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ARTICLE



Direct determination of 3-chloropropanol esters in edible vegetable oils using high resolution mass spectrometry (HRMS-Orbitrap)

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ABSTRACT

A series of refined edible oils derived from mixed seeds, peanuts, corn, sunflower and palm obtained from the local supermarket were analyzed for their content of 3-MCPD esters. A direct analytical method for the determination of 3-monochloropropane-1,2-diol esters (3-MCPD esters) was applied to investigate the major MCPD esters found in common edible oils; in particular seven types of monoesters and eleven types of diesters were detected. The limits of detection (LODs) for monoesters and diesters of 3-MCPD were in the range of 0.079–12.678 $\mu\text{g kg}^{-1}$ and 0.033–18.610 $\mu\text{g kg}^{-1}$ in edible oils, and the ranges of limits of quantitation (LOQs) were 0.979–38.035 $\mu\text{g kg}^{-1}$ and 0.100–55 $\mu\text{g kg}^{-1}$, respectively. The recoveries of 3-MCPD esters from oil samples were in the range of 80–100%, with RSD ranging between 1.9 and 11.8%. The concentration levels of total 3-MCPD diesters in vegetable oil samples were in the range from 0.106 up to 3.444 $\mu\text{g g}^{-1}$ whereas total monoesters ranged from 0.005 up to 1.606 $\mu\text{g g}^{-1}$.

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Introduction

Fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropanediol (2-MCPD) represent an important group of food processing contaminants that can be formed during the oil refining process especially during the deodorisation step. Deodorisation is a water steam distillation of volatile substances, in which a superheated water steam (180–260°C) is introduced under lowered pressure (300–2000 Pa) into vegetable oil (Šmidrkal et al. 2016). Vegetable oil contains, before deodorisation, triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids and chloride compounds (chlorides, hydrogen chloride) or organic chloride compounds. Chlorides come from parts of plants (pulp in cases of palm and olive) from which the oil was pressed and extracted; the free hydrogen chloride comes from bleaching earth, by which the oil was bleached (Collison 2010). The acylglycerols react with chlorine released from organic compounds naturally present in the oil, levels of which may be elevated by the uptake of chloride from inorganic fertilisers added to soil (Nagy et al. 2011). The quantity of available chlorine is the limiting factor for MCPD fatty acid ester formation during the

oil refining process (Ermacora and Hrcirik 2014). The major esterifying acids depend on the type of oil but the most common fatty acids are palmitic acid (hexadecanoic acid C16:0), stearic acid (octadecanoic acid C18:0), oleic acid (octadecenoic acid C18:1), linoleic acid (octadecadienoic acid C18:2) and linolenic acid (octadecatrienoic acid C18:3) (Ai et al. 2014; Orsavova et al. 2015). 3-MCPD and 2-MCPD can each form monoesters and diesters, and in the case of diesters positional isomers exist in which the two hydroxyl groups are esterified with different acids. The esters are formed in a similar ratio to that of the acids in the parent oil, although some factors such as volatility and deodorisation conditions can cause small differences.

The possible hydrolysis of 3-MCPD esters by enzymes in the human gut microbiota releases free 3-MCPD (Abraham et al. 2013; Seefelder et al. 2008), a compound that has been related with nephrotoxicity and the ability to affect male fertility (JEFCA 2002).

However, 3-MCPD has been classified as a possible human carcinogen (group 2B) in view of its potential to induce cancer in *in vivo* experiments (IARC 2012).

Thus, bearing in mind its potential toxicity, persistent nature and cumulative behaviour as well as the lunge consumption of refined vegetable oils, the development of rapid methods to analyze 3-MCPD related compounds is needed. In literature both direct and indirect analytical approaches coupled with chromatographic techniques and mass spectrometry measurements have been reported (Zelincova et al. 2006; Haines et al. 2011; Kuhlmann 2011; Ermacora and Hrnčirik 2012; Hori et al. 2012; MacMahon et al. 2013a, b; Li et al. 2015). Indirect methods are based either on acidic or alkaline transesterification reactions in order to release free chloropropanediol from the esterified form. After release, the free 3-MCPD is purified, derivatised and quantified using gas chromatography-mass spectrometry.

On the other hand, direct methods rely on each single fatty acid ester determination (including both monoesters and diesters) and final quantification by liquid chromatography-time of flight mass spectrometry (LC-MS) or liquid chromatography-time-of-flight mass spectrometry (LC-TOFMS). It requires quite easy sample clean-up (based usually on the solid phase extraction [SPE] technique) and enables complete information to be obtained about sample composition through LC-MS determination (Crews et al. 2013).

In response to the lack of reliability of indirect methodology, direct methods have been developed for 3-MCPD ester analysis. Direct methods allow a clear pattern of the 3-MCPD esters to be obtained, which gives useful information for establishing the toxicity of these compounds since the effects of fatty acid substitution, chain length and degree of unsaturation of 3-MCPD fatty acid esters on their overall toxicity are well known. Moreover, the sum of the limits of detection (LODs) for each ester in a direct method produces higher 3-MCPD detection limits than indirect methodology (Li et al. 2015).

Various approaches towards the mitigation of 3-MCPD esters in refined vegetable oils have been proposed and tested. These include prevention of their formation through careful selection of raw materials and modification of the refining conditions, or their elimination from fully refined oils and fats by applying various post-treatment technologies (Matthaus et al. 2011; Ramli et al. 2011; Strijowski et al. 2011; Zulkurnain et al. 2013).

Keeping in view of the potential toxicity, persistent nature and cumulative behaviour as well as the

consumption of refined vegetable oils, it is necessary to test and analyze these oils to ensure that the levels of 3-MCPD esters meet the agreed international requirements. Therefore, the aim of this study was to develop a direct method to determine 3-MCPD esters in edible vegetable oils by HRMS-Orbitrap. The developed method was carefully validated according to European commission decision 2002/657/EC and applied to 35 commercially available Italian oil samples.

The results obtained provide useful knowledge about the determination of these contaminants in oils, improving performance parameters for direct investigation such as linearity, limit of quantitation (LOQ) and reproducibility.

Materials and methods

Chemicals and reagents

Acetonitrile, 2-propanol and methanol (LC/MS grade) were obtained from Carlo Erba Reagents S.r.l. (Cornaredo, MI, Italy). Ammonium formate (LC/MS grade) was purchased from Sigma-Aldrich S.r.l. (Milano, Italy), formic acid (HPLC grade) was purchased from VWR International PBI S.r.l. (Milano, Italy). Ultra-pure water (LC/MS grade) was obtained from Merck KGaA (Darmstadt, Germany). PSA powder (50 μm particle size; 70 Å pore size) and C18 powder (50 μm particle size; 70 Å pore size) were purchased from Supelco by Sigma-Aldrich S.r.l. (Bellefonte, USA). The list of 3-MCPD ester compounds determined in this study is shown in Table 1. 3-MCPD ester standards, including rac 1-Lauroyl-3-chloropropanediol (1-LA), rac 1-Myristoyl-3-chloropropanediol (1-MY), rac 1-Linolenoyl-3-chloropropanediol (1-LN), rac-1-Linoleoyl-3-chloropropanediol (1-LI), rac-1-Oleoyl-3-chloropropanediol (1-OL), rac-1-Palmitoyl-3-chloropropanediol (1-PA), rac-1-Stearoyl-3-chloropropanediol (1-ST), rac-3-chloro-1,2-propanediol dilinoleate (LI-LI), rac-1-palmitoyl-2-linoleoyl-3-chloropropanediol (PA-LI), rac-1-oleoyl-2-linoleoyl-3-chloropropanediol (OL-LI), rac-1-oleoyl-2-linolenoyl-3-chloropropanediol (OL-LN), 1,2-Bis-palmitoyl-3-chloropropanediol (PA-PA), 1,2-Bis-oleoyl-3-chloropropanediol (OL-OL) were acquired from Toronto Research Chemicals (Toronto, Canada). Individual standard stock solutions of 3-MCPD esters were prepared at 25 mg/mL in ethyl

Table 1. Limit of detection (LOD) and limit of quantitation (LOQ) expressed as 3-MCPD equivalents (ppb) for 3-MCPD esters in the different oils analyzed.

Sample oil 3-MCPD ester	Peanut oil		Sunflower oil		Corn oil		Mixed seed oil		Palm oil	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
1-LA	1.539	4.618	12.678	38.035	6.430	19.291	0.720	2.161	3.490	10.469
1-LN	1.215	3.645	5.464	16.393	5.075	15.224	0.568	1.705	9.268	8.262
1-MY	1.677	5.030	1.013	3.040	1.109	3.326	3.301	9.903	5.445	16.334
1-LI	2.266	6.797	1.057	3.173	3.545	10.635	0.784	2.353	0.330	0.990
1-PA	3.150	9.450	1.119	3.357	3.257	9.771	0.789	2.367	0.356	1.068
1-OL	3.009	9.028	5.124	15.373	1.917	5.751	0.812	2.437	0.079	0.238
1-ST	2.126	6.378	0.996	2.988	3.788	11.364	0.850	2.55	0.350	1.050
LN-LN	0.198	0.593	4.787	14.361	0.034	0.101	0.404	1.212	0.739	2.218
LI-LI	0.196	0.589	4.757	14.270	0.033	0.100	0.402	1.205	0.735	2.204
OL-LN	0.185	0.555	17.986	53.958	3.278	9.834	2.986	8.958	1.254	3.762
PA-LI	0.228	0.683	0.272	0.816	2.251	6.753	2.953	8.860	0.837	2.510
OL-LI	0.196	0.589	1.856	5.566	3.167	9.500	3.019	9.056	1.110	3.329
PA-PA	0.250	0.750	0.267	0.801	2.214	6.642	3.005	9.015	0.799	2.397
OL-OL	0.187	0.561	2.015	6.045	3.687	11.061	2.841	8.523	1.214	3.642
LI-ST	0.218	0.653	0.260	0.780	2.152	6.457	2.824	8.471	0.800	2.400
PA-ST	0.226	0.679	0.270	0.811	2.236	6.709	2.934	8.802	0.831	2.493
OL-ST	0.195	0.586	1.844	5.531	3.147	9.441	3.000	8.999	1.103	3.308
ST-ST	0.216	0.649	0.258	0.775	2.139	6.416	2.806	8.418	0.795	2.385

acetate and stored at -20°C . The mixed standard stock solutions of 13 compounds (1 mg/mL for 1-LA, 1-MY, 1-LI, 1-LN, 1-OL, 1-PA, 1-ST, LI-LI, PA-LI, OL-LI, PA-PA and OL-OL) were prepared by appropriate dilution of individual stock solutions with methanol and used to build calibration curves for quantitative analysis of respective compounds. Furthermore, as no standards were available, semiquantitative analysis for 3-MCPD diesters LN-LN, OL-LN, LI-ST, PA-ST, OL-ST and ST-ST was performed using calibration curves obtained from LI-LI, OL-LI, PA-LI, PA-LI, OL-LI and PA-LI for diesters.

Oil samples

A total of 35 oil samples, including crude palm oil ($n = 1$), refined palm ($n = 1$) oil and various refined vegetable oils such as peanut oil ($n = 8$), corn oil ($n = 9$), sunflower oil ($n = 6$) and mixed seed oil ($n = 10$), were purchased from local supermarkets. We used one sample for each oil class as a matrix blank and for the preparation of blank spiked samples. All samples were analyzed in duplicate.

3-MCPD extraction

An aliquot of oil sample (15 mg) was accurately weighed into a 15 mL centrifuge tube, 3 mL of acetonitrile-2-propanol (1:1, v/v) were added and thoroughly vortexed for 30 s, then a mixture of 75 mg of PSA powder and 75 mg of C18 powder

were added. The mixture was thoroughly vortexed for 3 min and centrifuged (6000 rpm for 10 min). The supernatant was recovered, evaporated under nitrogen flow until dry. The dry extract was then resuspended with 200 μL of acetonitrile-2-propanol (1:1 v/v) and injected for UHPLC-MS/MS analysis (Li et al. 2015).

Method validation

The proposed method was validated following the guidelines of the European Commission (2002/657/EC) in terms of selectivity, matrix effects, linearity, LOD and LOQ, precision and accuracy. Linearity was evaluated using the calibration curve of each standard used at five concentration levels from 0.005, 0.020, 0.100, 0.500 and 1 $\mu\text{g g}^{-1}$. The accuracy was evaluated through recovery studies and calculated as follows: $[(\text{mean observed concentration}) / (\text{added concentration})] \times 100$. Recovery studies were conducted at 5 spiking levels for each oil category. The spiked samples were then extracted and analyzed as described above. Intraday precision (repeatability) was assessed by calculating the relative standard deviation (RSD_r), calculated from results generated under repeatability conditions of three determinations for concentration in a single day. Interday precision was calculated by the relative standard deviation (RSD_R) calculated from results generated under reproducibility conditions by one determination per concentration on three different

days. Sensitivity was evaluated by limit of detection (LOD) and limit of quantitation (LOQ) values. LOQ and LOD were defined as the concentration at which the S/N of the analyte is equal to 10 and 3, respectively. Five replicates were carried out for determination of LOD, LOQ, RSD_r and RSD_R .

High resolution mass spectrometry (HRMS-Orbitrap) analysis

UHPLC chromatographic analysis

Qualitative and quantitative profiles of 3-MCPD esters have been obtained using Ultra High Pressure Liquid Chromatograph (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a degassing system, a Dionex Ultimate 3000 a Quaternary UHPLC pump working at 1250 bar, an auto sampler device and a thermostated column compartment ($T = 40\text{ }^\circ\text{C}$) with a Gemini 3 μm ($100 \times 2.1\text{ mm}$) column (Phenomenex, Torrance, CA, USA). The injection volume was 10 μL . The eluent phase was formed as follows: phase A consisted of a mixture of 0.05% of formic acid and 2 mM ammonium formate in methanol/water (98:2, $v:v$), and the mobile phase B was composed of 2-propanol/water (98:2, $v:v$) with 0.05% formic acid and 2 mM ammonium formate. All 3-MCPD esters have been eluted using a 0.2 mL/min flow rate with a gradient programmed as follows: 0 to 5 min –0% of phase B, 3 min –15% of phase B, 10 min –25% of phase B, 15 min –30% of phase B, 20 min –50% of phase B, 30 min –83% of phase B, 31 min –0% of phase B and it stays at this condition for the next 9 min for equilibration of the column.

Orbitrap Q Exactive mass spectrometry analysis

For the mass spectrometry analysis a Q Exactive Orbitrap LC-MS/MS (Thermo Fisher Scientific, Waltham, MA, USA) was applied. An ESI source (HESI II, Thermo Fisher Scientific, Waltham, MA, USA) was operated in positive ion mode (ESI+) for all the analyzed compounds. Ion source parameters were: spray voltage +4.0 kV, sheath gas ($\text{N}_2 > 95\%$) 40, auxiliary gas ($\text{N}_2 > 95\%$) 15, capillary temperature 310 $^\circ\text{C}$, S-lens RF level 50, auxiliary gas heater temperature 305 $^\circ\text{C}$. All compounds were analyzed using HRMS-Orbitrap in Target SIM mode (Target Single Ion Monitoring) with the automatic gain control (AGC) target set at 5×10^5 , with a resolution of

35,000 FWHM (full width at half maximum), the msx count (maximum number of precursors to be multiplexing) set to 10 and the isolation window set to 2.0 m/z. The accuracy and calibration of the Q Exactive Orbitrap LC-MS/MS was checked weekly using a reference standard mixture obtained from Thermo Fisher Scientific. Data analysis and processing have been performed using the Xcalibur software v. 3.1.66.10 (Xcalibur, Thermo Fisher Scientific).

Results and discussion

Considering the possible risk of human exposure to 3-MCPD esters, the toxicological and chemical characterisation of these compounds has attracted more and more attention in recent years. In the present study, selected commercial vegetable oils available on the Italian market (35 samples) were analyzed in relation to 3-monochloropropane-1,2-diol (3-MCPD) ester content using a LC-MS spectrometry method on a high resolution mass spectrometer (HRMS-Orbitrap). In particular, 3-MCPD monoesters and diesters with the most abundant fatty acids in oils, such as linolenic, linoleic, palmitic, oleic and stearic were considered to characterise the profile of 3-MCPD fatty acid esters in oils as much as possible. To evaluate the performance of the method used for the analytical determination of 3-MCPD esters all oil matrices considered in the experimental plan were spiked, in duplicate, with different levels of each standard: $0.005\text{ }\mu\text{g g}^{-1}$, $0.02\text{ }\mu\text{g g}^{-1}$, $0.1\text{ }\mu\text{g g}^{-1}$, $0.5\text{ }\mu\text{g g}^{-1}$ and $1\text{ }\mu\text{g g}^{-1}$. The LODs and LOQs were determined by analysing the spiked oil samples and when the signal-to-noise ratio reached 3 and 10, respectively, the spiking concentration for that analyte was used to determine the LOD and LOQ, respectively. These values were then multiplied with the dilution factor of 20 and corrected for the recovery of the extraction step of the respective matrix. In [Table 1](#) reported LODs and LOQs were expressed as 3-MCPD equivalents (ng/g) for each compound and oil category. LOD and LOQ values for diesters LN-LN, OL-LN, LI-ST, PA-ST, OL-ST and ST-ST were performed using calibration curves obtained from LI-LI, OL-LI, PA-LI, PA-LI, OL-LI and PA-LI, respectively.

The coefficients of 3-MCPD ester converted to free 3-MCPD were calculated from the value derived from the molecular ratio of individual esters (1-LA: 0.376; 1-LN: 0.297; 1-MY: 0.343; 1-LI: 0.296; 1-PA:

0.316; 1-OL: 0.294; 1-ST: 0.292; LN-LN: 0.175; LI-LI: 0.173; OL-LN: 0.173; PA-LI: 0.180; OL-LI: 0.173; PA-PA: 0.188; OL-OL: 0.172; LI-ST: 0.172; PA-ST: 0.179; OL-ST: 0.172; ST-ST: 0.171) according to Li et al. (2015).

The LODs for monoesters and diesters of 3-MCPD, in the different types of oils analyzed, were in the range of 0.079–12.678 $\mu\text{g kg}^{-1}$ and 0.033–18.610 $\mu\text{g kg}^{-1}$ in edible oils, and the ranges of LOQs were 0.979–38.035 $\mu\text{g kg}^{-1}$ and 0.100–55 $\mu\text{g kg}^{-1}$, respectively. Previous literature data (Li et al. 2015) reported LODs and LOQs for 3-MCPD esters, measured in extra virgin olive oil, considered as blank samples; alternatively, the values of LODs and LOQs were obtained by the use of pure solvent not considering the type of oil analyzed (Yamazaki et al. 2013). The comparison of the results obtained in this study showed that LOD and LOQ values were lower with respect to those reported in the literature (Li et al. 2015) and, for this reason, our analytical method represents an improvement for direct determination of very low contaminated oils.

Table 2 reports the recovery values calculated as described in materials and methods, using vegetables oil spiked with standards at levels of 0.1 and 0.5 $\mu\text{g g}^{-1}$. The results of the recovery experiment showed that the overall average recoveries were 80–100% for monoesters and diesters, while precision parameters (Table 2), expressed as %RSD_r and %RSD_R, ranged from 1.9 to 11.8 and from 2.1 to 12, respectively. Considering these results, according to literature data, the method applied in this study was accurate and precise for the determination and surveillance of the 3-MCPD esters in vegetables oils.

Table 3 shows the list of 18 MCPD esters identified using Q Exactive Orbitrap LC-MS/MS, along with their retention time, accurate mass molecular formula and error (ppm). In general, the mass error for all the compounds ranged from –4.05 to 4.99 ppm for all studied ions and was largest for diester LI-LI whereas the lowest values was obtained for diester ST-ST.

Representative HRMS chromatograms for the standards included in the method were shown in Figure 1(a and b). In particular, LC-MS (positive ion-mode) extracted-ion chromatograms of 3-MCPD esters based on accurate mass with a mass window of 5 ppm were reported.

The content of 3-MCPD esters found in various oils purchased from a local supermarket, expressed as 3-MCPD equivalents, is shown in Tables 4 and 5. The concentration levels of total 3-MCPD diesters in vegetable oil samples were in the range from 0.106 up to 3.444 mg kg^{-1} while total monoesters ranged from 0.005 up to 1.606 mg kg^{-1} , these levels are in good agreement with the levels reported in the literature (Haines et al. 2011; Pinkston et al. 2011; Yamazaki et al. 2013; Li et al. 2015) for peanut oils while for sunflower, corn and palm oils values are different to those reported in the literature (Haines et al. 2011; Yamazaki et al. 2013). Our results highlighted that OL-OL, OL-LI, OL-LN were the predominant diester-bound species found respectively in peanut, corn and sunflower oils, whereas in mixed seed oils the diester composition was more heterogeneous considering that only for 50% of analyzed samples OL-LN was the most representative diester.

Our results highlighted that bound MCPD was detected almost ubiquitously with widely varying

Table 2. Recovery, repeatability (RSD_r) and reproducibility (RSD_R) of 3-MCPD esters in the different oils investigated.

Sample oil	Peanut oil			Sunflower oil			Corn oil			Mixed seed oil			Palm oil		
	Recovery (%)	RSD _r (%)	RSD _R (%)	Recovery (%)	RSD _r (%)	RSD _R (%)	Recovery (%)	RSD _r (%)	RSD _R (%)	Recovery (%)	RSD _r (%)	RSD _R (%)	Recovery (%)	RSD _r (%)	RSD _R (%)
3-MCPD ester															
1-LA	87	1.9	2.1	98	8.1	8.1	95	3.4	3.8	82	3.5	3.8	90	8.7	9.1
1-LN	87	9.7	10.2	98	3.1	3.5	95	9.2	10.1	82	8.3	8.9	90	9.0	9.1
1-MY	93	4.4	5.1	93	4.9	5.1	93	7.0	8.0	93	2.8	2.9	93	3.7	3.8
1-LI	98	6.5	7.1	93	3.8	4.9	97	10.3	10.3	98	6.5	7.1	92	2.0	3.1
1-OL	100	8.4	8.7	91	10.7	10.7	100	6.9	7.2	100	6.9	8.2	100	6.5	7.2
1-PA	89	5.9	6.5	95	4.2	5.4	90	7.3	7.6	98	2.5	3.0	97	4.0	4.4
1-ST	87	8.3	9.1	91	5.2	6.2	96	4.9	5.1	87	5.9	7.1	90	7.2	7.9
LI-LI	98	10.6	11.0	100	2.7	3.1	82	8.6	9.1	92	8.1	9.3	87	3.0	4.2
PA-LI	93	11.3	11.3	100	5.4	5.7	80	7.8	8.3	89	3.9	4.3	91	4.7	5.7
OL-LI	92	11.8	12.0	100	9.0	9.0	82	10.0	10.0	90	4.9	5.2	97	7.3	8.2
OL-LN	98	8.9	9.1	100	7.3	7.7	90	8.9	9.3	99	6.1	6.5	98	4.0	4.5
PA-PA	100	3.2	3.3	100	5.6	5.9	98	8.2	8.6	89	7.5	8.0	96	6.2	7.0
OL-OL	98	8.5	8.7	100	3.9	4.1	89	3.6	3.8	98	8.2	8.7	98	2.4	2.7

Table 3. The MS parameters for 3-MCPD esters.

Compounds	Abbreviation	Molecular formula	RT (min)	Theoretical mass (m/z)	Ionisation mode	Measured mass (m/z)	Accuracy (Δ ppm)
Monoesters							
1-Lauroyl-3-chloropropanediol	1-LA	C ₁₅ H ₂₉ ClO ₃	2.06	293.18780	M-H ⁺	293.18848	2.32
1-Linolenoyl-3-chloropropanediol	1-LN	C ₂₁ H ₃₅ ClO ₃	2.19	371.23475	M-H ⁺	371.23422	-1.43
1-Myristoyl-3-chloropropanediol	1-MY	C ₁₇ H ₃₃ ClO ₃	2.24	321.21910	M-H ⁺	321.21964	1.68
1-Linoleoyl-3-chloropropanediol	1-LI	C ₂₁ H ₃₇ ClO ₃	2.39	390.27695	M-NH ₄ ⁺	390.27740	1.16
1-Palmitoyl-3-chloropropanediol	1-PA	C ₁₉ H ₃₇ ClO ₃	2.39	349.25040	M-H ⁺	349.25003	-1.06
1-Oleoyl-3-chloropropanediol	1-OL	C ₂₁ H ₃₉ ClO ₃	2.65	375.26605	M-H ⁺	375.26572	-0.88
1-Stearoyl-3-chloropropanediol	1-ST	C ₂₁ H ₄₁ ClO ₃	3.42	377.28170	M-H ⁺	377.28113	-1.51
Diesters							
1,2-Dilinolenoyl-3-chloropropanediol	LN-LN	C ₃₉ H ₆₃ ClO ₄	9.06	648.47531	M-NH ₄ ⁺	648.47498	-0.51
1,2-Dilinoleoyl-3-chloropropanediol	LI-LI	C ₃₉ H ₆₇ ClO ₄	9.51	652.50661	M-NH ₄ ⁺	652.50397	-4.05
1-Oleoyl-2-Linolenoyl-3-chloropropanediol	OL-LN	C ₃₉ H ₆₇ ClO ₄	10.04	652.50661	M-NH ₄ ⁺	652.50964	4.64
1-Palmitoyl-2-Linoleoyl-3-chloropropanediol	PA-LI	C ₃₇ H ₆₇ ClO ₄	10.56	628.50661	M-NH ₄ ⁺	628.50696	0.56
1-Oleoyl-2-Linoleoyl-3-chloropropanediol	OL-LI	C ₃₉ H ₆₉ ClO ₄	10.96	654.52226	M-NH ₄ ⁺	654.52026	-3.06
1,2-Dipalmitoyl-3-chloropropanediol	PA-PA	C ₃₅ H ₆₇ ClO ₄	11.79	604.50661	M-NH ₄ ⁺	604.50952	4.81
1,2-Dioleoyl-3-chloropropanediol	OL-OL	C ₃₉ H ₇₁ ClO ₄	12.45	656.53791	M-NH ₄ ⁺	656.53815	0.36
1-Linoleoyl-2-Stearoyl-3-chloropropanediol	LI-ST	C ₃₉ H ₇₁ ClO ₄	12.50	656.53791	M-NH ₄ ⁺	656.53778	-0.20
1-Palmitoyl-2-Stearoyl-3-chloropropanediol	PA-ST	C ₃₇ H ₇₁ ClO ₄	14.04	632.53791	M-NH ₄ ⁺	632.53967	2.78
1-Oleoyl-2-Stearoyl-3-chloropropanediol	OL-ST	C ₃₉ H ₇₃ ClO ₄	14.45	658.55356	M-NH ₄ ⁺	658.55621	4.02
1,2-Distearoyl-3-chloropropanediol	ST-ST	C ₃₉ H ₇₅ ClO ₄	16.70	660.56921	M-NH ₄ ⁺	660.57251	4.99

concentrations according to MacMahon et al. (2013a). In fact, MacMahon reported that concentrations of bound 3-MCPD, in 94 refined oils, ranged from 0.005 to 7.2 mg kg⁻¹.

Some refined oils like peanut oils and corn oils contained bound 3-MCPD (sum of monoesters and diesters, expressed as 3-MCPD equivalents) in a relatively low concentration range between 0.130 and 1.420 mg kg⁻¹ whereas other refined oils (mixed seed oils, sunflower oils and palm oils) carried 3-MCPD total esters in a concentration range between 0.468 and 4.214 mg kg⁻¹. The total concentrations of bound 3-MCPD measured in palm oils, peanut oils and sunflower oils are comparable with those determined using indirect methodology by Kuhlmann (2011). In particular, Kuhlmann analysed 20 or more samples of palm and sunflower oils and 4 peanut oils, finding bound 3-MCPD concentrations ranging between 1.1 and 10 mg kg⁻¹ in palm oil, 0.1 and 2.1 mg kg⁻¹ in sunflower oil and 0.1 and 0.9 mg kg⁻¹ in peanut oils. As expected and according to Kuhlmann (2011) and MacMahon et al. (2013b), refined palm oil was characterised by a high concentration of bound 3-MCPD reaching the highest value of 4.214 mg kg⁻¹. In accordance with results reported by MacMahon, none of the oils contained appreciable concentrations (not found or not detectable) of the 3-MCPD diesters LN-LN and ST-ST whereas 3-MCPD monoester 1-LN was only seen in corn oils, in unrefined palm oil and only in two samples of mixed seed oils. Also

expectedly, low concentrations of 3-MCPD esters were observed in crude palm oil compared to refined palm oil (1.842 mg kg⁻¹ vs 4.260 mg kg⁻¹). This is in accordance with the literature (Matthäus et al. 2011), where it was reported that these contaminants were formed during the deodorisation process. An interesting result was the presence of monoester 1-LI and diesters LI-LI, OL-LN and PA-LI in refined palm oil and their absence in crude palm oil. This information could be useful since refined palm oil is a widespread ingredient in industrial food preparations.

All diesters investigated were found in refined palm oils and among them LI-ST was the most representative. As expected, the fatty acids pattern in 3-MCPD diesters corresponded to their natural abundance in respective oil according to literature data (Moravcova et al. 2012).

Interestingly, the monoesters, 1-LN, 1-LI, 1-PA, and 1-OL were found. Among these, 1-OL was the highest in mixed seeds, sunflower and corn oils and the concentrations ranged from 0.010 to 0.213 mg kg⁻¹ with the highest concentration in the mixed seed oil sample. In peanut oils 1-PA and 1-LI were the most representative monoesters and ranged from 0.044 to 0.197 mg kg⁻¹ and from 0.009 to 0.081 mg kg⁻¹, respectively.

The relative contribution of 3-MCPD esters in food is of key importance considering that the structure of 3-MCPD esters as monoesters or diesters may play a determinant role in the release of free 3-MCPD. Several studies reported in the literature

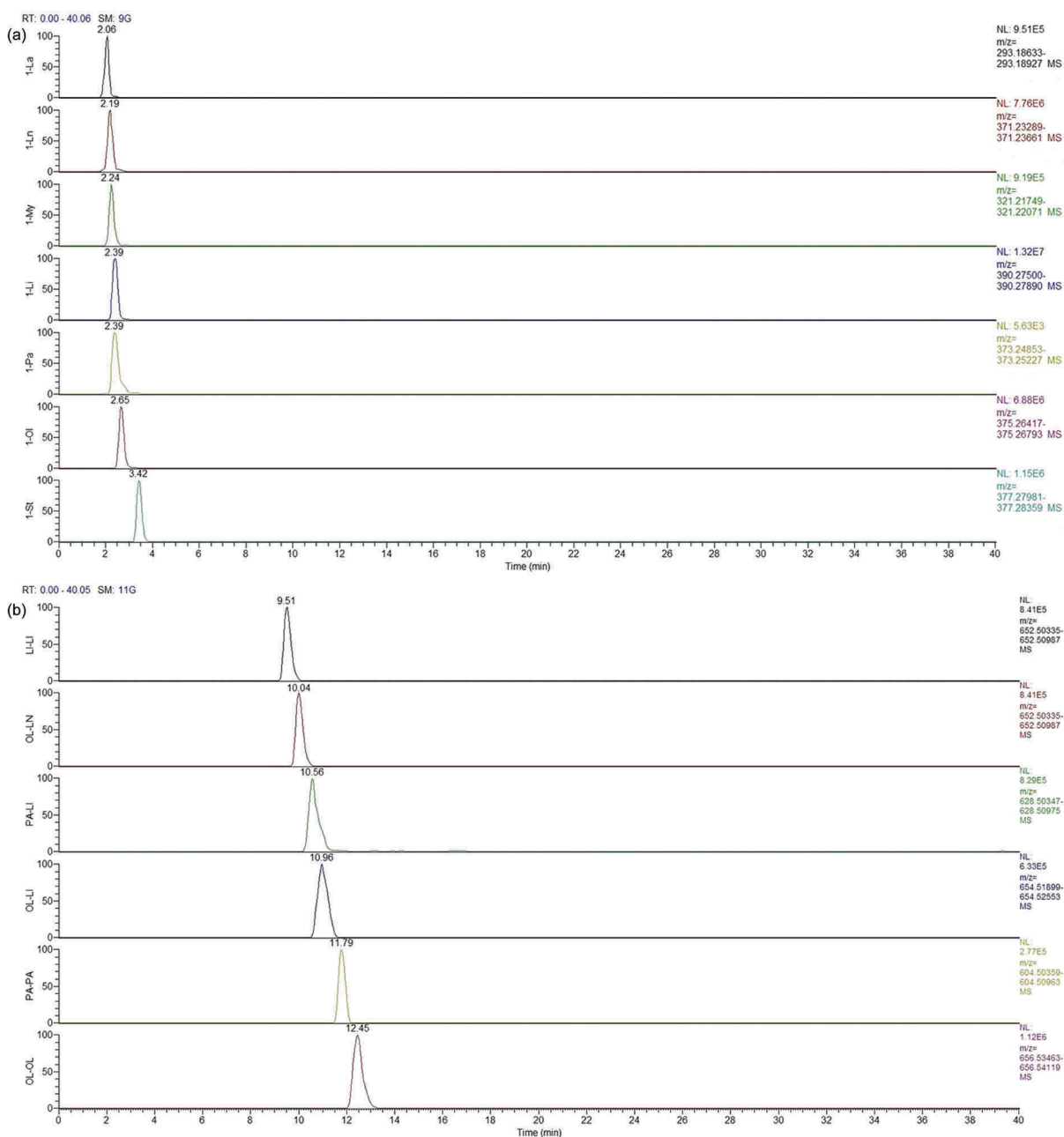


Figure 1. (a) Representative chromatograms of MCPD monoesters used as standards; (b) representative chromatograms of MCPD diesters used as standards.

(Schilter et al. 2011) show that 3-MCPD esters are suitable substrates for intestinal lipases due to their structural similarities with acylglycerol. This determines 3-MCPD release, which contributes to the overall dietary exposure to this compound. Furthermore, it is well recognised that 3-MCPD monoesters are more hydrophilic than diesters, which might have altered their absorption, distribution and metabolism. For example, monoesters could release free 3-MCPD more rapidly by lipase

hydrolysis in the human gastrointestinal tract and for this reason the relationship between 3-MCPD esters (monoesters and diesters) could be a fundamental parameter to evaluate the healthy properties of vegetable oils (Liu et al. 2017). On the other hand, in the literature it is well known that intestinal lipases prefer position sn-1 and sn-3 of acylglycerols and this justifies the more efficient release of 3-MCPD from the sn-1-monoesters than from the diesters.

Table 4. Levels of 3-MCPD esters expressed as 3-MCPD equivalents (ppm) in the vegetable oils (mixed seeds, peanuts and corn).

	1-LN	1-LI	1-PA	1-OL	Monoesters	LN-LN	LI-LI	OL-LN	PA-LI	OL-LI	PA-PA	OL-OL	LI-ST	PA-ST	OL-ST	ST-ST	Diesters	Total equivalents 3-MCPD ppm
Mixed seed oils																		
1	0.229	0.063	0.004	0.213	0.505	NF	0.079	0.257	0.402	0.424	0.135	0.750	0.468	0.080	0.092	NF	1.544	2.048
2	NF	0.007	NF	0.050	0.057	NF	0.052	0.188	0.092	0.112	NF	0.014	0.074	NF	ND	NF	0.518	0.575
3	NF	0.026	0.004	0.082	0.111	NF	0.112	0.352	0.224	0.570	NF	0.299	0.146	NF	ND	NF	1.703	1.814
4	NF	0.023	NF	0.055	0.078	NF	0.513	1.296	0.336	0.711	NF	0.349	0.160	NF	0.025	0.054	3.444	3.522
5	0.231	0.035	0.004	0.094	0.363	NF	0.073	0.239	0.354	0.379	0.114	0.477	0.344	0.082	0.075	NF	1.898	2.260
6	NF	0.042	0.004	0.099	0.146	NF	0.247	0.709	0.335	0.498	NF	0.467	0.296	0.067	0.037	0.054	2.709	2.854
7	NF	0.044	< LOQ	0.052	0.096	NF	0.014	0.094	0.477	0.437	0.128	0.637	0.312	0.089	0.103	NF	2.291	2.387
8	NF	0.005	NF	NF	0.005	NF	0.185	0.529	0.130	0.337	NF	0.109	0.123	0.066	ND	NF	1.479	1.484
9	NF	0.046	ND	0.145	0.191	NF	0.233	0.660	0.411	0.666	0.115	0.529	0.372	0.078	0.078	NF	3.142	3.333
10	NF	0.007	0.003	0.064	0.073	NF	0.005	0.069	0.086	0.059	NF	0.012	0.076	0.087	NF	NF	0.395	0.468
Peanut oils																		
1	NF	0.081	0.129	0.012	0.223	NF	NF	NF	NF	NF	NF	0.049	0.031	NF	0.026	NF	0.106	0.329
2	NF	0.016	0.100	0.028	0.144	NF	NF	NF	0.009	0.059	NF	0.134	0.126	NF	0.026	NF	0.354	0.498
3	NF	0.012	0.059	< LOQ	0.071	NF	NF	0.035	0.037	0.134	NF	0.184	0.170	NF	0.029	NF	0.589	0.661
4	NF	0.022	0.056	0.010	0.088	NF	0.102	NF	0.042	0.141	NF	0.179	0.176	NF	0.030	NF	0.671	0.759
5	NF	0.015	0.110	< LOQ	0.125	NF	0.097	0.049	0.059	0.208	NF	0.169	0.155	NF	0.029	NF	0.766	0.891
6	NF	0.020	0.118	0.010	0.148	NF	NF	NF	0.012	0.081	NF	0.116	0.106	NF	0.026	NF	0.341	0.490
7	NF	0.009	0.048	ND	0.057	NF	NF	NF	0.028	0.109	NF	0.143	0.128	NF	0.028	NF	0.436	0.493
8	NF	0.033	0.176	0.014	0.223	NF	NF	NF	0.019	0.085	NF	0.112	0.092	NF	0.027	NF	0.334	0.557
Corn oils																		
1	0.021	0.029	0.026	0.033	0.110	NF	0.127	0.087	0.038	0.161	ND	ND	ND	ND	NF	ND	0.413	0.523
2	0.020	0.038	0.028	0.038	0.124	NF	0.189	0.150	0.093	0.333	ND	0.013	0.018	NF	NF	ND	0.796	0.920
3	0.027	0.032	0.032	0.080	0.171	NF	0.080	0.041	0.027	0.071	ND	ND	ND	NF	NF	ND	0.219	0.389
4	0.026	0.021	0.031	0.079	0.156	NF	0.061	0.017	0.023	0.092	ND	ND	ND	NF	NF	ND	0.193	0.349
5	0.026	0.029	0.034	0.031	0.121	NF	0.095	0.060	0.018	0.099	ND	ND	ND	NF	NF	ND	0.272	0.392
6	0.028	0.052	0.055	0.043	0.178	NF	0.227	0.185	0.152	0.330	ND	0.036	ND	NF	ND	ND	0.931	1.109
7	0.025	0.017	0.046	0.042	0.130	NF	NF	NF	ND	ND	ND	ND	ND	NF	NF	ND	0.000	0.130
8	0.023	0.042	0.032	0.050	0.147	NF	0.329	0.305	0.185	0.313	ND	0.070	0.071	NF	NF	ND	1.272	1.420
9	0.033	0.047	0.059	0.057	0.196	NF	0.281	0.259	0.155	0.366	ND	0.032	ND	NF	ND	ND	1.093	1.289

NF, not found; ND, not detected.

Table 5. Levels of 3-MCPD esters expressed as 3-MCPD equivalent (ppm) in the vegetable oils (sunflower and refined palm).

	1-LN	1-LI	1-PA	1-OL	Monoesters	LN-LN	LI-LI	OL-LN	PA-LI	OL-LI	PA-PA	OL-OL	LI-ST	PA-ST	OL-ST	ST-ST	Diesters	Total equivalents 3-MCPD ppm
1	NF	0.015	0.018	0.080	0.113	NF	0.345	0.411	0.079	0.312	NF	0.110	0.090	NF	NF	NF	1.347	1.460
2	NF	0.015	0.100	0.155	0.270	NF	0.266	0.360	0.057	0.228	NF	0.111	0.072	NF	NF	NF	1.094	1.364
4	NF	0.019	0.010	0.037	0.067	NF	0.511	0.797	0.149	0.601	NF	0.162	0.131	NF	0.053	NF	2.404	2.471
5	NF	0.012	0.036	0.072	0.120	NF	0.369	0.556	0.099	0.371	NF	0.109	0.147	NF	0.050	NF	1.699	1.819
6	NF	0.015	0.024	< LOQ	0.039	NF	0.131	0.171	0.039	0.156	NF	0.069	0.041	NF	NF	NF	0.608	0.647
7	NF	0.007	0.145	0.103	0.254	NF	0.087	0.102	0.020	0.108	NF	0.063	0.046	NF	NF	NF	0.425	0.680
											Palm oils							
1 (unrefined)	0.181	NF	0.390	0.143	0.714	NF	NF	NF	NF	0.186	0.141	0.437	0.164	0.075	0.131	NF	1.135	1.848
2 (refined)	NF	0.354	0.590	0.662	1.606	NF	0.018	0.087	0.437	0.306	0.167	0.643	0.709	0.082	0.160	NF	2.609	4.214

NF, not found.

Other important information obtained from the direct method is the distribution of monoesters and diesters, considering that toxicological data reported in the literature show that the toxic effects of 3-MCPD monoester (palmitic) is double compared to those of the diester (di-palmitic), suggesting that the introduction of the second fatty acid group might reduce the toxicity of a 3-MCPD monoester (Liu et al. 2017). Moreover, it was also reported that the degree of unsaturation, chain length, number of substitutions and relative substitution locations of fatty acids might alter the toxicity of 3-MCPD esters. The real problem linked to 3-MCPD esters present in refined vegetable oil could be associated with chronic exposure, as reported by Wallace et al. (2016), considering that these oils are consumed as ingredients in a wide spectrum of different foods (cereal-derived products like bread crust, toast, biscuits, malt-derived products, coffee, grilled cheese and smoke treated products), continuous monitoring is needed to carry out exposure assessment studies.

Conclusions

The use of good quality dietary vegetable oil by the food industry contributes to the healthiness of finished food products, reducing the dietary intake of free 3-MCPD and the consequent relative exposure risk. On the other hand, considering the widespread use of various refined fats in different kinds of food, for an exposure evaluation, a huge number of contamination data for each food is required. This study led to the development of a rapid, quick and reproducible analytical method for the quantification of 3-MCPD esters. Moreover, this method allows the occurrence of 3-MCPD esters in edible vegetal oils to be established, highlighting that 3-MCPD esters were widespread contaminants being present in all analysed samples reaching the highest values in refined palm oil. Regarding qualitative information, the amounts of 3-MCPD monoesters in refined vegetable fat mixes were significantly lower than those of the 3-MCPD diesters, highlighting that the 3-MCPD esters consisted mainly of 3-MCPD diesters. Therefore, additional food occurrence data, as well as further bioavailability,

metabolism and toxicological studies on the 3-MCPD mono and diesters will be needed.

Disclosure statement

No potential conflict of interest was reported by the authors.

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