Experimental and theoretical determination of pesticide processing factors to model their behavior during virgin olive oil production

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A B S T R A C T

The purpose of the present work was the experimental evaluation of pesticides transfer to virgin olive oil during the production step and prediction of their processing factors, which could be eventually used for the calculation of maximum residue limits (MRLs) in olive oil from the MRLs set in olives. A laboratory-scale Abencor system was used for the production of olive oil from olives spiked with the 104 pesticides studied, three different chromatographic methods being used for the analysis of raw olives and the obtained olive oil: (i) gas chromatography-tandem mass spectrometry (GC–MS/MS) for GC-amenable pesticides; (ii) hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) for polar pesticides, and; (iii) reversed-phase liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for low to medium polarity pesticides. Processing factors experimentally calculated were correlated to their octanol-water partitioning coefficient (log K_{ow}), enabling the calculation of the equivalent MRLs in olive oil from the MRLs in olives, considering the percentage of oil extracted (oil yield) and the log K_{ow} of each pesticide.

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1. Introduction

Nowadays, the use of pesticides in crops is widely extended in order to counteract the adverse effects of pests, while, at the same time, increasing harvest yields to meet the food demands of a growing global population. However, even trace amounts of pesticides in food can cause health problems. To avoid an inadequate use of pesticides and an unreasonable transfer of them to the processed foodstuffs, several institutions worldwide have established guidelines for good agricultural practices and stringent regulations establishing the maximum concentration of pesticides allowed in foodstuffs. The maximum residue limits (MRLs) set by the European Union (EU), (European Commission, 2005) or the Codex Alimentarius Commission (REP15/CAC, 2015) are a couple of examples. These MRLs have been calculated individually for each foodstuff, depending on the physicochemical properties of the active substances, and on their toxicity. For selected compounds, a default value of $10 \, \mu g \cdot kg^{-1}$ (European Commission, 2008) has been established.

MRLs have not been set for some processed vegetables such as olive oil. In the particular case of virgin olive oils, a default conversion factor of 5 (viz. assuming a standard production yield of 20% olive oil from raw olives) was proposed for the first time in 2014 to convert the MRLs set in olives to an indicative maximum concentration level of pesticide residues authorized in virgin olive oil during official control (European Commission, 2014). However, the actual processing factor (PF) of each pesticide strongly depends on its relative solubility and affinity towards the aqueous phase or to the oil phase during olive oil production. In 2015, the EU differentiated between fat-soluble and fat insoluble compounds, setting processing factors of 5 and 1, respectively (European Commission, 2015). Thus, the MRL in olive oils should be estimated taking into account the concentration or dilution performed during olive oil production. In fact, annex VI of Regulation EC N° 396/2005 is expected to deliver PFs for each compound in several raw commodities. Unfortunately, to the best our knowledge this annex has not been yet established, (European Commission., 2017). Meanwhile, there is a particular concern from the European vegetable oil industry and its suppliers (Fediol, 2012).

Given the industrial two-phase extraction used in olive mills and the partitioning undergone by all the olive paste components during malaxation and centrifugation, the polarity of the compounds (e.g., the octanol-water partition coefficient (log K_{ow}))

may be a useful parameter to estimate the degree of transfer and, thus, to predict the PFs during olive oil production. Previous studies have calculated PFs in other vegetables crops including tomatoes (Abou-Arab, 1999), (Liu et al., 2014), cucumbers (Ramezani & Shahriari, 2015) and spinach (Bonnechère et al., 2012); apples (Li et al., 2015), (Taylor et al., 2013), raisins (Shabeer et al., 2015), grapes (Pazzirota, Martin, Mezcua, Ferrer, & Fernández-Alba, 2013) and oranges (Li et al., 2012); in cereals, such as soybeans (Zhao, Ge, Liu, & Jiang, 2014) and sorghum (Han et al., 2016). However, all these studies have been undertaken for a limited number of pesticides, and with the goal of studying the effect of different steps carried out in industrial processing, such as drying, washing, peeling, hulling, milling, water addition and other processes (Keikotlhaile, Spanoghe, & Steurbaut, 2010).

Nevertheless, scarce literature is available on the study of PFs in olive oil. To the best our knowledge, only one previous study has estimated the PFs for thirteen pesticides in olives and olive oils (Amvrazi & Albanis, 2008). In this study, the objective was to examine the effect of the amount of water added during olive oil production for pesticide reduction, revealing that an increase of water content yielded a decrease of the pesticides transferred. With this scenario in mind, the aim of this work is to examine and tentatively predict levels of pesticides during olive oil production. For this task, 104 commonly used pesticides was selected and the PF were calculated using a laboratory-scale olive mill (Abencor system). Different analytical methods based on gas chromatography-tandem mass spectrometry (GC–MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were used for the determination of pesticides in the initial spiked olives and in the olive oil produced by Abencor.

2. Experimental section

2.1. Reagents and pesticide standards

HPLC-grade solvents: methanol (MeOH), acetonitrile (MeCN), ethyl acetate (EtOAc) and n-hexane were purchased from Sigma-Aldrich (Madrid, Spain) as well as sodium chloride (NaCl), anhydrous magnesium sulfate ($MgSO_4$) and formic acid (HCOOH). Acetic acid (HOAc) was purchased from J.T. Baker (Center Valley, PA). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA) was used throughout the study to obtain the HPLC-grade water used during the analyses. EMR-Lipid sorbent employed to extract the pesticides was acquired from Agilent Technologies (Santa Clara, CA). A suite of 104 pesticides were selected (Table SD-1, Supplementary Data), including representative compounds from different families typically used in olive groves, including insecticides, herbicides, fungicides and acaricides. All the analytes are included in the latest Annex 1 (last Annex 1/2016) of European Regulation 396/2005, (European Commission, 2017). Analytical standards (>99% purity) of each pesticide were supplied by Sigma-Aldrich or Dr. Ehrenstorfer (Augsburg, Germany). Standard solutions of each pesticide (500 $\mu g \cdot mL^{-1}$) were prepared in MeOH or MeCN for the analysis by LC-MS/MS and in EtOAc or n-hexane for the compounds analyzed by GC–MS/MS. Solutions containing mixtures of the studied compounds (5 $\mu g\ mL^{-1}$) were prepared in MeCN or n-hexane and stored in amber glass vials with caps at $-20\ °C$ until use.

2.2. Sample treatment and laboratory-scale olive oil extraction from crushed olives

Olive samples were collected from an olive grove (Jaén, Spain), and stored at $-20\ °C$. Before the sample treatment, olives were crushed using a mill (Talleres Lopera, Priego de Córdoba, Spain).

To obtain virgin olive oil samples for the analyses, portions of olive samples were selected and processed by means of a lab-scale Abencor system (MC2 Ingeniería y Sistemas, S.L., Sevilla, Spain) to extract olive oil. Milled samples were weighed (ca. 150 g) and transferred to the malaxer, equipped with thermometer (MC2 model TB-100), where the crushed olive paste was shaken for 30 min at temperature less than 20.6 °C. Subsequently, the paste obtained was centrifuged for 1 min at 3500 rpm (Abencor centrifuge MC2 model CF-100). In this final step, the virgin olive oil was separated from pomace juice and water that constitute the olive, and frozen until the moment of analysis.

2.3. Procedures for pesticide determination in olive oil and olives

2.3.1. Sample treatment for pesticides with low or medium polarity
The "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) method for pesticides in fatty matrices (AOAC Official Method 2007.01, 2011), was used with the modification of using a novel sorbent (EMR-Lipid), which provides some advantages in terms of method precision and matrix effects (López-Blanco et al., 2016). In brief, the modification consisted of a two-step cleanup procedure of the acetonitrile extract, using (1) EMR-Lipid sorbent and (2) $MgSO_4$ and NaCl. The description of the procedure is detailed in the supplementary data section. For the compounds studied by LC-MS/MS, the method performance in terms of recovery rates was already assessed (López-Blanco et al., 2016), being the recovery rates used to correct and compensate analyte losses during analysis. In the case of pesticides analyzed by GC–MS/MS, the recovery rates obtained with this sample treatment procedure are included in Table SD-2 (Supplementary Data).

2.3.2. Sample treatment for polar pesticides
A method for polar pesticides, the so-called QuPPe (Quick Polar Pesticides), was adapted for pesticide analyses of highly polar compounds in both olive oil and olives (EU Reference Laboratories for Residues of Pesticides (EURLs), 2016). The detailed description is addressed in the Supplementary Data section. Briefly, an extraction with methanol (1% formic acid)/water (1:1 v/v) was performed, aided by a heating step to extract some compounds. The method performance in terms of recovery rates was already assessed (Nortes-Méndez et al., 2016), the recovery rates obtained being used to correct and compensate analyte losses during analysis.

2.4. Determination of pesticides in olive oil and olives using LC-MS/MS and GC-MS/MS

From the 104 pesticides selected, 60 pesticides (with low or medium polarity) were analyzed using QuEChERS and UHPLC-MS/MS (Thermo Scientific Quantiva, San José, CA) with a C18 column and a method described elsewhere and detailed in Supplementary Data (López-Blanco et al., 2016). Seven polar pesticides were analyzed with QuPPe and HPLC-MS/MS with a HILIC column (Nortes-Méndez et al., 2016), and the remaining 37 compounds were analyzed by GC–MS/MS using an ion trap instrument (Polaris Q-Ion Trap; Thermo Scientific, USA) with the method described in Supplementary Data.

Briefly, the reversed-phase UHPLC-MS/MS method consisted of a 20-min run on a short C18 column (2.1 mm i.d. × 50 mm, 1.8 μm), using a gradient of a mixture of water (0.1% formic acid) and acetonitrile (0.1% formic acid). The HILIC-MS/MS method employed a longer HILIC column (2.1 mm i.d. × 100 mm, 1,8 μm) for a total analysis time of 16 min. Mobile phase **A** was water (ammonium formate 100 mM, adjusted to pH 2.85 with formic acid)/acetonitrile (1:2 v/v), and mobile phase **B** was acetonitrile. Finally, the GC–MS/MS method was based on a temperature ramp from 70 to 300 °C (with different steps) in 43 min.