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Mitigation methods during physical refining for the reduction of 2- and 3-MCPD esters and glycidyl esters in (organic) high oleic sunflower and rapeseed oils

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ABSTRACT

2-monochloropropane-1,3-diol esters (2-MCPDE), 3-monochloropropane-1,2-diol esters (3-MCPDE), and glycidyl fatty acid esters (GE) are process contaminants formed during the production of refined edible oils and fats. The aim of this research was to develop mitigation strategies for the formation of these contaminants in organic oils, mainly high oleic (HO) sunflower and rapeseed oil, during physical refining. Different strategies such as a double refining with a high-low deodorization temperature, water washing and increasing amounts of bleaching earth were compared with a single physical refining (control treatment). Experiments were conducted in a pilot-plant refinery. Compared with the control treatment, double refining successfully reduced the concentration of formed of GE in HO sunflower and rapeseed oil, respectively by, 70% and 94%. In particular, the second degumming and bleaching were crucial for eliminating GE concentrations. Both 2- and 3-MCPDE were formed during temperature ramping, prior to the deodorization process and the concentrations of these contaminants remained stable throughout the refining process. Doubling the amount of bleaching earth led to an approximately 48% decrease of 2- and 3-MCPDE levels in final HO sunflower oil. No decrease was observed in rapeseed oil. The results of our studies provide useful insights which can directly be implemented by the (organic) vegetable oil industry.

1. Introduction

Vegetable oils and fats are important food ingredients. Usually, crude oils and fats are refined before using them in food production, with the aim to remove or reduce unwanted compounds, in this way increasing their quality and safety. However, during the refining process, undesired side reactions can also occur, leading to the formation of 2-monochloropropane-1,3-diol fatty acid esters (2-MCPDE), 3-monochloropropane-1,2-diol fatty acid esters (3-MCPDE) and glycidyl fatty acid esters (GE). The free forms of these compounds (2-MCPD, 3-MCPD and glycidol), which are completely released during digestion, has toxic properties (EFSA, 2017; EFSA Contam Panel, 2016). According to the International Agency for Research on Cancer (IARC), 3-MCPD is classified as a possible human carcinogen (category 2 B) while glycidol has been classified in the category 2 A as a probable human carcinogen (IARC, 2000; IARC, 2013).

Palm oil is one of the most susceptible oils for the formation of 2-MCPDE, 3-MCPDE and GE, though these processing contaminants can be formed in all kind of vegetable and animal oils and fats (Yung et al., 2023; Beekmann et al., 2022; Hew et al., 2020; Cheng et al., 2017; EFSA Contam Panel, 2016; Craft et al., 2013; Kuhlmann, 2011). The susceptibility of different vegetable oils and fats to the formation of 3-MCPDE is well studied. For instance, Matthäus, Freudenstein, et al. (2011) found that palm oil, corn oil, hazelnut oil and coconut oil had a higher capacity to form 3-MCPDE as compared to rapeseed oil, soybean oil and virgin olive oil. However, the possible formation of 2-MCPDE and GE in vegetable oils is less well studied.

In recent years, a need for reduction of processing contaminant levels in vegetable oils evolved. Food industry is faced with Maximum Levels (MLs) on 3-MCPDE and GE, that were established and revised in the EU in recent years. In 2018, the European Commission (EC) set MLs for the presence of GE in vegetable oils and fats of max. 1000 μg/kg. In 2020, these limits were revised by the EC, applying different MLs for 3-MCPDE: 1250 μg/kg of certain oils as rapeseed and sunflower oils and 2500 μg/kg for all non-specified oils and fats in the legislation (e.g. palm oil). Stricter limits apply for baby food (500 μg/kg for GE and 750 μg/kg

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for 3-MCPDE on all kind of oils and fats) (EC, 2023). These limits require the application of mitigation strategies to control (and reduce) the levels of these contaminants.

The formation of these unwanted, processing contaminants mainly occurs in the deodorization step in which the oils are treated at elevated temperatures for an extended period of time. Several previous studies have concluded that diacylglycerols (DAGs) and monoacylglycerols (MAGs) are the main precursors for GE formation while different chlorine compounds seems to be related with the formation of 2- and 3-MCPDE (Codex Alimentarius Commission, 2019; Destaillats et al., 2012; Gao et al., 2019; Hamlet et al., 2011; Matthäus & Pudel, 2022; Šmidrkal et al., 2016; Sulin et al., 2020). Different reaction pathways for the formation of these different processing contaminants imply that different approaches to control or mitigate them are needed. Therefore, a combined strategy could be promising to decrease the final levels of 2-, 3-MCPDE, and GE in refined oil (Oey et al., 2019; Codex Alimentarius Commission, 2019).

For oils with high gums and high acidity, the standard processing route is chemical refining to remove the hydratable gums and FFA. For products with high waxes content, winterization is done to remove them. This process step can be done on products physical or chemical refined.

Although chemical oil refining seems to be the best mitigation strategy to achieve the lowest concentrations of 2-, 3-MCPDE and GE in the refined palm oil (Yung et al., 2023; Oey et al., 2022; Matthäus & Pudel, 2022; Sulin et al., 2020), it is not always the preferred choice in the industry. During chemical refining, caustic soda is used for deacidification. Caustic soda binds the free fatty acids present on the oil creating a soapstock which is later washed out from the oil. The soapstock needs to be treated and disposed. This treatment also implies lower yield than physical refining. Usually losses on chemical refining are 2–3 times higher than physical refining, depending on starting acidity of the oil (AOCS, 2021; Gharby, 2022; Kovari et al., 2000).

Demand for organic food, including organic oils and fats, has been increasing in recent years and it is expected to growth further. The EC has specified a desired target of seeing 25% of total farmland dedicated to organic agriculture by 2030. Organic processing has to comply with specific process conditions that can limit the use of some of the established mitigation strategies for 2-MCPDE, 3-MCPDE and GE contaminants. For example, oils with higher amounts of gums, including sunflower oil and rapeseed oil, are usually treated with water degumming to remove the water-soluble gums after addition of an acid, usually citric acid or phosphoric acid (AOCS, 2021; Gharby, 2022). The use of phosphoric acid is not allowed on the production of organic oils by the vast majority of organic regulations (Bio Suisse, 2023; Japanese Agricultural Standard for Organic, 2022; Bioland, 2022; EC, 2021). Also some specific organic regulations as Bio Suisse (Bio Suisse, 2023), Bioland (Bioland, 2022) or Japanese Agricultural Standards (JAS, 2022) don't allow the use of chemical refining as refining method on organic oils and fats. Only few studies have been performed on mitigation of 2- and 3-MCPDE and GE in organic oils, specifically on organic palm oil that some pre- and post-treatments used during physical refining, such as a double refining with a high-low deodorization step, can help to reduce the formation of 2-, 3-MCPDE and GE. (Oey et al., 2022, 2020).

Therefore the research on mitigation strategies during the physical refining of different (organic) oils and fats to reduce the formation of 2-, 3-MCPDE and GE simultaneously is of great interest.

The aim of this study is to investigate mitigation strategies for the reduction of 2-, 3-MCPDE and GE in organic sunflower and organic rapeseed oil during physical refining conducted on a pilot scale. In this study different pre- and post-treatments were applied in HO sunflower oil and rapeseed oil to study their effect on the formation of 2-MCPDE and 3-MCPDE, with single physical refining as the control treatment.

2. Materials and methods

2.1. Materials

Crude organic HO sunflower oil originating from Ukraine was supplied by Spack B.V. (Nieuwe-Tonge, The Netherlands); this organic origin was certified by Skal Biocontrole (NL-BIO-01). Crude organic rapeseed oil originating from Ukraine was supplied by CARE Naturkost GmbH Co & KG (Sittensen, Germany); this organic origin was certified by Ecocert (DE-ÖKO-005). Both oils were organic certified on basis of Article 29 (1) of regulation (EC) No. 834/2007 and Regulation (EC) No. 889/2008. Citric acid and Dicalite® perlite 478 filter aid, purchased via Univar Solutions (Rotterdam, The Netherlands). Norit® SA 4 PAH-HF activated carbon was purchased from Cabot Norit Nederland B.V. (Amersfoort, The Netherlands). Pure-Flo® B80 natural bleaching earth was purchased from Oil-Dri (Ripley, Mississippi, USA).

3-chloro-1,2-propanediol-dipalmitate (PP-3-MCPD), 3-chloro-1,2-propanediol-dipalmitate-d5 (PP-3-MCPD-d5), 2-chloro-1,3-propanediol-dipalmitate (PP-2-MCPD), 2-chloro-1,3-propanediol-dipalmitate-d5 (PP-2-MCPD-d5), glycidyl palmitate (Gly-P), and glycidyl palmitate-d5 (Gly-P-d5) were bought in ampules of 1000 μg/mL in toluene from Chiron AS (Trondheim, Norway). Phenylboronic acid (PBA), sodium bromide (NaBr), anhydrous tetrahydrofuran (THF) containing 250 ppm BHT as inhibitor were purchased from Sigma-Aldrich (Schnelldorf, Germany). Sodium sulfate (Na_2SO_4), sodium hydrogen carbonate ($NaHCO_3$), and sulfuric acid (H_2SO_4) were purchased from Merck (Darmstadt, Germany). Acetone, iso-octane, methanol, and toluene were purchased from Actu-All Chemicals (Oss, The Netherlands). *n*-Heptane was purchased from VWR International (Fontenay-sous-Bois, France). PP-2-MCPD, PP-3-MCPD, Gly-P, PP-2-MCPD-d5, PP-3-MCPD-d5, Gly-P-d5 standards, H_2SO_4 in methanol solution, acidified NaBr solution, two $NaHCO_3$ solutions, Na_2SO_4 solution, H_2SO_4 solution, and PBA in acetone solution were prepared prior to the sample analysis.

2.2. Pilot plant experiments

Pilot plant experiments with the organic oils were conducted at Special Refining Company B.V. Details on the design and construction of the pilot plant can be found elsewhere (Oey et al., 2020). The pilot plant allows for conducting all refining stages; from degumming, through bleaching to deodorization, and for taking samples after every single refining step.

For each of the two oil types, one experiment was carried out consisting of one control treatment and four physical refining treatments. The control treatment was conducted simulating the standard refining conditions for these oils under regular industrial conditions, without any preventive measures. The four experimental treatment included: double refining (a rework of the control experiment); bleaching with a double quantity of bleaching earth, a water wash treatment before the degumming step, and a water wash treatment after the degumming step. Details of the experimental conditions of each of the four treatments are shown in Table 1. Prior to the beginning of each treatment, a preconditioning of the pilot plant was done. This preconditioning was similar to the control treatment and its objective was to "flush" the pilot plant. Between treatments the pilot plant was flushed with crude oil to remove any residuals of the previous treatment. Every experiment was conducted a single time (no replicates). Replicates were deemed redundant, as earlier pilot plant experiments on palm oil (Oey et al., 2020), showed that comparable 2-MCPDE, 3-MCPDE and GE results obtained when running the exact same experimental setup multiple times.

2.3. Analysis of 2-, 3-MCPDE, and GE

Sample preparation was performed following the AOCS Cd29a-13

Table 1

Experimental conditions of the four physical refining conditions (Treatment A - D) and one control treatment refined in the pilot plant.

Experiment	Water wash of crude oil	Degumming	Water wash	Bleaching	Deodorisation
Control treatment	–	70 °C, 20 min, 0.5% citric acid (from a 25% citric acid solution)	–	95 °C, 20 min, 1.0% Pure Flo® B80, 0.1% Norit®, 0.2% Dicalite 478®	260 °C (HO sunflower oil)/255 °C (rapeseed oil) for 60 min, strip-steam, 3 mbar vacuum. Then, cooling down until 30 °C
Treatment A – Double refining (re-refining of the control treatment)	–	Same as control	–	Same as control	220 °C for 60 min, strip-steam, 3 mbar vacuum. Then, cooling down until 30 °C
Treatment B - Double bleaching earth	–	Same as control	–	95 °C, 20 min, 2.0% Pure Flo® B80, 0.1% Norit®, 0.2% Dicalite 478®	Same as control
Treatment C - Water washing before degumming	70 °C, 20 min, 10% DI-water (water pre-heated to 70 °C)	Same as control	–	Same as control	Same as control
Treatment D -Water washing after degumming	–	Same as control	70 °C, 20 min, 10% DI-water (water pre-heated to 70 °C)	Same as control	Same as control

method with minor adjustments to improve the accuracy, sensitivity, and selectivity of the analytical method. The full details of the analytical method, including information on the adjustments and validation, can be found elsewhere (Oey et al., 2020, 2022). Briefly, a sample aliquot was taken and GE was converted to 3-monobromopropanediol esters (3-MBPDE) using an acid aqueous sodium bromide solution. Then, 2-, 3-MCPDE, and 3-MBPDE were extracted with n-heptane and subsequently, the transesterification was performed with sulfuric acid solution in methanol. After removal of the fatty acid methyl esters, the unbound 2-, 3-MCPD, and 3-MBPD were derivatized using PBA. After final clean-up, the PBA derivatives of 2-, 3-MCPD, and 3-MBPD are re-dissolved in iso-octane and analyzed by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). All results are expressed as mg/kg oil. As regards the variability of the analytical method for the analysis of 2-MCPDE, 3-MCPDE and GE, this was assessed during validation (Oey et al., 2020), and amounted 2–4% (repeatability, RSD_r) and 5–11% (reproducibility, RSD_{RL}).

3. Results and discussion

3.1. Physical refining control

Results of the control treatment (unmodified physical refining) performed on organic HO sunflower oil and organic rapeseed oil are shown in Fig. 1. For HO sunflower oil, at the end of the control treatment, 2-MCPDE, 3-MCPDE and GE concentrations were 0.35 mg/kg, 0.69 mg/kg and 1.11 mg/kg, respectively; for rapeseed oil, these concentrations were 0.09 mg/kg, 0.16 mg/kg and 0.63 mg/kg respectively.

The vast majority of 2- and 3-MCPDE was already formed during the temperature ramping to the start of the deodorization (at 260 °C for HO sunflower oil and 255 °C for rapeseed oil) and remained stable during the whole deodorization time. This behavior was also recently described by Oey et al. (2020). Similar findings for 3-MCPDE have also been reported by Hrncirik and van Duijn (2011) and Li et al. (2016) who also noticed that 3-MCPDE can be generated above 180 °C. After the temperature ramping to the final deodorization temperature was reached, most of the 2- and 3-MCPDE was formed and their concentrations only increased slightly during the entire deodorization time.

On the contrary, formation of GE during temperature ramping showed to be limited; at the start of the deodorization (0 min), 0.27 mg/kg GE was found in HO sunflower oil and 0.12 mg/kg GE in rapeseed oil. These concentrations increased substantially to 0.91 mg/kg and 0.65 mg/kg, respectively, after 40 min and to 1.14 mg/kg and 0.76 mg/kg, respectively, after 60 min at the deodorization temperature. Similar results were reported for sunflower oil and rapeseed oil by Bognar et al. (2018) who described that after deodorizing these oils above 220–240 °C higher concentrations than 0.5 mg/kg of GE may be formed. They also noticed that GE concentration increase with increasing the refining temperature and deodorization time. Experiments performed with (organic) palm oil also show that the deodorization conditions such as temperature and residence time affected the GE concentration (Craft et al., 2013; Hrncirik & van Duijn, 2011; Oey et al., 2020; Oey et al., 2022).

3.2. Effect of treatments on 2- and 3-MCPDE

2- and 3-MCPDE concentration of each treatment are represented in Fig. 2. Results obtained on HO sunflower oil show that the formation of 2- and 3-MCPDE decreases when pre-treatments are applied. When applying water washing before and after addition of citric acid in organic HO sunflower oil, the concentration of 2- and 3- MCPDE was decreased by 55 and 51% respectively. However, this effect was not seen on the treatments with rapeseed oil, showing even a very slight increase in 2- and 3-MCPDE concentration. The underlying reason of this slight increase is unclear. With doubling the amount of bleaching earth (compared to the control), a substantial decrease (approximately 48%) of 2- and 3-MCPDE concentration was observed for HO sunflower oil, but not in rapeseed oil. The double refining (rework) had no effect on the 2- and 3-MCPD concentration.

Previous studies (Hew et al., 2021; Pudel et al., 2011; Ramli et al., 2020; Ramli et al., 2011) also observed a 3-MCPDE decrease after water degumming using deionized water compared with no water degumming in palm oil (±76% less 3-MCPDE than the control treatment). Oey et al. (2022) found a reduction of approximately 17% when washing the palm oil before the addition of citric acid and a 36% reduction when performing the water washing after the addition of citric acid. Matthäus and Pudel (2013) found a 38% reduction of the 3-MCPDE concentration after water washing while Zulkurnain et al. (2013) found a reduction of this compound up to 80%, reporting a final concentration of 0.2 mg/kg after water washing on palm oil. Similar results were reported by Redeuil et al. (2021) on sunflower oil, who tested a combined scenario of water washing and long cooling. The reduction of 2-MCPDE is only scarcely investigated. 2-MCPDE was reduced for at least 50% by washing the crude oil in the experiments performed by Ramli et al. (2020) on palm oil and by Redeuil et al. (2021) on sunflower oil. Oey et al. (2022) found a reduction of approximately 18% when washing the oil before the addition of citric acid and 34% when performing the water washing after the addition of citric acid on organic palm oil. The results of our current study are comparable to those from previous studies.

Several studies have been done investigating effects of different kinds and amounts of bleaching earth on 2- and 3-MCPDE values in palm oil. According to Ramli et al. (2011), natural bleaching earths and acid

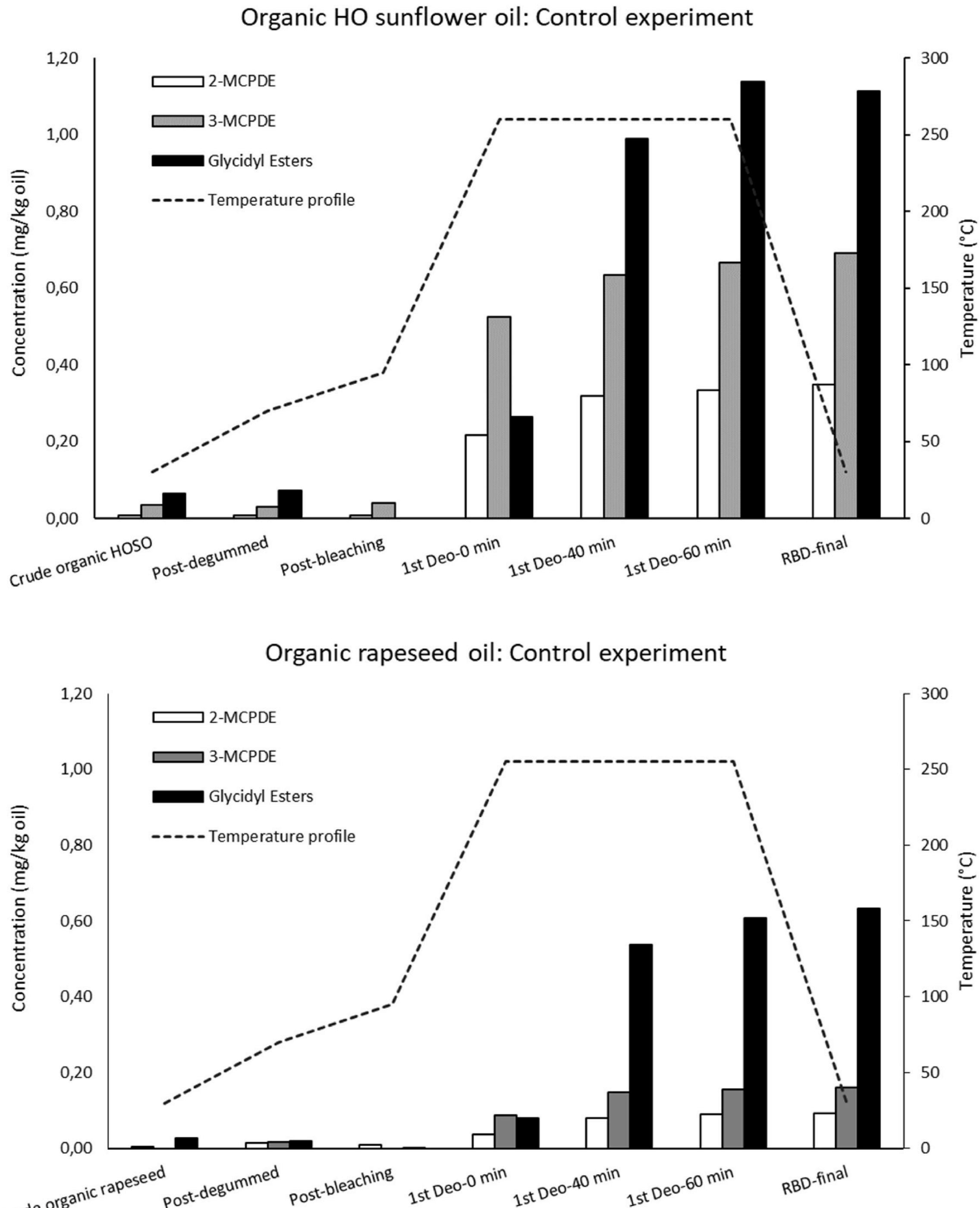

Fig. 1. Results of the control experiment for organic HO sunflower oil and organic rapeseed oil. The oil temperature profile for all experiments are shown as a reference (dashed line). The samples were collected and displayed chronologically.

activated earths with more neutral pH should be considered as options to reduce the formation of 3-MCPDE. Pudel et al. (2011), tested the effect of adding 0.7% and 1.5% of activated bleaching earth. According to their results, the amount of added bleaching earth had no significant effect on the formation of 3-MCPDE. On the other side, a study by Matthäus, Freudenstein, et al. (2011) showed a significant decrease of 3-MCPDE when 3% bleaching earth was used instead of 1%. In our experiments, doubling the amount of natural bleaching earth resulted in a decrease of 2- and 3-MCPDE concentrations of approximately 48% when compared to the control, which is in line with results of Matthäus, Freudenstein, et al. (2011).

Re-work of the refined product including dry degumming, bleaching and deodorization at 220 °C did not have a significant effect on the concentrations of 2- and 3-MCPDE in the double refined oils. This was also found by Oey et al. (2020) and Shimizu et al. (2013) in similar experiments with palm oil.

All the pre-treatment experiments tested in this study showed to be effective for the reduction of the 2- and 3-MCPDE in the refined oil product. The combination of water washing and bleaching with double quantity of bleaching earth could eventually reduce the 2- and 3-MCPDE concentration even more, but this has not been studied yet. Research on effects of combination strategies could help to determine even more

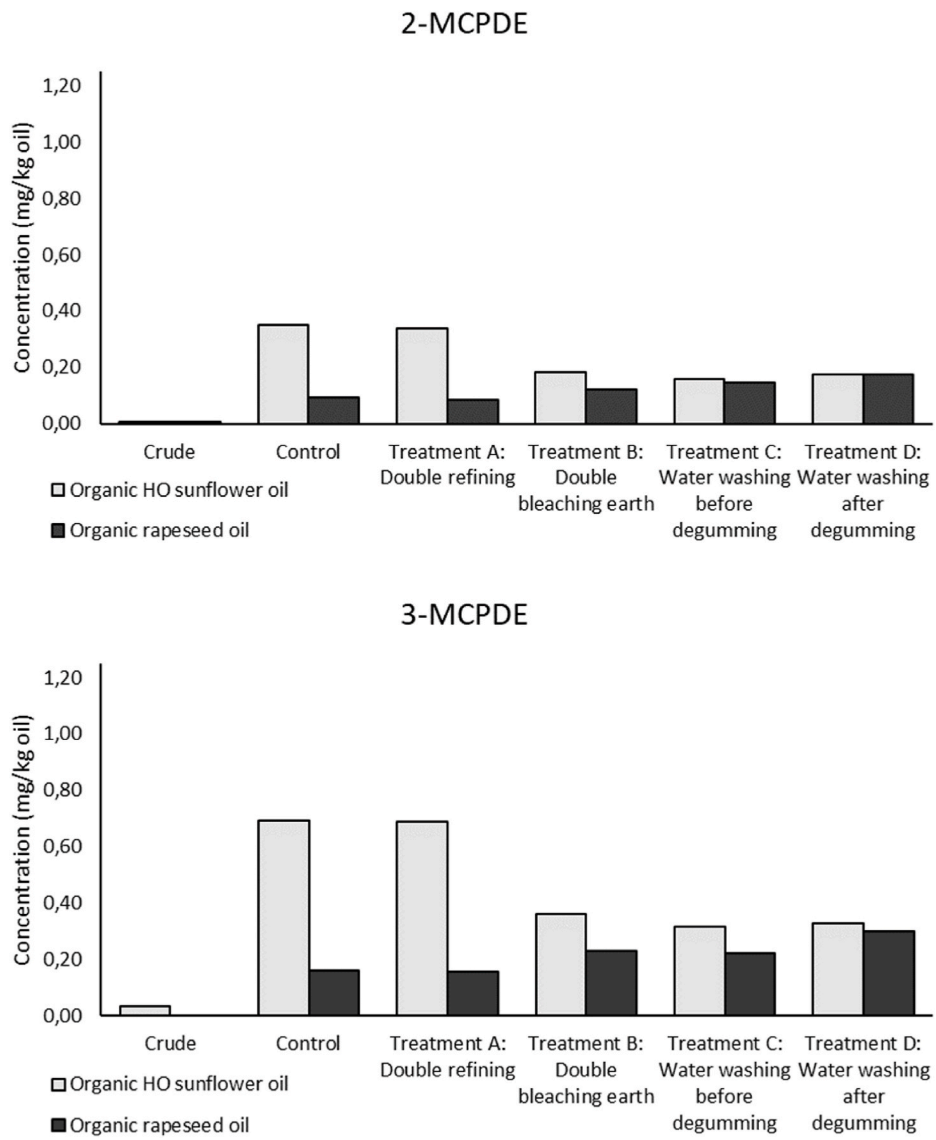

Fig. 2. 2- and 3-MCPDE results for different treatments (control, treatment A, B, C and D) performed on organic HO sunflower oil and organic rapeseed oil. The values represent those in the final oil obtained after completion of the entire refining process.

effective future strategies for lowering the 2- and 3-MCPDE concentrations to comply with the stricter limits for baby food (3-MCPDE maximum concentration of 0.75 mg/kg) or for infant formula (3-MCPDE maximum concentration of 0.125 mg/kg).

3.3. *Effect of different treatments on glycidyl esters*

The effects of the different pre- and post-treatments on GE formation in organic HO sunflower oil and organic rapeseed oil are shown in Fig. 3. Bleaching with double quantity of bleaching earth shows no changes on final GE concentrations when compared to the control treatment. The same was found for water washing: this treatment showed little to no effect on the GE concentrations. The re-work (treatment A) showed a substantial decrease of the GE concentration by 70% on rapeseed oil and 94% on HO sunflower oil.

Contrary to the current observations, in previous studies performed on palm oil by Oey et al. (2022) and Silvia et al. (2019), an increase in GE was observed when the palm oil was washed before deodorization. This could be explained due to the fact that the water washing process could hydrolyze the TAG into DAGs, which is a known precursor for the formation of GE, as stated by Silva et al. (2019). They observed an increase of 20% on DAGs concentration on water washed palm oil, topping at 3.0% of DAGs which corresponded with an increase of 25% of GE concentration, topping at 1.00 mg/kg. Redeuil et al. (2021) also reported higher GE values after performing a water washing on sunflower oil. However in our experiments with sunflower and rapeseed oil no significant changes on GE have been noticed after water washing. Differences in results between the different experiments could be due to the contact time of water with the oil. During our experiments, fats, such as palm oil, made emulsions easier than oils, such us sunflower oil and rapeseed oil, which could be originated by eventually cooling down of the product when water was added. This could imply longer reaction time between water and oil which would facilitate the hydrolysis of TAGs into DAGs. Another possible reason is that oils with higher FFAs or with more aggressive extraction methods are more unstable and - with contact with water - TAGs and DAGs hydrolyzes to DAGs and MAGs, respectively, easier.

After double refining a substantial decrease of the GE concentration by 70% on rapeseed oil and 94% on HO sunflower oil was observed. Such a reduction has also been noticed by Shimizu et al. (2012) who used acid-activated bleaching earth to remove GE from different types of vegetable oils. Oey et al. (2020) found a reduction of 87% of the GE

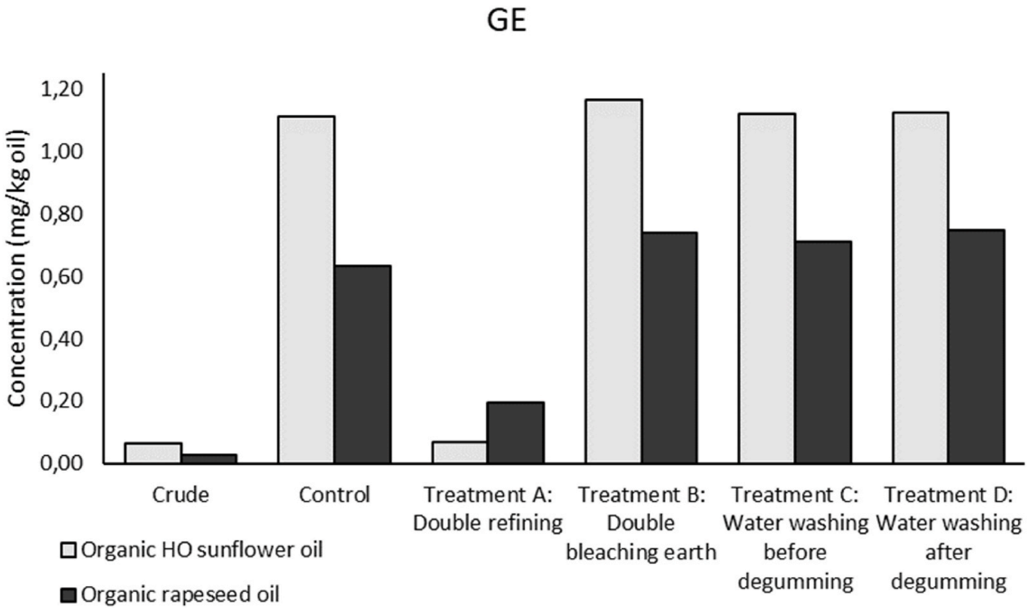

Fig. 3. GE on different treatments (control, treatment A, B, C and D) performed on organic HO sunflower oil and organic rapeseed oil. The values represent those in the final oil obtained after completion of the entire refining process.

concentration by double refining with a high-low deodorization temperature on palm oil where the combination of a second degumming followed with a bleaching step were critical for this reduction. This behavior can also be seen in Fig. 4, where the double refining with high-low temperature process is shown, detailing all steps in the entire process. After the second degumming and bleaching of the already refined organic HO sunflower oil and organic rapeseed oil, a decrease of, respectively, 94 and 70% of the GE concentration was observed. These low GE concentration were maintained throughout the second deodorization at a lower temperature of 220 °C. This would indicate that during the second degumming and bleaching some degradation or adsorption of GE occurs.

Double refining with high-low deodorization temperature seems to be the best strategy to achieve very low values of GE. This could be very beneficial for vegetable oil production in which low GE concentrations are required e.g. oils and fats destinated for baby food or infant formula.

A possibility to be considered is to avoid the second deodorization step by stopping after the second bleaching. In that case the lowest GE values would be achieved and the process would be cheaper and faster. This would only be possible when a separate bleacher dedicated for refined product would be used to avoid possible contamination with crude oil which can contain other kind of contaminants such as microorganisms. However, it should be investigated before implement the adapted process of the double refining experiment performed if the non-

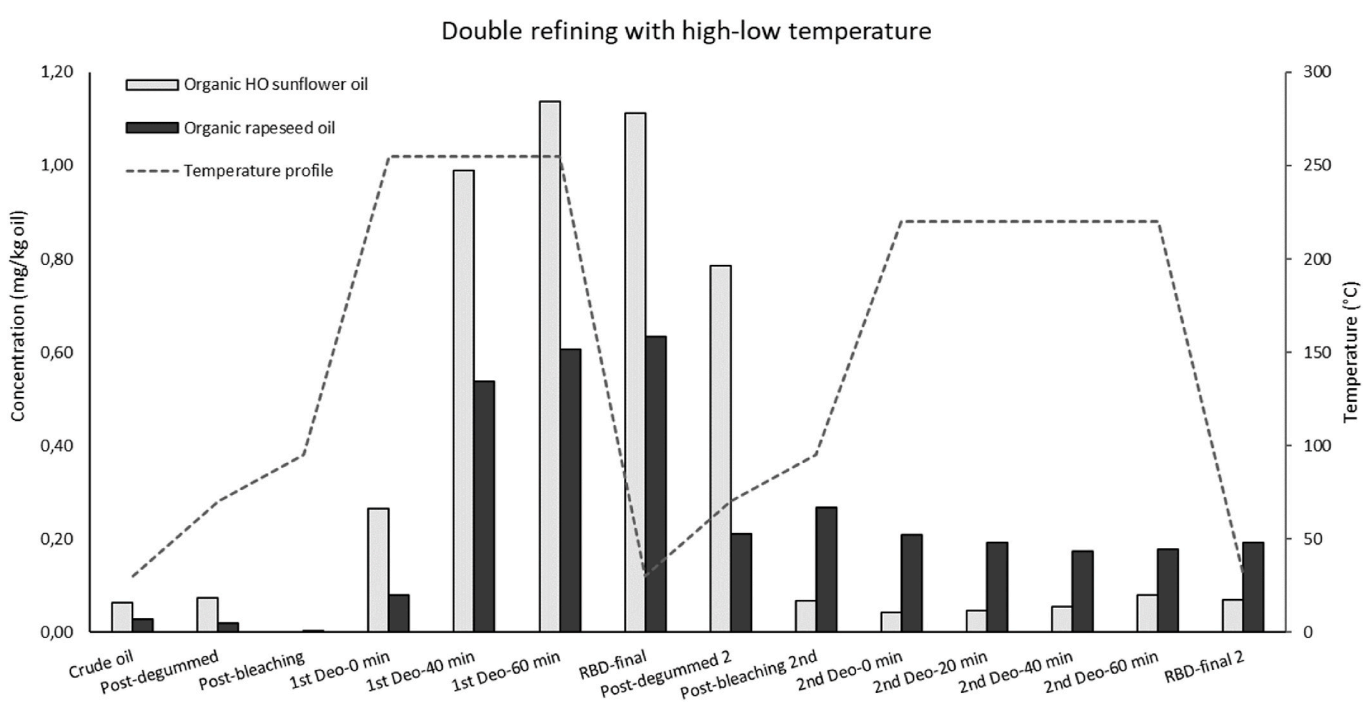

Fig. 4. GE evolution during double refining high-low temperature on organic HO sunflower oil and organic rapeseed oil (treatment A).

deodorization step after the second bleaching process has an impact on other quality parameters important on refined oils and fats such as neutral taste and odor as some bleaching earths can alter the taste. Moreover, the cost and benefits of such approach versus full double refining should be evaluated.

4. Conclusions

This is the first study reporting on the investigation of mitigation strategies on the formation of 2-, 3-MCPDE and GE which are applicable to the refining of organic HO sunflower and organic rapeseed oil.

This study revealed that different strategies need to be implemented for the reduction of the different kinds of processing contaminants during refining of vegetable oils. Double refining with a high-low deodorization temperature strategy showed to be the best mitigation strategy to reduce GE on the refined oils, resulting in a reduction of at least 70% of the GE concentration when compared to the control. This reduction of GE occurs mainly in the second bleaching process, just before the second deodorization step (low deodorization temperature).

For the reduction of MCPDE values, the best strategies are to avoid their formation before the deodorization step by washing the oil before bleaching or by increasing the amount of bleaching earth during the bleaching process. These strategies showed to result in a decrease of 2- and 3 MCPDE of at least 50% and 48%, respectively on organic HO sunflower oil.

CRediT authorship contribution statement

Cristina Mayayo: Writing – original draft, Visualization, Methodology, Investigation. **Sergio B. Oey:** Writing – review & editing, Methodology, Investigation. **H.J. van der Fels-Klerx:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Stefan P.J. van Leeuwen:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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