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Comparative analysis of ultrasound-accelerated alkaline and acid hydrolysis for the indirect determination of 3-monochloropropane-1,2-diol fatty acid esters

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ABSTRACT

Ultrasound-accelerated hydrolysis is effectively used for the first time in the release of 3-monochloropropane-1,2-diol (3-MCPD) from its fatty esters. Indirect determination of 3-MCPDE fatty acid esters was performed under alkaline and acid conditions using sodium methoxide and sulfuric acid, respectively, prior to GC-MS analysis. Dispersive liquid-liquid microextraction (DLLME) was used to derivatize the free 3-MCPD with n-heptafluorobutyrylimidazole (HFBI). Two asymmetrical screening designs were used to evaluate parameters affecting the methods' performances: time, temperature, hydrolytic agent volume, and concentration. A comparative study between both methods was performed. Research showed that comparable results are achieved in terms of linearity ($r^2 > 0.9995$), specificity, accuracy, and precision. Determination limits were lower for the alkaline method (67 ng g^{-1}). Twenty samples of four types of edible oil were assessed using both methods: extra virgin olive oil (EVO), refined sunflower oil, refined olive oil, and palm oil. Results obtained here were comparable and similar to those reported in the literature. Alkaline hydrolysis was the recommended approach and should be used in occurrence studies.

1. Introduction

Edible oils are widely consumed worldwide, directly and as an ingredient in many foodstuffs, and so their harmlessness is a crucial factor in food safety assurance (Hu et al., 2019). During edible oil manufacture, several processing stages ensure higher quality, nevertheless, undesirable food processing contaminants may be formed under those conditions (Hu et al., 2019; Xia et al., 2021). 3-monochloropropane-1,2-diol (3-MCPD) and its fatty acid esters (3-MCPDE) are food processing contaminants generated by heat and have been characterized and classified as possibly carcinogenic to humans (category 2B) by the International Agency for Research in Cancer (IARC, 2014). A tolerable daily intake of 2 μg kg^{-1} of body weight per day has been set up for 3-MCPD and 3-MCPDE (EFSA Panel on Contaminants in the Food Chain CONTAM et al., 2018). During the last decade, several works on the determination of 3-MCPDE have been published. These methods are commonly based on LC (direct method) or GC (indirect method). The direct method allows the individual and simultaneous determination of

Abbreviations: **3-MCPD**, 3-monochloropropane-1,2-diol; **3-MCPDd5**, deuterated 3-monochloropropane-1,2-diol; **3-MCPDE**, 3-monochloropropane-1,2-diol fatty acid esters; **AOCS**, American Oil Chemists' Society; **DLLME**, Dispersive liquid-liquid microextraction; **EFSA**, European Food Safety Authority; **EVO**, extra virgin olive oil; **FDA**, Food and Drug Administration; **IARC**, International Agency for Research in Cancer; **BSTFA**, N,O-Bis(trimethylsilyl)trifluoroacetamide; **HFBI**, n-heptafluorobutyrylimidazole; **PBA**, phenylboronic acid; **QC**, quality control substances; **RSD**, Relative standard deviation.

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each 3-MCPDE (Custodio-Mendoza et al., 2022, 2019; Gao et al., 2019). Although some novel strategies have been developed, currently direct methods have some drawbacks such as multiple sample preparation steps, large volumes of sample and organic solvents and the use of expensive standards are still required. Also, the total 3-MCPD cannot be determined but estimated from the 3-MCPDE contributions. On the other hand, the indirect method requires first releasing 3-MCPD from its esters and the derivatization of total 3-MCPD before GC–MS determination.

As for indirect methods, American Oil Chemists' Society has provided two official methods: AOCS Official Method Cd 29a-13 (AOCS, 2017a), based on the slow acid-catalyzed 3-MCPDE transesterification, and the AOCS Official Method Cd 29b-13 (AOCS, 2017a), based on a slow basic-catalyzed 3-MCPDE transesterification, that may be applied to different vegetable oils and fats. Even though these methods have proven their efficiency in many proficiency tests, they have the disadvantage of requiring long transesterification times of up to 16 h. Based on such methods, some alkaline (Sadowska-Rociek, 2020; Stauff et al., 2020; Zwagerman & Overman, 2016.) and acid (Ramli et al., 2011; Wöhrlin et al., 2015; Xu et al., 2020; Zelinkova et al., 2017; Zheng et al., 2021) methods were developed. The alkaline approach has been improved, as Almoselhy et al. (2021) propose with simultaneous extraction and phenylboronic acid (PBA) derivatization once 3-MCPD is released. Wang et al. (2016) reduced the alkaline transesterification time by vortex agitation down to 1 min. The major improvement in the acid approach is the lowering of the derivatization time down to 15 min, as reported by Zheng et al. (2021), or by simultaneous derivatization and solid-phase microextraction (SPME), as proposed by Xu et al. (2020). These transesterification approaches have been developed for different food matrices, including edible oils, oil-based foodstuffs, and other complex matrices. Nevertheless, transesterification and derivatization times remain long, negatively affecting the cost-effectiveness of such methods. Moreover, alkaline transesterification must be performed at slightly alkaline and low-temperature conditions to inhibit the transformation of MCPD into glycidol and other oxiranes, as described by Jędrkiewicz et al. (2016a).

As for 3-MCPD derivatization, official methods include PBA derivatization (AOCS, 2017a, 2017b). Other derivative reagents, including N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), and n-Heptafluorobutyrylimidazole (HFBI), have been proposed (Lee et al., 2016), but they require high temperatures and additional clean-up after derivatization. Conventional protocols, based on PBA derivatization, also require long reaction times at high temperatures, and an additional solvent exchange is needed to perform GC–MS analysis (AOCS, 2017a, 2017b; Lee et al., 2016). These drawbacks can be reduced using HFBI derivatization incorporated in dispersive liquid-liquid microextraction (DLLME) as proposed by Carro et al. (2013) for the determination of chloropropanols in aqueous samples, and simultaneous solid-phase microextraction (SPME) and PBA derivatization applied to the determination of 3-MCPDE in oil samples as proposed by Xu et al. (2020).

In this work, two indirect approaches have been successfully developed and validated following FDA guidelines (FDA, 2018, 2019). These methods are based on ultrasound-accelerated alkaline and acid transesterification of 3-MCPDE from edible vegetable oils followed by HFBI derivatization performed during DLLME prior GC–MS analysis. These methods were applied to 20 different edible oils, including virgin and refined olive and sunflower oils. The results of both methods are presented and compared to those published before by other authors.

To the best of our knowledge, this is the first study about the use of ultrasound to accelerate the esterification part of these approaches, and the combination of the HFBI derivatization during DLLME. This has positively impacted the cost-effectiveness and greenness of both methods.

2. Materials and methods

2.1. Chemicals and materials

Hypergrade acetonitrile (ACN), chloroform ($CHCl_3$), methanol (MeOH), ethyl acetate (EtOAc), n- Heptafluorobutyrylimidazole (HFBI), n-hexane, sodium methoxide (CH_3NaO) 0.5 mol/L in methanol, sulfuric acid, sodium hydrogen carbonate ($NaHCO_3$), and sodium sulfate (Na_2SO_4) were all purchased from Merck (Darmstadt, Germany) with a purity ≥99 %. Milli-Q water was obtained using a Millipore purification system (Millipore, Billerica, MA, USA). Oleic, linoleic, stearic and palmitic acid esters of 3-MCPD (Table 1) were selected based on fatty acid distribution of olive and sunflower oils and purchased from Toronto Research Chemical (Toronto, Ontario, Canada). Standard solutions of 3-MCPD and deuterated 3-MCPD (3-MCPDd5) were purchased from Merck (Darmstadt, Germany). Deuterated 3-MCPD esters were used as internal standards provided that the labeled part of each standard corresponded to 3-MCPD. Individual and working standard solutions were prepared in MeOH at the specific concentrations and stored at –20 ºC.

2.2. Samples

An EVO sample was used to develop and validate the proposed method. A total of 20 edible vegetable oil samples, including 5 EVO, 2 refined olive oil with 1º acidity (RO 1º), 2 refined olive oil with 0.4º acidity (RO 0.4º), 4 pomace olive oil (POO), 5 refined olive oil (RSO), and 2 red palm oil (RPO) were purchased from local supermarkets in Santiago de Compostela area, and stored in their original packet at 4 ºC until analysis.

2.3. Sample preparation

Oil samples were prepared as summarized in Fig. 1. First, the cleavage of 3-MCPDE was carried out by two ultrasound-accelerated approaches. Extract were then washed by a double LLE with hexane followed by the HFBI derivatization using a modification of the DLLME proposed by Carro et al. (2013) prior to GC–MS analysis.

Quality control samples were freshly prepared by adding the standard solution at concentrations of 0.5, 1.0 and 3.0 μg g^{-1} to an extra virgin olive oil (EVO) sample and keeping them stored at –20 °C for no longer than a week. IS concentration was set at 0.5 μg g^{-1}.

2.3.1. Ultrasound-accelerated alkaline transesterification

An aliquot of 0.3 g of oil was accurately weighed in a flask followed

Table 1
3-MCPD, 3-MCPD fatty acid esters standards and internal standards.

Standard	Abbreviation	Cas No.
rac 1,2-Dioleoyl-3-chloropropanediol	OLOL	69161.73-5
rac 1,2-Dioleoyl-3-chloropropanediol-d5	OLOLd5*	1246833-00-0
rac 1–2-Bispalmitoyl-3-chloropropanediol	PAPA	51930-97-3
rac 1–2-Bispalmitoyl-3-chloropropanediol-d5	PAPAd5*	1185057-55-9
rac 1-Oleoyl-2linoleoyl-3-chloropropanediol	OLLI	1336935-03-5
rac 1-Oleoyl-2-stearoyl-3-chloropropanediol	OLST	1336935-05-7
rac 1-Oleoyl-2-stearoyl-3-chloropropanediol-d5	OLSTd5*	1336935-05-7
rac 1-Palmitoyl-2-linoleoyl-3-chloropropanediol	PALI	1246833-87-3
rac 1-Palmitoyl-2-oleoyl-3-chloropropanediol	PAOL	1363153-60-9
rac-1,2-Dilinoleoyl-3-chloropropanediol	LILI	7487-96-0
rac 1-Oleoyl-3-chloropropanediol	OL	10311-82-7
rac 1-Oleoyl-3-chloropropanediol-d5	OLd5*	10311-82-7
rac 1-Palmitoyl-3-chloropropanediol	PA	30557-04-1
rac 1-Palmitoyl-3-chloropropanediol-d5	PAd5*	1346599-60-7
rac-1-Linoleoyl-3-chloropropanediol	LI	74875-98-2
rac-1-Stearoyl-3-chloropropanediol	ST	22094-20-8
rac-1-Stearoyl-3-chloropropanediol-d5	STd5*	22094-20-8
3-monochloropropane-1,2-diol	3-MCPD	96-24-2
3-monochloropropane-1,2-diol	3-MCPDd5*	342611-01-2

*used as internal standard

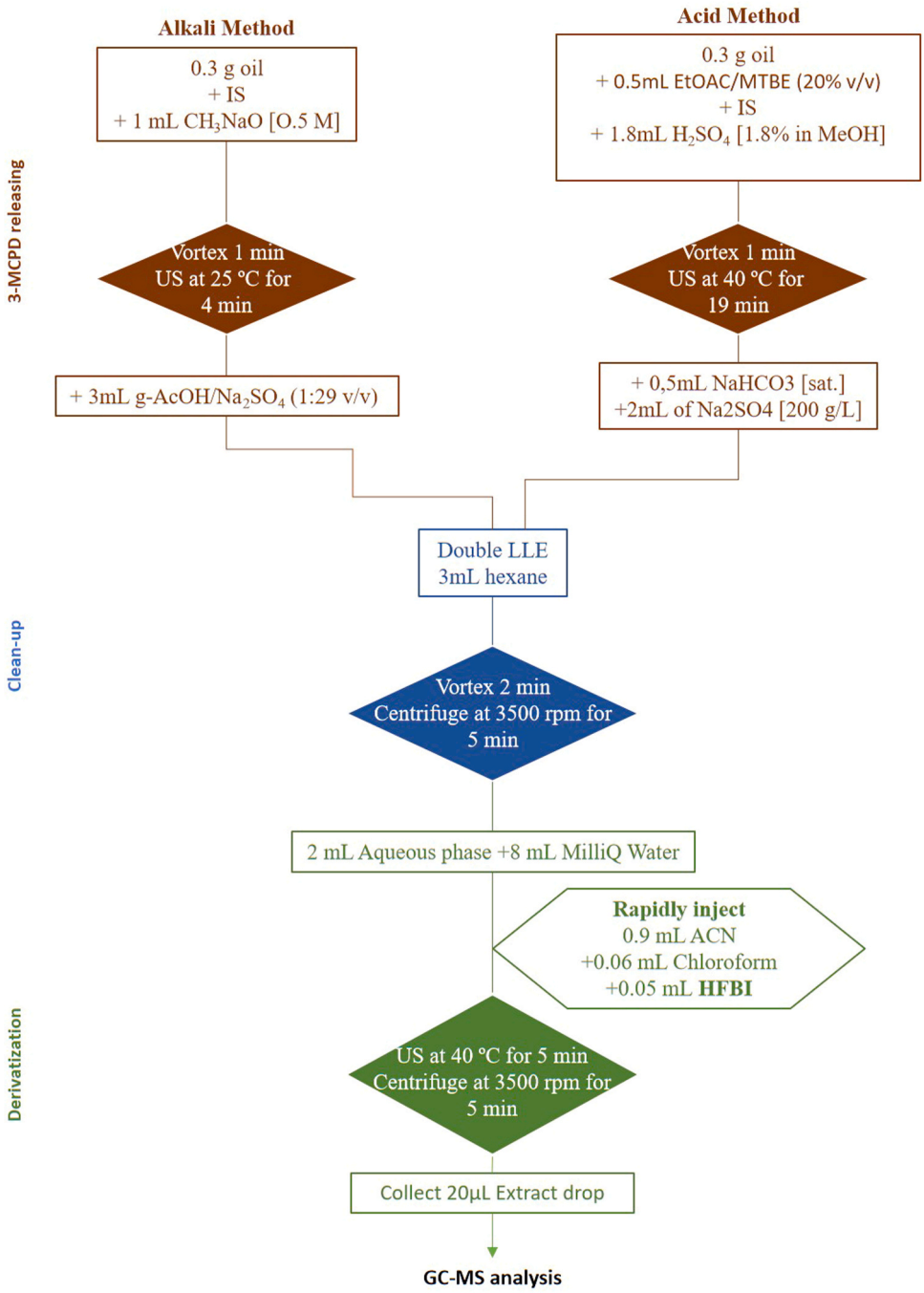

Fig. 1. Scheme of ultrasound-accelerated Alkaline and Acid transesterifications follow by HFBI derivatization in DLLME for the determination of total 3-MCPD in edible oils.

by the addition of the IS solution (3-MCPDd5) and 1 mL of sodium methoxide 0.5 M in methanol. The mixture was homogenized by vortex for 1 min and submitted to ultrasound (40 kHz & 130 W) at 25 °C for 4 min to release the 3-MCPD from its fatty acid esters. To stop the reaction 3 mL glacial acetic acid and sodium sulfate (1:1 v/v) solution was added and vortexed for 1 min

2.3.2. *Ultrasound-accelerated acid transesterification*

An aliquot of 0.3 g of oil was accurately weighed in a flask and diluted in 0.5 mL EtOAc/MTBE (20 % v/v), followed by the addition of the IS solution (3-MCPDd5) and 1.8 mL of sulfuric acid 1.8 % in methanol. The system was homogenized by vortex for 1 min and submitted to ultrasound (40 kHz & 130 W) at 40 °C for 19 min to release the 3-MCPD from its fatty acid esters. To stop the reaction, 0.5 mL saturated sodium hydrogen carbonate solution and 2 mL sodium sulfate solution (200 g/L) were added and vortexed for 1 min

2.3.3. *Clean-up and derivatization*

After 3-MCPDE cleavage by acid or base hydrolysis, the aqueous system containing total 3-MCPD for each method was separately washed with 3 mL hexane, vortexed for 1 min and centrifuged twice for 5 min at 3500 rpm, discarding the organic layer each time. A 2 mL aliquot of the cleaned aqueous phase was transferred into a conical-bottom tube alongside milliQ water to complete 10 mL. Then a mixture of 0.9 mL ACN, 60 µL chloroform, and 50 µL HFBI was rapidly injected into the aqueous phase to perform the simultaneous derivatization and

extraction of 3-MCPD by DLLME. The tube was incubated in an ultrasound bath at 40 ºC for 5 min and centrifuged for 5 min at 3500 rpm. After this, an organic micro-drop containing the 3-MCPD-HFBI and 3-MCPDd5-HFBI adducts was collected using a microsyringe and transferred into a chromatography vial with insert to be GC–MS analyzed.

2.4. Instrumental conditions

GC–MS analysis was carried out using a 7890B-5977B-MSD system equipped with an automatic liquid sampler (model 7650 A) from Agilent Technologies (CA, USA). The injection volume was set to 1 μL. The inlet with an ultra-inert double taper liner (model 5190–3983), from Agilent Technologies (CA, USA) was set at 280 ºC, with a flow of 24.2 mL min^{-1}, on splitless mode. A J&W HP-5MS column (30 m × 0.25 mm id × 0.25 μm) from Agilent Technologies (CA, USA) was used for analyte separation. The temperature program was set initially at 50 ºC and held for 2 min, then increased to 100 ºC at a rate of 3 ºC min^{-1}, and finally to 280 ºC at a rate of 40 ºC min^{-1}, holding it for 10 min, in a total analysis time of 30 min. A single quadrupole mass analyzer operated in the positive ion mode (EI +) (70 eV) was used with an electron impact source for determination of target compounds. The transfer line was set at 280 ºC, the source temperature was set at 230 ºC and the quadrupole temperature was set at 150 ºC. Under said conditions, 3-MCPD and 3-MCPDd5 eluted at around 15 min, 253 and 257 *m/z* were selected as quantifier ions and 289 and 453, and 294 and 456 *m/z* as confirmation ions, respectively. Data acquisition was performed by MassHunter Workstation Software (version B.07.00) from Agilent Technologies (CA, USA).

By the same token, a Centromix II-BL Centrifuge from J. P. Selecta (Barcelona, Spain), a 100 μL micro-syringe from Hamilton Company (Nevada, USA), a pH meter model Basic 20 from Crison Instruments (Barcelona, Spain,) a 2510EMTH ultrasonic bath from Branson Ultrasonics (Danbury, USA), and a Reax top vortex mixer from Instruments GmbH & Co (Schwalbach, Germany) were used to develop all experiments.

2.5. Methods validation

Both alkaline and acid transesterification methods were validated following the Food and Drug Administration (FDA) guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products (FDA, 2019) and Bioanalytical Method Validation Guidance for Industry (FDA, 2018). An EVO was selected as a blank sample to study all analytical features.

The specificity of the methods was based on the selection of a quantifier ion and two confirmation ions for 3-MCPD and 3-MCPDd5 and confirmed by the absence of interference in the retention region of the analytes. The lowest and highest amount of each analyte that can be quantitatively determined with acceptable precision and accuracy in this method lies between the lower (LLOQ) and the upper (ULOQ) limits of quantification. Limit of detection (LOD) and LLOQ were determined by analyzing analyte concentrations at which the signal-to-noise ratio was 3.3 and 10, respectively, while ULOQ was set at 3 μg g^{-1}. A standard addition calibration curve was built from LLOQ to ULLOQ in at least 6 levels of concentrations to assess the method's linearities. Sensitivity of the methods was assessed at LLOQ in terms of relative standard deviation (%RSD) for each analyte in triplicate. Accuracy of the optimized processes was assessed using quality control substances (QC) at three levels of concentration (at 0.5, 1 and 3 μg g^{-1}) within the linear range. Acceptable criteria for recovery were considered between 80 % and 120% of the nominal value, The method's precision was determined by intraday and interday assays in terms of %RSD in quintuplicate using the same QC.

3. Results and discussion

3.1. Optimization of ultrasound-accelerated alkaline transesterification method

Sample size directly affects both the effectiveness and the sensitivity of the method. It is important to study the exact amount at which the signal of the analyte is detected unequivocally with no matrix interferences. And on the other hand, a reduced sample size follows trends of green analytical chemistry and positively affects the cost-effectiveness of the method. For the alkaline approach, 0.1, 0.3 and 1 g of oil were assessed in triplicate (Fig. 1S.A). Results expressed as 3-MCPD/IS show that 0.3 and 1 g yield a similar analyte response, higher than those for 0.1 g, so it was decided to continue with 0.3 g sample size to reduce any matrix interference. The alkaline approach is usually performed by the effect of sodium methoxide in a 5 mol/L solution in methanol. The amount of reactive added varies among the published methods, from 0.04 M to saturated, and from 0.1 to 1.5 mL (Marc et al., 2016; Wang et al., 2016; Xu et al., 2020). In this work, 0.5, 1 and 2 mL of sodium methoxide 5 mol/L solution in MeOH solution were tested in triplicate. As may be seen in Fig. 1S.B, adding 1 and 2 mL of sodium methoxide 5 mol/L solution in MeOH gives a similar analyte response in terms of recovery, so 1 mL sodium methoxide was selected to reduce analyte dilution. Alkaline approaches are often performed at temperatures from 25 to 60 ºC which have also been tested in this study (Fig. 1S.C) in triplicate. The obtained results, expressed in terms of % recovery, show similar efficacies at any of the studied levels, so it was decided to go with 25 ºC to improve the cost- and energy- effectiveness of the method.

Other factors affecting the efficacies of the alkaline transesterification were studied simultaneously using an asymmetrical screening design $2^2 1^4 // 8$ in a total of 8 experiments. Two factors were studied at two levels: b_1, agitation mode using vortex and ultrasound for 1 min; b_2, neutralization solution using sodium sulfate and ammonium sulfate, and one factor was studied at four levels: b_3, salting-out effect with 0, 0.5, 1.0 and 1.8 g of NaCl addition. These assays were performed using spiked EVO samples and results are plotted in a total effect chart (Fig. 2). In said graph, the length of each bar is proportional to the influence of the factor level on the response. So, as it can be seen, the best conditions are achieved when using sodium sulphate as the neutralization agent, and no salting-out. Confoundingly, between vortex or ultrasound there is hardly any difference, but ultrasound agitation was selected due to the reproducibility, in terms of temperature control, of the experiments. At these conditions a kinetic study of the transesterification reaction was performed to study the degree of 3-MCPD

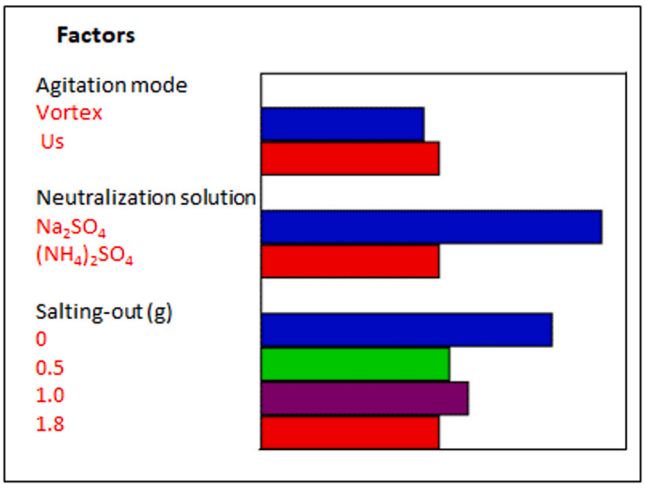

Fig. 2. Total effect chart for the asymmetrical $2^2 1^4 // 8$ screening design for optimizing alkaline transesterification.

released from 3-MCPD by means of sodium methoxide. The quintupled results of this study show that the equilibrium of the transesterification reaction is reached at 4 min (Fig. 2S) so it was decided to fix 4 min as the reaction time.

Most indirect approaches are based on the use of sodium methoxide (Almoselhy et al., 2021; Cui et al., 2021; Dingel & Matissek, 2015; Garballo-Rubio et al., 2017; Jędrkiewicz et al., 2016a, 2016b; Jędrkiewicz et al., 2017; Kuhlmann, 2019, 2021; Sadowska-Rociek, 2020; Stauff et al., 2020; Wang et al., 2016; Xu et al., 2020; Zwagerman & Overman, 2016) at different temperatures from –25–25 ºC. The use of sodium hydroxide has been proposed by Marc et al. (2016) and Jędrkiewicz et al. (2017). However, it requires hydrolysis times up to 960 min and temperatures down to –25 ºC, decreasing the cost-effectiveness of the method. Most of these processes perform stationary transesterification, leading to reaction times up to 1080 min (Kuhlmann, 2021). Vortex agitation has been proposed by Zwagerman & Overman (2016), and Cui et al. (2021). The use of vortex successfully decreased hydrolysis time down to 8.5 min (Zwagerman & Overman, 2016). Compared with the available bibliography, in this work a reduction in transesterification time is achieved thanks to the application of ultrasonic agitation, which translates to significant improvements in the total analysis time, cost and profitability of the method.

3.2. Optimization of ultrasound-accelerated acid transesterification method

Based on the results of the alkaline transesterification in terms of the amount of sample, only 0.1 and 0.3 g oil were studied (Fig. 3S.A). Results in terms of chromatographic area were higher at 0.3 g, as expected. This shows that sample mass has the same effect in both methods. Most acid transesterification approaches are widely performed by means of sulfuric acid. Most authors use a 1.8 % sulfuric acid in methanol solution. Volumes of this solution vary in accordance with the type and amount of sample, so in this case, three volumes including 0.9, 1.8 and 2.7 mL were assessed (Fig. 3S.B). Similar results were achieved using 1.8 mL and 2.7 mL in terms of recovery, so it was decided to use 1.8 mL of 1.8 % sulfuric acid for further experiments. To neutralize the sulfuric acid and stop the transesterification process the addition of 0.5 mL sodium hydrogen carbonate solution was used. Different concentrations including 50, 80, 100 mg/mL and a saturated solution were used to stop the reaction (Fig. 3S.C). All concentrations showed similar effectiveness stopping the acid transesterification in terms of recovery, so it was decided to continue using the 100 mg/mL solution. Furthermore, to extract the released 3-MCPD from the sample, 2 mL sodium sulfate was added to the system. Sodium sulfate concentrations at 100 and 200 g/L were assessed in terms of recovery percent (Fig. 3S.D). Recoveries closer to 100 % were achieved when using 200 g/L sodium sulfate. Most authors performed acid transesterification at 40 ºC with no agitation reaching reactions times from 1 to 16 h (Becalski et al., 2015; Sadowska-Rociek, 2019; Xu et al., 2020; Zelinkova et al., 2017). So, the effect of ultrasound agitation upon reaction time was assessed in terms of recovery by performing the acid transesterification at 15, 30, 45, and 60 min (Fig. 4S). As may be seen, after 30 min of ultrasound agitation, recoveries are similar to those of 16 h with no agitation. To study the effect of time and temperature over the acid transesterification a Doehlert design was performed in a total of 9 experiments, and it was evaluated in terms of recovery. Fig. 3 plots the two-dimensional estimated response surface for 3-MCPD released by acid hydrolysis which suggests optimal reaction conditions of 19 min of ultrasound at 40 ºC.

All previously published methods performed an acid approach with sulfuric acid (Arisseto et al., 2015; Becalski et al., 2015; Jiao et al., 2017; Ramli et al., 2011; Razak et al., 2012; Sadowska-Rociek, 2019; Wöhrlin et al., 2015; Xu et al., 2020; Zelinkova et al., 2017; Zheng et al., 2021), most of them with no agitation, which leads to hydrolysis times up to 960 min at temperatures between 40 and 50 ºC. Reaction time was successfully reduced by Zheng et al. (2021) using mechanical agitation

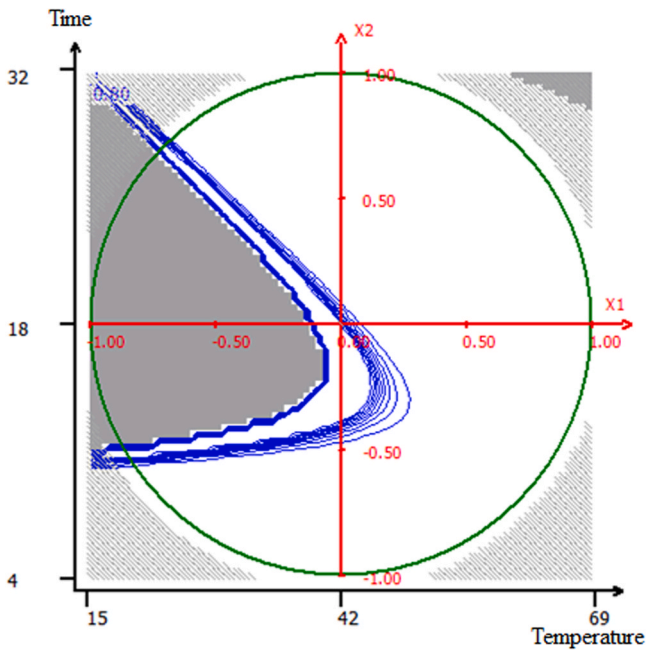

Fig. 3. Estimated response surface for 3-MCPD released by acid hydrolysis.

at 50 ºC in only 15 min. Similarly, the application of ultrasonic agitation allows the reduction of the reaction time, which is usually the longest step in indirect methods by acid hydrolysis, positively impacting the total reaction time and the yield of the method.

3.3. Clean-up and HFBI derivatization of released 3-MCPD

Clean-up and derivatization processes were identical for both approaches and optimized for them. Once 3-MCPD is released using either method, it is crucial to remove all reaction remnants that may interfere with the analyte determination. For this, a liquid-liquid extraction is often preferred. Hexane was proven to extract all lipids, fats and waxes originally present in the sample, as well as other non-polar compounds formed after the transesterification process. Performing two and three LLE cycles with 3 mL hexane achieved similar results (Fig. 5S.A) so it was decided to continue with two LLE cycles.

An aliquot of 2 mL of the clean extract was then transferred to a conical bottom tube and derivatized with HFBI using the Carro et al. (2013) method with slight modifications. The total aqueous volume was assessed at 7 mL and 10 mL in terms of recovery. The best results were achieved using 10 mL of aqueous phase, which is composed of the 2 mL clean extract and 8 mL milliQ water. This could be related to the fact that extract dilution significantly reduces the influence of any matrix interference that may remain, while the analyte is selectively and simultaneously derivatized by means of HFBI and extracted into the chloroform phase.

Most previously published methods performed PBA derivatization based on AOCS official methods (AOCS, 2017a, 2017b). However, other authors have proposed the use of cyclohexanone (Becalski et al., 2015) and HFBI (Cui et al., 2021; Jiao et al., 2017; Wang et al., 2016). But, conventional derivatization procedures involve stationary reactions which require lengthy times and additional cleanup steps prior to GC–MS analysis. By incorporating the Carro et al. (2013) procedure with slight modifications described above, both reaction time and determination limits are significantly reduced due to simultaneous derivatization and extraction. Thus, the methods developed here are performed in a total time of 20 and 32 min from sample collection to GC–MS data analysis.

3.4. Analytical features

Based on the FDA guidelines the analytical features of the alkaline and acid hydrolysis method were assessed (FDA, 2018, 2019). First, the selectivity and specificity of both processes are common (Table 1) since they are based on the selection of one quantifier and two qualifier ions at a specific retention time for 3-MCPD and 3-MCPDd5. Satisfactorily, analyte and the internal standard elute at different retention times with no matrix interferences observed for both methods (Fig. 4).

Determination limits, linearity, accuracy, and precision of both methods are presented in Table 3. It is notable that alkaline hydrolysis achieved 1.5 times lower LLOQ than acid hydrolysis. Moreover, both methods achieved excellent determination coefficients ($r^2 \geq 0.9995$) (Table S1). QC were used in assessing accuracy and precision for both methods. Accuracy results spanned 92.0–100.2 % of recoveries for the alkaline method and similarly from 98.5 % to 103.4 % for the acid method, both within acceptable criteria. Intraday and interday precision showed excellent results in terms of %RSD for both methods, with slightly lower % RSD in the acid method's intraday precision.

Previously published articles (Table S2) on the alkaline transesterification of 3-MCPDE reported quantification limits similar to or higher than the presented here (Cui et al., 2021; Garballo-Rubio et al., 2017; Jędrkiewicz et al., 2016a, 2016b, 2017; Kuhlmann, 2021; Marc et al., 2016; Shen et al., 2016; Wang et al., 2016; Zwagerman & Overman, 2016). Almoselhy et al. (2021) achieved lower quantification in edible oil limits than those reported here but used a 6-times larger sample size and a tandem mass spectrometer (MS/MS) detector. Sadowska-Rociek (2020) also reported lower limits in the analysis of dietary supplements based on fish oils. The proposed work demonstrates higher accuracy and precision compared to previous reports, as evidenced by the recoveries being closer to 100 % and the relative standard deviation (RSD) being ≤7.9 %. This indicates that the developed method yields results that are more consistent and reliable, with a smaller deviation from the true value.

As for the 3-MCPDE acid transesterification (Table 2), quantification limits reported here are similar or lower than those previously published (Arisseto et al., 2015; Jiao et al., 2017; Razak et al., 2012; Wöhrlin et al., 2015). This method is also more accurate and precise than prior studies. Xu et al. (2020) achieved lower quantification limits for both approaches in the analysis of edible oils using SPME with similar reaction times, accuracy and precision that those of the alkaline method but with lower recoveries and higher transesterification times than the acid approach reported here.

Table 2
Retention time, quantifier and qualifier ions and instrumental limits for total 3-MCPD.

Analyte	RT	Quantifier ion	Qualifier ions		LOD	LLOQ
	min	*m/z*	*m/z*	*m/z*	ng mL^{-1}	ng mL^{-1}
3-MCPD	12.74	253	289	453	3	5
3-MCPDd5	12.68	257	294	456	–	–

3-MCPD, 3-monochloropropane-1,2-diol; LOD, limit of detection; LLOQ, lower limit of quantification

3.5. Analysis of oil samples

These novel indirect protocols were used to determine total 3-MCPD from 20 different vegetable edible oils. Results of both methods are plotted in Table 4. As previously reported (Custodio-Mendoza et al., 2019), 3-MCPD was not present at any EVO sample processed with both methods. Refined olive oil yielded concentrations between 1.6 and 6.0 ng g^{-1} and 1.8–4.9 ng g^{-1} when using alkaline and acid methods. Among these oils RO 1º presented lower concentrations of 3-MCPD than those of RO 0.4º, as reported before, since these edible oils are mixtures of virgin and refined olive oils at different compositions: Virgin oil is the majority in RO 1º in contrast with RO 0.4º. POO presented the higher concentrations of analyte among olive oils, with concentrations among 5.3–6.8 and 5.3–6.4 ng g^{-1} when using alkaline and acid methods. Regarding sunflower oils, 3-MCPD varies from 2.0 to 3.3 ng g^{-1} and 2.1–2.7 ng g^{-1} when using alkaline and acid methods. The higher total 3-MCPD concentrations were found in red palm samples with concentration from 6.2 to 7.2 ng g^{-1} and 7.0–7.2 ng g^{-1} using alkaline and acid hydrolysis methods. These results are consistent with those previously reported by other authors, both by direct and indirect methods (Cui et al., 2021; Dingel & Matissek, 2015; Jędrkiewicz et al., 2016a).

Additionally, the results obtained from the analysis of the sample set using both alkaline and acid ultrasound-accelerated transesterification methods were subjected to a dependent t-test with a significance level (alpha) of 0.05 using the XLSTAT® software (Table S4). The calculated p-value for a one-tailed test was 0.0667, and for a two-tailed test, it was 0.13330. This indicates that there is no statistically significant difference between the means of the two methods. In other words, the results obtained from the two methods are not significantly different, and both methods yield comparable outcomes for the analyzed samples.

4. Conclusions

Two novel indirect approaches for total 3-MCPD have been

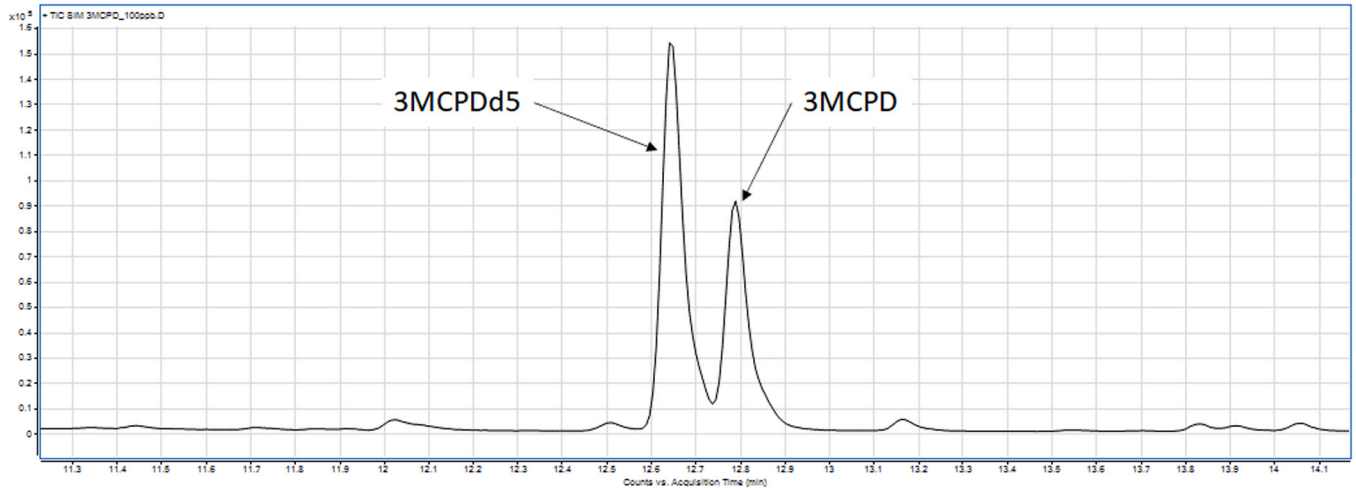

Fig. 4. Chromatogram of total 3-MCPD and 3-MCPDd5.

Table 3
Analytical features of the ultrasound-assisted transesterification methods for total 3-MCPD in edible oil.

Method	LOD	LLOQ	r^2	Accuracy (n = 3) % Recovery			Precision Intraday (n = 5) % RSD			Interday (n = 5) % RSD		
	μg g^{-1}	μg g^{-1}		L-Qc	M-QC	H-QC	L-Qc	M-QC	H-QC	L-Qc	M-QC	H-QC
Alkali	0.022	0.067	0.9995	100.2	93.5	92.0	7.9	7.7	2.1	3.7	6.0	4.2
Acid	0.030	0.100	0.9998	100.6	103.4	98.5	1.5	1.6	0.8	3.6	2.0	2.4

LOD, limit of detection; LLOQ, lower limit of quantification; %RSD, relative standard deviation; L-QC; low quality control at 0.5 μg g-1; M-QC; middle quality control at 1 μg g-1; high quality control at 3 μg g-1.

Table 4
Content of total 3-MCPD monoesters in edible oils.

Sample	Alkali		Acid	
	ug g^{-1}	±SD	ug g^{-1}	±SD
EVO_1	<LLOQ	–	<LLOQ	–
EVO_2	<LLOQ	–	<LLOQ	–
EVO_3	<LLOQ	–	<LLOQ	–
EVO_4	<LLOQ	–	<LLOQ	–
EVO_5	<LLOQ	–	<LLOQ	–
RO 1°_1	2.1	0.1	1.9	0.1
RO 1°_2	1.6	0.1	1.8	0.2
RO 0.4°_1	5.2	0.6	4.7	0.2
RO 0.4°_2	6.0	0.5	4.9	0.1
POO_1	6.2	0.8	5.8	0.1
POO_2	5.3	0.1	5.3	0.1
POO_3	6.5	0.1	6.2	0.2
POO_4	6.8	0.8	6.4	0.1
RSO_1	3.3	0.6	2.7	0.2
RSO_2	2.0	0.1	2.1	0.1
RSO_3	3.1	0.2	3.0	0.2
RSO_4	2.9	0.1	2.8	0.2
RSO_5	2.7	0.1	2.5	0.2
RPO_1	7.2	0.5	7.0	0.1
RPO_2	6.2	0.1	7.2	0.2

EVO, extra-virgin olive oil; RO 1°, refined olive oil 1°; RO 0.4°; refined olive oil 0.4°; POO, pomace olive oil; RSO, refined sunflower oil; RPO, red palm oil.

successfully developed and validated. 3-MCPD was released from 3-MCPDE by means of an alkaline and an acid transesterification. These reactions have been ultrasound-accelerated for the first time. The total 3-MCPD was HFBI-derivatized using an ultrasound-assisted dispersive liquid-liquid microextraction and analyzed by GC–MS. The optimization of both approaches was conducted, focusing on factors such as sample size, reagent amount, temperature, and agitation method. In the alkaline method, enhancements included optimizing the sample size to 0.3 g, selecting a 1 mL sodium methoxide solution, and using a cost-effective 25 ºC operating temperature. Sodium sulfate was employed as a neutralization agent without encountering salting-out issues. In the acidic transesterification, similar improvements were achieved, involving optimizing the sample mass to 0.3 g, selecting a 1.8 mL 1.8 % sulfuric acid solution, and using a 100 mg/mL sodium hydrogen carbonate solution to terminate the reaction. The addition of 2 mL of 200 g/L sodium sulfate improved extraction. Optimal conditions were determined as 19 min of ultrasound at 40 ºC. The results demonstrated that the alkaline method achieved a lower limit of quantification, while the acid method exhibited good accuracy and precision. Both methods showed comparable performance in terms of analytical features. Ultrasound significantly reduces reaction times, leading to faster, greener, and more cost-effective processes. Both methods were optimized using experimental design and validated based on FDA guidelines, achieving excellent sensitivity, specificity, accuracy, and precision - similar to or lower than those previously published.

Finally, 20 edible oil samples, including virgin and refined sunflower and olive oils and red palm oil, were analyzed using both methods. Results were compared and found to be similar to those previously reported. The variation in total 3-MCPD content among the oils studied appears to be linked to their processing levels. EVO has no detectable 3-MCPD, while mixed olive oil (RO 1° and 0.4°) exhibits varying 3-MCPD levels, with higher amounts in those with more refined oil (0.4°). RSO shows results similar to refined olive oil. POO, subjected to chemical solvent extraction after initial pressing, undergoes more processing and has higher 3-MCPD content. Similarly, RPO, which undergoes rigorous refining to remove impurities, also has elevated 3-MCPD levels.

From an overall comparison of both presented methodologies, it is observed that the alkaline approach requires smaller volumes of organic solvents, fewer distinct aqueous solutions, less waste production, and less reaction time and lower temperature. The analysis of 20 edible oils using both the ultrasound-accelerated alkaline and acid methods revealed no significant differences in the total 3-MCPD content, demonstrating that both methods provide comparable outcomes for the analyzed samples. The use of ultrasound-accelerated alkaline hydrolysis, after a proper matrix extension study, is recommended in total 3-MCPD occurrence studies from a greener perspective in food analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This work was supported by the Spanish Ministry of Science, Innovation, and Universities (Project RTI2018-096450-B-I00) and FEDER funds.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105764.

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