omethoate: Vapour Pressure (mmHg): 2.48E-05 (20°C) Solubility in Water (mg/l): 1.0E+06 log *Kow*: -0.74	 Dimethoate: Vapour Pressure (mmHg): 8.25E-06 (25°C) Solubility in Water (mg/l): 25000 (21°C) log *Kow*: 0.78	 Diazinon: Vapour Pressure (mmHg): 9.01E-05 (25°C) Solubility in Water (mg/l): 40.0 (20°C) log *Kow*: 3.81
 Fenthion: Vapour Pressure (mmHg): 1.05E-05 (25°C) Solubility in Water (mg/l): 7.5 (20°C) log *Kow*: 4.09	 Fenthion Sulfoxide: Vapour Pressure (mmHg): 5.51E-06 (25°C) Solubility in Water (mg/l): 3.72 log *Kow*: 1.92	 Fenthion Sulfone: Vapour Pressure (mmHg):- Solubility in Water (mg/l):- log *Kow*: 2.17
 Chlorpyrifos: Vapour Pressure (mmHg): 2.03E-05 (25°C) Solubility in Water (mg/l): 1.12 (24°C) log *Kow*: 4.96	 Azinphos Methyl : Vapour Pressure (mmHg): 1.60E-06 (25°C) Solubility in Water (mg/l): 20.9 (20°C) log *Kow*: 2.75	
 α-Endosulfan : Vapour Pressure (mmHg): 3.00E-06 (25°C) Solubility in Water (mg/l): 0.51 (20°C) log *Kow*: 3.83	 β-Endosulfan : Vapour Pressure (mmHg): 6.00E-07 (25°C) Solubility in Water (mg/l): 0.45 (20°C) log *Kow*: 3.83	 Endosulfan Sulfate: Vapour Pressure (mmHg): - Solubility in Water (mg/l): 0.48 (20°C) log *Kow*: 3.66
 Deltamethrin: Vapour Pressure (mmHg): 1.50E-08 (25°C) Solubility in Water (mg/l): 0.002 (25°C) log *Kow*: 6.20	 Cyhalothrin: Vapour Pressure (mmHg): 1.59E-09 (25°C) Solubility in Water (mg/l): 0.0008 (20°C) log *Kow*: 7.00	

[a] —, not found.

the large volume of water added during the olive oil extraction process. To confront these problems, a new horizontal centrifugal two-phase decanter was manufactured, to separate the oil from olive paste with a negligible amount of water addition. This technology led to both the production of better quality oil and the generation of negligible quantities of liquid wastes (*26, 27*).

In Greece, olives are cultivated primarily in southern Greece, mainly in two regions, Peloponnese (30% of the total olive production areas) and Crete (22%). The existing 3000 mills are virtually dispersed throughout the country, but still nearly 60% are located in these two regions. Most of these mills use three-phase centrifugation systems (70%), and only a small part still use pressure systems. Two-phase centrifugation systems are rare among the existing olive mills (<5%) (*28, 29*).

Studies on the behavior of pesticide residues through the technological transformation of oil production are scarce. These few studies have investigated the effect of washing on pesticide residues in olives and have determined the residue concentra-

Table 2. Nominal Concentrations (μg/kg) of Pesticides in Spiked Olive Batches (5 kg, $n = 3$) Processed into Oil with the Three Extraction Procedures Tested

pesticide	C1	C2	C3	C4	C5
fenthion	1256.5 ± 25.7	596.2 ± 51.6	301.4 ± 16.5	150.6 ± 9.4	59.0 ± 5.6
endosulfan	62.8 ± 1.3	29.8 ± 2.6	15.1 ± 0.8	6.0 ± 0.4	2.9 ± 0.3
λ-cyhalothrin	47.1 ± 1.0	29.8 ± 2.6	18.1 ± 1.0	9.0 ± 0.6	5.9 ± 0.6
deltamethrin	628.2 ± 12.9	298.1 ± 25.8	150.7 ± 8.2	60.2 ± 3.8	29.5 ± 2.8
azinphos methyl	785.3 ± 16.1	298.1 ± 25.8	150.7 ± 8.2	60.2 ± 3.8	29.5 ± 2.8
chlorpyrifos	62.8 ± 1.3	29.8 ± 2.6	18.1 ± 1.0	9.0 ± 0.6	2.9 ± 0.3
dimethoate	628.2 ± 12.9	298.1 ± 25.8	150.7 ± 8.2	75.3 ± 4.7	38.3 ± 3.7
diazinon	62.8 ± 1.3	29.8 ± 2.6	18.1 ± 1.0	9.0 ± 0.6	5.9 ± 0.6

tions in olive oil. According to these findings, the effect of olive washing on pesticides is limited, and the decrease of residues is not correlated with pesticide water solubility. Pesticide residue processing factors have been estimated for several insecticides (azinphos methyl, diazinon, dimethoate, methidathion, parathion, parathion methyl, quinalphos, fenthion, acephate, buprofezin, phosphamidon, formothion, and deltamethrin) (*19–23*). However, in the latter studies, either the olive oil extraction processes were not always specified or the oil separation was usually performed with no water addition (as in pressure and two-phase centrifugation systems) or dealt with high concentrations of pesticide residues in the olives processed. To our knowledge, no studies have been conducted to evaluate the effect of water added in the different extraction systems on persistant pesticide residues in olive oil.

The objective of this work was to determine the processing factors of eight pesticides (**Table 1**) detected with high frequency in olive oil in the Mediterranean region (*25*) through the olive oil production process and to perform a comparative examination regarding the processing factors of selected pesticides in olive oil obtained from three different extraction procedures that differed in water volume added during the oil separation. The main oxidative metabolites of dimethoate, fenthion, and endosulfan (omethoate, fenthion sulfoxide, fenthion sulfone, and endosulfan sulfate, respectively) that present higher toxicities than the parent compounds were also tested in the samples analyzed. For this scope, a multiresidue method for the determination of the selected pesticides in olives based on solid-phase extraction (SPE) techniques coupled to gas chromatography (GC) detection [electron capture detection (ECD) and nitrogen phosphorus detection (NPD)], previously developed in our laboratory for pesticide residues analysis in olive oil (*31*), was optimized for sample preparation and validated.

MATERIALS AND METHODS

Chemicals. Pesticide standards (purity 97.0−99.9%) were purchased from Riedel-de Haën (Seelze, Germany). All solvents used were of pestiscan grade and were obtained from Labscan (Dublin, Ireland). Stock standard solutions of each pesticide were prepared in acetone at 1000 μg/mL and stored in glass tapered bottles at −20 °C. Working standard solutions were obtained by appropriate dilution with acetone. Endrin (100 μg/L) and bromophos ethyl (200 μg/L) were used as internal standards in ECD and NPD analyses, respectively. Both internal standard solutions were prepared in acetone and were added just before the injection. Diol and ENVI-Carb solid SPE cartridges were purchased from Supelco (Bellefonte, PA).

The commercial formulations Lebaycid 50EC (Bayer, CropScience, Greece; 51%, w/v fenthion), Thiodan (Makhteshim, Israel; 47%, w/w, endosulfan), Karate 10CS (Syngenta Hellas; 10.05%, w/v, λ-cyhalothrin), Decis 2.5EC (Bayer CropScience SA, France; 2.5%, w/v, deltamethrin), Azinphos Methyl (20EC Lapafarm, Greece; 20%, w/v, azinphos methyl), Dursban 25WP (Dow Agrosciences LLC, United States; 25%, w/w, chlorpyrifos), Oligor 40EC (Cheminova, Lemving, Denmark; 40%, w/v, dimethoate), and Diazolin 60EC (Farma Chem SA; 60%, w/v, diazinon) were prepared in distilled water at concentrations of 50−300 mg/L in dark glass bottles of 2 L capacity. Working pesticide solutions applied to olive batches processed into oil in laboratory experiments were then prepared by dilution with distilled water.

Samples. *Validation of Analytical Methodology.* Olive and olive oil samples from organic cultivars of three different olive-producing areas in Greece (Peloponnese, Crete, and Preveza) were used for the validation of the methodology, to ensure diversity of olive and olive oil samples and purity from pesticide residues. Appropriate amounts of a pesticide working solution were spiked in suitable portions of samples to have a range of pesticide concentrations ranging between 5 and 500 μg/kg for recovery experiments and linearity studies. After agitation, the samples were allowed to equilibrate for 60 min prior to different extraction assays.

Laboratory Olive Oil Extraction Studies. Seventy-five kilograms of olives from an organic cultivation in Preveza was used in the oil extraction experiments performed in laboratory. Olives were collected by hand, and the leaves and stones were removed carefully before olive treatment with pesticide formulations.

Monitoring Study. Olive and olive oil samples used in monitoring study were collected during 30 olive oil extraction processes in conventional olive mills of three-phase centrifugation systems located at four main olive oil-producing areas in Greece (Peloponnese, $n = 15$; Crete, $n = 5$; Preveza, $n = 9$; and Chalkidiki, $n = 1$) during the olive crop period of 2004−2005. Olive samples that were collected (before washing process) weighed approximately 1 kg each, were analyzed for fat and moisture content right after their arrival to the laboratory, and were stored at −20 °C until further analysis. The determination of fat was performed by repeated extractions (Soxhlet method) of crashed olives with petroleum ether for 4 h. Subsequently, the petroleum ether was evaporated by means of a rotary evaporator. The determination of moisture was performed following AOAC method 926.12 (1997) proposed for the determination of moisture in oils and fats (*32*). The fat and moisture contents (%) determined in samples from Peloponnese were 30.5 ± 3.0 and 59.4 ± 6.4, respectively; in samples from Crete, they were 35.3 ± 3.8 and 45.9 ± 2.9, respectively; in samples from Preveza, they were 19.6 ± 2.6 and 58.7 ± 1.3, respectively; and in the sample from Chalkidiki, the fat content was 25.7 ± 0.1%, and the moisture content was 54.3 ± 0.0%.

Olive Processing. Twenty-five kilograms of olives from the organic cultivation in Preveza was divided into five equal batches, and each batch was sprayed homogeneously with a 250 mL solution of the mixture of the commercial formulations of the selected pesticides to have five fortified batches at five different concentrations (C1−C5; **Table 2**) (**Figure 1**). Pesticide applications were preformed using a hand pump spray bottle to control spraying and ensure homogeneity. The concentration levels of each target pesticide used in olive treatments were selected to be at low levels that were normally expected to persist in olives. The highest fortification (C1) for each pesticide studied was defined to be slightly higher than the specified MRL by the European Union. After 24 h, each batch (5 kg) was divided in three equal portions of approximately 1.5 kg and was processed into oil using three different procedures (P1, P2, and P3) that differ in water addition after paste malaxation as follows.

Olive Oil Extraction Process 1 (P1). Spiked olives (∼1.5 kg) were weighted, crushed, and kneaded at 30 °C for 45 min. After malaxation,

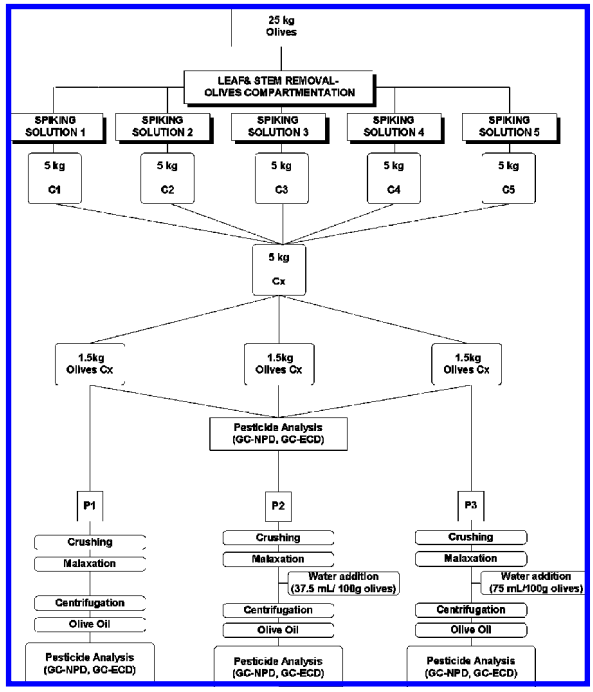


Figure 1. Schematic presentation of the experiment performed in triplicate at laboratory scale olive processing into oil. Five olive batches were spiked at five levels of concentration, and each batch was processed into oil following P1, P2, and P3 procedures.

the olive paste was centrifuged at 3000 rpm, and the oil was collected and weighted.

Olive Oil Extraction Process 2 (P2). Spiked olives (~1.5 kg) were weighted, crushed, and kneaded at 30 °C for 45 min. After malaxation, 37.5 mL of distilled water of 30 °C per 100 g of olives was added slowly in the olive paste during centrifugation at 3000 rpm, and the oil was collected and weighted.

Olive Oil Extraction Process 3 (P3). Spiked olives (~1.5 kg) were crushed and kneaded at 30 °C for 45 min. After malaxation, 75 mL of distilled water of 30 °C per 100 g of olives was added slowly in the olive paste during centrifugation at 3000 rpm, and the oil was collected and weighted.

Samples were processed into oil in a laboratory unit made up of stainless steel consisting of a crusher, a unit for paste malaxation, and a centrifugal separator. Pesticides determination was performed in samples of olives and of extracted olive oils, to estimate pesticide processing factors in olive oil. Each determination was performed in triplicate. Interpretation of the effects of processing related to the pesticides physical and chemical properties was based on the assumption that no pronounced interactions between the pesticides occurred.

Pesticide Analysis. The analysis of pesticides was based on the multiresidue method developed in our laboratory for the determination of 35 insecticides and herbicides in olive oil by SPE techniques coupled to GC detection (ECD and NPD) (*31*).

Sample Preparation for Olive Oil. An aliquot of 5.000 ± 0.001 g of olive oil was weighted in a 40 mL screw-capped glass tube and dissolved in 5 mL of *n*-hexane. The solution was extracted twice with 10 mL of acetonitrile (ACN), and the extracts were combined. An aliquot of 6 mL of the extract was subjected to an SPE cleanup procedure for GC-NPD, and 12 mL of the extract was subjected to an SPE cleanup procedure for GC-ECD as follows.

SPE Cleanup Prior GC-NPD Analysis. For pesticides analyzed by GC-NPD, an ENVI-Carb SPE cartridge was conditioned with 6 mL of ACN. The extract was applied to the cartridge by avoiding the drying of the column, and the elution was performed with 12 mL of ACN. The eluants were brought to dryness by the use of a rotary evaporator (water bath temperature, <40 °C); the residues were reconstituted in 0.5 mL of acetone that contained 200 μg/L bromophos ethyl (internal standard) and were analyzed by GC-NPD.

SPE Cleanup Prior GC-ECD Analysis. For pesticides analyzed by GC-ECD, an ENVI-Carb SPE cartridge was conditioned with 6 mL of ACN. The extract was applied to the column, and the cartridge was eluted with 12 mL of ACN and straightly with 12 mL of a mixture of ACN/toluene (95:5, v/v). The eluants were brought to dryness, and the residues were redissolved in 2 mL with *n*-hexane. A Diol-SPE cartridge was conditioned by the consecutive passing of 6 mL of methanol and 6 mL of *n*-hexane. Without allowing the column to dry, the 2 mL extract in *n*-hexane was passed through the Diol cartridge. The column was eluted with 6 mL of *n*-hexane and straight after with 6 mL of a mixture of hexane/ethyl acetate/methanol (95:2.5:2.5, v/v/v). The eluants were brought to dryness by the use of a rotary evaporator (water bath temperature, <40 °C), and the residues were reconstituted in 2 mL of acetone containing 100 μg/L endrin (internal standard) prior to GC-ECD analyses.

Extraction Procedure for Olives. Approximately 100 g of raw olives was weighted, crushed (with the kernel) by the means of a laboratory hammer mill, and homogenized in a blender. Two different procedures were tested for the optimization of olive sample preparation.

(a) Ten grams of the crushed and homogenized olives was mixed with 10 g of anhydrous Na_2SO_4 and was extracted with two doses of 20 mL of ACN in a vortex for 2 min and then in an ultrasonic system (FRITCH GMBH laborette 17, Germany, 40 kHz) at sonic power 110 W for 20 min (the temperature of water bath was kept at 30 °C). The extracts were passed through a funnel with anhydrous Na_2SO_4 and glass wool as a filter, and the funnel was washed with 5 mL of ACN. An aliquot of 12 mL of the combined extracts was subjected to a SPE cleanup procedure for GC-NPD, and 18 mL of the combined extracts was subjected to a SPE cleanup procedure for GC-ECD according to the procedure followed for olive oil extracts.

(b) Fifty grams of crushed and homogenized olives was lyophilized in a model LP3 lyophilizer (Jouan, Saint-Herblain, France). Five grams of the lyophilized sample was extracted with two doses of 20 mL of ACN, initially in a vortex for 2 min and then in the ultrasonic system at sonic power 110 W for 20 min (the temperature of water bath was kept at 30 °C). An aliquot of 12 mL of the combined extracts was subjected to a SPE cleanup procedure for GC-NPD, and 18 mL of the combined extracts was subjected to a SPE cleanup procedure for GC-ECD according to the procedure followed for olive oil extracts.

GC. Analyses were performed on a Shimadzu GC-14B gas chromatograph equipped with a 63Ni ECD and on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a NPD. In both GC analyses, the injection port was in splitless mode, and the splitter opened after 1 min (injection volume, 1 μL). Quantification was carried out on GC-NPD and GC-ECD by the internal standard method using standards in the matrix extract.

Analyses on GC-ECD were performed on a fused silica capillary column Zebron ZB-5, 30 m × 0.25 mm i.d. × 0.25 μm df film thickness, containing 5% phenyl−95% dimethylpolysiloxane (Phenomenex). The injector and detector were operated at 220 and 280 °C, respectively. The chromatographic temperature program was as follows: 100 °C for 1 min, raised to 210 °C (5 °C/min) and held for 16 min, then raised to 285 °C (3 C/min) and held for 10 min.

Analyses on GC-NPD were performed on a Zebron ZB-1, 30 m L × 0.32 mm i.d. column contained 100% methylpolysiloxane 1.00 μm df film thickness (Phenomenex). The injector and detector were operated at 220 and 280 °C, respectively. The chromatographic temperature program was as follows: 100 °C for 1 min, raised to 190 °C (15 °C/min) and held for 3 min, then raised to 270 °C (4 °C/min) and held for 15 min.

Statistical Analysis. One-way analysis of variance and Duncan posthoc tests were used to determine the statistical significance among the processing factors of the different oil extraction methods studied. Nonparametric analysis using Mann−Whitney U test was used to compare processing factors determined in real samples to those determined in laboratory experiments. Bivariate correlation among the physicochemical properties of pesticides studied as well as the water and fat contents of olive fruits and the processing factors determined was performed using Spearman correlation coefficients for nonparametric data and Pearson correlation coefficients for normal distributed

Table 3. Analytical Data of the Methods Used for Pesticides Determination

pesticide	t_R (min)	mLOD (μg/kg)	recovery (%)	RSD (%)	linear range (μg/kg)	R^2	MRL (μg/kg)
			olive oil				
			GC-NPD method				
omethoate	12.05	2.9	105.3	8.4	10−200	0.9943	−
dimethoate	15.02	1.5	97.2	8.9	5−200	0.9980	50 (olive oil, refined)[a]
diazinon	16.96	1.3	97.1	7.7	5−200	0.9976	−
fenthion	21.29	1.4	100.8	2.4	5−200	0.9982	1000[a]
fenthion sulfoxide	27.58	2.5	96.4	9.1	10−500	0.9935	
fenthion sulfone	28.26	0.4	98.6	9.1	5−500	0.9964	
chlorpyrifos	21.59	2.4	100.2	11.4	10−200	0.9990	−
azinphos methyl	34.25	14.0	101.5	6.9	50−500	0.9955	−
			GC-ECD method				
α-endosulfan	27.19	1.3	96.2	8.5	5−500	0.9974	−
β-endosulfan	31.57	1.7	95.4	8.4	5−500	0.9960	−
endosulfan sulfate	35.53	1.7	101.6	7.3	5−500	0.9972	−
λ-cyhalothrin	49.07	2.6	88.6	10.9	10−500	0.9997	−
deltamethrin	62.25	13.1	96.3	9.4	15−500	0.9916	−
			olives (method a: dehydration of the extracts by anhydrous sodium sulfate addition)				
			GC-NPD method				
omethoate	12.05	2.6	71.9	11.0	10−500	0.9871	200[b]
dimethoate	15.02	1.5	97.1	7.5	5−500	0.9944	2000[b], 1000[a]
diazinon	16.96	1.3	78.1	6.6	5−500	0.9874	20[b]
fenthion	21.29	2.5	75.3	8.7	10−500	0.9899	2000[b]
fenthion sulfoxide	27.58	5.6	72.0	11.0	20−500	0.9899	
fenthion sulfone	28.26	2.8	80.2	7.9	10−500	0.9786	
chlorpyrifos	21.59	2.0	89.4	11.9	10−500	0.9786	50[b]
azinphos methyl	34.25	5.3	88.4	9.4	20−500	0.9812	500[b]
			GC-ECD method				
α-endosulfan	27.19	0.8	79.4	10.9	5−500	0.9946	50[b]
β-endosulfan	31.57	1.1	84.9	9.1	5−500	0.9945	
endosulfan sulfate	35.53	1.1	99.0	7.0	5−500	0.9899	
λ-cyhalothrin	49.07	1.7	95.2	9.1	10−500	0.9892	20[b]
deltamethrin	62.25	6.0	110.7	7.6	20−500	0.9859	100[b]
			olives (method b: dehydration of olive samples by lyophilization)				
			GC-NPD method				
omethoate	12.05	2.0	86.0	10.3	10−500	0.9784	200[b]
dimethoate	15.02	1.2	76.4	6.3	5−500	0.9824	2000[b], 1000[a]
diazinon	16.96	1.2	72.0	2.8	5−500	0.9945	20[b]
fenthion	21.29	0.6	82.1	9.0	5−500	0.9954	2000[b]
fenthion sulfoxide	27.58	1.1	76.3	8.0	5−500	0.9888	
fenthion sulfone	28.26	1.3	77.1	7.2	5−500	0.9872	
chlorpyrifos	21.59	1.0	71.2	6.4	5−500	0.9788	50[b]
azinphos methyl	34.25	3.3	72.1	7.1	10−500	0.9921	500[b]
			GC-ECD method				
α-endosulfan	27.19	0.8	80.3	9.1	5−500	0.9972	50[b]
β-endosulfan	31.57	1.0	81.3	6.2	5−500	0.9897	
endosulfan sulfate	35.53	0.8	82.0	6.5	5−500	0.9896	
λ-cyhalothrin	49.07	1.0	92.3	8.7	5−500	0.9892	20[b]
deltamethrin	62.25	5.8	99.7	7.0	20−500	0.9936	100[b]

[a] Codex Alimentarius Commission 1996 (*3*). [b] EC 1976 (*2*); −, not specified.

data. All tests were performed at a 0.05 significance level. Analysis of data was performed using SPSS 15.0.

RESULTS AND DISCUSSION

Validation of Analytical Methodology. The analytical data of the methods validated for the selected pesticides in olive oil and olives are presented in **Table 3**. Method limits of detection (mLOD) and quantification (mLOQ) were calculated experimentally from a signal-to-noise ratio of 3.0 and 10.0, respectively, by spiking at low concentrations the olive and olive oil samples and subjecting them to the sample preparation reported. Blank extracts were used for the estimation of the background noise of the chromatographic analysis. Linearity of the methods was checked in the range 5−500 μg/kg by measuring the peak areas relative to that of the internal standard. Correlation coefficients were >0.99 in all cases, indicating a good linearity of both GC-NPD and GC-ECD methods for the quantification of target pesticides in the range studied (**Table 3**). The precision of the methods, expressed as repeatability (% RSD, $n = 9$), was evaluated by analyzing in triplicate olive oil samples fortified at 20, 50, and 100 μg/kg and olive samples fortified at 10, 50, and 100 μg/kg.

The development of the methodology used and its analytical performance for pesticides determination in olive oil have already been reported in our previous work (*31*). The optimum olive oil cleanup method was applied successfully for the residue determination of the selected pesticides in olive samples as well in this study. To optimize the method for the determination of the selected pesticides in olive samples, two different methods of dehydration were studied as follows: method a, where the dehydration step in the olive sample preparation was achieved by the addition of anhydrous Na_2SO_4, and method b, where the

Table 4. Average Processing Factors (F)[a] ± Coefficient of Variance (n = 15) as Determined in Samples Processed with the Three Olive Oil Extraction Systems Tested (P1, P2, and P3)

	P1	P2	P3	
pesticide	F_1 (n = 15)	F_2 (n = 15)	F_3 (n = 15)	ANOVA (p)
dimethoate	0.91 ± 0.17	0.72 ± 0.08	0.47 ± 0.08	<0.001
diazinon	3.33 ± 0.41	3.14 ± 0.24	2.74 ± 0.36	<0.001
fenthion	5.75 ± 1.84	5.14 ± 1.36	4.57 ± 1.21	0.109
total fenthion	5.83 ± 1.78	5.31 ± 1.23	4.83 ± 1.10	0.161
chlorpyrifos	2.94 ± 0.55	2.64 ± 0.58	2.35 ± 0.37	0.011
azinphos methyl	5.24 ± 0.39	5.20 ± 0.64	4.87 ± 0.76	0.215
α-endosulfan	3.31 ± 0.68	2.53 ± 0.65	1.95 ± 0.55	<0.001
β-endosulfan	3.06 ± 0.57	3.60 ± 0.96	3.32 ± 0.82	0.377
total endosulfan	3.78 ± 0.87	3.17 ± 0.92	2.48 ± 0.91	0.003
λ-cyhalothrin	2.37 ± 0.25	2.28 ± 0.24	2.22 ± 0.23	0.290
deltamethrin	3.67 ± 0.36	4.07 ± 0.67	3.83 ± 0.36	0.098

[a] F = concentration found in olive oil/concentration found in olives.

Table 5. Transfer of Pesticide in Olive Oil (%) ± Coefficient of Variance (n = 15) as Determined in Samples Processed with the Three Olive Oil Extraction Systems Tested (P1, P2, and P3)[a]

	% transferred			
pesticide	P1	P2	P3	ANOVA (p)
dimethoate	8.8 ± 0.8	8.4 ± 1.0	6.3 ± 1.1	<0.001
diazinon	33.3 ± 6.6	36.8 ± 4.4	36.2 ± 5.4	0.370
fenthion	58.6 ± 7.2	60.1 ± 17.1	60.0 ± 14.9	0.693
total fenthion	59.4 ± 6.9	62.0 ± 15.9	63.4 ± 13.0	0.378
chlorpyrifos	27.1 ± 9.1	31.0 ± 7.6	31.2 ± 5.8	0.988
azinphos methyl	50.0 ± 11.0	60.9 ± 9.8	64.9 ± 13.9	0.104
α-endosulfan	32.6 ± 9.9	29.4 ± 8.4	22.2 ± 12.4	0.038
β-endosulfan	36.9 ± 2.9	42.5 ± 10.4	45.2 ± 11.9	0.103
total endosulfan	38.0 ± 10.2	43.1 ± 8.4	48.9 ± 7.3	0.035
λ-cyhalothrin	23.4 ± 5.1	26.8 ± 4.6	29.4 ± 3.5	0.015
deltamethrin	36.2 ± 11.1	49.9 ± 5.1	50.6 ± 5.8	<0.001

[a] % transferred = processing factor (F) × oil yield (%) and oil yield (%) = mass (kg) of oil obtained/mass (kg) of olives processed × 100.

dehydration step in the olive sample preparation was achieved by lyophilization. Although with method a the concentration factor aimed at six times for the NPD method and 2.5 times for the ECD method, the mLOD of most pesticides studied were similar to those achieved with a method developed in olive oil where the concentration factors were three times for the NPD method and 1.5 times for the ECD method. This was due to the observed higher noise in olive extract chromatograms. Although recoveries of the less polar organophosphates were lower with method b, this method led to the lowest mLOD for all pesticides studied and the best analytical performance. The complete removal of the water content by the lyophilization of the sample concentrated residues at least one time by allowing a lower concentration factor of the final extract, which resulted in lower coextracted material and consequently lower noise and interferences in chromatograms. In addition, a significant higher precision (expressed as repeatability) was achieved by method b than by method a, due to interference of oil−water emulsifiers often formed during sample preparation by method a to pesticide recovery. As a consequence, method b was selected to be further used in the analysis of pesticides in olives.

Effect of Water Addition during the Olive Oil Production Process on the Processing Factors of Selected Pesticides. Three levels of water volume addition during oil extraction were studied as follows: no water addition (P1), 37.5 mL of water/100 g of olives (P2), and 70.0 mL of water/100 g of olives (P3). P3 was a simulation of the industrial three-phase oil extraction procedure. Different oil yields were obtained with the three different processes studied in the laboratory-scale olive oil extractions from fortified olives. These ranged from 6.88 to 12.79% with P1 (10.44 ± 2.44%, n = 15), from 9.00 to 13.02% with P2 (11.74 ± 1.56%, n = 15), and from 9.54 to 14.90% with P3 (13.29 ± 1.61%, n = 15). The highest oil yields were obtained with P3, and the lowest yields were obtained with the P1 procedure. In the P3 process, the paste becomes more fluid as a consequence of the addition of water, thus reducing the proportion of pulp and woody endocarp fragments and hence its viscosity.

The average processing factors (F) of the selected pesticides in olive oil and the average percentage (%) transfer of the active substances in oils obtained with the three olive oil extraction procedures are presented in **Tables 4** and **5**, respectively. Regression analysis among pesticides concentrations in olives and in olive oil indicated good linearity with high correlation coefficients ($r^2 > 0.97$) for all pesticides studied.

Processing factors were found to vary among the different pesticides studied. The pesticides determined belong to different chemical families and exhibit large differences among their physicochemical properties. As a result, the process of olive oil production affected their transfer into the oil phase in different ways, whereas the correlation observed among the processing factors determined and the main physicochemical properties (partition coefficient log K_{ow} and log S solubility in mg/L) of the pesticides studied were weak in all processes studied. The Pearson correlation coefficient among log F and log S (S = solubility in mg/L) was found −0.369 ($p < 0.001$) in samples from laboratory P1 and P2 oil extractions and −0.368 ($p < 0.001$) in samples from the laboratory P3 oil extraction. The positive correlation coefficients determined among log F values and log K_{ow} values of pesticides studied were 0.354 ($p < 0.001$) for the P1 and P2 oil extraction processes and 0.356 ($p < 0.001$) for the P3 oil extraction.

Dimethoate and α-endosulfan residues in olives were affected the most by the different olive oil extraction procedures used, and a significant decrease of F values and of the amounts transferred in olive oil was observed with the increase of water volume in the extraction procedure. However, these two pesticides are members of different chemical families with different physicochemical properties, whereas the observed water effect for dimethoate may be attributed to its high solubility in water, which leads to its pass into the water phase (processing waste waters) (*21, 25*); the lipophilic character of α-endosulfan does not allow a direct interpretation of the results (**Table 1**). In addition, endosulfan hydrolysis is alkaline and is not expected to take place during the olive oil extraction process. However, processing factors determined for α-endosulfan relative to β-endosulfan are in accordance with existing data on endosulfan degradation in the environment; β-endosulfan is more stable and less volatile than α-endosulfan, microbial hydrolysis degrades α-endosulfan faster than β-endosulfan, and β-endosulfan is hydrolyzed faster in chemical hydrolysis. The formation of endosulfan sulfate is accomplished in any case by enzymatic processes, whereas in remobilization experiments of endosulfan, α-endosulfan was reported to be more readily desorbed from sediments than β-endosulfan, with endosulfan sulfate somewhere in between (*33*).

Processing factors of diazinon and chlorpyrifos were affected less by the different olive oil extraction procedures by observing significant differences of F values at P1 and P3 processes. As can be seen in **Table 5**, the amount of these pesticides transferred in olive oil was the same in all olive oil extraction processes studied, and the observed differences of F values were due to the higher oil yields observed in the P3 process.

Table 6. Residues (μg/kg $\pm$ SD) of Fenthion Sulfoxide and Endosulfan Sulfate in Olives and Olive Oils Obtained with the Three Oil Extraction Procedures Tested[a]

Fenthion Sulfoxide							
		P1		P2		P3	
fenthion C_{olives}	C_{olives}	C_{oil}	conversion[b] (%)	C_{oil}	conversion (%)	C_{oil}	conversion (%)
1301.2 $\pm$ 87.3	ND	191.6 $\pm$ 20.7	1.88 $\pm$ 0.17	323.0 $\pm$ 59.5	3.00 $\pm$ 0.43	471.9 $\pm$ 44.9	4.91 $\pm$ 0.34
692.2 $\pm$ 44.1	ND	98.9 $\pm$ 12.6	1.92 $\pm$ 0.18	218.5 $\pm$ 26.3	3.64 $\pm$ 0.59	239.1 $\pm$ 23.5	4.63 $\pm$ 0.35
330.7 $\pm$ 21.7	ND	39.3 $\pm$ 17.8	1.12 $\pm$ 0.48	85.7 $\pm$ 7.8	3.13 $\pm$ 0.24	128.8 $\pm$ 15.8	5.32 $\pm$ 0.09
128.8 $\pm$ 7.6	ND	ND	–	ND	–	28.0 $\pm$ 3.1	2.42 $\pm$ 0.46
75.1 $\pm$ 13.3	ND	ND	–	ND	–	ND	–

Endosulfan Sulfate							
		P1		P2		P3	
endosulfan C_{olives}	C_{olives}	C_{oil}	conversion[c] (%)	C_{oil}	conversion (%)	C_{oil}	conversion (%)
50.5 $\pm$ 10.1	>BQL	17.2 $\pm$ 1.3	4.43 $\pm$ 0.63	11.9 $\pm$ 1.6	2.88 $\pm$ 0.18	19.8 $\pm$ 1.5	5.46 $\pm$ 1.16
31.0 $\pm$ 0.0	ND	9.7 $\pm$ 0.5	4.20 $\pm$ 0.11	18.6 $\pm$ 7.3	6.92 $\pm$ 2.71	8.9 $\pm$ 1.0	3.84 $\pm$ 0.41
12.1 $\pm$ 0.4	ND	9.40	7.60	5.2 $\pm$ 0.3	5.05 $\pm$ 0.22	5.7 $\pm$ 0.4	6.46 $\pm$ 0.52
4.4 $\pm$ 0.5	ND	6.30	–	ND	–	ND	–
2.7 $\pm$ 0.2	ND	ND	–	ND	–	ND	–

[a] * = of parent compound. [b] % fenthion in olives converted to fenthion sulfoxide in olive oil. [c] % endosulfan (sum of isomers) in olives converted to endosulfan sulfate in olive oil; ND, not detected; BQL, below method quantification limit.

Processing factors of azinphos methyl, λ-cyhalothrin, β-endosulfan, and deltamethrin were not affected significantly by the water addition used in the different laboratory olive oil extraction procedures. However, significantly higher amounts of these fat-soluble pesticides were transferred in olive oil by the P3 process that was found to lead in higher oil yields as compared with the P1 oil extraction process.

Processing factors of fenthion as well as its percent transfer in olive oil were not affected by the different oil extractions tested (**Tables 4** and **5**). However, fenthion sulfoxide (**Table 6**) was detected only in oils obtained from olives processed with P1 and P2 at high concentrations and in all oils obtained with P3 procedure and was found to increase with the increase of water in olive oil extraction and with the increase of the initial concentration of fenthion (parent compound) in olives. The percentage (%) of the initial quantity of fenthion (parent compound) on olives determined as fenthion sulfoxide in olive oil obtained by P1 process was found to be 1.64 $\pm$ 0.47%, whereas in olive oils obtained by P2 and P3 processes, these percentages were 3.26 $\pm$ 0.48 and 4.32 $\pm$ 1.21%, respectively.

Endosulfan sulfate in laboratory experiments was detected in all oils obtained by olives spiked with high concentrations and in one olive sample that was spiked with the highest concentration of endosulfan (parent compound) (**Table 6**). No significant differences on endosulfan sulfate concentrations in olive oil were observed among the different concentrations of the parent compound used on olives processed or the different extraction procedures studied.

Processing Factors of Selected Pesticides in Olive Oil Extraction Process by Three-Phase Centrifugation Systems. The methods developed for pesticides determination were applied in olive and olive oil samples collected during 30 olive oil extractions in conventional olive mills located at different olive oil-producing areas (Peloponnese, Crete, Preveza, and Chalkidiki). All mills used three-phase centrifugation systems for oil separation. The relative residue data of olive and olive oil samples analyzed are shown in **Table 7**. In 96.7% of samples analyzed, pesticide residues were detected. The highest detection rates were observed for residues of dimethoate, fenthion, and endosulfan. Except for one olive sample that contained omethoate (394.8 μg/kg) above the MRL (200 μg/kg), the other detected pesticides in olive samples were below MRLs for the commodity of olives (*2*). The obtained oil from olives containing omethoate above MRL contained negligible residues of this active ingredient (below the method quantification limit).

The calculable processing factors derived from positive detections above method quantification limits (F') and the processing factors determined in laboratory experiments with P3 oil extraction process that imitated the extraction process from conventional mills, where samples were collected (F_3), are also presented in **Table 7**.

Dimethoate. Processing factors for the parent compound dimethoate (F' = 0.29 $\pm$ 0.21) (**Table 7**) were found to be similar to those reported by other authors (F = 0.22−0.33) (*19–21*). Processing factors for dimethoate in the P3 olive oil extraction process (F_3 = 0.47 $\pm$ 0.08) were higher, and this difference can be attributed to the close system of the laboratory unit used for oil extraction that prevented evaporation losses and oxidation processes.

Omethoate, the main oxidative metabolite of dimethoate, was not detected in any sample analyzed in experimental olive oil extractions of fortified olives with dimethoate. In olive mill samples, omethoate was detected in both olive and olive oil samples analyzed. This more toxic than the parent compound metabolite was not expected to be concentrated in olive oil due to its high water solubility and volatility (*21*). However, when omethoate was detected in olives at concentrations below 100 μg/kg, processing factors in olive oil were found higher than those of dimethoate. A possible explanation might be that in open industrial systems of olive oil production the oxidation of dimethoate to omethoate could occur after oil separation. Moreover, in most olive samples with omethoate at concentrations below 100 μg/kg, higher levels of dimethoate were detected, whereas processing factors of total dimethoate (as sum of dimethoate and omethoate) calculated in olive mill samples and in laboratory extractions were not significantly different (**Table 7**).

The powerful effect of the water addition during oil separation determined in laboratory oil extractions for dimethoate was confirmed by a significantly negative correlation (Spearman R

Table 7. Mean Concentrations (C_M, above mLOQ), Positive Detections and Processing Factors of the Pesticides Detected in Olives, and Olive Oil Samples Obtained during Olive Oil Extraction in Conventional Olive Mills (Three-Phase Centrifugation Systems)

	olives ($n = 30$)		olive oil				
pesticide	C_M (μg/kg)	positive[a]	C_M (μg/kg)	positive	F'	F_3	p
omethoate	49.3	22 (4)	18.8	8 (1)	1.37 ± 0.41 ($n = 7$)	ND	–
dimethoate	71.9	27 (2)	31.6	18 (8)	0.29 ± 0.21 ($n = 10$)	0.47 ± 0.08	0.013
total dimethoate	99.1	27 (0)	30.7	22 (7)	0.42 ± 0.32 ($n = 14$)	0.47 ± 0.08	0.270
diazinon	2.0	1 (1)	2.3	4 (3)	–	2.74 ± 0.36	–
fenthion	68.9	14 (2)	164.0	19 (0)	4.39 ± 1.29 ($n = 11$)	4.57 ± 1.21	0.856
fenthion sulfoxide	22.6	13 (1)	51.8	18 (2)	3.52 ± 2.09 ($n = 11$)	–	–
fenthion sulfone	18.3	7 (0)	46.9	14 (4)	4.27 ± 2.54 ($n = 5$)	ND	–
total fenthion	87.1	15 (1)	231.6	20 (0)	3.77 ± 1.63 ($n = 14$)	4.83 ± 1.10	0.067
chlorpyrifos	13.1	3 (1)	27.4	6 (3)	2.55 ± 0.10 ($n = 2$)	2.35 ± 0.37	–
azinphos methyl	15.0	5 (4)	56.4	5 (4)	3.76 ($n = 1$)	4.87 ± 0.76	–
α-endosulfan	7.6	17 (3)	2.2	4 (4)	–	1.95 ± 0.55	
β-endosulfan	8.8	17 (7)	2.9	7 (7)	–	3.32 ± 0.82	–
endosulfan sulfate	9.1	17 (4)	14.2	20 (4)	1.85 ± 0.83 ($n = 13$)	–	–
total endosulfan	20.8	17 (2)	14.2	20 (4)	0.76 ± 0.34 ($n = 15$)	2.48 ± 0.91	0.000
λ-cyhalothrin	6.5	3 (2)	14.7	3 (0)	2.74 ($n = 1$)	2.22 ± 0.23	–
deltamethrin	ND	ND	ND	ND	–	3.83 ± 0.36	–

[a] In columns are shown the number of samples where the residue was detected. In parentheses are shown the number of samples that were positive and below the method quantification limit; F', processing factors calculated from residue data in surveyed samples; F_3, processing factors calculated in laboratory oil extractions with P3; ND, not detected; p is the two-tailed significance obtained from Mann−Whitney U test among F' and F_3 values (F' and F_3 = concentration in olive oil/concentration in olives).

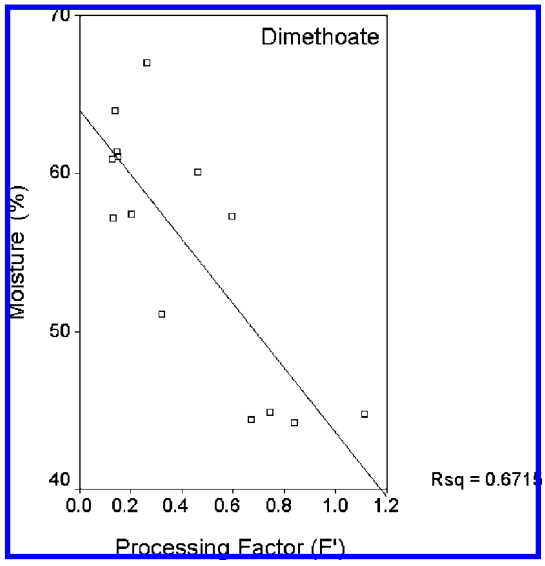


Figure 2. Correlation between the processing factors determined for dimethoate in samples collected from olive mills and the water content of the olive fruits processed into oil.

correlation coefficient, −0.709; $p < 0.001$) observed among the calculated processing factors determined from residue data of samples collected from olive mills (F') and the moisture of olive fruit processed (samples collected from olive mills) (**Figure 2**).

Diazinon. Processing factors for diazinon were calculated only experimentally because residues of diazinon in olives were below the method quantification limit (**Table 7**). Processing factors in laboratory-scale oil extractions ranged from 2.74 when P3 was followed to 3.33 with P1 (**Table 4**), and no significant differences were observed among the different concentrations studied. Similar processing factors for diazinon in olive oil process with no water addition (P1 in this study) have been reported by Cabras and co-workers (*22*) (3.3 when the concentration of diazinon in olives was from 680 to 1340 μg/kg and 5.6 when the concentration in olives was at 350 μg/kg) and Ferreira and Tainha (*19*) (3.03−5.00 when the concentration in olives was at 470−2500 μg/kg with lower F values to be observed at high concentrations in olives processed into oil). However, in this study, olives that were processed contained significantly lower levels of diazinon, and no differences were observed among the different concentrations studied.

Fenthion. Processing factors calculated from residues of fenthion (parent compound) in olive mill samples were similar to those calculated in laboratory with P3 and were in accordance with results reported in previous studies (*20–22*). Processing factors for fenthion sulfoxide and fenthion sulfone that were calculated from residue data from samples collected from olive mills in this study ($F' = 3.52 \pm 2.09$ for fenthion sulfoxide and $F' = 4.27 \pm 2.54$ for fenthion sulfone) (**Table 7**) were found to be higher than those derived from data reported by Cabras and co-workers (*20*) ($F = 0.53-1.31$, $n = 2$ for fenthion sulfoxide, and $F = 0.75-1.25$, $n = 2$ for fenthion sulfone). Fenthion sulfone was not detected in any sample (olives and olive oil) of the laboratory oil extractions performed, and fenthion sulfoxide was detected only in experimentally produced oils. Thus, it was not possible to estimate processing factors of fenthion metabolites during olive oil extraction in laboratory-scale experiments.

Chlorpyrifos. No data for the behavior of chlorpyrifos in olive oil production process were found in the literature. The processing factors of chlorpyrifos ($F' = 2.55 \pm 0.10$) in two olive oil extractions in olive mills were similar with those calculated in laboratory studies by the P3 process ($F_3 = 2.35 \pm 0.37$) (**Table 7**). The processing factors estimated for chlorpyrifos were lower than expected according to its main physicochemical properties (log K_{ow} and water solubility), and this trend could be correlated either with the chlorine substitutes on its molecular structure that may interact with olive cake components or with the low concentrations studied.

Azinphos Methyl. Although residues of azinphos methyl on olives and olive oil were detected in samples from five olive oil extractions in olive mills, processing factors could be calculated only in one, and that was found o be equal to 3.76 (**Table 7**). Processing factors calculated experimentally for azinphos methyl were higher ($F_3 = 4.87 \pm 0.76$), whereas those reported by Cabras et al. (*22*) ranged from 2.3 to 3.0. However, the transfer of this pesticide in olive oil with the

P1 procedure was similar to these reported data (ca. 50%, **Table 5**), and the difference in F may be due to the different levels of azinphos methyl used in olives processed into oil.

Endosulfan. Processing factors from α- and β-endosulfan residue data detected in samples obtained from olive mills could not be calculated (these were below the method quantification limit) (**Table 7**). However, positive detections of α-endosulfan in olive oil samples from olive mills were low relative to β-endosulfan, indicative of its dissipation during the olive oil extraction process, and this result is in accordance with results from laboratory experiments. Processing factors calculated for total endosulfan in laboratory olive oil extractions with P3 ($F_3 = 2.48 \pm 0.91$) were significantly higher as compared with those derived from residue data from olive mill samples ($F' = 0.76 \pm 0.34$) (**Table 7**) due to the initial content of total endosulfan in olives processed, which was significantly different from this in olive mill samples that contained higher concentrations of endosulfan sulfate and lower of α- and β-endosulfan. These results are supported by existing monitoring data of endosulfan residues in olive oil, where the most abundant residue is endosulfan sulfate, whereas α- and β-isomers are rarely detected (*24, 25*).

λ-Cyhalothrin. The λ-cyhalothrin processing factor calculated from residues detected in samples from one olive oil extraction process was similar with those calculated in laboratory studies (**Table 7**). No data for the processing factor of this pyrethroid pesticide in olive oil are available. However, the processing factors determined for λ-cyhalothrin as compared with those determined for deltamethrin (**Table 4**), which is a more apolar insecticide of pyrethroids, are in accordance with their physicochemical properties.

Deltamethrin. Deltamethrin was not detected in any of the samples collected from olive mills (**Table 7**). In laboratory-scale oil production, processing factors of deltamethrin were determined at 3.83 ± 0.36 with P3. Leandri et al. (*21*) reported a similar concentration of deltamethrin residues in olive oil (three times when olives processed contained residues at concentrations 34−38 μg/kg and higher up to six times when residues on olives were at 2−9 μg/kg).

Conclusions. In this work, processing factors of eight pesticides widely used in olive groves in Mediterranean countries were estimated in olive oils produced with three different extraction procedures that differ in the amount of water used in centrifugation system. For this purpose, a selective multiresidue method for the determination of multiclass pesticides in olives based on SPE techniques coupled to GC detection (NPD and ECD) was developed and validated. The lyophilization of olives led to higher mLOD and higher precision on pesticides analysis in olives by GC-NPD.

All pesticide residues studied, except for dimethoate, were found to concentrate in olive oil. Water addition in the olive oil extraction process was found to have a double effect on pesticide concentration in olive oil: (i) to increase oil yields and consequently the transfer of fat-soluble pesticides by not changing their final concentration in olive oil as observed for deltamethrin and λ-cyhalothrin (**Table 5**) and (ii) to decrease processing factors of pesticides with high water solubility and/or those susceptible to hydrolytic processes as observed for dimethoate, α-endosulfan, diazinon, and chlorpyrifos (**Table 4**).

Dimethoate and α-endosulfan processing factors in olive oil were affected the most by the water addition in olive oil extraction processes and water contents of olive fruit were found to affect processing factors of dimethoate in olive oil proportionally. In consequence, oil yield and water content of the variety of olive cultivations for oil production could affect processing factors of pesticide residues in olive oil. The latter result could be considered in pest control management by the use of pesticides as oil yields of different olive varieties have been mapped, and these data could be useful in such considerations (*34*).

Therefore, olive variety together with the virgin olive oil extraction technology used appear to be important factors on pesticide concentrations in olive oil. These results encourage studies for further investigation on the parameters in olive oil production experiments that may influence and eliminate pesticide residues in olive oil (e.g., malaxation temperature, microbial degradation, salinity, and metal-catalyzed hydrolysis).

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