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The concentration and probabilistic health risk assessment of pesticide residues in commercially available olive oils in Iran

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ABSTRACT

This study was undertaken to analyze 29 pesticides residues in 37 commercially olive oil collected samples from Iran's markets using Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach along with acetonitrile for the extraction, surface adsorbents for clean-up procedure, following with a gas chromatography-mass spectroscopy (GC-MS). In order to eliminate the matrix effect, the calibration curves were drawn using spiked samples with the Area under curve (AUC) portion calculation of pesticide residue to AUC internal standard (Triphenyl Methane (TPM)). Moreover, the probabilistic health risk assessment includes non-carcinogenic and carcinogenic risk were estimated by target hazard quotient (THQ), total target hazard quotient (TTHQ) and cancer risk (CR) using the Monte Carlo Simulation (MCS) method. The calibration curves were linear in the range of 10–1500 ng/g, and R^2 was higher than 0.994. All pesticides recoveries as average were in the range of 77.97–112.65%. The respective numbers attributed to LOD and LOQ were 3–5 ng/g and 8–15 ng/g. Results showed that 29.7% of samples were contaminated by pesticides which according to Iranian regulation, while in 7 cases banned pesticides were detected. Only 4 samples are noncompliant with EU regulation. The rank order of pesticides based on THQ was Heptachlor $>$ DDT $>$ Pretilachlor. Also, TTHQ for adults was 0.139; and children 0.467. The rank order of pesticides based on CR was Heptachlor $>$ DDT. Consumers (adults and children) are not at non-carcinogenic risk due to ingestion of oil olive content (THQ and TTHQ $<$ 1 value) but are at considerable carcinogenic (CR $>$ 1E-6). According to the observed profile of pesticide in olive oil samples, which are mostly banned according to Iranian regulation, further improvements in agriculture procedures of cultivated olive in Iran, as well as required assessments of imported olive oil, was recommended.

1. Introduction

Several types of pesticides including insecticides, herbicides, disinfectants, fungicides, are used for destroying, preventing, or controlling pests. They play an important role in the agricultural production by decreasing the adverse effects of weeds, and pests on crop productivity and quality (Gómez-Almenar &García-Mesa, 2015; López-Blanco et al., 2016; Sánchez et al., 2006). The residues of these pesticides can be remained among the harvest stage and cause further contamination in the some of the agriculture products, such as fruits fruit juices, vegetables, cereals and fish, rice, as well as olive and related derivatives from olive such as olive oil (Banias et al., 2017; Fuentes et al., 2010; Garcia-Reyes et al., 2007). Moreover, the extensive application of pesticides resulted in raised serious public concerns regarding the further adverse effects on the environment, safety, and quality of food products (Abtahi et al., 2013; Amirahmadi et al., 2017; Shoeibi et al., 2013; Yadolahi et al., 2012). In this context, several countries and organizations have decided to establish the maximum acceptable

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thresholds for the pesticides in foods (Ambrus &Yang, 2015; Ambrus, 2015; Handford et al., 2015; Nasreddine &Parent-Massin, 2002; Winter &Jara, 2015; Yeung et al., 2017).

Olive oil as an important product of the Mediterranean agri-food sector which provoking significant economic and political concern can be produced in Mediterranean areas and European Union countries such as Andalusia, Spain, Italy, and Greece (Banias et al., 2017; Garcia-Reyes et al., 2007; Sánchez et al., 2006). The contamination by trace amounts of pesticides in fruits and consequently extracted oil can cause raised severe public concerns regarding the food safety and also a major risk for consumer health (Garcia-Reyes et al., 2007). In this regard, determination of residues of these compounds is a matter of concern (Bargańska et al., 2014). Moreover, some regulations regarding maximum residue limits (MRLs) in olive and olive oil have been established by the European Union (Regulation EC No 396/2005) and the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) of the United Nations (Garcia-Reyes et al., 2007; López-Blanco et al. 2016, 2018; Sánchez et al., 2006). MRLs of pesticides in each country is varied based on the diversity of pesticides used, the climatic conditions and type of product (Tsipi et al., 2015). On the other hand, the determination of pesticide residues needs to be processed through a valid analytical method (Lehotay &Cook, 2015; Rathore &Nollet, 2016; Shoeibi et al., 2013; Valverde et al., 2017).

In this context, some chromatography techniques such as GC and HPLC equipped with vary of detectors (FID: Flame Ionization Detector, NPD: Nitrogen Phosphorus Detector, ECD: Electron Capture Detector) and (UV: Ultraviolet Detector, DAD: Diode Array Detector, MSD: Mass Spectrometry Detector), respectively, are developed to measure the pesticide content and further comparison with the established limits (Pelajić et al., 2016; Rathore &Nollet, 2016). Among them, the gas chromatograph with a mass spectrometer detector (GC-MASS) is capable of selective ion monitoring (SIM) (Soler &Pico, 2007). Additionally, the clean-up stage before analysis of each analyte is crucial, while in the case of pesticides, different methods are used for the purification and extraction. Among them, one of the most commonly used technique for pesticide detection in fruits and vegetables is the QuEChERS method (Bargańska et al., 2014; Rizzetti et al., 2016). QuECHERS method was first introduced by Anastassiades et al. (2003). This method is based on a cleanup method, which removes impurities such as excessive water, sugars, organic acids, lipids.

Monte Carlo Simulation (MCS) as one of the available models to measuring the uncertainties associated health risks (Fjeld et al., 2007; Hastings, 1970; Keramati et al., 2018; Shahrbabki et al., 2018), has been defined as a averages of variability in risk assessments and quantifying uncertainty via the united states environmental protection agency (USEPA) and national academy of the council (NRC) (NRC, 1994; USEPA, 1997).

In this study, for the first time, the residues of 29 types pesticides from different chemical groups were evaluated by a modified QuEChERS sample preparation and GC-MS technique in the 37 collected olive oil samples from Tehran, Iran. Also, the non-carcinogenic and carcinogenic risks for adults and children age groups due to oil olive ingestion was assessed using MCS method.

2. Materials and methods

2.1. Sampling, chemicals, and reagents

Total 37 samples of olive oil (28 domestic and 9 imported samples from Italy and Spain, all with glass packaging) were collected from different grocery stores in Tehran through June to August 2017. Ethyl acetate (organic trace analysis), acetonitrile, n-hexane, toluene, and sodium chloride (NaCl), all in analytical grade were purchased from Merck (Darmstadt, Germany), Anhydrous $MgSO_4$ was obtained from Sigma-Aldrich Co. (Japan). Moreover, C_{18} and PSA were purchased from Supelco Inc (Bellefonte, USA). $MgSO_4$ was used as a source of contamination from phthalates but did not intervene in the analysis. In order to prepare the $MgSO_4$ for analysis, it was heated at 500 °C for 5 h in a muffle furnace to remove the phthalates. The flasks were washed with water and detergent, and they were placed at sulfuric acid (20%) for 24 h, and they were thoroughly washed.

2.2. Extraction and preparation sample

The (QuEChERS) method was used for the preparation of samples, spiked samples and monitoring pesticides (Tuzimski &Rejczak, 2016). For this purpose, 2.5 g of olive oil was added to a falcon of 50 mL, and then 5 μL of internal standard (5 μg/mL TPM in ETAC) was added. The solution was kept in a cold and dark place for an hour. Then, 5 mL of deionized water was added to the solution and was stirred for one min (Mekonen et al., 2014). Then, 10 mL of acetonitrile and 5 mL of n-hexane were incorporated and agitated again. Afterward, 4 g of magnesium sulfate and 1 g of activated salt (NaCl) were added and stirred for 1.5 min. Then, the obtained solution was centrifuged at 4000 g for 10 min at −5 °C. Five mL of the supernatant was transferred to a 15 mL falcon containing sorbents; 0.4 g of C_{18}, 0.2 g of Primary Secondary Amine (PSA) and 1 g of magnesium sulfate. The prepared solution was stirred for 2 min then, centrifuged at 4000 g for 10 min at −5 °C, and 4 mL of the supernatant was transferred to a 5 mL flask. The samples were concentrated under a nitrogen atmosphere to the size of 0.3–0.5 mL. Then, samples were reconstituted until 1 mL with toluene and stirred for 3 min eventually, 2 μL of the final solution was injected into the GC-MS device.

The acetonitrile and magnesium sulfate dehydrate salt was used for the extraction of the analytes. Primary and secondary amine (PSA) and C_{18} were also used to remove non-polar substances such as lipids to prevent interference (Gilbert-López et al., 2009). To eliminate the matrix effects, analysis of spiked samples was used, and then the concentrations of pesticides were calculated by interpolation of the relative peak areas for each pesticide to internal standard peak area in the sample on the spiked calibration curve.

2.3. Single and mixed standard solution

The ethyl acetate was used for preparing a stock solution of the standards at a concentration of 1 mg/mL as an internal standard of triphenylmethane (TPM), and then was kept in a freezer at −20 °C. The mix stock solution was prepared from standards of 29 pesticides with a concentration of 10 μg/mL of each pesticide in ethyl acetate. In following, all pesticides were injected individually into the GC-MS device to determine the retention time of each pesticide and find their relevant chromatograms. The mixture of pesticides was injected into the GC-MS device in scan mode for providing SIM program. The diagnostic ions and retention times for the target pesticides and internal standard are reported in table one (Table 1).

2.4. Gas chromatography-mass spectrometry (GC-MS)

An Agilent Technologies 6890 N Network GC System chromatograph (Wilmington, USA) equipped with an SQ detector and an Agilent 7683 B autosampler (Agilent Technologies, USA) and an HP-5 capillary column (30 m × 0.25 mm I.D., 1 μm film thickness) was used for analysis of the samples.

Briefly, the oven temperature program was initially set at 75 °C and kept for 3 min at this temperature. The temperature was ramped to 120 °C at a rate of 25 °C/min. Then, the temperature was ramped to 300 °C at a rate of 5 °C/min and then held for 20 min. The temperature of injector port, MS transfer line, and MS heater were set at 250 °C. The splitless mode was used for operating the GC-MS device. The flow rate of the carrier gas (helium, 99.999%) was set to a constant of one mL/min.