ELSEVIER

Food Chemistry 79 (2002) 303–305

Food Chemistry

www.elsevier.com/locate/foodchem

Determination of organophosphorus pesticide residues in Cilento (Campania, Italy) virgin olive oil by capillary gas chromatography

Luca Rastrelli*, Katia Totaro, Francesco De Simone

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte don Melillo, 84084, Fisciano (SA), Italy

Received 4 July 2001; received in revised form 30 January 2002; accepted 30 January 2002

Abstract

In this study, the occurrence of 18 organophosphorus pesticide residues in Cilento (Campania, Italy) virgin olive oil was investigated. Sixty-five samples were taken from the major production areas during 1999–2000. In 31 samples, nine organophosphorus pesticides, namely azinphos-ethyl, chlorpyrifos-methyl, diazinon, dimethoate, fenthion, formothion, methidathion, parathion and parathion-methyl were found in concentrations ranging from 0.030 to 0.120 mg/kg. Acefate, carbophenothion, chlorpyrifos, malaoxon, malathion, methamidophos, omethoate, paraoxon-methyl, and pirimiphos-methyl were not detected in any sample. Analysis was carried out using capillary gas chromatography with a Nitrogen/Phosphorus Detector (NPD) detector, after sample extraction with *n*-hexane and clean-up by a single-step multi-cartridge system. Recoveries of 18 pesticides at three fortification levels (0.5, 1.0 and 2.5 mg/kg) were in the range 82–110%. Only two samples contained dimethoate residue that exceeded the FAO/WHO Codex Alimentarius maximum residue limits (MRLs).

Keywords: Cilento virgin olive oil; Protected designation of origin; Campanian (Italy) oil; Organophosphorus pesticide residues; Capillary gas chromatography

1. Introduction

''Cilento'' virgin olive oil is obtained from the fruit of six cultivars Pisciottana, Rotondella, Ogliarola, Frantoio, Salella, Leccino of olive tree (*Olea europea*) that grow mainly in the Cilento National Park (Campania region, Italy). The origin of this oil is guaranteed and this product is defined as a ''Protected Designation of Origin'' (PDO; EC, 1998) and presents a few characteristics of quality and of originality that are the result of the geographical influences and the human factor. Because of its nutritional and biological characteristics, Cilento virgin olive oil is one of the most important components of the Mediterranean diet (Ferro-Luzzi & Sette, 1989). Olive trees are attacked by several pests, mainly the olive fruit fly *Bactocera* (*Dacus*) *Oleae*, and receive treatment with several pesticides. Those more extensively used belong to the class of organophosphorus insecticides and are mainly fenthion, dimethoate, diazinon, parathion-methyl, methidathion, and azinphos-ethyl. Toxic residues in olive oil have been reported by several researchers (Lentza-Rizos, 1994; Morchio, Andreis, & Verga, 1992). Because pesticide residues in food constitute a significant health risk, and olive oil has a high consumption rate among people of the producing countries, the continuous control of pesticide residues in olive oil is of great importance.

The Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) has established maximum residue limits (MRLs) for pesticide residues in olives and olive oil (Codex Alimentarius Commission, 1996). Maximum levels for pesticide residues in and on certain products of plant origin, including olives, are also fixed by the European Community (EC, 1976) and by the Italian Department of Health (2000).

The target compounds studied, namely acefate, azinphos-ethyl, carbophenothion, chlorpyrifos, chlorpyrifos-methyl, diazinon, dimethoate, fenthion, formothion, malaoxon, malathion, methamidophos, methidathion, omethoate, paraoxon-methyl, parathion, parathion-methyl and pirimiphos-methyl, were selected for monitoring in Cilento virgin olive oil, due to their intensive use in olive tree treatment. They are also

* Corresponding author. Fax: +39-089964356.
E-mail address: rastrelli@unisa.it (L. Rastrelli).

0308-8146/02/$ - see front matter
PII: S0308-8146(02)00143-7

included in the pesticides for which MRLs have been established by the European Communities for olives (EC, 1991) and by the Codex Alimentarius Commission for olive oil.

Sixty-five samples of virgin olive oil were analysed, determination was carried out by capillary gas chromatography (GC) using NPD with capillary column. The results of this monitoring of virgin olive oil samples from Cilento National Park are reported.

2. Materials and methods

2.1. Samples

Sixty-five samples of virgin olive oil were collected from some of the major olive oil production areas of Cilento National Park (Campania), namely S. Mauro Cilento, Giungano, Montecorice, Pisciotta during 1999 (36 samples) and 2000 (29 samples). Virgin olive oil samples were collected in dark glass bottles. The samples were transported to the laboratory and kept for a short time at 4 °C until analysis.

2.2. Materials and reagents

All organic solvents were products of Carlo Erba, Milano (Italy). Compounds of purity 97–99% were products of Dr. Ehrenstorfer, Augsburg (Germany) and provided by Labservice Analytica, Bologna (Italy). Stock standard solutions of each organophosphorus pesticide were prepared in acetone at 1000 mg/l. An intermediate solution of the mixture of all pesticides was also made up in acetone containing ca. 50 mg/l of each one. Ready-to-use Extrelut-3 columns (Merck, Darmstadt, Germany) were used as received for the on-column partition. Sep-Pak silica cartridges (Water Associates, Milford, USA) and Sep-Pak C18 cartridges (Water Associates) were also used.

2.3. Extraction and cleanup

The extraction and the multi-cartridge method employed were based on the procedure of Di Muccio et al. (1990). A 6-g sample of oil was mixed with 10 ml of *n*-hexane. Of the above, 3 ml lipid solution were loaded onto the top of an Extrelut-3 column to which a silica-gel cartridge and a C18 silica cartridge had been connected (the two cartridges were linked together by means of a small glass connector). After 10 min, the lipidic extract was eluted under gravity with 5 ml portions of acetonitrile (saturated with hexane); the eluate was collected in a 50 ml Erlenmeyer flask, 4 ml MeOH added, and evaporated to dryness by a rotary evaporator (40–45 °C water-bath, reduced pressure). The last traces of solvent were removed in a gentle stream of nitrogen. The residue was redissolved in acetone (1 ml) and was transferred quantitatively into a 2 ml volumetric flask; 1 μl of the solution was injected into the GC–NPD.

2.4. GC analysis

The analysis of the 18 organophosphorus pesticides was carried out by capillary GC using a Chrompack model 9001 GC, with NPD, split/splitless injection port, and a CP-SIL 13CB fused silica capillary column by Chrompack (50 m×0.32 mm i.d., 0.4 μm film thickness).

The temperature programme applied in GC/NPD was as follows: 110 °C for 2 min, 110–250 °C at 6 °C/min, and 250 °C for 15 min. The temperature of the detector was 290 °C. The injection volume was 1 μl.

2.5. Recovery experiments, detection limits (DLs) and quantitative evaluation

Untreated oil samples were fortified, on average at 0.1, 1.0 and 2.5 mg/l, by adding intermediate pesticide solutions in hexane. Samples were allowed to equilibrate for 30 min prior to extraction, and were processed according to the earlier procedure. The recovery assays were replicated four times. The recovery values were not related to the spiking level. Data derived from these experiments are presented in Table 1. The DLs, calculated by using a signal-to-noise (S/N) ratio of 3, were in the range of 0.001–0.02 mg/kg. The quantity of each pesticide was calculated by applying the external standard method.

Table 1
Mean percent recovery±RSD of 18 organophosphorus pesticides in olive oil samples at 0.1, 1.0, and 2.5 mg/kg fortification levels ($n=4$)

Organophosphorus pesticide	Mean % recovery ±RSD at different fortification levels of (mg/kg)		
	0.5	1.0	2.5
Acefate	108±9	110±8	106±9
Azinphos-ethyl	82±12	83±10	83±9
Carbophenothion	103±3	98±2	97±4
Chlorpyrifos	95±4	94±3	91±5
Chlorpyrifos-methyl	95±6	88±2	93±4
Diazinon	92±9	92±11	94±15
Dimethoate	103±13	106±10	107±14
Fenthion	89±7	86±9	83±9
Formothion	93±5	88±9	93±9
Malaoxon	106±8	100±8	98±6
Malathion	86±6	82±8	88±8
Methamidophos	90±4	93±6	95±4
Methidathion	96±4	94±5	91±4
Paraoxon-methyl	83±4	90±6	97±7
Parathion	84±8	85±11	88±9
Parathion-methyl	85±16	83±12	86±8
Pirimiphos-methyl	95±5	88±4	89±8
Omethoate	106±8	109±7	110±4

Table 2
Organophosphorus pesticides in 65 samples of cilento virgin olive oil from individual growers, produced during 1999/2000[a]

Pesticide	Mean value (mg/kg)	Conc. range (mg/kg)	No. of positive samples
Acefate		N.D.[b]	–
Azinphos-ethyl	0.090	0.080–0.100	2
Carbophenothion		N.D.	–
Chlorpyrifos		N.D.	–
Chlorpyrifos-Methyl	0.080	0.050–0.090	4
Diazinon	0.083	0.064–0.101	3
Dimethoate	0.061	0.030–0.120	29
Fenthion	0.073	0.055–0.085	18
Formothion	0.082	0.082	1
Malaoxon		N.D.	–
Malathion		N.D.	–
Methamidophos		N.D.	–
Methidathion	0.063	0.051–0.085	3
Omethoate		N.D.	–
Paraoxon-methyl		N.D.	–
Parathion	0.080	0.060–0.100	2
Parathion-methyl	0.056	0.056	1
Pirimiphos-methyl		N.D.	–

[a] Number of samples without residues: 34. Number of samples with residues greater than the Codex MRL: 2.
[b] ND, not detectable.

3. Results and discussion

Organophosphorus pesticides are widely used in agriculture and animal production for the control of various insects. These compounds have higher acute toxicities than chlorinated pesticides and they have the advantage of being more rapidly degraded in the environment. A study of the possible contamination of virgin olive oil produced in the Cilento National park (Campanian region, Italy) with 18 organophosphorus pesticides was carried out during the crop years 1999–2000. The target compounds were selected among those commonly used for controlling the olive fruit fly (*Daucus oleae*) in Italy. Two of these insecticides, methamidophos and omethoate, are metabolites of acephate and dimethoate, respectively. The target pesticides were determined by GC, using capillary columns and NPD detectors. The calculation of the amount of the organophosphorus pesticides present was carried out by comparing the peak areas for unknown samples with the corresponding peaks for standards, according to established procedures.

Sixty-five samples of virgin olive oil, collected from individual growers at the locations mentioned, were analysed. The levels of the 18 organophosphorus pesticides in the virgin olive oil samples are presented in Table 2. Thirty-four samples contained no detectable residues. Dimethoate residues were detected in 29 samples at concentrations ranging from 0.030 to 0.120 mg/kg. Fenthion was detected in 18 samples at concentrations ranging from 0.055 to 0.085 mg/kg. Chlorpyrifos-methyl and azinphos-ethyl were detected in four samples at concentrations ranging from 0.050 to 0.100 mg/kg. Diazinon, parathion-methyl, and parathion were detected in three samples each. Methidathion and formothion were detected in two and one samples, respectively. Acefate, carbophenothion, chlorpyrifos, malaoxon, malathion, methamidophos, omethoate, paraoxon-methyl and pirimiphos-methyl, were not detected in any sample.

The most common organophosphorus pesticide residues found were dimethoate and fenthion. Fenthion was detected in 27% of olive oil samples, and dimethoate in 44% of the samples. These findings indicate that dimethoate is one of the most important residues in olive oil.

The results of our monitoring of organophosphorus pesticides indicate that, among 65 samples of Cilento virgin olive oil that were examined, only two samples were found to have dimethoate (0.120 and 0.092 mg/kg) in concentration above the MRL of Codex Alimentarius for olive oil (0.05 mg/kg). However, none of Campanian virgin olive oil samples contained residues higher than the maximum permissible levels recorded by the Italian Department of Health.

References

Codex Alimentarius Commission. (1996). *Codex alimentarius pesticide residues in food-maximum residues limits. 2nd ed., Vol. 2B.* Rome: FAO/ WHO Press.

Di Muccio, A., Ausili, A., Vergori, L., Camoni, I., Dommarco, R., Gambetti, L., Santilio, A., & Vergori, F. (1990). Single-step multicartridge cleanup for organophosphate pesticide Rrsidue determination in vegetable oil extracts by gas chromatography. *Analyst*, *115*, 1167–1169.

EC (European Communities) (1976). *Council Directive 76/895 of 23 November 1976. Relating to the fixing of maximum levels for pesticide residues in and on certain products of plant origin including fruit and vegetables, L340, 9.12.76* (Modifications 90/642 of 27 November 1990, L350, 14.12.90, 93/58 of 29 June 1993, L211, 95/38 of 17 July 1995, L197, 22.8.95, 96/32 of 21 May 1996, L144, 18.6.96). Off. J. Eur. Communities.

EC (European Communities) (1991). *Regulation 2568/91. On the characteristics of olive oil and oil-residue oil and on the relevant methods of analysis*. L248 5.9.91. Off. J. Eur. Communities..

EC (European Communities) (1998). *Regulation No. 1726 of 22 July 1998. On the protection of geographical indications and designations of origin for agricultural products and foodstuffs*. Off. J. Eur. Communities.

Ferro-Luzzi, A., & Sette, S. (1989). The Mediterranean diet: an attempt to define its present and past composition. *European Journal of Clinical Nutrition*, *43*, 13–29.

Italian Government, Department of Health (2000). *D.M. 19 May 2000. Testo unico sui limiti massimi di residui di sostanze attive contenute nei prodotti fitosanitari.*

Lentza-Rizos, Ch. (1994). Monitoring pesticide residues in olive products: organophosphorus insecticides in olives and oil. *J. AOAC*, *77*, 1096–1100.

Morchio, G., De Andreis, R., & Verga, G. R. (1992). Investigation of organophosphorus residues in vegetable oils with special reference to olive oil. *Riv. Ital. Sostanze Grasse*, *69*, 147–157.