Residues of 165 pesticides in citrus fruits using LC-MS/MS: a study of the pesticides distribution from the peel to the pulp

Enza Calvaruso\textsuperscript{a}, Gaetano Cammilleri\textsuperscript{a}, Andrea Pulvirenti\textsuperscript{b}, Gianluigi Maria Lo Dico\textsuperscript{a}, Giovanni Lo Cascio\textsuperscript{a}, Vita Giaccone\textsuperscript{a}, Valeria Vitale Badaco\textsuperscript{a}, Valentina Cipri\textsuperscript{a}, Mobilia Maria Alessandra\textsuperscript{c}, Antonio Vella\textsuperscript{a}, Andrea Macaluso\textsuperscript{a}, Calogero Di Bella\textsuperscript{a} and Vincenzo Ferrantelli\textsuperscript{a}

\textsuperscript{a}Istituto Zooprofilattico Sperimentale della Sicilia “A. Mirri”, Palermo, Italy; \textsuperscript{b}Dipartimento Scienze della Vita, Università degli studi di Modena e Reggio Emilia, Modena, Italy; \textsuperscript{c}Freelance Medical Doctor, Messina, Italy

ABSTRACT
A sensitive LC-ESI-MS/MS method was developed for the determination of 165 pesticides in 50 citrus fruit samples collected in Sicily. Moreover, an evaluation of pesticides levels in the citrus layers (peel, albedo, and pulp) was carried out. The method presented acceptable trueness, precision, and linearity with LOQ of 5\(\mu\)g/kg. The results obtained showed a high frequency of fungicides class pesticides in all the citrus samples examined (>95\%) with the highest concentrations in the peel (4468 \(\mu\)g/Kg). A significant difference of concentrations was found between the layers of the citrus fruits analysed (p < 0.05). In particular, the peel and albedo present higher pesticides significantly higher than the pulp. Our findings confirming the widespread use of these substances in citrus cultivation and suggesting the importance of pesticides analysis in all the citrus fruit layers separately, considering the different interactions between the physicochemical characteristics of the matrices and the pesticides.

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CONTACT
Gaetano Cammilleri gaetano.cammilleri86@gmail.com

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1. Introduction

Citrus fruits represent an important source of nutrition and health value since ancient time (Cicero et al. 2015; Salvo et al. 2016). Citrus fruits are one of the most produced fruit, and they are grown in more than 80 countries. Unfortunately, fungal pathogens mainly produce post-harvest diseases of citrus fruits. Thus, pesticides treatments are necessary to overcome this problem. The fungicides employed are toxic, and they can present some hazards to public health. Therefore, legal requirements of many countries are increasing, making it necessary to determine fungicide residues at very low levels. Green and blue molds, caused by *Penicillium digitatum* and *Penicillium italicum*, respectively are the most important postharvest diseases of citrus in all production areas (Palou et al. 2008). *Penicillium digitatum* and *Penicillium italicum* have a rapid reproduction; their spores are ubiquitous in the atmosphere and could be found on fruit surfaces. Therefore, the source of fungal inoculum in citrus groves and packing-houses is practically continuous during the season. (Kanetis et al. 2007). Chemical fungicides have been used for over 60 years by the fresh citrus industry and have reduced losses to acceptable levels. Plant protection products, commonly known as pesticides, are widely used in agriculture to increase the yield, improve the quality, and extend the storage life of food crops (Kmellár et al. 2010; Naccari et al. 2014; Cicero et al. 2017; Aiello et al. 2018). The European Union Council Directive describes the regulatory framework for which Maximum Residue Levels (MRLs) are set (EC 396/2005). Typical MRL is in the range of 0.01 and 5 mg/kg, depending on the pesticide and the commodity. Despite the high number of works on the presence of pesticides in citrus fruits (Lafuente and Tadeo 1985; Ortelli et al. 2005; Kanetis et al. 2007; Phetkul et al. 2014), further works are needed on the pesticides distribution from the pulp to the peel. Citrus peel has been reported to be a good source of pectin and dietary fibre, with an equilibrated proportion of soluble and insoluble fractions (Larrauri et al. 1994; Baker 1997). Commercial citrus fibre products are widely consumed, with several food industries dedicated to the production of sweets. The aim of the present work was to develop and validate a LC-MS/MS method for the simultaneous determination of 165 pesticides in citrus fruits collected in Sicily (Southern Italy) and investigate the pesticides distribution in the different layers (peel, albedo, and pulp).

2. Results and discussion

The most intense transition was used as a quantifier while the other were used as a qualifier peak for the confirmatory analysis. The Retention Times (RT) and MS/MS conditions for each compound are summarized in Table S1. The selectivity of the method was evaluated by injecting blank sample extracts. The absence of signal above a signal-to-noise ratio of 3 at the retention times of the target compounds showed that the method is free of interferences. The linearity assessment showed correlation coefficient ($r^2$) values for each pesticide studied $\geq 0.99$, indicating excellent linearity for the pesticides analysed. All the studied pesticides showed recovery ranges of 70–100% with RSDs $\leq 20\%$ at the concentration levels of 5, 10 and 25 μg/kg, in agreement with the DG-SANTE guidelines. The experimental LOQ obtained for each pesticides analysed
showed values between 0.71 and 5 µg/kg except for propoxur (5.97 µg/kg). The LOQs obtained were up to 10 times lower than the MRLs established by the EU legislations, suggesting that the proposed method is particularly favorable for the analysis of pesticides in food of plant origin (Vinale et al. 2016; Celano et al. 2018). The assessment of the pesticides distribution in citrus fruit layers allowed us to examined 20 samples of orange peel, 20 samples of orange albedo, 20 samples of orange pulp, 20 samples of lemon peel, 20 sample of lemon albedo, 20 samples of lemon pulp, 10 sample of mandarin peel and 10 samples of mandarin pulp. Unfortunately, we were unable to separate the albedo from the mandarin samples due to the extreme thinness of the layers. Twenty-seven (60%) samples analysed showed the presence of only one pesticide, whereas only 2 samples (4%) revealed the presence of 2 pesticides (Table S2). In particular, 12 (60%) samples of orange detected one pesticide, one (5%) sample detected two pesticides, 12 (60%) samples of lemon detected one pesticide, 3 (30%) samples of mandarin detected one pesticide, and one (10%) sample detected two pesticides. Orange samples showed the highest pesticides mean values (2491 ± 1024 µg/kg) with a maximum of 4468 µg/kg (imazalil). Imazalil (1-[2-(2.4-dichlorophenyl)-2-(2-propen-3-yl)-ethoxy]-1Himidazole) was the most detected pesticide in orange samples with a frequency of 83.3% confirming the wide use of this compound in citrus fruits cultivation due to its high activity against Penicillium spp. (Lafuente & Tadeo 1985). A greater presence of imazalil was even found in mandarine samples, with a mean value of 3841.25 ± 2145 µg/kg and a maximum of 4456 µg/kg.

Regarding the lemon samples, fenhexamide was the only pesticide found with the highest values in the peel 66 µg/kg; the mean value of fenhexamide in this layer was 30.25 ± 18.34 µg/kg. Fenhexamide is a highly effective systemic fungicide against Botrytis and several related fungi. It is a sterol biosynthesis inhibitor. Fenhexamid block the 3-ketoreductase encoded by erg27, a later step in ergosterol biosynthesis (Leroux et al. 2002). The different distribution of pesticides in citrus layers are shown in Figure S1. The box-plot represent the distribution of pesticides concentration (µg/Kg) for each citrus fruit layers analyzed (orange, mandarin, and lemon).

The three data groups were subjected to non-parametric and parametric statistical analysis. For orange, an ANOVA test was used, for mandarin a T test and for lemon a Kruskal Wallis test was used. The analysis of different layers of citrus fruits showed significant differences of imazalil levels between the layers of orange (p-value = 6.689e-05) and mandarine (p-value = 9.199 e-05.) samples. Furthermore, a significant difference on fenhexamide levels in the layers of the lemon samples was found (p-value = 9.192e-06). In fact, 58% of imazalil passes from peel to albedo, and only 6% passes to the pulp. In the case of the mandarin, just the 1.6% of imazalil passes from peel to pulp. Significant differences were even found for Carbendazim levels between pulp and peel of mandarin samples (p-value = 0.0034), where only 16% passes from peel to pulp. Finally, for lemon samples the 43% of fenhexamide passed from peel to albedo, and the 18% from albedo to pulp. Our findings indicate a low penetration of these fungicides in the pulp. This low penetration is probably due to physico-chemical characteristic of the analytes studied and the peels of the fruit samples analysed. It appears that at least some pesticides are absorbed from the soil by the tree roots and then concentrated in the fruit peel. Pesticides, if applied during the fruit formation
period, may also be directly absorbed into the peel (Muraldihara 1996). In fact, residue levels of both fungicides were considerably higher in the peel than in the whole fruit. Given these considerations and considering the absence of knowledge on the origin of the product, the use of citrus peels for culinary or beverage preparation is not recommended. In fact, the peel of citrus fruits is often utilized for the preparation of liquors, jams or cakes.

4. Conclusions

Citrus fruits represent an important source of nutrition, and medicinal value (Khan et al. 2010; Phetkul et al. 2014; Geraci et al. 2017; Celano et al. 2018; Ciriminna et al. 2018). Unfortunately, fungicides treatments are necessary to overcome this problem. The legal requirements of many countries are increasing, making it necessary to determine fungicide residues at very low levels.

As far we know, this work showed for the first time the importance of doing the analyses in all the citrus fruit layers separately, considering the different interactions between the physicochemical characteristics of the matrices and the pesticides.

Declaration of interest statement

All the authors declare no conflicts of interest.

Special issue statement

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