Food and Chemical Toxicology 47 (2009) 214–219

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Persistence and effects of rotenone on oil quality in two Italian olive cultivars

Teresa Casacchia [a,*], Adriano Sofo [b], Pietro Toscano [c], Luca Sebastianelli [d], Enzo Perri [c]

[a] Freelance nutritionist, via Lazio No. 24/A, 87100 Cosenza, Italy
[b] Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano No. 10, 85100 Potenza, Italy
[c] CRA, Centro di Ricerca per l'Olivicoltura e l'Industria Olearia, c. da Li Rocchi-Vermicelli, 87036 Rende, Cosenza, Italy
[d] Clinica Neurologica, Policlinico Tor Vergata, Viale Oxford No. 81, 00133 Roma, Italy

ARTICLE INFO

Article history:
Received 17 July 2008
Accepted 2 November 2008

Keywords:
Bactrocera olea Gmel.
Olea europaea L.
Olive oil quality
Parkinson's disease
Rotenone

ABSTRACT

The aims of this work are to assess the persistence of rotenone in oil and drupes of olive plants of cultivars Nocellara del Belice (NB) and Cassanese (CA), and to compare the quality of oils from rotenone-treated and untreated plants. Samples of drupes and oil were analyzed at 2, 12, 22 and 30 days after treatment. Rotenone levels in drupes of treated plants declined by about 50% after 22 days from treatment (0.037 mg kg^{-1} in NB and 0.039 mg kg^{-1} in CA), whereas the respective values in the oil were higher (0.209 mg kg^{-1} in NB and 0.229 mg kg^{-1} in CA) and had a lower decay half-life (4.02 days in NB and 4.71 days in CA). For both cultivars, no significant differences in oil physicochemical and nutritional parameters were found between the two treatments. The panel test of oils extracted after 22 days did not reveal significant differences in unpleasant aromatic notes nor defects between the two treatments. Our results confirm that serious doubts remain about the safety and healthiness of oils extracted from drupes treated with rotenone. This information could assess the real risk in the use of this product for plant protection in olive growing.

1. Introduction

The phytosanitary status of olive drupes significantly affects the quality of the finished product (Perri et al., 2002; Cerretani et al., 2005). For this reason, among the eligible products for the protection against olive pathogens, rotenone has been widely used in organic olive growing (Iannotta, 2003; Cabras et al., 2002).

Rotenone is an active ingredient extracted from *Derris elliptica* (Wall.) Benth. and, although its natural origin, represents a serious toxicological threat to food safety (Iannotta, 2003; Casacchia et al., 2007). This chemical is very persistent and quickly decomposes in residues that could accumulate in the final products (Cabras et al., 2002). Moreover, rotenone is extremely lipophilic and acts mainly by contact, causing death by suffocation in several orders of phytoparasitic insects, such as diptera, lepidoptera and coleopteran, with a consequent negative impact on the environment (Singer and Ramsay, 1994).

An experimental study based on a model of chronic poisoning demonstrated that the minimum effective dose of rotenone to induce a Parkinson-type syndrome in rats is 2.0–3.0 mg $kg^{-1}day^{-1}$ (Betarbet et al., 2000). Moreover, rotenone in rats inhibits the mitochondrial NADH dehydrogenase and causes a reduction in ATP synthesis, the inhibition of mitochondrial complex I, an increase of cell oxidative stress and the accumulation of cytotoxic proteins (Lee et al., 2000; Hoglinger et al., 2003; Kim et al., 2007). This suggests that there might be a direct relationship between exposure to rotenone and Parkinson's disease and/or other neurological diseases in humans (Heikkila and Sonsalla, 1987; Jenner, 2001). For all these reasons, the EU Commission has recently revoked the permission of rotenone as a plant protection product for olive (EC Decision 317/2008).

Rotenone is an efficacious product to control *Bactrocera olea* Gmel., commonly called olive fly (Iannotta, 2003; Cabras et al., 2002). In olive plants, attacks by *B. olea* cause severe changes in fruit physicochemical characteristics, with a negative effect on the quality and on organoleptic parameters of olive oils. In fact, when the degree of infestation of *B. olea* reaches 30–40%, oxidative and hydrolytic processes in olive oil sharply increase, so affecting negatively its quality (Scarpati et al., 1996; Servili et al., 2004; Cerretani et al., 2005). Peroxide number in olive oil extracted from fruits infested by *B. olea* is often significantly higher if compared to healthy drupes (Zunini et al., 1992). Oil fatty acids composition does not depend much on the degree of infestation by *B. olea*, but decreases in oleic acid and increases in γ-linolenic acid were observed in plants attacked by this pathogen (Parlati et al., 1990; Zunini et al., 1992). The panel test conducted on oils extracted from drupes with different degrees of attack presents slight decreases in scoring for plants at 25–50% infestation level and sharp falls (average score below 6) in plants at 75–100% infestation level (Cabras et al., 2002).

* Corresponding author. Tel.: +39 334 2127958.
E-mail addresses: teresa.casacchia@nutrizioneumana.it (T. Casacchia), adriano.sofo@unibas.it (A. Sofo), pietro.toscano@entecra.it (P. Toscano), sebastianelli@med.uniroma2.it (L. Sebastianelli), enzo.perri@entecra.it (E. Perri).

0278-6915/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2008.11.002

Considering the possible harmfulness of rotenone and the few studies on this subject, the objectives of this work are (a) to assess the persistence of rotenone in oil and in drupes of plants treated with the doses of principle active recommended by the producers, and (b) to compare the physicochemical characteristics of olive oils extracted from rotenone-treated and untreated plants. This information could assess the real risk in the use of this product for plant protection in olive growing.

2. Materials and methods

2.1. Study site and experimental design

The trials were conducted in 2006 on two cultivars of southern Italy (Nocellara del Belice and Cassanese) planted in two experimental fields of CRA – Research Center for Olive Growing (Rende, Cosenza, Italy) subjected to the same agronomic management. The cv Cassanese (CA) has low yields in oil and is quite sensitive to fly, while the cv Nocellara del Belice (NB) has high yields in oil and is more resistant to *B. olea* attack (International Olive Oil Council, 2000).

Olive plants were monitored from the end of August to detect the degree of infestation by *B. olea*. At the end of September, the period of highest *B. olea* incidence, an average percentage of active infestation of 10% was observed on a composite sample of drupes (200 drupes from 10 randomly chosen plants for each cultivar). For each cultivar, homogeneous ad uniform plants (having similar degree of infestation, age and fruit yield) were divided in two groups: 20 rotenone-treated plants and 20 untreated plants (control). The active ingredient Rotena 43® (Serbios, Rovigo, Italy), with a weight composition of 43 g L^{-1} was used with a dosage of 300–550 ml h^{-1}. The product was distributed in a single treatment carried out on 28 September 2006 using a manual spraying machine, giving about 2 l of 0.5% (w/w) solution per plant. Subsequently, for both cultivars and treatments, random samplings of drupes (5.5 kg) were carried out at 2, 12, 22 and 30 days after rotenone application. For each date of sampling, an aliquot of 500 g of drupes was used for the analysis of rotenone residues, while the remaining aliquot of 5 kg was milled in the same day using a continuous cycle – two phases mini-mill (Toscana Enologica Mori, Italy). After each milling, the mill was cleaned with jets of water under pressure. The oil so extracted was kept in dark-glass containers at a temperature of 4 °C.

2.2. Extraction and quantitative analysis of rotenone

For the extraction of rotenone from fruits, 10 g of sodium sulphate and 50 ml of acetonitrile were added to 25 g of drupes. The solution was kept under agitation for about 30 min and then an aliquot of 3 ml was put into a test tube and dried under a nitrogen flux. The residue so obtained was suspended in 1 ml of internal standard. For the extraction of rotenone from olive oil, 208.3 μl of 624 mg kg^{-1} internal standard were added to 5 g of oil and the solution so obtained was mixed for 30 min. An aliquot of 3 g of this solution was added to 5 ml of acetonitrile, sonicated in an ultrasonic bath for 5 min and loaded in a VersaPak® C_{18} Cartridge (Sigma Co., USA) with 40 ml of acetonitrile. The eluate was dried by rotavapor (R-3000, Büchi, Switzerland) and then suspended in 1 ml of acetonitrile for mass spectrometry analysis.

The quantification of rotenone in drupes and olive oil was carried out in a triple quadrupole mass spectrometer (Applied Biosystem/MDS Sciex API 2000TM LC/MS/MS System, Toronto, Canada; software: Analyst®, version 1.2) working in MRM conditions (Multiple Reaction Monitoring) (Cordaro et al., 2004; di Donna et al., 2004). The preparation of internal standard and calibration curves was performed following the method of di Donna et al. (2004). The results were expressed in a semi-logarithmic (ln) scale and correlation coefficients (R^2), decrease rate (k_d) and decay half-life ($t_{1/2}$) calculated on the basis of first-order elimination kinetics.

2.3. Physicochemical and nutritional parameters of oil

The evaluation of acidity, expressed as a percentage of oleic acid (in accordance with Italian law DM 509/87), and peroxide number (expressing the amount of oxygen able to oxidize fatty acids) was carried out according to the official method described in EC Regulation 1989/03.

The levels of α-tocopherol were determined on 0.6 g of oil brought to a final volume of 10 ml with hexane. The resulting solution was purified by filtering (45 μm-diameter pores) and then analyzed through HPLC (HPLC1100 Series Technologies Agilent, Agilent Technologies, Santa Clara, CA, USA) with the following settings: isocratic pump: flow 1.0 ml min^{-1}, stop time 20 min; solvent 100% hexane/ethyl acetate 80/20; max pressure limit 400 bars, min bar 0; Agilent Technologies Zorbax analytical column 4.6 m × 250 nm × 5 μm; FLD lamp: excitation 295 nm, emission 325 nm.

Polyphenols, expressed as mg gallic acid kg^{-1} oil (in accordance with EEC Regulation 2568/91), were determined on 1 g of oil loaded in VersaPak® C_{18} Cartridge (Sigma Co., USA). After washes with hexane, the phenolic component was recovered by adding to the eluate 10 ml of methanol, 50 μl of Folin–Ciocalteu reagent (Fluka Biochemical Co., USA), 3 ml of 1 M sodium carbonate and 5 ml of H_2O. The resulting solution was centrifuged at 4000 rpm for 10 min and the recovered supernatant was read spectrophotometrically at 725 nm (50 Varian Cary Bio UV, USA).

Oil classification was evaluated calculating the difference (ΔAbs) of specific extinctions read spectrophotometrically at 232 and 270 (UV–visible spectrophotometer model Cary50Bio, Varian Inc., Palo Alto, CA, USA). This type of analysis can detect the presence of any oxidative processes that produce a shift in double bonds causing formation of the corresponding dienic and trienic conjugated bonds (EEC Regulation 2568/91).

For the determination of fatty acids, 0.15 g of oil were added to 100 μl of a 0.2 N KOH/methanol solution. The solution was mixed for 10 min and the recovered supernatant was suspended in 250 μl of hexane and then analyzed by a gas–chromatograph (Series 6890, Agilent Technologies, Santa Clara, CA, USA) with the following settings: inlet: split gas He, heather 260 °C, pressure 25.11 psi, total flow 23.9 ml min^{-1}; oven and column: flow 0.9 ml min^{-1}, average velocity 21 cm s^{-1}, constant flow mode, capillary column Supelco SP 2340, 60.0 m × 250 μm × 0.20 μm, max temp 250 °C; detector: fid detector, heather 260 °C, H_2 flow 35 ml min^{-1}, air flow 350 ml min^{-1}, makeup flow (He) 29.1 ml min^{-1}.

2.4. Quantitative analysis of oil (panel test)

The method used for the panel test was developed by IOC (International Olive Council), according to EEC Regulation 2568/91. A group of experts panelist identified oil specific characteristics and flavors by testings (Morales et al., 1995; Angerosa, 2000; Angerosa et al., 2004). Panelist also monitored the eventual presence of any defects that might declass oil from the best-quality designation of 'extra-virgin' to other merchandise classes having lower quality.

2.5. Statistical analysis

The values of rotenone levels, physicochemical, nutritional and panel test parameters were represented as means of three measurements (±SE) for each sample of olive oil or drupes. Statistical analysis was performed using ANOVA. Significant differences between values of enzyme activity in CP and SP were determined at $P \leqslant 0.05$, according to Duncan's Multiple Range Test. Significant differences between rotenone-treated and untreated plants were determined at $P \leqslant 0.05$, according to Student's *t*-test.

Principal component analysis (PCA) was applied by Statistical Analysis System package (SAS Institute Inc., Canada) via the correlation matrix (PROC FACTOR) on the values of physicochemical (acidity, peroxide number), nutritional (α-tocopherol, polyphenols; myristic, palmitic, stearic, oleic, linoleic and linolenic acids) and quantitative (grass, floral, fruity, almond, apple and spicy) parameters of oils extracted, in order to highlight their variation as a function of the four treatments investigated (two cultivars and presence or absence of rotenone application). Each principal component was a linear combination of the original variables with coefficients equal to the eigenvectors of the correlation matrix. According to Kaiser's criterion, only components with eigenvalue >1 were retained. Confidence ellipses (90%) enclosing parameters of each oil type were plotted.

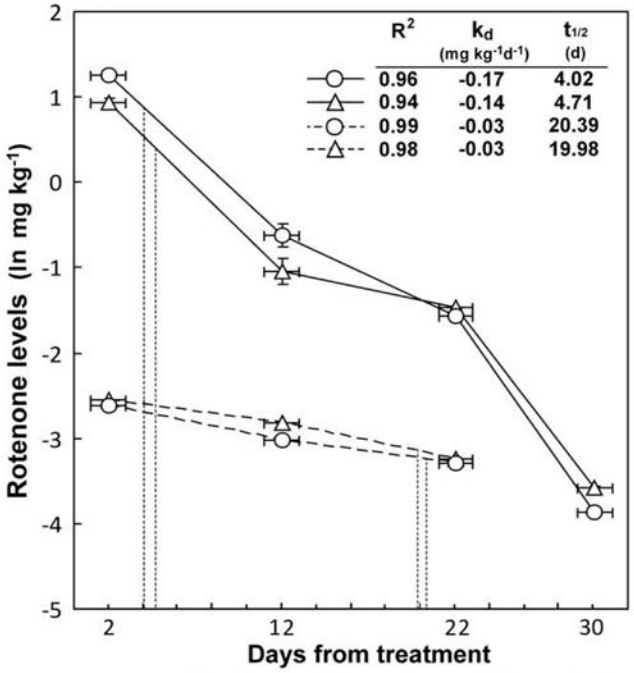
3. Results

3.1. Rotenone levels

In both cultivars, rotenone level in drupes of treated plants declined by 50% after 22 days from treatment (0.037 and 0.039 mg kg^{-1} in NB and CA, respectively) and was equal to zero after 30 days (Fig. 1). In the oil, the concentration of rotenone during the whole experimental period was higher than the corresponding values found in drupes, with values of 0.209 mg kg^{-1} in NB and 0.229 mg kg^{-1} in CA, after 22 days from treatment (Fig. 1). Both for oil and fruits, no significant differences in rotenone levels were found between the two cultivars.

3.2. Physicochemical parameters of oil

The values of acidity and peroxide number increased during the experimental period and, for both cultivars, no significant differences were found between rotenone-treated and untreated plants (Figs. 2A and 2B). After 30 days from treatment, peroxide number was significantly higher in CA (6.97 and 6.70 meq O_2 kg^{-1} in treated and untreated plants, respectively) that in NB (4.60 and 4.30 meq O_2 kg^{-1}, in treated and untreated plants, respectively), according to Student's *t*-test ($P \leqslant 0.05$) (Fig. 2B). The levels of α-tocopherol and polyphenols had parallel trends and decreased during the experimental period, showing a range from 190.18 to



Cultivar	Days from treatment	Rotenone in drupes (mg kg^{-1})	Rotenone in olive oil (mg kg^{-1})
Nocellara del Belice	2	0.073 ± 0.010	3.541 ± 0.032
	12	0.049 ± 0.006	0.539 ± 0.135
	22	0.037 ± 0.004	0.209 ± 0.070
	30	0.000 ± 0.000	0.021 ± 0.002
Cassanese	2	0.078 ± 0.006	2.541 ± 0.060
	12	0.060 ± 0.003	0.354 ± 0.155
	22	0.039 ± 0.002	0.229 ± 0.003
	30	0.000 ± 0.000	0.028 ± 0.005

Fig. 1. Concentration of rotenone in olive oil (continue lines) and drupes (dashed lines) of rotenone-treated plants of cultivars Nocellara del Belice (circles) and Cassanese (triangles) during the experimental period. The values represent the average (±SE) of three measurements for each sample of olive oil and drupes. In the graph above, the same values (±SE) were represented on a semi-logarithmic plot, in which linear correlation coefficients (R^2), decay rate (k_d) and decay half-life ($t_{1/2}$; dotted lines) were reported.

122.89 mg kg^{-1} for α-tocopherol and from 260.91 to 139.65 mg kg^{-1} for polyphenols (Figs. 2C–D). The differences in α-tocopherol content between NB and CA resulted to be significant only after 30 days (Fig. 1). The difference of specific extinctions (ΔAbs) was equal to zero for all the four oils analyzed.

The levels of myristic, palmitic, stearic, oleic, linoleic and linolenic acids did not show significant differences between rotenone-treated and untreated plants (Fig. 3) and their content remained within the limits normally expected for 'extra-virgin' olive oils (EC Regulation 1989/03). The only significant differences in fatty acids contents between treated and untreated plants regarded the levels of palmitic, stearic and linoleic acids in the last sampling date (Fig. 3). In fact, after 30 days from treatment, rotenone-treated NB plants had significantly higher levels of palmitic acid if compared to untreated plants (13.63 and 11.53 mg kg^{-1}, respectively), stearic acid (3.55 and 2.83 mg kg^{-1}, respectively) and linoleic acid (9.86 and 8.61 mg kg^{-1}, respectively) (Figs. 3B and 3C–E).

3.3. Panel test

The panel test data of oils extracted after 22 days after treatment (2 days after safety period suggested by rotenone producers) did not reveal significant differences in unpleasant aromatic notes, such as musty, rancid, fusty and muddy flavors, nor defects between rotenone-treated and untreated plants of both cultivars, with some few exceptions (indicated by asterisks in Fig. 4).

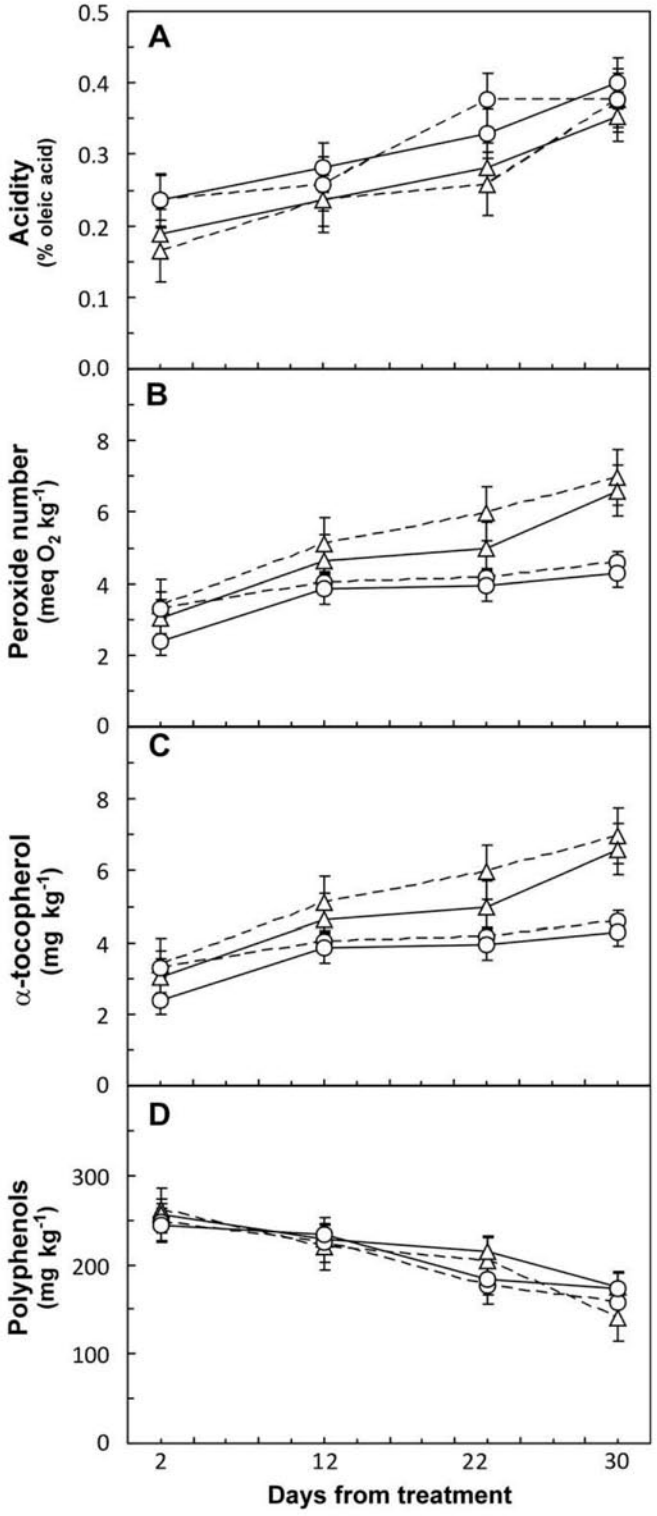


Fig. 2. (A) Acidity, (B) peroxide number, (C) α-tocopherol and (D) polyphenols in oils extracted from rotenone-treated (continue lines) and untreated drupes (dashed lines) of cultivars Nocellara del Belice (circles) and Cassanese (triangles) throughout the experimental period. The values represent the average (±SE) of three measurements for each olive oil sample.

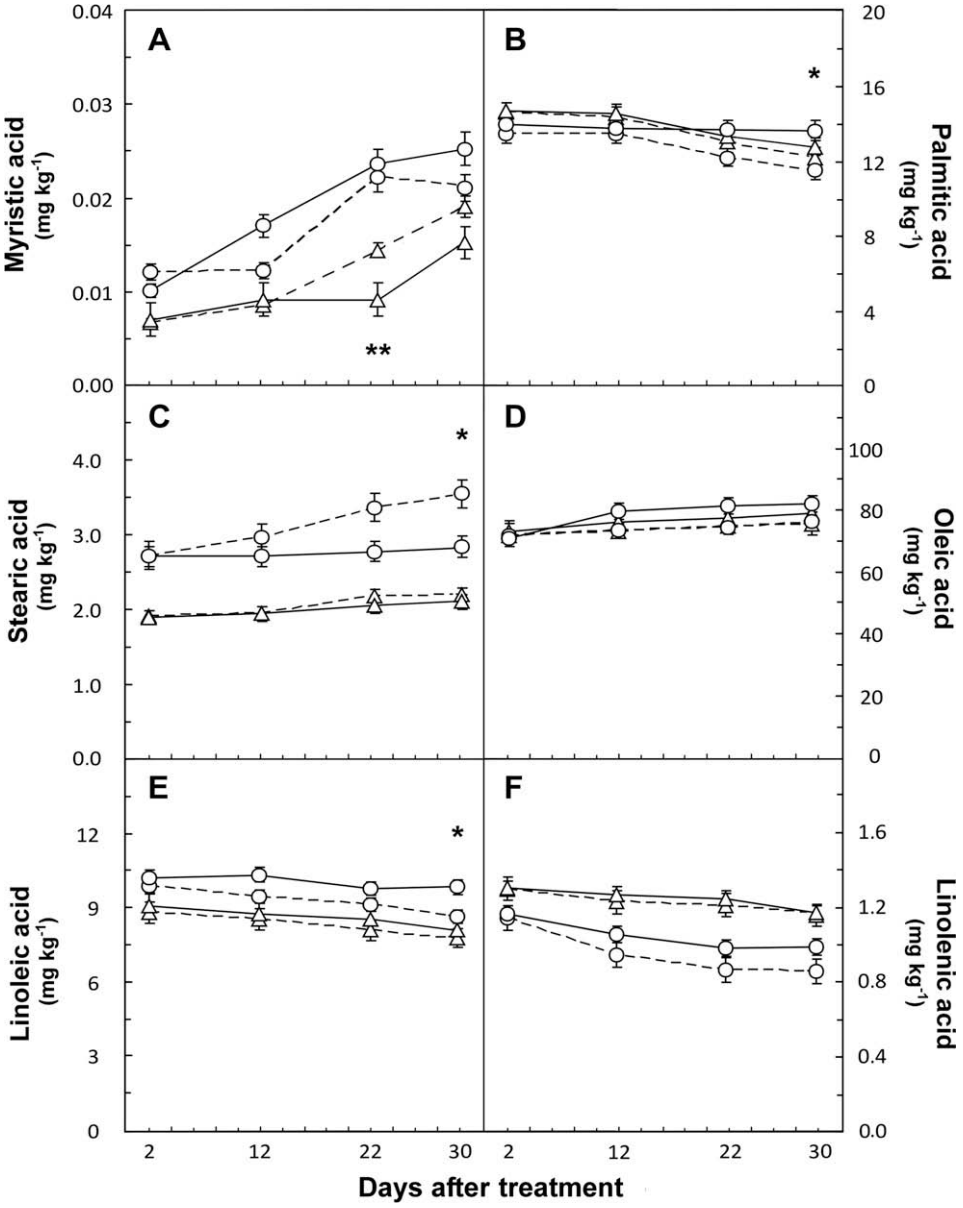


Fig. 3. Concentrations of (A) myristic acid, (B) palmitic acid, (C) stearic acid, (D) oleic acid, (E) linoleic and (F) linolenic acid in oils extracted from rotenone-treated (continue lines) and untreated drupes (dashed lines) of cultivars Nocellara del Belice (circles) and Cassanese (triangles) throughout the experimental period. The values represent the average (±SE) of three measurements for each olive oil sample. The asterisks (single for NB and double for CA) indicate significant differences between rotenone-treated and untreated plants ($P \leqslant 0.05$, according to Student's *t*-test).

The oils of both cultivars resulted to be 'extra-virgin', according to the sensorial parameters of EC Regulation 1989/03. The aromas 'grass' and 'floral' were more intense in NB, while 'fruity', 'almond', 'apple' and 'spicy' were more marked in CA (Fig. 4).

4. Discussion

Rotenone belongs to the list of active ingredients used in plant protection that must be evaluated for risks to human health and environment (EC Regulation 2229/02). Our study confirmed that treatments with rotenone do not affect the physicochemical and nutritional parameters of oils and that the few differences observed in oil physicochemical parameters (Figs. 1, 2 and 3) are rather due to differences between the cultivars and not to rotenone treatment. Furthermore, the values of UV spectrophotometric analysis ($\Delta Abs = 0$) and the results of the panel test (Fig. 4) demonstrate that both oils from rotenone-treated and untreated plants can be defined as 'extra-virgin'.

Similarities among the physicochemical, nutritional and quantitative parameters of oils extracted after 22 days from rotenone-treated and untreated NB and CA plants are shown in the score plot obtained by principal component analysis (Fig. 5), that gives a visual representation of intersample relationships. Oils were separated by cultivar on the first component (80.7% of variance explained) and by the application of rotenone on the second component (10.2% of variance explained (Fig. 5). The bivariate (90%) confidence ellipses enclosing the values of the different parameters considered for each oil (Fig. 5) confirmed that in both cultivars there are no significant difference between the average quality of oil extracted from rotenone-treated and untreated drupes.

The analysis of direct and retro-nasal aromatic olfactory characteristics of oils derived from both treatments revealed the presence

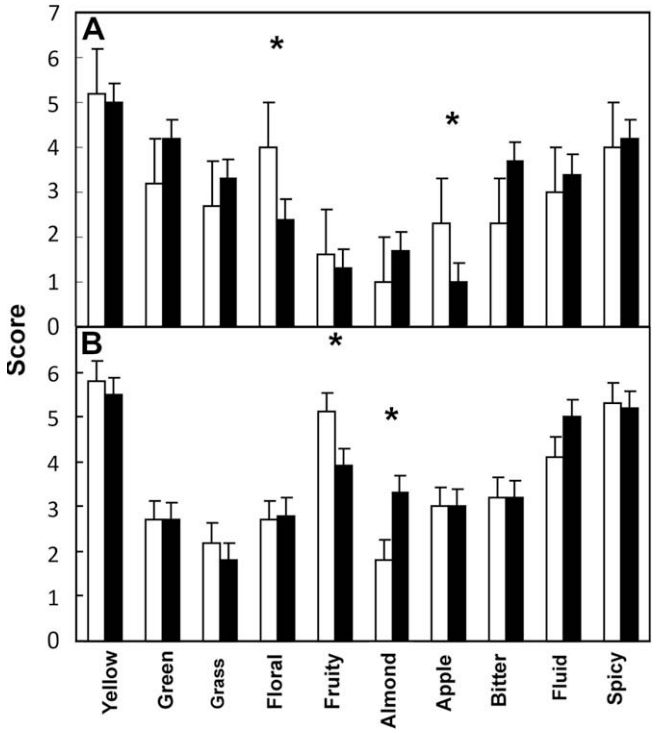


Fig. 4. Results of panel test in oils extracted from rotenone-treated (continue lines) and untreated drupes (dashed lines) of cultivars (A) Nocellara del Belice and (B) Cassanese after 22 days from treatment. The values represent the average (±SE) of three measurements for each olive oil sample. The asterisks indicate significant differences between rotenone-treated and untreated plants ($P \leqslant 0.05$, according to Student's *t*-test).

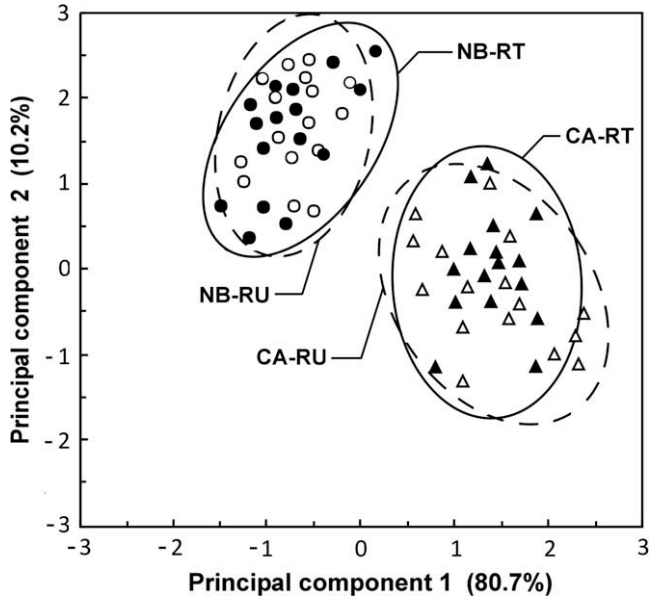


Fig. 5. Score plot obtained from principal component analysis (PCA) of physico-chemical, nutritional and quantitative parameters of oils extracted from rotenone-treated (full symbols, RT) and untreated drupes (empty symbols, RU) of cultivars Nocellara del Belice (circles, NB) and Cassanese (triangles, CA) after 22 days from treatment. The values represent the average of three measurements for each oil sample. Confidence ellipses (90%) enclosing parameters of each treatment are also shown.

of intense fruity notes (apple and almond) and floral notes (grass, floral and olive green fruity) (Fig. 4). Gustative analysis showed that in both treatments bitter taste, able to give to oil the feeling of freshness, is in harmonious balance with all the other parameters (Fig. 4). The analysis of tactile and kinesthetic characteristics of rotenone-treated and untreated oils, demonstrated that spicy taste and fluidity, which give pleasantness to the product, appear to be balanced with the fruity, floral and gustative notes (Fig. 4).

On the basis of our results (Fig. 1) and of previous studies (Adediran et al., 1999; Cabras et al., 2002) we hypothesize that molecular physicochemical characteristics, climatic conditions (mainly light, temperature, rainfall and wind) and cultivar type affected the mechanisms of degradation of rotenone. This can explain the pharmacokinetic data on disappearance of rotenone from drupes (Fig. 1), which showed a 50% decrease in the first 22 days after treatment (Fig. 1) and successively a fast decomposition in the last eight days of the experimental period. Our data is different from that of Cabras et al. (2002), which found that rotenone levels in drupes have a decay half-life of only 4 days. Before the non-admission of rotenone in olive growing, the EEC Regulation 2092/91 set a limit of 0.05 mg kg^{-1} of rotenone for drupes from organic olive groves and used for food. According to the companies supplying the active ingredient, this limit should be reached 20 days after treatment with the recommended doses. Cabras et al. (2002) observed that the levels of rotenone in drupes after 10 days from treatment are still above the legislative limit. Our results on the levels of rotenone in drupes demonstrated that after 22 days from treatment they are still close to the legislative limit of 0.05 mg kg^{-1} (0.037 and 0.039 mg kg^{-1} in NB and CA, respectively) (Fig. 1).

The oil/drupe ratios of rotenone levels observed (5.65 in NB and 5.87 in CA, at 22 days after treatment) were much higher than the values found by other authors (Cabras et al., 2002). The differences in rotenone content between drupes and oil (Fig. 1) were probably due to the lipophilic characteristics of this active ingredient, that in the first days after treatment is stored in the parenchimatic cells in which oil is synthesized (Iannotta, 2003). This compartimentalization is likely the cause of the lower decay half-life of rotenone in oil (4.02 days in NB and 4.71 days in CA), where it is easily enzimatically or photo-degraded, than in drupes (20.39 days in NB and 19.98 in CA), where it is preserved in cells (Fig. 1). Many pesticide producers declare that rotenone is safe because is quickly inactivated in an aqueous environment when exposed to light (Iannotta, 2003). Adediran et al. (1999) found that in oils kept in the dark and at a low temperature, the rate of degradation of rotenone is much slower than in oils maintained in the light and at room temperature. By contrast, the present study demonstrates the presence of this chemical in a natural agricultural environment and the potential danger of its high persistence in olive oil. In fact, although it has not yet been definitively established if rotenone and its secondary metabolites (e.g. deguelin) have significant effects on human health (Heikkila and Sonsalla, 1987; Jenner, 2001), recent studies in rats have shown that rotenone decay produces chemical compounds potentially toxic for human nervous system, inducing a pathology similar to Parkinson's disease (Greenamyre et al., 2003; Caboni et al., 2004).

In conclusion, serious doubts remain about the safety and healthiness of oils extracted from drupes treated with rotenone. Our results confirm that the definition of 'organic' in olive growing is often not sufficient to ensure the safety of production processes and final products. Our study also suggests the need of more attention and consideration by the legislature to the various competences involved in the fields of agricultural and food chemistry and to the development of directives aimed at the improvement of the quality of agricultural production.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors acknowledge Prof. Giovanni Sindona for providing internal standard, Dr. Caterina Briccoli Bati and Dr. Nino Iannotta for their assistance and Prof. Nicola Biagio Mercuri for medical suggestions.

References

Adediran, K.A., Stavroulakis, G., Petrakis, C., 1999. Monitoring the residual activity of rotenone in organic olive oil. Acta Horticult. 474, 691–694.

Angerosa, F., 2000. Sensory quality of olive oil. In: Harwood, J., Aparicio, R. (Eds.), Handbook of Olive Oil: Analysis and Properties. Springer, New York, USA, pp. 355–392.

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., Montedoro, G., 2004. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. J. Chromatogr. A 1054, 17–31.

Betarbet, R., Sherer, T.B., McKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J.T., 2000. Chronic systemic pesticide exposure reproduces feature of Parkinson's disease. Nat. Neurosci. 3, 1301–1306.

Caboni, P., Sherer, T.B., Zhang, N., Taylor, G., Me, N.H., Greenamyre, J.T., Casida, J.E., 2004. Rotenone, deguelin, their metabolites, and the rat model of Parkinson's disease. Chem. Res. Toxicol. 17, 1540–1548.

Cabras, P., Caboni, P., Cabras, M., Angioni, A., Russo, M., 2002. Rotenone residues on drupes and olive oil. J. Agric. Food Chem. 50, 2576–2580.

Casacchia, T., Caravita, M.A., Perri, E., Russo, A., Sindona, G., 2007. Rotenone in organic olive growing and possible risk for human health: extra virgin olive oil quality and safety. In: Proceedings of Giornata di Studio Congiunto SIB-SINU: Effetti Biochimici e Nutrizionali della Trasformazione degli Alimenti, Cesena, Italy, pp. 43–44. <http://www.sinu.it/attualita/Atti%202007.pdf)>.

Cerretani, L., Gómez-Caravaca, A.M., Bendini, A., Segura-Carretero, A., Fernández-Gutiérrez, A., Del Carlo, M., Compagnone, D., Cichelli, A., 2005. Influence of olive fruit fly (*Bactrocera oleae*) attack on the chemical quality and oxidative stability of extra virgin olive oils from Abruzzo region (Italy) produced with industrial mill. In: Proceedings of the 5th EuroFed Lipid Congress. Oils, Fats and Lipids: from Science to Applications – Innovations for a better World, Gothenburg, Sweden, Section OLIV-011, pp. 237–245.

Cordaro, M., di Donna, L., Grassi, G., Maiuolo, L., Mazzotti, F., Perri, E., Sindona, G., Tagarelli, A., 2004. High resolution electrospray and electrospray tandem mass spectra of rotenone and its isoxazoline cycloadducts. Eur. J. Mass Spectrom. 10, 691–697.

di Donna, L., Grassi, G., Mazzotti, F., Perri, E., Sindona, G., 2004. High-throughput assay of rotenone in olive oil using atmospheric pressure chemical ionization tandem mass spectrometry. J. Mass Spectrom. 39, 1437–1440.

Greenamyre, J.T., Betarbet, R., Sherer, T.B., 2003. The rotenone model of Parkinson's disease: genes, environment and mitochondria. Parkinsonism Rel. Dis. 9, 59–64.

Heikkila, R.E., Sonsalla, P.K., 1987. The use of the MPTP-treated mouse as an animal model of parkinsonism. Can. J. Neurol. Sci. 14, 436–440.

Hoglinger, G.U., Feger, J., Annick, P., Michel, P.P., Rarine, P., Champy, P., Ruberg, M., Wolfgang, W.O., Hirsh, E., 2003. Chronic systemic complex I inhibition induces a hypokinetic multisystem degeneration in rats. J. Neurochem. 84, 1–12.

Iannotta, N., 2003. La difesa fitosanitaria. In: Fiorino, P. (Ed.), Olea: Trattato di Olivicoltura, Edagricole Il Sole 24 Ore. Bologna, Italy, pp. 393–407.

International Olive Oil Council, 2000. World Catalogue of Olive Varieties. Agra Editrice, Rome, Italy.

Jenner, P., 2001. Parkinson's disease, pesticides and mitochondrial dysfunction. Trends Neurosci. 24, 245–246.

Kim, Y.G., Ko, H.H., Han, E.S., Lee, C.S., 2007. Lamotrigine inhibition of rotenone or 1-methyl-4-phenylpyridinium-induced mitochondrial damage and cell death. Brain Res. Bull. 71, 633–640.

Lee, H.J., Shin, S.Y., Chol, C., Lee, Y.H., Lee, S.J., 2002. Formation and removal of alpha-synuclein aggregates in cells exposed to mitochondrial inhibitors. J. Biol. Chem. 277, 5411–5417.

Morales, M.T., Alonso, M.V., Rios, J.J., Aparicio, R., 1995. Virgin olive oil aroma: relationship between volatile compounds and sensory attributes by chemometrics. J. Agric. Food Chem. 43, 2925–2931.

Parlati, M.V., Petruccioli, G., Pandolfi, S., 1990. Effects of the Dacus infestation on the oil quality. Acta Horticult. 286, 387–390.

Perri, E., Lombardo, N., Parlati, M.V., Fodale, A., Mula, R., Pellegrino, M., Salvo, F., Sparta G., 2002. Caratterizzazione di oli di oliva da agricoltura biologica provenienti dalla Sicilia. In: Proceedings of Convegno Internazionale di Olivicoltura, Spoleto, Italy, pp. 526–532.

Scarpati, M.L., Vita, G., Lo Scalzo, R., Gambacorta, A., 1996. Chemiotropic behavior of female olive fly (*Bactrocera oleae* Gmel.) on olea europaea L.. J. Chem. Ecol. 22, 1027–1036.

Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G.F., Morozzi, G., 2004. Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspect of production that affect their occurrence in the oil. J. Chromatogr. A 1054, 113–117.

Singer, T.P., Ramsay, R.R., 1994. The reaction site of rotenone and ubiquinone with mitochondrial NADH dehydrogenase. Biochim. Biophys. Acta 1187, 198–202.

Zunini, P., Evangelisti, F., Tiscornia, E., Petacchi, R., 1992. Influenza del tipo di infestazione da Dacus sulla composizione dell'olio ottenuto da *Olea europaea*. Rivista Italiana delle Sostanze Grasse 69, 541–546.