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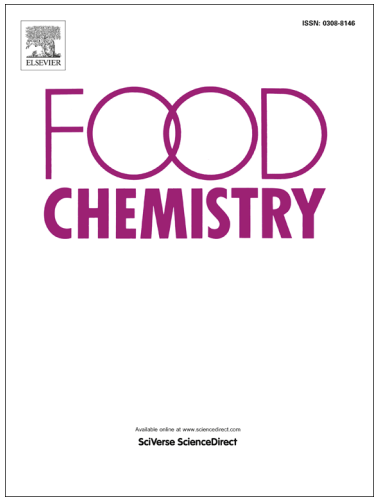

Occurrence and exposure of 3-monochloropropanediol diesters in edible oils and oil-based foodstuffs from the Spanish market

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PII: S0308-8146(18)31246-9
DOI: https://doi.org/10.1016/j.foodchem.2018.07.100
Reference: FOCH 23215

To appear in: *Food Chemistry*

Received Date: 15 March 2018
Revised Date: 11 July 2018
Accepted Date: 16 July 2018

Please cite this article as: Custodio-Mendoza, J.A., Carro, A.M., Lage-Yusty, M.A., Herrero, A., Valente, I.M., Rodrigues, J.A., Lorenzo, R.A., Occurrence and exposure of 3-monochloropropanediol diesters in edible oils and oil-based foodstuffs from the Spanish market, *Food Chemistry* (2018), doi: https://doi.org/10.1016/j.foodchem.2018.07.100

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Occurrence and exposure of 3-monochloropropanediol diesters in edible oils and oil-based foodstuffs from the Spanish market

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Abstract

During the industrial refining process of edible oils and the manufacture of oil-based foodstuff, contaminants such as 3- monochloropropanediol (3-MCPD) fatty acid diesters can be produced. One hundred samples of different edible oils and related fatty food purchased from local Spanish markets were analyzed to evaluate the occurrence of these contaminants. Data of seven 3-MCPD diesters together with corresponding total 3-MCPD equivalents are presented. The procedure is based on a modified QuEChERS protocol followed by LC-MS/MS analysis. Extra virgin olive oil (EVOO) and unrefined oils did not contain detectable levels of the target analytes. The highest levels of 3-MCPD diesters were found in palm oils, for 1,2-Dilinoleoyl-3-chloropropanediol (LILI) and 1-2-Bispalmitoyl-3-chloropropanediol (PAPA) with concentrations close to 10 mg kg^{-1} and in the lipid fraction of margarines (8.09, 3.77 and 3.72 mg kg^{-1} for LILI, PAPA and 1-Oleoyl-2-linoleoyl-3-chloropropanediol (OLLI), respectively).

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Keywords: 3-MCPD fatty acid diesters, edible oils, foodstuff, LC-MS/MS, processing contaminants.

Highlights

- Evaluation of presence and exposure of processing contaminants to oil consumers
- Seven 3-MCPD diesters determined in one hundred EVOO and other edible oils samples
- High content of 3-MCPD diesters was detected in pomace and refined olive oils
- Potato chips daily intake should be limited to reduce the health risk

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1. Introduction

Vegetable edible oils are commonly consumed directly and used as ingredients in foodstuff preparation being a vital source of macronutrients in human nutrition. Spain is the largest producer and exporter of olive oil in the world. According to the report on food consumption in Spain 2016, published in 2017 by the Ministry of Agriculture and Fisheries, Food and Environment, the per capita consumption is 8.51 L / person / year of total of olive oil which comprises the sum of three types of olive oil marketed: virgin olive oil (1.06 L / person / year), extra virgin olive oil (2.43 L / person / year) and refined olive oil (5.02 L / person / year). These data serve to quantify the household demand for olive oil in Spain. In addition, Spain produces almost half of the almost 3 million tons of olive oil consumed per year throughout the world. The potential benefits of olive oil are known for the prevention of risk factors that lead to different diseases, such as cardiovascular diseases, diabetes mellitus or overweight. Despite these data, palm oil is the most used due to the growing trend of consumption of prepared foods, such as industrial bakery products.

Edible oils are extracted from either plant seed or pulp by thermal, mechanical or chemical methods. To match the organoleptic properties for the consumer's acceptance, edible vegetable oils are often submitted to refining processes at high temperatures, forming both desirable and undesirable compounds (Destaillats et al., 2012; Hernandez et al., 2013; MacMahon, 2015). One of these latter is 3-MCPD, a food contaminant which can be formed from three routes: (1) acid hydrolysis of glycidol by hydrochloric acid, (2) thermal decomposition of glycidol in the presence of sodium chloride and (3) thermal decomposition of 3-MCPD esters (Jędrkiewicz et al., 2016a). Also, 3-MCPD esters are prone to be formed from acylglycerols at temperatures above 200ºC in the presence of organic or inorganic chlorinated compounds, typically occurring during the deodorization step of oils refining process (Destaillats et al., 2012). For this reason, unrefined edible oils (known as extra virgin) and mechanically processed oils are not expected to have these contaminants in opposition to the refined oils, (such as palm oil or grapeseed oil) (Destaillats et al., 2012; Yamazaki et al., 2013).

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3-MCPD has been classified by the International Agency for Research on Cancer (IARC) as “possible carcinogen to human” (Group 2B) (IARC, 2014) and the Office of Environmental Health Hazard Assessment (OEHHA) has also considered it as a substance that can cause cancer, birth defects, and other reproductive harm (Prop. 65). The Food Chain Contaminants Committee (CONTAM) of the European Food Safety Authority (EFSA) estimates that 3-MCPD is released from its esters within the human digestive tract. The toxicological significance of 3-MCPD esters and their possible contribution to dietary intake of free 3-MCPD is not well understood although they appear to be widely distributed in the food chain. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a Tolerable Daily Intake (TDI) of 2 μg kg^{-1} bw per day, but from the new scientific information a reduced TDI of 0.8 μg kg^{-1} bw per day has been recently recommended for 3-MCPD and its esters (EFSA, 2016). However, in the same year, the JECFA has established a provisional TDI of 4 μg kg^{-1} bw per day for 3-MCPD and 3-MCPD esters individually or in combination (FAO/WHO, 2016).

The availability of reliable analytical methodology in control laboratories is essential to establish maximum levels for contaminants in food and carry out assessment tasks health risks. Furthermore, three basic criteria, green analytical chemistry, metrological and economic should be considered in the chloropropanols determination (Jędrkiewicz et al., 2016b). Different advanced sample preparation techniques for chloropropanols in food were widely discussed by Jędrkiewicz and co-workers (Jędrkiewicz et al., 2014). 3-MCPD was determined in a wide range of heat processed foods (Xu et al., 2013; Jędrkiewicz et al., 2016a; EFSA, 2013) including bakery products (Racamonde et al., 2011), milk and soy beverages (Carro et al., 2013), coffee surrogates and malts (Divinova et al., 2007), meat products (Baer et al., 2010) or fish products (Merkle et al., 2018) by gas chromatography-mass spectrometry (GC-MS or GC-MS/MS) that require a previous derivatization step. In the last years, the determination of 3-MCPD free form and bound as fatty acid esters in foods with a high fat content (Zelinková et al., 2009a; Leigh et al., 2017) and infant formulas (Jędrkiewicz et al., 2016c; Leigh and MacMahon, 2017) has become of great importance (Jędrkiewicz et al., 2016a). Refined oils (olive, palm, sunflower oil), as well as margarine and derivatives are the main contributors to

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total dietary exposure among population groups, followed by products manufactured with these fats.

The European Commission has issued Recommendation 2014/661/EU calling for the active participation of representatives of industry, research and national and international health authorities to participate in monitoring the presence of these compounds in vegetable oils and fats. The presence of 3-MCPD diesters in edible oils, particularly olive oils from the Spanish market has not been reported so far. However, some studies performed in the last years showed the occurrence of 3-MCPD esters concerning refined fats and oils (Weißhaar, 2011; Chung et al., 2013; MacMahon et al., 2013a; Yamazaki et al., 2013; Li et al., 2015; Becalski et al., 2015; Jędrkiewicz et al., 2016d; Graziani et al., 2017; Ben Hammouda et al., 2017; Yan et al., 2018). Most data of 3-MCPD esters are presented as 3-MCPD equivalents (Weißhaar, 2011; Chung et al., 2013; Li et al., 2015; Becalski et al., 2015; Jędrkiewicz et al., 2016d; Graziani et al., 2017; Yan et al., 2018) when 3-MCPD esters are determined by indirect methods, based on a transesterification step to release free 3-MCPD before GC-MS quantification. Particular attention requires sample preparation to avoid unwanted reactions and transformations and compound loss. In addition, due to its characteristics, the derivatization of 3-MCPD is necessary for its determination by GC-MS (Jędrkiewicz et al., 2016a; Jędrkiewicz et al., 2017; Yan et al., 2018). Direct methods, based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), do not require any additional reaction and allow the simultaneous and individualized quantification of each MCPD esters, providing useful information to study the toxicity of these compounds (Graziani et al., 2017). A large number of standards is required and require complex sample preparation to minimize the matrix effect and provide accurate quantitative results (Hori et al., 2012; MacMahon et al., 2013a; MacMahon et al., 2013b; Yamazaki et al., 2013; Ermacora et al., 2014).

The current study aimed to monitor the presence of seven 3-MCPD diesters in three types of edible oils and oil-based foodstuffs, namely olive oil, vegetable seed oil, vegetable pulp oil, potato chips and margarines, with particular attention to products from the Spanish market. The analytical method used is based on LC-MS/MS using a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach, involving liquid-liquid partitioning and

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purification of the extract by dispersive solid-phase extraction (d-SPE) (Custodio-Mendoza et al., 2018). FDA guideline (FDA, 2015) and ISO 11843-2 (ISO, 2000) were used for validation of the method in olive oil, seed oils, vegetable pulp oils and oil-based foodstuffs. This evaluation provides information on the presence of these food processing contaminants and highlights the level of exposure of consumers.

2. Materials and methods

2.1. Samples collection

The samples used for this study were purchased in local markets and classified into four categories: olive oil (7 EVOO, 12 refined olive 1º, 15 refined olive 0.4º, 7 olive pomace and 2 fried EVOO), vegetable seed oil (11 sunflower oil, 6 sesame oil and 3 line oil, 3 soybean mixed with other oils, 3 corn oil and 7 other seed oils), vegetable pulp oil (7 oils) and oil-based foodstuffs (12 margarines and 6 potato chips). An EVOO sample was selected as the blank sample used both in analytical quality control (QC) preparation and validation of the method. For the other types of oils, bio-sesame oil, virgin linen oil, extra virgin avocado oil, potato chips fried in extra virgin olive oil and margarine made of soybean and coconut oil are used as blanks.

Most of the edible vegetable oil, potato chips and margarine samples were acquired from local stores in Santiago de Compostela, Spain. Pure palm oil was purchased online from www.amazon.com. One coconut and the red palm oil were acquired at a local store in Porto, Portugal. One avocado, one coconut, one corn, one canola, one safflower and one sesame oils, were purchased from several supermarkets in Villahermosa, Mexico. All the samples were stored in the dark at room temperature and in the original package.

All samples were analyzed in triplicate except for oil-based samples which have been analyzed in duplicate. Samples were studied in daily sequences. Acceptance of sequence results was based on FDA parameter in terms of QCs %RSD, which have been calculated between sequences.

2.2. Reagents and materials

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Individual standards of rac ,2-Dilinoleoyl-3-chloropropanediol (LILI), rac 1-Oleoyl-2linoleoyl-3-chloropropanediol (OLLI), rac 1,2-Dioleoyl-3-chloropropanediol (OLOL), rac 1-Oleoyl-2-stearoyl-3-chloropropanediol (OLST), rac 1-Palmitoyl-2-linoleoyl-3-chloropropanediol (PALI), rac 1-2-Bispalmitoyl-3-chloropropanediol (PAPA), rac 1-Palmitoyl-2-oleoyl-3-chloropropanediol (PAOL) and internal standards (IS) 1,2-Dioleoyl-3-chloropropanediol-d5 (OLOL-d5), rac 1-Oleoyl-2-stearoyl-3-chloropropanediol-d5 (OLST-d5), rac 1-2-Bispalmitoyl-3-chloropropanediol-d5 (PAPA-d5) were from Toronto Research Chemical (Toronto, Ontario, Canada). Ethyl acetate (EtOAc), methanol (MeOH), acetonitrile (ACN), tert-butyl methyl ether (MTBE), n-hexane, 2-propanol (IPA), diethyl ether (Et_2O), ammonium formate and formic acid were from Merck (Darmstadt, Germany) all LC–MS grade. Primary secondary amine (PSA) and silica SAX (Si-SAX) sorbents were from Agilent® (Santa Clara, CA, USA) and Z-sep+ bulk was from Supelco (Sigma-Aldrich Quimica SL, Madrid, Spain). Ultrapure water type I was from a Wasserlab, water purification system (Barbatáin, Spain).

2.3. Standard solutions

For each compound, individual standard stock solutions of 25 mg mL^{-1}, except for PAOL (10 mg mL^{-1}) were prepared in ethyl acetate (EtOAc) or methanol (MeOH), according to manufacturer's recommendations. Individual IS solutions were prepared at the concentration of 2.5 mg mL^{-1}. A stock mixture solution of all target analytes at a concentration of 50 µg g^{-1} was prepared by appropriate dilution of individual standard solutions in MeOH. Working solutions were used to prepare calibration curves by injecting in triplicate eight concentration levels: 25, 35, 50, 100, 250, 500, 750 and 1000 µg kg^{-1}, all with a fixed concentration of the IS of 250 µg kg^{-1}. All the solutions were stored in the dark at -20 ºC.

2.4. Sample preparation

The samples were prepared using a modified QuEChERS method according to the protocol described by Custodio-Mendonza et al. (2018). The protocol

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includes the use of different sorbents formulations containing Si-SAX or Z-sep+ combined with PSA to remove triglycerides and waxes to obtain cleaner extracts by d-SPE clean-up. The flow diagram of the sample procedure is shown in Fig. 1.

In case of margarines, a previous sample preparation to separate the aqueous phase was required by centrifugation (5 min at 3500 rpm) of the sample mixed with water; 0.1 mL of the organic phase was collected to continue the extraction process. For potatoes chips, it was necessary to grind the sample using a porcelain mortar with pistil; the ground sample was mixed with the extraction solvent and centrifuged at 3500 rpm for 5 min. The sample preparation protocol was performed as usual.

2.5. LC-MS/MS analysis

The quantification of 3-MCPD diesters in the evaluated samples was performed by HPLC-MS/MS using a Thermo Scientific™ TSQ Quantum™ Access MAX LC-MS equipped with a triple quadrupole mass spectrometer TSQ Quantum Access MAX (Thermo Fisher Scientific, San José, CA, USA) with a heated electrospray ionization source HESI-II, an Accela 1250 pump fitted with a degasser, and an autosampler. Separation of the different compounds was achieved in a Kinetex® C18 column (2.6µm, 100Å, 2.1 x 50mm) from Phenomenex (Torrance, USA). Mobile phases were: 2mM ammonium formate/0.05% formic acid in 92:8 MeOH/H_2O (mobile phase A) and 98:2 IPA/H2O (mobile phase B) (MacMahon et al., 2013b). The gradient program started at 75% mobile phase A/ 25% mobile phase B (held 2 min) increased to reach 100% mobile phase B in 6 min and decreased to reach 25% mobile phase B in 1 min (held 15 min) and keeping at 15 min for stabilization. The flow rate was 400 µL min^{-1} and the injection volume was 10 µL. The analytes were analyzed in less than 6 min but when oil samples were processed, stabilization time was kept at 22 minutes to ensure that any remaining matrix is eluted from the column.

To quantify the 3-MCPD diesters, the mass spectrometer was operated in positive mode Multiple Reaction Monitoring mode (MRM) with a spray voltage of 3000V. MS detector settings were as follows: ion transfer temperature 350°C

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and HESI vaporizer temperature 340°C. Nitrogen (>99.98%) was used as an auxiliary gas and sheath gas (pressure 10 and 35 arbitrary units respectively). Collision-induced-dissociation (CID) argon was set at 18 bar. For precursor and product ions identification, preliminary optimization was performed by continuous infusion of the individual compounds at 50-100 µL min^{-1} in mobile phase at initial conditions. The retention time of target compounds was determined by analyzing a mixture of standards under conditions described before. MS parameters are shown in Supplementary material, Table 1S.

2.6 Method validation and quality control

The method was validated according to FDA (FDA, 2015), ISO 11843-2 (ISO, 2000) in terms of linearity, trueness, accuracy, sensitivity, decision limit (CCα) and minimum detectable value (CCβ). The selectivity of the method was evaluated by injection of blank samples (virgin oils).

The linearity was assessed with the internal standard method. Eight-level calibration curves were prepared from 25 µg kg^{-1} except for PALI (50 µg kg^{-1}) and OLST (35 µg kg^{-1}) to 1000 µg kg^{-1} setting the internal standard concentration at 250 µg kg^{-1}. For those analytes that did not have deuterated species accessible when the method was developed, available deuterated 3-MCPD diesters in the laboratory were tested and the best were selected as surrogate standard in each case (Table 1S).

Fortified EVOO was used for QC purposes. QC samples spiked at three concentration levels were used to assess precision: 100 µg kg^{-1} (low); 500 µg kg^{-1} (middle); and 1000 µg kg^{-1} (high). Recoveries were evaluated using QC spiked at three concentration levels (100, 250 and 500 µg kg^{-1}) with the target 3-MCPD diesters and six replicates for each concentration. These QC samples were stored refrigerated at 4ºC. QCs solutions spiked at known concentrations of the target analytes in blank matrix samples (100 µg kg^{-1}, 500 µg kg^{-1} and 1000 µg kg^{-1}) were used through the analysis into each run in order to determine the acceptance of the sequence of samples regarding on relative standard deviation (%RSD) at each level (n=3). These QC samples were stored refrigerated at 4ºC. The QCs were used to provide information about accepting or rejecting the run to measure each sample batch.

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CCα and CCβ were calculated according to ISO 11843-2 (ISO, 2000) from regression lines prepared in the range of 0 to 50 µg kg^{-1}.

3. Results and discussion

3.1 Performance of the method

The modified QuEChERS procedure followed by LC-MS/MS analysis was previously evaluated (Custodio-Mendoza et al., 2018). Sample preparation was successfully performed for the several vegetable oil categories considered in this study. Si-SAX was capable of retaining most of triglycerides and waxes from the matrix, PSA successfully removed the remaining acylglycerides and some polar pigments while Z-sep+ retain the remaining pigments from most of the samples. However, it was necessary to include the addition of 50 mg of EnviCarb to remove all the remaining pigments in the case of palm oil. Calibration curves were constructed using the ratio of the chromatographic peak area of each 3-MCPD diester to the corresponding IS in a relation of analyte concentration. Excellent determination coefficients ($r^2 \geq 0.9960$) were obtained in the calibration ranges studied. Concerning selectivity, no interferences were observed in the chromatograms, in the region where the target analytes presented signals. Limits of quantification (LOQ) were established as the first point of calibration curves and varied from 25.0 to 50.0 µg kg^{-1} and were lower (Yamazaki et al., 2013; Ermacora et al., 2014) or similar (MacMahon et al., 2013b; Jędrkiewicz et al., 2016d) than those previously reported. The CCα and CCβ ranged from 9.4 to 20.1 µg kg^{-1}, and 17.7 to 37.9 µg kg^{-1}, respectively, with a probability of false positive and false negative equal to 0.05 (Table 1). Accuracy was evaluated by means of recovery of QCs at three concentration levels (100, 250 and 500 µg kg^{-1}) and expressed as %Recovery± SD (Supplementary Material, Fig.1S). Relative recoveries (Fig.1SA) ranged between 81.0±9.0% and 105.6±8.2% in all cases, except for PAPA (78.3±6.0%) and PALI (79.1±5.0%) at 500 µg kg^{-1}, and are in accordance with FDA guideline (FDA, 2015). Absolute recovery values (Fig.1SB) obtained for each compound ranged between 85.8±3.5 and 119.5±12.3%, except for OLST (71.4±1.3%) and

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PAPA at 100 µg Kg^{-1} (122.9±12.7%). These good results can be attributed to the absence of matrix effects showed for the compounds. The inter-day precision was evaluated for each compound in terms of %RSD using the QCs at three concentration levels (100, 500 and 1000 µg kg^{-1}). Table 1 shows the results obtained for the 3-MCPD diesters evaluated through replicate analytical series in the four different matrices, i.e. olive oil (n=16), vegetable seed oil (n=16), vegetable pulp oil (n=6) and oil-based foodstuff (n=10). Inter-day precision was satisfactory showing %RSD values below 3.8% in olive oil, 2.5% in seed oil, 13.0% in pulp oil and 10.0% in oil-based foodstuffs. The precision and accuracy results, similar to those of other authors (MacMahon et al., 2013b; Yamazaki et al., 2013; Ermacora et al., 2014) indicated that this method was highly accurate and reproducible according to the validation guidelines. The suitability of the method for the analysis of the compounds selected in this study was demonstrated.

This study has been focused on the analysis of seven 3-MCPD diesters of linoleic, oleic, stearic, and palmitic acid since they are the primary constituents of vegetable oils and the selected oil based foodstuffs, this is in accordance with Dubois who estimated that the determination of the ten most abundant 3-MCPD diesters would be enough to establish the total 3-MCPD diester content (Dubois et al. 2012).

3.2 Quality control

Control charts are an adequate tool for internal quality control, allowing the detection of random and systematic errors that affect the measurement and therefore the precision and accuracy of the method. In this study, the QCs at three concentration levels and different matrices were analyzed for consecutive days. Shewhart Charts were used for the control of bias with a central line representing the value of the statistic (mean) of measurements over time, upper and lower warning limits (UWL and LWL) calculated as 2×standard deviation (2SD) from the center line and upper and lower control limits (UCL and LCL) calculated as 3SD from the center line. Each point and bar represents the mean of experiments and the SD. As an example, Supplementary Material, Fig. 2S showed the control charts for the target 3-MCPD diesters in EVOO at 100 µg kg-1 within the period of the study. Two points which fell outside the UWL and

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LWL for OLST and one point fell outside the UWL for PAPA. All results are within the controlled area defined by the UCL and LCL meaning that the analysis has not been out of control at any time confirming the robustness of the method for the determination of 3-MCPD diesters in oil samples.

3.3 3-MCPD diesters occurrence levels in oils and oil-based foodstuff from the Spanish market

Table 2 shows the results concerning the occurrence of the target 3-MCPD diesters in 43 olive oil samples, and in Supplementary Material, Table 2S the results from 40 seed and pulp oils and 18 oil-based foodstuffs are presented. 76 positive samples were found containing one or more target analytes and 24 samples did not have detectable amounts of 3-MCPD diesters. Supplementary Material, Fig. 3S shows the sample distribution (%) (Fig. 3SA) and the occurrence (%) of each analyte in the analyzed samples (Fig. 3SB). OLOL was found in 56 samples, LILI in 54 samples, PAOL in 51 samples, OLLI in 18 samples, OLST and PAPA in 13 samples and PALI in 9 samples. The distribution of the target 3-MCPD diesters in the categorized oils and foodstuffs is shown in Fig. 4S. In this study, 3-MCPD diesters were not found in 7 EVOO, 4 soybean-based, 3 virgin linen, 3 coconut, 1 bio sesame, 1 virgin hemp, 1 virgin nut, 1 refined sunflower, 1 virgin avocado, 1 seeds oil 0.2º, 1 margarine and 1 potato chips fried with EVOO samples. Similarly, other authors reported that 3-MCPD diesters were not detected in unrefined oils, such as EVOO, sesame, coconut, sunflower or hemp oil (MacMahon et al., 2013a; Moravcova et al., 2012).

3.3.1 Oils

The presence of 3-MCPD diesters in the analyzed edible oil samples is shown in Table 2 and Supplementary Material, Table 2S. The results of the analysis o 3-MCPD diesters in three types of oils are presented in box plots (Fig. 2a, 2b and 2c). The rectangular box has one end at Q1 and the other end at Q3. The horizontal segment inside is the median value. The two vertical segments on each side of the box are called the whiskers, one down to the minimum value and one up to the maximum value.

None of the target compounds was detected in EVOO because it is produced by the mechanical process, extracting the juice from the olives without using

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chemical and industrial refining. In the olive oils, OLOL, PAOL and LILI are the 3-MCPD diesters found (Fig. 2a), coinciding with the composition of the most abundant fatty acids in olive oils, as observed by Yamazaki (Yamazaki et al., 2013) and MacMahon (MacMahon et al. 2013a). Two types of refined olive oils with different free acidities (expressed as oleic acid) were analyzed: 0.4º and 1º acidity, also designated as "mild flavor/intense flavor". The industry uses this classification in the labels of olive oils which are mixtures of refined and virgin oils; the products with higher content of refined olive oil have lower acidity and fewer flavors. Thus, it means that, in the samples analyzed in this study, the olive oil 1º has more virgin oil in its composition than the oil 0.4º. OLOL concentration ranged between 1.69 and 3.48 mg kg^{-1} for refined olive 1º, and values between 1.83 and 5.60 mg kg^{-1} of OLOL were obtained for refined olive 0.4º. PAOL showed contents in a range of 1.24 to 1.41 mg kg^{-1} for refined olive 1º and 1.28 and 1.62 mg kg^{-1} for refined olive 0.4º. LILI concentrations ranged between 1.30 and 1.56 mg kg^{-1} for refined olive 1º and 1.29 and 1.87 mg kg^{-1} for refined olive 0.4º. However, the highest concentrations of OLOL, PAOL and LILI were found in olive pomace, which consists on a chemical processing of the pomace resulting from the mechanical processing of olives for the production of virgin olive oil. The concentration ranges were between 3.07 and 10.89 mg kg^{-1} for OLOL, 0.59 and 1.81 mg kg^{-1} for PAOL and 1.66 and 1.96 mg kg^{-1} for LILI. Fig. 3 presents the MRM chromatograms of the three 3-MCPD diesters detected in olive pomace oil (sample number 7 from Table 2). Two samples of fried EVOO were also analyzed and the presence of PAOL and OLOL was observed, demonstrating that these compounds can be generated when EVOO is treated at high temperatures (Matthäus & Pudel, 2013). PAOL showed levels ranging from 0.74 to 2.44 mg kg^{-1} while OLOL was quantified in one sample (7.42 mg kg^{-1}).

Fig. 2b shows the concentration ranges found for the seven 3-MCPD diesters in the 30 seed oil samples. The most abundant diester was LILI with 19 positive samples and the concentration ranged from 6.98 mg kg^{-1} in sesame oil to 0.015 mg kg^{-1} in sunflower oil. OLLI was positive in twelve samples and the concentration ranged from 5.81 mg kg^{-1} in safflower oil from Mexico to 1.41 mg kg^{-1} in sunflower oil. OLOL was positive in ten samples and the concentration ranged from 7.36 mg kg^{-1} in sesame oil from Mexico to 0.22 mg kg^{-1} in

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sunflower oil. The least abundant diesters were OLST and PAOL (four positive samples), PALI and PAPA (three positive samples). It should be noted that the highest concentration for OLST was 7.37 mg kg^{-1} in sesame oil from Mexico and PALI was 6.55 mg kg^{-1} in sunflower oil. The levels we obtain for the eleven sunflower oils tested were comparable to the levels found in one sunflower sample analyzed by Dubois (Dubois et al., 2012) and Moravcova (Moravcova et al., 2012). Seed oils labelled as virgin or unprocessed (bio sesame, linen, hemp and nut) and soybean-based oil did not contain detectable levels of 3-MCPD diesters and are in agreement with data reported in literature for a soybean oil (Hori et al., 2012; Yamazaki et al., 2013) and two hemp oils (MacMahon et al., 2013a). Levels of LILI and OLOL found in the present study in corn oils were different from those obtained by MacMahon (MacMahon et al., 2013a). These authors also found other diesters of 3-MCPD. On the contrary, Hori et al. (Hori et al., 2012) reported undetectable levels of LILI and OLOL in corn oil.

Pulp oils are the least edible oils consumed in Spain in their unprocessed form, although many processed foods are made with them. The highest levels of 3-MCPD diesters were found in palm oils, especially for LILI and PAPA with concentrations close to 10 mg kg^{-1} (Fig. 2c) and these levels are similar to those reported by other authors (Hori et al., 2012; Moravcova et al., 2012; MacMahon et al., 2013a; Yamazaki et al., 2013).

Since the significant part of 3-MCPD is present in the form of diesters (85–93%) the quantification of ester-linked 3-MCPD was calculated and expressed as total amounts of 3-MCPD equivalents (Weißhaar, 2011; Graziani et al., 2017). The results are shown in Fig. 4, for the samples categorized. The content was variable, reaching the highest levels for palm oils (pulp oil) from 4.74 to 6.22 mg kg^{-1} and sesame oil (other seed oil) with 4.09 mg kg^{-1}. Levels in sunflower oils group varied from not detected to 2.05 mg kg^{-1}. These results reveal that the contents of 3-MCPD were similar than those reported by Graziani (Graziani et al., 2017). Olive oil category was found to have the lowest levels of total 3-MCPD from 0.53 to 1.22 mg kg^{-1} in refined olive oils and from 0.64 to 2.53 mg kg^{-1} in olive pomace (see Table 2). These results are comparable to those reported in literature by the most frequently used indirect methodology (Becalski et al., 2015; Li et al., 2015; Jędrkiewicz et al., 2016d; Yan et al., 2018) and direct methods (MacMahon et al., 2013a; Yamazaki et al., 2013). The values of

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3-MCPD found in refined olive and pomace oils may be due to the presence of precursors such as acylglycerols (Yan et al., 2018). 3-MCPD esters were detected in palm oil, at levels below 2.91 mg kg^{-1}, and in other oils, margarines and similar fats, the level found were between 0.048-0.61 mg kg-1 (Li et al., 2015; Jędrkiewicz et al., 2016d; EFSA, 2016).

3.3.2 Margarine and potato chips

To the best of our knowledge this is the first study on the presence of individual 3-MCPD diesters in margarines and potato chips. The direct method used in this study allows the individual determination of each fatty acid 3-MCPD diester in these oil-based foodstuffs (Fig. 2d). Large amounts of 3-MCPD diesters (8.09, 3.77 and 3.72 mg kg^{-1} were found for LILI, PAPA and OLLI, respectively) in the lipid fraction of margarines. The composition of margarine 7 (Supplementary Material, Table 2S) is sunflower oil (with a high content of linoleic acid) and palm oil, but its lipid fraction is meager (19%), which explains the low level of LILI (0.65 mg kg^{-1}) and the undetected levels of the other 3-MCPD diesters. The major components of margarine 9 are sunflower and olive oils and only presented 2.82 mg kg^{-1} of PAOL. Comparatively, lower levels of 3-MCPD diesters (>LOQ-0.64 mg kg^{-1}) were reported in the study of MacMahon et al. (MacMahon et al., 2013a) where five shortenings were analyzed. Probably, these differences are related to the composition and manufacture of shortenings with respect to margarines, especially the type of oily ingredients of margarines (sunflower, olive, linen, palm, soybean) and the percentage of the lipid fraction. The total bound 3-MCPD concentrations in lipid fractions of margarines were in the range of 0.11- 2.61 mg kg^{-1}. Several studies using indirect methodology for monitoring the concentration of total 3-MCPD esters showed similar results, ranged between 0.79–1.60 mg kg^{-1} (Li et al., 2015), 1.3-7.3 mg kg^{-1} (Jędrkiewicz et al., 2016d) both in five samples; 0.4–4.5 mg kg^{-1} in 37 samples (Weißhaar, 2011). The lower content was determined by Becalski et al. (Becalski et al., 2015) in 4 margarine samples (0.09-0.43 mg kg^{-1}) and one vegetable shortening (0.50 mg kg^{-1}). Potato chips also contained significant high amounts of the target 3-MCPD diesters (6.65, 5.19 or 4.44 mg kg^{-1} for OLST, OLOL and PAOL, respectively). When palm oils were used for frying

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potato chips (samples 4, 5 and 6), the highest levels of the target compounds where found (see Supplementary Material Table 2S). These results demonstrated that the vegetable origin of the frying oil is related to the presence of 3-MCPD diesters. Potato chips from sample 1 were fried with EVOO and no 3-MCPD diesters were detected. The range of concentration for total bound 3-MCPD in potato chips varied from no detected to 3.22 mg kg^{-1}. Lower concentrations were reported in potato chips ranging from 0.11 to 0.81 mg kg^{-1} (Arisseto et al., 2015) and 0.29 to 1.01 mg kg^{-1} (Zelinková et al., 2009b). However, in these studies they did not discriminate the different 3-MCPD diesters and they do not specify the type of oil. The variations on the levels of total 3-MCPD observed in this food group could be associated with variables such as the type of oil used, the temperature of frying, the individual content of 3-MCPD esters and the presence of potential precursors.

3.3.3 Dietary exposure

The per capita consumption of oil in Spain was 12.66 liters/person/year in 2016, especially olive oil (8.51 L of which 5.02 L were refined olive oil), and sunflower oil (3.21 L). The 3-MCPD exposure, according to the concentration obtained (Table 2, Supplementary Material Table 2S and Fig. 4) and the consumption data in Spain with a mean body weight of 70 kg, results on an average daily intake per day between 0.12 and 0.23 µg kg^{-1} bw per day for refined olive oil, and 0.25 µg kg^{-1} bw per day for refined sunflower oil, similar or less to the rest of Europe and below the TDI set by the EFSA (EFSA, 2016). This may be due to the fact that in Spain the main consumed oil is the olive oil (67% of the total, of which a 2.6 % is virgin olive oil). As shown in Supplementary Material Table 2S, five positive potato chips samples were obtained. Taking into account a body weight of 60 kg of young people, the consumption of an individual ration of potato chips of 30 g with a mean content of oil of 2.68 mg kg^{-1} would produce an intake of 3-MCPD of 1.34 µg kg^{-1} bw per day. This value exceeds the TDI proposed by EFSA (0.8 µg kg^{-1} bw per day) but not the one established by JECFA (4 µg kg^{-1} bw per day). Considering that potato chips are a very popular snack among adolescents, these results suggest a potential concern about the health risk of this consumer subgroup.

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4. Conclusions

Edible oils can be contaminated with 3-MCPD diesters which are processing contaminants. The presence of seven 3-MCPD diesters associated with the most abundant fatty acids in edible oils from the Spanish retail market (including different types of olive oil, produced in Spain for national consumption and exportation). To the best of our knowledge, this is the first study on 3-MCPD diesters occurrence in edible oils in Spain, especially in olive oils. This evaluation will provide information on the presence of these food processing contaminants and the level of exposure of consumers, which has great relevance for the national and European consumers. Particular relevant is the inexistence of data in the available literature to compare the levels of 3-MCPD diesters in different types of olive oils. 3-MCPD diesters were not detected in EVOO and other non-heat processed oils, while were detected in refined olive oil and other edible oils, especially palm oil. OLOL was the most abundant analyte among the total samples analyzed. The information on daily intake indicates that the consumption of EVOO does not pose a health risk due to the exposure to 3-MCPD. The systematics results obtained in this study can also contribute to enhancing the information related to the content of 3-MCPD dieters in edible oils and the establishment of new regulations. As a future approach we envisage the evaluation of the possible transfer of these contaminants to foods when using with other vegetable oils and potential assessment of mitigation strategies.

Acknowledgment

This work was supported by the Spanish Ministry of Science and Innovation (Project AGL-2014-53647-R) and FEDER funds.
JACM (ref. 330) acknowledge his IACOBUS grant to Xunta de Galicia, POCTEP Interreg 2014-2020, CCDRN and FEDER

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IMV (SFRH/BPD/111181/2015) also acknowledge her post-doctoral grant to FCT/MEC and the European Social Fund within the 2014–2020 Strategic Framework.

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References

Arisseto, A.P., Marcolino, P.F.C., Vicente, E. (2015). 3-Monochloropropane-1,2-diol fatty acid esters in commercial deep-fat fried foods. *Food Additives & Contaminants: Part A, 32*(9), 1431–1435.

Baer, I., de la Calle, B. and Taylor, P. (2010). 3-MCPD in food other than soy sauce or hydrolyzed vegetable protein (HVB). *Analytical & Bioanalytical Chemistry, 396*(1), 443–456.

Becalski, A., Feng, S., Lau, B. P-Y., Zhao, T. (2015). A pilot survey of 2- and 3-monochloropropanediol and glycidol fatty acid esters in foods on the Canadian market 2011–2013. *Journal of Food Composition and Analysis, 37*, 58–66.

Ben Hammouda, I., Zribi, A., Ben Mansour, A., Matthäus, B., Bouaziz, M. (2017) Effect of deep‐frying on 3‐MCPD esters and glycidyl esters contents and quality control of refined olive pomace oil blended with refined palm oil. *European Food Research and Technology, 243*, 1219–1227.

Carro, A. M., Gonzalez, P., Lorenzo, R. A. (2013). Simultaneous derivatization and ultrasound-assisted dispersive liquid-liquid microextraction of chloropropanols in soy milk and other aqueous matrices combined with gas-chromatography-mass spectrometry. *Journal of Chromatography A, 1319*, 35–45.

Custodio-Mendoza, J.A., Lorenzo, R.A., Valente, I.M., Almeida, M.A. Lage, J.A. Rodrigues, Carro, A.M. (2018) Development of a partitioned liquid-liquid extraction- dispersive solid phase extraction procedure followed by liquid chromatography-tandem mass spectrometry for analysis of 3-monochloropropane-1,2-diol diesters in edible oils. *Journal of Chromatography A, 1548,* 19–26.

Chung, H.Y., Chung, S.W.C., Chan, B.T.P., Ho, Y.Y., Xiao, Y. (2013). Dietary exposure of Hong Kong adults to fatty acid esters of 3-monochloropropane-1,2-diol. *Food Additives & Contaminants: Part A, 30*(9), 1508–1512.

Destaillats, F., Craft, B. D., Sandoz, L., & Nagy, K. (2012). Formation mechanisms of monochloropropanediol (MCPD) fatty acid diesters in refined

ACCEPTED MANUSCRIPT

palm (Elaeis guineensis) oil and related fractions. *Food Additives & Contaminants: Part A*, *29*(1), 29-3

Divinova, V., Dolezal, M. and Velisek, J. (2007). Free and Bound 3-Chloropropane- 1,2-diol in coffee surrogates and malts. *Czech Journal of Food Sciences, 25*(1), 39–47.

Dubois, M., Tarres, A., Goldmann, T., Empl, A.M., Donaubauer, A., Seefelder, W. (2012). Comparison of indirect and direct quantification of esters of monochloropropanediol in vegetable oil. *Journal of Chromatography A*. *1236*, 189–201.

EFSA, 2013. European Food Safety Authority (EFSA) (2013). Analysis of occurrence of 3-monochloropropane-1,2-diol (3-MCPD) in food in Europe in the years 2009-2011 and preliminary exposure assessment. *EFSA Journal 11*(9), 3381, 45 pp. Available on line: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3381/epdf Accessed 2018 January 10.

EFSA, 2016. EFSA Panel on Contaminants in the Food Chain (CONTAM) (2016). Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. *EFSA Journal 14*(5), 4426, 159 pp.

Ermacora, A., & Hrnčiřík, K. (2014). Development of an analytical method for the simultaneous analysis of MCPD esters and glycidyl esters in oil-based foodstuffs. *Food Additives & Contaminants: Part A*, *31*(6), 985-994.

FAO/WHO, 2016. Summary report of the eighty-third meeting of JECFA. Food and Agricultural Organization/World Health Organization. Available online: http://www.fao.org/3/abq821e.pdf Accessed 2018 January 10.

FDA, 2015. U.S. Department of Health and Human Services Food and Drug Administration, Guidelines for the Validation of Chemical Methods for the FDA FVM Program 2nd Edition US Food & Drug Administration Office of Foods and Veterinary Medicine 2015. Available on line:https://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf Accessed 2018 January 14.

ACCEPTED MANUSCRIPT

Graziani,G., Gaspari,A., Chianese, D., Conte, L., Ritieni, A. (2017) Direct determination of 3-chloropropanol esters in edible vegetable oils using high resolution mass spectrometry (HRMS-Orbitrap), *Food Additives & Contaminants: Part A, 34* (11),1893-1903.

Hernandez, E. M., & Kamal-Eldin, A. (2013). *Processing and nutrition of fats and oils*. Chicago, USA: Wiley-Blackwell.

Hori, K., Koriyama, N., Omori, H., Kuriyama, M., Arishima, T., & Tsumura, K. (2012). Simultaneous determination of 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils using liquid chromatography time-of-flight mass spectrometry. *LWT-Food Science and Technology*, *48*(2), 204-208.

IARC, 2014. International Agency for Research on Cancer (IARC). Agents classified by the IARC Monographs, Volumes 1–109. Available online: http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf Accessed 2017 December 1

ISO 11843-2, 2000. International Organization for Standardization. Capability of detection. Part 2: Methodology in the linear calibration case. Geneva, Switzerland.

Jędrkiewicz, R., Głowacz, A., Kupska, M.,Gromadzka J., & Namieśnik J.(2014). Application of modern sample preparation techniques to the determination of chloropropanols in food samples. *Trends in Analytical Chemistry 62*, 173–183

Jędrkiewicz, R., Kupska, M., Głowacz, A., Gromadzka J., & Namieśnik J. (2016a). 3-MCPD: A Worldwide Problem of Food Chemistry. Critical Reviews in Food Science & Nutrition, 56(14), 2268-2277.

Jędrkiewicz, R., Orłowski, A., Namieśnik, J., Tobiszewski, M. (2016b). Green analytical chemistry introduction to chloropropanols determination at no economic and analytical performance costs? *Talanta 147*, 282–288.

Jędrkiewicz, R., Głowacz, A., Gromadzka, A., Kloskowski, J., Namieśnik, J. (2016c). Indirect determination of MCPD fatty acid esters in lipid fractions of commercially available infant formulas for the assessment of infants' health risk. Food Analytical Methods, 9(12), 3460-3469.

ACCEPTED MANUSCRIPT

Jędrkiewicz, R., Głowacz, A., Gromadzka, J., Namieśnik, J. (2016d). Determination of 3-MCPD and 2-MCPD esters in edible oils, fish oils and lipid fractions of margarines available on Polish market. *Food Control, 59*, 487-492.

Jędrkiewicz, R., Głowacz, A., Gromadzka, A., Kloskowski, J., Namieśnik, J. (2017). Novel fast analytical method for indirect determination of MCPD fatty acid esters in edible oils and fats based on simultaneous extraction and derivatization. *Analytical and Bioanalytical Chemistry, 409*(17), 4267–4278.

Leigh, J., MacMahon, S. (2017). Occurrence of 3-monochloropropanediol esters and glycidyl esters in commercial infant formulas in the United States. *Food Additives & Contaminants: Part A, 34*(3), 356-370.

Li, C., Nie, S-P., Zhou, Y-Q., Xie, M-Y. (2015). Exposure assessment of 3-monochloropropane-1, 2-diol esters from edible oils and fats in China. *Food & Chemical Toxicology, 75*, 8–13.

MacMahon, S. Begley, T.H., Diachenko, G.W. (2013a). Occurrence of 3-MCPD and glycidyl esters in edible oils in the United States. *Food Additives & Contaminants: Part A, 30*(12), 2081-2092.

MacMahon, S., Begley, T. H., & Diachenko, G. W. (2013b). Analysis of processing contaminants in edible oils. Part 2. Liquid chromatography–tandem mass spectrometry method for the direct detection of 3-monochloropropanediol and 2-monochloropropanediol diesters. *Journal of Agricultural and Food Chemistry*, *61*(20), 4748-4757.

MacMahon, S. (Ed.). (2015). *Processing contaminants in edible oils: MCPD and glycidyl esters*. Urbana, IL: AOCS Press.

Matthäus, B., & Pudel, F. (2013). Mitigation of 3-MCPD and glycidyl esters within the production chain of vegetable oils especially palm oil. *Lipid Technology, 25*(7), 151–155.

Merkle, S., Ostermeyer, U., Rohn, S., Karl, H., Fritsche, J. (2018). Mitigation strategies for ester bound 2-3-MCPD and esterified glycidol in pre-fried breaded and frozen fish products. *Food Chemistry 245*, 196–204.

ACCEPTED MANUSCRIPT

Moravcova, E., Vaclavik, L., Lacina, O., Hrbek, V., Riddellova, K., Hajslova, J. (2012). Novel approaches to analysis of 3-chloropropane-1,2-diol esters in vegetable oils. *Analytical and Bioanalytical Chemistry, 402*, 2871–2888.

Racamonde, I., González, P., Lorenzo, R. A., Carro, A. M. (2011). Determination of chloropropanols in foods by one-step extraction and derivatization using pressurized liquid extraction and gas chromatography-mass spectrometry. *Journal of Chromatography A, 1218*(39), 6878–6883.

Weißhaar, R. (2011). Analysis and occurrence of dichloropropanol fatty acid esters and related process-induced contaminants in edible oils and fats. *European Journal of Lipid Science and Technology, 113*(3), 304–308.

Xu, X-M., He, H-L., Zhu, Y., Feng, L., Ying,Y., Huang, B-F., Shen, H-T., Han, J-L., Ren, Y-P. (2013). Simultaneous determination of 3-monochloropropane-1,2-diol and acrylamide in food by gas chromatography-triple quadrupole mass spectrometry with coupled column separation. *Analytica Chimica Acta*, *760*, 93–99.

Yamazaki, K., Ogiso, M., Isagawa, S., Urushiyama, T., Ukena, T., & Kibune, N. (2013). A new, direct analytical method using LC-MS/MS for fatty acid esters of 3-chloro-1, 2-propanediol (3-MCPD esters) in edible oils. *Food Additives & Contaminants: Part A*, *30*(1), 52-68.

Yan, J., Oeya, S.B., van Leeuwena, S.P.J., van Ruth, S.M. (2018). Discrimination of processing grades of olive oil and other vegetable oils by monochloropropanediol esters and glycidyl esters. *Food Chemistry 248,* 93–100.

Zelinková, Z., Doležal, M., Velíšek, J. (2009a). Occurrence of 3-chloropropane-1, 2-diol fatty acid esters in infant and baby foods. *European Food Research and Technology, 228*(4), 571-578.

Zelinková, Z., Doležal, M., Velíšek, J. (2009b). 3-chloropropane-1,2-diol fatty acid esters in potato products. *Czech. Journal Food Science 27*, 421–424

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Fig. 1. Flow diagram of 3-MCPD diesters extraction in vegetable oils.

Fig. 2. Box plots of the seven 3-MCPD diesters analyzed in four categories of oils (a) olive oil, (b) seed oil (c) pulp oil and (d) oil-based foodstuffs. Concentration expressed as mg Kg^{-1}.

Fig.3. MRM chromatograms of olive pomace oil 7 sample from Table 2 in which LILI, PAOL and OLOL were quantified.

Fig. 4. Box plot of 3-MCPD equivalents (mg kg^{-1}) determined in categorized samples: Refined Olive Oil 1º (ROO 1º), Refined Olive Oil 0.4 º (ROO 0.4º), Olive Pomace Oil (OPO), Refined Sunflower Oil (RSO), Other Seed Oil (OSO), Pulp Oil (PO), Margarine (MAR) and Potato Chips (PC).

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Table 1. Quality parameters: CCα, CCβ (for α = β = 0.05) and inter-day

COMPOUND	CCα	CCβ	OLIVE OIL (%RSD) (n=16)			SEED OIL (%RSD) (n=16)			PULP OIL (%RSD (n=6)	
	µg kg^{-1}	µg kg^{-1}	Level (µg kg^{-1})			Level (µg kg^{-1})			Level (µg kg^{-1})	
			100	500	1000	100	500	1000	100	500
LILI	9.4	17.7	1.4	0.9	1.7	1.7	1.4	1.7	10.1	5.2
OLLI	14.7	27.8	2.8	3.3	2.9	2.1	1.7	1.6	2.5	6.4
OLOL	9.5	18.0	2.8	2.9	3.8	2.5	1.5	1.8	1.8	3.9
OLST	20.1	37.9	2.7	2.7	2.6	1.7	1.8	1.9	6.0	3.3
PALI	17.1	32.3	1.0	3.0	3.2	1.7	1.7	1.6	4.3	3.2
PAOL	13.1	24.7	2.2	2.7	1.9	1.1	1.6	1.1	3.2	3.7
PAPA	9.6	18.2	2.7	2.5	2.4	1.4	1.5	1.9	5.1	4.3

precision for 3-MCPD diesters in different oil matrices

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Table 2. Occurrence of 3-MCPD diesters (Average concentration ± standard deviation) and 3-MCPD equivalent obtained in olive oils by LC-MS/MS (mg Kg^{-1})

OIL	LILI	OLLI	OLOL	OLST	PALI	PAOL	PAPA	3-M
irgin olive oil 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
irgin olive oil 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
irgin olive oil 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
irgin olive oil 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
irgin olive oil 5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
irgin olive oil 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
irgin olive oil 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
d olive oil 1º 1	1.30±0.02	n.d.	2.13±0.01	n.d.	n.d.	1.24±0.01	n.d.	0.8
d olive oil 1º 2	1.38±0.01	n.d.	1.87±0.02	n.d.	n.d.	1.36±0.01	n.d.	0.8
d olive oil 1º 3	1.34±0.01	n.d.	2.09±0.07	n.d.	n.d.	1.36±0.01	n.d.	0.8
d olive oil 1º 4	1.30±0.01	n.d.	2.06±0.06	n.d.	n.d.	1.35±0.01	n.d.	0.8
d olive oil 1º 5	1.56±0.23	n.d.	2.48±0.16	n.d.	n.d.	1.42±0.02	n.d.	0.9
d olive oil 1º 6	1.34±0.01	n.d.	2.19±0.09	n.d.	n.d.	1.33±0.01	n.d.	0.8
d olive oil 1º 7	1.47±0.22	n.d.	3.48±0.02	n.d.	n.d.	1.41±0.01	n.d.	1.1
d olive oil 1º 8	1.34±0.01	n.d.	1.76±0.06	n.d.	n.d.	1.33±0.02	n.d.	0.7
d olive oil 1º 9	n.d.	n.d.	3.42±0.05	n.d.	n.d.	n.d.	n.d.	0.5
d olive oil 1º 10	n.d.	n.d.	3.27±0.03	n.d.	n.d.	1.32±0.01	n.d.	0.8
d olive oil 1º 11	n.d.	n.d.	2.82±0.06	n.d.	n.d.	n.d.	n.d.	0.4
d olive oil 1º 12	n.d.	n.d.	1.69±0.30	n.d.	n.d.	1.34±0.01	n.d.	0.5
d olive oil 0.4º 1	1.38±0.01	n.d.	2.24±0.21	n.d.	n.d.	1.35±0.01	n.d.	0.8
d olive oil 0.4º 2	1.34±0.01	n.d.	1.83±0.07	n.d.	n.d.	1.34±0.01	n.d.	0.7
d olive oil 0.4º 3	1.54±0.01	n.d.	2.14±0.02	n.d.	n.d.	1.35±0.01	n.d.	0.8
d olive oil 0.4º 4	1.49±0.01	n.d.	2.34±0.05	n.d.	n.d.	1.30±0.01	n.d.	0.9
d olive oil 0.4º 5	1.62±0.01	n.d.	3.36±0.02	n.d.	n.d.	1.47±0.01	n.d.	1.1
d olive oil 0.4º 6	1.38±0.01	n.d.	4.23±0.04	n.d.	n.d.	1.38±0.02	n.d.	1.2
d olive oil 0.4º 7	1.52±0.01	n.d.	3.16±0.05	n.d.	n.d.	1.40±0.02	n.d.	1.0
d olive oil 0.4º 8	1.87±0.01	n.d.	3.70±0.27	n.d.	n.d.	1.31±0.01	n.d.	1.2
d olive oil 0.4º 9	1.43±0.01	n.d.	2.06±0.07	n.d.	n.d.	1.31±0.01	n.d.	0.8
d olive oil 0.4º 10	1.29±0.01	n.d.	1.96±0.06	n.d.	n.d.	1.28±0.01	n.d.	0.7
d olive oil 0.4º 11	n.d.	n.d.	3.91±0.02	n.d.	n.d.	1.29±0.32	n.d.	0.9
d olive oil 0.4º 12	n.d.	n.d.	5.60±010	n.d.	n.d.	n.d.	n.d.	0.9
d olive oil 0.4º 13	n.d.	n.d.	3.34±0.02	n.d.	n.d.	1.46±0.18	n.d.	0.8
d olive oil 0.4º 14	n.d.	n.d.	3.76±0.03	n.d.	n.d.	1.39±0.06	n.d.	0.9
d olive oil 0.4º 15	1.32±0.01	n.d.	2.70±0.03	n.d.	n.d.	1.62±0.34	n.d.	0.9
omace oil 1	n.d.	n.d.	7.54±0.05	n.d.	n.d.	1.81±0.43	n.d.	1.6
omace oil 2	n.d.	n.d.	4.76±0.10	n.d.	n.d.	1.56±0.10	n.d.	1.1
omace oil 3	n.d.	n.d.	3.07±0.04	n.d.	n.d.	0.59±0.02	n.d.	0.6
omace oil 4	n.d.	n.d.	4.85±0.01	n.d.	n.d.	1.13±0.05	n.d.	1.0
omace oil 5	1.66±0.08	n.d.	4.36±0.10	n.d.	n.d.	1.36±0.01	n.d.	1.2
omace oil 6	1.72±0.03	n.d.	7.41±0.09	n.d.	n.d.	1.58±0.01	n.d.	1.8
omace oil 7	1.96±0.04	n.d.	10.89±0.45	n.d.	n.d.	1.68±0.02	n.d.	2.5
VO oil 1	n.d.	n.d.	n.d.	n.d.	n.d.	0.74±0.03	n.d.	0.1
VO oil 2	n.d.	n.d.	7.42±0.48	n.d.	n.d.	2.44±0.25	n.d.	1.7

Analysis in triplicate; n.d. not detected.

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Highlights

- Evaluation of presence and exposure of processing contaminants to oil consumers
- Seven 3-MCPD diesters determined in one hundred EVOO and other edible oils samples
- High content of 3-MCPD diesters was detected in pomace and refined olive oils
- Potato chips daily intake should be limited to reduce the health risk

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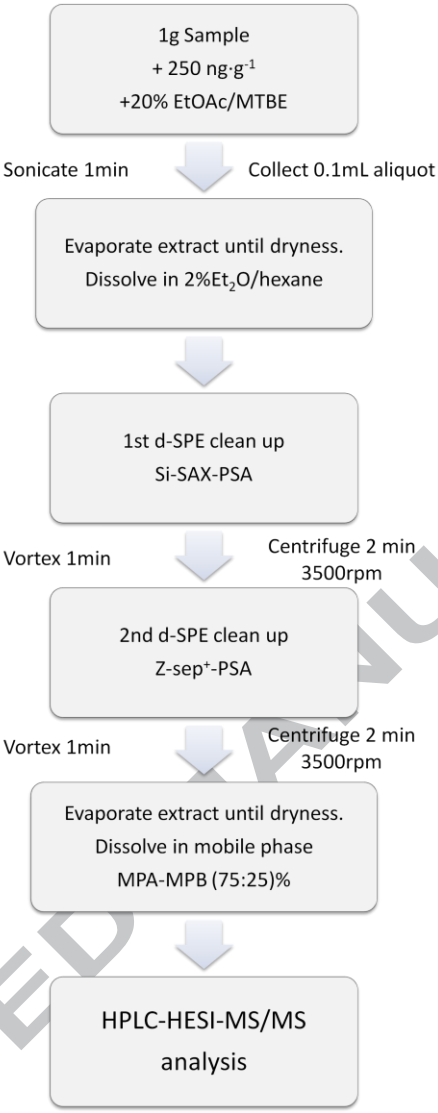

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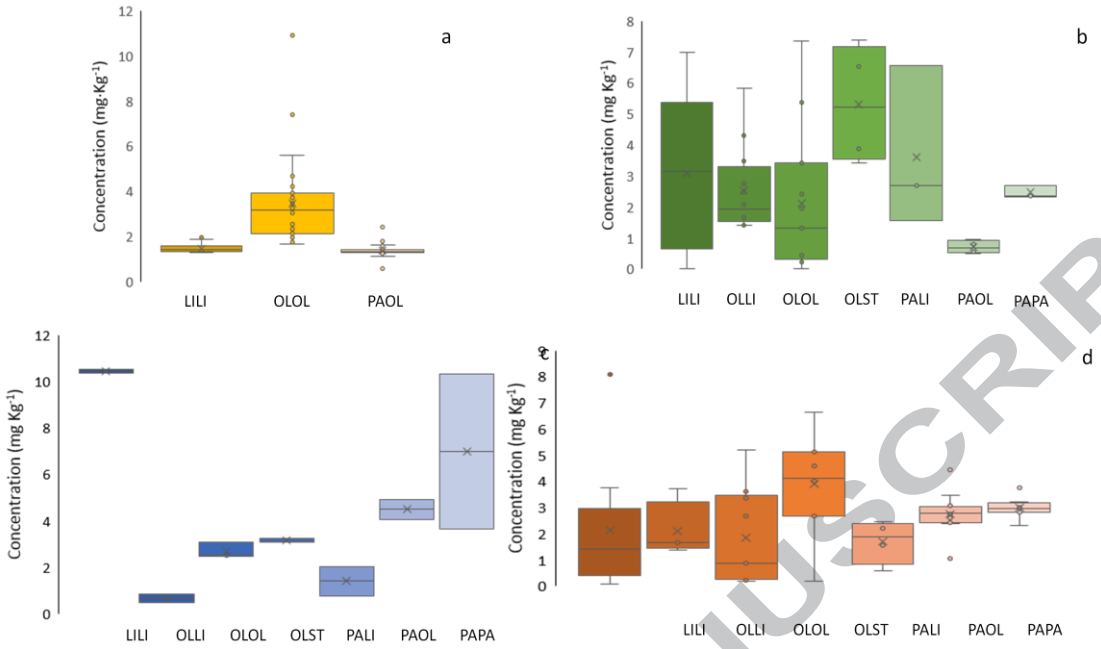

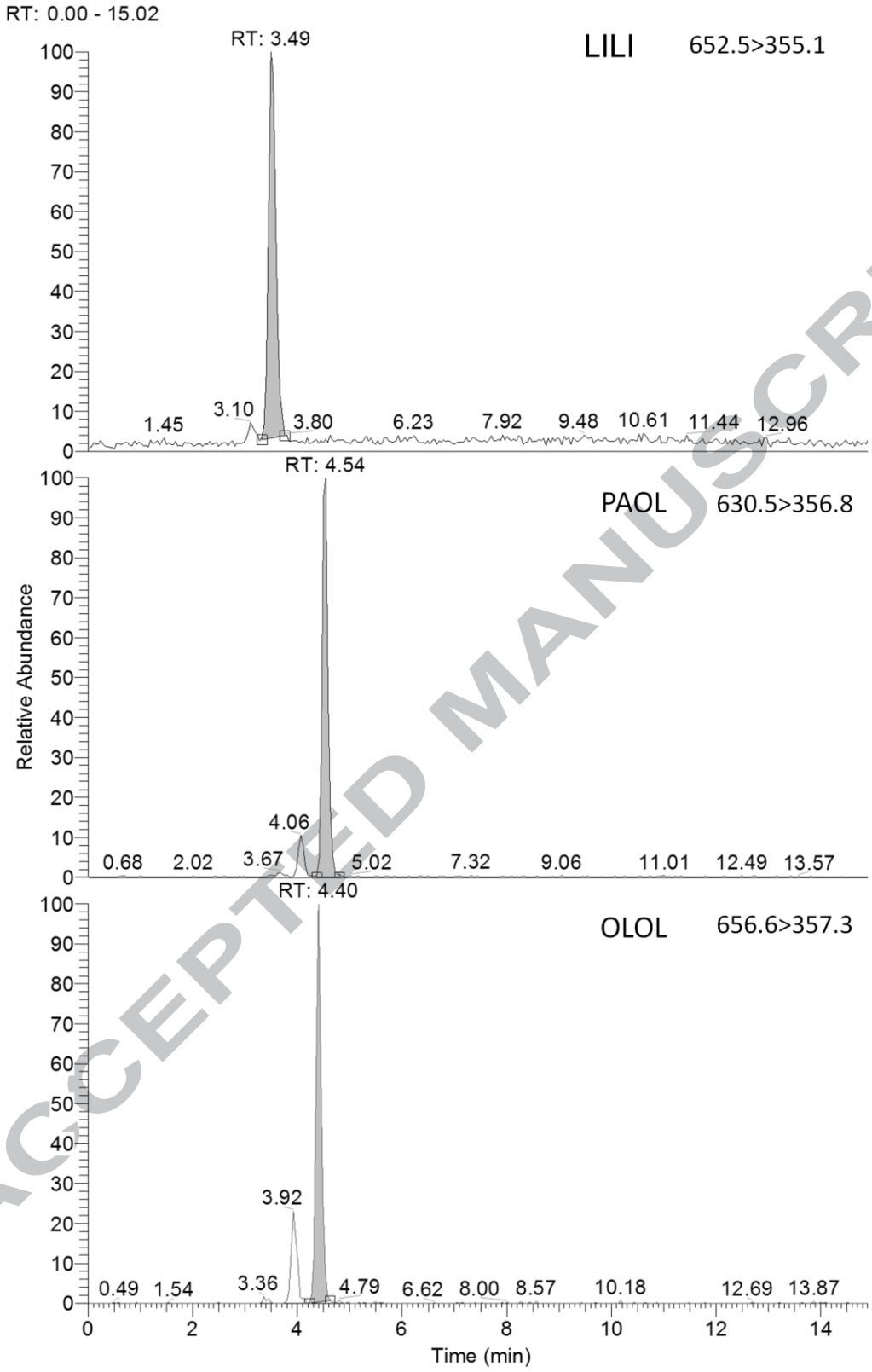

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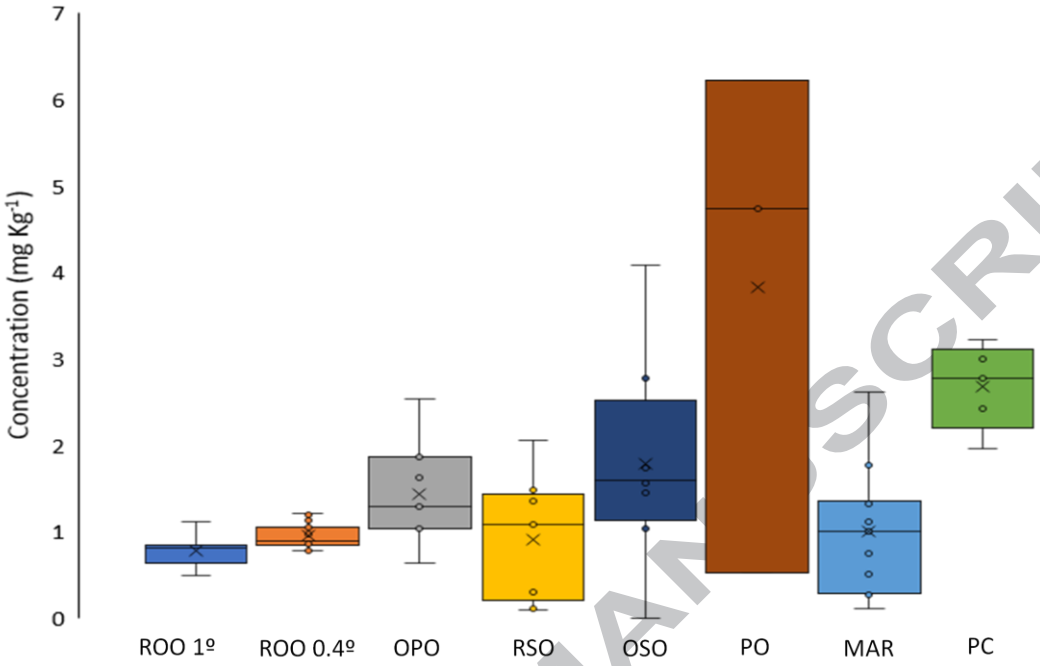

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